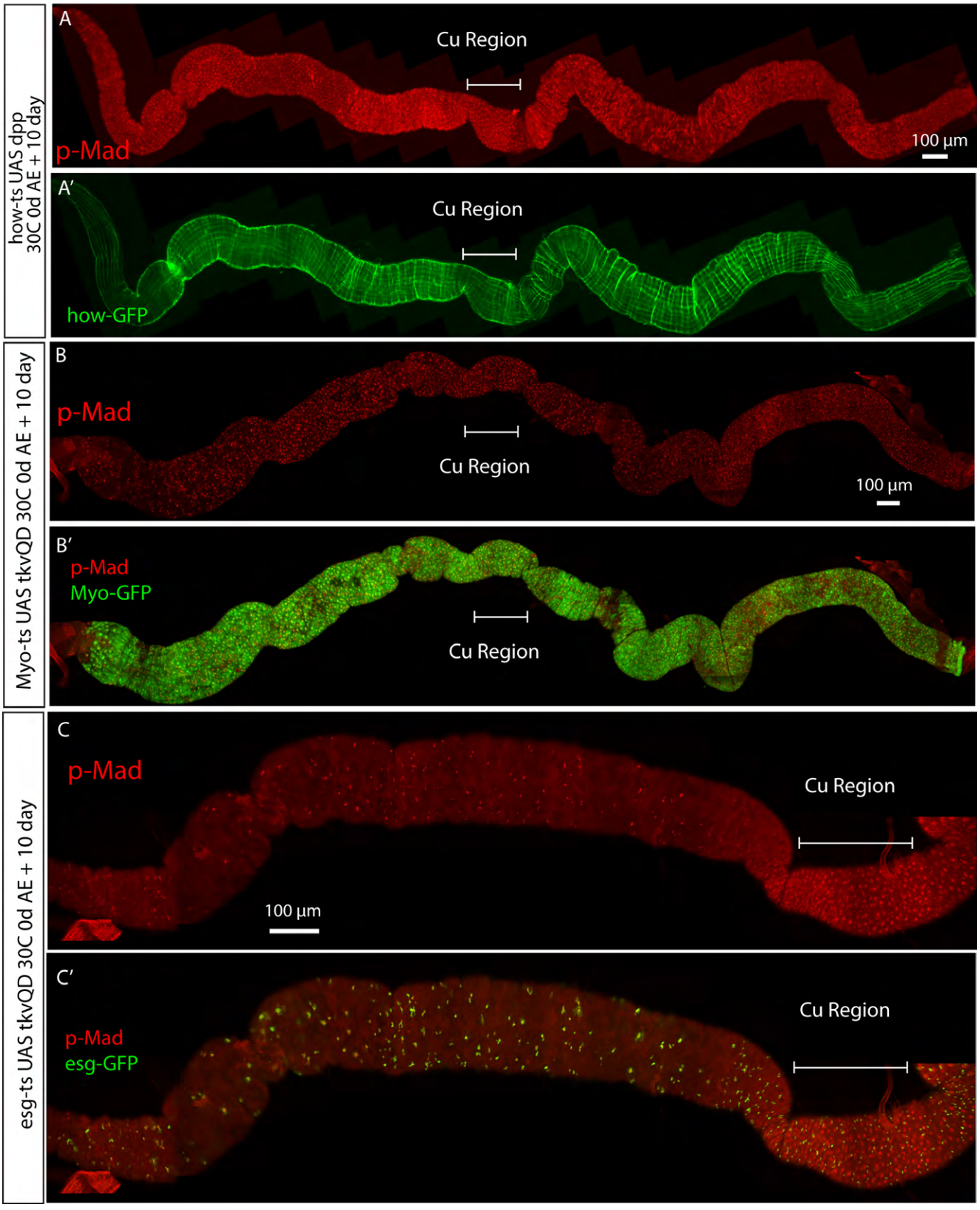

Figure S1. BMP pathway activation in the adult results in p-Mad staining in each cell type. (A-A') *how*^{ts} UAS-*dpp* UAS-GFP flies were kept at 30°C for 10 days after eclosion. p-Mad staining (red) is present in all cell types throughout the midgut. GFP (green) is expressed in the visceral muscle and trachea throughout the midgut. **(B-B')** *Myo*^{ts} UAS-dpp UAS-GFP flies were kept at 30°C for 10 days after eclosion. p-Mad staining (red) is present in enterocytes (ECs) throughout the midgut. GFP (green) is present in most ECs in the midgut. **(C'C')** *esg*^{ts}-UAS-*dpp* UAS-GFP flies were kept at 30°C for 10 days after eclosion. p-Mad staining (red) is present in ISCs/EBs throughout the midgut in addition to the copper cells. GFP is present in most ISCs/EBs in the midgut. For each image anterior is to the left.

