Supplemental Materials and Methods

Mosaic clonal analysis

GSC and SP clones were induced with the FLP/FRT-mediated mitotic recombination technique (Xu and Rubin, 1993) in files with following genotypes:

yw, hs-FLP/Y; ubiGFP, FRT82B/FRT82Biso (CTL);

yw, hs-FLP/Y; ubiGFP, FRT82B/FRT82B $pic^{GE28589}$;

yw, hs-FLP/Y; ubiGFP, FRT82B/FRT82B $pic^{EY01408}$;

yw, hs-FLP/Y; ubiGFP, FRT40A/FRT40Aiso(CTL);

yw, hs-FLP/Y; ubiGFP, FRT40A/FRT40A $mahj^{LL00674}$;

yw, hs-FLP/Y; ubiGFP, FRT42D/FRT42Diso(CTL);

yw, hs-FLP/Y; ubiGFP, FRT42D/FRT42D $Cul\text{-}4^{KG02900}$;

To induce clones, 0– to 2–day-old adult male flies were subjected to 1 hr heat shock at 37℃. Flies were kept at 25℃ and dissected and stained at 2–, 9– and 15–days after heat shock (AHS). GSC clones were identified as Vasa-positive, GFP-negative cells attaching to the hub cells.

Mouse strains and crosses

Wild type *C57/B6* mice were obtained from the Zhejiang Academy of Medical Science, China. *Ddb1*$^{flox/flox}$ and *Ddx4-Cre* mice were previously generated (Cang et al., 2006; Gallardo et al., 2007). Mice were maintained under SPF conditions in a controlled environment of 20–22℃, with a 12/12 h light/dark cycle, 50–70% humidity, and food and water provided ad libitum. Animal care and experimental procedures were in accordance with the Animal Research Committee guidelines of Zhejiang University. Mice that lacked *Ddb1* in their germ cells were generated by crossing *Ddx4-Cre* mice with previously reported *Ddb1*$^{flox/flox}$ mice. All mutant mouse strains were had a*C57/B6* background.

Mice fertility test

To test fertility, control and *Ddb1 cKO* male mice were mated with 10– to 12–week old fertile wild type females overnight. Successful mating was confirmed by the presence

of vaginal plugs. Zygotes were harvested from oviducts at 20 hours after hCG injection. And pronucleus formation was examined in the zygotes.

Hematoxylin and eosin (H&E) staining

Testes tissues were fixed overnight in 10% PBS buffered formalin, dehydrated using an ascending series of graded ethanol solutions, and then embedded in paraffin. Testes samples were serially sectioned at 5 μm thickness and stained with haematoxylin and eosin.

Light and phase-contrast microscopy

Fly testes were dissected in 1x phosphate-buffered saline (PBS) and washed several times. For an overall view, the testis was observed directly under light microscope. Shredded testes were observed on slides by a phase-contrast microscope after gently squashing them with a cover slip. All stages of germ cells during spermatogenesis are found in normal testes.

Immunofluorescence and antibodies

Fly testes were dissected in 1x PBS and fixed for 30 min in 4% paraformaldehyde. After washing three times in 1x PBS with 0.1% Triton X–100 (PBST) and blocking for 1hr in 5% bovine serum albumin (BSA), the samples were incubated with primary antibodies overnight at 4℃. After washing three times for 10 min in 0.1% PBST, the samples were incubated for 1 hr with secondary antibodies at room temperature followed by three times washing in 0.1% PBST. Testes were then stained with Hoechst 33342 (1.0 mg/ml, Invitrogen) for 5 min before mounting.

