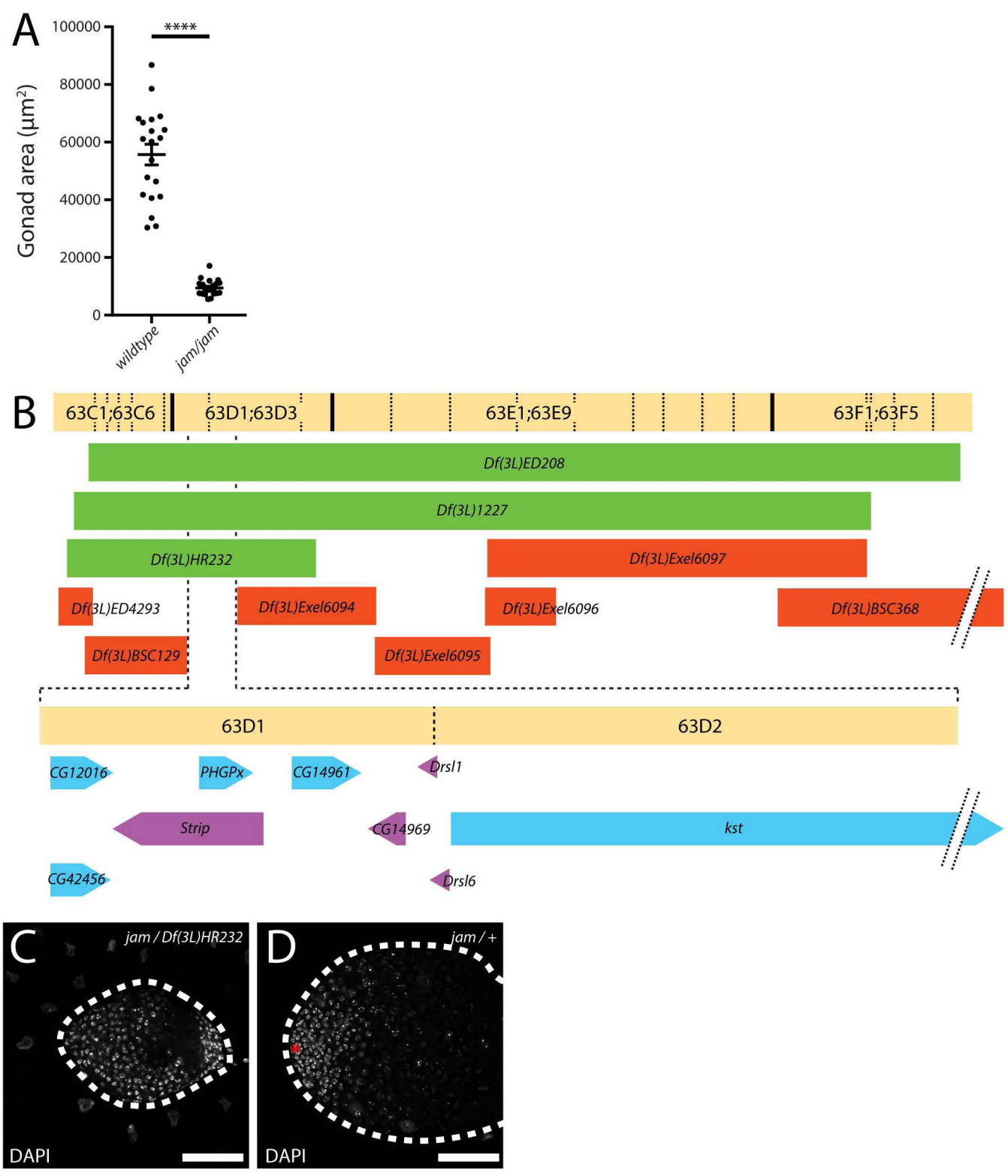

Figure S1. Deletion mapping to identify the lethal region containing *jam*. (A) Graph indicating the statistically significant reduction in area of *jam/jam* gonads compared to the *wildtype* ($p<0.0001$, represented by ****). Error bars = S.E.M. (B) Diagram representing cytological region 63C;63F of the *Drosophila* 3rd chromosome, and the coverage of deficiency stocks used to map the lethal region containing *jam*. Deficiency stocks that failed-to-complement *jam* are green, and those that complemented are red. (B,C) Gonads from transheterozygous *jam/Df(3L)HR232* (n=18/18) (C) and heterozygous *jam/+* (n=3/3) (D) animals, stained with DAPI. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50µm.

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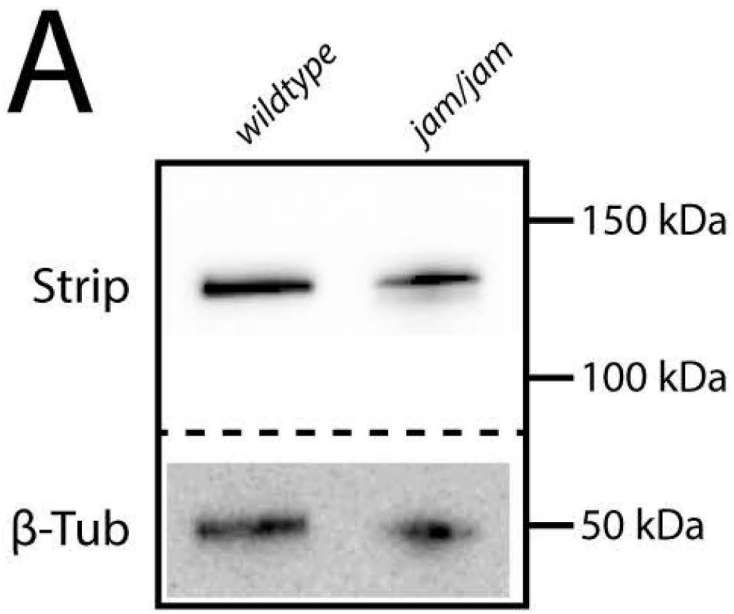

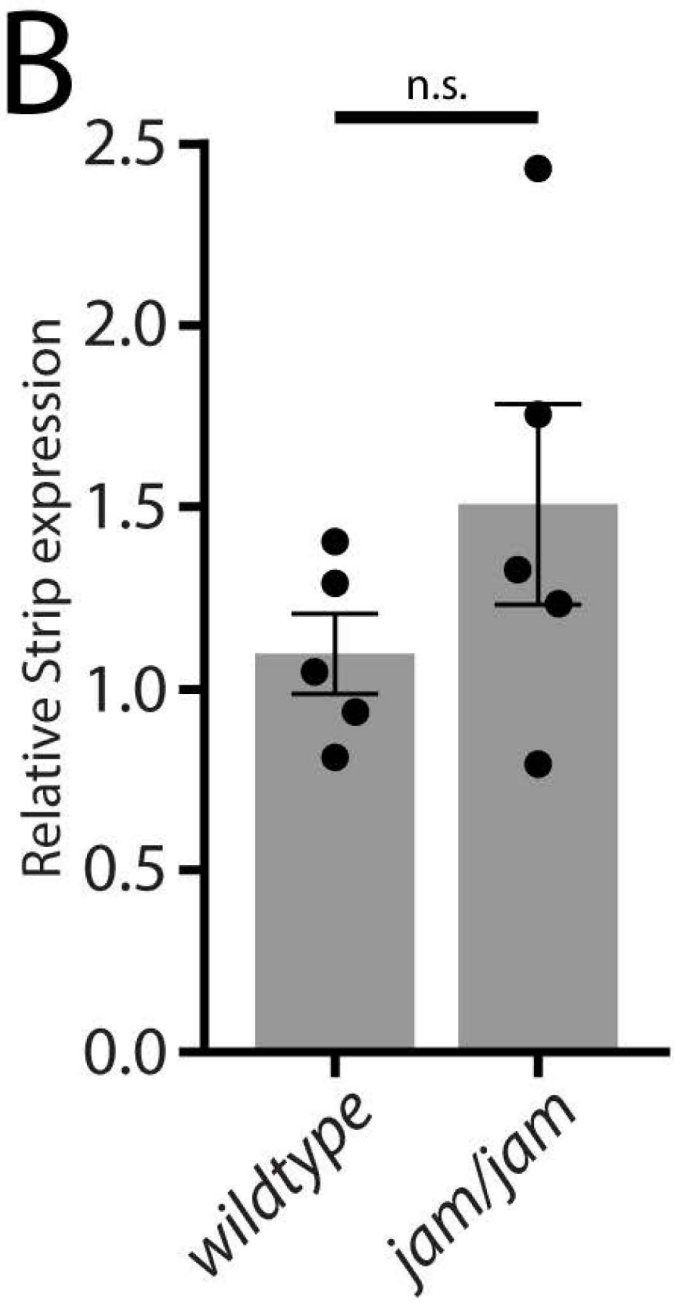

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Figure S2. Strip^{jam} is translatable. (A) Western blotting for Strip revealed the protein in both *wildtype* and *jam/jam* tissues, at roughly 130 kDa (performed 5 times). β-Tubulin was used as a loading control. (B) Quantification of Strip expression relative to the loading control revealed no significant difference between the *wildtype* and the mutant (Student's t-test, $p>0.05$).

