Supporting Information

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SI Materials and Methods

Fly Strains. w¹¹¹⁸ (wt), dad-GFP (J. Casanova, Institute de Biologia Molecular de Barcelona, Barcelona, Spain), UASshmiRdpp2 (B. Haley, University of California, Berkeley, CA), UASdmyc (C. Benassayag, Centre de Biologie du Développement, Université Toulouse III, Toulouse, France), *gbb1* and *gbb4* (K. Wharton, Brown University, Providence, RI), UAS-dsCol (S. Thor, Linkoping University, Linkoping, Sweden), *witA12*, and *wit B11. dppHR56* is a thermosensitive allele. *dppHR92/dppHR56* hypomorphic mutants were grown at 18 °C until the L1 stage before shifting to 29 °C. Other strains were provided by the Bloomington and the Vienna *Drosophila* RNAi stock centers.

Antibodies. Staining procedures were performed as described elsewhere (8, 45), using rabbit anti-GFP (1/500; Torrey Pines); anti- β -galactosidase (1/2,000; Promega); anti-Mad (1/3,000; C. Heldin, Ludwig Institute of Cancer Research, Uppsala, Sweden); anti-Dmyc (1/50; D. Stein, The University of Texas at Austin, Austin, TX); anti-H3P (1/200; Upstate Biotechnology); mouse anti-proPO (1/200; T. Trenczek, Justus-Liebig-University Giessen, Giessen, Germany); anti-P1 (1/30; I. Ando, Institut of Genetics, Biological Research Center of the Hungarian Academy of Science, Szeged, Hungary); and anti-Antp and anti-Dlp (1/50; Hybridoma Bank).

Plasmid Constructions. *dpp, tkv,* and *wit* genomic fragments, 2,318, 1,023, and 960 bp long, respectively, were PCR-amplified and cloned in pGEM-T Easy (Promega) to make RNA probes. Primers were as follows: *dpp* sense, 5'-CAAGGAGGCGCTCAT-CAAG-3'; *dpp* anti-sense, 5'-CACCAGCAGTCCGTAGTTGC-3'; *tkv* sense, 5'-TATCCACCGGATTGGAAAAA-3'; *tkv* antisense, 5'-GCCATGTCTGATGGCTACAA-3'; *wit* sense, 5'-CA-GTTGGGCGAAGAGAAGTC-3'; and *wit* anti-sense: 5'-CGC-ACTACACGCTAGGATGA-3'. T7 and SP6 RNA polymerases (Roche) were used for in vitro transcription of *dpp* and *wit* and *tkv*, respectively.

Fly Genotypes. The fly genotypes in Fig. 1 were as follows: dad-GFP (Fig. 1*A–B'*); wt (Fig. 1 *C* and *C'*); col > UAS-mCD8GFP > UAS-tkv^{DN} (Fig. 1*D*); wit^{B11}/wit^{A12} (Fig. 1*E*); col > UASmCD8GFP (Fig. 1*G*); col > UAS-mCD8GFP > UAS-tkv^{DN}

(Fig. 1*H*); col > UAS-mCD8GFP; dome-MESO (Fig. 1*K*); col > UAS-mCD8GFP > UAS-tkv^{DN}; dome-MESO (Fig. 1*L*); col > UAS-mCD8GFP (Fig. 1*N*); col > UAS-mCD8GFP > UAS-tkv^{DN} (Fig. 1*O*); and wit^{B11}/wit^{A12} (Fig. 1*M*-*P*). The fly genotypes in Fig. 2 were as follows: col > UAS-mCD8GFP (Fig. 2A); $dlp^{20}FRT2A/dlp^{20}$ FRT2A (Fig. 2B); col > UAS-Dicer2 (Fig. 2C); col > UAS-Dicer2 > UAS-dsdpp (Fig. 2D); col > UAS-Dicer2 > UAS-mCD8GFP > UAS-dscol; dome-MESO (Fig. 2E); and col > UAS-Dicer2 > UAS-dscol (Fig. 2G). The fly genotypes in Fig. 3 were as follows: col > UAS-Dicer2> UASdscol; dad-GFP (Fig. 3 A-A"); col > UAS-dicer2 > UASmCD8GFP > dscol (Fig. 3B-B''); HhF4-GFP (Fig. 3C-C'); col > GFP (Fig. 3D); HhF4-GFP; col > UAS-dicer2 > UAS-dscol (Fig. 3 E - E'; pcol > UAS-dicer2 > UAS-dscol > UAS-mCD8GFP (Fig. 3F); HhF4-GFP; pcol > UAS-tkv^{DN} (Fig. 3 G-G'); and col > UAS-tkv^{DN} > UAS-mCD8GFP (Fig. 3*H*). The fly genotypes in Fig. 4 were as follows: col > UAS-mCD8GFP (Fig. 4 A and B); $col > UAS-mCD8GFP > UAS-tkv^{DN}$ (Fig. 4C); col > UASmCD8-GFP > UAS-dmyc; dome-MESO (Fig. 4D); col > UASmCD8-GFP > UAS-dmyc (Fig. 4E); and col > UAS-mCD8- $GFP > UAS-tkv^{DN} > UAS-dsdmyc;$ dome-MESO (Fig. 4F). The fly genotypes in Fig. 5 were as follows: col > UAS-mCD8GFP > wg; dome-MESO (Fig. 5A); col > UAS-mCD8GFP > UAS-wg > UAS-dsmyc; dome-MESO (Fig. 5*B*); col > UAS-mCD8-GFP > UAS-dTCF^{DN}; dome-MESO (Fig. 5*C*); and col > UAS-mCD8-GFP > UAS-dTCF^{DN} > UAS-tkv^{DN} dome-*MESO* (Fig. 5*D*).

