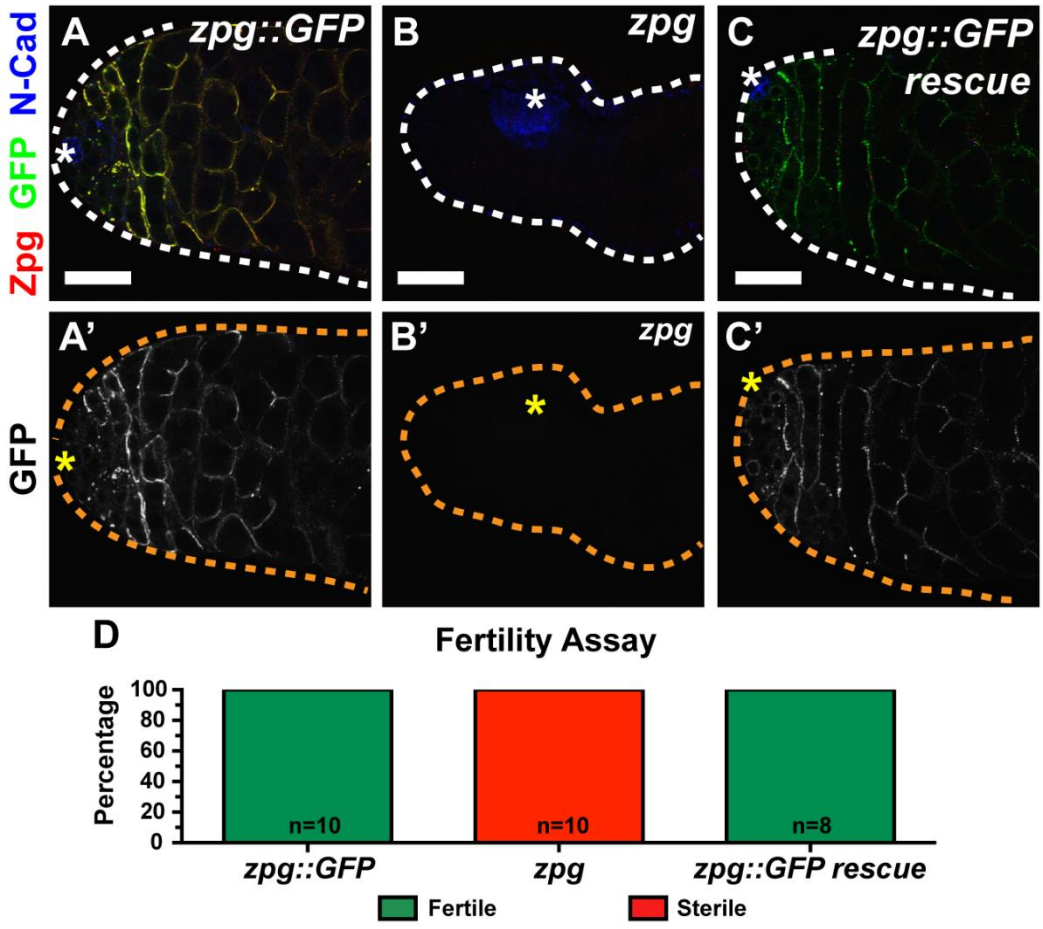

Figure S1 – A GFP-tagged, wild-type *zpg* transgene rescues fertility in *zpg* mutant testes. (A-C) Expression of a GFP-tagged *zpg* transgene under the putative endogenous promoter of *zpg* co-localized with endogenous Zpg (A). The *zpg::GFP* transgene (C) was able to rescue the *zpg* mutant (B) phenotype, observed by immunohistochemistry. (D) Fertility assays demonstrate that the *zpg::GFP* transgene restored fertility in *zpg* mutant males to wild-type levels. Testes were stained with antibodies to detect Zpg (red), GFP (green), and N-Cad (blue). Asterisk indicates the hub. Dashed lines outline the testis. Scale bars are 30µm in all panels.