For mice testis staining, the tissues were fixed in 4% paraformaldehyde, embedded in O.C.T. compound (Sakura Finetek USA Inc.), and stored at –80℃ before preparing 7 μm sections using a Leica CM1950 cryomicrotome (Leica Microsystems, Wttzlar, Germany). After blocking with 1% BSA in PBS, testes sections were incubated with primary antibodies diluted in blocking solution at room temperature for 1 h. After three washes with PBS, testes were labeled with secondary

antibodies for 45 mins. Slides were mounted using VectaShield with 4',6–diamidino–2–phenylindole (DAPI, Vector Laboratories). Images were captured on an LSM710 Zeiss confocal microscope and processed using Adobe Photoshop CS5 software.

The antibodies used were as follows: rat anti-Vasa (DSHB, 1:20); mouse anti-Eya (DSHB, 1:20); mouse anti-FasIII (DSHB, 1:50); rat anti-DE-cadherin (DSHB, 1:20); mouse anti-1B1 (DSHB, 1:75); rabbit anti-Bam C (a gift from DH Chen, 1:2000) (Yang et al., 2007); rabbit anti-Vasa (1:1000, Santa Cruz); rat anti- Zfh-1 (1:5000); rabbit anti-MVH (Abcam, 1:400); goat anti-PLZF *(R&*D, 1:500); and rabbit anti-WT1 (Santa Cruz Biotech, 1:500). Secondary antibodies conjugated to A488, Cy3, A594, or A647 (Molecular Probes and Jackson Immunologicals) were diluted at 1:1000.

Generation of Zfh-1 antibody

cDNA fragments of the *zfh-1* genes were subcloned into the pGEX vector systems to produce GST-fusion proteins containing a fragment of the Zfh-1 proteins (648-775 aa). Generation and affinity–purification of rat polyclonal antisera were conducted as described before (Lai et al., 1991).

