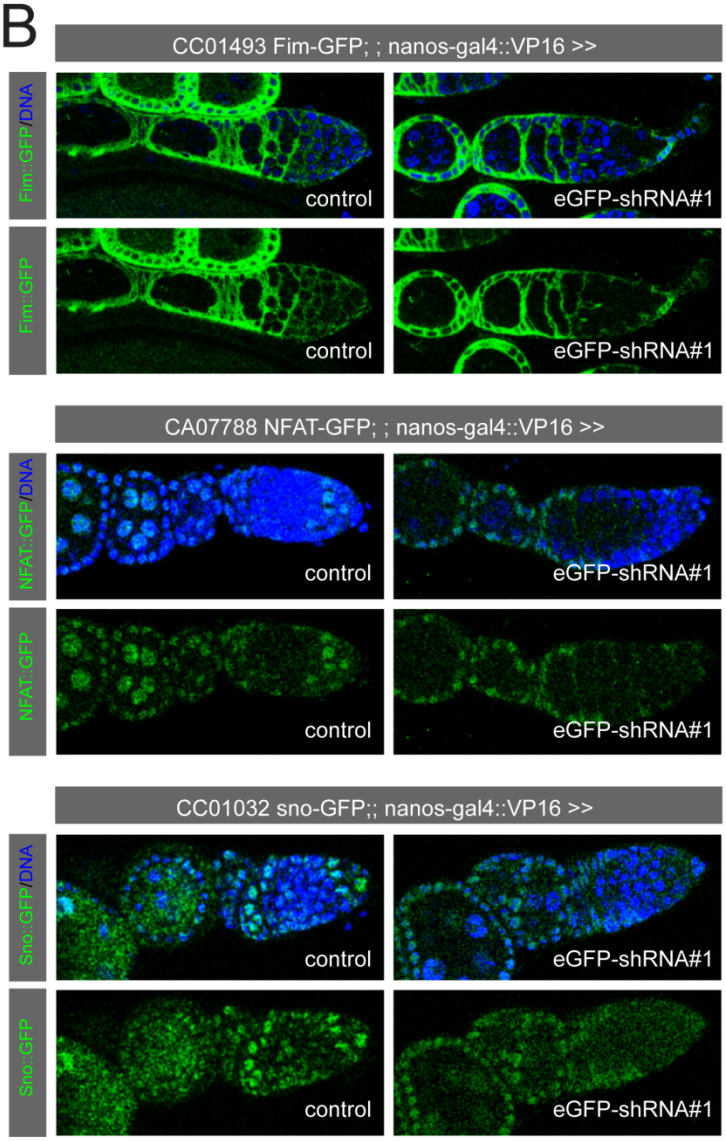
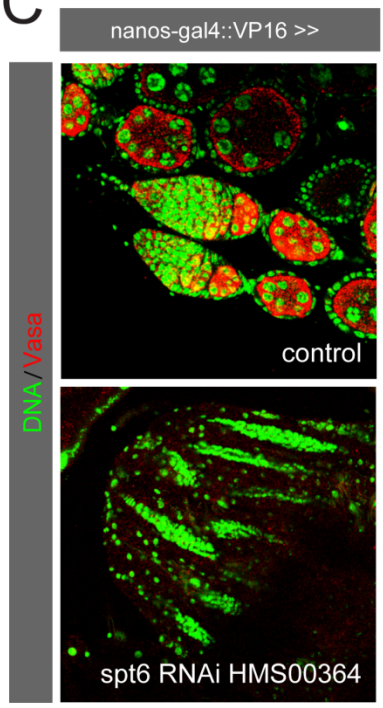
A

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

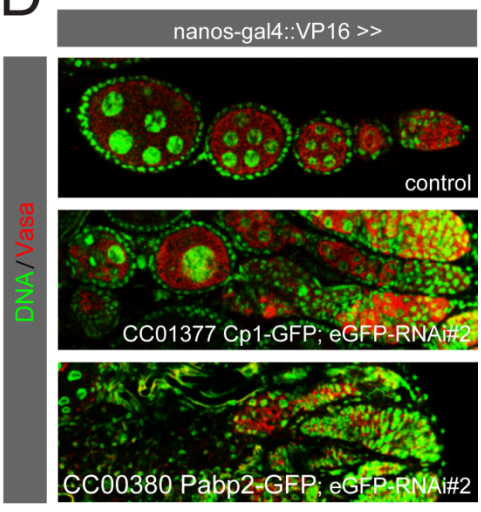


Figure S1 (A) Homozygous viable GFP traps with expression in the germline were selected from the Carnegie collection (Buszczak et al. 2007). EGFP-shRNAs were driven in the background of these traps using the germline-specific *nanos-GAL4*. The phenotype upon tag-mediated knockdown is indicated. (B) Examples 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.

