Muroyama et al., http://www.jcb.org/cgi/content/full/jcb.201601099/DC1

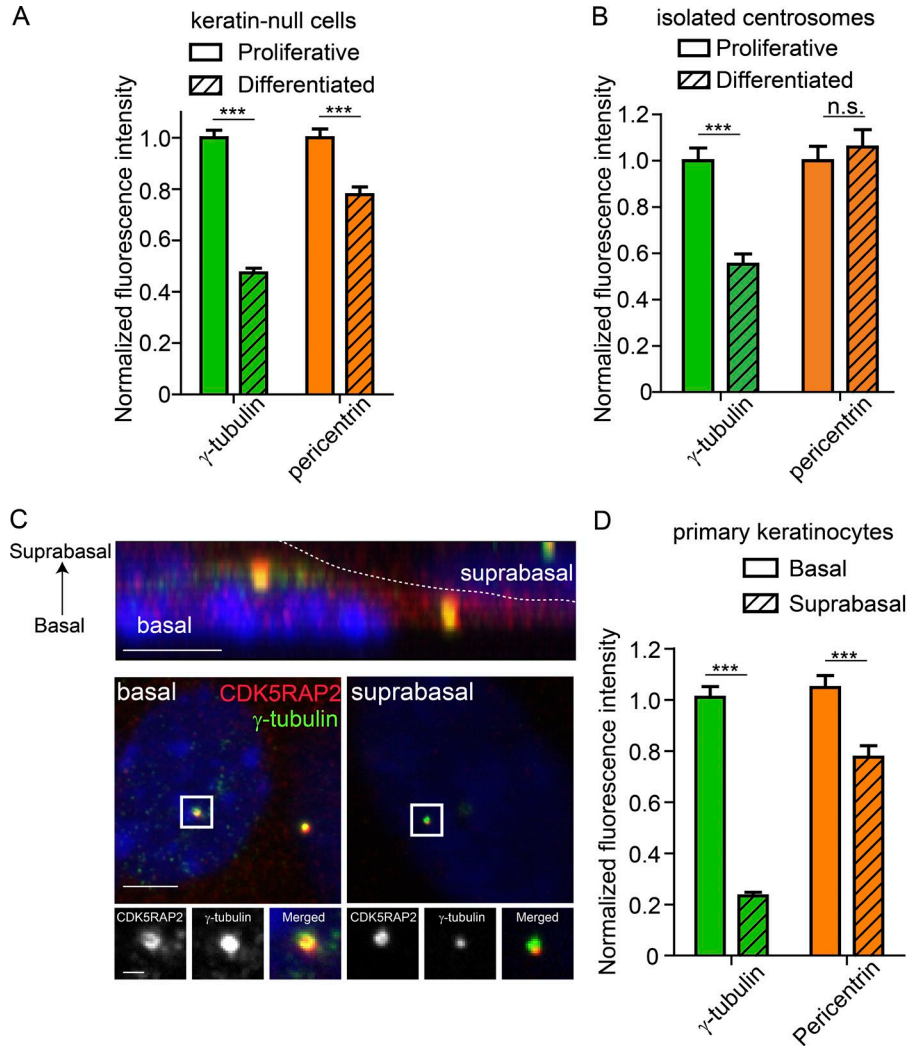


Figure S1. **Analysis of centrosomal proteins in cultured cells and after centrosome purification.** (A) Quantification of centrosomal γ-tubulin and pericentrin levels in proliferative and differentiated keratin-null cells (n = 150 centrosomes each from three independent experiments). (B) Quantification of γ-tubulin and pericentrin levels in isolated centrosomes obtained from either proliferative or differentiated keratin-null cells (n = 50 centrosomes each). (C) The top panel is an XZ image of a culture of primary keratinocytes showing a basal cell and a suprabasal cell. Bar, 5 µm. The bottom panel shows XY images of basal and suprabasal cells stained for CDK5RAP2 and γ-tubulin. Bar: 5 µm; (insets) 1 µm. (D) Quantification of γ-tubulin and pericentrin levels at the centrosome in basal and suprabasal cells from primary cultures of keratinocytes ($n \geq 94$ centrosomes from two independent experiments). n.s., not significant; ***, $P < 0.001$. Data are presented as mean ± SEM.

