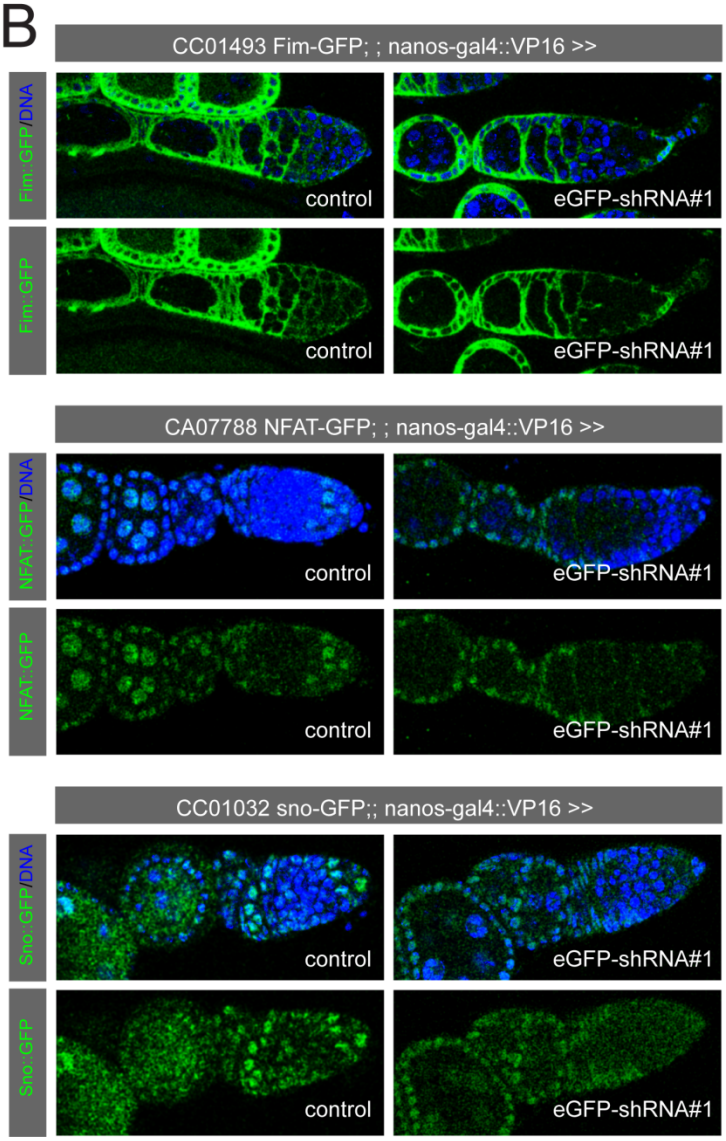
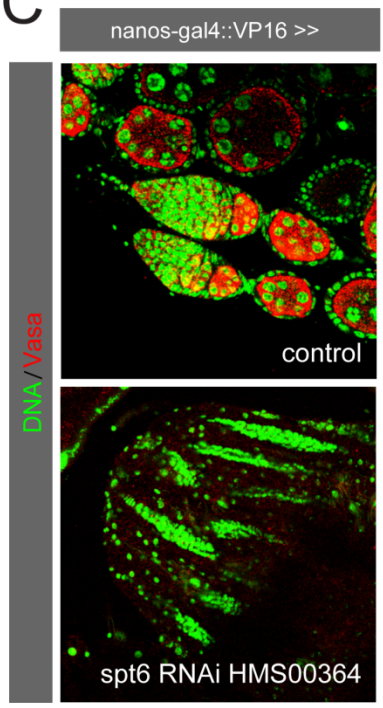
A

Trap number	gene name	phenotype in germline
BA00253	NetB	wild type
CA06750	Trxr-1	high fraction of empty ovarioles
CA06924	CAP	wild type
CA07692	Spt6	loss of germline cells
CA07788	NFAT	wild type
CB022888	lola	wild type
CC00380	Pabp2	loss of germline cells
CC00737	Tudor-SN	wild type
CC00791	vkg	wild type
CC01032	sno	wild type
CC01377	Cp1	degenerating egg chambers
CC01493	Fim	wild type

