Appendix

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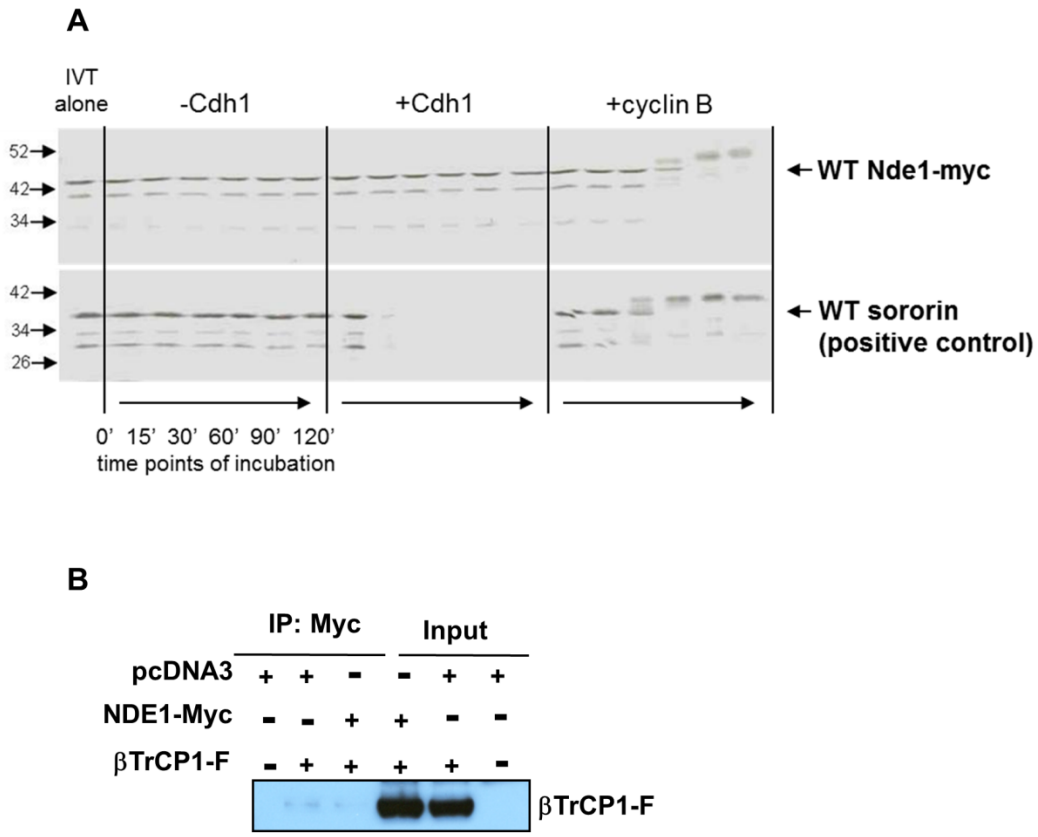
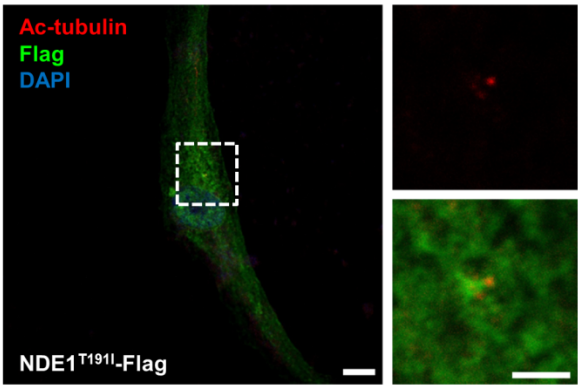


Appendix figure S1. NDE1 is not a substrate for APC/C-Cdh1 or β-TrCP1 targeted degradation.

