

Supplemental Information

Title: A role for keratins in supporting mitochondrial organization and function in skin keratinocytes

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List of elements:

Supplemental Figure 1. Low-magnification surveys of epoxy-embedded E18.5 back skin tissue sections (1 μ m thick) from *WT* and *Krt6a/Krt6b* null mouse littermates. Bars equal 50 μ m.

Supplemental Figure 2. Measurement of ROS using the Abcam DCFDA cellular ROS detection assay kit. (A) Skin keratinocytes were isolated from P1 *WT* and *Krt6a/Krt6b* null littermates and 5×10^4 cells were seeded in a 96-well plate for primary. The following day cells were given fresh media and 24 hours later, cells were labeled with DCFDA dye and Tert-Butyl Hydrogen Peroxide to induce oxidative stress. (B) Keratinocytes were cultured from *WT* and *Krt16* null littermates and cultured as described in (A). (C) Spontaneously immortalized keratinocytes from P1 *WT* and *Krt16* null mice were seeded at a density of 2.5×10^4 cells per well and assayed as described in (A). D=DCFDA, T=Tert-Butyl Hydrogen Peroxide. n= 3 independent experiments. Student's t test, significance set at $p < 0.05$.

Supplemental Figure 3. Data shown are derived from the experiments reported in Supplemental Videos 1-4, but represented as mitochondrial speed as a function of mitochondrial size. Also related to data presented in Figure 4.

Supplemental Video 1. Representative movie of keratinocytes in primary culture, isolated from the *WT* littermates of *Krt6a/Krt6b* null mice. Related to data presented in Figure 4 and Suppl. Figure 3. See main text for details.

Supplemental Video 2. Representative movie of keratinocytes in primary culture, isolated from *Krt6a/Krt6b* null mice. Related to data presented in Figure 4 and Suppl. Figure 3. See main text for details.

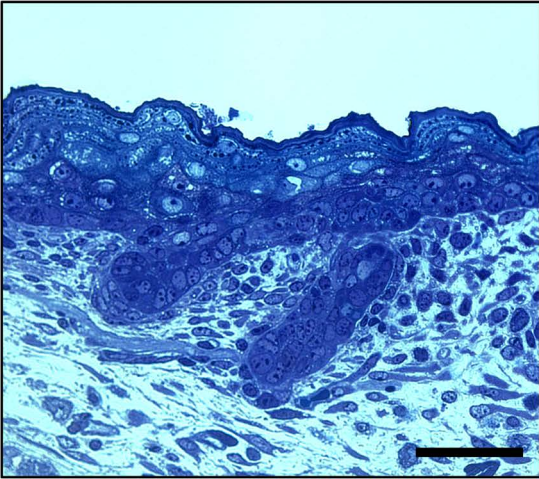
Supplemental Video 3. Representative movie of keratinocytes in primary culture, isolated from the *WT* littermates of *Krt16* null mice. Related to data presented in Figure 4 and Suppl. Figure 3. See main text for details.

Supplemental Video 4. Representative movie of keratinocytes in primary culture, isolated from *Krt16* null mice. Related to data presented in Figure 4 and Suppl. Figure 3. See main text for details.

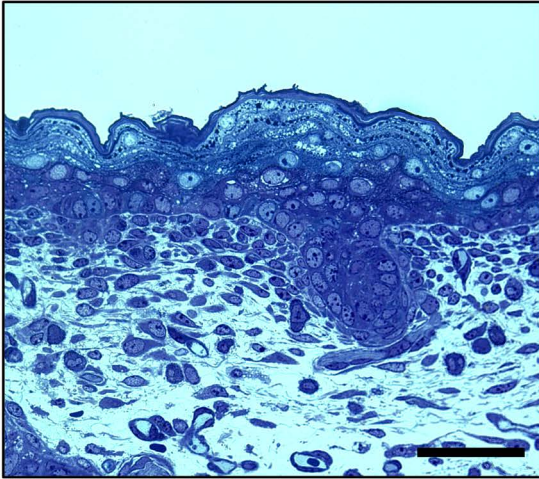
Supplemental Table 1. Forward and reverse oligonucleotide primers used for RT-qPCR assays.

Supplemental Figure 1. Steen et al.

