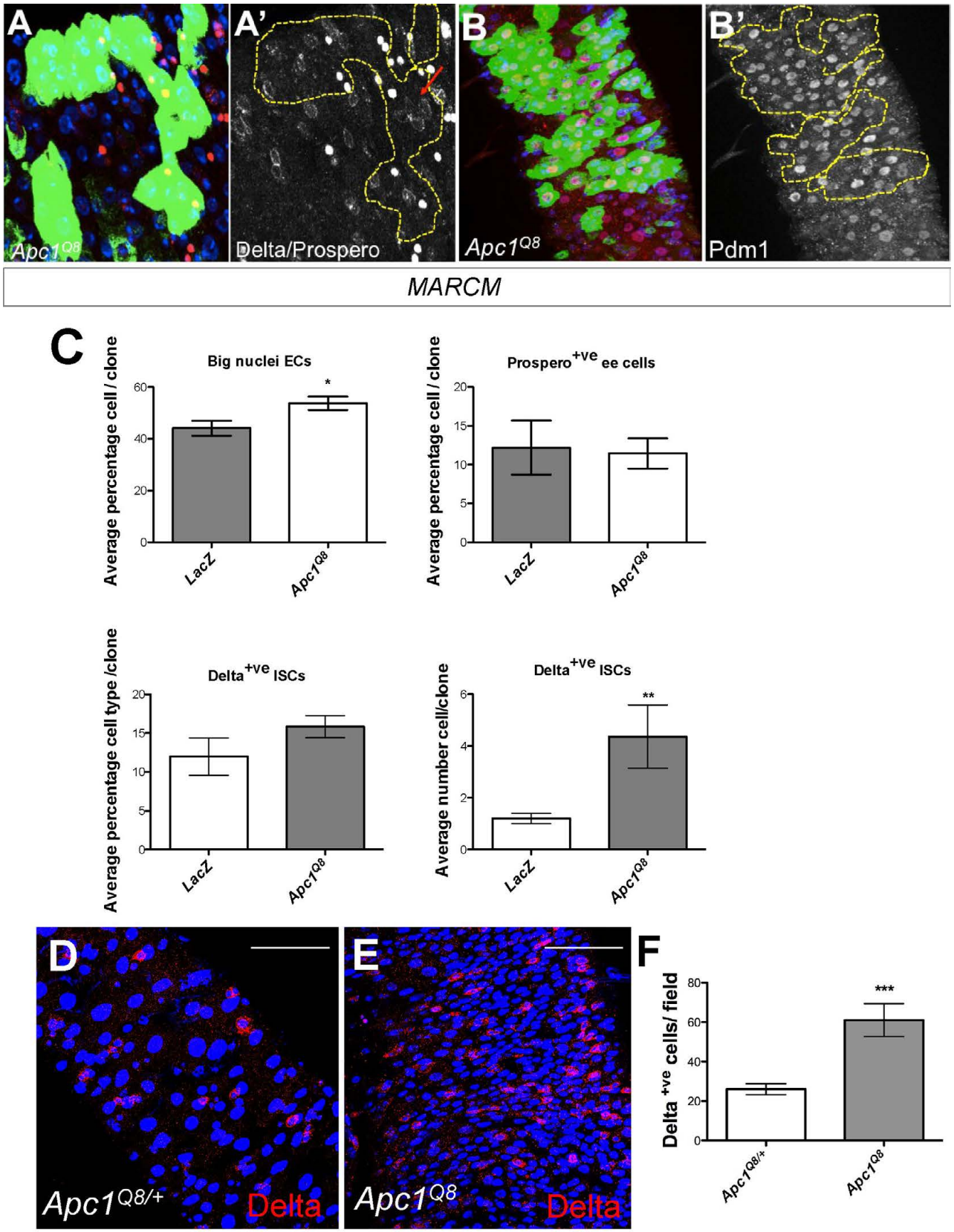

Fig. S1. Loss of *Apc1* does not affect ISC differentiation in the adult *Drosophila* midgut. (**A-B′**) 14-day-old $Apc1^{-/-}$ ($Apc1^{Q8}$) MARCM clones (GFP$^+$ or outlined by dashed line). Clones were immunostained for Delta and Prospero (A,A′, red or white) to label ISCs and enteroendocrine (ee) cells, respectively, or for Pdm1 (B,B′, red or white) to label enterocytes (ECs). (**C**) The average percentage of large nuclei ECs (*P=0.02, Student's t-test), Prospero$^+$ and Delta$^+$ cells and the average number of Delta$^+$ cells (lower right panel; **P=0.01, Student's t-test) in control (*lacZ*) and $Apc1^{Q8}$ MARCM clones. (**D**,**E**) Whole $Apc1^{Q8/+}$ (D) and $Apc1^{Q8}$ (E) midguts stained with anti-Delta (red). (**F**) Quantification of the total number of Delta$^+$ cells (***P<0.007, Student's t-test) within a pre-established field of $Apc1^{Q8/+}$ and $Apc1^{Q8}$ posterior midguts. DAPI (blue) was used to label nuclei. Scale bars: 40 μm.

