Supplemental Materials

Molecular Biology of the Cell

Kim *et al*.

Supplemental Figure 1

IF consensus motif alignment

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TYRSLLEGEGS          K10, H. sapiens
TYRTLLEGEES          K1, H. sapiens
TYRKLLEGEEC          K5, H. sapiens
TYRRLLEGEDA          K14, H. sapiens
TYRKLLEGEES          Vimentin, H. sapiens
TYRKLLEGEES          Desmin, H. sapiens
AYRKLLEGEEE          Lamin C, H. sapiens
AYRKLLEGEEE          Lamin B, H. sapiens
AYRKLLEGEEE          Lamin A, H. sapiens
AYRKLLEGEET          Neurofilament-L, H. sapiens
AYRKLLEGEEC          Neurofilament-H, H. sapiens
AYRKLLEGEET          Neurofilament-M, H. sapiens
TYRKLLEGEEN          GFAP, H. sapiens
TYRTLLEAENS          Nestin, H. sapiens
AYRKLLEGEET          Internexin, H. sapiens

AYDKLLVGEEA          Lamin D, D. melanogaster
AYDKLLVGEEA          Lamin C, D. melanogaster
AYDKLLVGEEA          Lamin B, D. melanogaster
AYDKLLVGEEA          Lamin A, D. melanogaster

RYRVLLNGANV          Ifc-1, C. elegans
RYRILLNGANV          Ifc-2, C. elegans
KYRELLDRSGD          Ifp-1, C. elegans
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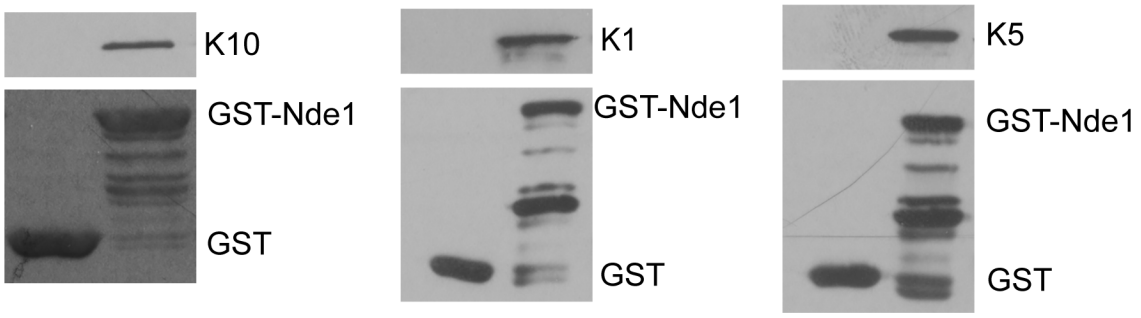
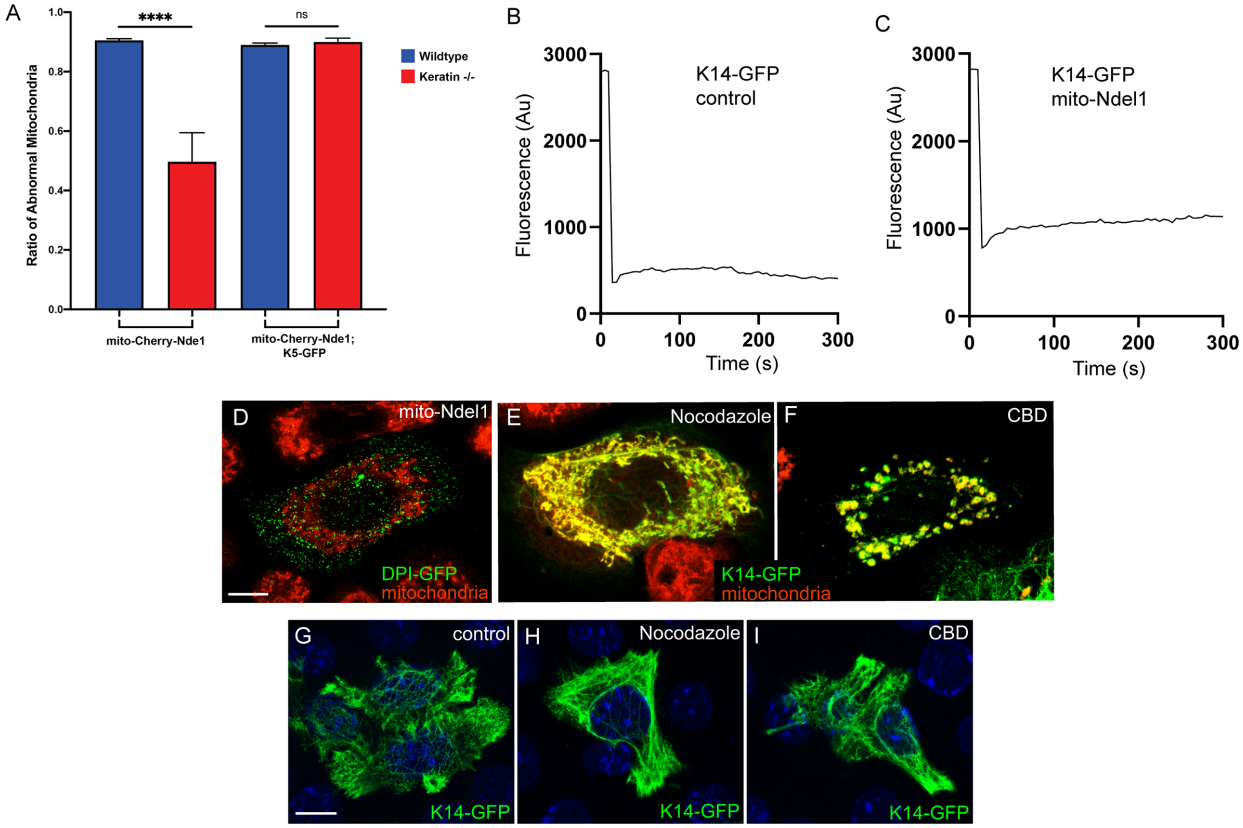
Supplemental Figure 1. Alignment of intermediate filament (IF) consensus motifs of diverse IF proteins.

