Expanded View Figures

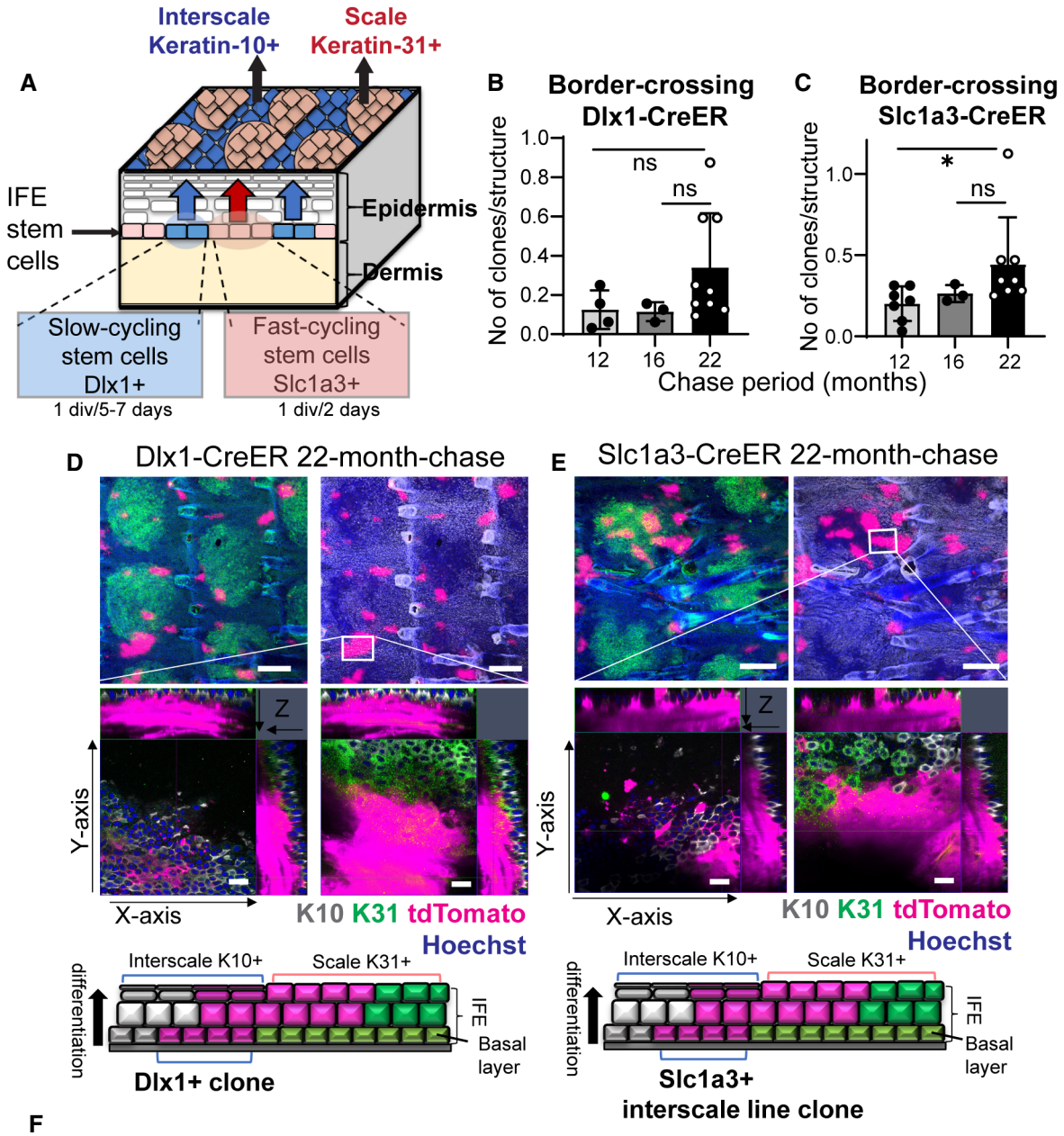
Figure EV1. Disruptions in the epidermal stem cell compartments in aging skin.

A Illustration of a mouse tail skin showing the slow- and fast-cycling stem cells in the interfollicular epidermis (IFE) and their distinct differentiation program, giving rise to the interscale and scale regions, respectively.

