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Supplemental Information

Plk1/Polo Phosphorylates Sas-4 at the Onset of Mitosis for an Efficient Recruitment of Pericentriolar Material to Centrosomes

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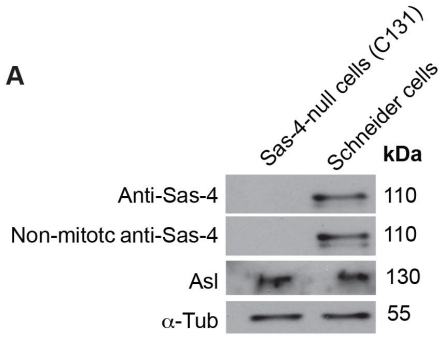
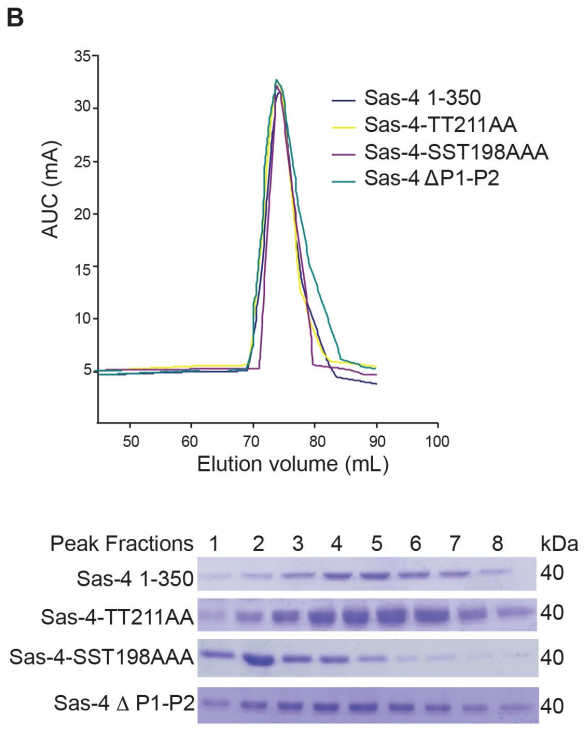
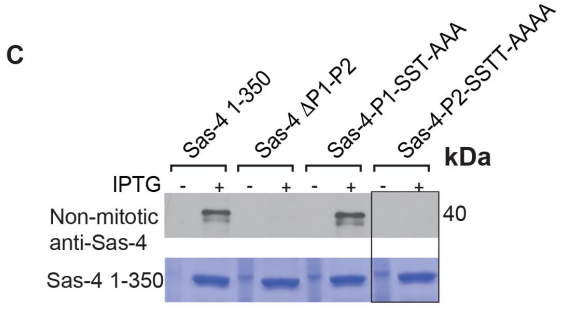
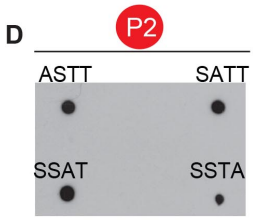
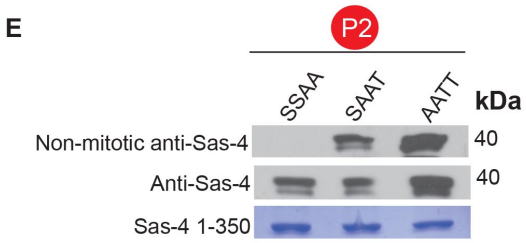
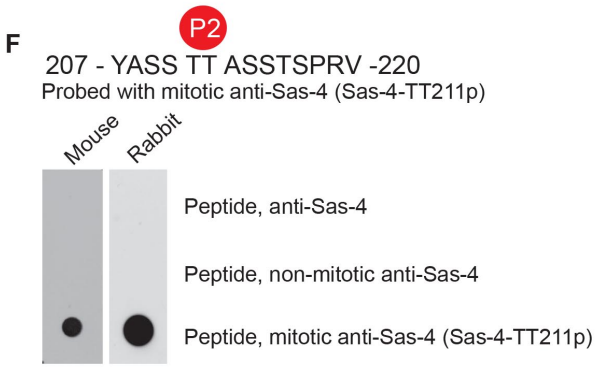

A
Sas-4-null cells (C131)
Schneider cells
kDa
Anti-Sas-4
110
Non-mitotc anti-Sas-4
110
Asl
130
α-Tub
55

