

Supplementary information to

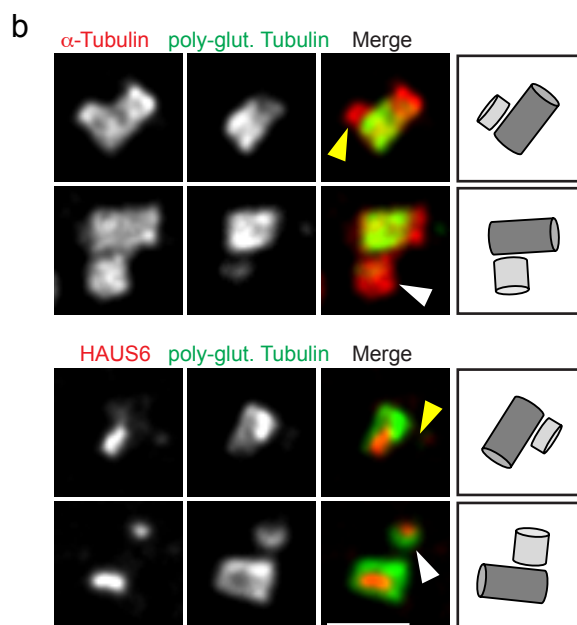
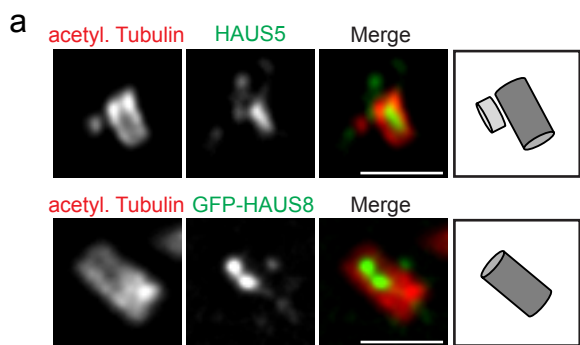
Sub-centrosomal mapping identifies augmin- γ TuRC as part of a centriole-stabilizing scaffold

Schweizer et al.

