



# A Role for POU3F3 in Myocyte Differentiation: Exploring New Frontier in Alveolar Rhabdomyosarcoma Development

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## INTRODUCTION

Children who are diagnosed with pediatric rhabdomyosarcoma (RMS), a mesenchymal-derived soft tissue cancer that comprises 3.5% of childhood cancers, are often delivered a bleak prognosis with little hope of a future. Despite significant advances illuminating transcription factor signaling in RMS onset and progression, research is still needed to precisely understand RMS pathogenesis on a molecular level in order to develop targeted treatment options. The goal of this project is to explore the role of POU3F3 in myogenesis, particularly in relation to cell fusion and myocyte differentiation.

## MATERIALS AND METHODS

### Immunofluorescence:

POU3F3 knockdown cells were differentiated in 2% horse serum. On day 2 of differentiation, cells were probed with POU3F3 primary and red immunofluorescent secondary antibody, allowing for imaging of POU3F3 localization during myoblast differentiation.

## MATERIALS AND METHODS

### Western Blot:

Three knock-down shPOU3F3 C2C12 cell-line constructs were tested. Western blots were performed that compared C2C12 control, POU3F3 overexpression, and POU3F3 knockdown cells.

### Crystal Violet:

After differentiation, POU3F3 knock-down and control cell lines were stained with crystal violet stain to visualize the effect of POU3F3 knockdown on differentiation.

## RESULTS

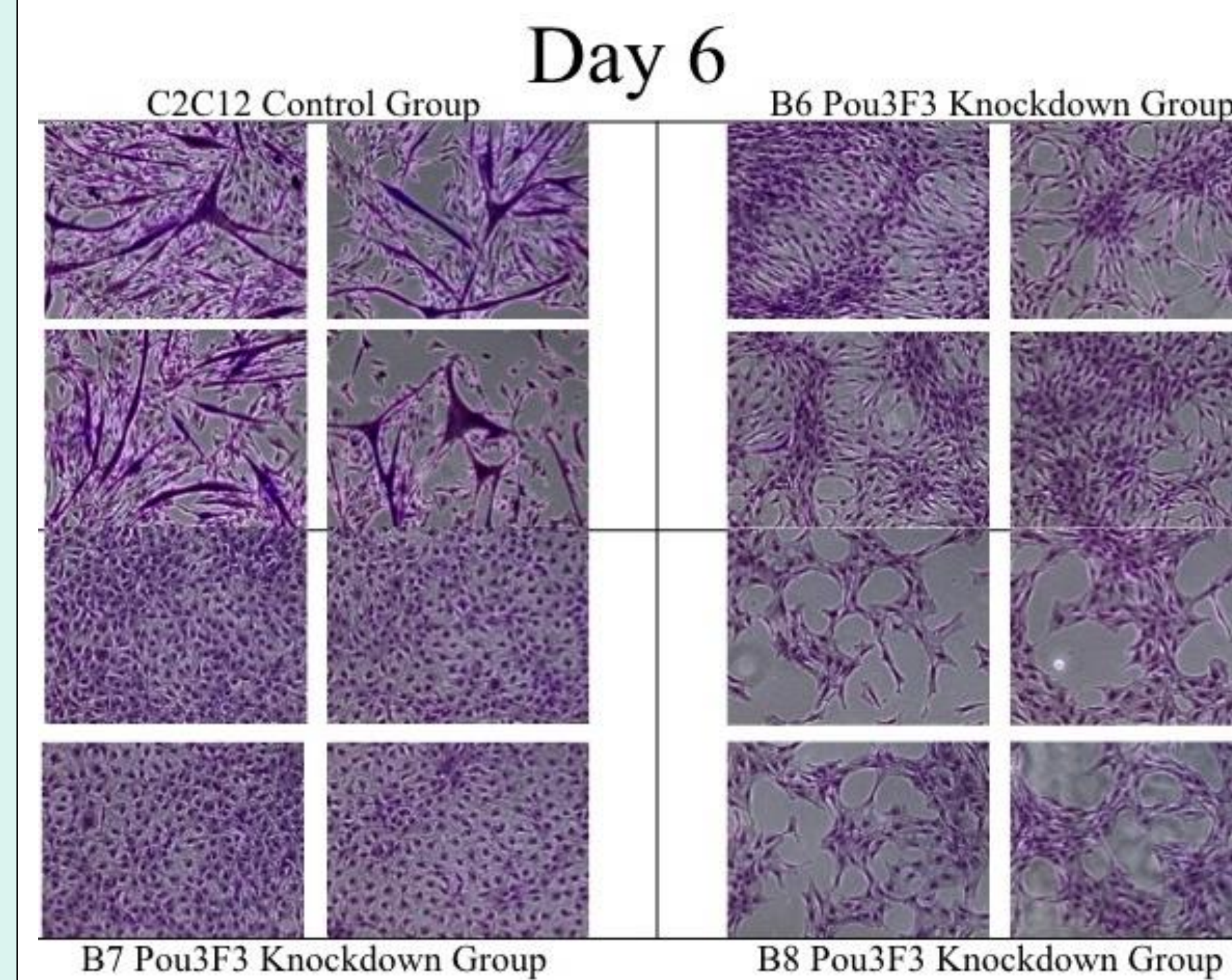
### Immunofluorescence:

