Biteau et al., Supplementary Material

Supplementary Experimental procedures

Electron microscopy

Guts were dissected and fixed overnight in Phosphate Buffer + 2.5% Glutaraldehyde, then stained using standard procedures (including post-fixation in 1.0% osmium tetroxide and staining with uranyl acetate and lead citrate). Images were captured using a Hitachi 7100 Transmission Electron Microscope with a digital MegaView III camera.

Quantification of gene expression by real time RT-PCR

RNA were extracted from pools of 5-6 dissected guts and real-time RT-PCR was performed as previously described (Wang et al., 2003). rp49 expression was used as normalization. The following primers were used: β-trypsin: 5'-TGG AGC TCT TGA TCT GGT TTC CGT-3' and 5'-TCG TCG CCA AGG TTT CCT CTT TCA-3'; ε-trypsin: 5'-TAG ACA CCG GGA TAC CTC ACA TCA-3'and 5'-TCC AGC ATT CGC GAG ATC CGT ATT-3'; ζ-trypsin: 5'-TTG TTC CAA GGC CAG CTT AAT GGC-3'and 5'-TTT CCC TGC GCT ACA AGG GTA TCA-3'; delta: 5'-TGA GCA CTT TCT CCT CGC ACA TCT-3'and 5'-AGG CTT GTA CTG CAA CCA GGA TCT-3'; rp49: 5′-TCC TAC CAG CTT CAA GAT GAC-3′ and 5′-CAC GTT GTG CAC CAG GAA CT-3′. Results are average +/- standard deviation of 3 to 4 independent samples.

Quantification of cell numbers in aging intestines

Confocal images of multiple 10.000μm^2 areas of the posterior midgut of young and old flies were acquired. Total number of cells and number of enteroendocrine were determined using Hoechst DNA staining and prospero inmmunostaining, respectively. At least five different guts were observed of each age, and more than 300 cells counted.

P-values were calculated using Student's t-test.

Lifespan measurement

Flies carrying UASHep were backcrossed more than 15 times into the w^{1118} background and kept as an unbalanced stock. Homozygous males (+/+ or UASHep/UASHep) were independently crossed to the same number of *yw ; esgGal,UASGFP/CyO* virgins. Progeny of the cross were collected 2 days after hatching and allowed to mate in bottles for 3 days. Females and males were then separated and transferred to cages (50-100 flies/cage). Food was changed every 3 days, and the number of dead flies was recorded. Survival curves were analyzed using SAS JMP7 statistical software and the log-rank test.

Supplementary Figure Legends

Figure S1: Increased numbers of esg$^+$ cells in aging flies.

A. Control shows endogenous β-galactosidase activity in the anterior midgut (A: anterior, P: posterior) of wild-type (OreR) flies.

B. ISCs and EBs labeled by XGal staining in flies carrying a P element insertion into the *esg* locus (P{lacW}esg^{k00606}).

C. ISCs and EBs labeled by XGal staining in flies carrying a transgenic esg-lacZ reporter construct (P{esg-LacZ C4-1}).

Figure S2: Tissue degeneration in the aging *Drosophila* gut.

A. Confocal images of aging guts from flies expressing GFP in ISCs and EBs (genotype: w^{1118}; esgGal4, UASGFP) showing age-related changes in the morphology of esg+ cells. ISCs and EBs are labeled by GFP expression (esgGal4>UASGFP, green) in the gut of young flies. Cell boundaries are labeled by immunostaining against Armadillo (membrane, red). EE cells are labeled by nuclear *pros* staining (nuclear, red).

B. Similar phenotype was observed in old wild-type flies. Confocal images of young (3d) and old (40d) guts from OregonR flies. ISCs are identified by the strong armadillo staining (red) and the small size of their nuclei (Hoechst staining, blue). EE cells are labeled by nuclear *pros* staining (red). Cross sections show the loss of tissue organization in the apico - basal (A-B) axis.

Figure S3: esg>GFP expression is rapidly lost in differentiating enterocytes.

esgGal4, UASGFP flies were kept on food containing BrdU for 2 days. Newly formed enterocytes (that have been dividing and/or endoreplicating during this period) are BrdU-positive, but GFP-negative (indicated by an arrowhead), showing that GFP is rapidly degraded in the differentiating cells.

Figure S4: Increased number of cells in the old intestinal epithelium.

Total number of cells and number of enteroendocrines cells in the gut of young (3d) and old flies (40d). The number of cells per area of 10.000μm^2 was determined by confocal microscopy by counting nuclei, stained using Hoechst (left panel), and EEs were identified as prospero positive cells (right panel).

Figures S5: Ultrastructural analysis of the aging intestinal epithelium.

A. Overview of the structure of the intestinal epithelium in young (2 day old) and old (60 day old) flies.

B. Higher magnification images showing the accumulation of cells along the basal membrane in the gut of old flies.

C. High magnification images showing the alteration of the brushed border in the older epithelium (note shorter and disorganized villi).

Figure S6: Oxidative Stress induces proliferation of ISCs and changes in Notch signaling.

A. Guts of 5-day-old esgGal4, UASGFP flies treated with 5mM Paraquat in Sucrose solution (5mM PQ 24h) or plain Sucrose solution (Mock) for 24 hours. Loss of epithelial organization in stressed animals is illustrated by *arm* staining (red). esg-positive cells in one representative cell cluster are indicated by arrowheads.

B. Increased rate of ISCs proliferation is observed in the gut of wild-type (esg>GFP) flies exposed to Paraquat. Flies were fed BrdU for 2 days and then treated with 5mM Paraquat with BrdU in sucrose solution for 48 hours. Arrowheads indicate ISCs that had recently divided.

C. Young flies expressing GFP in ISCs (esg>GFP) exposed to Paraquat (5mM). Note the formation of esg+ cell clusters with ectopic Delta expression. GFP, green; Dl, red; DNA, blue.

D. Young flies carrying a reporter for Notch activity (genotype: esgGal4, UASGFP; Su(H)-GBE-lacZ/+) exposed to Paraquat (5mM). Note the formation of esg+ cell clusters with ectopic Notch activity. GFP, green; lacZ, red; DNA, blue

E,F. Posterior midguts of young flies exposed to 5mM Paraquat for 48 hours or to the carrier alone (mock). Immunostaining against Delta (red) identifies ISCs and expression of the Notch reporter (Su(H)-GBE-lacZ; green) identifies EBs in control flies (E). In Paraquat-treated animals, cell clusters containing cells that express both *delta* and β-galactosidase can be observed (F).

Figure S7: Over-expression of Hep using esgGal4 leads to shortening of lifespan.

