Expanded View Figures

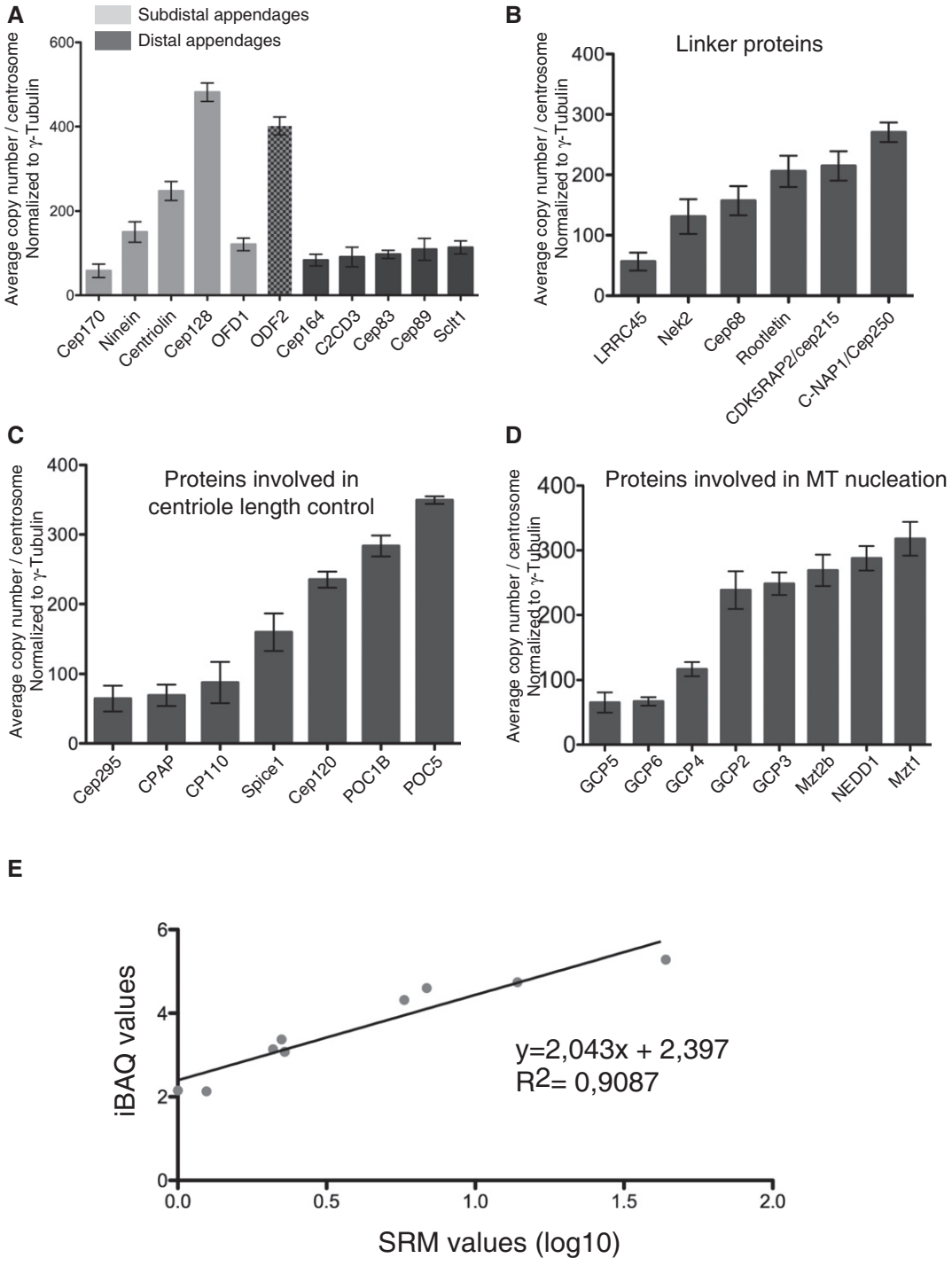
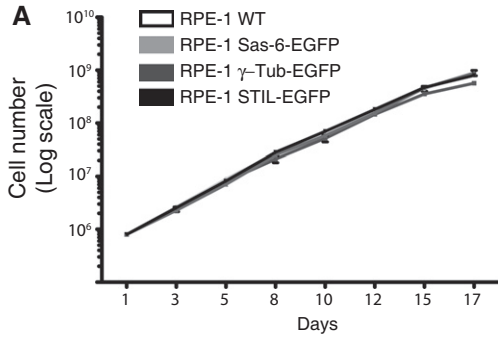


Figure EV1. Quantification of protein subsets within centrosome preparations.

