

REGULATION OF HUMAN TELOMERASE ALTERNATIVE SPLICING

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## **DEDICATIONS**

I dedicate my dissertation to my family and friends. I feel especially grateful and a deep sense of appreciation for my dearest parents, Siu Kwong and Dip Fan Wong, for their unconditional love, kindness, and support. Special gratitude goes to Deirdre Davis and Sarah R. Gonzales van Horn, who not only have been there for me throughout the entire doctorate program but have truly touched my life and made an impression on my heart. To the ones I love, I sincerely thank you.

REGULATION OF HUMAN TELOMERASE ALTERNATIVE SPLICING

By

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by

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## REGULATION OF HUMAN TELOMERASE ALTERNATIVE SPLICING

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### ABSTRACT

Telomerase adds TTAGGG repeats onto chromosome ends (telomeres). Since telomerase is expressed in ~90% of all human cancer cells while being absent in most somatic tissues, telomerase is an almost universal cancer therapeutic target. Yet, the clinical application of an effective telomerase inhibitor is still lacking. The pre-mRNA of the catalytic subunit of human telomerase (*hTERT*) may be alternatively spliced into 22 different isoforms. Only a small fraction of *hTERT* transcripts are spliced into the full length isoform, the form capable of being translated into function *hTERT* with reverse transcriptase activity. If telomerase activity is partially regulated at the level of RNA splicing, then telomerase activity may be modulated to

increase the amount of nonfunctional transcripts and decrease the amount of full-length *hTERT* and this would be a novel anti-cancer therapeutic approach.

A *hTERT* minigene was created to understand the cis-regulatory elements governing *hTERT* splicing. A 1.1kb region of 38 bp repeats (block 6) ~2 kb from the exon/intron junctions is essential for the exclusion of exons 7 and 8. Block 6 repeats suggested that RNA:RNA pairing may regulate splicing of *hTERT*. Mutations within the repeat sequence that abolish exon skipping were corrected by compensatory mutations in the pre-mRNA. To identify trans-acting regulators of *hTERT* alternative splicing, the minigene was modified into a dual-luciferase reporter for a selected 528 RNA-binding proteins/splicing factors siRNA screen. Our initial validation focused on 45 factors with enzymatic activity and resulted in CDC-Like Kinase 1 (CLK-1) as a potential *hTERT* splicing modulator. Knock-down of CLK-1 altered *hTERT* splicing, resulting in reduction in telomerase activity and telomere shortening. CLK-1 belongs to the Clk family of dual-specificity nuclear kinases that can auto-phosphorylate SR proteins. TG003, a chemical CLK-1/4 inhibitor, results in less full length *hTERT* splicing and a decrease in cancer cell telomerase activity. This approach provides a platform to identify additional *hTERT* splicing regulators that may be suitable drug targets to increase or decrease telomerase activity. Altogether, these results demonstrate the potential of manipulating *hTERT* splicing as a target for both chemotherapy and regenerative medicine.

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## PRIOR PUBLICATIONS

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Ling, J., Liao, H., Clark, R., Wong, M. S. & Lo, D. D. Structural constraints for the binding of short peptides to claudin-4 revealed by surface plasmon resonance. *The Journal of biological chemistry* **283**, 30585-30595, doi:10.1074/jbc.M803548200 (2008).

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## LIST OF ABBREVIATIONS

ALT – Alternative Lengthening of Telomeres

AVG - Average

BMD – Becker Muscular Dystrophy

BP - Basepair

cDNA – Complementary DNA

CLK-1 – CDC-Like Kinase 1

CTD – Carboxy-Terminal Domain

DDX1 – DEAD (Asp-Glu-Ala-Asp) Box Helicase 1

DDX3Y – DEAD (Asp-Glu-Ala-Asp) Box Helicase 3, Y-Linked

DDX41 – DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 41

DHX38 - DEAH (Asp-Glu-Ala-His) Box Polypeptide 38

DMD – Duchenne Muscular Dystrophy

DNA – Deoxyribonucleic Acid

E2A - *Equine rhinitis* A virus 2A peptide

EJC – Exon-Junction Complex

ESE - Exonic Splicing Enhancer

esiRNA - Endoribonuclease-Prepared Small Interfering RNA

ESS – Exonic Splicing Silencer

FAM120A – Family with Sequence Similarity 120A

HSP90B1 - Heat Shock Protein 90kDa Beta (Grp94), Member 1

*hTERT* – Human Telomerase Reverse Transcriptase

ISE – Intronic Splicing Enhancer

ISS – Intronic Splicing Silencer

mRNA – Messenger RNA

PCR – Polymerase Chain Reaction

PPM1G - Protein Phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup> Dependent, 1G

Pre-mRNA – Pre-messenger RNA

PRPF4B - Pre-mRNA Processing Factor 4B

PTC – Premature Termination Codon

RNA – Ribonucleic Acid

RPM – Rotations per Minute

SDS – Sodium Dodecyl Sulfate

SEAP - Secreted Embryonic Alkaline Phosphatase

SF1 – Splicing Factor 1

SF2/ASF - Serine/Arginine-Rich Splicing Factor 1

siRNA – Short Interfering RNA

shRNA - Short Hairpin RNA

SMARCA5 - SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin,  
Subfamily a, Member 5

SMN2 - Survival of Motor Neuron 2

snRNP – Small Nuclear Ribonucleic Particle

STDEV – Standard Deviation

T2A - *Thoseaasigna* Virus 2A peptide

TAA – Tumor Associated Antigen

TRAP – Telomeric Repeat Amplification Protocol

TRF – Telomere Restriction Fragment

UPL – Universal Probe Library

USP39 - Ubiquitin Specific Peptidase 39

VNTR – Variable Number of Tandem Repeat

# CHAPTER ONE

## INTRODUCTION

### **I. An Introduction to Alternative Splicing**

#### **The Basics of Alternative Splicing**

Alternative splicing affects about 95% of the genes in multicellular eukaryotes, allowing for the generation of over 100,000 proteins from about 20,000 protein-coding sequences and greatly expanding the coding capacity of eukaryotic genomes (Nilsen and Graveley, 2010). RNA splicing is the process of joining of the exon coding sequences by the removal of the noncoding interspersed intron sequences. Splicing occurs co-transcriptionally, where the splicing machinery called the spliceosome is recruited and assembled around the emerging splice sites as polymerase II transcribes the nascent pre-mRNA ((Schmidt et al., 2011), reviewed in (Goldstrohm et al., 2001). The splice sites are almost always indicated by nucleotides GU at the 5' end of the intron (the splice donor site) and nucleotides AG at the 3' end of the intron (the splice acceptor site) (Figure 1-1). The spliceosome is a large and dynamic ribonucleoprotein that is composed of five small nuclear ribonucleic particles (snRNP) core components (U1, U2, U4, U5, and U6) and about 300 other proteins (reviewed in (Jurica and Moore, 2003). Spliceosome components assemble in an ordered and step-wise manner at each 5' and 3' splice sites, branch point, and polypyrimidine tract in order to facilitate intron removal in the form of a lariat and subsequent exon ligation (Figure 1-1) (Beyer and Osheim, 1988; Tennyson et al., 1995).

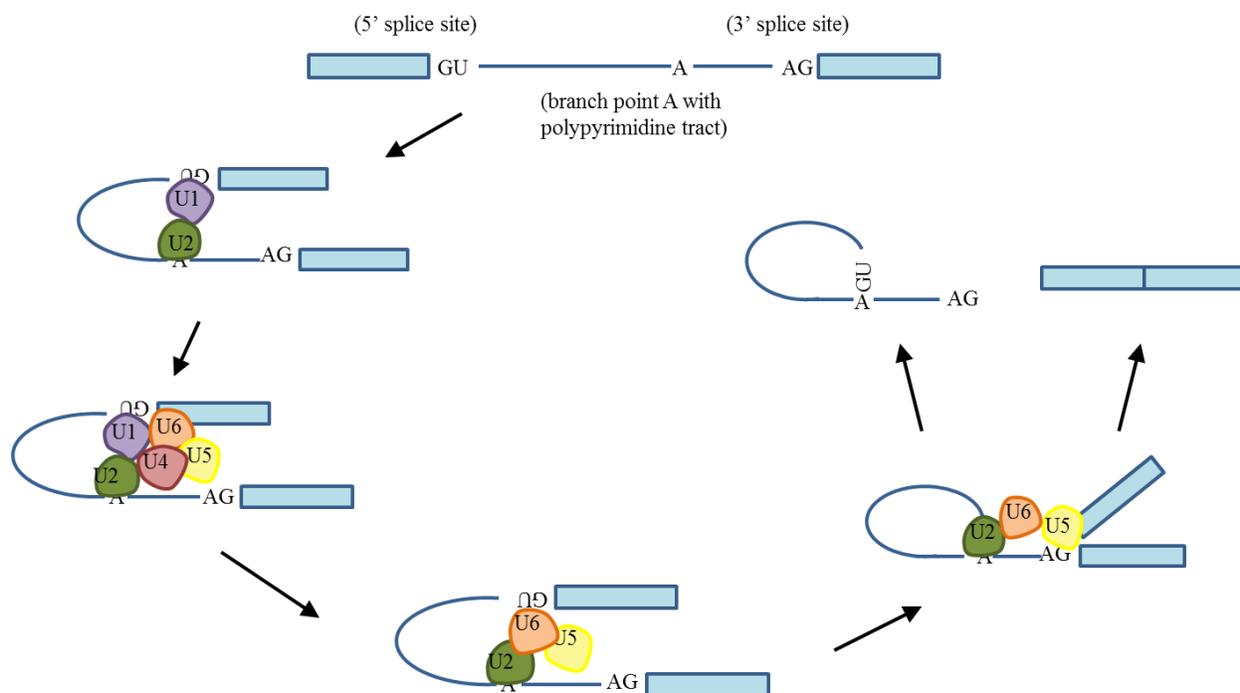


Figure 1-1. Diagram illustrating the step-wise process of RNA splicing. The U1 snRNP and U2 4snRNP of the spliceosome assembles at the 5' splice site and branch point A respectively to form complex A. Recruitment of U4-U5-U6 snRNPs forms complex B, which goes through several rearrangements to form the catalytically active complex C. The intron is released as a lariat, the exons are ligated together, and the snRNPs disassemble.

An exon may be constitutive (where it is always included in the mRNA of a gene) or alternative (where it may be included or excluded in the mRNA). The choice between strong splice sites (those that closely adhere to the consensus sequence that are preferentially recognized as the splice site) versus weak splice sites (those with suboptimal recognition sequences that have one or more mismatch from the consensus sequence) leads to preferential usage of particular splice sites, therefore alternative splicing occurs. Alternative splicing may result from the usage of alternative 5' splice sites or alternative 3' splice sites, the inclusion/exclusion of alternative exons, the choice of alternative mutually exclusion exons, the usage of cryptic splice sites that leads to intron retention, or the usage of alternative polyadenylation site. The usage of a splice site may be enhanced or suppressed by its proximity

to local cis-regulatory sequences such as exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) and intronic splicing silencers (ISSs) (reviewed in (Roca et al., 2013)). The cis-regulatory sequences are in turn bound by trans-acting factors, which are RNA-binding proteins or splicing factors.

| Basic Splicing Terminology        |  |
|-----------------------------------|--|
| Types of exon                     |  |
| Constitutive exon                 | An exon that is always included in the mRNA  |
| Alternative exon                  | An exon that may be included or excluded due to alternative splicing                         |
| Modes of alternative splicing     |  |
| Exon skipping                     | An exon is excluded or spliced out in the transcript   |
| Mutually exclusive exons          | One of two exons is included in the transcript   |
| Alternative 5' donor sites        | An alternative 5' splice junction is used where the 3' end of the upstream exon is changed   |
| Alternative 3' acceptor sites     | An alternative 3' splice junction is used where the 5' end of the downstream exon is changed |
| Intron retention                  | Intronic sequences are included in the transcript after splicing                             |
| Cis-acting elements               |  |
| Exonic splicing enhancers (ESE)   | Sequences within exons where activators bind   |
| Exonic splicing silencers (ESS)   | Sequences within exons where repressors bind   |
| Intronic splicing enhancers (ISE) | Sequences within introns where activators bind   |
| Intronic splicing silencers (ISS) | Sequences within introns where repressors bind   |
| Trans-acting proteins             |  |
| Activators                        | Proteins that bind to enhancer sequences to promote the usage of a splice junction           |
| Repressors                        | Proteins that bind to silencer sequences to reduce the usage of a splice junction            |

Table 1-1. Definition of the basic splicing terminologies.

There are more than 500 RNA-binding proteins/splicing factors that can participate in alternative splicing. These splicing regulatory proteins generally have both a RNA recognition motif (to bind single-stranded RNA) and a protein interaction domain (to interact with other proteins) (Sanford et al., 2005a). Although the members of the Ser/Arg-rich (SR) protein family

were initially thought of as splicing activators and the members of the heterogeneous nuclear ribonucleoprotein (hnRNP) protein family as splicing repressors, many trans-acting factors may act as either activators or inhibitors depending on the relative location of its binding to the splice site (Dirksen et al., 2000). These RNA binding proteins bind to the pre-mRNA with low specificity, which allows the proteins to bind and detach from the pre-mRNA during the splicing process. As a consequence of the low RNA binding specificity, the control of alternative splicing often relies on the cooperative binding of splicing factors to a short RNA motif sequence, which is often assisted by the phosphorylation state of the protein (Singh and Valcarcel, 2005). Splicing factors are often post-transcriptionally modified by phosphorylation, glycosylation, and/or methylation. Altering the phosphorylation status of a splicing factor can change its ability to interact with other proteins and/or binding to RNA as well, ultimately affecting splice site selection (Shen and Green, 2006; Xiao and Manley, 1997). In addition, changing the phosphorylation status of a splicing factor alters its subcellular localization, which can in turn change splice site selection (reviewed in (Stamm, 2008)). The reversible regulation of a splicing factor by phosphorylation will be discussed in a later section.

In a simplistic view, the outcome of alternative splicing is to either generate transcripts with protein-coding potential or transcripts that are destined for degradation by nonsense-mediated decay. The exon-junction complex (EJC) plays a major role in RNA surveillance and determining the fate of the spliced mRNA. The EJC is a complex of proteins that is deposited onto mRNA 20-24 nucleotides upstream of exon-exon junctions during RNA splicing. At the initial phase of translation, the ribosome scans the entire length of the processed mRNA and displaces the EJCs until it reaches the translation termination codon (Ishigaki et al., 2001; Lejeune et al., 2002). In the case where a premature termination codon (PTC) is used, the EJCs

downstream of the PTC will recruit non-sense mediated decay proteins, leading to the degradation of the PTC-containing mRNA (Culbertson and Neeno-Eckwall, 2005; Ishigaki et al., 2001; Maquat, 2004).

Besides splicing regulatory proteins and the EJC acting as RNA surveillance factors, the regulation of alternative splicing is intricately related to many other cellular processes. Splice site selection and pre-mRNA splicing are intimately connected to transcription, 5'-end capping, and 3'-end polyadenylation. Eukaryotic RNA polymerase II (Pol II) is a DNA-dependent RNA polymerase that is responsible for transcribing pre-mRNAs. A variety of proteins, including splicing factors, bind to the carboxy-terminal domain (CTD) of RNA Pol II subunit Rpb1 to facilitate pre-mRNA synthesis and coordinate co-transcriptional processing events (Darzacq et al., 2007). Splicing factors may provide a link between these processes by remaining bound to the pre-mRNA throughout the processing steps. In addition, RNA has the natural tendency to form stable secondary and tertiary structures as nascent pre-mRNA are being transcribed (Brion and Westhof, 1997; Conn and Draper, 1998; Fontana et al., 1993). Chromatin organization and histone modifications are yet additional layers of complexity in splicing regulation. DNA is tightly compacted into a chromatin structure by wrapping around nucleosomes, which are composed of nine histone proteins. In order for Pol II to gain access to DNA and for transcription to occur, these chromatin structure needs to partially disassemble and reassemble (reviewed in (de Almeida and Carmo-Fonseca, 2014). The interconnected network of co-transcriptional processing events, the role of RNA secondary structure, and the effects of chromatin structure on alternative splicing will be discussed in more details in a later section. Finally, deregulation of splicing may have detrimental consequences to the cell. Many diseases have been correlated with

deregulation of splicing or splicing factor expression, which will be discussed at the end of this section.

### **Regulation of Splicing**

In addition to the presence of cis-regulatory sequences and the abundance of trans-acting factors, there are many complex layers of alternative splicing regulation encompassing a large variety of mechanisms, including but not limited to exon/intron length, secondary structure the RNA, Pol II interactions, and chromatin structure. Furthermore, splicing is intricately related to other processing steps, such as transcription, 5'-end capping, 3'-end polyadenylation, and nuclear export (Graveley, 2005a; Moore, 2005).

Splicing factors are often regulated by post-transcriptional modifications, such as phosphorylation, glycosylation, and/or methylation. Splicing factors may be phosphorylated and dephosphorylated by different kinases and phosphatases to control their ability to interact with other proteins and/or binding to RNA as well, ultimately affecting splice site selection (Shen and Green, 2006; Xiao and Manley, 1997). Splicing factors shuttle between the nucleus and the cytoplasm accompanying the pre-mRNA as it is being processed and exported. This process is mediated by reversible phosphorylation (Huang and Steitz, 2005; Sanford et al., 2005b). As an example, diagramed in Figure 1-2, SF2/ASF is phosphorylated in the cytoplasm by SR protein kinases SRPK1 and SRPK2 at a serine residue of the RS domain. Phosphorylation of SF2/ASF facilitates its nuclear import and accumulation in nuclear speckles (a subnuclear compartment that is enriched in splicing factors). Inside the nuclear speckle, a family of cdc2-like nuclear kinases hyper-phosphorylates SF2/ASF, causing it to be released from speckles to participate in splicing events. During splicing, SF2/ASF is dephosphorylated by protein phosphatase 1 (PP1)

which helps facilitate its nuclear export with the processed mRNA. In response to stress and other cellular changes, different signal transduction pathways are activated that alters downstream kinase or phosphatase activities, which in turn can alter the phosphorylation status of splicing factors and their availability to participate in splicing events (van der Houven van Oordt et al., 2000).

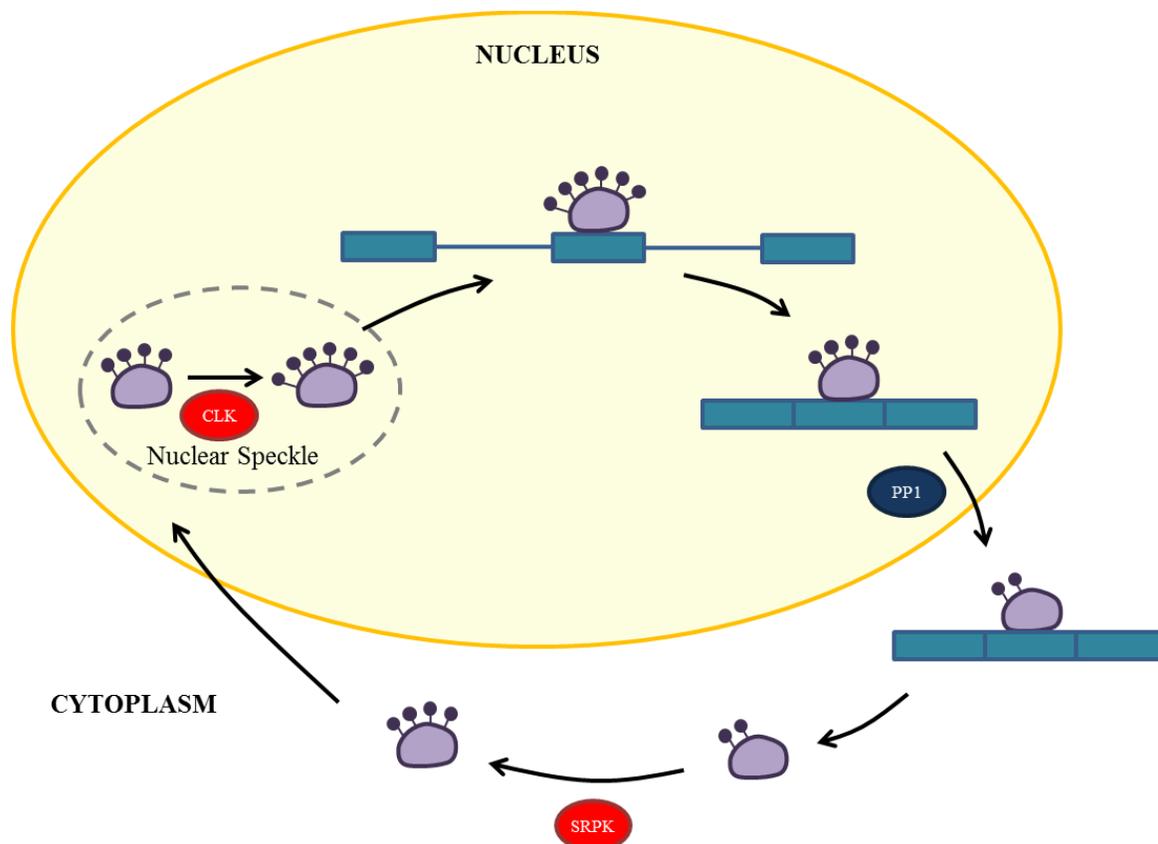


Figure 1-2. Phosphorylation of SF2/ASF facilitates with shuttling. SF2/ASF (depicted in purple) is hyper-phosphorylated (depicted by purple dots on a stick) by CLK protein in the nuclear speckle, which prepares it to participate in splicing events. Hyper-phosphorylated SF2/ASF binds to pre-mRNA and facilitates RNA splicing. PP1 dephosphorylates SF2/ASF to mediate its export into the cytoplasm along with the processed mRNA. There, SF2/ASF is recycled by rephosphorylation by SRPK, which allows the phosphorylated SF2/ASF to import back into the nuclear speckle of the nucleus.

The eukaryotic RNA Pol II CTD provides a docking site for a variety of proteins to bind and facilitate pre-mRNA synthesis and coordinate co-transcriptional processing events, including

the initiation of transcription, 5' end capping of the RNA transcript, 3' end formation by cleavage/polyadenylation, and RNA splicing (reviewed in (Kornblihtt et al., 2013)). Splicing of Pol II transcribed pre-mRNA is more efficient than splicing of pre-synthesized pre-mRNA or T7 polymerase transcribed pre-mRNA (Bird et al., 2004; Ghosh and Garcia-Blanco, 2000). Furthermore, RNA polymerase II elongation kinetics and pausing can also affect alternative splicing. Slow Pol II rate may promote the usage of weak splice sites by allowing more time for the spliceosome recognition and recruitment, whereas fast Pol II rate may preferentially use strong splice sites (de la Mata et al., 2003; Roberts et al., 1998). Using a mutant slow polymerase, more inclusion of the weak fibronectin ED-I exon was observed compared to using the wild-type polymerase (de la Mata et al., 2003; Munoz et al., 2009).

As the nascent pre-mRNA is being transcribed, RNA has the natural tendency to form stable secondary and tertiary structures (Brion and Westhof, 1997; Conn and Draper, 1998; Fontana et al., 1993). RNA secondary structure is another element in splicing regulation. RNA structure can interfere with spliceosome assembly and/or splicing enhancer/inhibitor binding by masking splice sites, branch sites, or RNA protein binding sites (reviewed in (Jacobs et al., 2012)). The formation of a RNA stem-loop structure near the 5' splice site in exon 7 of the gene termed survival of motor neuron 2 (SMN2) prevents snRNP U1 from binding to the splice site, therefore inducing exon 7 skipping (Singh et al., 2007). RNA secondary structure can also cause the "looping-out" of an exon, causing that exon to be skipped. Bioinformatic analyses revealed complementary motifs in introns upstream and downstream of skipping exons, supporting that base-pairing interactions between the complementary motifs can induce exon skipping (Howe and Ares, 1997; Miriami et al., 2003).

Chromatin organization and histone modifications provide additional layers of complexity in alternative splicing regulation. DNA wraps around nucleosomes to form chromatin structures. During transcription, chromatin needs to partially disassemble for Pol II to bind and then reassemble after transcription. Nucleosomes are preferentially positioned over exons where DNA methylation and histone modifications (especially H3K36me3) are enriched. Alternative exon nucleosome positioning correlates with exon inclusion (Tilgner et al., 2009). Intronic regions are depleted in nucleosome binding. Nucleosome positioning may enhance exon inclusion by causing Pol II pausing and increasing the time for recognition of splice sites (Gomez Acuna et al., 2013).

### **Splicing and Diseases**

While some RNA binding proteins are highly tissue-specific, most splicing factors are expressed in all tissue types with different expression levels in different tissues (Hartmann et al., 1999). Deregulation of splicing factor expression has been linked to a variety of human disorders such as muscular dystrophies, premature aging disorders, and cancer (Bonnal et al., 2012; Tazi et al., 2009; Wang and Cooper, 2007). Although some RNA binding proteins may be over- or under-expressed in cancer, others may have acquired mutations that alter its own splicing or its function in the splicing of downstream genes directly (Venables et al., 2009). Several splicing regulators, such as SRSF1, SRSF6, SRSF9, hnRNP A2/B1, and hnRNP H, have been shown to have oncogenic properties, whereas RBM5, RBM6, and RBM10 have been shown to act as tumor suppressors (reviewed in (Ladomery, 2013)). Recent genome-wide studies show that changes in splicing factor expression may activate splicing program changes in many cancer-related genes that subsequently promote cancer growth (Kim et al., 2008; Ritchie et al., 2008).

In addition to the changes in splicing factor function/abundance, mutations in splice site sequences or enhancer/inhibitor binding sequences can also change splice site selection to correlate with the development of disease. Several mutations are known to affect the splicing of oncogenes, tumor suppressors, and other cancer relevant genes (Srebrow and Kornblihtt, 2006; Venables, 2006). Several skeletal system diseases are associated with splice site mutations in genes, such as, COL1A1, SEDL, and LRP5, which leads to aberrant splicing. Over 90% of patients with osteogenesis imperfecta disorder (also known as brittle bone disease) correlate with a mutation in COL1A1 and COL1A2, which encodes two alpha chains of type I collagen (Pollitt et al., 2006). There are 102 splice site mutations identified in COL1A1, which may induce the usage of a cryptic splice site that leads to a reading frame shift and the production of truncated non-functional proteins (Marini et al., 2007).

### **Therapeutic Approaches to Correct Splicing**

Since splicing deregulation is associated with an array of human diseases, many have sought to develop splicing correction therapeutics. In particular, antisense oligonucleotides (AONs) have been implemented to redirect alternative splicing, restore splice site usage, and skip defective exon(s). AONs hybridize to a sense target sequence to form a DNA:RNA hybrid which sterically blocks the access of cellular machinery to pre-mRNA and masks splicing signals (Kole and Sazani, 2001). This antisense-mediated exon skipping therapeutic approach has been especially successful in treating Duchenne muscular dystrophy (DMD). DMD is a neuromuscular disorder that is caused by mutations in the DMD gene that results in the deletions of one or more exons, disrupts the open reading frame, and therefore produces a truncated and non-functional dystrophin protein (Emery, 2002; Hoffman et al., 1987). Dystrophin normally

links the cytoskeleton to the extracellular matrix and is responsible for maintaining muscle stability during contraction (Matsumura and Campbell, 1994). The truncated form of dystrophin is unable to maintain muscle fiber stability, leading to gradual muscle damage in DMD patients. Using AONs to skip a more dispensable parts of the gene in DMD patients, the reading frame of the DMD gene can be restored to produce a truncated but functional dystrophin that can convert the severe DMD case into the milder phenotype seen in Becker muscular dystrophy (BMD) (Matsuo et al., 1990; Matsuo et al., 1991). Although phase II and III clinical trials are underway for using AONs as a therapeutic approach for DMD, there are several challenges in using AONs as a mainstream gene therapy approach, including the heterogeneity of disease-causing mutations, the ability to target AONs to affect tissue, and an effective systemic delivery system.

## **II. An Introduction of Telomere Biology**

### **Telomeres and the End Replication Problem**

Telomeres are dynamic DNA-protein structures at the end of chromosomes that prevent chromosome ends from being recognized as DNA damage, such as double-stranded DNA breaks (Blackburn, 2001). Telomere repeats are bound by a shelterin protein complex that is composed of TRF1, TRF2, TIN2, RAP1, TPP1, and POT1 (Palm and de Lange, 2008). Together, the proteins of the shelterin complex recognize and bind to telomere repeats and promote the formation of a structure called a T-loop at telomeric ends by interacting with the 3' guanine-rich termini of the telomere overhang, therefore concealing overhangs and preventing telomere degradation at DNA checkpoints (Griffith et al., 1999; Stansel et al., 2001).

Initially, each human chromosome is capped by 15-20kb of telomeric TTAGGG repeats. DNA polymerases only work in the 5' to 3' direction and require a nucleic acid primer for semi-

conservative DNA replication (Watson, 1972). Due to incomplete replication of the DNA lagging strand at the ends of the chromosomes, telomeres shorten with every cellular division. This process is called the end-replication problem (Levy et al., 1992; Olovnikov, 1973). When a telomere becomes critically short, DNA damage signaling is induced resulting in cells undergoing a growth arrest termed replicative senescence (Figure 1-3) (Harley, 1991; Wright et al., 1996a). The limited proliferative capacity of cells is widely accepted as an “aging time clock” mechanism that cells use to prevent unlimited cell growth leading to the accumulation of mutations over time and potentially permitting cells to progress to malignancy (Harley, 1991; Kim et al., 1994; Shay and Bacchetti, 1997).

### **Telomerase and Cancer**

To overcome the limited growth due to replicative aging, cancer cells almost universally up-regulate or re-express telomerase to re-elongate or maintain telomeres sufficiently to avoid a DNA damage signal. Telomerase is a ribonucleoprotein composed of a catalytic component with reverse transcriptase activity (*hTERT*) that uses an RNA component (*hTR* or *hTERC*) as a template to elongate telomeres (Greider, 1990). Although telomerase is initially expressed in all cells during early fetal development, its expression is rapidly repressed to almost undetectable levels in somatic cells. Only a small subset of stem cells and proliferating progenitor cells maintain telomerase expression post-development (Wright et al., 1996b). Some normal cells, such as lymphocytes, may exhibit transient telomerase activity in response to stimulation (Chebel et al., 2009).

Re-expression of telomerase prevents the progressive shortening of telomeres and replicative senescence (Figure 1-3) (Shay and Bacchetti, 1997). It was shown that expression of

*hTERT* in telomerase-negative normal diploid cells results in cell immortalization by reconstituting telomerase activity without progressing cells to cancer (Bodnar et al., 1998; Morales et al., 1999). On the other hand, expression of a dominant negative mutant *hTERT* in telomerase expressing cancer cell lines results in decreased telomerase activity, progressive telomere shortening, and eventually to cell death (Colgin et al., 2000). Due to its crucial role in providing cells with unlimited ability to divide (immortality), telomerase activity has been detected in the vast majority (~90%) of cancer cells. The remainder telomerase-negative tumors often use a recombination-based mechanism named Alternative Lengthening of Telomeres (ALT), which relies on recombination-mediated elongation of telomeres (Bryan et al., 1997).

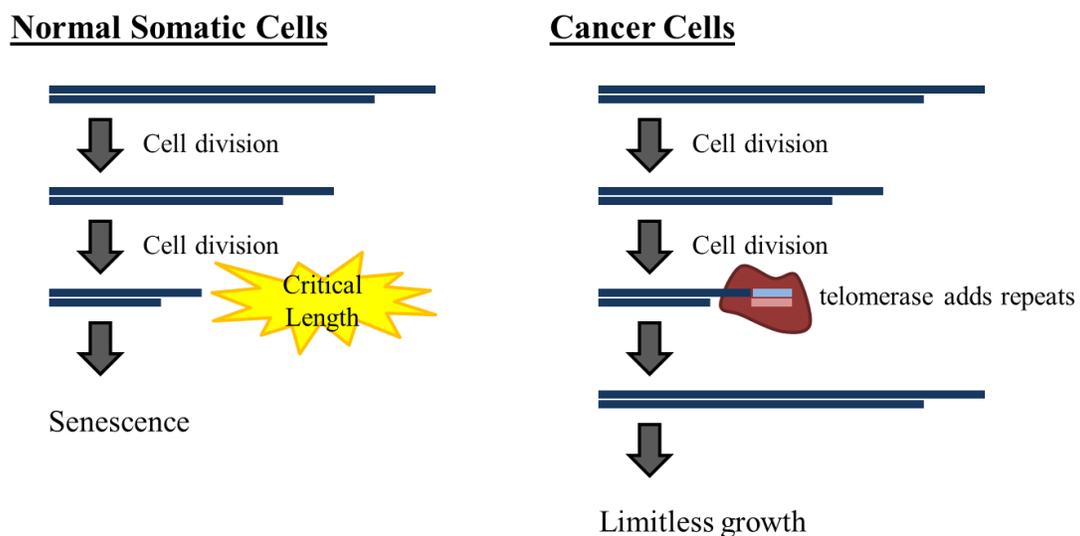


Figure 1-3. Telomerase expression provides cancer cells with immortality. In normal somatic cells, telomeres shorten with every cellular division. When a telomere reaches a critical length, cellular senescence is induced. In cancer cells, the expression of telomerase allows telomere to elongate and evade cellular senescence, so that cancer cells are immortal.

## Regulation of Telomerase

Telomerase is subject to an array of transcriptional, post-transcriptional, and epigenetic levels of control, but there is no consensus on the precise mechanism regulating telomerase repression during development and re-expression of telomerase in cancer cells. Regardless of whether the cells have telomerase activity or not, almost all cells have an excess amount of the *hTR* RNA template of telomerase. In contrast, *hTERT* is detected at relatively low levels in stem cells, progenitor cells, and even in cancer cells. This suggests that the regulation of telomerase activity primarily depends on regulating the expression of *hTERT* as the limiting factor for telomerase activity. The current best estimate for the number of catalytically active telomerase molecules per cell is 50-100 (Cohen et al., 2007), produced from approximately 20 mRNA molecules per cell (Yi et al., 2001).

Several transcription factors, including both activators and repressors, may interact with the *hTERT* promoter and regulate *hTERT* expression at the transcriptional level. CCCTC-binding factor (CTCF) was shown to bind to the promoter of telomerase-negative cells but not in telomerase-positive cells, suggesting that CTCF may repress *hTERT* expression in normal cells (Renaud et al., 2005). The proximal E-box upstream of the transcriptional start site may be bound by c-Myc, which acts as an activator of *hTERT* transcription and promote tumorigenesis in selected cancer cells (Cong et al., 1999). In addition, the G-rich sequence within the *hTERT* promoter may form G-quadruplexes, which are helical structures formed by stacking of GGGG tetrads (Gellert et al., 1962; Lim et al., 2010; Simonsson, 2001). G-quadruplex ligands may bind to these high-ordered structures and in turn alter *hTERT* transcription (Gomez et al., 2004; Lim et al., 2010).

Epigenetic modulation of the *hTERT* promoter may provide additional control of *hTERT* expression. A cluster of CpG sites found within the *hTERT* promoter suggests that *hTERT* may be controlled, at least in part, by DNA methylation, however there is no consensus on the effects of promoter methylation on *hTERT* expression. Histone 3-Lysine 9 (H3-K9) modification of the *hTERT* promoter may affect the ability of cMyc to bind to the E-box and subsequently alter *hTERT* expression (Iliopoulos et al., 2009). Acetylation of the *hTERT* promoter has been another avenue of epigenetic control of *hTERT* expression that has been examined (Choi et al., 2010; Meeran et al., 2010). More recently, the alternative splicing of *hTERT* as another level of *hTERT* control has gained attention and will be further discussed in a later section.

### **Therapeutic Approaches to Telomerase**

Telomerase expression is almost specific to cancer cells with the exception of progenitor cells and stem cells. Due to the accelerated proliferation rate of cancer cells, their telomeres are generally short in comparison to the longer telomeres seen in the rarely dividing progenitor cells and stem cells. Thus, inhibiting telomerase activity should result in telomere shortening leading to apoptosis of cancer cells while having little to no effects on progenitor and stem cells. For these reasons, telomerase has been well recognized as an attractive and almost universal target for cancer therapeutics. Significant efforts have been expended to develop cancer therapeutics targeting telomerase, yet the development of telomerase inhibitors has been largely unsuccessful. A variety of approaches have been undertaken to inhibit telomerase activity, such as antisense oligonucleotides, ribozymes, G-quadruplex stabilizers, natural compounds, small molecule inhibitors (BIBR1532), and RNA interference. Although many of these telomerase inhibitors have inhibitory effects in *in vitro* systems, they rarely progress into clinical trials due to lack of

potency, low specificity, and/or high toxicity. A major challenge remains to identify an effective therapeutic against telomerase is that telomerase is expressed at relatively low abundance even in cancer cells.

Immunization against tumor associated antigens (TAA) has been proposed more than a century ago but clinical application has largely failed due to the need to find specific TAAs for a specific type of tumor and the heterogeneity of the tumor (Klebanoff et al., 2011; Mocellin et al., 2004). Telomerase-based immunotherapy poses a new and promising avenue for immunization against cancer. TERT-derived peptide can be recognized by cytotoxic CD8<sup>+</sup> T lymphocytes in a MHC class I restricted manner (Vonderheide et al., 1999). Patients with solid tumors vaccinated with the telomerase vaccine produce an immunological response and pre-clinical models have shown tumor regression with telomerase vaccination (Brunsvig et al., 2011; Mavroudis et al., 2006; Nair et al., 2000; Peruzzi et al., 2010; Schmidt et al., 2006). The successes of small scale telomerase vaccination studies have led telomerase immunotherapy moving forward into advanced clinical trials.

Imetelstat (GRN163L) is a modified oligonucleotide which binds to the template region of the RNA component of telomerase, acting as an active site inhibitor by preventing telomere access to telomerase (Marian et al., 2010). Imetelstat has shown successes in inhibiting telomerase activity in xenograft models (Joseph et al., 2010). Although it has progressed into phase II clinical trials, Imetelstat has shown little promise whether it will achieve sufficient and sustained inhibition in patients to drive cancer cells into crisis. Development of additional telomerase inhibitors, used in combination with standard cancer therapy, may enhance the potential of telomerase as a therapeutic target.

