Expanded View Figures

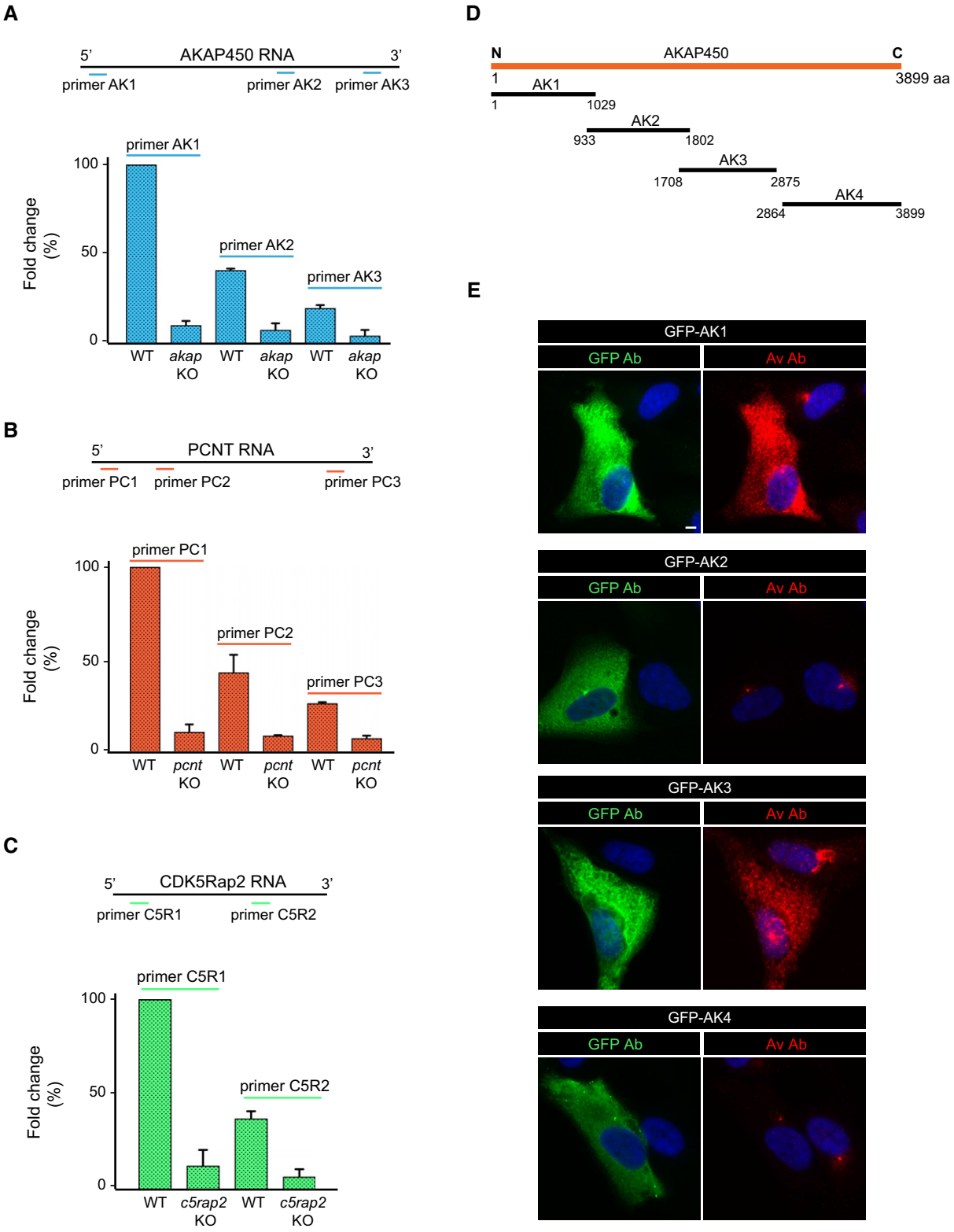

Figure EV1.

Figure EV1. Real-time RT–PCR quantification of mRNAs of AKAP450, PCNT, and CDK5Rap2 in knock-out cell lines and characterization of the anti-AKAP450 Av antibody.

