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Supplemental Information

Sox100B Regulates Progenitor-Specific Gene Expression and Cell Differentiation in the Adult *Drosophila* Intestine

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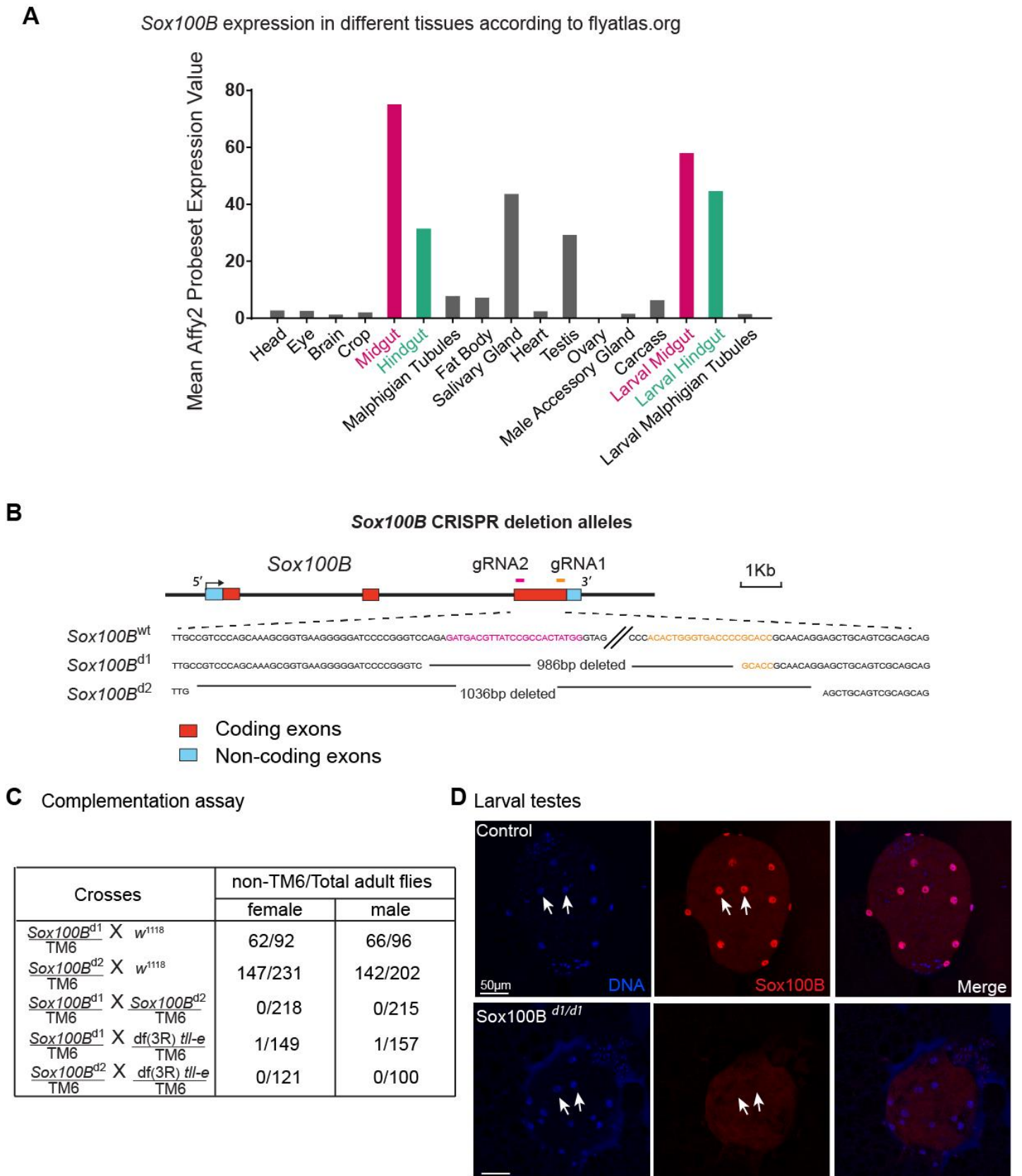


Figure S1. Expression pattern of *Sox100B* and validation of *Sox100B* CRISPR mutant alleles, Related to Figure 1.

