Legends to Supplemental Movies

Movie 1. Three-dimensional images of an EGFP$^+$ corneal LC.

A three-dimensional data set similar to that shown in Figure 4B (without counter-staining with PI) was reconstructed along the x-z plane, followed by rotation about the z-axis (180°) and the x-axis (360°). Note that while some of the fine dendritic processes extend upward in the z-axis, the LC occupies a much greater area in the x-y plane.

Movie 2. Spatial relationship of an EGFP$^+$ corneal LC to neighboring epithelial cells.

The data set shown in Figure 4B was processed to display 46 sequential x-y images separated by 0.5 μm spanning a total z-axis depth of 24 μm. The series begins superficially in the epithelium and proceeds towards to the stroma.

Movie 3. The spatial relationship of EGFP$^+$ corneal LCs to collagen bundles.

The data set shown in Figure 4C was processed to show rotation about the z-axis (360°). Note the location of the EGFP$^+$ LCs slightly above the collagen signals. This places the LCs within the epithelium, which lacks the abundant collagen bundles seen in the stroma.

Movies 4-5. Time-lapse images of baseline dSEARCH performed by multiple corneal LCs.

The data set shown in Figure 6 (Movie 4) and the data set from an independent experiment (Movie 5) were compiled to show dynamic movement of EGFP$^+$ corneal LCs. Note that most of the EGFP$^+$ LCs with the characteristic dendritic morphology exhibit dSEARCH in the epithelial compartment, while rapid migration is observed in smaller EGFP$^+$ cells possessing a polygonal shape primarily in the stromal compartment.

Movie 6. Time-lapse images of baseline behaviors of epidermal LCs *ex vivo*.

Confocal images recorded every 5 min in a skin sample freshly harvested from an I-Aβ-EGFP knock-in mouse were compiled to show the dynamic movement of $EGFP^+$ epidermal LCs.

Movies 7-8. Time-lapse images of *in vivo* behaviors of epidermal LCs in the steady state.

The data set shown in Figure 11 and the data set from an independent experiment were compiled to show steady state dSEARCH detectable in some of the $EGFP^+$ epidermal LCs in anesthetized mice. dSEARCH was noticeable in $EGFP^+$ LCs characterized by larger cell size and higher EGFP signal intensity.

Legends to Supplemental Movies

Movies 9-11. Time-lapse images of injury induced augmentation of dSEARCH and lateral movement in corneal LCs.

The three movies demonstrate injury-induced amplification of dSEARCH and lateral migration of $EGFP^+$ corneal LCs observed consistently in 8 independent imaging experiments. After 60 min recording of baseline behaviors, pinpoint thermal injury was produced at time 0 in an $EGFP^+$ LC indicated with an arrow. Note the increased activity of the dendrites and the propensity for lateral movement within the epithelium after injury.

Movie 12. Time-lapse images of injury-induced lateral migration by corneal LCs.

After 60 min recording of baseline behaviors, pinpoint thermal injury was produced at time 0 in and $EGFP^+$ LC indicated with an arrow. Two LCs in the vicinity of the injury site show immediate and drastic migratory responses. While these cells are seen migrating toward the site of injury, in general LC migration after injury occurred in random paths with no apparent directional bias (e.g., toward the injury site). Additionally, a large $EGFP^+$ stromal cell with an amorphous shape (indicated with an asterisk) exhibits an unusual response characterized by rapid migration toward the injury site followed by casting fine, mesh-like projections toward the neighboring LCs.

Movies 13-14. Time-lapse images of the impact of IL-1Ra on injury-triggered changes in corneal LC behaviors.

Data sets shown in Figure 16 (Movie 13) and from an independent experiment (Movie 14) were compiled to show the dynamic behavioral responses of corneal LCs to local injury in the presence of IL-1Ra, which was added to the circulating media at time -60 to achieve a concentration of 200 ng/ml. After 60 min recording of baseline behaviors, pinpoint thermal injury was produced at time 0 in an $EGFP^+$ LC indicated with an arrow. Note how the activity of the dendrites does not change appreciably after injury and how $EGFP^+$ LCs do not move significantly from their original positions. In Movie 13, a large, amorphously shaped $EGFP^+$ cell in the stroma is indicated with an asterisk.