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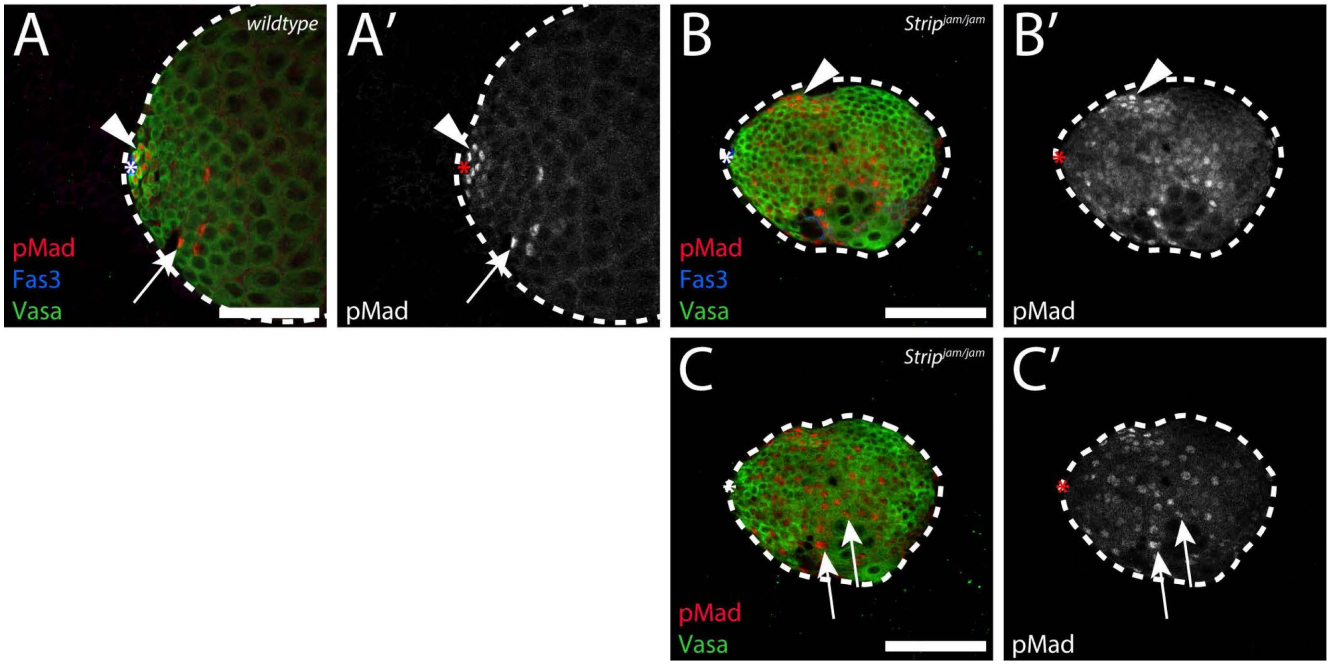

Figure S3. BMP signalling is disrupted in *Strip*$^{jam/jam}$ gonads. (A-B') *Wildtype* (n=21/21) (A,A') and *Strip*$^{jam/jam}$ (n=29/29) (B-C') gonads stained for pMad, Fas3, and Vasa. B and C are different Z-sections of the same gonad. Arrowheads and arrows indicate pMad-positive germline and somatic cells, respectively. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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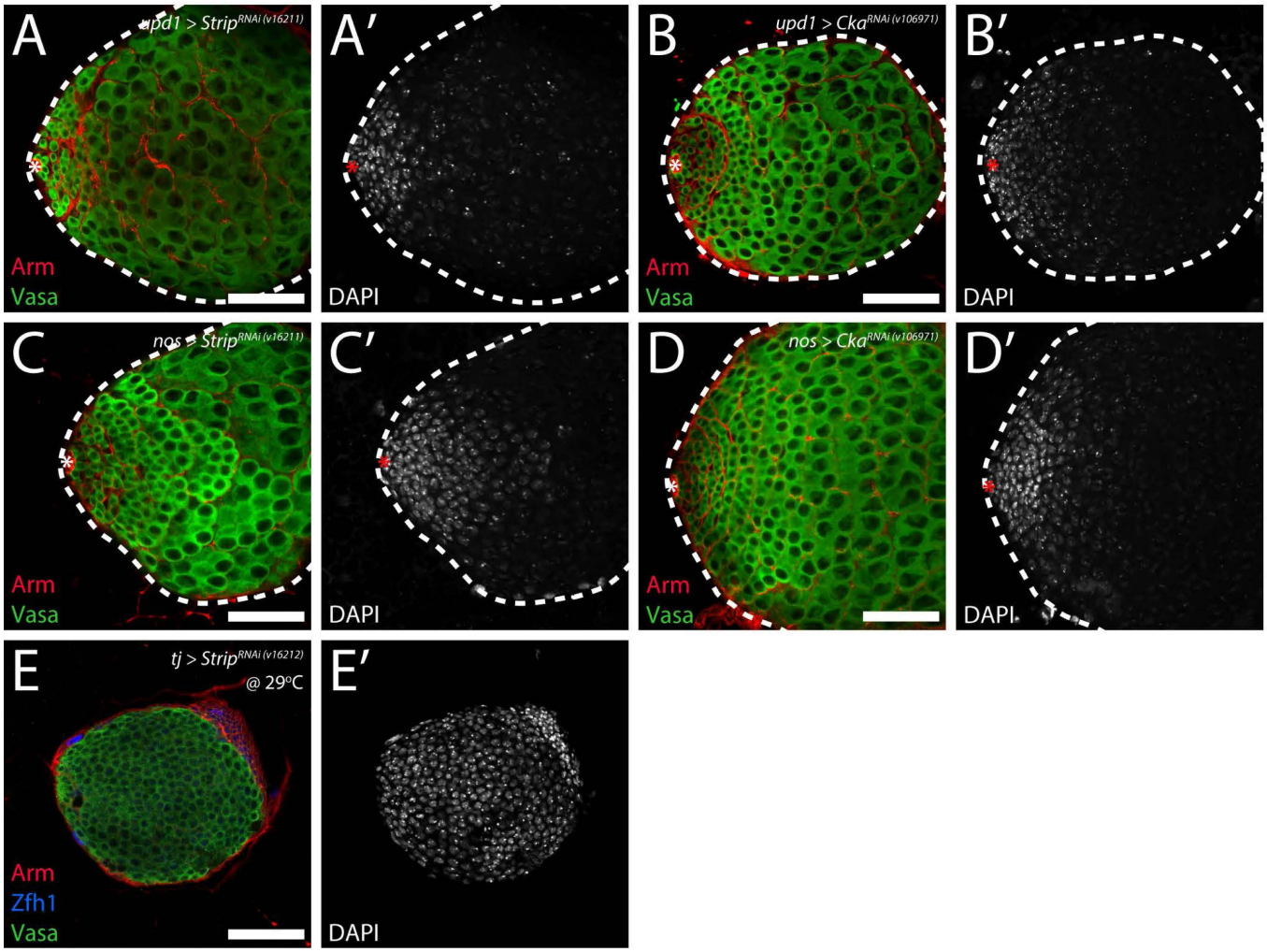

