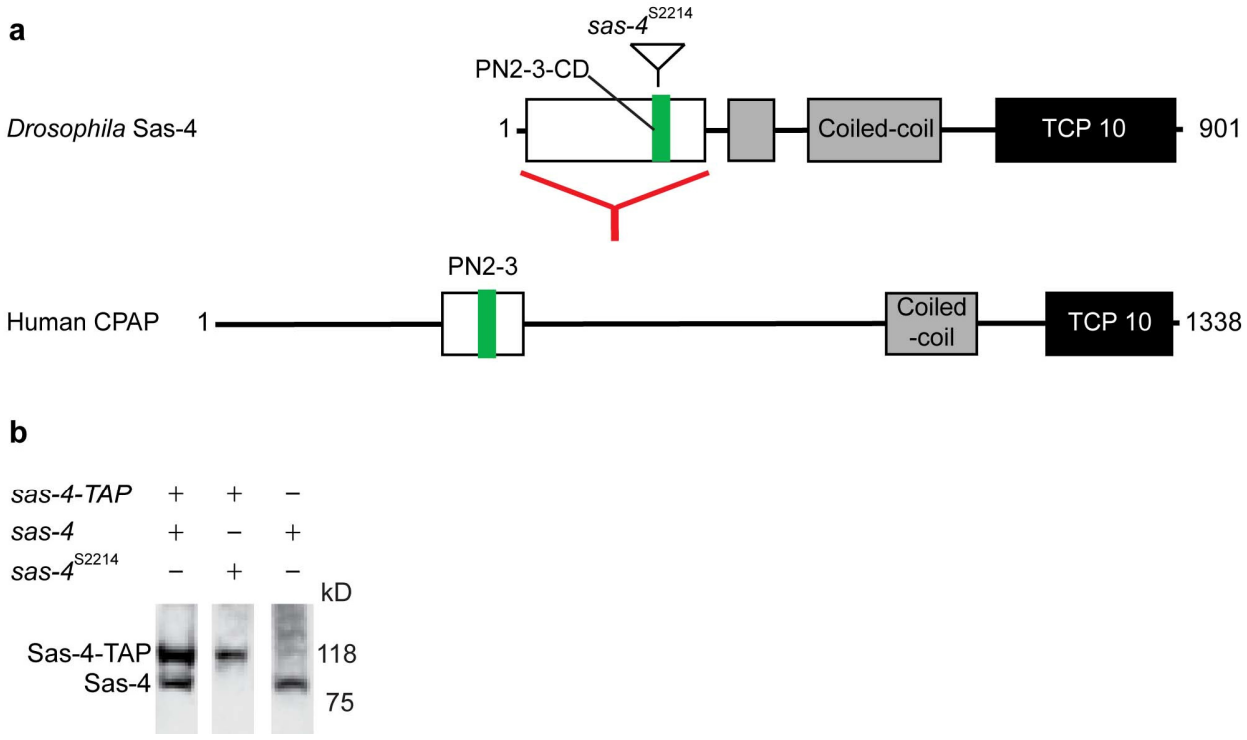
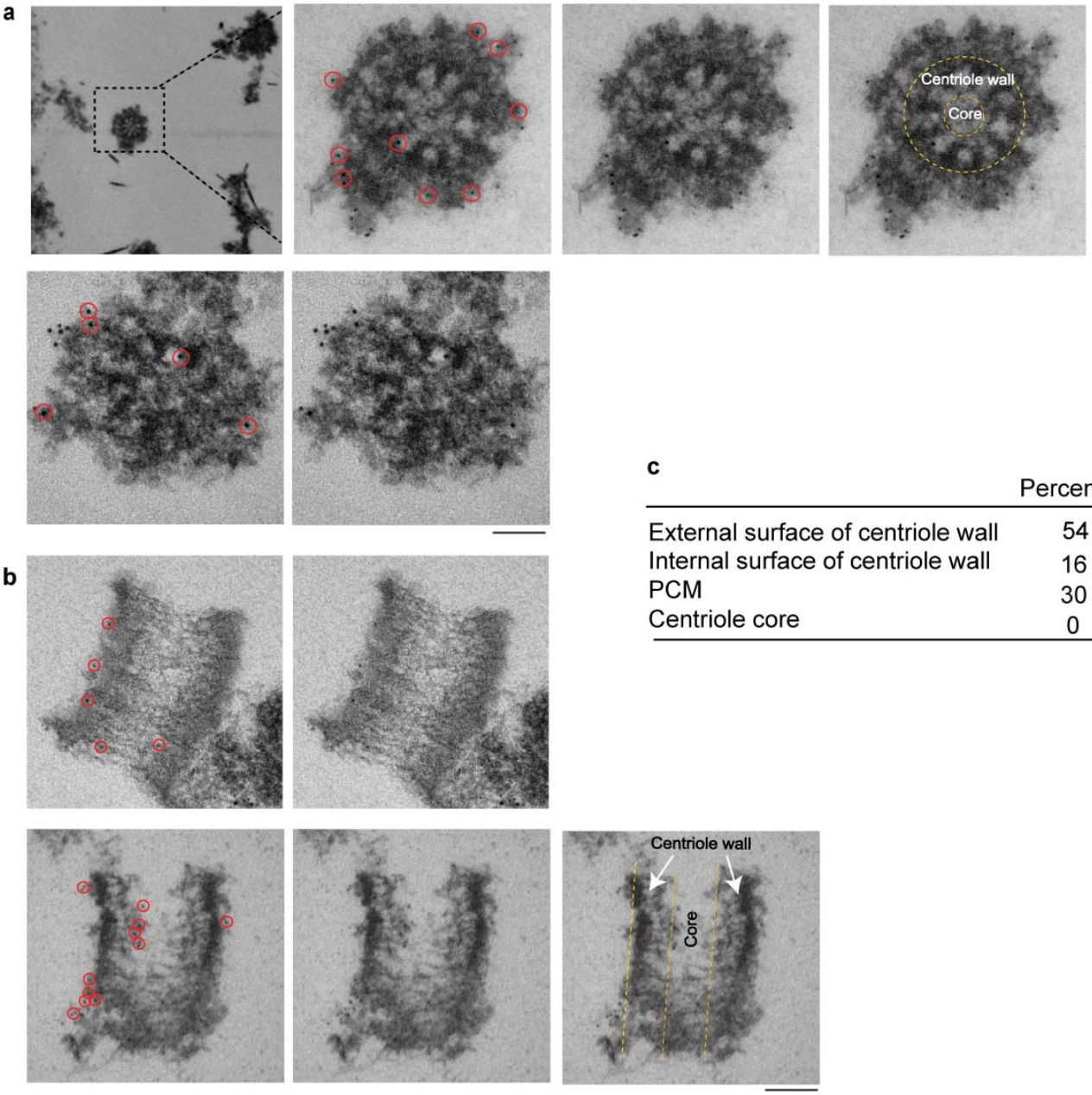