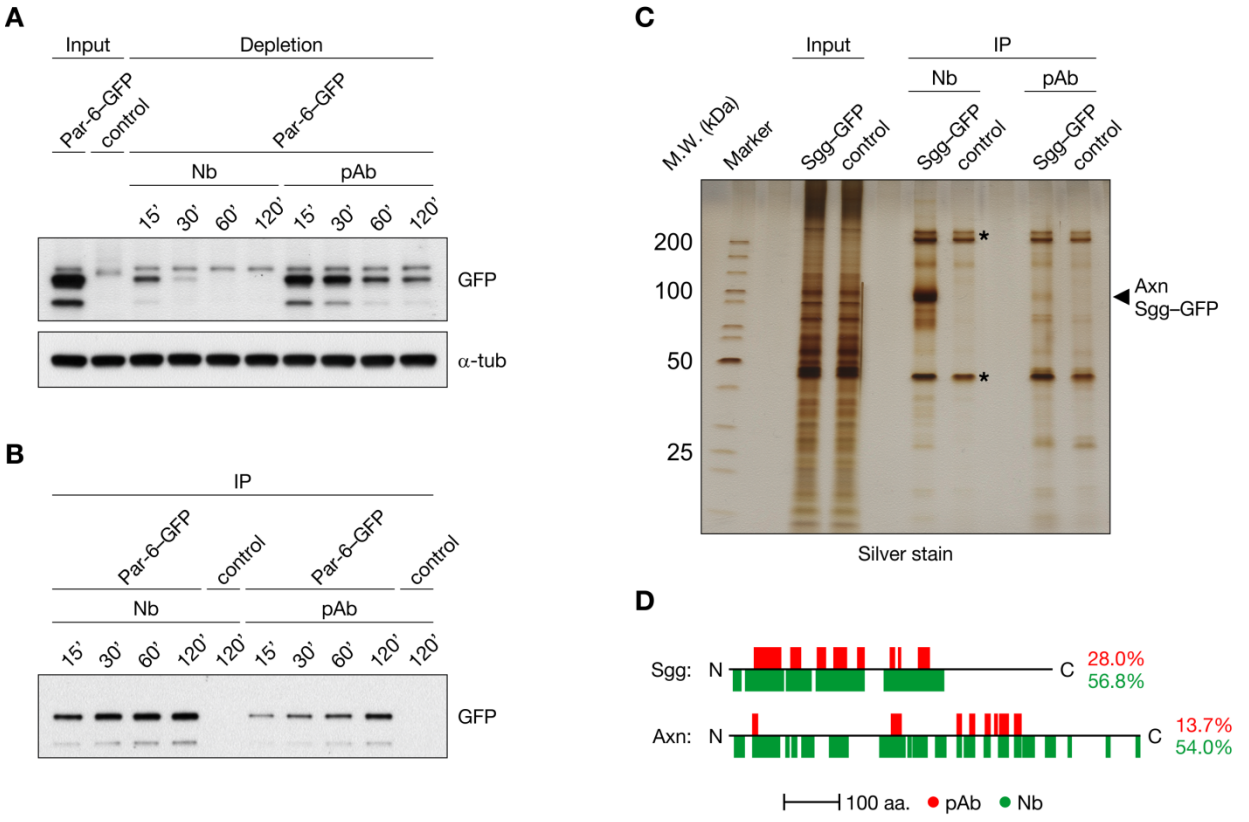


Figure S2 (A,B) Extracts from embryos expressing Par-6–GFP were incubated with either anti-GFP nanobodies (Nb) or anti-GFP polyclonal antibodies (pAb) for 15–120 min. w^- embryos not expressing Par-6–GFP were used as a control. (A) The depletion of Par-6–GFP from the extract was assayed by Western blot 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.