(A) *Sox100B* mRNA expression profile among various tissues. Expression data are from FlyAtlas, and expression profile in adult midgut and larval midgut are highlighted in red.

(B) Schematic diagram showing the generation of *Sox100B^{d1}* and *Sox100B^{d2}* mutant alleles using double gRNAs-mediated deletion using the CRISPR/Cas9 method. Sizes of deleted regions are shown in the diagram.

(C) Lethality assay performed by counting non-TM6 surviving adult flies both in female and male F1 progenies for the indicated crosses.

(D) Representative images of Sox100B antibody staining in L3 stage larval testes of both wildtype and *Sox100B^{d1/d1}* mutant flies. Pigment cells are indicated by arrows.

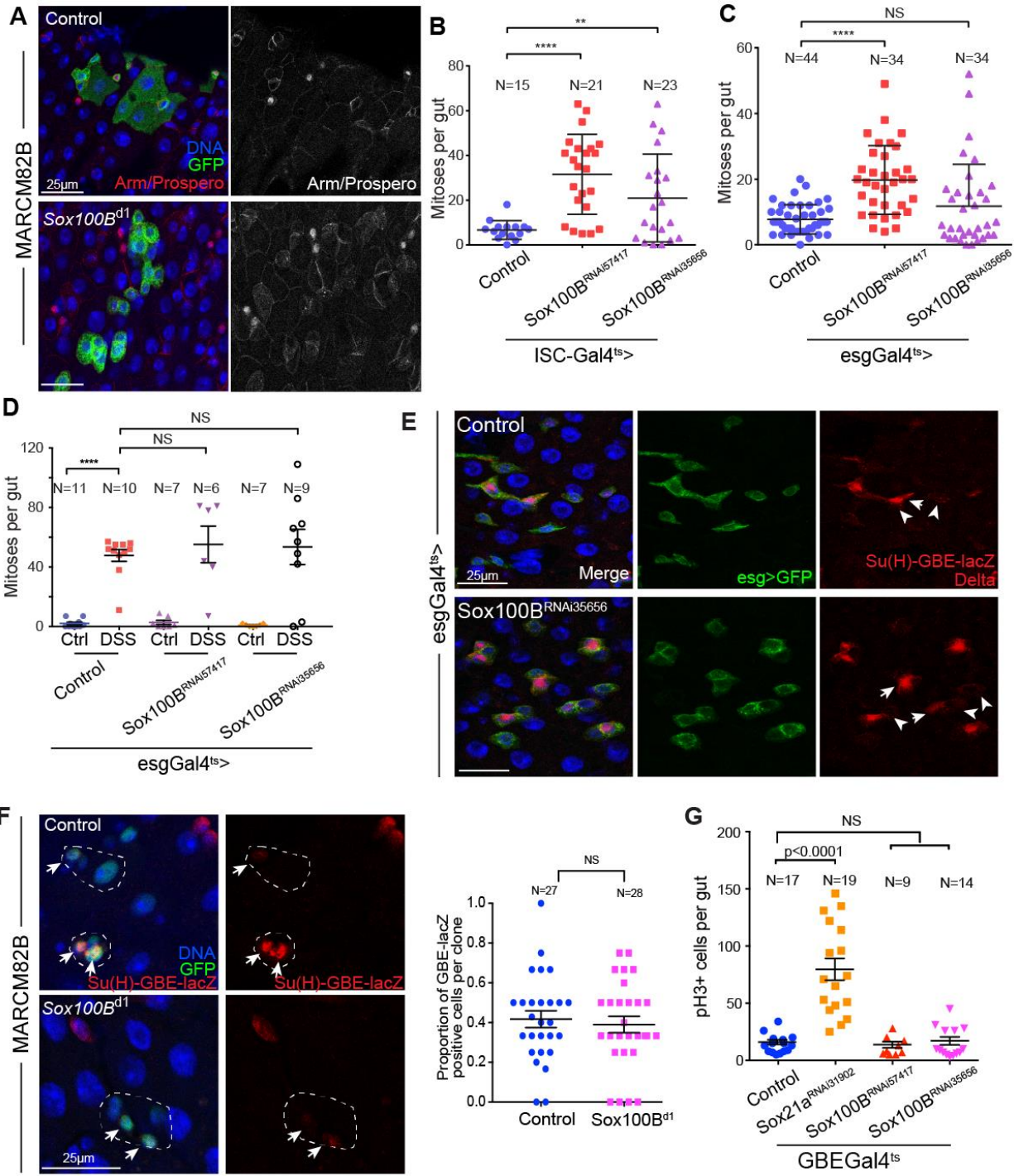


Figure S2. Sox100B is not required for ISC maintenance/proliferation, but is required for ISC differentiation, Related to Figure 2.

(A) Confocal images of Armadillo (Arm) staining to outline cell size in control and Sox100B^{d1} MARCM clones. Arm and Prospero staining are distinguished by cellular localization (Arm, membrane staining; Prospero, nuclear staining).

(B) Quantification of pH3-positive mitotic cells in control and ISC-specific Sox100B^{RNAi}-expressing guts 7days after induction.

(C) Quantification of pH3-positive mitotic cells in control and ISC/EB-specific Sox100B^{RNAi}-expressing guts 7days after induction.

