Supplemental Data

BMP Signaling Is Required for Controlling Somatic Stem Cell Self-Renewal in the *Drosophila* Ovary

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Supplemental Results

Generation of Stocks for the Positively Marked Mosaic Lineage (PMML) Technique

We used an insertion of AyGAL4 at 52B (AyGAL4-25) (Ito et al., 1997) as the starting stock to generate two FRT containing lines. We crossed y, w; AyGAL4-25 flies to a Delta 2-3 Transposase stock and scored for imprecise excisions that removed either the *white* or *yellow* transgene of AyGAL4. We identified $15y^{-}w^{+}$ and $4y^{+}w^{-}$ excisions. We found that one $y^{-}w^{+}$ event (line 34) deleted not only the *white* gene, but also the first FRT. Out of the $4y^{+}w^{-}$ excisions, we found that one, line 3-2, effectively removed the yellow gene, the second FRT and the GAL4 gene. When given a source of FLP recombinase and a UAS reporter gene, the combination of $y^{+}34$ (yellow-FRT-GAL4) and $w^{+}3$-2 (white-Actin5C-FRT) could produce positively marked lineages.

For optimal detection, we generated multiple inserts of UAS-EGFP throughout the genome as well as a membrane localized version of EGFP. For a plasma membrane localized EGFP, we synthesized two complimentary oligonucleotides (ATGGGCTCCTCCAAGTCCAAGCCCAAGGACCCCTCCCAGCGG) encoding the *v-src* myristyolation sequence (MGSSKSKPKDPSQR) annealed them together and ligated the double stranded fragment in frame to the N-terminus of EGFP. This *srcEGFP* fusion was then cloned into pUAST and transformed into flies by standard methods (Rubin and Spradling, 1982).

Supplemental Experimental Procedures

To determine if mutations in *dpp* and *gbb* affect *Dad-lacZ* expression, the following stocks were generated and used: (1) *Dad-lacZ*/+; (2) dpp^{hr56}/dpp^{hr4}*; Dad-lacZ*/+ ; (3) gbb^{4}/gbb^{D4}*; Dad-lacZ*/+; (4) gbb^{4}/gbb^{D20}*; Dad-lacZ*/+ ; (5) *hs-bam*/*Dad-lacZ*. For examining *dpp* or *gbb* agametic germarial phenotypes, late-stage pupae were subjected to four 2-hour heatshock treatments at 37°C with 8-12 hour intervals and continued to be cultured at 18°C. Then, adult females with these genotypes were isolated and continued to be cultured at 29°C for an additional 10 days: (1) *hs-bam/*+; (2) dpp^{hr56}/ dpp^{e90}*; hs-bam/*+; (3) dpp^{hr56}/ dpp^{hr4}*; hs-bam/*+; (4) gbb^{4}/ gbb^{D4}*; hs-bam/*+; (5) gbb^{4}/ gbb^{D20}*; hs-bam/*+.

