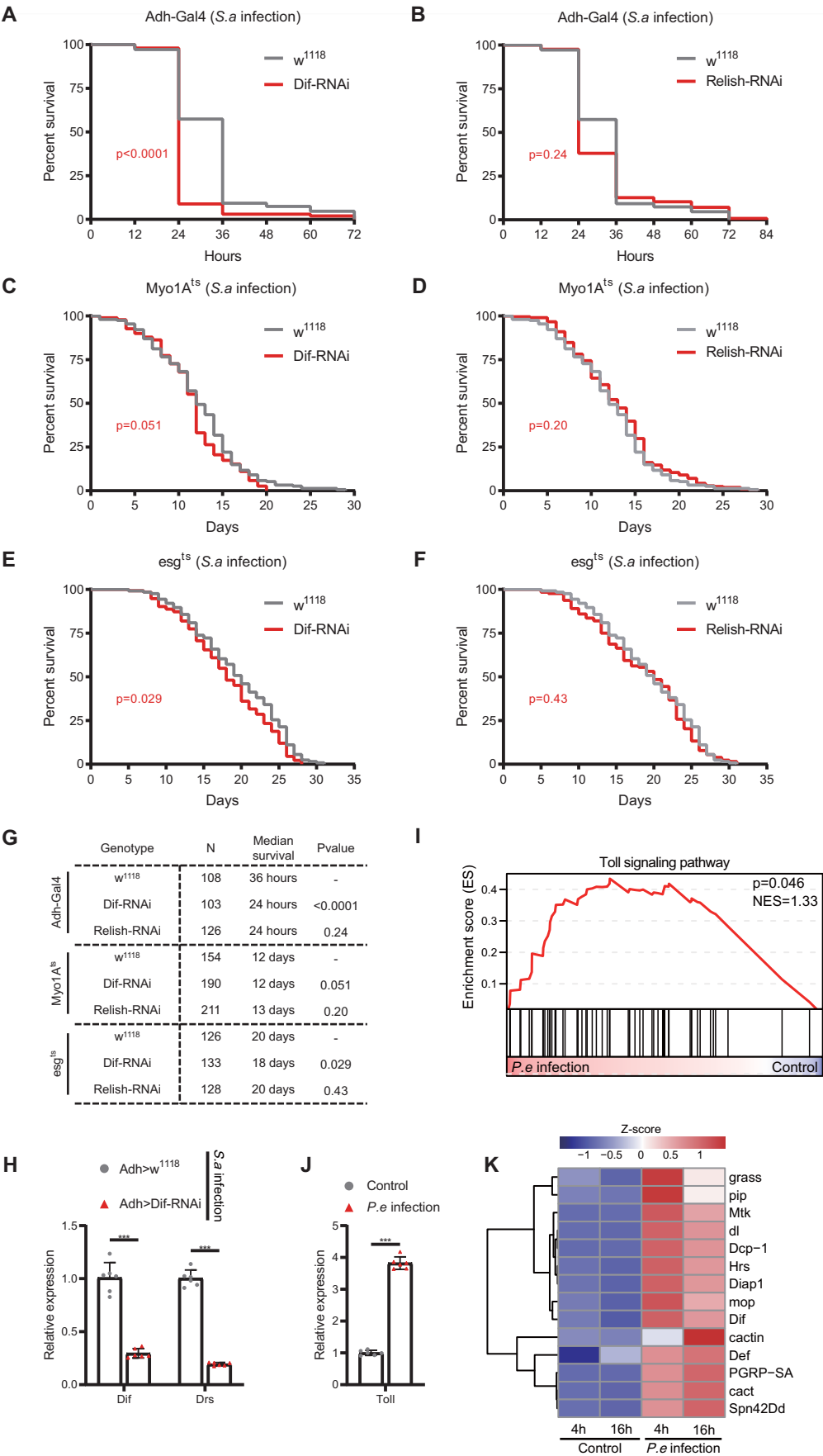


Expanded View Figures

Figure EV1. Toll signaling is not essential for intestinal immunity against infection.

