Figure S1

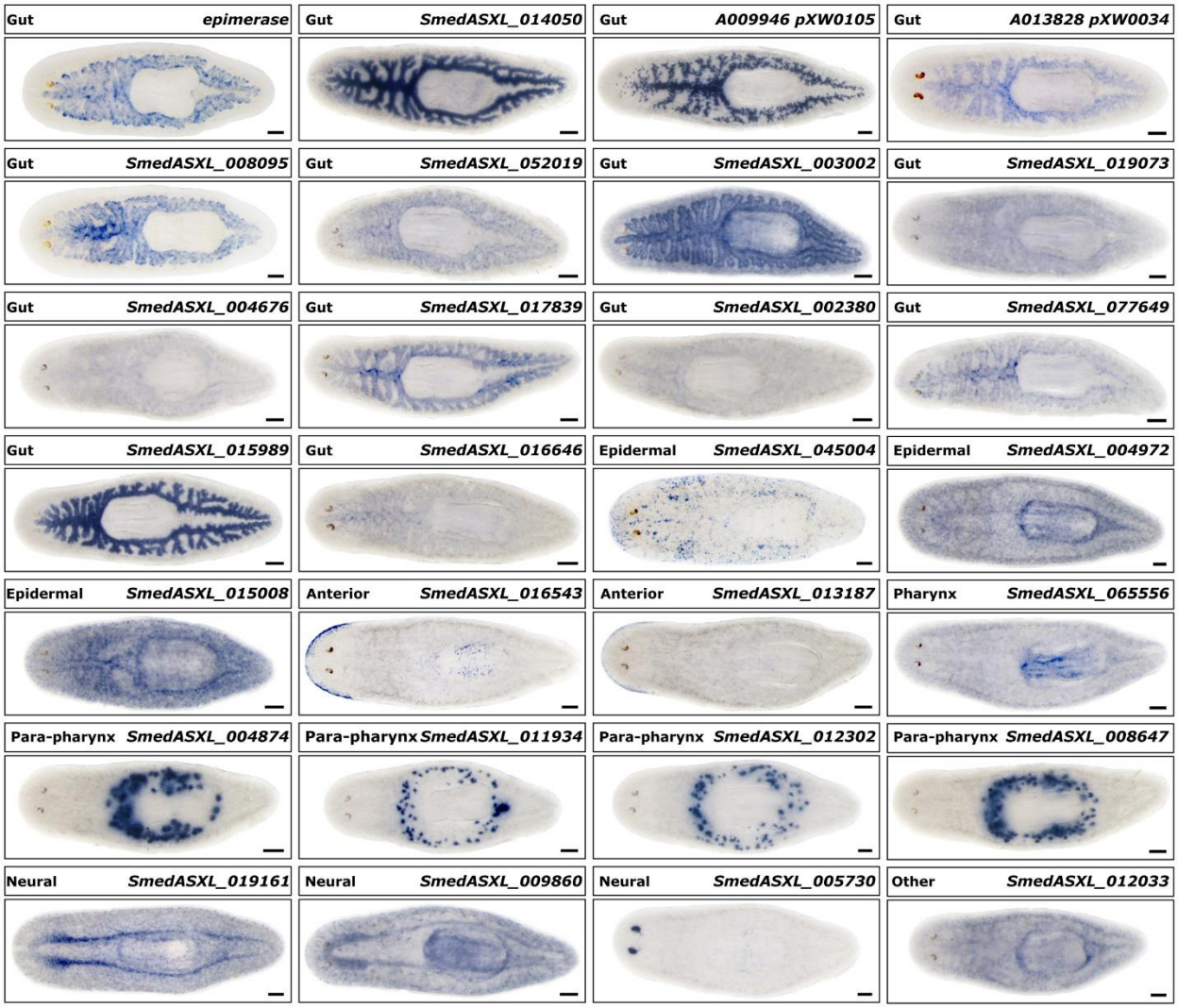

Figure S1. Expression Patterns of Pigment Cell Marker Candidates.

Expression patterns were determined by whole mount *in situ* hybridization (WISH). All genes shown have down-regulation in depigmented animals in whole worm RNAseq dataset. 14 genes show gut expression patterns, 3 show epidermal expression patterns, 2 show anterior expression patterns, 1 shows pharyngeal expression pattern, 4 show para-pharyngeal expression patterns and 3 show neural expression patterns. Scale bar: 100µm.