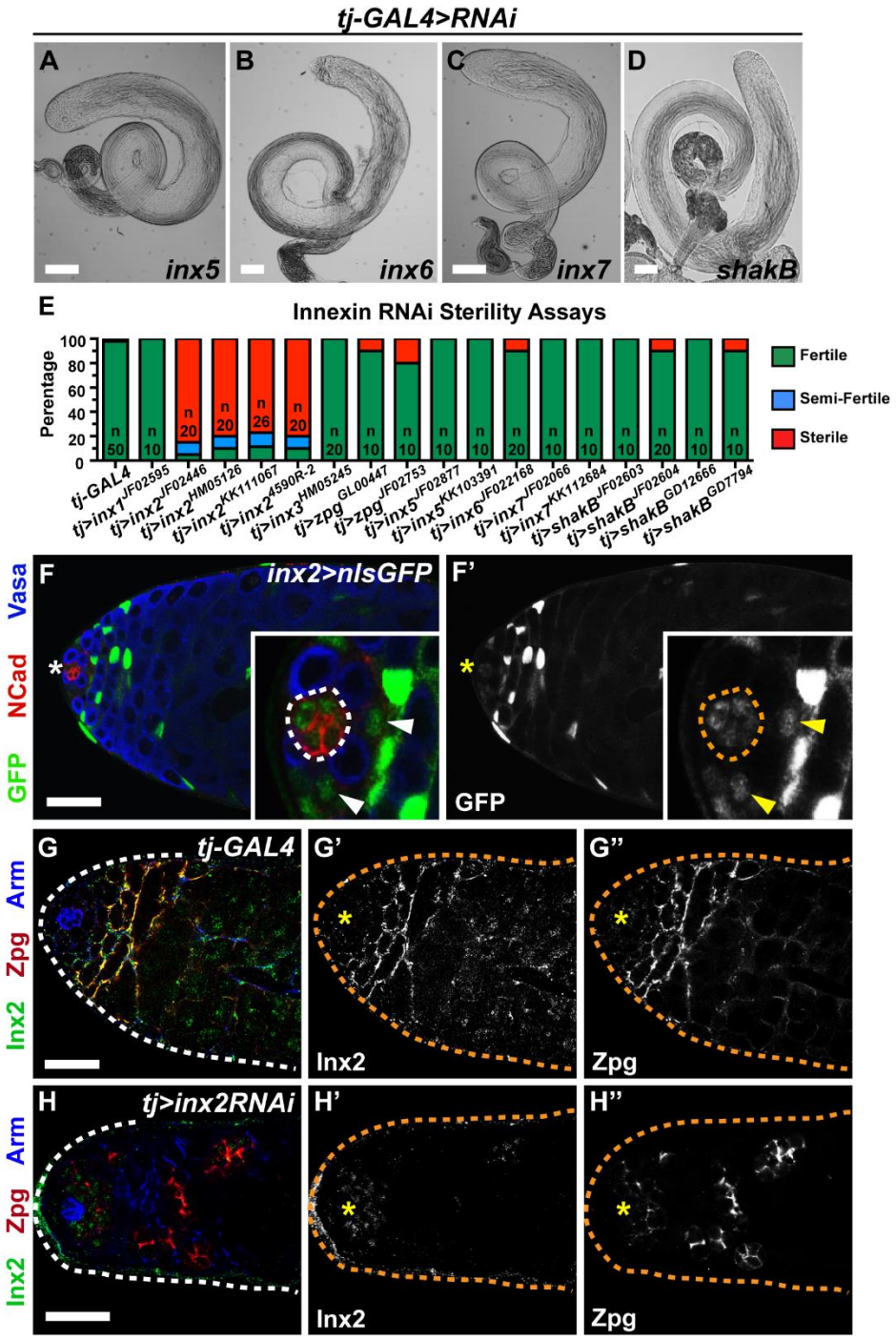

Figure S2 – Inx2 alone is required in the soma for fertility and Inx2 is expressed early in spermatogenesis. (A-D) Representative DIC images of testes in which Innexins 5, 6 ,7 and 8 (*shaking-B)* were knocked down in the soma using RNAi lines (see materials and methods) driven with tj-GAL44. No visible phenotypes were observed with these knockdowns. (E)

