

Supplementary Materials for

Diffusible fraction of niche BMP ligand safeguards stem-cell differentiation

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The PDF file includes:

Table S1, Figures S1 to S6
Legends for Supplementary Movie S1, S2, Source Data, Supplemental Data1

Alleles	<i>mCherry-dpp</i> ^{*1}	<i>GFP-dpp</i> ^{*1}	<i>mGL-dpp</i> ^{*1}	<i>mSC-dpp</i> ^{*1}	<i>HA-dpp</i> ^{*1}	<i>GFP-dpp</i>
Sources	CRISPR knock-in. Fereres et al., 2019	CRISPR knock-in. Gift from Thomas Kornberg	This study, Rasouliha et al., 2023	This study, Rasouliha et al., 2023	Matsuda et al., 2021	Matsuda et al, 2021
Phenotypes	Homozygous viable Potential generation of non-tagged Dpp fraction ^{*2}	Homozygous viable Potential generation of non-tagged Dpp fraction ^{*2}	Homozygous semi-lethal Rescuable with <i>pPA dpp 8391/X</i>	Homozygous semi-lethal Rescuable with <i>pPA dpp 8391/X</i>	None	Haploinsufficient Partially rescuable with <i>pPA dpp 8391/X</i> (patterning defects)
Tag location	AA465	AA465	AA485	AA485	AA485	AA485
GSC phenotypes after expression of MT under hub driver (FasIIIGal4)	Not tested	Reduced pMad with mCD8-MT	Reduced pMad with mCD8-MT	Not tested	Reduced pMad with HAtrap	Not tested
De-differentiation phenotypes after expression of MT under hub driver (FasIIIGal4)	Not tested	Accelerated dedifferentiation with Nrv-MT trap	Accelerated dedifferentiation with Nrv-MT trap	Not tested	Not tested	Not tested

^{*1} Alleles used in this study

^{*2} Tags placed at AA465 may be cut out at the last furin processing site

Table S1. Comparison of fluorescent tagged *dpp* alleles.

Fluorescent tags are inserted after the indicated amino acid (AA) in *dpp* isoformE (NM_164488.2). Fereres et al., 2019 (54); Rasouliha et al., 2023 (57); Matsuda et al., 2021 (20).

mSC: mScarlet, mGL:mGreen Lantern.

