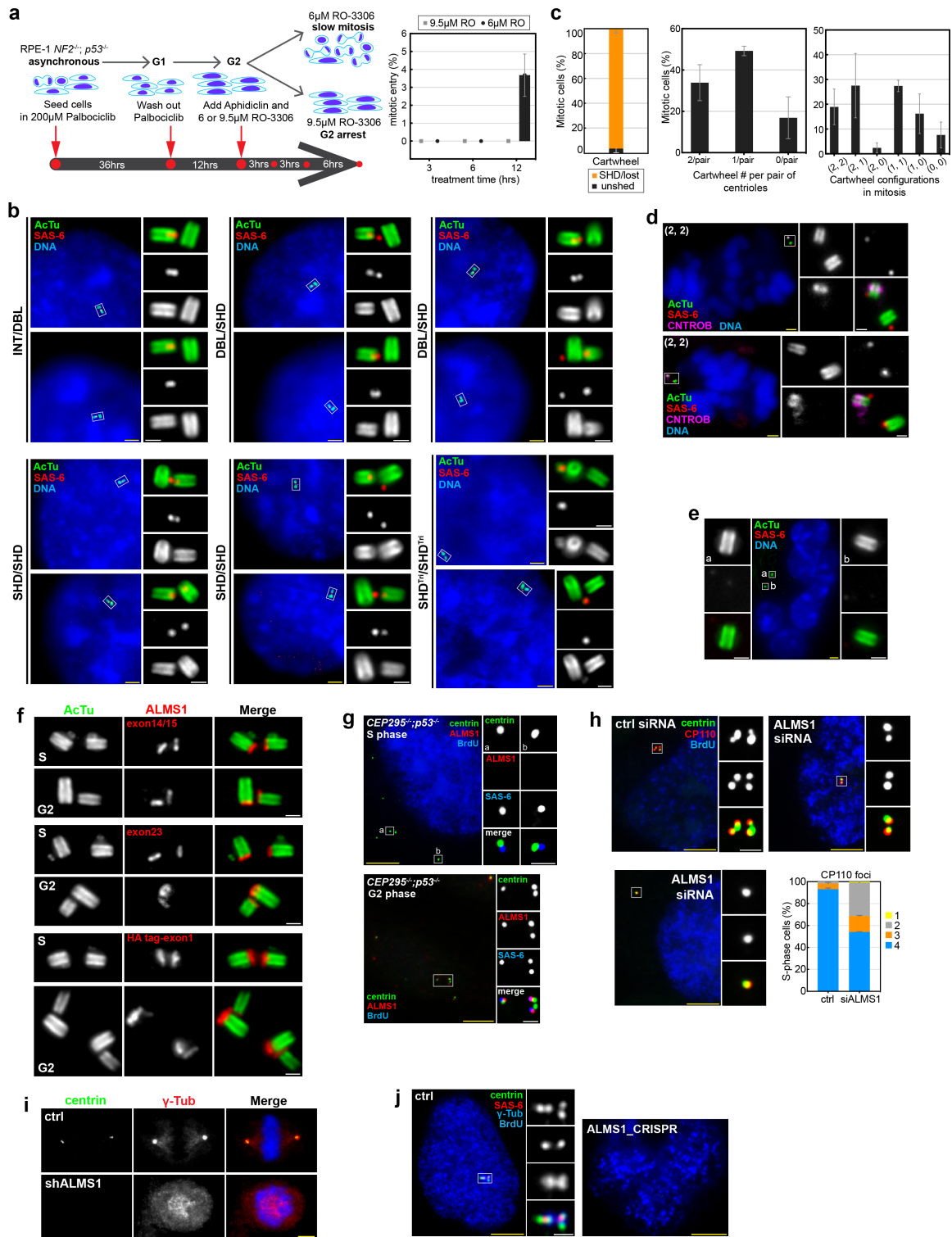


Supplementary Fig. S1



Supplementary Fig. S1: G2/M cartwheel dynamics and its association with ALMS1

(a) The experimental scheme of G2 cells synchronously progressing toward mitosis for a time course of 12 hrs in the presence of partial (6 μ M) or strong (9.5 μ M) CDK1 inhibition. Percentages of cells that had undergone mitotic entry after CDK1 inhibition at indicated time points were recorded based on time-lapse microscopy (right). While no mitotic entry was seen under strong CDK1 inhibition, ~4% of cells had entered mitosis by the 12h timepoint under partial CDK1 inhibition. Data are mean $\pm$ s.d., with N = 3 independent biological replicates. Source data are provided as a Source Data file.

