

Supplemental Materials

Molecular Biology of the Cell

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Supplementary Figure 1. Nipped-A is required in the germ line and soma

(A-A') Control, (B-B') germline-depleted *Nipped-A* using RNAi #2 (C-C') and RNAi #3 germaria stained with Vasa (blue) and 1B1 (red). Germaria depleted of *Nipped-A* shows accumulation of single cells (yellow dashed line) (n=25 for all). 1B1 channel is shown in A', B', and C'.

(D-D') Control and (E-E') germline-depleted *Nipped-A* in *Nipped-A* GFP-trap stained with GFP (green), Vasa (blue), and 1B1 (red). Germline-depleted *Nipped-A* germaria shows reduced GFP expression in the germarium (yellow dashed line). GFP channel is shown in D' and E'.

(F) Control and (G) soma-depleted *Nipped-A* ovaries stained with DAPI (green), Vasa (blue), and 1B1 (red) in 4x and 10x magnification, respectively. Ovaries depleted of *Nipped-A* show that *Nipped-A* is also required in the soma for egg chamber formation and germline maintenance (n=10 ovary pairs for both).

Statistical analysis performed with Chi-square for (F-G). Scale bar for all images is 20 μ m.

Supplementary Figure 2. Nipped-A is required for timely cell cycle progression

(A-A') Germline-depleted *bam* and (B-B') *Nipped-A* germaria stained with CycA (green) and Vasa (blue). Germline-depleted *Nipped-A* germaria shows accumulation of a lower percentage of CycA-positive germ cells (yellow arrow). CycA channel is shown in A' and B'.

(C-C') Germline-depleted *bam* and (D-D') *Nipped-A* germaria stained with CycB (green), Vasa (blue), and 1B1 (red). Germline-depleted *Nipped-A* germaria shows accumulation a higher percentage of CycB-positive germ cells (yellow dashed line). CycB channel is shown in C' and D'.

(E) Quantitation of the percentage of germ cells positive for G2 phase cell cycle markers in germline-depleted *bam* and *Nipped-A* germaria (18.58 \pm 6.68% CycA-positive germ cells per germaria in *Nipped-A* RNAi compared to 41.62 \pm 11.05% CycA-positive germ cells per germaria in *bam* RNAi control; 81.20 \pm 11.32% CycB-positive germ cells in *Nipped-A* RNAi compared to 63.21 \pm 14.38% CycB-positive germ cells in *bam* RNAi control; n=20 for all, **** indicates P-value<0.0001, *** indicates P-value=0.0002).

Statistical analysis performed with Student t-test. Scale bar for all images is 20 μ m.

Supplementary Figure 3. Loss of germline Tip60 complex members, but not SAGA complex members, leads to accumulation of single germ cells

(A-A') Control and germline-depleted Tip60 complex members (B-B') *Act87E*, (C-C') *Brd8*, (D-D') *DMAP1*, (E-E') *Eaf6*, (F-F') *Mrg15*, (G-G') *pont*, and (H-H') *rept* germaria stained with Vasa (blue) and 1B1 (red). Tip60 complex member-depleted germaria show accumulation of single cells (yellow dashed line), no significant accumulation (*Act87E*), or complete germline-loss (*rept*) (n=25, P-value=0.3110 for *Act87E* and P-value<0.0001 for all others). 1B1 channel is shown in A', B', C', D', E', F', G', and H'.

(I) Quantitation of the number of single cells in control and germline-depleted SAGA complex members *Ada2b*, *Ada3*, *Ataxin7*, *Gcn5*, *Sgf29*, and *Spt3* germaria showing that loss of SAGA Complex members in the germ line does not lead to accumulation of single cells (3.04 \pm 0.68 in *UAS-Dcr2;nosGal4*, 3.36 \pm 1.08 in *Ada2b* RNAi, 3.24 \pm 0.66 in *Ada3* RNAi, 3.24 \pm 0.97 in *Ataxin7* RNAi, 3.12 \pm 0.93 in *Gcn5* RNAi, 3.52 \pm 0.82 in *Sgf29* RNAi, and 3.28 \pm 0.98 in *Spt3* RNAi; n=25 for all, P-value=0.5870).

