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Supplemental material

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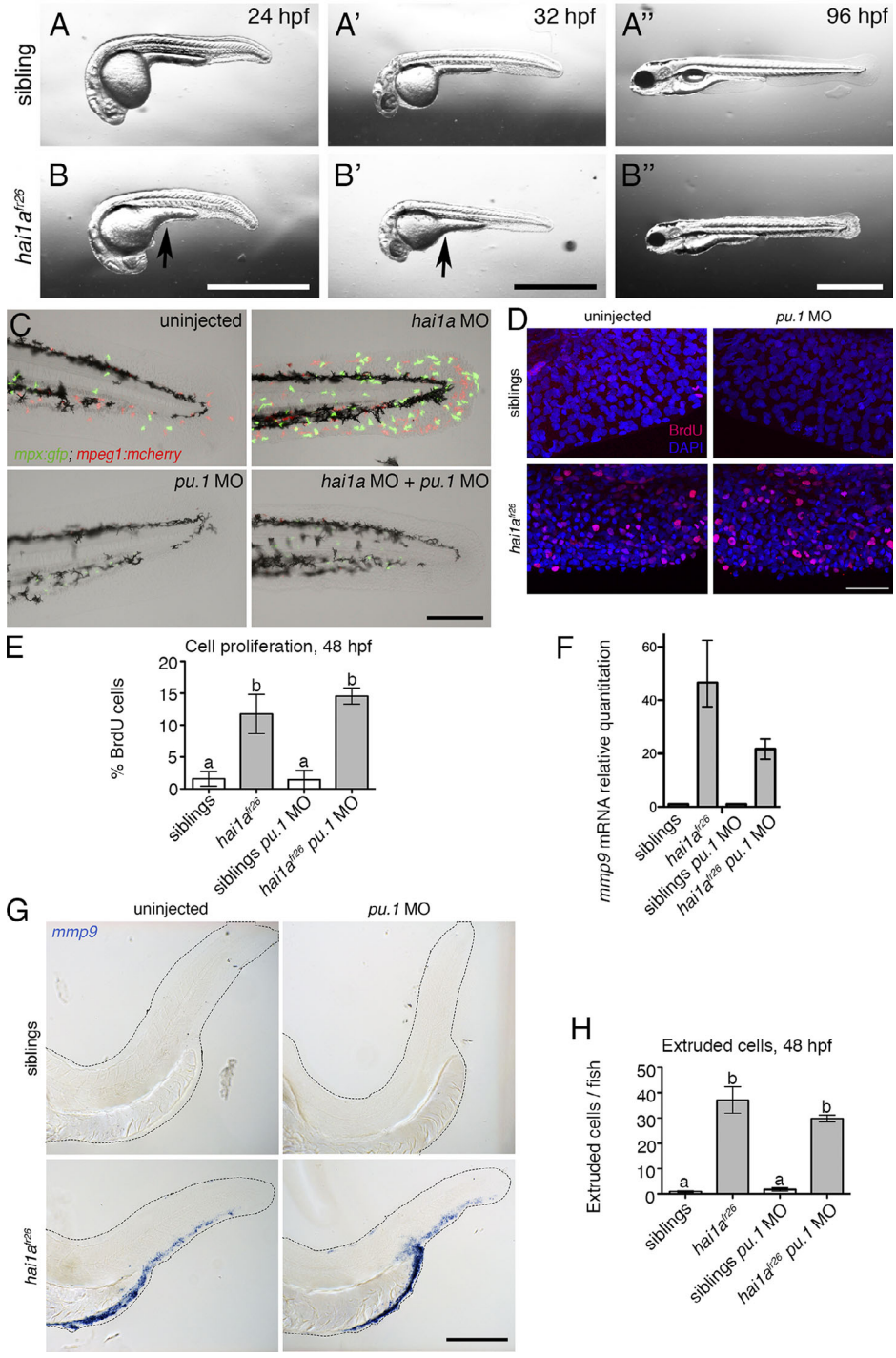