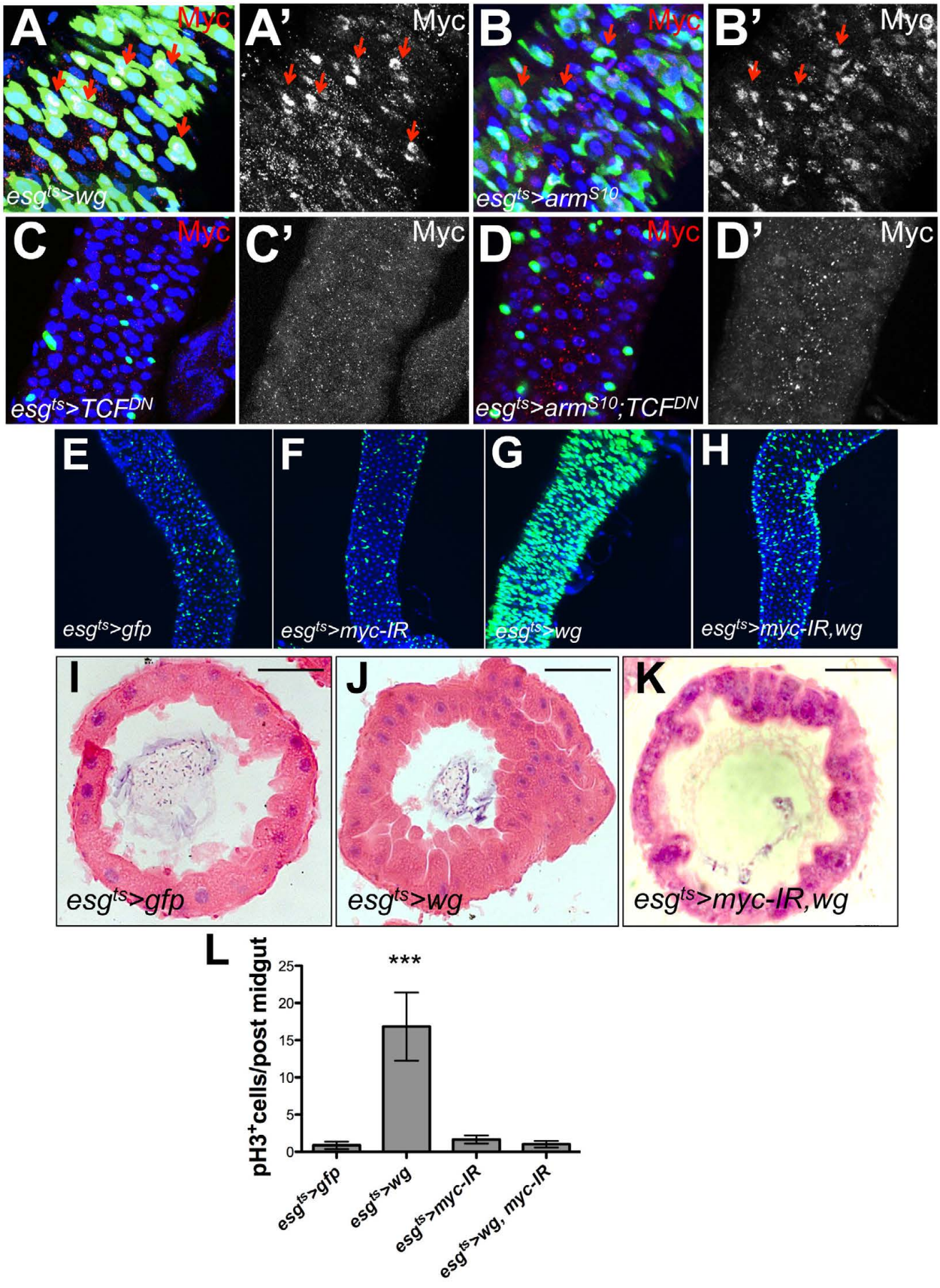

Fig. S2. Myc upregulation mediates Wnt-dependent hyperproliferation in the *Drosophila* midgut. (**A-D′**) Posterior midguts of the indicated genotypes after 14 days of transgene overexpression and stained with anti-GFP (green) to label *esg*$^+$ cells and anti-Myc (red or white). (**E-H**) Immunofluorescence of midguts of the indicated genotypes after 14 days of transgene overexpression and stained with anti-GFP (green) to label *esg*$^+$ cells. DAPI (blue) labels nuclei. (**I-K**) Immunohistochemistry and H+E staining of midguts as in E,G,H. (**L**) Quantification of pH3$^+$ cells in midguts of the indicated genotypes after 14 days of transgene expression. ***P<0.0001, one-way ANOVA with Bonferroni's multiple comparison test. Scale bars: 40 μm.

