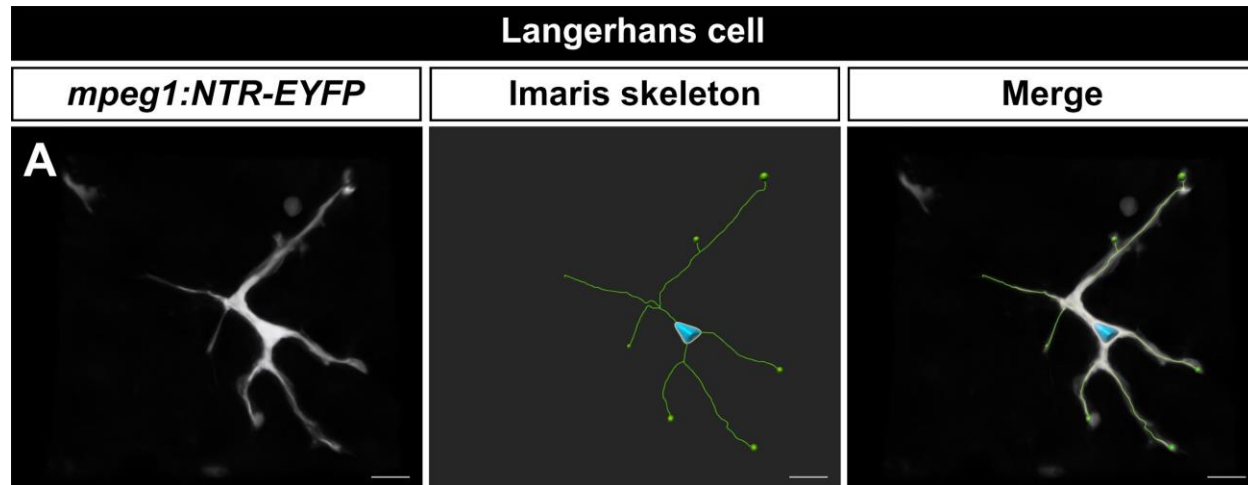


582 SUPPLEMENTAL FIGURES

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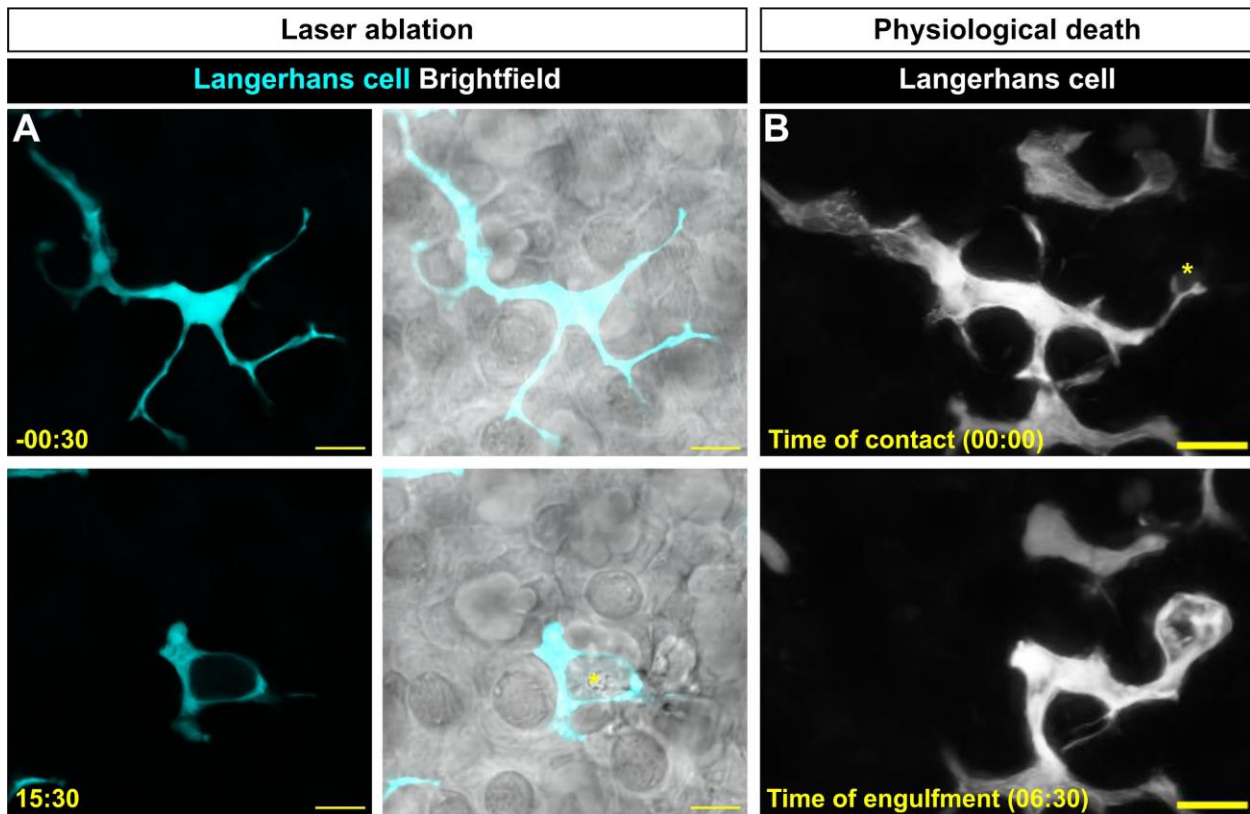
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585 **Supplemental Figure 1. Skeletonization of a Langerhans cell. A.** Representative image of *Tg(mpeg1:NTR-EYFP)*  
586 skeletonized using Imaris Filaments module. See also Supplemental Video 1. Scale bar in **(A)** denotes 10 microns.