Supplemental Figure 2



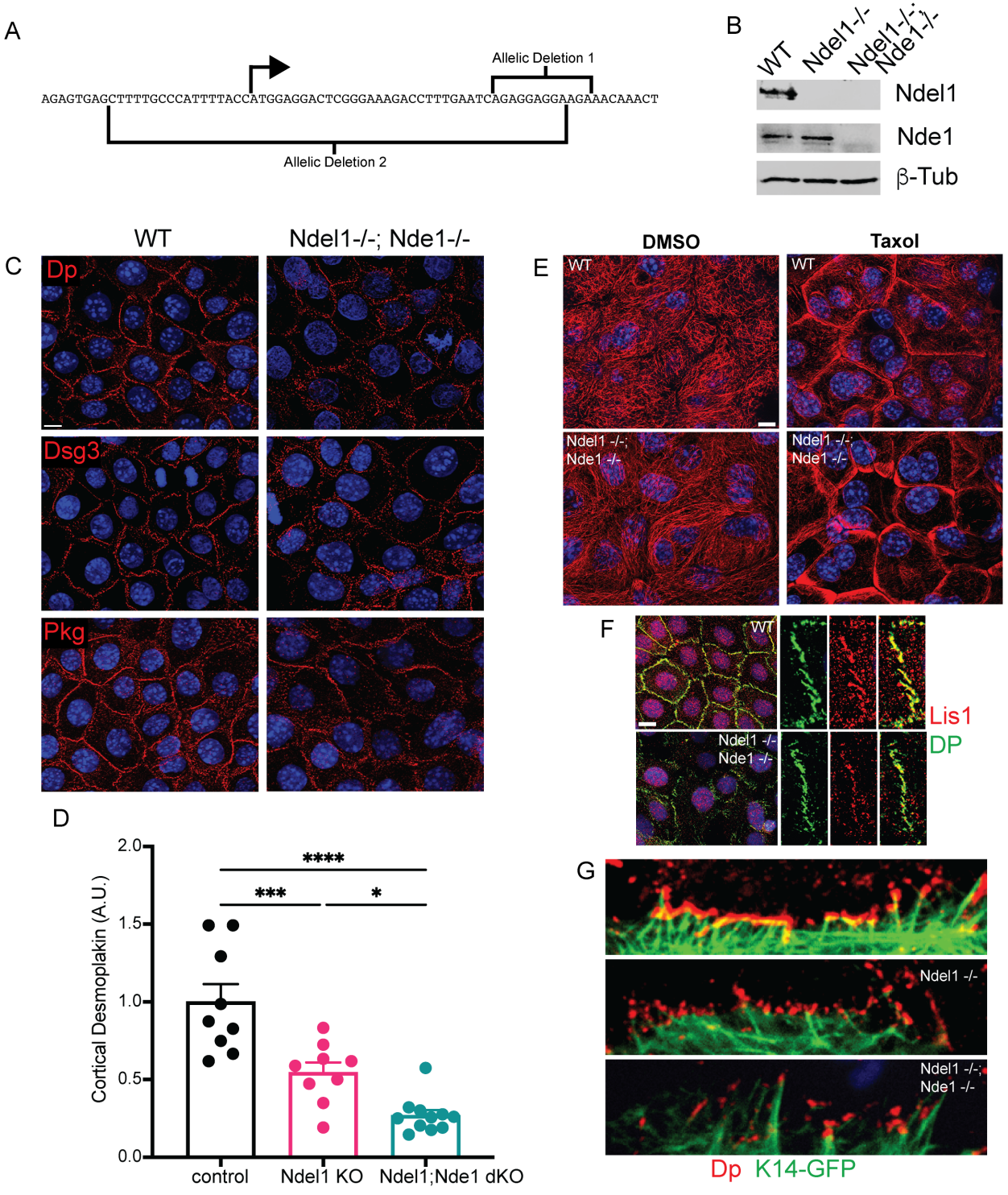
Supplemental Figure 2. Interaction of Keratins 10, 1, and 5 with Nde1. Purified K10, K1, or K5 was added to beads bound to either GST or GST-Nde1.

Supplemental Figure 3



Supplemental Figure 3. Mitochondrial-targeting assay. (A) Quantitation of the frequency of abnormal mitochondrial morphology in keratinocytes. As indicated, either wild-type cells of keratin null cells were transfected with the mito-Cherry-Nde1 construct. Note that in keratin null cells, mitochondrial morphology defects are decreased. Re-expression of Krt5-GFP in these cells, which restores the keratin network, results in more penetrant defects in mitochondrial morphology in response to mito-Cherry-Nde1 expression. n=4; ANOVA analysis was $p<0.0001$. Post Tukey's multiple comparisons were $p<0.00001$ for wild type verses keratin null and $p>0.9999$ for the rescue. Mito-Cherry-Nde1 and mito-Cherry-Ndel1 gave similar results. Mito-Cherry-Nde1 was used because of slightly higher transfections efficiencies. (B,C) Representative traces of K14-GFP FRAP experiments in control and mito-Ndel1 expressing cells. (D) WT keratinocytes were transfected with DPI-GFP (green) and mito-Ndel1. Cells were treated with MitoTracker-Red to label mitochondria. We did not observe any co-localization of DP with the mitochondria. (E,F) WT keratinocytes were transfected with mito-Ndel1 and keratin 14-GFP (green) and treated with either nocodazole (to disrupt microtubules), or ciliobrevin D (CBD, to inhibit dynein). Mitochondria were labeled with MitoTracker Red. (G-I) K14-GFP localization in control keratinocytes and in keratinocytes treated with either nocodazole or CBD. Note that drug treatment itself does not cause filament disruption. Scale bar is 10 μm.