Figure S2

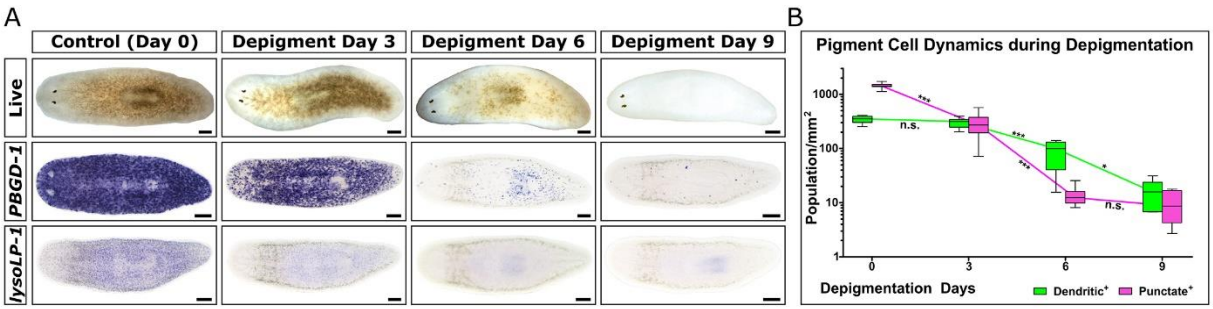

Figure S2. Pigment Cell Dynamics During Light Induced Depigmentation.

A. Planarian pigmentation is gradually but completely lost over the course of 9 days. Loss in both dendritic$^+$ and punctate$^+$ pigment cell population, detected by WISH, is observed alongside depigmentation. Punctate$^+$ pigment cell loss happens prior to dendritic$^+$ cell loss. **B.** During light induced depigmentation, punctate$^+$ pigment cell population drops first. Significant decreases were observed from D0 to D3, and from D3 to D6, but remained at similar low levels from D6 to D9. Dendritic$^+$ pigment cell population does not change initially, and only drops at later time points during depigmentation. No significant changes were observed from D0 to D3. Significant decreases of dendritic$^+$ pigment cells were observed from D3 to D6, and from D6 to D9. Scale bar: 100µm. p-values, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n. s. not significant.

