Schoborg et al., http://www.jcb.org/cgi/content/full/jcb.201509054/DC1

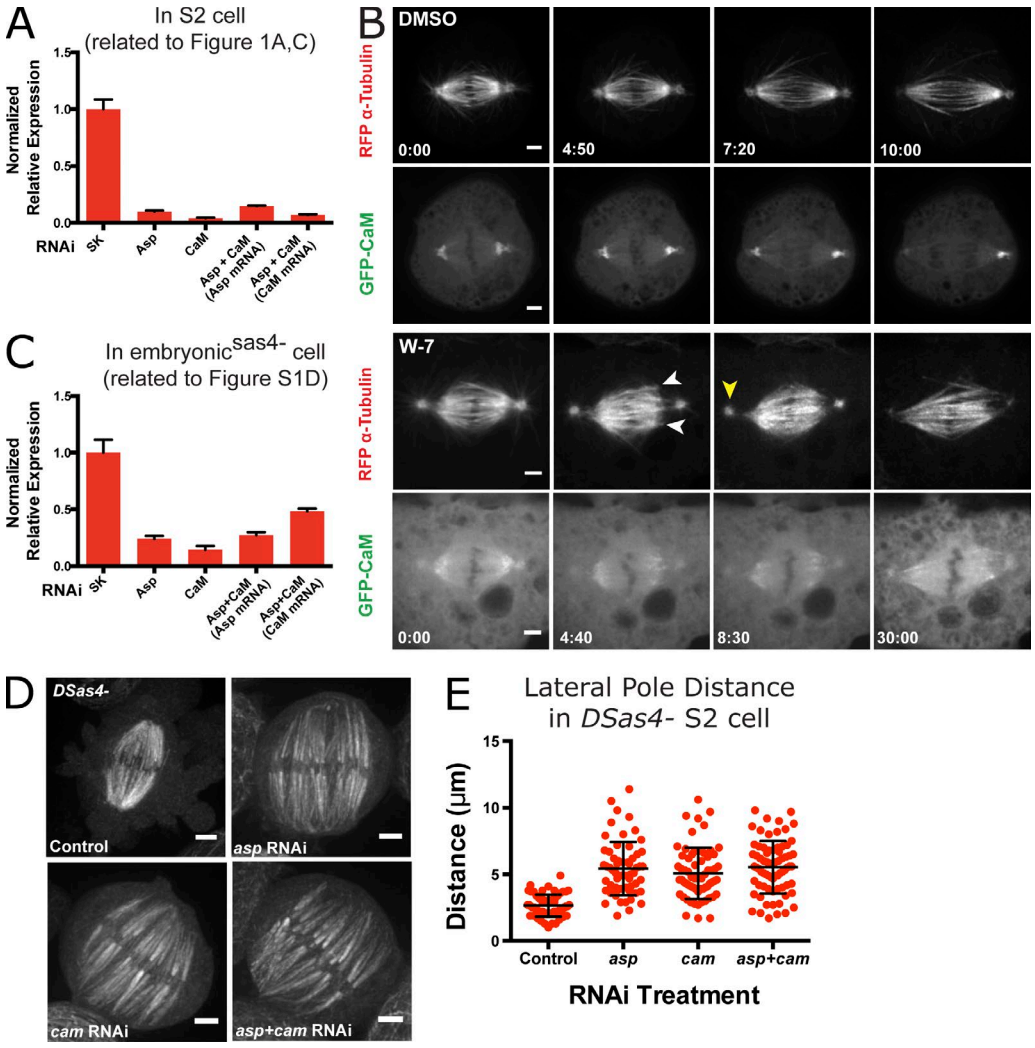


Figure S1. **CaM and Asp localization and function.** (A) Quantitative PCR analysis of transcript levels after knockdown in the experiment outlined in Fig. 1 (three biological replicates, error bars are SEM). (B) Time-lapse imaging of an S2 cell expressing RFP-α-tubulin and GFP-CaM treated with either DMSO (top two panels) or 200 µm W-7 (bottom two panels). White arrowheads denote unfocused pole, and yellow arrowhead denotes partially detached centrosome. Note failure of anaphase onset in W-7–treated cells. (C) Quantitative PCR analysis of transcript levels after knockdown in the *sas4*$^{-/-}$ experiment (D and E) (three biological replicates, error bars are SEM). (D) *sas4*$^{-/-}$ cells expressing Jupiter::GFP treated with *asp*, *cam*, or *asp+cam RNAi*. (E) Measurement of lateral pole distance after the indicated RNAi treatment in *sas4*$^{-/-}$ cells ($n > 60$, error bars are SD). In A and C, SK, control RNAi. Bars, 2 µm.