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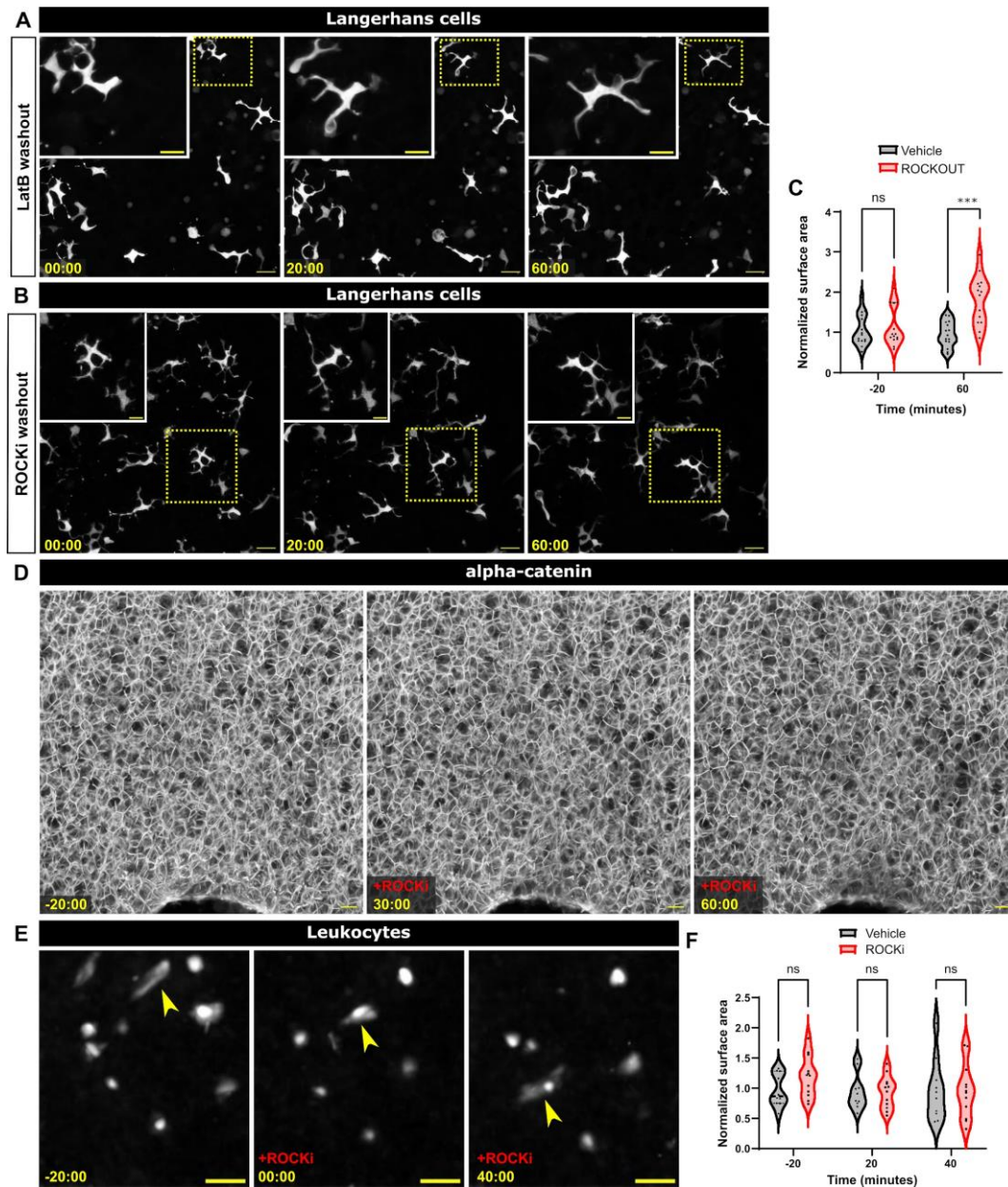
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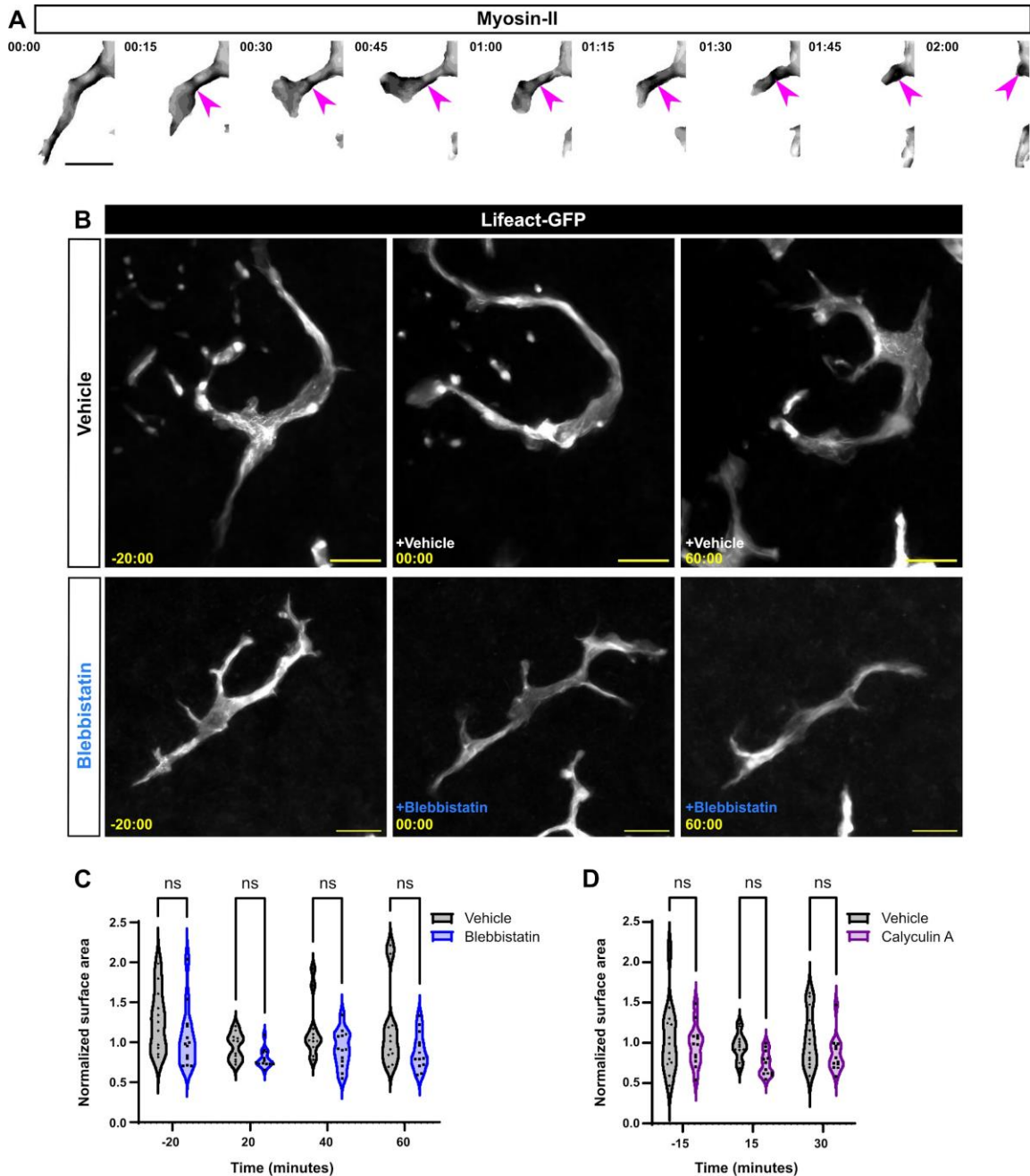
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**Supplemental Figure 2. Langerhans cells undergo shape changes during engulfment of large cellular debris.**  
**A.** Stills of *Tg(mpeg1:NTR-EYFP)*-positive Langerhans cell showing fluorescent only (left) and fluorescent+brightfield composite (right) images before and after laser ablation. Asterisk depicts location of laser ablation. **B.** Stills of *Tg(mpeg1:NTR-EYFP)* showing “natural” engulfment of large debris in the absence of laser ablation. Asterisk depicts future site of phagocytosis. Timestamps denote mm:ss. Scale bars denote 10 microns.