We confirmed that in knockdown C2C12 cells, POU3F3 localizes like control C2C12, in the periphery. Previous research has shown that in overexpressed POU3F3 C2C12 cell lines, POU3F3 localizes to the nucleus. The implication of the disconnect between POU3F3 location in controls and knock-downs versus overexpressed cell lines is an area that is an opportunity for further research.

## RESULTS

### Crystal Violet:

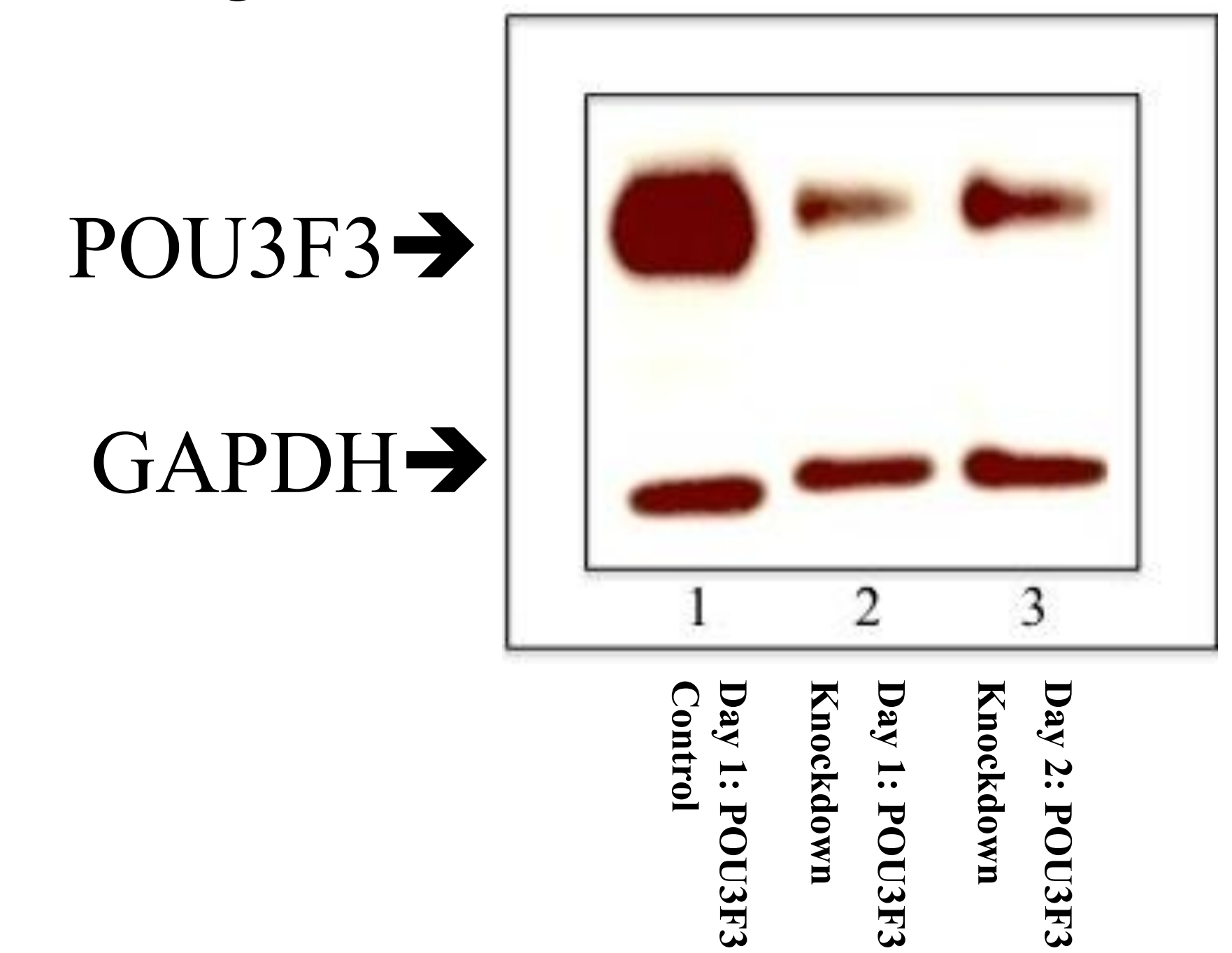
Crystal violet staining suggests that POU3F3 participates in a myoblast differentiation, as the control cells fuse into myotubes, while POU3F3-silenced cells do not.