Summary of fertility assays carried out with various RNAi lines targeting every fly innexin driven by tj-GAL4 in the soma (n=number of independent fertility assays). Inx2 was the only innexin that was consistently required for fertility. (F-F') Expression of nls-GFP under the control of the *inx2* promoter. Reporter expression can be detected weakly in hub cells and CySCs, before increasing in early somatic cells. Arrowheads indicate CySCs. (G) control and (H) *tj>inx2RNAi* testes that were stained with antibodies to detect Inx2 (green) and Zpg (red) proteins show that RNAi mediated knockdown substantially reduced Inx2 expression (Armadillo in blue was used to highlight the soma and the hub). Asterisk indicates the hub. Dashed lines outline the hub in F'F', and the testis in G-H. Scale bars A-D are 100µm and 30µm in all other panels.

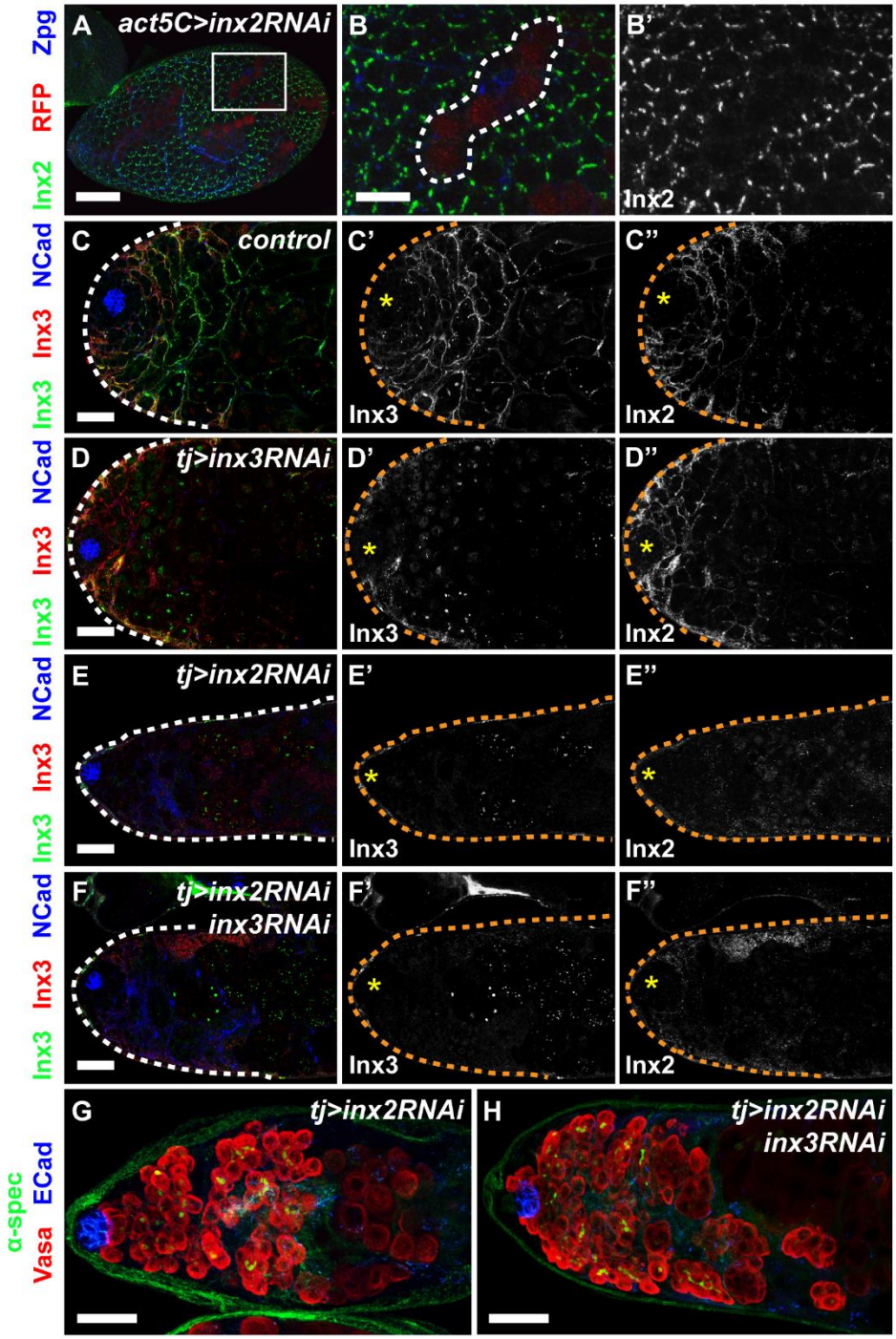

