SUPPLEMENTARY METHODS AND MATERIALS

Living Skin Equivalent (LSE)

Human primary keratinocytes were obtained from breast reduction or abdominoplasty (Cascade Biologics, subsidiary of LifeTechnologies, Carlsbad CA), or from inner or outer arm skin biopsy (kindly gifted from Marcia Simon, Stony Brook University). All donor cells were expanded once in Cascade Biologics Epilife Medium with Human Keratinocyte Growth Supplement (HKGS), stored in liquid nitrogen and applied at P3. The keratinocyte cells used in these experiments consisted of at least two young donor cells (aged 32y and 30-40y) and at least five aged donor cells (aged 53-66y). Of these, all of the donor cells are from sun-protected areas. Human neonatal fibroblasts from a single donor were grown in Cascade Biologics Medium 106 with Low Serum Growth Supplement (LSGS) and stored similarly.

LSEs were assembled and grown in-house according to Aho *et al.* (2012). The cultures presented here are representative of growth for these age groups at the particular time points. For each culture, P3 keratinocytes (~150,000 cells per 50 ul) were applied on top of a dermal matrix composed of ~75,000 P <5 neonatal fibroblasts embedded into bovine collagen (Organogenisis, Canton MA; 1 mg ml^{-1}). LSE cultures were grown first submerged in growth medium for 96h followed by incubation in differentiation medium for 72h prior to air exposure. Samples were harvested at 7 or 11 days post-air exposure.

Lentiviral Constructs

All lentiviral overexpressing constructs were designed in-house and prepared by LifeTechnologies (Carlsbad, CA). The truncated promoter (Supplementary Figure 1) was produced synthetically (Blue Heron Biotechnology, Bothell, WA), bringing the necessary distal and proximal elements together for targeted basal cell expression (Di Nunzio *et al.*, 2008), and the correct sequence was confirmed. Briefly, pENTR221 containing CDKN2A (NCBI accession number NM_000077)(Open Biosystems; IHS1380-97652206) or lacZ, pENTR5' K14 promoter, and pLenti6.4/R4R2/V5-DEST, containing a 5' V5 epitope tag and WPRE stabilizing fragment, were used in MultiSite Gateway® LR recombination reactions to assemble the final constructs. All four constructs were infected separately into 293T cells for viral production, after which lentivirus was concentrated to a usable titer.

For knockdown of p16INK4A in keratinocytes, lentiviral particles were produced from the destination vector pLenti6.4-CMV-MSGW/miR-NM000077_630 by LifeTechnologies (Carlsbad, CA) and concentrated similarly. The miR RNAi sequences are shown in Figure S2.

For all lentiviral infections, keratinocytes were incubated with lentiviral particles and 6 ng ml-1 polybrene for ten minutes at room temperature prior to loading onto the dermal matrix.

ttccta

tgtcccgggc tccggagctt ctattcctga tccctgcata agaaggagac atggtggtgg

tggtggtggg tgggggtggt ggggcacaga ggaagccggt actgggctct gcaccccatt

cccgctccca gatccctctg gacacagcat ttttctccag tgagcacagc ctccccttgc

cccacagcca acagcaacat gcctcccaac aaaagcatct gtccctcagc caaaacccct

gttgcctctc tctggggaaa ttgtagggct gggccagggt ggggggacca ttctctgcag

ggagattagg agtgtctgtc aggggcgggt ggagcggggt ggggccctgg cttactcaca

tccttgagag tcctttgctg gcagatttgg ggagcccaca gctcagatgt ctgtctcagc

attgtcttcc aagctcctag gccacagtag tgggggggctc ccttctctgg cttcttcttt

ggtgacagtc aaggtggggt tgggggtgac agagggtcct gcttctcttc taggagcagt

tgatcccagg aagagcattg gagcctccag caggggctgt tggggcctgt ctgaggagat

aggatgcgtc aggcagcccc agacacgatc acattcctct caacatgcct gccggggtct

gtggagccta ggggctgatg ggagggtggg gtgggggccg gaagcccagg gtccgatggg

aaagtgtagc ctgcaggccc acacctcccc ctgtgaatca cgcctggcgg gacaagaaag

cccaaaacac tccaaacaat gagtttccag taaaatatga cagacatgat gaggcggatg

agaggaggga cctggctggg agttggcgct agcctgtggg tgatgaaagc caaggggaat

ggaaagtgcc agacccgccc cctacccacg agtataaagc actcgcatcc ctttccaatt

tacccgagca ccttctcttc actcagccaa ctgctcgctc gctcacctcc ctcctctgca cc

Figure S1. The truncated K14 promoter sequence used in constructs for lentiviral infection targeting the basal layer of epidermis.

tgctgatctatgcgggcatggttactgttttggccactgactgacagtaaccacccgcatagat

cctgatctatgcgggtggttactgtcagtcagtggccaaaacagtaaccatgcccgcatagatc

Figure S2. miR RNAi sequences used in the construct for lentiviral infections in p16INK4A gene silencing experiments (LifeTechnologies, Carlsbad, CA).

Immunohistochemistry (IHC) and Immunoblotting (Western)

One half of each epidermal LSE 6mm biopsy sample was taken for IHC analysis. Samples were immediately fixed in neutral buffered formalin for 3 hours then processed for embedding in paraffin by AML Laboratories (Baltimore, MD). Unstained fixed sections were deparaffinized and one set of slides was stained with hematoxylin and eosin (H&E). Primary antibodies, listed in Table S1, were used following the manufacturer's instructions and tested for the best dilution. Antigen retrieval (AR) was done in 10 mM sodium citrate, pH6.1, when required. The Superpicture Immunohistochemistry Kit (LifeTechnologies, Carlsbad, CA) was used for the development of stain, counterstained with hematoxylin and mounted with **ClearMount**™ (LifeTechnologies, Carlsbad, CA) solution.

Cy5 conjugated secondary antibodies from Jackson ImmunoResearch Laboratories (West Grove, PA) were also used according to the manufacturer's instructions. Those used were goat anti-mouse IgG (115-175-003) and goat anti-rabbit IgG (111-175-003).

Antigen	Antibody Source	Species	IHC	Western
caspase 14	Abcam #26991	Rabbit pAb whole antiserum	ND	1:2000
loricrin	Abcam #24722	Rabbit polyclonal	1:25,000	1:3000
Ki-67	Thermo Scientific Neomarkers #RB-9043-P	Rabbit pAb purified IgG	1:5000	ND
p16 (JC8)	Santa Cruz #56330	Mouse monoclonal	ND	1:200
p16INK4A	CINtec histology kit	Mouse anti-human	no dilution	1:10
V5	Invitrogen #46-0705	Mouse monoclonal	1:500	ND
tubulin, alpha	Thermo Scientific Neomarkers #MS-581-P	Mouse monoclonal (clone DM1A)	ND	1:1000

Table S1. Antibodies used for IHC and Western blotting analysis of LSEs.

A portion of each epidermal LSE sample was dissected, processed and immunoblotted for protein detection according to Aho *et al.* (2012), using the antibodies and dilutions listed in Table S1. The amount of protein used per well is listed separately in each figure.