Figure S4. *Strip* and *Cka* are dispensable in the hub and germline cells. (A-D') Gonads from animals with hub or germline lineage expression of RNAi against *Strip* (v16211) or *Cka* (v106971), stained for Arm and Vasa, and with DAPI. (A-B') Hub cell specific expression of RNAi against *Strip* (n=28/28) (A,A') and *Cka* (n=7/7) (B,B') driven using *upd1-GAL4*. (C-D') Early germline cell specific expression of RNAi against *Strip* (n=14/14) (C,C') and *Cka* (n=14/14) (D,D') driven using *nos-GAL4*. (E,E') Gonads with somatic lineage expression of RNAi against *Strip* (v16212) via *tj-GAL4* , stained for Arm, Zfh1, and Vasa, and with DAPI. The phenotype induced via homozygosity for $Strip^{jam}$ or knockdown of *Strip* using *C587-GAL4* is replicated using *tj-GAL4*. Note that the *nos-GAL4* driver stock also includes a *UAS-GAL4* construct to strengthen expression. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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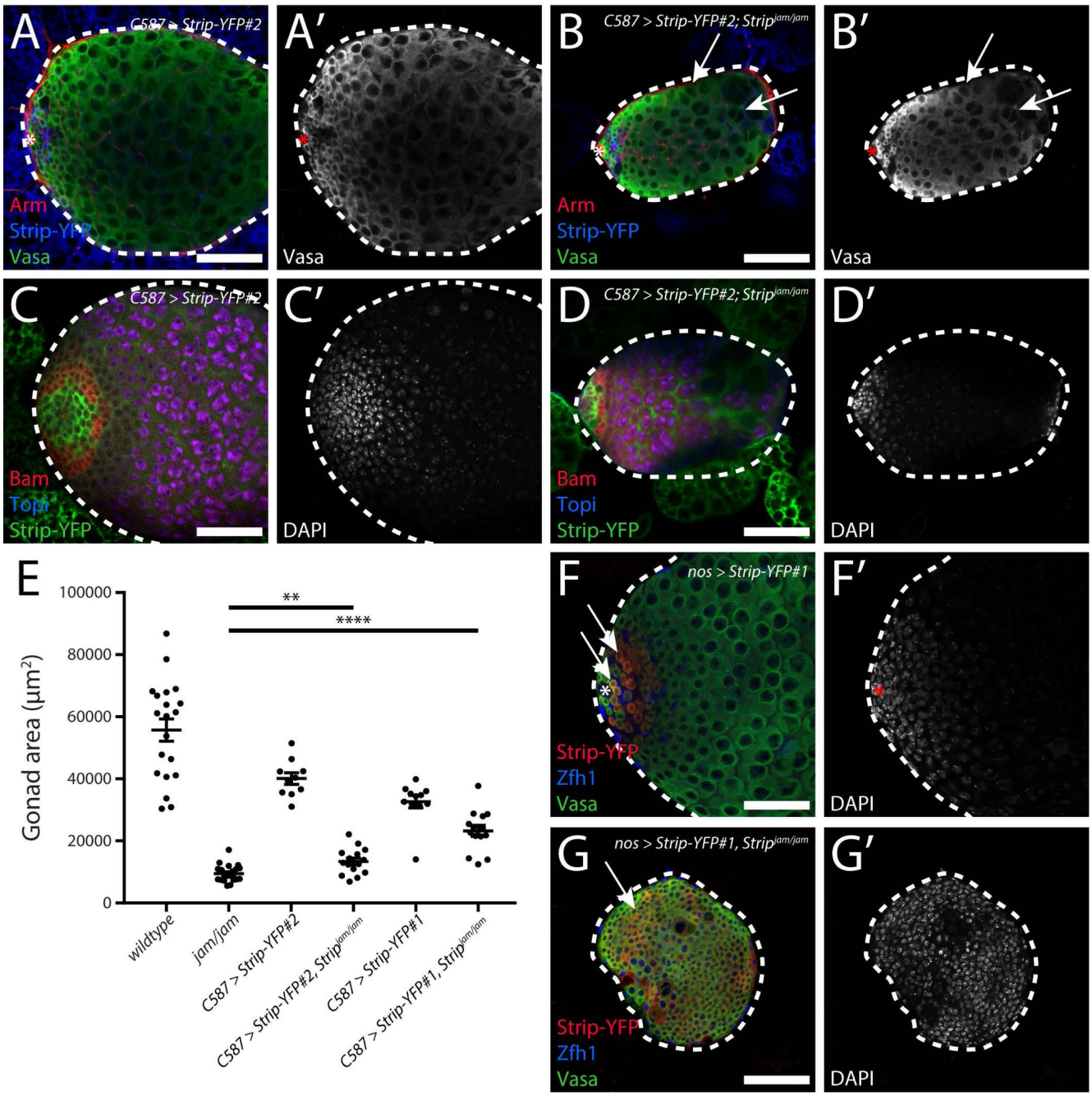

Figure S5. Somatic cell expression of *Strip-YFP* partially rescues *Strip$^{jam/jam}$*. (A-D') Gonads from animals with somatic lineage expression of *Strip-YFP#2* in both *wildtype* (n=23/23) (A,A',C,C') and *Strip$^{jam/jam}$* (n=42/42) (B,B',D,D') backgrounds, stained for Arm and Vasa, or for Bam and Topi with DAPI. Arrows indicate individualised, yet differentiated, germline cells. Note the apparent magenta staining in C and D is due to stronger than usual background staining in the Bam (red) channel. (E) Graph indicating the statistically significant increase in the area of *Strip$^{jam/jam}$* gonads after somatic lineage expression of *Strip-YFP#1* ($p<0.0001$, represented by ****) and *Strip-YFP#2* ($p<0.01$, represented by **). (F-G') Gonads from animals expressing *Strip-YFP#1* in the somatic lineage in both *wildtype* (n=9/9) (E,E') and *Strip$^{jam/jam}$* (n=16/16) (F,F') backgrounds, stained for Zfh1 and Vasa. Arrows indicate Strip-YFP expression in the germline cells. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50µm.

