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Supplemental information

Hair follicles modulate skin barrier function

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Ford, Benedeck_Fig S1

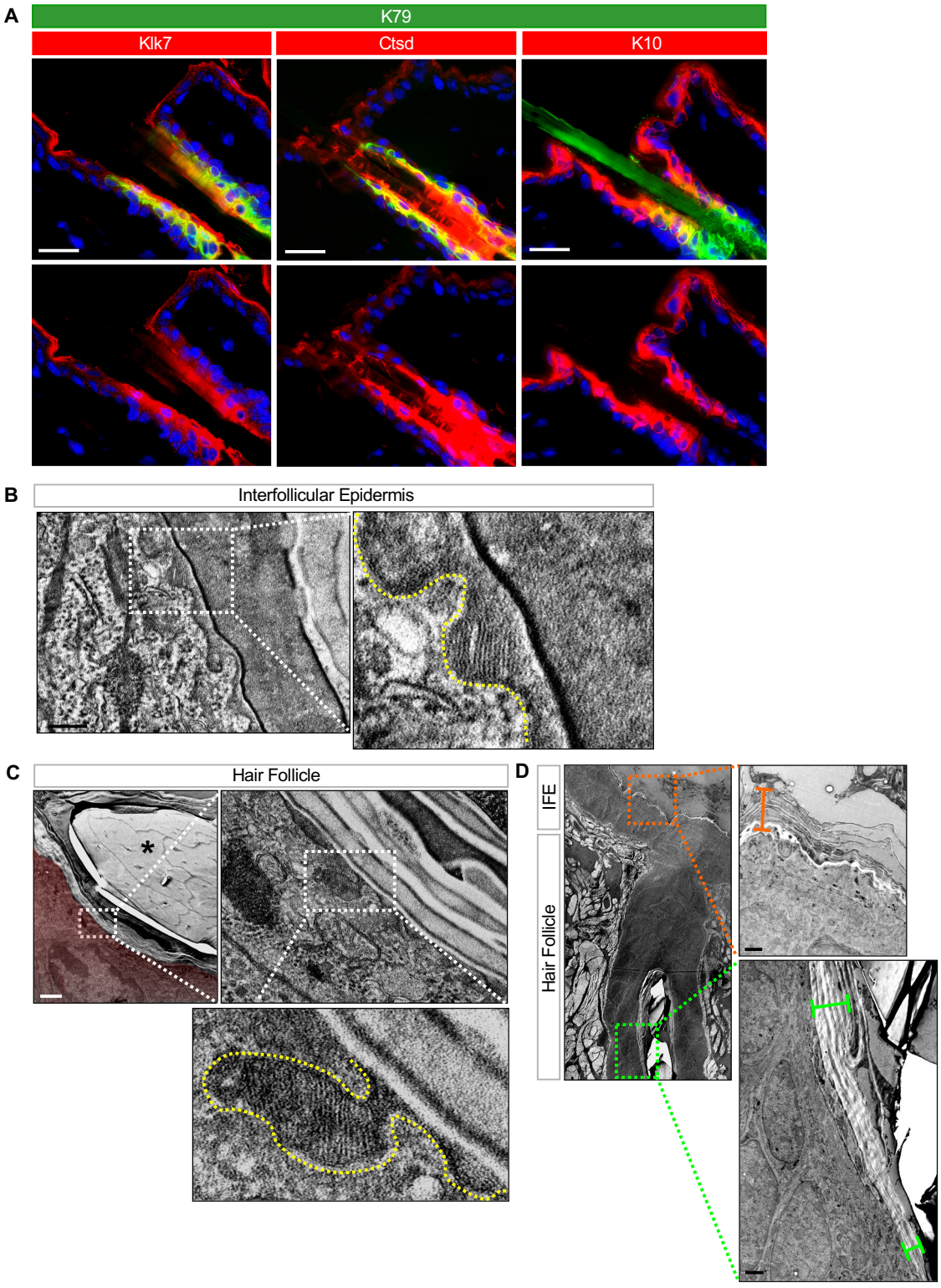

Figure S1. Differentiation- and barrier-associated proteins are present in the uHF

A. Upper panels, co-localization of Klk7, Ctsd and K10 (red) with K79 (green) in the uHF. Lower panels are single channel views.