To determine if BMP downstream components are required in SSCs for their maintenance, marked mutant SSC clones were generated by FLP-mediated mitotic recombination according to the previously described procedures (Song and Xie, 2002; Song and Xie, 2003). The following genotypes were generated and analyzed: (1) *hs-FLP; FRT_{40A} / FRT_{40A} armadillo* (*arm*)*-lacZ*; (2) *hs-FLP; FRT_{40A} tkv^{4}/ FRT_{40A} arm-lacZ*; (3) *hs-FLP; FRT_{40A} mad^{12}/ FRT_{40A} arm-lacZ*; (4) *hs-FLP; FRT_{G13} sax^{4}/ FRT_{G13} arm-lacZ*; (5) *hs-FLP; FRT_{82B} Med^{26}/ FRT_{82B} arm-lacZ*; (6) *hs-FLP; FRT_{82B} Med^{4F33}/ FRT_{82B} arm-lacZ*; (7) *hs-FLP; FRT_{82B} put^{135}/ FRT_{82B} arm-lacZ*. To generate twin spots of homozygous follicle cells on the surface of egg chambers, the females of desirable genotypes were heat-shocked twice for one hour with an interval of 8 hours, and then the ovaries were isolated five days later for immunostaining. To determine if BMP signaling regulates SSC survival, the MARCM system (Lee and Luo, 1999) was used to generate positively labeled mutant clones that also expressed *UAS-p35* and marked SSC clones were analyzed according to our published procedures (Song and Xie, 2002). For *p35* expression in $punt^{135}$ or Med^{26} mutant SSCs, the following genotypes were generated and used: (1) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP; FRT_{82B} tub-gal80/ FRT_{82B}* ; (2) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP/UAS-p35; FRT_{82B} tub-gal80/ FRT_{82B}* ;(3) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP; FRT_{82B} tub-gal80/ FRT_{82B} put^{135}*; (4) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP/UAS-p35; FRT_{82B} tub-gal80/ FRT_{82B} put^{135}*; (5) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP; FRT_{82B} tub-gal80/ FRT_{82B} Med^{26}*; (6) *hs-FLP UAS-srcEGFP; actin-gal4 UAS-EGFP/UAS-p35; FRT_{82B} tub-gal80/ FRT_{82B} Med^{26}*.

To constitutively express an activated BMP receptor *tkv** or *sax** in the IGS cells, SSCs and early follicle cell progenitors, the females with genotypes *c587/+; UAS-tkv*/+* and *c587/+; UAS-sax*/+* were generated and used. For overexpression of *tkv** or *sax** in positively GFP labeled SSCs, the females with following genotypes were generated and heat shocked at 37°C once for 1 hour for two consecutive days: (1) *hs-FLP UAS-srcEGFP; FRT_{52B}(y) UAS-EGFP/ FRT_{52B}(w); Dad-lacZ/+*; (2) *hs-FLP UAS-srcEGFP; FRT_{52B}(y) UAS-EGFP/ FRT_{52B}(w); Dad-lacZ/UAS-tkv** ; (3) *hs-FLP UAS-srcEGFP; FRT_{52B}(y) UAS-EGFP/ FRT_{52B}(w); Dad-lacZ/UAS-sax** ; (4) *hs-FLP UAS-srcEGFP; FRT_{52B}(y) UAS-EGFP/ FRT_{52B}(w)*; (5) *hs-FLP UAS-srcEGFP; FRT_{52B}(y) UAS-EGFP/ FRT_{52B}(w); UAS-tkv*/+*. The *UAS-srcEGFP* is used to express membrane-tagged EGFP to highlight cell outlines. Their ovaries were isolated and immunostained 1, 2 and 3 weeks after the last heat-shock.

To investigate if hyperactive BMP signaling can rescue SSC loss phenotypes caused by defective Wg and Hh signaling, the females with following genotypes were generated and heat shocked at 37°C twice for 1 hour with an interval of 8-10 hours for three consecutive days: (1) *hs-FLP UAS-srcEGFP; smo^{3} FRT_{40A} / FRT_{40A} tub-gal80 ; actin-gal4 UAS-EGFP/+*; (2) *hs-FLP UAS-srcEGFP; smo^{3} FRT_{40A} / FRT_{40A} tub-gal80 ; actin-gal4 UAS-EGFP/UAS-tkv**; (3) *hs-FLP UAS-srcEGFP; smo^{D16} FRT_{40A} / FRT_{40A} tub-gal80 ; actin-gal4 UAS-EGFP/+*; (4) *hs-FLP UAS-srcEGFP; smo^{D16} FRT_{40A} / FRT_{40A} tub-gal80 ; actin-gal4 UAS-EGFP/ UAS-tkv**; (5) *hs-FLP tub-gal80 FRT_{19A}/ dsh^{3} FRT_{19A}; actin-gal4 UAS-EGFP/+*; (6) *hs-FLP tub-gal80 FRT_{19A}/ dsh^{3} FRT_{19A}; actin-gal4*

*UAS-EGFP/UAS-tkv**. Their ovaries were analyzed 1, 2 and 3 weeks after the last heat-shock.

Supplemental References

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