Movies 15-16. Time-lapse images of IL-1-induced amplification of dSEARCH in corneal LCs.

The data set shown in Figure 19 (Movie 15) and the data set from an independent experiment (Movie 16) were compiled to show the dynamic behavioral responses of corneal LCs to IL-1. After 60 min recording of baseline behaviors, IL-1α was added to the circulating media at time 0 to achieve a final concentration of 5 pg/ml. Note the increase in the activity of the dendrites after addition of IL-1 and the relative absence of lateral movement by LCs.

Movie 17. Time-lapse images of dendrite hyper-elongation in corneal LCs triggered by IL-1 at a high concentration.

The data set shown in Figure 22 was compiled to show the dynamic behavioral responses of corneal LCs to IL-1. After 60 min recording of baseline behaviors, recombinant IL-1α was added to the circulating medium at time 0 to achieve a final concentration of 100 pg/ml. Not the profound elongation of the dendrites in the absence of significant lateral movement.

Movies 18-19. Time-lapse images of TNFα-induced changes in corneal LC behaviors.

Data sets shown in Figure 23 (Movie 18) and from an independent experiment (Movie 19) were compiled to show the dynamic behavioral responses of corneal LCs to TNFα. After 60 min recording of baseline behaviors, recombinant TNFα was added to the circulating culture media at time 0 to achieve a final concentration of 50 pg/ml. Note an increase in dendrite activity and lateral movement similar to the changes seen after tissue injury.

Movies 20-21. Time-lapse images of *ex vivo* behaviors of epidermal LCs after skin organ culture.

The same data set shown in Figure 27A (Movie 20) and the data set from an independent experiment (Movie 21) were compiled to show the dynamic behaviors of $EGFP^+$ epidermal LCs after prolonged skin organ culture. Ear skin samples were harvested and cultured for 16 hours before imaging by confocal

microscopy. Note the increased dendrite activity and lateral movement observed after prolonged culture.

Movie 22. Time-lapse images of *ex vivo* behaviors of epidermal LCs after local TNFα injection.

Imaging data were compiled to show the dynamic behaviors of $EGFP^+$ epidermal LCs after injection of TNFα. Sixteen hours after s.c. injection of recombinant TNFα into the ear, skin samples were harvested and imaged by confocal microscopy. Note the provoked dSEARCH and motile activities of the $EGFP^+$ epidermal LCs.

Movie 23. Time-lapse images of exacerbated *in vivo* behaviors of epidermal LCs in hapten-treated skin.

The same data set shown in Figure 31 was compiled to show the provoked motile activities of $EGFP^+$ epidermal LCs recorded *in vivo* 30 hours after the application of DNFB. An overlap of dendrite-associated EGFP signals between neighboring LCs is indicated with an arrow.

Legends to Supplemental Movies

Movies 24-25. Time-lapse images of *in vivo* behaviors of epidermal LCs after tape stripping.

The stratum corneum of the ear skins in I-Aβ-EGFP knock in mice were removed by repeated tape-stripping. After 18 hours, $EGFP^+$ epidermal LCs were imaged by intravital confocal microscopy. Note that while some dSEARCH activity is appreciable, the cells retain a more immature morphology and exhibit no little lateral movement.

Movies 26-27. Time-lapse images of *in vivo* behaviors of $EGFP^+$ epidermal LCs after tape-stripping plus infection with *Escherichia coli*.

The stratum corneum was removed from the ear skin anesthetized I-Aβ-EGFP knock-in mice, and fluorescent *E. coli* cells were applied to the tape-stripped surface. LCs in the epidermis were then imaged by intravital confocal microscopy 18 hours later. Note the mature morphology and the active dSEARCH and lateral migration of the LCs. While the bacteria did, in fact, fluorescence in all experiments, they rested on the surface of the tape-stripped skin while the LCs were positioned in the suprabasal layers of the epidermis. Thus, the two cell populations occupied different positions in the z-axis. The imaging volume was chosen to monitor the LCs specifically, but in some cases bacteria can be seen as they transiently drift into the imaging volume.