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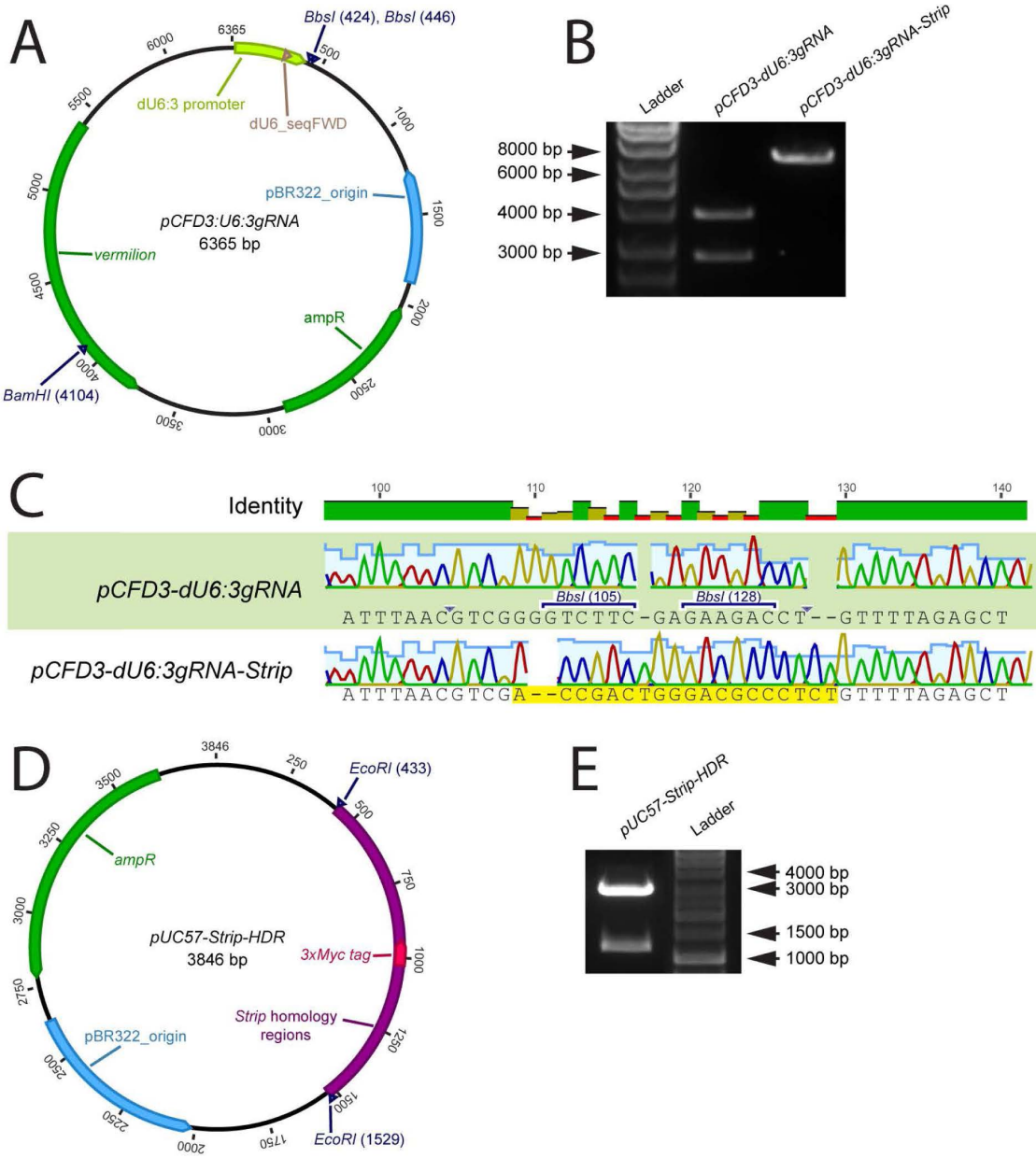

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Figure S6. CRISPR/Cas9 epitope tagging of Strip. (A-C) *pCFD3:U6:3-gRNA* was modified to contain the gRNA sequence for targeting *Strip*. (A) Plasmid map of unmodified *pCFD3:U6:3-gRNA*. The desired gRNA sequence was inserted into the *BbsI* sites. "dU6:3 promoter" is the gRNA promoter sequence, and "dU6_seqFWD" denotes the position of the forward primer used for sequencing. (B) Gel electrophoresis of unmodified and modified *pCFD3:U6:3-gRNA* digested with *BamHI* and *BbsI* restriction endonucleases. Loss of BbsI sites indicates successful insertion of the gRNA sequence. (C) Sequence data for unmodified *pCFD3:U6:3-gRNA* and modified *pCFD3:U6:3-gRNA-Strip*. The *pCFD3:U6:3-gRNA-Strip* sequence was aligned to the *pCFD3:U6:3-gRNA* reference sequence. The modified sequence reflects the gel electrophoresis result, showing successful insertion of the gRNA sequence (highlighted in yellow), and loss of the *BbsI* cut sites. (D,E) A repair template consisting of a homology region for *Strip* and a *3×Myc tag* at the appropriate site was synthesised in a *pUC57-simple* (*pUC57*) backbone. (D) Plasmid map of *pUC57-Strip-HDR*. (E) Gel electrophoresis of *pUC57-Strip-HDR* digested with *EcoRI* to release the 1090bp repair template. In each plasmid map "pBR322_origin" refers to the plasmid origin of replication sequence and "*ampR*" refers to the ampicillin resistance gene. Plasmid maps were generated in Geneious v.8 (Kearse et al., 2012). (F-G'') *Strip-Myc*$^{2\text{-}38\text{-}4\text{-}1}$ gonads (n=9/9) stained for the Myc epitope tag, Zfh1, and Vasa, at both regular (F-F'') and higher (G-G'') magnifications. Arrows indicate patches of strong Strip-Myc expression. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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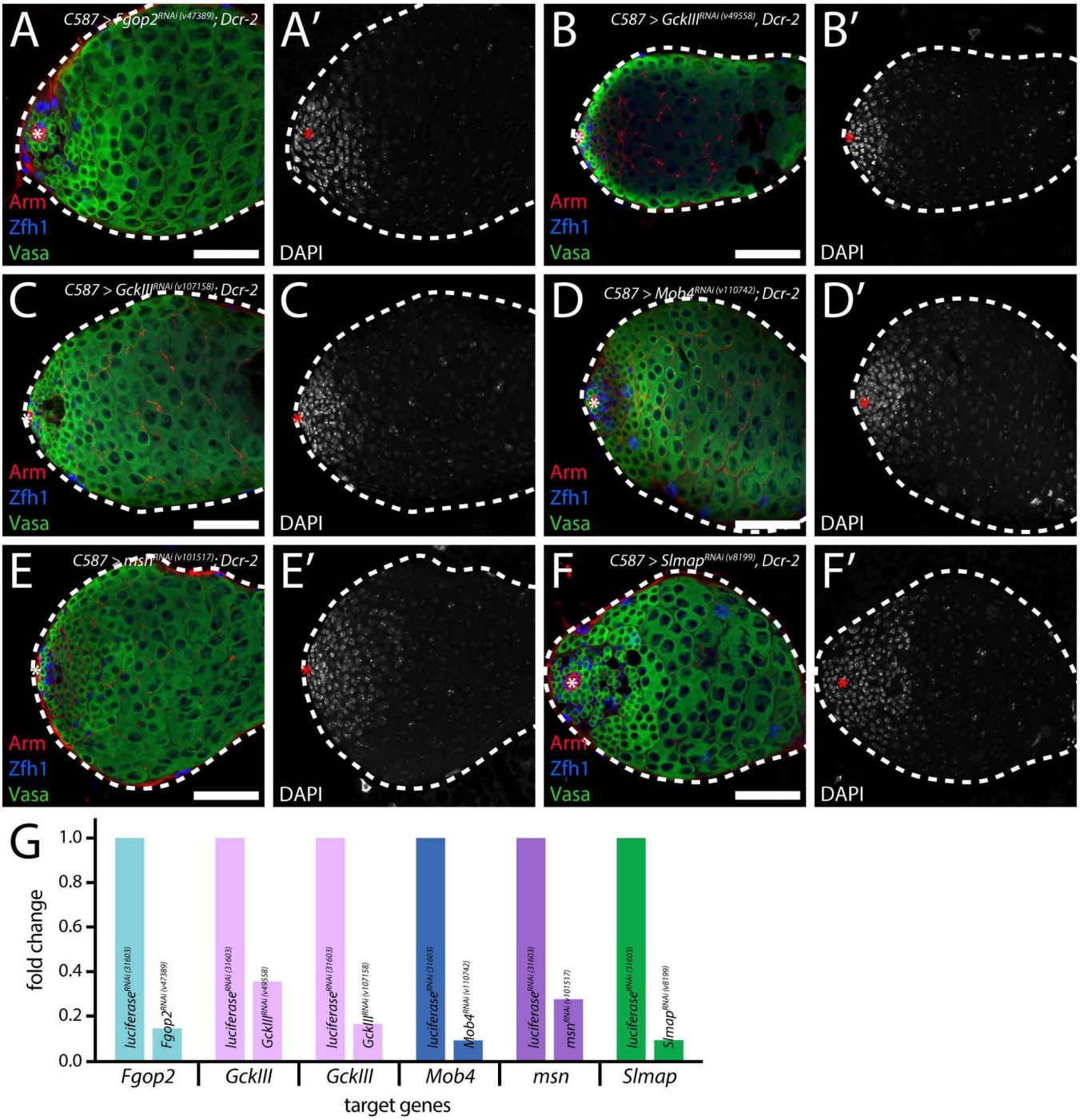