B, C The total number of boundary-crossing clones in Dlx1^{CreER} and Slc1a3^{CreER} lineage-tracing mice. Dlx1^{CreER} mice and chase time: $N = 4$ (12 months), $N = 3$ (16 months), and $N = 9$ (22 months). Slc1a3^{CreER} mice and chase time: $N = 7$ (12 months), $N = 3$ (16 months), and $N = 8$ (22 months). One-way ANOVA, Dunn's multiple comparisons test. ns, not significant; $^{*}P < 0.05$. Data show mean ± SD. N reflects biological replicates, which are summarized from at least two independent experiments.

D, E Confocal imaging of representative clones at 2-year-chase, stained with K10 and K31. White boxes indicate areas that are enlarged in the lower panels. Z-stack images show that the clones are originating from the basal layer and expanding into the upper differentiated layers. Cartoons summarize the sagittal view of the clones. Scale bar: 200 μm (upper panels), 20 μm (lower panels). K10 and K31 intensities were adjusted to similar levels between samples.

F Classification of the border-crossing clones from Dlx1^{CreER} and Slc1a3^{CreER} lineage-tracing mice at 16-month- and 22-month-chases. Images of these clones were represented in Fig 1I and EV1D and E.



F

		Dlx1 16-m-chase		Dlx1 22-m-chase		Slc1a3 22-m-chase	
	Category	Clone no	%	Clone no	%	Clone no	%
1	clone contains K10+ cells only	1	6.7	2	9.5	0	0
2	clone contains K31+ cells only	0	0	2	9.5	3	20
3	clone contains K10 and K31 double positive cells	11	73.3	12	57.1	6	40
4	clone contains either K10+ or K31+ cells (no overlap)	3	20	5	23.8	6	40
	Total clone number	15	100	21	100	15	100

Figure EV1.

Figure EV2. Isolation of slow- and fast-cycling epidermal stem cells from H2B-GFP mice and changes in their signature gene expression during aging.

A, B Confocal imaging from wholemount tail epidermis showing GFP expression level in the basal cells before and after 6 weeks of doxycycline chase in the 3-month-old (A) and 2-year-old mice (B). Scale bar: 200 μm.

C FACS plots show isolation strategy of epidermal basal cells (α6-integrin^{high}/CD34^{-}/Sca-1^{+}).

D FACS histograms illustrate isolation of label-retaining cells (LRCs) and non-label-retaining cells (nLRCs) based on their GFP signal peaks representative of the number of cell divisions from young (2-month-old) and old (2-year-old) H2B-GFP mice at 2 weeks doxycycline chase.

E Graph describes LRC or nLRC defined from their GFP dilution in (D) as percentages of basal interfollicular epidermal (IFE) cells in young and old H2B-GFP mice. $N = 3$ mice per group.