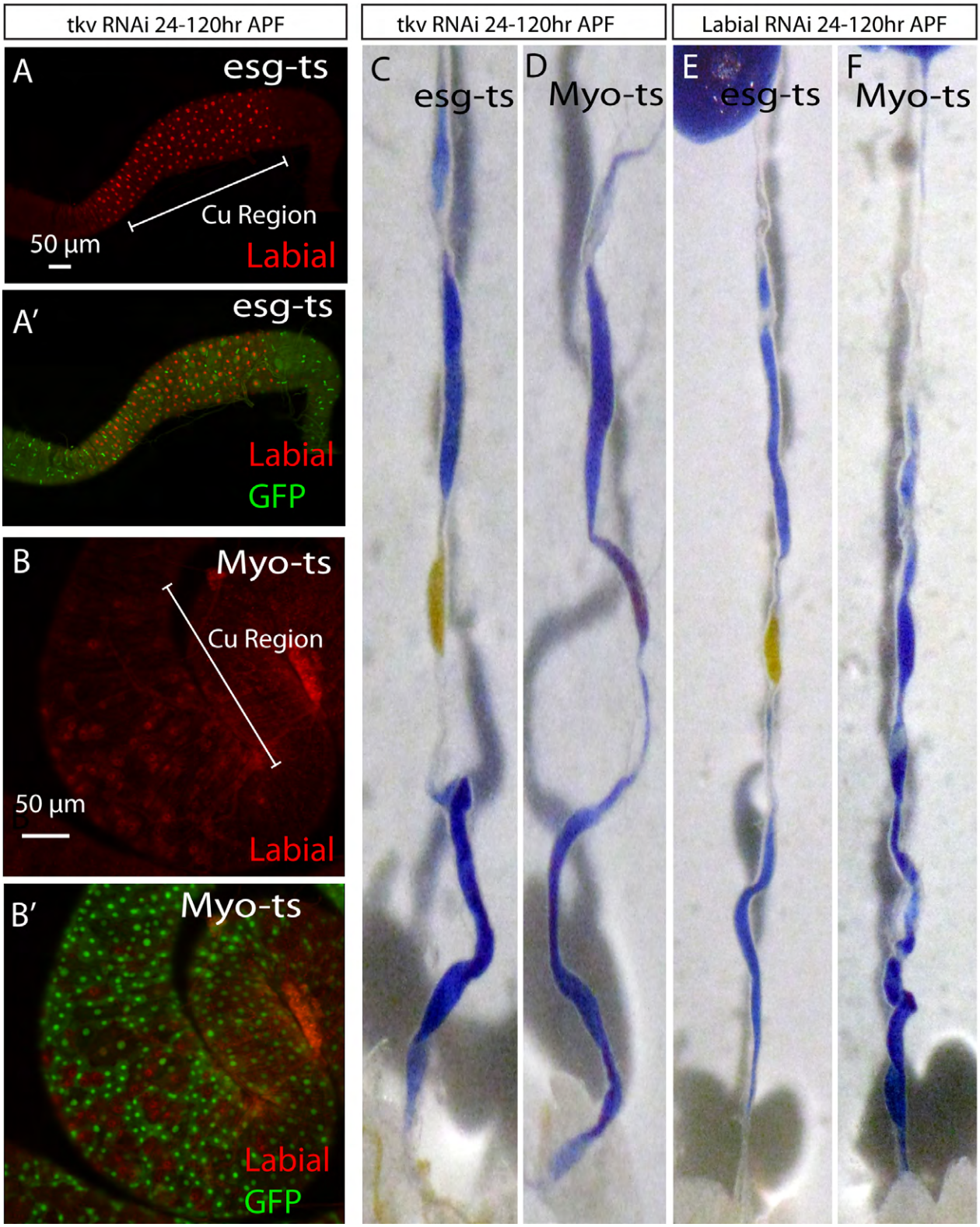

Figure S2. Activation of BMP during pupation by specific Gal4 lines. (A-A') Intestine from a *how*^{ts} UAS-*dpp*, UAS-GFP pupae 24 hours after being moved to 30°C. GFP (green) can be seen in the muscle throughout the pupal midgut. p-Mad staining (red) is visible in all regions of the pupal midgut. **(B)** Intestine from *Myo*^{ts} UAS-GFP pupae 24 hours after being moved to 30°C. GFP (green) is expressed in polyploid cells of the pupal midgut. **(C)** Intestine from an *esg*^{ts}-UAS GFP pupae 24 hours after being moved to 30°C. GFP is expressed in diploid cells scattered throughout the pupal midgut. **(D)** Intestine from *Myo*^{ts} UAS-*tkv*^{QD}, UAS-GFP pupae 24 hours after being moved to 30°C GFP (green) is expressed in polyploid cells of the pupal midgut. **(D')** p-Mad staining is visible in all large cells of the pupal midgut. **(E)** Intestine