Supplemental Materials and Methods

Quantification of mRNA levels: Differences in mRNA levels of the NHE1 transporter and the sPLA2 isoform, sPLA2f, were measured by real-time, quantitative PCR (Q-PCR). Briefly, total RNA was isolated from mouse skin using STAT 60 (TEL-TEST, Inc., Friendswood, TX), TRIzol (Invitrogen, Carlsbad, CA), or RNeasy Mini RNA isolation kit (Qiagen, Valencia, CA). cDNA was prepared by reverse transcription with Tetro cDNA Synthesis Kit (Bioline, Taunton, MA), and mRNA expression levels were measured by Q-PCR, using SYBR Green Master Mix (Applied Biosystems (ABI), Foster City CA) on an ABI machine 7300 or 5300. Primers: Mouse NHE1: F: 5'- TTTCCCCGATTTCCTTCTCT - 3'; Mouse NHE1: R: 5'- GCGTGTAAGACCTGGGACAT – 3' and Mouse sPLA2g2f: F: 5'- CCCCATCCAGTCCTTAGTCA -3'; Mouse sPLA2g2f: R: 5'- ACTTCTGGGCAGGAGTCAGA -3'. Relative expression of the mRNAs was compared to the housekeeping enzyme, GAPDH. Results for the *in vivo* studies were presented as percentage of vehicle-treated control, after setting vehicle-treated sites as 100%. For melanocyte marker, total RNA was extracted from the back skin of 3 to 4 month SKH1 or SKH2/J mice with RNA STAT-60 (TEL-TEST, INC.). cDNA was synthesized at 20 ul of final reaction volume with SuperScript III First-Strand Synthesis System for RT-PCR Kit (Invitrogen) using 5 ug of total RNA. 1 ul of cDNA was amplified with Phusion Hot Star DNA Polymerase (FINNZYMES) in the presence of various primers: TYR(forward) 5'-GGC CAG CTT TCA GGC AGA GGT-3' (reverse) 5'-TGG TGC TTC ATG GGC AAT C-3'; TYP1(forward) 5'-CCC TAG CCT ATA TCT CCC TTT T-3' (reverse) 5'-TAC CAT CGT GGG GAT AAT GGC-3'; DCT (forward) 5'-GTC CTC CAC TCT TTT ACA GAC G-3' (reverse) 5'-ATT CGG TTG TGA CCA ATG GGT-3'; S16 (forward) 5'-TGC GGT GTG GAG CTC GTG CTT-3' (reverse) 5'-GCT ACC AGG CCT TTG AGA TGG-3'. PCR products were analyzed in a 2% of Argarose gel.

Primary melanocyte cultures: The dorsal back skin from 2-day old SKH1 pups was separated and incubated in HBSS with dispase overnight at 4°C. The thinner white epidermis was separated from the dermis and further incubated in 0.05% trypsin/EDTA for 10-12 minutes at 37 degrees. Cells were pelleted and cultured in 154 media with 0.07 mM Ca^{2+} plus a human keratinocyte growth supplement. The media was changed after 3-4 days to 254 media with a human melanocyte growth supplement. The cells were maintained at 37°C in 5% CO_2 atmosphere. SKH1 primary melanocytes then were plated in 8 well chamber slides and cultured at 37°C, 5% CO_2 for 3 days, and stained with anti-TYR or anti-TYP1 antibodies prior to imaging in a confocal microscope.

Cell and organotypic culture

Primary human keratinocytes were isolated from neonatal foreskins (pigment type I/II or pigment type III/IV) and grown in 0.07 mM Ca^{2+} 154CF medium (Life Technologies, Carlsbad, CA) supplemented with HKGS. Suspensions of second passage keratinocytes (~$2.21x10^5/cm^2$ insert) were seeded onto Cellstart CTS (Life Technologies, Carlsbad, CA) coated inserts (EMD Millipore, PET, 0.4um, Billerica, MA) in CnT-07 media (CELLnTEC, Switzerland), according to manufacturer's protocol. Three days after seeding (D3), the media was switched to CnT-02-3D (CELLnTEC, Switzerland). On D4, the HEE were air-exposed by feeding the bottom of the insert with CnT-02-3D. From D4 onward, HEE were fed daily with CnT-02-3D until harvested. HEE were grown in a dry incubator (30-50% RH) at 37°C and 5% CO_2. A dial hydrometer (Fisher Scientific, Hampton, NH) was used to measure incubator humidity. Low incubator humidity was maintained by removal of the water pan. Because media was changed daily, significant changes in osmolarity were not detected using this protocol, as measured by a micro-osmometer (Precision Systems, Natick, MA). 12-well inserts were used for transepithelial electrial resistance (TER) measurements (see below). These studies were conducted according to

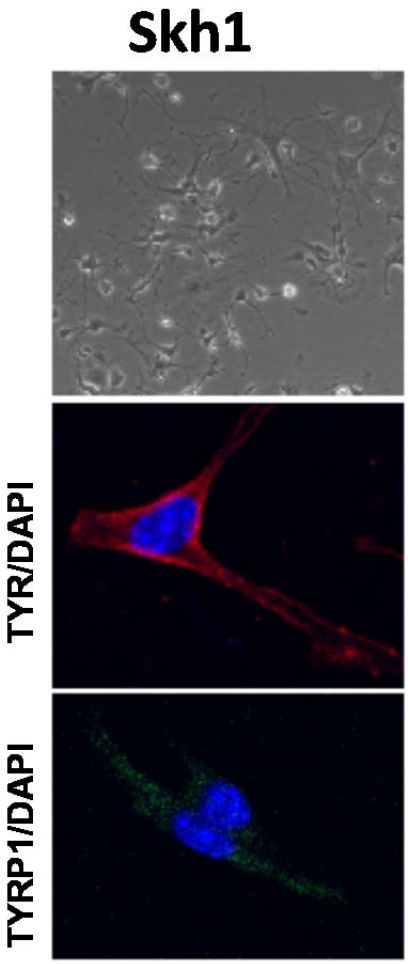
Declaration of Helsinki Principles, and a protocol approved by the UCSF-SFVAMC Committee on Human Research.