A–D Histograms show quantifications for subsets of centrosomal proteins; results presented are means ± SEM. Data are extracted from Fig 3 but arranged to facilitate comparisons of proteins with similar localizations and/or functions. Grouped together are distal and subdistal appendage proteins (A; note that ODF2 is believed to associate with both structures), centrosomal linker proteins (B), proteins involved in centriole length control (C), and core components involved in MT nucleation (D).

E Correlation analysis shows excellent correspondence between values determined by iBAQ and SRM.



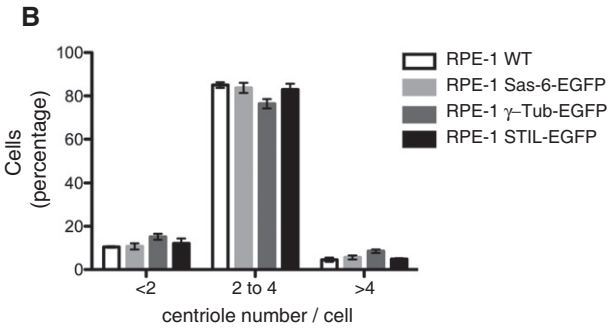


Figure EV2. Cell cycle properties of EGFP-tagged cell lines.

A EGFP-tagging of centrosomal proteins does not detectably affect cell proliferation. The indicated cell lines were grown under standard conditions, and cell numbers counted at the indicated times. Note that all growth curves are nearly identical.

B EGFP-tagging of centrosomal proteins does not detectably affect centriole number. Cells of the indicated genotype were grown on coverslips, and centriole numbers were counted after staining with anti-CP110 antibodies (immunofluorescence microscopy). Histogram shows average of two experiments; results presented are means ± SEM. Note that the vast majorities of cells showed between 2 and 4 centrioles (as expected for G1- and G2-phase cells, respectively), with no discernible differences between cell lines.