Figure S1

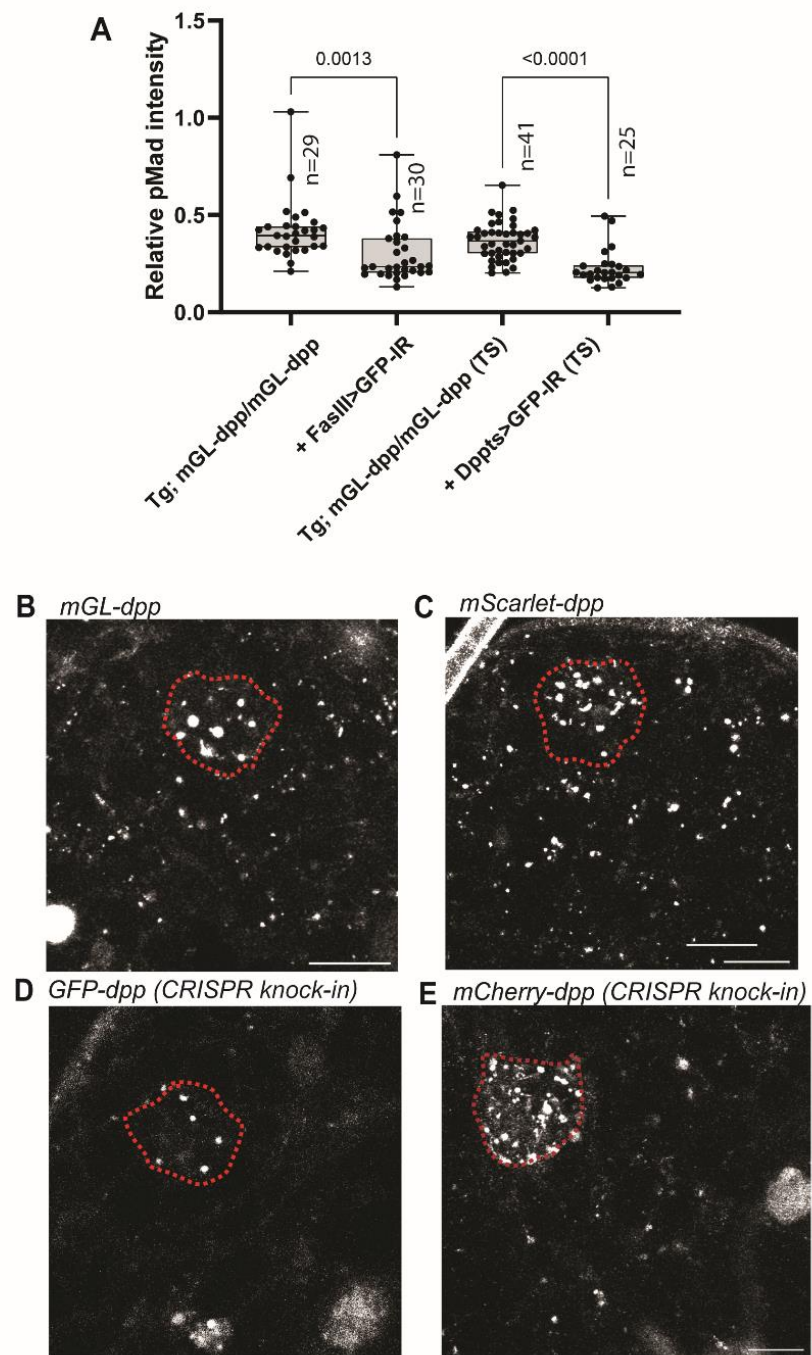


Figure S1. Comparison of fluorescent patterns of tagged *dpp* alleles.

A) Quantification of pMad intensity in GSCs (relative to somatic cyst cells or CCs) of indicated genotypes. *fasIII*Gal4 or *dpp*Gal4 (with *tub*Gal80^{ts}) driven GFP (mGL) knock-down in homozygous *mGL-dpp* with *pPA dpp 8391/X*. Temperature shift (TS) was performed at 29 degrees for 2 days before dissection. p-values were calculated by Šídák's multiple comparisons tests and provided on the graph. Fixed samples were used. Numerical values are provided in [Source Data file](#). “n” indicates the number of scored GSCs. Box plots show 25–75% (box), minimum to maximum (whiskers) with all data points. **B-E)** Representative images comparing testis tips isolated from homozygous *mGL-dpp* with *pPA dpp 8391/X* (**B**), homozygous *mScarlet(mSC)-dpp* with *pPA dpp 8391/X* (**C**), homozygous *GFP-dpp* CRISPR knock-in (**D**), and homozygous *mCherry-dpp* CRISPR knock-in (**E**). Scale bars represent 10 μm. The hub is encircled by red broken lines. Live tissues were used for imaging for **B-E**.