A. Representative survival curves of fly populations at 25^{o}C on standard yeast and molasses - based food. esgGal4>Hep males (top panel) and females (lower panel) show significant lifespan reduction compared to their wild-type counterparts.

B. Lifespan analysis of the same fly populations.

Figure S8: Notch restricts Hep-mediated ISC proliferation.

A. Confocal images of the posterior midgut of flies expressing Hep and Notch^{RNAi} in ISCs and EBs using the TARGET system. Cross sections show that, after 5 days of induction at 29ºC, ISCs massively accumulate in the apicobasal axis. green: GFP; blue: DNA.

B. These cells also express high level of delta protein (detected by immunostaining, red), further confirming their ISC identity. Higher magnification images of the boxed areas for esgG80>Hep and esgG80>N^{RNAi};Hep show the differences in morphology of esg+ cells between these two genetic conditions. Note that tumors of cells co-expressing Hep and N^{RNAi} appear as early as 2 days after transfer to 29^{o}C.

Figure S9: Delta/Notch signaling restricts Paraquat-induced stem cell proliferation.

Flies were fed BrdU for 2 days in a sucrose solution supplemented or not with 5mM paraquat. Over-expression of Delta restricts stress-induced ISC proliferation and mis-

differentiation of their daughter cells compared to control flies, whereas expression of N^{RNAi} exacerbates the paraquat-induced loss of epithelial organization.

Figure S10: Inhibition of gamma-secretase using DAPT inhibits Hep-induced mis-differentiation.

esgGal4,UASGFP/UASHep;tubGal80^{ts} flies were fed food supplemented with 0.5mM DAPT at 29^{o}C for 2 days. DAPT treatment leads to either reduction of JNK-induced mis-differentiation or to the formation of ISC and EE tumors.

Figure S11: JNK signaling is activated in stressed and aging Delta heterozygotes flies.

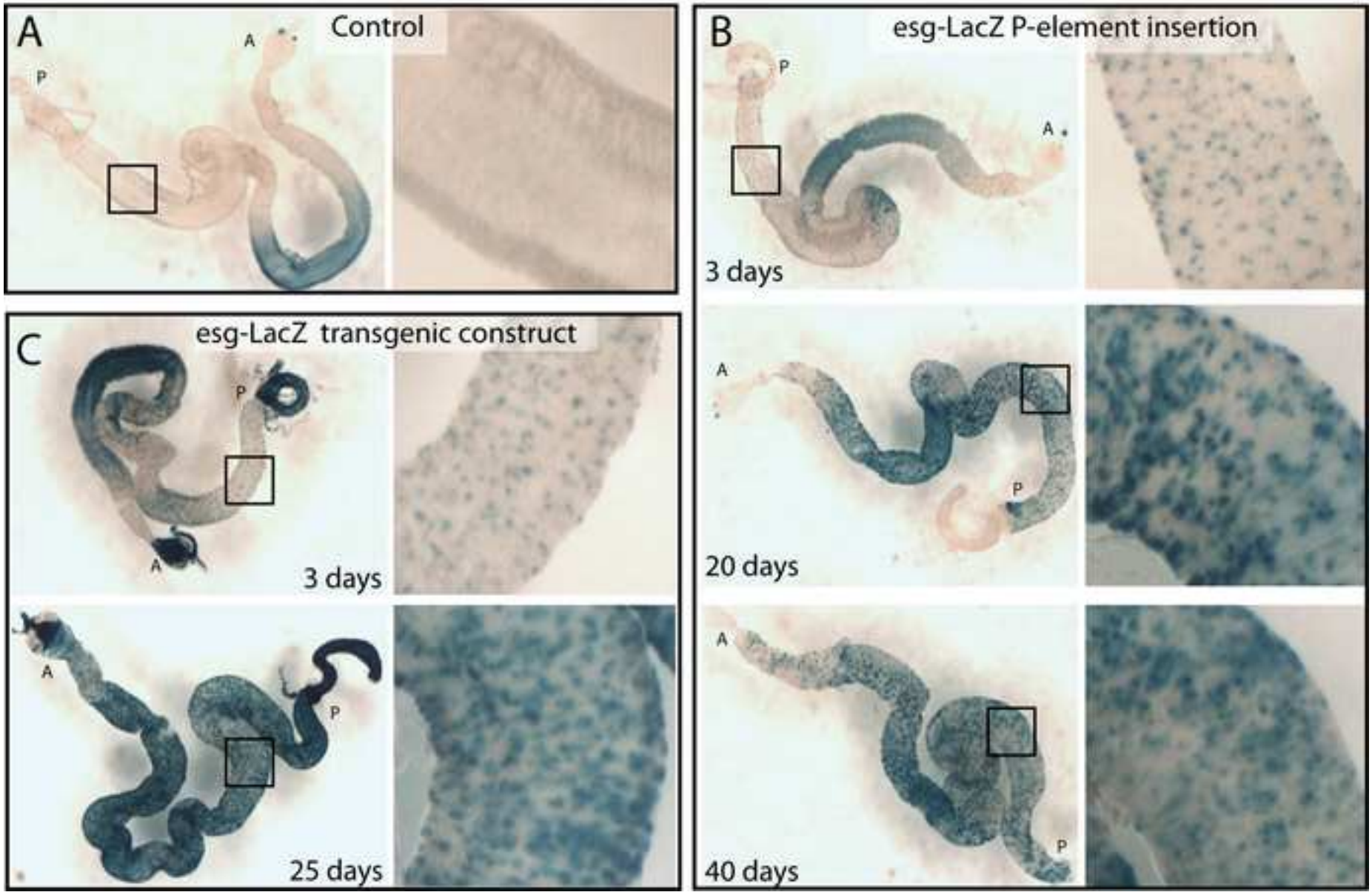
A, B. puc^{E69}/+ and puc^{E69}/dl^{7} flies were exposed to 0.5mM Paraquat for 24h (A) or aged for 20 days (B). Similar β-galactosidase expression levels were observed in *dl*^{7} heterozygotes and control flies. Also note the absence of tissue degeneration in the 20-day-old puc^{E69}/dl^{7}, as seen using the armadillo immunostaining, similar to the protection observed in *Dl*^{05151}/+ flies (Fig.6D).