### **III. An Introduction of Alternative Splicing in Telomerase**

#### **Telomerase Splicing Isoforms**

The 42 kb telomerase (*hTERT*) gene on human chromosome 5p15.33 containing 16 exons can be spliced into multiple isoforms (Kilian et al., 1997). To date, 22 isoforms of *hTERT* has been identified (Hrdlickova et al., 2012). Besides the full length transcript with all 16 exons, none of the identified alternative spliced forms has reverse transcriptase activity (Saeboe-Larssen et al., 2006; Yi et al., 2000). Whether the other isoforms of *hTERT* can be translated into proteins and have functions related to telomerase or not *in vivo* remains largely unexplored. The alternatively spliced isoforms within the reverse transcriptase domain include minus alpha, minus beta, or both (minus alpha beta) (Figure 1-4). The minus alpha splicing isoform uses an alternative 3' splice acceptor site 36 bp into exon 6, resulting in an in-frame transcript that is translated into a dominant negative protein without reverse transcriptase activity (Colgin et al., 2000; Yi et al., 2000). Overexpression of the minus alpha transcript inhibits telomerase activity in telomerase positive cell lines that either results in cell death or senescence (Colgin et al., 2000). The minus beta splicing isoform skips exons 7 and 8, creating a frame-shift and encounters a pre-mature stop codon in exon 10. The minus beta isoform is the major alternatively spliced component of *hTERT* transcripts in cultured cancer cells and its steady state level is at least 5 times less than its rate of production since it is subject to degradation by non-sense mediated decay (data not shown). Although minus beta is subject to non-sense mediated decay, its transcripts may be translated into protein and overexpression of the minus beta protein may offer a growth advantage to breast cancer cells (Listerman et al., 2013).

Only a small fraction of the *hTERT* transcripts are spliced into the full length form that can generate the catalytically active protein. A working hypothesis to explain these results is that

the transcriptional machinery is unable to reduce transcription to a level that produces only 1-2 mRNA molecules per cell, so that ~20 mRNA molecules per cell might represent the lower limit of transcriptional regulation. The cell then disposes of this excess transcription by alternatively splicing most into non-functional forms in order to reduce the number to the 1-2 mRNA full length molecules sufficient to produce the very low levels of protein needed to maintain telomere length in cancer cells. Very little is currently known about the regulation of extremely low abundance proteins, such as telomerase, even though this gene was identified over 15 years ago.

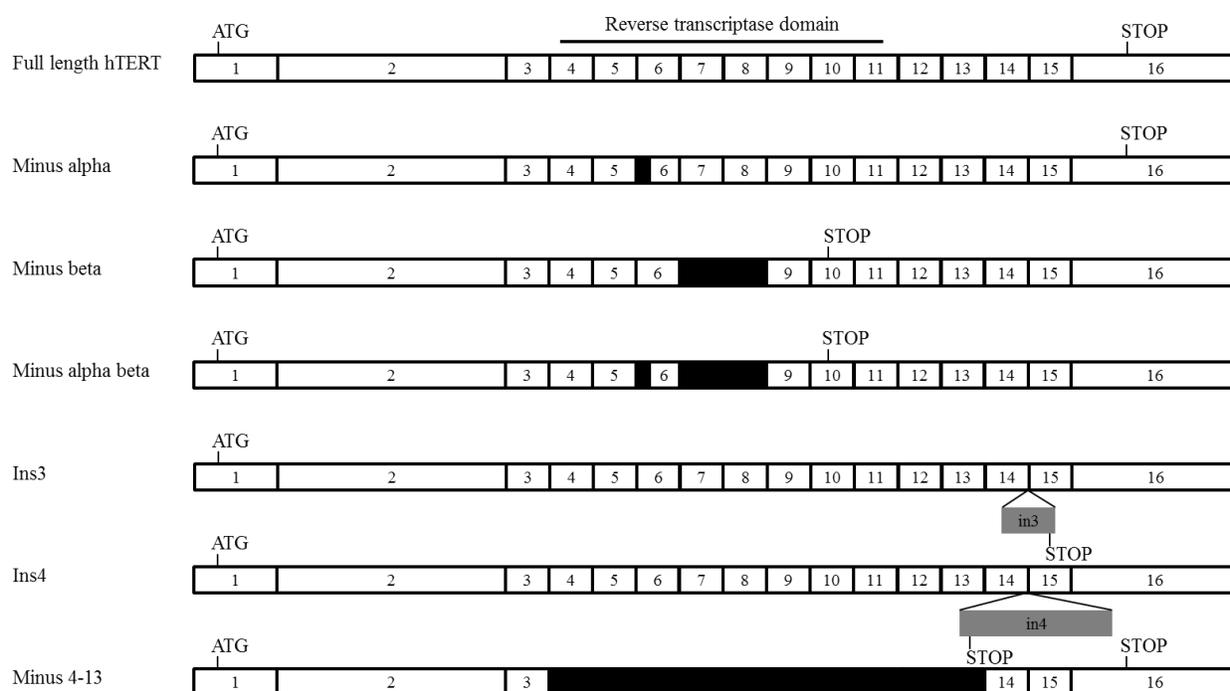


Figure 1-4. Diagram illustrating the exons and introns of human telomerase (not to scale). Functional telomerase with reverse transcriptase activity is made from the full length transcript containing all 16 exons. The splicing isoforms within the RT domain (minus alpha and minus beta) are indicated. The minus alpha splicing isoform creates a dominant-negative protein. The minus beta splicing isoform creates a frame-shift and a premature stop codon as indicated in exon 10. Ins3 and ins4 both have intron retention and can produce protein with unknown function. Minus 4-13 is the predominant isoform of *hTERT* seen in telomerase-negative cells.

## **Telomerase Splicing During Development, in Normal Cells, and in Cancer cells**

During tissue development, telomerase activity disappears before transcripts because of a dramatic shift in splicing pattern from full length *hTERT* to mostly minus beta and other isoforms without reverse transcriptase activity (Ulaner et al., 2001). This shift in *hTERT* splicing is a highly regulated process that is both tissue- and time- dependent. *hTERT* was traditionally believed to be transcriptionally silenced in somatic cells post-development because the common method of examining *hTERT* splicing uses primers that spans from exon 5 to 9, leading to the false assumption that no transcription of *hTERT* occurs in somatic cells after development. More recently, it was determined in our laboratory that *hTERT* transcription rate in somatic cells remains largely the same during and after development (data not shown). Rather, a large proportion of the pre-mRNA in normal somatic cells are spliced into an isoform with the skipping of exons 4-13, which was not previously detected using primers restricted within the reverse transcriptase domain (Figure 1-4). This suggests that alternative splicing plays a key role in the regulation of telomerase activity during development.

In addition to transcriptional changes, *hTERT* splicing in cancer cells largely reverts back to the splicing pattern seen during development in which the pre-mRNA is spliced into mostly the minus beta isoform with only a smaller proportion of full length transcripts that can be translated into functional reverse transcriptase. Telomerase alternative splicing in a variety of cancer cell lines, including DLD-1 (colorectal adenocarcinoma), HeLa (cervical carcinoma), 293FT (embryonic kidney cells), RKO-1 (colon carcinoma), H1299 (non-small cell lung cancer), HT29 (colorectal adenocarcinoma), and HCT116 (colorectal carcinoma) are shown in figure 1-5. The ratio of transcripts spliced into each of the isoforms varies slightly between each line with an overall similar splicing pattern as cells in developing tissues. Consistent with the common theme

in cancer, the alternative splicing of *hTERT* appears to be reprogrammed in cancer cells to return to its embryonic status.

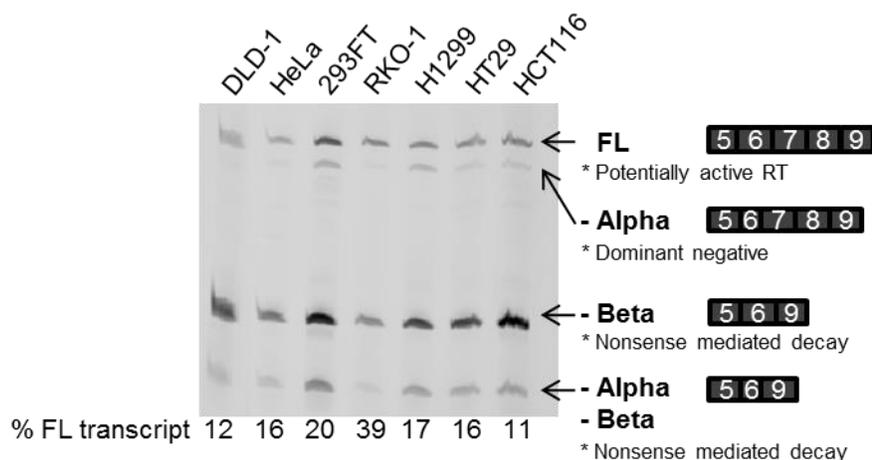


Figure 1-5. Telomerase splicing is differentially regulated in different cancer cell lines. PCR analysis of endogenous *hTERT* splicing, showing a similar splicing pattern among cancer cell lines. “Full length” splicing includes the functional full length transcript (other potential isoforms might contain changes outside this assayed region). Minus beta is the dominant isoform in all cell lines, but variations in the distribution of each splice variant is cell line specific.

### Therapeutic Approaches to Telomerase Splicing

Although there is a positive correlation between *hTERT* mRNA expression and telomerase activity in cancer cells and B lymphocytes, cells from normal tissues have been shown to express *hTERT* mRNA even though telomerase activity is not detected in these cells (Hu and Insel, 1999; Kolquist et al., 1998; Meyerson et al., 1997; Ramakrishnan et al., 1998; Ulaner et al., 1998). This suggests that transcription of *hTERT* alone cannot explain the presence or absence of telomerase activity in cells. Previous reports demonstrate that telomerase activity may in part regulated by alternative splicing of *hTERT* (Kilian et al., 1997; Ulaner et al., 2001). This presents a therapeutic opportunity to inhibit telomerase activity by manipulating *hTERT* alternative splicing to produce less full length transcripts and more non-functional isoforms. An

in depth understanding of the regulation of telomerase in normal development and its aberrant expression in cancer cells should provide additional targets for the development of novel therapeutics.

## CHAPTER TWO

### DEVELOPMENT OF A TELOMERASE MINIGENE

The work presented in this chapter has been published in Cell Reports, volume 3, issue 4, p1028-1035. This work is reproduced with the permission of Cell Reports. Copyright 2013. Experiments were performed by Sze Wong unless otherwise noted in the text and/or figure legends.

#### **Introduction**

To begin to address what regulates *hTERT* alternative splicing, we constructed an *hTERT* minigene containing ~150-300 intronic sequences flanking *hTERT* exons 5-10. Surprisingly, it failed to produce significant amounts of alternatively spliced products. It was previously known that large long-lived mammals regulate telomerase differently from many small short-lived mammals (Gomes et al., 2011). Therefore we carefully examined the telomerase gene and identified several intronic sequences that were conserved in primates but not in other mammalian species. These included an 1100 bp VNTR (variable number tandem repeats) composed of 38 bp repeats in intron 6, and an ~ 260bp sequence that was present as a direct repeat in both introns 6 and 8 (flanking the exons removed in the major nonfunctional isoform that eliminates exons 7 and 8). Each of these elements was a kilobase or more from any splice junction. Including these elements in the minigene reconstituted alternative splicing. Exon-skipping oligonucleotides complementary to a region at the beginning of the 260 nt direct repeat decreased the amount of functional splice forms transcribed from the endogenous gene, providing validation of these novel mechanisms involved in splicing and that this may be an important target for telomerase

therapy. Understanding the detailed mechanism(s) by which these regulatory sequences function to direct splicing choices may identify new telomerase inhibitor targets that could be used in combination with standard cancer chemotherapy and/or other telomerase inhibitors to reduce the probability of cancer recurrence (e.g. durable responses), as well as interventions to increase telomerase activity in stem cells for regenerative medicine.

## **Results**

### **Telomerase minigene requires conserved distant intronic sequences for proper splicing**

We constructed a minigene containing exons 5 to 10 and 150-300 flanking nucleotides (Fig. 2-1). The regions immediately downstream of exons 6 and 8 were unusually GU rich, so we included 325-362 bp from these regions. This minigene containing 3669 bp was inserted into the pcDNA/FRT vector, and stably transfected into a HeLa clone selected for a single FRT site introduced by Invitrogen's Flp-In system to reduce the variability arising from random genomic incorporation. Alternative splicing was determined using a minigene-specific forward primer. The basic minigene only produced 5-9 and minus alpha splicing, and therefore was unable to recapitulate the endogenous telomerase splicing pattern.

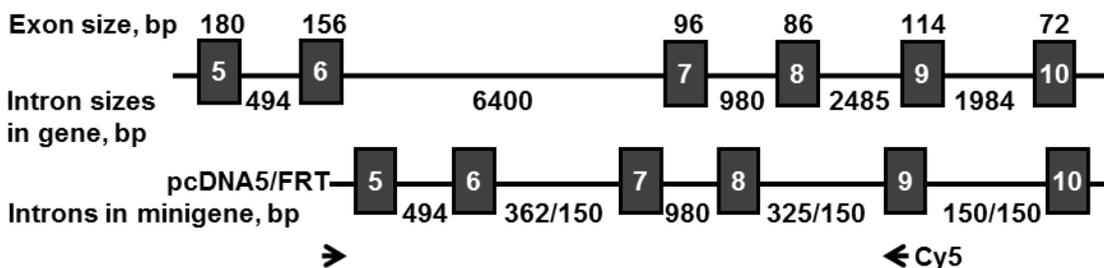


Figure 2-1. Diagram of human telomerase from exon 5 to exon 10 with exon sizes indicated above and intron sizes below (top) and the sequences included in the telomerase basic minigene (construct 1, c1)(bottom). All exonic sequences from exon 5 to exon 10 are included in the minigene, whereas only flanking intronic sequences are included. The values before and after the forward slash (/) indicate the 5' and 3' intronic sequences adjacent to the exons that were used. The minigene was inserted into pcDNA5/FRT and is driven by a CMV promoter. The gene specific primer used to make cDNA and the reverse cy5-labelled primer for PCR is the same as the primers used for endogenous TERT analysis. However, the forward PCR primer lies within the pcDNA5/FRT vector just before exon 5, making it minigene-specific.

The human telomerase gene contains a block of 26 short repeats with a strong 38 nucleotide consensus sequence ~1900nt downstream of exon 6 (Fig. 2-2). This block of repeats in intron 6 (“Block 6”) is immediately followed by a 256 nucleotide direct repeat in intron 6 (“DR6”), which has 85% sequence similarity with a 258 nucleotide direct repeat in intron 8 (“DR8”). DR8 is ~1500nt downstream of exon 7. These 3 elements are highly conserved among old world primates (Figure 2-3, Figure2-4), suggesting a functional role in regulation. A separate and unrelated second block of repeats ~1270 nt downstream from DR6 is also conserved among primates but the number of repeats varies greatly and therefore was not included in the minigene in this initial study. The endogenous *hTERT* splicing isoforms were restored when B6, DR6, and DR8 were incorporated into the minigene (“full” minigene) (Figure 2-5).

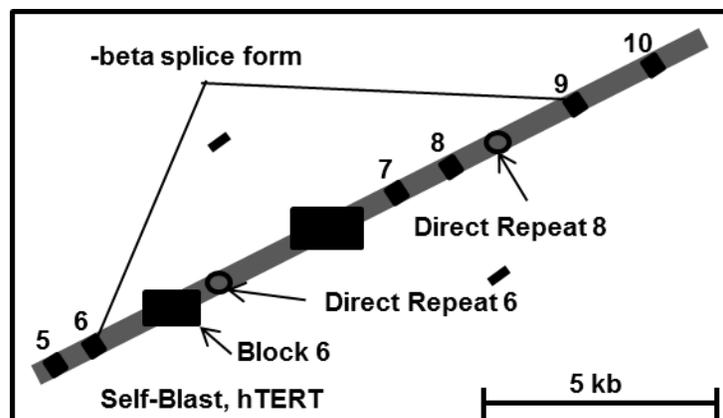


Figure 2-2. Self-blast alignment of the nucleotide sequence of human telomerase with itself shows a block of repeats in intron 6 (“block 6”), a direct repeat in intron 6 (“DR6”), and a direct repeat in intron 8 (“DR8”).

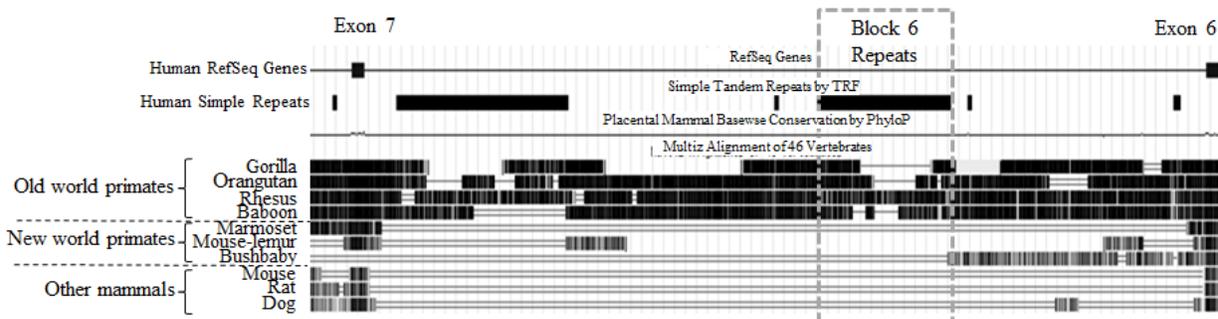


Figure 2-3. Block 6 repeats are only present in old-world primates. TERT sequences from different species were aligned against the human sequence using the UCSC genome browser<sup>20</sup>. The human Block 6 repeats are outlined using a grey dashed box. Variable numbers of these repeats are present in different old-world primates, but they are not present in other mammals.

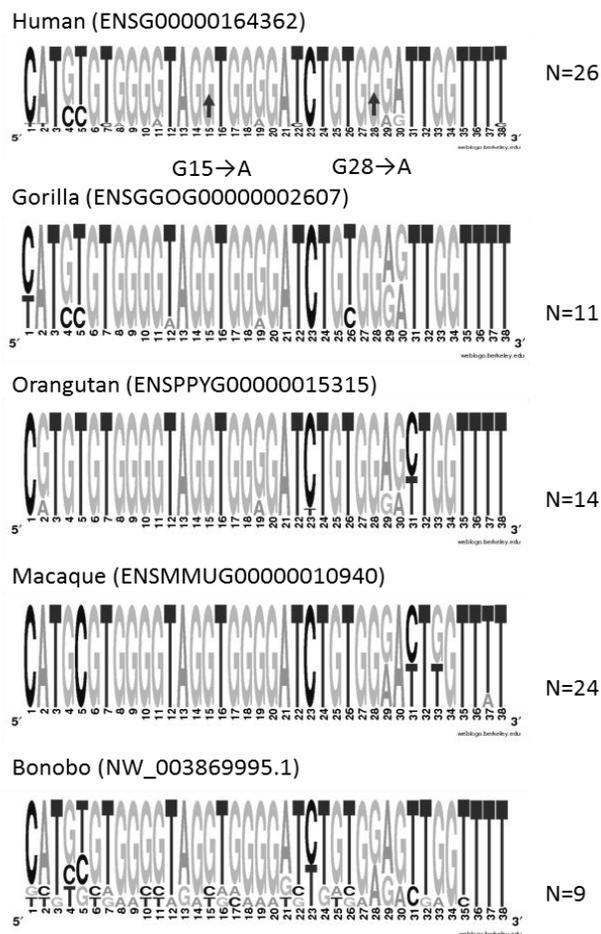


Figure 2-4. Conservation of the 38 bp consensus repeat. The sequence logo (Crooks et al., 2004) is shown for old-world primates along with the number of repeats reported for each species. The two single nucleotide mutations introduced in the human sequence, each of which abolished minus-beta splicing, are indicated by arrows in the top panel.

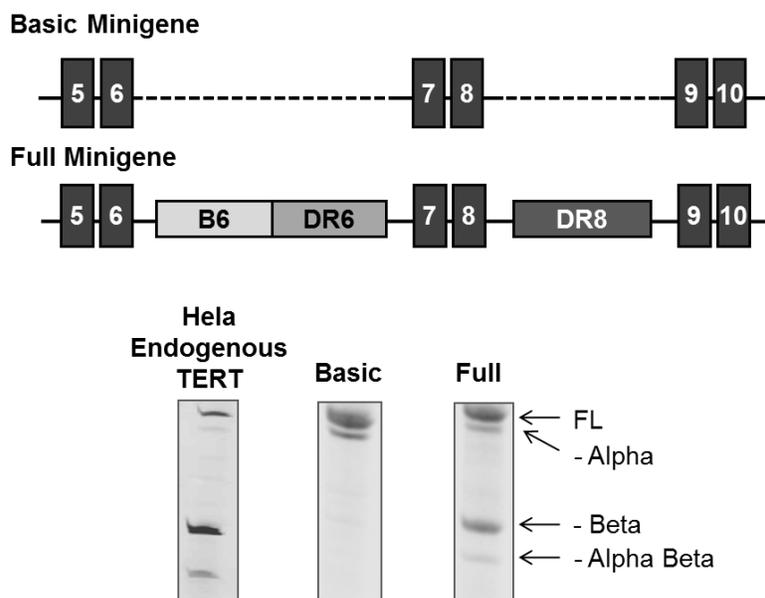


Figure 2-5. Conserved elements in full minigene restores *hTERT* splicing pattern. The basic minigene stably expressed in HeLa cells only produced full length and minus alpha splicing. Three conserved elements (block 6, direct repeat 6, and direct repeat 8) were included in the full minigene to restore the splicing pattern that is similar to endogenous *hTERT* splicing.

### Block 6 of repeats is necessary to promote minus beta splicing

The addition of just the direct repeats in intron 6 and 8 to the basic minigene failed to produce significant amounts of minus beta splicing (Figure 2-6, construct c3). In contrast, the addition of block 6 alone to the basic minigene (Figure 2-6, construct c4) was sufficient to enhance exon 7 and 8 skipping to produce a high proportion of minus beta splicing. The combination of block 6 and the direct repeat in intron 6 produced exclusively minus beta splicing (Figure 2-6, construct c5), while the combination of block 6 and the direct repeat in intron 8 shifted splicing towards greater inclusion of all the exons (Figure 2-6, construct c6). Thus, while the direct repeats by themselves are not sufficient to produce the skipping of exons 7 and 8 (minus beta, construct c3), they modulate the ability of block 6 to shift splicing towards non-functional forms.

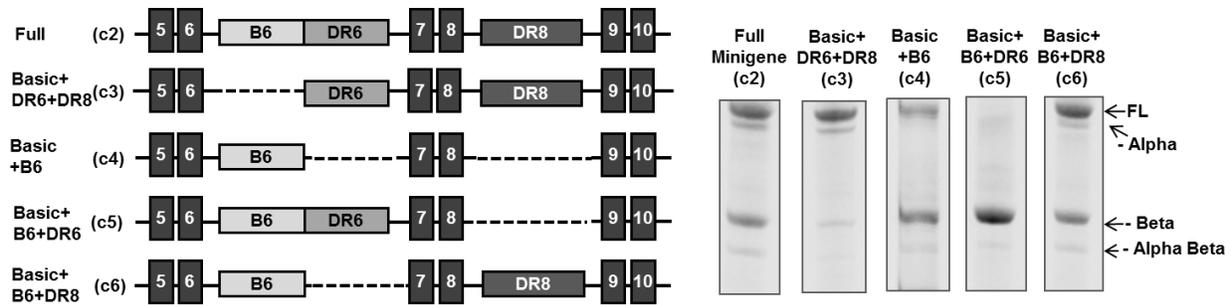


Figure 2-6. The effects of direct repeats 6 and 8 on *hTERT* splicing. DR6 and DR8 (construct c3) do not generate significant exon skipping in the absence of B6 (construct c2). B6 without DR6 and DR8 is sufficient to produce minus beta splicing (c5). Adding DR6 to B6 (c5) modifies its activity and virtually eliminates all 5-9 products that include all of the exons. Adding only DR8 to B6 (c6) shifts splicing in the opposite direction and increases the amount of 5-9 splicing.

### Direct repeats in introns 6 and 8 refine telomerase alternative splicing

Switching the positions of direct repeats 6 and 8 did not change splicing of the *hTERT* minigene (Figure 2-7, constructs c2 vs c7). Similarly, there was little difference when the direct repeats were substituted for each other. Replacing DR6 with DR8 (Figure 2-8, constructs c5 vs c8) or replacing DR8 with DR6 (Figure 2-8, constructs c6 vs c9) did not significantly change the splicing pattern. This suggests that it is the intronic location rather than the sequence differences between DR6 and DR8 that influences their effect on splicing.

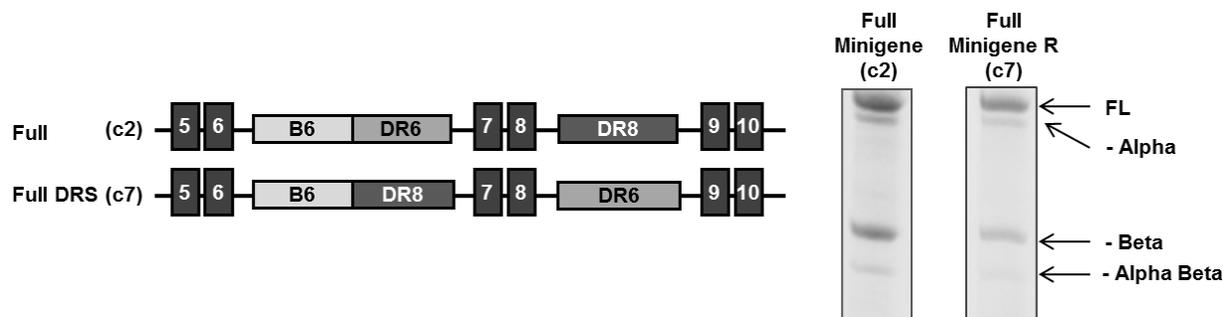


Figure 2-7. Switching the position of DR6 and DR8 in the presence of B6 does not change the ratio of 5-9 to minus beta splicing (c7 vs c2).

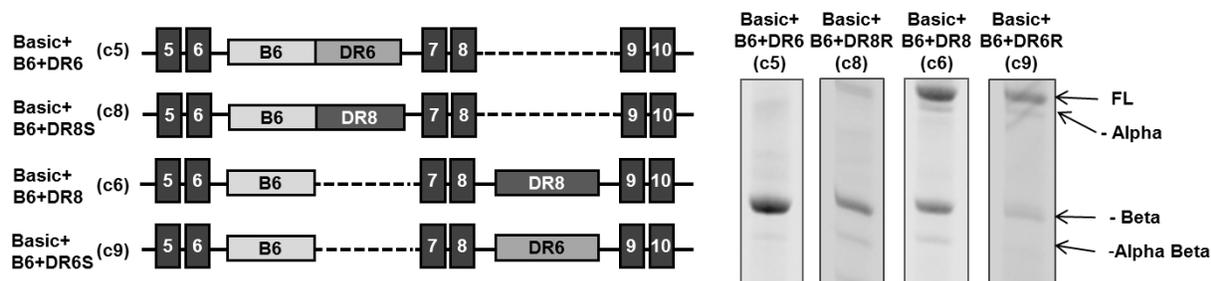


Figure 2-8. Position of DR6 and DR8 determines function. DR6 and DR8 produce similar effects when positioned as the direct repeat only in intron 6 (constructs 5 vs 8) or only in intron 8 (constructs 6 vs 9). It is thus the position of the direct repeats rather than their sequence difference that influence splice choice. All constructs were analyzed in Hela FRT clone 8 cells.

### Antisense oligonucleotide supports the validity of the minigene model

Minigenes lack all of the intronic sequences of the endogenous gene, and can sometimes fail to reflect the regulation of the endogenous gene. Because the direct repeat in intron 8 suppressed the minus beta promoting effects of the direct repeat in intron 6 in the minigene, we tested whether interfering with its structure might change the splicing of the endogenous TERT gene. Figure 2-9 demonstrates that an antisense oligonucleotide (DR8+19, a 20-mer beginning at nt19 of the DR8 sequence) directed against DR8 shifted the splicing of endogenous *hTERT* to mostly minus beta splicing. This confirms that the behavior of the minigene is recapitulating important aspects of endogenous regulation.

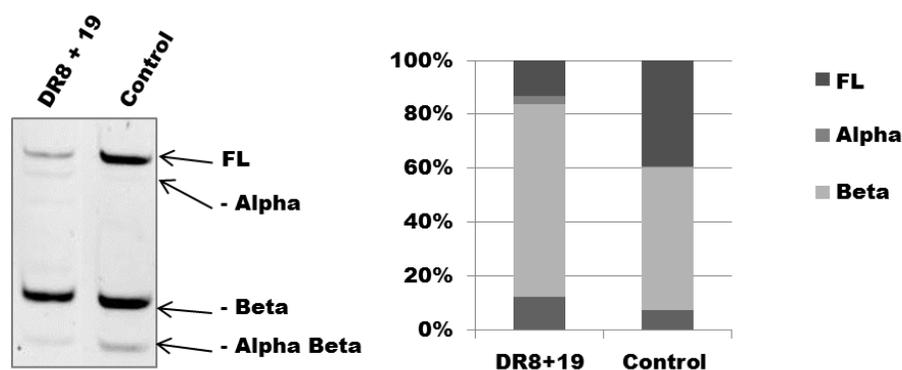


Figure 2-9. Anti-sense oligonucleotide against DR8 shifts *hTERT* splicing. Introduction of the oligonucleotide DR8+19 dramatically shifts splicing of the endogenous telomerase mRNA towards the production of the non-functional minus beta isoform. Cells were treated with 100nM DR8+19 oligonucleotide or vehicle control. Right side shows the quantification.

## Conclusions

We used a minigene system to identify the pre-mRNA sequence elements required for in vivo *hTERT* (human telomerase) splicing regulation. In contrast to most alternative splicing events that have been studied, the splicing of *hTERT* is regulated by long-range interactions rather than by the intronic/exonic elements adjacent to the splice sites. We identified three elements that were highly conserved among primates that were embedded in introns 6 and 8. These elements surround the alternative exons 7 and 8, where the elimination of these exons results in the major non-functional minus beta telomerase (*hTERT*) isoform. Only when these elements were included in the telomerase minigene did we observe the various splicing isoforms seen with endogenous gene. CHIP-seq data suggests that there are many potential splicing factor binding sites at long distances from intron/exon junctions (Yeo et al., 2009), but few specific examples of regulation-at-a-distance have been previously reported. To our knowledge these elements in the telomerase gene represent the first examples in mammals of specific distant sequences that influence splice choice by a mechanism other than introducing cryptic splice sites

(Dominski and Kole, 1993; Friedman et al., 1999; Parra et al., 2012; Pros et al., 2009; Salem et al., 2012).

The direct repeat in introns 6 and 8 have 85% sequence similarity, but their effects on telomerase splicing is very different. When these elements were switched, we determined that it is the position of the element rather than the nucleotide sequence that primarily governed its effect on telomerase splicing. This suggests that if the direct repeats function by recruiting similar RNA binding proteins based on their sequences, the interactions of these binding proteins with other neighboring proteins may dictate the splicing decision. This is similar to studies demonstrating that the effects of SR and hnRNP binding are highly dependent on their position with respect to splice sites (Llorian et al., 2010; Yeo et al., 2009). The mechanisms and factors involved in telomere maintenance throughout evolution are extraordinarily diverse, indicating the lack of constraints to evolving multiple specific mechanisms for protecting the ends of the chromosomes from degradation (Fisette et al., 2010). Even among mammals there is considerable diversity in the size and use of telomeres (Gomes et al., 2011). The conservation among higher primates, but not other mammals of the elements we have discovered, may be surprising but is consistent with the evolutionary diversity of known telomere maintenance mechanisms.

There are large numbers of different avenues for exploiting these observations to develop therapeutic interventions. The minigene we have created can now be used to screen for small molecules, oligonucleotides, and siRNAs that alter the splicing pattern. Because of the limited efficiency of oligonucleotides as therapeutic agents, we believe that identifying small molecules that affect minor splicing components that have enzymatic activity is likely to be the best long-term approach. The demonstration that the oligonucleotide DR8+19 acts on the endogenous gene

to decrease the production of full-length functional telomerase establishes the validity of the minigene as a reporter system and the initial feasibility of this approach.

Oligonucleotides are not ideal therapeutic agents, but the only current telomerase therapy in clinical trials uses an oligonucleotide. The potential for combining two oligonucleotides that work by entirely different mechanisms and which should have at least additive effects needs to be explored. However, this approach is complicated by the observation that oligonucleotides that alter *hTERT* splicing have unexplained toxic effects. An oligonucleotide targeted at the start of exon 6, which caused a relatively limited decrease in the fraction of “full-length” (including exons 6-7-8-9) telomerase caused cell death within three days (Brambilla et al., 2004). This effect was independent of progressive telomere shortening (Folini et al., 2007; Folini et al., 2005), which would require long periods of treatment. The oligonucleotide that we used to demonstrate the relevance of the direct repeat in exon 8 to the endogenous gene (DR8+19) was effective at concentrations that did not cause the cells to die. However, it did inhibit their ability to divide during the 3-4 days during which the effect persisted. Since cell division is required for telomere shortening based on telomerase inhibition, we were unable to continuously treat with this oligonucleotide over long periods of time to demonstrate that it produced telomere shortening. Assuming that the unexplained toxic effects of these oligonucleotides do not produce unacceptable side effects due to toxicity to stem or other cells, it is possible that they would nonetheless be additive to Imetelstat and produce a greater degree of therapeutic efficiency. There are many “telomeropathies” due to haploinsufficiency for telomerase during development (Garcia et al., 2007), and increasing evidence suggests that telomere shortening in stem cells may contribute to the failure to adequately maintain tissues in late life. There may be many minor or previously uncharacterized spliceosome factors that influence these long-range

interactions in different directions, where some factors would decrease full length functional telomerase and would be important for the treatment of cancer while others would increase functional telomerase and would provide therapeutics for regenerative medicine. Increasing our understanding of the regulation of telomerase at the level of splicing may provide additional pathways to the current approaches for manipulating the function of this critical ribonucleoprotein that influences human health at multiple levels.

**CHAPTER THREE**  
**MECHANISM OF BLOCK 6 REGULATION OF TELOMERASE SPLICING BY**  
**RNA:RNA PAIRING**

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**Introduction**

RNA:RNA pairing is a splicing mechanism used for mutually exclusive exon choice in a few insects (such as Dscam in *D. melanogaster* and related species) (Graveley, 2005b), and until very recently it had not been seen in mammals (Pervouchine et al., 2012), where it has been recently reported to control exon choice for a single gene. Human splicing factor (SF1, zinc finger protein 162:ZFM162) is the only other mammalian gene for which RNA:RNA pairing has been demonstrated. Previously, we have identified a region within intron 6 of *hTERT* pre-mRNA that contains a variable number of 38bp repeats, that lies >1kb from exon-intron junctions, and is only conserved in old but not new world primates or other mammals. This block of repeats in intron 6 is necessary to promote exons 7 and 8 skipping, therefore producing the major non-functional “minus beta” TERT isoform. Although once believed to be “junk” DNA, variable number of tandem repeats (VNTRs) dispersed throughout the genome have been found to have several diverse biological functions (Gemayel et al., 2010).

In this study, we show that the block of repeat promotes exon skipping through RNA:RNA pairing between the repeat sequences and the distal portion of the TERT pre-mRNA. We first mutated the repeat sequence to abolish exon skipping and then restored exon skipping by creating compensatory mutations in the pre-mRNA. RNA:RNA pairing through a repetitive sequence deep within an intron that shows conservation in only a small mammalian clade represents completely novel aspects of the RNA:RNA pairing mechanism. This study therefore establishes a novel form of alternative splicing regulation by RNA:RNA pairing through short repetitive sequences and provides additional insights on functions for conserved elements embedded deep within introns.

## **Results**

### **Block 6 requires a minimum number of repeats to function**

The minimum number of repeats present in the primate genomes we examined was 9 repeats in bonobo. The block 6 sequence in the full minigene was replaced with varying number of the consensus repeat sequence in order to determine the minimum number required to increase minus beta splicing. With 1 or 5 repeats, the minigenes did not induce minus beta splicing. However, 11 or more repeats did restore the effect (Figure 3-1). Predicted binding sites for several abundant splicing factors were found in the 38 nt consensus repeat sequence. However, minimal to no effects on endogenous *hTERT* splicing was observed upon knockdown of these factors (data not shown).

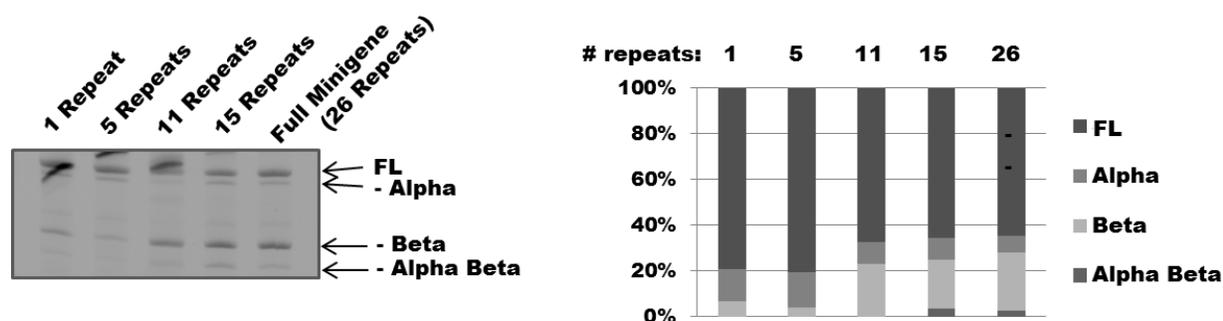


Figure 3-1. Minimum of 11 repeats are necessary to produce minus beta splicing. Left side shows splicing products of HeLa FRT clone 8 cells stably transfected with minigenes containing varying numbers of block 6 consensus repeats. Right side shows the quantification. <11 repeats failed to produce significant amounts of minus beta splicing.