Figure S2

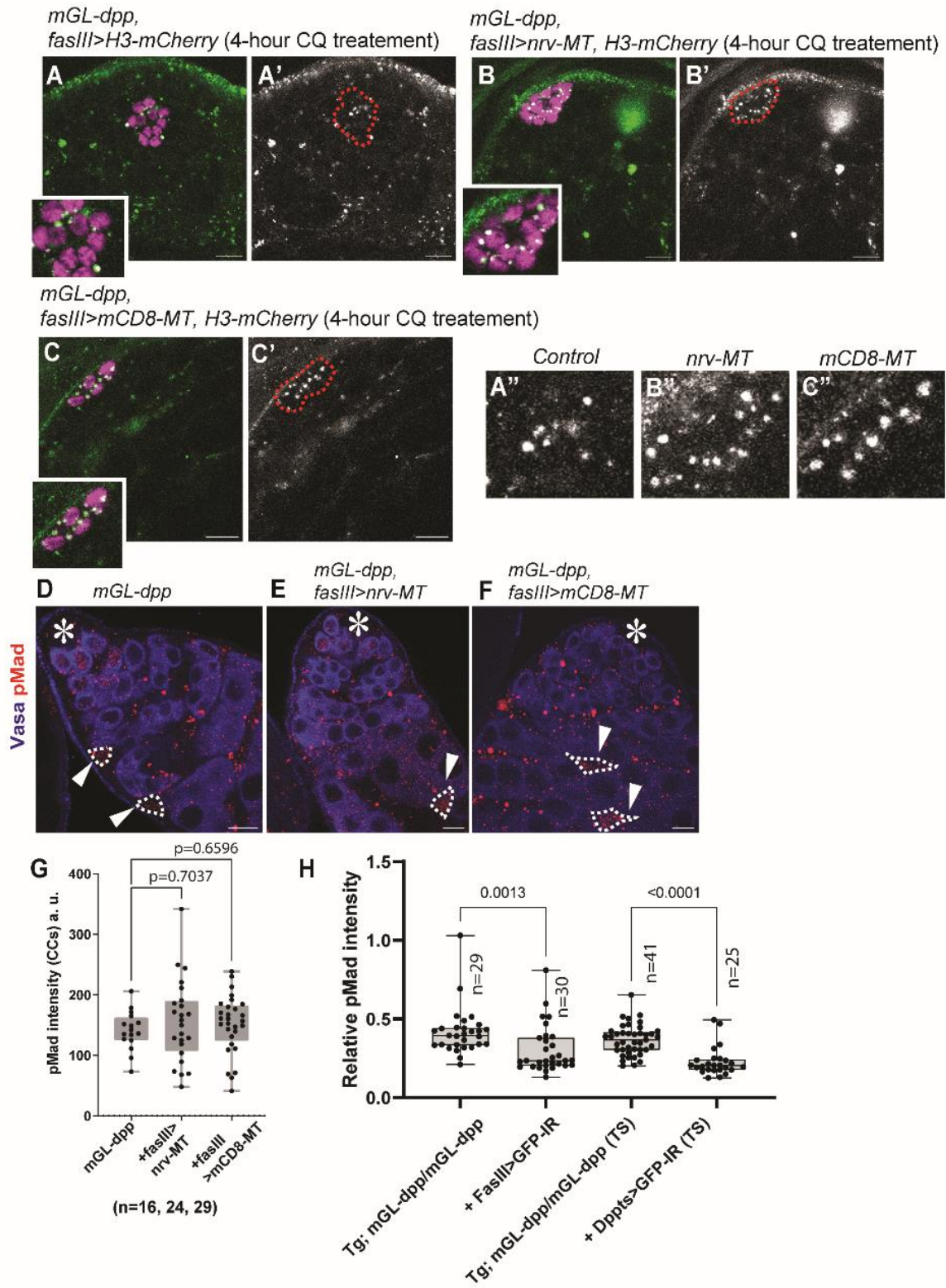


Figure S2. Characterization of morphotrap phenotypes.

A-C) Representative images of *mGL-dpp* signal after 4-hour CQ treatment in the testes of indicated genotypes. Magnified hub areas are shown in left corner of **A-C**. The hub is encircled by red broken lines in **A'-C'**. Magnified images of hub area are shown in **A''-C''**. Live samples were used. **D-F)** Representative images of pMad staining in somatic cyst cells (CCs) (marked by broken lines and arrowheads) of indicated genotypes. **G)** Quantification of pMad intensity in CCs of indicated genotypes. p-values were calculated by Šidák's multiple comparisons tests and provided on the graph. Fixed samples were used for **G**.

Asterisks indicate approximate location of the hub. All scale bars represent 10 μm . “n” indicates the number of scored GSCs. Box plots show 25–75% (box), minimum to maximum (whiskers) with all data points. Numerical values are provided in [Source Data file](#).

