Expanded View Figures

Figure EV1. H2B-GFP LRC and non-LRC IFE domains in mouse tail skin from lineage-traced mice (Associated with Fig 1).

A Whole mount view (top) and sections (bottom) of mouse tail skin from Dlx1-/Slc1a3-/AspmCreER x K5-tTA/pTRE-H2BGFP mice showing H2BGFP label retaining cells (LRCs) after doxycycline (doxy) chases. Scale bar = 50 or 200 μm as indicated on panels. Interscales regions have been previously shown enriched in LRCs (Mascre *et al*, 2012; Gomez *et al*, 2013; Sada *et al*, 2016). Here, scale and interscale regions are tentatively outlined based on the known H2B-GFP LRC clustering, the 3-hair follicle grouping patterns and DAPI staining.

B Cartoon showing the microscopic view for whole mount and sections.

C Top Panels: Schematics of tet-off (left) versus tet-on (right) inductions used for comparison with (Piedrafita *et al*, 2020). Bottom Panels: Tail skin sections from K5-tta x and K14-rta x pTRE-H2BGFP mice stained with interscale (K10) marker (as indicated). "Mosaicism" denotes areas of the IFE devoid of H2BGFP due to deficiency in transgene expression. High vs. low exposure images demonstrate enrichment of LRCs in the interscale areas in both mouse models. Better contrast is obtained in the K5-tTa (our preferred) mouse model compared to the K14-rTa (Piedrafita *et al*, 2020) and a minimum of 2-week chase is required to note differences.

D Tail skin sections stained for LRC/non-LRC markers as in Fig 1D, exhibit preferred overlap with H2BGFP LRC or non-LRCs IFE domains. Dotted lines indicate scale or interscale, depending on which is written within the image.

Source data are available online for this figure.