### Single nucleotide mutations in the repeat sequence disrupt block 6 function

When various numbers of 38 nt repeats were analyzed using the Mfold web server (Zuker, 2003), the large majority of the putative structures were dominated by the staggered overlap shown in figure 3-2, in which one repeat interacted with two adjacent half-repeats. We introduced two different single mutations into the 38 bp repeat, G28→A (predicted to be found in the top loop of the self-complementary repeat structure) and G15→A (predicted to be located in two different double stranded regions, disrupting neither of them). Both of these mutations either completely or almost completely abolished the ability of 13 repeats to produce minus beta splicing (Figure 3-2b).

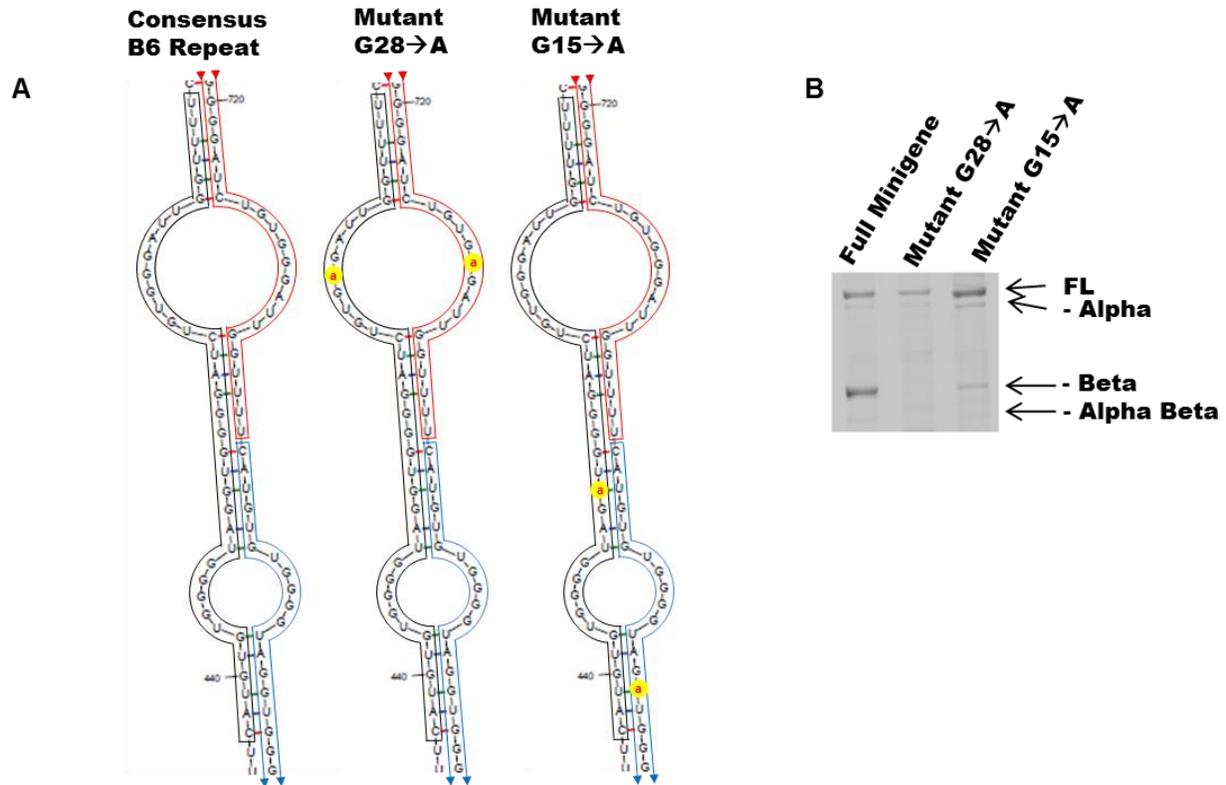


Figure 3-2. Mutant repeats abolish minus beta splicing. (A) Secondary RNA folding structures predicted by Mfold for B6 self-interactions for the consensus sequence and two mutants. The structures with the lowest free energies are shown. The consensus 38 bp repeat can self-fold into a highly structured staggered secondary structure. One complete (black) and two half consensus repeats (red and blue) are highlighted. Neither mutant  $G28 \rightarrow A$  nor mutant  $G15 \rightarrow A$  altered this structure and both self-folded similarly to the consensus repeat. The mutated nucleotides are in red and highlighted in yellow. (B) Mutations that do not alter the secondary structure of self-folding abolish minus beta splicing.

### Block 6 repeats are predicted to pair with TERT pre-mRNA

The 38bp repeats in telomerase are a VNTR (variable number of tandem repeats) containing 18 to 38 repeats with minimal sequence variability in humans (Leem et al., 2002). The sequence is conserved but the number of repeats varies greatly in different primate species (bonobo having the least with 9 repeats). We previously showed that 11 but not 5 repeats could induce exons 7 and 8 skipping, suggesting that a minimal number of repeats are necessary (Wong et al., 2013). Mfold analysis suggests that RNA:RNA pairing between the repeats and

distal portions of TERT pre-mRNA could bring exon 6 and 9 closer together than in a linear configuration (Figure 3-3), which is lost with fewer than 9 repeats. Two different single-nucleotide mutations within the repeat sequence (G28→A and G15→A) each abolished the ability of the repeats to induce exon 7 and 8 skipping (Wong et al., 2013). This suggests that the tandem repeats in TERT intron 6 may rely on RNA:RNA pairing to distal portions of TERT pre-mRNA as a mechanism to promote minus beta splicing in human TERT.

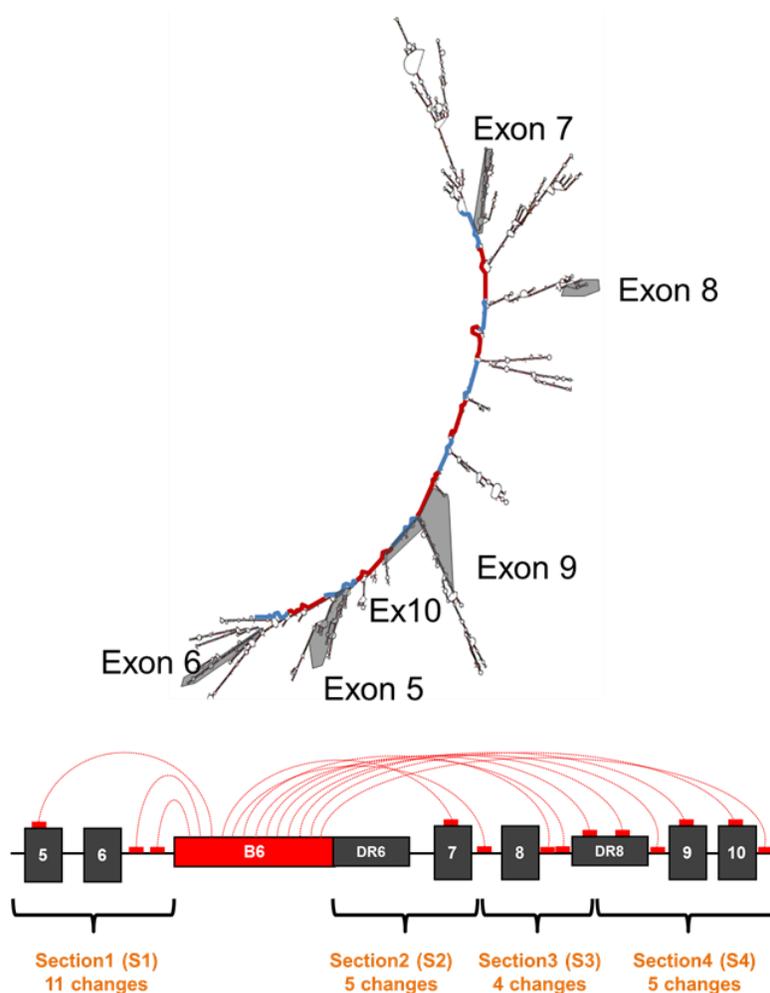


Figure 3-3. The block 6 repeats are predicted to hybridize along the distal portions of the TERT pre-mRNA. Mfold analysis predicts that the block 6 repeats (marked by alternating black and gray to represent each 38 nucleotide repeat) hybridize along the distal portions of the TERT pre-mRNA to create an elongated structure that puts exon 6 and exon 9 closer together than exons 6 and 7, thereby potentially promoting minus beta splicing. Bottom panel shows a linear representation of the pre-mRNA with the predicted approximate location of each repeat hybridizing along the TERT pre-mRNA indicated as light gray boxes. The borders of the sections were determined by useful restriction sites. The number of compensatory mutations introduced within each section is indicated.

### Derivation of compensatory mutations

A single mutation in the 38bp repeat would produce multiple mismatches with many potential targets. This combined with the inability of Mfold to accurately predict large structures posed a challenge to determine which compensatory mutations might restore splicing. Mfold

produces numerous secondary folding structures per input sequence based on thermodynamic determinations (Zuker, 2003). Certain predicted secondary structures (in particular, conformations produced by exonic sequences) were relative stable, appearing in all output structures. Other regions were folded into multiple and therefore probably less stable structures. Table 3-1 summarizes the nucleotides predicted to base pair with the mutated nucleotides of the repeat sequence and the percent of occurrences of such base pairing in multiple Mfold output structures in different segments corresponding to useful restriction sites. Segments containing the predicted complementary mutations were synthesized (Gene Oracle, Mountain View, CA) (Figure 3-3) and then used to systematically replace the original sequence in the minigenes containing either mutant or consensus repeats. The high GC content of segment 2 prevented its synthesis so it was not available for replacement studies.

| Section 1<br>ex5-in5-ex6-in6 |                     | Section 2<br>in6-ex7-in7 |                     | Section 3<br>in7-ex8-in8 |                     | Section 4<br>in8-ex9-in9-ex10 |                     |
|------------------------------|---------------------|--------------------------|---------------------|--------------------------|---------------------|-------------------------------|---------------------|
| Pairing nucleotide           | % output structures | Pairing nucleotide       | % output structures | Pairing nucleotide       | % output structures | Pairing nucleotide            | % output structures |
| 1332C                        | 24%                 | 3839C                    | 69%                 | 4498C                    | 100%                | 4768C                         | 100%                |
| 580C                         | 40%                 | 3767C                    | 69%                 | 4470C                    | 100%                | 5390C                         | 55%                 |
| 526C                         | 24%                 | 3626C                    | 72%                 | 4376T                    | 100%                | 5304T                         | 90%                 |
| 799C                         | 7%                  | 3054C                    | 97%                 | 3943T                    | 28%                 | 5343C                         | 21%                 |
| 53T                          | 7%                  | 2712C                    | 69%                 |                          |                     | 5380C                         | 14%                 |
| 632C                         | 3%                  |                          |                     |                          |                     |                               |                     |
| 447C                         | 7%                  |                          |                     |                          |                     |                               |                     |
| 202C                         | 7%                  |                          |                     |                          |                     |                               |                     |
| 105T                         | 3%                  |                          |                     |                          |                     |                               |                     |
| 555C                         | 7%                  |                          |                     |                          |                     |                               |                     |
| 90C                          | 3%                  |                          |                     |                          |                     |                               |                     |
| 467T                         | 3%                  |                          |                     |                          |                     |                               |                     |

Table 3-1. High frequency conserved interactions predicted by Mfold. Mfold analysis suggested that the consensus 38bp block of repeats in TERT intron 6 consistently interacts with particular residues of the TERT pre-mRNA. Compensatory mutations were designed for the nucleotides showing the highest frequency among the output structures. The exact borders of the sections shown above do not correspond to the borders of the regions synthesized, which were chosen so that convenient restriction sites could be used to introduce the modified pre-mRNA.

## Compensatory mutations restore function of mutant repeats

Neither segments 1, 3 or 4 alone compensated the mutant repeats. However, combining segments 3 and 4 resulted in abundant minus beta splicing in repeat mutant G15→A (Figure 3-4). Furthermore, the compensatory segments 3 and 4 abolished the ability of wild-type repeats to produce minus beta splicing (Fig. 3-5). The fact that compensatory mutations restore splicing using mutant repeats and abolish minus beta splicing using wild-type repeats provides the formal proof that RNA:RNA pairing is regulating TERT splicing. Segment 4 spans exon 9, thereby potentially influencing splice site availability or exposure of splicing factor binding sites. While segments 3 and 4 together restores minus beta splicing in repeat mutant G15→A, neither segments 1, 3 or 4 alone or in any combination restores minus beta splicing in repeat mutant G28→A. This further suggests that specific RNA:RNA pairings are necessary to promote minus beta splicing.

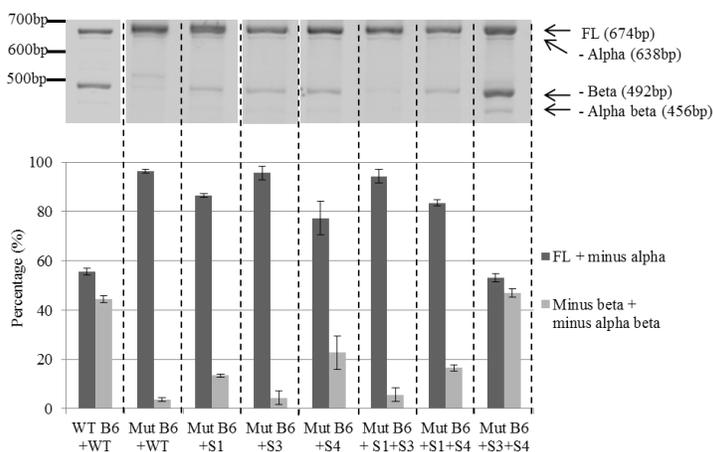


Figure 3-4. Compensatory mutations within sections 3 and 4 together restore minus beta splicing of the mutant block 6 repeats. The splicing of minigenes containing wild-type or mutant repeats, with or without replacement of sections containing compensatory mutations, are shown together with the quantification of the full-length and minus beta isoforms. Alternative splicing is abolished using a block 6 repeat sequence containing a single-nucleotide mutation, which is restored by introducing compensatory mutations when sections 3 and 4 are combined. Data are from HeLa FRT clone 8 cells stably transfected with the minigenes (n=3). The results are

replicated in three separate transfections in two HeLa FRT clones. Minus alpha is a minor alternative splice form that skips the first 36 nt of exon 6.

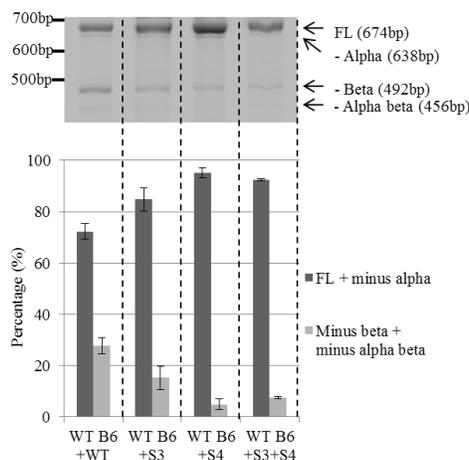


Figure 3-5. Compensatory mutations within sections 3 and 4 abolish the exon skipping function of the wild-type block 6 repeats. The splicing of minigenes containing wild-type or mutant repeats, with or without replacement of sections containing compensatory mutations, are shown together with the quantification of the full-length and minus beta isoforms. The ability of the wild-type block 6 repeats to promote minus beta splicing is abolished when compensatory mutations in both sections 3 and 4 are introduced to the minigene. Data are from HeLa FRT clone 8 cells stably transfected with the minigenes (n=3). The results are replicated in three separate transfections in two HeLa FRT clones.

## Conclusions

Prior studies of RNA:RNA pairing have relied on the identification of sequences that are conserved across large evolutionary distances, and usually located close to intron/exon junctions. The lack of conservation of the block 6 repeats, being restricted to old world primates, was unexpected. This is somewhat typical of telomere biology in general, where the constraints appear to be very flexible. While there are many conceptual analogies for their function, the specific solutions to telomere sequence and the proteins that bind and regulate telomere structure vary dramatically between eukaryotes (fission yeast, budding yeast, plants, insects, mammals). For example, although the crystal structures are related, there is virtually no sequence conservation between the *S. cerevisiae* Est1-Stn1-Ten1 complex and the mammalian CST-Stn1-Ten1 complex<sup>18</sup>. Telomerase expression correlates with body size where smaller short-lived

animals tend to express telomerase ubiquitously while larger long-lived animals repress telomerase expression<sup>19</sup>. The pattern of TERT splicing across mammals remains largely unknown. It will be interesting to identify the different splicing patterns and the mechanisms that regulate them in other mammalian species. The involvement of a repetitive element in RNA:RNA pairing is also currently unique to TERT. The wide distribution of VNTRs throughout the genome raises the possibility for their involvement with other splicing events. Altogether, our results suggest that a minimum of nine 38bp repeats is necessary for RNA:RNA pairing in human telomerase to either change the proximity of exon 6 and 9 splice junctions and/or expose the necessary docking sites for splicing factors or the spliceosome for splice site selection. We have demonstrated the first example of RNA:RNA pairing using a relatively non-conserved repetitive element regulating exon skipping in a mammalian gene. The prevalence of similar regulation in other genes remains to be determined. Manipulating the factors regulating RNA:RNA pairing and the splicing of telomerase may provide additional therapeutic approaches for inhibiting telomerase activity in cancer cells.

## CHAPTER FOUR

### DETERMINE SPLICING REGULATORS OF TELOMERASE

#### Introduction

With the advance of technology, several genome-wide studies have examined the differential expression of splicing factors and alternative exon inclusions in cancer versus normal cells to decipher specific cancer-associated splicing events as biomarkers and targets for cancer therapeutics (Brosseau et al., 2014). A pattern emerges in which a deregulated splicing factor may alter the splicing of multiple oncogene and tumor suppressor genes that in turn feed into signal transduction pathways to collectively promote cancer progression. Due to the low abundance nature of the *hTERT* transcript, there is little to no information available on *hTERT* splicing in the open access databases from genome-wide studies.

Nonetheless, telomerase remains an attractive cancer therapeutic target due to its potential as a universal cancer target that is mostly specific to cancer cells. In order to modulate *hTERT* alternative splicing as a cancer therapeutic, we need to identify drug-targetable trans-acting factors that can bind to the cis-acting regulatory sequences (block 6, direct repeat 6, and/or direct repeat 8) that are necessary for minus beta splicing. The previously described *hTERT* minigene was modified into a dual-luciferase reporter for a siRNA screen targeting 528 RNA-binding and/or splicing-related proteins.

As expected, knockdown of many (~2/3) RNA-binding and/or splicing-related proteins modulated *hTERT* splicing. We had anticipated high amount of false positives due to the use of a minigene system and the number of targets general splicing factors have. We validated the effects of knocking down 45 RNA-binding proteins that have enzymatic activity in endogenous

*hTERT* splicing, reasoning that enzymes are better suited as drug targets. We further validated potential *hTERT* modulators by long term knockdown using shRNAs and chose CDC-like kinase 1 (CLK-1) as a candidate for an *hTERT* splicing modulator from the preliminary study. Clk-1 belongs to the Clk family of dual-specificity nuclear kinases that can autophosphorylate SR proteins on tyrosine, serine, and threonine residues. Of the 4 members of the CLK protein family, CLK-1 and CLK-4 are ubiquitously expressed, have almost identical amino acid sequences, and have functional redundancy. A chemical library screen study has previously identified TG003 (a benzothiazole compound) as a kinase inhibitor that targets both CLK-1 and CLK-4 (Hagiwara, 2005). TG003 treated cells showed dephosphorylation of SRSF4 (SRp75) that resulted in accumulation of SRSF4 in nuclear speckles, which in turn altered splicing patterns of effector genes (Hagiwara, 2005). Short-term TG003 treatment in HeLa and HCC827 cells resulted in reduced telomerase activity, suggesting that CLK-1/4 may be bonafide *hTERT* splicing modulators.

## **Results**

### **Modification of the *hTERT* minigene into a dual-luciferase reporter**

The *hTERT* minigene previously described was modified into a dual-luciferase reporter to be used in a selected siRNA screen. Exon 5, intron 5, intron 9, and exon 10 were excluded from this minigene to reduce the size of the final constructs and it was previously determined that the elements governing minus beta splicing (block 6) is within intron 6 (Figure 4-1). This reduced minigene was shown to produce similar ratios of minus beta to full length as the original minigene (data not shown). A renilla luciferase reporter was embedded in-frame within exon 8 using the AvrII restriction site follow by an E2A (*equine rhinitis A virus 2A*) peptide and a

firefly luciferase reporter was inserted directly after exon 9 followed by a T2A (*Thoseaasigna* virus 2A) peptide (Figure 4-1). Renilla luciferase will only be expressed with the full length isoform, while firefly luciferase will only be expressed with a frame-shift introduced by minus beta splicing. 2A peptides are self-cleaving peptides that impairs the normal peptide bond formation between the 2A glycine and 2B proline, resulting in co-translational cleavage (Ryan et al., 1991). The usage of the 2A peptides was used to generate luciferases at equal molar ratios with *hTERT*. The dual-luciferase minigene stably transfected into cells resulted in a firefly to renilla luciferase (beta to full length) ratio of 12:1, which is close to the endogenous *hTERT* beta to full length ratio (data not shown).

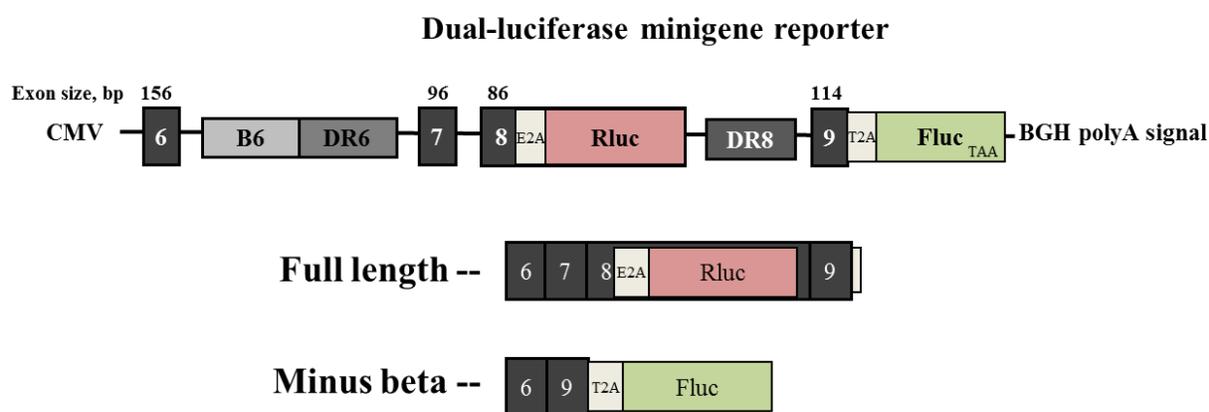


Figure 4-1. Design of the dual-luciferase minigene reporter where E2A-renilla luciferase is embedded in-frame within exon 8 and T2A-firefly luciferase directly follows exon 9. Renilla luciferase will only be expressed in the full length transcript. Due to a frame shift introduced by minus beta splicing, firefly luciferase will only be expressed in minus beta transcripts and not in full length transcripts.

### Cell confluency affects telomerase splicing and activity

It was observed that the cell confluency at the time of sample collection affects telomerase splicing. Since it was anticipated that siRNA knockdown of RNA-binding proteins may induce toxicity or cellular senescence, we sought out to determine the relationship between cell confluency and *hTERT* by seeding different number of HeLa cells and determining *hTERT*

splicing and telomerase activity after 72 hours. As shown in Figure 4-2, there is an overall increase in *hTERT* levels (as measured by primers spanning exons 15 and 16), accompanied by modest increase in both exons 7 and 8 containing transcripts (including full length) and transcripts with exons 7 and 8 skipping (minus beta and minus alpha beta) (Figure 4-2 top). Even though exons 7 and 8 containing transcripts increased over increased cell confluency, telomerase activity surprisingly decreased over increased cell confluency (Figure 4-2 bottom). This suggest that that the increase in exons 7 and 8 containing transcripts observed may be due to an increase in another *hTERT* isoform that cannot produce functional *hTERT* rather than an increase in the full length transcript.

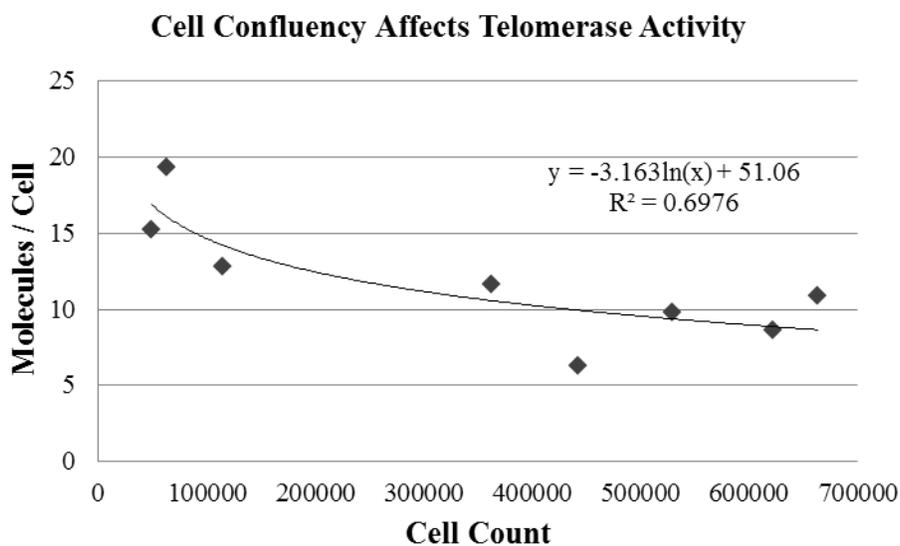
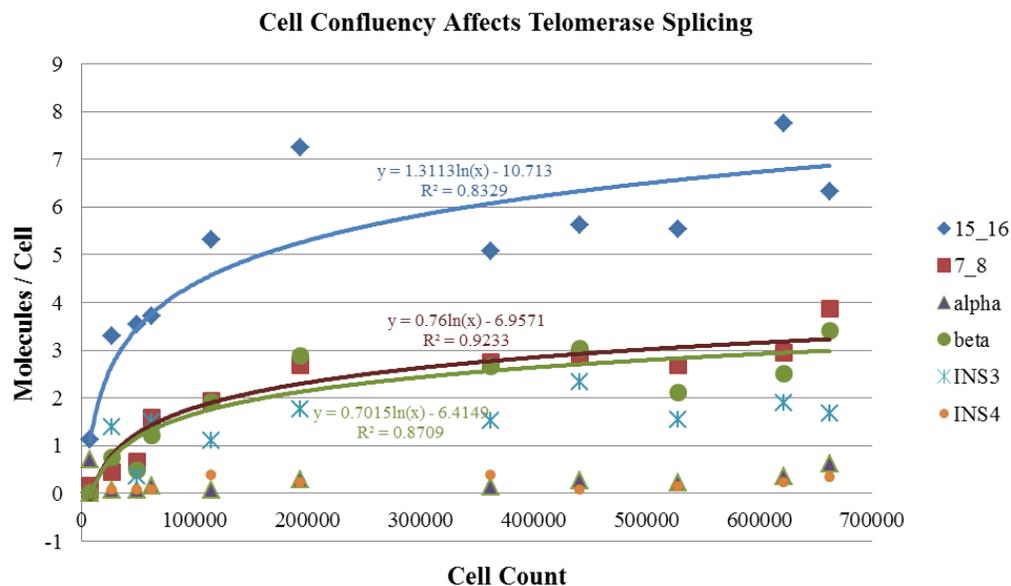


Figure 4-2. The effects of cell confluency on *hTERT* splicing and telomerase activity. Top, graph shows that overall *hTERT* transcription is increased with increase confluency, along with increases in both exons 7/8 containing transcripts and minus beta isoform. Bottom, graph shows a slight decrease in telomerase activity with increasing cell confluency.

### Selected siRNA screen against 528 RNA-binding proteins/splicing factors identified numerous *hTERT* splicing modulators

The dual-luciferase minigene reporter was stably transfected into HeLa and MCF-7 cells. In addition, a lentivirus with a secreted embryonic alkaline phosphatase (SEAP) was introduced to the reporter cells to correct for cell confluency. Since only live cells can produce SEAP mRNA and protein, measuring the amount of secreted SEAP concentration in the media can provide an indirect measurement of cell viability. It was previously determined that knockdown of hnRNP H1 in HeLa cells promotes minus beta splicing in endogenous *hTERT* (Figure 4-3), therefore hnRNP H1 was used as a positive control for the siRNA screen (pools of 4 siRNAs per gene). The ratio of minus beta to full length (firefly luciferase to renilla luciferase), minus beta (firefly luciferase) normalized to viability (SEAP), and full length (renilla luciferase) normalized to viability (SEAP), all of which are normalized to the “cell only” control, are provided in the appendix. As expected, many (~2/3) of the selected genes altered *hTERT* splicing when knocked-down in the cells.

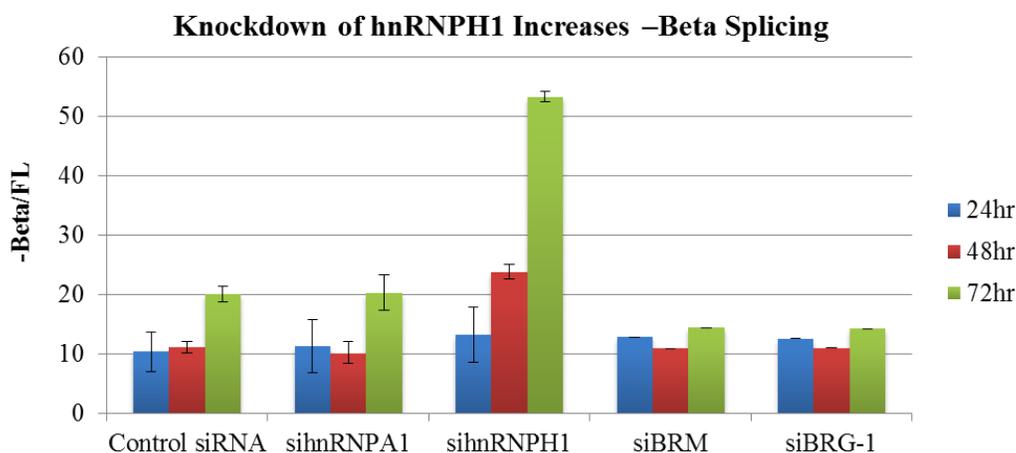


Figure 4-3. Knockdown of hnRNP H1 increases *hTERT* minus beta splicing. siRNA treatment of HeLa cells with different RNA-binding proteins/splicing factors suggest that knockdown of hnRNP H1 can dramatically increase the minus beta to full length splicing ratio in 72 hours.

**Further validations on 45 RNA-binding proteins/splicing factors with enzymatic activity identified CLK-1 as a potential *hTERT* modulator**

To begin narrowing down potential *hTERT* modulators, we preferentially validated the effects of knocking down 45 RNA-binding proteins that have enzymatic activity in endogenous *hTERT* splicing, reasoning that enzymes are better suited as drug targets. The pools of 4 siRNAs used in the initial screen were transfected into HeLa cells to examine the effects of knockdown of the genes on endogenous *hTERT*. From the 45 selected genes, 11 of them altered endogenous *hTERT* splicing (CLK-1, DDX1, DDX3Y DDX41, DHX38, FAM120A, HSP90B1, PPMIG, PRPF4B, SMARCA5, USP39) and were further validated using shRNAs to knockdown those genes in long term culture. For each gene, knockdown efficiency was examined by qPCR or ddPCR, *hTERT* splicing by ddPCR, telomerase activity by ddTRAP, and telomere length by TRF. With moderate gene knockdown (~30-40%), CLK-1 knockdown induced less full length *hTERT* splicing that resulted in decrease telomerase activity, and a moderate decrease in telomere length in HCC827 non-small cell lung cancer cells (Figure 4-4 a-d).

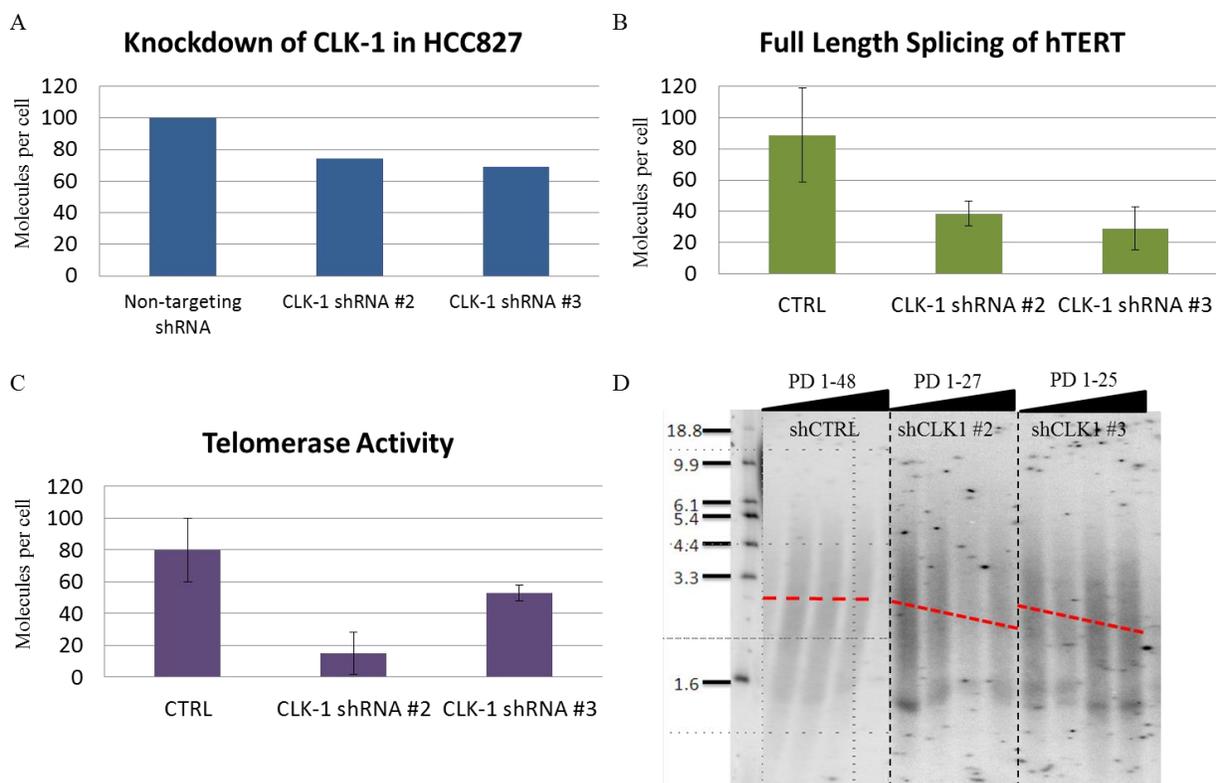


Figure 4-4. Effects of CLK-1 knockdown in HCC827 non-small cell lung cancer cells. A, knockdown efficiency of CLK-1 by 2 different shRNAs were assessed to be a modest knockdown of 30-40%. B, ddPCR results showing that full length splicing is decreased in CLK-1 knockdown cells. C, ddTRAP shows reduced telomerase activity in CLK-1 knockdown cells. D, TRF results show telomere length decrease over time in cells with CLK-1 knockdown.

### CLK-1 is overexpressed in all cancer cell lines examined compared to normal BJ fibroblast cells

More splicing proteins are up-regulated in cancer cells than down-regulated, suggesting a potential cancer dependency or addiction of splicing factor over-expression to promote splicing of a subset of genes that may enhance tumor growth (Grosso et al., 2008; Patry et al., 2003). Indeed, Western blotting shows a higher CLK-1 protein level in all cancer cells examined when compared to normal BJ fibroblast cells.

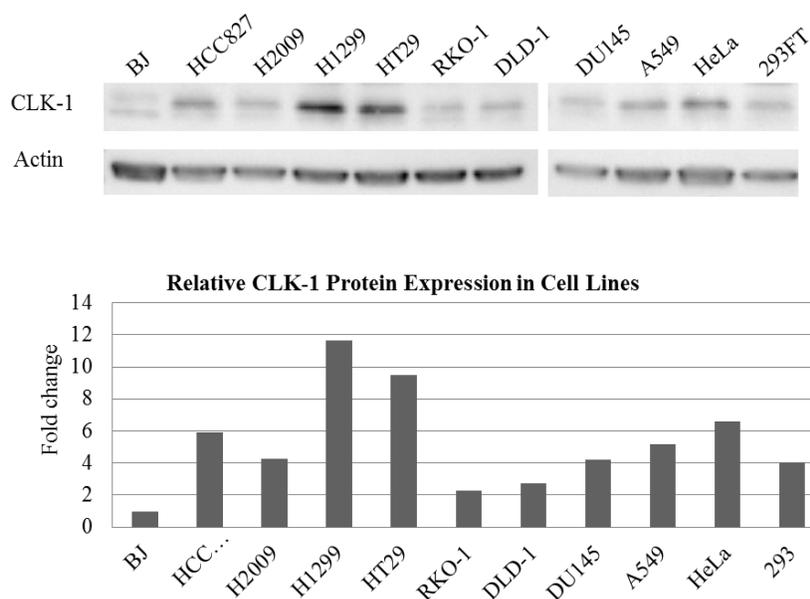


Figure 4-5. CLK-1 is overexpressed in cancer cells. Western blotting with anti-CLK-1 antibody shows CLK-1 is overexpressed in cancer cells when compared to actin. The graph shows CLK-1 signal normalized to actin.