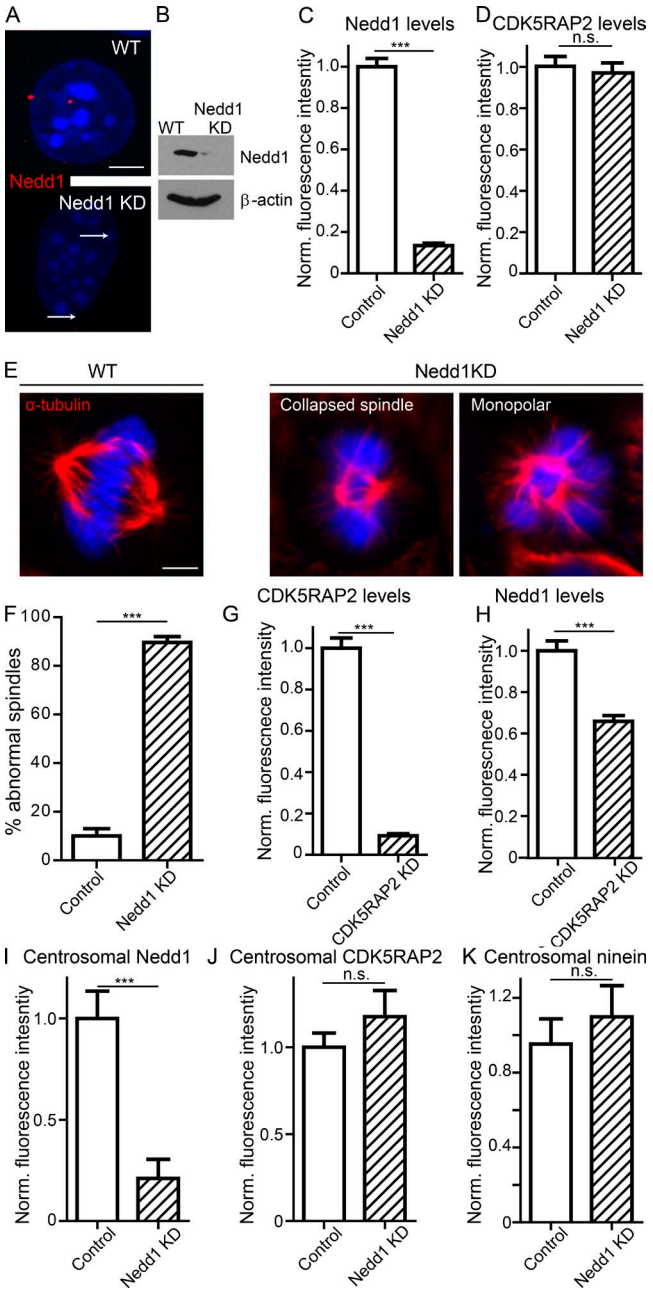
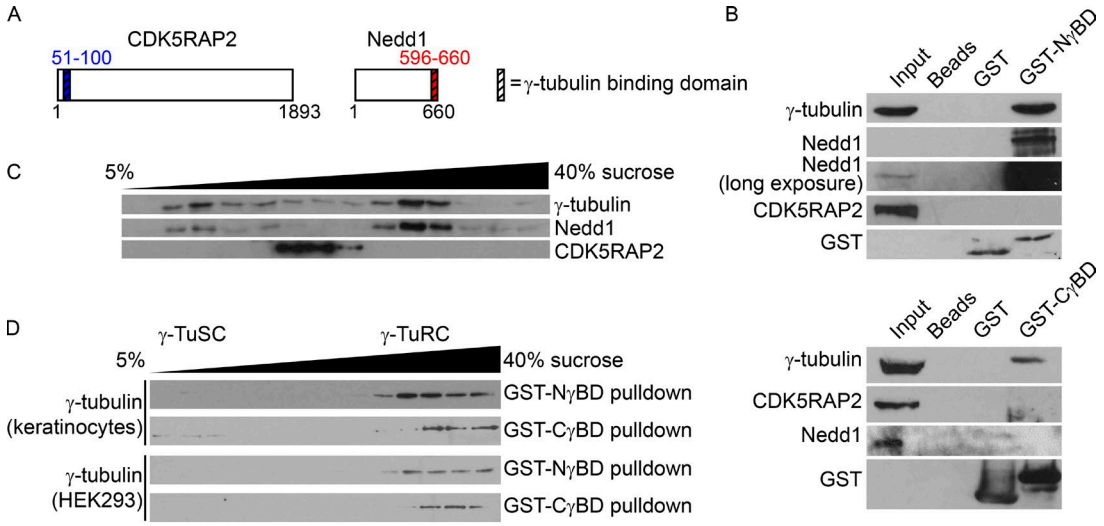


