Yang et al., https://doi.org/10.1083/jcb.201701024

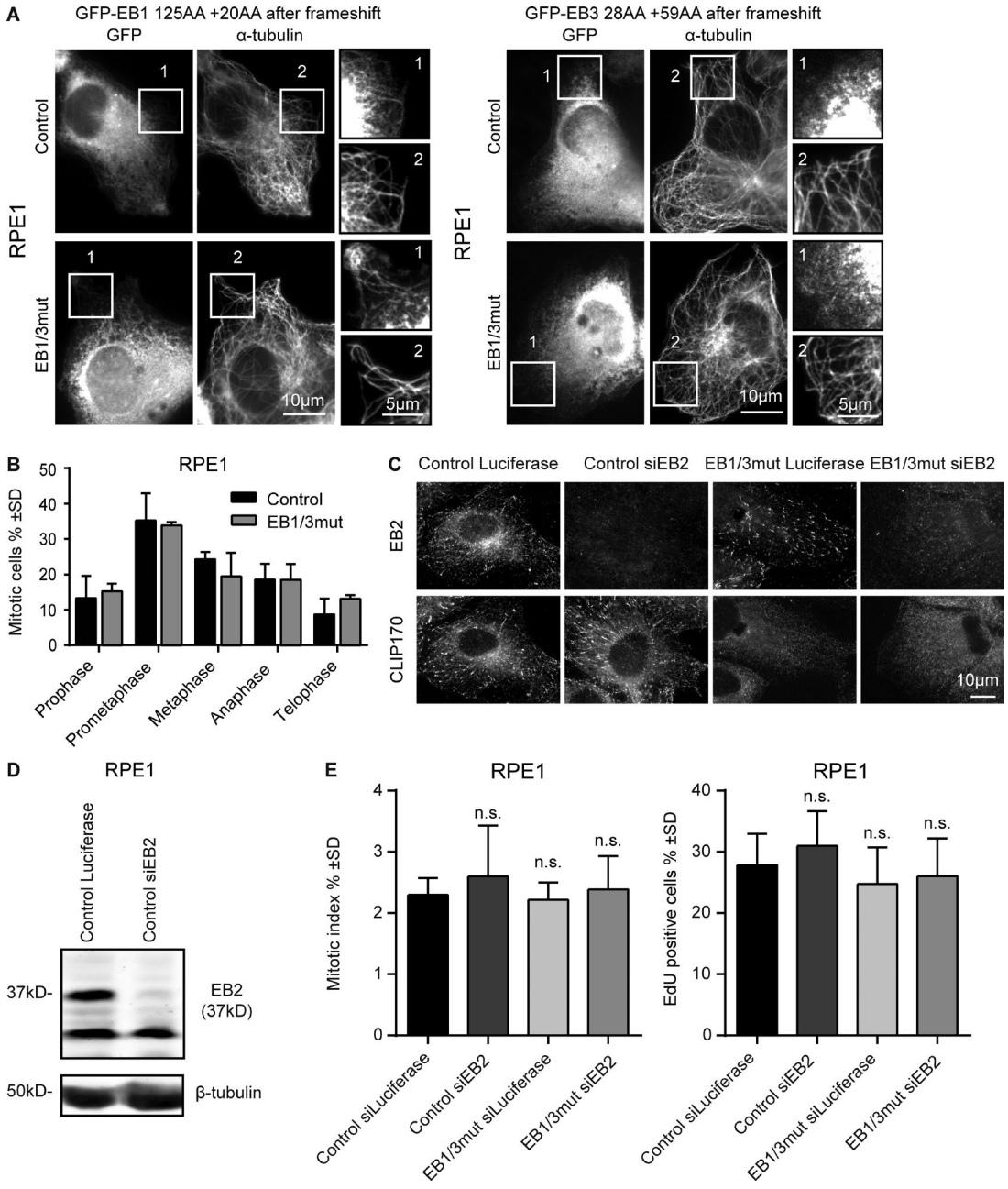

Figure S1. **Characterization of cell division after disruption of EB-encoding genes in RPE1 cells.** (A) Immunostaining for α-tubulin in control and EB1/3mut RPE1 cells expressing EB1 and EB3 fragments, which would result from the missense mutations observed in EB11/3mut RPE1 cells. Enlarged images of the boxed areas indicated by numbers are shown on the right. (B) Quantification of mitotic stages in control and EB1/3mut RPE1cells. Three independent experiments with 3,000 cells per condition were analyzed. No significant differences were detected (Student's *t* test). (C) Immunostaining for EB2 and CLIP170 in control and EB1/3mut RPE1 cells transfected with the indicated siRNAs. (D) Western blot illustrating EB2 expression in RPE1 cells transfected with siRNAs against luciferase (control) or EB2. (E) Quantification of the mitotic index and EdU-positive RPE1 control or EB1/3mut cells transfected with the indicated siRNAs. $n \geq 3$ experiments, with 1,000 cells per experiment. n.s., $P > 0.05$ (Mann-Whitney *U* test).