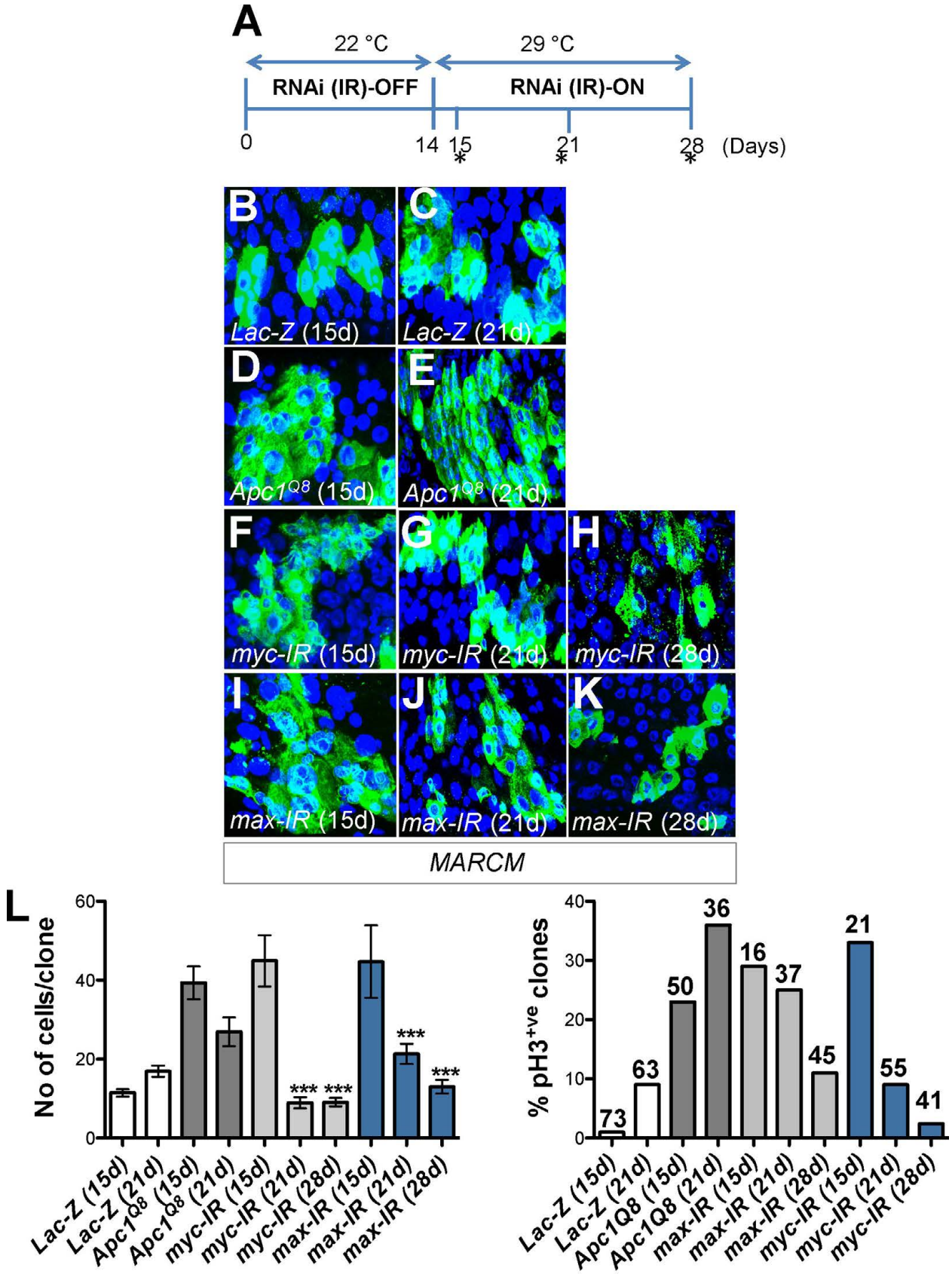

Fig. S3. Myc and Max depletion regresses pre-established intestinal hyperproliferation by *Apc1* loss. (**A**) Experimental setup. Flies were maintained at 22°C for the first 14 days after clonal induction (ACI). This was followed by incubation at 29°C for 1, 7 or 14 days. Cohorts were analyzed at the time points indicated by asterisks. (**B-K**) Control (*lacZ*) (B,C) and $Apc1^{Q8}$ MARCM clones (D,E) were analyzed 15 and 21 days ACI and compared with 14-day-old $Apc1^{Q8}$ MARCM clones in which temperature-sensitive RNAi transgenes for *myc* or *Max* (*myc-IR* and *max-IR*, respectively) were induced for an additional 1 (F,I; 15d), 7 (G,J; 21d) or 14 days (H,K; 28d) at 29°C. Clones are labeled with GFP (green) and DAPI (blue) labels nuclei. (**L**) Quantification of the number of cells per clone (left) and percentage of clones with $pH3^+$ cells (right) for the different conditions described in B-K. Note that knockdown of *myc* or *Max* regressed the size of $Apc1^{Q8}$ clones (L). ***$P<0.0001$, one-way ANOVA with Bonferroni's multiple comparison test. Numbers inside bars indicate the total number of clones scored.