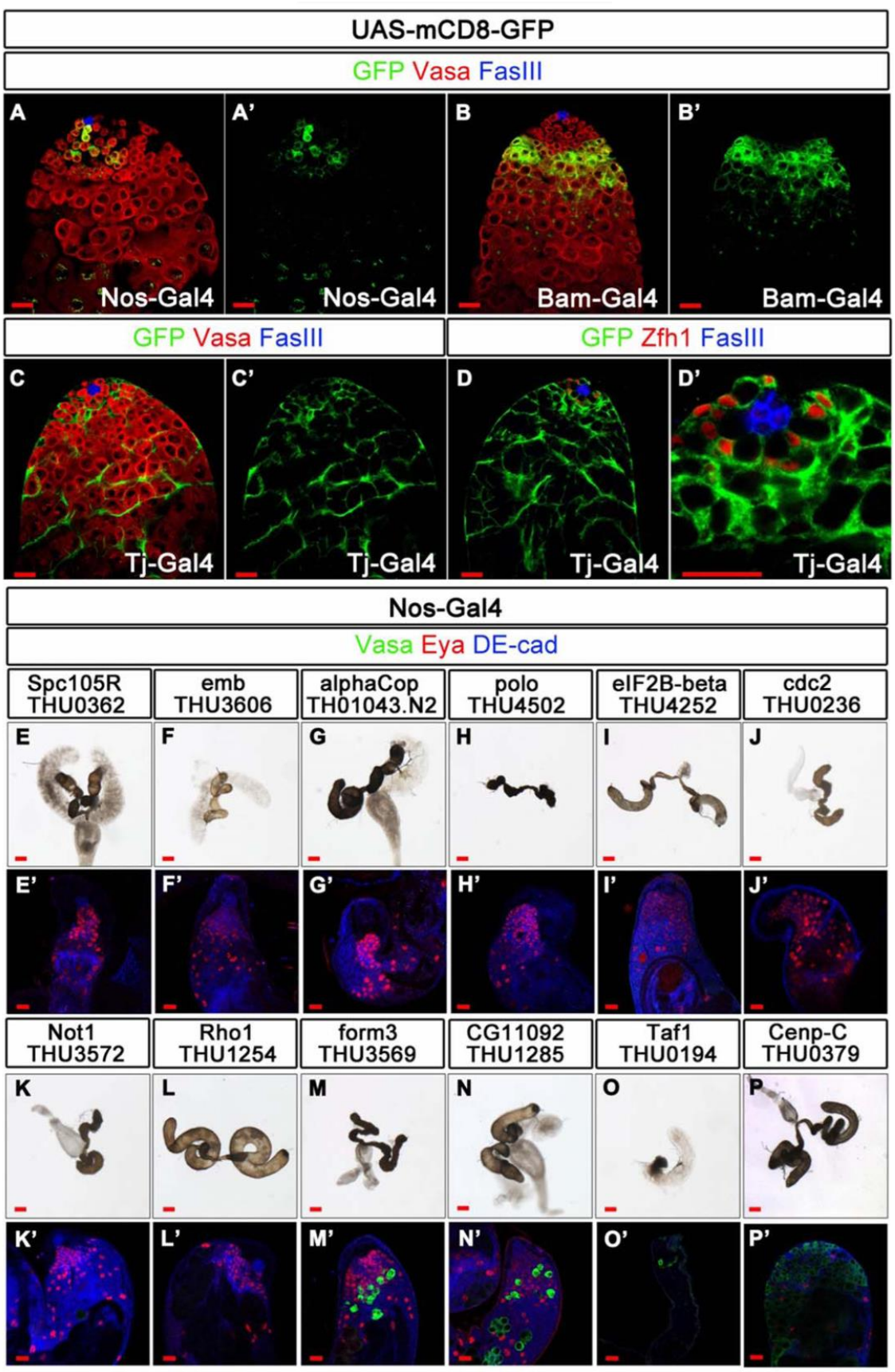

Figure S1. Gal4 expression patterns in the testis and quality controls of the screen. (A–D and A′–D′) The expression patterns of *nos-Gal4* (A and A′), *bam-Gal4* (B and B′) and *tj-Gal4* (C, C′, D and D′) in testes were visualized by crossing to *UAS-mCD8-GFP*. *Nos-Gal4* is mainly expressed in early germ cells including GSCs; *bam-Gal4* is mainly expressed in TA–spermatogonia; *tj-Gal4* is expressed in cyst cells especially in early cyst cells. The testis shown in D' is an enlarged view of the top region in D. (E–P and E′–P′) 12 genes that have two independent RNAi lines show similar phenotypes when both lines were crossed to *nos-Gal4* (data of one RNAi line for each gene were shown). (E–P) Light microscopy view and (E′–P′) confocal view of the testes with indicated gene knockdown. Anti-GFP show the Gal4 expression patterns (green in A–D and A′–D′), Anti-Vasa labels germ cells (red in A–Cand green in E′–P′), anti-FasIII shows the hub cells (blue in A–D and D′), anti-Zfh1 labels the CySCs (red in D and D′), anti-Eya marks the differentiated cyst cells (red in E′–P′), anti-DE-cad labels the cyst cells and hub cells (blue in E′–P′). Scale bars: 100μM for E–P; 20μM for others.

