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Supplementary Information for

The aging skin microenvironment dictates stem cell behavior

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Supplementary Figure Legends

Figure S1. Single cell RNAseq identifies well-defined epithelial cell populations. T-distributed Stochastic Neighbor Embedding (tSNE) plots from 10x genomics scRNAseq. Data are from young (2 month) and aged (24 month) mice back skin epithelia (young & aged data are combined in top panel). Cells cluster into specific populations, which whose identities were assigned according to their expression of established markers (bottom panels).

Figure S2. No major age-related changes in proliferation and cell cycle transcripts of bulge stem cells from telogen-phase HFs. (*A*) Immunofluorescence of INTEGRIN □6 (ITG□6) in red and KERATIN10 (K10) in green, suggesting no detectable K10 staining within the older bulge of aged skin where two-bulges are still maintained. Bu #1: the older bulge. Bu #2, the newer bulge. (*B*) qPCR validation of candidate ECM genes that are decreased (Fgf18, Ltbp1) or increased (Mmp9, Timp3) in aged bulge compared to young. N = 3. Data are presented as mean ± SEM. Paired t test was performed, **p<0.01. (*C*) FACS quantification of % bulge HFSCs (lineage-, ITG□6+, SCA1-, CD34+) among all basal cells (lineage-, ITG□6+) in young verses aged hairy and aged bald skin. N = 6. Data are presented as mean ± SEM. Paired t test was performed, *p<0.05, **p<0.01. (*D*) Immunofluorescence for proliferation marker KI67 in white reveals no significant age-related differences in HFSCs of telogen-phase HFs. INTEGRIN □6 (ITG□6) in green. * mark the hair shaft of HF. At least five independent biological replicates were analyzed; shown are representative images. Scale bar = 20μm. (*E*) Heatmap of bulk RNAseq data showing hierarchical clustering of cell cycles transcripts, which do not change significantly between young (yg) and aged HFSCs. Red denotes increased expression while blue denotes decreased expression.

Figure S3. Wound-induced lineage infidelity in migrating HFSCs is preserved in aged skin. (*A*) Immunofluorescence for HFSC marker KERATIN 24 (K24) in green shows that epidermis and upper HF are removed by dremel wounding but HF bulges remain intact. Shown are images one-day post injury. Note that scab has formed above the bulge marked by intensive DAPI staining. Scale bar = 50μm. (*B*) Immunofluorescence for isthmus marker LRIG1 shows re-growth of isthmus (arrows) and tissues above it in both young and aged skin, 5d post dremel wounding. Scale bar = 50μm. (*C*) Immunofluorescence for epidermal transcription factor KLF5 and hair follicle transcription factor SOX9, showing dual expression (arrows) in the regenerating area above the bulge of both young and aged HFs. Scale bar = 20μm. Percentages of KLF5+SOX9+ double positive cells are quantified below the images. N = 5. Data are presented as mean ± SEM. Paired t test was performed, N.S., not significant. For (*A*)-(*C*), three to five independent biological replicates were performed, shown are representative images.

Figure S4. *In vitro* culture overrides intrinsic difference between young and aged HFSCs. (*A*) Schematics depict *in vitro* culturing of FACS-purified bulge HFSCs, known to induce lineage infidelity, compared to *in vivo* partial-thickness (dremel) wounding, where HFSCs also undergo (transient) lineage infidelity. (*B*) qRT-PCR showing comparable expression of lineage infidelity signature genes in young and aged cultured HFSCs, including epidermal stem cell (EpdSC) markers (*Klf5, Tfap2c*), HFSC markers (*Sox9, Grem1*), and stress specific marker microRNA-21 (*miR-21*). Dashed line indicates baseline for log2 fold change plotted. N = 5. Data are presented as mean ± SEM. Paired t test was performed, N.S., not significant. (*C*) Quantification of colony numbers formed from FACS-purified bulge HFSCs isolated and cultured from young and aged telogen-phase skins. N = 5. Data are presented as mean ± SEM. Paired t test was performed, **p<0.01. N.S., not significant.