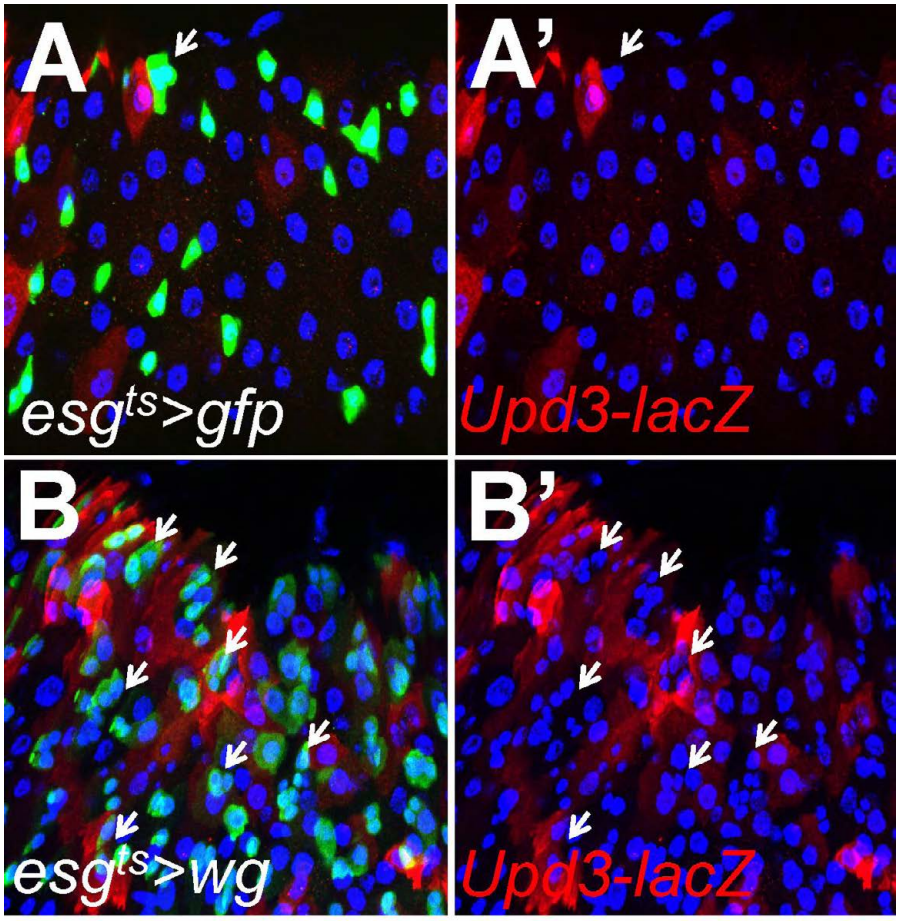

Fig. S4. Overexpression of Wg in ISCs leads to Upd3 upregulation in ECs. *upd3-lacZ* reporter expression (red) in posterior midguts following 14 days of *esg*^{ts}*>gfp* (**A**,**A′**) or *esg*^{ts}*>wg* (**B**,**B′**) expression. Arrows point to ISCs/EBs labeled with *esg>gfp* (green). Overexpression of Wg in ISCs resulted in non-autonomous activation of *upd3* in ECs.