A–C qPCR analysis of AKAP450 (A), PCNT (B), and CDK5Rap2 (C) mRNA expression in both WT and KO cell lines. For AKAP450 and PCNT, three pairs of primers specific to different regions of mRNAs were used while two pairs of primers were used for CDK5Rap2 (as indicated). mRNA levels normalized to GADPH were then referred to the respective mRNA level of WT cells amplified with the most 5′ pair of primers. Data are expressed as the mean ± SD of two independent experiments (three replicates each).

D Schematic diagram showing the position of AKAP450 truncated mutants used in (E). Numbers represent amino acid positions in the full-length protein.

E RPE-1 cells transfected with plasmids coding for different AKAP450 deletion mutants fused to GFP (as indicated). Merged images of cells labeled for GFP (green) or AKAP450-Av (red) and DAPI are shown. Scale bars, 5 μm.

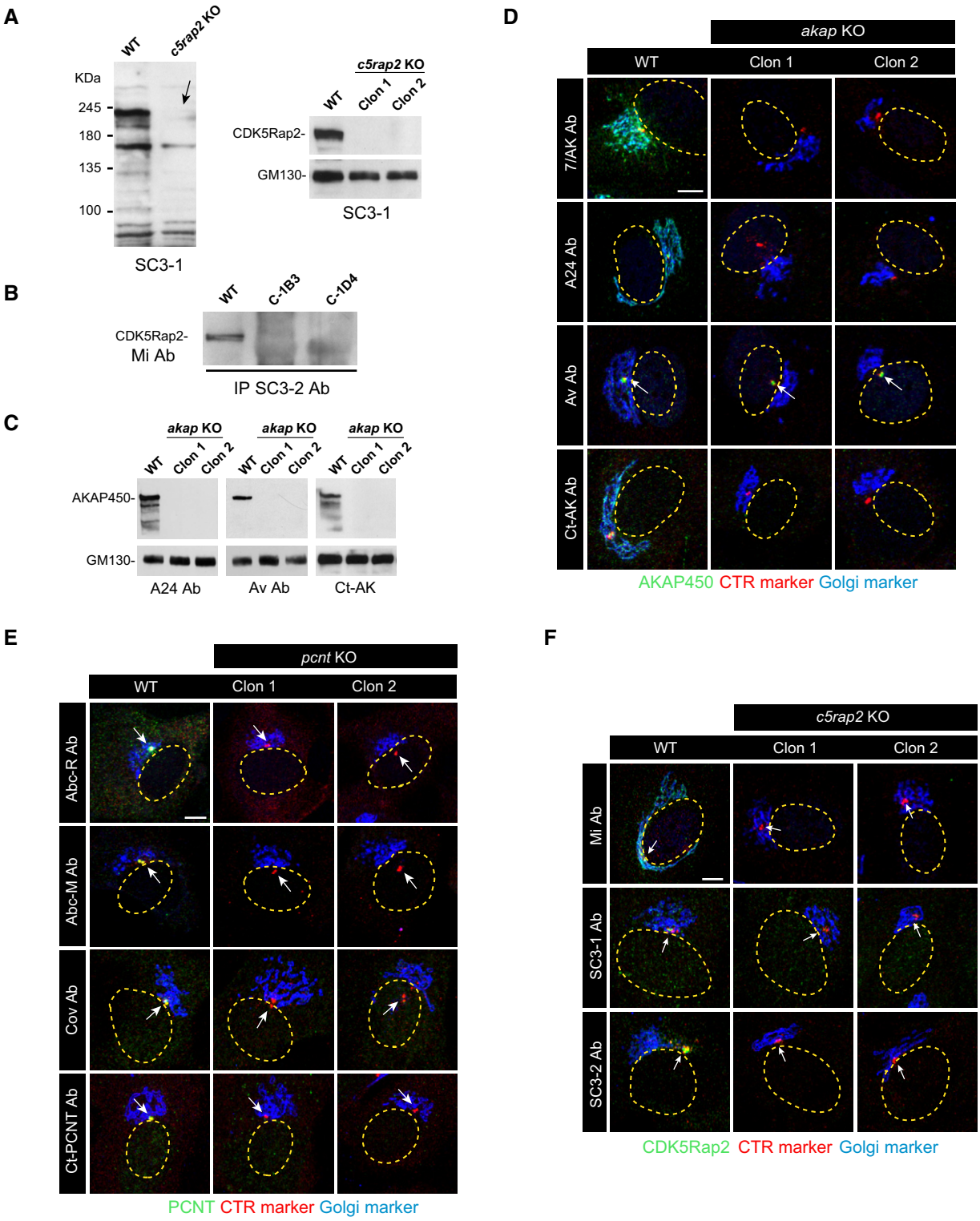

Figure EV2.

Figure EV2. Further characterization of *akap9*, *cdk5rap2*, and *pcnt* knock-out cell lines.

