## Materials and Methods

### Protein expression and purification

The TCP domain (residues 710-897) of *D. melanogaster* Sas-4 was cloned into the pET-28b (Novagen) vector containing an N-terminal 6*His-tag. The protein was over-expressed in *E. coli* BL21(DE3) (Novagen) host cells induced by 0.4 mM IPTG overnight at 25 °C in LB medium. After cell lysis and centrifugation, the protein was affinity purified from supernatant using a HisTrap column (GE Healthcare). Notably, in order to prevent TCP precipitation, 1 M imidazole was added to samples shortly after elution. The protein was finally polished by a HiLoad 26/60 Superdex 75 column (GE Healthcare) using elution buffer containing 20 mM Tris-HCl, pH 8.5, 400 mM KCl, 1 M imidazole and 2 mM DTT. Selenomethionine(SeMet)-labeled TCP protein was prepared through the strategy of methionine metabolism pathway inhibition with M9 as the growth medium. The sample was expressed and purified essentially the same as the wild type protein.

Both native and SeMet-labeled fly TCP samples used for crystallization were concentrated to ~25 mg/ml under buffer: 20 mM Tris-HCl, pH 8.5, 400 mM KCl, 1 M imidazole and 2 mM DTT. Concentrated samples were aliquoted and stored at -80$^{o}$C for future use.

For GST-pull down assays, full length TCP domain of wild type and TCP variants were cloned into pGEX vector (Amersham biosciences) and expressed as a GST-fusion protein. For gel filtration assays, the GST tag was cleaved before size exclusion analyses using Superdex 75 columns.

### Crystallization, data Collection and structure determination

Crystallization was performed using the sitting-drop vapor diffusion method by mixing 1 µl protein with 1 µl crystallization solution at 18 °C. Due to the high salt/imidazole content of the protein sample, the reservoir solution was prepared by mixing 75% crystallization solution and 25% 5 M imidazole-HCl, pH 8.0. Crystals usually appeared after 3-5 days under the crystallization condition of 10% PEG3350 and 0.2M ammonium acetate. For data collection, suitable crystals were flash-frozen in liquid nitrogen under the cryo-protectant condition composed of 80% reservoir solution and 20% glycerol.

Data collection was performed at BL17U at Shanghai Synchrotron Radiation Facility (SSRF). Both native and SeMet datasets were collected at the wavelength of 0.9792 Å. The data were processed with HKL2000 (http://www.hkl-xray.com), and

the phase was determined by the program PHENIX (43) based on the selenium single-wavelength anomalous dispersion method. Model building and refinement were formed with the programs COOT(1) and PHENIX, respectively. Data collection and structural refinement statistics are summarized in table 1.

**Yeast two-hybrid assays**

Yeast two-hybrid interaction assays were performed using the Matchmaker Gold yeast two-hybrid system (Clontech) following the manufacturer's instructions. In brief, full length Ana2 was amplified and introduced into pGBKT7 as prey. Indicated cDNA fragments of SAS4's TCP domain were amplified by PCR and sub-cloned into pGADT7 as baits. Surface point mutants of TCP were generated using the "QuickChange Mutagenesis" strategy (Stratagene). All constructs were verified by sequencing. Both prey and bait plasmids were co-transformed into AH109 yeast strains. After growth selection, positive colonies growing on DDO plates were picked and re-streaked to obtain isolated colonies. The isolated clones were then inoculated into liquid DDO medium for overnight culture at 30 °C. For each clone, ~200 µl yeast cells with a starting $OD_{600nm}$ of 2 were serially diluted in five-fold intervals. About 10 µl of cells for each dilution point were dot-blotted onto QDO plates for growth test.

**Circular Dichroism spectroscopy**

Circular Dichroism spectroscopy was carried out at 25°C using an Applied Photo-physics Pistar π-180 spectropolarimeter with a 1 mm path-length cell and a bandwidth of 4.0 nm. The TCP protein was prepared in PBS buffer (10mM $Na_2HPO_4$, 2mM $KH_2PO_4$, 30mM NaCl, pH 7.0) at a concentration of 0.05 mg/ml. Spectra were recorded from 255 to 195 nm at an interval of 1 nm and were repeated three times. Each spectrum was the average of three scans and all resultant spectra were obtained by subtraction of the spectrum of the buffer. The results were expressed as CD in delta ellipticity ($M^{-1}\cdot cm^{-1}$).

**Transgenic flies and stable cell lines**

Transgenic flies and Sas-4 constructs were previously described but with second chromosome specific pattB-UAST vector (2, 3). P[lacW]l(3)s2214 (*Sas-4*$^{s2214}$) was obtained from the Bloomington Stock Center. All Sas-4 constructs for *in vivo* expression were sub cloned into pUAS vectors in a manner that the cloned proteins were expressed using a Sas-4 promoter rather than overexpressed using the GAL4 system. For Ana-1 imaging experiments, flies were crossed with transgenics expressing Ana-1 as Ana-1-tdT. Ana2 mutant flies for the Ana2 antibody validation is

a kind gift from Dr. Hongyan Wang. GST-TCP was constructed by insertion of a Sas-4 TCP domain into the EcoRI site of pGEX2 (Stratagene).

For BAC recombineering and stable transfection, the BAC RP11-756A22, harboring human CPAP/CENPJ, was obtained from the BACPAC Resources Center (http://bacpac.chori.org). The LAP (EGFP-IRES-Neo)-tagging-cassette was PCR-amplified using primers carrying 50-nt-homology arms to the C-terminus of CPAP. Recombineering and stable transfection of the modified BAC was performed as described previously (4). Briefly, both a plasmid carrying two recombinases and the purified tagging cassette, were introduced into the *E. coli* stain containing the BAC vector using electroporation. Precise incorporation of the tagging cassette was confirmed by PCR and sequencing. DNA of the GFP-tagged BAC was isolated from bacteria using the Nucleobond PC100 kit (Macherey-Nagel, Germany). Subsequently, HeLa Kyoto cells were transfected using Effectene (Qiagen) and cultivated in selection media containing 400 µg/ml geneticin (G418, Invitrogen). Finally, the pools of HeLa cells stably expressing wild type or mutated CPAP-GFP were analyzed by western blot and immunofluorescence using our anti-CPAP antibodies to verify correct protein size and localization of the tagged transgene.

For siRNA-resistant versions and point mutations, first, a siRNA-resistant version against 5'-CCAAACAACUUCAUUCAUU-3' (Dharmacon siRNA, D-010209-02) of the GFP-tagged CPAP BAC was generated using the BAC counter selection procedure as described previously (5). Next, additional point-mutations (E1235V and K1243A/I1245A, respectively) were inserted by applying a BAC-modification technique called "BAC intronization". Here, point mutations are inserted at any specific site via an artificial intron carrying the desired modification as well as a second resistance marker. Final constructs were validated by sequencing and used to generate stable Hela BAC cell lines. RNAi treatment conditions for the HeLa cells were done as previously described (6).

**Differentiation and characterization of human iPS cell-derived neural progenitor cells**

Feeder-free human iPS cell line (clone hiPSC4) was differentiated to long-term self-renewing neural progenitor cells (NPCs) using a modification of a previously published protocol (7, 8).

Briefly, day 2 embryoid bodies from hiPSC4 were treated for 4 days with 10 µM SB431542 and 5 µM dorsomorphin (Sigma-Aldrich, Taufkirchen, Germany). Rosette structures that were obtained on day 7-10 of differentiation were isolated,

dissociated into single cells with 0.05% Trypsin/EDTA and plated on plates coated with 0.001% poly-ornithine and 1 µg/ml laminin. Cells were cultured in NPC medium composed of DMEM/F12 (Gibco, Life Technologies, Darmstadt, Germany), 1% N2 supplement (Gibco), 0.05x B27 supplement (Gibco), 1.6 g/L glucose (Sigma-Aldrich), 20 µg/ml insulin (Sigma-Aldrich), 20 ng/ml basic fibroblast growth factor (bFGF, Peprotech, Hamburg, Germany) and 20 ng/ml epidermal growth factor (EGF, Sigma-Aldrich) until cells reached confluency. Afterwards, NPCs were expanded and passaged every 4 days with medium change every other day.

To verify the progenitor character of NPCs, cells were stained with antibodies against SOX1 (clone EPR4766, 1:1000, Abcam, Cambridge, UK) and nestin (clone 10C2, 1:200, Millipore, Darmstadt, Germany) and analyzed on an Axiovert fluorescence microscope (Carl-Zeiss, Oberkochen, Germany) equipped with the image processing software Axiovision 4.5. The expression of an additional NPC marker, polysialylated neuronal cell adhesion molecule (PSA-NCAM), was assessed by flow cytometry using specific antibodies (clone 2-2B, 1:100, Millipore, Darmstadt, Germany).

The potential of iPS cell-derived NPCs to differentiate into three major neural cell lineages (neurons, oligodendrocytes and astrocytes) was evaluated by immunocytochemistry. Neuronal differentiation of NPCs was induced by cultivating the cells in bFGF- and EGF-depleted NPC medium for 3-4 weeks prior to immunocytochemical staining with polyclonal TUJ1 (1:1000, Santa Cruz, Heidelberg, Germany) and Map2 antibodies (1:1000, Santa Cruz). Astrocyte differentiation of NPCs was carried out in DMEM/F12 medium supplemented with 10% fetal bovine serum (Gibco) while oligodendrocytes were generated by culturing NPCs in DMEM/F12 medium containing 40 ng/ml 3,3',5-triiodo-L-thyronine (T3, Sigma), 20 ng/ml EGF and 2x B27 supplement. At day 8-14 of differentiation astrocytes were detected with antibodies against glial fibrillary acidic protein (GFAP, clone G-A-5, 1:200, Sigma) and oligodendrocytes were stained on day 21 with antibodies against the marker O4 (1:500, Sigma).

### *Drosophila* embryonic and S2 cell extracts

As described previously *Drosophila* embryonic extracts were prepared from 0-8 hour embryos (9). The extract buffer contained 80 mM K-PIPES, pH 6.8, 1 mM $MgCl_2$, 1 mM $Na_3EGTA$, 14% sucrose, 100 mM KCl, 1 mM PMSF, protease inhibitor cocktail, and EDTA-free Roche complete tablets. A high-speed lysate (HSL) was prepared by centrifuging the extract for 30 minutes at 200,000 g. Similar procedures

were used for *Drosophila* S2 cells. Stable *Drosophila* S2 or C131 cell lines expressing Td-Tomato tagged Sas-4-TCP variants of β9-10 mutants constructs encompassing E792V (Sas-4-TCP-1, patient mutation), V800A/I802A (Sas-4-TCP-2) and L841K (Sas-4-TCP-3) were established by co-transfecting plasmids containing a Sas-4 construct with a hygromycin-resistant plasmid (Invitrogen).

**Immuno-purification of Sas-4 complexes**

As previously described, protein G beads were coated with an anti-Sas-4 antibody overnight at 4°C (2, 3). The antibody-coated beads and *Drosophila* cell extracts were mixed and incubated at 4°C for 4h, twice washed with extract buffer containing 0.1% Triton X-100, and then twice washed with extract buffer. For eluting the complexes, the antibody specific epitope was used as previously described (2).