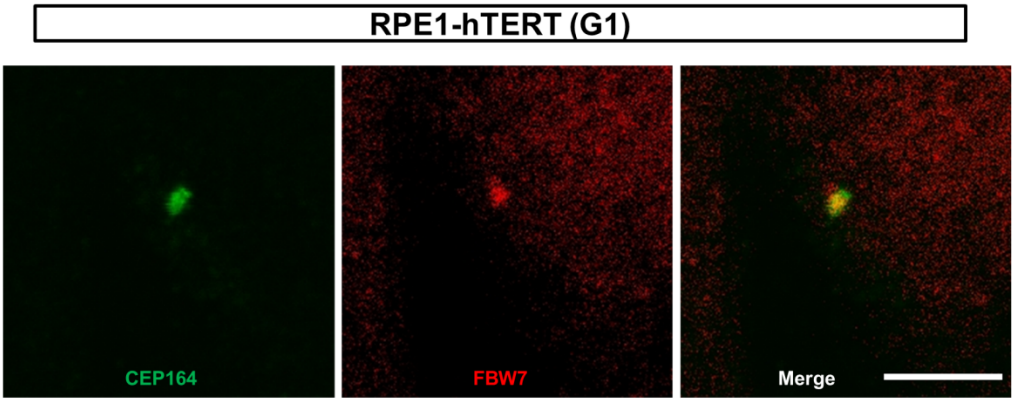
(A) In vitro-translated ^{35}S-labeled mouse NDE1 or Sororin (positive control) were added to interphase egg extract supplemented with or without recombinant Cdh1 (+Cdh1 or –Cdh1), or with recombinant non-degradable cyclin B (+CycB) to drive the extract into mitosis. Samples were collected at 0, 15, 30, 60, 90, and 120 minutes and analyzed by SDS-PAGE.

(B) HEK293T cells were transfected with pCDNA3 (lane 1), Flag-tagged β-TrCP1 (F-β-TrCP1, lane 2), or NDE1-myc plus F-β-TrCP1 (lane 3). NDE1-myc was immunoprecipitated with α-Myc and complexes were immunoblotted with α-Flag. Expression of F-β-TrCP1 in whole cell lysates is shown in lanes 4-6.

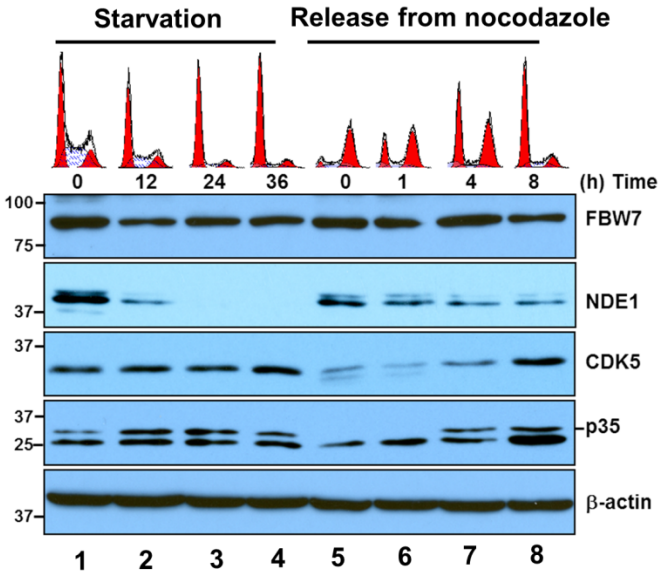


Appendix figure S2. Transfected Flag-tagged NDE1^{T191I} accumulates at the basal body and suppresses ciliary length.

RPE1-hTERT cells were transfected with Flag-tagged NDE1^{T191I} (NDE1^{T191I}-Flag) or 48 h and serum-starved for 24 h following transfection to allow cilia formation. Cilia were visualized using a mouse monoclonal antibody (611B) against acetylated tubulin (shown in red), whereas NDE1 was labeled by rabbit α-Flag (shown in green). Scale bar: 5 μm.

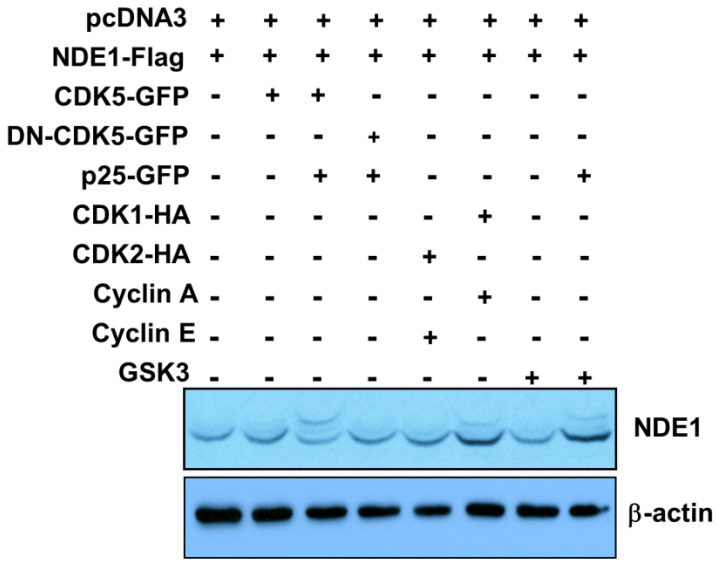


Appendix figure S3. FBW7 co-localizes with CEP164. RPE1-hTERT cells were serum-starved for 24h and stained with mouse α-FBW7 (red) and rabbit α-CEP164 (green). Scale bar: 10 μm.