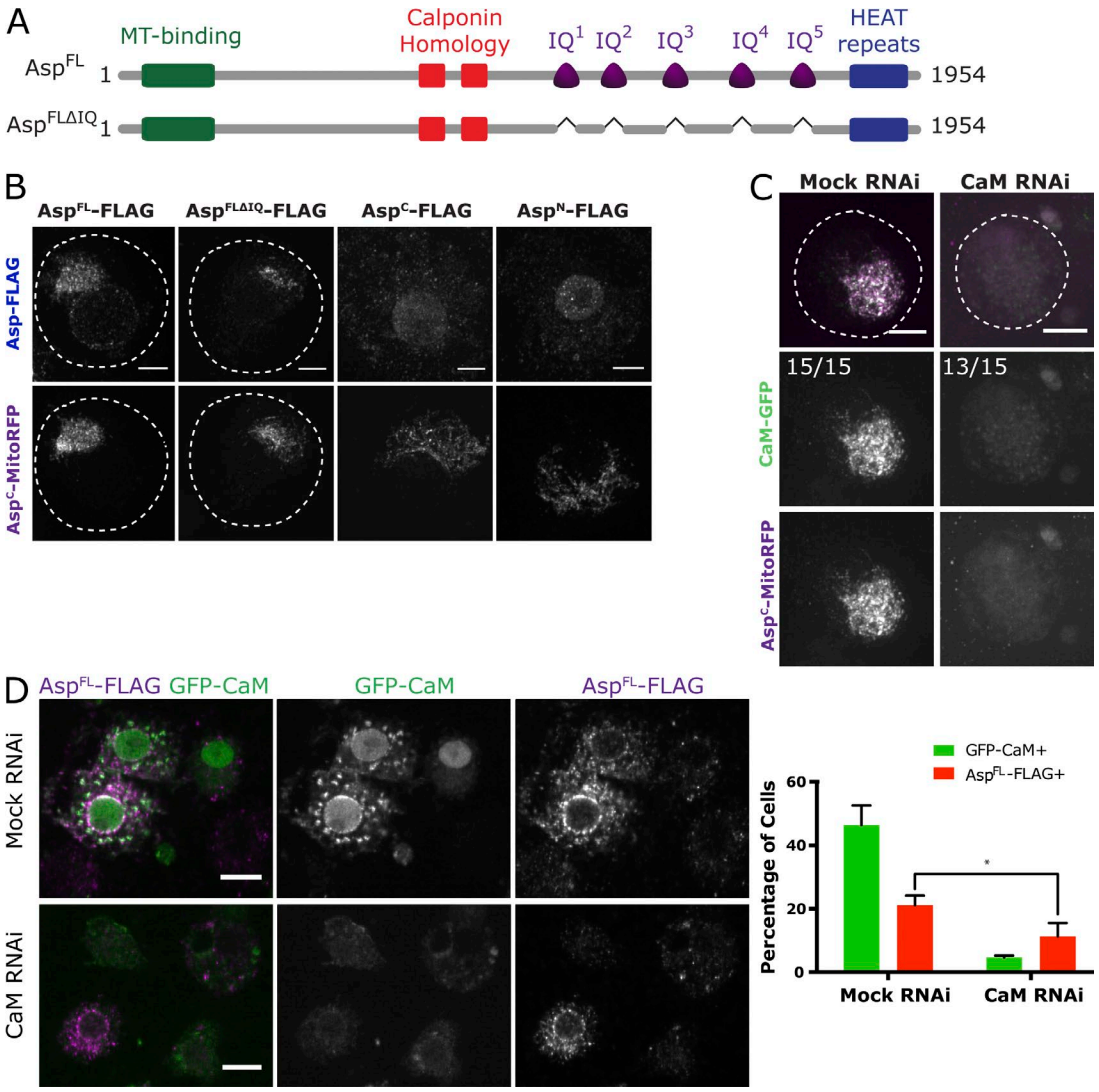


Figure S2. **Asp dimerization analysis and stability after *cam* depletion.** (A) Schematic of the individual IQ motifs in the C terminus of Asp (IQ^1–IQ^5) deleted to create $Asp^{FL\Delta IQ}$. (B) Dimerization analysis in S2 cells using the mitochondria targeting assay. The Asp^C fragment was targeted to mitochondria, and cells were cotransfected with FLAG-tagged Asp^{FL}, $Asp^{FL\Delta IQ}$, Asp^C, and Asp^N. Note the interaction between Asp^C and each of Asp^{FL}-FLAG and $Asp^{FL\Delta IQ}$-FLAG. (C) Asp^C fragment behavior after mock or CaM RNAi treatment. All mock-treated cells (n = 15) display both Asp^C signal and mitochondria localization (left), which is lost in most cells (13/15) on CaM depletion (right). This suggests that Asp^C is unstable without CaM present. (D) Stability of the Asp^{FL} fragment in mock or CaM RNAi–treated interphase S2 cells. Representative 40× images of transfected