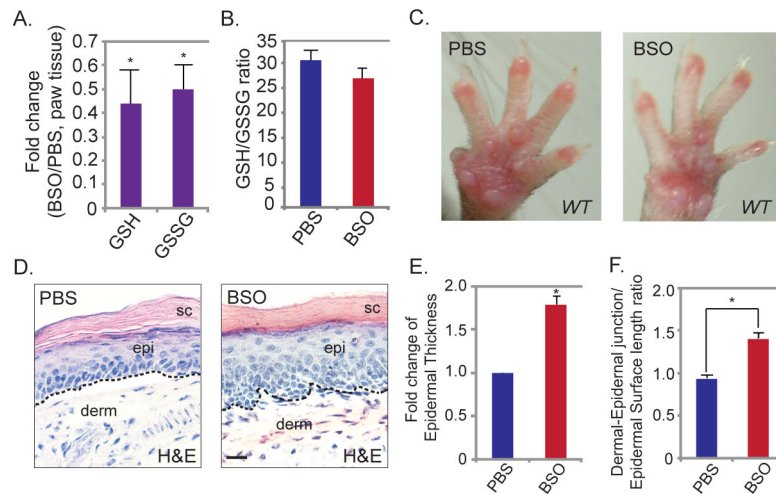
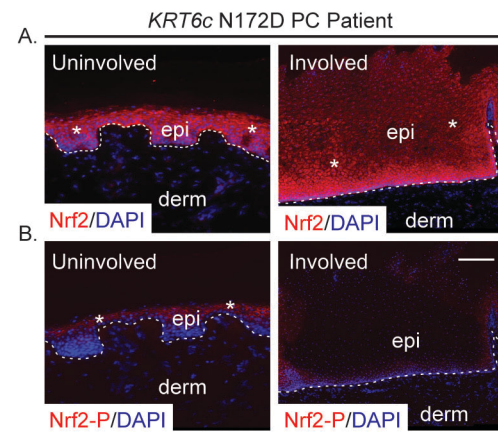


Role of oxidative stress and dysfunctional Nrf2 in pachyonychia congenita.



Supplemental Figure 1. Systemic BSO treatment results in increased oxidative burden and epidermal changes in male murine paw skin. (A) Fold change of GSH and GSSG in paw tissue from 1 month old male *WT* mice treated with BSO relative to vehicle (PBS) treated controls. (B) GSH to GSSG ratio for the same mice. For A and B, data represents the mean \pm SEM of three biological replicates. Student's t test, *P value < 0.05. (C, D) Images and hematoxylin and eosin (H&E) staining of paw skin from one month old male *WT* mice treated with BSO or vehicle (PBS). sc = stratum corneum, epi= epidermis, derm = dermis, and dotted line represents the dermo-epidermal junction. Scale bar = 50 μ m. (E) Fold change of epidermal thickness of paw skin of BSO treated mice relative to vehicle (PBS) treated controls. PBS treated controls are set to one. (F) Dermo-epidermal junction/epidermal surface length ratio of paw skin of BSO and vehicle (PBS) treated mice. For (E) and (F), data calculated from three measurements for two images from three mice per experimental group and represents the mean \pm SEM. Student's t test, *P value <0.05.

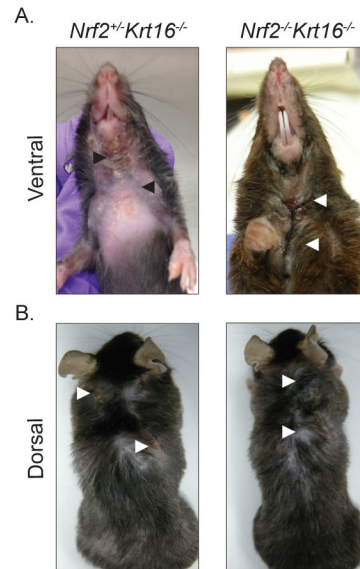
Role of oxidative stress and dysfunctional Nrf2 in pachyonychia congenita.



Supplemental Figure 2. Status of Nrf2 in human PC patient with a *KRT6c* N172 deletion. Indirect immunofluorescence staining for Nrf2 and Nrf2-P in PC patient skin. Nrf2-P = phospho-Nrf2, DAPI = nuclear staining, epi = epidermis, derm = dermis; scale bar = 50 μ m. Asterisks mark areas of increased immunofluorescent signal. Dotted line marks the dermo-epidermal junction. Uninvolved: non-lesional skin, involved: lesional skin; from the same individual.

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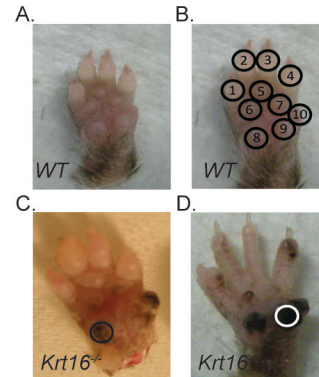


Supplemental Figure 3. Macroscopic appearance of one month old *Nrf2^{+/-}Krt16^{-/-}* and *Nrf2^{-/-}Krt16^{-/-}*. (A) Ventral surface. (B) Dorsal surface. Arrowheads mark affected areas.

