Supplemental Information

Ms. Title: **Keratin 16 null mice develop palmoplantar keratoderma, a hallmark feature of pachyonychia congenita and related disorders**

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Supplemental Experimental Procedures

Protein extraction and Western Blotting

Dorsal tongue epithelium was isolated and total protein extracted in RIPA buffer. Equal amounts of protein were separated on a 10% SDS-PAGE gel, transferred to nitrocellulose membranes, blocked in 5% milk in TBS-T, and incubated overnight in primary antibody solution (5% milk in TBS-T). After rinsing with TBS-T, membranes were incubated with secondary antibody and developed with the PICO/FEMTO kit (Thermo Scientific, Rockford, IL) according to the

manufacturer's protocol. Images were collected using a FluorChemQ system (Alpha Innotech).

Antibodies used in this study: Krt17 (1:5000; McGowan and Coulombe, 1998), Krt5 (1:2000; Covance, Princeton, NJ), alpha-tubulin (1:5000; Sigma, St. Louis, MO), Goat-anti-rabbit HRP and Goat-anti-mouse HRP (1:2000; Sigma, St. Louis, MO).

Transmission Electron Microscopy

Tongues from newborn pups were fixed in 2% formaldehyde/2% glutaraldehyde in 0.1M cacodylate buffer pH 7.4 overnight at 4°C, postfixed in osmium tetroxide, counterstained with uranyl acetate, and embedded in epoxy resin as previously described (Paladini and Coulombe, 1998). Thin sections were cut and examined using a Hitachi HU-12A transmission electron microscope.

References:

1. McGowan KM, Coulombe PA (1998) Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. *J Cell Biol* 143:469-486.
2. Paladini RD, Coulombe PA (1998) Directed expression of keratin 16 to the progenitor basal cells of transgenic mouse skin delays skin maturation. *J Cell Biol* 142:1035-1051.

Supplemental Figure Legends

Supplemental Figure S1

(**a**) *Krt16*$^{-/-}$ mice are born at approximately Mendelian ratios. At birth, all genotype are visually indistinguishable. (**b**) Toluidine Blue dye exclusion assays show no gross epidermal barrier defects in newborn *Krt16*$^{-/-}$ mice. (**c**) *Krt16*$^{-/-}$ pups at P0 weigh less than their littermates. Asterisk indicates a p-value < 0.03, Student's T-test.

Supplemental Figure S2

(**a**) H&E stained cross-sections of tongues from 4-month-old mice. Note the increased thickness and altered papilla architecture in *Krt16*$^{-/-}$. Scale bar, 50μm. (**b**) Transmission electron microscopy at P0 reveals early signs of cell lysis in the anterior column in a subset of *Krt16*$^{-/-}$ filiform papillae (asterisk). AC = anterior column, BC = buttress column, PC = posterior column. Control image: 3000x magnification, *Krt16*$^{-/-}$ image: 2000x magnification.

Supplemental Figure S3

(**a**) Occasionally, generalized hyperkeratosis (black arrowheads) is observed in 3-week-old *Krt16*$^{-/-}$ front paws. At this point, there is no evidence of PPK formation. (**b**) Nail morphology is normal in adult *Krt16*$^{-/-}$ mice. nb = nail bed. Scale bar, 100μm. (**c**) TUNEL staining reveals the absence of apoptosis in both

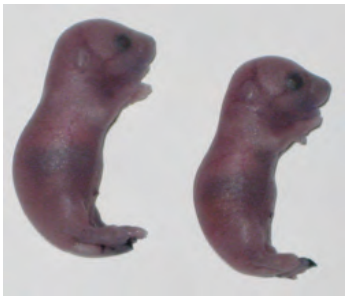
established *Krt16*$^{-/-}$ front paw calluses and control tissue. DNAse I digested positive control tissue from the same experiment is shown for comparison. The dotted line represents the epidermal/dermal junction. Scale bar, 50μm. (**d**) *Krt16*$^{-/-}$ mice spent more time resting and less time walking than control animals (experimental time frame of 30 min). Asterisks indicate a p-value < 0.002, Student's T-test.