Figure S12: Interaction between JNK signaling and Notch-mediated proliferation and differentiation

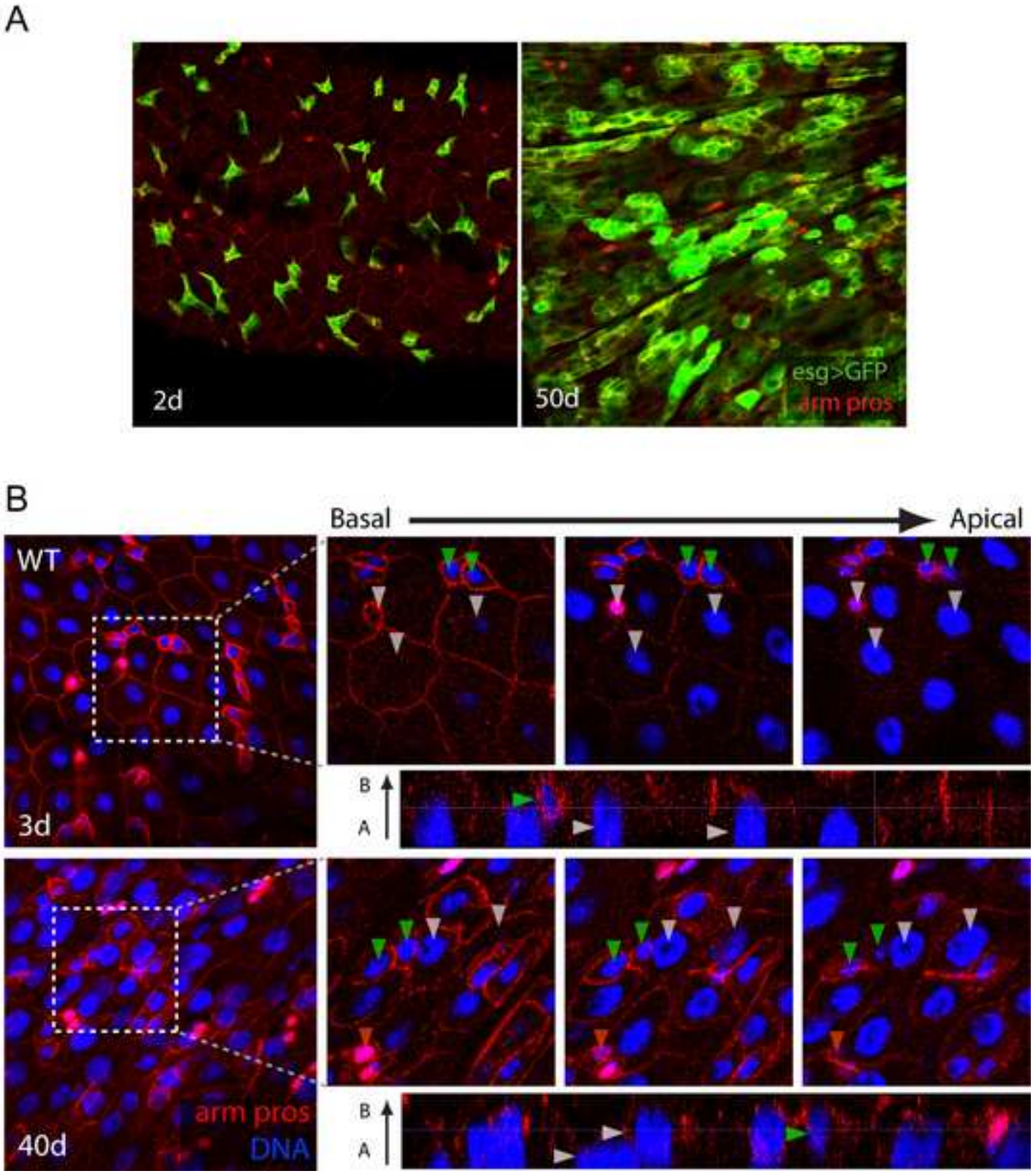
A. Expression of Notch Intra-Cellular Domain (NICD) using esgGal4 induces differentiation of ISCs into ECs, resulting in ISC loss, as described before (Ohlstein and Spradling 2007). Co-over-expression of Hep in this background doesn't change the fate of ISCs.

B. Co-expression of N^{RNAi} and Bsk^{DN} leads to increased proliferation and formation of ISC and EE tumors, similar to the ones observed when N^{RNAi} is expressed alone, showing that JNK signaling is not required for normal cell division or EE differentiation in Notch loss-of-function conditions.