Figure S1. **Epidermal defects in the *hai1a*^{fr26} allele are independent of inflammation.** Related to Fig. 1. **(A and B)** Representative bright field images of the *fr26* phenotype, which differs from that of the *hi2217* phenotype by a thickening of the yolk sac extension (arrows), apparent at 24 and 32 dpf, but resolved by 96 hpf. Scale bars, 1 mm. **(C)** Injection of the *pu.1* MO at the one-cell stage eliminates both *mpx*-positive neutrophils (green) and *mpeg1*-positive macrophages (red) in both control and *hai1a* morphant caudal fin fold at 48 hpf. Scale bar, 200 µm. **(D and E)** Injection of the *pu.1* MO does not affect cell proliferation. Representative images of BrdU-labeled cells (D, red) in the caudal fin fold of 48 hpf siblings and *hai1a*^{fr26} mutants with or without *pu.1* MO injection. Scale bar = 50 µm. Quantification of proliferating cell numbers (E) shows no significant difference between uninjected and *pu.1* MO-injected embryos. *n* = 4 or 5 fish per condition, in two independent experiments. **(F and G)** Elimination of the myeloid cell lineage has no significant effect on *mmp9* transcript levels or localization. Q-RT-PCR detecting *mmp9* levels (F) relative to respective sibling controls indicates a reduction in *mmp9* levels in the *pu.1* MO–injected embryos. Data are from a single representative experiment, *n* = 20 fish per condition. In situ hybridization with a probe against *mmp9* (G) reveals that the majority of the *mmp9* transcript (blue) is found in the epidermis, and that there is no obvious difference in localization or intensity of staining in *pu.1* MO–injected embryos compared with uninjected controls. Scale bar, 200 µm. **(H)** Counting of extruded cell numbers at 48 hpf reveals no significant difference between uninjected *hai1a*^{fr26} mutants and mutants injected with the *pu.1* MO. *n* = 54–80 embryos per condition, in two independent experiments. For quantifications and statistical significances, see legend of Fig. 1.