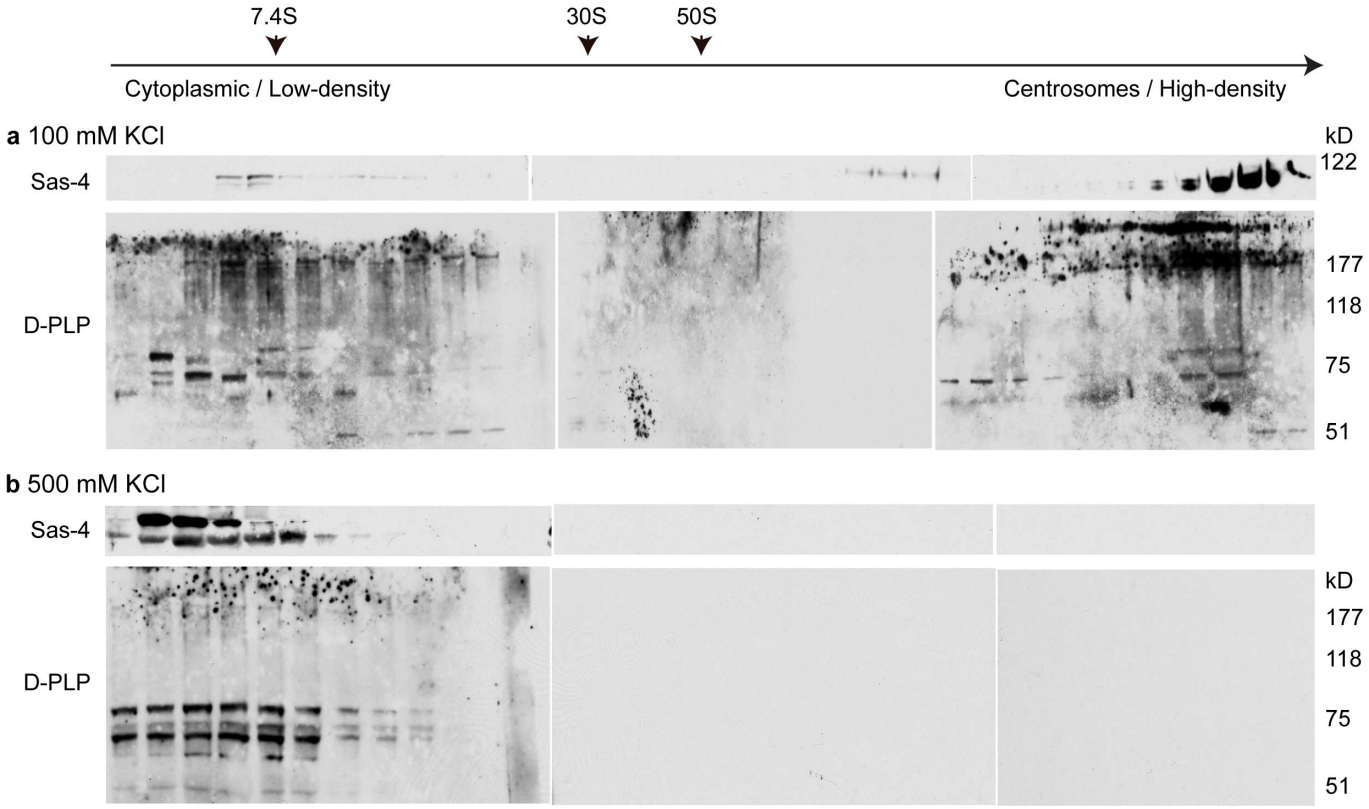
Supplementary Figure S1. Sas-4 genes and antibody. (**a**) Schematics of *Drosophila* Sas-4 and human CPAP genes. The PN2-3 domain, the conserved segment in PN2-3 that is essential for tubulin binding (green box, PN2-3-centriole duplication), and *Sas-4*^{s2214}*'s P*-element insertion site are indicated[19,22]. A monoclonal antibody that recognizes amino acids 2-150 of Sas-4 is shown (red). (**b**) The anti-Sas-4 antibody, shown in (**a**), recognizes Sas-4 protein in Western blots. Bands corresponding to Sas-4 and Sas-4-TAP (Sas-4 with a Tandem Affinity Protein tag) are detected in embryonic extracts of wild type or Sas-4-TAP transgenic flies. Embryonic extracts of *Sas-4* null mutants rescued by Sas-4-TAP lack a wild type Sas-4 band and only have the band corresponding to Sas-4-TAP.