B. TEM of the IFE, with the right magnified panel showing secreted lamellar material (outlined in yellow) at the granular layer apical side. Scale bar, 200 nm.

C. TEM of hair follicle epithelium. Asterisk, hair shaft. Red, presumably K79+ granular cell abutting the hair shaft. Right and bottom magnified panels show lamellar material (outlined in yellow) being secreted into the interstitial space between the granular and cornified layer.

D. TEM of IFE-uHF interface, with magnified views of the IFE (top right) and hair follicle (bottom right). Brown line, IFE cornified layers. Green lines, hair follicle cornified layers. Note that the follicular cornified layers become thinner at the proximal end (bottom).

Scale bar for A, 50 μm. Scale bar for B, 200 nm. Scale bar for C, D, 1 μm.

Related to Figure 1.

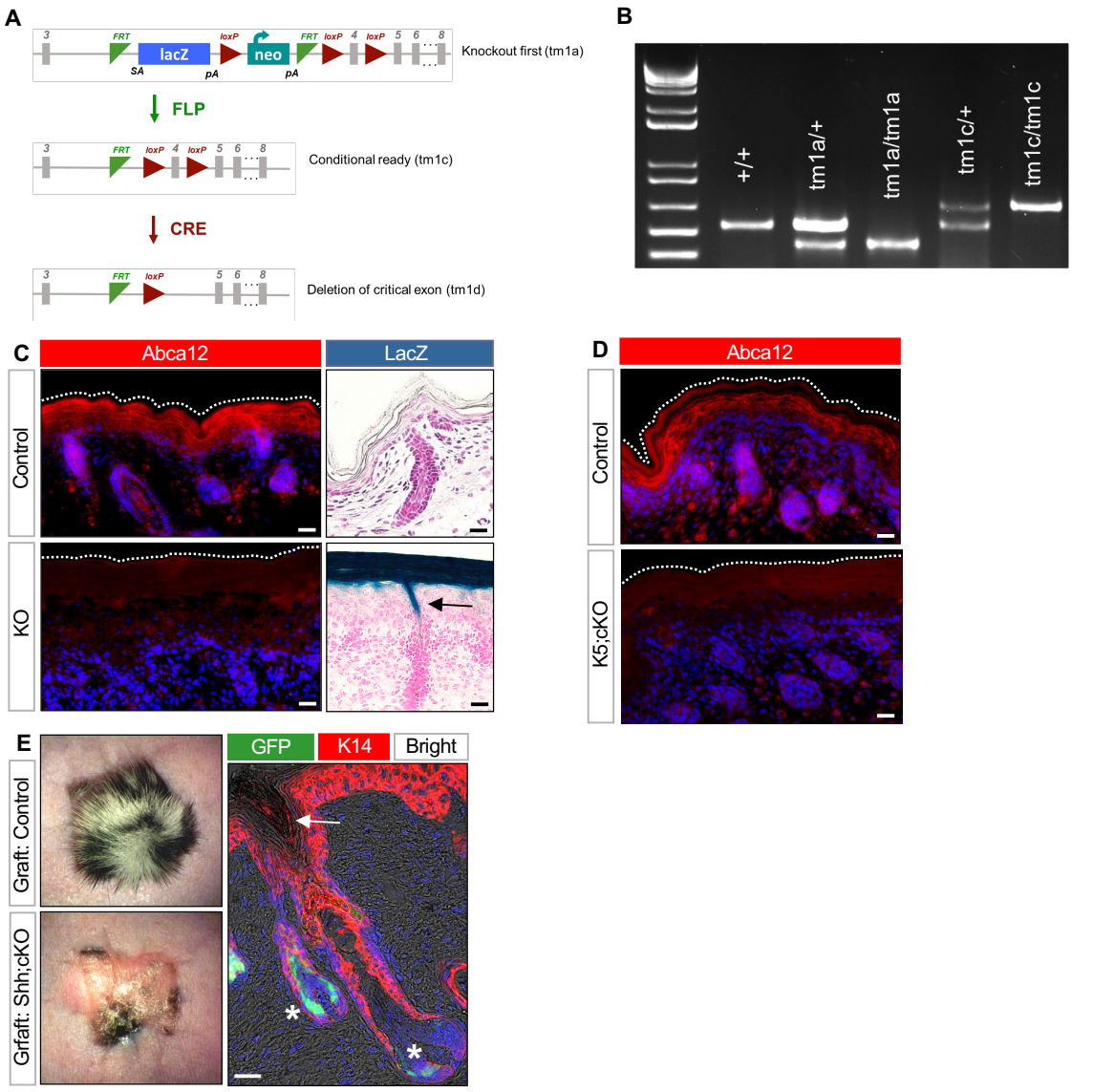

Figure S2. Mutant mice following constitutive or conditional deletion of *Abca12*

A. Schematic of *Abca12* KO allele containing a *LacZ* insertion between exons 3 and 4 (tm1a). Following FLP-mediated recombination, the KO allele is converted into a cKO allele with *loxP* sites flanking exon 4 (tm1c). After Cre-mediated recombination, exon 4 is removed, resulting in a non-functional gene (tm1d).

B. Genotyping strategy to identify wild-type (+), KO (tm1a) and condition (tm1c) alleles of *Abca12*. Please see methods section for primer sequences and PCR conditions.

C. IHC staining for Abca12 (red, left panels) and LacZ staining (blue, right panels) in control and *Abca12* KO skin. Arrow, *Abca12* promoter-driven LacZ expression in the uHF of developing follicles.

D. IHC staining for Abca12 (red) in control and *K5;Abca12-cKO* skin.

E. Gross images of nude mice grafted with control or *Shh;cKO* skin (left). Right, *Shh;cKO* graft, with IHC staining to detect *Shh* promoter-driven GFP (green, asterisk), identifying follicles of donor origin[47]. Arrow, hyperkeratotic uHF impeding hair shaft emergence, visible by brightfield overlay.

Scale bar, 50 μm.

Related to Figure 2.