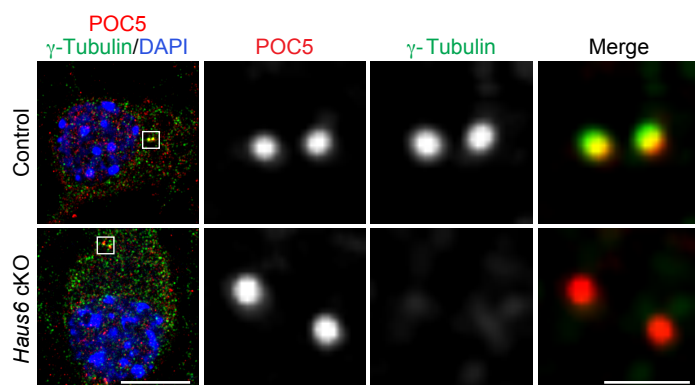
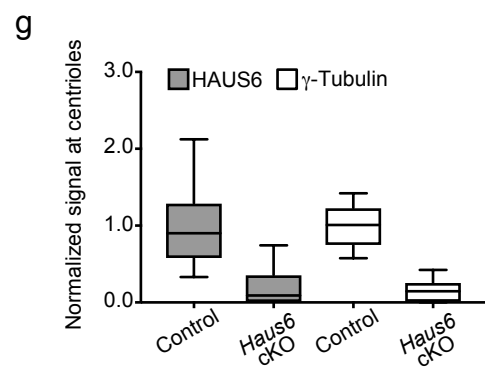
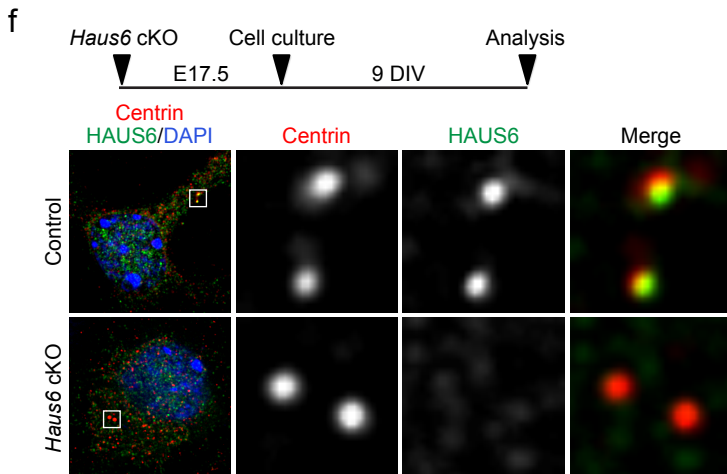
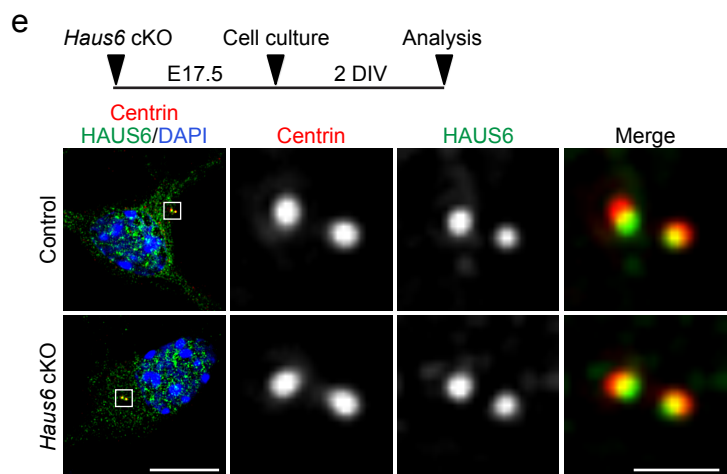
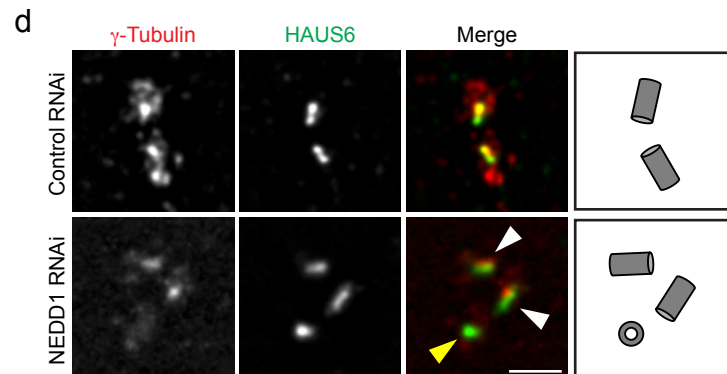
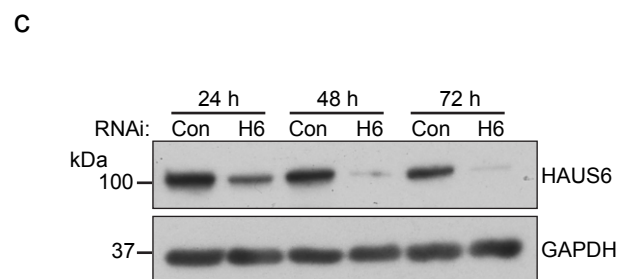