File S1

Proteins identified by mass spectrometry

All proteins identified by LC-MS/MS in the immunoprecipitation experiments summarized in Figure 2F and Figure S2D. Worksheet names specify the bait and antibody reagent (Nb, anti-GFP nanobodies; pAb, anti-GFP polyclonal control antibodies) used in each experiment. The following data are provided for each hit: rank, number of unique and total peptides, average peptide cross-correlation score (XCorr), and the number of unique peptides found for the protein in the w^- control.

File S1 is available for download at http://www.genetics.org/content/suppl/2011/12/14/genetics.111.136465.DC1 as an Excel file.

Table S1 ***Drosophila*** **S2R+ protein trap lines and the subcellular distribution of their mCherry signal**

Cell line	Subcellular localization of mCherry	Inverse PCR	MS
NPTC1	Control cell line with no visible signal		
NPTC2	Control cell line with no visible signal		
NPTC3	Control cell line with no visible signal		
NPTC4	Control cell line with no visible signal		
NPTC5	Control cell line with no visible signal		
NPTC6	Control cell line with no visible signal		
NPT001	Weak cytoplasmic signal		
NPT002	Weak cytoplasmic signal		
NPT003	Weak cytoplasmic signal		
NPT004	Cytoplasmic signal		
NPT005	Cytoplasmic signal and endoplasmic reticulum	X:13711049 (*Clic*)	Clic
NPT006	Weak signal		
NPT007	Weak signal		
NPT008	Cytoplasmic signal		
NPT009	Weak cytoplasmic signal		
NPT010	Weak cytoplasmic signal		
NPT011	Weak signal		
NPT012	Weak signal		
NPT014	Cytoplasmic signal		
NPT015	Cytoplasmic signal		
NPT106	Weak cytoplasmic signal		
NPT017	Cytoplasmic signal and cleavage furrow	N/A (potentially multiple insertions)	Fim
NPT018	Cytoplasmic signal		
NPT019	Weak signal		
NPT020	Weak cytoplasmic signal		
NPT021	Weak signal		
NPT022	Weak cytoplasmic signal	3R:11236593 (*atx2*)	
NPT023	Weak cytoplasmic signal		
NPT024	Weak signal		
NPT025	Weak cytoplasmic signal		
NPT026	Weak cytoplasmic signal		
NPT027	Weak signal		
NPT208	Weak signal		
NPT029	Weak signal		
NPT030	Weak signal		
NPT031	Weak signal		
NPT032	Weak signal		
NPT033	Weak signal		
NPT034	Weak cytoplasmic signal		
NPT035	Weak cytoplasmic signal		
NPT036	Weak signal		
NPT037	Weak cytoplasmic signal		
NPT038	Weak signal		
NPT039	Weak signal		
NPT040	Weak signal		
NPT041	Weak signal		

NPT042	Weak signal	
NPT043	Weak signal	
NPT044	Weak signal	
NPT046	Weak cytoplasmic signal	
NPT047	Weak signal	
NPT048	Weak signal	
NPT049	Weak signal	
NPT050	Peri-Golgi	
NPT052	Weak signal	
NPT101	Endoplasmic Reticulum	
NPT102	Endoplasmic Reticulum	3L:9844863 (*gap1*)
NPT103	Cytoplasmic signal	
NPT104	Cytoplasmic signal	

The insertion site as mapped by inverse PCR and the trapped protein as identified by LC-MS/MS (MS) are indicated, if determined.

Table S2 Oligos used to generate EGFP-shRNAs. Capitalized letters indicate target-specific sequences

Construct	Forward oligo	Reverse oligo
#1	ctagcagtCGGCATCAAGGTGAACTTCAAtagttatattcaagcata TTGAAGTTCACCTTGATGCCGgcg	aattcgcCGGCATCAAGGTGAACTTCAAtatgcttgaatataactaT TGAAGTTCACCTTGATGCCGactg
#2	ctagcagtCAAGGACGACGGCAACTACAAtagttatattcaagcat aTTGTAGTTGCCGTCGTCCTTGgcg	aattcgcCAAGGACGACGGCAACTACAAtatgcttgaatataacta TTGTAGTTGCCGTCGTCCTTGactg
#3	ctagcagtCGGCCACAAGTTCAGCGTGTCtagttatattcaagcata GACACGCTGAACTTGTGGCCGgcg	aattcgcCGGCCACAAGTTCAGCGTGTCtatgcttgaatataacta GACACGCTGAACTTGTGGCCGactg