### A chemical inhibitor of CLK-1 reduced telomerase activity in cancer cells

CLK-1 and CLK-4 have almost identical amino acid sequences and are known to have functional redundancy. To further pursue CLK-1 as an *hTERT* splicing regulator, we chose to use a known chemical inhibitor of CLK-1, TG003, which inhibits both CLK-1 and CLK-4 activity at  $IC_{50}$  of 20nM and 15nM, respectively (Hagiwara, 2005). The effectiveness of TG003 treatment was examined by assaying the splicing of *Clk-1* itself (Figure 4-6). Consistent with other splicing factors, CLK-1 is an auto-regulated protein. When the cellular concentration of active CLK-1 is high, the skipping of exon 4 is induced so that less functional CLK-1 is produced and vice versa. Treatment of HeLa cells with TG003 decreased the amount of active CLK-1 in the cells and is compensated by the splicing of *clk-1* pre-mRNA into more full length functional *clk-1* as seen in Figure 4-6.

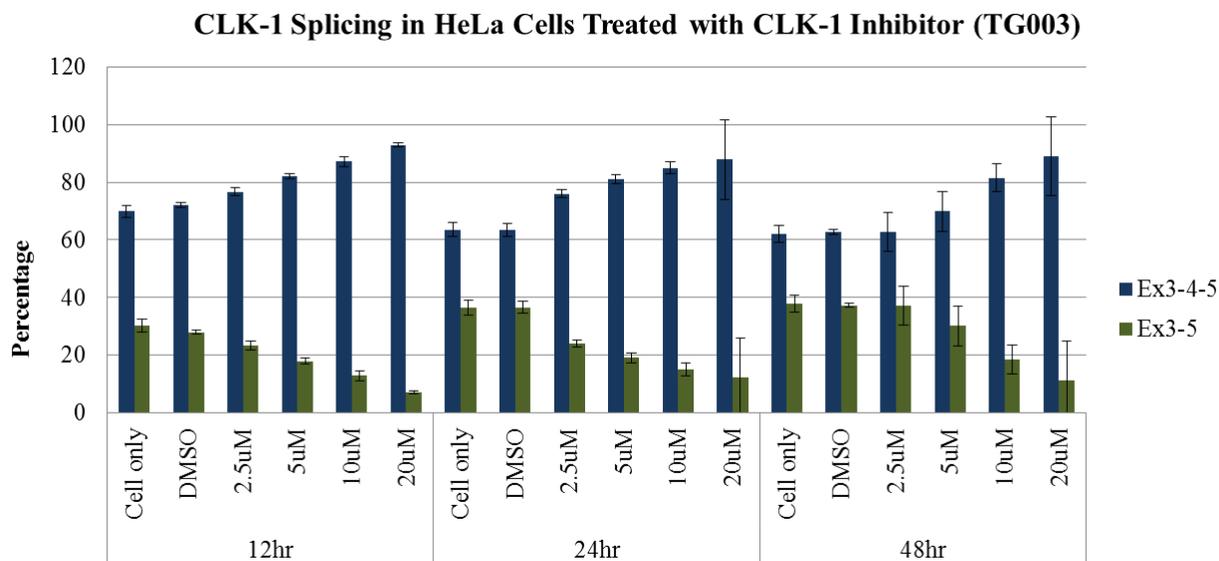


Figure 4-6. CLK-1 inhibitor treatment activates CLK-1 autoregulation by increasing functional Clk-1 splicing. Treatment of HeLa cells with TG003 (CLK-1 inhibitor) at various concentrations over shows more full length Clk-1 transcripts (Ex3-4-5) are made to compensate for the decreased CLK-1 activity in the cells.

Short-term treatment of HeLa cells and HCC827 non-small cell lung cancer cells both resulted in a rapid decrease in telomerase activity (Figure 4-7). The application of TG003 CLK-1 inhibitor as a telomerase inhibitor in long-term inhibition of telomerase activity remains to be seen.

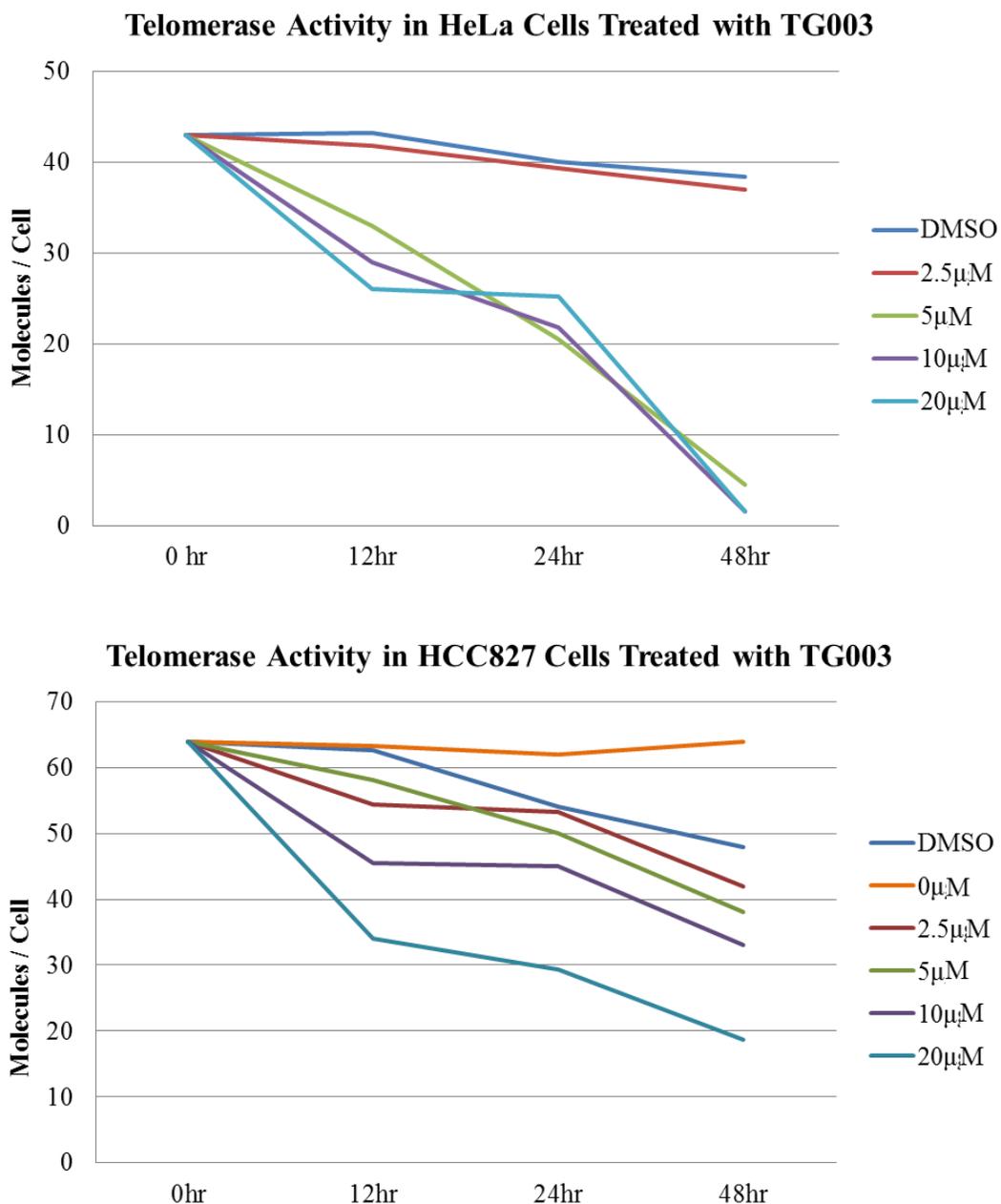


Figure 4-7. HeLa and HCC827 cells treated with TG003 results in decreased telomerase activity. Top shows HeLa cells with decreased telomerase activity when treated with all concentrations of TG003 examined. Bottom shows more dramatic decrease in telomerase activity in HCC827 cells with increase TG003 concentrations.

#### **Additional *hTERT* splicing modulators**

Although the siRNA screen followed by validation gave an initial candidate to pursue, some of the hits from the siRNA screen produced dramatically different results during the

validation process. One of the major contributing factors to such contradictory results could be the non-specific binding of siRNAs that could produce false-positives or false-negatives. To ensure the validity of our initial results, the selected 45 RNA-binding proteins/splicing factors with enzymatic activity were screened again using endoribonuclease-prepared siRNAs (esiRNAs) (Figure 4-8). esiRNAs are a large pool of siRNAs that are made by cleavage of a long double-stranded RNA that is homologous to the target gene-of-interest so that many siRNAs all target the same gene and may provide higher specificity and more effective gene silencing than the traditional usage of a single siRNA. Knockdown of the genes that resulted in a change in telomerase activity will be further pursued as potential telomerase activators or inhibitors.

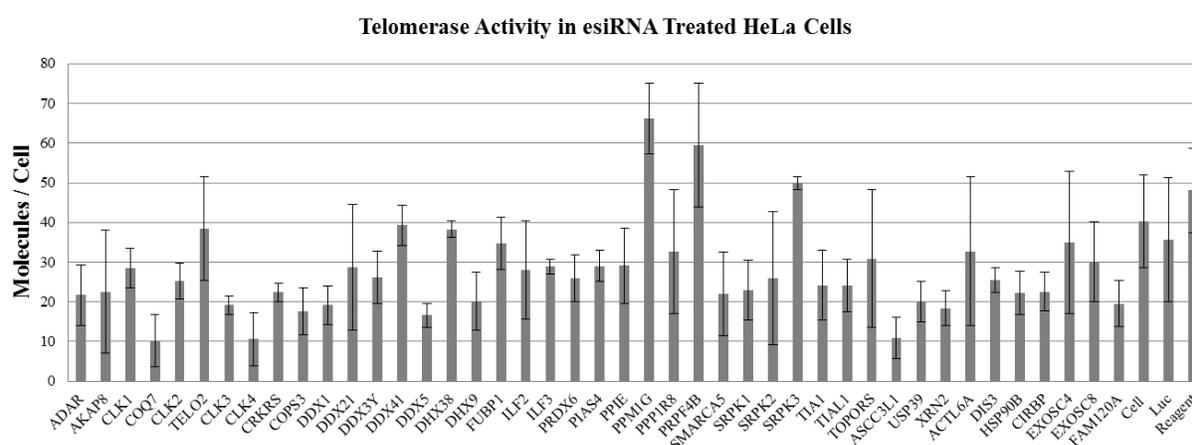


Figure 4-8. esiRNA knockdown of 45 selected RNA-binding proteins/splicing factors with enzymatic activity. Graph shows the endogenous telomerase activity in HeLa cells after knockdown of selected genes with esiRNAs. esiRNAs provide higher specificity and more effective gene silencing than transitional siRNAs.

## Conclusions

In order to identify drug-targetable telomerase inhibitors, we conducted a 528 selected RNA-binding protein/splicing factor siRNA screen using a dual-luciferase minigene reporter that can distinguish full length and minus beta splicing. As expected, almost 2/3 of the splicing factors examined altered the *hTERT* dual-luciferase minigene reporter splicing. A change in a

splicing factor will likely alter the splicing of many genes, which could both directly and indirectly affecting *hTERT* splicing. In addition, the minigene composed of only ~7kb nucleotides will likely have different exposed splicing factor binding sites that may normally be hidden in endogenous *hTERT*, rendering numerous false positive hits.

The major challenge for drug development will be to manipulate a splicing factor that regulates a limited number of splicing events or one that feeds into a signal transduction pathway to specifically influence a small subset of splicing events. Since kinases and phosphatases significantly contribute to alternative splicing regulation and they are potentially more desirable drug targets, we focused our validations in a subset of 45 splicing factors from the original siRNA screen that have kinase activity. Due to potential specificity problems with using siRNAs, we had performed the same screen of the 45 splicing factors using esiRNA. Even though our initial screen and validations suggests CLK-1 to be a strong candidate for *hTERT* splicing regulator, the esiRNA screen showed that CLK-4 had a bigger impact on *hTERT* splicing than CLK-1. This suggests that careful evaluation of the screen data with rigorous follow up validations are needed to choose future candidates to pursue. Regardless, the chemical inhibitor we chose, TG003, inhibits both CLK-1 and CLK-4 and show promise in inhibiting telomerase activity in short-term cancer cells treatment.

## CHAPTER FIVE

### DISCUSSIONS AND FUTURE DIRECTIONS

Alternative splicing is a highly regulated process that requires the cooperation of multiple proteins and complexes. The complexity of higher eukaryotic genes adds additional challenges to studying alternative splicing. For example, *DSCAM* (*Down syndrome cell adhesion molecule*) is a well-studied *Drosophila melanogaster* gene that has 24 exons and encodes for more than 38,000 mRNA variants via alternative splicing. The sizes of eukaryotic pre-mRNAs with both exons and introns make cloning virtually impossible. For a more manageable system to study, minigenes are often used to examine the splicing of a particular exon of interest in a gene with its immediately surrounding regulatory elements. While using minigenes have led to many discoveries, long distance regulatory elements as in the case of *hTERT* is often negated in these systems. Combined with the knowledge of sequence conservation amongst primates, we were able to create an *hTERT* minigene that closely resembles the splicing pattern observed in endogenous *hTERT* and identified long-range intronic regulatory elements that are crucial for *hTERT* alternative splicing.

In order to study alternative splicing in a more physiological-like environment with the intact exons and introns along with the gene-specific promoter and 5' & 3' UTR, bacterial artificial chromosomes (BAC) can be used. BAC is a functional fertility plasmid (F-plasmid) that can retain large inserted DNA sizes including flanking regulatory sequences and subsequently be transfected into mammalian cells so that complete genes can be transferred and integrated into the genome of the cell. Recombineering techniques also allow gene manipulation in BACs to examine the effects of mutations and deletions in a whole gene context. Although BACs have

been successfully used to study alternative splicing of some genes (Ciotta et al., 2011; Delaloy et al., 2006), our efforts using BACs to study *hTERT* splicing was ineffective. As discussed, *hTERT* is subject to an array of promoter regulations. Although the *hTERT* BAC was successfully generated, its expression was silenced *in vivo*. Several manipulation of the promoter region by recombineering was unable to lift the repression, therefore the usage of the *hTERT* BAC was not progressed in our current studies.

Variable number tandem repeats (VNTRs) have been observed throughout the genome with diverse biological functions (Gemayel et al., 2010). The repeat sequence varies in length from 5bp (microsatellites) to longer repeat sequence (minisatellites) as observed in the block 6 repeats in *hTERT* (38bp). In this study, we have demonstrated that the block 6 repeats uses RNA:RNA pairing with distal portion of the *hTERT* pre-mRNA sequences to regulate exons 7 and 8 skipping by making mutant repeats that abolish function and restore function by making compensatory mutations. This provides one of the first examples of VNTRs participating in RNA:RNA pairing to regulate splicing in a mammalian gene. A caveat of the study is that it was performed using the *hTERT* minigene system. The endogenous *hTERT* intron 6 alone is 6360bp. Only 2912bp of intron 6 was included in the minigene. The precise RNA secondary structure the block of repeats forms *in vivo* is expected to be different than the one predicted in our study. Furthermore, *in vivo* RNA folding is a dynamic interplay between the RNA binding proteins coating the RNA and the adaptation of the more stable secondary structure as nascent RNA is being transcribed. Confirmation of whether RNA:RNA pairing is the mechanism governing minus beta splicing in endogenous *hTERT* will require determination of the RNA secondary structure of *hTERT in vivo*, which is beyond the scope of this study. In addition to the block of repeats in intron 6 we examined, there are three additional block of repeats within the *hTERT*

pre-mRNA. A second block of repeat in intron 6 with is composed of repeats of 36bp long. Two blocks of repeats are in intron 2 with repeats of 61bp and 42bp long (Leem et al., 2002). The consensus sequences of each of the four blocks of repeats are independent of one another. We chose to study the 38bp block of repeat in intron 6 due to its conservation with primates and that it is located within intron 6. It would be of interest to determine whether the other blocks of repeats have similar functions in modulating *hTERT* splicing.

Through alternative splicing, *hTERT* pre-mRNA can generate at least 22 isoforms. Our study has focused on manipulating *hTERT* splicing to shift full length splicing to the minus beta isoform because minus beta is the predominate isoform of *hTERT* in telomerase-positive cells. In theory, shifting of the full length splicing to any other isoform should achieve the same goal of inhibiting telomerase activity since only the full length transcript is capable of being translated into *hTERT* protein with reverse transcriptase function. Therefore, shifting full length splicing into other isoforms is an area that remains to be explored. Besides minus alpha, the identified dominant negative isoform, the possible function(s) of the other *hTERT* isoforms is unknown. The function of an alternative splicing isoform is often determined by overexpression in cell lines. Although this provides an initial clue as to possible function(s), the physiological relevance of such assessments needs to be carefully examined since the expression level of such studies are often dramatically different than *in vivo*.

The advancement of deep-sequencing and splice-sensitive microarray technologies has greatly accelerated the discovery of the pre-mRNAs targeted by specific RNA binding proteins. RT-PCR remains the only sensitive method for validation of alternative splicing candidates from genome-wide studies, particularly for low-abundance transcripts (Pan et al., 2008). However, experimental biases and artifacts are often introduced in several steps of the sample processing.

During cDNA synthesis, 5' and/or 3' end bias often occurs. Different polymerases chosen for the PCR reaction often introduce different degrees of preferences towards short products over long products due to their processivity. These problems are especially augmented in the examination of *hTERT* which is a low-abundance transcript. To rectify some of these challenges, we used a mixture of oligo(dT) and a random hexamer primer during cDNA synthesis to reduce the 5' and/or 3' end bias and used digital droplet technology to analyze *hTERT* splicing patterns. Using ~50 cells of input material, the PCR reaction is divided equally amongst ~20,000 droplets generated. The reaction is run to saturation using high cycle numbers. The machine then counts all of the droplets and determines the number of positive and negative droplets. Due to the high number of droplets generated, the number of molecules can be determined because it assumes that the molecule population will follow the Poisson distribution. Since the ddPCR does not rely of amplification cycle number, it can provide absolute quantification of the starting copy number. Although the digital droplet technology can eliminate PCR product length biases, the extreme sensitivity of the assay does require high precision and caution in processing the samples to reduce error. In addition, the lack of exclusive splicing events in any of the 22 identified isoforms of *hTERT* makes distinguishing one specific isoform from another virtually impossible. For example, our analysis of the “full length” isoform using primers spanning exons 7 and 8 at a minimum would detected full length *hTERT*, ins3, and ins4 (see Figure 1-4). It was determined that roughly 50% of the 7/8 signal detected may be contributed by ins3 and ins4 (see Figure 4-2). The inability to isolate full length signal alone remains a challenge for this study.

Alternative splicing is involved in every aspect of the life of a cell. Even though aberrant splicing may produce an isoform that favors disease development, that isoform is often also present in normal cells as well and not unique to a disease state. It is usually a shift in the

splicing pattern that changes the abundance of a particular isoform over the others. Alternative splicing is often tissue-specific where one isoform of a gene may be expressed in one cell type and another isoform in a different cell type. Therefore, it becomes crucial to distinguish between tissue-specific splicing and disease-specific splicing to identify the splicing events that are actually relevant to a disease state. Tumors are composed of a heterogeneous cell population. Without using a normal control with matching tissue type, an exon profiling array identified 366 changes in exon exclusion or inclusion, many of which were later identified as tissue-specific changes instead of cancer-specific changes (Brosseau et al., 2014). Using laser capture microdissection (LCM), Brosseau and coworkers carefully dissected normal controls that matched the composition of the tumor tissue and identified eight alternative splicing events within the cancer tissue and five alternative splicing events in the tumor microenvironment that defined the ovarian cancer signature (Brosseau et al., 2014). Telomerase have additional function in cells besides regulating telomere length. Telomerase activity can often be detected briefly during cellular stress in culture conditions. It poses an interesting basic biology question to address whether *hTERT* splicing is controlled differently for different functions it has in the cell, such as during time of stress or cancer development, and whether shifting of the *hTERT* splicing program in cancer is directed by or is a consequence of other cancer driver gene reprogramming. Regardless if *hTERT* alternative splicing is a driver or passenger event, understanding how *hTERT* alternative splicing can be manipulated provides a unique prospect to inhibiting telomerase activity which may lead to cancer cell death.

## **CHAPTER SIX**

### **MATERIALS AND METHODS**

#### **Cell culture**

HeLa cervical carcinoma, H1299 lung adenocarcinoma, 293FT embryonic kidney cells, DLD-1 colon carcinoma, RKO-1 colon carcinoma, HT29 colon adenocarcinoma, and HCT116 colorectal carcinoma were cultured at 37<sup>0</sup>C in 5% CO<sub>2</sub> in 4:1 DMEM:Medium 199, containing 10% calf serum (HyClone, Logan, UT).

#### **Generation of cells containing FRT site**

The Flp-In System (Invitrogen, Carlsbad, CA) was used to generate HeLa cells (purchased from American Type Cell Culture (ATCC)) with a stably integrated FRT site. The population of transfected cells were subcloned and individual FRT stably integrated clones were screened for single insertion by southern blotting. Expression level of the FRT site was determined by beta galactosidase staining. Three HeLa clones (#1, #8, #12) were used to confirm the results observed.

#### **Construction of minigene plasmids**

Human telomerase sequences were inserted in the pcDNA5/FRT expression vector (Invitrogen, Carlsbad, CA) using a variety of restriction sites.

#### **RT-PCR and splicing analysis**

Using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA), RNA was extracted from HeLa cells stably expressing the minigene. Complementary DNA was made using Biorad iScript cDNA Synthesis Kit. A minigene specific forward primer 5'-CTGGCTAACTAGAGAACCCACTGC-3' and a cy5-labelled reverse primer 5'-AGGCTGCAGAGCAGCGTGGAGAGG-3' were used to examine the splicing of the hTERT minigene using Taq polymerase PCR (Promega, Madison, WI). The reaction was initially denatured at 94<sup>0</sup>C for 3 minutes, then denatured at 94<sup>0</sup>C for 30 seconds, annealed at 61<sup>0</sup>C for 30 seconds, and extended at 72<sup>0</sup>C for 1 minute for 30 cycles, with a final extension at 72<sup>0</sup>C for 10 minutes. The PCR product was resolved on a 5% denaturing polyacrylamide gel and visualized at 650nm.

#### **Annealing oligonucleotides to generate consensus and mutant block 6 repeats**

In separate reactions, equal molar ratios of the top and bottom strands of the terminating ends containing restriction sites and the overlapping mutant repeats were annealed using 1x annealing buffer (10mM Tris pH 8.0, 50mM NaCl, 1mM EDTA), heated to 95<sup>0</sup>C for 5 minutes and then gradually cooled to room temperature. The sequence and strategy are shown in the Supplementary Methods. After purifying the annealed DNA using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), the terminating ends were combined with the overlapping consensus or mutant repeats at a 1:40:1 ratio. After ligating the mixture overnight at room temperature using T4 DNA ligase (Fermentas, Canada), ligation products were separated on a 1% agarose gel. Gel purified size classes were then digested with restriction enzymes, and ligated to the minigene vector.

### **Antisense oligonucleotide**

The 2-O-methyl oligonucleotide DR8+19 oligonucleotide (5'–CGAUCUCAACUCACUGCAAC – 3') containing 50% phosphothioate linkages was synthesized by Integrated DNA Technologies, Inc. The oligonucleotide (100nM) was introduced into the cells by reverse transfection using Lipofectamine RNAiMAX (Life Technologies Corporation, CA). Transfected cells were collected after 48 hours and analyzed by RT-PCR.

### **Replacement of sections containing compensatory mutations**

Three sections containing compensatory mutations were synthesized by Gene Oracle (Mountain View, CA). A TERT minigene was previously constructed by inserting portions of the human telomerase sequences into the pcDNA5/FRT expression vector (Invitrogen, Carlsbad, CA). The sequence of the minigene is available upon request. Pre-existing unique restriction sites in the minigene were used to replace the wild-type sections with the sections containing compensatory mutations to avoid additional sequence changes. Section 1 was flanked by a 5' MluI site and a 3' PacI site. Section 3 was flanked by a 5' FspAI site and a 3' BssHII site. Section 4 was flanked by a 5' BssHII site and a 3' SphI site (Fig. 8).

### **siRNA Screen Transfection**

siRNA (0.1nmol) were resuspended with 100ul 1x siRNA buffer to make 1uM stock. 200ul of media was added to outer edges of 30 plates to buffer evaporation. Cells were trypsinized and prepared at 80,000 cells per mL. 300ul RNAiMAX was mixed with 56.1mL OptiMEM. Using a multichannel pipette, 75.2ul RNAiMax+OptiMEM mixture were added to each well. 4.8ul of 1uM stock siRNA was added to each well using robot. The plates were incubated for 20 minutes

at room temperature. Meanwhile, 8000 cells per 100ul were added to 30 plates. After incubation, 20ul of transfection mixture was added to 3 plates containing cells. The plates were incubated at 37°C for 72 hours. One day prior to assay, media was changed with 100ul fresh media added.

### **Great EscAPe SEAP Fluorescence Detection Kit**

The experiment was performed according to manufacturer's instructions. 1mg reagent was dissolved in 1320ul water. 20ul of the conditioned media was transferred to black plates and incubated at 65°C for 15 minutes. The plates were cooled to room temperature. 4ul of the prepared reagent was added to each well and the plates were read at 360nm/440nm.

### **Luciferase assay**

The siRNA transfected cells were washed with 100uL 1xPBS. 35uL of 1x passive lysis buffer was added and incubated at room temperature for 15 minutes with gentle rocking. 35ul of LARII was added and the firefly luciferase signal was recorded. 35ul of Stop-Glo with luciferin was added and the renilla luciferase signal was recorded.

### **shRNA production and transfection**

The retroviral-vector based shRNA constructs were purchased from OpenBiosystems . To produce viruses, 0.75ug of DNA was mixed with 100ul Buffer EC, 0.26ug PMD2G, 0.49ug PsPAX2, and 8ul Enhancer and incubated at room temperature for 5 minutes. 15ul Effectene were added to the mixture and incubated for 10 minutes at room temperature. During incubation, 293FT cells were trypsinized and prepared at 1,000,000 cells per ml. 600ul media X was added to the DNA-Effectene mixture and immediately added to one well of a 6-well plate. 1,000,000

cells were seeded on top of the transfection mixture and 600ul of media was added. The cells were incubated at 37<sup>0</sup>C. After 24 hours, the transfection mix was removed, 2mL of fresh media was added, and the plate returned to 37<sup>0</sup>C. Another 24 hours later, the media was collected and passed through a 0.45um filter into a 15mL tube. The virus can be frozen at -80<sup>0</sup>C or used immediately. To apply the virus onto cells, seed Hela cells in a 6 well plate and incubate overnight. Aspirate media, add 1 mL of the prepared virus and 1 mL of fresh media to the cells, and incubate overnight. 24 hours later, change media, wait one day and add puromycin for selection.

### **Quantitative Polymerase Chain Reaction**

Quantitative PCR was carried out using the human Universal Probe Library (Cat. 04683633001) and TaqMan Master (Cat. 04535286001) from Roche following the manufacture's manual. The experiments were repeated 2-3 times, and the relative expression level of each gene was normalized to GAPDH, HPRT, and PP1A. The primers and probe for each gene were selected by using ProbeFinder software provided by Roche (<https://www.roche-applied-science.com/sis/rtPCR/upl/adc.jsp>).

### **Digital Droplet Polymerase Chain Reaction**

Primers and probes were purchased from Sigma and Roche (Universal ProbeLibrary) respectively, and were diluted to 10 μM for use in PCR reactions. The sequences were as follow:

| <u>Primer</u>   | <u>Sequence (5' – 3')</u>           |
|-----------------|-------------------------------------|
| Ex15/16 F (#37) | GGGTCACTCAGGACAGCCCAG               |
| Ex16 R (#37)    | GGGCGGGTGGCCATCAGT                  |
| Ex3/14 F (#24)  | AGAACAGGCTCTTTTTCTACCG              |
| Ex3/14 R (#24)  | CAGCTCCCATTTTCATCAGC                |
| Ex7 F (#52)     | ACAGTTCGTGGCTCACCTG                 |
| Ex8 R (#52)     | TCTTCGACGTCTTCCTACGC                |
| Ex6 F (#58)     | CAAGAGCCACGTCCTACGTC                |
| Ex9 R (#58)     | CAAGAAATCATCCACCAAACG               |
| INS3 F          | AGAGATGGAGCCACCCCGCA                |
| INS3 R          | AGCGACATCCCTGGGGGAAAAC              |
| INS4 F          | TGAAAGCCAAGAACGCAGGTAT              |
| INS4 R          | TAAGCCCAGATTCACTCAGTCTCC            |
| <br>            |                                     |
| <u>Probe</u>    | <u>Sequence (5' – 3')</u>           |
| UPL #24         | GGGAGCTG                            |
| UPL #37         | TGCCCTGG                            |
| UPL #52         | GGGAGGAG                            |
| UPL #58         | GGATGGAG                            |
| INS3 probe      | [6FAM]AGCTTTCCGGTGTCTCCTGG[BHQ1]    |
| INS4 probe      | [6FAM]CTGCCTGCTGGTGTAGTGTGTCA[BHQ1] |

For QX100 ddPCR reaction, 2ul of the cDNA reaction was mixed with 10ul of 2x QX100 Reaction Super Mix, 1  $\mu\text{L}$  of the appropriate forward primer for a given splice variant, 1  $\mu\text{L}$  of the appropriate reverse primer for a given splice variant, 0.1  $\mu\text{L}$  of the respective probe, and 5.9  $\mu\text{L}$  of DEPC-treated water. The 20  $\mu\text{L}$  sample mixture was loaded into the sample well of the droplet generation cartridge. 70  $\mu\text{L}$  of QX100 droplet generation oil was added into oil well of the droplet generation cartridge. The droplet generation cartridge was assembled with the gasket and placed into the droplet generation machine. After droplet generation, droplets (final volume of 40  $\mu\text{L}$ ) were transferred into a 96 well twin.tec skirted plate (Eppendorf) and the plate was sealed using an Easy Pierce Heat Sealing Foil (Thermo Scientific) and the ALPS 50V Microplate Sealer (Thermo Scientific). The sealer was set to 165<sup>0</sup>C and the plate was sealed once for 2 seconds, turned 180<sup>0</sup>, and sealed again for 2 seconds.

Samples were incubated at 95<sup>0</sup>C for 10 minutes, followed by 40 cycles of denaturation at 94<sup>0</sup>C for 30 seconds and extension at 60<sup>0</sup>C for 1 minute. After 40 cycles, the samples were heated to 95<sup>0</sup>C for 30 seconds and stored at 12<sup>0</sup>C until reading. Samples were read using the QX100 droplet reader (Biorad) and analyzed using Quantalife software. All plates were run with a no template control as the background for determining the positive droplet threshold.

### **Digital Droplet Telomeric Repeat Amplification Protocol**

Cells were harvested into 1.5 mL microcentrifuge tubes and pelleted. Cells were lysed in NP-40 lysis buffer (10 mM Tris-HCl, pH 8.0; 1 mM MgCl<sub>2</sub>; 1 mM EDTA; 1% v/v NP-40; 5 mM 2-mercaptoethanol; 0.1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF)) for at least 30 minutes on ice. The concentration of lysate was adjusted to 1250 cells/uL. 1  $\mu\text{L}$  of

lysate was added to a telomerase extension master mix with 1X TRAP buffer (10x: 200mM Tris-HCl, pH 8.3, 15mM MgCl<sub>2</sub>, 630mM KCl, 0.5% Tween 20, and 10mM EGTA), 0.05 mM dNTPs, 0.2 μM TS primer (5'-AATCCGTCGAGCAGAGTT-3'), 20 μg BSA and DEPC-treated H<sub>2</sub>O to make a final extension reaction volume of 50 μL. The extension reaction was incubated at 25<sup>0</sup>C for 40 minutes, inactivated at 95<sup>0</sup>C for 5 minutes and stored at 4<sup>0</sup>C.

For QX200 ddPCR reaction, 2 μL of the extension reaction was added to 20 μL of QX200 reaction mix containing 11 μL 2X QX200 Evagreen Reaction SuperMix, 0.11 μL TS, 0.11 μL ACX (5'-GCGCGGCTTACCCTTACCCTTACCCTAACC-3'), and 8.8 μL DEPC-treated water. 20 μL of the final reaction mix was loaded into a droplet generation cartridge and 70 μL of QX200 Evagreen droplet generation oil was added to the oil wells of the cartridge. The droplet generation cartridge was assembled with the gasket and placed into the droplet generation machine.

Samples were incubated at 95<sup>0</sup>C for 5 minutes, followed by 40 cycles of denaturation at 95<sup>0</sup>C for 30 seconds, annealing at 54<sup>0</sup>C for 30 seconds, and extension at 72<sup>0</sup>C for 30 seconds. After 40 cycles, the samples stored at 12<sup>0</sup>C until reading. Samples were read using the QX200 droplet reader (Biorad) and analyzed using Quantalife software. All plates were run with a lysis buffer control to set the background for determining the positive droplet threshold.

Quantalife uses Poisson statistics to calculate a concentration given the ratio of positive to negative droplets. The background concentration given by the lysis buffer control is then subtracted from each sample to give a corrected concentration (molecules/μL). This is then

multiplied by 20 to give the total products generated. The total products generated is then divided by 50 (the number of cell equivalents that was added into the reaction) to give the molecules/cell equivalent.

### **Telomere Restriction Fragment**

Cells (1-2,000,000) were lysed in 987ul lysis buffer that is composed of 50mM Tris-HCl, pH 7.4, 20mM EDTA, and 1% SDS. 13ul of Proteinase K (20mg/ml) were added to each cell lysate and incubated overnight at 37<sup>0</sup>C with gentle shaking. Proteinase K was inactivated by incubating the samples at 65<sup>0</sup>C for 10 min. 10ul RNaseA (1mg/ml) was added and the reaction incubated for 2 hours at 37<sup>0</sup>C. To saturate the solution, 180ul of 6M NaCl was added and vigorously shook for 30 seconds, spun down at 12,000 rpm for 7 minutes, and then the supernatant was transferred to a new microcentrifuge tube. 2x 100% EtOH, mix was added, and spun down at 5000g for 5 minutes. The supernatant was removed, 1ml of 70% EtOH, was added and spun down at 5000g for 5 minutes. Supernatant was removed and the pellet was air dried. DNA pellet was resuspended in 50ul water.

2ug of prepared DNA was digested with 1.5ul of enzyme mixture (1 unit of each of the following: HinfI, HaeI, AluI, MspI, RsaI, and HhaI), 3ul of 10X NEB buffer 4, and water for a total volume of 30ul. The reaction was incubated overnight in a water bath at 37<sup>0</sup>C. An extra-large 0.7% agarose gel was prepared. The digested DNA sample was loaded onto gel along with a TRF ladder. The loaded gel was ran at 70V for 19 hours. The gel was visualized under UV light. The gel was placed in denaturation solution (1.5M NaCl and 0.5M NaOH) and incubated

for 20 minutes with gentle rocking. The gel was placed facing down on 2 pieces of Whatman paper with saran wrap on top and dried using a gel dryer (50°C for 2 hours).

The probe was prepared by mixing 1ul of the pre-annealed oligonucleotide template, 3.125ul of 8x adjusted buffer, 1ul of 1.25mM dATP & dTTP, 1uL Klenow, and 5uL  $\alpha^{32}\text{P}$ -dCTP. The reaction was incubated for 30-45 minutes at room temperature and then heated to 95°C for 5 minutes. 0.5uL UDG (1U/ul) was added, incubated at 37°C for 15 minutes, and then heated to 95°C for 10 minutes.

Once dried, the gel was neutralized for 20 minutes in neutralization solution (1.5M NaCl and 0.5M Tris-HCl, pH 8.0). The gel was transferred to a cylindrical hybridization tube and pre-hybridized with 15mL hybridization solution (6ml 20xSSC, 1ml 10x Denhardt, 0.5ml 20% SDS, and 12.5ml water) for 30 minutes at 42°C. 5ul of the prepared probe was added to the hybridized gel and incubated overnight at 42°C. The hybridization buffer with probe was removed and the gel was washed in 2x SSC for 15 minutes at 42°C. Then the gel was washed twice in 0.1x SSC + 0.1% SDS for 10 minutes at 42°C. Finally, the gel was washed in 2x SSC at 42°C and exposed to film for at least 1 day and visualized.

### **TG003 treatment**

TG003 was purchased from Sigma (#T5575). 10mM stock of TG003 was prepared in DMSO. Cells were trypsinized, counted, seeded, and incubated overnight. The desired concentration of TG003 was added to the cells. Fresh media with fresh drug was added every 2 days.

## **Western Blotting**

Cells were lysed in Laemmli SDS reducing buffer (50 mM Tris·HCl (pH 6.8), 2% SDS, and 10% glycerol) and boiled at 95°C for 10 minutes. Protein concentration was measured using the Pierce BCA Protein Assay Kit according to the manufacturer's instruction. 20-30ug of lysate was mixed with 3ul BPB-BME and 1x SDS sample buffer and loaded onto SDS/PAGE gel and ran at 120V for 30 minutes. The gel was transferred to PVDF membrane and blocked with 5% skim milk in PBST for 1 hour. Primary anti-CLK1 antibody (1:5000 in PBST) was added and incubated with rocking for 1 hour or overnight. The membrane was washed 3 times with 10mL PBST. Secondary antibody was added at 1:10,000 in 5% skim milk for 30 minutes. The membrane was washed 3 times with 10mL PBST. Super Signal West Pico Kit was used to visualize protein bands according to manufacturer's instructions.

## **esiRNA Transfection**

Mission esiRNA for 45 selected splicing factors with enzymatic activity were synthesized and purchased from Sigma-Aldrich. HeLa cells were seeded at 125,000 cells/well in 6 well plates one day prior to transfection. Just prior to transfection, the HeLa cells were given 1.5ml fresh media X. Transfection mixtures were prepared by adding 2.5ul of 200ng/ul esiRNA (final concentration 0.25ng/ul) to 494.5ul Opti-MEM media (Gibco at Life Technologies, cat#31985-062) and then adding 3ul Lipofectamine RNAiMAX (Invitrogen, cat#13778-150). The transfection mixtures were gently mixed and incubated at room temperature for 20 minutes. After incubation, the transfection mixtures were gently added to the cells. The cells were incubated at 37°C for 72 hours. Each gene was done in triplicate with esiRNA to luciferase as a negative control. At the time of collection, the cells were trypsinized, collected and counted. 250,000 cells were transferred to a separate Eppendorf tube for ddTRAP and the remainder cells were used for RNA

extraction. Both samples were centrifuged at 1000rpm for 5 minutes and the supernatant was removed.