from an *esg*^{ts} UAS-*tkv*^{QD}, UAS-GFP pupae 24 hours after being moved to 30°C. GFP is expressed in diploid cells scattered throughout the pupal midgut. **(E')** p-Mad staining is visible in all small *esg* positive cells of the pupal midgut. For each image anterior is to the left.

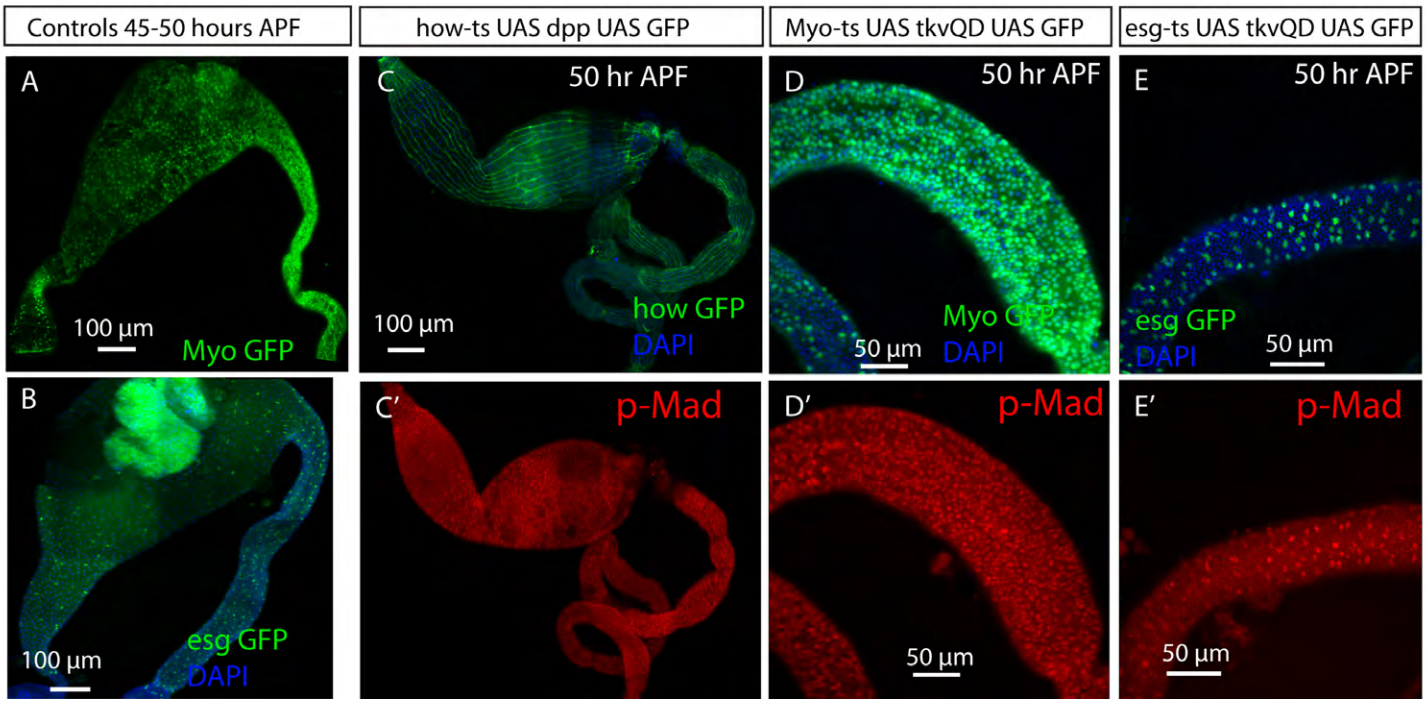

Figure S3. Myo-Gal4 and esg-Gal4 are specific to different cell types during metamorphosis. (A) Intestine from a newly eclosed *esg*^{ts} UAS-*tkv* RNAi, UAS-GFP reared at 30°C between 24 hours APF. GFP (green) is expressed in diploid cells scattered throughout the pupal midgut. **(A')** Labial (red) åis expressed in copper cells. **(B)** Intestine from a newly eclosed *Myo*^{ts}-UAS *tkv* RNAi, UAS-GFP fly reared at 30°C between 24 hours APF and eclosion. The intestine lacks copper cells and Labial (red) is weakly present in the CCR. GFP (green) is expressed in adjacent