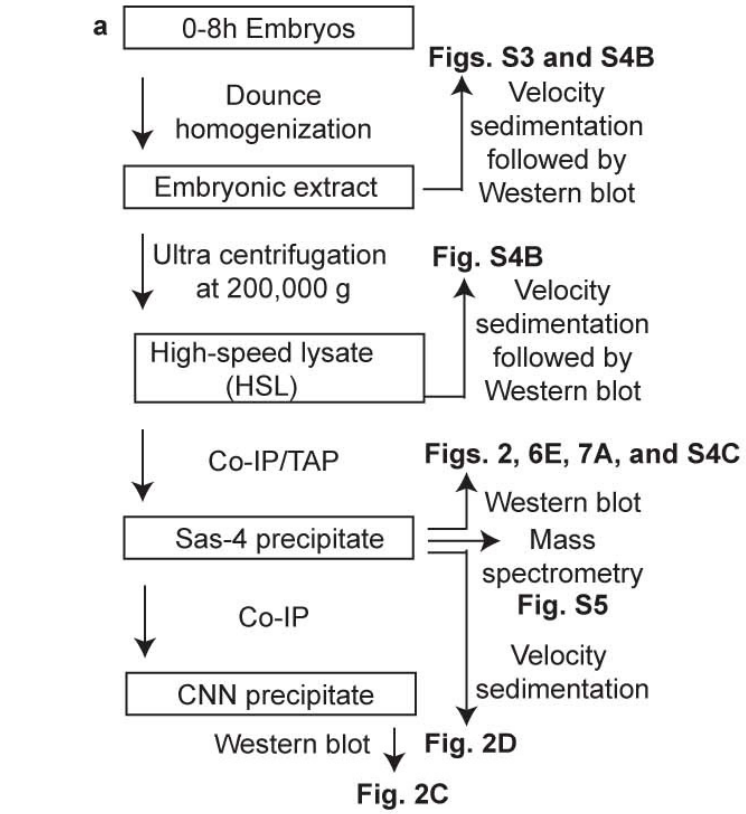
c

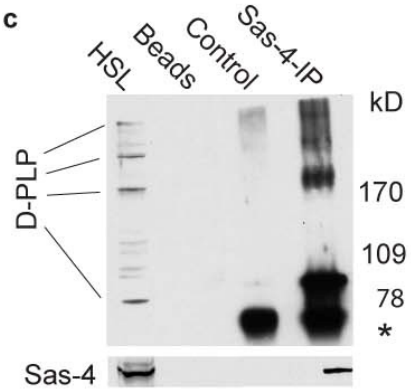
	Percent (%)
External surface of centriole wall	54
Internal surface of centriole wall	16
PCM	30
Centriole core	0