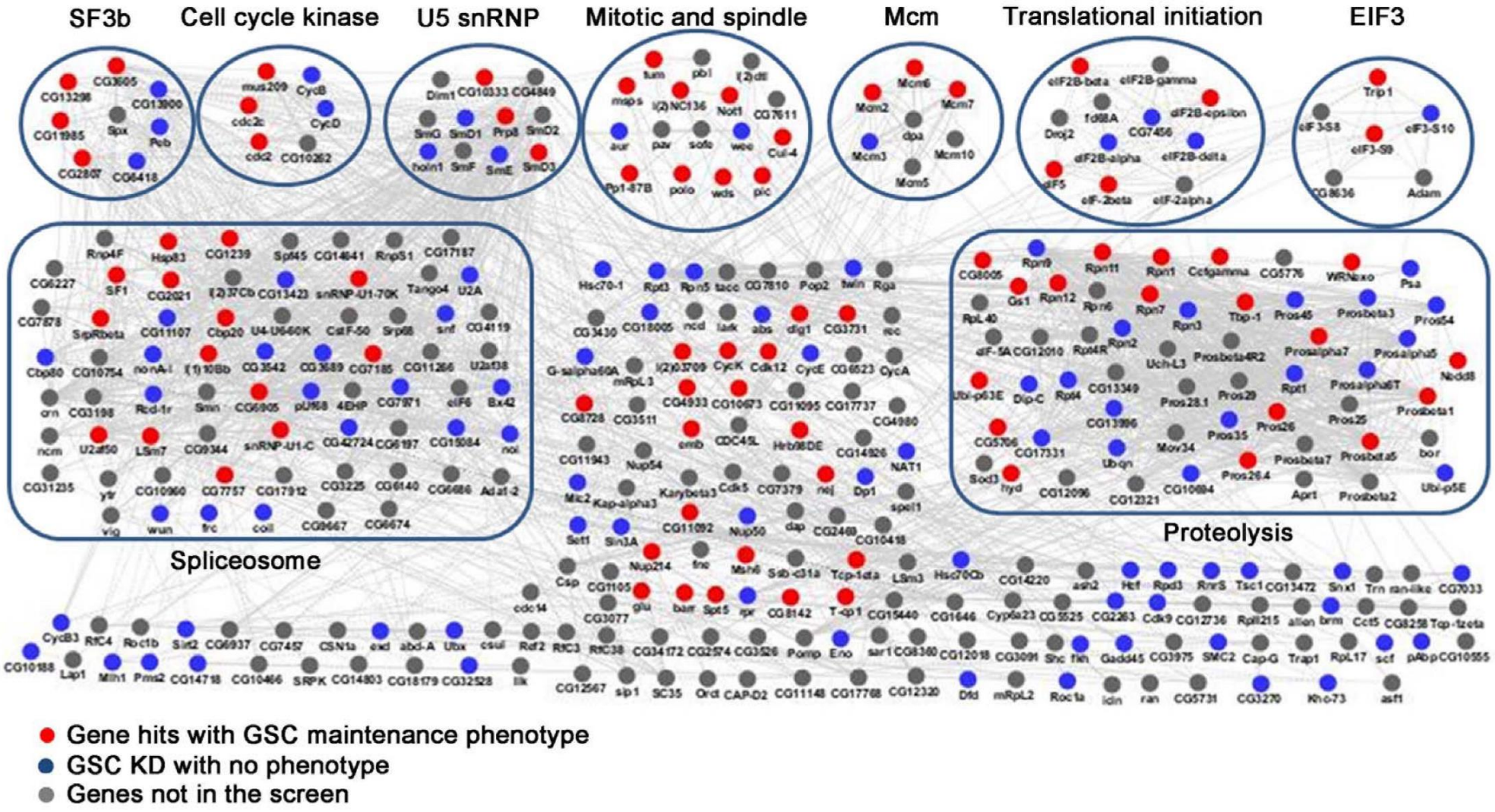

Figure S2. A regulatory network generated by COMPLEAT analysis. Genes are shown as nodes; red nodes indicate genes identified in this screen. Blue nodes are components of complexes not identified in this screen. Gray nodes indicate genes that have not been analyzed in this study. Edges denote interactions between proteins. Solid edges reflect interactions supported by direct experimental evidence from the fly proteins and dotted edges represent putative interactions supported by experimental evidence from the homologous proteins of other species.