## APPENDIX

Appendix. Results of siRNA screen against 528 RNA-binding proteins/splicing factors.

| Gene Name  | Minus beta / full length |       |                |       |              |       | Minus beta / viability |       |                |       |              |       | Full length / viability |       |                |       |              |       |
|------------|--------------------------|-------|----------------|-------|--------------|-------|------------------------|-------|----------------|-------|--------------|-------|-------------------------|-------|----------------|-------|--------------|-------|
|            | HeLa Screen #1           |       | HeLa Screen #2 |       | MCF-7 Screen |       | HeLa Screen #1         |       | HeLa Screen #2 |       | MCF-7 Screen |       | HeLa Screen #1          |       | HeLa Screen #2 |       | MCF-7 Screen |       |
|            | AVG                      | STDEV | AVG            | STDEV | AVG          | STDEV | AVG                    | STDEV | AVG            | STDEV | AVG          | STDEV | AVG                     | STDEV | AVG            | STDEV | AVG          | STDEV |
| Cell only  | 1.00                     | 0.00  | 1.00           | 0.00  | 1.00         | 0.00  | 1.00                   | 0.00  | 1.00           | 0.00  | 1.00         | 0.00  | 1.00                    | 0.00  | 1.00           | 0.00  | 1.00         | 0.00  |
| siCTRL     | 1.27                     | 0.24  | 1.58           | 0.31  | 0.97         | 0.21  | 0.80                   | 0.12  | 0.47           | 0.09  | 0.76         | 0.30  | 0.64                    | 0.08  | 0.31           | 0.08  | 0.80         | 0.31  |
| UBB        | 1.40                     | 0.41  | 0.90           | 0.34  | 2.21         | 0.89  | 0.14                   | 0.05  | 0.05           | 0.03  | 0.97         | 0.45  | 0.10                    | 0.03  | 0.06           | 0.03  | 0.45         | 0.23  |
| hnmpH1     | 3.63                     | 1.15  | 6.22           | 1.75  | 2.40         | 0.65  | 0.58                   | 0.09  | 0.31           | 0.08  | 0.70         | 0.22  | 0.16                    | 0.03  | 0.05           | 0.02  | 0.31         | 0.16  |
| media only | 1.01                     | 0.28  | 0.62           | 0.15  | 1.16         | 0.25  | 0.19                   | 0.05  | N/A            | N/A   | N/A          | N/A   | 0.20                    | 0.04  | N/A            | N/A   | N/A          | N/A   |
| ABT1       | 1.04                     | 0.07  | 0.97           | 0.10  | 0.81         | 0.09  | 0.87                   | 0.01  | 0.53           | 0.05  | 0.98         | 0.05  | 0.84                    | 0.06  | 0.55           | 0.07  | 1.23         | 0.15  |
| ACIN1      | 1.08                     | 0.15  | 0.84           | 0.15  | 1.70         | 0.36  | 0.21                   | 0.01  | 0.15           | 0.01  | 0.52         | 0.27  | 0.19                    | 0.01  | 0.18           | 0.02  | 0.29         | 0.10  |
| ACTL6A     | 0.66                     | 0.22  | 0.65           | 0.09  | 0.82         | 0.20  | 0.97                   | 0.32  | 0.41           | 0.02  | 0.88         | 0.24  | 1.48                    | 0.05  | 0.65           | 0.07  | 1.07         | 0.11  |
| ADAR       | 0.95                     | 0.19  | 1.04           | 0.10  | 0.76         | 0.22  | 0.95                   | 0.22  | 0.22           | 0.04  | 1.13         | 0.42  | 1.00                    | 0.13  | 0.21           | 0.02  | 1.51         | 0.48  |
| AKAP17A    | 1.36                     | 0.61  | 1.40           | 0.52  | 1.92         | 0.20  | 1.27                   | 0.15  | 0.59           | 0.22  | 0.63         | 0.12  | 1.02                    | 0.32  | 0.44           | 0.20  | 0.34         | 0.09  |
| AKAP8      | 0.71                     | 0.11  | 0.82           | 0.08  | 1.47         | 0.29  | 0.81                   | 0.09  | 0.43           | 0.03  | 1.18         | 0.52  | 1.18                    | 0.34  | 0.53           | 0.01  | 0.79         | 0.27  |
| ALYREF     | 1.20                     | 0.09  | 0.90           | 0.11  | 1.17         | 0.19  | 0.43                   | 0.02  | 0.27           | 0.06  | 0.75         | 0.16  | 0.36                    | 0.01  | 0.31           | 0.11  | 0.65         | 0.20  |
| AQR        | 0.81                     | 0.07  | 1.05           | 0.29  | 0.71         | 0.21  | 2.30                   | 0.11  | 1.24           | 0.22  | 1.06         | 0.40  | 2.85                    | 0.24  | 1.24           | 0.38  | 1.55         | 0.62  |
| BCAS2      | 0.86                     | 0.21  | 0.74           | 0.15  | 0.88         | 0.20  | 1.06                   | 0.11  | 0.78           | 0.01  | 1.03         | 0.51  | 1.26                    | 0.17  | 1.08           | 0.20  | 1.14         | 0.41  |
| BUB3       | 0.54                     | 0.08  | 0.57           | 0.10  | 0.89         | 0.25  | 0.63                   | 0.08  | 0.24           | 0.04  | 0.98         | 0.31  | 1.22                    | 0.35  | 0.42           | 0.03  | 1.14         | 0.35  |
| BUD13      | 3.11                     | 0.90  | 2.66           | 0.27  | 0.98         | 0.09  | 1.15                   | 0.30  | 0.85           | 0.24  | 1.24         | 0.07  | 0.37                    | 0.05  | 0.33           | 0.13  | 1.27         | 0.10  |
| BUD31      | 1.03                     | 0.33  | 0.82           | 0.14  | 1.10         | 0.16  | 0.97                   | 0.28  | 0.50           | 0.04  | 1.79         | 0.80  | 0.95                    | 0.09  | 0.61           | 0.05  | 1.73         | 0.95  |
| C14orf166  | 0.78                     | 0.16  | 0.57           | 0.15  | 0.84         | 0.24  | 0.79                   | 0.12  | 0.34           | 0.06  | 1.07         | 0.35  | 1.02                    | 0.06  | 0.63           | 0.08  | 1.31         | 0.34  |
| C16orf80   | 2.24                     | 0.43  | 1.64           | 0.33  | 0.77         | 0.01  | 1.27                   | 0.18  | 0.70           | 0.22  | 1.67         | 0.29  | 0.57                    | 0.04  | 0.43           | 0.12  | 2.16         | 0.34  |
| C19orf43   | 2.14                     | 0.35  | 1.88           | 0.25  | 1.18         | 0.30  | 0.87                   | 0.02  | 0.34           | 0.13  | 1.38         | 0.52  | 0.41                    | 0.07  | 0.18           | 0.04  | 1.15         | 0.15  |
| C1QBP      | 1.00                     | 0.10  | 0.80           | 0.06  | 1.21         | 0.17  | 0.47                   | 0.06  | 0.24           | 0.04  | 0.92         | 0.06  | 0.47                    | 0.04  | 0.30           | 0.02  | 0.78         | 0.11  |

|          |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| C22orf28 | 3.01 | 1.39 | 2.88 | 0.80 | 2.46 | 0.69 | 1.01 | 0.44 | 0.47 | 0.13 | 1.24 | N/A  | 0.34 | 0.02 | 0.17 | 0.02 | 0.43 | 0.06 |
| C2orf49  | 1.16 | 0.37 | 1.16 | 0.18 | 1.43 | 0.18 | 1.11 | 0.11 | 0.51 | 0.11 | 1.54 | 0.16 | 1.01 | 0.24 | 0.45 | 0.08 | 1.08 | 0.03 |
| CCDC12   | 1.43 | 0.17 | 1.87 | 0.37 | 1.48 | 0.41 | 0.48 | 0.08 | 0.29 | 0.07 | 2.60 | 1.23 | 0.33 | 0.03 | 0.15 | 0.02 | 1.51 | 0.72 |
| CCDC94   | 1.34 | 0.43 | 1.32 | 0.16 | 1.79 | 0.35 | 1.11 | 0.27 | 0.72 | 0.28 | 1.86 | 0.50 | 0.84 | 0.08 | 0.56 | 0.25 | 1.10 | 0.46 |
| CCNA1    | 0.89 | 0.06 | 0.93 | 0.20 | 1.28 | 0.13 | 0.98 | 0.13 | 0.49 | 0.03 | 0.95 | 0.36 | 1.09 | 0.11 | 0.54 | 0.11 | 0.73 | 0.19 |
| CCNK     | 1.04 | 0.10 | 1.57 | 0.06 | 1.02 | 0.12 | 0.93 | 0.20 | 0.36 | 0.05 | 1.46 | 0.51 | 0.89 | 0.19 | 0.23 | 0.04 | 1.40 | 0.33 |
| CD2BP2   | 0.84 | 0.06 | 0.69 | 0.08 | 0.76 | 0.18 | 0.59 | 0.07 | 0.30 | 0.02 | 0.98 | 0.27 | 0.71 | 0.09 | 0.44 | 0.07 | 1.30 | 0.25 |
| CDC40    | 2.21 | 0.14 | 2.28 | 0.19 | 0.98 | 0.23 | 0.69 | 0.09 | 0.37 | 0.07 | 0.82 | 0.15 | 0.31 | 0.02 | 0.16 | 0.02 | 0.87 | 0.23 |
| CDC5L    | 3.01 | 0.72 | 2.86 | 0.58 | 1.96 | 0.52 | 0.78 | 0.16 | 0.25 | 0.04 | 1.06 | 0.35 | 0.26 | 0.02 | 0.09 | 0.02 | 0.55 | 0.12 |
| CDK11A   | 0.85 | 0.18 | 1.15 | 0.30 | 0.87 | 0.16 | 0.87 | 0.16 | 0.40 | 0.09 | 0.88 | 0.18 | 1.03 | 0.06 | 0.35 | 0.03 | 1.02 | 0.21 |
| CDK12    | 0.60 | 0.10 | 0.53 | 0.07 | 0.98 | 0.34 | 0.31 | 0.06 | 0.16 | 0.05 | 0.71 | 0.24 | 0.52 | 0.01 | 0.29 | 0.05 | 0.74 | 0.12 |
| CELF1    | 0.92 | 0.16 | 0.85 | 0.18 | 0.94 | 0.10 | 0.90 | 0.05 | 0.55 | 0.05 | 0.83 | 0.15 | 0.99 | 0.13 | 0.66 | 0.10 | 0.88 | 0.17 |
| CELF2    | 0.66 | 0.18 | 0.44 | 0.05 | 0.83 | 0.16 | 0.69 | 0.13 | 0.39 | 0.00 | 0.82 | 0.14 | 1.06 | 0.13 | 0.90 | 0.10 | 1.00 | 0.08 |
| CELF3    | 1.59 | 0.29 | 2.02 | 0.24 | 1.18 | 0.36 | 0.84 | 0.07 | 0.55 | 0.08 | 1.14 | 0.07 | 0.54 | 0.08 | 0.27 | 0.01 | 1.02 | 0.25 |
| CELF4    | 0.60 | 0.05 | 0.62 | 0.10 | 1.10 | 0.15 | 0.90 | 0.07 | 0.50 | 0.06 | 1.34 | 0.41 | 1.49 | 0.10 | 0.81 | 0.13 | 1.22 | 0.31 |
| CELF5    | 0.91 | 0.15 | 0.84 | 0.06 | 1.02 | 0.30 | 0.40 | 0.08 | 0.18 | 0.02 | 0.79 | 0.26 | 0.44 | 0.04 | 0.21 | 0.04 | 0.78 | 0.17 |
| CELF6    | 0.99 | 0.17 | 0.95 | 0.04 | 0.83 | 0.23 | 0.58 | 0.10 | 0.20 | 0.03 | 0.75 | 0.19 | 0.59 | 0.01 | 0.21 | 0.02 | 0.93 | 0.28 |
| CHERP    | 1.76 | 0.49 | 1.33 | 0.13 | 1.25 | 0.27 | 0.69 | 0.19 | 0.30 | 0.08 | 1.39 | 0.51 | 0.40 | 0.02 | 0.22 | 0.04 | 1.12 | 0.37 |
| CIR1     | 1.16 | 0.26 | 1.64 | 0.25 | 1.32 | 0.56 | 0.88 | 0.18 | 0.48 | 0.05 | 1.04 | 0.34 | 0.75 | 0.02 | 0.30 | 0.03 | 0.86 | 0.30 |
| CIRBP    | 0.62 | 0.21 | 0.43 | 0.01 | 0.91 | 0.22 | 1.02 | 0.40 | 0.45 | 0.08 | 1.88 | 0.63 | 1.65 | 0.25 | 1.06 | 0.19 | 2.07 | 0.55 |
| CLASRP   | 1.20 | 0.47 | 1.33 | 0.64 | 1.08 | 0.05 | 0.74 | 0.09 | 0.22 | 0.04 | 0.94 | 0.10 | 0.68 | 0.26 | 0.19 | 0.08 | 0.89 | 0.15 |
| CLK1     | 1.37 | 0.26 | 1.75 | 0.45 | 1.02 | 0.23 | 1.00 | 0.17 | 0.57 | 0.22 | 1.20 | 0.39 | 0.73 | 0.03 | 0.32 | 0.05 | 1.17 | 0.29 |
| CLK2     | 1.83 | 0.18 | 2.02 | 0.25 | 1.28 | 0.12 | 0.93 | 0.12 | 0.60 | 0.04 | 1.35 | 0.27 | 0.51 | 0.07 | 0.30 | 0.04 | 1.04 | 0.12 |
| CLK2     | 1.82 | 0.22 | 2.04 | 0.27 | 1.21 | 0.22 | 0.95 | 0.08 | 0.56 | 0.09 | 1.38 | 0.39 | 0.52 | 0.02 | 0.27 | 0.03 | 1.14 | 0.26 |
| CLK3     | 1.39 | 0.18 | 1.19 | 0.21 | 0.88 | 0.25 | 0.44 | 0.10 | 0.19 | 0.03 | 0.85 | 0.16 | 0.31 | 0.03 | 0.16 | 0.02 | 1.00 | 0.22 |
| CLK4     | 0.66 | 0.08 | 0.59 | 0.01 | 0.61 | 0.13 | 0.80 | 0.12 | 0.37 | 0.04 | 0.94 | 0.22 | 1.21 | 0.06 | 0.63 | 0.06 | 1.58 | 0.34 |
| CPSF1    | 3.90 | 1.02 | 4.27 | 0.57 | 1.10 | 0.40 | 0.64 | 0.04 | 0.37 | 0.06 | 1.08 | 0.28 | 0.17 | 0.04 | 0.09 | 0.01 | 1.05 | 0.34 |
| CPSF2    | 2.16 | 0.68 | 1.61 | 0.22 | 1.26 | 0.41 | 0.57 | 0.14 | 0.22 | 0.03 | 0.94 | 0.37 | 0.27 | 0.03 | 0.14 | 0.01 | 0.83 | 0.53 |
| CPSF3    | 2.72 | 0.29 | 5.32 | 0.24 | 1.70 | 0.69 | 0.47 | 0.08 | 0.27 | 0.02 | 1.37 | 0.41 | 0.17 | 0.02 | 0.05 | 0.00 | 0.88 | 0.35 |
| CPSF4    | 1.31 | 0.36 | 1.17 | 0.17 | 1.21 | 0.30 | 0.59 | 0.13 | 0.26 | 0.05 | 1.12 | 0.30 | 0.45 | 0.05 | 0.23 | 0.02 | 0.94 | 0.23 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| CPSF6   | 2.20 | 0.88 | 1.57 | 0.16 | 1.24 | 0.44 | 1.06 | 0.33 | 0.41 | 0.10 | 1.27 | 0.43 | 0.50 | 0.07 | 0.26 | 0.06 | 1.08 | 0.31 |
| CRNKL1  | 4.01 | 0.16 | 4.13 | 1.39 | 1.75 | 0.28 | 1.09 | 0.03 | 0.43 | 0.06 | 0.91 | 0.56 | 0.27 | 0.01 | 0.11 | 0.02 | 0.57 | 0.45 |
| CSDA    | 0.72 | 0.26 | 0.29 | 0.03 | 1.16 | 0.24 | 0.65 | 0.15 | 0.29 | 0.09 | 1.09 | 0.47 | 0.94 | 0.17 | 1.01 | 0.36 | 1.01 | 0.57 |
| CSN3    | 1.19 | 0.51 | 0.95 | 0.11 | 1.00 | 0.23 | 1.03 | 0.25 | 0.48 | 0.11 | 1.36 | 0.22 | 0.91 | 0.19 | 0.51 | 0.09 | 1.30 | 0.47 |
| CSTF3   | 1.17 | 0.32 | 1.14 | 0.13 | 1.85 | 0.56 | 1.29 | 0.28 | 0.55 | 0.19 | 1.26 | 0.45 | 1.12 | 0.16 | 0.50 | 0.21 | 0.71 | 0.31 |
| CTNBL1  | 1.01 | 0.40 | 0.68 | 0.10 | 1.27 | 0.30 | 0.59 | 0.15 | 0.49 | 0.03 | 0.88 | 0.12 | 0.61 | 0.10 | 0.73 | 0.10 | 1.31 | 0.88 |
| CWC15   | 1.56 | 0.45 | 1.24 | 0.12 | 1.70 | 0.20 | 1.29 | 0.31 | 0.66 | 0.14 | 0.90 | 0.21 | 0.84 | 0.14 | 0.53 | 0.07 | 0.54 | 0.14 |
| CWC22   | 3.45 | 0.51 | 3.79 | 0.45 | 1.73 | 0.36 | 1.85 | 0.23 | 0.52 | 0.09 | 0.83 | 0.01 | 0.54 | 0.04 | 0.14 | 0.01 | 0.50 | 0.10 |
| CWC27   | 0.85 | 0.17 | 1.02 | 0.19 | 1.43 | 0.08 | 0.71 | 0.14 | 0.48 | 0.24 | 1.02 | 0.01 | 0.84 | 0.05 | 0.50 | 0.30 | 0.71 | 0.04 |
| CWF19L1 | 1.14 | 0.31 | 0.87 | 0.07 | 1.40 | 0.31 | 0.87 | 0.18 | 0.35 | 0.06 | 2.77 | 0.80 | 0.78 | 0.10 | 0.40 | 0.04 | 2.08 | 0.86 |
| DAZAP1  | 1.24 | 0.28 | 0.96 | 0.04 | 1.04 | 0.30 | 1.42 | 0.19 | 0.63 | 0.06 | 2.01 | 0.58 | 1.18 | 0.25 | 0.66 | 0.06 | 2.03 | 0.86 |
| DDB1    | 2.36 | 0.64 | 1.33 | 0.14 | 0.97 | 0.20 | 1.06 | 0.33 | 0.55 | 0.05 | 1.52 | 0.70 | 0.45 | 0.05 | 0.42 | 0.07 | 1.68 | 1.09 |
| DDIT3   | 1.19 | 0.07 | 0.95 | 0.27 | 1.28 | 0.21 | 0.75 | 0.01 | 0.22 | 0.08 | 0.88 | 0.41 | 0.63 | 0.04 | 0.23 | 0.07 | 0.71 | 0.41 |
| DDX1    | 2.11 | 0.12 | 1.57 | 0.22 | 1.02 | 0.22 | 1.07 | 0.17 | 0.37 | 0.07 | 1.94 | 0.89 | 0.51 | 0.06 | 0.24 | 0.08 | 2.01 | 1.18 |
| DDX17   | 1.18 | 0.19 | 1.27 | 0.31 | 1.30 | 0.34 | 1.24 | 0.30 | 0.50 | 0.10 | 2.70 | N/A  | 1.05 | 0.13 | 0.40 | 0.02 | 0.88 | N/A  |
| DDX19B  | 1.73 | 0.30 | 1.07 | 0.08 | 0.86 | 0.27 | 0.56 | 0.12 | 0.21 | 0.02 | 0.85 | 0.75 | 0.32 | 0.02 | 0.20 | 0.02 | 1.54 | 0.82 |
| DDX21   | 1.47 | 0.77 | 1.05 | 0.15 | 1.17 | 0.32 | 1.00 | 0.38 | 0.47 | 0.13 | 1.93 | 1.18 | 0.72 | 0.18 | 0.46 | 0.16 | 1.99 | 1.31 |
| DDX23   | 3.51 | 1.05 | 4.08 | 0.79 | 2.31 | 0.20 | 1.00 | 0.09 | 0.48 | 0.09 | 0.77 | 0.08 | 0.30 | 0.08 | 0.12 | 0.03 | 0.33 | 0.01 |
| DDX39A  | 0.99 | 0.03 | 0.48 | 0.12 | 0.83 | 0.18 | 0.97 | 0.11 | 0.48 | 0.09 | 1.34 | 0.52 | 0.98 | 0.09 | 1.03 | 0.29 | 1.64 | 0.69 |
| DDX39B  | 0.98 | 0.49 | 0.77 | 0.13 | 1.38 | 0.34 | 0.46 | 0.15 | 0.26 | 0.02 | 0.67 | 0.30 | 0.50 | 0.10 | 0.35 | 0.06 | 0.49 | 0.15 |
| DDX3X   | 0.49 | 0.17 | 0.23 | 0.03 | 0.48 | 0.10 | 1.42 | 0.31 | 0.74 | 0.14 | 1.30 | 0.69 | 2.97 | 0.40 | 3.18 | 0.21 | 1.79 | 0.58 |
| DDX3Y   | 2.66 | 0.89 | 1.40 | 0.24 | 1.66 | 0.25 | 1.30 | 0.50 | 0.54 | 0.04 | 1.69 | 0.37 | 0.49 | 0.06 | 0.40 | 0.10 | 1.58 | 0.94 |
| DDX41   | 0.66 | 0.09 | 0.53 | 0.11 | 1.03 | 0.23 | 1.17 | 0.27 | 0.57 | 0.08 | 0.69 | 0.59 | 1.78 | 0.36 | 1.09 | 0.09 | 1.15 | 0.14 |
| DDX46   | 1.60 | 0.46 | 1.26 | 0.30 | 1.44 | 0.42 | 1.08 | 0.11 | 0.42 | 0.12 | 1.05 | 0.49 | 0.72 | 0.21 | 0.36 | 0.17 | 0.79 | 0.47 |
| DDX49   | 2.59 | 0.83 | 1.64 | 0.16 | 2.15 | 0.65 | 1.17 | 0.52 | 0.48 | 0.03 | 5.13 | 0.50 | 0.44 | 0.05 | 0.29 | 0.02 | 2.88 | 0.08 |
| DDX5    | 1.31 | 0.52 | 0.94 | 0.03 | 1.01 | 0.33 | 0.83 | 0.29 | 0.31 | 0.07 | 2.24 | 0.84 | 0.64 | 0.07 | 0.33 | 0.09 | 1.65 | 0.42 |
| DDX54   | 2.03 | 0.64 | 1.47 | 0.18 | 1.31 | 0.07 | 0.95 | 0.23 | 0.36 | 0.08 | 0.97 | 0.41 | 0.48 | 0.05 | 0.25 | 0.09 | 0.75 | 0.34 |
| DDX6    | 2.27 | 0.56 | 1.98 | 0.21 | 1.38 | 0.27 | 1.04 | 0.33 | 0.55 | 0.04 | 1.38 | 0.45 | 0.45 | 0.03 | 0.28 | 0.01 | 1.03 | 0.41 |
| DEK     | 1.97 | 0.12 | 1.17 | 0.19 | 0.81 | 0.24 | 1.60 | 0.06 | 0.56 | 0.05 | 0.98 | 0.47 | 0.81 | 0.08 | 0.49 | 0.12 | 1.33 | 0.85 |
| DGCR14  | 2.56 | 0.37 | 1.95 | 0.17 | 1.33 | 0.35 | 1.08 | 0.20 | 0.42 | 0.09 | 1.29 | 0.38 | 0.43 | 0.12 | 0.22 | 0.06 | 1.04 | 0.50 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| DHX15   | 1.78 | 0.34 | 1.01 | 0.26 | 1.17 | 0.27 | 0.80 | 0.22 | 0.30 | 0.04 | 1.95 | 0.21 | 0.45 | 0.08 | 0.31 | 0.08 | 1.73 | 0.71 |
| DHX16   | 1.75 | 0.49 | 1.06 | 0.21 | 1.57 | 0.27 | 1.54 | 0.39 | 0.79 | 0.14 | 0.74 | 0.66 | 0.90 | 0.15 | 0.76 | 0.16 | 0.72 | 0.30 |
| DHX35   | 1.90 | 0.55 | 1.57 | 0.11 | 1.19 | 0.28 | 0.86 | 0.18 | 0.46 | 0.02 | 0.83 | 0.43 | 0.46 | 0.07 | 0.29 | 0.01 | 0.75 | 0.53 |
| DHX38   | 2.21 | 0.64 | 1.73 | 0.13 | 1.25 | 0.33 | 1.10 | 0.31 | 0.44 | 0.11 | 2.31 | 0.82 | 0.50 | 0.04 | 0.25 | 0.04 | 1.91 | 0.82 |
| DHX8    | 1.18 | 0.53 | 0.61 | 0.12 | 0.87 | 0.29 | 1.82 | 0.94 | 0.70 | 0.27 | 1.23 | 0.48 | 1.51 | 0.11 | 1.24 | 0.70 | 1.51 | 0.79 |
| DHX9    | 1.21 | 0.59 | 0.56 | 0.04 | 1.29 | 0.46 | 1.15 | 0.40 | 0.54 | 0.10 | 1.42 | 0.53 | 1.00 | 0.16 | 0.97 | 0.21 | 1.17 | 0.62 |
| DIDO1   | 1.06 | 0.32 | 0.18 | 0.01 | 0.40 | 0.06 | 1.07 | 0.24 | 0.21 | 0.03 | 1.11 | 0.40 | 1.03 | 0.14 | 1.16 | 0.15 | 2.10 | 0.35 |
| DIS3    | 1.32 | 0.35 | 1.20 | 0.22 | 1.10 | 0.14 | 0.80 | 0.11 | 0.33 | 0.08 | 0.49 | 0.13 | 0.63 | 0.18 | 0.27 | 0.03 | 0.44 | 0.12 |
| DNAJC17 | 1.56 | 0.18 | 0.98 | 0.19 | 1.13 | 0.14 | 1.53 | 0.36 | 0.84 | 0.17 | 1.62 | 0.65 | 0.97 | 0.15 | 0.87 | 0.17 | 1.46 | 0.66 |
| DNAJC6  | 1.89 | 0.67 | 1.50 | 0.01 | 1.06 | 0.28 | 1.66 | 0.44 | 0.37 | 0.07 | 1.64 | 1.45 | 0.90 | 0.17 | 0.25 | 0.04 | 2.48 | 1.22 |
| DNAJC8  | 2.22 | 0.37 | 2.67 | 0.22 | 2.21 | 0.80 | 1.31 | 0.25 | 0.55 | 0.06 | 1.70 | 0.80 | 0.59 | 0.07 | 0.21 | 0.03 | 0.81 | 0.46 |
| EEF1A1  | 1.30 | 0.50 | 0.79 | 0.04 | 1.21 | 0.36 | 0.64 | 0.18 | 0.20 | 0.04 | 0.98 | 0.46 | 0.51 | 0.06 | 0.25 | 0.04 | 0.85 | 0.49 |
| EFTUD2  | 2.73 | 0.66 | 1.10 | 0.11 | 1.39 | 0.23 | 0.99 | 0.31 | 0.39 | 0.05 | 1.85 | 0.71 | 0.36 | 0.06 | 0.35 | 0.01 | 1.34 | 0.50 |
| EIF2S2  | 4.09 | 0.35 | 2.44 | 0.38 | 0.93 | 0.20 | 0.94 | 0.20 | 0.28 | 0.00 | 0.97 | 0.40 | 0.23 | 0.03 | 0.12 | 0.02 | 1.10 | 0.61 |
| EIF3A   | 0.67 | 0.10 | 0.42 | 0.05 | 1.08 | 0.30 | 1.10 | 0.12 | 0.37 | 0.04 | 2.60 | 0.76 | 1.65 | 0.18 | 0.90 | 0.21 | 2.42 | 0.41 |
| EIF3E   | 0.59 | 0.17 | 0.30 | 0.02 | 0.93 | 0.23 | 0.78 | 0.20 | 0.24 | 0.03 | 1.87 | 0.81 | 1.33 | 0.12 | 0.79 | 0.06 | 2.17 | 1.37 |
| EIF3I   | 1.13 | 0.29 | 0.75 | 0.11 | 1.18 | 0.39 | 0.95 | 0.20 | 0.44 | 0.11 | 1.69 | 0.45 | 0.85 | 0.12 | 0.59 | 0.10 | 1.47 | 0.36 |
| EIF3L   | 1.44 | 0.10 | 1.02 | 0.06 | 1.46 | 0.45 | 0.88 | 0.16 | 0.29 | 0.05 | 1.83 | 0.64 | 0.61 | 0.12 | 0.29 | 0.06 | 1.35 | 0.74 |
| EIF3M   | 1.15 | 0.27 | 1.48 | 0.08 | 1.67 | 0.40 | 0.69 | 0.14 | 0.31 | 0.06 | 1.77 | 0.42 | 0.60 | 0.10 | 0.21 | 0.04 | 1.12 | 0.43 |
| EIF4A2  | 1.31 | 0.40 | 0.95 | 0.05 | 0.96 | 0.33 | 1.11 | 0.27 | 0.39 | 0.04 | 0.93 | 0.60 | 0.87 | 0.13 | 0.41 | 0.05 | 1.09 | 0.88 |
| EIF4A3  | 1.98 | 0.57 | 1.03 | 0.15 | 1.26 | 0.39 | 0.44 | 0.13 | 0.06 | 0.04 | 1.36 | 0.36 | 0.22 | 0.03 | 0.06 | 0.03 | 1.14 | 0.45 |
| EIF4G3  | 1.26 | 0.26 | 0.89 | 0.11 | 1.23 | 0.35 | 0.99 | 0.25 | 0.39 | 0.08 | 1.68 | 0.41 | 0.79 | 0.09 | 0.44 | 0.09 | 1.43 | 0.53 |
| ELAVL1  | 2.04 | 0.79 | 1.16 | 0.17 | 1.20 | 0.23 | 1.11 | 0.25 | 0.48 | 0.06 | 1.62 | 0.95 | 0.59 | 0.19 | 0.42 | 0.11 | 1.46 | 1.10 |
| ELAVL2  | 1.16 | 0.50 | 0.43 | 0.05 | 1.38 | 0.45 | 1.43 | 0.61 | 0.40 | 0.10 | 1.65 | 0.80 | 1.23 | 0.05 | 0.93 | 0.21 | 1.26 | 0.78 |
| ELAVL3  | 1.17 | 0.21 | 1.47 | 0.19 | 1.26 | 0.23 | 1.20 | 0.23 | 0.82 | 0.15 | 2.46 | 1.67 | 1.03 | 0.08 | 0.56 | 0.08 | 1.89 | 1.03 |
| ELAVL4  | 1.63 | 0.75 | 1.57 | 0.33 | 1.90 | 0.09 | 0.95 | 0.33 | 0.59 | 0.18 | 2.18 | 0.53 | 0.62 | 0.13 | 0.40 | 0.16 | 1.15 | 0.26 |
| ELMOD3  | 0.80 | 0.07 | 0.66 | 0.31 | 1.63 | 0.12 | 0.40 | 0.03 | 0.19 | 0.06 | 1.13 | 0.44 | 0.50 | 0.06 | 0.31 | 0.15 | 0.70 | 0.27 |
| EP400   | 0.49 | 0.20 | 0.74 | 0.28 | 2.37 | 0.55 | 0.84 | 0.35 | 0.45 | 0.11 | 1.65 | 0.88 | 1.76 | 0.39 | 0.63 | 0.07 | 0.71 | 0.33 |
| ERCC3   | 0.53 | 0.21 | 0.57 | 0.08 | 1.54 | 0.26 | 0.40 | 0.13 | 0.22 | 0.05 | 1.66 | 0.33 | 0.79 | 0.16 | 0.41 | 0.17 | 1.10 | 0.31 |
| ERVW-1  | 0.72 | 0.26 | 0.86 | 0.11 | 2.05 | 0.32 | 0.59 | 0.18 | 0.59 | 0.04 | 1.79 | 0.40 | 0.84 | 0.14 | 0.70 | 0.12 | 0.90 | 0.33 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| ESRP1   | 1.27 | 0.06 | 2.02 | 0.68 | 1.73 | 0.06 | 0.75 | 0.12 | 0.53 | 0.15 | 1.46 | 0.45 | 0.59 | 0.12 | 0.28 | 0.12 | 0.84 | 0.27 |
| ESRP2   | 2.83 | 0.51 | 5.10 | 2.34 | 1.32 | 0.25 | 0.91 | 0.08 | 0.66 | 0.30 | 0.86 | 0.05 | 0.33 | 0.04 | 0.13 | 0.04 | 0.67 | 0.15 |
| EWSR1   | 0.84 | 0.14 | 1.41 | 0.14 | 1.53 | 0.24 | 0.55 | 0.07 | 0.38 | 0.10 | 0.94 | 0.35 | 0.66 | 0.12 | 0.27 | 0.06 | 0.60 | 0.14 |
| EXOSC1  | 0.45 | 0.18 | 0.40 | 0.00 | 1.33 | 0.41 | 0.58 | 0.15 | 0.31 | 0.17 | 1.31 | 0.32 | 1.36 | 0.30 | 0.79 | 0.42 | 1.05 | 0.38 |
| EXOSC10 | 1.04 | 0.24 | 1.68 | 0.24 | 1.59 | 0.30 | 1.17 | 0.30 | 0.50 | 0.20 | 1.65 | 0.20 | 1.12 | 0.06 | 0.29 | 0.10 | 1.06 | 0.22 |
| EXOSC2  | 0.72 | 0.27 | 0.68 | 0.07 | 1.10 | 0.19 | 0.89 | 0.35 | 0.48 | 0.01 | 1.69 | 0.48 | 1.25 | 0.13 | 0.71 | 0.08 | 1.56 | 0.49 |
| EXOSC3  | 1.09 | 0.48 | 1.24 | 0.03 | 1.46 | 0.33 | 0.89 | 0.20 | 0.56 | 0.29 | 8.71 | 0.60 | 0.90 | 0.31 | 0.46 | 0.25 | 6.82 | 0.72 |
| EXOSC4  | 1.31 | 0.49 | 1.80 | 0.32 | 1.60 | 0.35 | 0.41 | 0.11 | 0.24 | 0.06 | 1.50 | 0.31 | 0.32 | 0.06 | 0.14 | 0.05 | 0.97 | 0.28 |
| EXOSC5  | 0.61 | 0.15 | 0.63 | 0.06 | 1.78 | 0.32 | 0.93 | 0.31 | 0.58 | 0.14 | 1.79 | 0.88 | 1.51 | 0.17 | 0.94 | 0.30 | 0.99 | 0.37 |
| EXOSC7  | 1.07 | 0.36 | 0.95 | 0.16 | 1.64 | 0.34 | 0.80 | 0.33 | 0.27 | 0.08 | 2.07 | 0.54 | 0.75 | 0.14 | 0.28 | 0.07 | 1.27 | 0.28 |
| EXOSC8  | 0.57 | 0.25 | 0.59 | 0.12 | 1.70 | 0.32 | 0.41 | 0.10 | 0.23 | 0.00 | 2.15 | 0.92 | 0.78 | 0.15 | 0.39 | 0.07 | 1.30 | 0.61 |
| EXOSC9  | 0.74 | 0.12 | 0.91 | 0.12 | 0.97 | 0.09 | 1.06 | 0.07 | 0.74 | 0.06 | 1.31 | 0.16 | 1.45 | 0.25 | 0.83 | 0.18 | 1.36 | 0.27 |
| FAM120A | 1.35 | 0.57 | 0.90 | 0.17 | 1.61 | 0.44 | 0.98 | 0.44 | 0.32 | 0.11 | 1.11 | 0.32 | 0.73 | 0.05 | 0.35 | 0.06 | 0.69 | 0.11 |
| FAM131B | 2.09 | 0.17 | 1.92 | 0.76 | 0.60 | 0.07 | 0.49 | 0.01 | 0.15 | 0.01 | 0.66 | 0.08 | 0.24 | 0.02 | 0.08 | 0.03 | 1.09 | 0.06 |
| FAM207A | 0.39 | 0.09 | 0.50 | 0.05 | 0.50 | 0.11 | 0.98 | 0.06 | 0.45 | 0.06 | 0.71 | 0.17 | 2.58 | 0.58 | 0.90 | 0.10 | 1.42 | 0.10 |
| FAM32A  | 1.09 | 0.30 | 1.26 | 0.15 | 1.02 | 0.14 | 0.95 | 0.14 | 0.58 | 0.11 | 1.73 | 0.16 | 0.89 | 0.14 | 0.46 | 0.06 | 1.71 | 0.21 |
| FAU     | 0.53 | 0.10 | 0.72 | 0.05 | 1.57 | 0.23 | 1.40 | 0.33 | 0.60 | 0.16 | 1.56 | 0.37 | 2.66 | 0.60 | 0.84 | 0.25 | 1.03 | 0.33 |
| FIP1L1  | 1.23 | 0.43 | 1.56 | 0.26 | 1.32 | 0.22 | 0.57 | 0.15 | 0.21 | 0.06 | 1.62 | 0.62 | 0.48 | 0.12 | 0.14 | 0.05 | 1.30 | 0.63 |
| FKBP3   | 1.06 | 0.46 | 0.81 | 0.08 | 0.90 | 0.13 | 0.76 | 0.26 | 0.31 | 0.04 | 1.43 | 0.39 | 0.74 | 0.09 | 0.38 | 0.08 | 1.62 | 0.55 |
| FMR1    | 0.86 | 0.20 | 1.13 | 0.17 | 0.97 | 0.21 | 0.63 | 0.10 | 0.27 | 0.08 | 1.63 | 0.32 | 0.74 | 0.12 | 0.24 | 0.04 | 1.94 | 0.52 |
| FRG1    | 1.40 | 0.52 | 1.09 | 0.08 | 1.66 | 0.28 | 1.12 | 0.40 | 0.31 | 0.09 | 1.76 | 0.41 | 0.81 | 0.03 | 0.28 | 0.07 | 1.09 | 0.33 |
| FUBP1   | 1.18 | 0.50 | 1.38 | 0.19 | 2.17 | 0.30 | 0.77 | 0.23 | 0.39 | 0.09 | 1.32 | N/A  | 0.69 | 0.14 | 0.29 | 0.10 | 0.56 | N/A  |
| FUBP3   | 0.92 | 0.27 | 0.80 | 0.06 | 1.14 | 0.08 | 0.78 | 0.09 | 0.46 | 0.12 | 0.80 | 0.11 | 0.92 | 0.39 | 0.59 | 0.19 | 0.71 | 0.15 |
| FUS     | 0.73 | 0.14 | 0.55 | 0.01 | 0.78 | 0.09 | 0.73 | 0.07 | 0.25 | 0.10 | 1.00 | 0.36 | 1.02 | 0.10 | 0.46 | 0.18 | 1.26 | 0.35 |
| GCFC1   | 0.47 | 0.06 | 0.48 | 0.09 | 0.77 | 0.18 | 0.70 | 0.06 | 0.28 | 0.04 | 1.09 | 0.59 | 1.52 | 0.08 | 0.60 | 0.12 | 1.37 | 0.49 |
| GCFC2   | 1.00 | 0.19 | 0.74 | 0.09 | 1.05 | 0.21 | 0.81 | 0.21 | 0.39 | 0.08 | 0.96 | 0.48 | 0.81 | 0.06 | 0.52 | 0.07 | 0.93 | 0.51 |
| GINS1   | 1.80 | 0.69 | 1.45 | 0.34 | 1.23 | 0.27 | 0.89 | 0.27 | 0.41 | 0.14 | 0.73 | 0.06 | 0.46 | 0.08 | 0.22 | 0.06 | 0.61 | 0.09 |
| GINS2   | 1.72 | 0.22 | 1.29 | 0.20 | 0.74 | 0.04 | 1.60 | 0.22 | 0.70 | 0.19 | 0.67 | 0.06 | 0.87 | 0.11 | 0.48 | 0.02 | 0.91 | 0.11 |
| GINS3   | 1.17 | 0.29 | 0.60 | 0.04 | 0.93 | 0.06 | 1.35 | 0.30 | 0.57 | 0.13 | 0.87 | 0.04 | 1.04 | 0.05 | 0.80 | 0.04 | 0.94 | 0.08 |
| GNB2L1  | 1.48 | 0.52 | 1.23 | 0.25 | 1.32 | 0.32 | 1.67 | 0.48 | 0.59 | 0.14 | 2.20 | 0.22 | 1.15 | 0.12 | 0.48 | 0.04 | 1.75 | 0.55 |