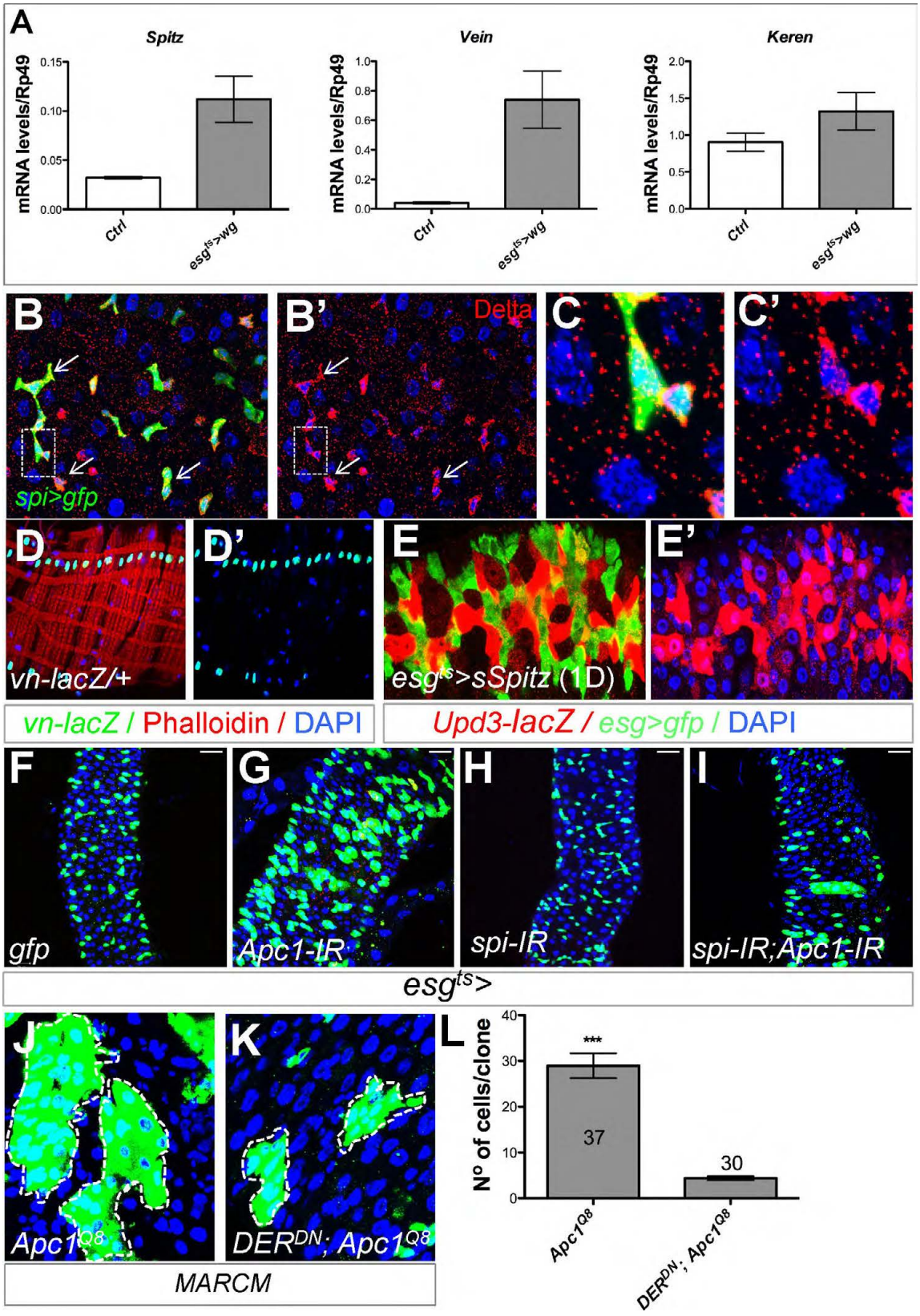

Fig. S5. Spitz/Egfr signaling mediates *Apc1*-dependent midgut hyperproliferation. (**A**) RT-qPCR of the EGF ligands *spitz*, *vein* and *Keren* in control (Ctrl) and *esg*^{ts}*>wg* midguts after 14 days of transgene expression. (**B-C′**) Midguts expressing *gfp* under the control of the *spitz-gal4* reporter (*spi>gfp*) were stained with anti-GFP (B,C, green) and anti-Delta (B-C′, red). (C,C′) Magnified views of the boxed areas in B,B′. Arrows point to *spi>gfp*/Delta^{+} ISCs. *spitz* was expressed in Delta^{+} ISCs and enteroblasts (Delta^{-}) (C,C′). (**D**,**D′**) *vein* expression in the visceral muscle (phalloidin, red) as monitored by a *vein-lacZ* reporter line (green). (**E**,**E′**) *upd3-lacZ* expression (red) in response to overexpression of secreted Spitz in progenitor cells (*esg*^{ts}*>sSpitz*, green). *upd3-lacZ* is almost exclusively expressed in large nuclei, *esg*$^{-}$ ECs. DAPI (blue) labels nuclei. (**F-I**) Immunofluorescence of midguts overexpressing *gfp* (F), RNAi for *Apc1* (G; *Apc1-IR*), RNAi for *Spitz* (H; *spi-IR*) or combined *spitz* and *Apc1* RNAi (I; *spi-IR; Apc1-IR*) during 14 days in progenitor (*esg*^{ts}) cells (anti-GFP, green). (**J**,**K**) 14-day-old *Apc1*^{Q8} MARCM clones (J) and clones with combined expression a dominant-negative form of the EGF receptor (*DER*^{DN}*; Apc1*^{Q8}) (K); clones are labeled with GFP and outlined by dashed line. (**L**) Quantification of the number of cells per clone from J,K. ***P<0.0001, Student's t-test. Numbers in bars indicate the total number of clones scored. Scale bars: 20 μm.