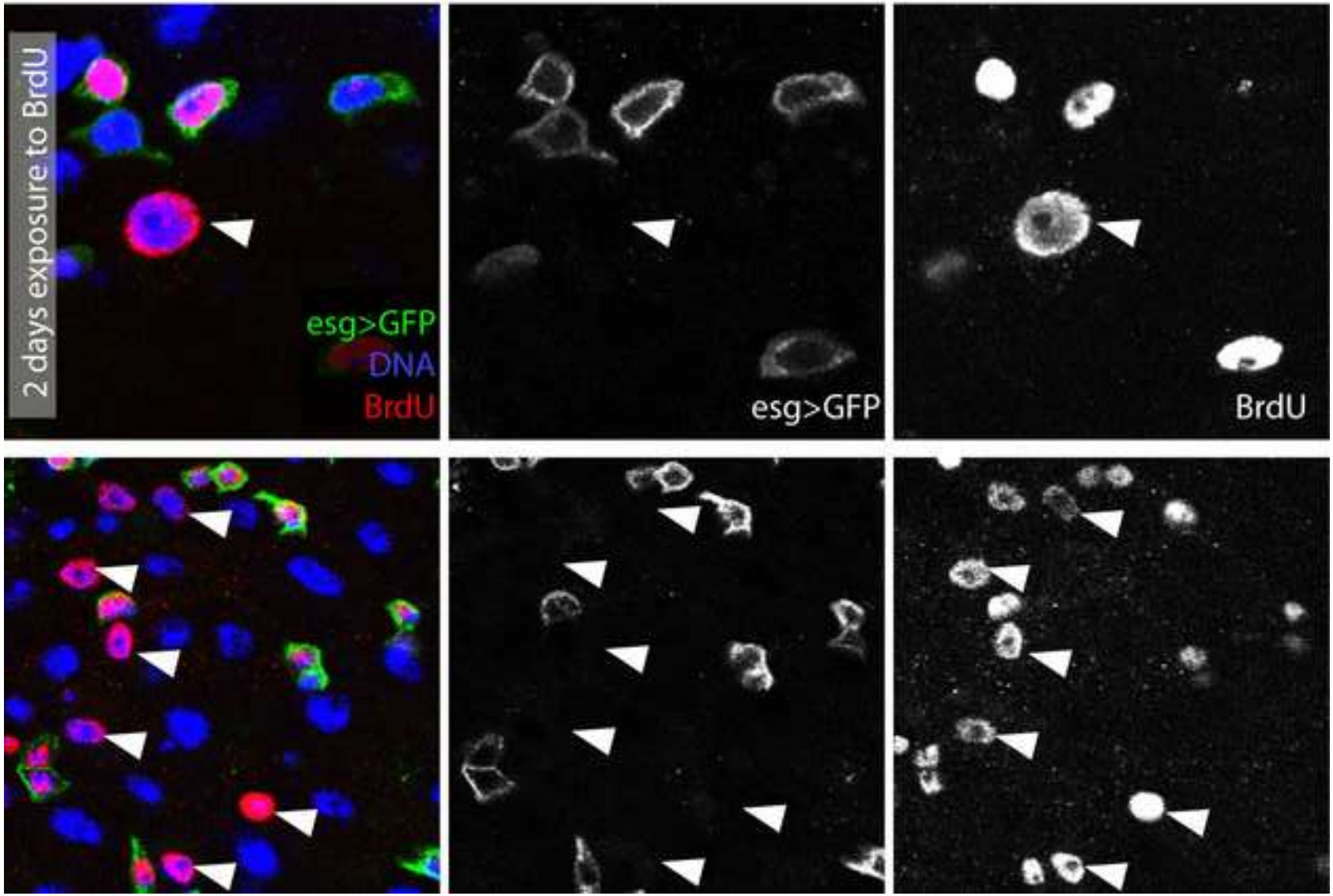
Biteau et al., Supplementary Figure 1



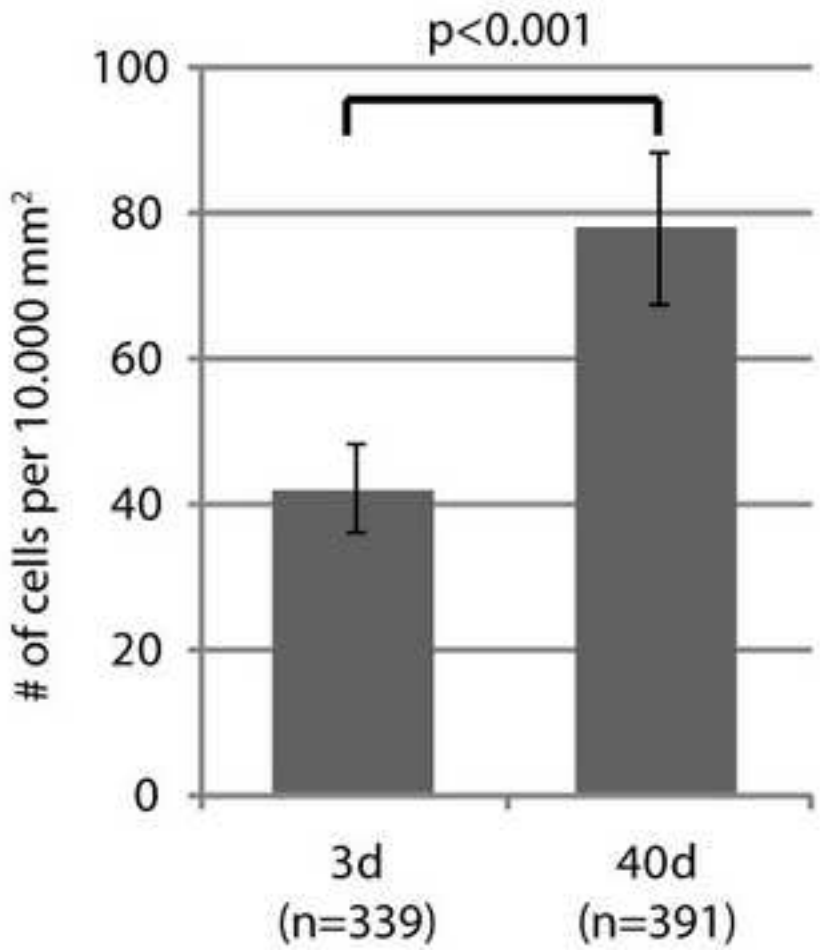
Biteau et al., Supplementary Figure 2

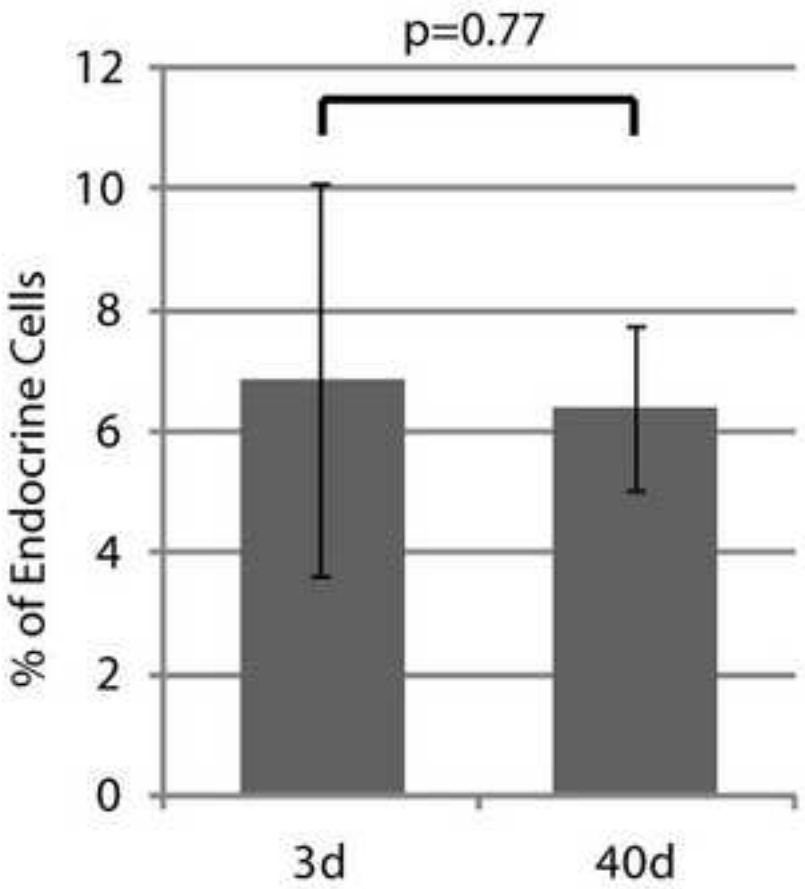