A WB characterization of the anti-CDK5Rap2 antibody SC3-1 used in this study (left). A representative WB of RPE-1 WT cells and the two selected *c5rap2* knock-out clones probed with the SC3-1 antibody is shown at right. The arrow points to the specific band.

B Immunoprecipitation of cell extracts from WT and *c5rap2* KO cell lines using the anti-CDK5Rap2 antibody SC3-2. Beads were tested for the presence of CDK5Rap2 by WB with the anti-CDK5Rap2 (Mi) antibody.

C Representative WBs of RPE-1 WT cells and the two selected *akap9*-mutated clones probed with three anti-AKAP450 antibodies (A24, Av, and Ct-AK) as indicated.

D–F Confocal IF images of RPE-1 WT cells and RPE-1 *akap* KO (D), *pcnt* KO (E), and *c5rap2* KO (F) cells with different antibodies as indicated. Arrows point to centrosomes.

Data information: GM130 was used as a loading control in (A and C). The yellow dashed line indicates the nucleus contour. Scale bars: 5 μm.

Source data are available online for this figure.

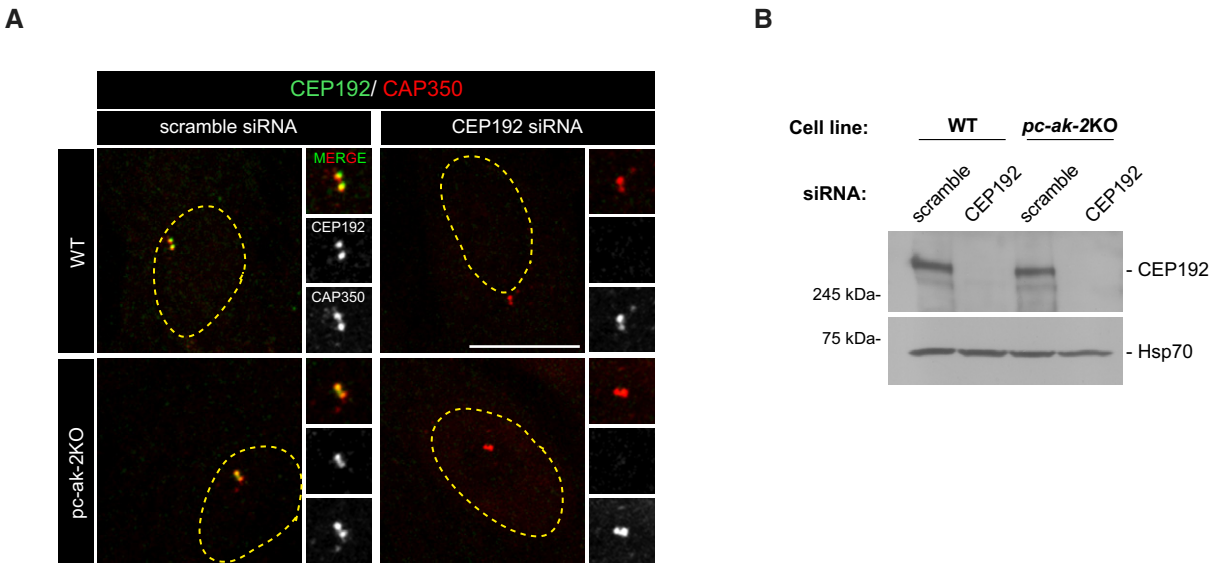

Figure EV3. Efficiency of CEP192 siRNAs.