(J-J') Control and (K-K') *pgcGFP* with *nosGAL4*-driven *UAS-Tip60^{DN}* germaria stained with GFP (green), Vasa (blue), and 1B1 (red). Germaria with germline expression of *Tip60^{DN}* accumulate a higher number of Pgc-positive germ cells (n=30 for both, P-value=0.0009). Pgc expression is marked by GFP (yellow dashed circle/line in control and mutant, respectively). GFP channel is shown in J' and K'.

Statistical analysis performed with Student t-test for all except for one-way ANOVA for (I). Scale bar for all images is 20 μ m.

Supplementary Figure 4. Delayed differentiation is not caused by DNA damage response or nucleolar stress in *Nipped-A* depleted germ cells

(A-A') *UAS-Dcr2;nosGAL4*, (B-B') germline-depleted *bam* and (C-C') *Nipped-A* germaria stained with pH2Av (green), Vasa (blue), and 1B1 (red). GSC daughters in *UAS-Dcr2;nosGAL4* (white circle) are frequently not pH2Av-positive. Germline-depleted *Nipped-A* germaria do not accumulate pH2Av-positive germ cells compared to *bam* RNAi germaria (n=20 for all, P-value=0.4380). pH2Av channel is shown in A', B', and C'.

(D-D') *nosGAL4;p53R-GFP*, (E-E') *p53R-GFP* with germline-depleted *bam* and (F-F') *Nipped-A* germaria stained with GFP (green), Vasa (blue), and 1B1 (red). p53 activity is elevated in *SETDB1* depleted germ cells and 16-cell cysts of *nosGAL4* germaria (yellow dashed line). Frequency of GFP-positive germ cells per germaria is not significantly lower in germline-depleted *Nipped-A* germaria compared to *bam* RNAi germaria (n=20 for all, P value=0.6464 for *Nipped-A* and *bam* RNAi). GFP channel is shown in D', E', and F'.

(G-G') *UAS-Dcr2;nosGAL4*, (H-H') Germline-depleted *bam* and (I-I') and *Nipped-A* germaria stained with Fibrillarin (red), Vasa (blue), and DAPI (green). *Nipped-A* depleted germ cells do not undergo nucleolar stress. Fibrillarin channel is shown in G', H', and I'.

Statistical analysis performed with Student t-test. Scale bar for all images is 20 μ m.

Supplementary Figure 5. Ectopic expression of *bam* does not rescue germline-depletion of *Nipped-A*

(A-A') *nosGAL4*, (B-B') *nosGAL4>hs-bam*, (C-C') germline-depletion of *bam*, (D-D') *bam* RNAi with *hs-bam*, (E-E') germline-depletion of *Nipped-A*, and (F-F') *Nipped-A* RNAi with *hs-bam* germaria stained with Vasa (blue) and 1B1 (red). With ectopic expression of *bam*, both *nosGal4* and *bam* RNAi germaria consistently have fusome formation (yellow arrow). *Nipped-A* RNAi with ectopic expression of *bam* does not give significantly change the frequency of germaria with fusomes (white arrow). 1B1 channel is shown in A', B', C', D', E', and F'.