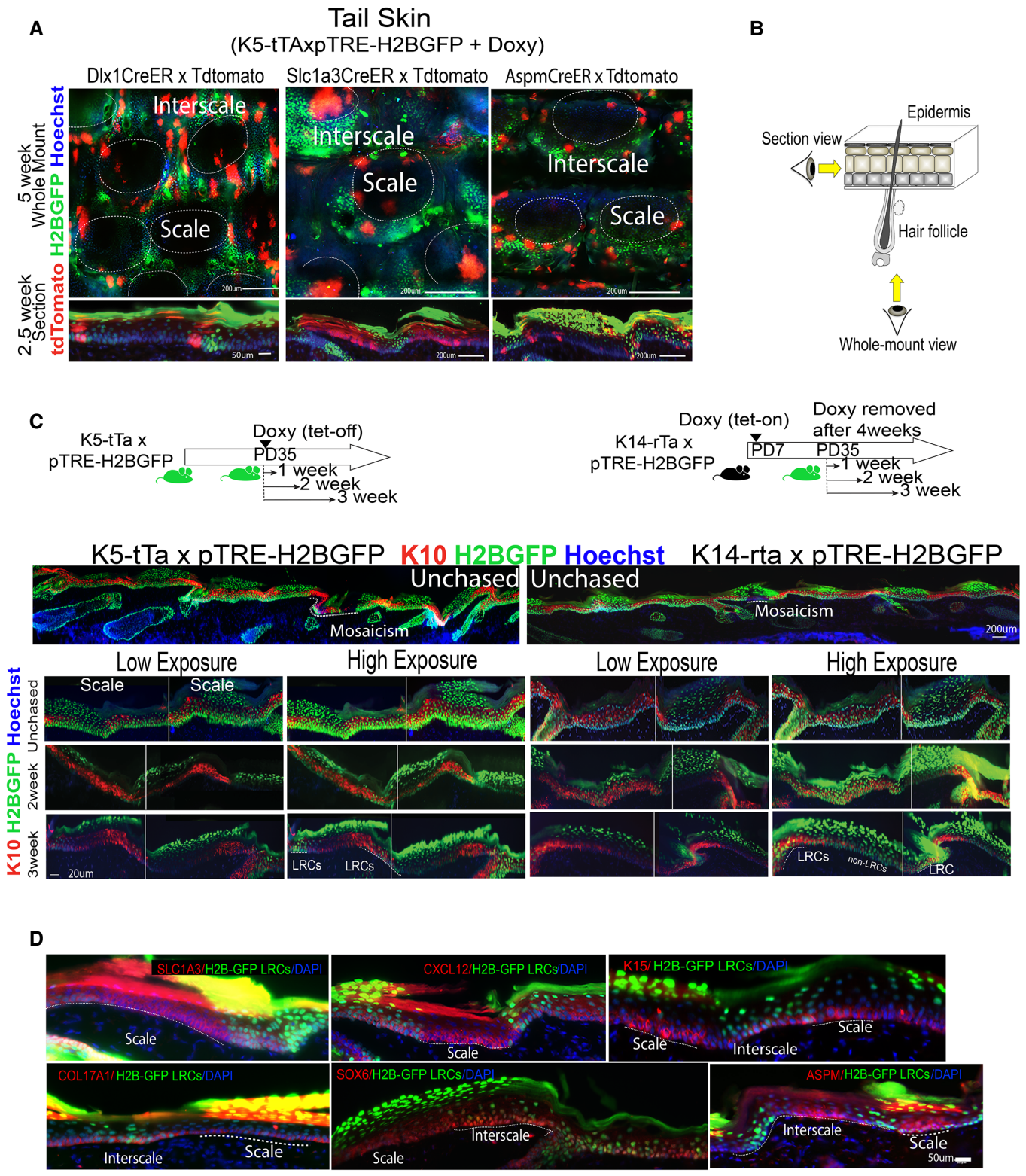
A
Tail Skin
(K5-tTAxpTRE-H2BGFP + Doxy)
Dlx1CreER x Tdtomato
Slc1a3CreER x Tdtomato
AspmCreER x Tdtomato
5 week Whole Mount H2BGFP Hoechst
2.5 week Section tdTomato
Interscale
Scale
200um
50um
B
Epidermis
Section view
Hair follicle
Whole-mount view
C
K5-tTa x pTRE-H2BGFP
Doxy (tet-off)
PD35
1 week
2 week
3 week
Doxy (tet-on)
Doxy removed after 4weeks
K14-rTa x pTRE-H2BGFP
PD7
PD35
1 week
2 week
3 week
K5-tTa x pTRE-H2BGFP K10 H2BGFP Hoechst K14-rta x pTRE-H2BGFP
Unchased
Mosaicism
200um
Low Exposure
High Exposure
K10 H2BGFP Hoechst
Unchased
2week
3week
Scale
20um
LRCs
non-LRCs
LRC
D
SLC1A3/H2B-GFP LRCs/DAPI
CXCL12/H2B-GFP LRCs/DAPI
K15/ H2B-GFP LRCs/DAPI
COL17A1/ H2B-GFP LRCs/DAPI
SOX6/H2B-GFP LRCs/DAPI
ASPM/H2B-GFP LRCs/DAPI
Scale
Interscale
50um

Figure EV1.

Figure EV2. H2B-GFP LRC and non-LRC IFE domains in mouse non-tail body regions from lineage-traced mice (Associated with Fig 1).

A Schematic of tamoxifen and doxy chase times for various body skin regions.

B Back and ear skin sections from unchased K5-tTA x pTRE-H2BGFP mice. Mosaicism is due to deficiency in transgene expression in ear skin. Scale bars = 200 μm.

C, D Whole mount (C) and section (D) view of back from lineage-traced mice showing 2-week chased H2B-GFP (green) postinjection with high tamoxifen (TM) dose. Scale bars = 200 μm (C) or as indicated (D). Dashed line in (C) indicates non-LRC patches devoid of H2BGFP signal. Dotted lines in (D) indicated basement membrane.