**Sucrose gradient linear and discontinues velocity sedimentation**

Sucrose was dissolved in extract buffer containing either 100 or 500 mM KCl. Continuous gradients of 15-60% sucrose were generated with a Gradient Master (Biocomp). Discontinuous gradients were manually poured. Centrifugation at 243,000 g for 13 hr at 4°C was performed using an SW-40 rotor for continuous gradient experiments (Beckman Coulter).

**Western blot**

Samples were resolved in 8% or 12% acrylamide gels. Proteins were transferred to nitrocellulose membranes and incubated with primary antibodies overnight at 4°C followed by secondary antibodies at RT for 1h. Super Signal West Pico or Femto Chemilluminescent substrate (Pierce) was used for detection of peroxidase activity. Molecular masses were determined by comparison to molecular standards (Thermo Fisher).

**Monoclonal antibodies**

A cDNA fragment containing amino acids 319-389 of human CPAP was cloned into a pET vector (Invitrogen) and expressed in *E. coli.* The purified proteins were then injected into mice. Hybridomas were prepared and the monoclonal antibody was collected using standard protocols. For Ana2 monoclonal antibodies the previously published peptides were used to immunize the animals (10).

For Western blots and antibody dilution: the monoclonal mouse anti-Sas-4 (1:500), rabbit-Bld-10 (1:3000 T. Megraw), rabbit-Cep135 (1:3000 Tang), Mouse anti-misato (1:5000 Sana Cruz), anti-Cnn (1:5000, C. Kaufman), mouse anti-γ-tubulin (1:5000, Sigma-Aldrich), mouse or rabbit anti-β-tubulin (1:5000, Sigma), rabbit anti-

Asl (1:5000) (11), mouse anti-CPAP (1:50), mouse anti-Ana2 (1:50) and Peroxidase conjugated secondary antibodies were used at 1:5000 (Vector Labs).

For immunofluorescence: rat anti-α-tubulin (1:200, Chemicon), mouse anti-γ-tubulin (1:200), the above-described monoclonal mouse anti-Sas-4 (1:100), mouse anti-Ana2 (1:50), rabbit anti-Asl (1:200), and rabbit anti-CP-190 (1:200), rabbit anti-Cep152 (Nigg, 1:500), mouse anti-PCNT (Abcam 1:200) were used. Secondary antibodies, alexa conjugated goat/donkey anti-mouse or rabbit were used at 1:200 (ImmunoResearch). DAPI (1 µg/ml, Sigma) stained DNA.

**Electron and light microscopy**

*Drosophila* S2 cells were grown on concanavalin A-coated cover slips for 1h at 25°C and then fixed with 2% glutaraldehyde (Electron Microscopy Sciences) and processed for electron microscopy as previously described (11, 12). For light microscopy, dark pupal testes were dissected in PBS and permeabilized with 0.1% Triton X-100 in PBS for 10 min. Blocking was done with 1% BSA, 0.1% Triton X-100 in PBS for 45 min. Antibody labeling was performed for 1h at RT or overnight at 4ºC followed by three washes in PBS. Confocal images were collected using an Olympus Fluoview FV 1000 scanning confocal microscope. Images were processed using Adobe Photoshop.

**Centrosome binding assay**

Sas-4 or Sas-4-TCP variant complexes were prepared as described before. Discontinuous sucrose gradient sedimentation was performed in SW-55 tubes (Beckman Coulter) and a sedimentation assay followed by Western analysis were performed as previously described (3). Bld-10 positive salt-stripped centrosomes were prepared using fly embryonic extracts (Fig. S7) as previously described (3).

**References**


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**Supplementary Figure Legends:**

**Supplementary Figure S1.** Sequence alignment of TCP domains among Sas-4 orthologues from vertebrates to invertebrates. Predicted secondary structure elements of *D. melanogaster* Sas-4 TCP domains are indicated above the alignment. Conserved residues are highlighted by red front. An asterisk marks the residues mutated in this study.

**Supplementary Figure S2.** Testes of Sas-4ΔTCP flies lack detectable centrosomes and Asl is predominantly present in the cytoplasm.

(**A**) In control testes, developing and elongated centrioles are labeled by Asl throughout the testis. The elongated centrioles within the dashed box are enlarged in the inset. In Sas-4ΔTCP flies, Asl positive centrosomes are absent. Fas III labels stem cells niche. Scale bar, 10 μm.

(**B**) Spermatogonium cells of control **(i)** and Sas-4ΔTCP **(ii)** flies. In contrast to controls, Sas-4ΔTCP cells lack centrosomes but centrosomal proteins Sas-4-GFP and Asl (magenta) are predominantly present in cytoplasm. Scale bar, 1 μm.

(**C**) Heterozygotes of control **(i)** and Sas-4ΔTCP **(ii)** spermatogonium cells. In the absence of TCP domain, Sas-4-GFP is present in the cytoplasm and fails to target the endogenous centrosomes efficiently. Sas-4-GFP is also labeled by anti-Sas-4 antibody (red). Endogenous centrosomes are labeled by Asl (magenta) Arrows mark the centrosomes. Scale bar, 1 μm.

**Supplementary Figure S3.** Circular dichroism and mass spectrometry analyses of Sas-4 TCP.

**(A)** Circular dichroism spectrum of Sas4-TCP. A single peak near 215 nM suggests an all-β fold of TCP.

**(B)** MALDI-TOF-MS of Sas4-TCP before (top) and after (bottom) crystallization.

**Supplementary Figure S4.** Crystal packing of TCP monomers.

**(A, B)** TCP β1-15 forms a continuous "head-to-tail" polymer in the crystal. β15 forms an anti-parallel β-sheet with β1' from the adjacent molecule. The β1-15 polymer is shown in both ribbon view **(A)** and electrostatic surface view **(B)**.

**(C)** Overlay of TCP β1-15 polymers for 3-dimensional packing. Note the 3-fold symmetry among different layers of β1-15 polymers.

**Supplementary Figure S5.** 3-dimensional structure-based fold comparison of TCP.

**(A)** Summary of top five hits based on Dali server search. TCP displays the highest structural similarity with an engineered OspA (PDB ID: 2FKJ).

**(B, C)** Structural and sequence alignments between TCP (blue) and the engineered OspA (green). Note that most matches are clustered into the six artificially engineered β-repeats of OspA.

**Supplementary Figure S6. (A)** Sas-4 variants could target endogenous centrosomes in *Drosophila* S2 cells. Control (cells expressing wild type Sas-4) and Sas-4-TCP-3 were normally recruited to endogenous centrosomes **(i** and **iv)**. Sas-4-TCP-1 **(ii)** and Sas-4-TCP-2 **(iii)** variants targeted the endogenous centrosomes faintly, but were also found dispersed within the cytoplasm. Endogenous centrosomes are stained with Asl. Graph at the right represents the quantification. Scale bar, 1 μm.

**(B)** Control and Sas-4-TCP-3 completely restores centrosome formation and recruits Asl in acentrosomal, Sas-4 null *Drosophila* C131 cells. Cells expressing Sas-4-TCP-1 or Sas-4-TCP-2 showed aberrant centrosome formation but induced centrosome-like foci along with cytoplasmic diffusion. The centrosome-like foci induced by TCP-1 and 2 variants recruit only moderate to weak levels of Asl. Graphs at the right represent the quantification and Asl intensity levels. Scale bar, 1 μm.

**(C)** Electron microscopic examination of centrosome structures induced by TCP variants. Scale bar, 200 nm.

**Supplementary Figure S7. (A)** Coomassie blue staining of purified Sas-4 TCP, TCP1- and TCP2-variants respectively.

**(B)** Size exclusion chromatography of Sas-4 TCP, TCP-1 and TCP-2 reveals that they elute same manner *in vitro*. TCP variants are color-coded and calibration standards are given with their apparent molecular weight and elution volume.

**Supplementary Figure S8.** Velocity sedimentation of *Drosophila* embryonic extracts with **(A)** 100 mM or **(B)** 500 mM KCl on a 15-60% sucrose gradient. Centrosomes sink to the bottom of the gradient. At high-salt condition, centrosomes are positive for Bld-10 but not for Sas-4 or Asl indicating that some of the components are stripped in this condition. These Bld-10 positive stripped centrosomes were used for the cell-free binding assay in **Fig. 2D.**

**Supplementary Figure S9.** TCP domain's β9-10 surface is essential for centrosome formation.

**(A)** TCP variants in Sas-4 null (*sas-4*$^{s2214}$) flies show similar levels of Sas-4 protein expression.

**(B)** Whole mount testes staining. In control (expressing full-length wild type Sas-4) and Sas-4-TCP-3 testes, Asl (magenta) labels developing and elongated centrioles throughout the testis. The elongated centrioles within the dashed box are enlarged in the inset. In Sas-4-TCP-1 and 2 testes, Asl was present only in spermatogonia but mostly diffused within cytoplasm (Panel at the upper right). Dotted lines mark the testis boundary. Fas III (green) labels the stem cell niche area. Scale bar, 10 μm.

**(C)** Quantification of centrosome number in control and Sas-4 variants.

**(D)** Ana2-antibody validation by Western blot. Equivalent numbers of control and ana2 null brains were loaded. Ana2 antibody recognizes a protein band at the size of

Ana2 protein that is absent from the ana2 null mutants. Misato was used as a loading control.

**Supplementary Figure S10.** TCP mutant flies do have Bld-10 and Ana-1 positive centrioles

**(A-D)** Spermatogonium and spermatocyte centrosomes of control and Sas-4-TCP-3 normally contain centriolar markers Bld-10 (Magenta) and Ana-1 (Green) that recruit Sas-4 (Green, A and B) and Asl (Magenta, C and D). Whereas, a significant number of Sas-4-TCP-1 and 2 variants cells contain Bld-10 or Ana-1 positive centrioles but fail to recruit Sas-4 or Asl effectively. Insets in **(A)** and **(B)** show centriole positive **(i)** or negative **(ii)** cells in Sas-4-TCP-1 and Sas-4-TCP-1. Ana-1 has been imaged from Ana-1-tdt expressing flies. Scale bars, 1 and 0.5 μm respectively.

**(E)** Quantification of Bld-10/Ana-1 positive centriole containing cells in control and Sas-4 variants.

**Supplementary Figure S11. (A)** Western blot analysis to validate CPAP antibody and RNAi resistant BACs expressing CPAP. siRNA that targets CPAP (Targeting) consistently depletes endogenous CPAP leaving RNAi resistant CPAP-GFP intact. Mock siRNA or non-targeting siRNA has no effect on either of the CPAP variants. These specific signals are detected by CPAP antibody. Increasing amount of proteins loaded.

**(B)** CPAP antibody recognizes centrosomes that are co-labeled with Cep152. RNAi resistant CPAP BACs induces centrosome formations labeled by CPAP and Cep152, whereas RNAi non-resistant CPAP BACs fail to produce centrosomes when the cells are treated with CPAP-targeting siRNA. Arrows point to the centrosomes. Scale bar, 1 μm.

**Supplementary Figure S12. (A)** Schematics of differentiation and characterization of human iPS cell-derived neural progenitor cells

**(B)** Nestin and Sox1 positive neural progenitors (i and ii). Potential of iPS cell-derived NPCs to differentiate into three major “neural cell lineages”, neurons (Tuj-1 positive, iii), astrocytes (GFAP positive, iv) and oligodendrocytes (O4 positive, v). Scale bar 5 μm.

**Supplementary Figure S13.** CPAP TCP-1 variant has a conserved function playing a role in tethering PCM complexes in neural progenitor centrosomes.