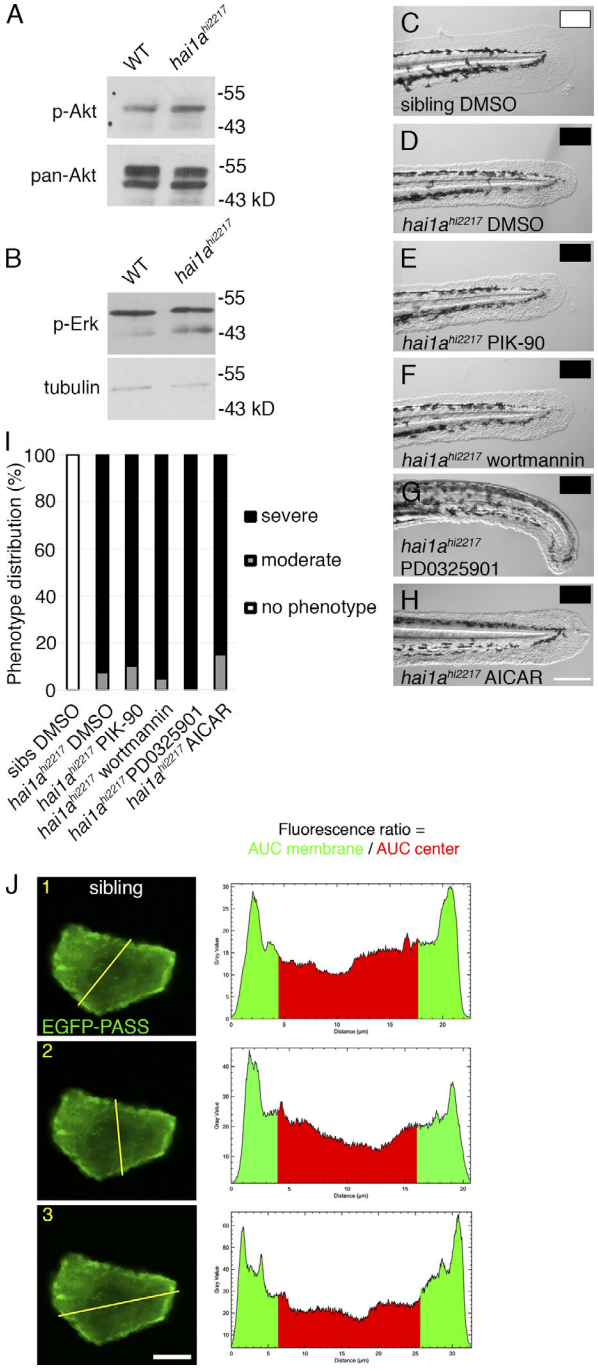


Figure S2. **Independence of epidermal defects of *hai1a*^{hi2217} mutants from PI3K/Akt, MEK/ERK, or Ampk, and quantification of subcellular EGFP-PASS distribution.** Related to Fig. 2. **(A)** Immunoblot demonstrating that pAkt is not elevated in *hai1a*^{hi2217} mutants at 48 hpf. Following development with a pAkt-specific antibody, the blot was reprobed using a pan-Akt antibody as loading control. *n* = 20 pooled tails per condition; image is representative of two independent experiments. **(B)** Immunoblot demonstrating that pErk is not elevated in *hai1a*^{hi2217} mutants at 48 hpf. After development with an anti-pErk (1/2) antibody, the blot was reprobed using a tubulin antibody as a loading control. *n* = 20 pooled tails per condition; image is representative of two independent experiments. **(C–H)** Representative bright-field images of the caudal fin fold at 48 hpf, treated with vehicle (DMSO), Akt inhibitors (PIK-90 and wortmannin), MEK1 and MEK2 inhibitor (PD0325901), or Ampk agonist (AICAR). All of the drug treatments failed to rescue the epidermal phenotype. Black and white boxes correspond with the phenotypic categories shown in I. Scale bar, 200 µm. **(I)** Phenotypic distribution of embryos treated with drugs targeting the PI3K, MEK, or Ampk pathways, sorted into severe, moderate, or no phenotype. *n* = 20–44 fish per condition, in two independent experiments. **(J)** Determination of EGFP-PASS membrane-cytoplasm fluorescence ratios, as summarized in Fig. 2 O, exemplified for an epidermal cell of an untreated sibling. Using ImageJ, a line 10 pixels wide was drawn through a single plane image of the cell, and fluorescence intensity values along the line were used to generate a plot profile. Using the extracted xy coordinates of the plot profile, the area under the curve (AUC) was calculated for the 20% closest to the membranes at each end of the line (green), as well as for the middle 60% of the cell (red). The total AUC for the membrane portion was divided by the AUC of the central portion to generate a ratio. This was repeated three times per cell with lines in different orientations, and the average ratio per cell was determined. Single plane of a z-stack, scale bar, 10 µm.