## RESULTS

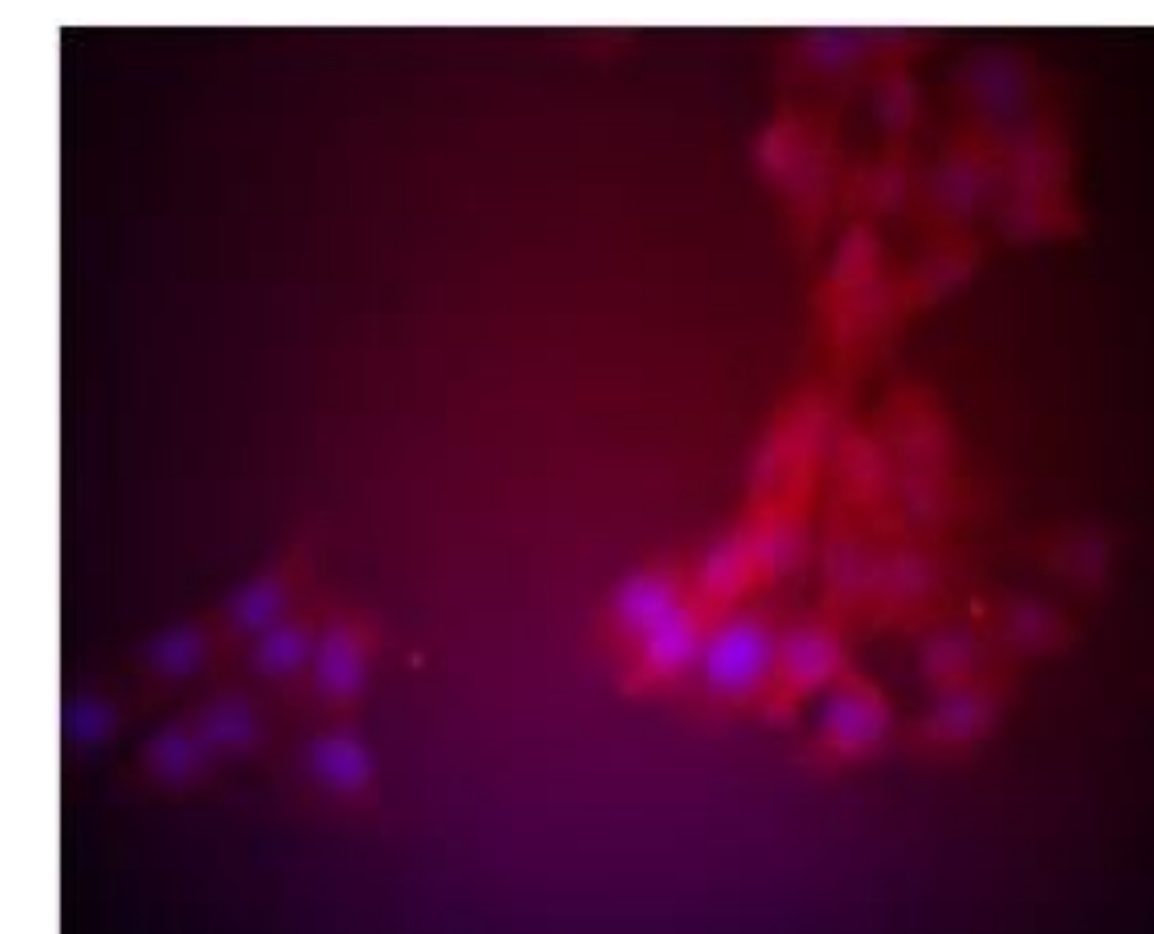
### Western Blot:

Western Blot analysis confirmed that POU3F3 knockdown was successful, and provides a platform for further POU3F3 interrogation.