Figure S2. **Validation of Nedd1 and CDK5RAP2 knockdowns.** (A) Immunofluorescence analysis of Nedd1 in WT and Nedd1 KD cells. Arrows indicate centrosomes. Bar, 5 µm. (B) Western blot of Nedd1 levels in lysates prepared from WT and Nedd1 KD cells. (C) Quantification of centrosomal Nedd1 levels in control and Nedd1 KD cells (n = 100 centrosomes from two independent experiments). (D) Quantification of centrosomal CDK5RAP2 levels in control and Nedd1 KD cells (n = 100 centrosomes from two independent experiments). (E) Images of mitotic figures from WT and Nedd1 KD cells. Bar, 5 µm. (F) Quantification of percentage of cells with abnormal mitotic spindles in control and Nedd1 KD cells (n = 3 independent experiments with 50 cells per experiment). (G) Quantification of centrosomal CDK5RAP2 levels in control and CDK5RAP2 KD cells ($n \geq 62$ centrosomes from two independently derived cell lines). (H) Quantification of centrosomal Nedd1 levels in control and CDK5RAP2 KD cells ($n \geq 60$ centrosomes from two independently derived cell lines). (I–K) Nedd1 (I), CDK5RAP2 (J), and ninein (K) levels in centrosomes isolated from control and Nedd1 KD cells. n.s., not significant; ***, $P < 0.001$. Data are presented as mean ± SEM.



E

Protein	Unique peptides	Total Spectrum Count	% Protein Coverage
Tubulin gamma-1 chain	40	150	93
Gamma-tubulin complex component 3	38	111	49
Gamma-tubulin complex component 2	26	81	38
Gamma-tubulin complex component 6	23	45	18
T-complex protein 1 subunit alpha	20	38	56
T-complex protein 1 subunit zeta	18	37	43
T-complex protein 1 subunit eta	17	38	41
T-complex protein 1 subunit epsilon	14	29	45
T-complex protein 1 subunit delta	10	24	33
T-complex protein 1 subunit beta	12	22	36
T-complex protein 1 subunit gamma	13	20	34
Actin, cytoplasmic 1	14	22	30
T-complex protein 1 subunit 1 theta	14	25	29
Gamma-tubulin complex component 4	11	22	27
Tubulin beta chain	11	16	33
Cytoplasmic dynein 1 heavy chain	10	11	2.9
Gamma-tubulin complex component 5	8	11	11
Tubulin alpha-1B chain	7	12	24
Nedd1	4	12	6.7
Mitotic-spindle organizing protein 2B	3	12	24
Nucleoside diphosphate kinase 7	6	9	26
RuvB-like 1	3	3	11
Mitotic-spindle organizing protein 2A	2	11	24
Mitotic-spindle organizing protein 1	2	3	33
Beta-centractin	1	2	2.9
RuvB-like 2	1	1	2.2