|            |      |      |      |      |      |      |      |      |      |      |       |       |      |      |      |      |       |       |
|------------|------|------|------|------|------|------|------|------|------|------|-------|-------|------|------|------|------|-------|-------|
| GPATCH1    | 1.41 | 0.52 | 1.44 | 0.28 | 1.69 | 0.29 | 0.70 | 0.24 | 0.32 | 0.03 | 1.16  | 0.78  | 0.50 | 0.10 | 0.22 | 0.02 | 0.71  | 0.48  |
| GRSF1      | 0.97 | 0.31 | 1.00 | 0.17 | 2.44 | 0.75 | 0.47 | 0.14 | 0.15 | 0.01 | 0.95  | 0.25  | 0.49 | 0.06 | 0.15 | 0.02 | 0.43  | 0.21  |
| GTF2I      | 0.99 | 0.52 | 0.73 | 0.08 | 1.55 | 0.30 | 1.00 | 0.41 | 0.45 | 0.03 | 33.92 | 14.44 | 1.05 | 0.14 | 0.62 | 0.09 | 24.85 | 16.37 |
| GULP1      | 2.11 | 0.13 | 0.84 | 0.07 | 1.02 | 0.15 | 1.77 | 0.09 | 0.90 | 0.04 | 1.25  | 0.33  | 0.85 | 0.08 | 0.92 | 0.09 | 1.26  | 0.48  |
| HCFC1      | 0.82 | 0.33 | 0.66 | 0.13 | 0.84 | 0.14 | 1.01 | 0.18 | 0.49 | 0.17 | 1.53  | 0.27  | 1.35 | 0.44 | 0.74 | 0.16 | 1.84  | 0.23  |
| HDAC2      | 0.85 | 0.40 | 0.49 | 0.12 | 1.02 | 0.21 | 0.76 | 0.23 | 0.33 | 0.07 | 0.76  | 0.37  | 0.96 | 0.20 | 0.68 | 0.09 | 0.72  | 0.22  |
| HIST1H2AC  | 1.31 | 0.51 | 0.90 | 0.16 | 1.23 | 0.27 | 0.84 | 0.23 | 0.37 | 0.03 | 1.70  | 1.57  | 0.67 | 0.11 | 0.42 | 0.09 | 1.26  | 0.90  |
| HIST2H2AA4 | 1.55 | 0.63 | 1.17 | 0.43 | 1.23 | 0.14 | 1.15 | 0.37 | 0.40 | 0.06 | 1.01  | 0.22  | 0.77 | 0.15 | 0.36 | 0.08 | 0.84  | 0.28  |
| HMGB1      | 0.58 | 0.05 | 0.55 | 0.06 | 0.75 | 0.14 | 0.70 | 0.17 | 0.32 | 0.04 | 0.72  | 0.13  | 1.20 | 0.21 | 0.59 | 0.13 | 0.99  | 0.25  |
| HMGB3      | 1.38 | 0.33 | 1.18 | 0.08 | 1.16 | 0.16 | 0.50 | 0.11 | 0.23 | 0.02 | 1.28  | 0.83  | 0.37 | 0.06 | 0.20 | 0.03 | 1.05  | 0.54  |
| HNRNPA0    | 0.94 | 0.39 | 0.65 | 0.16 | 1.62 | 0.37 | 0.61 | 0.22 | 0.31 | 0.04 | 1.65  | 0.10  | 0.67 | 0.10 | 0.49 | 0.07 | 1.06  | 0.26  |
| HNRNPA1    | 0.60 | 0.30 | 0.39 | 0.03 | 1.06 | 0.14 | 1.07 | 0.35 | 0.56 | 0.03 | 0.59  | 0.19  | 1.90 | 0.35 | 1.45 | 0.15 | 0.58  | 0.24  |
| HNRNPA2B1  | 1.10 | 0.24 | 0.98 | 0.14 | 1.14 | 0.16 | 0.88 | 0.11 | 0.43 | 0.12 | 1.01  | 0.23  | 0.81 | 0.09 | 0.44 | 0.13 | 0.89  | 0.18  |
| HNRNPA3    | 0.88 | 0.21 | 0.79 | 0.04 | 2.00 | 0.62 | 0.67 | 0.14 | 0.26 | 0.06 | 0.94  | 0.18  | 0.77 | 0.03 | 0.33 | 0.07 | 0.48  | 0.06  |
| HNRNPAB    | 0.93 | 0.41 | 0.64 | 0.17 | 3.78 | 0.85 | 0.75 | 0.23 | 0.42 | 0.10 | 1.37  | 0.13  | 0.83 | 0.13 | 0.70 | 0.34 | 0.37  | 0.05  |
| HNRNPC     | 1.10 | 0.42 | 1.36 | 0.30 | 2.63 | 0.35 | 0.47 | 0.14 | 0.25 | 0.05 | 0.86  | 0.02  | 0.44 | 0.10 | 0.18 | 0.04 | 0.31  | 0.03  |
| HNRNPCL1   | 1.30 | 0.44 | 1.37 | 0.46 | 2.60 | 0.53 | 0.65 | 0.10 | 0.47 | 0.08 | 0.76  | 0.16  | 0.53 | 0.14 | 0.37 | 0.12 | 0.31  | 0.14  |
| HNRNPD     | 0.65 | 0.05 | 0.67 | 0.12 | 1.35 | 0.18 | 1.39 | 0.18 | 0.43 | 0.07 | 0.59  | 0.10  | 2.15 | 0.28 | 0.67 | 0.22 | 0.45  | 0.14  |
| HNRNPF     | 5.56 | 0.58 | 6.48 | 2.23 | 2.41 | 1.22 | 1.57 | 0.18 | 0.56 | 0.13 | 0.83  | 0.10  | 0.28 | 0.02 | 0.09 | 0.02 | 0.44  | 0.27  |
| HNRNPH1    | 1.59 | 0.23 | 2.00 | 0.41 | 3.14 | 1.39 | 0.93 | 0.05 | 0.44 | 0.10 | 0.85  | 0.13  | 0.59 | 0.08 | 0.22 | 0.02 | 0.31  | 0.11  |
| HNRNPH2    | 1.02 | 0.27 | 1.02 | 0.10 | 0.95 | 0.08 | 1.06 | 0.22 | 0.53 | 0.04 | 0.72  | 0.31  | 1.06 | 0.12 | 0.53 | 0.06 | 0.77  | 0.39  |
| HNRNPH3    | 0.84 | 0.38 | 0.52 | 0.04 | 2.59 | 0.66 | 0.99 | 0.34 | 0.34 | 0.07 | 0.88  | 0.34  | 1.22 | 0.15 | 0.66 | 0.10 | 0.30  | 0.06  |
| HNRNPK     | 5.27 | 1.71 | 4.44 | 0.77 | 2.00 | 0.30 | 1.60 | 0.42 | 0.61 | 0.13 | 1.59  | 0.32  | 0.31 | 0.06 | 0.14 | 0.03 | 0.82  | 0.25  |
| HNRNPL     | 1.34 | 0.39 | 0.90 | 0.18 | 1.20 | 0.20 | 0.36 | 0.08 | 0.12 | 0.04 | 0.59  | 0.51  | 0.27 | 0.02 | 0.14 | 0.01 | 0.56  | 0.56  |
| HNRNPM     | 0.41 | 0.16 | 0.23 | 0.02 | 3.30 | 1.32 | 0.42 | 0.14 | 0.25 | 0.04 | 1.08  | 0.27  | 1.05 | 0.18 | 1.10 | 0.25 | 0.38  | 0.20  |
| HNRNPR     | 1.23 | 0.15 | 1.23 | 0.26 | 1.01 | 0.10 | 0.90 | 0.10 | 0.36 | 0.09 | 0.72  | 0.09  | 0.73 | 0.04 | 0.31 | 0.12 | 0.72  | 0.15  |
| HNRNPU     | 2.09 | 0.75 | 1.52 | 0.29 | 0.78 | 0.19 | 0.71 | 0.29 | 0.23 | 0.05 | 0.70  | 0.16  | 0.34 | 0.02 | 0.15 | 0.05 | 0.90  | 0.06  |
| HNRNPUL1   | 1.52 | 0.15 | 1.38 | 0.31 | 2.67 | 0.97 | 0.74 | 0.19 | 0.13 | 0.02 | 0.58  | 0.12  | 0.48 | 0.07 | 0.10 | 0.04 | 0.26  | 0.16  |
| HNRNPUL2   | 1.68 | 0.60 | 0.87 | 0.11 | 1.42 | 0.32 | 1.12 | 0.36 | 0.22 | 0.05 | 0.94  | 0.65  | 0.69 | 0.14 | 0.25 | 0.05 | 0.67  | 0.48  |
| HNRPDL     | 1.02 | 0.21 | 0.81 | 0.02 | 2.67 | 1.05 | 1.29 | 0.36 | 0.61 | 0.05 | 0.64  | 0.11  | 1.26 | 0.12 | 0.75 | 0.08 | 0.26  | 0.07  |

|          |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| HNRPLL   | 0.78 | 0.32 | 0.76 | 0.15 | 2.84 | 0.46 | 1.30 | 0.21 | 0.94 | 0.16 | 1.42 | 0.21 | 1.81 | 0.54 | 1.29 | 0.45 | 0.51 | 0.10 |
| HSP90B1  | 1.21 | 0.48 | 0.68 | 0.03 | 1.37 | 0.32 | 0.95 | 0.30 | 0.29 | 0.03 | 2.18 | 0.73 | 0.81 | 0.12 | 0.42 | 0.03 | 1.59 | 0.35 |
| HSPA1A   | 0.78 | 0.10 | 0.75 | 0.07 | 1.96 | 0.58 | 0.82 | 0.09 | 0.34 | 0.15 | 0.57 | 0.11 | 1.06 | 0.12 | 0.47 | 0.22 | 0.30 | 0.08 |
| HSPA1B   | 0.66 | 0.06 | 0.63 | 0.05 | 0.77 | 0.14 | 0.56 | 0.11 | 0.19 | 0.06 | 0.69 | 0.22 | 0.85 | 0.17 | 0.30 | 0.10 | 1.01 | 0.44 |
| HSPA5    | 0.73 | 0.10 | 0.46 | 0.11 | 1.29 | 0.34 | 1.06 | 0.16 | 0.45 | 0.09 | 1.12 | 0.34 | 1.48 | 0.39 | 1.03 | 0.33 | 0.80 | 0.38 |
| HSPA8    | 1.24 | 0.31 | 1.22 | 0.10 | 1.88 | 0.51 | 0.78 | 0.20 | 0.36 | 0.10 | 0.68 | 0.14 | 0.64 | 0.10 | 0.29 | 0.09 | 0.37 | 0.07 |
| HTATSF1  | 0.88 | 0.29 | 0.66 | 0.04 | 3.08 | 0.67 | 0.96 | 0.19 | 0.53 | 0.15 | 0.92 | 0.19 | 1.13 | 0.20 | 0.80 | 0.18 | 0.31 | 0.11 |
| IFT46    | 0.80 | 0.39 | 1.34 | 0.46 | 2.45 | 0.29 | 1.09 | 0.33 | 0.74 | 0.21 | 1.58 | 0.96 | 1.45 | 0.28 | 0.60 | 0.26 | 0.66 | 0.40 |
| IGF2BP1  | 2.02 | 0.35 | 2.15 | 0.14 | 0.94 | 0.11 | 0.91 | 0.25 | 0.37 | 0.16 | 0.76 | 0.25 | 0.45 | 0.06 | 0.17 | 0.07 | 0.81 | 0.19 |
| IGF2BP3  | 0.96 | 0.15 | 0.98 | 0.20 | 1.96 | 0.28 | 0.68 | 0.03 | 0.32 | 0.06 | 0.97 | 0.10 | 0.72 | 0.12 | 0.33 | 0.09 | 0.50 | 0.08 |
| IK       | 0.97 | 0.09 | 0.92 | 0.23 | 0.92 | 0.21 | 1.01 | 0.09 | 0.34 | 0.21 | 0.55 | 0.01 | 1.04 | 0.01 | 0.43 | 0.36 | 0.53 | 0.02 |
| ILF2     | 0.89 | 0.21 | 0.63 | 0.08 | 1.41 | 0.34 | 0.79 | 0.14 | 0.23 | 0.06 | 0.66 | 0.08 | 0.90 | 0.12 | 0.36 | 0.09 | 0.48 | 0.10 |
| ILF3     | 0.91 | 0.16 | 0.70 | 0.15 | 1.96 | 0.44 | 0.84 | 0.20 | 0.16 | 0.03 | 0.57 | 0.11 | 0.91 | 0.08 | 0.24 | 0.09 | 0.30 | 0.08 |
| INTS1    | 2.05 | 0.59 | 1.31 | 0.07 | 1.16 | 0.28 | 1.35 | 0.37 | 0.41 | 0.09 | 1.04 | 0.31 | 0.66 | 0.08 | 0.32 | 0.08 | 0.91 | 0.32 |
| INTS3    | 0.82 | 0.12 | 0.65 | 0.11 | 0.98 | 0.27 | 0.82 | 0.14 | 0.58 | 0.15 | 2.02 | 1.33 | 1.00 | 0.04 | 0.90 | 0.18 | 1.99 | 0.94 |
| INTS6    | 1.71 | 0.30 | 1.61 | 0.36 | 1.20 | 0.30 | 0.86 | 0.09 | 0.30 | 0.01 | 0.78 | 0.17 | 0.51 | 0.07 | 0.19 | 0.04 | 0.65 | 0.02 |
| IQGAP1   | 1.36 | 0.11 | 1.67 | 0.20 | 1.27 | 0.09 | 0.91 | 0.15 | 0.39 | 0.10 | 1.15 | 0.34 | 0.67 | 0.06 | 0.24 | 0.08 | 0.90 | 0.25 |
| ISY1     | 1.53 | 0.26 | 0.85 | 0.12 | 0.73 | 0.09 | 0.67 | 0.14 | 0.19 | 0.01 | 0.69 | 0.14 | 0.44 | 0.08 | 0.22 | 0.04 | 0.95 | 0.24 |
| IVNS1ABP | 2.67 | 0.50 | 2.25 | 0.36 | 1.49 | 0.19 | 1.10 | 0.12 | 0.28 | 0.03 | 1.60 | 0.33 | 0.42 | 0.06 | 0.13 | 0.03 | 1.07 | 0.17 |
| KHDRBS1  | 1.06 | 0.03 | 0.97 | 0.18 | 2.82 | 0.59 | 0.56 | 0.01 | 0.25 | 0.06 | 0.84 | 0.35 | 0.53 | 0.02 | 0.26 | 0.09 | 0.32 | 0.18 |
| KHDRBS2  | 1.70 | 0.25 | 1.33 | 0.39 | 0.95 | 0.17 | 0.82 | 0.21 | 0.36 | 0.02 | 0.86 | 0.05 | 0.50 | 0.18 | 0.29 | 0.11 | 0.90 | 0.16 |
| KHDRBS3  | 1.10 | 0.18 | 0.65 | 0.07 | 1.43 | 0.06 | 0.99 | 0.17 | 0.37 | 0.03 | 1.05 | 0.19 | 0.90 | 0.10 | 0.57 | 0.06 | 0.73 | 0.13 |
| KHSRP    | 2.40 | 0.17 | 2.11 | 0.47 | 0.44 | 0.06 | 0.75 | 0.08 | 0.21 | 0.03 | 0.32 | 0.03 | 0.32 | 0.06 | 0.10 | 0.01 | 0.73 | 0.10 |
| KIAA1429 | 1.62 | 0.24 | 1.11 | 0.05 | 1.55 | 0.42 | 1.83 | 0.25 | 0.61 | 0.12 | 1.49 | 0.26 | 1.14 | 0.19 | 0.56 | 0.13 | 0.99 | 0.25 |
| KIAA1967 | 2.40 | 0.60 | 1.60 | 0.18 | 3.63 | 1.63 | 0.66 | 0.15 | 0.21 | 0.03 | 0.94 | 0.28 | 0.28 | 0.05 | 0.13 | 0.01 | 0.31 | 0.18 |
| KIF16B   | 1.27 | 0.09 | 1.70 | 0.39 | 1.08 | 0.35 | 1.15 | 0.20 | 0.40 | 0.10 | 0.85 | 0.35 | 0.90 | 0.11 | 0.23 | 0.01 | 0.77 | 0.10 |
| KIN      | 0.92 | 0.25 | 0.51 | 0.06 | 1.98 | 0.69 | 0.76 | 0.17 | 0.26 | 0.06 | 0.80 | 0.14 | 0.84 | 0.06 | 0.51 | 0.13 | 0.42 | 0.09 |
| KLHDC8A  | 1.32 | 0.36 | 1.50 | 0.29 | 1.72 | 0.26 | 0.89 | 0.29 | 0.22 | 0.01 | 2.60 | N/A  | 0.68 | 0.12 | 0.15 | 0.02 | N/A  | N/A  |
| KPNA2    | 1.37 | 0.14 | 1.05 | 0.35 | 0.70 | 0.07 | 1.12 | 0.09 | 0.41 | 0.08 | 0.97 | 0.13 | 0.82 | 0.08 | 0.41 | 0.11 | 1.40 | 0.27 |
| LGALS3   | 0.86 | 0.11 | 0.57 | 0.14 | 0.73 | 0.06 | 1.03 | 0.10 | 0.41 | 0.02 | 0.89 | 0.22 | 1.22 | 0.28 | 0.75 | 0.19 | 1.23 | 0.29 |

|         |      |      |      |      |      |      |      |      |      |      |       |      |      |      |      |      |       |      |
|---------|------|------|------|------|------|------|------|------|------|------|-------|------|------|------|------|------|-------|------|
| LPAR1   | 1.55 | 0.68 | 0.76 | 0.21 | 0.86 | 0.35 | 1.30 | 0.27 | 0.33 | 0.05 | 1.92  | 0.34 | 0.91 | 0.24 | 0.46 | 0.12 | 2.52  | 1.27 |
| LSM1    | 1.26 | 0.22 | 0.64 | 0.17 | 0.89 | 0.11 | 0.84 | 0.10 | 0.31 | 0.06 | 0.89  | 0.51 | 0.68 | 0.10 | 0.53 | 0.23 | 1.04  | 0.68 |
| LSM10   | 0.79 | 0.25 | 0.60 | 0.11 | 1.42 | 0.29 | 0.91 | 0.12 | 0.34 | 0.08 | 0.78  | 0.36 | 1.19 | 0.22 | 0.60 | 0.21 | 0.56  | 0.28 |
| LSM11   | 0.79 | 0.15 | 0.66 | 0.13 | 1.81 | 0.50 | 0.39 | 0.05 | 0.12 | 0.02 | 1.04  | 0.25 | 0.51 | 0.11 | 0.20 | 0.06 | 0.63  | 0.34 |
| LSM2    | 1.95 | 0.34 | 1.61 | 0.12 | 2.62 | 0.25 | 0.44 | 0.04 | 0.19 | 0.04 | 0.78  | 0.24 | 0.23 | 0.05 | 0.12 | 0.02 | 0.29  | 0.07 |
| LSM3    | 1.43 | 0.49 | 0.97 | 0.09 | 1.50 | 0.61 | 1.06 | 0.19 | 0.43 | 0.10 | 0.93  | 0.35 | 0.77 | 0.12 | 0.45 | 0.14 | 0.72  | 0.40 |
| LSM4    | 3.42 | 0.61 | 3.53 | 0.85 | 1.63 | 0.75 | 0.86 | 0.10 | 0.36 | 0.03 | 1.43  | 0.20 | 0.25 | 0.05 | 0.10 | 0.02 | 1.01  | 0.45 |
| LSM5    | 0.93 | 0.26 | 0.94 | 0.29 | 1.14 | 0.24 | 0.70 | 0.12 | 0.33 | 0.06 | 1.74  | 0.77 | 0.77 | 0.10 | 0.37 | 0.08 | 1.48  | 0.34 |
| LSM6    | 1.66 | 0.14 | 1.42 | 0.38 | 0.94 | 0.16 | 0.71 | 0.04 | 0.32 | 0.05 | 1.57  | 0.44 | 0.43 | 0.05 | 0.23 | 0.04 | 1.64  | 0.30 |
| LSM7    | 2.10 | 0.03 | 1.99 | 0.60 | 1.11 | 0.07 | 1.16 | 0.08 | 0.51 | 0.04 | 1.67  | 0.49 | 0.55 | 0.04 | 0.27 | 0.06 | 1.48  | 0.34 |
| LSMD1   | 0.74 | 0.05 | 0.47 | 0.16 | 0.93 | 0.27 | 0.41 | 0.03 | 0.15 | 0.03 | 0.99  | 0.27 | 0.56 | 0.03 | 0.34 | 0.08 | 1.17  | 0.56 |
| LUC7L   | 0.94 | 0.25 | 0.87 | 0.06 | 1.07 | 0.15 | 0.38 | 0.08 | 0.21 | 0.03 | 0.90  | 0.19 | 0.40 | 0.01 | 0.25 | 0.03 | 0.84  | 0.12 |
| MAGOH   | 1.43 | 0.11 | 0.80 | 0.24 | 1.10 | 0.16 | 0.96 | 0.05 | 0.47 | 0.08 | 1.62  | 0.98 | 0.67 | 0.05 | 0.61 | 0.14 | 1.44  | 0.74 |
| MAGOHB  | 0.69 | 0.21 | 0.84 | 0.08 | 1.28 | 0.21 | 0.94 | 0.21 | 0.36 | 0.09 | 14.88 | 1.68 | 1.42 | 0.48 | 0.42 | 0.09 | 12.84 | 1.56 |
| MATR3   | 1.30 | 0.16 | 0.80 | 0.10 | 1.04 | 0.26 | 1.10 | 0.05 | 0.53 | 0.15 | 1.47  | 0.08 | 0.85 | 0.09 | 0.65 | 0.10 | 2.46  | 1.40 |
| MBD5    | 1.20 | 0.20 | 1.10 | 0.17 | 1.01 | 0.08 | 0.75 | 0.13 | 0.23 | 0.04 | 1.08  | 0.62 | 0.63 | 0.12 | 0.21 | 0.01 | 1.05  | 0.55 |
| MBNL1   | 0.59 | 0.17 | 0.60 | 0.12 | 0.86 | 0.10 | 0.56 | 0.08 | 0.25 | 0.04 | 1.01  | 0.14 | 0.99 | 0.26 | 0.41 | 0.04 | 1.19  | 0.17 |
| MBNL2   | 1.90 | 0.30 | 1.43 | 0.47 | 1.37 | 0.45 | 1.22 | 0.06 | 0.52 | 0.09 | 1.53  | 0.47 | 0.65 | 0.11 | 0.37 | 0.05 | 1.12  | 0.07 |
| MBNL3   | 1.55 | 0.09 | 1.43 | 0.30 | 1.10 | 0.27 | 0.81 | 0.04 | 0.36 | 0.05 | 2.15  | 0.79 | 0.52 | 0.01 | 0.26 | 0.06 | 1.93  | 0.26 |
| MEX3B   | 1.27 | 0.36 | 1.71 | 0.94 | 1.27 | 0.17 | 0.74 | 0.05 | 0.43 | 0.13 | 1.43  | 0.07 | 0.62 | 0.17 | 0.28 | 0.10 | 1.13  | 0.10 |
| MFAP1   | 3.52 | 0.23 | 1.85 | 0.39 | 1.33 | 0.29 | 1.02 | 0.14 | 0.44 | 0.07 | 1.38  | 0.52 | 0.29 | 0.03 | 0.25 | 0.09 | 1.01  | 0.18 |
| MFSD11  | 0.65 | 0.08 | 0.91 | 0.07 | 1.13 | 0.11 | 0.76 | 0.09 | 0.58 | 0.08 | 1.85  | 0.04 | 1.16 | 0.10 | 0.64 | 0.04 | 1.64  | 0.13 |
| MKI67IP | 0.76 | 0.18 | 0.80 | 0.11 | 0.84 | 0.14 | 0.82 | 0.06 | 0.39 | 0.02 | 1.45  | 0.39 | 1.12 | 0.19 | 0.49 | 0.06 | 1.70  | 0.18 |
| MOV10   | 1.96 | 0.09 | 1.13 | 0.33 | 1.05 | 0.17 | 1.26 | 0.19 | 0.50 | 0.08 | 1.10  | 0.16 | 0.65 | 0.12 | 0.46 | 0.08 | 1.07  | 0.24 |
| MSI1    | 1.34 | 0.20 | 0.80 | 0.14 | 0.80 | 0.23 | 1.41 | 0.12 | 0.87 | 0.28 | 2.31  | 0.26 | 1.08 | 0.22 | 1.07 | 0.22 | 2.96  | 0.50 |
| MSI2    | 1.25 | 0.30 | 0.99 | 0.11 | 0.84 | 0.19 | 1.01 | 0.23 | 0.48 | 0.06 | 0.89  | 0.11 | 0.83 | 0.17 | 0.49 | 0.06 | 1.11  | 0.28 |
| MYEF2   | 1.79 | 0.16 | 1.22 | 0.35 | 1.11 | 0.31 | 1.35 | 0.13 | 0.35 | 0.04 | 1.13  | 0.19 | 0.76 | 0.13 | 0.30 | 0.06 | 1.09  | 0.41 |
| NAA38   | 2.25 | 0.12 | 2.41 | 0.59 | 1.18 | 0.40 | 0.95 | 0.10 | 0.57 | 0.19 | 1.38  | 0.40 | 0.42 | 0.04 | 0.23 | 0.03 | 1.28  | 0.63 |
| NCBP1   | 0.76 | 0.16 | 0.56 | 0.13 | 0.96 | 0.33 | 0.65 | 0.03 | 0.20 | 0.02 | 1.10  | 0.24 | 0.88 | 0.14 | 0.37 | 0.07 | 1.17  | 0.14 |
| NCBP2   | 0.77 | 0.07 | 0.57 | 0.23 | 1.11 | 0.23 | 0.70 | 0.04 | 0.26 | 0.04 | 0.77  | 0.09 | 0.91 | 0.07 | 0.49 | 0.11 | 0.71  | 0.15 |

|         |      |      |      |      |      |      |      |      |      |      |       |      |      |      |      |      |       |      |
|---------|------|------|------|------|------|------|------|------|------|------|-------|------|------|------|------|------|-------|------|
| NCL     | 0.51 | 0.11 | 0.43 | 0.04 | 0.99 | 0.10 | 0.92 | 0.15 | 0.33 | 0.04 | 1.08  | 0.58 | 1.84 | 0.12 | 0.77 | 0.00 | 1.07  | 0.48 |
| NDUFA1  | 1.98 | 0.40 | 1.27 | 0.03 | 1.15 | 0.23 | 1.17 | 0.10 | 0.35 | 0.12 | 1.40  | 0.25 | 0.60 | 0.08 | 0.27 | 0.09 | 1.23  | 0.23 |
| NHP2L1  | 3.07 | 0.37 | 2.06 | 0.50 | 2.01 | 0.68 | 1.60 | 0.24 | 0.97 | 0.13 | 1.88  | 0.65 | 0.52 | 0.06 | 0.48 | 0.09 | 0.94  | 0.01 |
| NONO    | 1.21 | 0.20 | 0.80 | 0.17 | 1.48 | 0.65 | 1.39 | 0.34 | 0.96 | 0.41 | 1.45  | 0.12 | 1.15 | 0.19 | 1.18 | 0.40 | 1.13  | 0.53 |
| NOSIP   | 1.03 | 0.16 | 0.71 | 0.30 | 0.81 | 0.14 | 0.66 | 0.07 | 0.26 | 0.08 | 0.81  | 0.09 | 0.65 | 0.15 | 0.38 | 0.07 | 1.02  | 0.11 |
| NOVA1   | 3.41 | 0.96 | 2.74 | 0.70 | 0.95 | 0.16 | 1.39 | 0.24 | 0.54 | 0.12 | 0.82  | 0.32 | 0.42 | 0.05 | 0.20 | 0.05 | 2.03  | 1.89 |
| NOVA2   | 1.82 | 0.32 | 1.79 | 0.60 | 1.12 | 0.48 | 1.21 | 0.08 | 0.39 | 0.07 | 0.70  | 0.26 | 0.68 | 0.12 | 0.23 | 0.06 | 1.68  | 1.51 |
| NSRP1   | 1.72 | 0.24 | 0.97 | 0.30 | 2.44 | 0.34 | 0.80 | 0.13 | 0.18 | 0.04 | 0.69  | 0.19 | 0.47 | 0.02 | 0.19 | 0.03 | 0.29  | 0.11 |
| NUDT21  | 1.21 | 0.27 | 0.53 | 0.06 | 1.16 | 0.30 | 0.76 | 0.04 | 0.24 | 0.06 | 2.23  | 1.16 | 0.64 | 0.10 | 0.46 | 0.12 | 1.85  | 0.48 |
| NUFIP1  | 1.23 | 0.44 | 1.37 | 0.10 | 1.26 | 0.11 | 0.69 | 0.19 | 0.30 | 0.10 | 1.02  | 0.31 | 0.58 | 0.07 | 0.22 | 0.09 | 0.81  | 0.20 |
| NUFIP2  | 1.90 | 0.76 | 1.21 | 0.17 | 1.30 | 0.26 | 0.50 | 0.15 | 0.24 | 0.08 | 20.53 | 2.84 | 0.27 | 0.04 | 0.21 | 0.09 | 17.94 | 3.16 |
| NUMA1   | 0.74 | 0.18 | 0.71 | 0.19 | 1.13 | 0.31 | 0.91 | 0.07 | 0.36 | 0.07 | 1.72  | 0.40 | 1.27 | 0.24 | 0.52 | 0.06 | 1.53  | 0.19 |
| NXF1    | 1.60 | 0.04 | 0.66 | 0.15 | 1.49 | 0.40 | 0.15 | 0.03 | 0.06 | 0.01 | 1.49  | 0.76 | 0.09 | 0.01 | 0.09 | 0.03 | 0.95  | 0.29 |
| PABPC1  | 1.21 | 0.20 | 1.02 | 0.12 | 1.38 | 0.23 | 1.59 | 0.10 | 0.53 | 0.05 | 2.12  | 0.55 | 1.34 | 0.17 | 0.52 | 0.08 | 1.52  | 0.15 |
| PABPC3  | 0.95 | 0.17 | 0.71 | 0.04 | 1.51 | 0.05 | 0.56 | 0.02 | 0.28 | 0.08 | 0.62  | 0.17 | 0.60 | 0.11 | 0.39 | 0.10 | 0.41  | 0.10 |
| PABPC4  | 3.24 | 0.51 | 2.91 | 0.74 | 1.13 | 0.14 | 1.42 | 0.09 | 0.61 | 0.09 | 4.56  | 2.35 | 0.45 | 0.08 | 0.21 | 0.03 | 4.32  | 2.11 |
| PABPN1  | 0.95 | 0.31 | 0.47 | 0.09 | 0.51 | 0.12 | 1.12 | 0.20 | 0.47 | 0.09 | 0.99  | 0.07 | 1.22 | 0.19 | 0.99 | 0.06 | 1.98  | 0.37 |
| PARP1   | 2.13 | 0.44 | 1.43 | 0.31 | 1.00 | 0.40 | 0.83 | 0.08 | 0.32 | 0.04 | 0.64  | 0.27 | 0.39 | 0.05 | 0.24 | 0.07 | 0.65  | 0.26 |
| PCBP1   | 2.04 | 0.72 | 1.50 | 0.48 | 1.76 | 0.63 | 0.73 | 0.12 | 0.31 | 0.07 | 1.17  | 0.86 | 0.38 | 0.11 | 0.22 | 0.09 | 0.62  | 0.22 |
| PCBP2   | 5.40 | 0.30 | 4.46 | 1.31 | 1.68 | 0.64 | 0.67 | 0.11 | 0.17 | 0.04 | 0.97  | 0.17 | 0.12 | 0.02 | 0.04 | 0.01 | 0.64  | 0.26 |
| PCBP3   | 3.64 | 0.30 | 2.34 | 0.50 | 1.49 | 0.38 | 1.33 | 0.21 | 0.45 | 0.11 | 1.30  | 0.20 | 0.37 | 0.08 | 0.20 | 0.08 | 0.90  | 0.11 |
| PCBP4   | 0.74 | 0.29 | 0.49 | 0.08 | 0.77 | 0.19 | 0.90 | 0.05 | 0.32 | 0.01 | 2.16  | 0.87 | 1.33 | 0.48 | 0.67 | 0.09 | 2.74  | 0.45 |
| PHF5A   | 3.65 | 0.31 | 4.03 | 1.56 | 1.81 | 0.49 | 0.94 | 0.01 | 0.37 | 0.10 | 1.29  | 0.53 | 0.26 | 0.02 | 0.10 | 0.04 | 0.78  | 0.41 |
| PHRF1   | 2.53 | 0.49 | 2.11 | 0.31 | 1.41 | 0.19 | 1.23 | 0.20 | 0.47 | 0.08 | 0.75  | 0.14 | 0.49 | 0.03 | 0.22 | 0.02 | 0.55  | 0.17 |
| PIAS1   | 2.11 | 0.35 | 1.71 | 0.80 | 0.94 | 0.20 | 0.99 | 0.05 | 0.31 | 0.09 | 2.00  | 0.66 | 0.48 | 0.06 | 0.19 | 0.05 | 2.09  | 0.23 |
| PIAS4   | 1.13 | 0.31 | 0.92 | 0.35 | 1.67 | 0.55 | 0.92 | 0.02 | 0.31 | 0.12 | 1.35  | 0.31 | 0.85 | 0.23 | 0.36 | 0.13 | 0.85  | 0.28 |
| PLRG1   | 1.78 | 0.22 | 1.46 | 0.39 | 1.12 | 0.33 | 0.83 | 0.11 | 0.58 | 0.22 | 2.39  | 1.18 | 0.47 | 0.11 | 0.40 | 0.11 | 2.06  | 0.39 |
| PNN     | 0.90 | 0.39 | 0.51 | 0.06 | 1.15 | 0.46 | 1.49 | 0.37 | 0.65 | 0.02 | 1.11  | 0.28 | 1.58 | 0.26 | 1.20 | 0.28 | 1.09  | 0.57 |
| POLDIP3 | 0.92 | 0.19 | 0.55 | 0.12 | 1.42 | 0.19 | 0.96 | 0.07 | 0.88 | 0.43 | 1.20  | 0.28 | 1.16 | 0.09 | 0.95 | 0.05 | 0.85  | 0.19 |
| POLR2A  | 0.99 | 0.22 | 0.34 | 0.01 | 1.29 | 0.40 | 0.12 | 0.04 | 1.86 | 2.09 | 1.07  | 0.20 | 0.12 | 0.00 | 0.62 | 0.72 | 0.73  | 0.23 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| POLR2B  | 1.59 | 0.14 | 0.79 | 0.03 | 1.20 | 0.22 | 0.30 | 0.03 | 0.26 | 0.04 | 0.77 | 0.09 | 0.20 | 0.02 | 0.25 | 0.06 | 0.67 | 0.19 |
| PPAN    | 0.60 | 0.03 | 0.47 | 0.08 | 0.74 | 0.02 | 1.12 | 0.06 | 4.99 | 1.88 | 0.82 | 0.26 | 1.99 | 0.15 | 5.34 | 2.29 | 1.11 | 0.38 |
| PPIE    | 1.06 | 0.27 | 1.04 | 0.32 | 1.12 | 0.05 | 1.09 | 0.23 | 0.77 | 0.00 | 0.94 | 0.08 | 1.02 | 0.15 | 0.67 | 0.09 | 0.84 | 0.10 |
| PPIH    | 1.33 | 0.16 | 0.85 | 0.03 | 1.20 | 0.34 | 1.53 | 0.07 | 0.78 | 0.13 | 0.85 | 0.03 | 1.16 | 0.17 | 0.81 | 0.03 | 0.75 | 0.21 |
| PPIL1   | 0.76 | 0.21 | 0.58 | 0.07 | 0.91 | 0.23 | 0.75 | 0.17 | 0.33 | 0.07 | 1.01 | 0.08 | 0.95 | 0.12 | 0.56 | 0.07 | 1.14 | 0.25 |
| PPIL2   | 1.53 | 0.26 | 0.87 | 0.06 | 0.75 | 0.09 | 1.01 | 0.21 | 0.43 | 0.13 | 0.81 | 0.11 | 0.65 | 0.02 | 0.39 | 0.02 | 1.08 | 0.02 |
| PPIL3   | 1.68 | 0.39 | 1.49 | 0.01 | 1.31 | 0.15 | 1.12 | 0.19 | 0.42 | 0.05 | 1.17 | 0.08 | 0.62 | 0.04 | 0.28 | 0.03 | 0.85 | 0.12 |
| PPM1G   | 1.55 | 0.27 | 0.94 | 0.19 | 1.29 | 0.27 | 0.78 | 0.16 | 0.43 | 0.06 | 1.13 | 0.21 | 0.51 | 0.03 | 0.37 | 0.02 | 0.88 | 0.05 |
| PPP1R8  | 1.90 | 0.15 | 1.05 | 0.00 | 1.48 | 0.26 | 0.48 | 0.09 | 0.22 | 0.01 | 0.65 | 0.05 | 0.24 | 0.03 | 0.17 | 0.05 | 0.44 | 0.04 |
| PPWD1   | 1.52 | 0.09 | 1.16 | 0.28 | 1.24 | 0.21 | 1.47 | 0.24 | 0.81 | 0.13 | 1.24 | 0.22 | 0.93 | 0.06 | 0.61 | 0.18 | 0.98 | 0.05 |
| PQBP1   | 0.95 | 0.05 | 0.51 | 0.02 | 0.87 | 0.17 | 0.90 | 0.03 | 0.48 | 0.02 | 0.77 | 0.33 | 0.94 | 0.04 | 0.66 | 0.38 | 0.87 | 0.24 |
| PRCC    | 0.78 | 0.04 | 0.54 | 0.10 | 0.97 | 0.05 | 1.11 | 0.03 | 0.98 | 0.01 | 1.00 | 0.31 | 1.49 | 0.09 | 1.48 | 0.18 | 1.05 | 0.36 |
| PRKRA   | 1.04 | 0.06 | 1.31 | 0.36 | 1.16 | 0.15 | 0.85 | 0.15 | 0.43 | 0.05 | 1.05 | 0.19 | 0.81 | 0.06 | 0.31 | 0.02 | 0.93 | 0.25 |
| PRPF18  | 1.72 | 0.14 | 1.21 | 0.28 | 1.51 | 0.21 | 1.35 | 0.08 | 0.65 | 0.03 | 0.97 | 0.10 | 0.78 | 0.11 | 0.55 | 0.10 | 0.66 | 0.15 |
| PRPF19  | 1.73 | 0.29 | 1.34 | 0.01 | 1.38 | 0.21 | 0.78 | 0.14 | 0.29 | 0.02 | 0.88 | 0.07 | 0.42 | 0.07 | 0.18 | 0.03 | 0.65 | 0.13 |
| PRPF3   | 4.42 | 0.57 | 3.51 | 0.12 | 1.49 | 0.26 | 1.68 | 0.40 | 0.64 | 0.07 | 0.94 | 0.10 | 0.37 | 0.02 | 0.15 | 0.02 | 0.64 | 0.14 |
| PRPF31  | 4.27 | 0.34 | 2.17 | 0.09 | 1.05 | 0.12 | 0.95 | 0.11 | 0.42 | 0.09 | 0.64 | 0.05 | 0.22 | 0.02 | 0.15 | 0.01 | 0.61 | 0.02 |
| PRPF38A | 4.28 | 0.13 | 1.86 | 0.01 | 1.96 | 0.21 | 0.90 | 0.24 | 0.34 | 0.02 | 0.84 | 0.05 | 0.20 | 0.04 | 0.14 | 0.07 | 0.43 | 0.02 |
| PRPF38B | 1.79 | 0.42 | 0.91 | 0.06 | 1.40 | 0.34 | 0.75 | 0.12 | 0.40 | 0.14 | 0.92 | 0.05 | 0.42 | 0.05 | 0.33 | 0.03 | 0.68 | 0.16 |
| PRPF4   | 1.98 | 0.54 | 1.71 | 0.23 | 1.03 | 0.04 | 1.11 | 0.16 | 0.48 | 0.10 | 0.87 | 0.27 | 0.54 | 0.08 | 0.23 | 0.05 | 0.85 | 0.28 |
| PRPF40A | 4.30 | 1.58 | 3.51 | 0.81 | 2.10 | 0.51 | 1.22 | 0.45 | 0.32 | 0.10 | 1.24 | 0.35 | 0.29 | 0.04 | 0.09 | 0.04 | 0.63 | 0.25 |
| PRPF40B | 1.14 | 0.26 | 1.19 | 0.11 | 0.90 | 0.14 | 0.64 | 0.16 | 0.31 | 0.04 | 0.68 | 0.06 | 0.57 | 0.09 | 0.26 | 0.05 | 0.78 | 0.16 |
| PRPF4B  | 2.32 | 0.13 | 1.72 | 0.79 | 1.37 | 0.32 | 0.80 | 0.01 | 0.42 | 0.02 | 0.92 | 0.18 | 0.34 | 0.01 | 0.27 | 0.15 | 0.64 | 0.05 |
| PRPF6   | 1.64 | 0.58 | 1.57 | 0.09 | 1.38 | 0.14 | 1.14 | 0.11 | 0.51 | 0.06 | 1.12 | 0.31 | 0.76 | 0.29 | 0.33 | 0.05 | 0.81 | 0.20 |
| PRPF8   | 3.69 | 0.71 | 1.77 | 0.05 | 1.34 | 0.04 | 1.01 | 0.07 | 0.75 | 0.14 | 1.10 | 0.27 | 0.30 | 0.03 | 0.28 | 0.13 | 0.82 | 0.22 |
| PSEN1   | 1.29 | 0.23 | 1.78 | 0.05 | 1.15 | 0.12 | 0.59 | 0.09 | 0.30 | 0.02 | 0.68 | 0.05 | 0.47 | 0.15 | 0.17 | 0.00 | 0.60 | 0.11 |
| PSIP1   | 2.64 | 0.77 | 1.89 | 0.05 | 1.17 | 0.14 | 1.39 | 0.19 | 0.62 | 0.09 | 1.11 | 0.10 | 0.48 | 0.05 | 0.28 | 0.11 | 0.96 | 0.19 |
| PSPC1   | 1.74 | 0.18 | 1.81 | 0.11 | 1.48 | 0.27 | 1.48 | 0.28 | 0.58 | 0.17 | 0.79 | 0.11 | 0.75 | 0.13 | 0.23 | 0.05 | 0.55 | 0.17 |
| PTBP1   | 0.35 | 0.10 | 0.14 | 0.01 | 0.43 | 0.01 | 1.28 | 0.10 | 0.55 | 0.11 | 1.39 | 0.12 | 3.73 | 1.62 | 2.95 | 0.52 | 3.27 | 0.34 |
| PTBP2   | 2.64 | 0.45 | 1.47 | 0.28 | 1.17 | 0.16 | 1.72 | 0.53 | 0.70 | 0.09 | 0.97 | 0.09 | 0.62 | 0.04 | 0.47 | 0.04 | 0.85 | 0.21 |