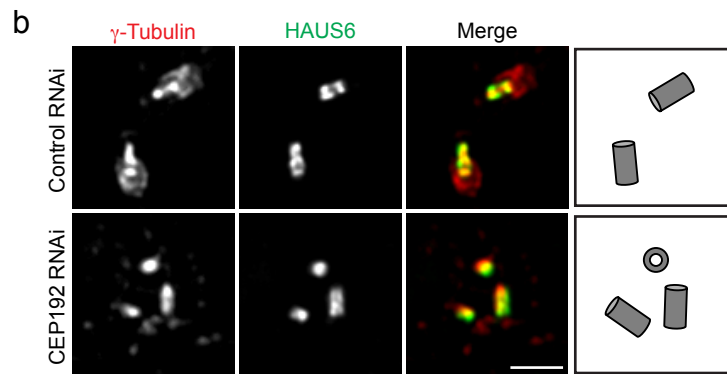
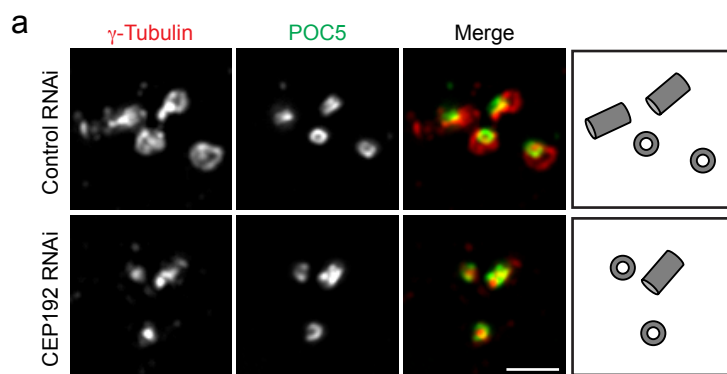
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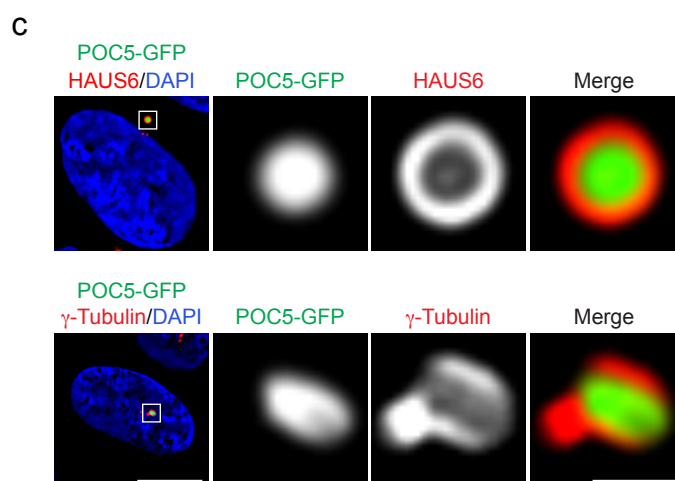
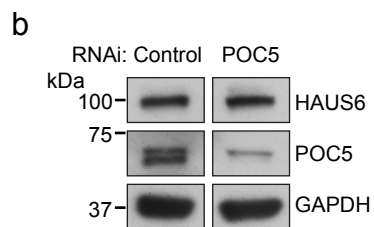
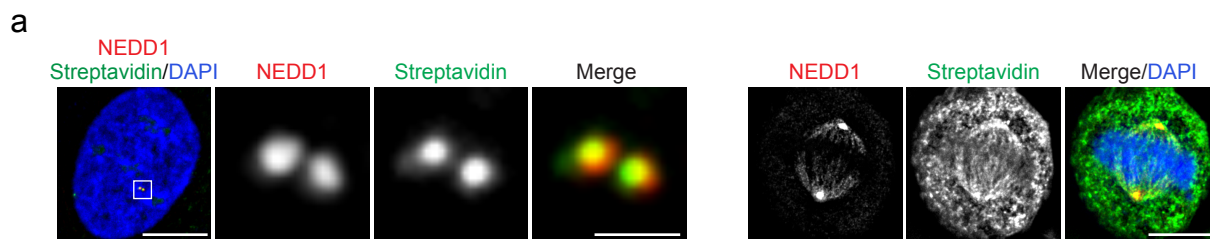