Figure S3. **CDK5RAP2 and Nedd1 form distinct complexes with γ-TuRCs.** (A) Diagrams of CDK5RAP2 and Nedd1 highlighting the positions of their γ-tubulin binding domains. (B) Pull-down assays using GST-NγBD and GST-CγBD. Associated proteins were analyzed by Western blotting for the indicated proteins. (C) Fractionated keratinocyte lysate showing migration of endogenous proteins. (D) The top panel shows pull-downs from keratinocyte lysate using either GST-NγBD or GST-CγBD centrifuged through a sucrose gradient and blotted for γ-tubulin. Note that both binding domains associate exclusively with γ-TuRC–sized complexes. The bottom panel shows pull-downs from HEK293 lysate using either GST-NγBD or GST-CγBD run through a sucrose gradient and blotted for γ-tubulin. Note the similarity to pull-downs from keratinocytes. (E) Mass spectroscopy analysis of Nedd1-γBD–associated γ-TuRCs. Note the presence of all known γ-TuRC components, but not CDK5RAP2.

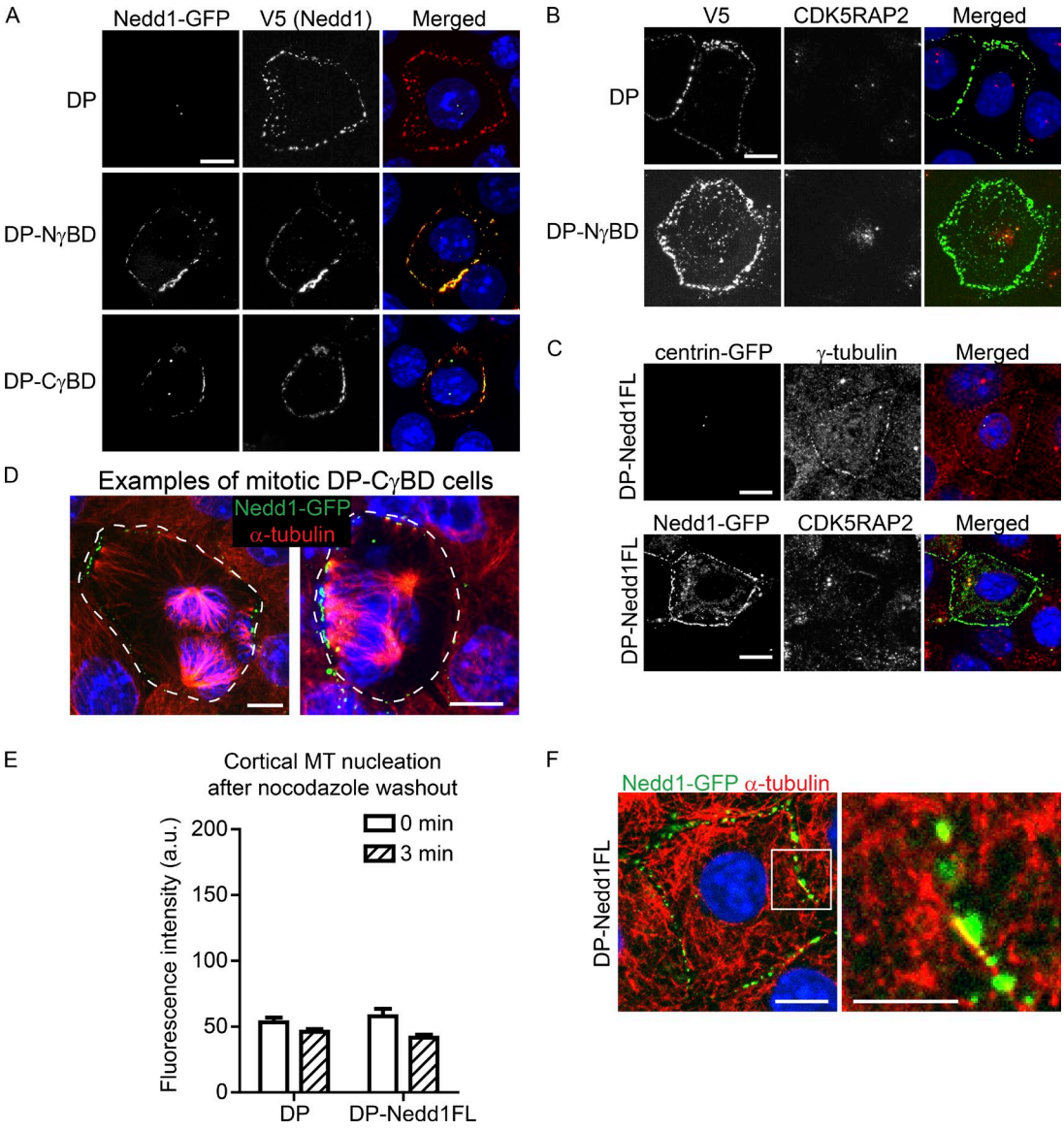