Biteau et al., Supplementary Figure 3

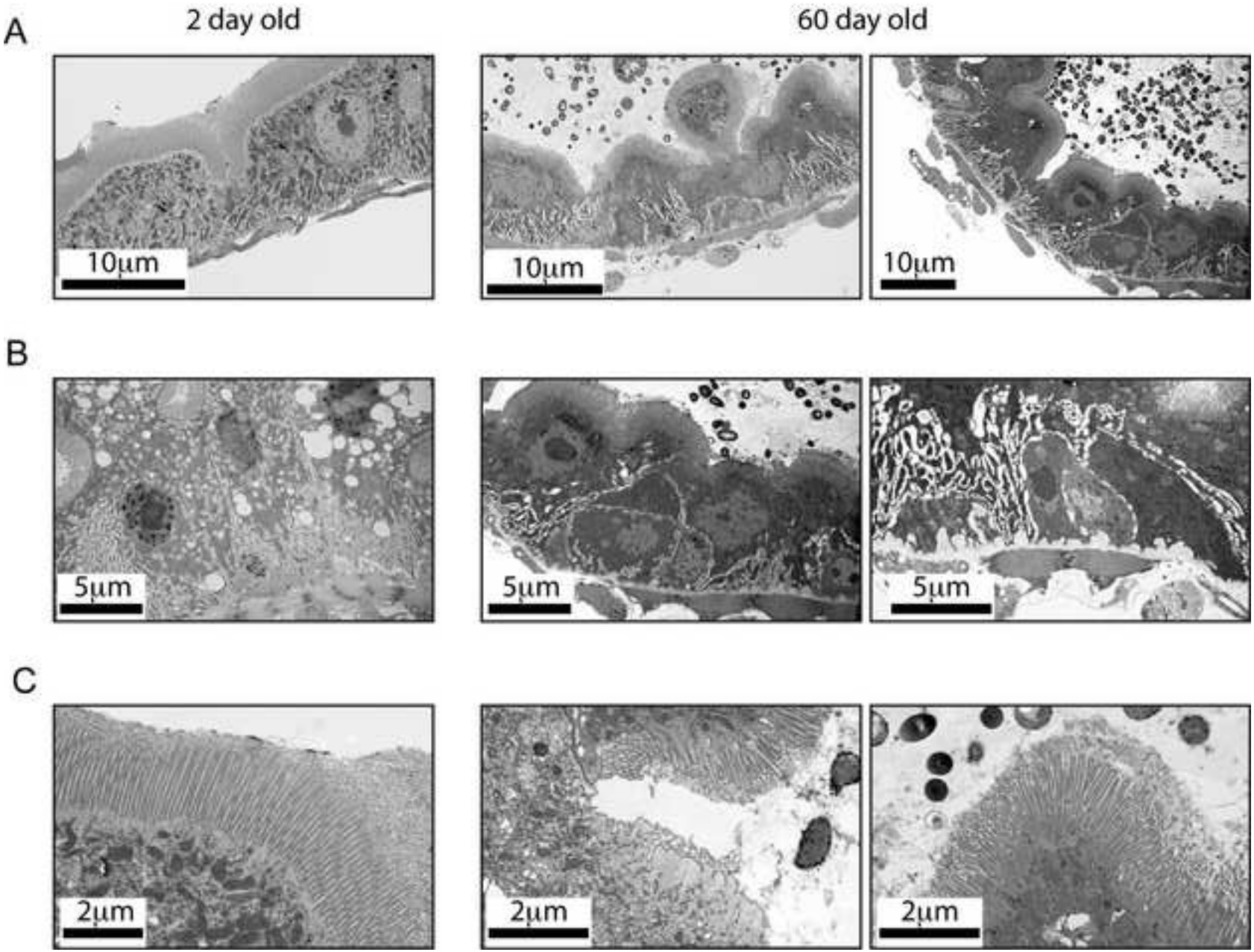


Biteau et al., Supplementary Figure 4

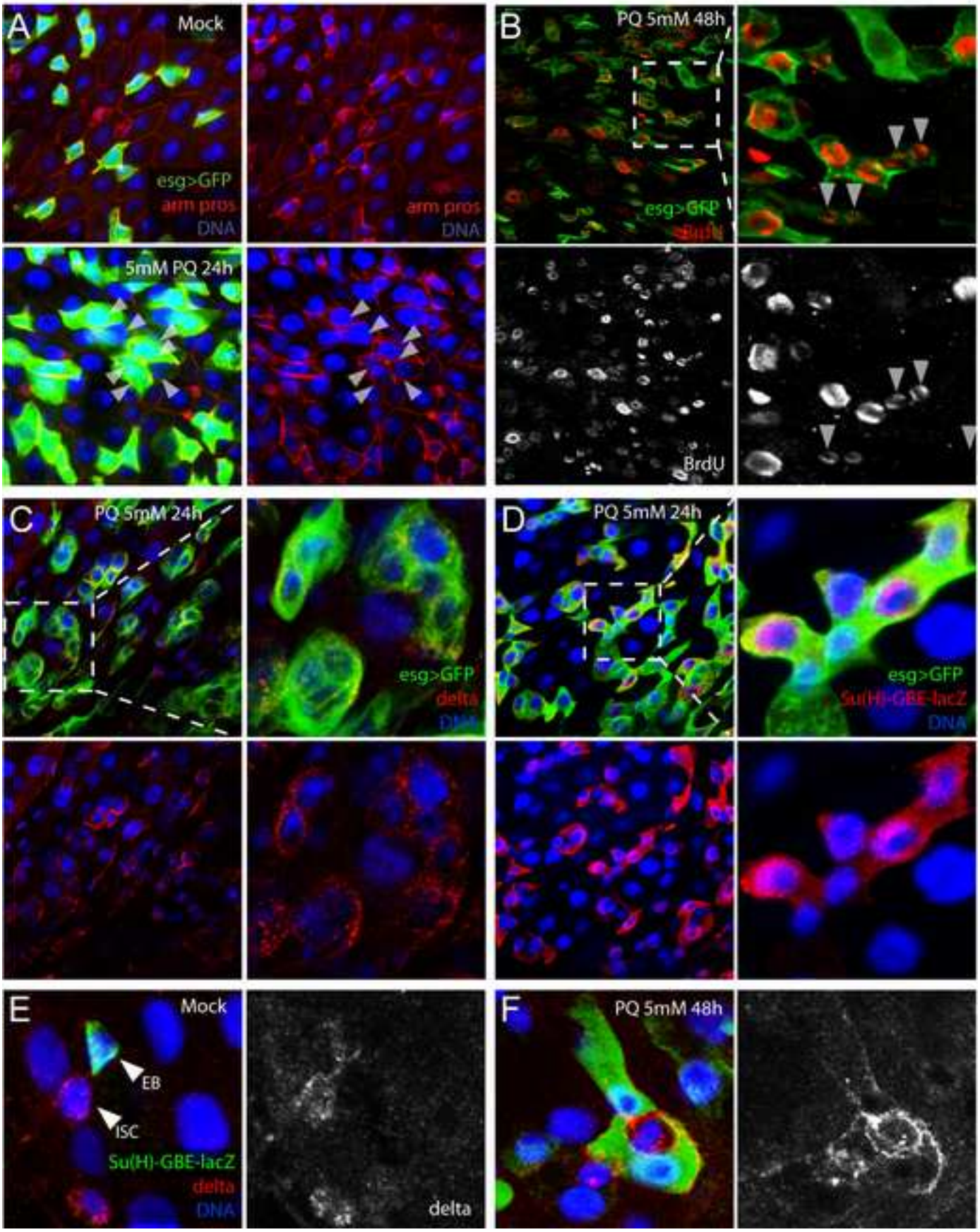




Biteau et al., Supplementary Figure 5