**(A)** Validation of RNAi resistant BACs expressing CPAP TCP-1 variant in neural progenitor cells. RNAi resistant CPAP TCP-1 BAC is resistant to CPAP-targeting SiRNA (Targeting). CPAP-targeting SiRNA treatment depletes endogenous CPAP. CPAP non-targeting SiRNA was used as a control. Both low **(i)** and high-exposure **(ii)** Western blots are shown.

**(B)** Quantification of centrosomes in control and TCP1 variants upon treatment of cells with CPAP-targeting siRNA

**(C-E)** Upon treatment with CPAP-targeting siRNA (Targeting), Control (i) but not CPAP TCP-1 restore the centrosome biogenesis that recruits a normal amount of centrosomal proteins (magenta) γ-tubulin, Cep152 and PCNT. CPAP TCP-1 expressing centrosomes recruit only moderate levels of γ-tubulin (n=17), Cep152 (n=16) and PCNT (n=16). Intensity measurements are given at the right. Scale bar, 1 μm**.**

**Supplementary Figure S14.** Model of Sas-4 mediated PCM assembly during centrosome biogenesis.

**(A)** Sas-4 contains a TCP domain (yellow). **(B)** TCP domain of Sas-4 mediates the formation of a ternary complex that includes Sas-4 (blue), Ana2 (magenta), and Bld-10 (orange). **(C)** Sas-4-PCM complex is tethered to centrioles *via* TCP-Ana-2-Bld-10 interaction.

**Supplementary Movie Legends:**

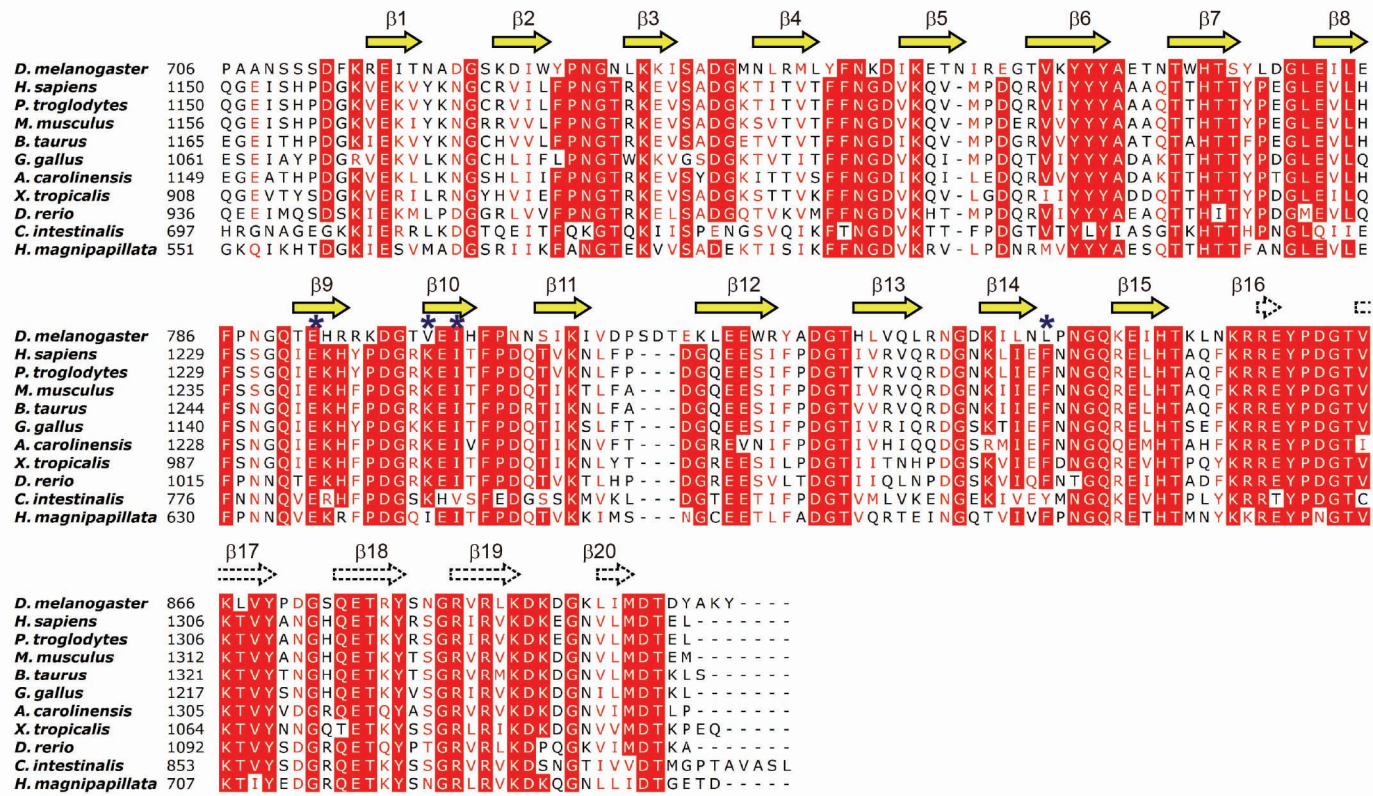
**Video S1**: Movement of Sas-4ΔTCP in *sas-4* null

**Video S2**: Movement of Sas-4-TCP-1 variant in *sas-4* null

**Video S3**: Movement of Sas-4-TCP-2 variant in *sas-4* null

**Video S4**: Movement of Sas-4-TCP-3 variant in *sas-4* null

# Fig. S1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D. melanogaster 706 PAANSSSDFKREITNADGSKDIWYPNGNLKKISADGMNLRMLYFNKDIKETNIREGTVKYYYAETNTWHTSYLDGLEILE
H. sapiens 1150 QGEISHPDGKVEKVYKNGCRVILFPNGTRKEVSADGKTITVTFFNGDVKQV-MPDQRVIYYYAAQTTHTTYPEGLEVLH
P. troglodytes 1150 QGEISHPDGKVEKVYKNGCRVILFPNGTRKEVSADGKTITVTFFNGDVKQV-MPDQRVIYYYAAQTTHTTYPEGLEVLH
M. musculus 1156 QGEISHPDGKVEKIYKNGRRVVLFPNGTRKEVSADGKSVTVTFFNGDVKQV-MPDERVVYYYAEAQTTHTTYPEGLEVLH
B. taurus 1165 EGEITHPDGKIEKVYKNGCHVVLFPNGTRKEVSADGETVTVTFFNGDIKQV-MPDGRVVYYYAATQTAHTTFPEGLEVLH
G. gallus 1061 ESEIAYPDGRVEKVLKNGCHLIFLPNGTWKKVGSDGKTVTITFFNGDVKQI-MPDQTVIYYYADAKTTHTTYPDGLEVLQ
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M. musculus 1312 KTVYANGHQETKYTSGRVRVKDKDGNVLMDTEM-------
B. taurus 1321 KTVYTNGHQETKYTSGRVRMKDKDGNVLMDTKLS------
G. gallus 1217 KTVYSNGHQETKYVSGRIRVKDKDGNILMDTKL-------
A. carolinensis 1305 KTVYVDGRQETQYASGRVRVKDKDGNVIMDTLP-------
X. tropicalis 1064 KTVYNNGQTETKYSSGRLRIKDKDGNVVMDTKPEQ-----
D. rerio 1092 KTVYSDGRQETQYPTGRVRLKDPQGKVIMDTKA-------
C. intestinalis 853 KTVYSDGRQETKYSSGRVRVKDSNGTIVVDTMGPTAVASL
H. magnipapillata 707 KTIYEDGRQETKYSNGRLRVKDKQGNLLIDTGETD-----

Fig. S2

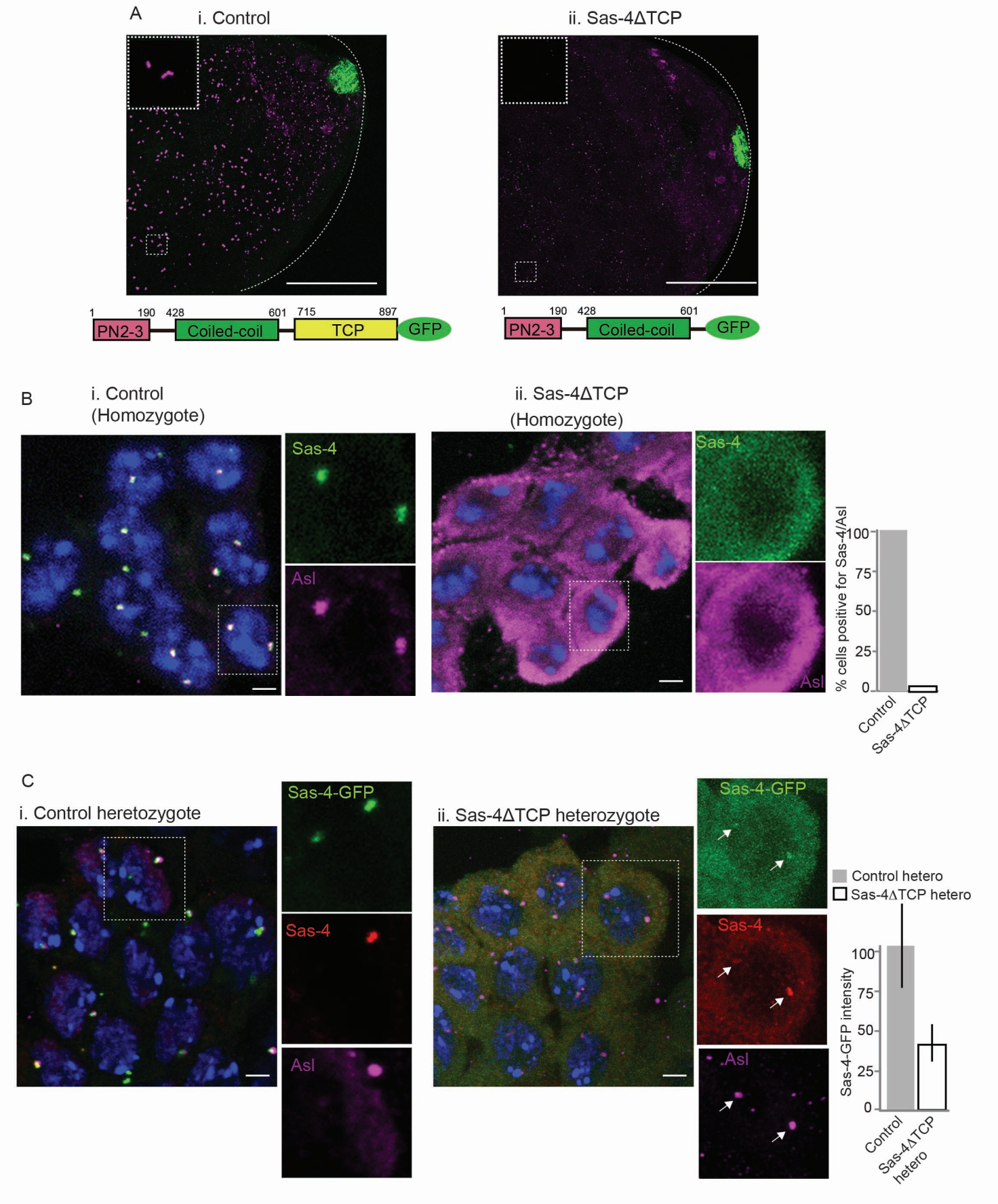
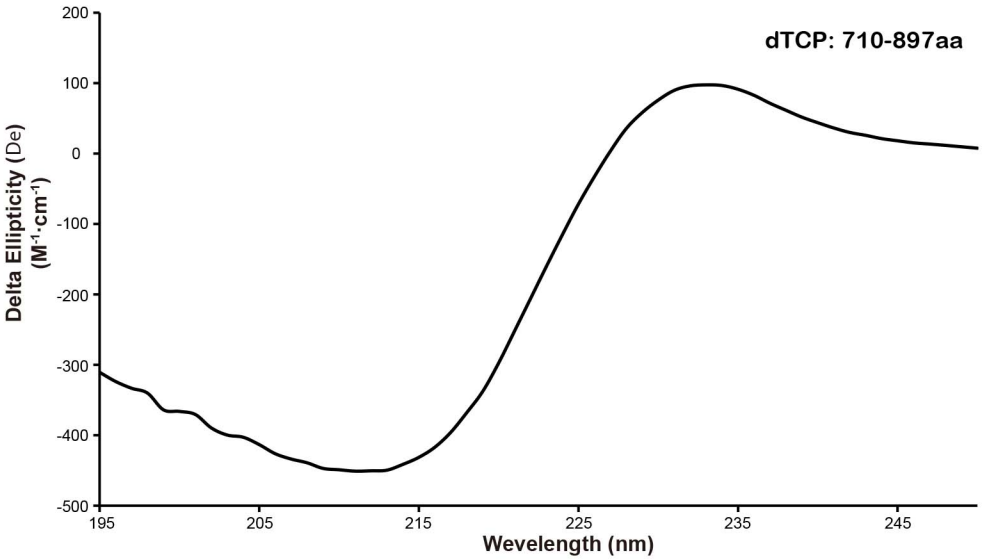