(D) Quantification of pH3-positive mitotic cells in control and ISC/EB-specific Sox100B^{RNAi}-expressing guts in response to 2day DSS treatment.

(E) Representative confocal images of posterior midguts in control and ISC/EB-specific Sox100B^{RNAi}-expressing flies. Note that the Notch reporter Su(H)-GBE-lacZ (cytoplasmic staining, indicated by white arrows) labelled EBs seems largely unaffected and the typical nested pattern of ISC/EB pairs are maintained after knocking down *Sox100B* in both ISCs and EBs. ISCs are identified by membrane Delta staining (indicated by white arrowheads).

(F) Representative images and quantification of Su(H)-GBE-lacZ positive EBs (indicated by white arrows) in control and Sox100B^{d1} homozygous MARCM clones 4days after clone induction.

(G) Quantification of pH3-positive mitotic cells in EB-specific Sox100B^{RNAi} and Sox21a^{RNAi} expressing guts 10days after induction. EB-specific *Sox100B* knockdown does not lead to high ISC proliferation, while *Sox21a* knockdown leads to drastic ISC proliferation which underlies the tumor formation phenotype observed when depleting *Sox21a* in EBs.

In B-D, F and G, values are presented as average \pm s.e.m and p-values are calculated using unpaired two-tailed Student's t-test, ** p<0.01; *** p<0.001; **** p<0.0001.