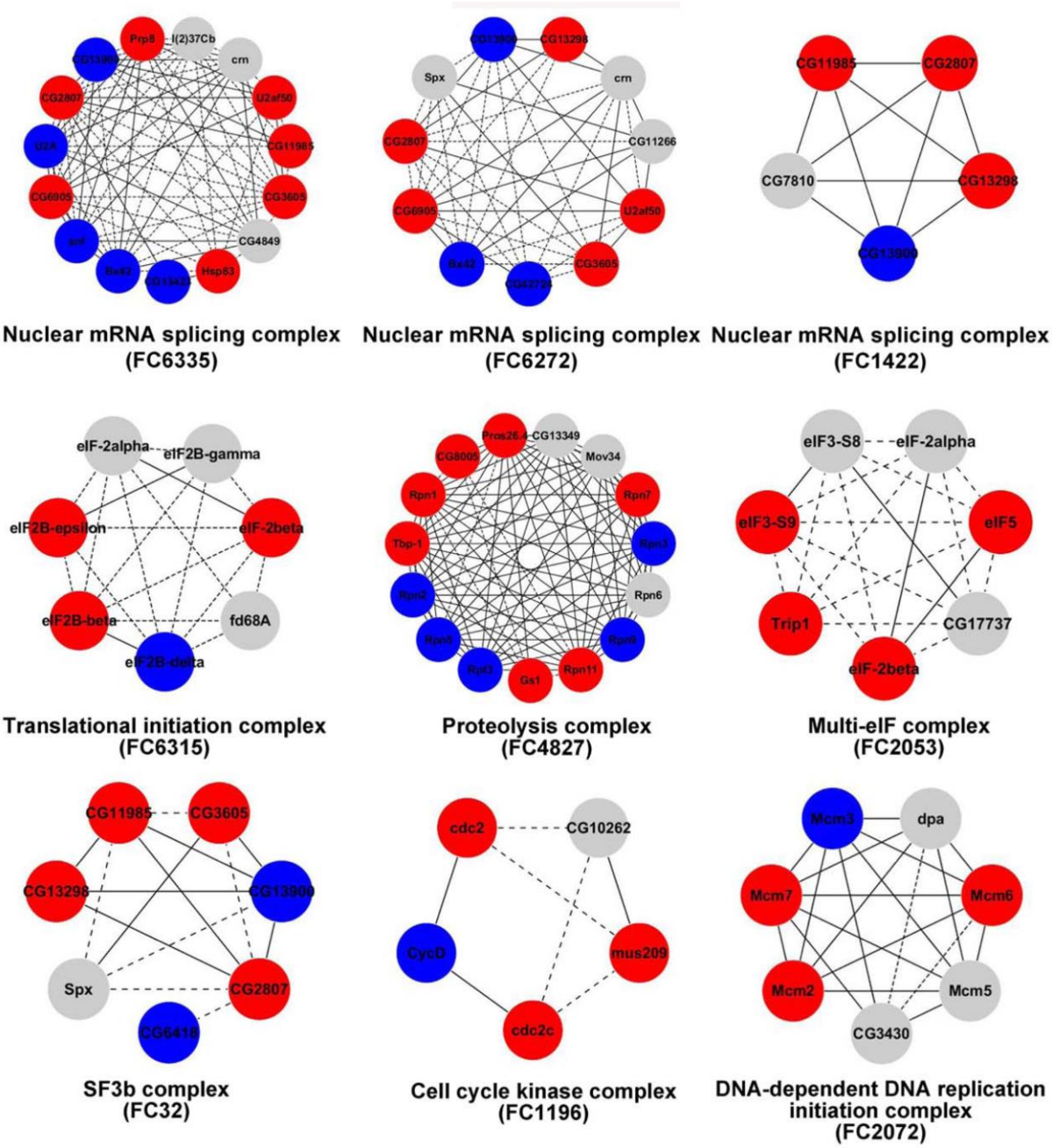

Figure S3. Representative complexes identified in the screen using COMPLEAT analysis. Red nodes indicate genes identified in the screen, blue nodes indicate genes that were not identified in this screen. Gray nodes indicate genes that have not been analyzed in this study. The full list of complex analysis is shown in Table S3.