A
i. Control
ii. Sas-4ΔTCP
1
190
428
601
715
897
PN2-3
Coiled-coil
TCP
GFP
B
i. Control
(Homozygote)
ii. Sas-4ΔTCP
(Homozygote)
Sas-4
Asl
% cells positive for Sas-4/Asl
100
75
50
25
0
Control
Sas-4ΔTCP
C
i. Control heretozygote
ii. Sas-4ΔTCP heterozygote
Sas-4-GFP
Sas-4
Asl
Control hetero
Sas-4ΔTCP hetero
Sas-4-GFP intensity
Control
Sas-4ΔTCP hetero

Fig. S3

A


dTCP: 710-897aa
Delta Ellipticity (De)
(M-1·cm-1)
200
100
0
-100
-200
-300
-400
-500
195
205
215
225
235
245
Wevelength (nm)

B

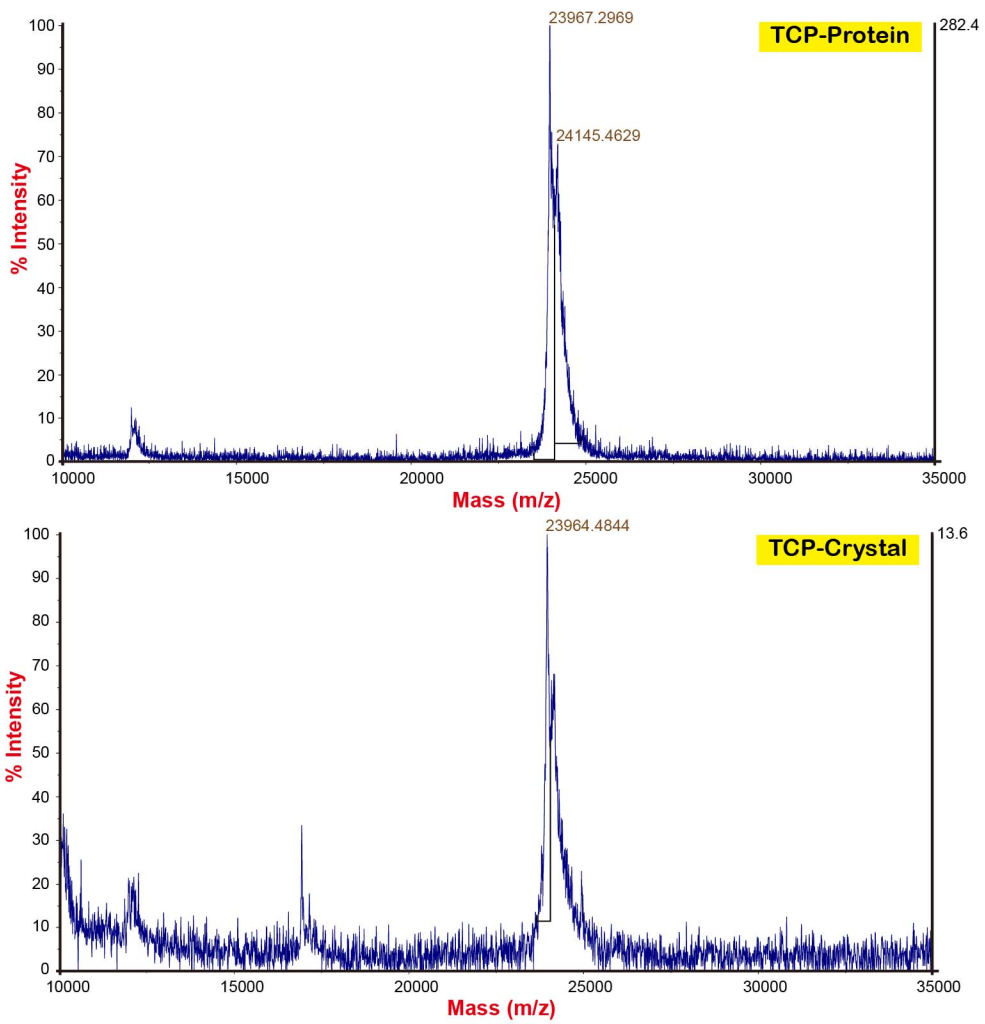

TCP-Protein
282.4
23967.2969
24145.4629
% Intensity
100
90
80
70
60
50
40
30
20
10
0
10000
15000
20000
25000
30000
35000
Mass (m/z)
TCP-Crystal
13.6
23964.4844
% Intensity
Mass (m/z)

Fig. S4

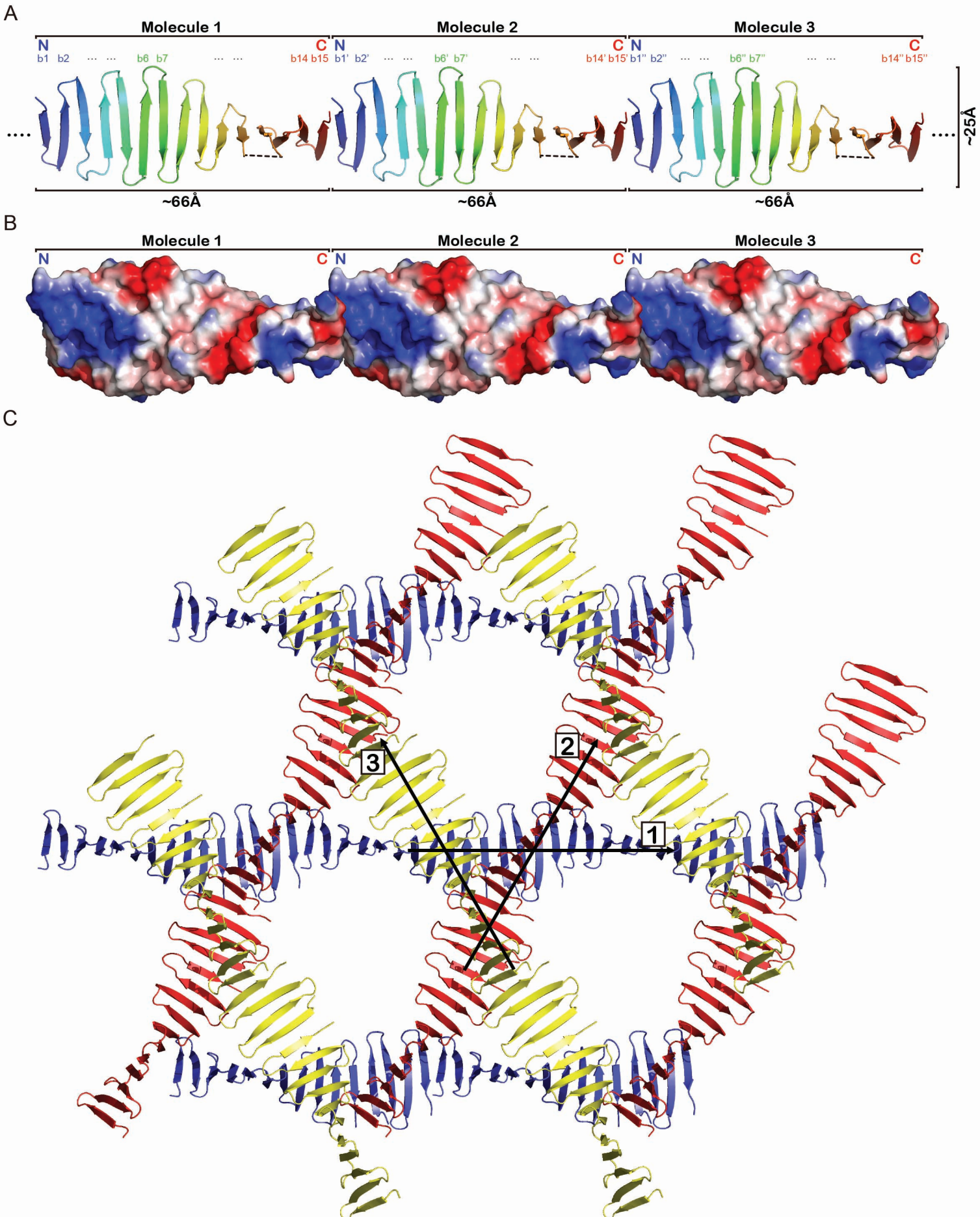
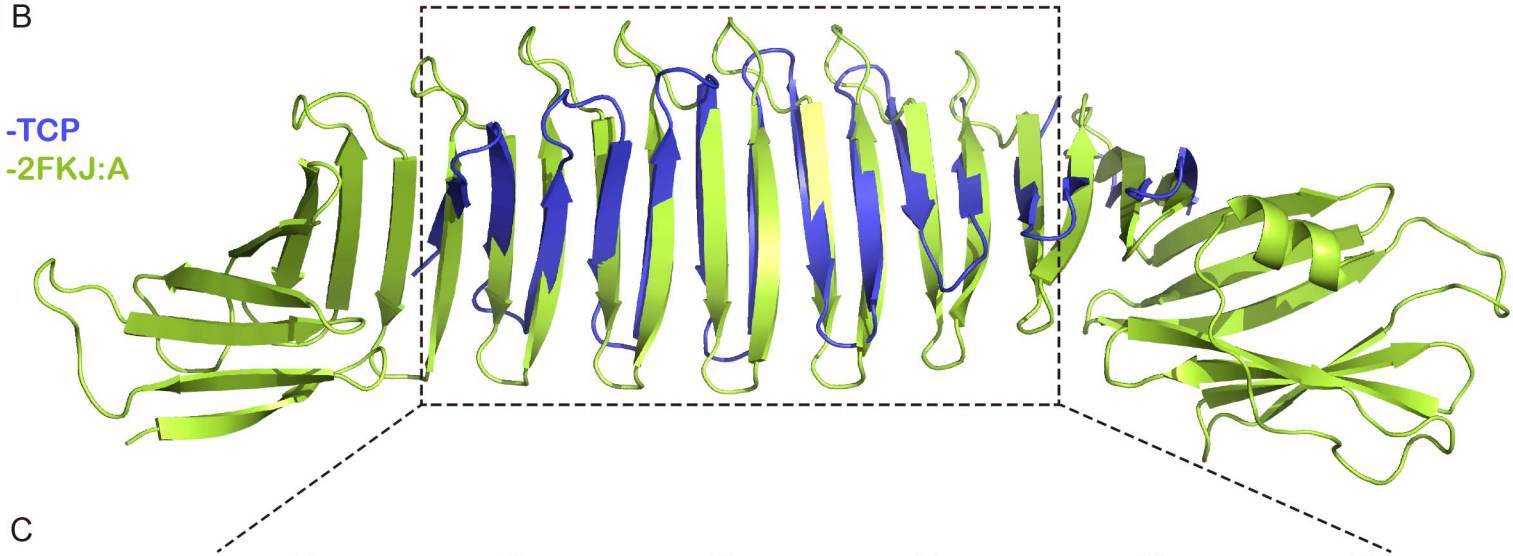