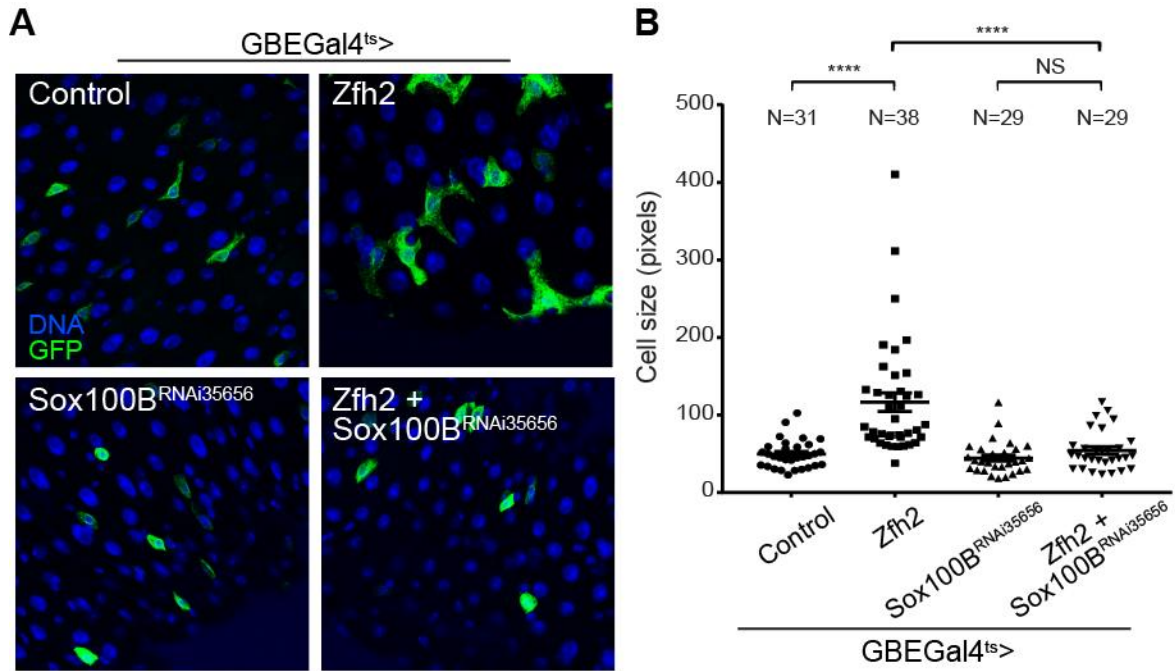


Figure S3. Sox100B is required for zfh2-mediated EB cell growth, Related to Figure 2.

(A-B) Representative images showing cell size of EBs and quantification of individual EB for the indicated genotypes. GBEGal4-driven GFP expression is used for quantifying cell size. Note that Zfh2-mediated EB size increase is abolished when Sox100B is knocked down.

In A-B, values are presented as average \pm s.e.m and p-values are calculated using unpaired two-tailed Student's t-test, ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