Figure S4. **Analysis of cells expressing cortically targeted CDK5RAP2 and Nedd1.** (A) Cells were transfected with DP (control), DP-NγBD, or DP-CγBD together with full-length Nedd1-GFP. Images show colocalization of Nedd1-GFP with either DP-NγBD or DP-CγBD. Bar, 10 µm. (B) Cells were transfected with DP (control) or DP-NγBD and then stained for V5 (marker of DP fusions) and CDK5RAP2. Note that there is no detectable accumulation of CDK5RAP2 with cortical Nedd1. (C) Cells transfected with DP fused to full-length Nedd1 (DP-Nedd1FL) recruit γ-tubulin (top), but not CDK5RAP2 (bottom), to cell junctions. Bar, 10 µm. (D) Images of cells transfected with DP-CγBD in mitosis where cortical sites became apparent spindle poles. Bar, 10 µm. (E) Quantification of the fluorescence intensity of α-tubulin at the cell cortex after nocodazole washout in control and DP-Nedd1FL–expressing cells ($n \geq 50$ cells from two independent experiments). Note that DP-Nedd1FL does not promote nucleation. (F) Image of a cell transfected with DP-Nedd1FL. Note the absence of cortically associated MTs. Bars: (main) 10 µm; (inset highlighting cortical region) 5 µm. Data are presented as mean ± SEM.

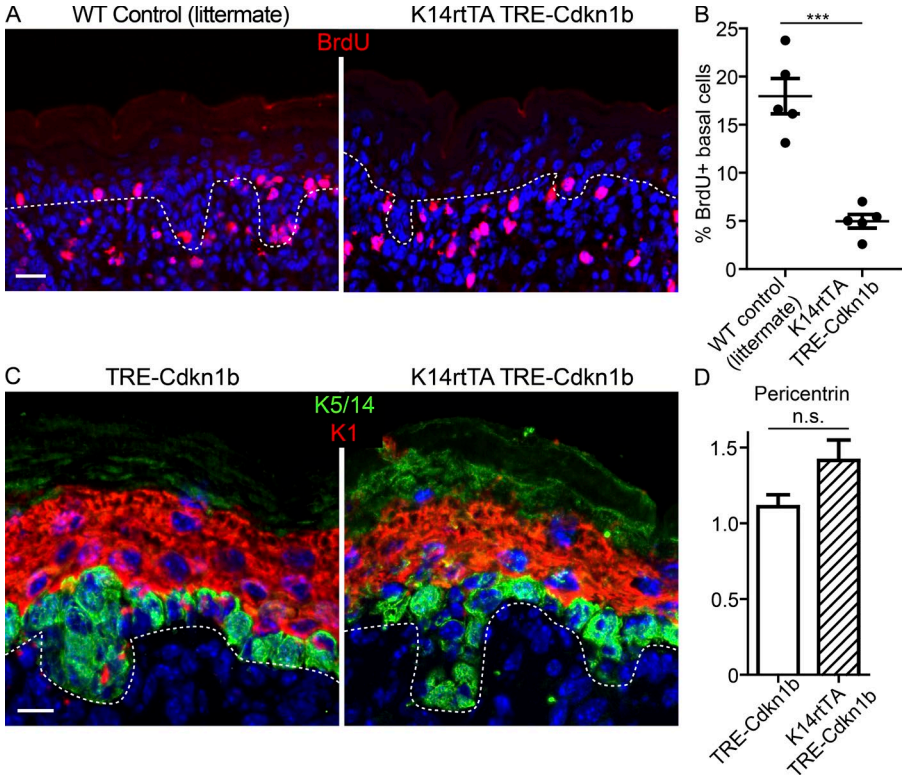
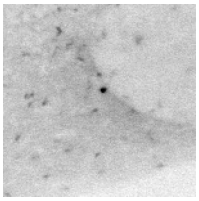
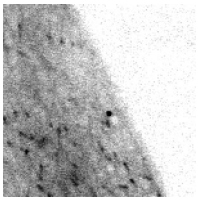