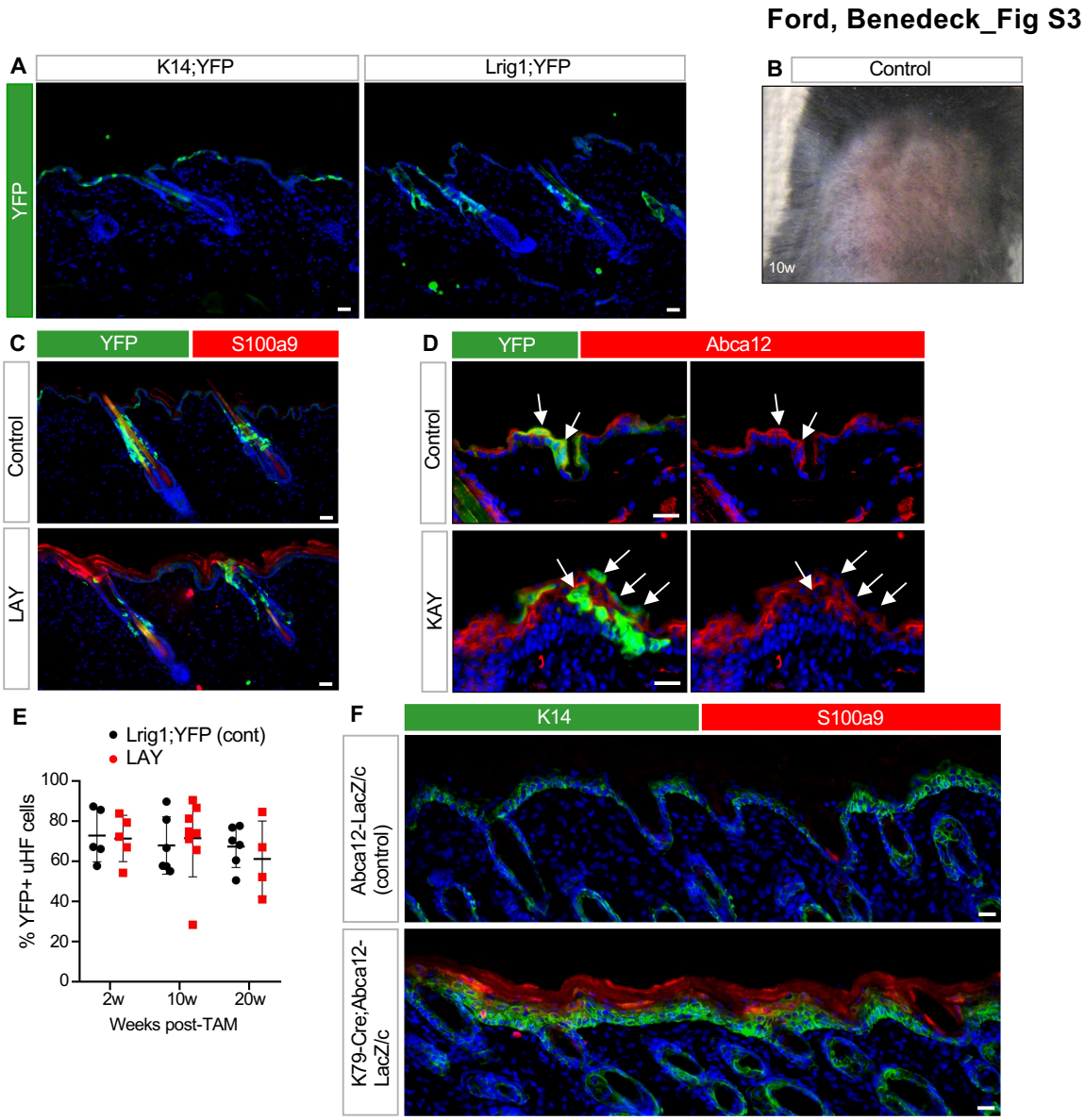

Figure S3. Targeting *Abca12* deletion to different skin compartments and upregulation of S100a9

A. Mice expressing K14-CreERT and a YFP reporter (K14;YFP) exhibit recombination primarily in the IFE (left, green staining), whereas mice expressing Lrig1-CreERT2 and YFP reporter (Lrig1;YFP) exhibit recombination primarily in the uHF (right).

B. Gross image of shaved dorsal skin from an LA littermate control mouse for Figure 2H, 10 weeks post-TAM.

C. IHC for S100a9 in *Lrig1-CreERT2;Abca12-c/c;ROSA-YFP* (LAY) mice (bottom). Control mice are similar to LAY mutants, but possess one wild-type copy of *Abca12* (*Abca12-c/+*) (top). S100a9 upregulation (red) in the IFE of LAY skin does not overlap with YFP+ uHF cells (green).

D. IHC for YFP (green) and Abca12 (red) in KAY and control mice, 2 weeks post-TAM. Arrows indicate labeled control cells expressing Abca12 (top panels) or labeled mutant cells lacking Abca12 (bottom panels). A corresponding single-channel view of Abca12 staining is shown for each image (right).

E. Quantitation of recombined YFP+ cells in the uHF of LAY (red) or control (black) mice, showing that mutant cells persist within the uHF, up to 20 weeks post-TAM. n ≥ 4 mice per genotype, per timepoint. Horizontal lines indicate mean +/- SD.

F. IHC showing upregulated S100a9 (red) in the IFE of mutant *K79-Cre;Abca12-LacZ/c* skin at P7 (bottom). Littermate control skin lacks *Cre* (top).

Scale bar, 50 μm. Related to Figures 2, 3 and 5.