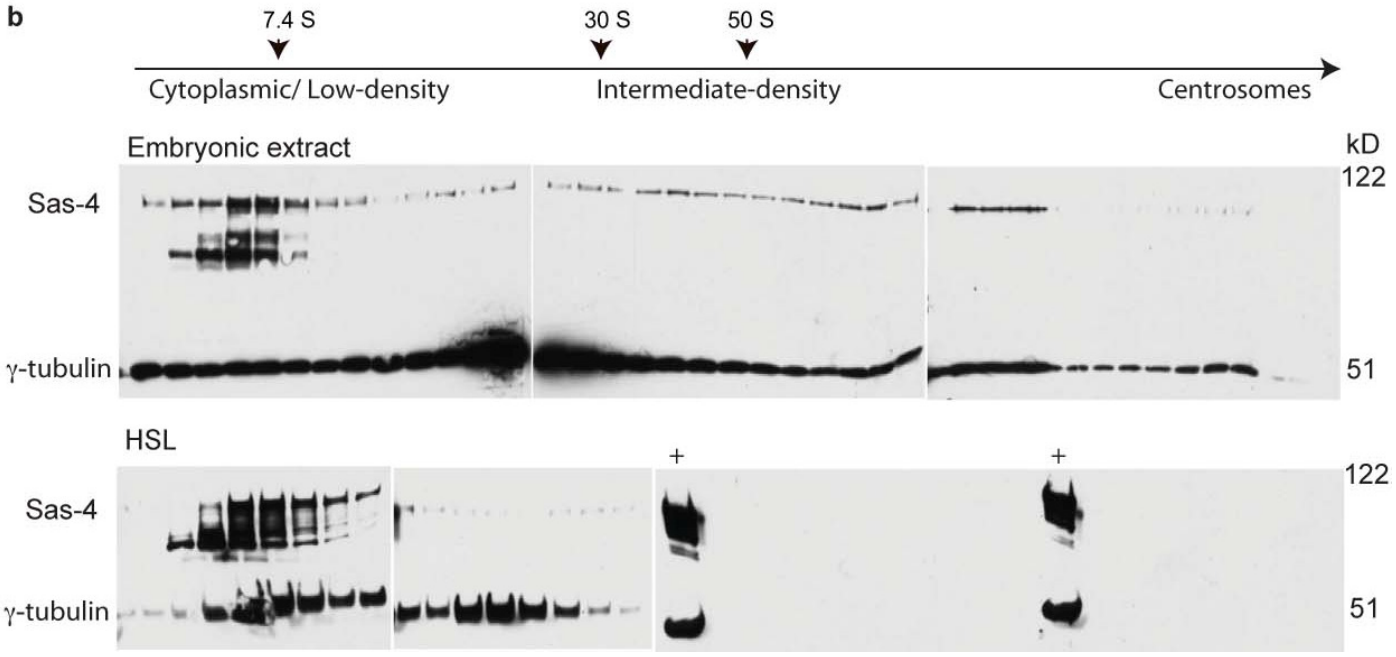
Supplementary Figure S2. Sas-4 is localized to the centriole and to the PCM at the vicinity of the centriole wall. Pre-embedding immunogold-labeling of isolated fly centrosomes using our anti-Sas-4 antibody in (**a**) Cross-section and (**b**) longitudinal-section. Red circles highlight the immunogold-labeling. The same images without red circles are also given in the adjacent figures. Dashed yellow and orange lines define the external and internal surfaces of the centriole wall, respectively. (**c**) Quantification of Sas-4 immuno-gold particles by location in centrosomes (n > 130). Scale bar, 100 nm.



Supplementary Figure S3. Low salt treatments of embryonic extracts allow isolation of intact centrosomes whereas high salt treatments strip PCM from the centrosomes, allowing isolation of "stripped-centrosomes". *Drosophila* embryonic extracts (see Supplementary Figure S4a) were separated along a 15-60% linear sucrose gradient with (**a**) 100 mM KCl or (**b**) 500 mM KCl. Fractions were subjected to SDS-PAGE and immunoblotted for the presence of Sas-4 or D-PLP. (a) Under low salt conditions, the anti-Sas-4 and anti-D-PLP antibodies label the low-density, cytoplasmic complexes (~7 S) and intact centrosomes. (**b**) Under high salt conditions, the anti-Sas-4 and anti-D-PLP antibodies label only the ~7 S fractions since PCM has been stripped from the centrosomes. This treatment affects adherence of PCM proteins to the centriole and does not affect the centriole itself, as evidenced by unchanging levels of the centriolar protein Sas-6 between untreated and treated centrosomes (see Fig. 7).

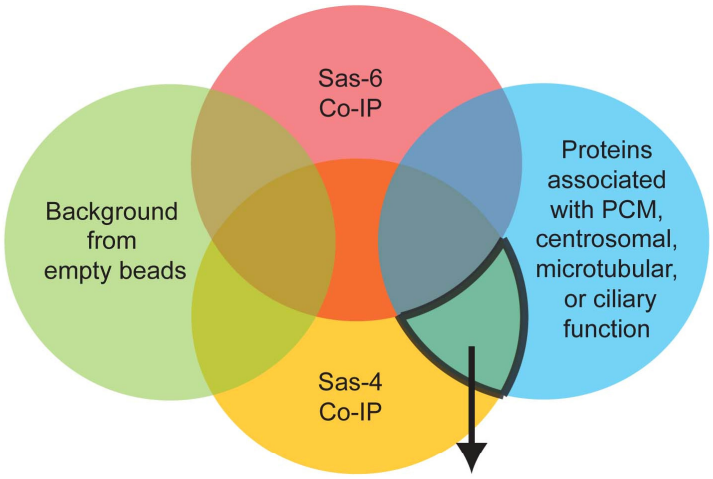

a
0-8h Embryos
Dounce homogenization
Embryonic extract
Ultra centrifugation at 200,000 g
High-speed lysate (HSL)
Co-IP/TAP
Sas-4 precipitate
Co-IP
CNN precipitate
Western blot
Fig. 2C
Figs. S3 and S4B
Velocity sedimentation followed by Western blot
Fig. S4B
Velocity sedimentation followed by Western blot
Figs. 2, 6E, 7A, and S4C
Western blot
Mass spectrometry
Fig. S5
Velocity sedimentation
Fig. 2D


c
HSL
Beads
Control
Sas-4-IP
kD
D-PLP
170
109
78
*
Sas-4


b
7.4 S
30 S
50 S
Cytoplasmic/ Low-density
Intermediate-density
Centrosomes
Embryonic extract
kD
Sas-4
122
γ-tubulin
51
HSL
+
+
Sas-4
122
γ-tubulin
51