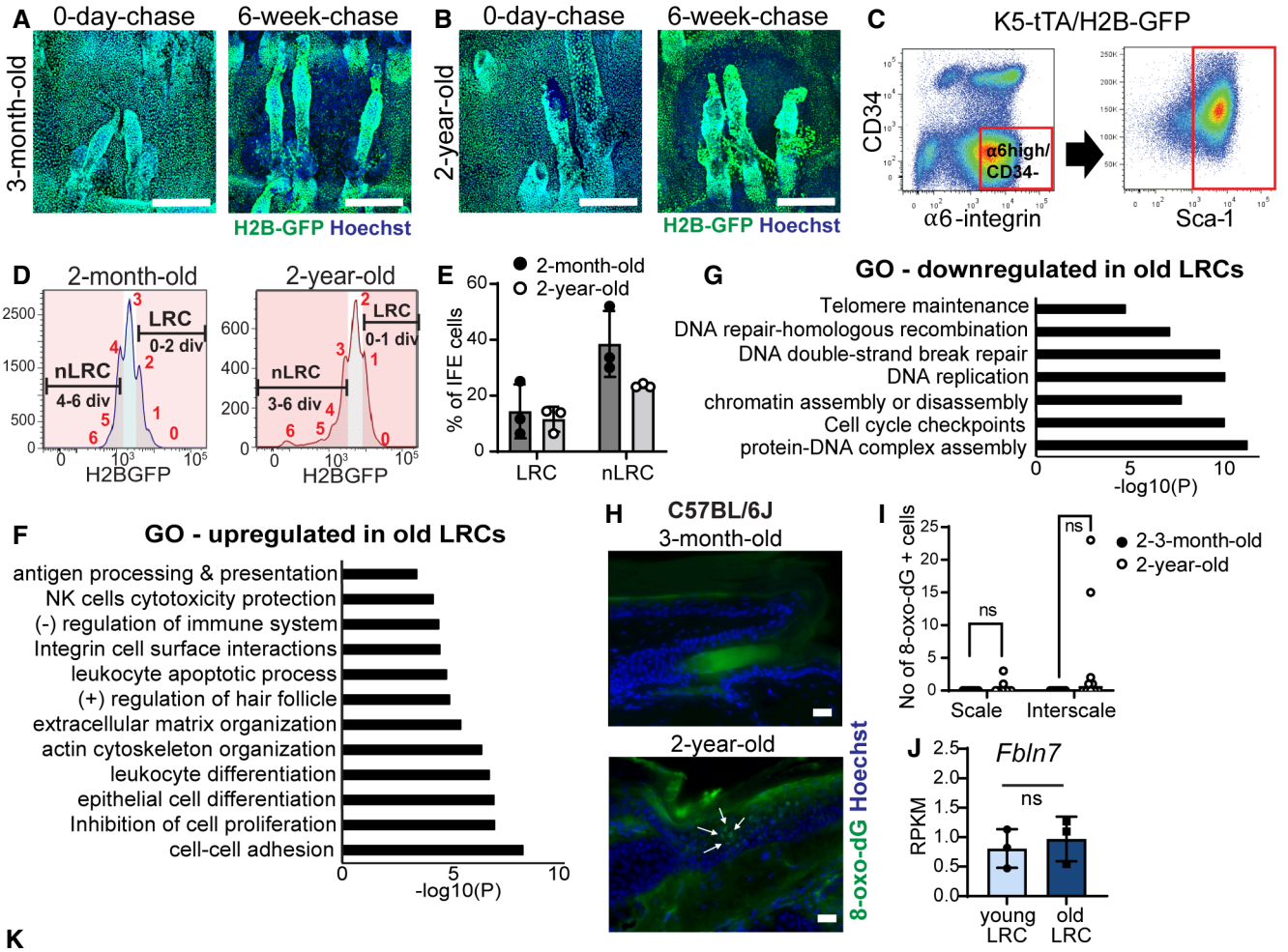
F, G Gene ontology (GO) analysis obtained from ≥twofold differentially regulated genes ($P < 0.05$) in 2-year-old LRCs compared to 2-month-old mice.

H Immunostaining of DNA oxidation marker 8-oxo-dG in tail epidermis of 3-month-old versus 2-year-old C57BL6J mice. White arrows indicate positively stained cells in the interscale region. Scale bar: 20 μm.

I Graph summarizes the number of 8-oxo-dG positively stained cells in the scale or interscale regions of 2–3-month-old ($N = 7$) or 2-year-old mice ($N = 10$). Mann–Whitney test. ns, not significant. *N* reflects number of biological replicates summarized from two independent experiments.

J Fbln7 gene expression in 2-month- versus 2-year-old LRCs. $N = 3$ mice per group (*t*-test). Data show mean ± SD.

K Table shows 6 ECM genes significantly upregulated in the 2-year-old (aged) nLRCs and their known function in the skin (Abreu-Velez & Howard, 2012; Theocharidis *et al*, 2016; Kuwatsuka & Murota, 2019; Pasmatzi *et al*, 2019; Strafella *et al*, 2019). These genes were shortlisted from the 466 genes increased in the aged nLRC (Dataset EV1) according to ECM structural protein and matricellular protein category and RPKM values of ≥1 in 2-year-old nLRC. 2 y; 2-year-old. 2 m; 2-month-old.



K

Genes increased in aged nLRC	Fold change (2y/2m)	T-test: P-value	nLRC 2m (RPKM)	nLRC 2y (RPKM)	Known functions in the skin
Col4a2	23.5762	0.04253	0.0517	1.21878	structural protein of the basement membrane; wound healing & embryogenesis
Col6a1	18.1978	0.03358	0.16955	3.08549	dermal matrix assembly & fibroblast migration; inhibits wound-induced hair growth
Lgals1	5.57646	0.00187	0.78555	4.3806	cell-matrix interactions for cell migration, converting dermal fibroblasts to myofibroblasts
Postn	4.33597	0.00999	5.45772	23.6645	promotes tissue remodelling, dermal fibrosis; associated with skin inflammation
Fbln7	3.60398	0.01841	0.42346	1.52615	none
Col8a1	2.15626	0.02203	0.51807	1.1171	dermal collagen synthesis; associated with atopic dermatitis

Figure EV2.