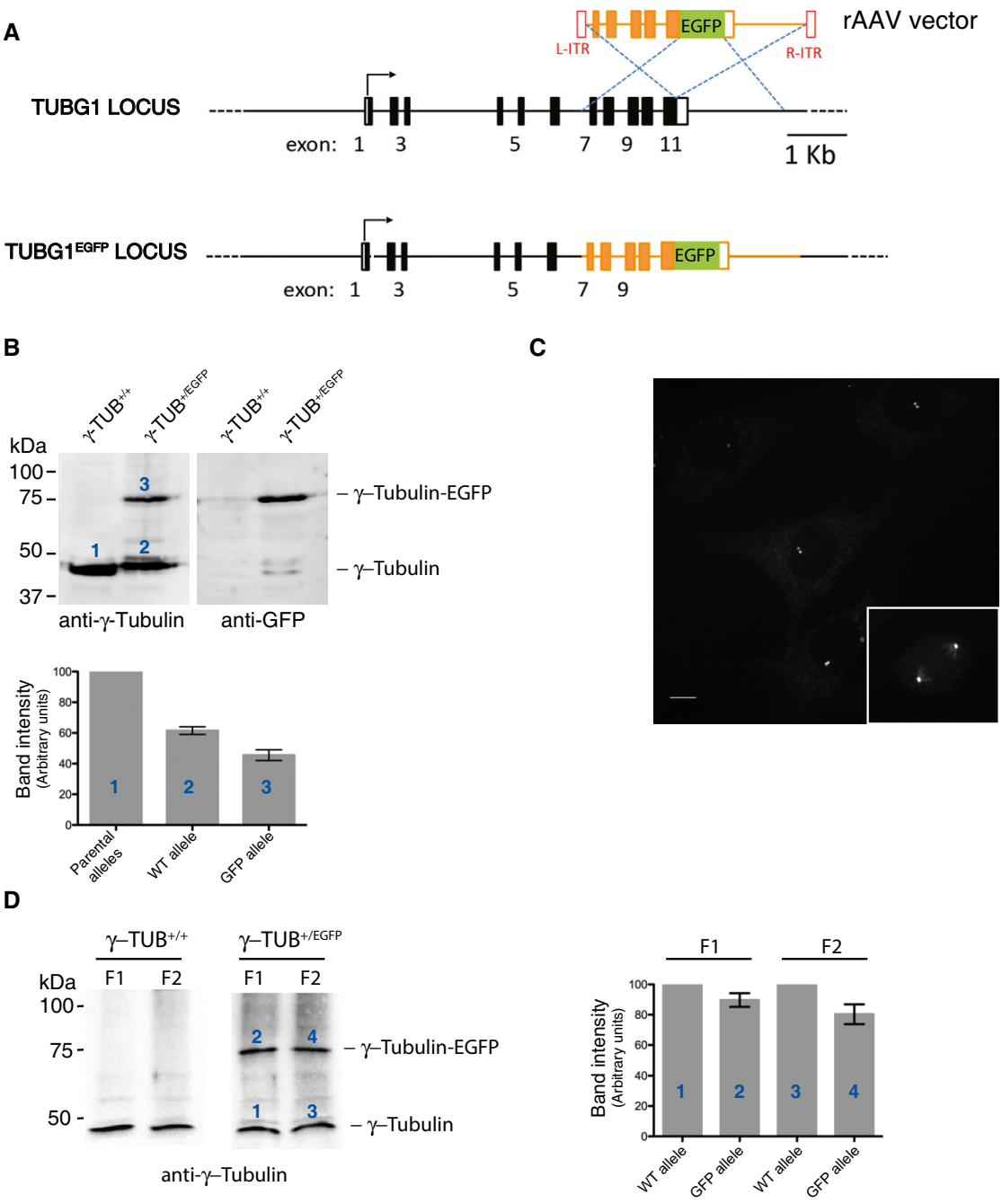


Figure EV3. Generation of RPE-1 cells expressing endogenously tagged γ-tubulin-EGFP.

A Schematic representation of wild-type (WT) TUBG1 locus and the targeted allele obtained after rAAV-mediated homologous recombination. Black rectangles represent exons and black lines introns. After insertion of the EGFP coding sequence, cells expressing γ-tubulin-EGFP were selected by FACS.

B Western blot analysis of parental RPE-1 and γ-tubulin-EGFP-expressing RPE-1 cells. Protein extracts were probed with anti-γ-tubulin (left panel) and anti-GFP (right panel) antibodies. Histogram shows quantification of the numbered bands; average of two experiments. Results presented are means ± SEM. Band intensity for WT alleles was set to 100%.

C Maximum projection fluorescence microscopy image showing asynchronously growing RPE-1 cells expressing γ-tubulin-EGFP. Inset shows mitotic cell (metaphase). Scale bar: 10 μm.

D Centrosomes were purified from parental RPE-1 cells (left panel) or γ-tubulin-EGFP-expressing RPE-1 cells (right panel), using a previously described procedure (Blomberg-Wirschell & Doxsey, 1998). In brief, starting with material derived from 2 × 15 cm plates, method 1 was employed with minor modifications. The first two centrosomal fractions (F1, F2; 0.5 ml each) were collected and concentrated by TCA precipitation prior to Western blot analysis. Membranes were probed by anti-γ-tubulin antibody. Histogram shows quantification of the numbered bands; average of two experiments. Results presented are means ± SEM. Band intensity for WT alleles was set to 100%. Note that both centrosome fractions (F1 and F2) contained WT γ-tubulin and EGFP-tagged γ-tubulin in similar proportions.