TER Measurements

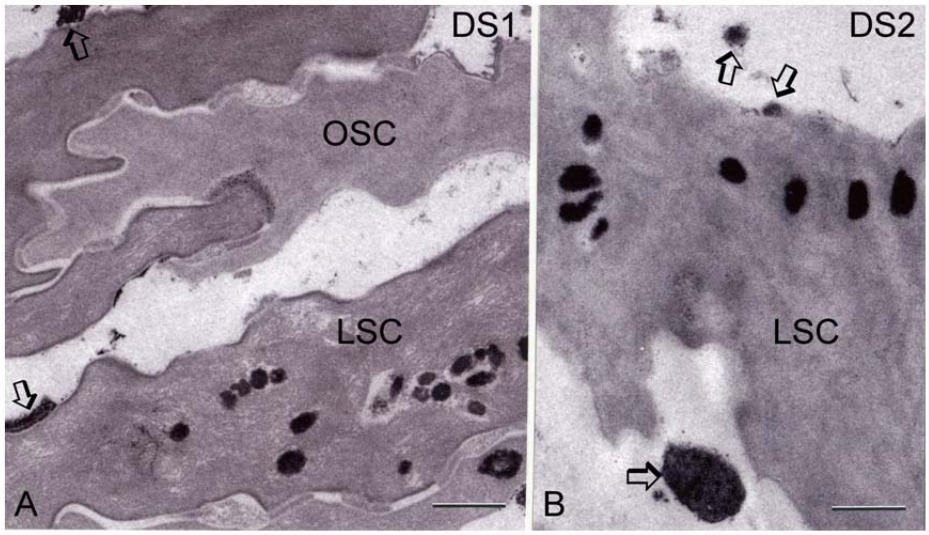
Transepithelial electrical resistance (TER) measurements were recorded with EVOM (World Precision Instruments, Sarasota, FL) according to manufacturer's instructions. Measurements were performed using fresh CnT-02-3D (0.5/mL) on top of the transwell, and 1 mL below the transwell. After measurements were completed, the apical media was aspirated, and samples were processed for microscopy, as described below.

Quantitation and localization of acidic microdomains:

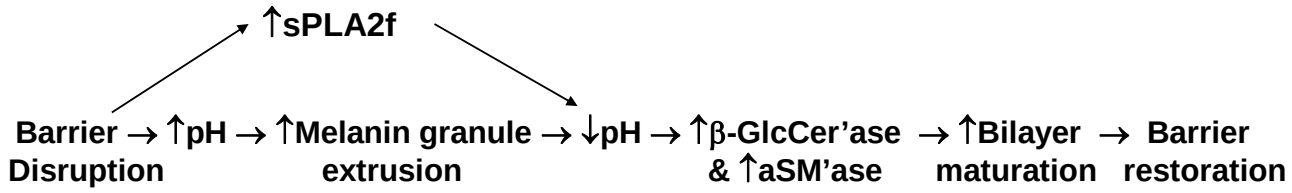
Biopsies from SKH1 and SKH2/J mice were obtained following the VA approved animal care protocols, and immediately after excision, the samples were cut in half. One half was incubated with in a 20uM solution of the pH sensitive dye SNARF-5F (Life Sciences, Eugene, Oregon) in PBS for 30 minutes and the other half was kept in dye free PBS for autofluorescence measurements. After incubation with SNARF-5F, samples were placed SC-side downward on a coverglass, and mounted in an inverted Axiovert 200 microscope (Zeiss, New York, NY). Z-stacks of ratiometric pH images were taken using the Meta 510 descanned detector, as described previously (Gunathilake*, et al.*, 2009). Autofluorescence intensity measurements were done on a dye-free portion of the sample, using the same laser power, detector settings, and at the same depths that was used for SNARF-5F imaging. At each depth, the average autofluorescence levels of the two channels were subtracted from the SNARF-5N images, and the average ratio between the two channels was calculated at every depth, as described (Gunathilake*, et al.*, 2009).



Supplemental Figure 1: SKH1 Mouse Skin Contains Melanocytes, But Lacks Enzymes of Eumelanin Synthesis. A. Phase contrast image of cultured primary melanocytes from neonatal SKH1 epidermis. **B&C.** Confocal immunofluorescence microscopy image of SKH1 primary melanocytes harvested from 2 day old pups, visualized with anti-TYR and anti-TYRP1 antibodies.



Supplemental Figure 2: Pigment Granule Persistence with Focal Extrusion in the SC of Darkly-Pigmented Humans. A&B. Skin biopsies from two different dark-skinned (DS 1 & 2) humans. Note pigment granule persistence in the cytosol of corneocytes in the lower stratum corneum (LSC), with gradual disappearance of granules in the outer SC (**A**, OSC). Some granules appear to be extruded and fragmented within the extracellular spaces. Osmium tetroxide post-fixation. Mag bars = 0.5 μm.



Suppl. Fig. 3: Proposed pH-Related Mechanisms That Account for Enhanced Permeability Barrier Function in Pigmented Skin