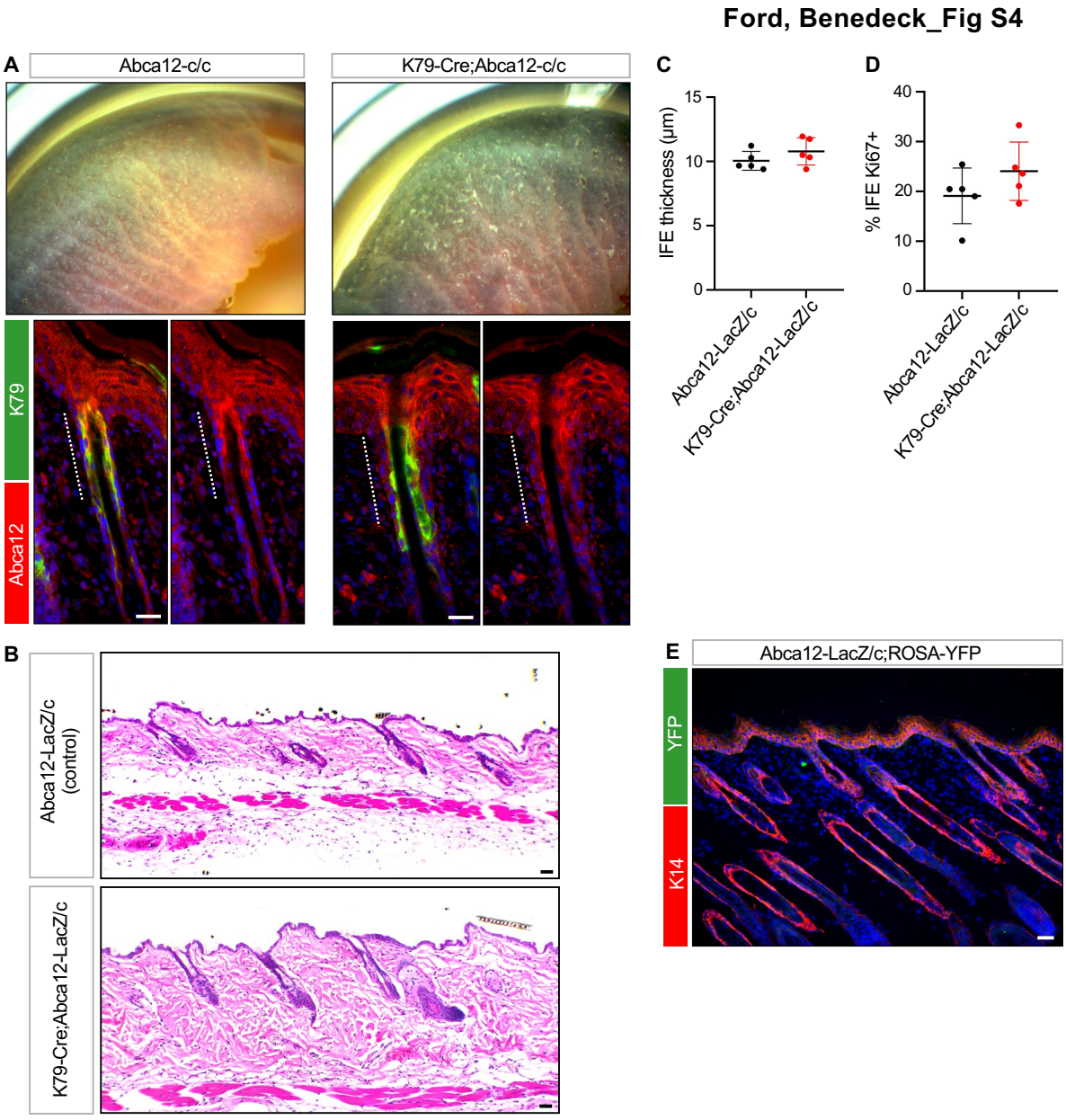

Figure S4. Adult *K79-Cre;Abca12-LacZ/c* mice do not exhibit overt skin defects

A. Top, gross photos showing subtle skin flaking in a *K79-Cre;Abca12-c/c* mutant pup at P4 (right panel), with a littermate pup lacking Cre as a control (left panel). Bottom, IHC showing strong Abca12 expression (red) that overlaps with K79 (green) in the developing uHF (dotted lines) in control skin at P4 (left panels). Mutant skin at P4 exhibits heterogeneous Abca12 staining in the uHF (right panels). A corresponding single-channel view of Abca12 staining is shown for each image.

B. Skin histology from a 9 week old *K79-Cre;Abca12-LacZ/c* mouse (bottom) and control littermate lacking Cre (top).

C. Quantitation of IFE thickness in mutant mice (red) or controls (black) at 9 weeks of age. n = 5 mice per genotype.

D. Same as (C), but with quantitation for basal IFE proliferation.

E. IHC staining of P6 *Abca12-LacZ/c*;*ROSA-YFP* pup lacking Cre. This is a littermate control for animals depicted in Figure 7H.

Horizontal lines indicate mean +/- SD. Scale bar, 50 µm. Related to Figure 7.