A IF images of WT and *pc-ak*-2KO RPE-1 cells transfected with either scramble or CEP192 siRNAs as indicated. Cells were double-stained for CEP192 and CAP350 as a centrosomal marker. High-magnification images of centrosomes are shown at right. The yellow dashed line indicates the nucleus contour. Scale bars: 5 μm.

B Representative WB of WT and *pc-ak*-2KO cells transfected with either scramble or CEP192 siRNAs and revealed with the anti-CEP192 antibody. Hsp70 was used as loading control.

Source data are available online for this figure.

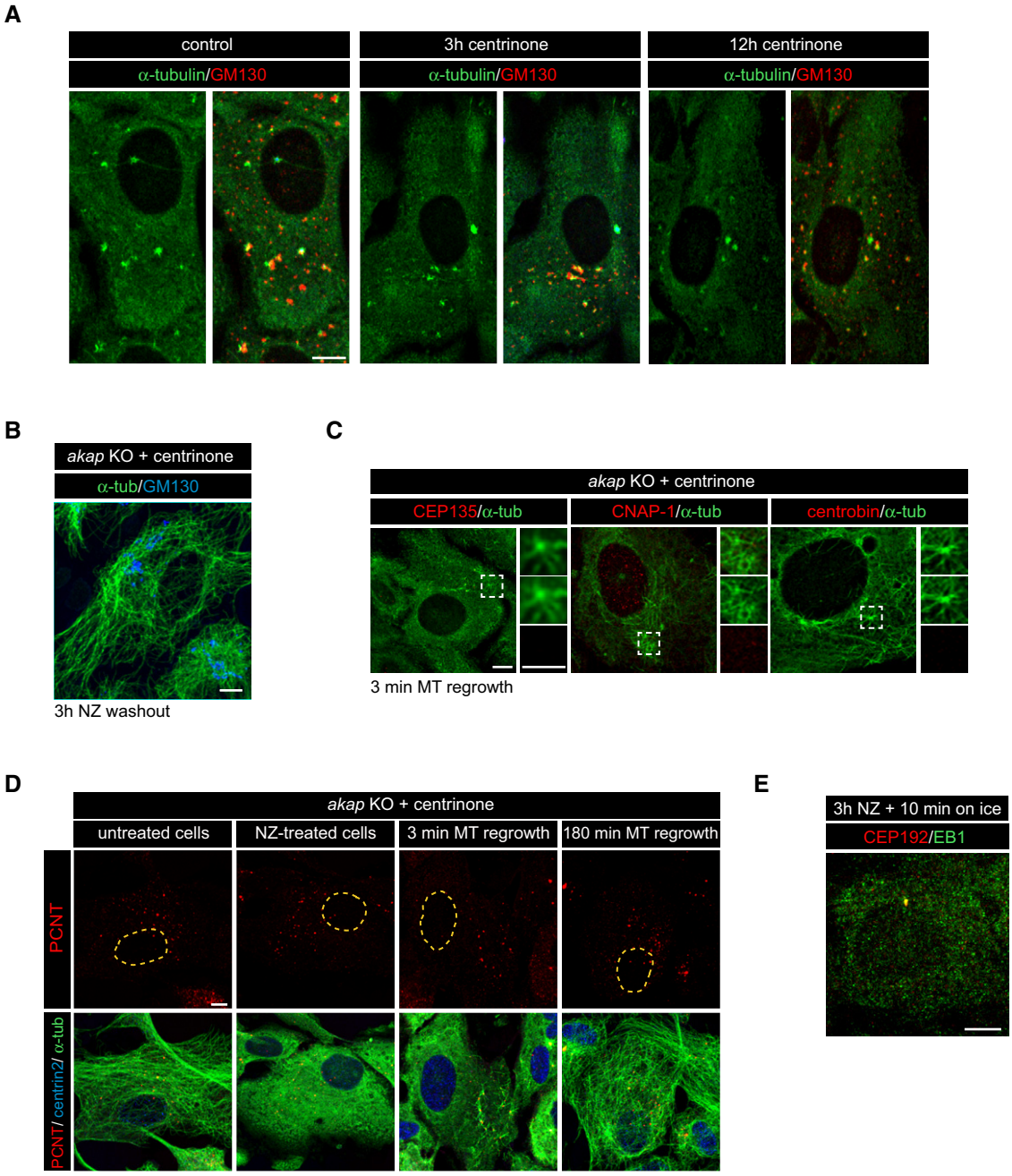

Figure EV4. Further characterization of centrinone-treated cells.