enterocytes. **(C and E)** Midguts from *esg*^{ts}-UAS tkv RNAi (C) or *esg*^{ts}-UAS *lab* RNAi (E) flies reared at 30°C between 24 hours APF and eclosion. Flies fed bromophenol blue after eclosion show normal acidification of the CCR. (**D and F)** Midguts from *Myo*^{ts}-UAS *tkv* RNAi (D) or *Myo*^{ts}-UAS *lab* RNAi (F) flies reared at 30°C between 24 hours APF and eclosion. Midguts form flies fed bromophenol blue show no evidence of acidification. For each image anterior is to the left.

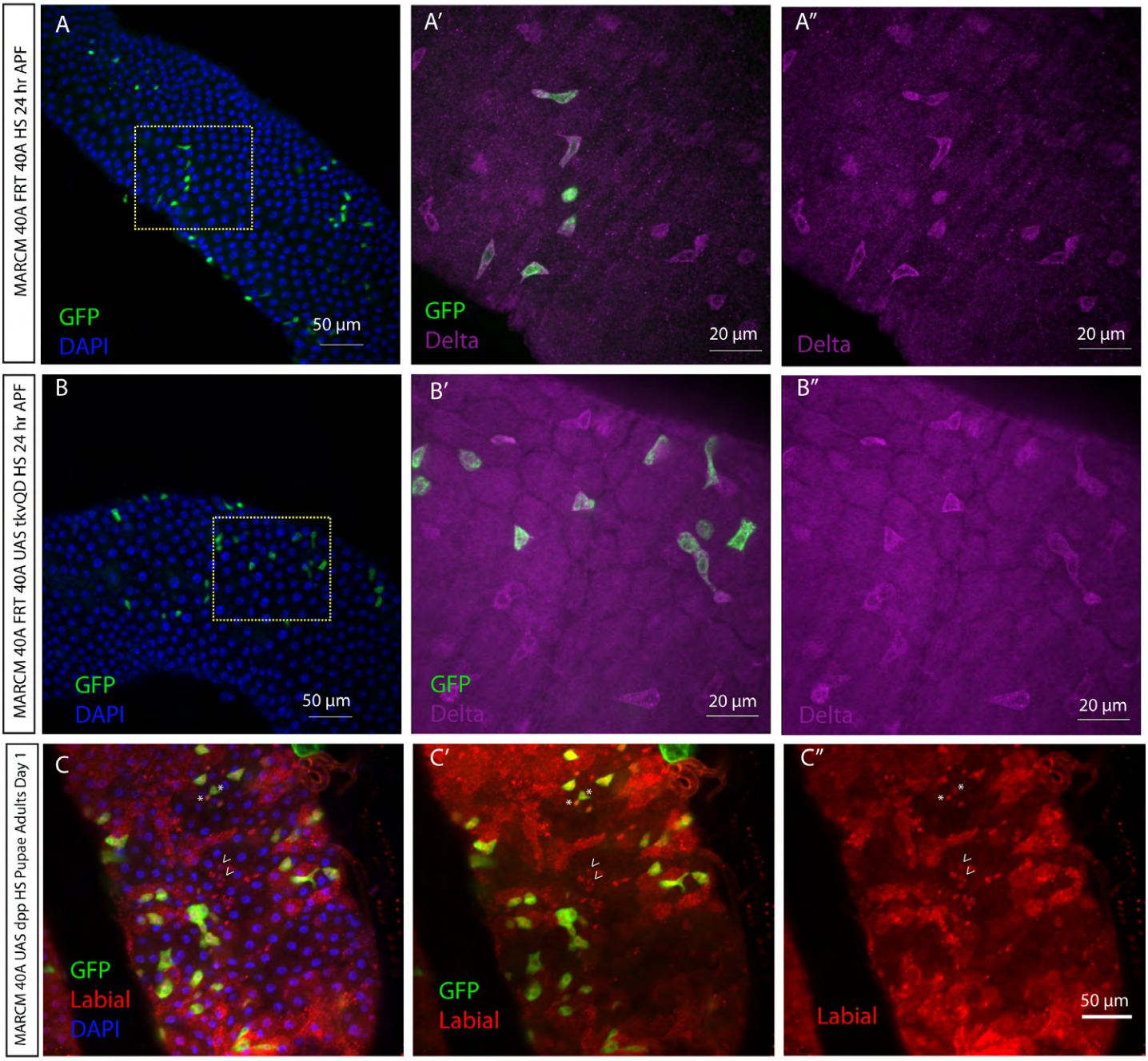

Figure S4. Heat shock of MARCM 40A flies during pupation marks ISCs. (A-A'') MARCM 40A UAS-*tkv*^{QD}, UAS-GFP flies were reared at 18°C and then heat shocked for one hour at 24 hours APF. Upon eclosion, clones, marked by GFP (green), are consist of one or two diploid cells that stain positive for the ISC marker Delta (magenta). **(B-B'')** MARCM 40A UAS-GFP flies were