E Images of skin from back, ear and paw immuno-stained for some markers from Fig 1D. Dotted lines in (E) indicates ROI in basement membrane. Scale bar is as indicated per row.

F, G (F) Quantification of images like those in (E) with n = 2–5 mice and 6–7 images per mouse showing marker heterogeneity and (G) overlap with H2BGFP LRCs vs. non-LRCs domains. *P*-values were calculated by two-tail Student's *t*-test.

Source data are available online for this figure.

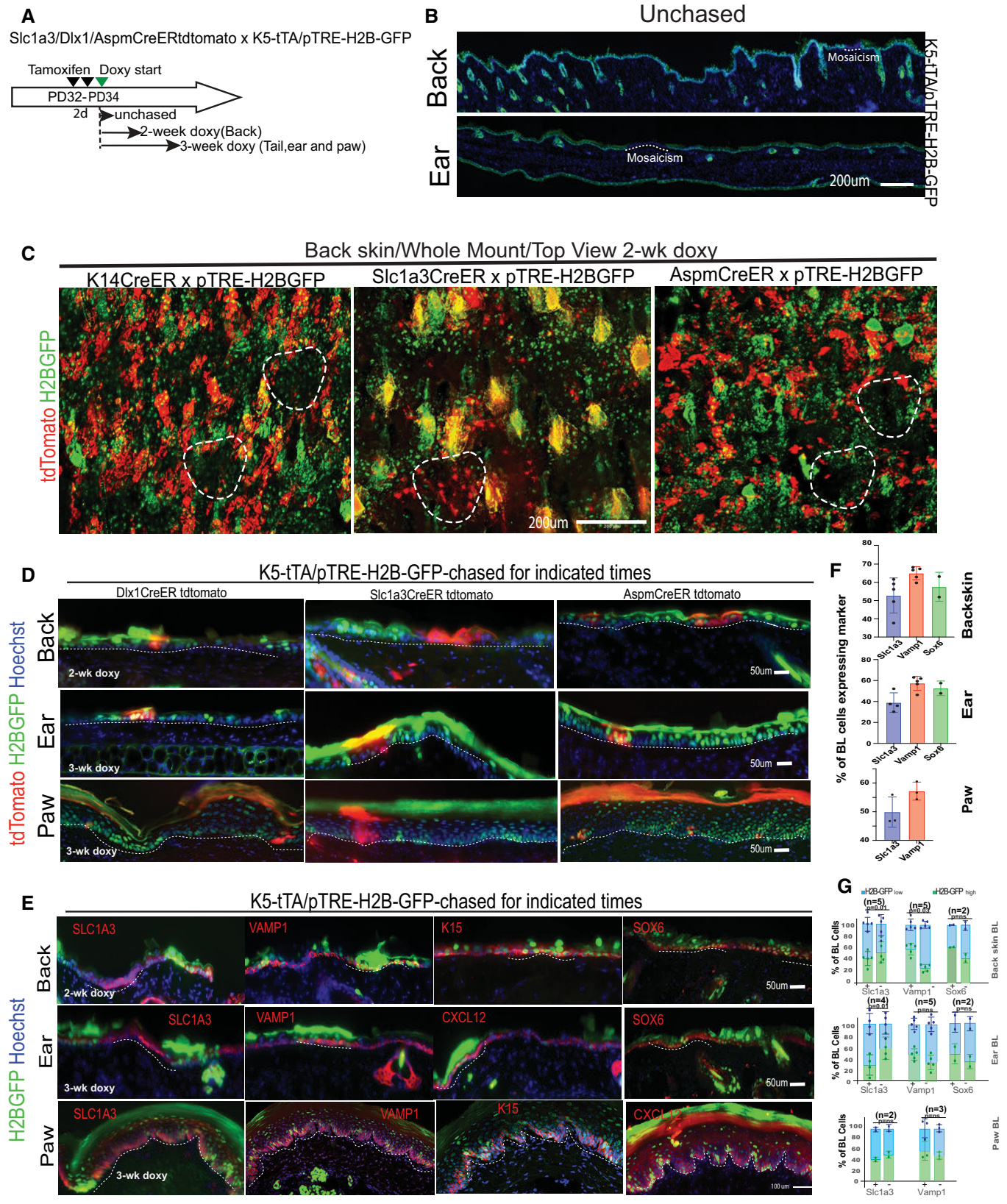
A
Slc1a3/Dlx1/AspmCreERtdtomato x K5-tTA/pTRE-H2B-GFP
Tamoxifen
Doxy start
PD32-PD34
2d
unchased
2-week doxy(Back)
3-week doxy (Tail,ear and paw)
B
Unchased
Back
Ear
Mosaicism
Mosaicism
200um
K5-tTA/pTRE-H2B-GFP
C
Back skin/Whole Mount/Top View 2-wk doxy
K14CreER x pTRE-H2BGFP
Slc1a3CreER x pTRE-H2BGFP
AspmCreER x pTRE-H2BGFP
tdTomato H2BGFP
200um
D
K5-tTA/pTRE-H2B-GFP-chased for indicated times
Dlx1CreER tdtomato
Slc1a3CreER tdtomato
AspmCreER tdtomato
tdTomato H2BGFP Hoechst
Back
Ear
Paw
2-wk doxy
3-wk doxy
3-wk doxy
50um
50um
50um
F
% of BL cells expressing marker
Backskin
Ear
Paw
Slc1a3
Vamp1
Sox6
E
K5-tTA/pTRE-H2B-GFP-chased for indicated times
H2BGFP Hoechst
Back
Ear
Paw
SLC1A3
VAMP1
K15
SOX6
CXCL12
2-wk doxy
3-wk doxy
3-wk doxy
50um
50um
100 um
G
H2B-GFP low
H2B-GFP high
% of BL Cells
Back skin BL
Ear BL
Paw BL
Slc1a3
Vamp1
Sox6