(A) Lifespan curves of female flies of the indicated genotypes systemically infected with the bacteria *S.a* at 25 °C. The *Adh-Gal4* driver induces gene expression in the fat body. *w¹¹¹⁸*, *n* = 108; *Dif-RNAi*, *n* = 103. (B) Lifespan curves of female flies of the indicated genotypes systemically infected with the bacteria *S.a* at 18 °C. The *Adh-Gal4* driver induces gene expression in the fat body. *w¹¹¹⁸*, *n* = 108; *Relish-RNAi*, *n* = 126. (C) Lifespan curves of female flies of the indicated genotypes orally infected with the bacteria *S.a* at 29 °C. The *Myo1A-Gal4* driver induces gene expression in the ECs. *w¹¹¹⁸*, *n* = 154; *Dif-RNAi*, *n* = 190. (D) Lifespan curves of female flies of the indicated genotypes orally infected with the bacteria *S.a* at 29 °C. The *Myo1A-Gal4* driver induces gene expression in the ECs. *w¹¹¹⁸*, *n* = 154; *Relish-RNAi*, *n* = 211. (E) Lifespan curves of female flies of the indicated genotypes orally infected with the bacteria *S.a* at 29 °C. The *esg-Gal4* driver induces gene expression in ISCs/EBs. *w¹¹¹⁸*, *n* = 126; *Dif-RNAi*, *n* = 133. (F) Lifespan curves of female flies of the indicated genotypes orally infected with the bacteria *S.a* at 29 °C. The *esg-Gal4* driver induces gene expression in ISCs/EBs. *w¹¹¹⁸*, *n* = 126; *Relish-RNAi*, *n* = 128. (G) Summary of statistics of the lifespan shown in (A–F). (H) RT-qPCR analysis of *Dif* and its target *Drs* in the flies with indicated genotypes upon *S.a* infection at 25 °C for 24 h. *n* = 6 (three independent biological replicates with two technical replicates each). (I) Gene set enrichment analysis (GSEA) revealed an enrichment in the Toll signaling pathway in the intestine upon *P.e* infection at 4 and 16 h post-challenge. (J) RT-qPCR analysis of *Toll* in *ISC^{ts}* > *w¹¹¹⁸* flies with or without *P.e* infection at 29 °C for 1 day (after 10 days of normal food). *n* = 6 (three independent biological replicates with two technical replicates each). (K) Heatmap showing expression of a subset of the genes involved in the Toll signaling pathway in the intestine upon *P.e* infection at 4 and 16 h post-challenge (using dataset [GSE128489](#)). Heatmap displays log2(FPKM + 1) data scaled using a Z-score. Data information: In (H, J), data are presented as mean ± SEM. Statistical significance in (H, J) was determined using a two-tailed unpaired *t*-test (**p* < 0.05; ***p* < 0.01; ****p* < 0.001). Exact *p* values are provided in Table EV1.