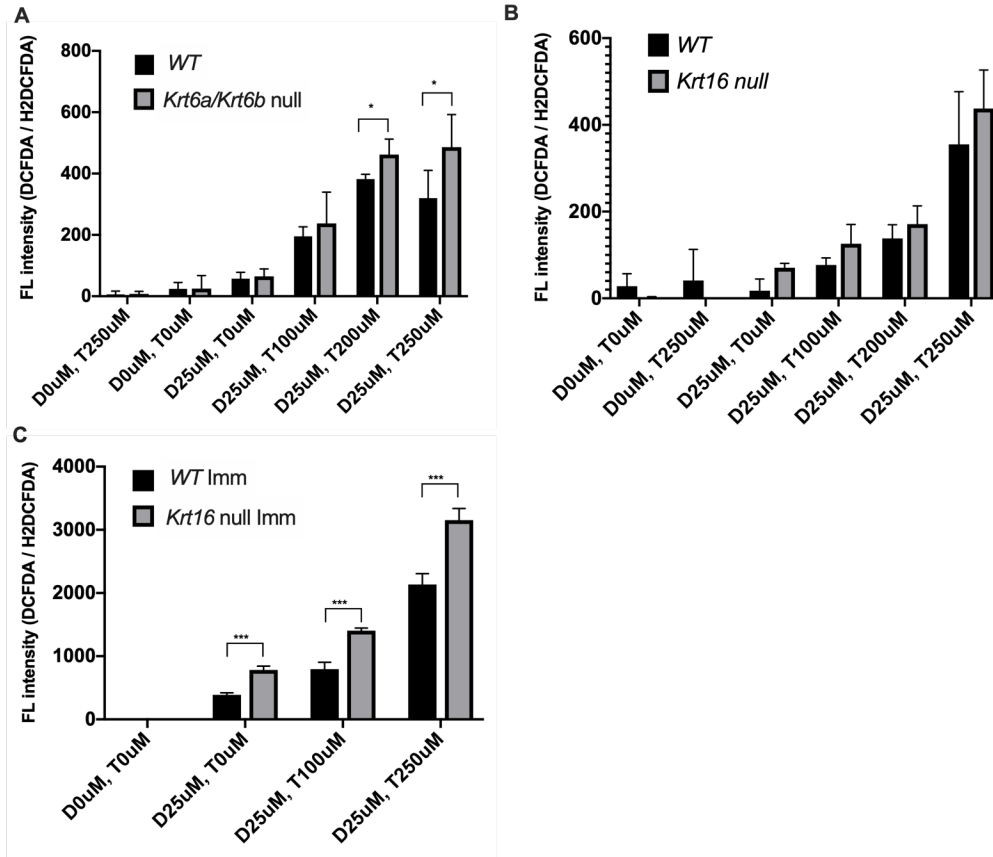
Krt6a/Krt6b WT E18.5



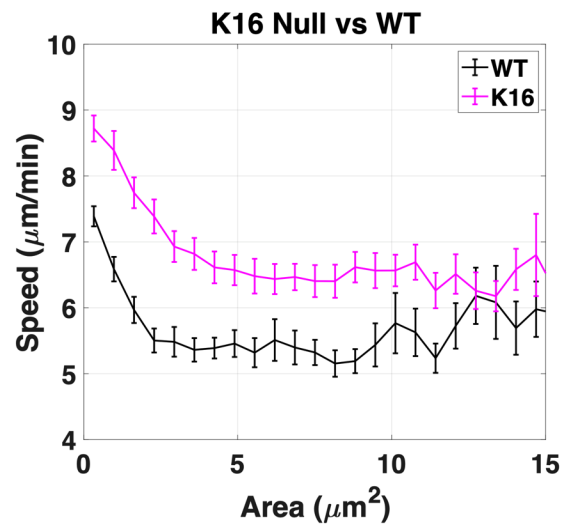
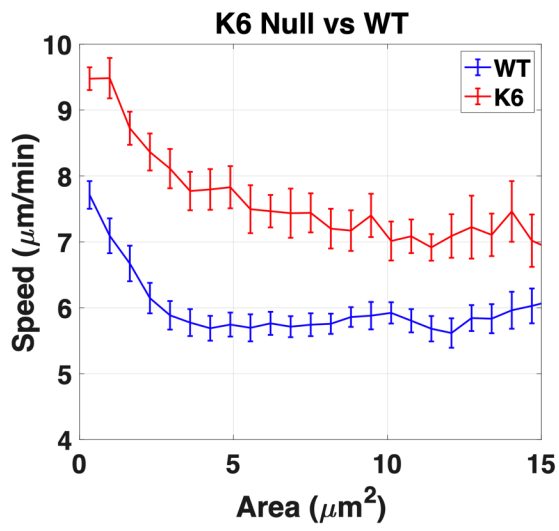
Krt6a/Krt6b null E18.5



Supplemental Figure 2. Steen et al.



Supplemental Figure 3. Steen et al.



Supplemental Table 1. Forward and reverse oligonucleotide primers used for RT-qPCR assays (listed in order they appear in the text). *lowercase letters represent the Flag tag sequence.

Molecule	Forward (5' – 3')	Reverse (5' – 3')
<i>CoX4</i>	ATTGGCAAGAGAGCCATTCTAC	CACGCCGATCAGCGTAAGT
<i>Pyruvate Dehydrogenase</i>	AGGAGGGAATTGAATGTGAGGT	ACTGGCTTCTATGGCTTCGAT
<i>Succinate Dehydrogenase</i>	GCTGCGTTCTTGCTGAGACA	ATCTCCTCCTTAGCTGTGGTT
<i>Tim23</i>	GAAGGTGGCGGAAGAAGTAGC	GGGGGTTCATAACCAGTCAGC
<i>COR2</i>	AAAGTTGCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
<i>Drp1</i>	CCAGCTGCCTCAGATCGTCG	CCGGGTGACCACACCAGTTC
<i>Mfn1</i>	CGTCGAGAACCTCCATGGGC	TCGTCCACGTCAGCCTCTCA
<i>Mfn2</i>	CTGGGCCCAAGAATAGCCG	GCGATGAGTCTCCAGCCCAC
<i>Opa1</i>	AGGGTCCGGCGAGATGATGA	CACCATCCTCTGCAGCCCAG
<i>K16-flag*</i>	CACCCAGCATGGCCACCT	TTActatcgtcgtcatccttgaatcGTCCTGG