Figure S3

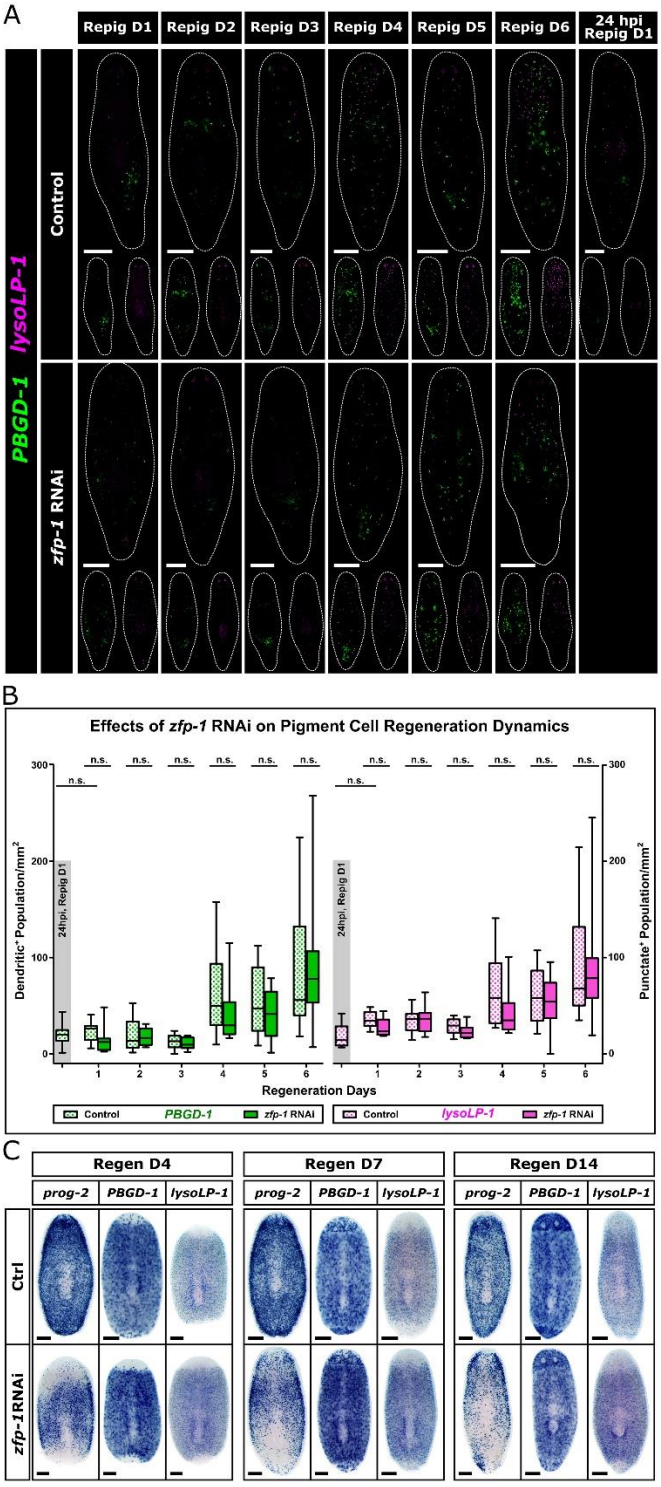

Figure S3. The effect of *zfp-1* RNAi on the population of dendritic and punctate markers during repigmentation and regeneration.

A. Fluorescent *in situ* hybridization of dendritic^{+} (*PBGD-1*) and punctate^{+} (*lysoLP-1*) pigment cells during repigmentation (Day 1 to Day 6) in control and *zfp-1* RNAi animals. 24hpi Repig D1: Animals were lethally irradiated at Repig D0 and fixed 24 hours after irradiation, equivalent to Repig D1 time point. Establishing a baseline of dendritic^{+} and punctate^{+} population during repigmentation in absence of entire neoblast population. **B.** Quantification of pigment cell populations in repigmenting control and *zfp-1* RNAi animals as shown in A. The difference in the population of dendritic^{+} and punctate^{+} pigment cells is non-significant between control and *zfp-1* RNAi animals at all time point. **C.** Whole mount *in situ* hybridization of *PBGD-1*$^{+}$ and *lysoLP-1*$^{+}$ pigment cells during head and tail regeneration in control and *zfp-1* RNAi animals. Scale bars: 250µm. n. s. not significant.