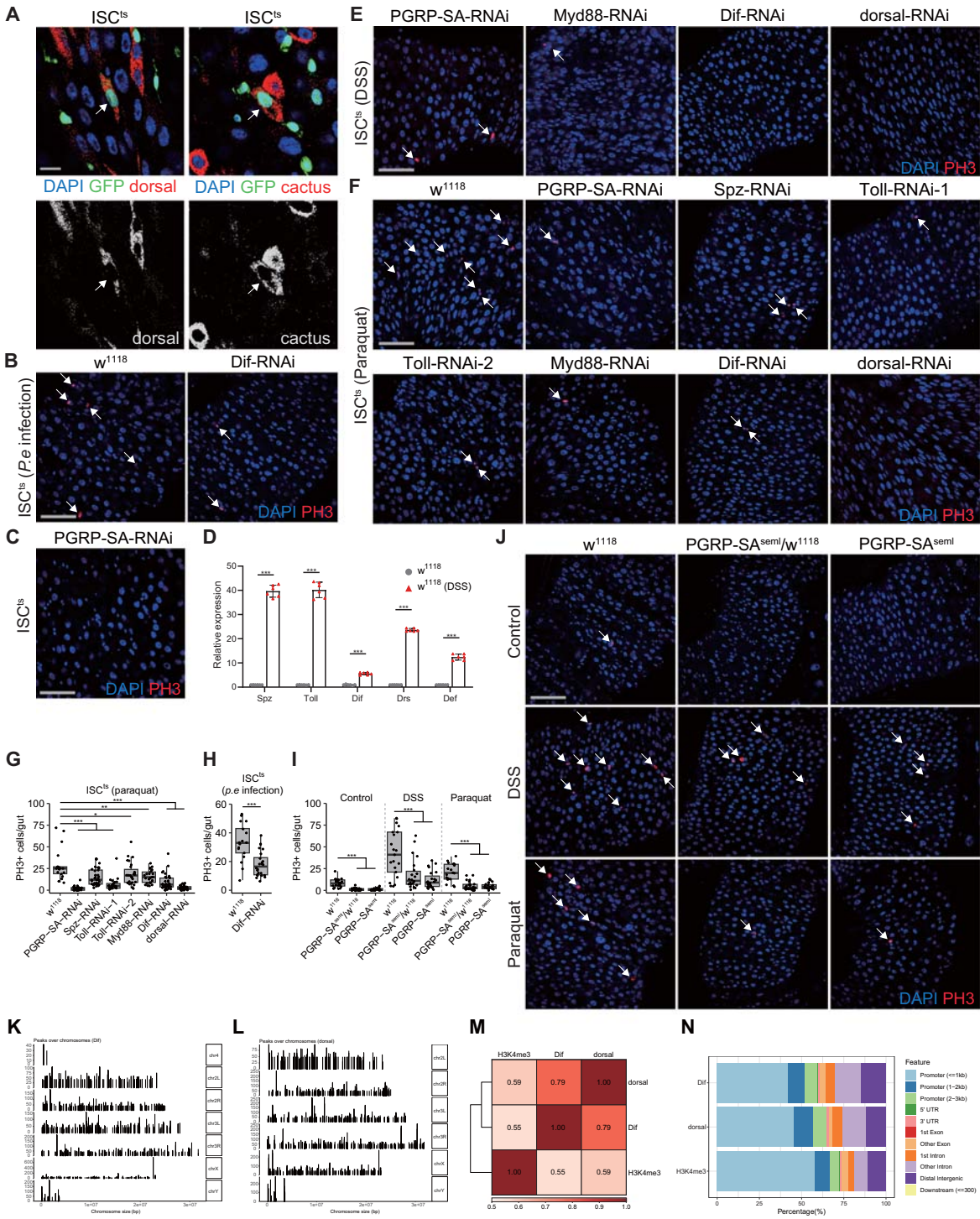


Figure EV2. Intestinal regeneration requires the Toll signaling pathway.

(A) Representative images of the posterior midgut of *ISC^{ts}* flies. dorsal or Cactus staining (red), Nuclei (blue), and ISC (green). The white arrows show dorsal-positive or Cactus-positive ISCs. Scale bar: 10 μ m. (B) Representative images of the posterior midgut of flies with indicated genotypes upon *P.e* infection at 29 °C for 1 day (after 10 days of normal food). PH3 staining (red), Nuclei (blue). The white arrows show PH3-positive cells. Scale bar: 50 μ m. (C) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 10 days. PH3 staining (red), Nuclei (blue). The white arrows show PH3-positive cells. Scale bar: 50 μ m. (D) RT-qPCR analysis of Toll pathway genes (*Spz*, *Toll*, and *Dif*) and their targets (*Drs* and *Def*) in the midgut of *w¹¹¹⁸* flies with or without 3% DSS treatment at 25 °C for 1 day. *n* = 6 (three independent biological replicates with two technical replicates each). (E) Representative images of the posterior midgut of flies with indicated genotypes with 3% DSS treatment at 29 °C for 1 day (after 10 days of normal food). PH3 staining (red), Nuclei (blue). The white arrows show PH3-positive cells. Scale bar: 50 μ m. (F) Representative images of the posterior midgut of flies with indicated genotypes with 5 mM Paraquat treatment at 29 °C for 1 day (after 10 days of normal food). PH3 staining (red), Nuclei (blue). The white arrows show PH3-positive cells. Scale bar: 50 μ m. (G) Quantification of PH3-positive cells per adult midgut of the indicated genotypes with 5 mM Paraquat treatment at 29 °C for 