Figure EV2.

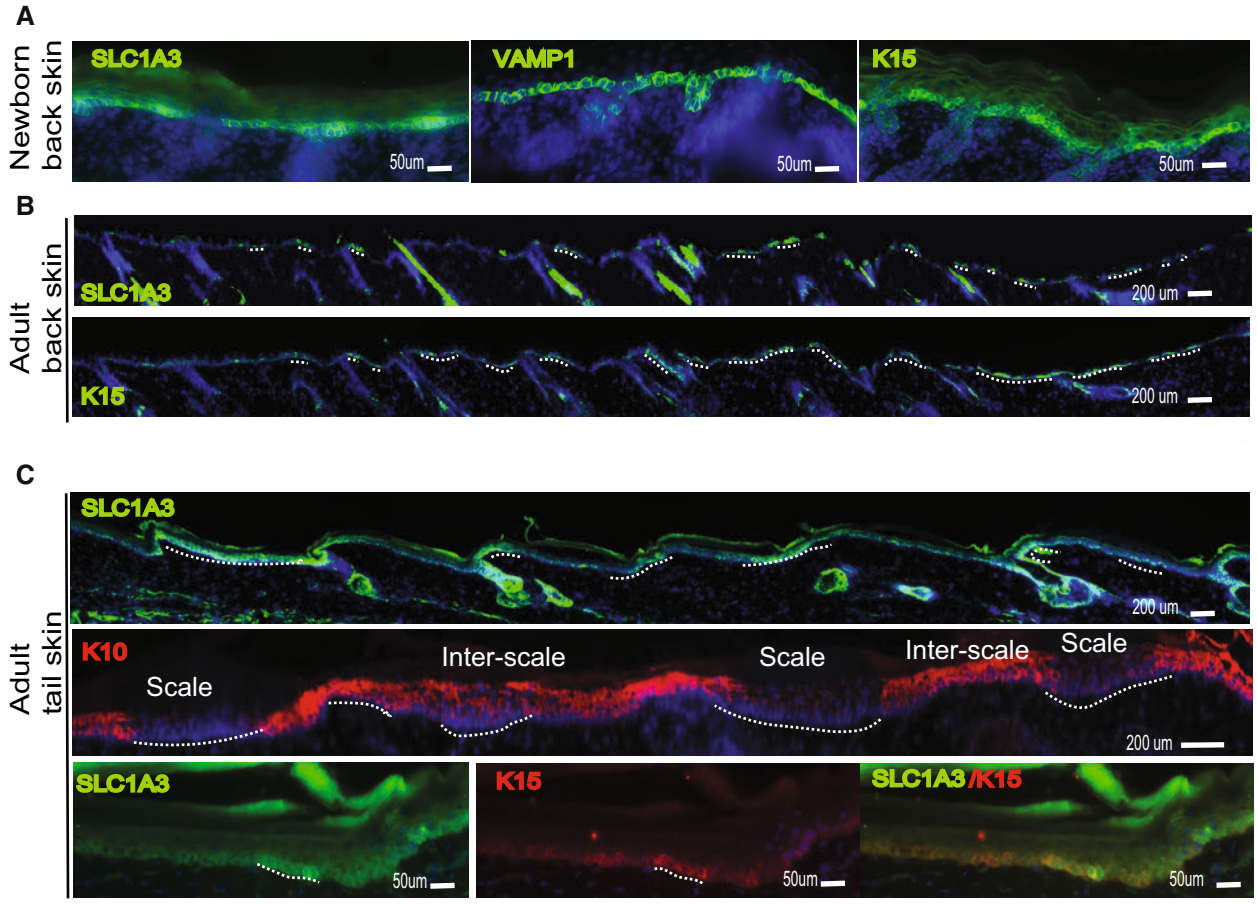

Figure EV3. Expression of select markers in newborn versus adult mouse skin (Associated with Fig 1).

A–C Immunostaining for select markers analyzed in Fig 1D shown here in mouse skin from newborn (A) vs. adult back (B) and adult tail (C) skin. Markers show more uniform expression in newborn back skin. Scale = 50 or 200 μm as indicated on panels.

Source data are available online for this figure.