Figure S3 – Inx3 is not required in the soma for spermatogenesis, but Inx2 is required in the soma for the localization of Inx3. (A-B) Clonal overexpression of *inx2RNAi* in developing egg chambers. At 2 days post-clone induction, Inx2 protein levels were greatly reduced in clones, compared to neighbouring cells based on Inx2 antibody staining. (C-F)

Control, *inx3RNAi*, *inx2RNAi*, and *inx2RNAi+inx3RNAi* testes stained for Inx3 (green), Inx2 (red), and N-Cad (blue). (C) Inx3 and Inx2 colocalized in control testes. (D) Upon knockdown of Inx3, Inx3 staining was reduced, but Inx2 remained similar to controls. (E) Somatic knockdown of Inx2 disrupted Inx3 localization, in addition to reducing Inx2 protein levels. (F) Knockdown of both Inx2 and Inx3 in the soma greatly decreased levels of both Inx2 and Inx3. (H) *tj>inx2RNAi* and *tj>inx2RNAi, inx3RNAi* testes show that double-knockdown of Inx2 and Inx3 in the soma results in a phenotype similar to that obtained from knockdown of Inx2 alone (Vasa in red; E-Cad in blue; α-spectrin in green). Asterisk indicates the hub. Dashed lines outline the clone in B, the testis in C-F. Scale bars are 30μm in all panels.

