Supporting Information

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SI Materials and Methods

***dpp* mRNA Quantification in Transfected S2R$^+$ cells and in *st-dpp* Embryos.** RNA was extracted from S2R$^+$ cells transfected with pMT-Dpp-HA or pMT-Dpp-Δa-HA (see *Materials and Methods* for transfection methods) and from embryos derived from crossing $y^{67c23}w^{118}$ females to *st2-dpp* or *st2-dpp-Δa* homozygous males using TRIzol (Life Technologies) according to the manufacturer's instructions. For RNA extraction from S2R$^+$ cells, two additional rounds of acid phenol extraction were included before RNA precipitation. RNA was treated with DNase I (Roche) for 30 min at room temperature, followed by DNase I inactivation by heat for 45 min at 75 °C. RNA extractions were performed in triplicate for each set of experiments.

From each RNA sample, cDNA was generated in duplicate (plus one no-reverse transcriptase control) by using eAMV reverse transcriptase (Sigma), oligo-dT$_{23}$ primers (Sigma), and 1 μg of total RNA. Quantitative PCR (qPCR) analysis was performed by using SYBR green on a Chromo 4 real-time PCR machine (MJ Research). Raw *dpp* or *st2-dpp* levels were normalized to the levels of the reference gene *rp49* measured in the same cDNA sample. The following primers were used for qPCR amplification:

RP49:

5′-CCCAAGGGTATCGACAACAGA-3′ and 5′-CGATGTTGGGCATCAGATACTG-3′

dpp (S2R+ cells):

5′-GGCAAGGTGCCGAAGGCGTG-3′ and 5′-GTCATCTCCTGGTAGTTCTTCAG-3′

st2-dpp transgenes (embryos):

5′GGGAAATCGCGAGCGAGAGAGCA-3′ and 5′-ACAATCTTTTGGGGGAGCATGGGGG-3′

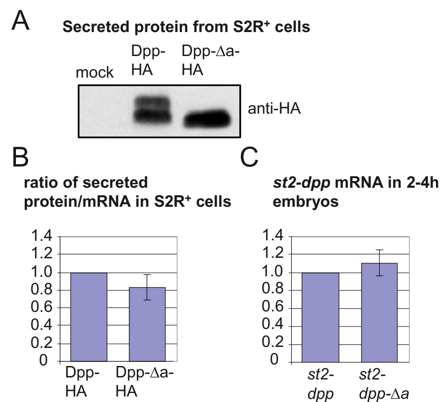

Fig. S1. Quantification of Dpp-Δa levels in tissue culture and 2- to 4-h embryos. (*A* and *B*) Expression of the same levels of *dpp-HA* and *dpp-Δa-HA* mRNA in S2R$^+$ cells results in equal extracellular accumulation of Dpp-HA and Dpp-Δa-HA protein in the supernatant. S2R$^+$ cells were transiently transfected with Copper-inducible vectors encoding Dpp-HA and Dpp-Δa-HA. The levels of secreted HA-tagged forms of Dpp-HA and Dpp-Δa-HA were quantified by Western blotting, with one representative blot shown in *A*. The level of *dpp* mRNA in transfected S2R$^+$ cells was quantified by qPCR and correlated with the resultant level of extracellular Dpp protein (*B*). The results indicate that expression levels of wild-type Dpp and Dpp-Δa are equivalent. Thus, the Δa mutation in *dpp* does not intrinsically affect any of the various stages of gene expression involved in production of Dpp protein. Results represent the mean of three independent experiments; error bar shows SEM. (*C*) Quantification of mRNA levels from *st2-dpp* and *st2-dpp-Δa* transgenes in 2- to 4-h embryos carrying one copy of the transgene in a wild-type background. mRNA levels were assessed by qPCR analysis by using primers specific for the *st2-dpp* transgenes. Graph shows the mean of three independent experiments; error bar is SEM. The results demonstrate that *st2-dpp* and *st2-dpp-Δa* embryos analyzed in Fig. 4 express similar levels of *dpp* mRNA from the transgene. In conjunction with the tissue culture data (*A* and *B*), these results suggest that Dpp and Dpp-Δa extracellular protein levels are similar in *st2-dpp* and *st2-dpp-Δa* embryos.

Fig. S2. The collagen IV and heparan sulfate proteoglycan binding sites on Dpp overlap. (*A*) Mutagenesis of the S1 and S3 processing sites in the previously generated heparan-sulfate proteoglycan binding site mutant, Dpp-ΔN (1), demonstrates that mature Dpp-ΔN arises from cleavage at the S3 site. Secreted HA-tagged forms were analyzed by Western blotting. Mutation of S3 (S3*) causes a size shift, whereas mutation of S1 (S1*) has no effect. (*B*) Amino acid sequence of Dpp in the region of the furin processing sites (S1, S2, S3) directly upstream of the HA-tagged ligand domain. The sequences of S1/S3 forms of mature wild-type Dpp, the collagen IV binding site mutants, Dpp-Δa and Dpp-Δc, and the HSPG binding site mutant, Dpp-ΔN, are shown below. Note that, because of its processing at the S3 site, Dpp-ΔN is very similar in sequence to Dpp-Δa and Dpp-Δc. Note that Dpp-ΔN also carries amino acids HA instead of QP at the positions directly following the S3 cleavage site, as shown. (*C*) GST-pulldown between GST-VkgC and wild-type or deletion mutants of Dpp-HA. The Western blot shown is the full version of the result presented in Fig. 1*B*. Dpp-ΔN shows a similar reduction in binding to the collagen IV NC1 domain as Dpp-Δa and Dpp-Δc.

1. Akiyama T, et al. (2008) Dally regulates Dpp morphogen gradient formation by stabilizing Dpp on the cell surface. *Dev Biol* 313:408–419.