Figure S5. Neonatal dermis rejuvenates aged HFSCs to youthful levels in hair follicle regeneration. Immunofluorescence of HFSC marker K24 in green and isthmus marker LRIG1 in white showing regenerated HFs derived from grafts of neonatal dermal cells combined with young and aged HFSCs. Arrows denote K24 in regenerated HFs from grafts of both young and aged HFSCs. INTEGRIN □6 (ITG□6) in red outlines HFs. Five independent biological replicates were performed, shown are representative images. Scale bar = 20μm.

Legends for Supplementary movies

Movie S1. Young adult hair follicle organizes into two-bulge structure at second telogen. Immunofluorescence of E-CADHERIN in green showing the hair follicle including the bulges where the HFSCs reside, P-CADHERIN in white showing the hair germ where the primed HFSCs reside. Five independent biological replicates were performed, shown are representative images.

Movie S2. Hair follicle in aged hairy mouse back skin only has one bulge. Immunofluorescence of E-CADHERIN in green showing the hair follicle including the bulges where the HFSCs reside, P-CADHERIN in white showing the hair germ where the primed HFSCs reside. Note only one bulge in the hair follicle. Five independent biological replicates were performed, shown are representative images.

Movie S3. Hair follicle in aged bald mouse back skin only has one bulge. Immunofluorescence of E-CADHERIN in green showing the hair follicle including the bulges where the HFSCs reside, and P-CADHERIN in white showing the hair germ where the primed HFSCs reside. Note only one bulge in the hair follicle. Five independent biological replicates were performed, shown are representative images.

Movie S4. Sensory neurons wrap above the hair follicle bulge in young animal. Immunofluorescence of LRIG1 in white showing the hair follicle isthmus, and TUJ1 in red showing the sensory neurons targeting to the region below isthmus and above bulge in young skin. Five independent biological replicates were performed, shown are representative images.

Movie S5. Sensory neurons mis-target to the bulge in aged animal. Immunofluorescence of LRIG1 in white showing the hair follicle isthmus, and TUJ1 in red showing the sensory neurons mis-targeting to the bulge in aged skin. Five independent biological replicates were performed, shown are representative images.

Legends for Supplementary datasets

Dataset S1. List of genes differentially expressed for young verses aged bulge HFSCs.

Dataset S2. List of genes differentially expressed for young verses aged EpdSCs.

Figure S1

scRNAseq identifies well-defined cell populations in young and aged skin epithelium