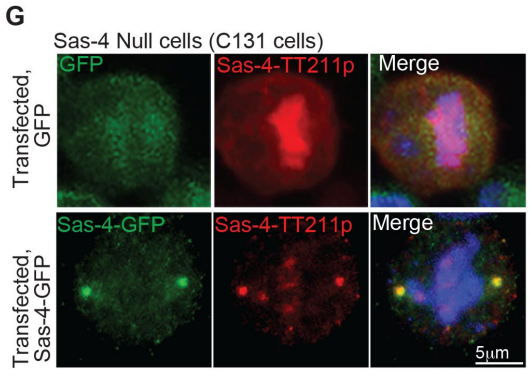

B
AUC (mA)
Elution volume (mL)
Sas-4 1-350
Sas-4-TT211AA
Sas-4-SST198AAA
Sas-4 ΔP1-P2
Peak Fractions 1 2 3 4 5 6 7 8
kDa
Sas-4 1-350
Sas-4-TT211AA
Sas-4-SST198AAA
Sas-4 Δ P1-P2
40


C
Sas-4 1-350
Sas-4 ΔP1-P2
Sas-4-P1-SST-AAA
Sas-4-P2-SSTT-AAAA
kDa
IPTG
Non-mitotic anti-Sas-4
Sas-4 1-350
40


D
P2
ASTT
SATT
SSAT
SSTA


E
P2
SSAA
SAAT
AATT
kDa
Non-mitotic anti-Sas-4
40
Anti-Sas-4
40
Sas-4 1-350


F
P2
207 - YASS TT ASSTSPRV -220
Probed with mitotic anti-Sas-4 (Sas-4-TT211p)
Mouse
Rabbit
Peptide, anti-Sas-4
Peptide, non-mitotic anti-Sas-4
Peptide, mitotic anti-Sas-4 (Sas-4-TT211p)


G
Sas-4 Null cells (C131 cells)
GFP
Sas-4-TT211p
Merge
Transfected, GFP
Sas-4-GFP
Sas-4-TT211p
Merge
Transfected, Sas-4-GFP
5μm

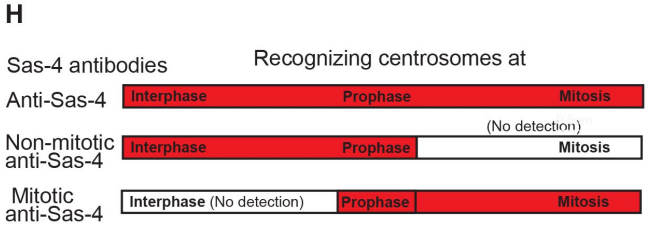

H
Sas-4 antibodies
Recognizing centrosomes at
Anti-Sas-4
Interphase
Prophase
Mitosis
Non-mitotic anti-Sas-4
(No detection)
Interphase
Prophase
Mitosis
Mitotic anti-Sas-4
Interphase (No detection)
Prophase
Mitosis

Figure S1. *In vitro* validation of anti-Sas-4, non-mitotic anti-Sas-4 and Sas-4-TT211p (mitotic specific Sas-4) antibody. Related to figures 1, 2 and 5.

(**A**) Western bot analysis to validate the Sas-4 antibodies using Schneider cells and Schneider cells lacking Sas-4 protein (C131 cells). The anti-Sas-4 antibody and the non-mitotic anti-Sas-4 antibody recognize Sas-4 only in the Schneider cells and not in the C131 cells. Tubulin and Asterless (Asl) are used as loading controls.
(**B**) Fast protein liquid chromatography (FPLC) analysis of purified Sas-4 1-350 variants. All of the variants elute at similar elution volume and have a similar area under the curve (AUC). Coomassie staining of peak fractions (fractions 1 to 8) is shown below.
(C) The non-mitotic anti-Sas-4 is specific for -P2 site. Coomassie blue staining of recombinant Sas-4 1-350 is given at the bottom panel. Plus sign (+) denotes IPTG induction of recombinant protein expression. Minus sign (-) denotes un-induced loading control.(**D**) Dot-blot shows that the non-mitotic anti-Sas-4 is able to recognize Sas-4 peptides (207-220aa) with single amino acid replacements at the -P2 site.
(**E**) The non-mitotic anti-Sas-4 is specific for Sas-4-TT211 as it fails to recognize double amino acid replacements at the -P2 site. Note in contrast to the non-mitotic anti-Sas-4, anti-Sas-4 recognizes all forms of the protein. Coomassie blue staining of recombinant Sas-4 1-350 is given at the bottom panel.
(**F**) Generation of mouse monoclonal and rabbit polyclonal anti-Sas-4 antibodies (Sas-4-TT211p) raised against phosphorylated peptide. This phosphorylated peptide (207-YASSpTpTASSTSPRV-220) is a specific substrate only for the mitotic anti-Sas-4 (Sas-4-TT211p).
(**G**) Schneider C131 cells (lacking Sas-4 protein) expressing –GFP (green, control) or Sas-4-GFP (green). The Sas-4-TT211p antibody (red) labels Sas-4 at mitotic centrosomes only in cells expressing Sas-4-GFP, showing that it is specific for Sas-4. Sas-4-TT211p seems to non-specifically label the DNA. Data not shown for Sas-4-TT211p raised in mouse.
(**H**) Schematic representation of the three different Sas-4 antibodies to compare the localization of Sas-4 at interphase and mitosis. The anti-Sas-4 antibody that recognizes centrosomal Sas-4 at interphase, prophase and metaphase (First bar). The non-mitotic anti-Sas-4 antibody recognizes Sas-4 only at the interphase and prophase (Middle bar); however, the mitotic anti-Sas-4 (Sas-4-TT211p) antibody recognizes Sas-4 only at pro-metaphase and metaphase of the cell cycle (Bottom bar).