A Non-treated, 3-h centrinone-treated or 12-h centrinone-treated RPE-1 cells were subjected to a MT regrowth assay, fixed 3 min after drug washout, and double-labeled for α-tubulin and GM130.

B Complete MT network recovery of *akap* KO centriole-less centrinone-treated cells 3 h after removal of NZ. Cells were stained with anti-α-tubulin and anti-GM130 antibodies.

C Centrinone-treated *akap* KO cells were subjected to a MT regrowth assay and double-stained with the indicated antibodies. High magnifications of the boxed areas are shown as merged images (top) or as individual green or red labelings (middle and bottom panels).

D Centrinone-treated *akap* KO cells were treated with NZ and, at the indicated time points after NZ washout, fixed, and labeled for α-tubulin (green), PCNT (red), and centrin 2 as a centrosomal marker (blue). Single labeling images for PCNT are shown at the top and the corresponding merged images at the bottom.

E RPE-1 cells treated with NZ for 3 h and incubated for 10 min on ice after NZ removal were double-labeled for EB1 and CEP192. No EB1 signal was visible throughout the cytoplasm demonstrating the complete depolymerization of MTs under these conditions.

Data information: Scale bars: 5 μm (except in A, scale bar: 7.5 μm).

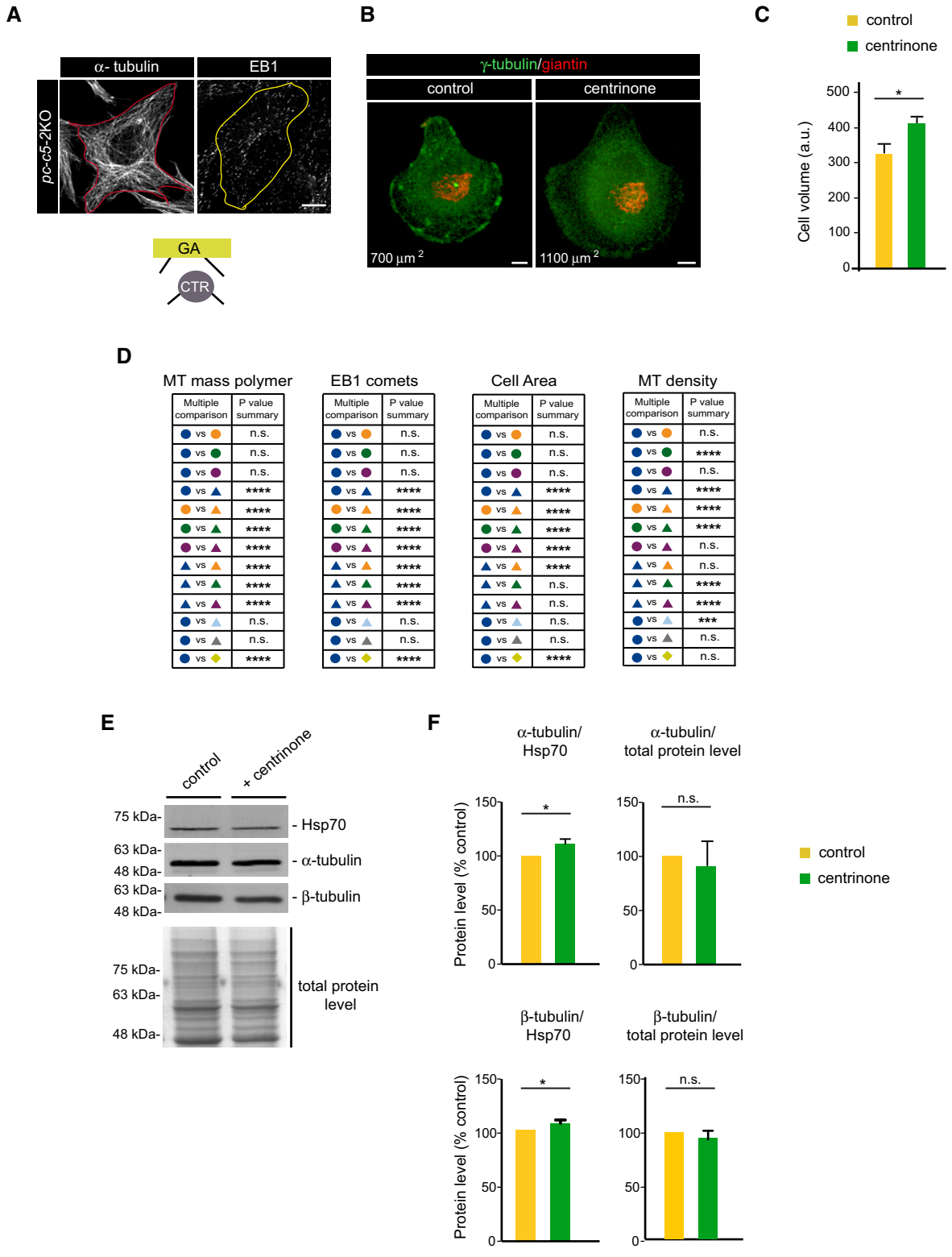
A
α- tubulin
EB1
pc-c5-2KO
GA
CTR
B
γ-tubulin/giantin
control
centrinone
700 µm 2
1100 µm 2
C
control
centrinone
Cell volume (a.u.)
500
400
300
200
100
0
*
D
MT mass polymer
EB1 comets
Cell Area
MT density
Multiple comparison
P value summary
E
control
+ centrinone
75 kDa-
63 kDa-
48 kDa-
- Hsp70
- α-tubulin
- β-tubulin
total protein level
F
α-tubulin/ Hsp70
α-tubulin/ total protein level
β-tubulin/ Hsp70
β-tubulin/ total protein level
Protein level (% control)
150
100
50
0
*
n.s.
control
centrinone

Figure EV5.