Figure EV3. Skin histology and cell proliferation assessment in *Fbln7* knockout mice.

A, B Fibulin 7 immunostaining in 3-month- (A) and 1-year-old tail section (B) in *Fbln7* WT versus KO mice. Dotted box areas were enlarged in the lower panels. White arrows indicate fibulin 7 basement membrane staining. Signal in the uppermost stratum corneum is background (asterisk). Scale bar: 50 μm.

C–E Fibulin 7 intensity quantification per basal epidermal stem cell/basement membrane, normalized to WT (C, D) or in plain intensity measurement (E). a.u., arbitrary unit. Data show mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant (Mann–Whitney test). $N = 4$ mice per group in 3-month-old, $N = 6$ WT and 4 KO in 1-year-old mice (C, D). $N = 4$ (3-month-old) and 6 WT mice (1-year-old) (E).

F Hematoxylin and eosin staining from tail sections of 2-month- and 2-year-old mice. Scale bar: 50 μm. Het, heterozygous.

G, H Epidermal thickness measurements from the scale and interscale areas of 2- to 3-month-old (G) and 1-year-old (H) mice. No significant changes were observed among the *Fbln7* WT, het, and KO mice ($N = 5$ WT, $N = 6$ het, $N = 6$ KO in 2- to 3-month-old mice; and $N = 3$ in 1-year-old WT/het/KO). Data show mean ± SD. Mann–Whitney test.

I Wholemount immunostaining from tail epidermis labeled with BrdU and Hoechst nuclear staining. Scale bar: 200 μm.

J, K Quantitation of BrdU+ cells per mm^2 structure area in 2- to 3-month-old mice (J) and 1-year-old mice (K). For 2- to 3-month-old mice, $N = 8$ (WT), $N = 11$ (het), $N = 12$ (KO). For 1-year-old mice, $N = 5$ (WT), $N = 2$ (het), and $N = 8$ (KO). $P = 0.057$ in 2- to 3-month-old *Fbln7* WT versus KO mice from Welch's *t*-test.

Data information: All graphs indicate mean ± SD. *N* reflects the number of biological replicates summarized from at least two independent experiments.