A
Molecule 1
Molecule 2
Molecule 3
N
C
b1 b2 ... ... b6 b7 ... ... b14 b15
b1' b2' ... ... b6' b7' ... ... b14' b15'
b1'' b2'' ... ... b6'' b7'' ... ... b14'' b15''
~66Å
~66Å
~66Å
~25Å
B
Molecule 1
Molecule 2
Molecule 3
N
C
C
1
2
3

Fig. S5

A

| No. | Chain | Z-score | RMSD | $L_{ali}$ | $N_{res}$ | %ID | Description |
|---|---|---|---|---|---|---|---|
| 1 | 2FKJ:A | 12.7 | 3.8 | 134 | 361 | 8 | Outer Surface Protein A |
| 2 | 2W6T:B | 8.4 | 10.6 | 130 | 754 | 3 | Ferripyoverdine Receptor |
| 3 | 3PRN:A | 7.5 | 10.7 | 112 | 289 | 5 | Porin |
| 4 | 1MPR:A | 7.5 | 6.0 | 126 | 421 | 6 | Maltoporin |
| 5 | 3SY9:D | 7.5 | 5.9 | 126 | 361 | 4 | Histidine Porin OPDC |

B




C

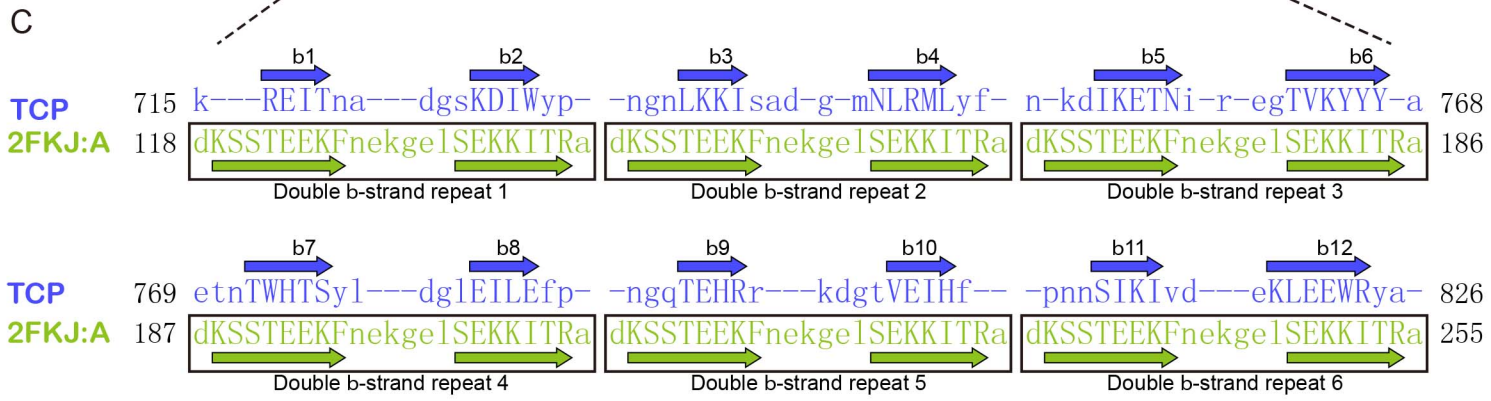


Fig. S6

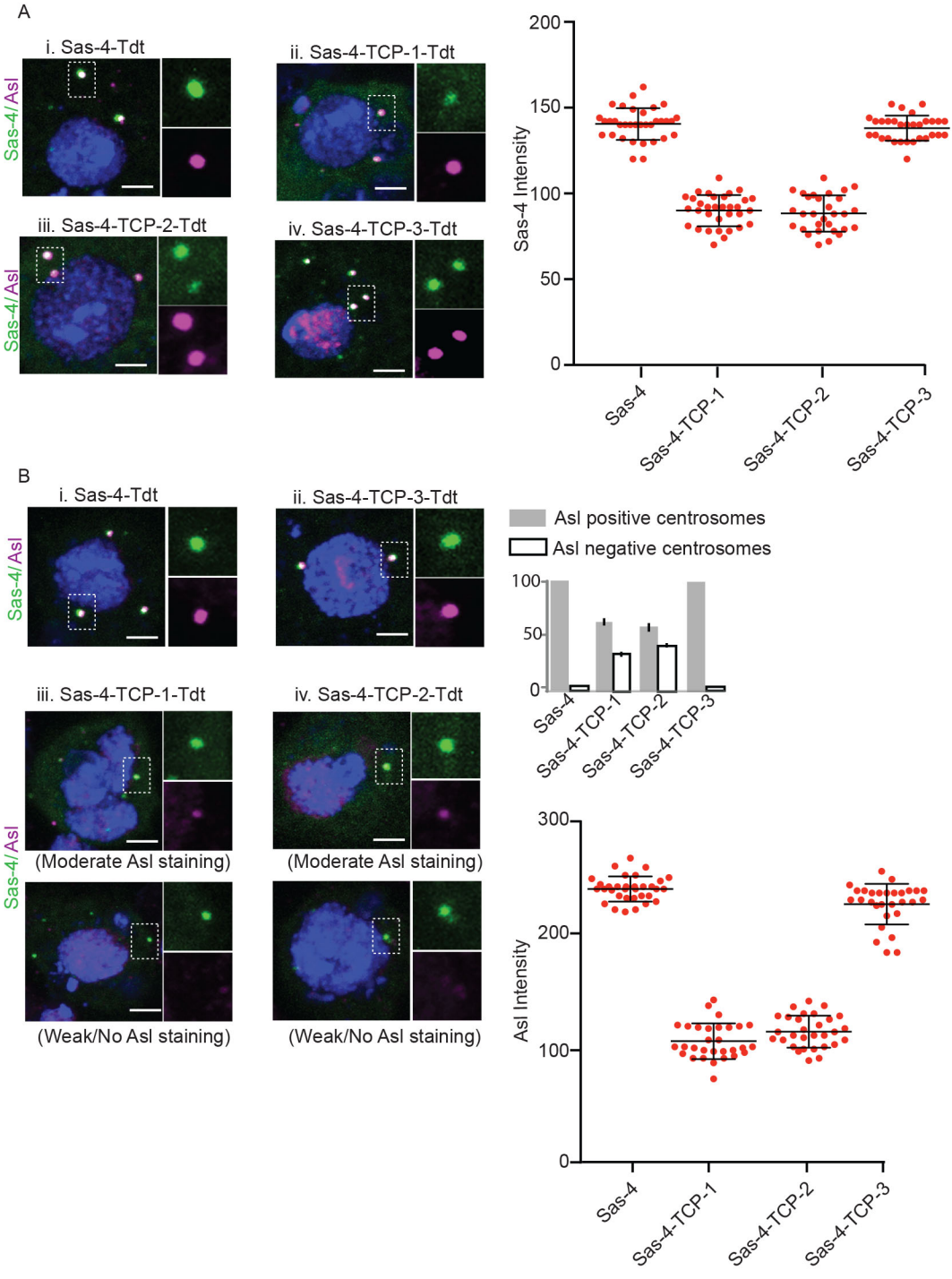
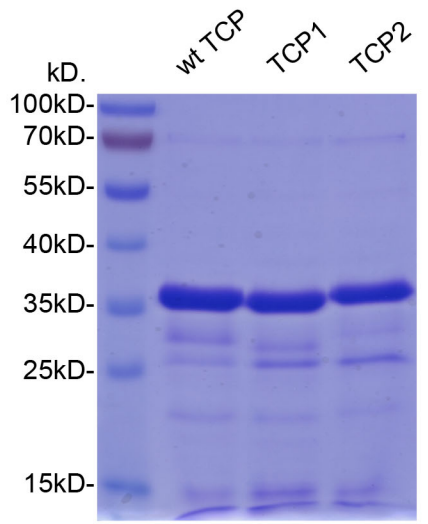

A
i. Sas-4-Tdt
ii. Sas-4-TCP-1-Tdt
iii. Sas-4-TCP-2-Tdt
iv. Sas-4-TCP-3-Tdt
Sas-4/Asl
Sas-4 Intensity
200
150
100
50
0
Sas-4
Sas-4-TCP-1
Sas-4-TCP-2
Sas-4-TCP-3
B
i. Sas-4-Tdt
ii. Sas-4-TCP-3-Tdt
iii. Sas-4-TCP-1-Tdt
iv. Sas-4-TCP-2-Tdt
(Moderate Asl staining)
(Weak/No Asl staining)
Asl positive centrosomes
Asl negative centrosomes
100
50
0
Asl Intensity
300
200
100
C
i. Sas-4
ii. Sas-4-TCP-1
iii. Sas-4-TCP-2