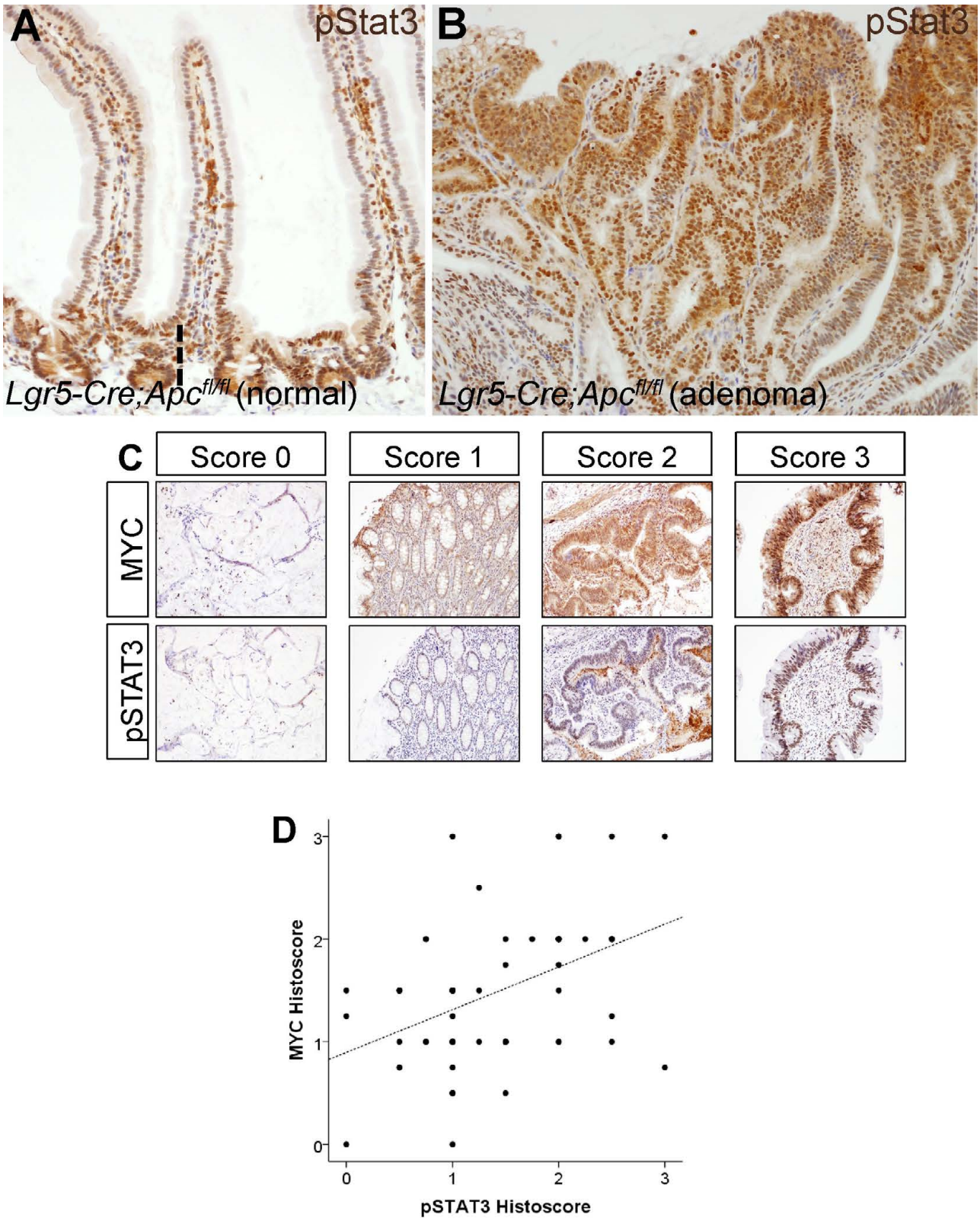

Fig. S6. Stat3 activation in mouse and human colorectal tumor samples. (**A**,**B**) Tissue sections of small intestine from *Lgr5-CreERT2 Apc*$^{fl/fl}$ mice stained with anti-p-Stat3 to look at the activated form of the protein. Examples of normal (A) and transformed (B) areas of the intestine are shown. The dashed line in A indicates the proliferative 'crypt' domain. (**C**,**D**) c-Myc/p-Stat3 correlation analysis in human colorectal TMA. (C) Examples of each of the scores associated with a staining intensity. (D) c-Myc/p-Stat3 staining correlation. Statistical analysis used Spearman's rank correlation coefficient.