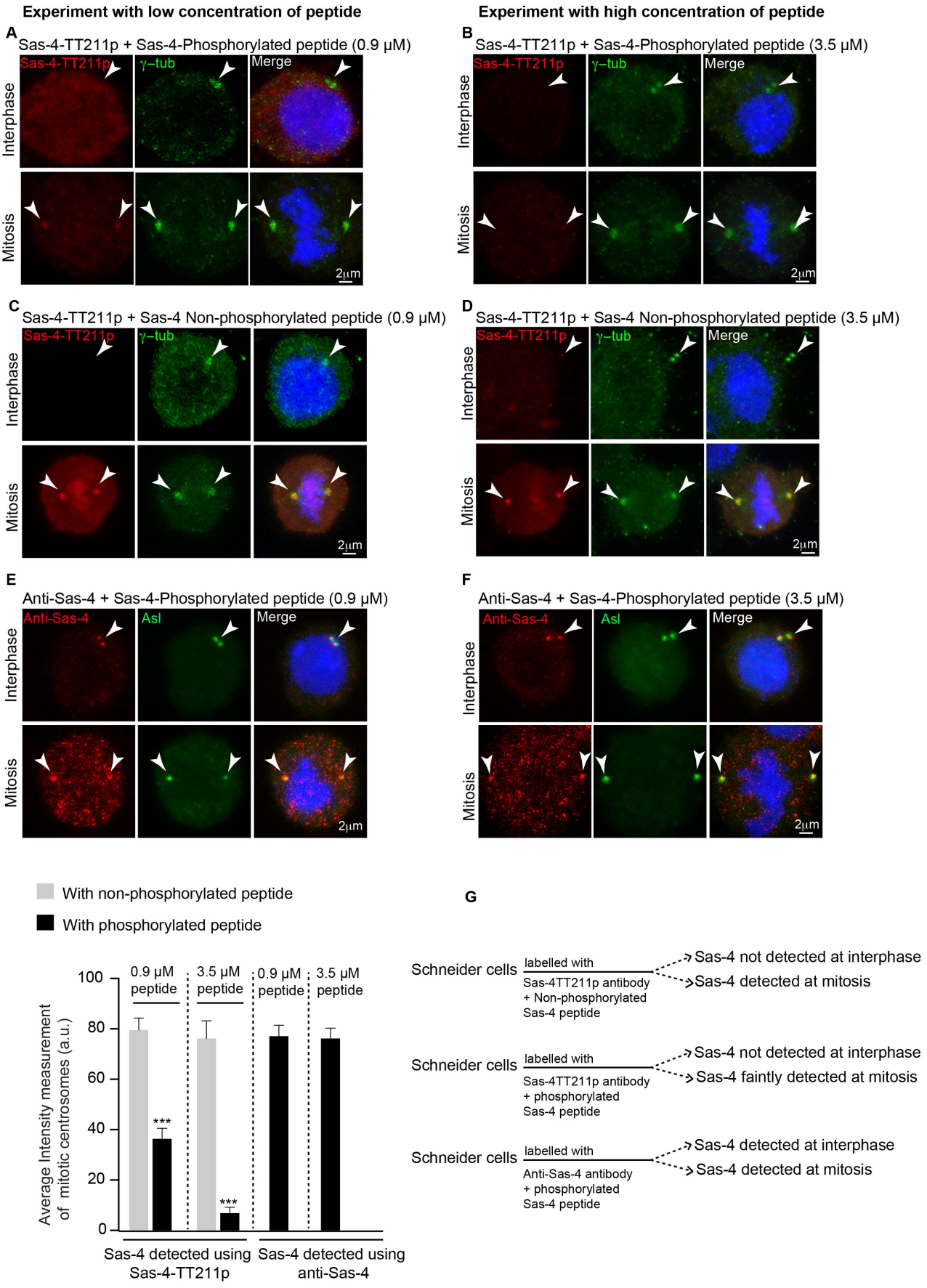


Figure S2. Sas-4 peptide-based competition experiment in Schneider cells. Related to Figures 2 and 5.

(**A-D**) Characterization of Sas-4-TT211p (red, mitotic-specific Sas-4 antibody) in the presence of increasing concentrations of Sas-4 phosphorylated peptide (207-YASS**TT**ASSTSPRV-220, 0.9 µM and 3.5 µM) or Sas-4 non-

phosphorylated peptide (207-YASSTTASSTSPRV-220, 0.9 μM and 3.5 μM). In the presence of higher concentration of Sas-4 phosphorylated peptide, Sas-4-TT211p failed to recognize mitotic Sas-4 (**A-B**). γ-tubulin (green) labels centrosomes. In contrast, Sas-4-TT211p normally labels Sas-4 at mitotic centrosomes in the presence of Sas-4 non-phosphorylated peptide (**C-D**). In either case, Sas-4-TT211p did not recognize Sas-4 at centrosomes of interphase cells. (**E-F**) Anti-Sas-4 antibody (red, whose epitope differs from Sas-4-TT211p) used as a control, recognizes Sas-4 at both interphase and mitosis in the presence of increasing concentrations of Sas-4 phosphorylated peptide. Asl (green) labels centrosomes. The arrowheads mark the centrosomes. The bar graph below represents the average intensity measurements of Sas-4 at mitotic centrosomes. At least 60 mitotic centrosomes *(n=60)* were analyzed from three independent experiments. ANOVA, ***$P<0.0001$. Error bars represent mean ± s.e.m.
(**G**) Experimental scheme of peptide-based competition experiment.