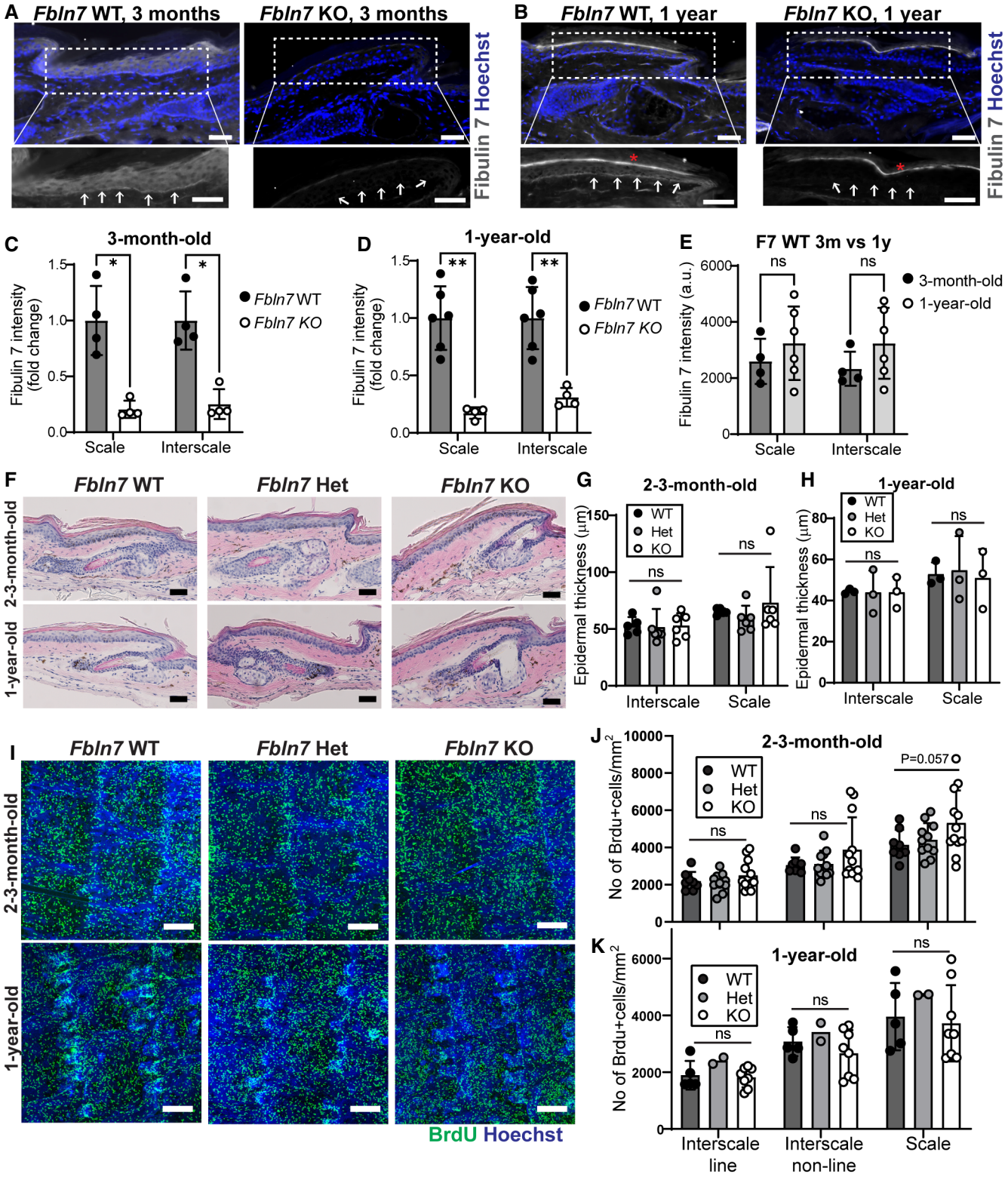

A
Fbln7 WT, 3 months
Fbln7 KO, 3 months
Fibulin 7 Hoechst
B
Fbln7 WT, 1 year
Fbln7 KO, 1 year
Fibulin 7 Hoechst
C
3-month-old
Fibulin 7 intensity (fold change)
Fbln7 WT
Fbln7 KO
Scale
Interscale
D
1-year-old
Fibulin 7 intensity (fold change)
Fbln7 WT
Fbln7 KO
Scale
Interscale
E
F7 WT 3m vs 1y
Fibulin 7 intensity (a.u.)
ns
3-month-old
1-year-old
Scale
Interscale
F
Fbln7 WT
Fbln7 Het
Fbln7 KO
2-3-month-old
1-year-old
G
2-3-month-old
Epidermal thickness (µm)
WT
Het
KO
ns
Interscale
Scale
H
1-year-old
Epidermal thickness (µm)
WT
Het
KO
ns
Interscale
Scale
I
Fbln7 WT
Fbln7 Het
Fbln7 KO
2-3-month-old
1-year-old
BrdU Hoechst
J
2-3-month-old
No of Brdu+cells/mm2
WT
Het
KO
ns
P=0.057
K
1-year-old
No of Brdu+cells/mm2
WT
Het
KO
ns
Interscale line
Interscale non-line
Scale

Figure EV3.

Figure EV4. *Fbln7* knockout does not affect the maintenance of slow-cycling stem cells or wound healing in young mice.

A Dlx1^{CreER} lineage tracing in the *Fbln7* WT and Het backgrounds. Low-dose tamoxifen was administered once at 2 months of age and samples were collected after 1-week, 3-month, and 1-year chases. Wholemount staining of tail epidermis with tdTomato, K10, and Hoechst. Scale bar: 200 μm.

B The number of Dlx1^{CreER} clones in the scale or interscale (both line and non-line) of *Fbln7* WT mice for 1-week ($N = 4$), 3-month ($N = 4$), 1-year chase ($N = 3$) and *Fbln7* het mice for 1-week ($N = 5$), 3-month ($N = 3$), and 1-year ($N = 3$) chase. ns, not significant. Mann–Whitney test.