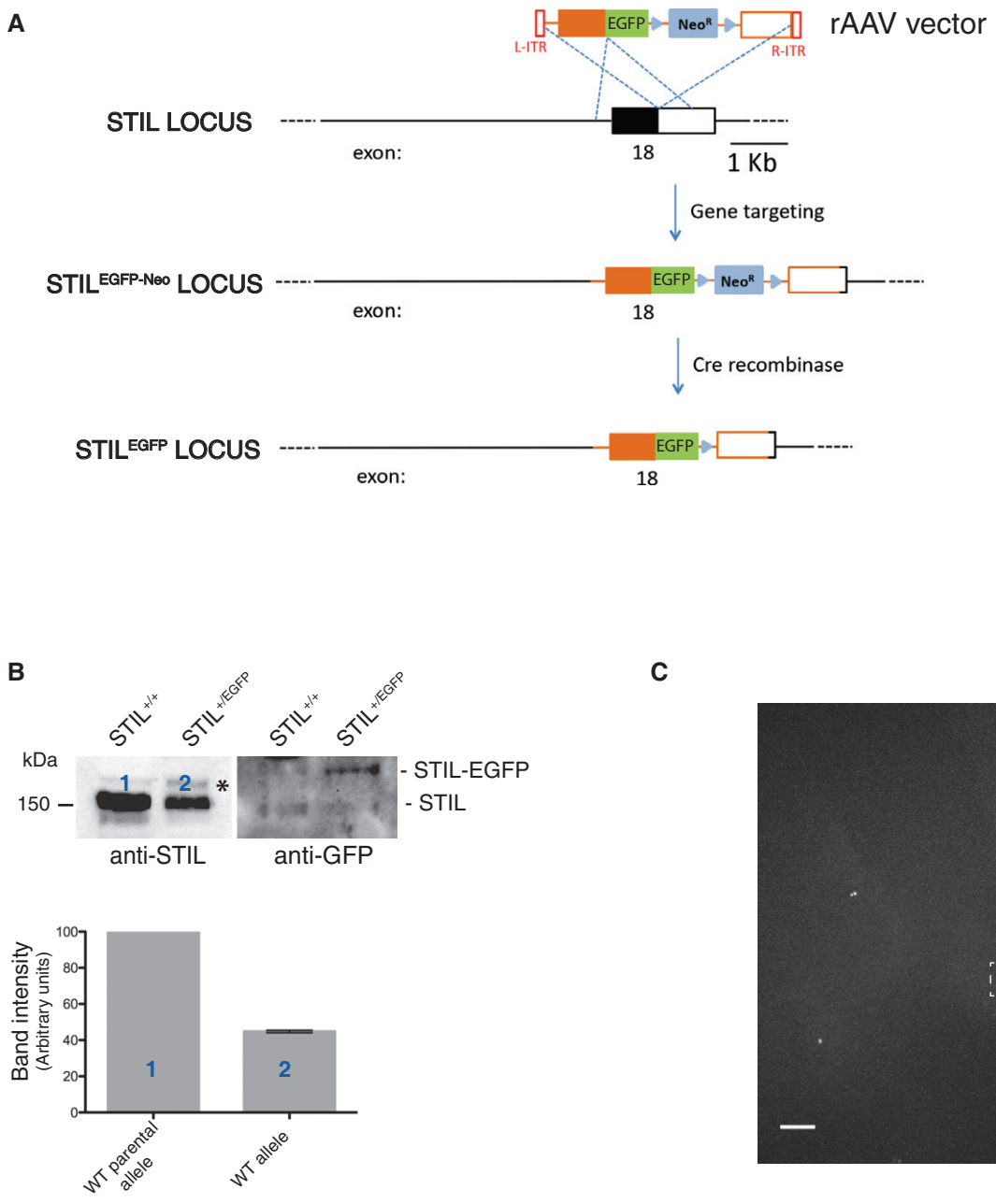


Figure EV4. Generation of RPE-1 cells expressing endogenously tagged STIL-EGFP.

A Schematic representation of WT STIL locus and the targeted STIL allele obtained after rAAV-mediated homologous recombination. Black rectangles represent exons and black lines introns. After insertion of the EGFP coding sequence and the G418 resistance cassette, cells were selected by addition of G418 to the medium. Then, the neomycin cassette was removed using an adenovirus-expressed Cre recombinase (Vector Biolabs, Malvern, PA, USA), and clones were isolated by serial dilution (loss of resistance to G418 was confirmed by replica plating). Finally, positive cells were confirmed by fluorescence microscopy.

B Western blot analysis of parental RPE-1 and STIL-EGFP-expressing RPE-1 cells. Protein extracts were probed with anti-STIL (left panel; asterisks denotes background band) and anti-GFP (right panel) antibodies. Note that only the anti-GFP antibody recognizes STIL-EGFP; lack of reactivity by the anti-STIL antibody almost certainly reflects interference of the EGFP tag with the epitope recognized by this antibody (this antibody was raised against a peptide corresponding to the C-terminus of STIL (last 50 amino acids), and involvement of the C-terminus in the fusion with EGFP makes interference plausible). Histogram shows quantification of the numbered bands; average of two experiments. Results presented are means ± SEM. Band intensity for WT alleles was set to 100%.

C Maximum projection fluorescence microscopy image showing asynchronously growing RPE-1 cells expressing STIL-EGFP. Scale bar: 10 μm. The inset shows an enlargement of the boxed interphase centrosome.