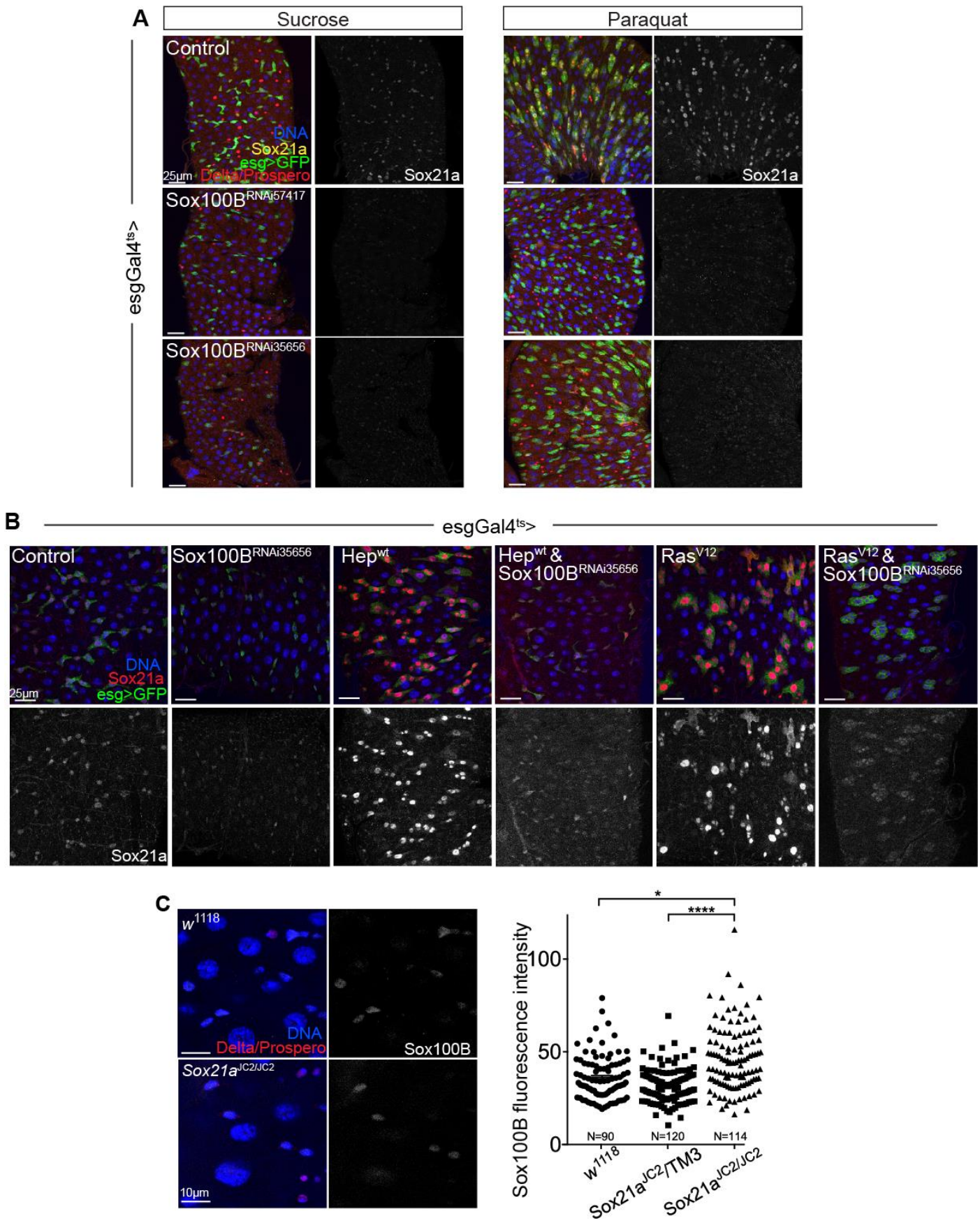


Figure S4. Sox100B is required for stress-induced Sox21a expression, Related to Figure 3.

(A) Representative confocal images of posterior midgut showing that Paraquat-mediated Sox21a induction is strongly impaired after 7day Sox100B knocking down in ISCs and EBs.

(B) Representative confocal images of posterior midgut showing that JNKK/Hep and Ras^{V12}-mediated Sox21a induction is strongly impaired in *Sox100B*^{RNAi} expressing ISCs and EBs. Transgene expression is induced for 24hour and guts are then dissected for Sox21a staining.

In A-B, *esgGal80^{ts}* is used to knock down *Sox100B* specifically in ISCs and EBs, with or without overexpression of JNKK/Hep or an active form of Ras (Ras^{V12}).

(C) Quantification of Sox100B fluorescence intensity in 5day old *Sox21a*^{JC2} null mutant flies indicates that Sox21a is not required for Sox100B expression. p-values are calculated using unpaired two-tailed Student's t-test, ** p<0.01; **** p<0.0001.