cells shows Asp^{FL}-FLAG primarily at the nuclear periphery, with weaker staining in the nucleoplasm. GFP-CaM localizes primarily to the nucleoplasm but also overlaps with Asp^{FL}-FLAG in the nuclear periphery. Bar graph (right) displays the percentage of GFP-CaM– and Asp^{FL}-FLAG–positive cells per treatment. Data are from three biological replicates ($n \geq 200$ cells for each replicate; error bars are SD). *, P = 0.03, two-tailed unpaired t test. Bars: (B and C) 5 µm; (D) 10 µm.

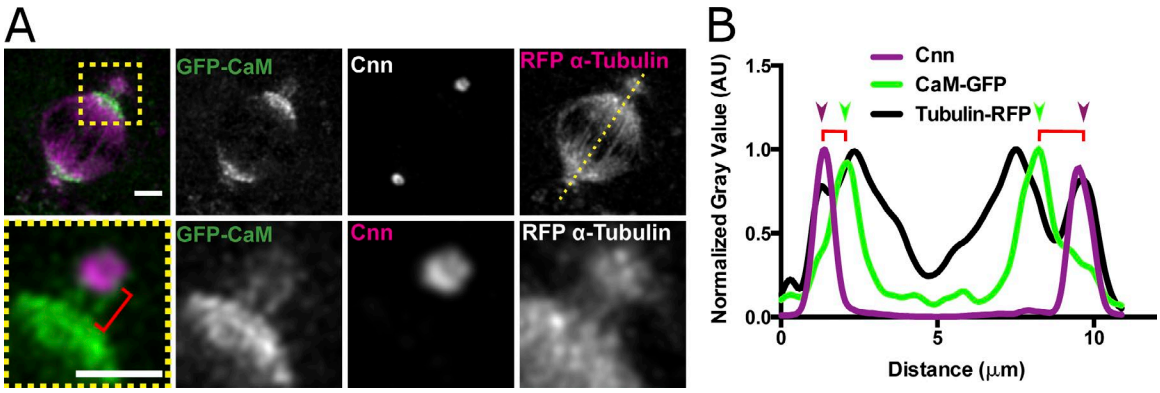


Figure S3. **CaM localization to metaphase spindles in S2 cells.** (A) S2 cell expressing GFP-CaM and RFP-α-tubulin, stained for centrosomin (Cnn). Yellow boxed region denotes inset (bottom). Red brackets denote distance between GFP-CaM signal at the pole and centrosomes. (B) Line scan showing signal intensity along the dotted yellow line in A. Note the separation between the CaM pole signal and centrosome. Bars, 2 μm.