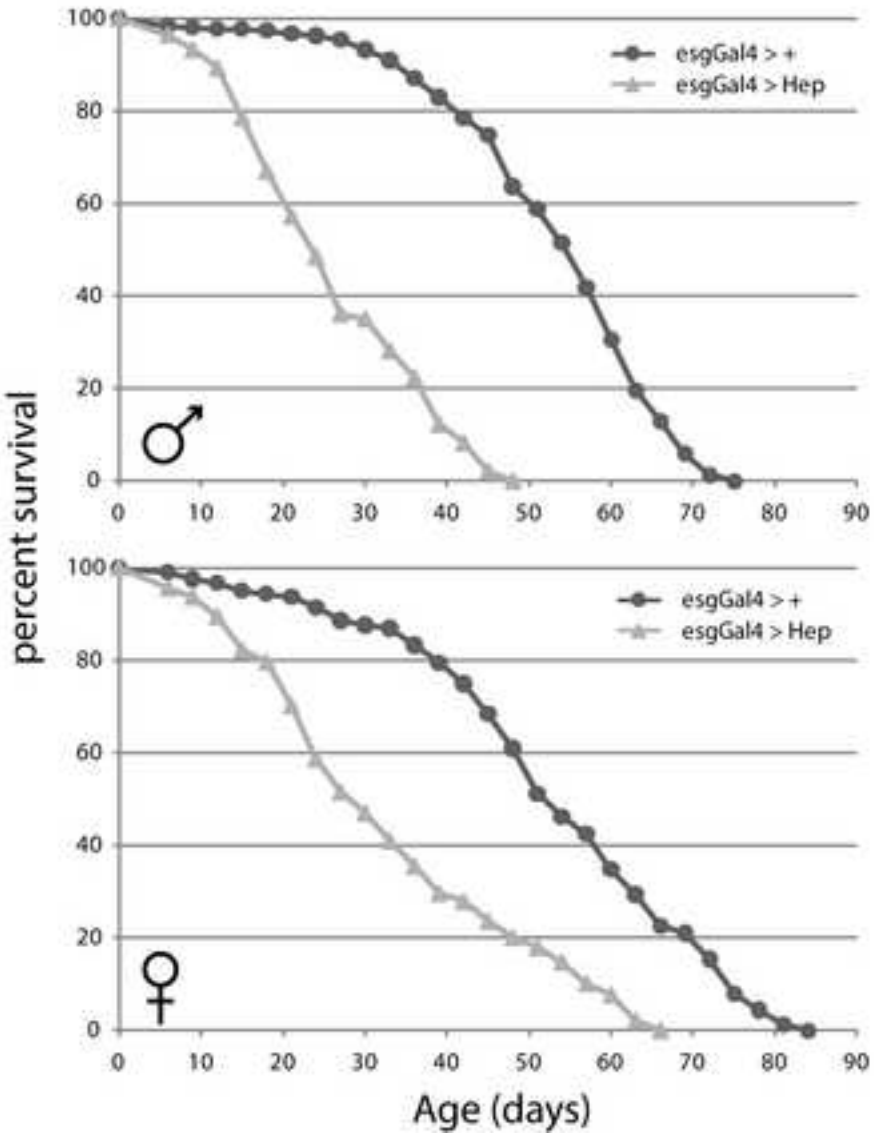


Biteau et al., Supplementary Figure 6



Biteau et al., Supplementary Figure 7

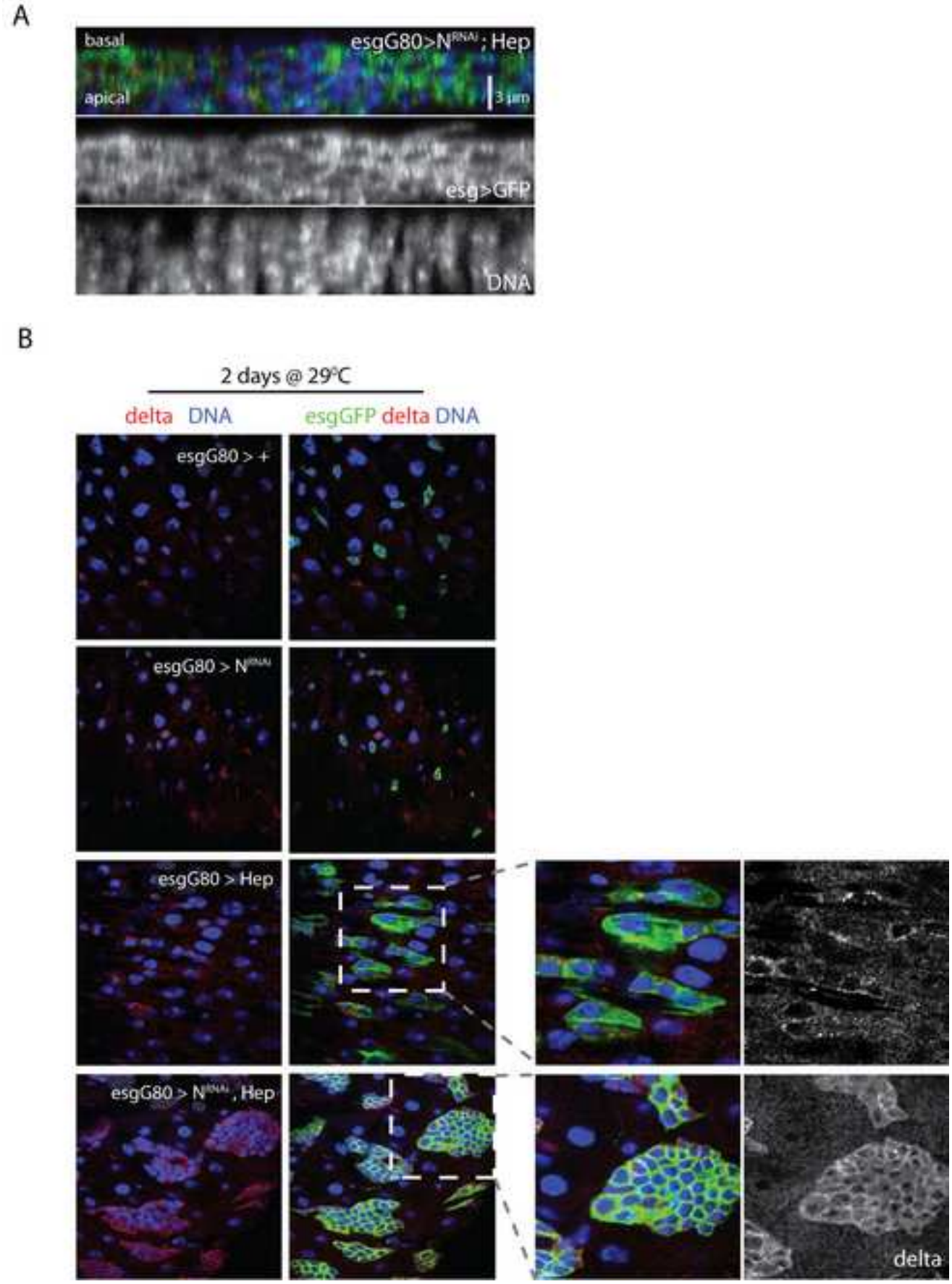
A



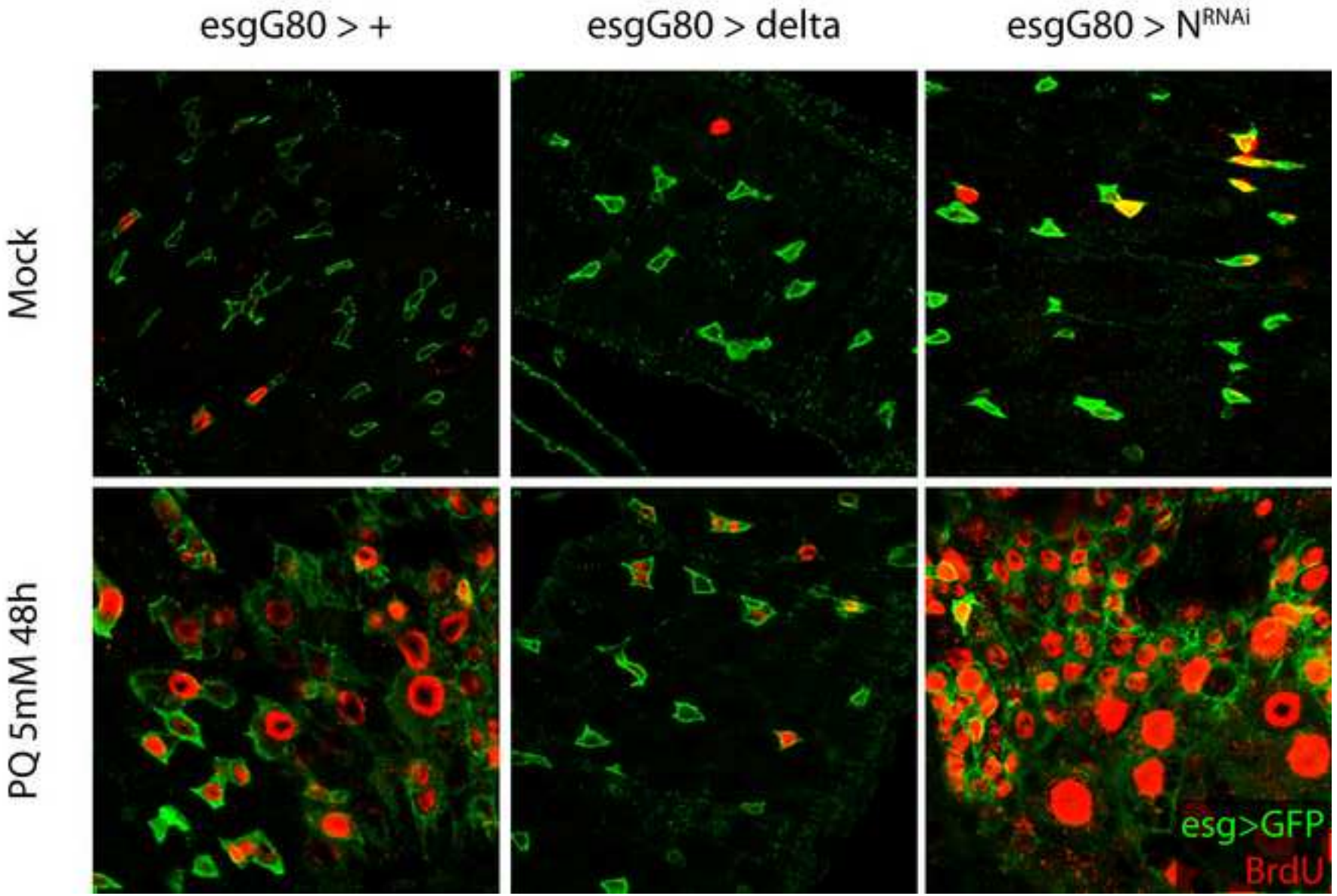
B

		Mean lifespan (days)	n	Log rank X^2	p-Value
♂	esgGal4 > GFP	52.1	291	367.0	<0.0001
	esgGal4 > GFP, Hep	25.7	153		
♀	esgGal4 > GFP	51.4	262	54.7	<0.0001
	esgGal4 > GFP, Hep	38.4	189		