Kerns et al., Suppl Figure 3.

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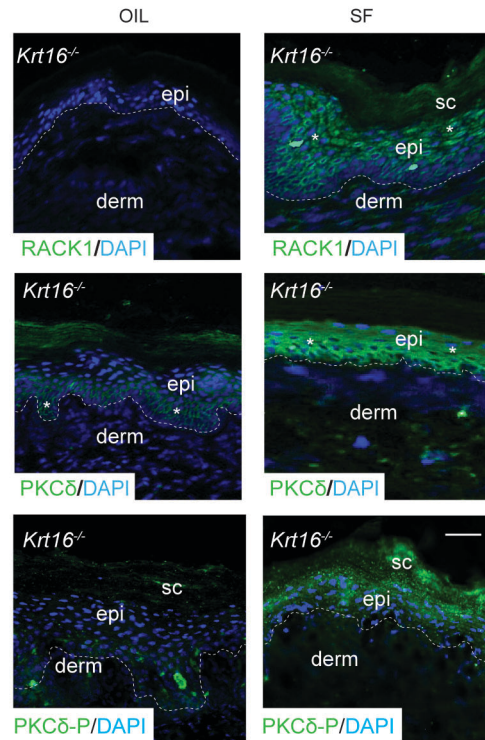
Role of oxidative stress and dysfunctional Nrf2 in pachyonychia congenita.



Supplemental Figure 4.
PPK Index: Assessment of Paw Lesion Severity. (A) Representative image of an unaffected paw of a one month old male *WT* mouse and (B) superimposed with areas of pressure points to be assessed, labeled 1-10. Representative images with example areas highlighted demonstrating approximately 50% affected (C) and 100% affected (D). "Affected" was determined by degree of hyperpigmentation. Assessors (n = 5) were blinded.

Kerns et al., Suppl Figure 4.

**Role of oxidative stress and dysfunctional Nrf2
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Supplemental Figure 5. Topical treatment with sulforaphane (SF) results in increase of RACK1, PKCδ, and PKCδ-P. Representative images of indirect immunofluorescence staining for RACK1, PKCδ, PKCδ-P in *Krt16*^{-/-} paw skin treated with SF or jojoba oil (vehicle). PKCδ-P = phospho-PKCδ, DAPI = nuclear staining, sc = stratum corneum, epi = epidermis, derm = dermis. Scale bar = 50 μm. Dotted line marks the dermo-epidermal junction. Scale bar = 50 μm. n=10 mice per experimental group.

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Company	Antibody	Catalog Number
Abcam plc.	GCLM	ab124827
	GS	ab91591
Cell Signaling Technology, Inc.	PKC δ -P	#9374
	Keap1	#4617S
	pSmad2	#3101
NeoBiolab	Nrf2-P	PAR2020
Santa Cruz Biotechnology, Inc.	PKC δ	sc-213
	GCLC	sc-28965
	RACK1	sc-17754
	β -actin	sc-47778
	Nrf2	sc-13032

Supplemental Table 1. Antibodies, sources, and catalog numbers.

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Target	Forward	Reverse
Actin	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	3'-TAAAACGCAGCTCAGTAACAGTCCG-5'
GCLC	5'-ACATCTACCACGCAGTCAAGGACC-3'	3'-CTCAAGAACATCGCCTCCATTAG-5'
GCLM	5'-GCCCGCTCGCCATCTCTC-3'	3'-GTTGAGCAGGTTCCCGGTCT-5'
GS	5'-CAAAGCAGGCCATAGACAGGG-3'	3'-AAAAGCGTGAATGGGGCATAAC-5'
G6PD	5'-ACCATCTGGTGGCTGTTCC-3'	3'-CATTGATGTGGCTGTTGAAGG-5'
HO-1	5'-CTGTGTAACCTCTGCTGTTCC-3'	3'-CCACACTACCTGAGTCTACC-5'
Keap1	5'-CATCCACCCTAAGGTCATGGA-3'	3'-GACAGGTTGAAGAACTCCTCC-5'
ME1	5'-GATGATAAGGTCTTCCTCACC-3'	3'-TTACTGGTTGACTTTGGTCTGT-5'
ME2	5'-TTCTTAGAAG CTGCAAAGGC-3'	3'-TCAGTGGGAAGCTTCTCTT-5'
NQO-1	5'-AGGATGGGAGGTAAGTCTGAATC-3'	3'-AGGCGTCCTCCTTATATGCTA-5'
Nrf2	5'-TCTCCTCGCTGGAAAAGAA-3'	3'-AATGTGCTGGCTGTGCTTTA-5'
p21	5'-CGAGAACGGTGGAACTTTGAC-3'	3'-CAGGGCTCAGGTAGACCTTG-5'
RACK1	5'-AGGGCCACAATGGATGGGTA-3'	3'-CAGCTTCCACATGATGATGGTC-5'

Supplemental Table 2. Oligonucleotide primer sequences.

Kerns et al., Suppl Table 2.