(b) Images of different stages of cartwheel transformation in G2 cells that slowly progressed toward mitosis under partial CDK1 inhibition. Representative images of cells hosting intact cartwheel (INT), dumbbell-like cartwheel (DBL), split-and-shed cartwheel (SHD), or shed cartwheel in centriole triplets (SHD^{Tri}) were shown.

(c) Quantification of cartwheel shedding or loss in cells that were progressing in mitosis under partial CDK1 inhibition. Left, quantification of mitotic centrioles that maintained their cartwheel in the lumen (unshed) or had shed or lost the cartwheel (SHD/lost). Middle, quantification of the cartwheel number per centriole pair in mitotic cells, ranging from 2 to 0 per pair. Right, quantification of the cartwheel configuration from two pairs of centrioles in the same cell, giving rise to 6 combinations as indicated. Data is mean $\pm$ s.d., with N = 3 independent biological replicates. Source data are provided as a Source Data file.

(d) UExM images of a mitotic cell showing the relative position of the shed cartwheel with the mother and daughter centrioles in the (2, 2) configuration as indicated in (c). CNTROB was used to mark disengaged daughter centrioles (magenta). Note that all shed cartwheels were outside the daughter lumen.

(e) UExM images of a G1 cell that had gone through disruptive mitosis (multiple nuclei) due to partial CDK1 inhibition and had lost the intact cartwheel (no SAS-6 signals). All DNA was shown in blue (DAPI).

(f) UExM analyses of ALMS1 localization using antibodies against ALMS1 epitopes encoded by exon14/15, exon23, or the HA epitope endogenously tagged at exon1 of ALMS1. In all cases, ALMS1 was absent at S-phase procentrioles but was localized to the proximal end of G2 procentrioles.

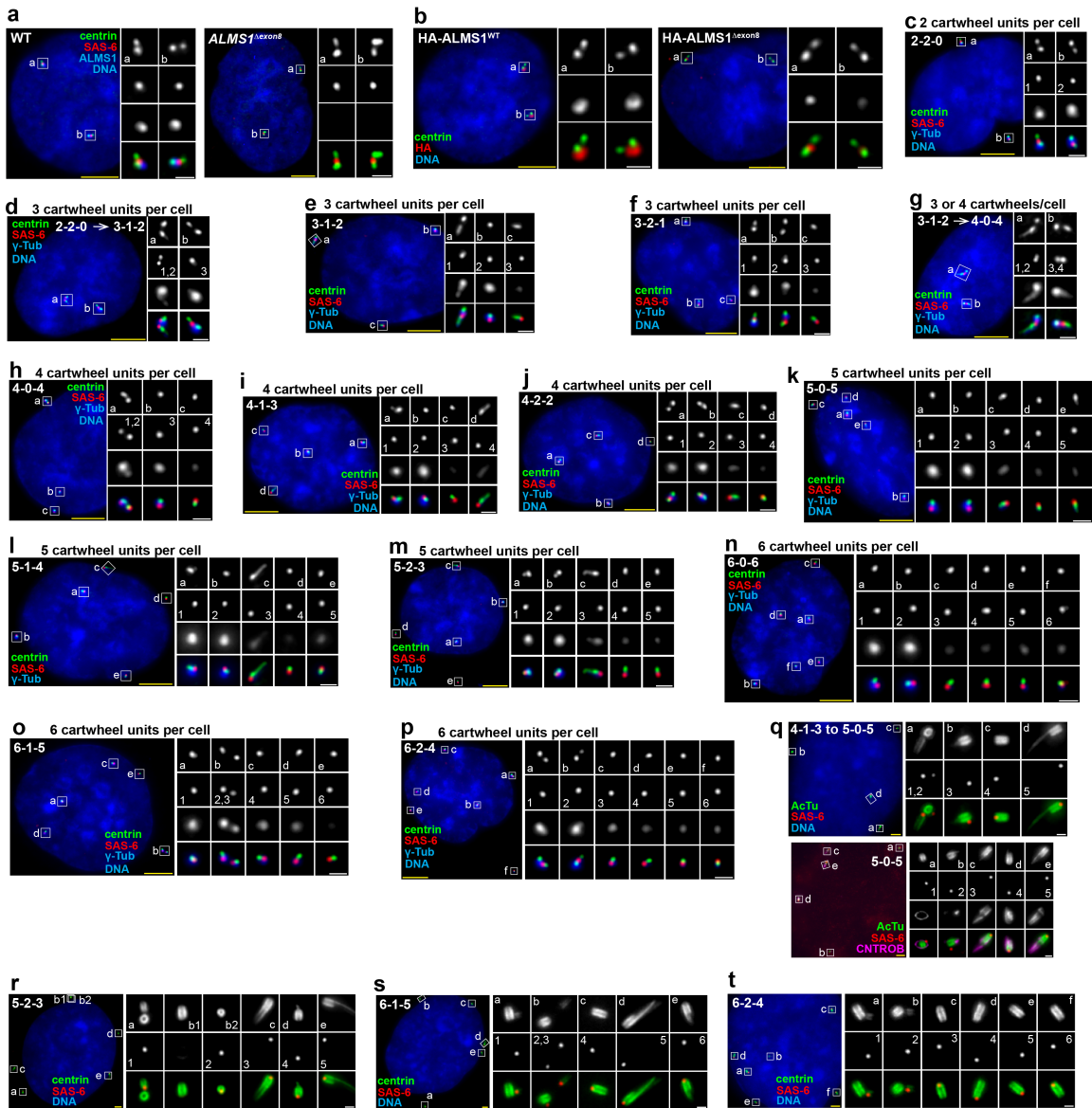
(g) ALMS1 localization at newborn centrioles formed in the absence of preexisting centrioles in *p53*^{-/-}; *CEP295*^{-/-} cells. Centrin and SAS-6 labeled all newborn centrioles in S or G2, whereas ALMS1 marked only those in G2.