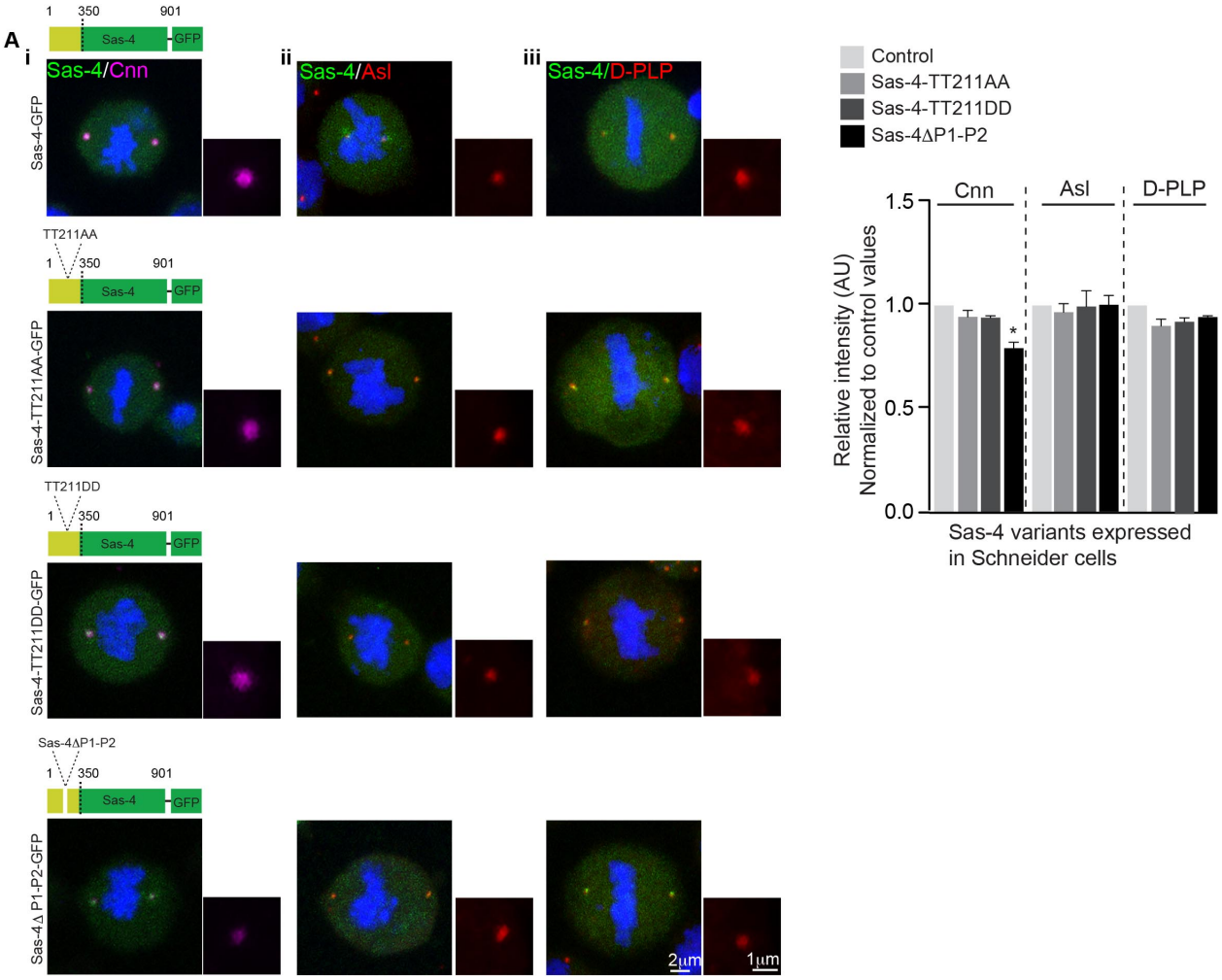


Figure S3. The Sas-4ΔP1-P2 mutation seems to mildly perturb the recruitment of Cnn to mitotic centrosomes in *Drosophila* Schneider cells. Related to figures 3 and 6.

(**A**) Schneider cells expressing different Sas-4 variants as -GFP (green). Sas-4ΔP1-P2 expressing cells showed mild defects in Cnn (**i**, magenta) recruitment at mitotic centrosomes. The bar graph on the right represents the relative intensity of PCM proteins at mitotic centrosomes. At least 40 mitotic centrosomes *(n=40)* were analyzed from three independent experiments. ANOVA, **P*<0.01. Error bars represent mean ± s.e.m.

