

Supporting Information

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SI Materials and Methods

Fly Strains. *w*¹¹¹⁸ (*wt*), *dad*-GFP (J. Casanova, Institute de Biologia Molecular de Barcelona, Barcelona, Spain), UAS-*shmiRdpp2* (B. Haley, University of California, Berkeley, CA), UAS-*dmyc* (C. Benassyag, Centre de Biologie du Développement, Université Toulouse III, Toulouse, France), *gbb1* and *gbb4* (K. Wharton, Brown University, Providence, RI), UAS-*dsCol* (S. Thor, Linköping University, Linköping, Sweden), *wit*^{*AI2*}, 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'-CAAGGAGGCGTCATCAAG-3'; *dpp* anti-sense, 5'-CACCAGCAGTCCGTAGTTGC-3'; *tkv* sense, 5'-TATCCACCGGATTGAAAAA-3'; *tkv* anti-sense, 5'-GCCATGTCTGATGGCTACAA-3'; *wit* sense, 5'-CAGTTGGGCGAAGAGAAGTC-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* > UAS-*mCD8GFP* (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. 2*A*); *dip*²⁰*FRT2A/dlp*²⁰ *FRT2A* (Fig. 2*B*); *col* > UAS-*Dicer2* (Fig. 2*C*); *col* > UAS-*Dicer2* > UAS-*dsdpp* (Fig. 2*D*); *col* > UAS-*Dicer2* > UAS-*mCD8GFP* > UAS-*dscol*; *dome*-MESO (Fig. 2*E*); and *col* > UAS-*Dicer2* > UAS-*dscol* (Fig. 2*G*). The fly genotypes in Fig. 3 were as follows: *col* > UAS-*Dicer2* > UAS-*dscol*; *dad*-GFP (Fig. 3*A–A''*); *col* > UAS-*dicer2* > UAS-*mCD8GFP* > *dscol* (Fig. 3*B–B''*); *HhF4*-GFP (Fig. 3*C–C'*); *col* > GFP (Fig. 3*D*); *HhF4*-GFP; *col* > UAS-*dicer2* > UAS-*dscol* (Fig. 3*E–E''*); *pcol* > UAS-*dicer2* > UAS-*dscol* > UAS-*mCD8GFP* (Fig. 3*F*); *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. 4*C*); *col* > UAS-*mCD8GFP* > UAS-*dmyc*; *dome*-MESO (Fig. 4*D*); *col* > UAS-*mCD8GFP* > UAS-*dmyc* (Fig. 4*E*); and *col* > UAS-*mCD8GFP* > UAS-*tkv*^{DN} > UAS-*dsdmyc*; *dome*-MESO (Fig. 4*F*). The fly genotypes in Fig. 5 were as follows: *col* > UAS-*mCD8GFP* > *wg*; *dome*-MESO (Fig. 5*A*); *col* > UAS-*mCD8GFP* > UAS-*wg* > UAS-*dmyc*; *dome*-MESO (Fig. 5*B*); *col* > UAS-*mCD8GFP* > UAS-*dTCF*^{DN}; *dome*-MESO (Fig. 5*C*); and *col* > UAS-*mCD8GFP* > UAS-*dTCF*^{DN} > UAS-*tkv*^{DN} *dome*-MESO (Fig. 5*D*).

The fly genotypes in Fig. S1 were as follows: *tkv1/tkv8* (Fig. S1*A, E, and I*); *dppHR92/dppHR56* (Fig. S1*B, F, and J*); *punt10460/punt135* (Fig. S1*C*); *gbb1/gbb4* (Fig. S1*D*); 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 *dip*²⁰*FRT2A* (Fig. S2*C* and *D*). The fly genotypes in Fig. S3 were as follows: *col* > UAS-*mCD8GFP* (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. S4*A*); *col* > UAS-*mCD8GFP* > UAS-*Col* (Fig. S4*B*); *col* (Fig. S4*C*); *col* > UAS-*col* (Fig. S4*D*); *col* > UAS-*mCD8GFP*; *dome*-MESO (Fig. S4*E*); and *col* > UAS-*col* > UAS-*mCD8GFP*; *dome*-MESO (Fig. S4*F*).