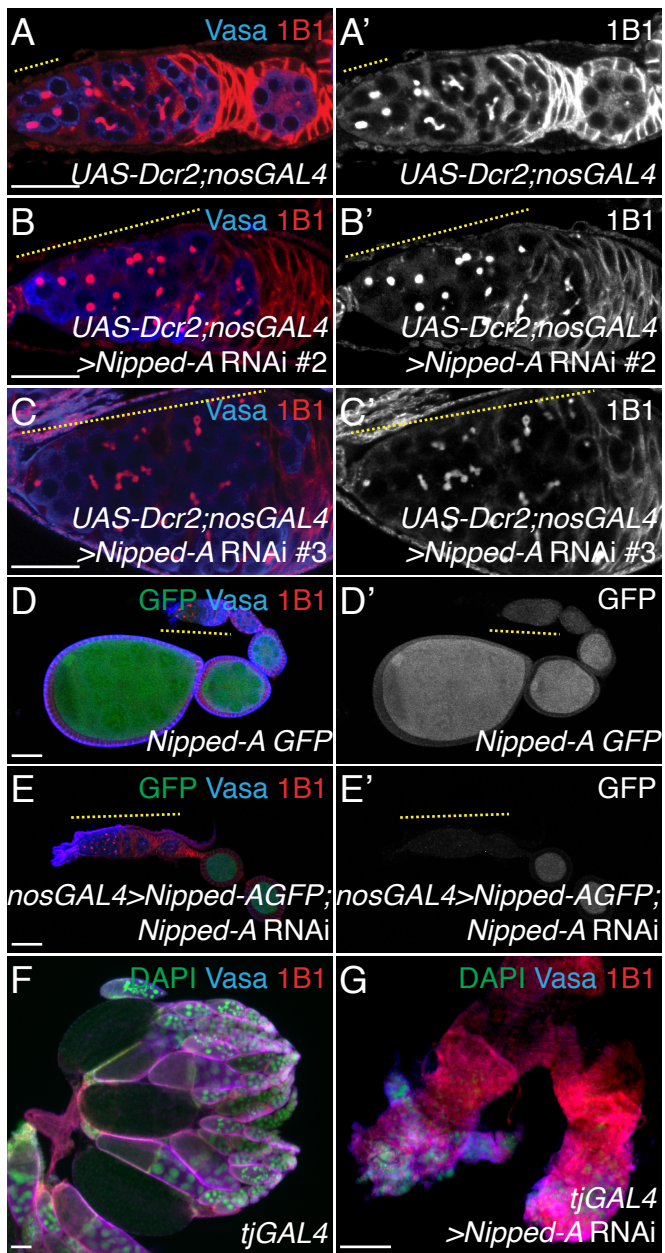
(G) Quantitation of the fraction of germaria with fusomes in germline-depleted *bam* and *Nipped-A* germaria with and without ectopic expression of *bam*. Ectopic expression of *bam* in *bam* RNAi rescues the differentiation defect, while in *Nipped-A* RNAi germaria it does not (77% germaria with fusomes in *bam* RNAi;*hs-bam* compared to 0% germaria with fusomes in *bam* RNAi control; 60% germaria with fusomes in *hs-bam/Nipped-A* RNAi compared to 40% germaria with fusomes in *Nipped-A* RNAi control; n=30 for all, **** indicates P-value<0.0001, ns indicates P-value=0.2292).

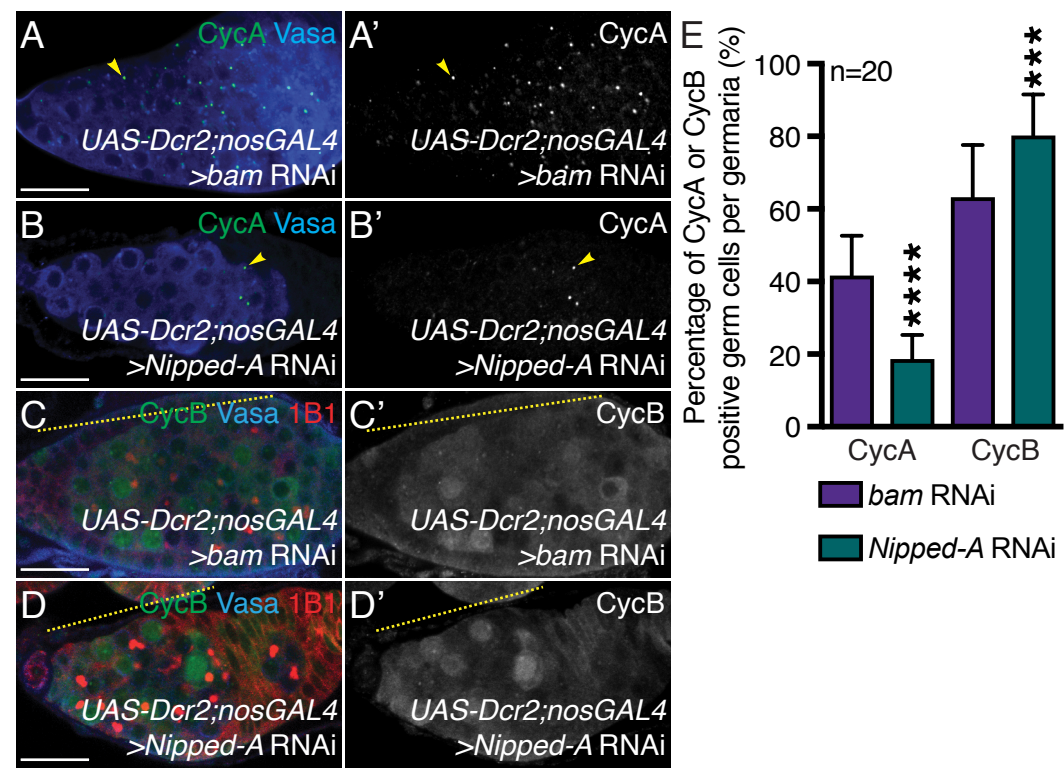
Statistical analysis performed with Chi-square. Scale bar for all images is 20 μ m.