Figure S4

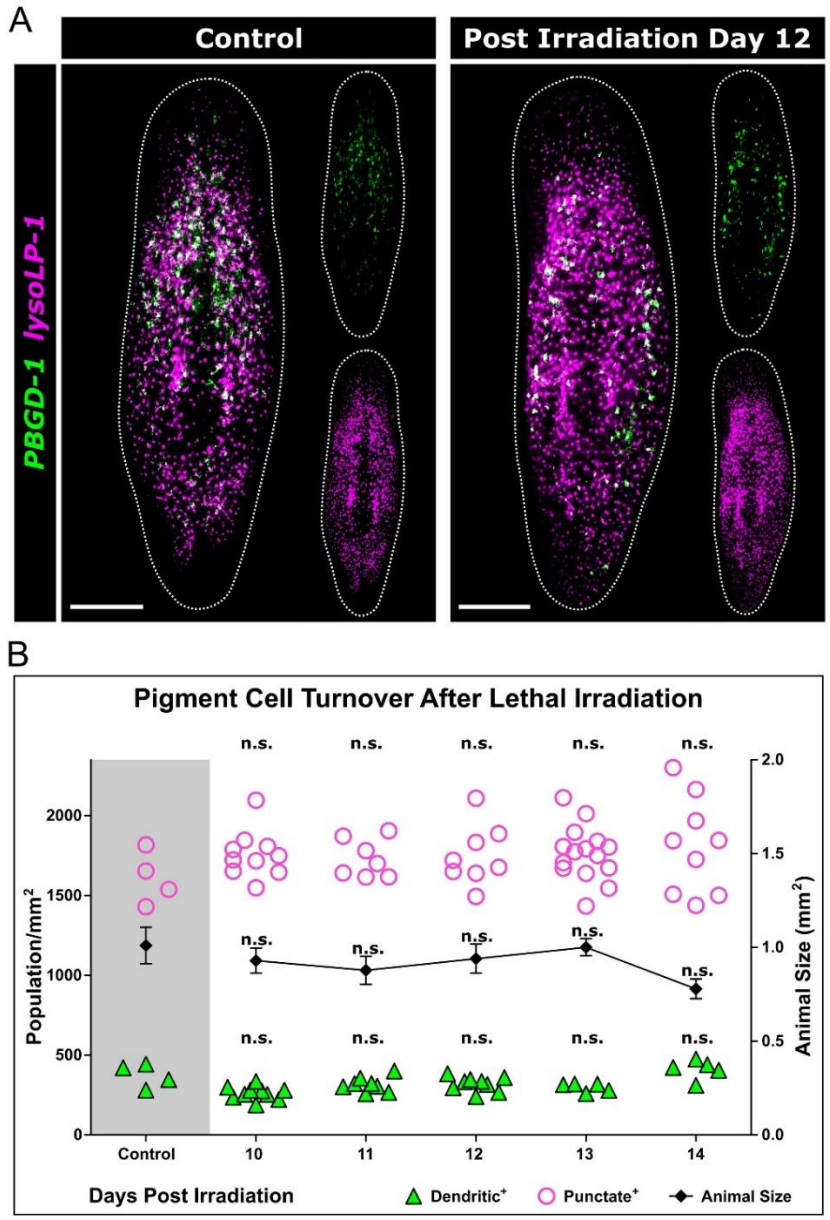

Figure S4. Retention of Dendritic⁺ and Punctate⁺ Cells After Lethal Irradiation Suggests Slow Turn Over Rates of Planarian Pigment Cells.

A. Detected by double fluorescent *in situ* hybridization (dFISH), both dendritic⁺ and punctate⁺ population remained comparable to that of control group 14 days after exposure to lethal dosage of γ-irradiation. **B.** No significant changes in either dendritic⁺ or punctate⁺ population density can be observed from animals D10 to D14 after lethal irradiation in comparison to control group. During this time, the animals did not shrink in size significantly, either. Thus, the unchanged pigment cell density is not due to the reduction in animal size, but rather due to the unchanged absolute numbers of pigment cells. Scale bars: 250µm. n. s. not significant.