Figure S7. Somatic cell knockdown of other STRIPAK components does not affect spermatogenesis. (A-H') Gonads from animals with somatic lineage expression of RNAi against STRIPAK complex components, alongside *Dcr-2*, in an otherwise *wildtype* background, stained for Arm, Zfh1, and Vasa, and with DAPI. (A,A') RNAi (v47389) against *Fgop2* (n=4/4). (B,B') RNAi (v49558) against *GckIII* (n=16/16). (C,C') RNAi (v107158) against *GckIII* (n=6/6). (D,D') RNAi (v110742) against *Mob4* (n=45/46). (E,E') RNAi (v101517) against *msn* (n=12/12). (F,F') RNAi (v8199) against *Slmap* (n=42/45). (G) Efficacy of STRIPAK RNAi lines measured via RT-qPCR. Pictured is the amount of target mRNA after knockdown of the STRIPAK component genes, relative to that from a *luciferase* knockdown control (n>10 animals per genotype). Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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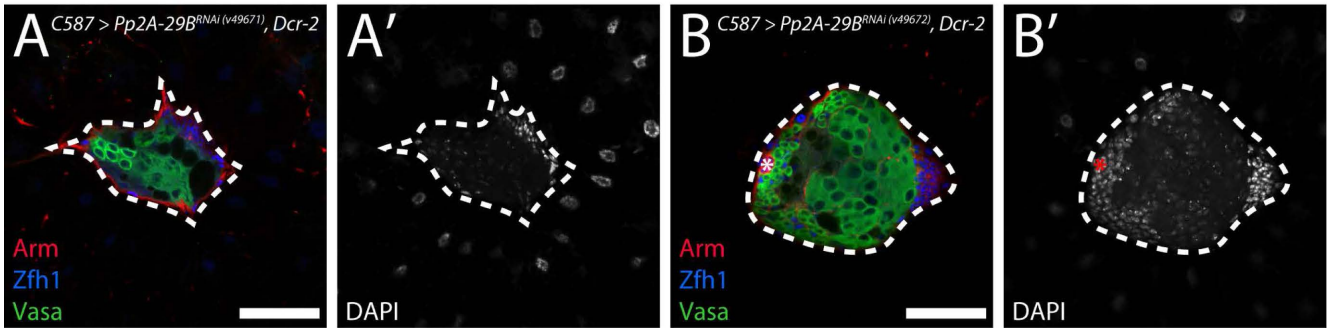

Figure S8. Somatic cell knockdown of *Pp2A-29B* leads to early germline cell loss. (A-B') Gonads from animals with somatic lineage expression of *Dcr-2*, alongside RNAi against *Pp2A-29B* v49671 (n=9/9) (A,A') and v49672 (n=9/9) (B,B'), stained for Arm, Zfh1, and Vasa, and with DAPI. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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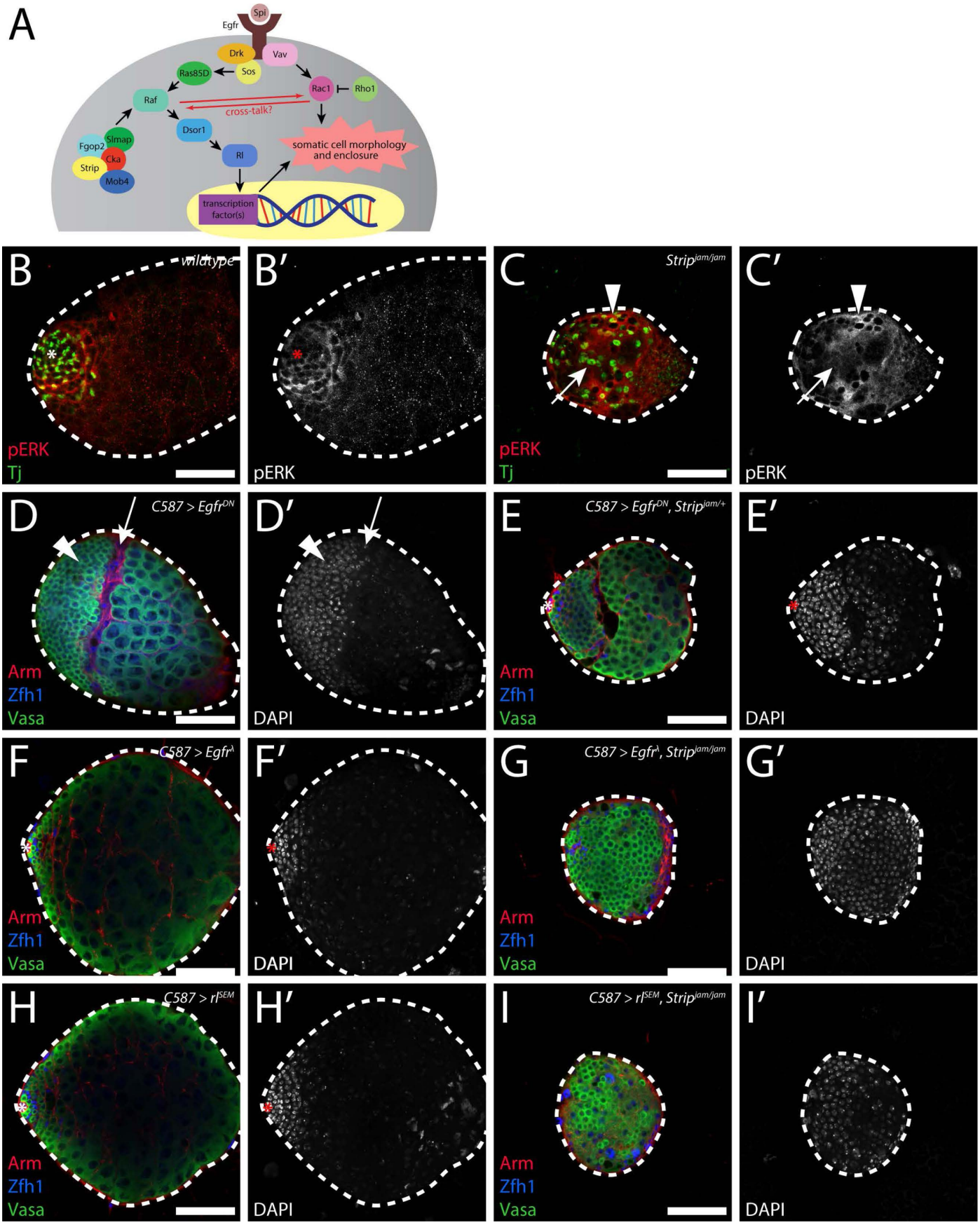