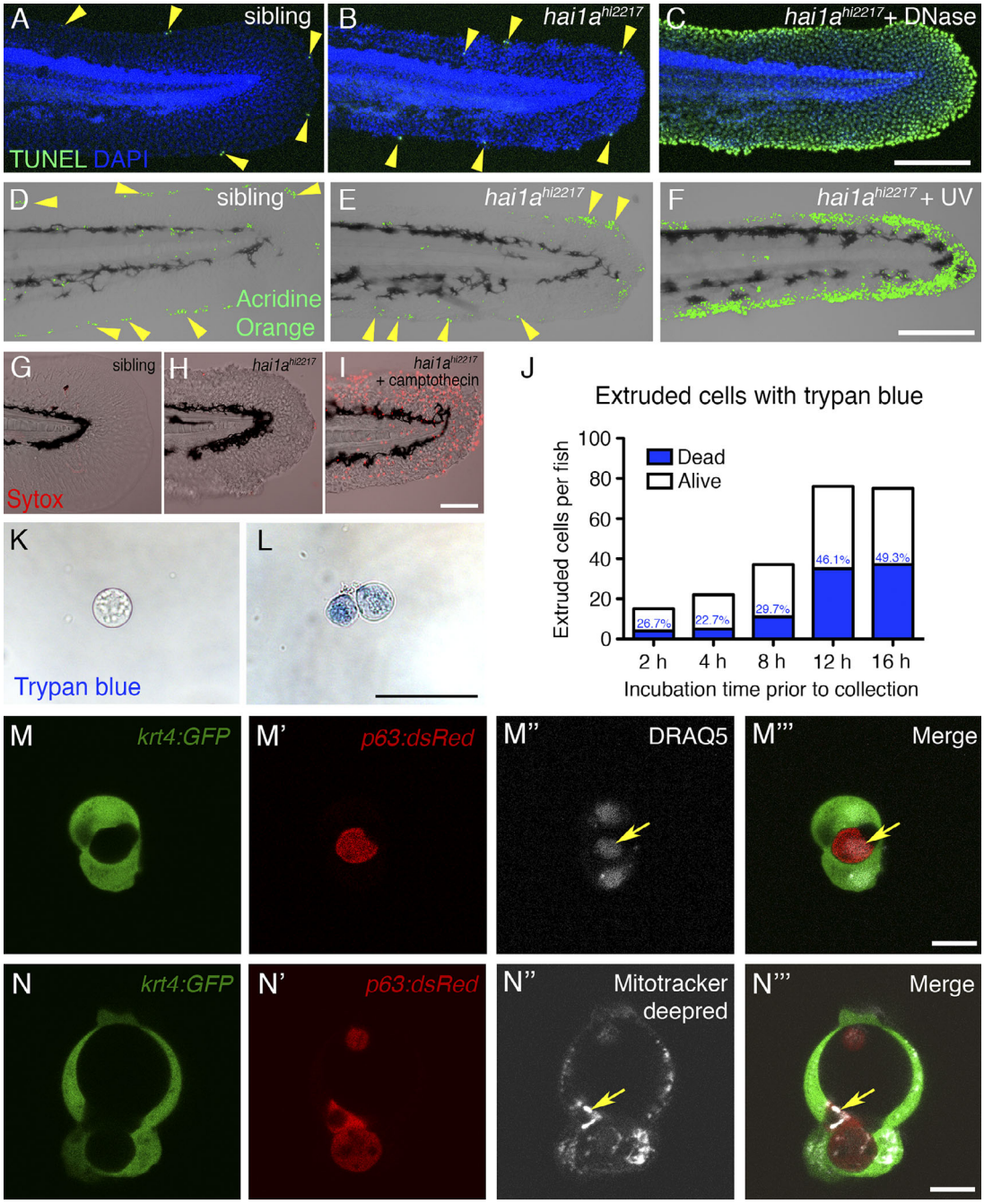


Figure S3. **Extruded cells are alive, intact, and metabolically active.** Related to Figs. 3 and 4. **(A–C)** TUNEL to label dying cells (green) is very similar in 48 hpf siblings and *hai1a*^{hi2217} mutants, with few TUNEL-positive cells found at the edge of the fin fold. As a positive control, embryos were treated with DNase before the TUNEL reaction. Scale bar, 100 µm. **(D–F)** Acridine orange to label apoptotic cells (green) in live 48 hpf embryos shows a very similar staining pattern to TUNEL. As a positive control, embryos were exposed to UV for 10 s and allowed to recover for 6 h before imaging. Scale bar, 100 µm. **(G–I)** Sytox staining (red) to label dead cells in live 48 hpf embryos. Embryos were treated with topoisomerase inhibitor camptothecin for 6 h before imaging as a positive control. Scale bar, 100 µm. **(J)** Extruded cells were collected after various incubation times and labeled with trypan blue to mark dead cells before counting. Shorter incubation times yielded fewer total cells, but also a smaller proportion of dead cells, which increased with longer incubation times. Cell death could be due to anoikis, or simply due to the lack of growth factors and energy sources in the E3 incubation medium. **(K–L)** Representative bright field images of recovered live (K) and dead (L) cells. *n* = 39 or 40 embryos per condition, in two independent experiments. **(M)** Extruded cells were recovered from the medium of *krt4:GFP;p63:dsRed* 48 hpf *hai1a*^{hi2217} embryos and stained with DRAQ5 (white), a cell-permeable nuclear dye. The basal cell at the center of this cluster contains a nucleus (arrow), and is therefore not merely a fragment of a dead basal keratinocyte. Single plane of a z-stack. Scale bar, 10 µm. **(N)** Cells recovered from the medium were treated with mitoTracker (white) to label active mitochondria. Mitochondrial labeling was observed in both peridermal cells and engulfed basal cells (arrow). Single plane of a z-stack. Scale bar, 10 µm.