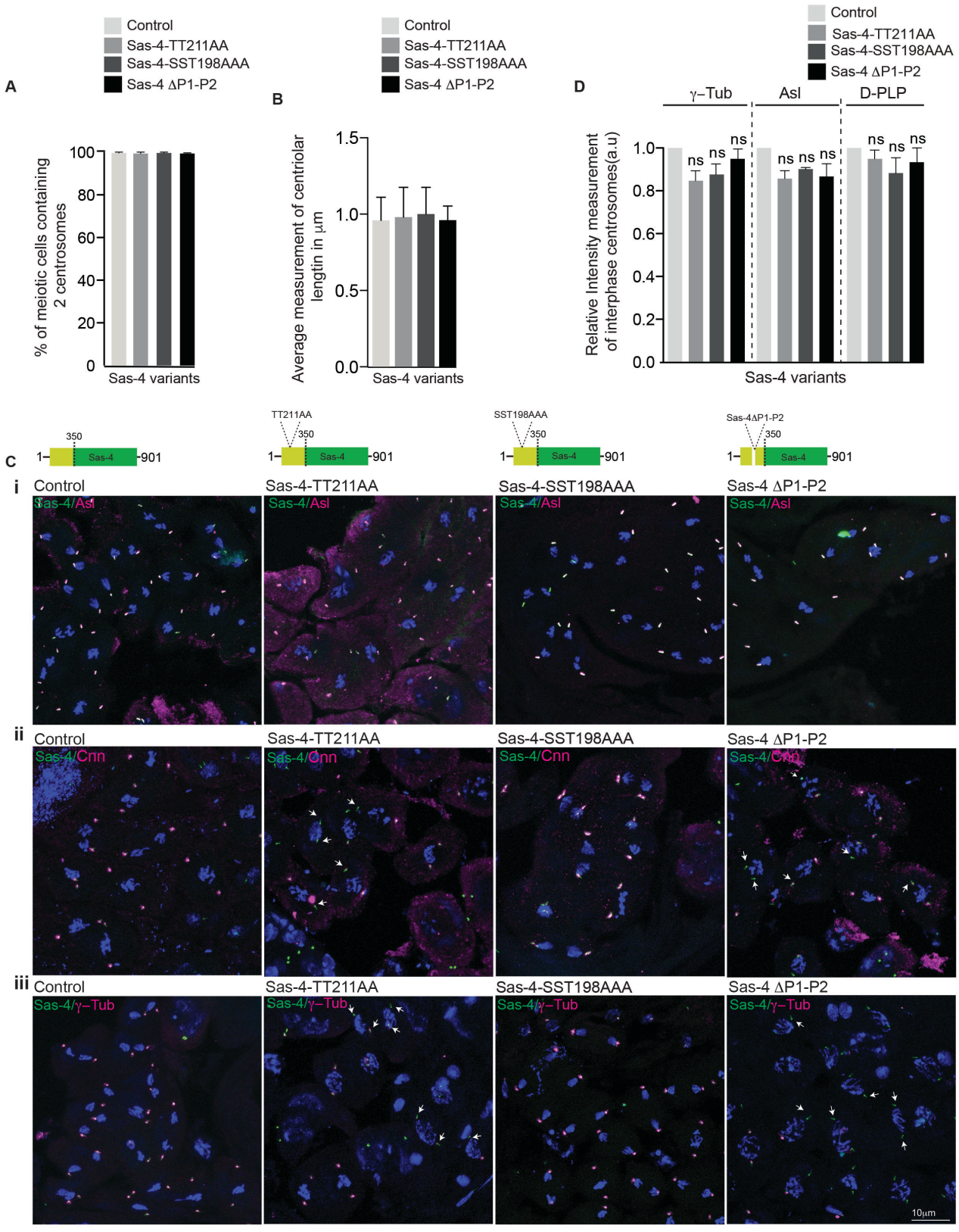


Figure S4. Sas-4 mutants used in this study do not alter centriolar numbers, lengths but perturb an efficient PCM recruitment. Related to figures 4 and 6.

(**A**) None of the Sas-4 variants display aberrant numbers of centrioles. At least 120 meiotic centrosomes (*n*=120) from testes were analyzed for each condition from three independent experiments. ANOVA. Error bars represent mean ± s.e.m.

(**B**) Flies expressing different Sas-4 variants show similar centriolar lengths. At least 80 meiotic centrosomes (*n*=80) from testes were analyzed for each condition from three independent experiments. ANOVA. Error bars represent mean ± s.e.m.

(**C**) Low magnification immunofluorescence images of meiotic cells of testes dissected from various Sas-4 variants. Note normal recruitment of Asl (magenta) in all of the Sas-4 variants **(i).** Defective recruitment of Cnn **(ii,** magenta**)** and γ-Tub **(iii,** magenta**)** is observed only in Sas-$4^{TT211AA}$ and Sas-$4^{\Delta P1\text{-}P2}$ transgenic flies.

(**D**) Bar graph representing the relative intensities of γ-tubulin, Asl and D-PLP at interphase centrosomes of mature spermatocytes expressing various Sas-4 variants. Note that these protein recruitments are not affected in any of the Sas-4 mutants. At least 60 meiotic centrosomes (*n*=60) from testes were analyzed for each condition from three independent experiments. ANOVA. Error bars represent mean ± s.e.m.