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**Supplemental Figure 3. Effects of LatB and ROCK inhibition of Langerhans cells.** **A.** Stills of *Tg(mpeg1:NTR-EYFP)*-positive Langerhans cells depicting normal cell motility after LatB washout. **B.** Stills of *Tg(mpeg1:NTR-EYFP)*-positive Langerhans cells depicting normal cell motility after Y-27632 washout. Dotted boxes in **(A,B)** denote regions magnified in insets. **C.** Quantification of surface area covered after ROCKOUT treatment, an alternative inhibitor of ROCK. Data shown are compiled from two individual experiments,  $n = 15$  cells from  $N = 6$  scales for vehicle treatment and  $n = 14$  from  $N = 5$  scales for ROCKOUT treatment. **D.** Stills of *Gt(ctnna1-Citrine)*-positive cells depicting normal tissue morphology after ROCK inhibition. **E.** Stills of *Tg(lck:lck-GFP)*-positive leukocytes showing no changes in cell morphology after ROCK inhibition. **F.** Violin plots of surface area covered by *Tg(lck:lck-GFP)*-positive cells after ROCKi. Data shown are compiled from two individual experiments,  $n = 10$  cells from  $N = 5$  scales for vehicle treatment and  $n = 12$  from  $N = 4$  scales for ROCKi treatment. \*\*\* =  $p < 0.0001$ . Mann-Whitney U tests were used to determine significance between groups at each time point in **(C)**. Two-way ANOVA followed by Bonferroni correction was used in **(F)**. Timestamps denote mm:ss. Scale bars in **(A, B, D)** denote 20 microns, scale bars in **(A, inset; B, inset; E)** denote 10 microns.