Figure S5

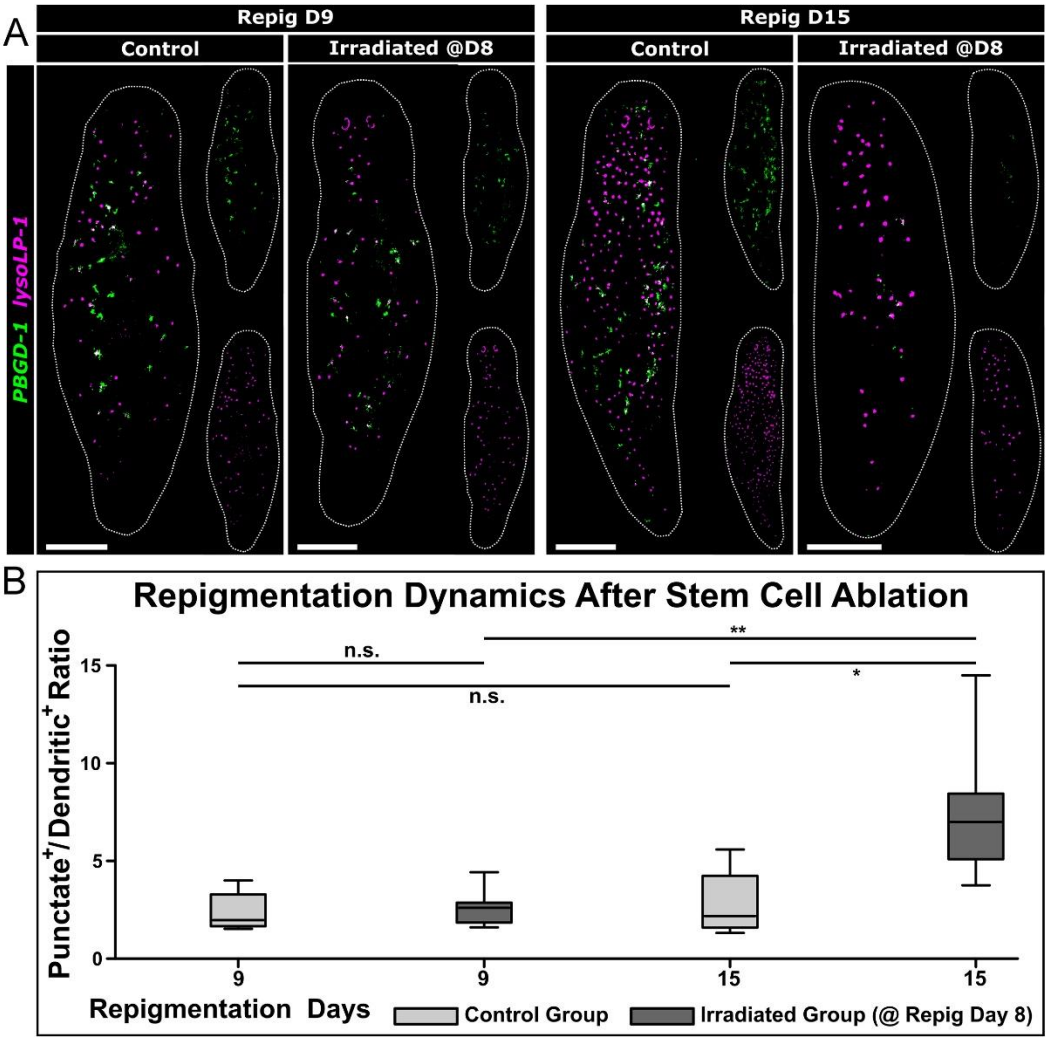

Figure S5. Dynamics of pigment cells during repigmentation + lethal irradiation.

A. Pigment cell lineage progresses toward punctate-positive stage without presence of stem cells. Animals were lethally irradiated on Day 8 of repigmentation, and pigment cell lineage progression were shown by double fluorescent *in situ* hybridization with dendritic (*PBGD-1*) and punctate (*lysoLP-1*) markers. **B.** Population dynamics of dendritic^{+} and punctate^{+} cells following repigmentation and irradiation. Statistics: two-tailed, unpaired *t*-test. Scale bars: 250µm. *p*-values, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n. s. not significant.

Figure S6

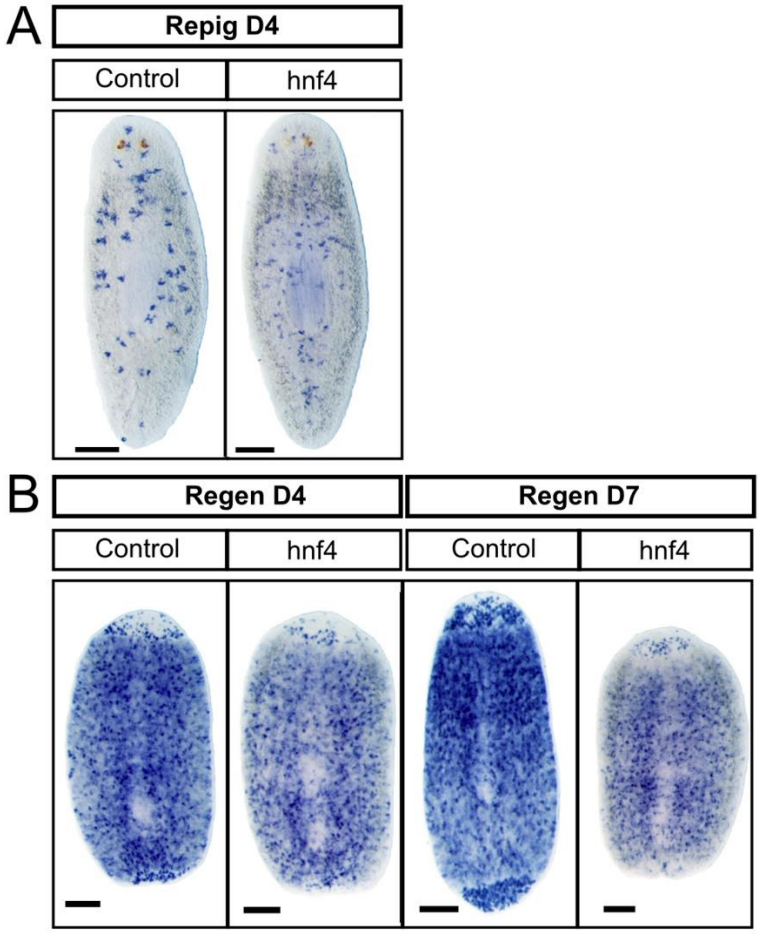

Figure S6. Effects of *hnf4(RNAi)* on repigmentation and regeneration of pigment cells.

A. Whole mount *in situ* hybridization of *PBGD-1*$^{+}$ pigment cells in repigmenting control and hnf4(RNAi) planarians. Following RNAi of hnf4, the number of *PBDG-1*$^{+}$ cells on day 4 of repigmentation remains the same, but the staining intensity is decreased compared to controls. Planarians were exposed to constant red light (625 nm) rather than white light for the final three days of the depigmentation protocol to optimize worm health. **B.** Whole mount *in situ* hybridization of *PBGD-1*$^{+}$ pigment cells in regenerating control and hnf4(RNAi) planarians. Scale bars: 250µm.