1 day (after 10 days of normal food). *n* = 17, 28, 36, 26, 27, 25, 36, 29 (from left to right). (H) Quantification of PH3-positive cells per adult midgut of the indicated genotypes upon *P.e* infection at 29 °C for 1 day (after 10 days of normal food). *w¹¹¹⁸*, *n* = 18; *Dif-RNAi*, *n* = 24. (I) Quantification of PH3-positive cells per adult midgut of the indicated genotypes with or without 3% DSS or 5 mM Paraquat treatment at 29 °C for 1 day (after 10 days of normal food). *n* = 21, 28, 24, 21, 22, 23, 20, 28, 23 (from left to right). (J) Representative images of the posterior midgut of flies with indicated genotypes with or without 3% DSS or 5 mM Paraquat treatment at 29 °C for 1 day (after 10 days of normal food). PH3 staining (red) and Nuclei (blue). The white arrows show PH3-positive cells. Scale bar: 50 μ m. (K) Distribution of *Dif* CUT&Tag signals on different chromosomes. (L) Distribution of dorsal CUT&Tag signals on different chromosomes. (M) Heatmap depicts Pearson correlation of *Dif*, dorsal, and H3K4me3 CUT&Tag signals. (N) Distribution of *Dif*, dorsal, and H3K4me3 CUT&Tag peaks across different functional genome regions. Data information: In (D), data are presented as mean $\pm$ SEM. In (G–I), box plots indicate median (center line), 25th–75th percentiles (box) and minima/maxima within 1.5 $\times$ interquartile range (whiskers); outliers are shown as individual points. Statistical significance was determined using a two-tailed unpaired *t*-test (D, G, H) or one-way ANOVA followed by Tukey's multiple comparisons test (I) ($p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Exact *p* values are provided in Table EV1.