(h) U2OS cells treated with siRNA for 72 hrs were examined for centriole duplication in S phase (BrdU). Failure of procentriole formation in *siALMS1* cells in S phase, as revealed by centrin and CP110 staining. Quantification is shown. Data are mean $\pm$ s.d., with N = 3 independent biological replicates. Source data are provided as a Source Data file.

(i) Mitotic defects of *shALMS1* cells revealed by centrin, γ -tubulin, and DAPI staining. Note that no prominent γ -tubulin foci that normally indicate microtubule asters were seen in these mitotic-arrested cells, a phenotype not explainable by centrosome loss.

(j) Failure of cartwheel and procentriole formation in S-phase cells (BrdU) deleted of ALMS1 by CRISPR-mediated gene targeting at exon 1. All scale bars are 1 μ m (white) and 5 μ m (yellow).

Supplementary Fig. S2



Supplementary Fig. S2: Characterization of G2/M cartwheel dynamics under *ALMS1* ^{Δ ex8} mutations

(a) *ALMS1* ^{Δ ex8} cells in G2 showing normal looking centrioles that had duplicated, like WT cells. Antibodies against exon8 epitopes of ALMS1 were used.

(b) *ALMS1* ^{Δ ex8} mutant proteins, revealed by endogenously tagging HA epitopes to exon 1, were expressed and recruited to centrioles, despite at a lower level, as compared to HA-tagged *ALMS1*^{WT}.

(c-p) Immunofluorescence images of cartwheel amplification seen in *ALMS1* ^{Δ ex8} cells arrested in G2. The cartwheel, ranged from 2 to 6 units per cell, exhibited 12 specific configurations as illustrated in Figure 2b. Representative images revealing the transition from the 2-2-0 to 3-1-2 or from the 3-1-2 to 4-0-4 configurations were shown (d & g)

(q-t) UExM images of 4-1-3 to 5-0-5 (q; top), 5-0-5 (q; bottom), 5-2-3 (r), 6-1-5 (s), and 6-2-4 (t) configurations in intact *ALMS1* ^{Δ ex8} cells. Note the difference in the relative position of the cartwheel to the mother or daughter (CNTROB); it is at the side of the mother or in the lumen of the daughter.