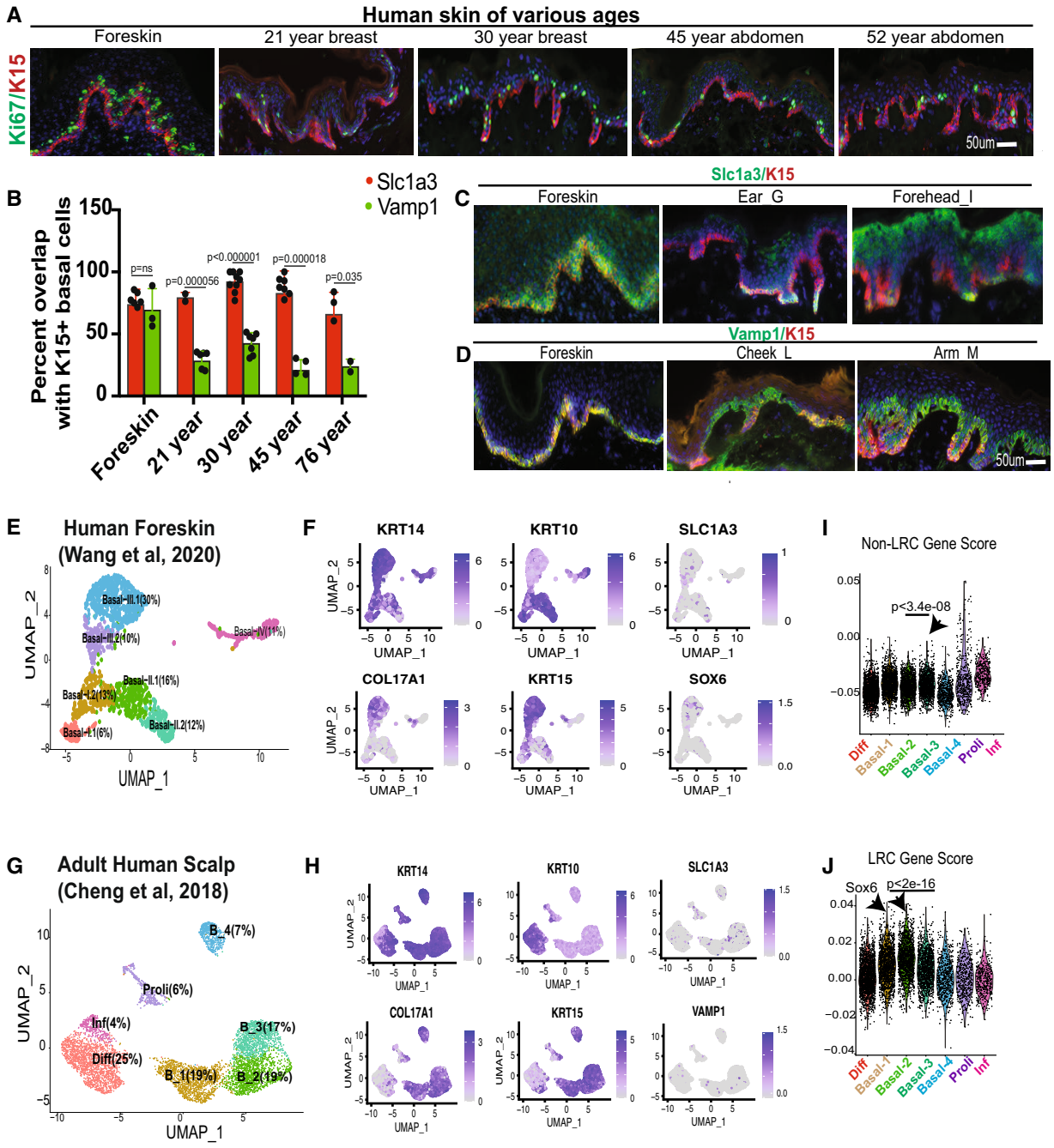

Figure EV4. Human skin LRC/non-LRC marker staining and scRNA-seq analysis (Associated with Fig 2).

A Ki67 staining of human skin at various ages. Scale = 50 μm for all the panels.

B Quantification demonstrates preferential colocalization of K15 with Slc1a3 (non-LRC/scale marker), but not with Vamp1 (LRC/interscale marker) in human skin of all ages, except in newborn foreskin. Error bars are SDs. 2–9 images were quantified from two different samples at every age. *P*-values were calculated by two-tailed Student's *t*-test.

C, D Examples of staining in newborn foreskin and other adult body regions suggests more pronounced heterogeneity in the adult. Scale = 50 μm for all the panels.

E–H (E, G) Seurat generated clusters from scRNA-seq extracted from indicated studies shown as UMAP plots. (F, H) Feature plots show expression of specific markers in clusters from (E) and (G), respectively.

I, J Non-LRC and LRC gene enrichment score analysis in cell clusters from (Cheng *et al*, 2018). *P*-values were calculated using pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction.

Source data are available online for this figure.

Figure EV5. Mouse back skin scRNA-seq of sorted basal LRCs, Mid-LRCs, Non-LRCs (Associated with Figs 3 and 4).

A–C (A) mRNA or gene count and percent mitochondrial genes reflecting the quality of selected cells used for scRNA-seq analysis are shown as violin plots for the (A) two mouse replicates and (B) three sorted IFE cell samples and (C) ten clusters used in our scRNA-seq experiment.

D, E High correlation between UMAP plots (D) and clusters in gene expression matrix (E) from the two mouse replicates.

F–H Gene expression changes in sorted basal cells from microarray (Sada *et al*, 2016) (F), scRNA-seq (G) and qRT-PCR (H); SEM and P-values are indicated. $N = 3$ in (F), two mice in (G) and 4–5 mice (NT is not tested) in (H). Note low or undetectable levels of many markers in (G). Two-tailed Student's *t*-test.

