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Supplementary Materials for

Mitochondrial Reactive Oxygen Species Promote Epidermal Differentiation and Hair Follicle Development

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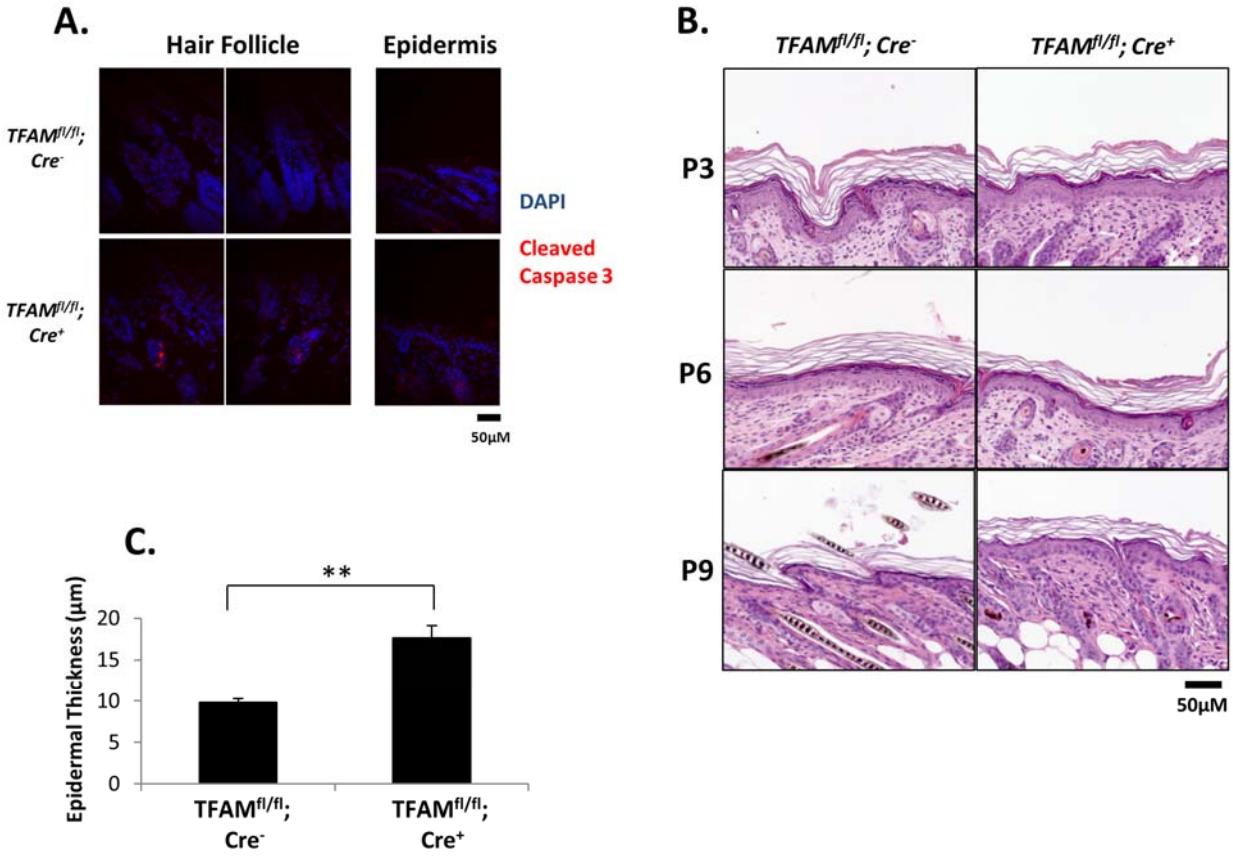

Figure S1: Analysis of epidermal apoptosis and epidermal thickness in control and *TFAM* cKO skin. (A) Immunofluorescent staining of P9 control and *TFAM* cKO hair follicles and epidermis demonstrating early onset of catagen in *TFAM* cKO mice (representative of 2 mice per genotype). (B) Images (40X) of skin sections from control and *TFAM* cKO mice stained with hematoxylin and eosin (representative of 3 mice per genotype). (C) Quantification of epidermal thickness as measured from the bottom of the basal layer to the top of the granular layer. The epidermises of 3 control and 3 *TFAM* cKO mice (P9) were measured at 10 points each. Graph represents mean ± SEM. ***P*<0.01.