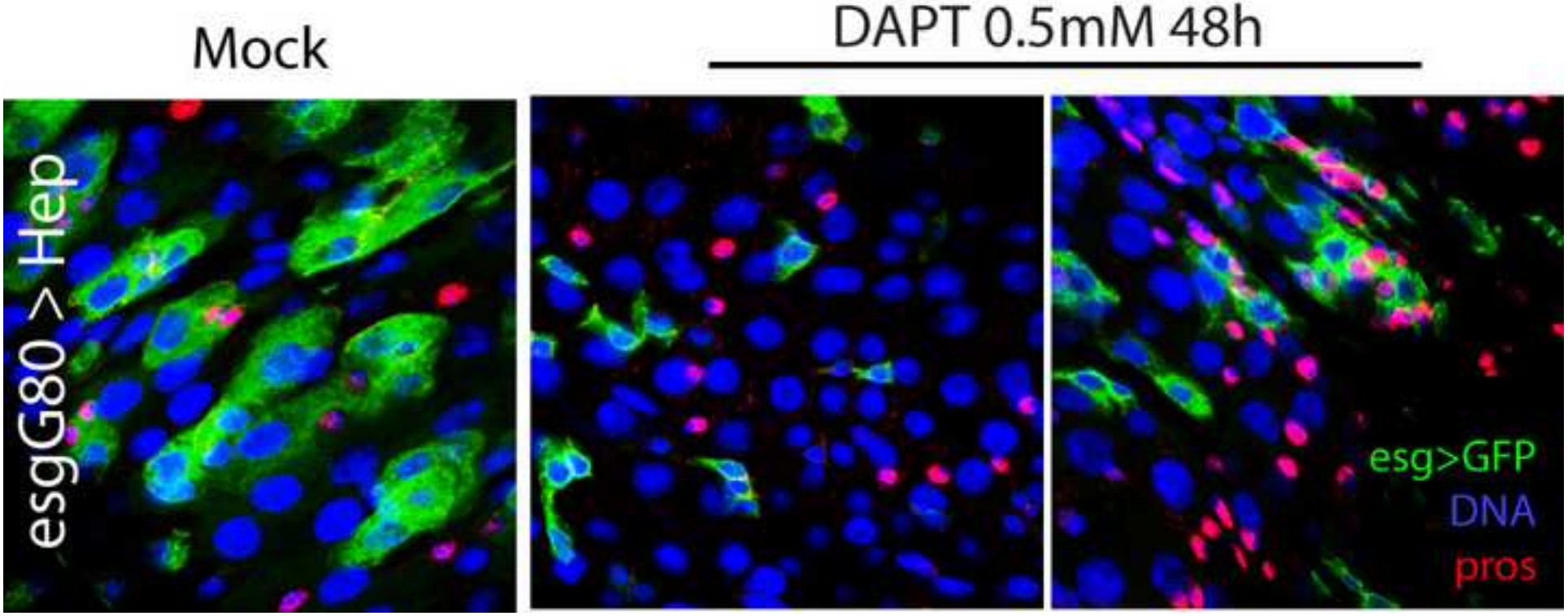
Biteau et al., Supplementary Figure 8



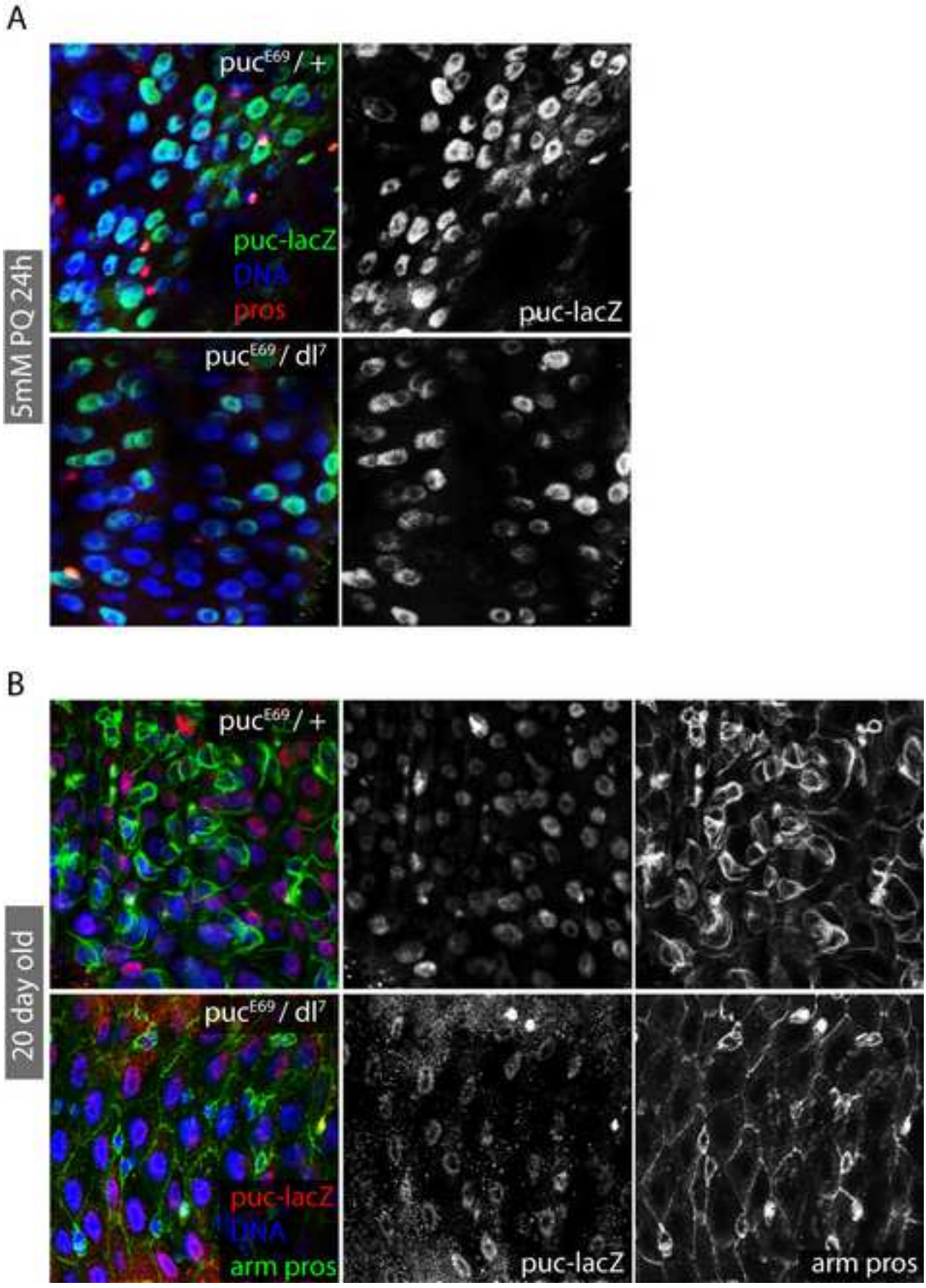
Biteau et al., Supplementary Figure 9



Biteau et al., Supplementary Figure 10



Biteau et al., Supplementary Figure 11



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