reared at 18°C and then heat shocked for one hour at 24 hours APF. Upon eclosion, clones, marked by GFP (green) consist of one or two diploid cells that stain positive for Delta (magenta). **(C-C'')** MARCM 40A UAS-*dpp*, UAS-GFP flies were reared at 18°C and then heat shocked for one hour at 24 hours APF. Upon eclosion, clones, marked by GFP (green), consist of one or two diploid cells that are nuclear Labial positive (red) (*). Because *dpp* ligand can diffuse outside of the marked cells, GFP negative diploid Labial positive cells (<) are present. . DAPI (blue) marks nuclei in **(A)**, **(B)**, and **(C)**. For each image anterior is to the left.

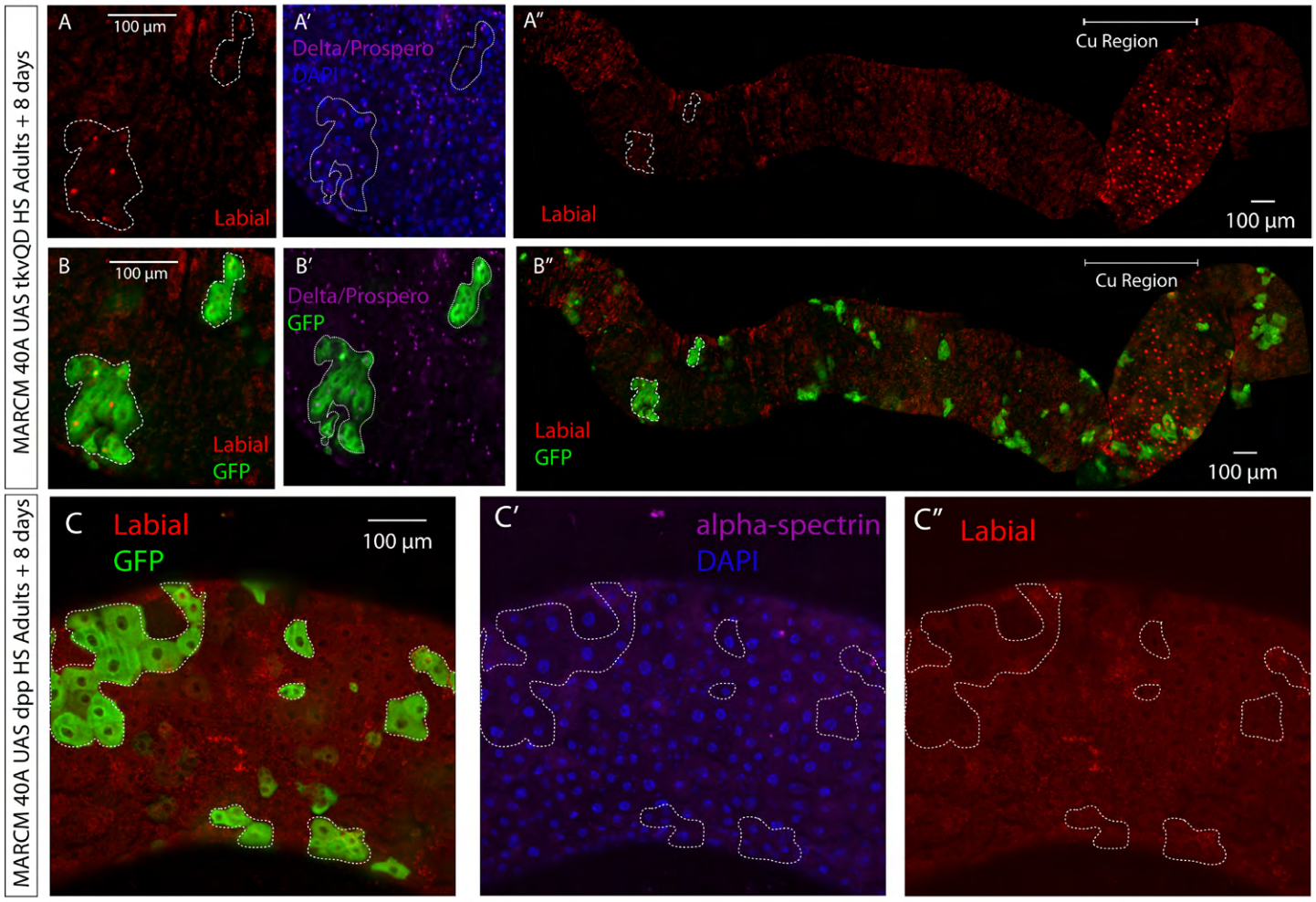

Figure S5. BMP activating clones induced in adulthood do not produce copper cells. (A-A'', B-B'') MARCM 40A UAS-*tkv*^{QD}, UAS-GFP flies were reared at 18°C and then heat shocked for 1 hour on day 3 after eclosion. **(A, A')** 8 days after clone induction, GFP marked clones (green) **(B, B')** outside the CCR contain Prospero positive enteroendocrine cells that are immune-positive for Labial and polyploidy enterocytes immuno-negative for Labial. **(A'', B'')** Low magnification view demonstrating that GFP marked clones (green) within the CCR contain cells positive for nuclear Labial (red). Clones shown in **(A)** and **(B)** are highlighted by white dotted lines. **(C-C'')** MARCM 40A UAS-*dpp* flies were reared at 18°C and then heat shocked for 1 hour on day 3 after eclosion. 8 days after clone induction, GFP marked clones (green) contain differentiated progeny **(C)** that are immuno-negative for **(C')** alpha-spectrin (orange) and **(C'')** Labial (red). DAPI (blue) marks nuclei in **(A')** and **(C')**. White dotted lines mark clone boundaries in all panels. For each image anterior is to the left.