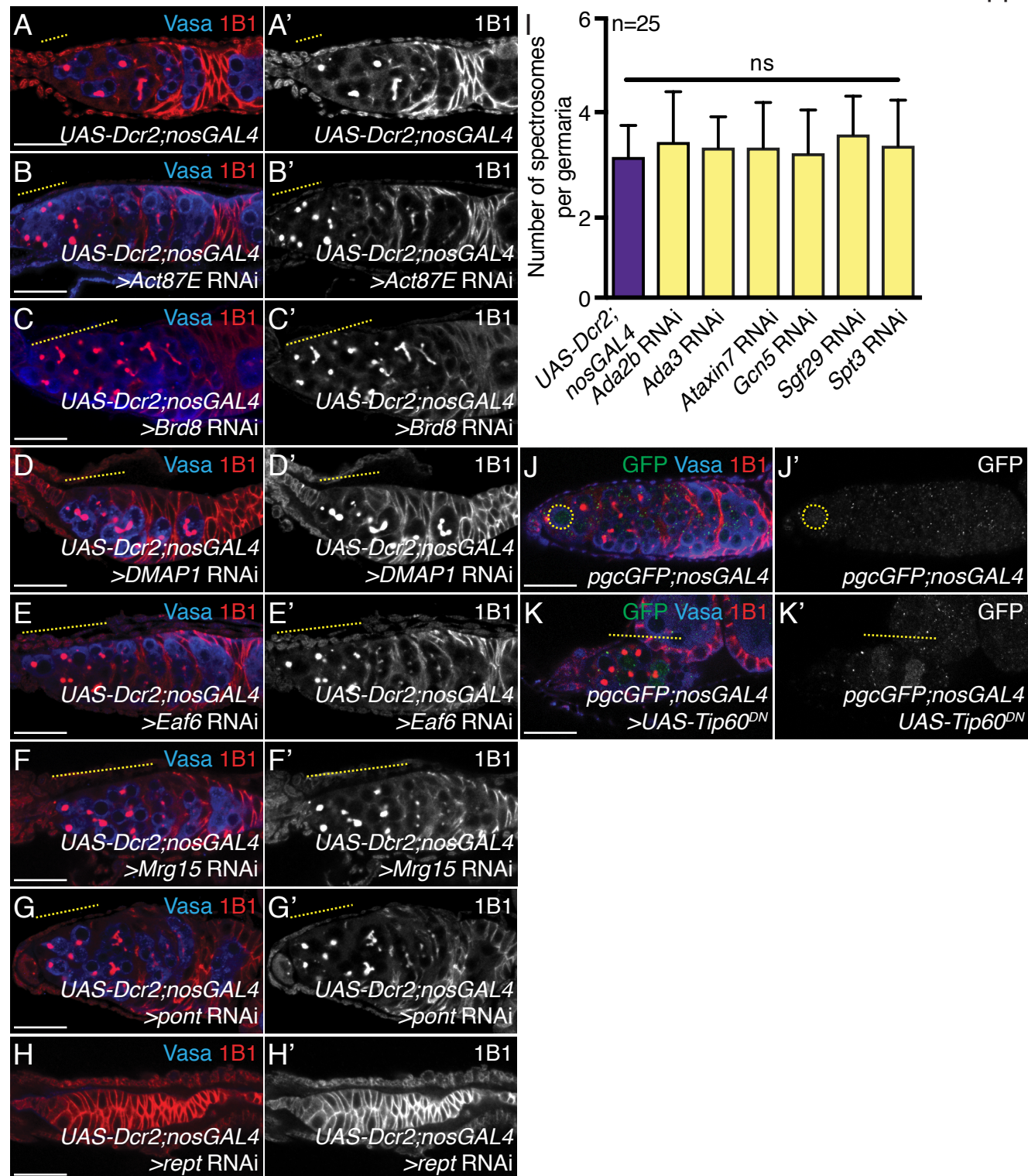
Supplementary Figure 6. Ectopic expression of *bgn* partially rescues germline-depletion of *Nipped-A*