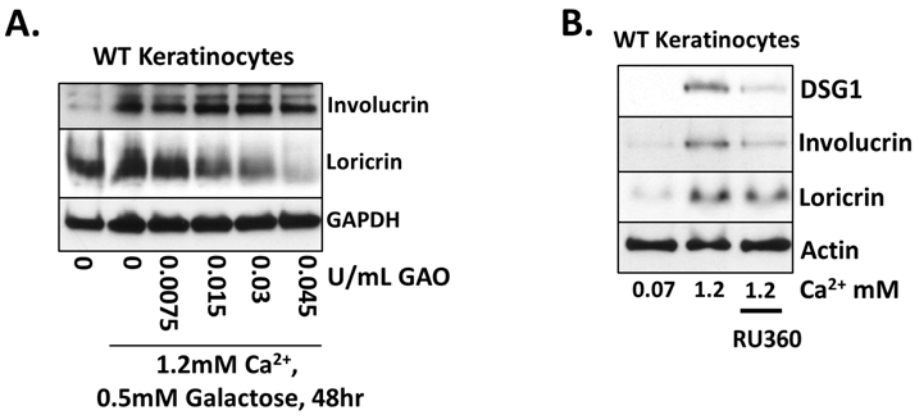

Figure S2: Sensitivity of control keratinocyte differentiation to exogenous H_2O_2 and inhibition of mitochondrial calcium uptake. (A) Western blot analysis of cellular lysates from control keratinocytes treated with 1 mM $CaCl_2$ and 0.5mM galactose for 48 hours in the presence or absence of the indicated amount of galactose oxidase (representative of 3 independent experiments). (B) Western blot analysis of cellular lysates from control keratinocytes left untreated or treated with 1.2 mM $CaCl_2$ for 48 hours in the presence or absence of 50 μM RU360 (representative of 3 independent experiments).

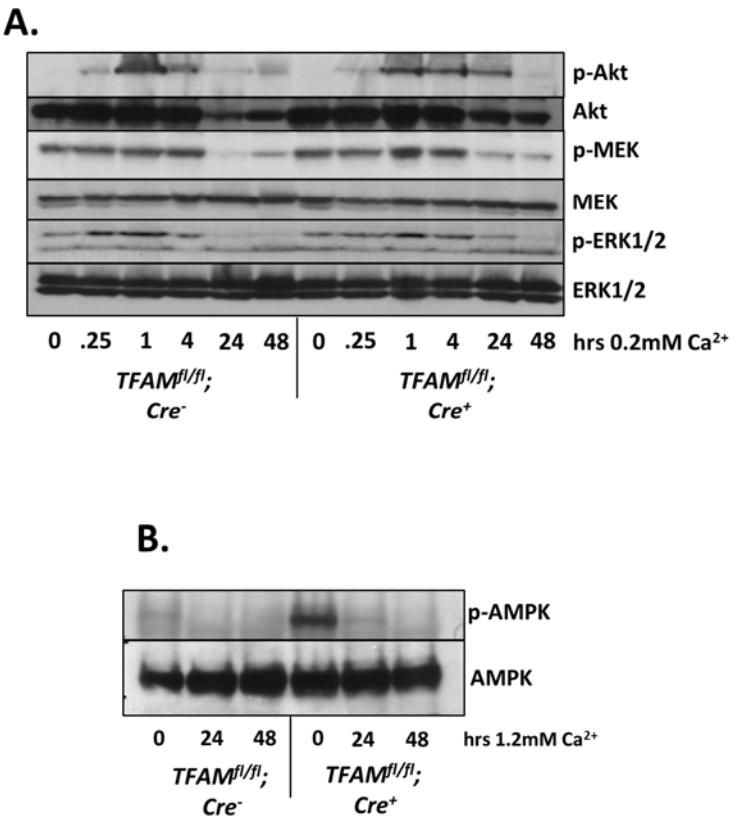

Figure S3: Analysis of cellular signaling pathways in control and *TFAM* cKO keratinocytes. (A,B) Western blot analysis of cellular signaling pathway activation in control and *TFAM* cKO keratinocytes at the indicated intervals after calcium switch (representative of 3 independent experiments).