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Figure S9. *Strip* does not genetically interact with the EGFR signalling pathway in the gonad. (A) Diagram of the bifurcated EGFR signalling pathway regulating *Drosophila* spermatogenesis. STRIPAK complex members have been shown to act as positive regulators of the pathway in *Drosophila* S2 cells, as well as adult wing and eye tissues. (B-C') *Wildtype* (n=26/26) (B,B') and $Strip^{jam/jam}$ (n=29/29) (C,C') gonads stained for pERK and Tj. Arrow indicates a pERK-positive somatic cell; arrowhead indicates a pERK-negative somatic cell. No obvious change in pERK levels was observed, although somatic cells in the mutant sporadically did not express pERK. (D-I') Gonads from animals with somatic lineage expression of various transgenes, stained for Arm, Zfh1, and Vasa, and with DAPI. (D-E') Gonads from animals expressing $Egfr^{DN}$ in the somatic lineage in both *wildtype* (n=17/21) (D,D') and $Strip^{jam/+}$ (n=19/22) (E,E') backgrounds. Arrows indicate non-enclosing somatic cells; arrowheads indicate supernumerary early germline cells. Being additionally heterozygous for $Strip^{jam}$ did not enhance the $Egfr^{DN}$-induced phenotype. (F-G') Gonads from animals expressing $Egfr^{\lambda}$ in the somatic lineage in both *wildtype* (n=7/7) (F,F') and $Strip^{jam/jam}$ (n=13/13) (G,G') backgrounds. $Egfr^{\lambda}$ expression did not alter the mutant phenotype. (H-I') Gonads from animals expressing rl^{SEM} in the somatic lineage in both *wildtype* (n=12/12) (H,H') and $Strip^{jam/jam}$ (n=6/6) (I,I') backgrounds. rl^{SEM} expression did not alter the mutant phenotype. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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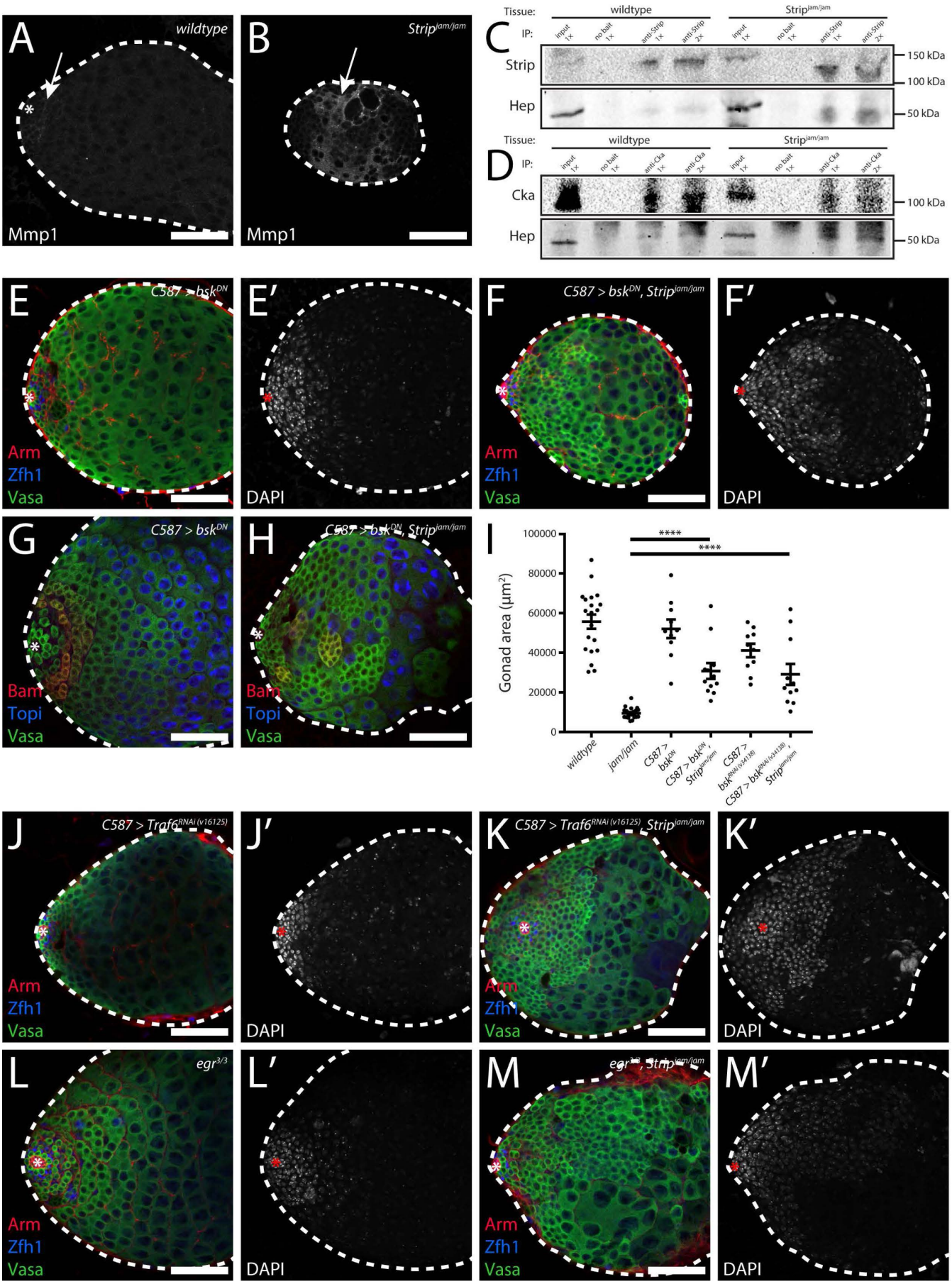

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Figure S10. Hep physically interacts with both Strip and Cka in both *wildtype* and *Strip$^{jam/jam}$* gonads.

(A,B) *Wildtype* (n=10/10) (A) and *Strip$^{jam/jam}$* (n=12/12) (B) gonads stained for Mmp1. Arrows indicate Mmp1 expression. (C,D) In both *wildtype* and *Strip$^{jam/jam}$* tissue extracts, Hep immunoprecipitated when baited with either anti-Strip (C) or anti-Cka (D) (each performed 2 times – further assays were limited by our having only small quantities of these non-commercial antibodies, which also partly explains our inability to obtain "cleaner" blots, alongside stripping and re-probing of each blot contributing to a degradation of signal quality). (E-H', J-M') Gonads from animals with somatic lineage expression of various transgenes, stained variously for Arm, Zfh1, Bam, Topi, and Vasa, and with DAPI. (E-F') Expressing *bsk^{DN}* in both *wildtype* (n=20/20) (E,E',G) and *Strip$^{jam/jam}$* (n=34/35) (F,F',H) backgrounds rescues somatic and germline morphologies, as well as germline differentiation marker expression. (I) Graph indicating the statistically significant increase in the area of *Strip$^{jam/jam}$* gonads after somatic lineage expression of either RNAi against *bsk* and *bskDN* (both $p<0.0001$, represented by ****). (J-K') Expressing RNAi against *Traf6* in both *wildtype* (n=10/10) (J,J') and *Strip$^{jam/jam}$* (n=16/16) (K,K') backgrounds. (L-M') Gonads from animals homozygous for *egr^{3}* in *wildtype* (n=7/7) (L,L') and *Strip$^{jam/jam}$* (n=21/21) (M,M') backgrounds, stained for Arm, Zfh1, and Vasa, and with DAPI. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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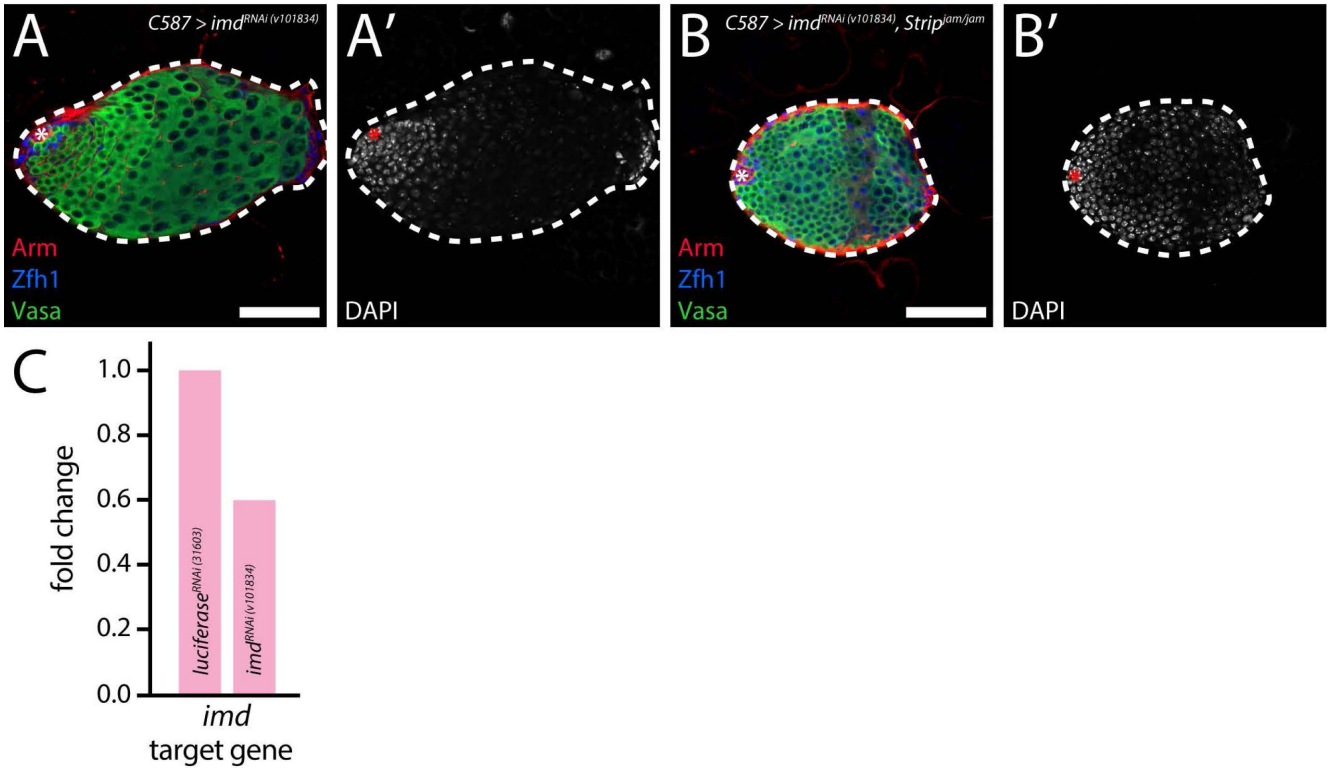