Supplemental Figure S1

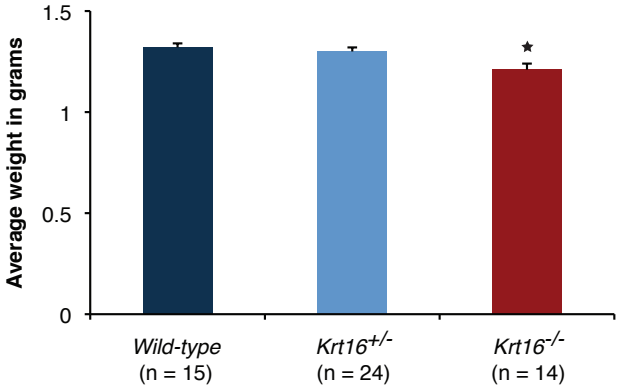
a

Genotype	E18.5 (total)	%	Overall (total)	%
WT	39 (114)	34	68 (236)	29
Krt16$^{+/-}$	55 (114)	48	121 (236)	51
Krt16$^{-/-}$	20 (114)	18	47 (236)	20

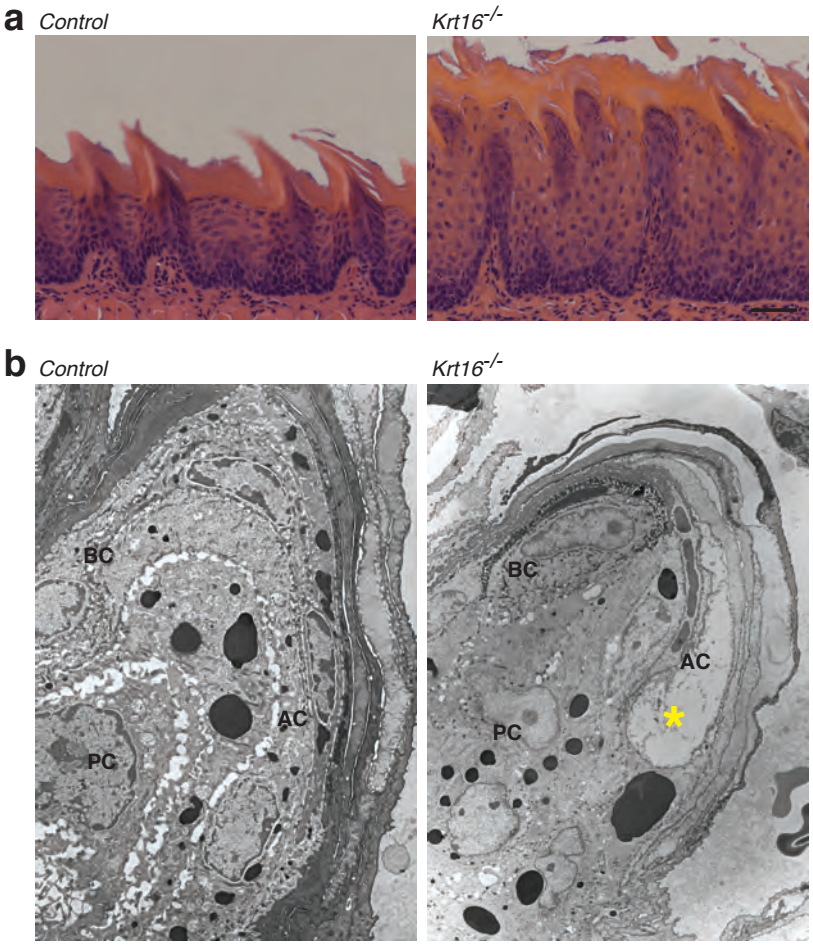
b



c



Supplemental Figure S2



Supplemental Figure S3