Supplementary Figure S4. High-speed lysate (**a**) Processing scheme of embryonic extracts and their uses in biochemistry experiments. (**b**) A high-speed lysate (HSL) from embryonic extracts contains structures that are less dense than ~30S. In embryonic extracts (that were not exposed to high-speed centrifugation) subjected to 15-60% velocity sucrose gradient sedimentation, Sas-4 and γ-tubulin are found in low-density fractions (cytoplasmic complexes) that sediment around 7 S, in intermediate-density fractions, and in high-density fractions (centrosomes). In embryonic HSLs, Sas-4 and γ-tubulin are observed only in low-density fractions (less than ~30S). This embryonic HSL, which is free of centrosomes, was used for the co-immunoprecipitation assays, described in the main text. For the two gels in HSL that cover the intermediate and high-density fractions, every twelfth lane was loaded with a sample of embryonic extract as a positive control (indicated by +). Note, in these two gels, no signal is observed in the intermediate or high-density fractions. (**c**) D-PLP co-immunoprecipitates with Sas-4. The anti-D-PLP antibody recognizes a series of high molecular weight bands in wild type embryonic extract HSLs, as previously reported[18], and detects a ~78 kD band. This band may be a degradation product of D-PLP due to extensive biochemical processing or an isoform present in embryonic extracts. This band is specifically D-PLP, as it co-purifies with other D-PLP high molecular weight bands in multiple, separate experiments (e.g., Figs. 2D and S3 and data not shown). We use this ~78 kD band to represent D-PLP labeling, as it is the most prominent band in our experiments. This Western blot is not meant to be quantitative and no conclusion can be made on the level of Sas-4 relative to D-PLP in the precipitate. Uncoated beads do not precipitate D-PLP from Sas-4-GFP HSLs (Bead). The anti-GFP antibody does not precipitate D-PLP from wild type HSL (Control) but does precipitate D-PLP from HSL that contains Sas-4-GFP (Sas-4-IP). The asterisk points out the IgG heavy chain band that is detected by the anti-rabbit secondary antibody. The smaller Western blot directly below the main blot shows Sas-4 precipitation.