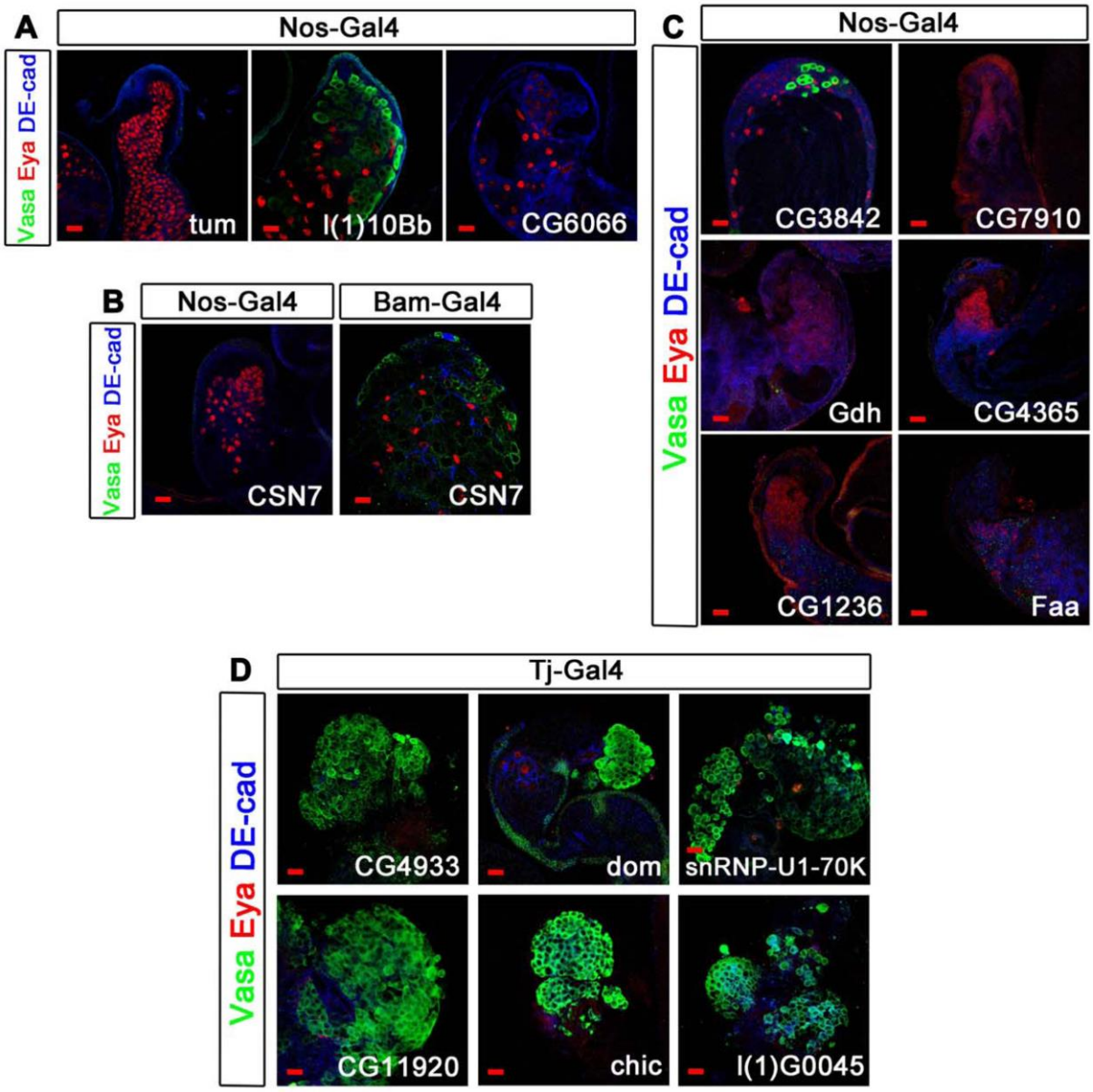

Figure S4. A few RNAi examples mentioned in the main text but did not show in the main figures. The indicated genes were knockdown with listed Gal4s. Anti-Vasa labels germ cells (green), anti-Eya marks the differentiated cyst cells (red), anti-DE-cad labels the cyst cells and hub cells (blue). Scale bars: 20 μM.