Supplementary Figure 1 Augmin localizes to the centriole lumen late during the cell cycle. **(a)** Centrioles of parental U2OS cells or U2OS cells stably expressing EGFP-HAUS8 in ExM stained for acetylated α -tubulin and HAUS5 or GFP (EGFP-HAUS8). **(b)** Centrioles of U2OS cells in ExM stained for α -tubulin and poly-glutamylated tubulin or HAUS6 and poly-glutamylated tubulin. Yellow arrowheads point to daughter centrioles that lack HAUS6/poly-glutamylation, white arrowheads point to poly-glutamylated daughter centrioles (that have HAUS6 in the lumen). Scale bar (all panels), 2 μ m. Cartoons illustrate configurations of mother (dark grey) and daughter (light grey) centrioles.



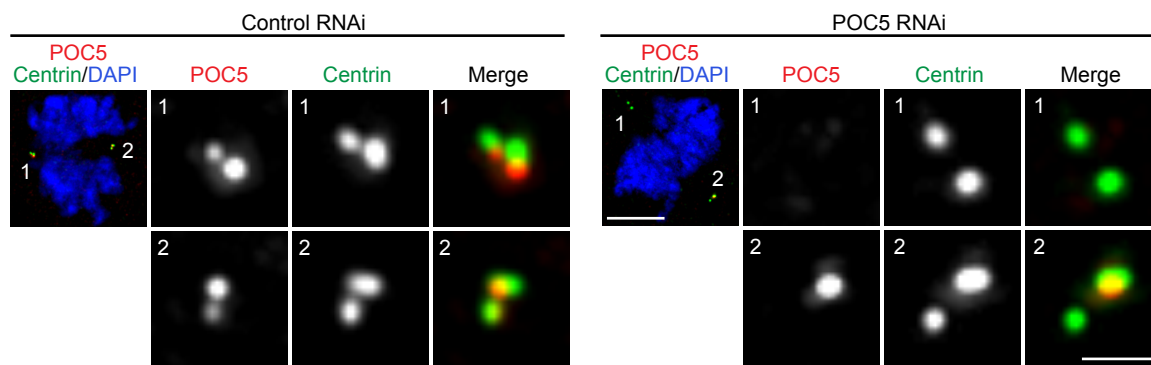
Supplementary Figure 2 γ TuRC centriole localization depends on CEP192 and augmin.

(a) Centrioles of control and CEP192 RNAi U2OS cells in U-ExM stained for γ -tubulin and POC5. (b) Centrioles of control and CEP192 RNAi U2OS cells in U-ExM stained for γ -tubulin and HAUS6. (c) Western Blot analysis of HAUS6 in extracts from control (Con) and HAUS6 RNAi (H6) U2OS cells at different time points after siRNA transfection. GAPDH was used as loading control. (d) Centrioles of control and NEDD1 RNAi U2OS cells in ExM stained for γ -tubulin and HAUS6. White arrowheads point to centrioles that have γ -tubulin in the lumen, yellow arrowhead points to a centriole that is depleted of luminal γ -tubulin, but has HAUS6 in the lumen. Scale bar (a,b,d), 2 μ m. Cartoons illustrate centriole configurations. (e) Murine control and *Haus6* cKO neurons, dissected from E17.5 embryos and cultured for 2 days (2 DIV) as depicted in the schematic, were stained for centrin, HAUS6 and DNA. (f) Murine control and *Haus6* cKO neurons, dissected from E17.5 embryos and cultured for 9 days (9 DIV) as depicted in the schematic, were stained for centrin, HAUS6 and DNA, or for POC5, γ -tubulin and DNA. Scale bar in (e) and (f), 10 μ m (cells) or 1 μ m (magnified centrioles). (g) Relative fluorescence signals for HAUS6 and γ -tubulin at centrioles in control and *Haus6* cKO neurons at 9 DIV. The box plot contains pooled data from 2 independent experiments, 16-20 centrioles analyzed per condition and experiment. Boxes extend from the 25th to the 75th percentile, the horizontal line indicates the median, whiskers depict the 10th and 90th percentiles. Source data are provided as a Source Data file.