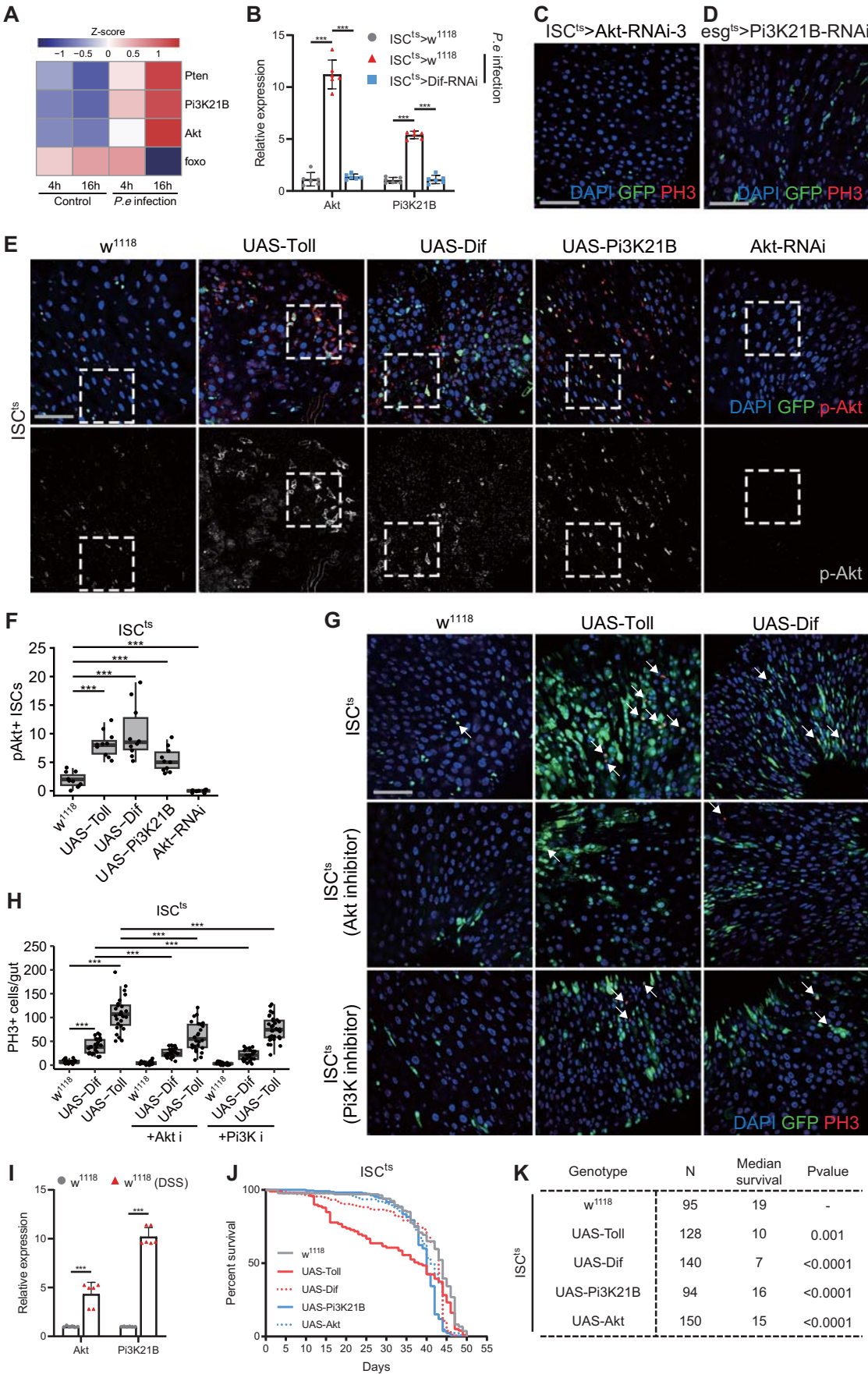


Figure EV3. Toll signaling promotes ISC proliferation by activating the PI3K/Akt pathway.

(A) Heatmap showing expression of *Pten*, *Pi3K21B*, *Akt*, and *foxo* in the intestine upon *P.e* infection at 4 and 16 h post-challenge (using dataset [GSE128489](#)). Heatmap displays $\log_2(\text{FPKM} + 1)$ data scaled using a Z-score. (B) RT-qPCR analysis of *Akt* and *Pi3K21B* in *ISC^{ts} > w¹¹¹⁸* and *ISC^{ts} > Dif* flies with or without *P.e* infection at 29 °C for 1 day (after 10 days of normal food). $n = 6$ (three independent biological replicates with two technical replicates each). (C) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 10 days. PH3 staining (red), Nuclei (blue), and ISC (green). The white arrows show PH3-positive cells. Scale bar: 50 μm . (D) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 10 days. PH3 staining (red), Nuclei (blue), and *esg > GFP* (green). The white arrows show PH3-positive cells. Scale bar: 50 μm . (E) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 8 days. p-Akt staining (red), Nuclei (blue), and ISC (green). The white box indicates the region enlarged in Fig. 3D. Scale bar: 50 μm . (F) Quantification of p-Akt-positive ISCs in the posterior midgut of flies with the indicated genotypes after 8 days at 29 °C. $n = 10$ independent biological samples. (G) Representative images of the posterior midgut of flies with indicated genotypes with or without 50 mM Akt or PI3K inhibitor treatment at 29 °C for 10 days. PH3 staining (red), Nuclei (blue), and ISC (green). The white arrows show PH3-positive cells. Scale bar: 50 μm . (H) Quantification of PH3-positive cells per adult midgut of the indicated genotypes at 29 °C for 10 days. “Akt i” and “PI3K i” denote Akt and PI3K inhibitors, respectively. $n = 28, 25, 27, 24, 26, 26, 25, 24, 32$ (from left to right). (I) RT-qPCR analysis of *Pi3K21B* and *Akt* in the midgut of *w¹¹¹⁸* flies with or without 3% DSS treatment at 25 °C for 1 day. $n = 6$ (three independent biological replicates with two technical replicates each). (J) Lifespan curves of female flies of the indicated genotypes at 29 °C. The number of flies ranged from 94 to 150. (K) Summary of statistics of the lifespan shown in (J). Data information: In (B, I), data are presented as mean $\pm$ SEM. In (F, H), box plots indicate median (center line), 25th–75th percentiles (box) and minima/maxima within $1.5 \times$ interquartile range (whiskers); outliers are shown as individual points. Statistical significance in (B, F, H, I) was determined using a two-tailed unpaired *t*-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Exact *p* values are provided in Table EV1.