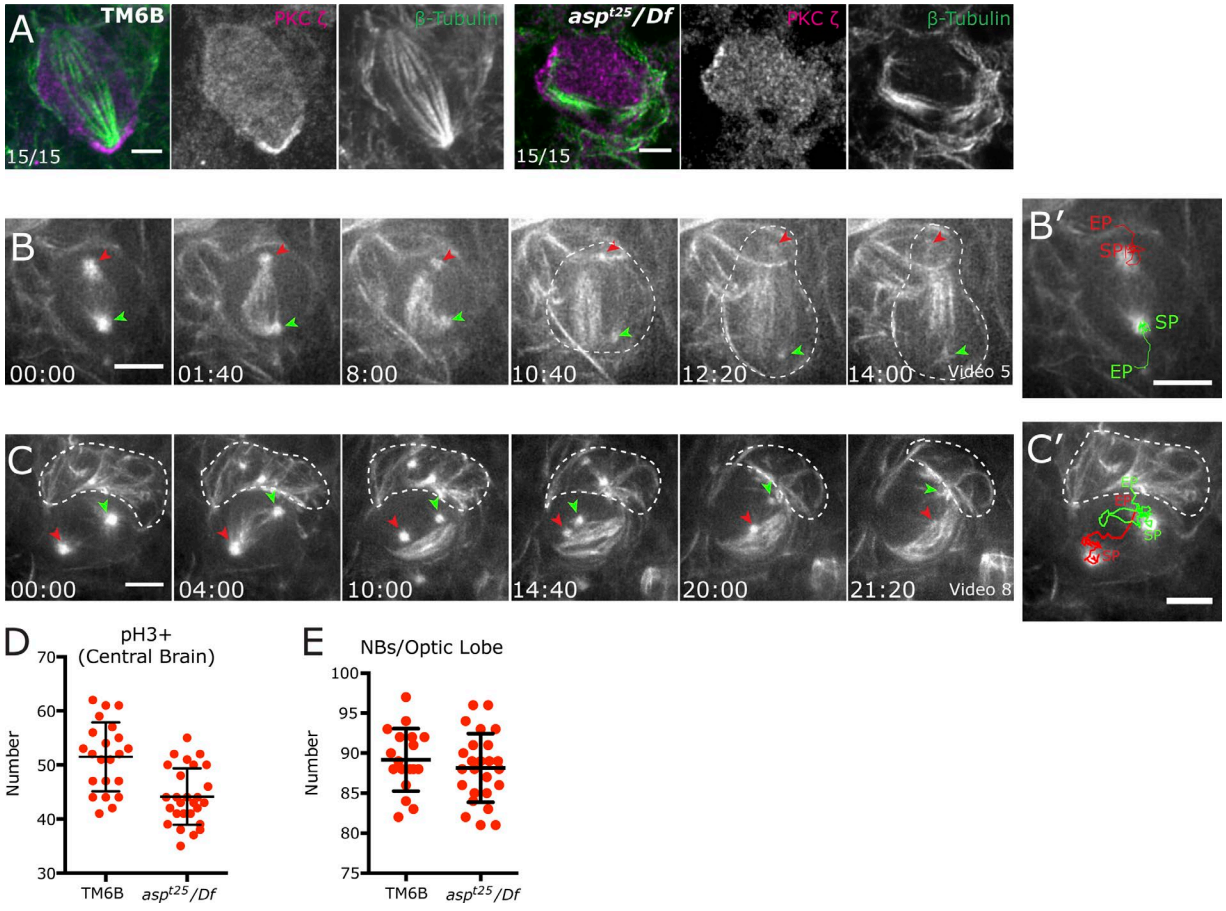
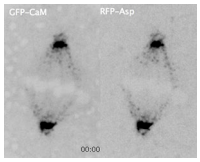
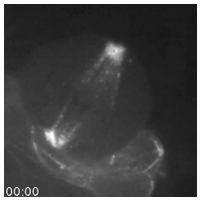