Figure S3

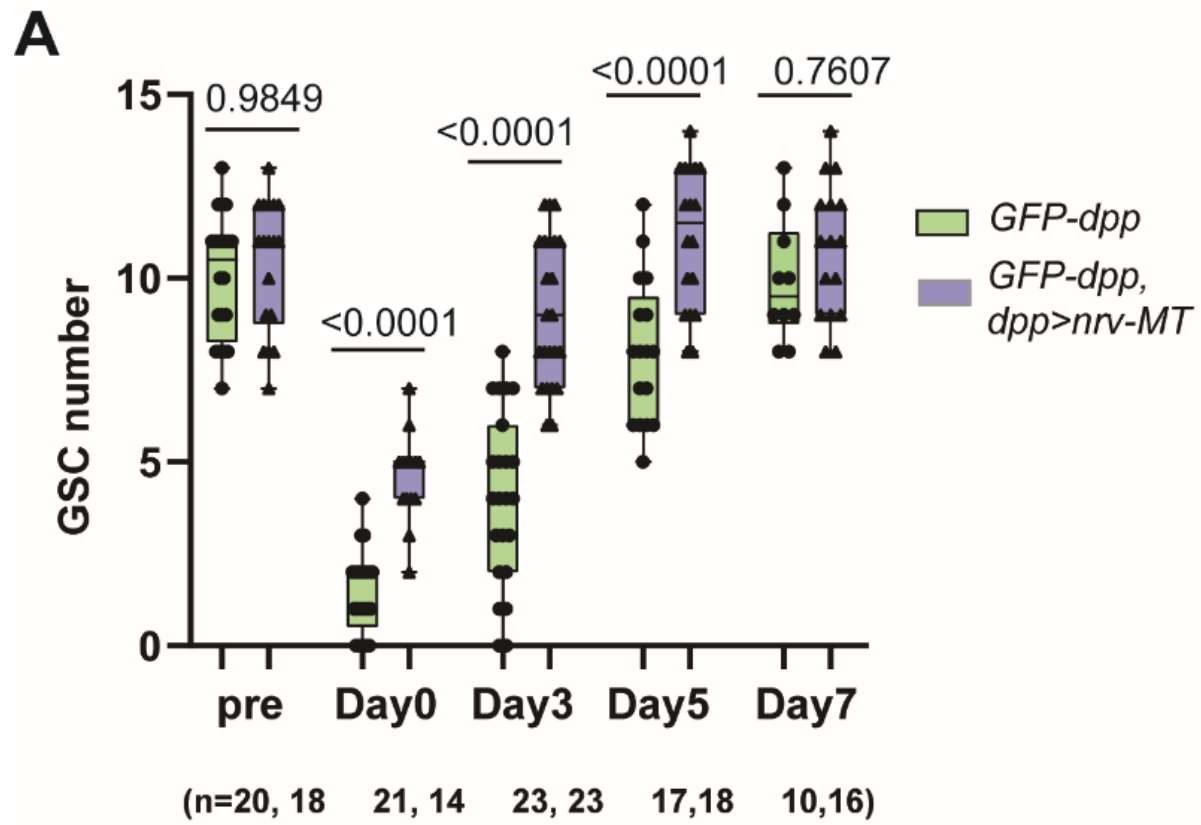


Figure S3. Morphotrap using alternative genotypes shows similar effect on de-differentiation.

A) Changes in GSC number during recovery from forced differentiation of GSCs. For trapping Dpp, Nrv-MT was expressed under the control of the dppGal4 driver in homozygous *GFP-dpp* CRISPR knock-in background. Homozygous *GFP-dpp* CRISPR knock-in flies are viable and fertile and were used for the control. P-values were calculated by Šídák's multiple comparisons tests and provided on the graph. Fixed samples were used for scoring. “n” indicates the number of scored testes. Box plots show 25–75% (box), minimum to maximum (whiskers) with all data points. Numerical values are provided in [Source Data file](#).