Fig. S7

A


kD.
wt TCP
TCP1
TCP2
100kD-
70kD-
55kD-
40kD-
35kD-
25kD-
15kD-

B

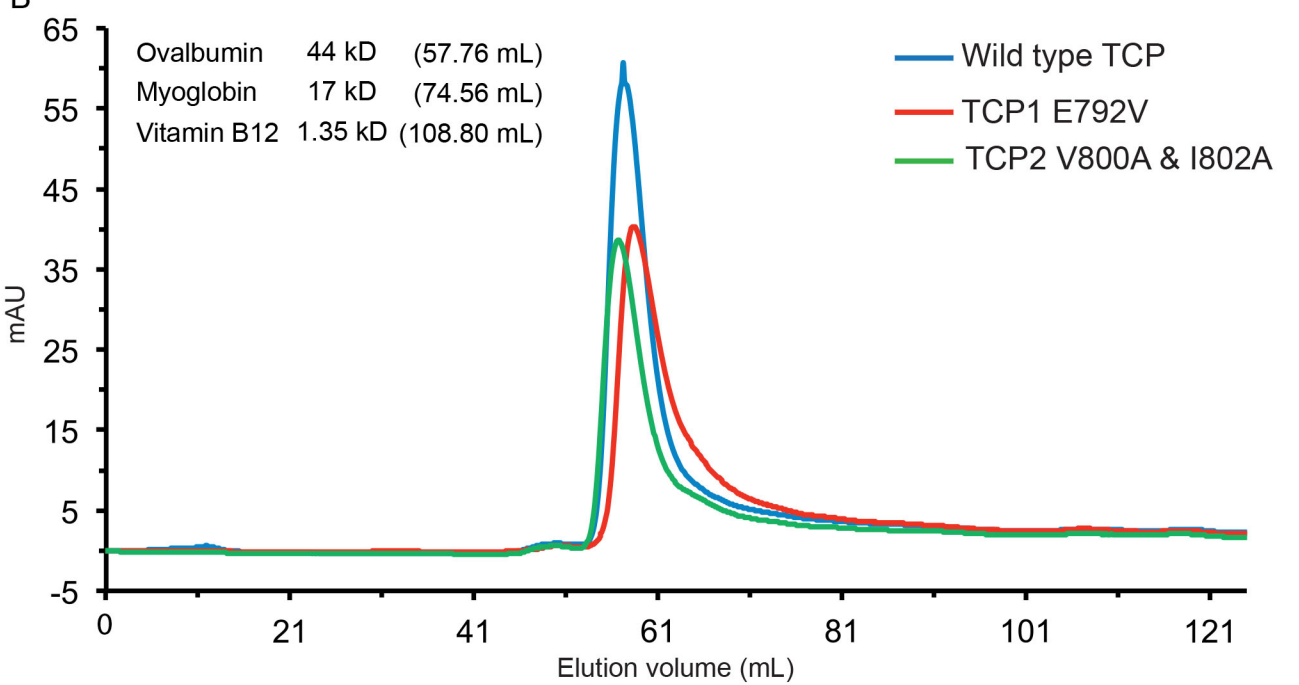

Ovalbumin 44 kD (57.76 mL)
Myoglobin 17 kD (74.56 mL)
Vitamin B12 1.35 kD (108.80 mL)
Wild type TCP
TCP1 E792V
TCP2 V800A & I802A
mAU
65
55
45
35
25
15
5
-5
0
21
41
61
81
101
121
Elution volume (mL)

Fig. S8

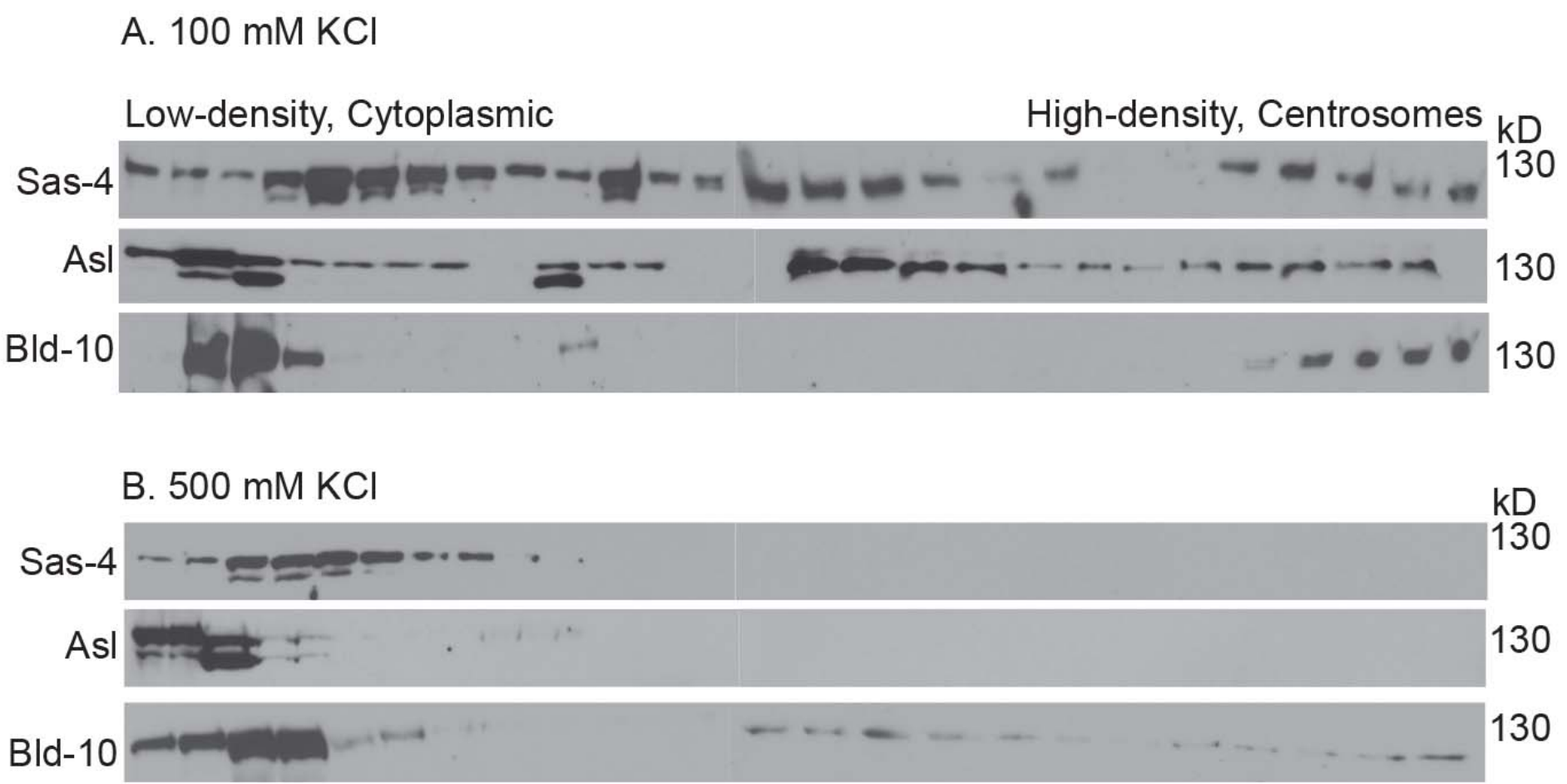

A. 100 mM KCl
Low-density, Cytoplasmic
High-density, Centrosomes
kD
Sas-4
Asl
Bld-10
130
130
130
B. 500 mM KCl
kD
Sas-4
Asl
Bld-10
130
130
130

Fig. S9

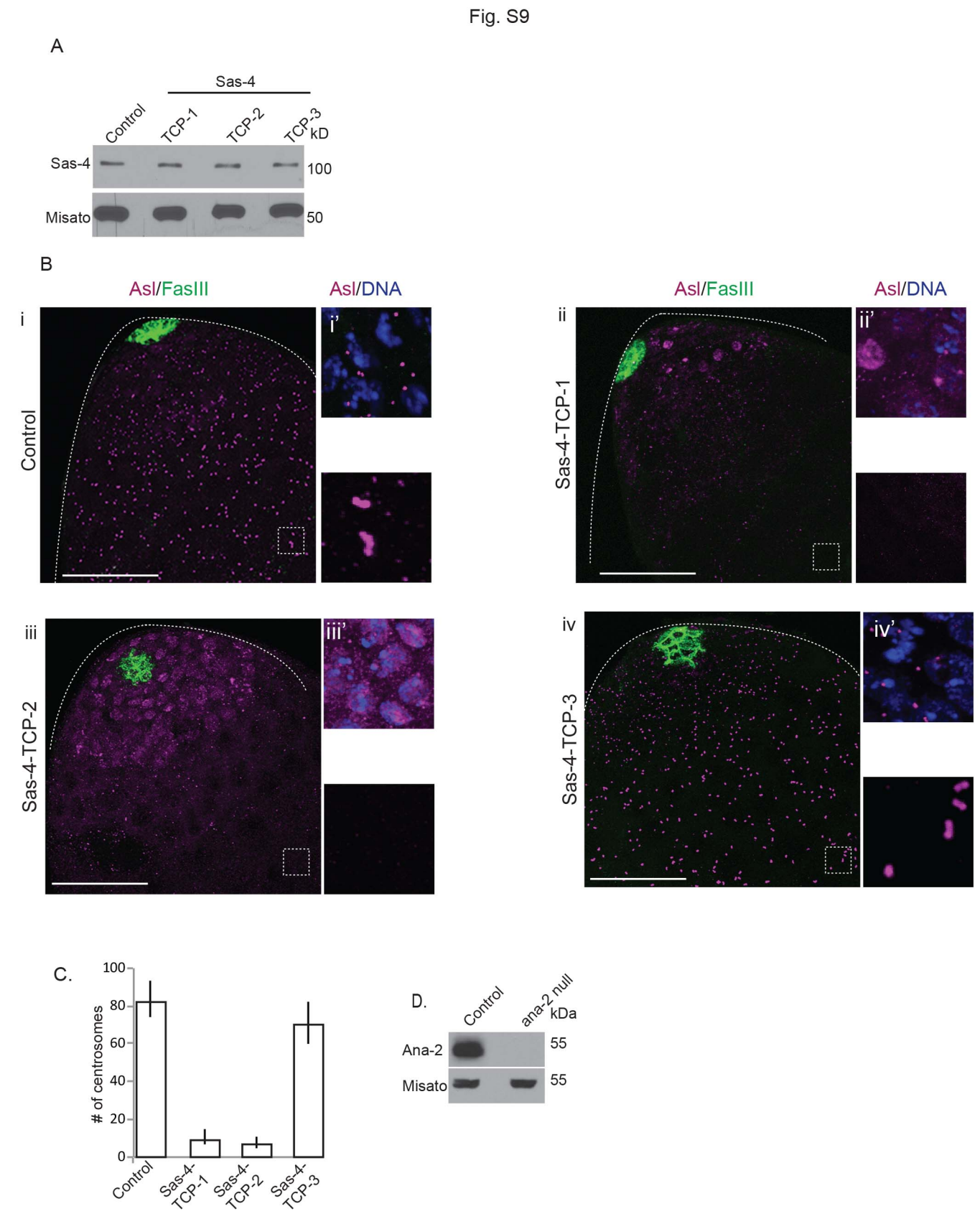

A
Sas-4
Control
TCP-1
TCP-2
TCP-3
kD
Sas-4
100
Misato
50
B
Asl/FasIII
Asl/DNA
i
i'
Control
ii
ii'
Sas-4-TCP-1
iii
iii'
Sas-4-TCP-2
iv
iv'
Sas-4-TCP-3
C.
# of centrosomes
100
80
60
40
20
0
Control
Sas-4-TCP-1
Sas-4-TCP-2
Sas-4-TCP-3
D.
Control
ana-2 null
kDa
Ana-2
55
Misato
55