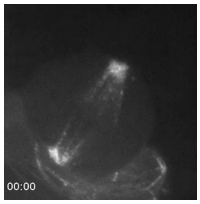
Figure S4. **Characterizing head size and spindles in *asp* mutants.** (A) Full panel from Fig. 7 D, showing individual grayscale images for aPKC (PKC ζ; magenta) and β-tubulin (green) in WT (TM6B) and *asp*^{t25}*/Df* mutant NBs. (B) Live-cell imaging of an *asp*^{t25}*/Df* mutant NB showing transient loss of centrosome attachment (arrowheads) but correct centrosome inheritance; centrosome trajectory tracks are shown in B′. (C) Live-cell imaging of an *asp*^{t25}*/Df* mutant NB showing centrosome detachment (arrowheads) and subsequent double inheritance by the GMC. The GMC cluster is outlined. Centrosome trajectory tracks are shown in C′. (D) Number of NBs with pH3-positive nuclei ($n > 22$ optic lobes; error bars are SD). (E) Total number of NBs/optic lobes from WT (TM6B) and *asp*^{t25}*/Df* mutants, based on deadpan staining ($n > 20$ optic lobes, error bars are SD). For B′ and C′, EP, end point; SP, start point at prophase. Bars: (A) 3 μm; (B, B′, C, and C′) 5 μm.



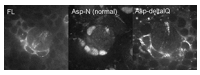
Video 1. **S2 cell expressing GFP-CaM and RFP-Asp.** Frames were acquired every 5 s and displayed at 13 fps.



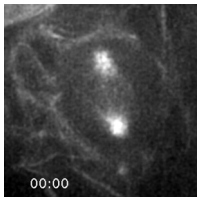
Video 2. **Mitotic NBs expressing GFP-CaM.** Frames were acquired every 4 s and displayed at 13 fps.



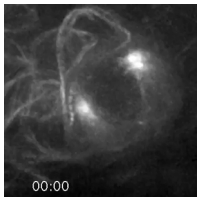
Video 3. **Mitotic NBs expressing GFP-CaM.** Same NB as described in Video 2, but metaphase duration only to highlight streaming. Frames were acquired every 4 s and displayed at 13 fps.



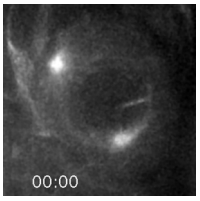
Video 4. **NB expressing asp^{FL}, asp^{N}, or $asp^{FL\Delta IQ}$.** Frames were acquired every 2 s and displayed at 13 fps.



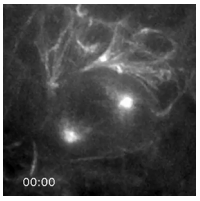
Video 5. **NB from an asp^{t25}/Df mutant expressing tubulin-GFP.** Note correct centrosome inheritance. Frames were acquired every 20 s and displayed at 13 fps.



Video 6. **NB from an asp^{t25}/Df mutant expressing tubulin-GFP.** Note that the NB inherits both centrosomes. Frames were acquired every 35 s and displayed at 13 fps.



Video 7. **NB from an *asp*^{t25}*/Df* mutant expressing tubulin-GFP.** Note centrosome swapping. Frames were acquired every 20 s and displayed at 13 fps.



Video 8. **NB from an *asp*^{t25}*/Df* mutant expressing tubulin-GFP.** Note the GMC inherits both centrosomes. Frames were acquired every 20 s and displayed at 13 fps.