Figure S4

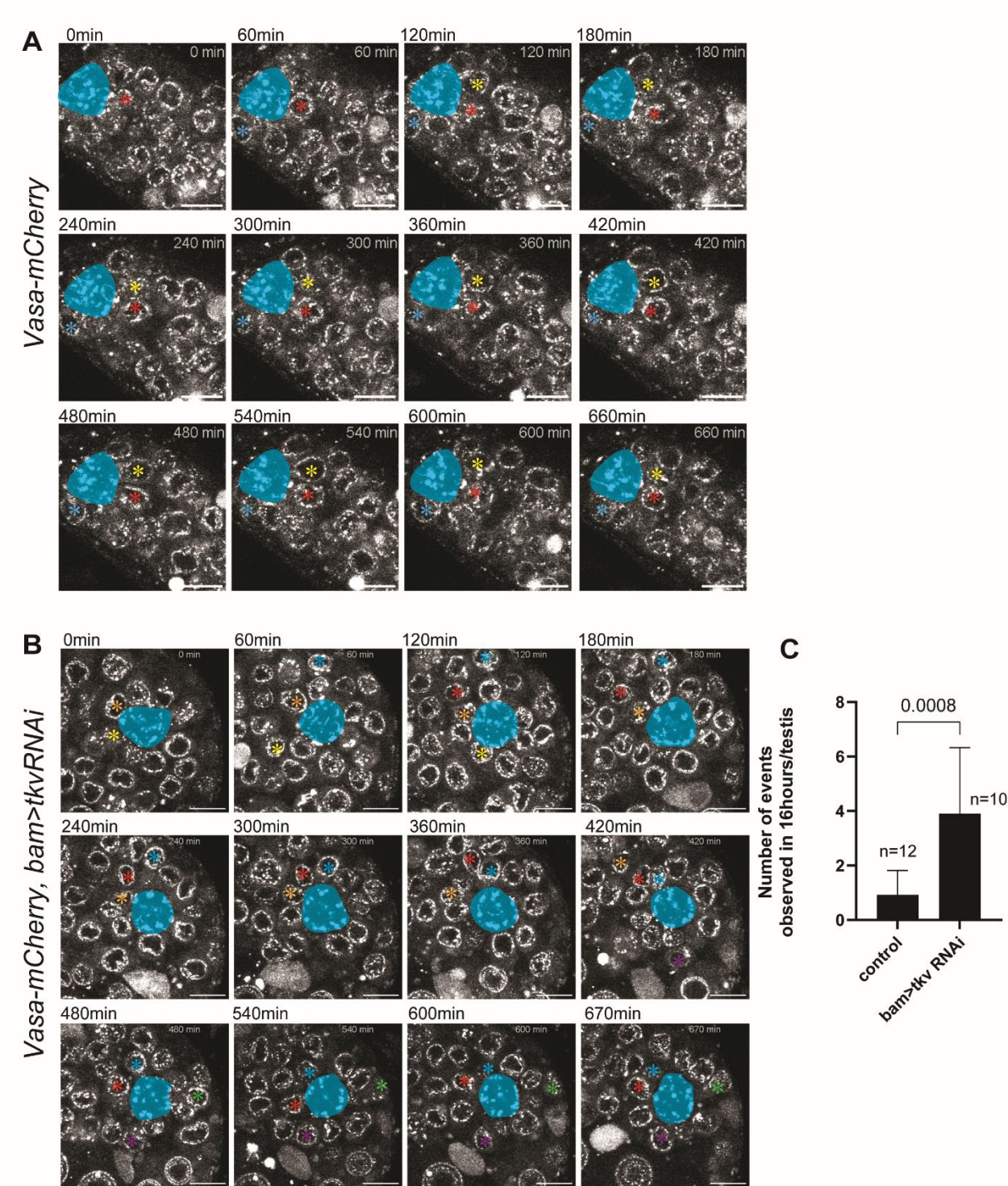


Figure S4. Live imaging of Tkv knock-down testis shows high frequency of non-asymmetric events.

A, B) Representative time-lapse live series of testis tip of indicated genotypes. Cells traced for entire imaging period are indicated by asterisks in each different color. In A, all marked GSCs stayed attached in the niche throughout the imaging period. In B, all marked cells show non-asymmetric behavior (yellow and orange cells leave from the hub, while other marked cells de-differentiate). Hub area is filled in turquoise blue. All scale bars represent 10 μ m. Corresponding movies are provided as supplemental materials ([Supplementary Movie S1](#) and [Supplementary Movie S2](#)). **C)** Number of non-asymmetric events observed in indicated genotypes in 16-hour imaging periods. Vasa-mCherry flies were used for the control. “n” indicates the number of time-lapse series analyzed. Data are means and standard deviation. The p-value was calculated by student-t-test. Numerical values are provided in [Source Data file](#).