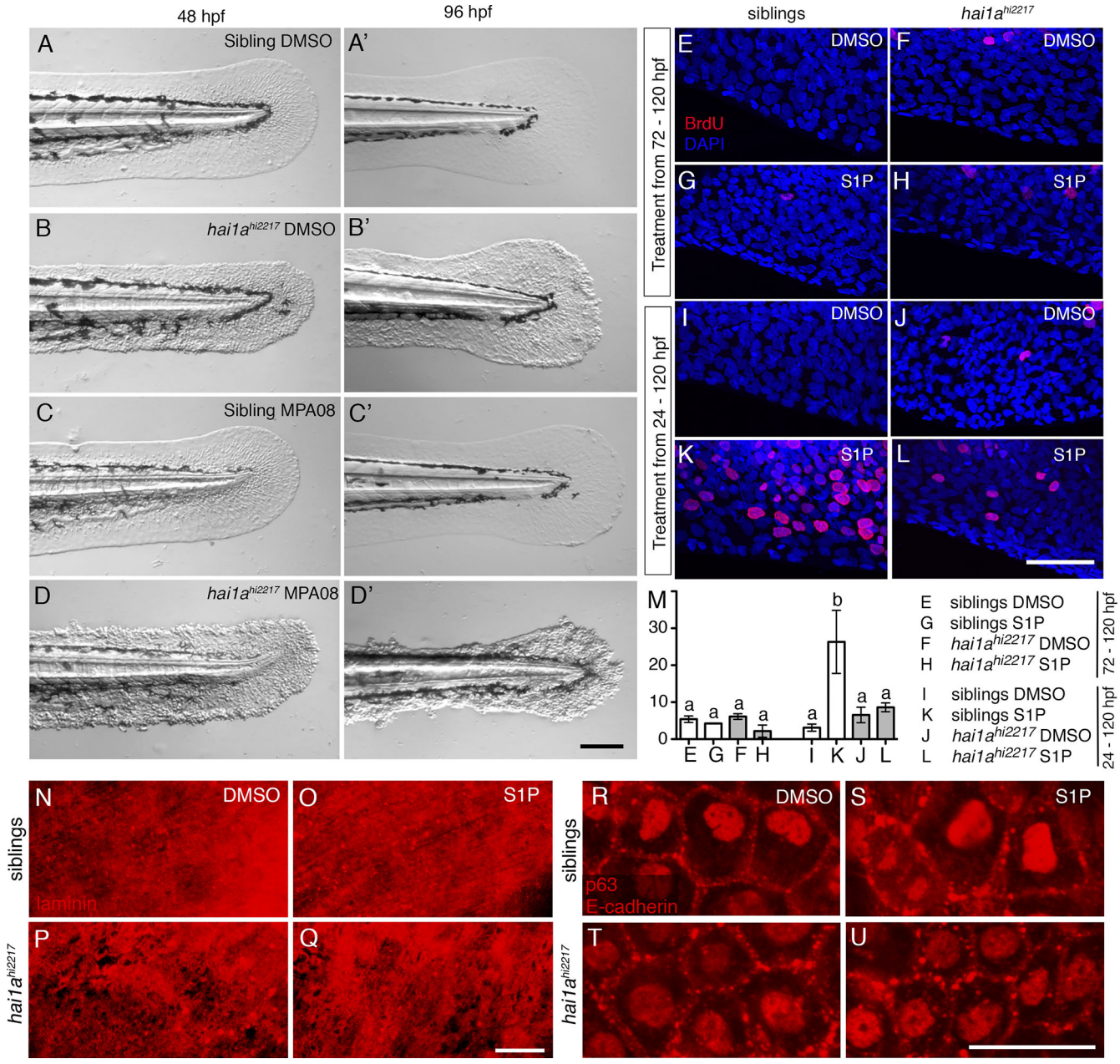


Figure S4. **Blockage of cell extrusion by inhibiting sphingosine kinase impairs healing in the *hai1a*^{hi2217} mutant, while exogenous S1P has no effect on proliferation or cell adhesion.** Related to Figs. 6 and 7. **(A–D)** Representative bright field images of sibling controls and *hai1a*^{hi2217} mutants treated with MPA08 from 24 hpf. At 48 hpf (A–D), inhibition of S1P production by MPA08 had no morphological effect on siblings or mutants. By 96 hpf (A′–D′), the vehicle-treated *hai1a*^{hi2217} mutants (B′) regained the normal fin fold shape and are recovering the epidermal morphology. In contrast, *hai1a*^{hi2217} mutants treated with MPA08 until 96 hpf (D′) continued to worsen and failed to recover the normal morphology. Scale bar, 200 µm. **(E–L)** Representative whole-mount images of BrdU (red) incorporation in the caudal fin fold in sibling control and *hai1a*^{hi2217} mutant embryos following