C The number of Slc1a3^{CreER} clones in the interscale non-line or interscale line regions for 1-week, 3-month or 1-year chase. *Fbln7* WT mice for 1-week ($N = 5$), 3-month ($N = 3$), and 1-year chase ($N = 3$). *Fbln7* KO mice for 1-week ($N = 5$), 3-month ($N = 4$), and 1-year ($N = 6$) chase.

D Quantitation of the area of Slc1a3^{CreER} clones per structural area from the same experiment as in (C).

E, F Representative pictures from tail wounds of 2-month versus 2-year-old C57BL/6J wild-type mice (E) and measurements of wound area over time (F). $N = 6$ (2-month-old) and $N = 5$ (2-year-old). Scale bar: 4 mm. $^{**}P < 0.01$; $^{*}P < 0.05$.

G, H Representative pictures from tail wound healing experiment in 2- to 3-month-old *Fbln7* mice (G) and the wound area quantitation over time (H). $N = 4$ (WT), $N = 8$ (Het), and $N = 7$ (KO). Scale bar: 4 mm.

Data information: All graphs show mean ± SD. *N* reflects the number of biological replicates summarized from at least two independent experiments. Mann–Whitney test was performed for (B–D). two-way ANOVA (Tukey's multiple comparison test) was performed for (F, H).

