Galletta et al., http://www.jcb.org/cgi/content/full/jcb.201501120/DC1

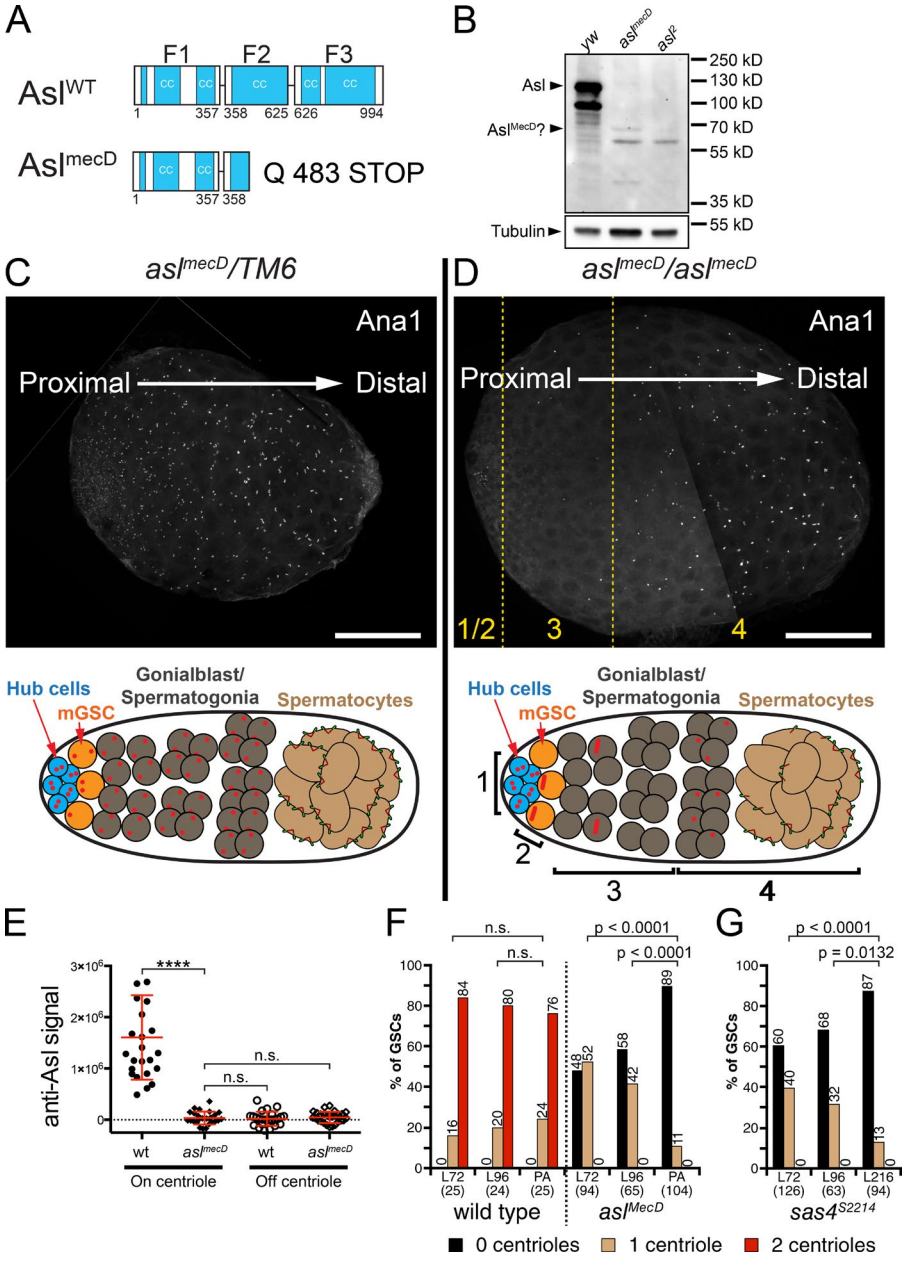


Figure S1. **Characterization of *asl*^{mecD} mutant testes.** (A) Schematic of the wt (Asl^{WT}) and Asl^{mecD} proteins. Predicted coiled coils (CCs) and subdivisions (F1, F2, and F3) for Y2H screening are indicated. (B) Western blots of extracts from *yw* control, *asl*^{mecD}, and *asl*^{2}*/Df(3R)5177* testes probed with anti-Asl antiserum or anti–α-tubulin as a loading control. The image has been significantly overexposed, resulting in saturation of the Asl signal, to reveal the faint bands seen in *asl*^{mecD}. (C) Projection of equatorial confocal slices through a control testis. Representation of the cell types in the *Drosophila* testis (bottom): hub cells (blue), mGSCs (orange) containing centrioles (red), gonialblast (gray), and mature SCs (brown) containing giant centrioles. (D) Similar projection of an *asl* mutant testis. The four distinct regions where we find populations of remnant, Asl-free, centrioles are indicated. Zone 1, hub cells; zone 2, mGSCs; zone 3, proximal germline cells with very few remnant centrioles; and zone 4, distal germline cells with a variable number of remnant centrioles. Multiple images were tiled to cover the entire testis. Schematic (bottom) as in C. Note the distribution and lengths of centrioles. (E) Measurements of the amount of Asl present at centrioles in wt (*asl*^{mecD}*/TM6*) or *asl* (*asl*^{mecD}*/asl*^{mecD}) mGSCs. As a control, measurements were taken at random cytoplasmic locations off the centriole. Mean ± SD is presented in red. Comparison by unpaired *t* tests, with Welch's correction when appropriate. ****, P < 0.0001; n.s., not significant. (F) Percentage of mGSCs with the indicated number of centrioles in wt and *asl*^{MecD} testes at three different developmental times, larvae 72 h after egg laying (L72), larvae 96 h after egg laying (L96), and PAs. As development proceeds, fewer *asl* mGSCs have centrioles, indicating centrioles are being lost over time. Comparisons are by Fisher's exact test. P-values are indicated. n.s., not significant. (G) Percentage of mGSCs with the indicated number of centrioles in *sas-4* testes at three different developmental times, larvae 72 (L72), 96 (L96), and 216 h (L216) after egg laying. As development proceeds, fewer *sas-4* mGSCs have centrioles, indicating centrioles are being lost over time. Comparisons are by Fisher's exact test. P-values are indicated. Bars, 25 µm.