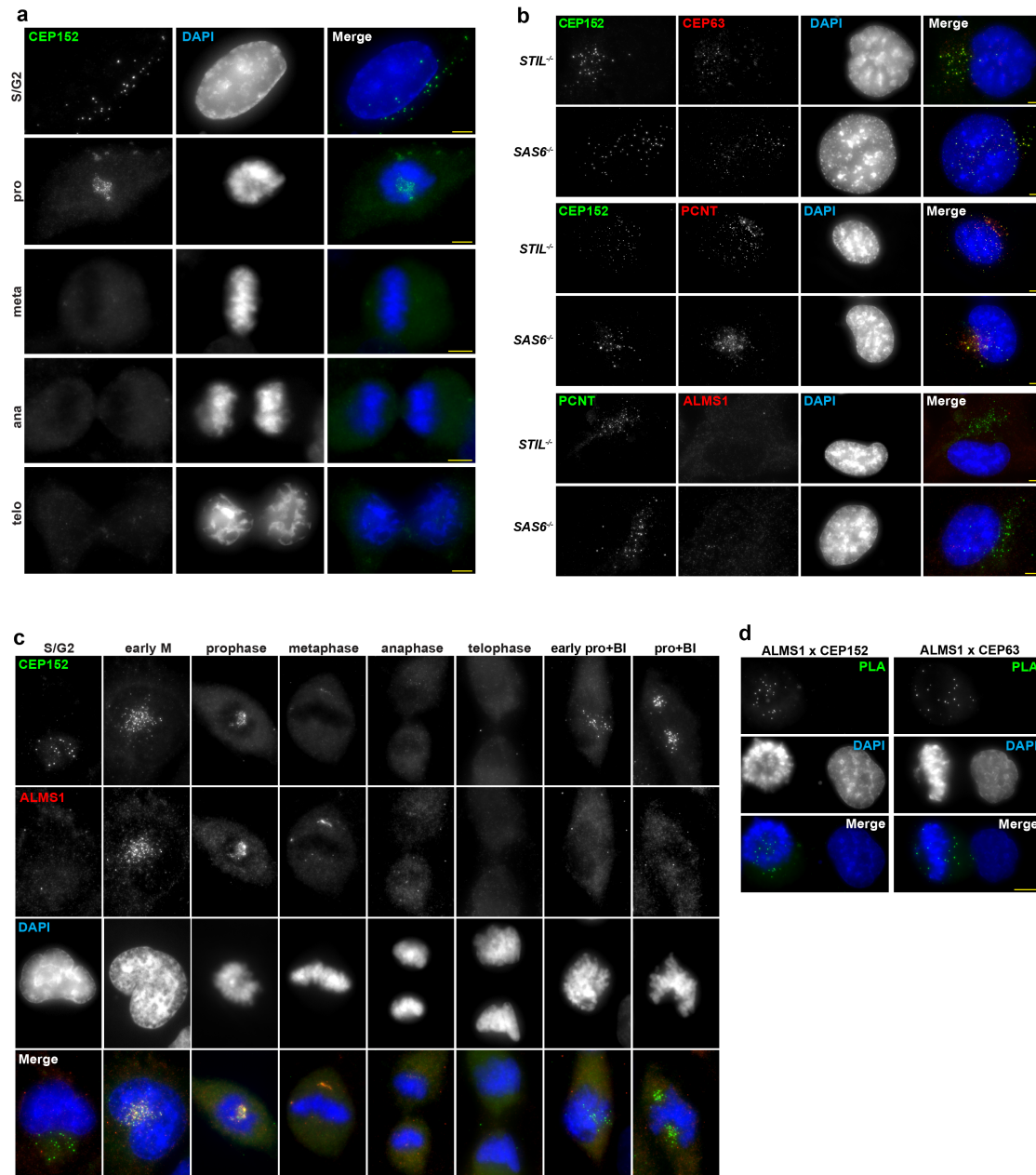
(q) the 5-0-5, the cell at the top was in the transition from the 4-1-3 to 5-0-5. In the bottom cell, CNTROB labeling (magenta) marks disengaged daughters and newly formed granddaughters all carrying the cartwheel in the lumen (box c-e).

(r) The 5-2-3: the two centrioles shown separately in b1 and b2 boxes represent a pair of engaged centrioles (a doublet) where the daughter, boxed by b2, is pointing toward the viewer.

(s) The 6-1-5: note that cartwheel shedding and centriole disengagement occurred at one of the granddaughter centrioles, with the shed cartwheel (#2) attaching to the side of the mother, whereas the other unit (#3) remained inside the lumen of the disengaging daughter centriole.

(t) The 6-2-4: two great-granddaughters (boxes a & b)—each growing from the shed cartwheel at the side of the mother—and 4 disengaging daughters or granddaughters (boxes c-f) each carrying a cartwheel unit in the lumen were shown. All DNA was shown in blue (DAPI). All scale bars are 1 μ m (white) and 5 μ m (yellow).

Supplementary Fig. S3



Supplementary Fig. S3. ALMS1 is physically associated with the disassembly of CEP152-CEP63-PCNT composites during the G2/M transition.