Figure S5

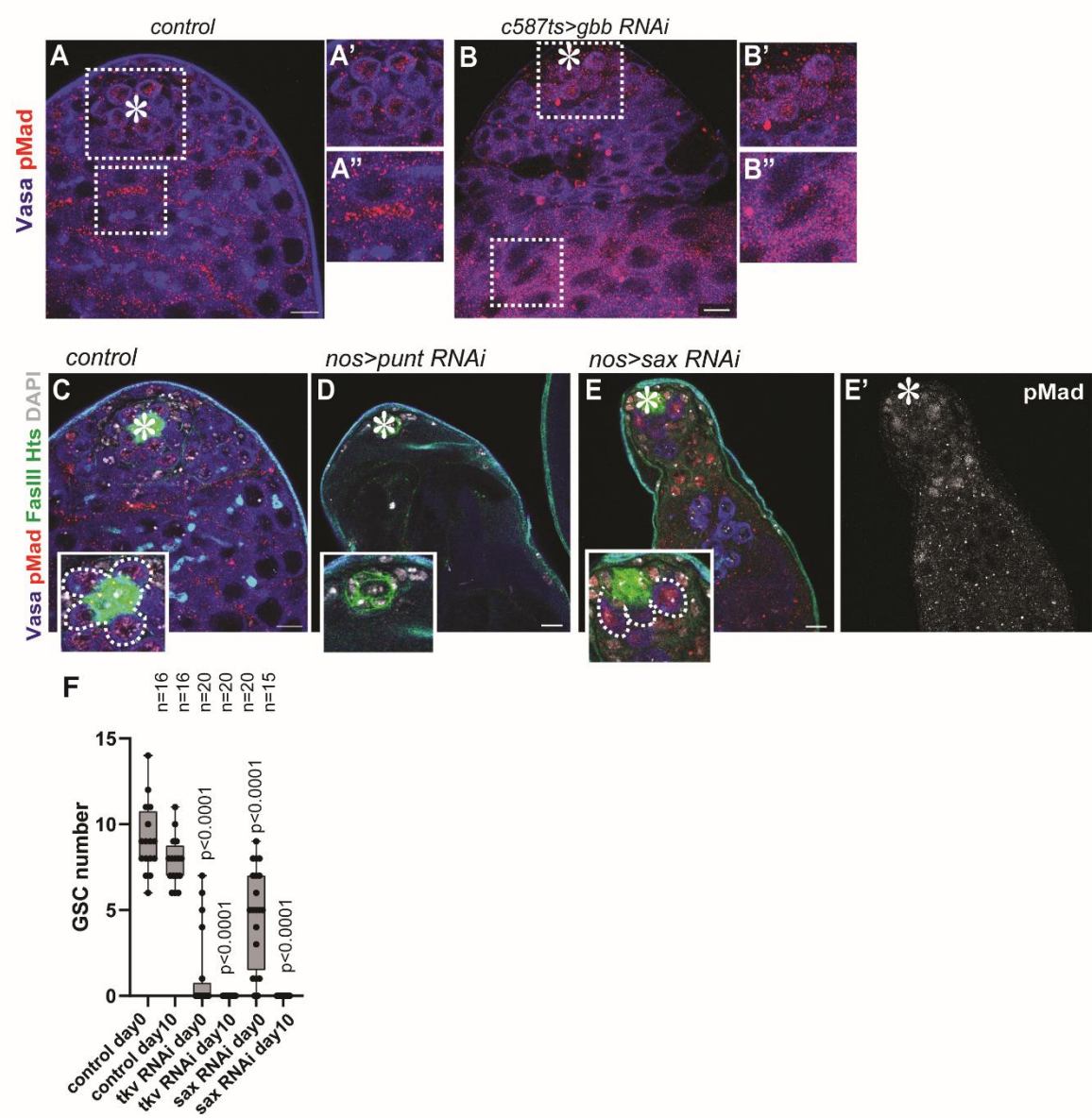


Figure S5. Sax and Punt show distinct effects on GSC maintenance.

A-B) Representative pMad staining images of testis tips with knock-down of *gbb* under the somatic cell specific driver, *c587Gal4^{ts}* after 4-days of temperature shift (29 degree). Squared regions in **A**, **B** are magnified in right panels. **A'** and **B'** show pMad signal in GSCs, which was intact in *gbb* RNAi testes. **A''** and **B''** show pMad signal in CCs, which was not detectable in *gbb* RNAi testes. **C-E)** Representative images of testis tips of no-Gal4 control (**C**) and with knock-down of *punt* *TRiP.GLV21066* (**D**) or *sax* *TRiP.HMJ02118* (**E**) under the control of *nosGal4* driver. Insets show magnified region around the hub. Punt RNAi shows the testis without any Vasa positive germ cells. White broken lines encircle GSCs in inset **C** and **E**, showing GSCs with *sax* RNAi are pMad positive (**E**). **F)** GSC number in indicated age of testes of indicated genotypes. P-values were calculated by Šídák's multiple comparisons tests and provided on the graph. "n" indicates number of scored testes. Numerical values are provided in [Source Data file](#). Scale bars represent 10 μ m. Asterisks indicate approximate location of the hub. Fixed samples were used for all images.