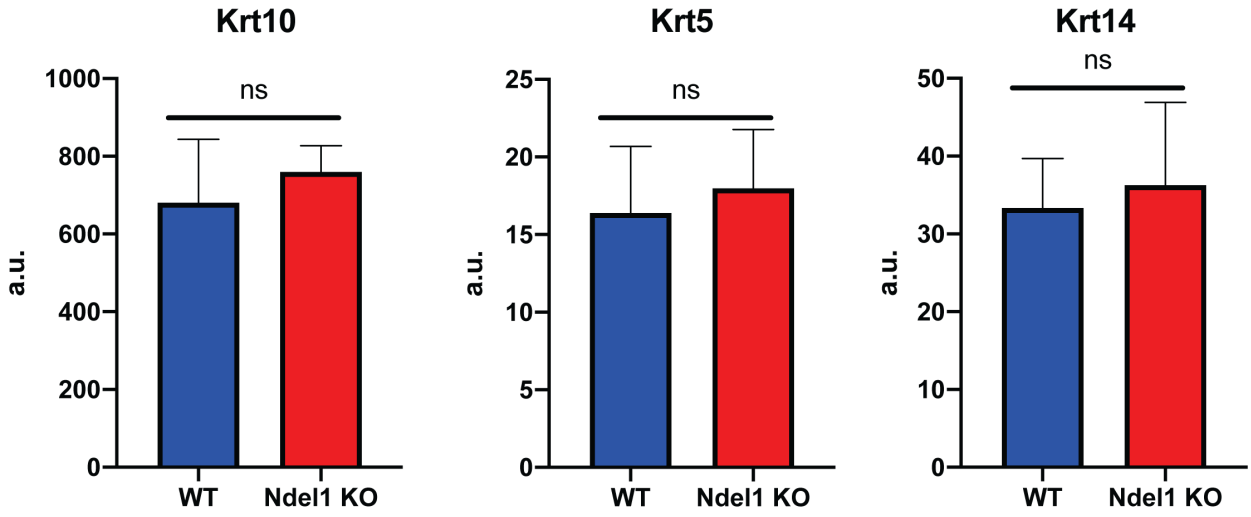
Supplemental Figure 4



Supplemental Figure 4. Generation of Ndel1;Nde1-/- cells. (A) Diagram of the region targeted by CRISPR and the two independent mutations generated in the clonal line used. (B) Western blot of lysates from control, Ndel1 null and Ndel1;Nde1 double null cell. β-tubulin is used as a

loading control. (C) Immunofluorescence of desmosomal proteins, as indicated, in control and Nde1;Nde1 double null cells. Scale bar is 10 μm. (D) Quantitation of cortical DP levels in control, Ndel1 KO and Ndel1/Nde1 double knockout cells. n=3 experiments, with 3-4 images examined for each. ANOVA analysis, p-value<0.0001. Tukey's multiple comparisons test gave p-values of: p=0.0008 for control verses Ndel1 KO, p<0.0001 for control verses the Ndel1;Nde1 dKO, p=0.0323 for the Ndel1 null verses the Ndel1;Nde1 dKO. (E) Microtubule reorganization to the cell cortex is normal in Nde1-/-;Ndel1-/- cells. Taxol was used to stabilize microtubules, which promotes their cortical organization in keratinocytes. (F) Localization of Lis1 (red) and DP (green) in control and Ndel1/Nde1 double null keratinocytes. Note that while overall cortical levels of desmosomes are lower, Lis1 is still detected at the cortex in the mutant cells. (G) K14-GFP (green) organization at the desmosomes, marked by DP (red) in control, Ndel1 null and Ndel1/Nde1 double null keratinocytes.

Supplemental Figure 5



Supplemental Figure 5. Quantitation of keratin levels in whole cell lysates from WT and Ndel1 KO epidermis.