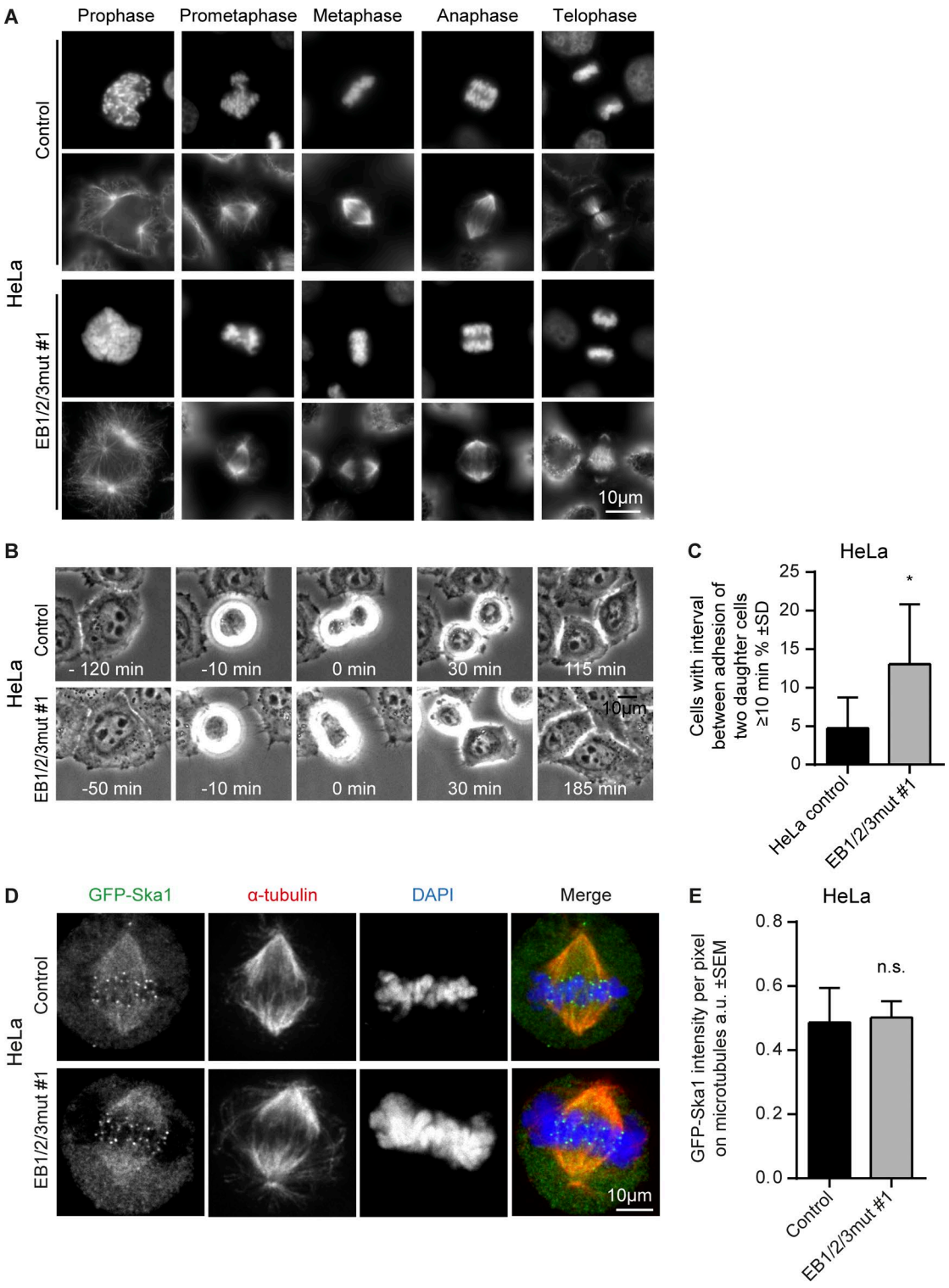

Figure S2. **Characterization of cell division after disruption of EB-encoding genes in HeLa cells.** (A) Immunostaining for α-tubulin and DAPI in control and EB1/2/3mut HeLa cells at different stages of mitosis. (B) Selected frames of phase-contrast live cell imaging of control cells and EB1/2/3mut HeLa cells. 0 min corresponds to the first frame in anaphase. (C) Quantification of the percentage of cells displaying a more than 10 min time interval between the adherence of the two daughter cells after mitosis in control and EB1/2/3mut HeLa cells. 160 and 495 cells were analyzed; *, $P < 0.05$ (Mann-Whitney *U* test). (D) Immunostaining for α-tubulin and DAPI in control and EB1/2/3mut HeLa cells expressing GFP-Ska1 construct. (E) Quantification of intensity of GFP-Ska1 on the spindle microtubules in control and EB1/2/3mut HeLa cells. $n > 30$; n.s., $P > 0.05$ (Mann-Whitney *U* test).