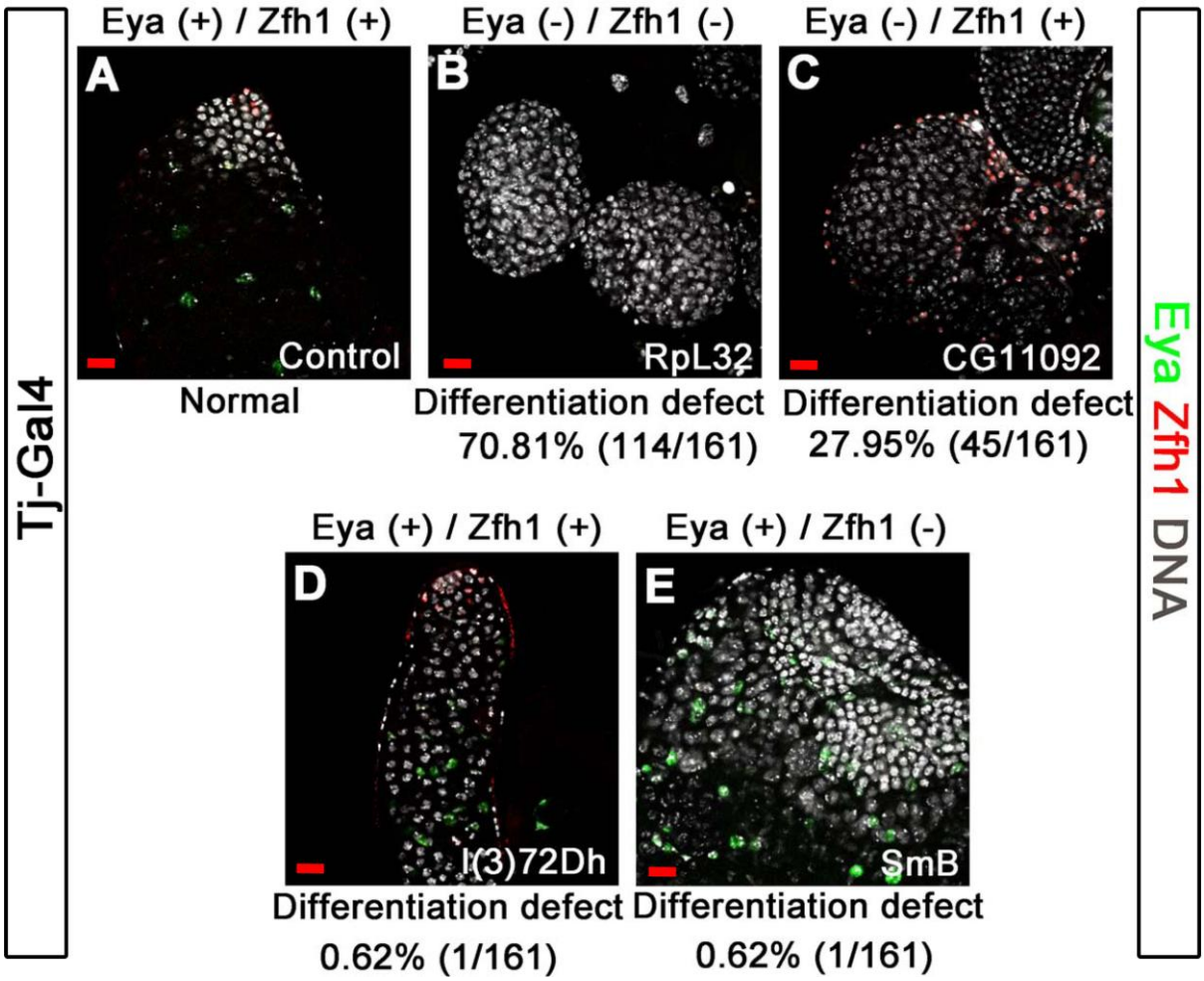

Figure S5. The typical patterns of CysC and cyst cell markers in the testes with differentiation defects resulted from the *tj-Gal4* driven RNAi screening. The indicated genes were knockdown with *tj-Gal4*. Anti-Zfh1 labels CysCs (red), anti-Eya marks the differentiated cyst cells (green), DNA was labeled with Hoechst 33342 (gray). Scale bars: 20 μM. The proportion of the lines that have similar Zfh1 and Eya patterns in the total testes with differentiation defects were labeled under the images. In total, 161 lines show the differentiation defects in the tj-Gal4 screen.

Table S1. Detailed information of the RNAi lines used in this study. Transgenic RNAi lines from *THFC* used for the GSC self-renewal screen.

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Table S2. Phenotype annotation of genes identified in the screen. Sheet1: The detailed phenotypes of the 221 genes required for GSC self-renewal and differentiation. Sheet2: List of 12 genes that have a second independent RNAi line. Phenotypes of the male progeny produced by crossing the second RNAi lines with *nos-Gal4*. Sheet3: Tumor formation frequency and patterns of Eya and Zfh-1 in testes from the *tj-Gal4* screen.

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Table S3. Full list of complexes required for GSC self-renewal and differentiation were generated by COMPLEAT analysis.