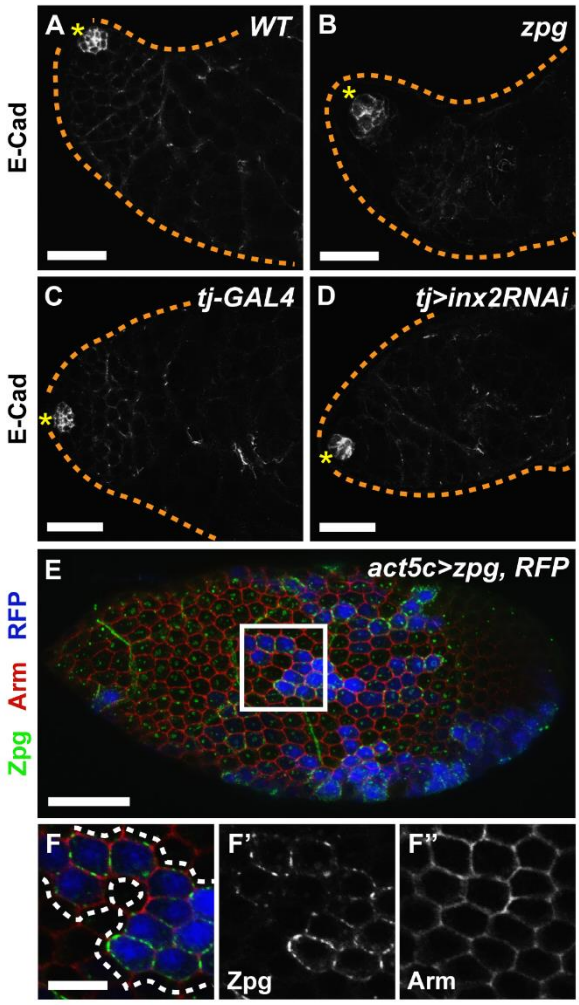

Figure S4 – Cadherin expression is not misregulated upon changes in *zpg* or *inx2* expression. (A-B) Wild-type and *zpg* testes stained for E-Cad. In both wild-type (A) and *zpg* testes (B), E-Cad was enriched in the hub and could be detected in differentiating germline cysts. (C-D) In control testes and upon somatic knockdown of Inx2, E-Cad staining showed a similar staining pattern, with enrichment in the hub, and lower levels of expression outside of the niche. (E) Overview of a stage 9 developing egg chamber. A heat-shock-inducible *act5c-GAL4* drives expression of RFP (blue) and a *UASt-zpg*^{cDNA} transgene (green). Armadillo (red) labels cell-cell junctions. (F) Inset of clone from E. Expression of Zpg (F') does not visibly alter levels of Armadillo within clones (F''). Asterisk indicates the hub. Dashed lines outline the testis in A-D, the clone in F. Scale bars are 30µm in A-E, 10µm in F.

Fly Stocks

The following lines were used: the somatic drivers *tj-Gal4* and *c587-Gal4*; the germline driver *nos-Gal4-VP16*; *zpg$^{z\text{-}2533}$,st/TM3,Sb*; *zpg$^{z\text{-}5352}$/TM6B*; *hs-flp/FM7;FRT2a/TM3,Ser; His2a::RFP/TM3,Ser*; *UAS-mCD8::GFP*; *w^{1118}*; *tud^{1}/CyO*, *tud^{B42}/CyO*, and *tud^{B45}/CyO*, (Arkov et al., 2006); *UAS-tkv^{Act},UAS-sax^{Act}/CyO* (Haerry et al., 1998); *hs-flp; +; Dr/TM3,Sb*; *w^{-}; +; act5c-GAL4-FRT-stop-FRT-RFP/TM3, Sb*; UAS-anti-*inx1* RNAi JF02595; UAS-anti-*inx2* RNAi JF02446; UAS-anti-*inx2* RNAi HM05126; UAS-anti-*inx2* RNAi KK111067; UAS-anti-*inx2* RNAi 4590R-2; UAS-anti-*inx3* RNAi HM05245; UAS-anti-*zpg* RNAi GL00447; UAS-anti-*zpg* RNAi JF02753; UAS-anti-*inx5* RNAi JF02877; UAS-anti-*inx6* RNAi JF02168; UAS-anti-*inx7* RNAi JF02066; UAS-anti-*inx7* RNAi KK112684; UAS-anti-*shakB* RNAi JF02603; UAS-anti-*shakB* RNAi JF02604; UAS-anti-*shakB* RNAi GD12666; and UAS-anti-*shakB* RNAi GD7794. The *innexin2* enhancer trap line, *inx2>nlsGFP* P01999, was obtained from the Cooley lab (Quiñones-Coello et al., 2007).