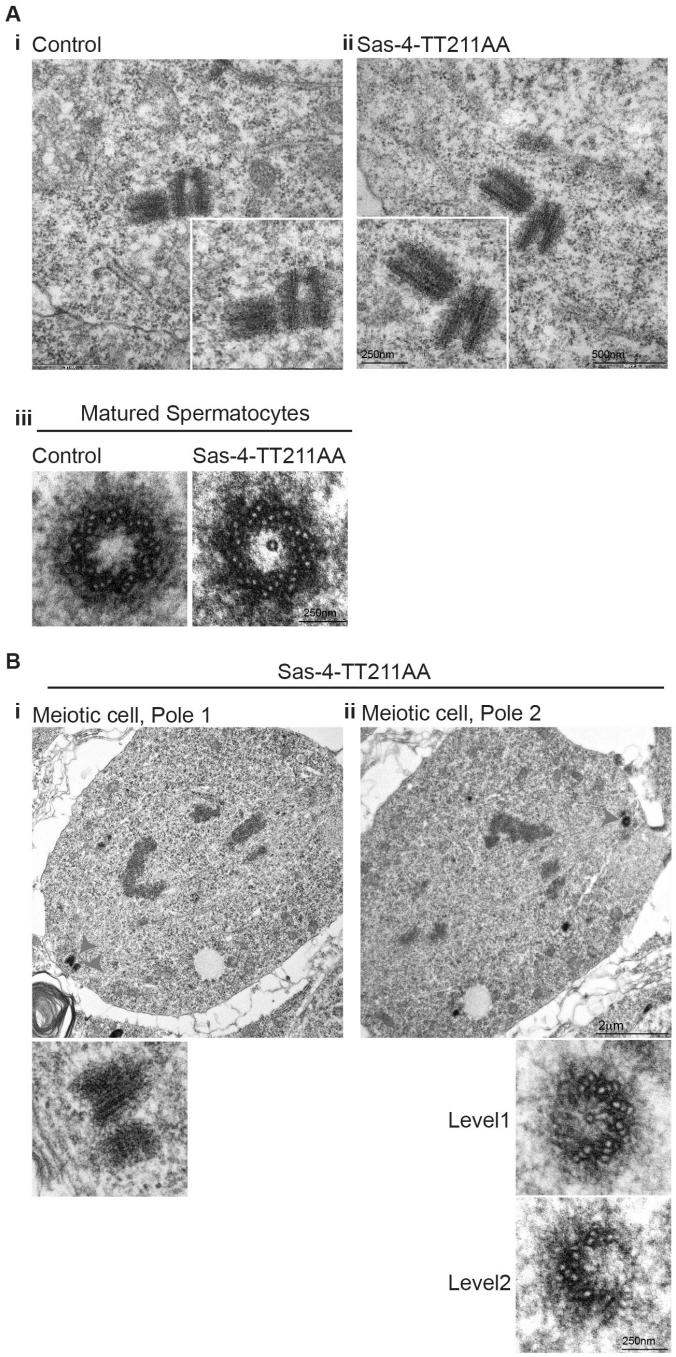


Figure S5. Sas-4^{TT211AA} expressing transgenic flies display structurally normal centrioles. Related to figures 4 and 6.

(**A**) EM micrographs showing normally looking centriole pairs in spermatocytes of control (Sas-4 wild type) **(i)** and Sas-4^{TT211AA} transgenic flies **(ii)**. Cross-sectioned centriolar structures of control (Sas-4 wild type) and Sas-4^{TT211AA} transgenic flies show no structural abnormalities **(iii).**

(B) Similarly, no defects are found in centrosomes of dividing spermatocytes of control (Sas-4 wild type) and Sas-4^{TT211AA} transgenic flies. Serial sectioning EM shows spindle poles (Arrowheads, **i** and **ii**) at two different slices. Cross sections show no defects in the over all structure of centrioles. Level 1 and level 2 are two adjacent sections. Note that the missing part of centriolar structure containing region of the level 1 is imaged at the level 2.