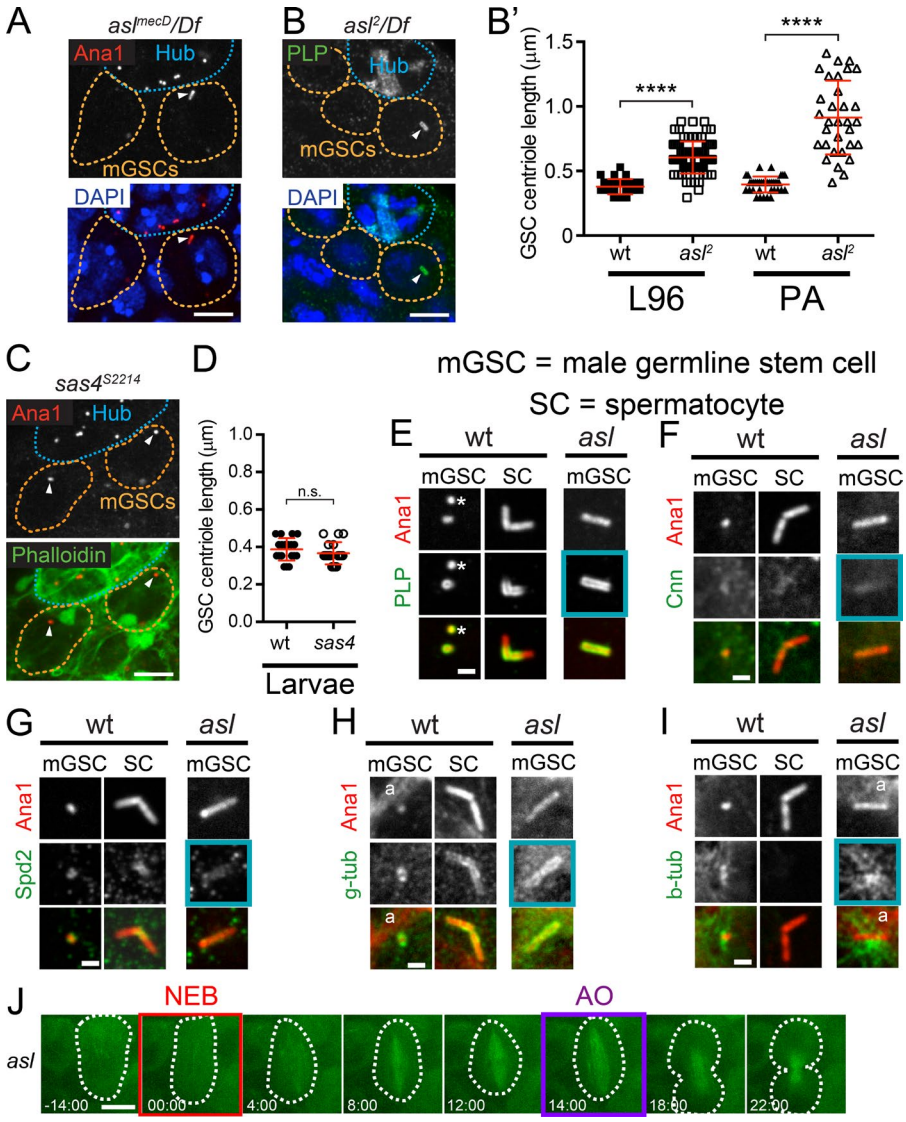


Figure S2. **mGSC long centrioles are a consequence of loss of Asl and recruit PCM components during interphase in *asl* mutants.** (A–C) Remnant centrioles in mGSCs. DAPI (blue) or phalloidin (green) was used to facilitate identification of cell types. mGSCs (orange) and hub cells (blue) are indicated. Arrowheads mark centrioles in mGSCs. (A) Representative example of the long remnant centrioles (Ana1::tdTomato, red, arrowhead) found in *asl^{mecD}/Df(3R)5177* mGSCs. (B) Representative example of the long remnant centrioles (anti-PLP, green, arrowhead) found in *asl²/Df(3R)5177* mutant mGSCs. (B′) Centriole length (Ana1::tdTomato length) in mGSCs in wt (*yw*) and *asl²/Df(3R)5177* mutants in larvae 96 h after egg laying (L96) or PAs. Mean ± SD, red. Comparison by unpaired *t* tests, with Welch's corrections when appropriate. ****, P < 0.0001. (C) Representative example of normal-length centrioles (anti-PLP, green, arrowhead) found in *sas-4^{S2214}* homozygous mGSCs. (D) Measurement of Ana1::tdTomato length in mGSCs in *sas-4* mutants demonstrating they are not longer than control. Mean ± SD is presented in red. Comparison by unpaired *t* tests. n.s., not significant. (E–I) Interphase centrioles (Ana1::tdTomato, red) from wt (*asl^{mecD}/TM6B*) and *asl* (*asl^{mecD}/ asl^{mecD}*) mGSCs or wt mature SCs. Asterisks mark centrioles in the adjacent hub cells. (E) PLP (anti-PLP, green) localizes to centrioles in wt mGSCs, wt SCs, and asl mGSCs (blue box). (F) Cnn (anti-Cnn, green) weakly localizes to centrioles in wt mGSCs, wt SCs, and asl mGSCs (blue box). (G) Spd2 (anti-Spd2, green) weakly localizes to centrioles in wt mGSCs, wt SCs, and asl mGSCs (blue box). (H) γ-Tubulin (anti–γ-tubulin, green) localizes to centrioles (Ana1::tdTomato, red) in wt mGSCs, wt SCs, and asl mGSCs (blue box). Actin "a" from phalloidin staining is indicated. (I) Microtubules (anti–β-tubulin, green) and centrioles (Ana1::tdTomato, red) in wt mGSCs, wt SCs, and asl mGSCs (blue box). Actin "a" from phalloidin staining is indicated. (J) Selected frames from Video 3. mGSCs without centrioles in an *asl* mutant expressing GFP::α-tubulin (microtubules, green) and Ana1::tdTomato (red). Times are in minutes relative to NEB (red box). Anaphase onset (AO) is indicated by the purple box. Bars: (A–C) 5 µm; (E–I) 1 µm; (J) 5 µm.