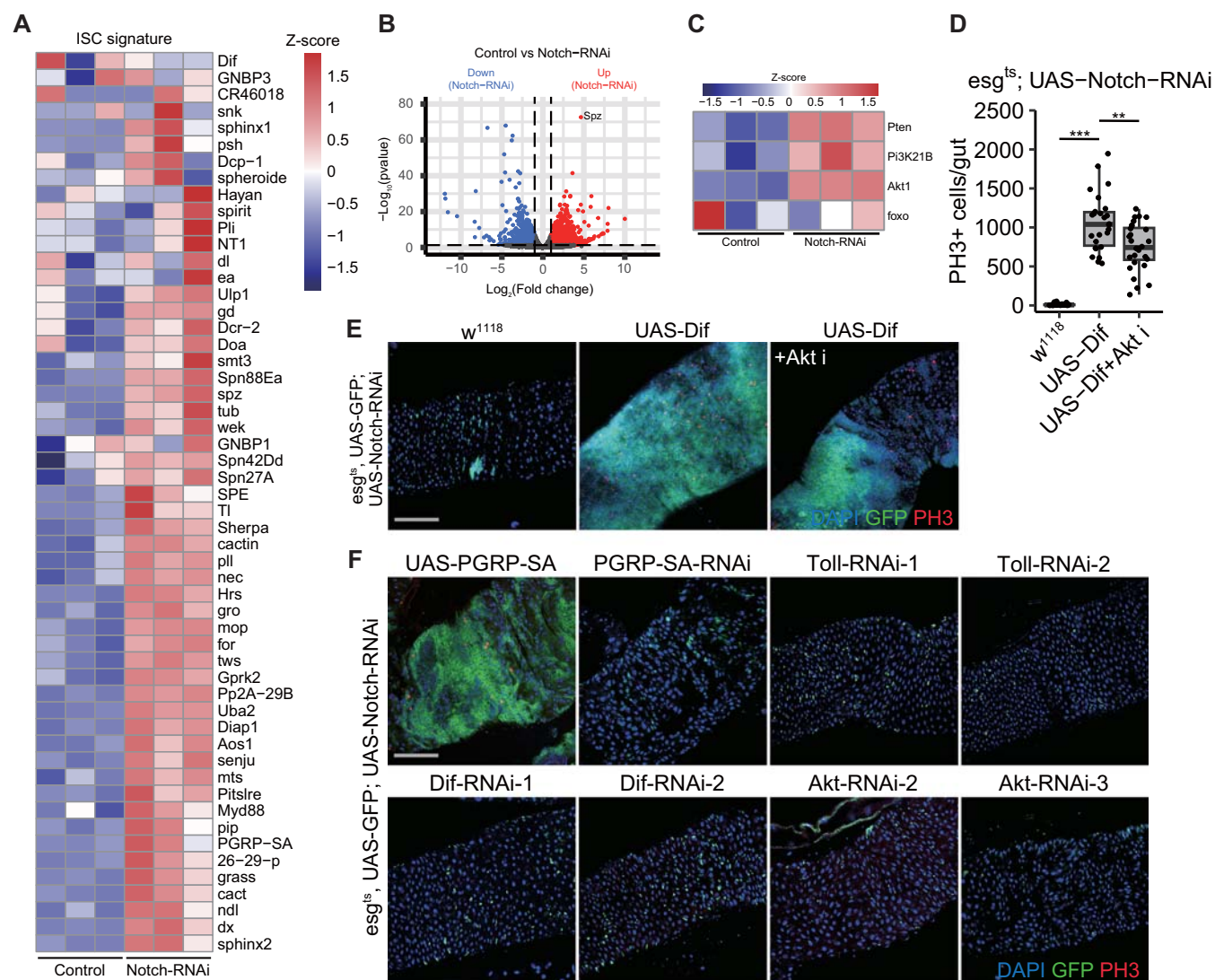


Figure EV4. Toll signaling promotes intestinal tumor growth via the PI3K/Akt pathway.