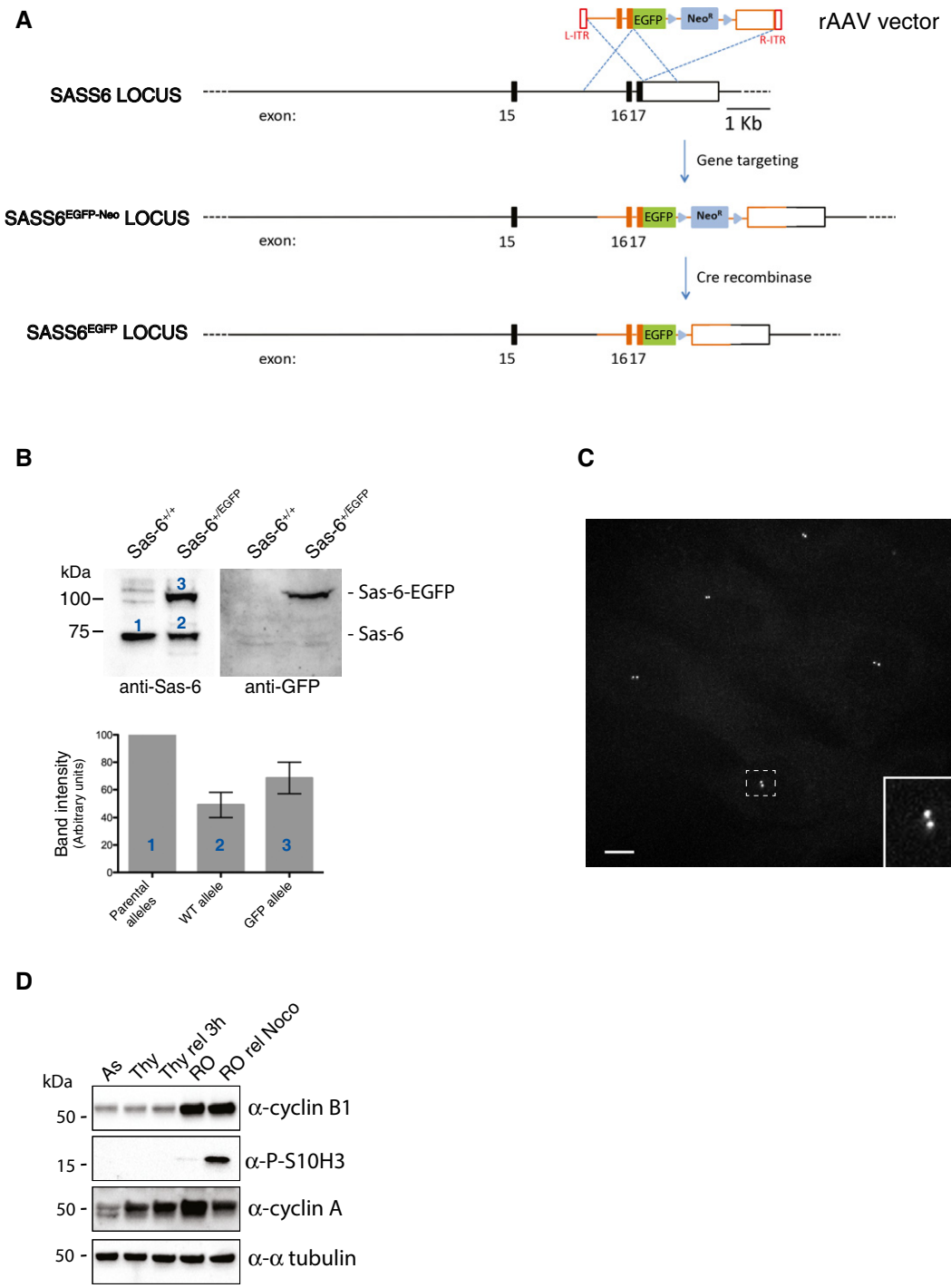


Figure EV5. Generation of RPE-1 cells expressing endogenously tagged Sas-6-EGFP.

A Scheme of WT SASS6 locus and the targeted SASS6 allele obtained by rAAV-mediated homologous recombination. Black rectangles represent exons and black lines introns. After insertion of the EGFP coding sequence and the G418 resistance cassette, cells were selected by addition of G418 in the medium. Then, the neomycin cassette was removed using an adenovirus-expressed Cre recombinase (Vector Biolabs, Malvern, PA, USA), and clones were isolated by serial dilution (loss of resistance to G418 was confirmed by replica plating). Finally, positive cells were confirmed by fluorescence microscopy.

B Western blot analysis of parental RPE-1 and Sas-6-EGFP-expressing RPE-1 cells. Protein extracts were probed with anti-Sas-6 (left panel) and anti-GFP (right panel) antibodies. Histogram shows quantification of the numbered bands. Results presented are means ± SEM. Band intensity for WT alleles was set to 100%.

C Maximum projection fluorescence microscopy image showing asynchronously growing RPE-1 cells expressing Sas-6-EGFP. Scale bar: 10 μm. The inset shows an enlargement of the boxed interphase centrosome.

D Efficacy of cell cycle synchronization was demonstrated by subjecting cell lysates to Western blotting with the indicated antibodies against cell cycle markers.