B



C



D

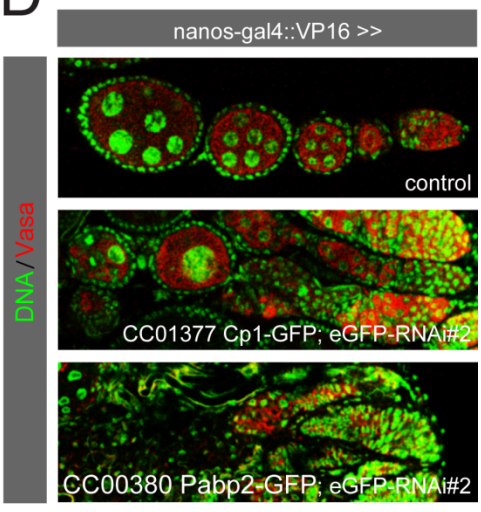


Figure S1 (A) Homozygous viable GFP traps with expression in the germline were selected from the Carnegie collection (Buszczak et al. 2007). EGFP-shRNAs were driven in the background of these traps using the germline-specific *nanos-GAL4*. The phenotype upon tag-mediated knockdown is indicated. (B) Examples of GFP traps that showed depletion of GFP signal in the germline but failed to show any detectable phenotype upon tag-mediated knockdown. Ovarioles stained for GFP and DAPI are shown; endogenous GFP fluorescence is shown for CC01493. (C) *Spt6* was knocked down in the germline using a *Spt6*-specific shRNA construct driven by *nanos-GAL4*, and ovaries were stained for Vasa and DAPI. The gene-specific knockdown is indistinguishable from tag-mediated knockdown (Figure 1D). (D) The indicated EGFP-shRNAs were driven by *nanos-GAL4* in the background of the *Cp1–GFP* or *Pabp2–GFP* traps, and ovaries were stained for Vasa and DAPI. The phenotypes resemble those in Figure 1, G and I, respectively.

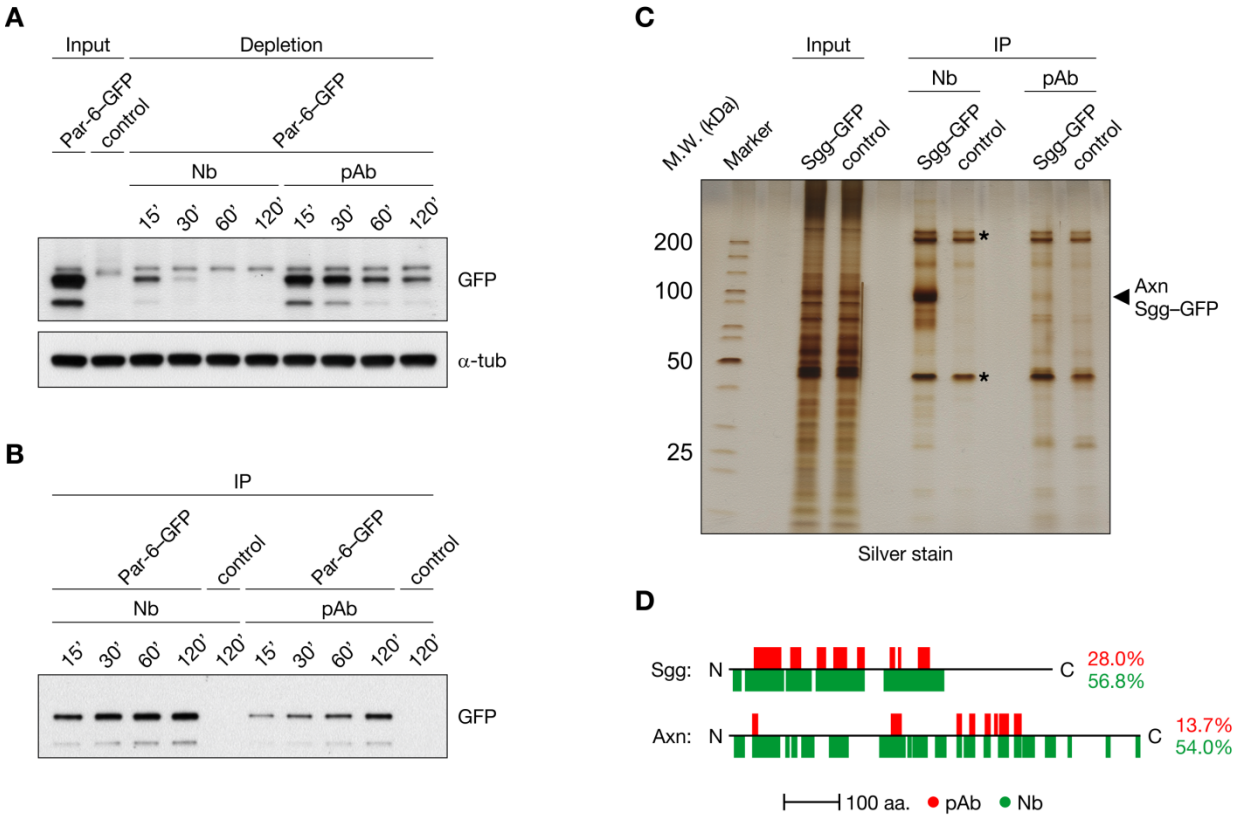


Figure S2 (A,B) Extracts from embryos expressing Par-6–GFP were incubated with either anti-GFP nanobodies (Nb) or anti-GFP polyclonal antibodies (pAb) for 15–120 min. w^- embryos not expressing Par-6–GFP were used as a control. (A) The depletion of Par-6–GFP from the extract was assayed by Western blot analysis. (B) Western blot analysis of the immunoprecipitates. (C,D) Embryos bearing a YFP trap in *shaggy* (*sgg*) were lysed and subjected to immunoprecipitation using either anti-GFP nanobodies (Nb) or anti-GFP polyclonal antibodies (pAb). (C) Silver stain of the immunoprecipitates. Asterisks indicate contaminants, presumably cytoskeletal components such as myosins and actin, which occasionally precipitate out or stick to the beads. (D) Peptide coverage maps of the Sgg bait and its binding partner Axin (Axn), obtained by LC-MS/MS after in-solution digestion of the immunoprecipitates prepared using either nanobodies (green) or polyclonal control antibodies (red). Percentages indicate the overall peptide coverages of the proteins.