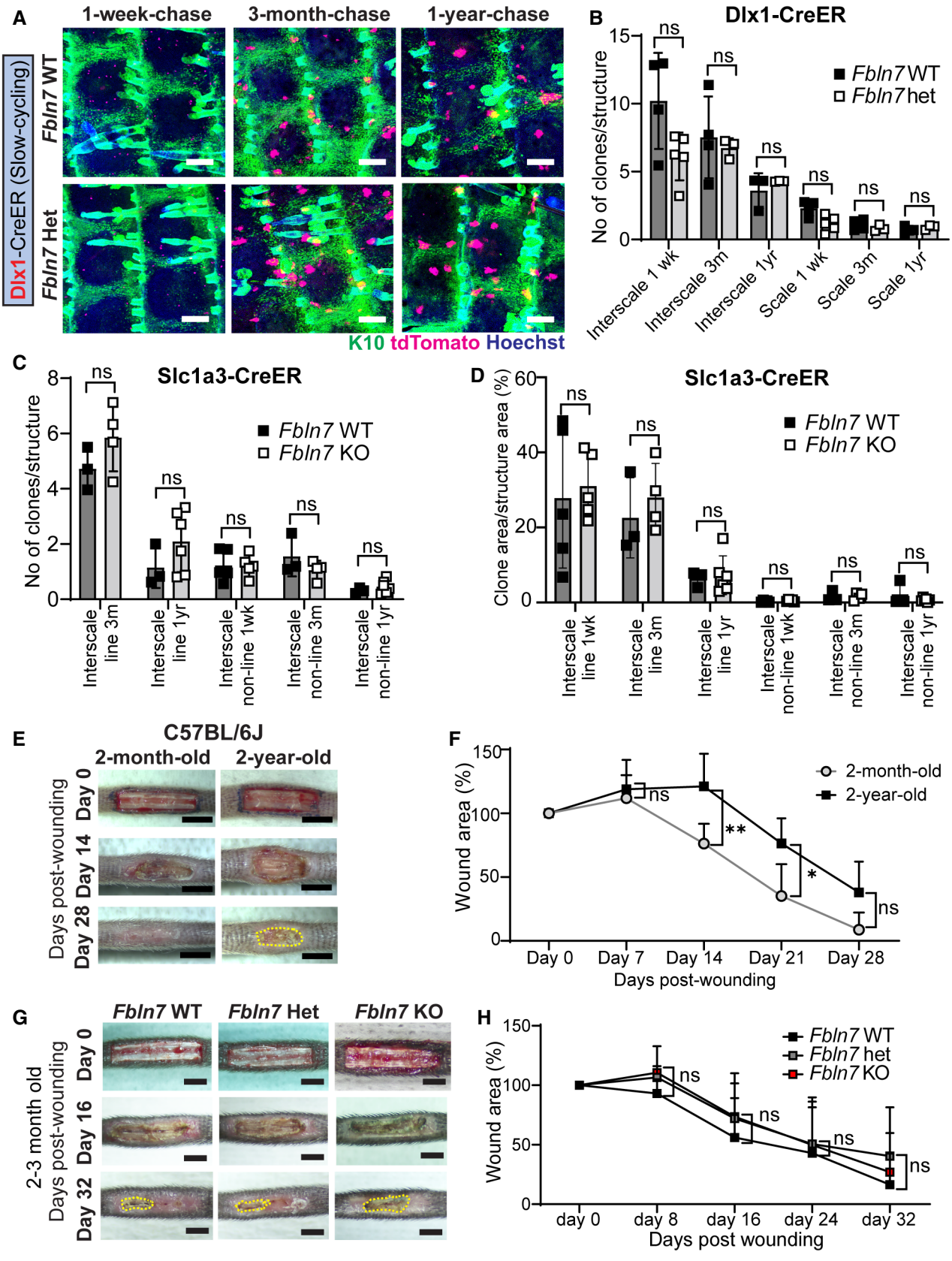
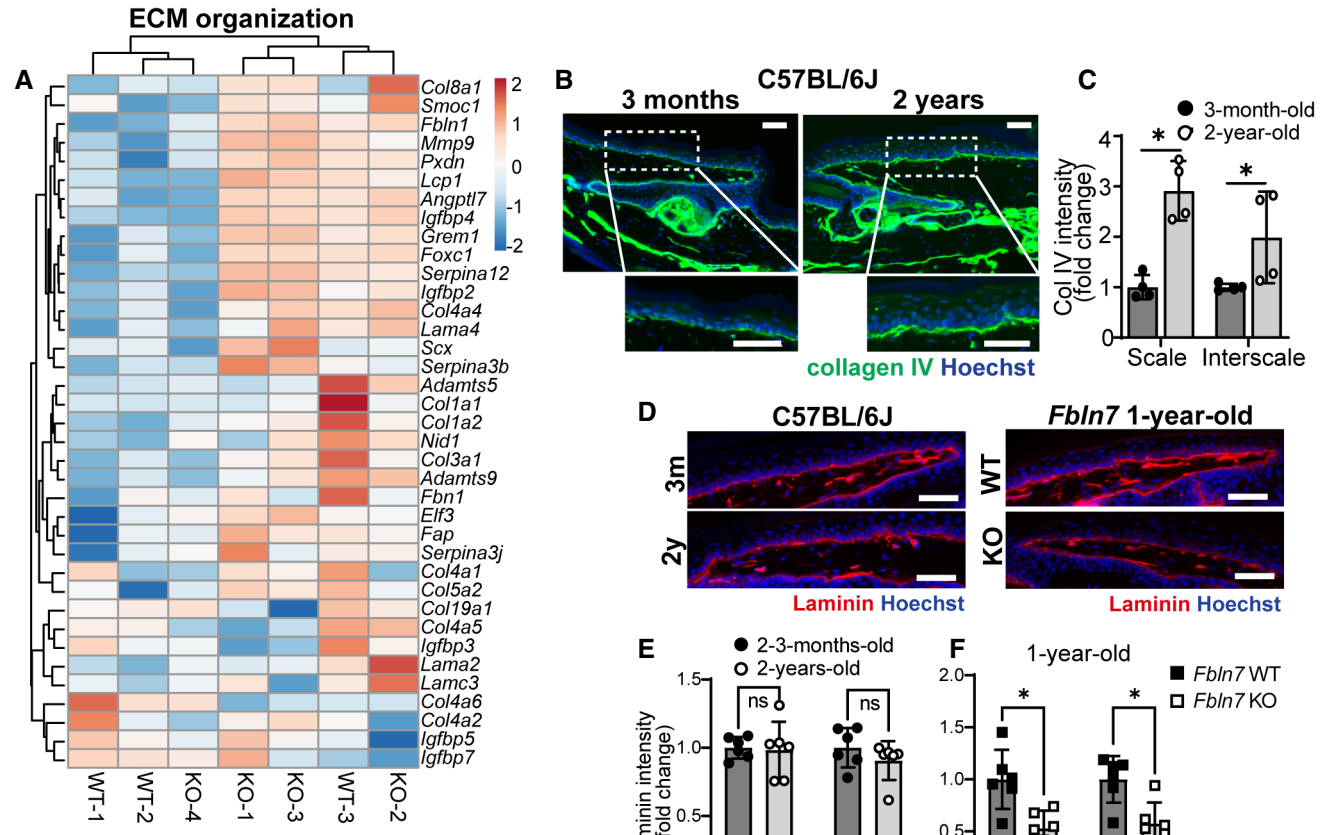