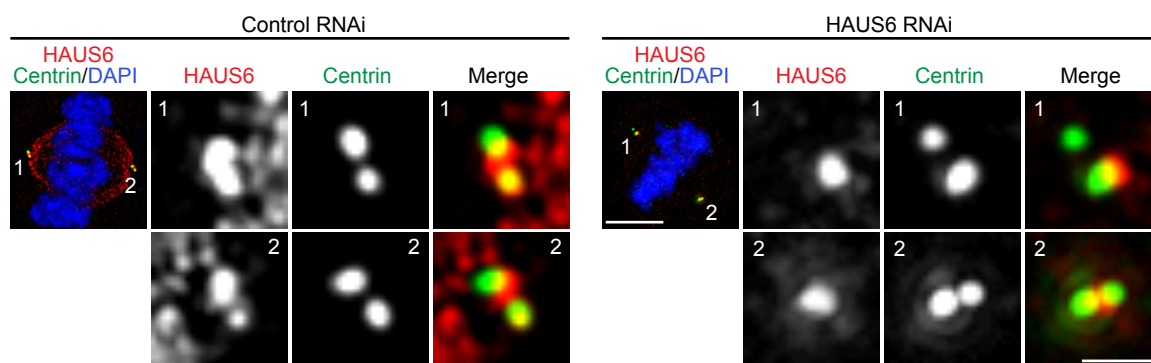


Supplementary Figure 3 Identification of POC5 as a proximity interactor of HAUS6. **(a)** Interphase or mitotic U2OS cells stably expressing BirA-HAUS6 were incubated with biotin and stained for NEDD1, biotin (568-conjugated streptavidin) and DNA. **(b)** Western Blot analysis of POC5 in extracts from control and POC5 RNAi U2OS cells. GAPDH was used as loading control. **(c)** U2OS cells stably expressing POC5-GFP stained for GFP (POC5-GFP), HAUS6 or γ -tubulin and DNA. Scale bar (all panels), 10 μ m (cells) or 1 μ m (magnifications).

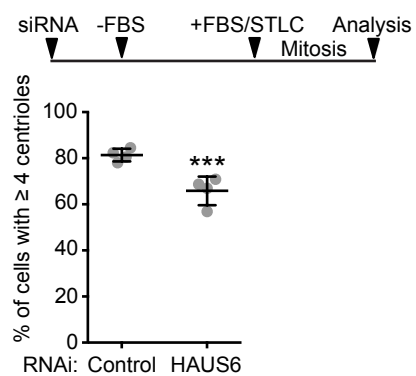
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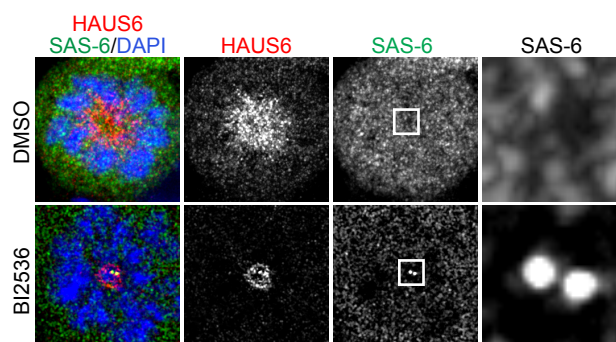
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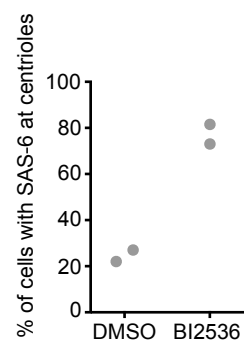
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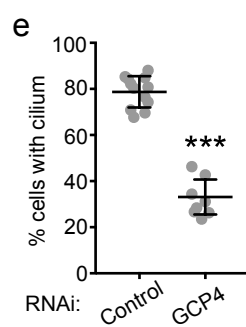
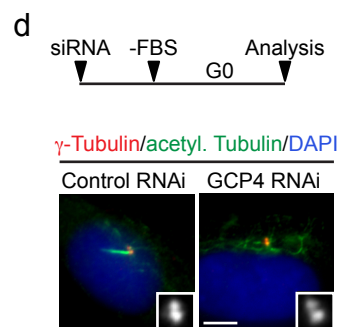
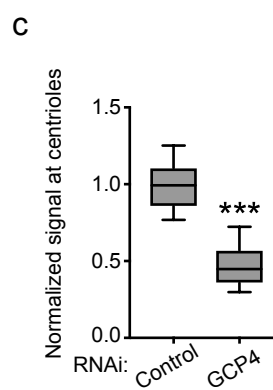
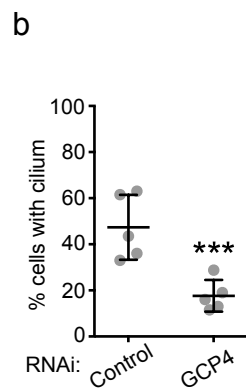
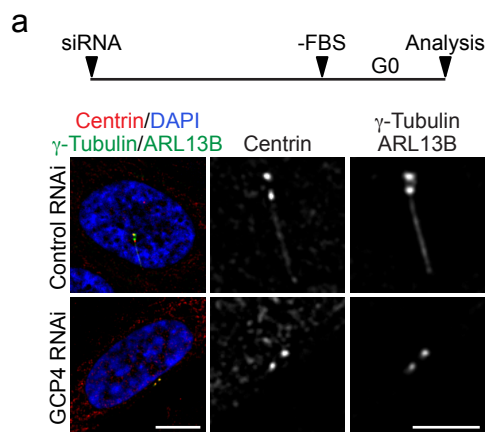
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e