Fig. S10

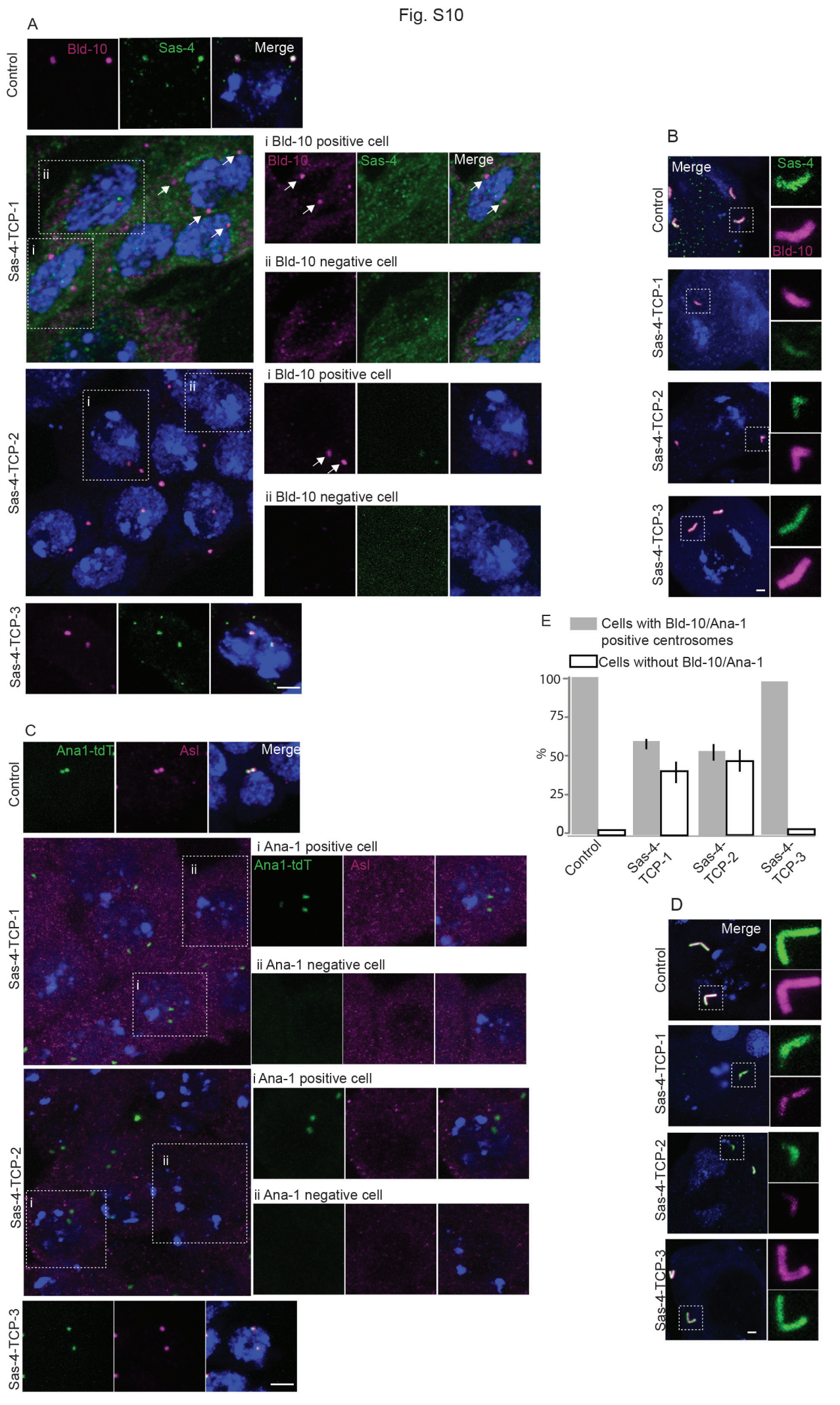
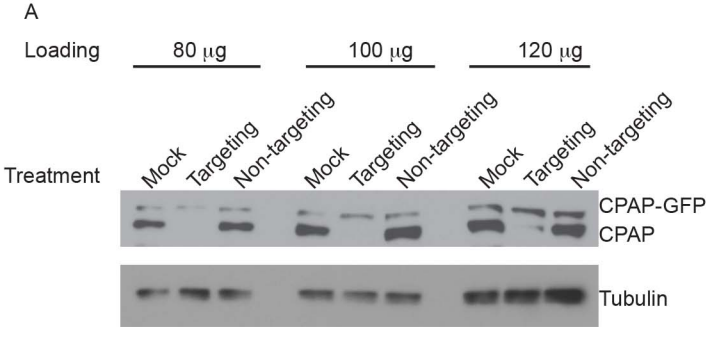

A
Control
Bld-10
Sas-4
Merge
Sas-4-TCP-1
ii
i
i Bld-10 positive cell
ii Bld-10 negative cell
Sas-4-TCP-2
Sas-4-TCP-3
B
Control
Merge
Sas-4
Bld-10
Sas-4-TCP-1
Sas-4-TCP-2
Sas-4-TCP-3
E
Cells with Bld-10/Ana-1 positive centrosomes
Cells without Bld-10/Ana-1
100
75
50
25
0
%
Control
Sas-4-TCP-1
Sas-4-TCP-2
Sas-4-TCP-3
C
Ana1-tdT
Asl
Merge
i Ana-1 positive cell
ii Ana-1 negative cell
D

Fig. S11


A
Loading
80 μg
100 μg
120 μg
Treatment
Mock
Targeting
Non-targeting
Mock
Targeting
Non-targeting
Mock
Targeting
Non-targeting
CPAP-GFP
CPAP
Tubulin

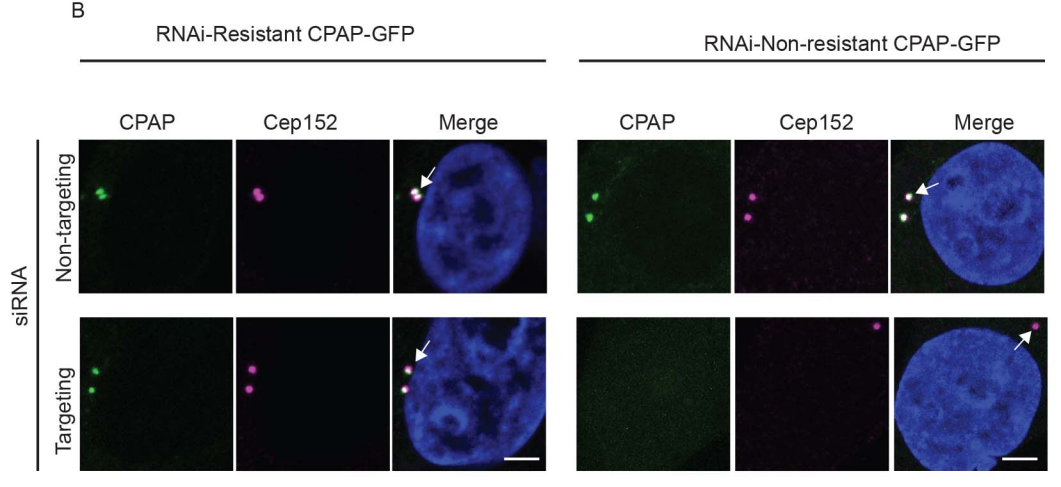

B
RNAi-Resistant CPAP-GFP
RNAi-Non-resistant CPAP-GFP
CPAP
Cep152
Merge
CPAP
Cep152
Merge
siRNA
Non-targeting
Targeting

Fig. S12

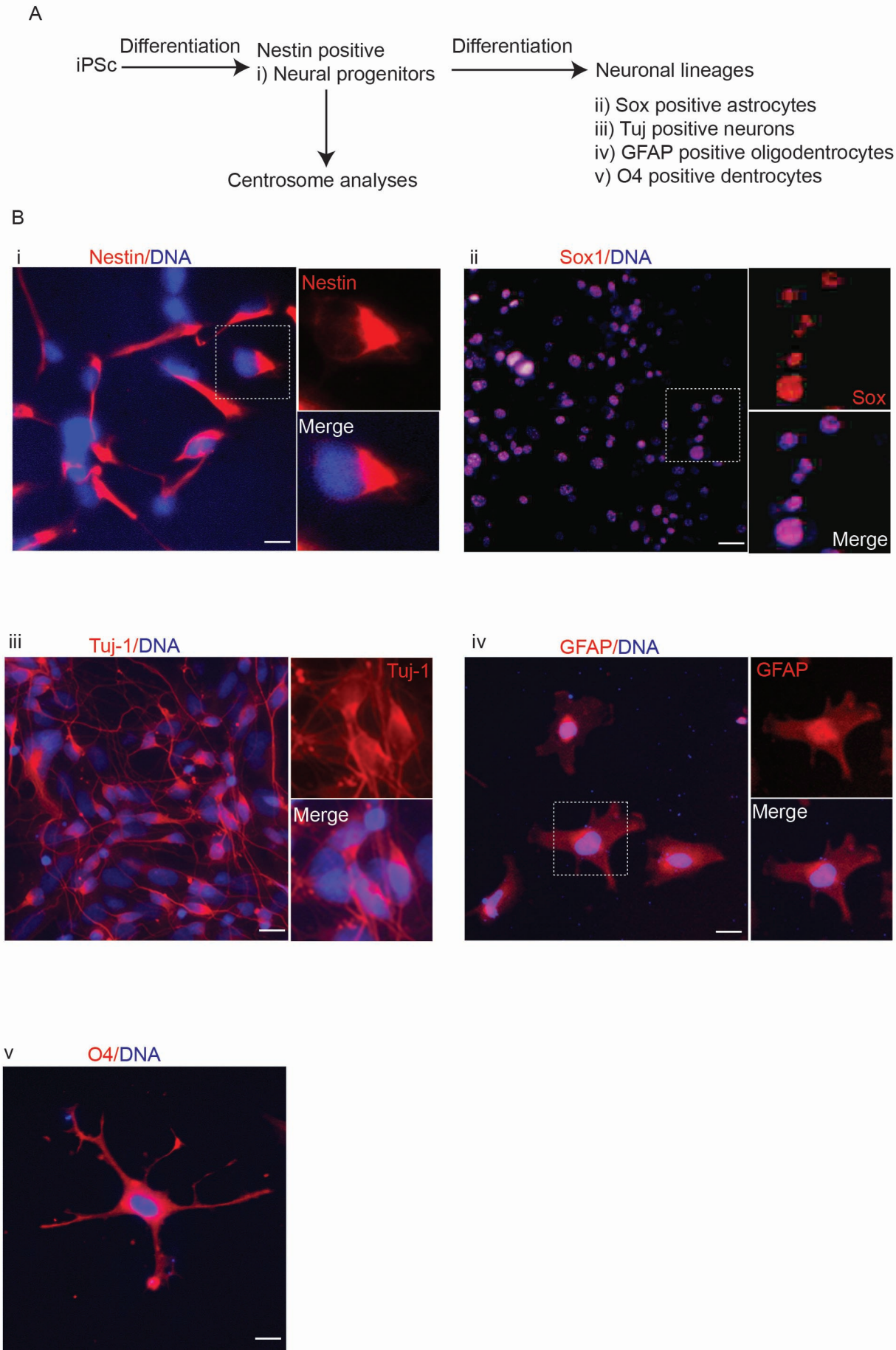
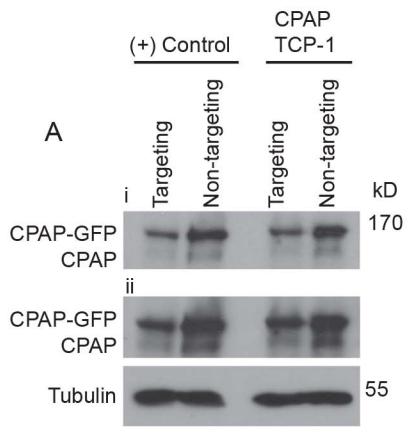
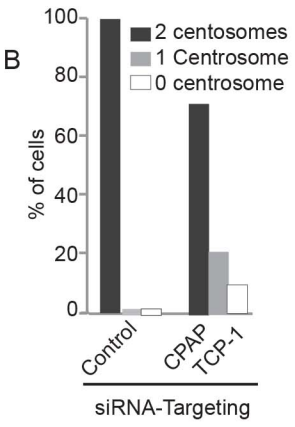
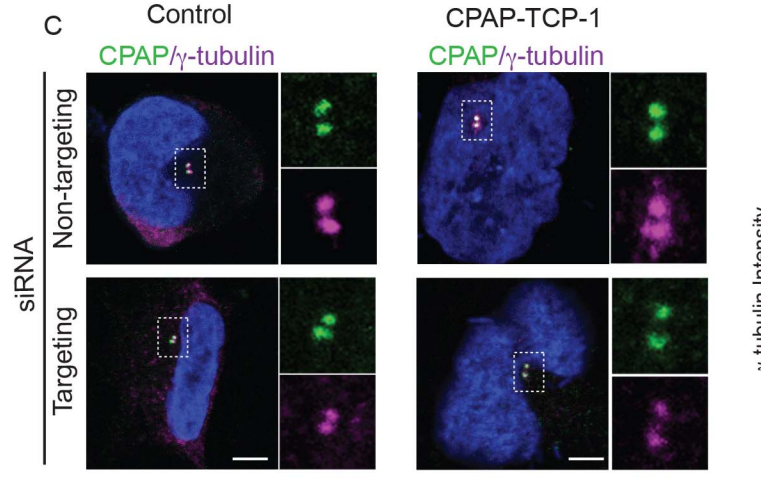
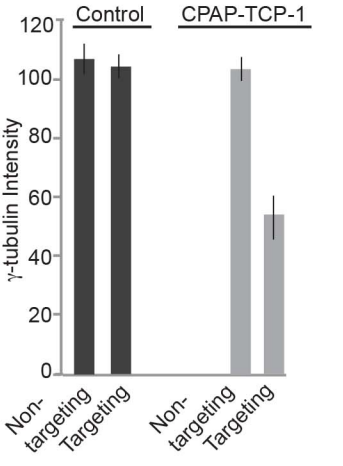