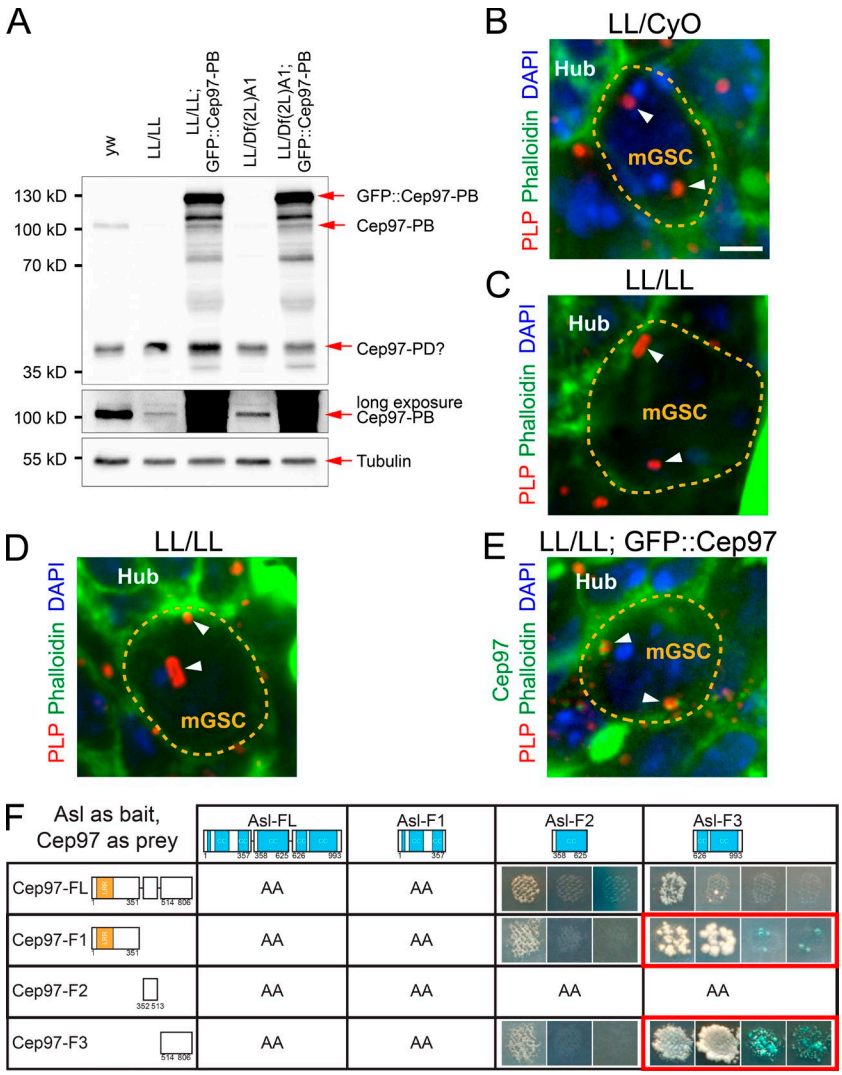


Figure S3. **Cep97 is required for proper centriole length control and interacts with Asl.** (A) Cep97-PB is dramatically reduced in *cep97*^{LL01167} flies. Western blots of protein extracts from adult testes probed with anti-Cep97 (top two panels) or anti–α-tubulin (bottom). The middle panel has been overexposed to reveal the small amount of Cep97-PB that remains in mutant testes. Sizes in kilodaltons are indicated on the left of the blot. GFP::Cep97-PB, Cep97-PB, a band of the approximate size of the Cep97-PD isoform, and the tubulin loading control are indicated. (B–E) mGSCs (dashed line) in adult testis stained for PLP (red), phalloidin (green), and DAPI (blue). All flies were the progeny of homozygous *cep97*^{LL01167} mothers and heterozygous fathers. Hub is labeled. Centrosomes in the mGSCs are indicated by arrowheads. (B) *LL/CyO* mGSC as a control with normal centrioles. (C) An *LL/LL* mGSC with a long apical centrosome. (D) An *LL/LL* mGSC with a long nonapical centrosome. (E) Transgenic expression of GFP::Cep97 in *LL/LL* rescues centriole length in mGSCs. Bar, 2 µm. (F) Full-length, as well as subfragments of, Asl and Cep97 were tested for direct protein–protein interaction by Y2H screening. The indicated combinations of bait and prey were tested for interaction by replica plating on test plates. Each cell of the table contains an image of the replicated colony on test plates (see Materials and methods) as follows: from left to right, DDO (growth indicates both bait and prey plasmids are present), QDO (growth indicates an interaction), DDOXA, and QDOXA (growth and blue color indicates an interaction). See Materials and methods for details on plate composition. Asl F3 interacts with both Cep97-F1 and Cep97-F3 (red boxes). AA indicates that one or both protein fragments autoactivated the Y2H reporters on their own and could not be tested.