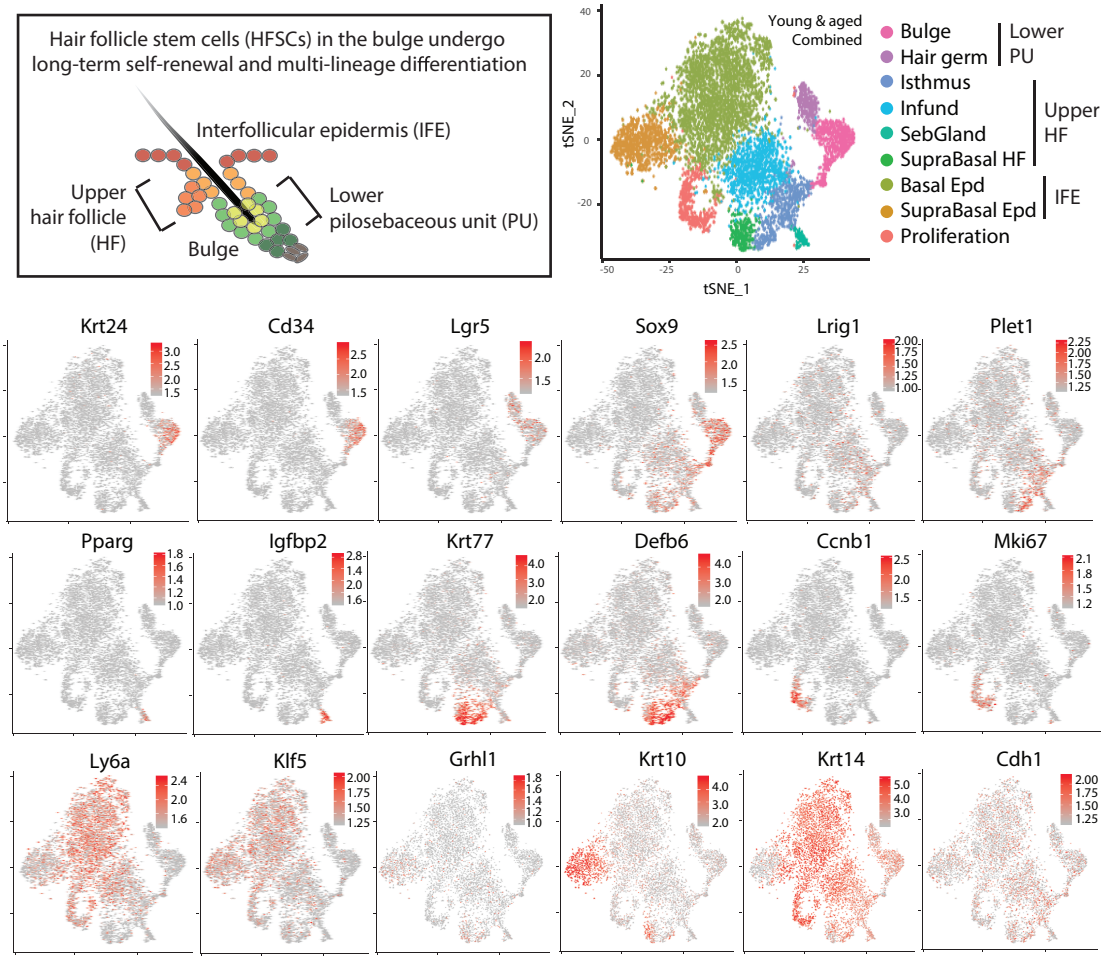


Figure S2

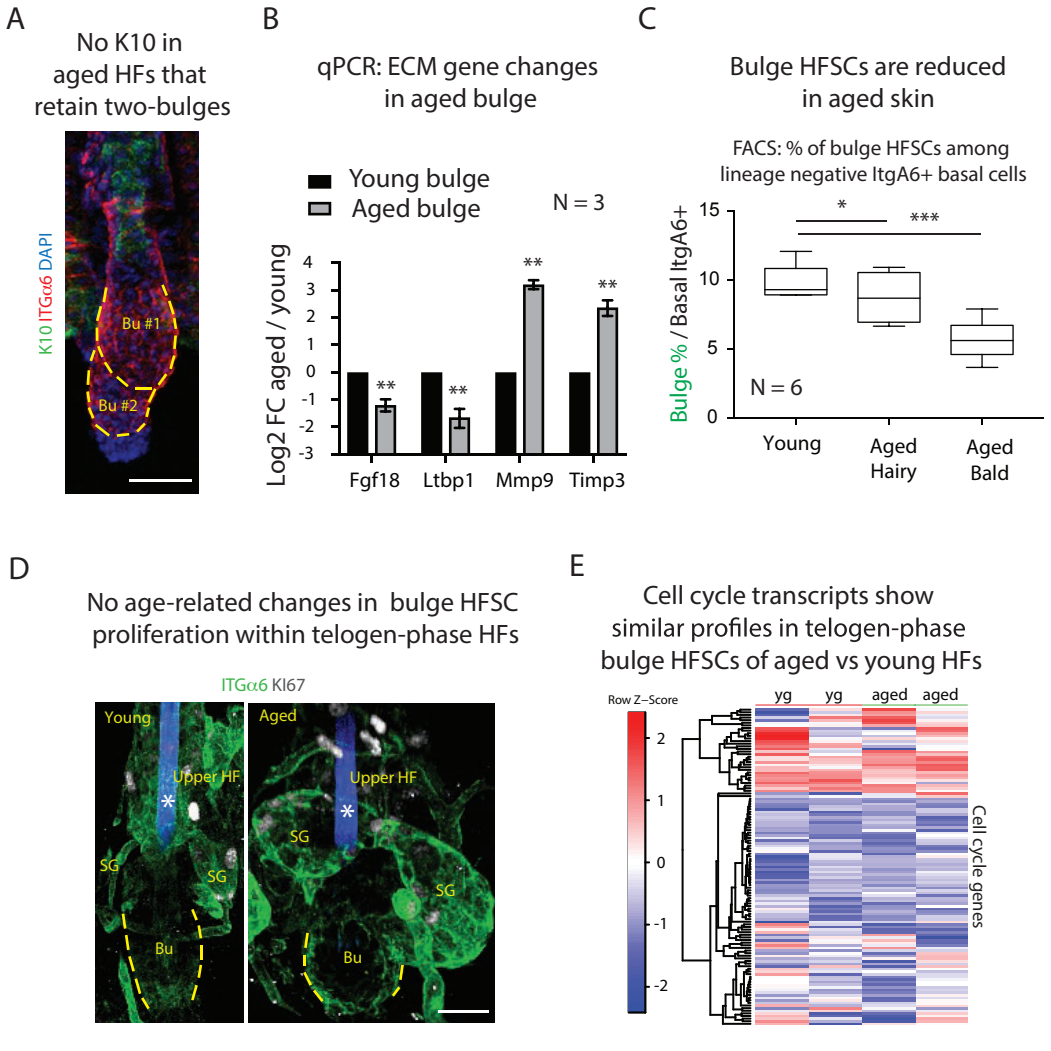
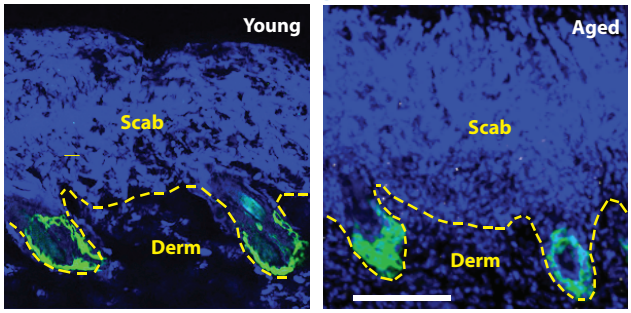