Figure S7

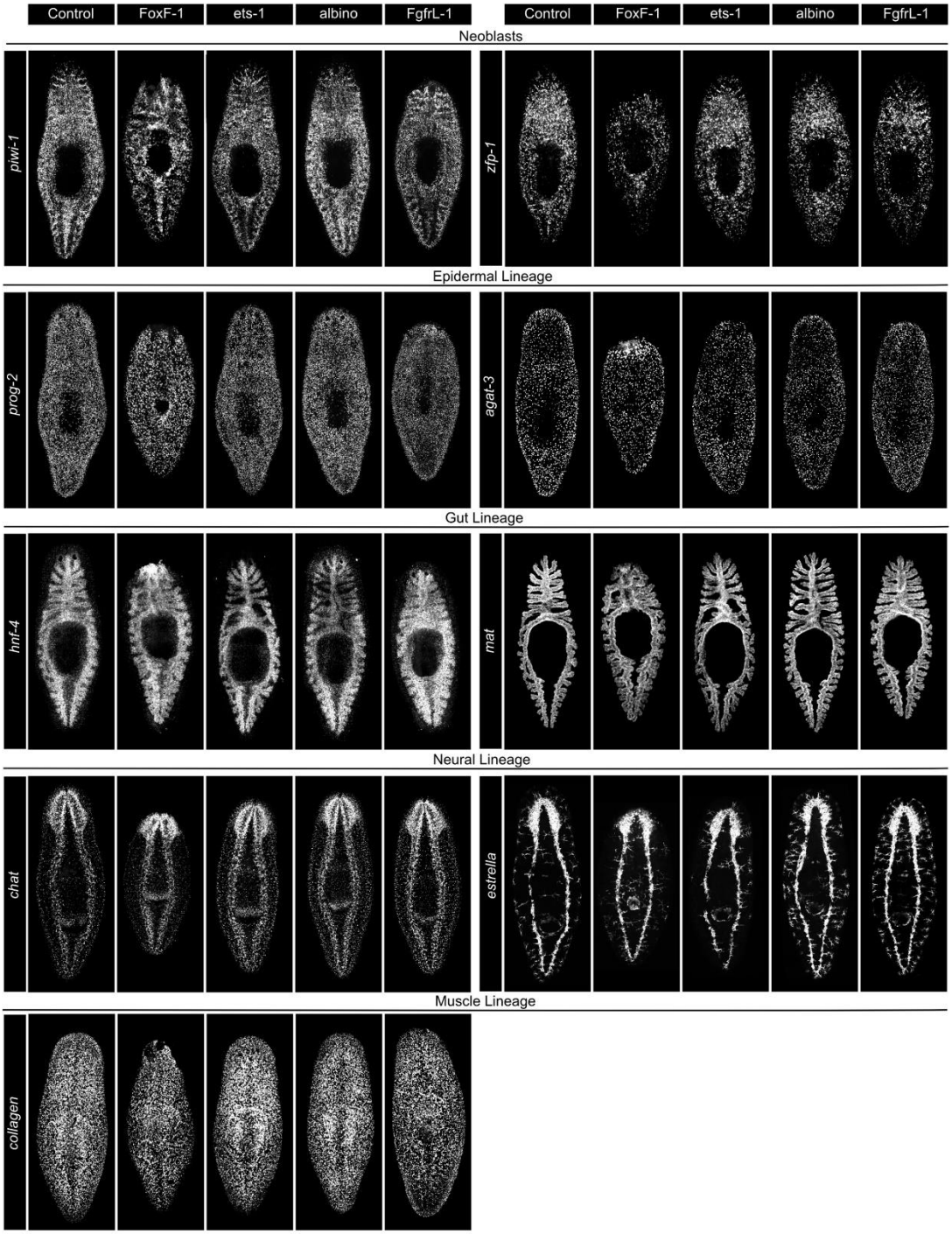

Figure S7. Assay of multiple cell types in RNAi conditions for *ets-1, foxF-1, fgfrL-1,* and *albino*.

Each row indicates various markers of cell type and each column is RNAi to a given gene. Stains were performed at the end point of animal health and sometimes on the precipice of head regression and animal lysis. Despite this, we did not find overt effects on any given cell type.

Table S1

Click here to Download Table S1

Table S2

Click here to Download Table S2