Figure EV5. Impact of centrinone treatment on cell size and overall MT network.

A Confocal images showing MT network in *pc-c5-2*KO RPE-1 cells fixed and stained with antibodies against α-tubulin (left) or EB1 (right). The red and yellow lines indicate the contour of the cell. A schematic representation of active MTOCs is shown at the bottom. Scale bars, 5 μm.

B Examples of control and centrinone-treated cells plated on individual crossbow-shaped micropatterned coverslips and labeled for γ-tubulin (green) and giantin (red). Numbers in μm^2 represent the approximate cell area depending on the pattern size. Scale bars, 5 μm.

C Quantitative analysis of cell volume by FACS analysis in control and centrinone-treated RPE-1 cells. Arbitrary units (a.u.) in forward scatter (FSC-H). Bars represent mean values ± SD of three independent experiments.

D Summary tables showing the most relevant data about statistical significance in the multiple comparisons test run for experimental data shown in Fig 7C–E.

E Control and centrinone-treated RPE-1 cell extracts (30 μg) were analyzed by WB with the indicated antibodies. At the bottom, a similar amount of cell extract was loaded onto gels and stained with Coomassie Blue for total protein normalization.

F Bar charts show α-tubulin (top) and β-tubulin (bottom) levels quantified and normalized against Hsp70 levels or against total protein levels, from three WB experiments as that showed in (E). Error bars represent mean values ± SD.

Data information: For statistical analysis in (D), one-way ANOVA followed by Tukey's multiple comparisons test was employed, and for statistical analysis in (C and F), we used unpaired two-tailed Student's *t*-test. *$P < 0{,}05$; ***$P < 0.001$; ****$P < 0.0001$; n.s., non-significant.

Source data are available online for this figure.