Figure S11. IMD signalling is dispensable during JNK signalling in *Strip*$^{jam/jam}$ gonads. (A-B') Gonads from animals with somatic lineage expression of RNAi against *imd* in both *wildtype* (n=7/7) (G,G') and *Strip*$^{jam/jam}$ (n=16/16) (H,H') backgrounds, stained for Arm, Zfh1, and Vasa, and with DAPI. (C) Efficacy of RNAi against *imd* measured via RT-qPCR. Pictured is the amount of *imd* mRNA after *imd* knockdown relative to that in a *luciferase* knockdown control (n>10 animals per genotype). Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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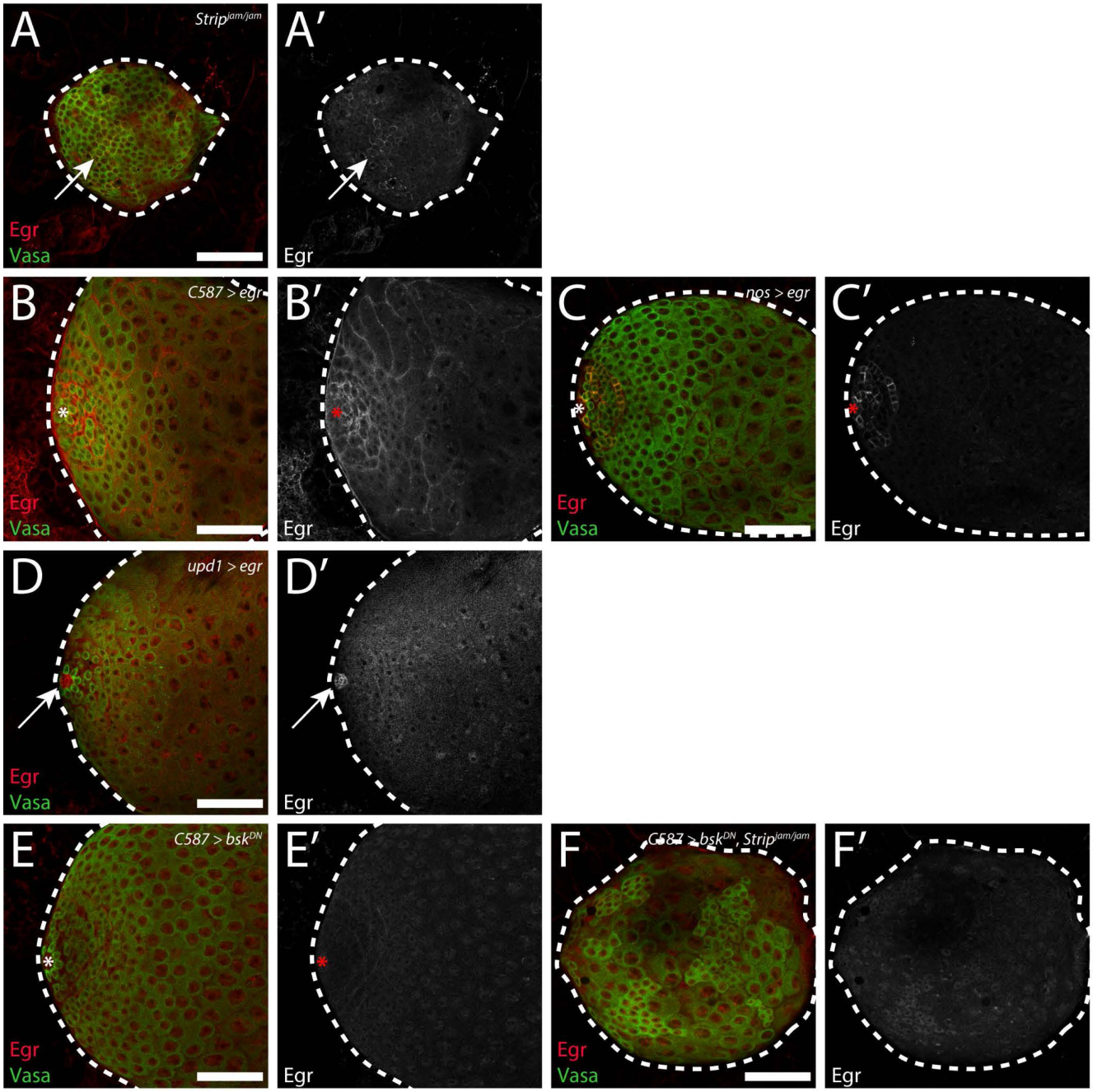

Figure S12. Egr expression is elevated in *Strip*$^{jam/jam}$ gonads. (A,A') *Strip*$^{jam/jam}$ gonads stained for Egr and Vasa. Arrows indicate concentrations of Egr expression in the cytoplasm of some germline cells. (B-F') Gonads from animals expressing various transgenes, stained for Egr and Vasa. (B-D') Expressing *egr* in the somatic (n=5/5) (B,B'), germline (n=11/11) (C,C'), and hub (n=12/12) (D,D') lineages in a *wildtype* background. Inducing *egr* expression does not alter the gonad phenotype. (E-F') Expressing *bsk*^{DN} in the somatic lineage in both *wildtype* (n=10/10) (E,E') and *Strip*$^{jam/jam}$ (n=13/13) (F,F') backgrounds. Where possible, an asterisk marks the hub, except for D,D', where an arrow marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.s

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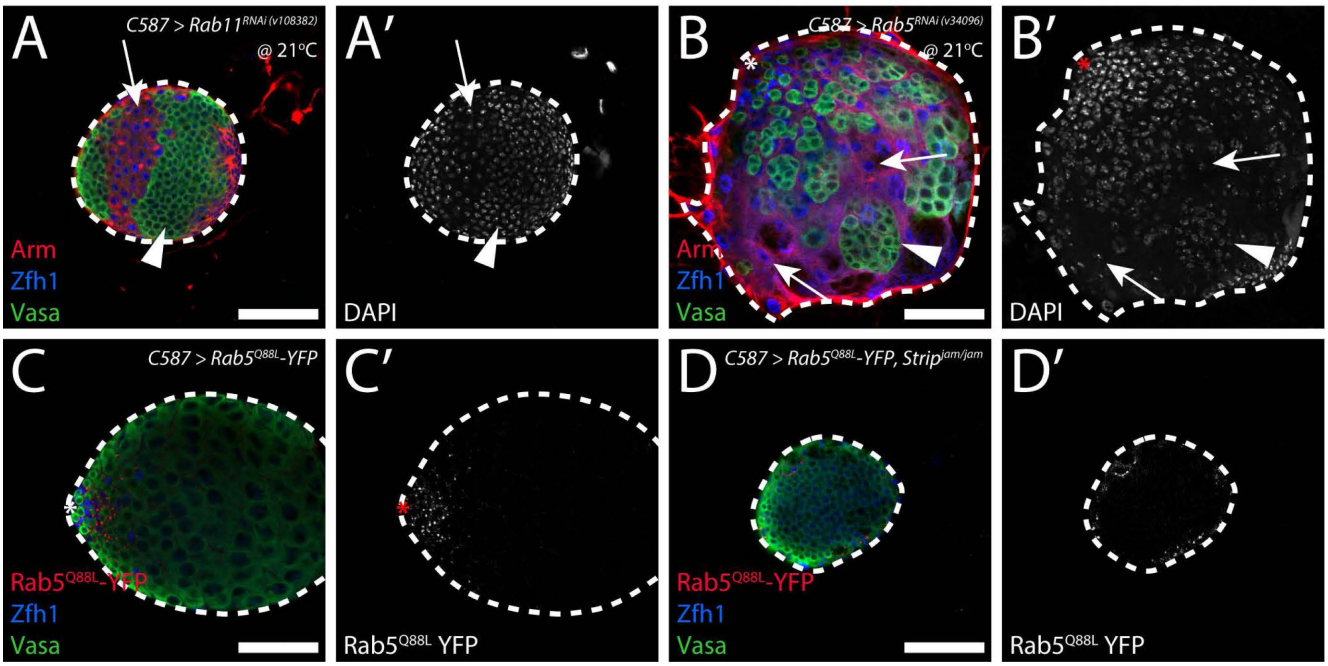