Genetics

Crosses were set up at room temperature, flipped each day, and raised at 25°C on standard media. To generate *zpg* flies, *zpg$^{z\text{-}2533}$/TM3,Sb* flies were crossed to *zpg$^{z\text{-}5352}$/TM6B* flies; heterozygous siblings were used as a control. *tudor* flies were generated using *tud^{B42}/CyO* or *tud^{B45}/CyO* crossed to *tud^{1}/CyO*, which were then crossed to *w^{1118}* males to generate germ cell-less flies. *CA-BMP* flies were generated by crossing *nos-Gal4::VP16* flies to *UAS-tkv^{Act},UAS-sax^{Act}/CyO* flies. RNAi knockdowns, were performed using *UAS-Dcr2; tj-Gal4/CyO* crossed to the corresponding UAS-anti-innexin-RNAi line. For *inx2RNAi* phenotypic analysis, *UAS-JF02446* was used as it provided the strongest knockdown based on testis morphology and sterility assays. Controls were *UAS-Dcr2; tj-Gal4/CyO* males. Clones were *hs-flp, c587-GAL44>UAS-mCD8::GFP; +; His2a::RFP, FRT2a/TM3,Ser* crossed to w^{-}; _; FRT2a for control clones and to *w^{-}; +; zpg$^{z\text{-}5352}$,FRT2a/TM6B* for mutant clones. Progeny were raised at 25°C, clones were induced in males less than 1 day post-eclosion (DPE), using two 60-minute heat shocks at 37°, 90-minutes apart, and aged for 4-5 days at room temperature. Clones in

the ovary follicular epithelium were *hs-flp; +; act5c-GAL4-FRT-stop-FRT-GAL4-RFP/TM3, Sb* crossed to *UAS-JF02446* for the Inx2 knockdown experiment and w^{-}*; UASt-zpg*^{cDNA} for the Zpg over-expression experiment. Clones were induced in females <1DPE as above and aged for three days prior to dissection.

Fertility Assays

Fertility assays were performed using single males, 5DPE, crossed to three w^{1118} virgin females, aged 15 days, and scored as sterile if no larvae/pupae were present, and semi-sterile if <50 larvae/pupae were present.

Immunostaining

Primary antibodies used were rabbit-anti-Vasa (R. Lehmann, P. Lasko, 1:5000), guinea-pig-anti-Traffic jam (D. Godt, 1:3500), rabbit-anti-Zfh-1 (R. Lehmann, 1:1000), guinea pig-anti-Zfh-1 (J. Skeath, 1:500), rabbit-anti-Stat92E (E. Bach, 1:500), rabbit-anti-Inx3 (J. Davies, 1:1000), rabbit-anti-Boule (S. Wasserman 1:1000), mouse-anti-Cheerio/Filamin (L. Cooley, 1:1000), rabbit-anti-Zpg (1:20000), guinea pig-anti-Inx2 (1:1000), rat-anti-DN-Cadherin (Developmental Studies Hybridoma Bank (DSHB), 1:50), rat-anti-DE-Cadherin (DSHB, 1:50), mouse-anti-spectrin (DSHB, 3A9, 1:5), mouse-anti-Bam (DSHB and D. McKearin, Bam, 1:50), mouse-anti-Coracle (DSHB, C566.9 and C615.16 1:500), mouse-anti-Armadillo (DSHB, N2 7A1, 1:1000), mouse-anti-Eya (DSHB, eya10H6, 1:500), mouse-anti-GFP (Invitrogen, A11120, 1:1000), and rat-anti-dsRed (Chromotek, 5f8, 1:1000).

Molecular Biology

A 6.15kb rescue fragment, encompassing the *zpg* genomic locus and an additional 1.5kb upstream and downstream of the locus (Tazuke *et al*., 2002) was cloned into a pattB using *Bam*HI. A short linker sequence (LAAA) was inserted after the last amino acid of Zpg (Arai et al., 2001) followed by a GFP cassette. The construct sequence was verified prior to injection (BestGene) into *Drosophila* w^{1118} flies. Transformants were generated using *attP40* integration sites on the second chromosome.

For *UASt-zpg*^{cDNA}, a vector containing the cDNA of *zpg* was obtained from the Berkley *Drosophila* Genome Project, care of the *Drosophila* Genomics Resource Centre (cDNA clone RE18536). The *zpg* cDNA was removed from the vector and cloned into a *UASt-pattB* vector. The construct sequence was verified prior to injection (BestGene) into *Drosophila w*^{1118} flies. Transformants were generated using *attP40* integration sites on the second chromosome.