I Violin plots showing expression of genes extracted from refs (Dekoninck *et al*, 2020; Haensel *et al*, 2020) utilized to assign cell identity to each cell cluster.

J Violin plot representation for the LRC/non-LRC marker expression in the given clusters across the samples showing generally low expression or weak differences in clusters.

K Feature plots of cluster-defining markers and LRC/non-LRC genes.

L Scores extracted from previous publication as indicated (Dekoninck *et al*, 2020; Haensel *et al*, 2020; Wang *et al*, 2020) demonstrate correlations among our 3 SC populations with the other databases (Dataset EV1). *P*-value was calculated using pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction.

M Cell cycle scoring of cells in various clusters as defined by cell-cycle phase-specific gene lists using Addmodulescore (see Materials and Methods) function in Seurat.

N LRCs and non-LRCs gene scores from previous microarray of back skin (Sada *et al*, 2016) detect both gene sets in all single-cell derived clusters with some enrichment. *P*-value was calculated using pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction.

Source data are available online for this figure.

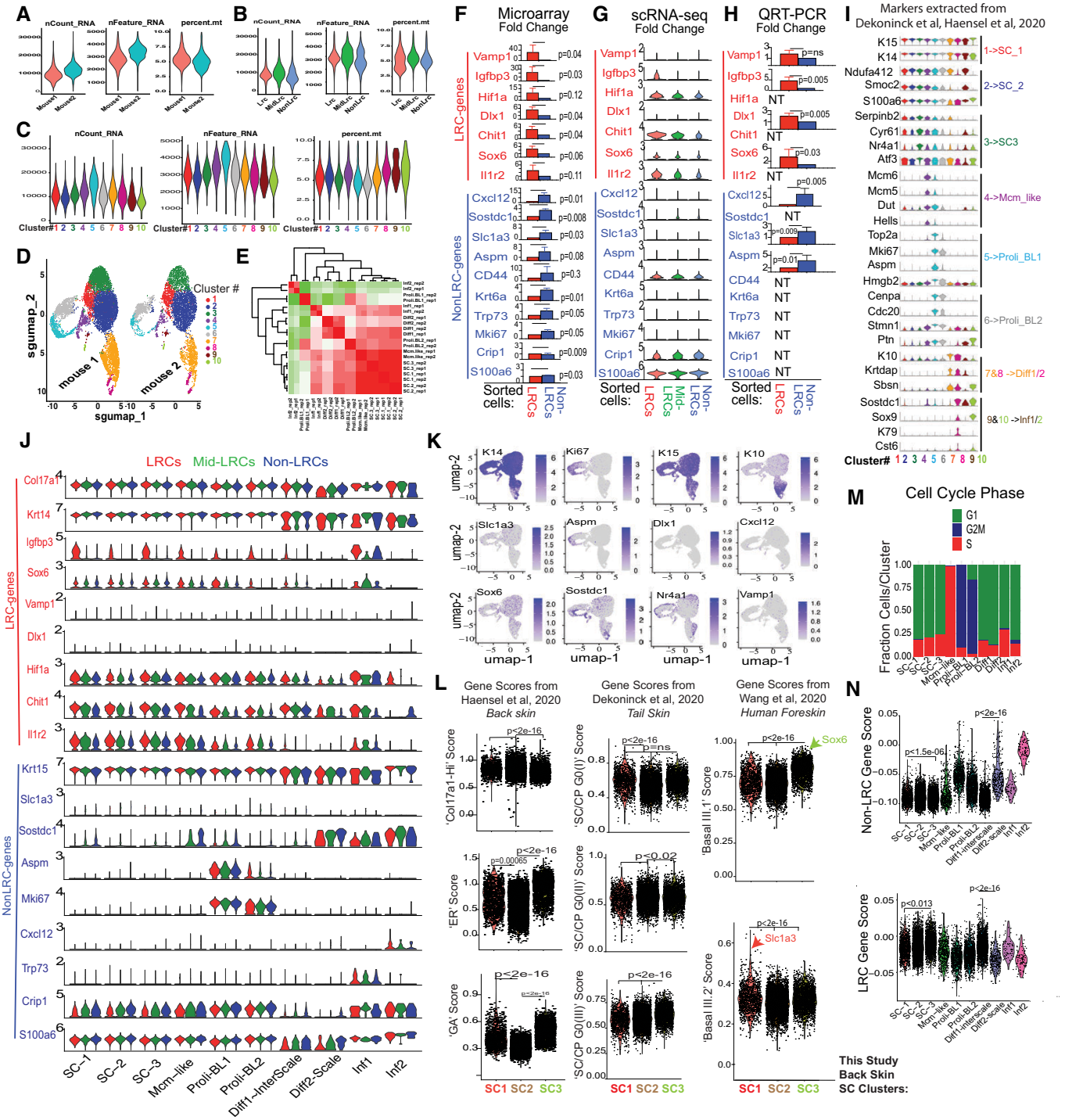
A
nCount_RNA
nFeature_RNA
percent.mt
Mouse1
Mouse2
B
Lrc
MidLrc
NonLrc
C
Cluster#1 2 3 4 5 6 7 8 9 10
D
Cluster #
sgumap_2