The fly genotypes in Fig. S1 were as follows: tkvI/tkv8 (Fig. S1 A, E, and I); dppHR92/dppHR56 (Fig. S1 B, F, and J); punt10460/punt 135 (Fig. S1C); gbbI/gbb4 (Fig. S1D); and col > UAS-dicer2 > UAS-dsdpp (Fig. S1 G–K). The fly genotypes in Fig. S2 were as follows: wt (Fig. S2 A and B) and $dlp^{20}FRT2A$ (Fig. S2 C and D). The fly genotypes in Fig. S3 were as follows: col > UASmCD8GFP (Fig. S3 A–A' and C–C') and col > UAS-mCD8GFP > UAS-tkv^{DN} (Fig. S3 B–B' and D–D'). The fly genotypes in Fig. S4 were as follows: col > UAS-mCD8GFP (Fig. S4A); col > UAS-mCD8GFP > UAS-Col (Fig. S4B); col (Fig. S4A); col > UAS-mCD8GFP > UAS-Col (Fig. S4B); col (Fig. S4C); col > UAS-col (Fig. S4D); col > UAS-mCD8GFP; dome-MESO (Fig. S4E); and col > UAS-col > UAS-mCD8GFP; dome-MESO (Fig. S4F).



Fig. S1. The BMP signaling pathway controls PSC cell number. (A–G and I–K) LGs of hypomorphic mutants for either tkv (A, E, and I), dpp (B, F, and J), punt (C), or gbb (D) or expressing dsdpp RNA in the PSC (col > dicer2 > dsdpp) (G and K), stained for Col (A–D), proPO (E–G), and P1 (I–K); nuclei are labeled by Topro (blue). (Scale bar: 80 µm.) (H) An increased number of PSC cells is observed in tkv and dpp mutants. (L) Crystal cell numbers relative to the volume of the anterior lobes.



Fig. S2. Activity of the BMP pathway is impaired in *dlp* mutant LGs. (*A*–*D*) Immunostaining of wt (*A* and *B*) and *dlp* mutant (*C* and *D*) LGs for P-Mad (white in *A* and *C*; and green in *B* and *D*) and Col (red in *B* and *D*). A strong decrease in P-Mad accumulation is observed in *dlp* mutant PSCs. White stars indicate pericardial cells. Topro (blue) labels nuclei (*B* and *D*). (Scale bar: 20 µm.)

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Fig. S3. Inactivation of BMP signaling leads to a strong decrease of *dpp* expression in PSC cells. (*A–B'*) Fluorescent in situ hybridizations for *dpp* (red) in *col* > *GFP* (*A*) and *col* > *tkv*^{DN} > *GFP* (*B*) LGs. *dpp* is down-regulated in the oversized PSC (*B*), which forms in absence of Dpp signaling, while maintained in other LG cells (white asterisk). (*C* and *D*) Dlp immunostaining of *col* > *GFP* (*C*) and *col* > *tkv*^{DN} > *GFP* (*D*) LGs. The PSC region is shown in green (*A'*, *B'*, *C'*, and *D'*). Topro (blue) labels nuclei. (Scale bar: 20 μ m.)



Fig. S4. No LG defects are observed upon Col overexpression in PSC cells. (A–F) Immunostaining of wt (A, C, and E) and col > col (B, D, and F) LGs for crystal cells [proPO (A and B)], plasmatocytes [(P1 (C and D)], and prohemocytes [LacZ (E and F)]. (G and H) No change in either the PSC cell number (G) or crystal cell index (H) is observed. Nuclei are labeled by Topro (blue). (Scale bar: 80 μ m.)

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