A
1-week-chase
3-month-chase
1-year-chase
Dlx1-CreER (Slow-cycling)
Fbln7 WT
Fbln7 Het
K10 tdTomato Hoechst
B
Dlx1-CreER
No of clones/structure
Fbln7WT
Fbln7het
ns
Interscale 1 wk
Interscale 3m
Interscale 1yr
Scale 1 wk
Scale 3m
Scale 1yr
C
Slc1a3-CreER
No of clones/structure
Fbln7 WT
Fbln7 KO
Interscale line 3m
Interscale line 1yr
Interscale non-line 1wk
Interscale non-line 3m
Interscale non-line 1yr
D
Slc1a3-CreER
Clone area/structure area (%)
Fbln7 WT
Fbln7 KO
Interscale line 1wk
Interscale line 3m
Interscale line 1yr
Interscale non-line 1wk
Interscale non-line 3m
Interscale non-line 1yr
E
C57BL/6J
2-month-old
2-year-old
Days post-wounding
Day 0
Day 14
Day 28
F
Wound area (%)
2-month-old
2-year-old
Day 0
Day 7
Day 14
Day 21
Day 28
Days post-wounding
G
Fbln7 WT
Fbln7 Het
Fbln7 KO
2-3 month old
Days post-wounding
Day 0
Day 16
Day 32
H
Wound area (%)
Fbln7 WT
Fbln7 het
Fbln7 KO
day 0
day 8
day 16
day 24
day 32
Days post wounding

Figure EV4.

Figure EV5. Transcriptome of *Fbln7* KO mice and fibulin 7-binding assays indicate fibulin 7 mechanism of action through ECM regulation.

A Heatmap of ≥twofold upregulated or downregulated genes related to ECM from gene ontology analysis in Fig 4B and C. Scale reflects *Z*-score.
B Collagen IV immunostaining in tail sections of 3-month-old versus 2-year-old C57BL/6J mice. Dotted box regions are enlarged.
C Quantification of Collagen IV basement membrane intensity per cell in 2-year-old mice normalized to 3-month-old mice in C57BL/6J mice (B). $*P < 0.05$. $N = 4$ mice per age group (biological replicates).
D Laminin immunostaining in tail sections of 3-month-old versus 2-year-old C57BL/6J mice (left panels) and 1-year-old *Fbln7* WT versus KO (right panels).
E, F Quantification of laminin basement membrane intensity per cell in 2-year-old mice normalized to 3-month-old mice in C57BL/6J mice (E) or in *Fbln7* KO mice normalized to WT mice (F). $N = 6$ per group for all mice. ns; not significant.
G Collagen XVII immunostaining in tail sections of 3-month-old versus 2-year-old C57BL/6J mice (left panels) and 1-year-old *Fbln7* WT versus KO (right panels).
H, I Quantification of Collagen XVII intensity per cell in 2-year-old mice normalized to 3-month-old mice in C57BL/6J mice (H) or in *Fbln7* KO mice normalized to WT mice (I). $N = 7$ (2–3 month-old) and $N = 10$ (2-year-old) C57BL/6J mice; $N = 6$ per group in *Fbln7* mice. $**P < 0.01$.
J Shortlisted fibulin 7-binding protein candidates (from Fig 5D and E) and their reported functions.
K Solid-phase binding assays using recombinant fibulin 7 as liquid phase and purified or recombinant ECM proteins as solid phase. *X*-axis shows increasing doses of fibulin 7 (μg/well). Bovine serum albumin (BSA) was used as the control liquid phase and added in the same amounts as fibulin 7. Data are from four technical repeats in two independent experiments.

Data information: All graphs show mean ± SD with Mann–Whitney test. *N* reflects the number of biological replicates summarized from at least two independent experiments. All scale bars: 50 μm.



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Fibulin 7- binding proteins	Known function
Collagen Ⅳ, Fibulin 1	Structural integrity of basement membranes.
Periostin	Chronic inflammatory diseases. Interacts with Fibronectin,Tenascin C, Integrins, IL-1/-6. Keratinocyte proliferation.
Ccdc80	Cell adhesion. JAK2/STAT signaling. Associated with atopic dermatitis.
Tenascin C	Chronic inflammation, wound healing, modulates proliferation.

Figure EV5.