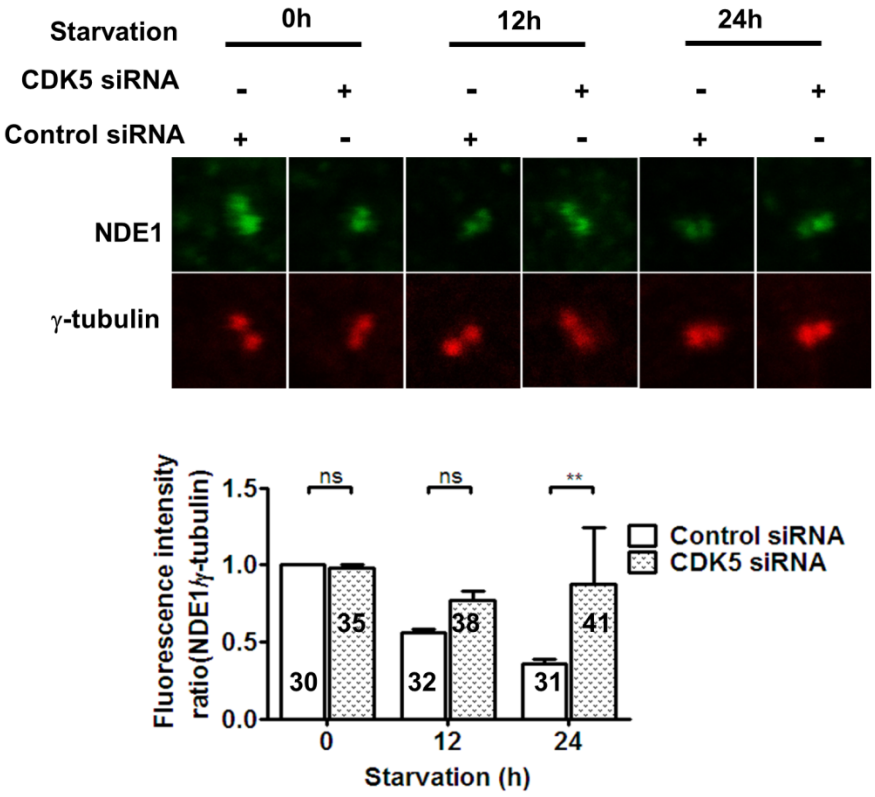
Appendix figure S4. NDE1 and CDK5 are reciprocally expressed in cycling cells.

NDE1 levels are reduced, whereas CDK5, and p35 levels are increased when cells are synchronized at G0/G1 or transit from mitosis to G1. BALB/C 3T3 cells were serum-starved for the indicated time points (lanes 1-4) or arrested in G2/M by nocodazole treatment 200 ng/ml for 18 h and then released in media containing 10% FBS to enter G1 (lanes 5-8). Whole cell lysates were prepared at the indicated time points following serum starvation or release from nocodazole and probed with FBW7, NDE1, CDK5, p35 or β-actin. Cell cycle profiles at all time-points were obtained by flow cytometry.



Appendix figure S5. Activated CDK5 phosphorylates NDE1.

HEK293T cells were transfected with indicated plasmids, total cell lysates were separated in SDS PAGE containing 50 μM Phos-tag™ reagent and immunoblotted using α-NDE1.



Appendix figure S6. Depletion of CDK5 minimizes downregulation of NDE1 at the centrosome/basal body of resting cells. Quantification of fluorescence intensity ratio of NDE1/γ-tubulin (green/red) signals at the centrosome at 0, 12 and 24 h serum starved of transfected cells with a scrambled (control) or CDK5-specific siRNA (CDK5 siRNA#1) as indicated. The range of fluorescence intensity per pixel in a box was 0-255 (total number of cells from 3 independent transfections is indicated on graph). Data represent mean ± SEM. One way ANOVA followed by Newman-Keuls post-test was used to determine significant difference among groups. **: $p<0.01$, ns indicates no significance.