(A) Heatmap showing expression of Toll pathway genes in ISCs of the Notch-depleted tumor intestine (using dataset [GSE112331](#)). Heatmap displays DESeq2 normalized counts scaled using a Z-score. (B) Volcano plot showing differentially expressed genes (blue and red points) identified by RNA-seq in ISCs (*Notch-RNAi* versus control) (using dataset [GSE112331](#)). The most upregulated significant gene (*Spz*) is shown. (C) Heatmap showing expression of *Pten*, *Pi3K21B*, *Akt*, and *foxo* in ISCs of the Notch-depleted tumor intestine (using dataset [GSE112331](#)). Heatmap displays DESeq2 normalized counts scaled using a Z-score. (D) Quantification of PH3-positive cells per adult midgut of the indicated genotypes at 29 °C for 4 days. $n = 33, 26, 28$ (from left to right). "Akt i" denotes Akt inhibitor. (E) Representative images of the posterior midgut of flies with indicated genotypes with or without 100 mM Akt inhibitor at 29 °C for 4 days. PH3 staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 100 μm . (F) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 8 days. PH3 staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 100 μm . Data information: In (D), box plots indicate median (center line), 25th–75th percentiles (box) and minima/maxima within $1.5 \times$ interquartile range (whiskers); outliers are shown as individual points. Statistical significance in (D) was determined using a two-tailed unpaired *t*-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Exact *p* values are provided in Table EV1.



◀ **Figure EV5. Toll signaling regulates intestinal tumor growth via the PI3K/Akt pathway.**

(A) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 16 days. PH3 staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 100 μ m. (B) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 16 days. PH3 staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 100 μ m. (C) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 16 days. TUNEL staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 50 μ m. (D) Quantification of TUNEL-positive cells in the posterior midgut of flies with the indicated genotypes after 16 days at 29 °C. $n = 15, 11, 12, 13, 12$ (from left to right). (E) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 16 days. DCP1 staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 50 μ m. (F) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 8 days. p-Akt staining (red), Nuclei (blue), and *esg > GFP* (green). Scale bar: 10 μ m. Data information: In (D), box plots indicate median (center line), 25th–75th percentiles (box) and minima/maxima within 1.5 $\times$ interquartile range (whiskers); outliers are shown as individual points. Statistical significance in (D) was determined using a two-tailed unpaired *t*-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Exact *p* values are provided in Table EV1.