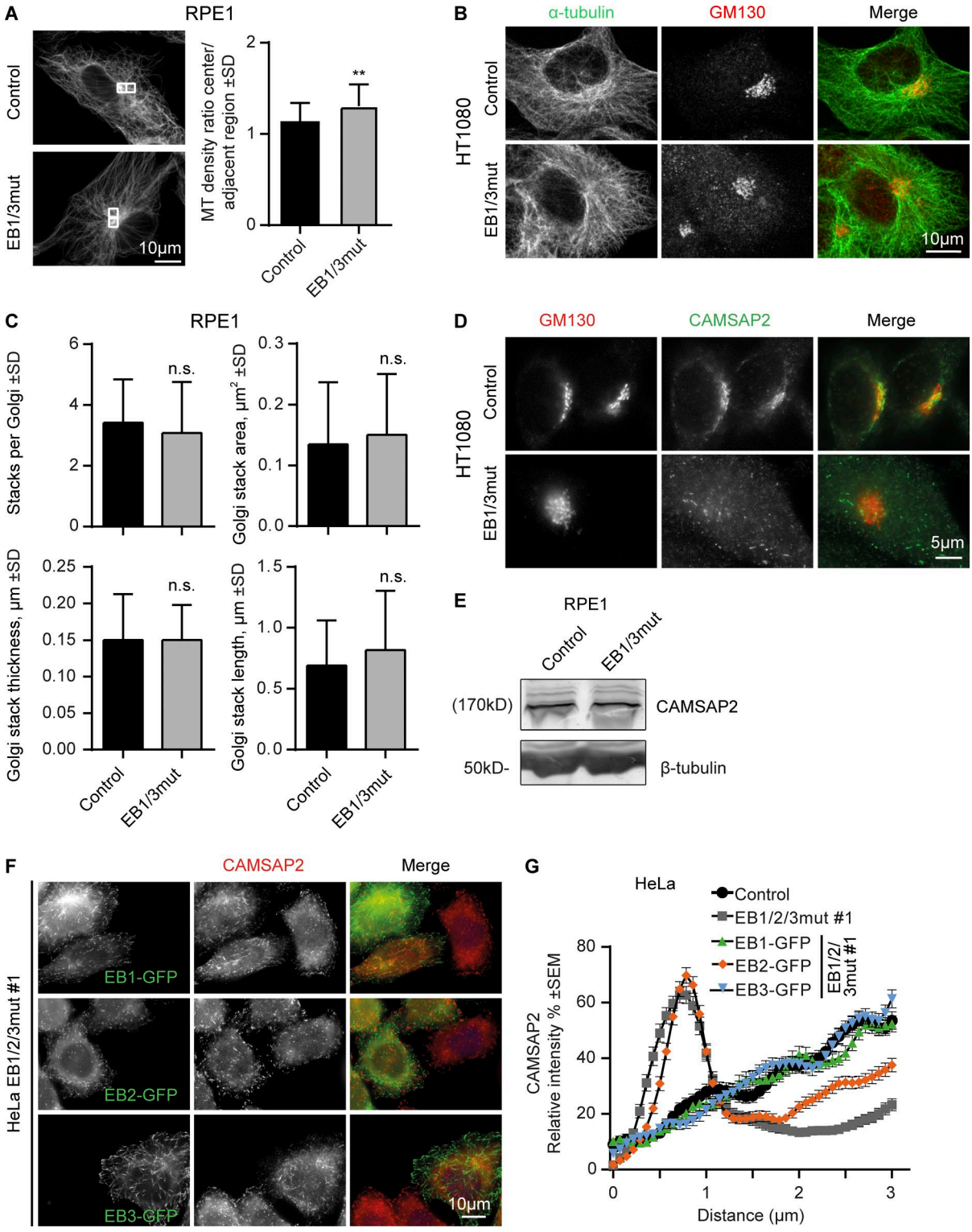

Figure S3. **Effects of EB disruption on MT and Golgi organization and the distribution of CAMSAP2-decorated MT minus ends.** (A) Immunostaining and quantification of the ratio of MT density around the centrosome and an adjacent cell region in the indicated RPE1 cells. 30 control and 35 EB1/3mut cells were analyzed. **, $P < 0.01$ (Mann-Whitney *U* test). (B and D) Immunostaining for GM130 and α-tubulin (B) or CAMSAP2 (D) in control and EB1/3mut HT1080 cells. (C) Quantification of Golgi stack number, Golgi stack area, thickness and length measured in EM images. 28 images of control and EB1/3mut RPE1 cells were analyzed. (E) Western blot analysis of CAMSAP2 expression in control and EB1/3mut PRE1 cells. (F) Immunostaining for CAMSAP2 in EB1/2/3mut HeLa cells expressing the indicated constructs. (G) Quantification the relative intensity of CAMSAP2 staining within a 3-μm distance from the cell edge (as depicted by yellow arrows in Fig. 5 A) in control and EB1/2/3mut cells expressing the indicated constructs. Ninety to 105 cells were analyzed per condition.