treatment with S1P. Application of S1P for 2 d (72–120 hpf) had no effect on proliferation in embryos (E–H), but longer exposure of 4 d (24–120 hpf) led to an increase in proliferating cells even in sibling controls (I–L). Scale bar, 50 µm. **(M)** Quantification of BrdU incorporation at 120 hpf following treatment with S1P. *n* = 5 embryos per condition, in two independent experiments. **(N–U)** Whole-mount immunofluorescence images for laminin and E-cadherin/p63 at 120 hpf in control and *hai1a*^{hi2217} mutant embryos treated with S1P. S1P application for 2 d (72–120 hpf) did not affect cell–matrix or cell–cell adhesion, as visualized by unaltered abundance and distribution of the basement membrane component laminin (red, M–P) and the cell–cell adhesion molecule E-cadherin (red, together with nuclear p63, Q–T). Scale bars, 50 µm.

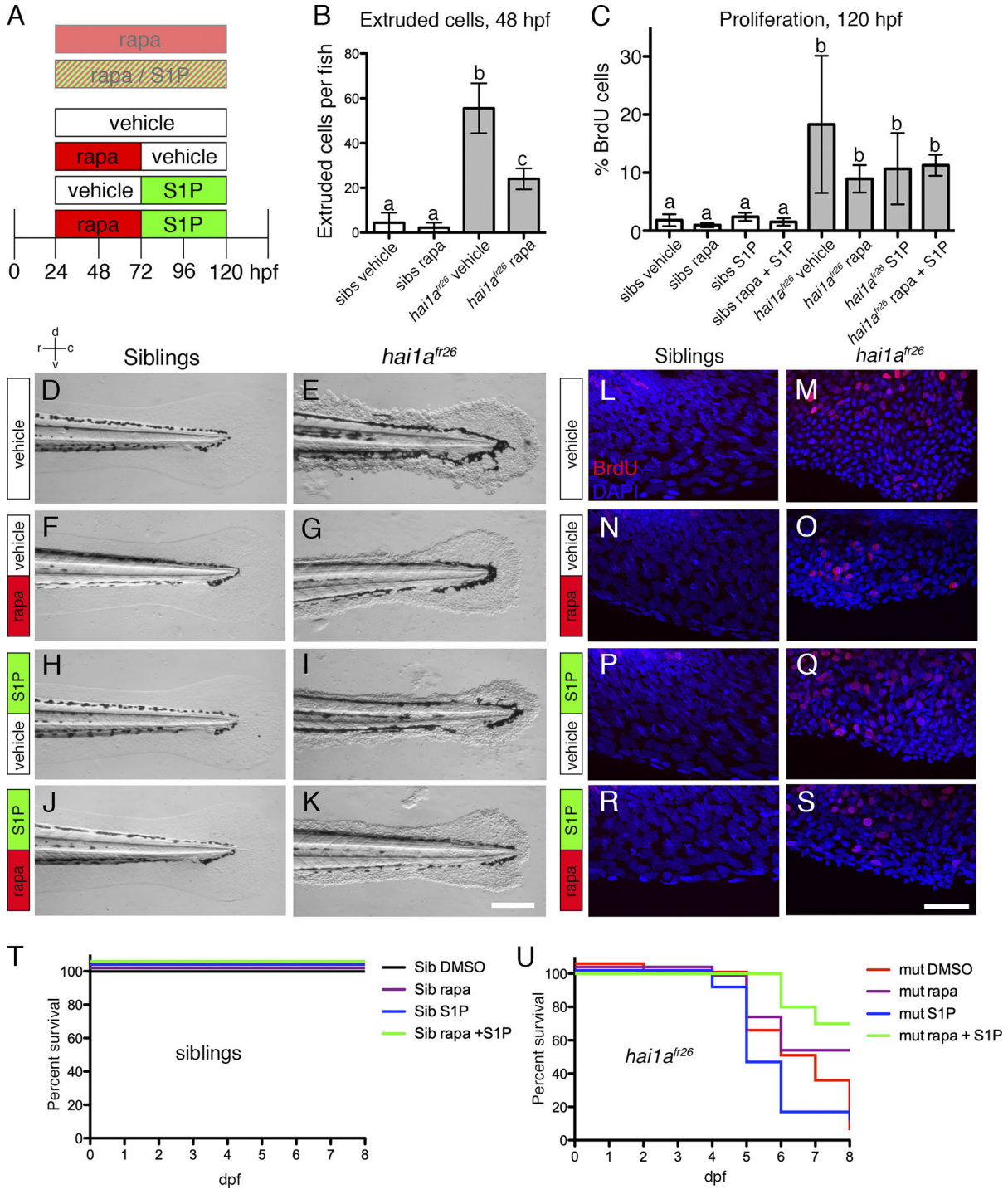
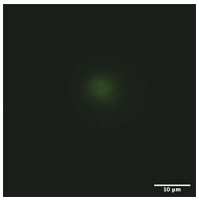
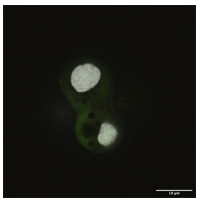