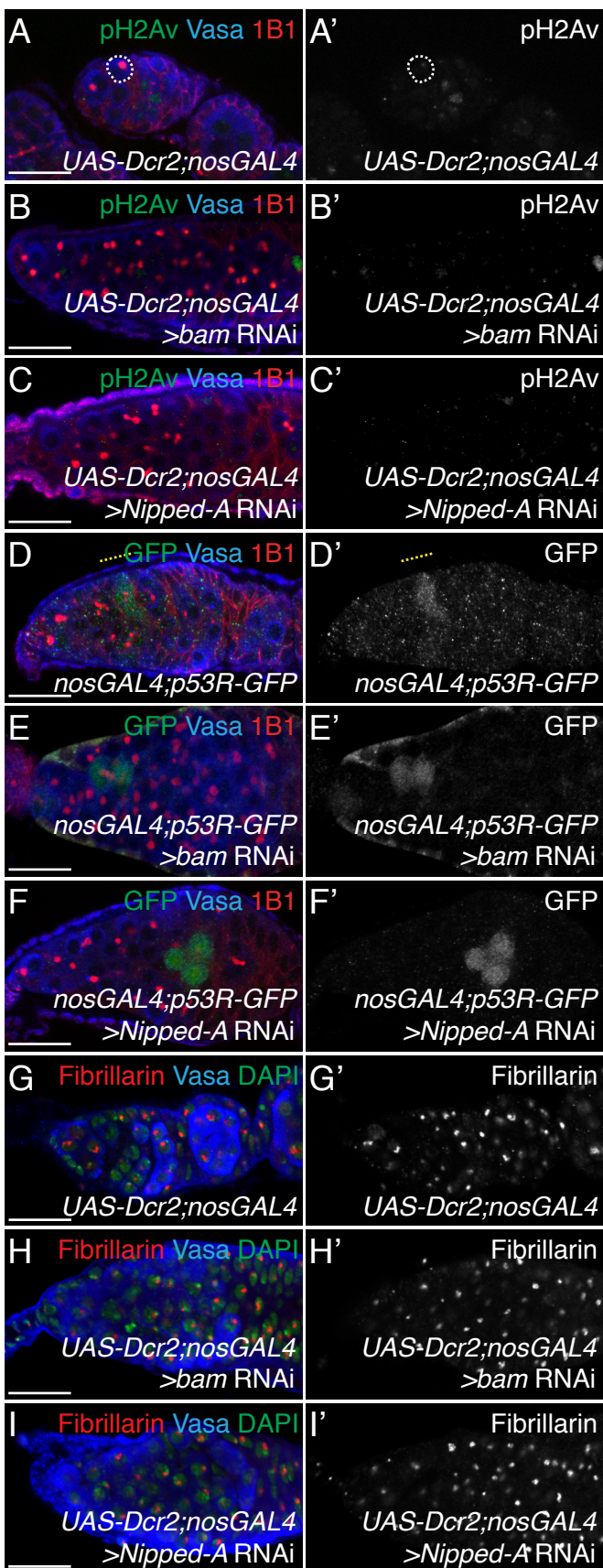
(A-A') Germline-driven *bgn^{EP}*, (B-B') depletion of *Nipped-A*, and (C-C') rescue ovaries stained with Vasa (blue) and 1B1 (red). Germline-driven *bgn^{EP}* and rescue show 2 or more egg chambers budding off the germaria (yellow dashed lines). Rescue ovaries make strings of repeating egg chambers that eventually die (yellow arrow). Germaria depleted of *Nipped-A* occasionally make egg chambers but those that do, die (white arrow). 1B1 channel is shown in A', B', and C' (n=50).

Statistical analysis performed with Chi-square for (B-C'). Scale bar for all images is 20 μ m.









+ heat shock