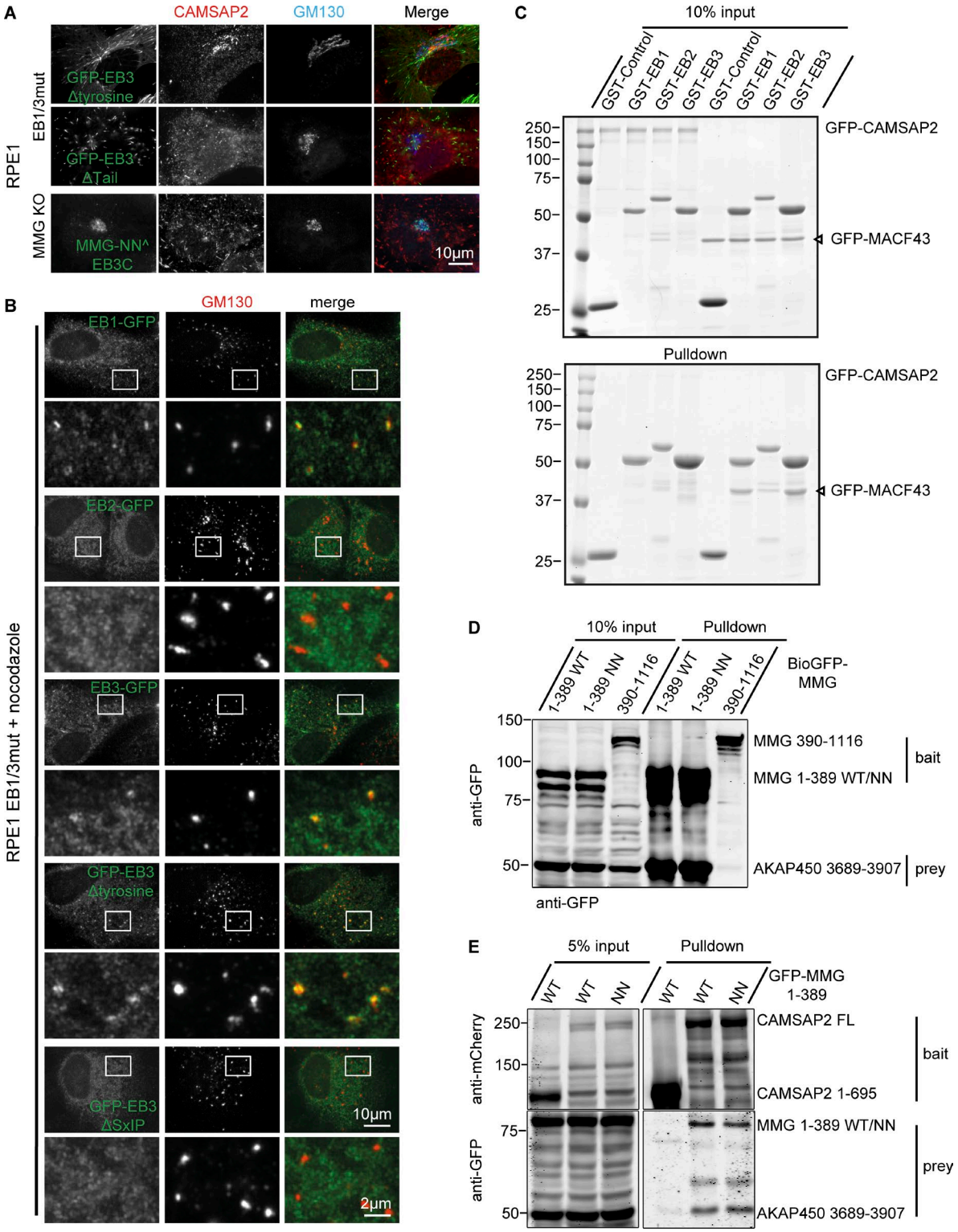

Figure S4. **Recruitment of EBs and CAMSAP2 to the Golgi and biochemical characterization of the interactions between AKAP450, MMG, CAMSAP2, and EB1.** (A) Immunostaining for CAMSAP2 and GM130 in EB1/3mut and MMG knockout RPE1 cells expressing the indicated constructs. (B) Immunostaining for GM130 in EB1/3mut RPE1 cells transfected with the indicated GFP-tagged constructs and treated with 14 µM nocodazole for 3 h. Enlarged portions of the boxed areas are shown below each row of panels. (C) Coomassie blue–stained gel of GST-EB pull down assays with purified GFP-CAMSAP2 and GFP-MACF43. GFP-MACF43 serves here as a positive control; it is the C-terminal 43 amino acid long peptide of MACF2 with a single SxIP motif, dimerized by the addition of a leucine zipper (Honnappa et al., 2009). (D) Streptavidin pull-downs with the extracts of HEK293T cells coexpressing Bio-GFP-myomegalin (MMG) 1–389 WT, Bio-GFP-myomegalin 1–389 NN, or Bio-GFP-myomegalin 390–1116 together with GFP-AKAP450 3689–3907 and BirA, analyzed by Western blotting with anti-GFP antibodies. (E) Streptavidin pull-down with the extracts of HEK293T cells coexpressing Bio-mCherry-CAMSAP2 1–695 or full length (FL) together with GFP-AKAP450 3689–3907, GFP-myomegalin 1–389 WT, or the LP/NN mutant and BirA, analyzed by Western blotting with antibodies against GFP or mCherry.