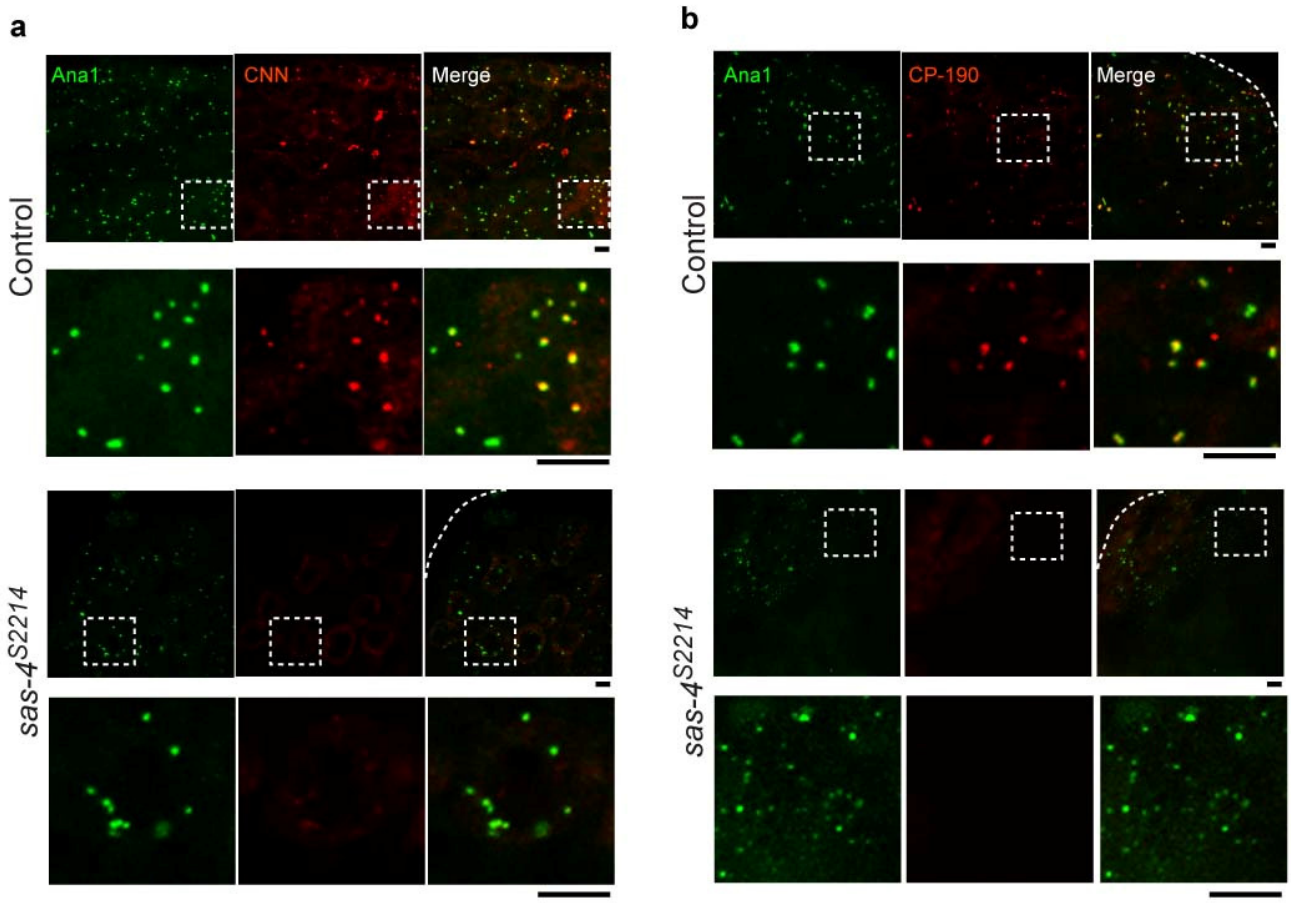
a



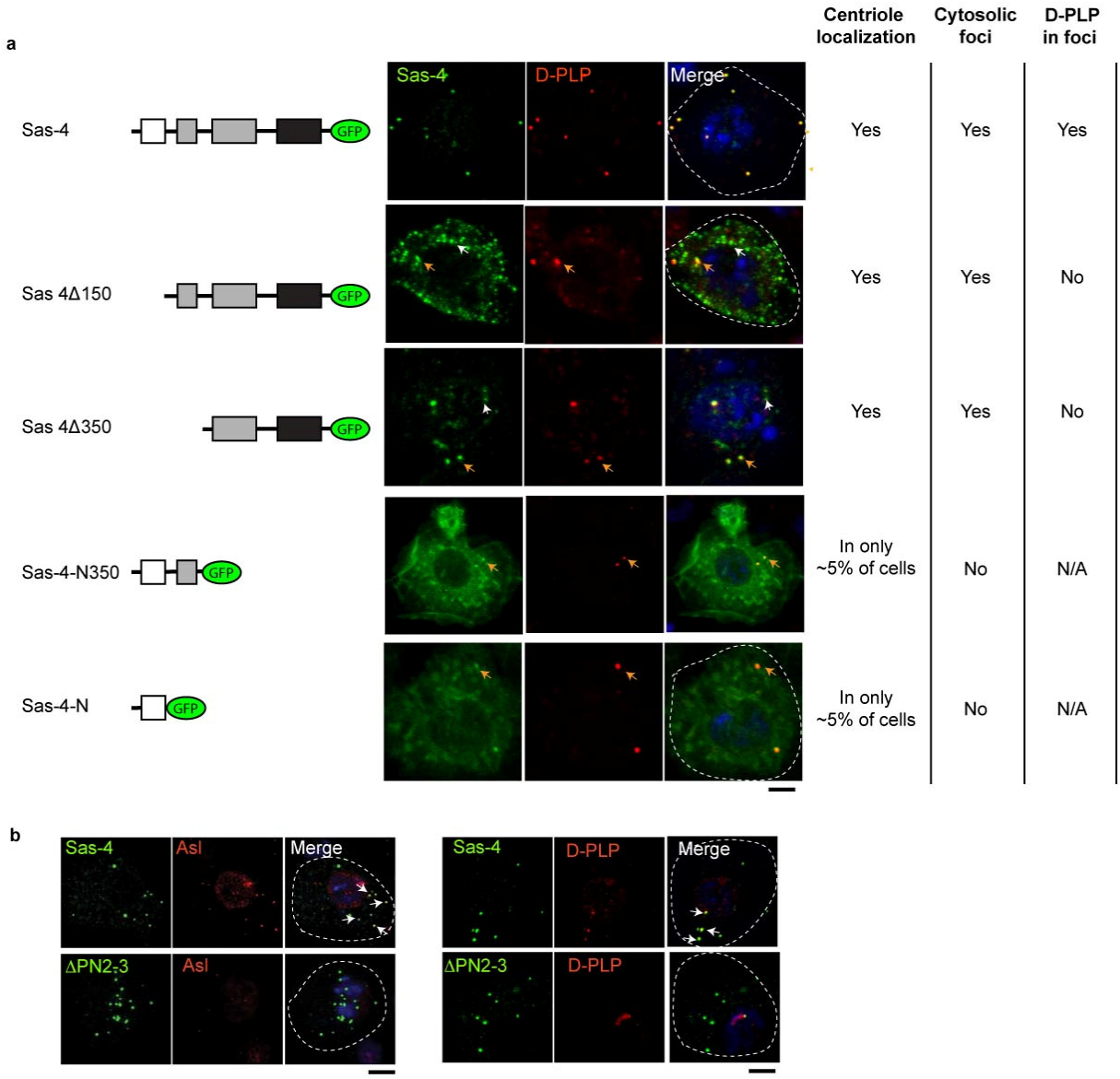
b

ID	Name	# of peptides detected	Verified by Western
	Centrosomal Proteins		
CG10061	Sas-4	13	Yes
CG4832	CNN	2	Yes
CG6735	D-PLP	1	Yes
CG6384	CP-190	3	Yes
CG14025	Ninein	2	No
CG9201	Grip 128	1	Yes
CG13162	Ana3	2	Yes
CG31291	Hs-CCCAP	2	No
CG 12307	Polo kinase	2	No
	Proteins associated with centrosomal, microtubular, or ciliary function		
CG3723	Ciliary dynein heavy chain 9	1	No
CG12052	Ciliary, flagellar motility, Lola	2	No
CG18584	Outer dense fiber-associated protein	1	No
CG7831	Ncd kinesin	1	No
CG4767	Tetkin	1	No
CG16896	Poc18 WD repeat-containing protein 67	2	No
CG8590	Chromosome-associated kinesin KIF4A	1	No