A
No K10 in aged HFs that retain two-bulges
K10 ITGα6 DAPI
Bu #1
Bu #2
B
qPCR: ECM gene changes in aged bulge
Young bulge
Aged bulge
N = 3
Log2 FC aged / young
Fgf18
Ltbp1
Mmp9
Timp3
C
Bulge HFSCs are reduced in aged skin
FACS: % of bulge HFSCs among lineage negative ItgA6+ basal cells
Bulge % / Basal ItgA6+
N = 6
Young
Aged Hairy
Aged Bald
D
No age-related changes in bulge HFSC proliferation within telogen-phase HFs
ITGα6 KI67
Young
Aged
Upper HF
SG
Bu
E
Cell cycle transcripts show similar profiles in telogen-phase bulge HFSCs of aged vs young HFs
Row Z-Score
yg
yg
aged
aged
Cell cycle genes

Figure S3

A

Day 1 post wounding:
partial-thickness dremel wounding preserves
bulge HFSCs in young and aged wounds

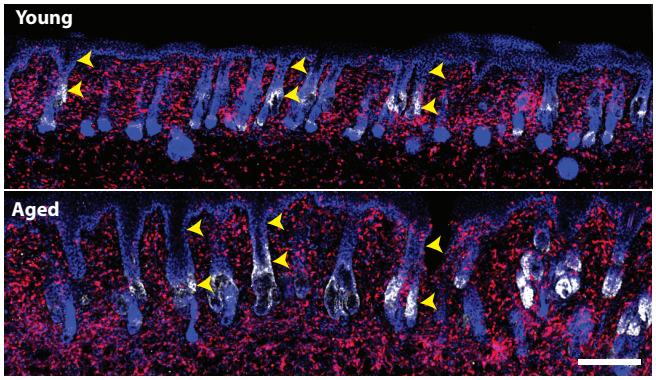
K24 DAPI



B

Day 5 post wounding:
Initiation of hair follicle regeneration is comparable
between young and aged wounds