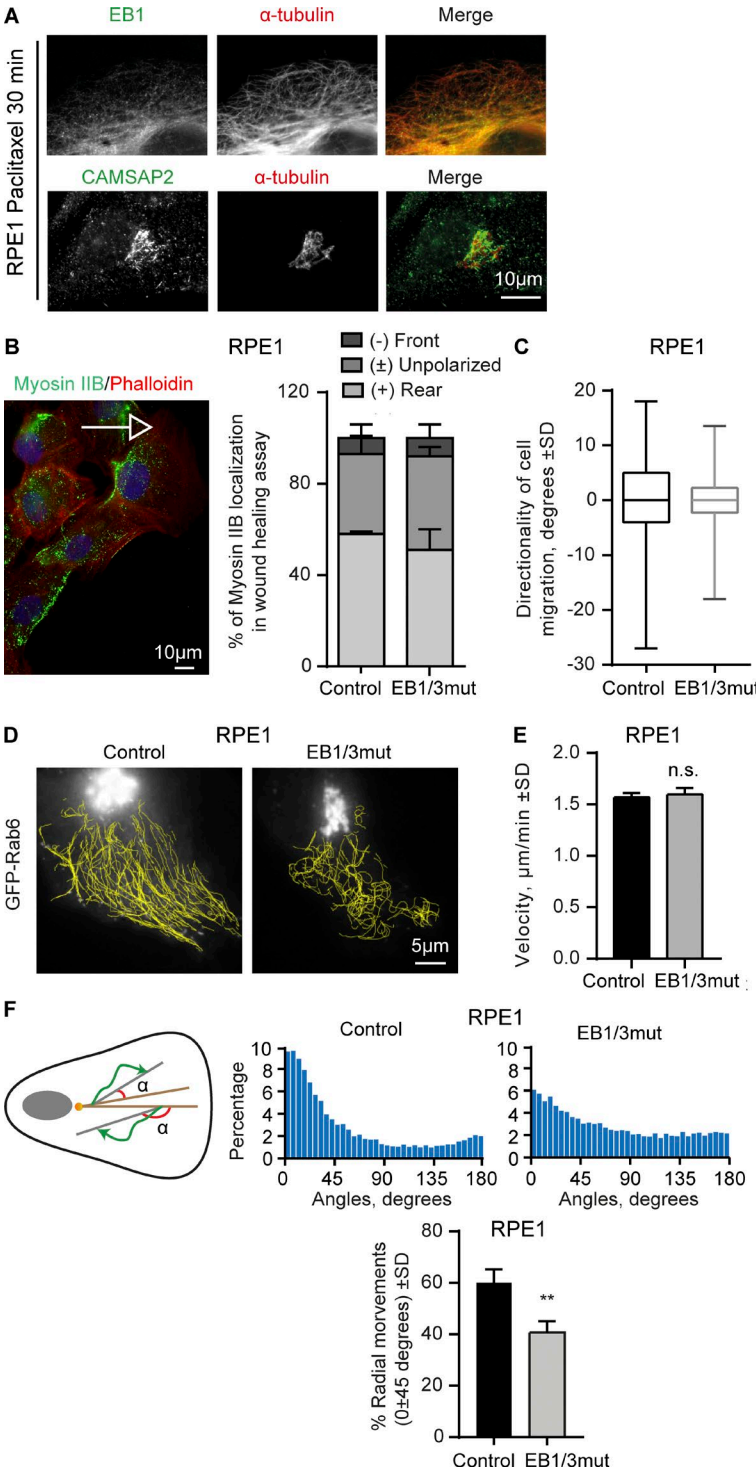

Figure S5. **Polarized distribution of Myosin IIB and the directionality of cell migration are not perturbed in EB1/3mut RPE1 cells.** (A) Immunostaining for EB1 and α-tubulin (top) or CAMSAP2 and GM130 (bottom) in RPE1 cells treated with 5 μM paclitaxel for 30 min. (B) Immunostaining for myosin IIB and F-actin (phalloidin) in a monolayer wound healing assay and quantification of myosin IIB localization relative to the monolayer wound. 196 control and 195 EB1/3mut RPE1 cells were analyzed. No significant differences were observed (Mann-Whitney *U* test). (C) Quantification of movement directionality during migration in sparse control and EB1/3mut RPE1 cultures. The angle between the vectors of cell displacement was measured in three subsequent frames. 0°, movement vectors were parallel between frames 1 and 2, and frames 2 and 3. Positive angle: counterclockwise deviation from 0°; negative angle: clockwise deviation from 0°. 51 control and 60 EB1/3mut RPE1 cells were analyzed. No significant differences were observed (Mann-Whitney *U* test). (D) Representative images of GFP-Rab6A (labeled as GFP-Rab6) vesicle tracks in control and EB1/3mut RPE1 cells. (E) Velocity of GFP-Rab6 vesicle movements. $n = 3$ experiments, representing in total 10,000 vesicle tracks in 10–12 cells per experiment. (F) Quantification of the radiality of GFP-Rab6 tracks in control and EB1/3mut RPE1 cells. Track angles range from 0° to 180°, where 0° corresponds to a particle moving along the radius away from the reference point (from the center of the Golgi to the cell periphery), and 180° corresponds to a particle moving radially toward the cell center. Percentage of tracks with the angle between 0° and 45° is shown on the right. $n = 3$ experiments, with 10–12 cells per experiment. n.s., $P > 0.05$; **, $P < 0.01$ (Mann-Whitney *U* test).

Provided online as a PDF is table S1, which shows mutations in EB genes from different clones.

References

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