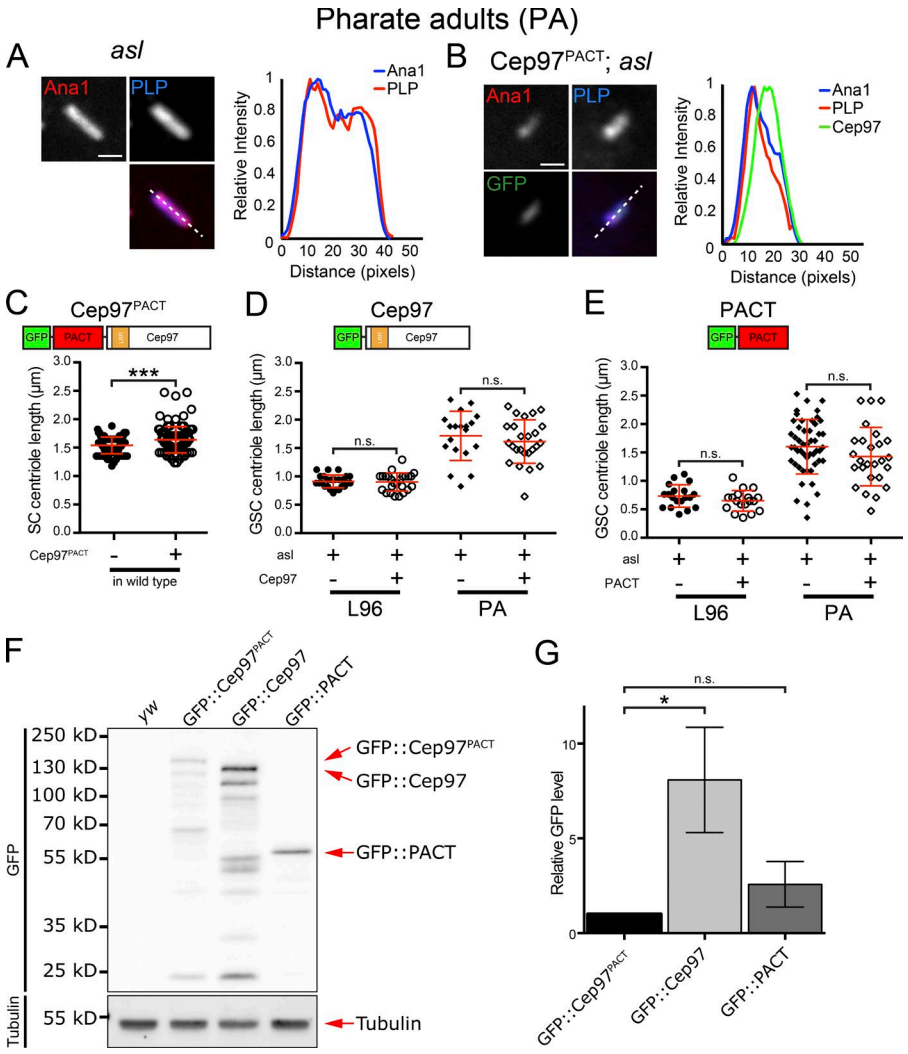


Figure S4. **Cep97 suppresses centriole elongation in *asl* mutants.** (A) A representative long centriole in a PA *asl* mutant mGSCs. PLP (anti-PLP, blue) and Ana1::tdTomato (red) on. (B) A representative example where Cep97^{PACT} suppresses centriole elongation in an *asl* mutant mGSC. PLP (anti-PLP, blue), Cep97^{PACT} (green), and Ana1::tdTomato (red) from mGSC centrioles in *asl* mutant mGSCs. (C) Measurements of the length of Ana1::tdTomato in wt spermatocytes in meiosis II. Expression of Cep97^{PACT} does not shorten these centrioles. Rather it makes them slightly longer. Comparison by unpaired *t* tests, with Welch's correction. ***, P < 0.001. (D) Measurements of the length of Ana1::tdTomato in *asl* mutant mGSC centrioles with and without expression of GFP::Cep97 at the indicated times during development. Expression of GFP::Cep97 does not suppress the *asl* mutant long centriole phenotype. n.s., not significant. (E) Measurements of the length of Ana1::tdTomato in *asl* mutant mGSC centrioles with and without expression of GFP::PACT at the indicated times during development. Expression