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Table S4. Full list of genes encoding WD40 domain proteins that were identified in this screen.

CG NO.	FlyBase ID	Fly Symbol	Interaction (with pic)	Domains	Human Orthologs	Ensembl Gene ID	Domains	Putative CUL4 CRL Substrate Recepto	Reference (PMID)
CG10080	FBgn0034641	mahj	PPI: DPiM coAP complex	WD40	VPRBP	ENSG00000145041	WD40	Yes	16964240;16949367;23062609;17588513
				WD40	RBBP4	ENSG00000162521	WD40	Yes	17588513
CG4236	FBgn0263979	Caf1	PPI: Predicted from huma	WD40	RBBP7	ENSG00000102054	WD40	Yes	17079684;21228219;17588513
				WD40	WDR26	ENSG00000162923	WD40	Yes	17588513
				WD40	TWF1	ENSG00000151239			
CG17293	FBgn0032030	Wdr82		WD40	TWF2	ENSG00000247596			
				WD40	WDR82	ENSG00000164091	WD40	Yes	17588513
				WD40	TBL3	ENSG00000183751			
CG17437	FBgn0040066	wds	InterologFinder: Predicted	WD40	WDR5	ENSG00000196363	WD40	Yes	17041588;17588513
				WD40	WDR5B	ENSG00000196981	WD40	Yes	17588513
CG5018	FBgn0263605	l(3)72Dn		WD40	CIRH1A	ENSG00000141076	WD40	NO	
CG3820	FBgn0010660	Nup214		WD40	NUP214	ENSG00000126883	WD40	NO	
CG5033	FBgn0028744	CG5033		WD40	BOP1	ENSG00000261236	WD40	NO	
CG6015	FBgn0038927	CG6015		WD40	CDC40	ENSG00000168438	WD40	NO	
CG7961	FBgn0025725	alphaCop		WD40	COPA	ENSG00000122218	WD40	NO	
CG5519	FBgn0261119	Prp19		WD40	PRPF19	ENSG00000110107	WD40	NO	
CG7989	FBgn0262560	wcd		WD40	UTP18	ENSG00000011260	WD40	NO	
CG4878	FBgn0034237	eIF3-S9		WD40	EIF3B	ENSG00000106263	WD40	NO	
CG8882	FBgn0015834	Trip1		WD40	EIF3I	ENSG00000084623	WD40	NO	