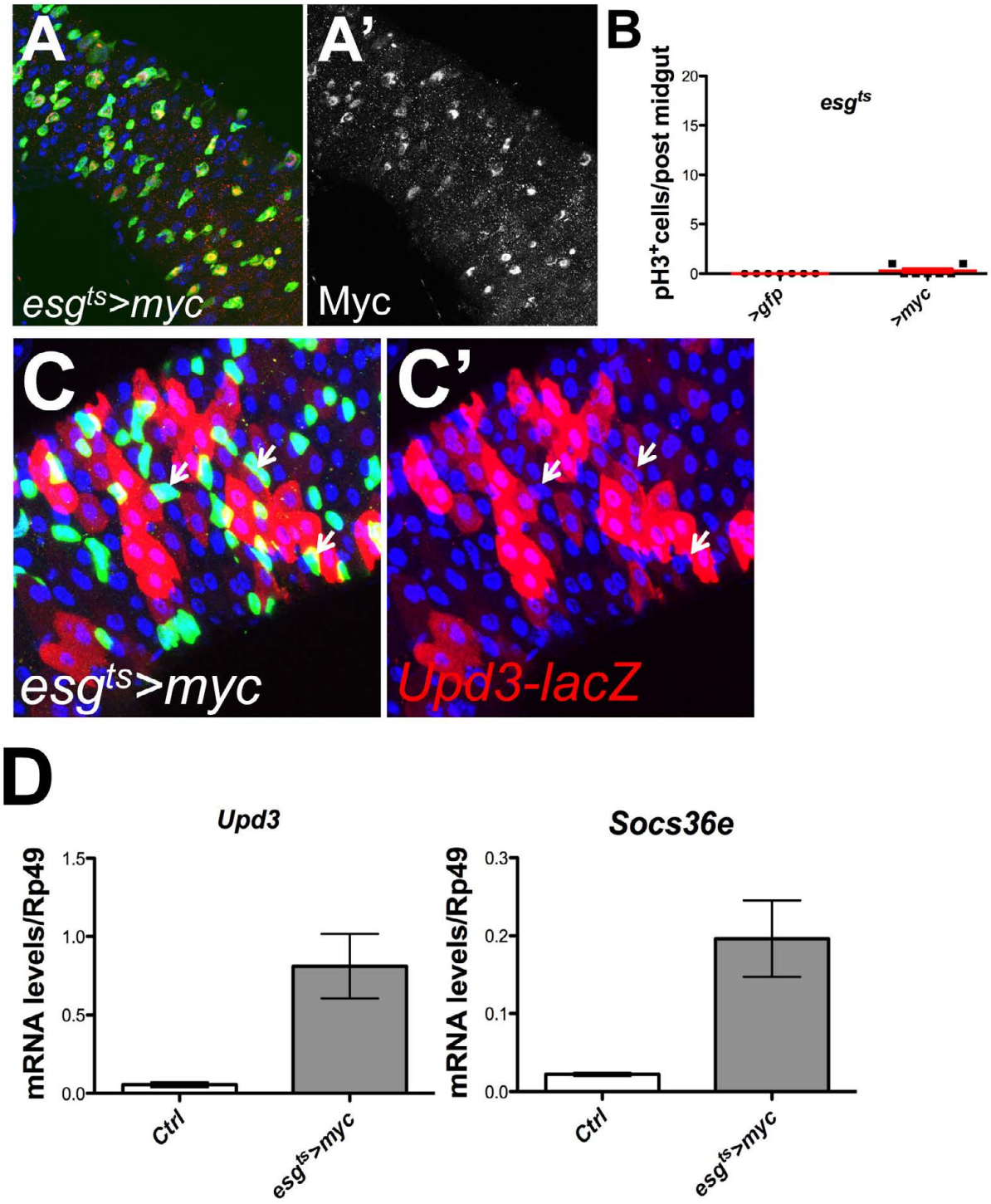

Fig. S7. Myc overexpression recapitulates only some of the phenotypes of high Wnt signaling in the midgut. (**A**,**A**′) Posterior midguts overexpressing Myc in progenitor cells for 14 days (*esg*^{ts}*>myc*) and stained with anti-GFP (green; A) and anti-Myc (red A and white A′). (**B**) Quantification of ISC proliferation from aged-matched *esg*^{ts}*>gfp* and *esg*^{ts}*>myc* intestines represented by the number of pH3^{+} cells/posterior midgut. (**C**,**C**′) Non-cell-autonomous *upd3* expression in ECs in *esg*^{ts}*>myc* midguts monitored with the *upd3-lacZ* reporter (red). Arrows point to *esg*$^{+}$ cells. (**D**) RT-qPCR for *upd3* and *Socs36e* in control and *esg*^{ts}*>myc* midguts after 14 days of transgene expression.