of GFP::PACT does not suppress the *asl* mutant long centriole phenotype. Linescans (dashed lines and graphs) were taken as in Fig. 6. Comparison by unpaired *t* tests, with Welch's correction when appropriate. n.s., not significant. (F) Expression levels of transgenic GFP fusions used in Figs. 8 and S4. Western blot of extracts from testis from adults of the indicated genotypes probed with anti-GFP or antitubulin antibodies. (G) Analysis of the relative expression levels of the transgenic GFP fusions used in Figs. 8 and S4. Three independent Western blots, as in G, using three independently produced extracts of the indicated genotype, were analyzed for the signal of the highest molecular mass band. This signal was normalized for loading relative to the amount of tubulin and then the fold change relative to GFP::Cep97^{PACT} was determined. The mean of the fold change ± SD is presented. Comparison by unpaired *t* tests, with Welch's correction. *, P ≤ 0.05; n.s., not significant. Bars, 1 µm.

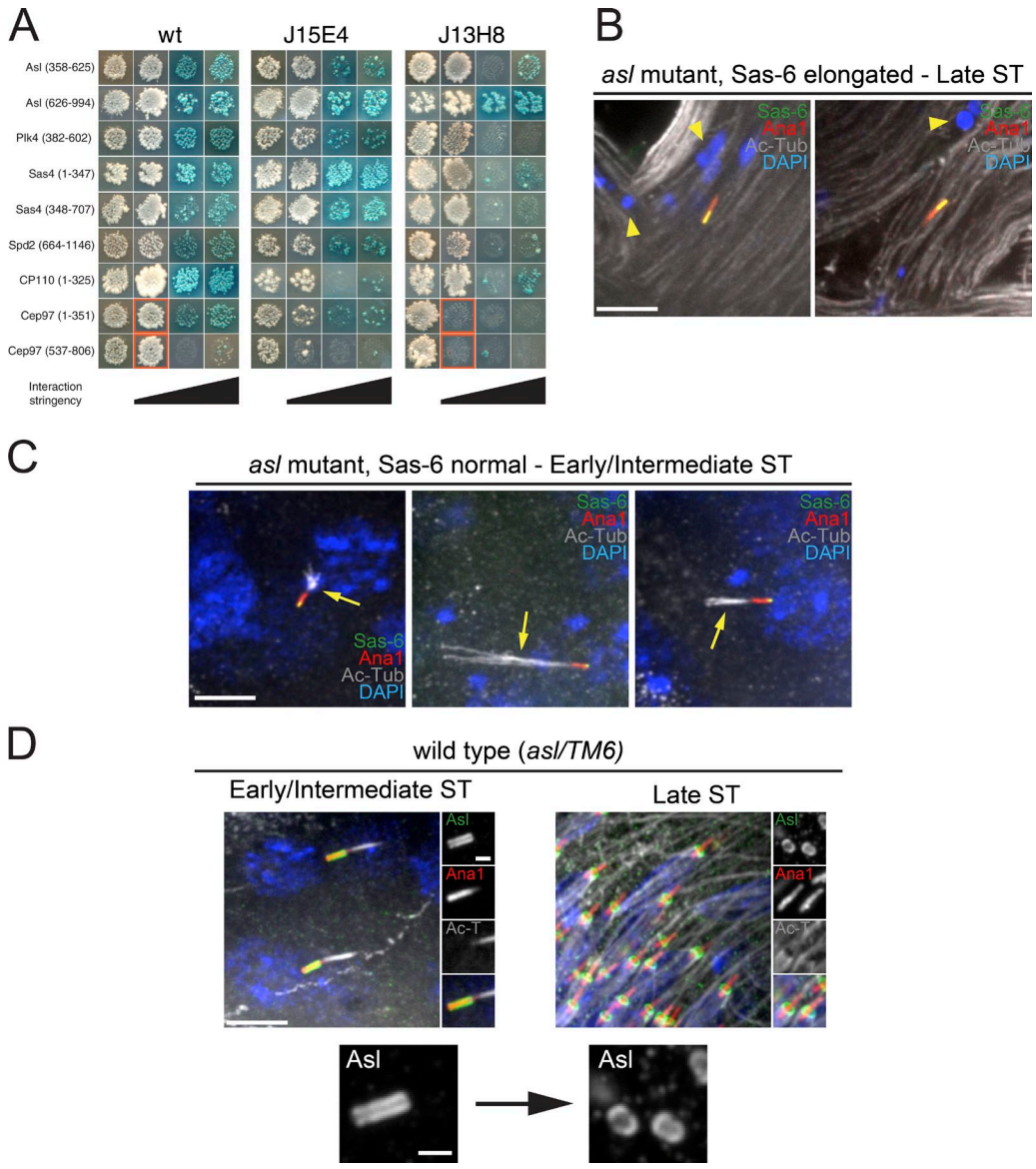
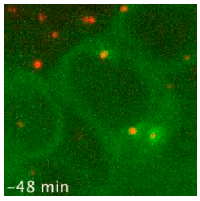
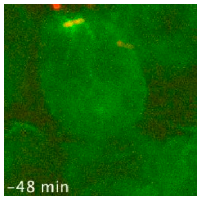