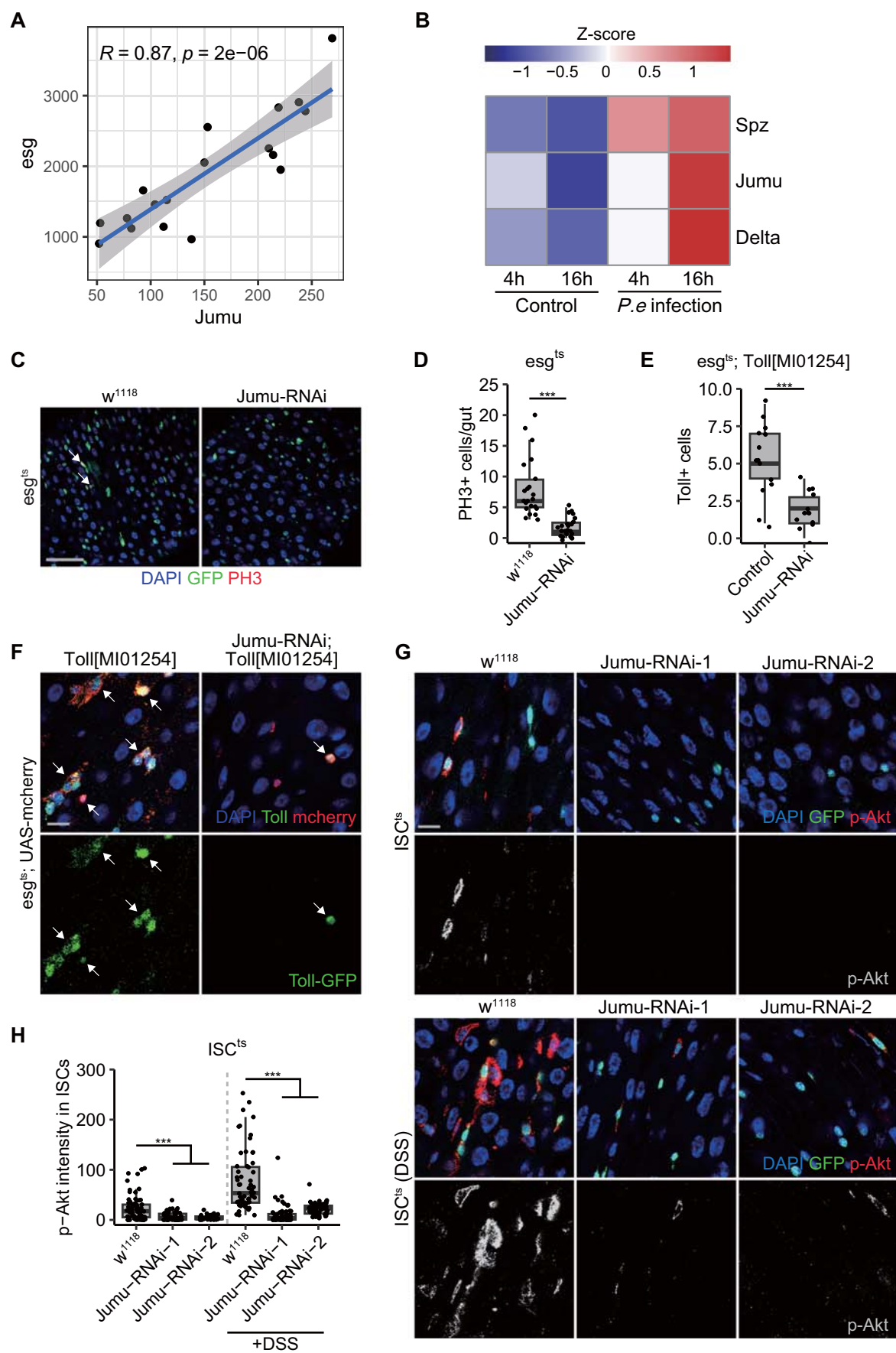


Figure EV6. Jumu/Spz/Toll cascade drives stem cell proliferation through Akt.

(A) Scatter plot showing expression correlation between *esg* and *Jumu*. Each point represents an individual sample from RNA-seq data, with *Jumu* expression on the x-axis and *esg* expression on the y-axis. (B) Heatmap showing expression of *Spz*, *Jumu* and *Delta* in the intestine upon *P.e* infection at 4 and 16 h post-challenge (using dataset [GSE128489](#)). Heatmap displays $\log_2(\text{FPKM} + 1)$ data scaled using a Z-score. (C) Representative images of the posterior midgut of flies with indicated genotypes at 29 °C for 10 days. PH3 staining (red), Nuclei (blue), and *esg* > *GFP* (green). The white arrows show PH3-positive cells. Scale bar: 50 μm . (D) Quantification of PH3-positive cells per adult midgut of the indicated genotypes at 29 °C for 10 days. *w¹¹¹⁸*, *n* = 22; *Jumu-RNAi*, *n* = 23. (E) Quantification of Toll-positive cells in the posterior midgut of flies with indicated genotypes at 29 °C for 10 days. *n* = 14 independent biological samples. (F) Representative images of the midgut of flies with indicated genotypes at 29 °C for 10 days. Nuclei (blue), *Toll-GFP* (green), *esg* > *mCherry* (red). The white arrows show GFP-positive ISCs. Scale bar: 10 μm . (G) Representative images of the posterior midgut of flies with indicated genotypes with or without 3% DSS treatment at 29 °C for 1 day (after 10 days of normal food). p-Akt staining (red), Nuclei (blue), and ISC (green). Scale bar: 10 μm . (H) Intensity statistics of p-Akt in the posterior midgut of flies with indicated genotypes with or without 3% DSS treatment at 29 °C for 1 day (after 10 days of normal food). *n* = 73, 57, 50, 60, 64, 66 (from left to right). Data information: In (D, E, H), box plots indicate median (center line), 25th–75th percentiles (box) and minima/maxima within 1.5 $\times$ interquartile range (whiskers); outliers are shown as individual points. Statistical significance in (D, E, H) was determined using a two-tailed unpaired *t*-test (**p* < 0.05; ***p* < 0.01; ****p* < 0.001). Exact *p* values are provided in Table EV1.