Supplementary Figure 4 Centriole numbers are not reduced in cycling POC5 RNAi or HAUS6 RNAi cells. **(a)** Mitotic control or POC5 RNAi U2OS cells stained for POC5, centrin and DNA. Magnifications show centriole staining at each of the two spindle poles. **(b)** Mitotic control or HAUS6 RNAi U2OS cells stained for HAUS6, centrin and DNA. Magnifications show centriole staining at each of the two spindle poles. **(c)** Quantifications of the percentage of control or HAUS6 RNAi RPE1 cells with ≥ 4 centrioles (centrin foci) after prolonged mitotic arrest following the depicted treatment. Data points are from 4 independent experiments, 100-500 cells analyzed per condition and experiment. The horizontal line depicts the mean, error bars represent standard deviations ($p = 7.5495e-15$, generalized linear model with binomial distribution). **(d)** Control RNAi U2OS cells after 24 h in mitosis without (DMSO) or with (BI2536) PLK1 inhibition, stained for HAUS6, SAS-6 and DNA. **(e)** Quantifications of the percentage of cells as in **(d)** with SAS-6 at centrioles. Data points are from 2 independent experiments, 100-200 cells analyzed per condition and experiment. *** $p < 0.001$. Scale bar **(a,b,d)**, 10 μm (cells) or 1 μm (magnifications). Source data are provided as a Source Data file.



Supplementary Figure 5 γ TuRC is required for ciliogenesis. (a) Serum-starved control and GCP4 RNAi RPE1 *p53* KO cells stained for centrin, γ -tubulin, ARL13B and DNA. Scale bar, 10 μ m (cells) or 5 μ m (magnifications of centrioles/cilia). The experimental design is depicted schematically. (b) Quantifications of the percentage of ciliated cells as in (a). Data points are from 5 independent experiments, 200-302 cells analyzed per condition and experiment. The horizontal line depicts the mean, error bars represent standard deviations ($p < 2.22\text{e-}16$, generalized linear model with binomial distribution). (c) Relative fluorescence intensity for γ -tubulin at centrioles in cells as in (a). The box plot contains pooled data from 3 independent experiments, 51-56 cells analyzed per condition and experiment. Boxes extend from the 25th to the 75th percentile, the horizontal line indicates the median, whiskers depict the 10th and 90th percentiles ($p < 2.22\text{e-}16$, mixed effects linear model). (d) Serum-starved control and GCP4 RNAi BJ cells stained for γ -tubulin, acetylated α -tubulin (acetyl. Tubulin) and DNA. Insets show magnifications of centrosomal γ -tubulin staining. The experimental design is depicted schematically. Scale bar, 5 μ m (e) Quantifications of the percentage of ciliated cells as in (d). Data points are from 12 independent experiments, 64-206 cells analyzed per condition and experiment. The horizontal line depicts the mean, error bars represent standard deviations ($p < 2.22\text{e-}16$, generalized linear model with binomial distribution). *** $p < 0.001$. Source data are provided as a Source Data file.

Supplementary table 1:

primer name	primer sequence (5'-3')
mAug6KO_FW	CAACCCGAGCAACAGAAACC
mAug6KO_Rev	CCTCCCACCAACTACAGACC
26994	GCTGGAAGATGGCGATTAGC
30672	TCAGCCTGGTTACAAGGAACA
oIMR7338	CTAGGCCACAGAATTGAAAGATCT
oIMR7339	GTAGGTGGAAATTCTAGCATCATCC