Figure S13. Endocytosis components are necessary during spermatogenesis, but do not interact genetically with Strip. (A-B') Gonads from animals with somatic lineage expression of various transgenes, stained for Arm, Zfh1, and Vasa, and with DAPI. Expressing RNAi against *Rab11* (n=13/13) results in dissociated somatic cells and ectopic early germline cells (A,A'). Arrows indicate non-enclosing somatic cells; arrowheads indicate supernumerary early germline cells. Expressing RNAi against *Rab5* (n=16/16) results in overproliferating cysts and some dissociated somatic cells (B,B'). Arrows indicate non-enclosing somatic cells; arrowheads indicate an overproliferating cyst. Note the apparent magenta staining is due to stronger than usual background staining in the Zfh1 (blue) channel. (C-D') Gonads from animals with somatic lineage expression of YFP tagged $Rab5^{Q88L}$, in both *wildtype* (n=35/35) (C,C') and $Strip^{jam/jam}$ (n=16/16) (D,D') backgrounds, stained for Zfh1 and Vasa. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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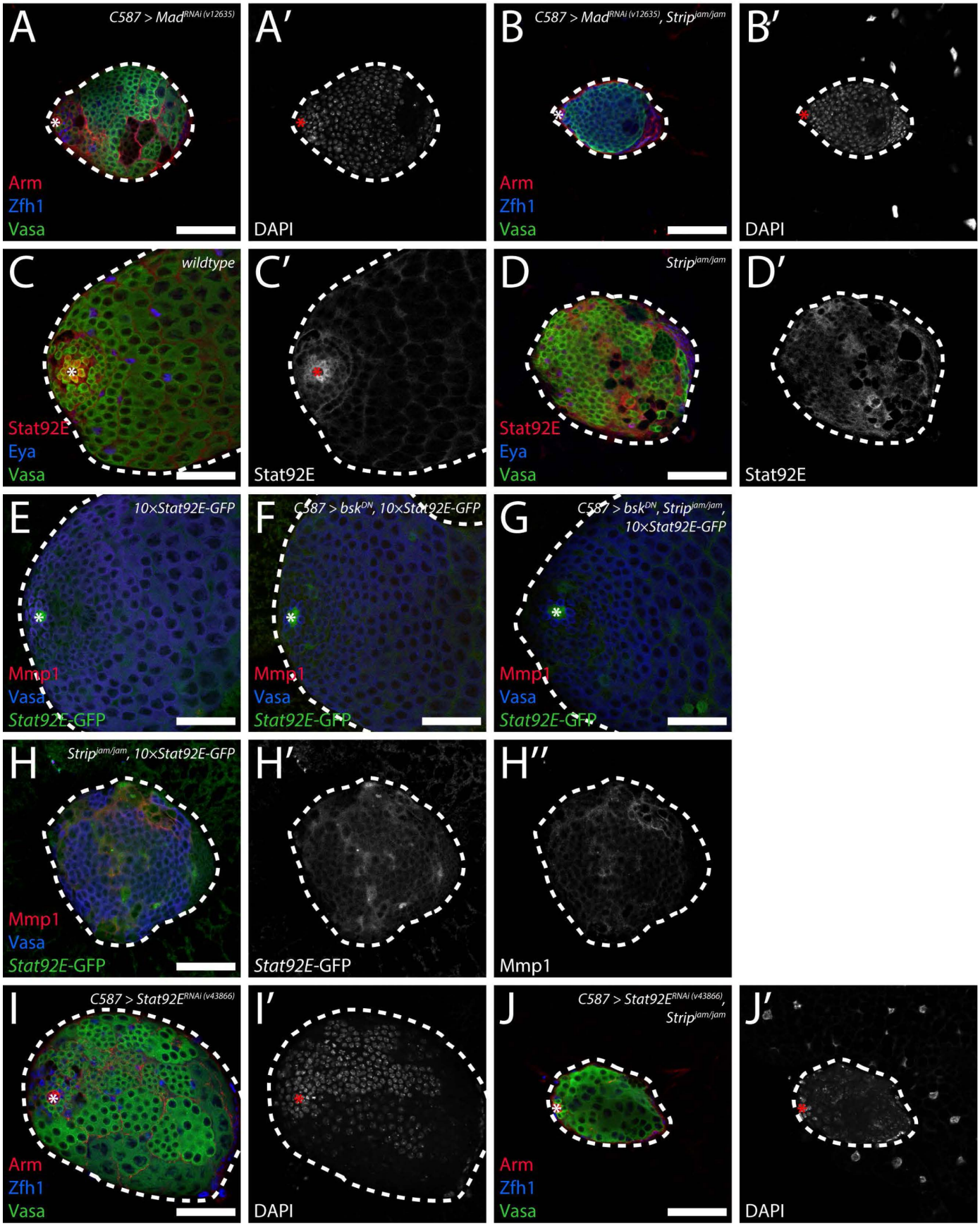

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Figure S14. Jak-STAT and TGF-β signalling components do not genetically interact with *Strip*. (A-B'') Gonads from animals with somatic lineage expression of RNAi against *Mad*, stained for Arm, Zfh1, and Vasa, and with DAPI. In a *wildtype* background (n=9/9) (A,A''), *Mad* knockdown led to ectopic spermatogonia TA divisions, and in a $Strip^{jam/jam}$ background (n=10/10) (B,B'') it did not rescue the mutant phenotype. (C-D'') *Wildtype* (n=9/9) (C,C'') and $Strip^{jam/jam}$ (n=11/11) (D,D'') gonads stained for Stat92E, Eya, and Vasa. Stat92E expression appeared greater in the somatic cells of $Strip^{jam/jam}$ gonads. (E-H'') Gonads from animals heterozygous for *10×Stat92E-GFP*, stained for Mmp1 and Vasa. With no additional transgene expression (n=5/5) (E) or with somatic cell expression of bsk^{DN} (n=6/6) (F) in a *wildtype* background, or with somatic cell expression of bsk^{DN} in a $Strip^{jam/jam}$ background (n=6/6) (G), GFP expression appears to be limited to the hub, while in a $Strip^{jam/jam}$ background with no additional transgene expression (n=7/7) (H-H'') GFP is strongly expressed in the somatic cells and co-localises with Mmp1. (I-J'') Gonads from animals with somatic lineage expression of RNAi against *Stat92E*, stained for Arm, Zfh1, and Vasa, and with DAPI. In both *wildtype* (n=6/9) (I,I'') and $Strip^{jam/jam}$ (n=14/24) (J,J'') backgrounds, *Stat92E* knockdown led to early germline cell loss, and it did not rescue the mutant phenotype. Where possible, an asterisk marks the hub. Dotted lines outline the gonads. Scale bars = 50μm.

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