Supporting Information

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SI Materials and Methods

RNAi in *Drosophila* S2 Cells. The experiments were carried out using a stable S2 cell line containing Flag-Mothers against decapentaplegic (Mad) controlled by a metallothionine promoter that is inducible by $CuSO_4$. S2 cells were transfected with dsRNA using DharmaFect 4 (Thermo Scientific) following manufacturer's procedures. The transfection was repeated 4 d later. Forty-eight hours after the second dsRNA transfection, cells at a density of 0.5×10^6 per well were seeded into 24-well plates, cultured overnight, and treated with 1 nM decapentaplegic (Dpp) for 1 h. To induce Flag-Mad expression, 0.5 mM $CuSO_4$ was added 30 min or 1 h before the Dpp treatment. Cells were harvested in RIPA buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1% (vol/vol) Nonidet P-40, 0.5% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) SDS] and analyzed by Western blots.

Generation of Constructs for Tagged Proteins. ***Flag–Lis-1.*** The coding region of *Lissencephaly-1* (*Lis1*), which was amplified from a *Drosophila* ovarian cDNA library, was cloned into pENTR TOPO cloning vector and completely sequenced. These pENTR vectors subsequently were recombined with Flag-tagged destination vectors (Dr. T. Murphy, Carnegie Institution of Washington, Baltimore) using LR Clonase (Invitrogen).

Punt-V5. PCR-amplified *punt* cDNA was cloned into the pSH vector at EcoR-I and Bam HI sites. The V5 tag is at the C terminus. The pSH vector is controlled by the metallothionine-inducible promoter.

Thickveins-HA. The *thickveins* (*tkv*) coding region was PCR-cloned into the XhoI-XbaI site of pMK33, with an HA tag at the C terminus. Thk-HA also is an inducible system controlled by the metallothionine promoter.

HA-Mad. The *mad* coding region was cloned into pMK33 at the KpnI-XbaI site, with the HA tag at the N terminus.

Coimmunoprecipitation in S2 Cells. S2 cells were transfected with cDNA expression vectors using Effectene (Qiagen). *Lis-1* cDNA was driven by an actin promoter; all other expression vectors were driven by metallothionine promoters and were induced by $CuSO_4$ (0.5 mM; 4 h). Cells were lysed in 0.5% (vol/vol) Nonidet P-40, 50 mM Hepes (pH 7.2), 100 mM KCL, 6.5% (vol/vol) glycerol, 1 mM Na3PO4, 20 mM Na pyrophosphate, 20 mM NaF, 20 mM beta-glycerophosphate, 0.5 mM DTT, and protease inhibitors. Immunoprecipitations were carried out overnight at 4 °C with anti-FLAG agarose beads (Sigma), anti-HA affinity matrix (Roche), and anti-V5 (Invitrogen) with protein A agarose beads (Roche). The beads were washed three times with the lysis buffer, and the bound proteins were subjected to SDS/PAGE and Western blot analysis. All coimmunoprecipitation experiments were performed at least three times.

Immunofluorescence Staining. S2 cells grown on coverslips were fixed by incubation with 4% (vol/vol) paraformaldehyde in PBS for 10 min at room temperature and permeabilized by 0.2% (vol/vol) Triton X-100 in PBS for 7 min. After blocking by 10% FBS in PBS for 1 h, anti-phospho-Smad1 (Cell Signaling) was added at 1:1,000 dilution and incubated at 4 °C overnight. Anti-rabbit IgG conjugated to Alexa 488 (Invitrogen) was used as the secondary antibody at 1:500 dilution. Coverslips were mounted by ProLong Gold (Invitrogen), and immunofluorescence images were acquired by confocal microscopy (Leica).

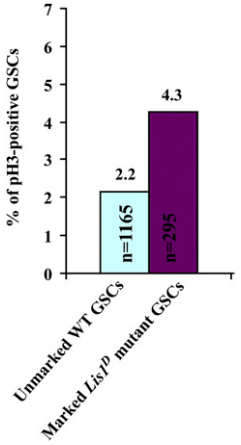

Fig. S1. Requirement of Lis1 for M-phase progression of germline stem cells (GSCs). Phosphorylated histone H3 (pH3) staining was used to label mitotic control and *Lis1*-mutant GSCs. There appeared to be more *Lis1* mutant than control GSCs in the M phase.