|        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| PTBP3  | 2.73 | 0.79 | 2.47 | 0.85 | 1.36 | 0.06 | 0.65 | 0.09 | 0.27 | 0.08 | 0.54 | 0.12 | 0.25 | 0.04 | 0.12 | 0.05 | 0.40 | 0.10 |
| PUF60  | 0.86 | 0.05 | 0.79 | 0.40 | 0.53 | 0.04 | 0.62 | 0.00 | 0.36 | 0.05 | 1.01 | 0.13 | 0.72 | 0.04 | 0.54 | 0.25 | 1.87 | 0.37 |
| QKI    | 1.31 | 0.13 | 1.21 | 0.13 | 1.05 | 0.18 | 0.91 | 0.19 | 0.34 | 0.06 | 0.65 | 0.10 | 0.67 | 0.19 | 0.27 | 0.03 | 0.63 | 0.14 |
| QPCT   | 1.54 | 0.17 | 0.77 | 0.05 | 1.10 | 0.22 | 1.44 | 0.04 | 0.51 | 0.09 | 0.85 | 0.10 | 0.88 | 0.07 | 0.51 | 0.05 | 0.79 | 0.14 |
| QPCTL  | 1.18 | 0.16 | 0.70 | 0.18 | 1.10 | 0.20 | 1.02 | 0.26 | 0.32 | 0.05 | 0.64 | 0.08 | 0.77 | 0.26 | 0.36 | 0.03 | 0.59 | 0.14 |
| RALY   | 2.05 | 0.29 | 1.52 | 0.27 | 1.15 | 0.23 | 0.90 | 0.23 | 0.27 | 0.00 | 0.76 | 0.05 | 0.41 | 0.03 | 0.16 | 0.01 | 0.69 | 0.17 |
| RALYL  | 0.84 | 0.11 | 0.48 | 0.14 | 1.28 | 0.27 | 0.53 | 0.03 | 0.23 | 0.05 | 0.61 | 0.17 | 0.63 | 0.04 | 0.52 | 0.18 | 0.47 | 0.04 |
| RAVER1 | 1.09 | 0.14 | 0.58 | 0.11 | 0.86 | 0.17 | 0.31 | 0.06 | 0.09 | 0.00 | 0.57 | 0.44 | 0.27 | 0.05 | 0.14 | 0.00 | 0.76 | 0.74 |
| RAVER2 | 1.18 | 0.21 | 0.69 | 0.15 | 1.04 | 0.10 | 1.02 | 0.23 | 0.47 | 0.06 | 0.89 | 0.06 | 0.82 | 0.10 | 0.53 | 0.02 | 0.82 | 0.11 |
| RBBP7  | 1.80 | 0.58 | 0.67 | 0.06 | 1.09 | 0.21 | 1.38 | 0.43 | 0.77 | 0.07 | 0.89 | 0.06 | 0.73 | 0.10 | 0.87 | 0.18 | 0.83 | 0.11 |
| RBFOX1 | 1.03 | 0.41 | 1.04 | 0.04 | 0.94 | 0.19 | 0.83 | 0.18 | 0.25 | 0.14 | 1.54 | 0.34 | 0.87 | 0.23 | 0.24 | 0.13 | 1.68 | 0.45 |
| RBFOX2 | 1.87 | 0.64 | 1.31 | 0.44 | 1.44 | 0.36 | 0.61 | 0.19 | 0.24 | 0.06 | 0.52 | 0.08 | 0.33 | 0.01 | 0.19 | 0.09 | 0.43 | 0.15 |
| RBFOX2 | 1.81 | 0.47 | 1.48 | 0.66 | 1.22 | 0.03 | 0.62 | 0.09 | 0.24 | 0.08 | 0.42 | 0.08 | 0.35 | 0.04 | 0.17 | 0.06 | 0.34 | 0.07 |
| RBFOX3 | 0.51 | 0.12 | 0.41 | 0.03 | 0.89 | 0.13 | 0.92 | 0.26 | 0.39 | 0.14 | 1.98 | 0.43 | 1.81 | 0.29 | 0.97 | 0.41 | 2.28 | 0.71 |
| RBM10  | 2.20 | 0.43 | 2.29 | 1.28 | 1.05 | 0.09 | 0.57 | 0.08 | 0.21 | 0.09 | 0.69 | 0.23 | 0.26 | 0.02 | 0.10 | 0.04 | 0.67 | 0.24 |
| RBM11  | 0.75 | 0.17 | 0.58 | 0.08 | 0.78 | 0.06 | 1.08 | 0.04 | 0.52 | 0.02 | 1.03 | 0.11 | 1.49 | 0.34 | 0.83 | 0.05 | 1.32 | 0.04 |
| RBM12  | 0.81 | 0.09 | 1.35 | 0.90 | 1.25 | 0.08 | 0.58 | 0.12 | 0.29 | 0.13 | 0.85 | 0.13 | 0.71 | 0.14 | 0.25 | 0.12 | 0.68 | 0.06 |
| RBM12B | 2.19 | 0.41 | 1.22 | 0.29 | 1.33 | 0.36 | 1.28 | 0.39 | 0.30 | 0.05 | 1.11 | 0.13 | 0.52 | 0.22 | 0.20 | 0.03 | 0.87 | 0.22 |
| RBM14  | 0.20 | 0.03 | 0.16 | 0.05 | 0.20 | 0.01 | 0.69 | 0.07 | 0.32 | 0.10 | 0.64 | 0.08 | 3.50 | 0.82 | 2.07 | 0.85 | 3.10 | 0.22 |
| RBM15  | 1.45 | 0.23 | 2.36 | 0.92 | 1.62 | 0.14 | 0.89 | 0.01 | 0.37 | 0.17 | 1.28 | 0.29 | 0.63 | 0.11 | 0.15 | 0.02 | 0.80 | 0.18 |
| RBM15B | 1.47 | 0.29 | 1.15 | 0.09 | 1.22 | 0.27 | 0.96 | 0.18 | 0.36 | 0.07 | 0.84 | 0.09 | 0.66 | 0.16 | 0.25 | 0.02 | 0.71 | 0.17 |
| RBM17  | 1.33 | 0.12 | 1.32 | 0.56 | 1.93 | 0.15 | 0.43 | 0.04 | 0.22 | 0.08 | 0.67 | 0.27 | 0.32 | 0.03 | 0.18 | 0.08 | 0.35 | 0.15 |
| RBM18  | 0.91 | 0.20 | 0.29 | 0.01 | 0.79 | 0.12 | 1.31 | 0.23 | 0.55 | 0.08 | 0.71 | 0.14 | 1.37 | 0.11 | 1.49 | 0.23 | 0.91 | 0.20 |
| RBM19  | 0.78 | 0.07 | 0.53 | 0.07 | 0.79 | 0.12 | 1.01 | 0.17 | 0.51 | 0.03 | 0.74 | 0.08 | 1.23 | 0.08 | 0.99 | 0.15 | 0.95 | 0.20 |
| RBM20  | 1.03 | 0.07 | 1.29 | 0.53 | 0.96 | 0.04 | 1.02 | 0.14 | 0.54 | 0.25 | 0.62 | 0.05 | 0.99 | 0.17 | 0.42 | 0.12 | 0.66 | 0.03 |
| RBM22  | 1.44 | 0.05 | 1.04 | 0.33 | 0.88 | 0.03 | 1.28 | 0.10 | 0.76 | 0.26 | 1.81 | N/A  | 0.89 | 0.10 | 0.73 | 0.08 | 2.06 | N/A  |
| RBM23  | 1.51 | 0.30 | 0.91 | 0.03 | 0.97 | 0.27 | 2.19 | 0.36 | 0.70 | 0.06 | 1.02 | 0.24 | 1.36 | 0.09 | 0.65 | 0.12 | 0.91 | 0.06 |
| RBM24  | 1.20 | 0.14 | 2.02 | 1.00 | 1.18 | 0.12 | 0.85 | 0.04 | 0.53 | 0.20 | 1.33 | 0.71 | 0.72 | 0.10 | 0.27 | 0.06 | 1.15 | 0.64 |
| RBM25  | 2.53 | 0.57 | 2.51 | 1.07 | 2.34 | 0.11 | 0.46 | 0.11 | 0.24 | 0.09 | 0.64 | 0.15 | 0.18 | 0.02 | 0.10 | 0.05 | 0.27 | 0.06 |
| RBM26  | 0.78 | 0.10 | 1.13 | 0.33 | 0.84 | 0.22 | 1.02 | 0.08 | 0.53 | 0.09 | 0.99 | 0.31 | 1.33 | 0.25 | 0.48 | 0.06 | 1.21 | 0.38 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| RBM27   | 0.90 | 0.01 | 0.41 | 0.07 | 0.65 | 0.10 | 0.79 | 0.03 | 0.28 | 0.03 | 0.70 | 0.20 | 0.86 | 0.04 | 0.68 | 0.06 | 1.06 | 0.14 |
| RBM28   | 0.66 | 0.06 | 0.36 | 0.08 | 0.76 | 0.09 | 0.54 | 0.00 | 0.21 | 0.01 | 0.58 | 0.25 | 0.78 | 0.08 | 0.58 | 0.13 | 0.75 | 0.30 |
| RBM3    | 0.97 | 0.12 | 0.58 | 0.20 | 1.81 | 0.14 | 1.06 | 0.14 | 0.45 | 0.34 | 1.03 | 0.27 | 1.09 | 0.02 | 0.88 | 0.79 | 0.58 | 0.19 |
| RBM33   | 2.10 | 0.40 | 1.41 | 0.15 | 1.39 | 0.28 | 0.73 | 0.11 | 0.42 | 0.12 | 0.76 | 0.33 | 0.35 | 0.03 | 0.26 | 0.00 | 0.53 | 0.13 |
| RBM34   | 1.55 | 0.31 | 1.02 | 0.16 | 0.86 | 0.15 | 0.76 | 0.27 | 0.13 | 0.05 | 0.61 | 0.07 | 0.43 | 0.07 | 0.11 | 0.04 | 0.73 | 0.14 |
| RBM38   | 0.99 | 0.29 | 0.66 | 0.06 | 1.01 | 0.14 | 0.66 | 0.10 | 0.18 | 0.03 | 0.68 | 0.10 | 0.64 | 0.25 | 0.22 | 0.01 | 0.69 | 0.20 |
| RBM39   | 2.36 | 0.68 | 2.25 | 0.85 | 2.49 | 0.32 | 0.97 | 0.24 | 0.42 | 0.13 | 0.75 | 0.11 | 0.42 | 0.05 | 0.19 | 0.04 | 0.31 | 0.08 |
| RBM4    | 0.68 | 0.33 | 0.76 | 0.35 | 0.69 | 0.02 | 0.96 | 0.55 | 0.54 | 0.15 | 0.94 | 0.06 | 1.37 | 0.27 | 0.78 | 0.33 | 1.36 | 0.13 |
| RBM41   | 1.35 | 0.24 | 0.76 | 0.06 | 1.04 | 0.23 | 0.91 | 0.12 | 0.54 | 0.15 | 0.87 | 0.07 | 0.67 | 0.06 | 0.52 | 0.02 | 0.86 | 0.21 |
| RBM42   | 2.13 | 0.27 | 1.66 | 0.66 | 1.48 | 0.58 | 1.27 | 0.15 | 0.87 | 0.34 | 1.42 | 0.28 | 0.60 | 0.11 | 0.53 | 0.03 | 1.01 | 0.26 |
| RBM43   | 0.71 | 0.04 | 0.53 | 0.10 | 1.22 | 0.10 | 0.73 | 0.09 | 0.42 | 0.08 | 0.79 | 0.08 | 1.03 | 0.06 | 0.80 | 0.09 | 0.65 | 0.11 |
| RBM44   | 0.58 | 0.11 | 0.37 | 0.13 | 0.65 | 0.06 | 0.76 | 0.00 | 0.34 | 0.06 | 0.92 | 0.08 | 1.27 | 0.37 | 0.86 | 0.33 | 1.41 | 0.10 |
| RBM45   | 1.01 | 0.15 | 1.21 | 0.56 | 1.41 | 0.19 | 0.55 | 0.02 | 0.35 | 0.11 | 1.27 | 0.48 | 0.55 | 0.09 | 0.32 | 0.16 | 0.91 | 0.37 |
| RBM46   | 1.42 | 0.36 | 0.68 | 0.04 | 0.96 | 0.15 | 1.34 | 0.52 | 0.65 | 0.03 | 0.87 | 0.08 | 0.91 | 0.04 | 0.79 | 0.24 | 0.92 | 0.14 |
| RBM47   | 1.22 | 0.39 | 1.17 | 0.06 | 2.05 | 0.27 | 0.54 | 0.17 | 0.24 | 0.05 | 2.81 | 0.65 | 0.45 | 0.12 | 0.20 | 0.05 | 1.39 | 0.33 |
| RBM48   | 1.39 | 0.04 | 1.50 | 1.24 | 1.16 | 0.10 | 0.80 | 0.17 | 0.65 | 0.47 | 1.31 | 0.12 | 0.58 | 0.11 | 0.73 | 0.53 | 1.12 | 0.06 |
| RBM4B   | 0.98 | 0.42 | 1.05 | 0.23 | 0.90 | 0.06 | 0.71 | 0.40 | 0.55 | 0.19 | 0.85 | 0.06 | 0.69 | 0.16 | 0.51 | 0.06 | 0.94 | 0.05 |
| RBM5    | 0.45 | 0.07 | 0.51 | 0.20 | 0.61 | 0.02 | 0.57 | 0.09 | 0.33 | 0.12 | 0.93 | 0.19 | 1.28 | 0.05 | 0.66 | 0.11 | 1.52 | 0.32 |
| RBM6    | 1.74 | 1.07 | 2.07 | 0.99 | 2.36 | 0.09 | 1.19 | 0.35 | 0.63 | 0.21 | 1.07 | 0.05 | 0.78 | 0.24 | 0.32 | 0.10 | 0.46 | 0.02 |
| RBM7    | 0.99 | 0.20 | 1.32 | 0.57 | 1.79 | 0.08 | 0.82 | 0.07 | 0.46 | 0.15 | 1.10 | 0.40 | 0.86 | 0.26 | 0.37 | 0.12 | 0.61 | 0.21 |
| RBM8A   | 0.62 | 0.28 | 0.53 | 0.15 | 0.99 | 0.09 | 0.53 | 0.09 | 0.29 | 0.08 | 0.98 | 0.36 | 0.93 | 0.33 | 0.55 | 0.15 | 0.97 | 0.30 |
| RBMS1   | 1.41 | 0.44 | 0.63 | 0.17 | 1.03 | 0.02 | 1.04 | 0.12 | 0.28 | 0.10 | 0.93 | 0.10 | 0.77 | 0.14 | 0.45 | 0.17 | 0.90 | 0.11 |
| RBMX    | 1.02 | 0.31 | 0.83 | 0.43 | 1.31 | 0.05 | 0.99 | 0.23 | 0.38 | 0.12 | 0.64 | 0.11 | 1.00 | 0.24 | 0.52 | 0.27 | 0.49 | 0.09 |
| RBMX2   | 0.88 | 0.49 | 0.84 | 0.20 | 1.15 | 0.05 | 0.55 | 0.18 | 0.26 | 0.07 | 0.74 | 0.17 | 0.69 | 0.19 | 0.31 | 0.04 | 0.65 | 0.16 |
| RBMXL2  | 1.03 | 0.24 | 1.04 | 0.19 | 1.35 | 0.17 | 0.44 | 0.12 | 0.21 | 0.01 | 1.33 | 0.17 | 0.42 | 0.04 | 0.20 | 0.03 | 0.99 | 0.12 |
| RBMX1A1 | 2.13 | 0.25 | 3.31 | 1.23 | 1.53 | 0.22 | 0.54 | 0.04 | 0.24 | 0.09 | 0.95 | 0.42 | 0.26 | 0.03 | 0.07 | 0.02 | 0.65 | 0.39 |
| RBP7    | 1.48 | 0.31 | 1.55 | 0.34 | 1.32 | 0.05 | 0.91 | 0.14 | 0.36 | 0.13 | 0.68 | 0.13 | 0.63 | 0.15 | 0.23 | 0.08 | 0.51 | 0.09 |
| RDBP    | 0.83 | 0.07 | 0.63 | 0.16 | 0.87 | 0.06 | 0.53 | 0.04 | 0.33 | 0.12 | 0.95 | 0.12 | 0.64 | 0.01 | 0.51 | 0.06 | 1.08 | 0.10 |
| REXO1   | 1.55 | 0.14 | 1.53 | 0.34 | 1.07 | 0.01 | 0.91 | 0.28 | 0.51 | 0.08 | 0.92 | 0.29 | 0.59 | 0.16 | 0.34 | 0.05 | 0.85 | 0.26 |
| REXO2   | 1.33 | 0.33 | 1.19 | 0.40 | 1.05 | 0.07 | 0.66 | 0.11 | 0.43 | 0.15 | 1.01 | 0.39 | 0.50 | 0.07 | 0.36 | 0.08 | 0.95 | 0.35 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| RNF113A | 0.80 | 0.10 | 0.55 | 0.03 | 0.93 | 0.32 | 0.66 | 0.11 | 0.50 | 0.20 | 0.69 | 0.12 | 0.82 | 0.10 | 0.91 | 0.42 | 0.83 | 0.39 |
| RNGTT   | 1.08 | 0.10 | 1.46 | 0.21 | 1.34 | 0.13 | 0.66 | 0.08 | 0.48 | 0.17 | 0.82 | 0.07 | 0.61 | 0.10 | 0.32 | 0.09 | 0.61 | 0.04 |
| RNPC3   | 1.08 | 0.09 | 1.05 | 0.36 | 1.19 | 0.15 | 1.19 | 0.08 | 0.64 | 0.18 | 0.93 | 0.15 | 1.11 | 0.03 | 0.63 | 0.13 | 0.79 | 0.11 |
| RNPS1   | 1.81 | 0.78 | 1.06 | 0.33 | 1.70 | 0.15 | 1.00 | 0.33 | 0.38 | 0.13 | 0.71 | 0.23 | 0.58 | 0.09 | 0.38 | 0.16 | 0.42 | 0.15 |
| RPL22   | 1.64 | 0.43 | 1.50 | 0.79 | 1.16 | 0.06 | 0.71 | 0.11 | 0.31 | 0.11 | 0.79 | 0.13 | 0.44 | 0.08 | 0.23 | 0.13 | 0.68 | 0.09 |
| RPL23A  | 0.76 | 0.10 | 0.70 | 0.27 | 2.45 | 0.21 | 2.47 | 0.27 | 1.66 | 0.90 | 0.86 | 0.12 | 3.26 | 0.15 | 2.32 | 1.02 | 0.35 | 0.06 |
| RPL31   | 2.00 | 0.75 | 2.07 | 1.04 | 1.50 | 0.05 | 1.46 | 0.26 | 0.73 | 0.26 | 0.65 | 0.02 | 0.79 | 0.26 | 0.38 | 0.10 | 0.44 | 0.01 |
| RPL5    | 1.39 | 0.33 | 1.52 | 0.69 | 1.87 | 0.28 | 1.67 | 0.29 | 0.88 | 0.17 | 1.37 | 0.47 | 1.21 | 0.10 | 0.63 | 0.17 | 0.72 | 0.18 |
| RPS10   | 0.74 | 0.08 | 0.85 | 0.35 | 1.20 | 0.07 | 1.05 | 0.10 | 0.72 | 0.32 | 1.14 | 0.09 | 1.41 | 0.07 | 0.87 | 0.38 | 0.95 | 0.03 |
| RPS11   | 1.71 | 0.73 | 1.69 | 0.61 | 1.35 | 0.16 | 1.03 | 0.24 | 0.52 | 0.25 | 1.29 | 0.35 | 0.64 | 0.16 | 0.31 | 0.14 | 0.96 | 0.26 |
| RPS12   | 0.68 | 0.14 | 0.73 | 0.23 | 0.95 | 0.15 | 1.39 | 0.30 | 0.50 | 0.19 | 0.60 | 0.05 | 2.04 | 0.12 | 0.68 | 0.12 | 0.64 | 0.05 |
| RPS13   | 0.88 | 0.58 | 0.89 | 0.38 | 0.99 | 0.02 | 1.82 | 0.73 | 1.36 | 0.29 | 0.81 | 0.12 | 2.31 | 0.56 | 1.68 | 0.67 | 0.82 | 0.12 |
| RPS15A  | 0.61 | 0.10 | 0.58 | 0.21 | 1.03 | 0.08 | 2.53 | 0.26 | 1.50 | 0.70 | 0.89 | 0.33 | 4.27 | 1.02 | 2.76 | 1.70 | 0.88 | 0.34 |
| RPS16   | 0.84 | 0.17 | 0.97 | 0.41 | 1.72 | 0.06 | 1.71 | 0.06 | 0.75 | 0.32 | 0.54 | 0.17 | 2.09 | 0.36 | 0.82 | 0.41 | 0.31 | 0.10 |
| RPS17   | 1.10 | 0.12 | 1.20 | 0.53 | 1.40 | 0.10 | 1.50 | 0.08 | 0.97 | 0.52 | 0.73 | 0.25 | 1.38 | 0.20 | 0.79 | 0.31 | 0.53 | 0.20 |
| RPS18   | 1.10 | 0.22 | 1.20 | 0.52 | 1.17 | 0.13 | 0.95 | 0.07 | 0.35 | 0.14 | 1.28 | 0.12 | 0.89 | 0.15 | 0.30 | 0.04 | 1.10 | 0.02 |
| RPS19   | 0.91 | 0.06 | 1.05 | 0.53 | 1.49 | 0.10 | 1.05 | 0.18 | 0.68 | 0.25 | 0.47 | 0.12 | 1.15 | 0.12 | 0.68 | 0.17 | 0.31 | 0.06 |
| RPS25   | 1.75 | 0.41 | 1.19 | 0.38 | 1.38 | 0.17 | 1.10 | 0.08 | 0.75 | 0.26 | 1.10 | 0.30 | 0.65 | 0.13 | 0.63 | 0.16 | 0.81 | 0.28 |
| RPS29   | 1.03 | 0.12 | 1.04 | 0.20 | 1.96 | 0.28 | 1.50 | 0.29 | 0.98 | 0.43 | 1.31 | 0.23 | 1.47 | 0.35 | 0.93 | 0.37 | 0.67 | 0.03 |
| RPS3    | 1.35 | 0.54 | 1.85 | 0.67 | 2.34 | 0.32 | 0.86 | 0.11 | 0.87 | 0.47 | 0.93 | 0.26 | 0.71 | 0.29 | 0.48 | 0.28 | 0.39 | 0.04 |
| RPS3A   | 0.89 | 0.04 | 1.38 | 0.32 | 1.49 | 0.26 | 1.48 | 0.27 | 1.16 | 0.22 | 0.96 | 0.05 | 1.67 | 0.30 | 0.89 | 0.32 | 0.60 | 0.04 |
| RPS4X   | 0.81 | 0.12 | 1.41 | 0.35 | 1.18 | 0.13 | 0.83 | 0.01 | 0.63 | 0.15 | 1.00 | 0.07 | 1.04 | 0.16 | 0.48 | 0.21 | 0.82 | 0.15 |
| RPS4Y1  | 0.83 | 0.20 | 1.01 | 0.07 | 1.01 | 0.11 | 0.35 | 0.08 | 0.17 | 0.04 | 0.84 | 0.20 | 0.43 | 0.04 | 0.17 | 0.04 | 0.83 | 0.10 |
| RPS5    | 1.00 | 0.07 | 0.99 | 0.37 | 1.26 | 0.13 | 1.21 | 0.04 | 0.61 | 0.11 | 1.52 | 0.33 | 1.21 | 0.05 | 0.70 | 0.36 | 1.19 | 0.13 |
| RPS7    | 0.70 | 0.07 | 0.69 | 0.09 | 1.30 | 0.20 | 0.94 | 0.20 | 0.93 | 0.17 | 1.02 | 0.10 | 1.36 | 0.35 | 1.36 | 0.21 | 0.79 | 0.09 |
| RPS8    | 1.02 | 0.03 | 1.45 | 0.11 | 1.41 | 0.19 | 1.80 | 0.14 | 2.01 | 0.76 | 0.94 | 0.21 | 1.76 | 0.08 | 1.37 | 0.42 | 0.66 | 0.02 |
| RPS9    | 0.72 | 0.13 | 1.07 | 0.29 | 1.13 | 0.03 | 1.38 | 0.05 | 1.09 | 0.13 | 0.85 | 0.00 | 1.96 | 0.32 | 1.07 | 0.27 | 0.75 | 0.03 |
| RUVBL1  | 3.02 | 0.45 | 2.35 | 0.07 | 0.91 | 0.02 | 1.03 | 0.15 | 0.46 | 0.10 | 0.88 | 0.02 | 0.34 | 0.01 | 0.19 | 0.04 | 0.96 | 0.03 |
| RUVBL2  | 0.53 | 0.04 | 0.34 | 0.04 | 1.18 | 0.09 | 0.68 | 0.04 | 0.31 | 0.05 | 0.82 | 0.11 | 1.30 | 0.04 | 0.90 | 0.08 | 0.67 | 0.05 |
| S100A8  | 1.07 | 0.22 | 0.94 | 0.28 | 1.43 | 0.18 | 1.66 | 0.23 | 0.86 | 0.16 | 2.10 | 0.52 | 1.60 | 0.42 | 0.98 | 0.34 | 1.39 | 0.19 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S100A9  | 0.64 | 0.19 | 0.68 | 0.19 | 1.30 | 0.09 | 0.49 | 0.06 | 0.25 | 0.03 | 1.08 | 0.32 | 0.79 | 0.23 | 0.40 | 0.14 | 0.84 | 0.29 |
| SAFB    | 0.49 | 0.21 | 0.28 | 0.10 | 0.53 | 0.04 | 0.54 | 0.08 | 0.26 | 0.03 | 0.78 | 0.13 | 1.21 | 0.34 | 1.01 | 0.44 | 1.55 | 0.24 |
| SAFB2   | 1.71 | 0.44 | 1.72 | 0.36 | 0.93 | 0.09 | 0.80 | 0.13 | 0.45 | 0.10 | 1.06 | 0.05 | 0.48 | 0.06 | 0.28 | 0.11 | 1.08 | 0.09 |
| SART1   | 3.29 | 1.04 | 5.07 | 2.31 | 1.34 | 0.14 | 0.66 | 0.04 | 0.52 | 0.12 | 1.10 | 0.12 | 0.22 | 0.09 | 0.11 | 0.04 | 0.78 | 0.06 |
| SART3   | 1.09 | 0.04 | 1.10 | 0.21 | 1.24 | 0.00 | 0.95 | 0.08 | 0.82 | 0.24 | 1.10 | 0.24 | 0.87 | 0.05 | 0.76 | 0.22 | 0.89 | 0.20 |
| SCAF1   | 2.03 | 0.60 | 1.42 | 0.33 | 1.37 | 0.19 | 0.66 | 0.15 | 0.42 | 0.10 | 0.51 | 0.11 | 0.33 | 0.06 | 0.30 | 0.08 | 0.38 | 0.11 |
| SCAF11  | 1.70 | 0.39 | 1.02 | 0.25 | 0.94 | 0.14 | 1.01 | 0.08 | 0.40 | 0.02 | 0.82 | 0.10 | 0.61 | 0.11 | 0.42 | 0.13 | 0.86 | 0.06 |
| SCAF8   | 0.96 | 0.23 | 1.10 | 0.49 | 1.51 | 0.20 | 0.68 | 0.08 | 0.73 | 0.28 | 0.70 | 0.29 | 0.72 | 0.14 | 0.68 | 0.18 | 0.48 | 0.27 |
| SDE2    | 0.57 | 0.14 | 0.62 | 0.17 | 0.88 | 0.19 | 1.16 | 0.50 | 0.67 | 0.09 | 0.83 | 0.20 | 1.99 | 0.39 | 1.13 | 0.36 | 0.98 | 0.33 |
| SF1     | 1.06 | 0.24 | 1.23 | 0.44 | 1.07 | 0.10 | 1.04 | 0.05 | 0.54 | 0.14 | 0.75 | 0.13 | 1.02 | 0.22 | 0.49 | 0.26 | 0.69 | 0.20 |
| SF3A1   | 2.30 | 0.15 | 2.61 | 0.75 | 2.16 | 0.20 | 1.31 | 0.16 | 0.77 | 0.17 | 0.71 | 0.08 | 0.57 | 0.10 | 0.31 | 0.10 | 0.32 | 0.07 |
| SF3A2   | 3.45 | 0.48 | 3.62 | 0.85 | 1.79 | 0.09 | 0.74 | 0.05 | 0.31 | 0.07 | 0.79 | 0.10 | 0.22 | 0.02 | 0.09 | 0.04 | 0.44 | 0.07 |
| SF3A3   | 5.17 | 0.31 | 5.76 | 2.73 | 1.78 | 0.10 | 0.96 | 0.03 | 0.67 | 0.14 | 1.04 | 0.08 | 0.19 | 0.01 | 0.13 | 0.04 | 0.57 | 0.03 |
| SF3B1   | 3.35 | 1.27 | 3.25 | 0.78 | 3.37 | 0.75 | 1.03 | 0.31 | 0.59 | 0.06 | 0.81 | 0.04 | 0.31 | 0.03 | 0.19 | 0.05 | 0.21 | 0.02 |
| SF3B14  | 6.26 | 1.73 | 6.89 | 2.17 | 1.62 | 0.15 | 1.70 | 0.06 | 2.15 | 1.54 | 0.88 | 0.11 | 0.29 | 0.09 | 0.32 | 0.20 | 0.54 | 0.00 |
| SF3B2   | 5.09 | 0.92 | 4.50 | 0.15 | 1.33 | 0.13 | 0.61 | 0.04 | 0.24 | 0.13 | 1.09 | 0.10 | 0.12 | 0.02 | 0.05 | 0.03 | 0.82 | 0.05 |
| SF3B3   | 2.72 | 0.40 | 3.07 | 1.07 | 1.02 | 0.11 | 0.59 | 0.02 | 0.32 | 0.19 | 1.24 | 0.20 | 0.22 | 0.03 | 0.10 | 0.05 | 1.21 | 0.08 |
| SF3B4   | 6.02 | 2.21 | 6.08 | 0.57 | 1.07 | 0.08 | 1.31 | 0.21 | 0.56 | 0.03 | 0.72 | 0.07 | 0.23 | 0.05 | 0.09 | 0.01 | 0.68 | 0.14 |
| SF3B5   | 4.12 | 0.68 | 3.59 | 0.68 | 1.48 | 0.14 | 0.72 | 0.12 | 0.23 | 0.05 | 0.63 | 0.05 | 0.17 | 0.00 | 0.07 | 0.03 | 0.41 | 0.04 |
| SFPQ    | 2.13 | 0.41 | 2.97 | 0.89 | 1.63 | 0.28 | 0.69 | 0.15 | 0.23 | 0.03 | 0.75 | 0.07 | 0.32 | 0.04 | 0.08 | 0.03 | 0.44 | 0.11 |
| SKIV2L  | 0.89 | 0.20 | 0.73 | 0.17 | 0.90 | 0.11 | 0.95 | 0.08 | 0.50 | 0.17 | 0.68 | 0.04 | 1.12 | 0.35 | 0.71 | 0.29 | 0.74 | 0.13 |
| SKIV2L2 | 1.49 | 0.18 | 1.01 | 0.35 | 1.23 | 0.22 | 1.03 | 0.02 | 0.38 | 0.03 | 0.74 | 0.21 | 0.70 | 0.08 | 0.41 | 0.17 | 0.60 | 0.10 |
| SLC43A2 | 1.72 | 0.31 | 1.10 | 0.05 | 1.59 | 0.18 | 1.17 | 0.06 | 0.36 | 0.07 | 1.26 | 0.06 | 0.69 | 0.09 | 0.33 | 0.08 | 0.81 | 0.17 |
| SLU7    | 1.97 | 0.28 | 2.06 | 0.87 | 0.74 | 0.07 | 2.06 | 0.13 | 0.96 | 0.15 | 0.80 | 0.14 | 1.06 | 0.12 | 0.55 | 0.33 | 1.07 | 0.12 |
| SMARCA5 | 1.55 | 0.25 | 1.61 | 1.11 | 1.40 | 0.26 | 0.88 | 0.08 | 0.52 | 0.04 | 0.86 | 0.03 | 0.57 | 0.06 | 0.42 | 0.23 | 0.63 | 0.09 |
| SMC1A   | 1.10 | 0.31 | 0.72 | 0.24 | 0.94 | 0.03 | 0.55 | 0.02 | 0.21 | 0.02 | 0.69 | 0.00 | 0.53 | 0.16 | 0.32 | 0.13 | 0.74 | 0.04 |
| SMC2    | 1.37 | 0.26 | 1.02 | 0.13 | 1.21 | 0.04 | 0.96 | 0.06 | 0.47 | 0.07 | 1.11 | 0.09 | 0.72 | 0.15 | 0.47 | 0.12 | 0.92 | 0.04 |
| SMNDC1  | 4.08 | 0.60 | 3.00 | 0.61 | 1.55 | 0.18 | 1.17 | 0.31 | 0.54 | 0.10 | 1.05 | 0.13 | 0.29 | 0.04 | 0.19 | 0.07 | 0.67 | 0.04 |
| SMU1    | 2.67 | 0.15 | 2.37 | 0.71 | 1.03 | 0.10 | 0.58 | 0.02 | 0.25 | 0.05 | 1.01 | 0.25 | 0.22 | 0.01 | 0.11 | 0.02 | 0.97 | 0.21 |
| SNIP1   | 0.69 | 0.03 | 0.55 | 0.06 | 1.32 | 0.10 | 0.92 | 0.13 | 0.57 | 0.18 | 1.51 | 0.21 | 1.35 | 0.25 | 1.06 | 0.46 | 1.15 | 0.17 |