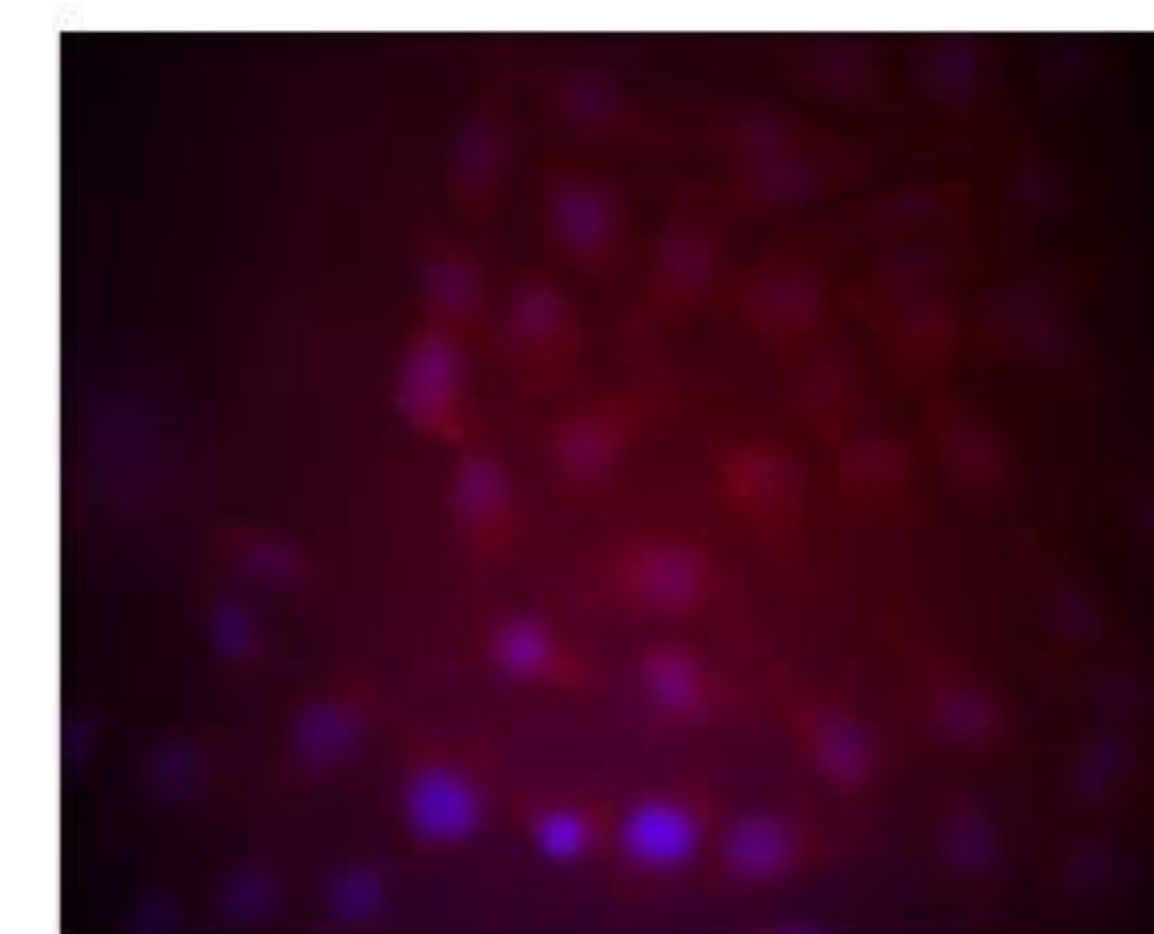


## RESULTS

### Immunofluorescence:



C2C12 POU3F3 DAPI



POU3F3 Knockdown

## CONCLUSION

These initial results suggest that POU3F3 participates in muscle differentiation. Next, the Galindo lab will be probing POU3F3 function in myogenesis in greater depth, insights they will next apply to RMS.

## CONTACT

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