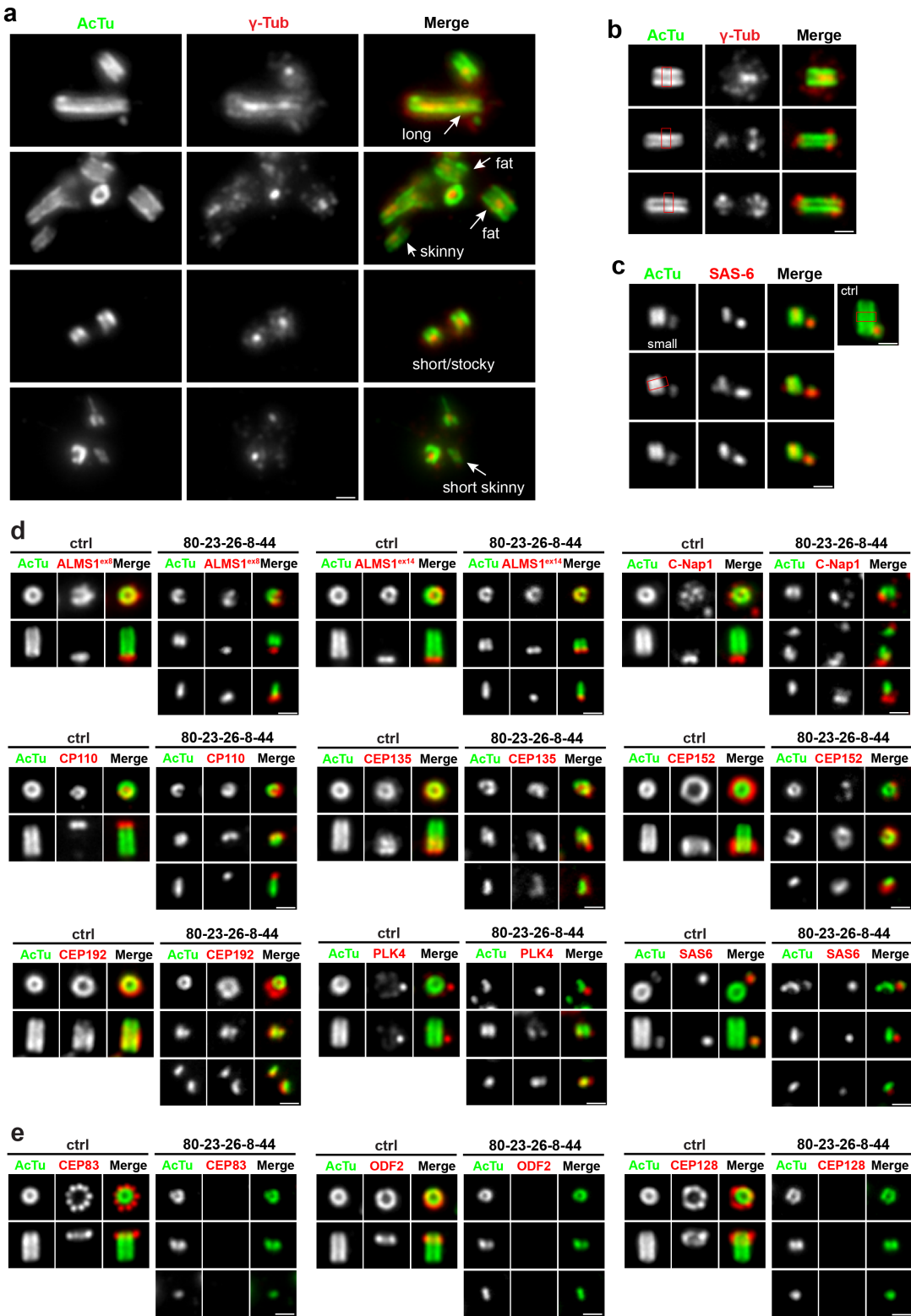
(a) Cell cycle dynamics of CEP152 puncta shown in SAS-6^{-/-} cells.

(b) CEP152, CEP63, and PCNT form composites that are absent of ALMS1. Antibodies used were indicated.

(c) Representative images of ALMS1 localization during the disassembly of CEP152 composites in mitosis.

(d) Representative images of PLA reactions that detect complex formation of ALMS1 with CEP152 (left) or CEP63 (right) in mitotic and interphase cells present in the same field. All DNA was shown in blue (DAPI). All scale bars are 5 μ m (yellow).

Supplementary Fig. S4



Supplementary Fig. S4. Depletion and addback of ALMS1 creates centrioles with diverse shapes that are transmittable.

(a) Centrioles with various shapes or sizes formed after ALMS1 depletion and add-back, compared under the same magnification.

(b and c) Centrioles with different shapes exhibiting distinct patterns of γ -tubulin (B) or SAS-6 (C) localization. Red boxes mark the normal width of control centrioles

(d and e) Control and tiny centrioles were examined for various centriolar markers as indicated. Note that tiny centrioles cannot recruit distal or sub-distal appendages (E). All scale bars are 1 μ m (white).