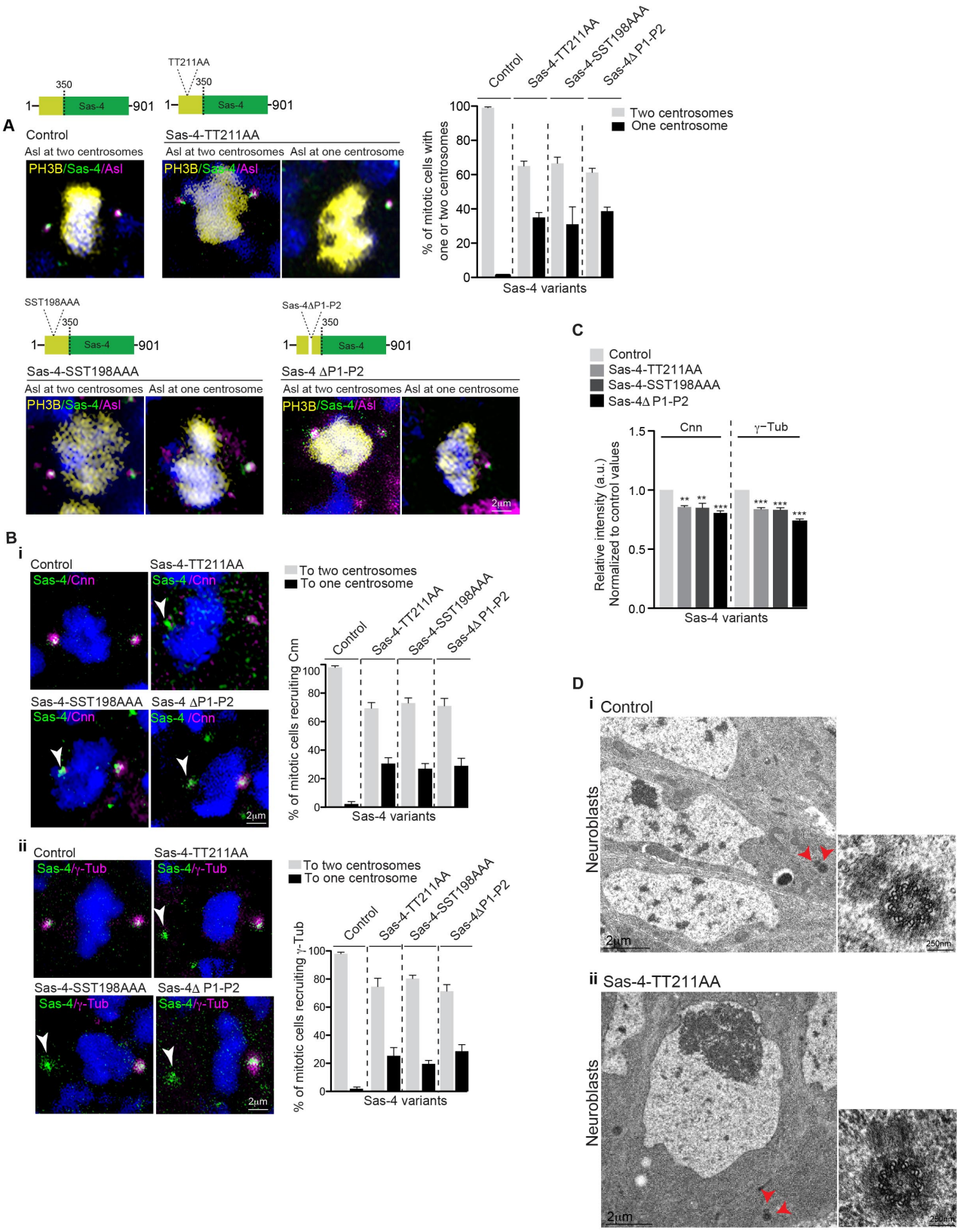


Figure S6. Sas-4-TT211 is required for an efficient recruitment of Cnn and γ-tubulin to the mitotic centrosomes of larval brain cells. Related to figures 6 and 7.

(**A**) Distribution of one and two-centrosome containing cells in various Sas-4 mutants as judged by the presence of Asl and Sas-4. Note Asl (magenta) recruitment appears to be unaffected. Anti-phospho-Histone H3 antibody (PH3B,

yellow) was used to identify mitotic cells. The bar graphs at right represent the percentage of mitotic cells recruiting Asl to either one or two centrosomes. At least 100 mitotic centrosomes (*n*=100) were analyzed for each condition from three independent experiments. ANOVA. Error bars represent mean ± s.e.m. Schematics representing the different Sas-4 mutations have been shown at the top for clarity.

(B) Reduced recruitment of Cnn (**i,** magenta) and γ-tubulin (**ii**, magenta) in two-centrosome containing mitotic cells expressing Sas-4^{TT211AA}, Sas-4^{SST198AAA} and Sas-4$^{\Delta P1\text{-}P2}$. The bar diagrams represent the percentage of mitotic cells recruiting Cnn and γ-tubulin to either one centrosome or both of the centrosomes. At least 80 mitotic centrosomes *(n=80)* were analyzed in each condition from three independent experiments. ANOVA. Error bars represent mean ± s.e.m.

(C) Bar graph representing the relative intensities of Cnn and γ-tubulin at mitotic centrosomes of larval neuroblasts expressing different Sas-4 mutations. Both Cnn and γ-tubulin recruitment is perturbed in flies expressing the different Sas-4 mutations. At least 80 mitotic centrosomes (*n*=80) from neuroblasts were analyzed for each condition from three independent experiments. ANOVA. ****P*<0.0001, ***P*<0.001. Error bars represent mean ± s.e.m.

(D) EM micrographs showing normally looking centriole pairs in interphase cells of larval neuroblasts of control (Sas-4 wild type) **(i)** and Sas-4^{TT211AA} transgenic flies, red arrow heads **(ii)**. Cross-sectioned centriolar structures of control (Sas-4 wild type) and Sas-4^{TT211AA} transgenic flies shows no structural abnormalities**.**