|          |      |      |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|----------|------|------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| SNRNP200 | 4.50 | 1.14 | 4.66  | 0.32 | 3.60 | 1.16 | 1.50 | 0.44 | 0.71 | 0.11 | 1.40 | 0.50 | 0.33 | 0.03 | 0.15 | 0.03 | 0.42 | 0.23 |
| SNRNP25  | 0.76 | 0.18 | 0.68  | 0.18 | 0.97 | 0.27 | 1.84 | 0.37 | 0.44 | 0.06 | 0.94 | 0.33 | 2.43 | 0.20 | 0.68 | 0.23 | 0.97 | 0.19 |
| SNRNP27  | 2.21 | 0.63 | 1.71  | 0.34 | 1.50 | 0.20 | 1.14 | 0.20 | 0.31 | 0.08 | 1.10 | 0.10 | 0.53 | 0.10 | 0.19 | 0.10 | 0.68 | 0.08 |
| SNRNP35  | 1.88 | 1.03 | 2.20  | 0.23 | 1.42 | 0.45 | 1.12 | 0.39 | 0.57 | 0.10 | 0.97 | 0.22 | 0.64 | 0.12 | 0.26 | 0.07 | 0.72 | 0.24 |
| SNRNP40  | 1.66 | 0.39 | 1.96  | 0.03 | 2.10 | 0.71 | 1.11 | 0.55 | 0.71 | 0.16 | 1.17 | 0.27 | 0.65 | 0.16 | 0.36 | 0.08 | 0.59 | 0.17 |
| SNRNP48  | 1.45 | 0.36 | 1.54  | 0.04 | 1.33 | 0.41 | 1.23 | 0.37 | 0.34 | 0.09 | 1.46 | 0.59 | 0.84 | 0.04 | 0.22 | 0.05 | 1.10 | 0.33 |
| SNRNP70  | 2.16 | 0.36 | 2.51  | 0.41 | 3.01 | 0.52 | 1.23 | 0.28 | 0.53 | 0.10 | 1.23 | 0.10 | 0.57 | 0.05 | 0.22 | 0.08 | 0.42 | 0.12 |
| SNRPA    | 1.42 | 0.41 | 1.35  | 0.25 | 2.56 | 0.24 | 1.10 | 0.29 | 0.58 | 0.14 | 1.76 | 0.38 | 0.78 | 0.02 | 0.44 | 0.14 | 0.69 | 0.14 |
| SNRPA1   | 2.87 | 0.70 | 2.79  | 0.65 | 1.99 | 0.33 | 1.60 | 0.30 | 1.07 | 0.13 | 1.72 | 0.18 | 0.57 | 0.09 | 0.40 | 0.14 | 0.88 | 0.19 |
| SNRPB    | 5.33 | 1.17 | 4.07  | 0.29 | 2.68 | 0.38 | 1.74 | 0.44 | 0.87 | 0.17 | 0.97 | 0.35 | 0.33 | 0.09 | 0.22 | 0.06 | 0.37 | 0.15 |
| SNRPB2   | 0.50 | 0.07 | 0.47  | 0.08 | 0.87 | 0.04 | 0.44 | 0.02 | 0.27 | 0.03 | 0.86 | 0.28 | 0.89 | 0.14 | 0.60 | 0.18 | 0.99 | 0.35 |
| SNRPC    | 2.95 | 0.22 | 3.50  | 0.53 | 2.38 | 0.15 | 1.00 | 0.11 | 1.15 | 0.24 | 0.81 | 0.14 | 0.34 | 0.04 | 0.34 | 0.13 | 0.34 | 0.04 |
| SNRPD1   | 5.19 | 1.62 | 4.08  | 1.07 | 1.92 | 0.31 | 1.04 | 0.26 | 0.33 | 0.07 | 1.07 | 0.14 | 0.20 | 0.02 | 0.09 | 0.03 | 0.57 | 0.13 |
| SNRPD2   | 9.26 | 1.45 | 10.64 | 1.58 | 2.37 | 0.36 | 1.68 | 0.41 | 0.70 | 0.18 | 1.17 | 0.29 | 0.18 | 0.04 | 0.07 | 0.01 | 0.49 | 0.05 |
| SNRPD3   | 6.61 | 1.00 | 8.59  | 0.46 | 3.98 | 1.60 | 2.12 | 0.51 | 1.16 | 0.09 | 1.37 | 0.17 | 0.32 | 0.07 | 0.14 | 0.02 | 0.38 | 0.15 |
| SNRPE    | 3.94 | 0.92 | 2.87  | 0.39 | 1.98 | 0.25 | 1.24 | 0.37 | 0.59 | 0.18 | 1.01 | 0.05 | 0.31 | 0.02 | 0.21 | 0.08 | 0.51 | 0.04 |
| SNRPF    | 3.90 | 0.31 | 1.72  | 0.28 | 3.31 | 0.28 | 1.51 | 0.36 | 0.64 | 0.06 | 1.00 | 0.29 | 0.38 | 0.06 | 0.38 | 0.07 | 0.31 | 0.11 |
| SNRPG    | 2.74 | 0.94 | 1.54  | 0.35 | 2.21 | 0.08 | 1.91 | 0.56 | 0.64 | 0.02 | 1.71 | 0.30 | 0.70 | 0.03 | 0.43 | 0.11 | 0.78 | 0.16 |
| SNRPN    | 1.77 | 0.64 | 1.54  | 0.46 | 1.48 | 0.18 | 0.67 | 0.21 | 0.44 | 0.06 | 1.42 | 0.33 | 0.39 | 0.10 | 0.30 | 0.09 | 0.98 | 0.33 |
| SNW1     | 2.69 | 0.89 | 2.00  | 0.10 | 1.72 | 0.04 | 1.02 | 0.25 | 0.73 | 0.06 | 1.97 | 0.95 | 0.38 | 0.03 | 0.36 | 0.04 | 1.14 | 0.56 |
| SPEN     | 1.83 | 0.09 | 2.35  | 0.66 | 0.86 | 0.06 | 0.36 | 0.03 | 0.24 | 0.03 | 0.62 | 0.17 | 0.20 | 0.01 | 0.11 | 0.03 | 0.73 | 0.25 |
| SPPL3    | 1.59 | 0.50 | 1.35  | 0.20 | 1.61 | 0.15 | 1.13 | 0.23 | 0.58 | 0.09 | 1.08 | 0.27 | 0.73 | 0.12 | 0.43 | 0.04 | 0.66 | 0.11 |
| SREK1    | 1.39 | 0.11 | 0.98  | 0.51 | 1.20 | 0.08 | 0.73 | 0.06 | 0.43 | 0.04 | 1.05 | 0.09 | 0.53 | 0.04 | 0.54 | 0.30 | 0.85 | 0.11 |
| SRP19    | 0.73 | 0.16 | 0.83  | 0.01 | 1.28 | 0.20 | 0.92 | 0.17 | 0.54 | 0.06 | 1.07 | 0.08 | 1.26 | 0.08 | 0.65 | 0.07 | 0.85 | 0.20 |
| SRP54    | 2.43 | 0.44 | 2.38  | 0.28 | 1.43 | 0.28 | 0.92 | 0.14 | 0.50 | 0.05 | 2.08 | 1.56 | 0.38 | 0.04 | 0.21 | 0.05 | 1.39 | 0.92 |
| SRP68    | 1.32 | 0.13 | 0.70  | 0.15 | 0.94 | 0.09 | 0.80 | 0.17 | 0.27 | 0.03 | 0.83 | 0.37 | 0.62 | 0.19 | 0.40 | 0.04 | 0.87 | 0.33 |
| SRP9     | 1.07 | 0.42 | 1.19  | 0.39 | 0.94 | 0.29 | 0.57 | 0.08 | 0.27 | 0.03 | 0.95 | 0.28 | 0.57 | 0.13 | 0.25 | 0.10 | 1.12 | 0.53 |
| SRPK1    | 0.97 | 0.08 | 1.06  | 0.28 | 1.04 | 0.23 | 0.44 | 0.07 | 0.29 | 0.05 | 0.79 | 0.16 | 0.46 | 0.05 | 0.29 | 0.09 | 0.77 | 0.19 |
| SRPK2    | 1.28 | 0.43 | 1.10  | 0.16 | 1.02 | 0.11 | 0.57 | 0.12 | 0.34 | 0.05 | 0.70 | 0.17 | 0.46 | 0.09 | 0.32 | 0.09 | 0.69 | 0.19 |
| SRPK3    | 1.32 | 0.25 | 1.10  | 0.23 | 1.36 | 0.13 | 1.29 | 0.30 | 0.78 | 0.18 | 1.29 | 0.23 | 0.97 | 0.04 | 0.72 | 0.18 | 0.95 | 0.16 |

|        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| SRRM1  | 0.99 | 0.13 | 0.92 | 0.10 | 1.26 | 0.22 | 0.71 | 0.13 | 0.37 | 0.05 | 0.65 | 0.02 | 0.71 | 0.07 | 0.41 | 0.08 | 0.52 | 0.08 |
| SRRM2  | 1.75 | 0.21 | 1.75 | 0.67 | 1.01 | 0.16 | 0.61 | 0.06 | 0.30 | 0.03 | 1.22 | 1.00 | 0.35 | 0.01 | 0.18 | 0.06 | 1.34 | 1.30 |
| SRRM4  | 1.16 | 0.06 | 0.72 | 0.32 | 1.48 | 0.30 | 0.69 | 0.07 | 0.28 | 0.06 | 0.90 | 0.33 | 0.60 | 0.04 | 0.42 | 0.11 | 0.65 | 0.32 |
| SRRT   | 0.58 | 0.21 | 0.59 | 0.15 | 0.53 | 0.13 | 1.16 | 0.31 | 0.60 | 0.13 | 0.97 | 0.26 | 2.03 | 0.16 | 1.07 | 0.35 | 1.91 | N/A  |
| SRSF1  | 1.85 | 0.20 | 1.09 | 0.44 | 1.21 | 0.03 | 0.64 | 0.03 | 0.34 | 0.05 | 1.02 | 0.01 | 0.35 | 0.03 | 0.37 | 0.20 | 0.84 | 0.02 |
| SRSF1  | 1.90 | 0.28 | 1.01 | 0.04 | 0.98 | 0.21 | 0.82 | 0.11 | 0.45 | 0.08 | 0.91 | 0.30 | 0.44 | 0.12 | 0.45 | 0.07 | 0.91 | 0.11 |
| SRSF10 | 1.54 | 0.60 | 1.25 | 0.08 | 1.03 | 0.20 | 1.01 | 0.26 | 0.28 | 0.01 | 1.69 | 0.11 | 0.69 | 0.13 | 0.23 | 0.02 | 1.68 | 0.33 |
| SRSF11 | 2.51 | 0.43 | 4.25 | 1.44 | 1.41 | 0.18 | 0.50 | 0.01 | 0.33 | 0.04 | 0.75 | 0.06 | 0.20 | 0.03 | 0.08 | 0.03 | 0.53 | 0.05 |
| SRSF2  | 2.50 | 0.23 | 3.02 | 1.49 | 0.91 | 0.09 | 0.66 | 0.09 | 0.29 | 0.06 | 0.61 | 0.02 | 0.26 | 0.02 | 0.11 | 0.06 | 0.65 | 0.07 |
| SRSF2  | 2.90 | 0.90 | 2.37 | 0.62 | 0.98 | 0.13 | 0.83 | 0.10 | 0.25 | 0.04 | 0.58 | 0.02 | 0.31 | 0.10 | 0.11 | 0.04 | 0.57 | 0.11 |
| SRSF2  | 2.29 | 0.75 | 1.97 | 0.50 | 1.00 | 0.15 | 0.75 | 0.22 | 0.43 | 0.06 | 2.43 | 1.10 | 0.33 | 0.02 | 0.22 | 0.06 | 2.47 | 1.24 |
| SRSF3  | 0.49 | 0.15 | 0.35 | 0.10 | 0.66 | 0.05 | 1.20 | 0.16 | 0.43 | 0.14 | 0.61 | 0.02 | 2.59 | 0.55 | 1.39 | 0.86 | 0.94 | 0.12 |
| SRSF3  | 0.40 | 0.10 | 0.30 | 0.08 | 0.55 | 0.08 | 1.28 | 0.15 | 0.69 | 0.08 | 1.21 | 0.19 | 3.34 | 1.04 | 2.30 | 0.35 | 2.03 | 0.33 |
| SRSF4  | 1.07 | 0.11 | 1.10 | 0.38 | 1.21 | 0.20 | 0.83 | 0.10 | 0.32 | 0.02 | 0.51 | 0.05 | 0.78 | 0.10 | 0.33 | 0.15 | 0.43 | 0.08 |
| SRSF4  | 0.84 | 0.16 | 0.87 | 0.23 | 1.01 | 0.07 | 0.69 | 0.09 | 0.42 | 0.04 | 0.55 | 0.06 | 0.82 | 0.05 | 0.50 | 0.13 | 0.54 | 0.03 |
| SRSF5  | 2.32 | 0.43 | 2.12 | 0.32 | 1.45 | 0.23 | 1.23 | 0.18 | 0.49 | 0.09 | 0.79 | 0.03 | 0.53 | 0.03 | 0.24 | 0.08 | 0.54 | 0.09 |
| SRSF5  | 2.43 | 0.33 | 2.03 | 0.42 | 1.47 | 0.07 | 1.53 | 0.32 | 0.64 | 0.15 | 1.03 | 0.26 | 0.63 | 0.05 | 0.34 | 0.15 | 0.70 | 0.16 |
| SRSF6  | 1.06 | 0.14 | 1.20 | 0.62 | 1.51 | 0.34 | 0.74 | 0.06 | 0.38 | 0.05 | 0.89 | 0.16 | 0.71 | 0.11 | 0.37 | 0.19 | 0.60 | 0.08 |
| SRSF6  | 0.99 | 0.30 | 0.96 | 0.12 | 1.49 | 0.19 | 0.81 | 0.16 | 0.49 | 0.06 | 0.86 | 0.25 | 0.89 | 0.43 | 0.51 | 0.03 | 0.57 | 0.10 |
| SRSF7  | 3.34 | 0.70 | 4.84 | 0.37 | 3.24 | 0.55 | 0.89 | 0.25 | 0.43 | 0.06 | 1.02 | 0.31 | 0.26 | 0.02 | 0.09 | 0.02 | 0.31 | 0.05 |
| SRSF7  | 4.29 | 0.65 | 5.23 | 0.95 | 2.30 | 0.28 | 0.85 | 0.14 | 0.36 | 0.02 | 0.84 | 0.03 | 0.20 | 0.03 | 0.07 | 0.02 | 0.35 | 0.05 |
| SRSF8  | 0.69 | 0.16 | 0.40 | 0.06 | 1.03 | 0.13 | 1.47 | 0.24 | 0.54 | 0.08 | 1.19 | 0.67 | 2.16 | 0.19 | 1.35 | 0.20 | 1.21 | 0.72 |
| SRSF9  | 2.17 | 0.52 | 2.26 | 0.95 | 1.65 | 0.17 | 0.76 | 0.24 | 0.40 | 0.02 | 1.06 | 0.24 | 0.35 | 0.03 | 0.20 | 0.07 | 0.64 | 0.13 |
| SRSF9  | 1.83 | 0.07 | 1.49 | 0.29 | 1.44 | 0.07 | 0.82 | 0.13 | 0.45 | 0.11 | 1.09 | 0.25 | 0.45 | 0.07 | 0.30 | 0.09 | 0.76 | 0.20 |
| SRSF9  | 2.04 | 0.57 | 1.63 | 0.35 | 1.60 | 0.32 | 0.75 | 0.15 | 0.45 | 0.11 | 0.79 | 0.20 | 0.38 | 0.09 | 0.29 | 0.11 | 0.51 | 0.19 |
| SSB    | 2.19 | 0.37 | 1.64 | 0.19 | 1.12 | 0.17 | 0.66 | 0.08 | 0.34 | 0.06 | 0.77 | 0.00 | 0.30 | 0.02 | 0.21 | 0.06 | 0.74 | 0.12 |
| STRBP  | 0.97 | 0.25 | 0.94 | 0.17 | 0.75 | 0.08 | 0.94 | 0.22 | 0.55 | 0.04 | 0.97 | 0.26 | 0.97 | 0.02 | 0.60 | 0.12 | 1.31 | 0.46 |
| SUGP1  | 1.10 | 0.17 | 1.32 | 0.35 | 0.83 | 0.07 | 0.77 | 0.09 | 0.24 | 0.03 | 0.57 | 0.03 | 0.70 | 0.03 | 0.20 | 0.08 | 0.66 | 0.08 |
| SUGP1  | 0.96 | 0.39 | 1.20 | 0.70 | 0.91 | 0.14 | 0.75 | 0.17 | 0.26 | 0.03 | 0.55 | 0.05 | 0.85 | 0.25 | 0.26 | 0.11 | 0.58 | 0.15 |
| SUGP2  | 1.41 | 0.32 | 1.60 | 0.27 | 1.34 | 0.06 | 0.75 | 0.19 | 0.41 | 0.04 | 1.44 | 0.46 | 0.53 | 0.03 | 0.26 | 0.07 | 1.05 | 0.34 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| SYF2    | 1.89 | 0.40 | 1.82 | 0.63 | 1.00 | 0.08 | 0.90 | 0.17 | 0.37 | 0.05 | 0.80 | 0.12 | 0.48 | 0.02 | 0.22 | 0.10 | 0.79 | 0.07 |
| SYNCRIP | 0.66 | 0.17 | 0.36 | 0.07 | 0.89 | 0.18 | 0.56 | 0.10 | 0.27 | 0.04 | 0.92 | 0.01 | 0.90 | 0.36 | 0.75 | 0.21 | 1.06 | 0.25 |
| TAF15   | 0.76 | 0.16 | 0.71 | 0.07 | 0.81 | 0.10 | 1.24 | 0.39 | 0.51 | 0.13 | 1.08 | 0.18 | 1.60 | 0.17 | 0.73 | 0.24 | 1.36 | 0.34 |
| TAF6    | 1.30 | 0.12 | 1.14 | 0.05 | 1.53 | 0.36 | 0.79 | 0.09 | 0.34 | 0.05 | 1.04 | 0.14 | 0.61 | 0.01 | 0.30 | 0.04 | 0.72 | 0.27 |
| TARDBP  | 1.40 | 0.25 | 1.19 | 0.28 | 1.58 | 0.25 | 0.96 | 0.14 | 0.45 | 0.10 | 1.44 | 0.99 | 0.69 | 0.08 | 0.41 | 0.21 | 0.87 | 0.56 |
| TCERG1  | 0.94 | 0.15 | 0.76 | 0.12 | 1.02 | 0.17 | 1.00 | 0.13 | 0.63 | 0.15 | 1.16 | 0.47 | 1.10 | 0.34 | 0.87 | 0.33 | 1.10 | 0.20 |
| TDRD3   | 1.03 | 0.19 | 0.59 | 0.04 | 0.93 | 0.08 | 0.95 | 0.10 | 0.51 | 0.10 | 1.08 | 0.24 | 0.93 | 0.12 | 0.88 | 0.24 | 1.15 | 0.17 |
| TET1    | 0.78 | 0.14 | 0.65 | 0.12 | 0.69 | 0.08 | 1.12 | 0.18 | 0.86 | 0.17 | 1.36 | 0.44 | 1.47 | 0.27 | 1.40 | 0.55 | 2.00 | 0.72 |
| TFIP11  | 0.89 | 0.22 | 0.75 | 0.16 | 0.76 | 0.10 | 0.53 | 0.08 | 0.25 | 0.02 | 0.48 | 0.07 | 0.64 | 0.26 | 0.34 | 0.09 | 0.64 | 0.16 |
| THOC1   | 1.63 | 0.28 | 1.42 | 0.33 | 1.65 | 0.29 | 0.83 | 0.14 | 0.44 | 0.07 | 0.85 | 0.24 | 0.51 | 0.03 | 0.33 | 0.14 | 0.52 | 0.13 |
| THOC2   | 1.79 | 0.24 | 1.93 | 0.22 | 2.02 | 0.34 | 0.85 | 0.13 | 0.60 | 0.12 | 0.63 | 0.18 | 0.47 | 0.01 | 0.32 | 0.10 | 0.32 | 0.10 |
| THOC3   | 1.49 | 0.14 | 0.85 | 0.10 | 0.96 | 0.13 | 1.14 | 0.25 | 0.73 | 0.18 | 0.78 | 0.13 | 0.78 | 0.22 | 0.88 | 0.28 | 0.76 | 0.05 |
| THOC5   | 1.82 | 0.17 | 1.63 | 0.22 | 1.30 | 0.23 | 0.93 | 0.11 | 0.40 | 0.09 | 1.42 | 0.38 | 0.51 | 0.03 | 0.24 | 0.05 | 1.15 | 0.53 |
| THOC6   | 1.32 | 0.13 | 1.30 | 0.09 | 1.70 | 0.55 | 0.56 | 0.09 | 0.42 | 0.06 | 0.68 | 0.12 | 0.43 | 0.07 | 0.32 | 0.02 | 0.50 | 0.19 |
| THOC7   | 3.03 | 0.97 | 2.33 | 0.89 | 1.19 | 0.22 | 1.38 | 0.24 | 0.43 | 0.12 | 2.33 | 0.92 | 0.48 | 0.12 | 0.19 | 0.05 | 1.92 | 0.46 |
| TIA1    | 1.30 | 0.46 | 1.51 | 0.53 | 1.15 | 0.12 | 1.08 | 0.17 | 0.55 | 0.01 | 0.86 | 0.18 | 0.89 | 0.27 | 0.40 | 0.18 | 0.75 | 0.08 |
| TIAL1   | 1.11 | 0.46 | 0.57 | 0.11 | 0.75 | 0.14 | 1.14 | 0.04 | 0.62 | 0.09 | 1.09 | N/A  | 1.14 | 0.44 | 1.13 | 0.40 | 1.27 | N/A  |
| TNPO1   | 1.39 | 0.12 | 1.31 | 0.16 | 0.77 | 0.10 | 0.61 | 0.06 | 0.48 | 0.12 | 3.56 | N/A  | 0.44 | 0.08 | 0.37 | 0.14 | 4.30 | N/A  |
| TOP1MT  | 1.29 | 0.66 | 1.64 | 0.15 | 1.66 | 0.42 | 0.86 | 0.31 | 0.57 | 0.12 | 0.68 | 0.13 | 0.70 | 0.12 | 0.35 | 0.04 | 0.42 | 0.05 |
| TOPORS  | 1.03 | 0.28 | 1.01 | 0.18 | 1.20 | 0.31 | 1.09 | 0.28 | 0.79 | 0.19 | 1.35 | 0.10 | 1.07 | 0.26 | 0.77 | 0.06 | 1.17 | 0.28 |
| TPR     | 0.60 | 0.11 | 0.68 | 0.08 | 1.07 | 0.31 | 0.54 | 0.09 | 0.35 | 0.14 | 1.12 | 0.08 | 0.92 | 0.26 | 0.51 | 0.15 | 1.11 | 0.31 |
| TPX2    | 1.69 | 0.88 | 1.56 | 0.34 | 1.59 | 0.38 | 0.63 | 0.22 | 0.17 | 0.08 | 0.74 | 0.25 | 0.43 | 0.16 | 0.11 | 0.05 | 0.52 | 0.31 |
| TRA2A   | 1.41 | 0.18 | 1.28 | 0.22 | 0.95 | 0.23 | 1.07 | 0.05 | 1.38 | 1.10 | 0.59 | 0.04 | 0.76 | 0.07 | 1.01 | 0.63 | 0.65 | 0.19 |
| TRA2B   | 1.22 | 0.20 | 1.10 | 0.31 | 1.61 | 0.12 | 0.86 | 0.09 | 0.43 | 0.06 | 0.74 | 0.08 | 0.71 | 0.08 | 0.42 | 0.16 | 0.46 | 0.09 |
| TRNT1   | 1.13 | 0.17 | 1.28 | 0.09 | 1.47 | 0.45 | 1.07 | 0.16 | 0.82 | 0.23 | 0.70 | 0.12 | 0.95 | 0.07 | 0.63 | 0.14 | 0.50 | 0.13 |
| TTF2    | 0.77 | 0.20 | 0.63 | 0.02 | 1.09 | 0.39 | 0.44 | 0.04 | 0.34 | 0.04 | 0.57 | 0.05 | 0.58 | 0.10 | 0.55 | 0.05 | 0.58 | 0.27 |
| TUBA1B  | 1.33 | 0.44 | 1.26 | 0.15 | 1.45 | 0.21 | 0.45 | 0.14 | 0.23 | 0.08 | 0.67 | 0.19 | 0.35 | 0.14 | 0.19 | 0.08 | 0.48 | 0.19 |
| TUBA4A  | 0.59 | 0.12 | 0.84 | 0.17 | 0.75 | 0.21 | 0.68 | 0.16 | 0.36 | 0.12 | 0.56 | 0.04 | 1.15 | 0.19 | 0.43 | 0.08 | 0.78 | 0.18 |
| TUBB    | 0.80 | 0.11 | 0.63 | 0.05 | 1.10 | 0.29 | 0.47 | 0.06 | 0.31 | 0.07 | 0.53 | 0.24 | 0.60 | 0.13 | 0.49 | 0.09 | 0.46 | 0.11 |
| TXNL4A  | 2.38 | 0.61 | 2.63 | 0.35 | 1.16 | 0.34 | 1.24 | 0.12 | 0.76 | 0.14 | 0.81 | 0.09 | 0.53 | 0.08 | 0.29 | 0.02 | 0.75 | 0.26 |

|         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| U2AF1   | 2.94 | 0.79 | 3.85 | 0.27 | 5.05 | 0.10 | 0.79 | 0.12 | 0.77 | 0.25 | 1.14 | 0.15 | 0.27 | 0.05 | 0.20 | 0.05 | 0.32 | 0.14 |
| U2AF1L4 | 0.83 | 0.31 | 0.80 | 0.05 | 0.83 | 0.17 | 0.85 | 0.19 | 0.61 | 0.22 | 1.17 | 0.01 | 1.09 | 0.25 | 0.75 | 0.23 | 1.60 | 0.22 |
| U2AF2   | 2.57 | 0.61 | 2.65 | 0.32 | 2.15 | 0.89 | 1.20 | 0.23 | 2.32 | 2.28 | 1.18 | 0.49 | 0.47 | 0.03 | 0.95 | 1.00 | 0.60 | 0.28 |
| U2SURP  | 1.21 | 0.41 | 0.93 | 0.26 | 1.57 | 0.23 | 0.58 | 0.12 | 0.28 | 0.04 | 0.61 | 0.08 | 0.51 | 0.18 | 0.33 | 0.13 | 0.36 | 0.05 |
| UBL5    | 3.12 | 0.68 | 3.85 | 0.78 | 2.37 | 0.37 | 0.69 | 0.06 | 0.54 | 0.14 | 0.44 | 0.06 | 0.23 | 0.04 | 0.14 | 0.04 | 0.19 | 0.05 |
| UNK     | 0.66 | 0.14 | 0.69 | 0.04 | 0.80 | 0.20 | 0.37 | 0.04 | 0.35 | 0.07 | 0.57 | 0.17 | 0.57 | 0.07 | 0.51 | 0.10 | 0.77 | 0.36 |
| USP39   | 1.93 | 0.53 | 1.54 | 0.01 | 1.26 | 0.45 | 0.59 | 0.11 | 0.27 | 0.07 | 0.58 | 0.11 | 0.31 | 0.04 | 0.17 | 0.05 | 0.53 | 0.28 |
| VIM     | 0.67 | 0.13 | 0.72 | 0.06 | 0.76 | 0.27 | 0.65 | 0.19 | 0.57 | 0.06 | 0.60 | 0.13 | 0.97 | 0.21 | 0.80 | 0.13 | 0.82 | 0.15 |
| WBP11   | 0.68 | 0.24 | 0.62 | 0.01 | 2.11 | 0.63 | 0.54 | 0.06 | 0.41 | 0.06 | 0.54 | 0.16 | 0.86 | 0.27 | 0.66 | 0.10 | 0.29 | 0.18 |
| WBP4    | 1.02 | 0.34 | 1.60 | 0.19 | 1.32 | 0.46 | 0.57 | 0.07 | 0.51 | 0.05 | 0.78 | 0.29 | 0.59 | 0.14 | 0.32 | 0.05 | 0.63 | 0.32 |
| WDR25   | 0.70 | 0.05 | 0.50 | 0.07 | 1.02 | 0.13 | 0.84 | 0.06 | 0.35 | 0.01 | 1.17 | 0.72 | 1.21 | 0.16 | 0.70 | 0.07 | 1.11 | 0.55 |
| WDR33   | 1.05 | 0.36 | 1.83 | 0.32 | 1.50 | 0.65 | 0.48 | 0.10 | 0.43 | 0.12 | 0.89 | 0.30 | 0.49 | 0.19 | 0.25 | 0.11 | 0.68 | 0.40 |
| WDR83   | 0.99 | 0.18 | 0.65 | 0.19 | 0.79 | 0.22 | 0.44 | 0.01 | 0.18 | 0.03 | 0.72 | 0.08 | 0.45 | 0.07 | 0.31 | 0.16 | 0.94 | 0.25 |
| WTAP    | 1.14 | 0.29 | 1.18 | 0.17 | 0.65 | 0.21 | 0.85 | 0.22 | 0.85 | 0.76 | 0.56 | 0.02 | 0.75 | 0.01 | 0.76 | 0.72 | 0.93 | 0.35 |
| XAB2    | 3.56 | 0.97 | 2.97 | 0.36 | 1.70 | 0.55 | 1.19 | 0.23 | 0.72 | 0.02 | 0.71 | 0.05 | 0.34 | 0.03 | 0.25 | 0.03 | 0.46 | 0.19 |
| XRCC5   | 2.68 | 0.85 | 1.59 | 0.20 | 1.15 | 0.23 | 1.38 | 0.49 | 0.49 | 0.11 | 1.15 | 0.32 | 0.51 | 0.05 | 0.31 | 0.05 | 1.02 | 0.29 |
| XRCC6   | 1.11 | 0.15 | 0.69 | 0.10 | 0.99 | 0.24 | 0.47 | 0.03 | 0.30 | 0.00 | 0.79 | 0.14 | 0.42 | 0.04 | 0.45 | 0.06 | 0.82 | 0.12 |
| XRN2    | 0.83 | 0.30 | 0.55 | 0.06 | 0.67 | 0.15 | 0.97 | 0.26 | 0.55 | 0.20 | 0.52 | 0.07 | 1.20 | 0.16 | 0.98 | 0.28 | 0.69 | 0.13 |
| YBX1    | 0.72 | 0.22 | 1.19 | 0.10 | 1.09 | 0.30 | 0.62 | 0.15 | 0.40 | 0.02 | 0.62 | 0.22 | 0.90 | 0.28 | 0.34 | 0.04 | 0.58 | 0.15 |
| ZC3H13  | 1.02 | 0.11 | 1.41 | 0.07 | 1.09 | 0.31 | 0.93 | 0.16 | 0.64 | 0.10 | 0.60 | 0.18 | 0.90 | 0.05 | 0.45 | 0.09 | 0.61 | 0.34 |
| ZC3H15  | 0.92 | 0.24 | 0.80 | 0.07 | 0.86 | 0.15 | 0.79 | 0.09 | 0.35 | 0.11 | 0.92 | 0.19 | 0.87 | 0.14 | 0.44 | 0.17 | 0.98 | 0.07 |
| ZC3H18  | 1.45 | 0.05 | 1.08 | 0.42 | 0.94 | 0.30 | 0.99 | 0.09 | 0.43 | 0.13 | 3.62 | 1.17 | 0.68 | 0.07 | 0.40 | 0.03 | 3.84 | 0.43 |
| ZCCHC8  | 0.68 | 0.12 | 0.75 | 0.02 | 0.97 | 0.31 | 0.68 | 0.14 | 0.51 | 0.04 | 0.83 | 0.11 | 1.01 | 0.14 | 0.68 | 0.04 | 0.94 | 0.33 |
| ZCRB1   | 0.98 | 0.11 | 0.87 | 0.29 | 1.03 | 0.12 | 1.01 | 0.20 | 0.45 | 0.11 | 0.99 | 0.04 | 1.02 | 0.09 | 0.55 | 0.23 | 0.97 | 0.15 |
| ZFP36L1 | 0.69 | 0.17 | 1.04 | 0.05 | 1.11 | 0.31 | 0.72 | 0.04 | 0.59 | 0.22 | 1.41 | 0.10 | 1.08 | 0.20 | 0.57 | 0.23 | 1.36 | 0.49 |
| ZFR     | 1.73 | 0.08 | 1.97 | 0.20 | 1.22 | 0.41 | 0.73 | 0.23 | 0.60 | 0.11 | 1.35 | 0.70 | 0.42 | 0.11 | 0.31 | 0.09 | 1.21 | 0.62 |
| ZMAT2   | 2.35 | 0.63 | 1.92 | 0.17 | 1.86 | 0.58 | 0.81 | 0.10 | 0.54 | 0.09 | 0.63 | 0.06 | 0.36 | 0.11 | 0.29 | 0.07 | 0.42 | 0.16 |
| ZMAT5   | 1.15 | 0.24 | 1.01 | 0.25 | 0.84 | 0.01 | 0.79 | 0.10 | 0.24 | 0.04 | 0.82 | 0.24 | 0.69 | 0.06 | 0.26 | 0.08 | 0.97 | 0.28 |
| ZNF207  | 2.05 | 0.42 | 2.19 | 0.19 | 1.79 | 0.53 | 0.58 | 0.11 | 0.42 | 0.05 | 0.43 | 0.10 | 0.28 | 0.01 | 0.20 | 0.03 | 0.27 | 0.15 |
| ZNF282  | 1.12 | 0.18 | 1.24 | 0.18 | 1.27 | 0.17 | 0.65 | 0.08 | 0.31 | 0.06 | 0.86 | 0.11 | 0.58 | 0.04 | 0.25 | 0.03 | 0.68 | 0.10 |

|        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| ZRANB2 | 1.82 | 0.30 | 1.19 | 0.08 | 1.06 | 0.34 | 0.95 | 0.06 | 0.51 | 0.03 | 0.48 | 0.10 | 0.53 | 0.08 | 0.42 | 0.01 | 0.50 | 0.23 |
| ZRSR2  | 0.97 | 0.42 | 1.02 | 0.19 | 1.42 | 0.57 | 0.96 | 0.45 | 0.61 | 0.25 | 1.37 | 0.20 | 1.01 | 0.19 | 0.58 | 0.13 | 1.12 | 0.56 |

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