A
iPSc
Differentiation
Nestin positive
i) Neural progenitors
Differentiation
Neuronal lineages
ii) Sox positive astrocytes
iii) Tuj positive neurons
iv) GFAP positive oligodentrocytes
v) O4 positive dentrocytes
Centrosome analyses
B
i
Nestin/DNA
Nestin
Merge
ii
Sox1/DNA
Sox
Merge
iii
Tuj-1/DNA
Tuj-1
Merge
iv
GFAP/DNA
GFAP
Merge
v
O4/DNA

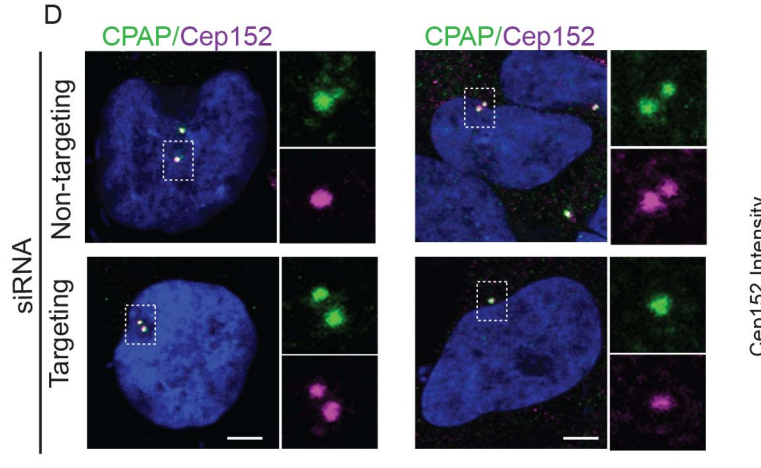
Fig. S13

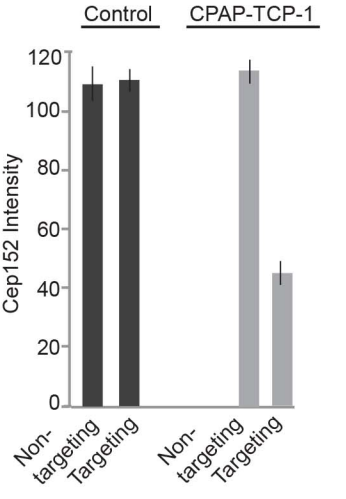

(+) Control
CPAP TCP-1
A
Targeting
Non-targeting
Targeting
Non-targeting
i
kD
CPAP-GFP
CPAP
170
ii
CPAP-GFP
CPAP
Tubulin
55

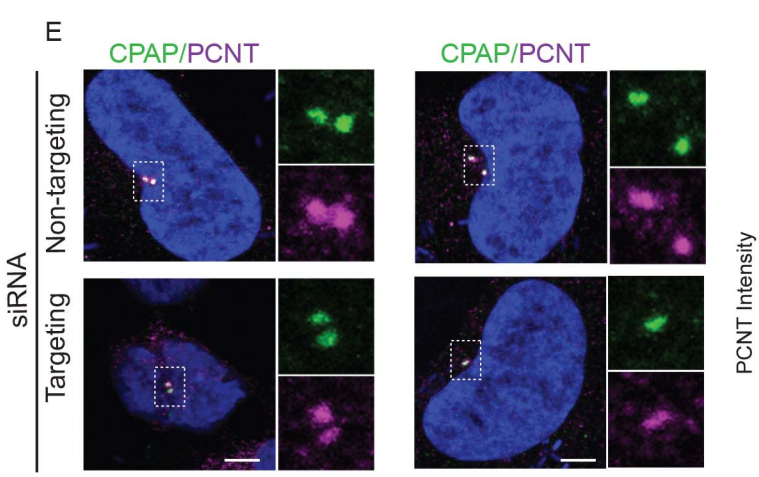

B
2 centosomes
1 Centrosome
0 centosome
% of cells
100
80
60
40
20
0
Control
CPAP TCP-1
siRNA-Targeting


C
Control
CPAP-TCP-1
CPAP/γ-tubulin
CPAP/γ-tubulin
siRNA
Non-targeting
Targeting


Control
CPAP-TCP-1
γ-tubulin Intensity
120
100
80
60
40
20
0
Non-targeting
Targeting
Non-targeting
Targeting


D
CPAP/Cep152
CPAP/Cep152
siRNA
Non-targeting
Targeting


Control
CPAP-TCP-1
Cep152 Intensity
120
100
80
60
40
20
0
Non-targeting
Targeting
Non-targeting
Targeting


E
CPAP/PCNT
CPAP/PCNT
siRNA
Non-targeting
Targeting

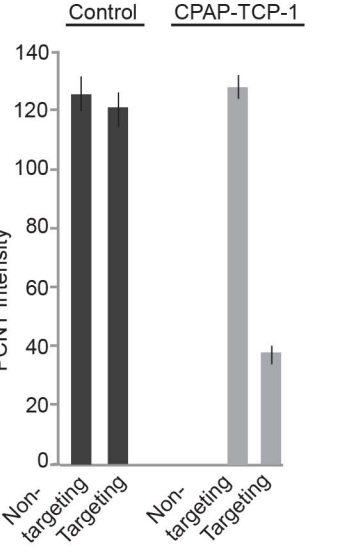
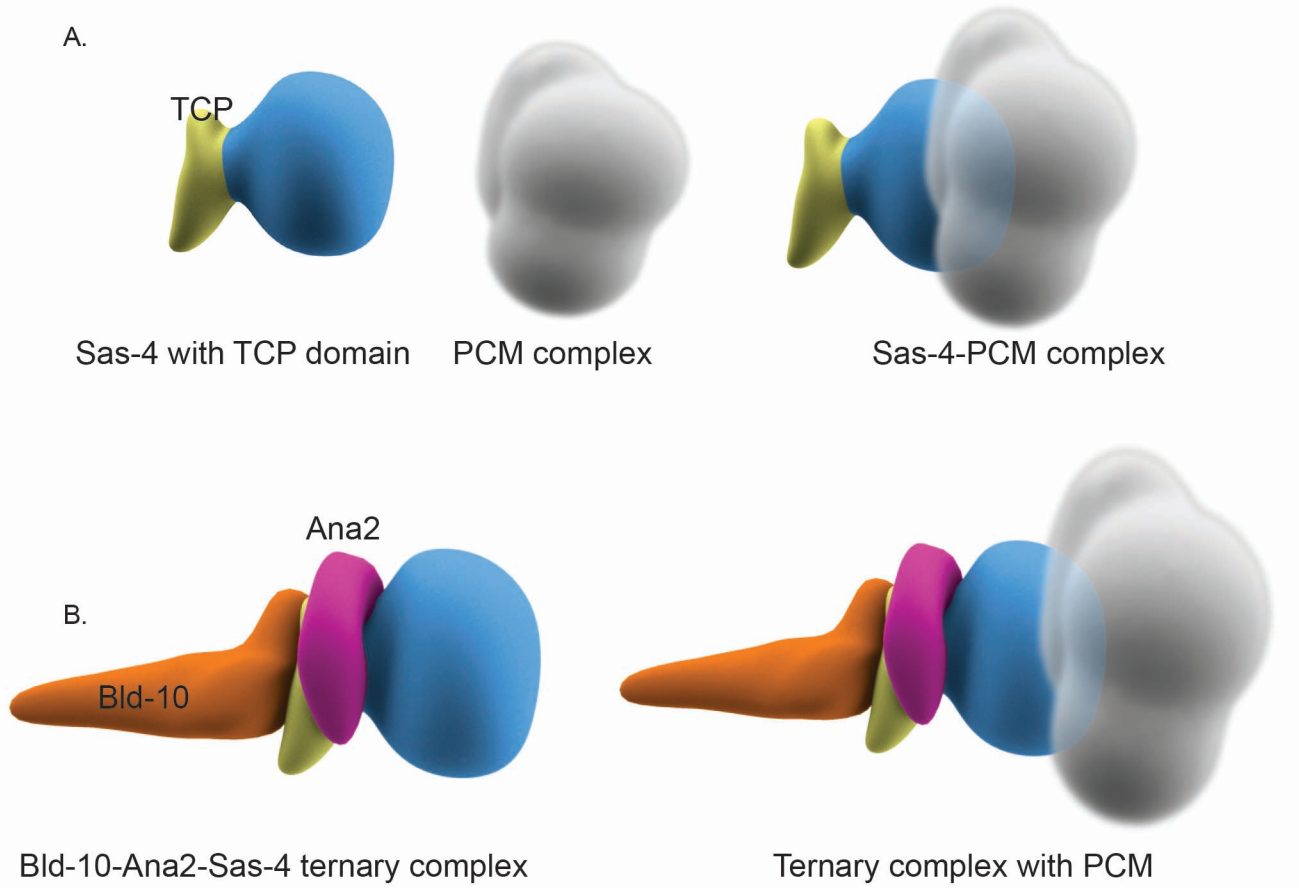

Control
CPAP-TCP-1
PCNT Intensity
140
120
100
80
60
40
20
0
Non-targeting
Targeting
Non-targeting
Targeting

Fig. S14


A.
TCP
Sas-4 with TCP domain
PCM complex
Sas-4-PCM complex
Ana2
B.
Bld-10
Bld-10-Ana2-Sas-4 ternary complex
Ternary complex with PCM
Cartwheel spoke
C.
Sas-4 tethers PCM complexes
*via* a possible co-ordinated function
of ternary complex
Centrosome