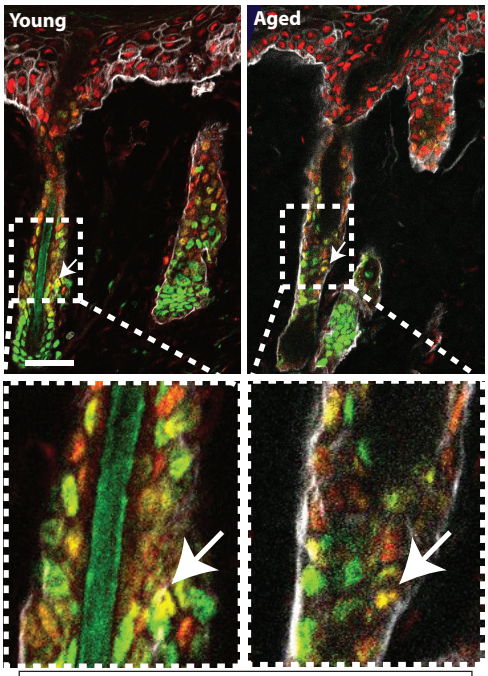
CD45 LRIG1 DAPI



C

Day 7 post wounding:
Lineage infidelity in young and aged wounds

SOX9 KLF5 ITGα6



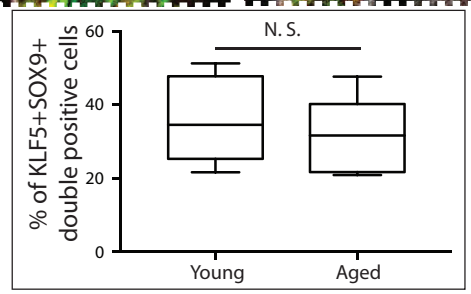


Figure S4

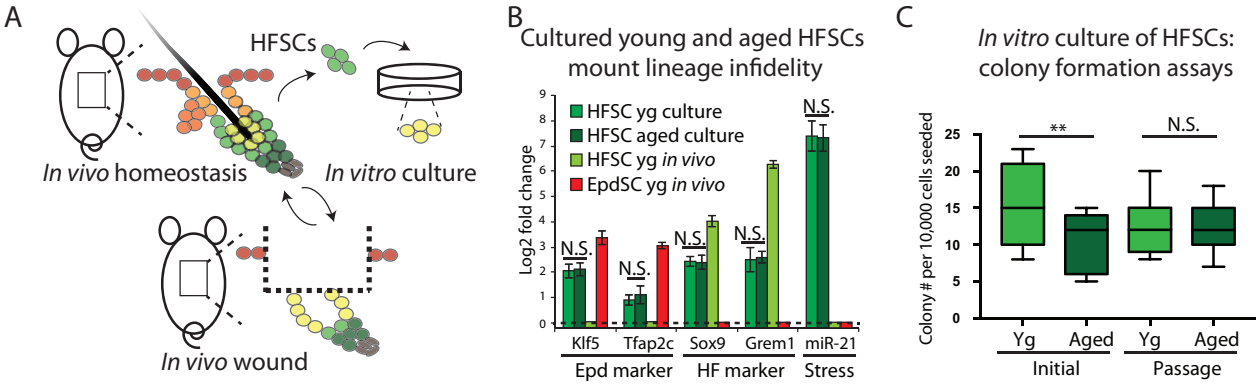

A
HFSCs
In vivo homeostasis
In vitro culture
In vivo wound
B
Cultured young and aged HFSCs mount lineage infidelity
HFSC yg culture
HFSC aged culture
HFSC yg in vivo
EpdSC yg in vivo
Log2 fold change
N.S.
Klf5
Tfap2c
Sox9
Grem1
miR-21
Epd marker
HF marker
Stress
C
In vitro culture of HFSCs: colony formation assays
Colony # per 10,000 cells seeded
**
N.S.
Yg
Aged
Yg
Aged
Initial
Passage

Figure S5

Regenerated HFs from chamber grafts of HFSCs combined with neonatal dermal cells