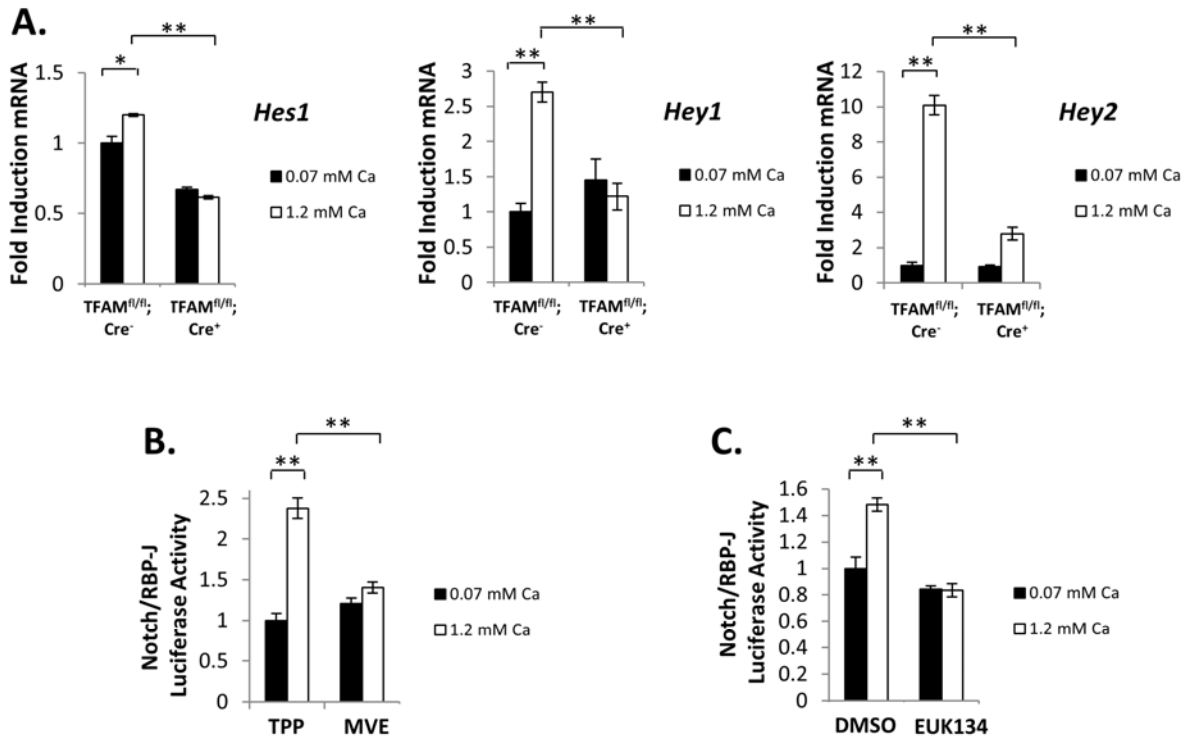

Figure S4: Sensitivity of Notch signaling to ROS during keratinocyte differentiation. (A) qRT-PCR quantification of the mRNA abundance for Notch target genes in control or *TFAM* cKO keratinocytes left untreated or treated with 1.2 mM $CaCl_2$. Graphs show means relative to undifferentiated control cells ± SEM. N=3 independent keratinocyte pools *P<0.05 **P<0.01. (B) Notch/RBP-J luciferase reporter activity in lysates of control keratinocytes treated with 1.2 mM $CaCl_2$ for 0 or 48 hours in the presence of 1 µM TPP or 1 µM MVE. Graph shows means relative to undifferentiated TPP-treated cells ± SEM. N=3 independent keratinocyte pools **P<0.01). (C) Notch/RBP-J luciferase reporter activity in lysates of control keratinocytes treated with 1.2 mM $CaCl_2$ for 0 or 48 hours in the presence of 50 µM EUK134 or DMSO as vehicle control. Graph shows means relative to undifferentiated DMSO-treated cells ± SEM. N=3 independent keratinocyte pools **P<0.01.