Figure S5. **Analysis of Cdkn1b-expressing epidermis.** (A) Representative images of backskins from K14rtTA TRE-Cdkn1b and control littermates, stained for BrdU incorporation. Bar, 20 µm. (B) Quantification of BrdU+ basal cells in K14rtTA TRE-Cdkn1b mice and control littermates (n = 5 mice each from two independent litters). (C) Immunofluorescence analysis of proliferation markers (keratin 5/14, green), which marks basal cells, and differentiation markers (keratin 1, red). Note that K14rtTA TRE-Cdkn1b basal cells are keratin 5/14 positive and do not express keratin 1. Bar, 10 µm. (D) Quantification of the basal/dermal ratio of centrosomal pericentrin levels in control (TRE-Cdkn1b) and K14rtTA Tre-Cdkn1b mice (n = 2 mice each). n.s., not significant; ***, $P < 0.001$. Data are presented as mean ± SEM. Dashed lines indicate the basement membrane.



Video 1. **MT dynamics in proliferative keratinocytes.** Time-lapse of Eb1-GFP; centrin-GFP basal primary keratinocyte. The movie was acquired on a microscope (DMI6000; Leica Biosystems) at 37°C and 5% CO_2 using a 63× Plan-Apo 1.4–0.6 NA oil objective at one frame per second. Total movie time is 1 min.



Video 2. **MT dynamics in differentiated keratinocytes.** Time-lapse of Eb1-GFP; centrin-GFP suprabasal primary keratinocyte. The movie was acquired on a microscope (DMI6000; Leica Biosystems) at 37°C and 5% CO_2 using a 63× Plan-Apo 1.4–0.6 NA oil objective at one frame per second. Total movie time is 1 min.

Video 3. **MT dynamics at cortical sites in a DP-NγBD-expressing cell.** Time-lapse of Eb1-GFP in a keratinocyte expressing DP-NγBD. Region depicted is at the cortex of the cell. The movie was acquired on a microscope (DMI6000; Leica Biosystems) at 37°C and 5% CO_2 using a 63× Plan-Apo 1.4–0.6 NA oil objective at one frame per second. Total movie time is 1 min.

Video 4. **MT dynamics at cortical sites in a DP-CγBD-expressing cell.** Time-lapse of Eb1-GFP in a keratinocyte expressing DP-CγBD. Region depicted is at the cortex of the cell. The movie was acquired on a microscope (DMI6000; Leica Biosystems) at 37°C and 5% CO_2 using a 63× Plan-Apo 1.4–0.6 NA oil objective at one frame per second. Total movie time is 1 min.