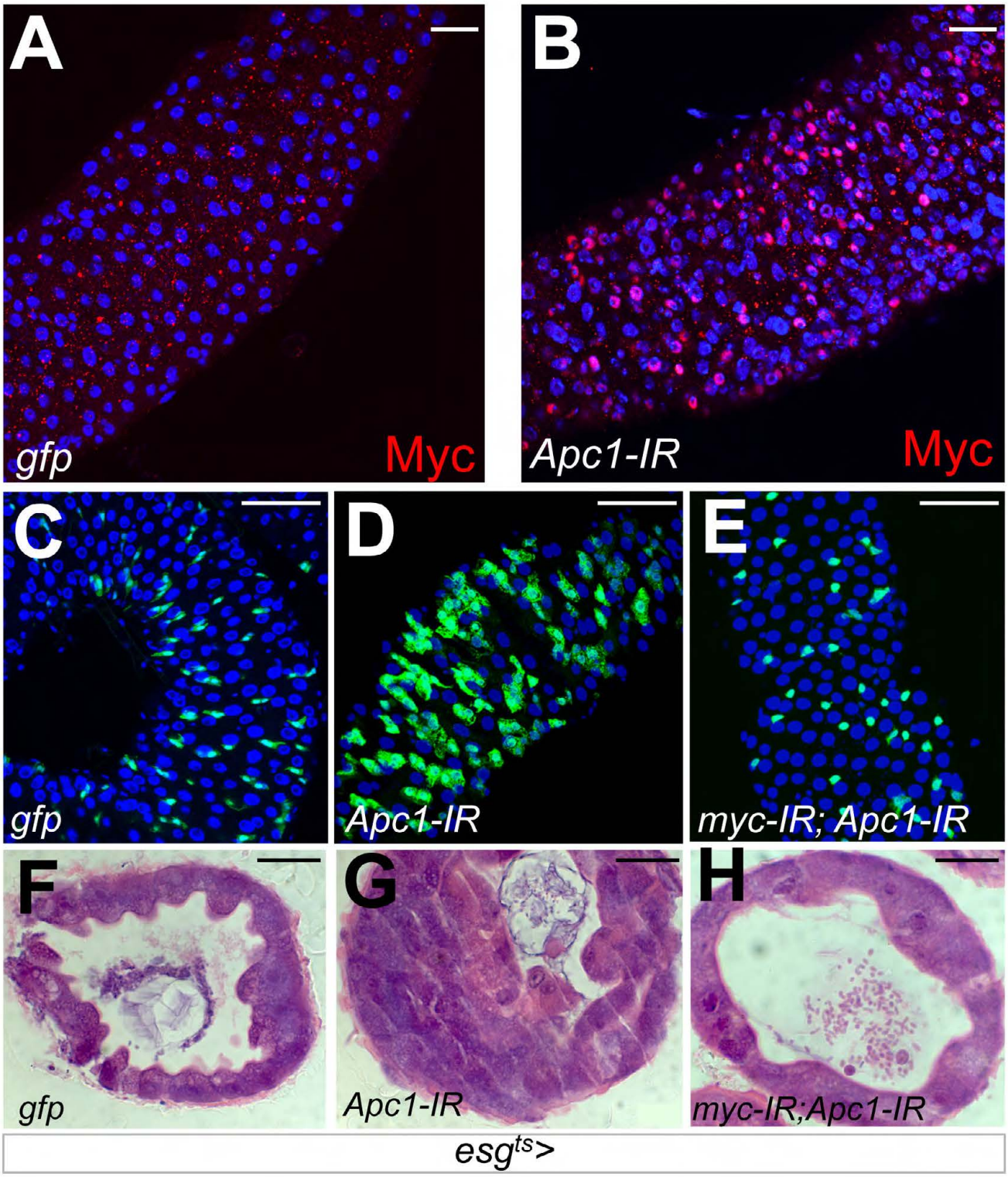

Fig. S8. Myc-dependent ISC proliferation in *esg^{ts}>Apc1-IR* midguts. (**A**,**B**) Posterior midguts of the indicated genotypes after 14 days of transgene overexpression, stained with anti-Myc (red). DAPI (blue) labels nuclei. (**C-E**) Immunofluorescence of midguts of the indicated genotypes after 14 days of transgene overexpression, stained with anti-GFP (green) to label *esg*$^{+}$ cells. (**F-H**) Immunohistochemistry and H+E staining of midguts as in C-E. Scale bars: 20 μm.

Table S1. Primer sequences

Primer	Sequence
RpL32 f	AGGCCCAAGATCGTGAAGAA
RpL32 r	TGTTGCACCAGGAACTTCTTGAA
upd f	CCACGTAAGTTTGCATGTTG
upd r	CTAAACAGTAGCCAGGACTC
upd2 f	ACTGTTGCATGTGGATGCTG
upd2 r	CAGCCAAGGACGAGTTATCA
upd3 f	AGGCCATCAACCTGACCAAC
upd3 r	ACGCTTCTCCATCAGCTTGC
Socs36e f	ATGACCGTGCACTCGCAAAT
Socs36e r	CCTCGTAGCGGTCCATCTTG
spitz f	TACCAGGCATCGAAGGTTTC
spitz r	GACCCAGGCTCCAGTCACTA
vein f	GTGAAGTTGCCTGGATTCGT
vein r	CTACAGGGAGCGACTGATGC
Keren f	CGAGCCATCAATCTCCTTGT
Keren r	AACGATGGCACCTGCTTTAC