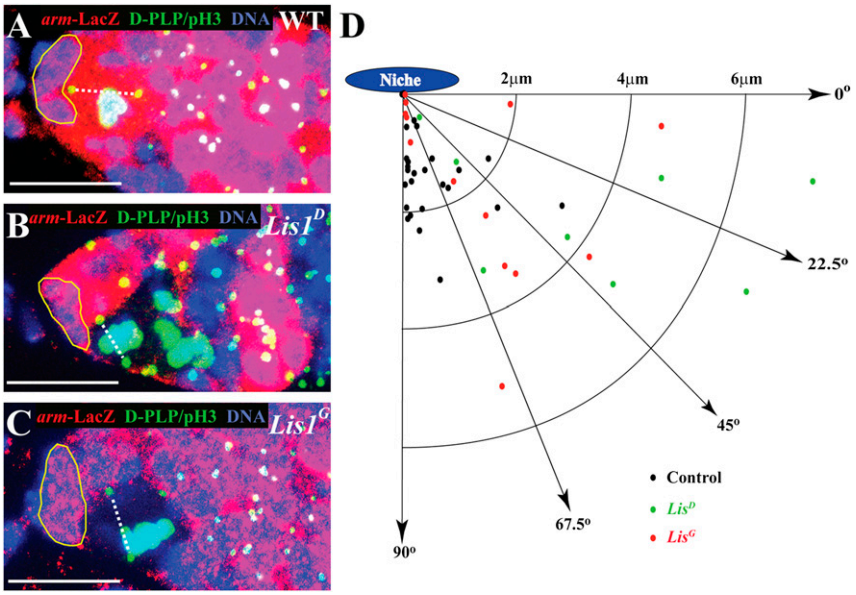

Fig. S2. Requirement of Lis1 for orienting the mitotic spindle of GSCs. The centriolar protein, Drosophila pericentrin-like *protein* (D-PLP), and pH3 were used to visualize two centrosomes and mitotic chromosomes of control and *Lis1* mutant GSCs, highlighting two endpoints of the mitotic spindle *(*1). (*A*) An unmarked wild-type GSC with a mitotic spindle oriented perpendicular to the niche (yellow line) is indicated by a white broken line linking two centrosomes. One of the two centrosomes almost always is located at the anterior side of the GSC close to the niche junction. (*B* and *C*) Marked $Lis1^D$ mutant GSCs (*B*) and $Lis1^G$ mutant GSCs (*C*) with their mitotic spindles misoriented to the niche (yellow lines) are indicated by a broken white lines. Spindles in the *Lis1* mutant GSCs, often are misoriented relative to the niche surface, with neither of the two centrosomes anchored to the apical side. (*D*) Quantitative data for spindle orientation and distance from the apical centrosome to the niche surface. 0° represents the spindle oriented parallel to the surface of the niche; 90° indicates the spindle oriented perpendicular to the surface of the niche. The spindle orientation is determined by drawing a line between the two centrosomes in a mitotic GSC. The numbers on the 0° axis represent the distance between the most anterior centrosome and the niche surface.

1. Stevens NR, Raposo AA, Basto R, St Johnston D, Raff JW (2007) From stem cell to embryo without centrioles. *Curr Biol* 17:1498–1503.

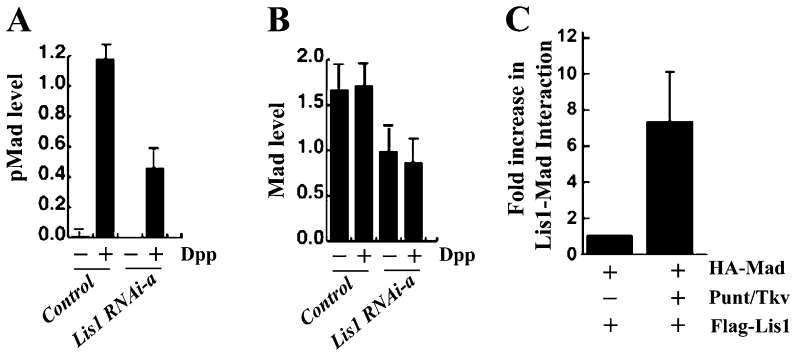

Fig. S3. Requirement of Lis1 for Mad phosphorylation and stability through direct interaction. (*A*) Quantitative data show that Lis1 is required for maintaining phosphorylated Mad (pMad) expression. Reduction of Lis1 expression by RNAi-mediated knockdown results in reduced pMad expression. (*B*) Quantitative data show that Lis1 is required for maintaining Mad expression. Reduction of Lis1 protein expression by RNAi-mediated knockdown results in reduced Mad expression. (*C*) Quantitative data show that BMP receptors Punt and Tkv enhance the interaction between Mad and Lis1.

Table S1. *Hu Li-tai Shao (hts)* appears to be dispensable for maintaining GSC self-renewal in the *Drosophila* ovary

	One week after clone induction			Two weeks after clone induction		
Genotype	% of germaria carrying marked GSCs	normalized %	number of germaria counted	% of germaria carrying marked GSCs	normalized %	number of germaria counted
FRT_{42D} control	21.8	100	165	20.0	91.7	70
FRT_{42D} *hts*^{1}	22.7	100	110	27.5	121.1	127
FRT_{42D} *hts*^{2}	26.2	100	99	23.1	88.1	91

The control and mutant GSC clones are marked by *armadillo-lacZ*. *hts*^{1} and *hts*^{2} represent strong/null and moderate alleles, respectively (1). *hts*^{1} mutant GSCs were shown previously to have misoriented spindles (2). FRT42D is Flippase-recognition target at the chromosomal location 42D.

1. Yue L, Spradling AC (1992) hu-li tai shao, a gene required for ring canal formation during Drosophila oogenesis, encodes a homolog of adducin. *Genes Dev* 6(12B):2443–2454.
2. Deng W, Lin H (1997) Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in Drosophila. *Dev Biol* 189:79–94.