sgumap_1
mouse 1
mouse 2
E
F
Microarray
Fold Change
LRC-genes
NonLRC-genes
Vamp1
Igfbp3
Hif1a
Dlx1
Chit1
Sox6
Il1r2
Cxcl12
Sostdc1
Slc1a3
Aspm
CD44
Krt6a
Trp73
Mki67
Crip1
S100a6
Sorted cells:
LRCs
Non-LRCs
G
scRNA-seq
Fold Change
Mid-LRCs
H
QRT-PCR
Fold Change
NT
p=ns
I
Markers extracted from
Dekoninck et al, Haensel et al, 2020
K15
K14
Ndufa412
Smoc2
S100a6
Serpinb2
Cyr61
Nr4a1
Atf3
Mcm6
Mcm5
Dut
Hells
Top2a
Mki67
Aspm
Hmgb2
Cenpa
Cdc20
Stmn1
Ptn
K10
Krtdap
Sbsn
Sostdc1
Sox9
K79
Cst6
1->SC_1
2->SC_2
3->SC3
4->Mcm_like
5->Proli_BL1
6->Proli_BL2
7&8 ->Diff1/2
9&10 ->Inf1/2
Cluster# 1 2 3 4 5 6 7 8 9 10
J
LRCs Mid-LRCs Non-LRCs
Col17a1
Krt14
Krt15
Slc1a3
Sostdc1
Aspm
Mki67
Cxcl12
Trp73
Crip1
S100a6
SC-1
SC-2
SC-3
Mcm-like
Proli-BL1
Proli-BL2
Diff1-InterScale
Diff2-Scale
Inf1
Inf2
K
umap-2
umap-1
K14
Ki67
K15
K10
Slc1a3
Aspm
Dlx1
Cxcl12
Sox6
Sostdc1
Nr4a1
Vamp1
L
Gene Scores from
Haensel et al, 2020
Back skin
Gene Scores from
Dekoninck et al, 2020
Tail Skin
Gene Scores from
Wang et al, 2020
Human Foreskin
'Col17a1-Hi' Score
'ER' Score
'GA' Score
'SC/CP G0(I)' Score
'SC/CP G0(II)' Score
'SC/CP G0(III)' Score
'Basal III.1' Score
'Basal III.2' Score
p<2e-16
p=ns
p=0.00065
p<0.02
Sox6
Slc1a3
SC1 SC2 SC3
M
Cell Cycle Phase
G1
G2M
S
Fraction Cells/Cluster
N
Non-LRC Gene Score
LRC Gene Score
p<1.5e-06
p<0.013
This Study
Back Skin
SC Clusters:

Figure EV5.