CG3064	Futsch microtubule organization	1	No
CG1258	Pavarotti	2	No
CG32670	Rab9Fb	1	No
CG6386	Ballchen, Serine/threonine-protein kinase	1	No

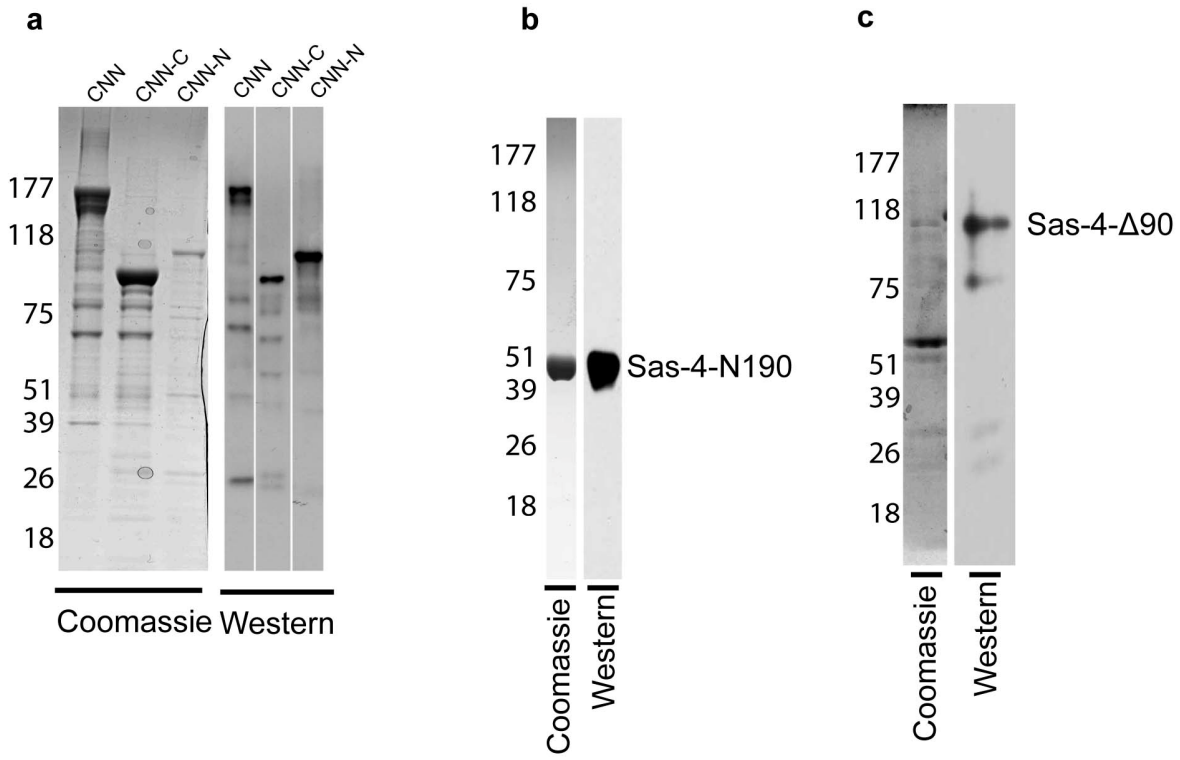
Supplementary Figure S5. Mass-spectrometry of Sas-4 complexes. (**a**) A Venn diagram showing the four categories of proteins identified by mass-spectrometry from wild-type HSLs. (**b**) A list of proteins obtained by subtracting the background proteins detected with beads alone and from the Sas-6 immunoprecipitate from the LC MS/MS of Sas-4 co-immunoprecipitates. Known PCM proteins and proteins with centrosomal, microtubule, or ciliary functions are listed separately in the table. Tubulin and 14-3-3 proteins were excluded.