Figure S5. **Combinatorial drug treatment regimen using rapamycin and S1P does not fully rescue the *hai1a*^{fr26} phenotype.** Related to Fig. 7. **(A)** Schematic indicating the time course of different tested drug treatment regimes, similar to Fig. 7. Rapamycin treatment in any of the depicted combinations caused developmental defects, notably a lack of swim bladder development. **(B)** Quantification of extruded cell numbers at 48 hpf after rapamycin treatment from 24 hpf. Application of rapamycin significantly reduced numbers of extruded cells in mutants, while having no effect on sibling controls. *n* = 40 embryos per condition in two independent experiments. **(C)** BrdU quantification at 120 hpf in embryos treated with rapamycin and/or S1P, relative to the total number of cells. Combined treatment with rapamycin and S1P had no advantage over the individual drugs. *n* = 6 embryos per condition in two independent experiments. **(D–K)** Bright field images of the fin fold of sibling controls and *hai1a*^{fr26} mutants at 120 hpf. Scale bar, 200 µm. **(L–S)** Representative images of BrdU incorporation (red) in 120 hpf embryos treated with rapamycin and/or S1P. Scale bar, 50 µm. **(T and U)** Survival curves of drug-treated embryos. Sibling control embryos survived for the duration of the experiment to 8 dpf (T); however, none of the embryos treated with rapamycin developed a swim bladder and therefore could not survive to adulthood. Similarly, rapamycin treatment allowed mutant embryos to survive longer than controls (U); however, they also failed to develop a swim bladder. *n* = 20 fish per condition in two independent experiments. For quantifications and statistical significances, see legend of Fig. 1.



Video 1. **Z-stack of the single plane image in Fig. 3 D, showing extrusion on the surface of an embryo.** Green = *krt4:GFP* (periderm); red = *p63:dsRed* (basal keratinocytes); blue = DAPI; white = phalloidin (actin dye). Frame rate: three frames per second.



Video 2. **Z-stack of extruded cell cluster recovered from the embryo medium, showing a single basal keratinocyte surrounded by several peridermal cells.** Green = *krt4:GFP* (periderm); red = *p63:dsRed* (basal keratinocytes); white = DRAQ5 (membrane-permeable nuclear dye). Frame rate: three frames per second.

Provided online is one table as a PDF. Table S1 lists the results of each Tukey's multiple comparison test following one-way ANOVA.