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 612 **Supplemental Figure 4. Myosin perturbation and its effects on Langerhans cell shape.** **A.** Stills of macrophage-  
 613 specific myosin (inverted grayscale) during dendrite retraction (see Materials and Methods for details). Magenta  
 614 arrowheads indicate higher levels of myosin during dendrite retraction. **B.** Stills of *Tg(mpeg1:Lifeact-GFP)*-positive  
 615 cells treated with vehicle (**B, top**) or para-amino blebbistatin (**B, bottom**). **C.** Violin plots of Langerhans cell surface  
 616 area, normalized to time of treatment with vehicle or para-amino blebbistatin. Data are representative of two  
 617 individual experiments,  $n = 11$  from  $N = 4$  scales cells for vehicle treatment and  $n = 13$  cells from  $N = 3$  scales for  
 618 blebbistatin treatment. **D.** Violin plots of Langerhans cell surface area, normalized to time of treatment with vehicle or  
 619 Calyculin A. Data are representative of two individual experiments,  $n = 13$  cells from  $N = 6$  scales for vehicle  
 620 treatment and  $n = 12$  cells from  $N = 5$  scales for Calyculin A treatment. Two-way ANOVA followed by Bonferroni post-  
 621 tests revealed no significant differences. Timestamps in **B** denote mm:ss. Scale bars in **(A, B)** denote 10 microns.

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## SUPPLEMENTAL VIDEO LEGENDS

**Supplemental Video 1.** Time-lapse microscopy of Langerhans cell (cyan) extending and retracting protrusions among epidermal cell membranes (white). Video was bleach-corrected. Scale bar denotes 10 microns.

**Supplemental Video 2.** Time-lapse microscopy of Langerhans cell (cyan) engulfing *Tg(p2rx3a:mCherry)*+ debris (magenta). Scale bar denotes 10 microns.

**Supplemental Video 3.** Time-lapse microscopy of Langerhans cell (white) engulfing cellular debris generated after laser-induced damage of keratinocytes. Yellow asterisk denotes site of ablation. Scale bar denotes 10 microns.

**Supplemental Video 4.** Time-lapse microscopy of Langerhans cell labeled with Lifeact-EGFP. Scale bar denotes 10 microns.

**Supplemental Video 5.** Time-lapse microscopy of Langerhans cell labeled with Lifeact-EGFP (false-colored) engulfing *Tg(p2rx3a:mCherry)*+ debris (white). Scale bar denotes 10 microns.

**Supplemental Video 6.** Time-lapse microscopy of Langerhans cell labeled with Lifeact-EGFP (false-colored) engulfing cellular debris generated after laser-induced damage of keratinocytes. Yellow asterisk indicates site of ablation. Red arrowhead indicates Lifeact-EGFP accumulation during engulfment. Scale bar denotes 10 microns.

**Supplemental Video 7.** Time-lapse microscopy of Langerhans cells (white) treated with vehicle or ROCK inhibitor. Scale bar denotes 20 microns.

**Supplemental Video 8.** Time-lapse microscopy of Langerhans cell (white) engulfing cellular debris generated after laser-induced damage of keratinocytes. Cells are treated with vehicle or ROCK inhibitor. Scale bar denotes 10 microns.

**Supplemental Video 9.** Time-lapse microscopy of Langerhans cells (cyan) reacting to epidermal wounds (epidermal cells labeled in white). Cells are treated with vehicle or ROCK inhibitor. Scale bar denotes 100 microns.