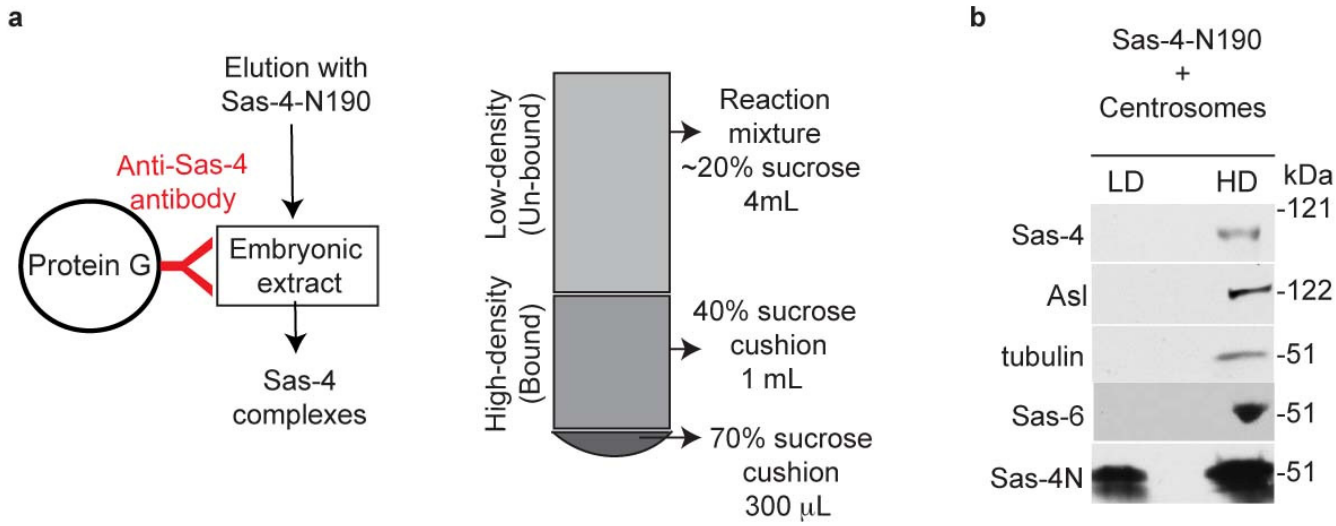
Supplementary Figure S6. *sas-4* mutants have centrioles that lack PCM proteins. Centrioles in wild-type spermatocytes are marked for the pan-centriolar protein Ana1 (**a** and **b**,). Wild-type centrioles are also immuno-positive for two PCM proteins, CNN (**a**) and CP-190 (**b**). Although positive for Ana1, centrioles of *sas-4* mutants are immuno-negative for CNN and CP-190. In other words, centrioles in *sas-4* mutants lack the PCM proteins CNN and CP-190. Scale bar, 2 µm (**c**) Sas-6-GFP protein expression using an endogenous promoter is near physiological levels. Western analysis of endogenous Sas-6 (control) and transgenic Sas-6 expressing (Sas-6-GFP) testes using anti-Sas-6 antibody {Gopalakrishnan, 2010 #504}. Each lane contains the equivalent of two testes.



Supplementary Figure S7. Localization of Sas-4 variants and their effects on foci induction. a) Representative images of S2 cells that express GFP-tagged full-length Sas-4 or truncated versions of Sas-4. In cells transfected with Sas-4, multiple foci are induced by Sas-4 (green) and are co-labeled with D-PLP (red). In cells transfected with constructs that lack N-terminal portions of Sas-4, there are Sas-4-positive but D-PLP-negative foci (white arrows). In cells transfected with constructs that have only N-terminal portions of Sas-4, Sas-4 is mostly diffused throughout the cytoplasm. Native centrioles can be identified as Sas-4- and D-PLP-positive foci (yellow arrows). The columns summarize each construct's capacity to localize to centrioles, to form foci, or to co-localize with D-PLP. Scale bar, 1 μm. **b**) Transfection of S2 cells with Sas-4 induces multiple foci (green) that contain Asl or D-PLP (red). Arrowheads indicate foci that are intensely co-labeled. The dotted line marks the cell's plasma membrane. In cells transfected with a variant of Sas-4 that lacks its PN2-3 domain ("ΔPN2-3"), there is induction of Sas-4-positive foci, which do not contain Asl or D-PLP. Scale bar, 1 μm



Supplementary Figure S8. Purified recombinant proteins used in Fig. 5. Coomassie and Western blots of CNN and its variants as GST fusion proteins (**a**), as well as recombinant Sas-4-N190 (**b**), and Sas-4-Δ90 (**c**). Sas-4-N190 includes only the N-terminal amino acids up to 190 [2] and Sas-4-Δ90 includes Sas-4's C-terminal amino acids beginning at number 90. The CNN variants include CNN's C-terminal (amino acids 517-1048) or N-terminal (17-558) half.



Supplementary Figure S9. Cell-free binding experiments. (**a**) Experimental design: Sas-4-PCM complexes from embryonic HSL are precipitated with beads coated with the anti-Sas-4 antibody and were eluted using recombinant Sas-4-N190 (Fig. S8A). Binding of purified Sas-4-PCM complexes, Sas-4-N190, or Sas-4-Δ90 to centrosomes or stripped-centrosomes was assayed by discontinuous sucrose gradient. (**b**) Purified Sas-4-N190 binds to centrosomes. Therefore, Sas-4-N190 cannot operate in tethering the initial layer of PCM to a centriole but it is capable of adding additional PCM to a centrosome that already contains seeding PCM.