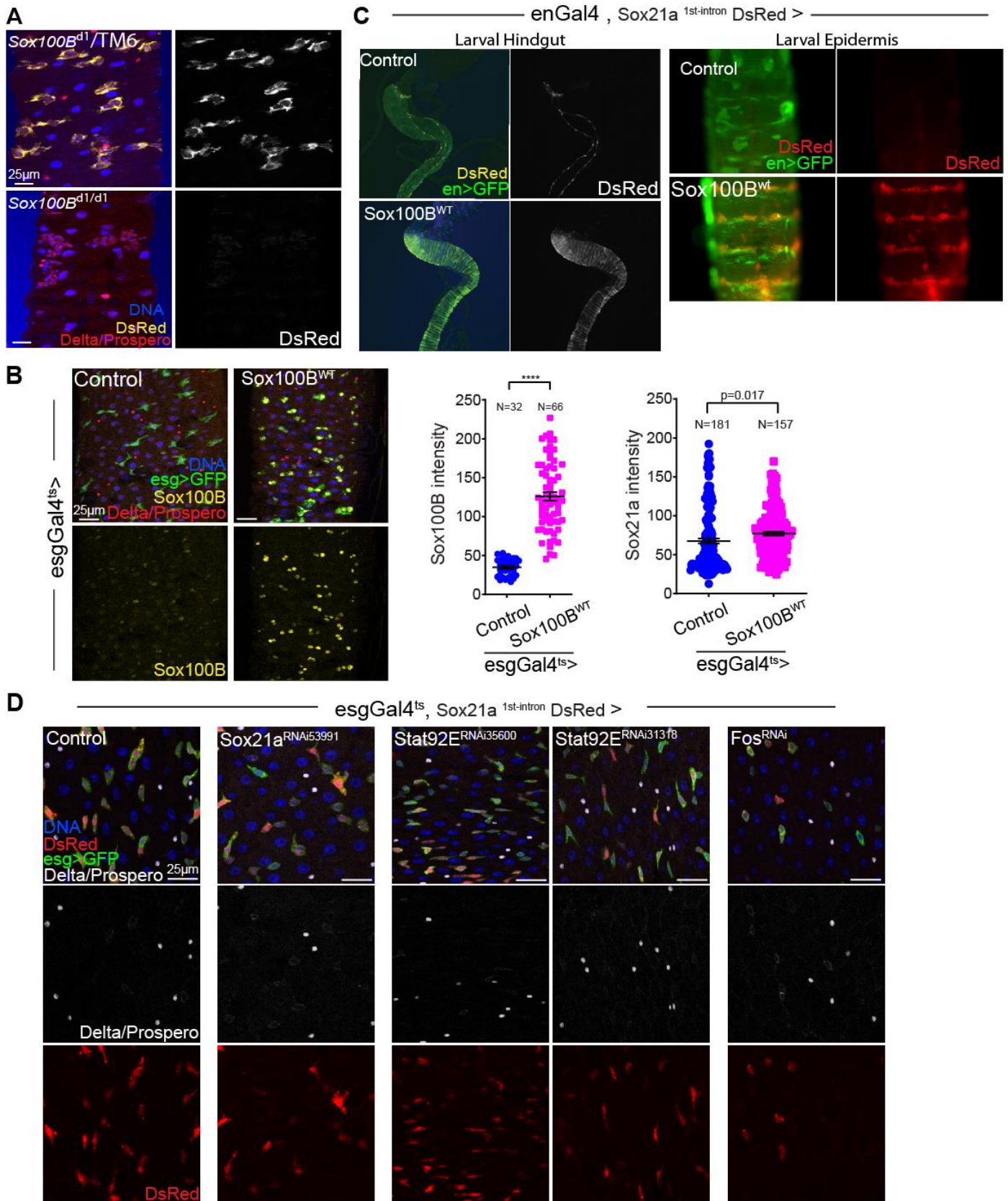


Figure S5. Additional characterization of the Sox21a^{1st-intron} DsRed reporter, Related to Figure 4.

(A) Representative images Sox21a^{1st intron} DsRed reporter expression in L3 stage larval posterior midguts of Sox100B^{d1} heterozygous and homozygous mutant flies.

(B) Validation of UAS-Sox100B^{WT} transgene when Sox100B is specifically expressed in adult ISCs and EBs using the esgGal4^{ts} driver for 24h. Sox100B immunostaining intensity is quantified in esg>GFP-positive cells. Quantification of Sox21a in Sox100B over-expressing ISCs/EBs shows that Sox21a expression is minimally induced under these conditions.

(C) DsRed reporter expression in L3 stage wildtype and Sox100B-overexpressing larval hindgut and larval epidermis. Note that DsRed reporter is only expressed in the boundary cells in the control hindgut while detected in the entire enGal4-driven Sox100B-expressing domain. Ectopic reporter expression is detected in enGal4-driven Sox100B-expressing domain of epidermis.

(D) Representative confocal images of adult posterior midguts showing that Sox21a^{1st intron} DsRed is not affected when *fos*, *Stat92E* and *Sox21a* was knocked down using the ISC/EB-specific esgGal4^{ts} driver.

In A, B and D, Delta and Prospero staining are distinguished by cellular localization (Delta, membrane staining; Prospero, nuclear staining). In B, values are presented as average \pm s.e.m and p-values are calculated using unpaired two-tailed Student's t-test, **** p<0.0001.