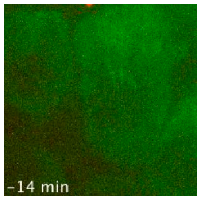
Figure S5. **Cep97 binding mutations in Asl and additional data, related to Fig. 9.** (A) Y2H analysis of the interaction between Asl (626–994) reverse Y2H mutants (J13H8–E760G, E796G, V871A, J15E4–Q687R, and L812P) and all its interactors (unpublished data). Images of the replicated patches on test plates (see Materials and methods) in each group are as follows from left to right: DDO (growth indicates both bait and prey plasmids are present), QDO (growth indicates an interaction), DDOXA, and QDOXA (growth and blue color indicates an interaction). The increase in stringency (requirement for a more robust interaction to drive reporter production) across test plates is indicated below each column. Eliminated or reduced interaction with Cep97 is indicated by a red box. No mutant met the criteria of disrupting both Asl–Cep97 interactions without significantly interfering with other Asl-interacting proteins. (B and C) Basal bodies in asl^{mecD} spermatids labeled with Ana1::tdTomato (red) and GFP::Sas-6 (green); axonemes are labeled with acetylated tubulin (Ac-tub, gray) and nuclei are labeled with DAPI (blue). (B) Remnant centrioles in *asl* mutant spermatids with elongated region of Sas-6. Additional examples of remnant centrioles in late spermatids that are not attached to a nucleus or nucleating an axoneme. Note the small, unshaped nuclear fragments (arrowheads). (C) Additional examples of remnant centrioles in *asl* mutants with a normal "dot" of Sas-6 at the proximal end with disrupted and flared axonemes in an early/intermediate spermatid. Arrows highlight flared axonemes. (D) Localization of Asl (anti-Asl, green) to basal bodies (Ana1::tdTomato, red), nucleating axonemes (anti–Ac-tub [Ac-T], gray), in early (left) and late (right) spermatids. In early spermatids, Asl localizes along the entire length of the basal body. As spermatogenesis proceeds, Asl localization changes to form a ring surrounding the end of the basal body. Bars: 5 µm; (insets) 1 µm.



Video 1. **Mitosis in a wt mGSC.** Stills of this video can be found in Fig. 5 F. wt mGSCs expressing GFP::α-tubulin (microtubules) and Ana1::tdTomato. Images are projections of multiple confocal stacks. Frames are displayed at 2-min intervals over 66 min at 7 frames/s.



Video 2. **Mitosis in an *asl* mGSC with a single, elongated centriole.** Stills of this video can be found in Fig. 5 F. *asl* mutant mGSC expressing GFP::α-tubulin (microtubules) and Ana1::tdTomato. Images are projections of multiple confocal stacks. Frames are displayed at 2-min intervals over 70 min at 7 frames/s.



Video 3. **Mitosis in an *asl* mGSC with no centrioles.** Stills of this video can be found in Fig. S2 J. *asl* mutant mGSC expressing GFP::α-tubulin (microtubules) and Ana1::tdTomato. Images are projections of multiple confocal stacks. Frames are displayed at 2-min intervals over 40 min at 7 frames/s.