Figure S6

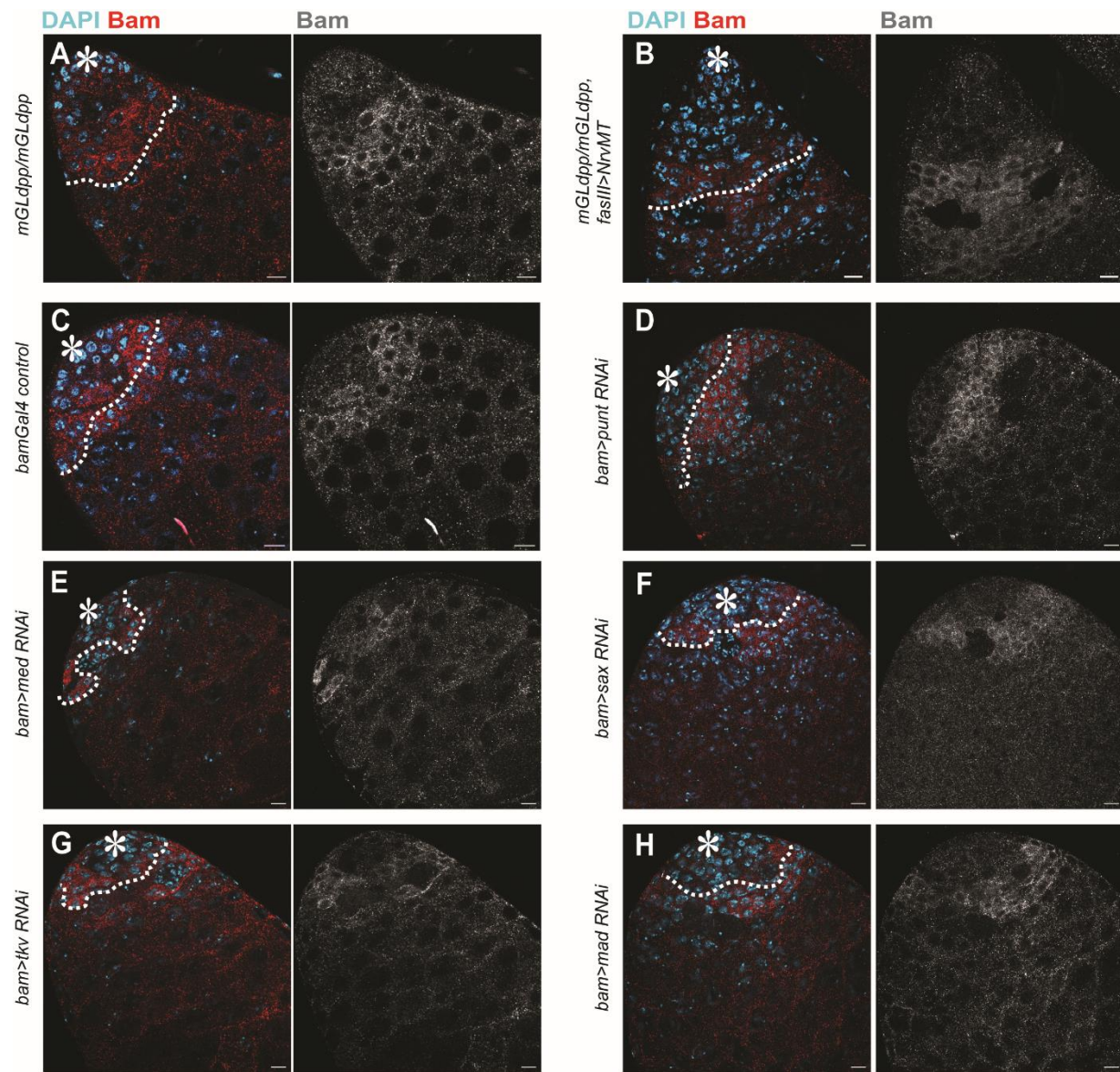


Figure S6. BMP signal in SGs is required for timely upregulation of Bam.

A-H) Representative Bam staining images of indicated genotypes. Boundary between 8-cell SGs and 16-cell SGs are divided by white broken lines. Asterisks indicate approximate location of the hub. All scale bars represent 10 μm . Fixed samples were used for all images.

Legends for other supplemental materials

Supplementary Movie S1

A representative time-lapse movie of a testis tip (corresponding to [Figure S4A](#)). Time-interval: 10min. Scale bar: 10 μ m.

Supplementary Movie S2

A representative time-lapse movie of a testis tip (corresponding to [Figure S4B](#)). Time-interval: 10min. Scale bar: 10 μ m.

Source Data

Numerical values of all graphs are provided in this excel spreadsheet.

Supplementary Data1

Catalog number of commercial reagents used in this study is provided in this excel spreadsheet.