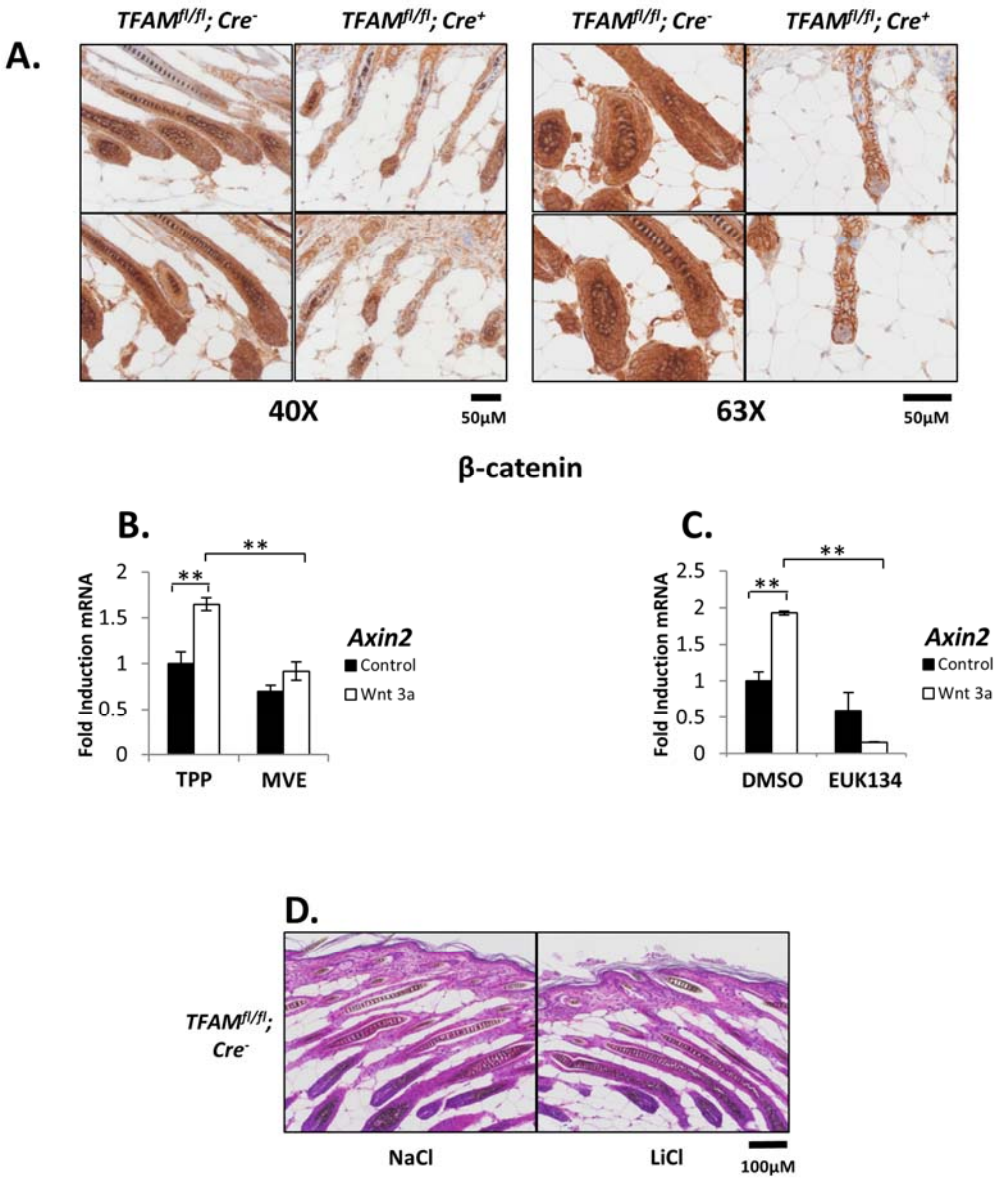

Figure S5: Sensitivity of Wnt–β-catenin signaling to ROS in epiermal keratinocytes. (A) Skin sections from control and cKO mice at P9 stained for β-catenin (representative of 2 mice per genotype). (B, C) Fold change of *Axin2* mRNA induction in control keratinocytes after treatment with Wnt3a or control conditioned media in the presence of (B) 1 µM TPP or 1 µM MVE or (C) 50 µM EUK134 or DMSO as vehicle control. Graphs show means relative to control cells with control treatment ± SEM. N=3 independent keratinocyte pools **P<0.01. (D) Back skin sections from P7 control mice stained with hematoxylin and eosin. Mothers of litters were injected with either 200 mg/kg LiCl or an equimolar dose of NaCl daily beginning at P0 (representative of 4 mice per treatment).