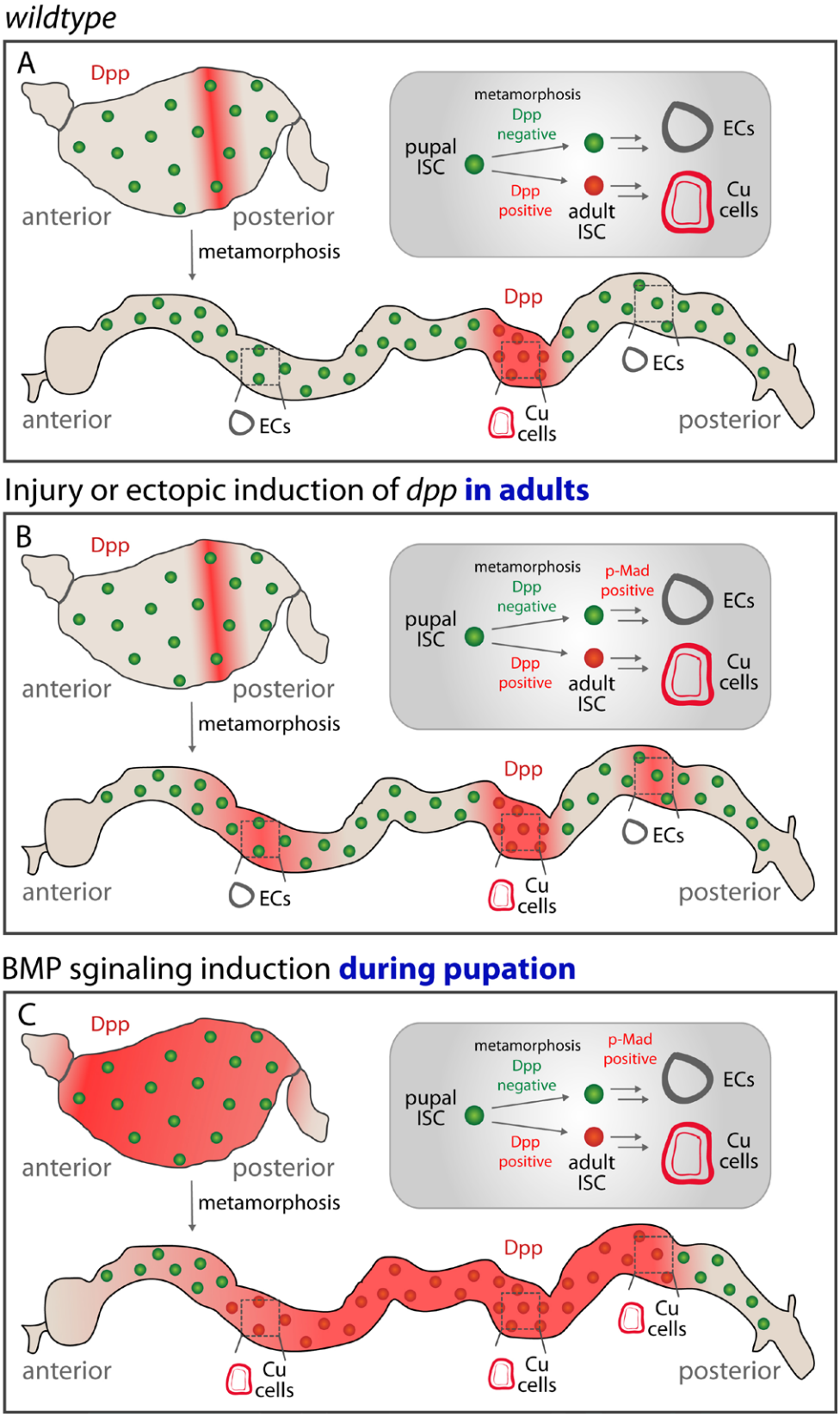

Figure S6. BMP pathway activation during pupation changes adult stem cell response to BMP signals. (A) A band of cells in a wildtype pupal midgut receives a BMP signal. Pupal ISCs in this region eclose as copper cell ISCs. In the presence of a BMP signal copper cell ISCs cells divide and maintain the CCR. **(B)** In wildtype flies, widespread injury or ectopic induction of the BMP signaling pathway results in BMP pathway activation outside of the CCR. Cells outside of the region do not change identity and copper cells remain restricted to the CCR. **(C)** Ectopic induction of the BMP signaling in the anterior and posterior midgut during 24-48 hours APF results in transformation of pupal AMPs into copper cells. Adult ISCs outside of the CCR are now competent to produce copper cells upon BMP pathway activation.