Aging Suppresses Skin-Derived Circulating SDF1 to Promote Full-Thickness Tissue Regeneration

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Introduction

- Wound repair two biological processes:
  - Scar formation
  - Tissue regeneration

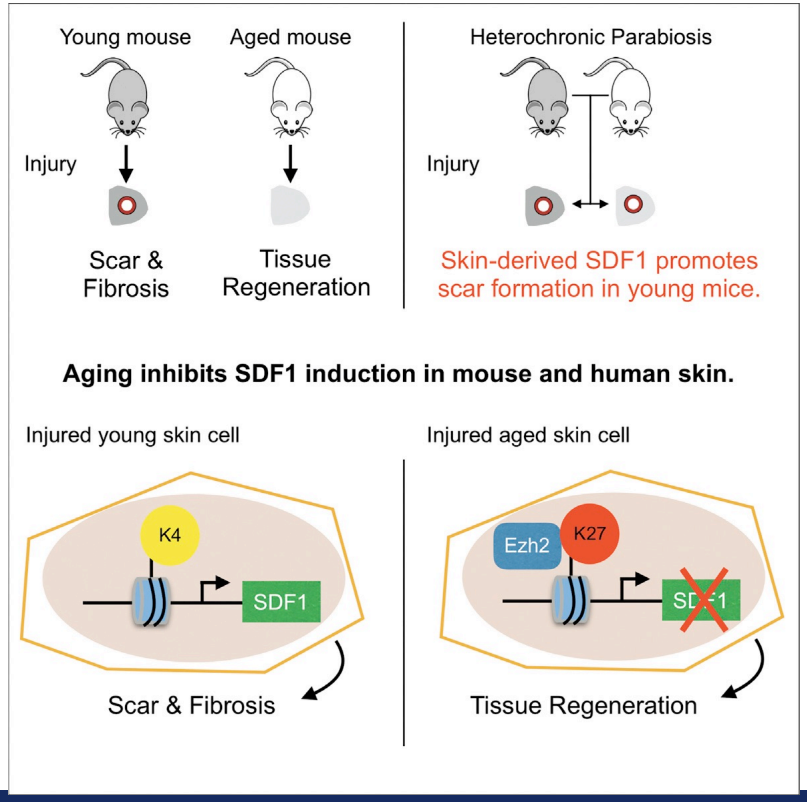
- Amphibians regenerate lost limbs
- Mammals repair injured skin with scar formation
  - Exception: liver regeneration, pediatric traumatic digit tip amputation, fetal skin wounds

- Human skin wounds invariably form scars
- Aging: slows skin re-epithelialization & rate of wound repair
- Strength of re-epithelized skin remains the same of any age

- Skin wounds in elderly close with thinner scar formation
- Incidence of keloid and hypertrophic scar formation peaks in second decade of life and decreases with age

Introduction

- Surgical wounds in elderly heal with thinner scars than wound in young patients
- ***Exposure of aged mice to blood from young mice by parabiosis***

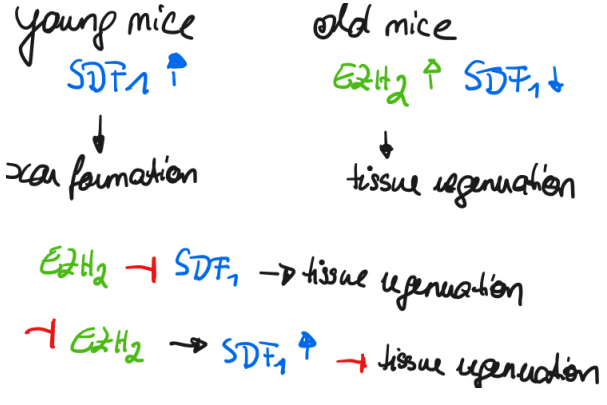


SDF1 stromal derived factor 1

- CXC motif chemokine 12 (CXCL12)
- Binds chemokine receptor type 4 and 7
- Expressed by several cell types (osteoblasten, fibroblasten, endothelial cells)
- Important in stem cell migration and proliferation
- Higher levels in young mice
- Genetic deletion of SDF enhanced tissue regeneration

EZH2

- Histone methyltransferase
- Histone methylation – suppress activity of certain genes



- Change due to circulating factor in blood?
- Identification of this factor
- SDF1, generated mouse that lacked SDF1 protein in the skin
- How does getting old shut of SDF1 production?
- EZH2 inhibition?
- Findings also true in human skin?

Methods

- Injury models
- Murine excisional back wound model
- Parabiosis
- Histology and Immunohistochemistry
- Real-time RT-PCR
- Chromatin Immunoprecipitation (ChIP)
- ELISA
- Pharmacologic inhibitor experiments

Methods

Animals

- C57BL/6 female mice (18 month) national institute on Aging
- C57BL/6 male mice (18 month) Jackson Labs
- C57BL/5 female mice (1 month)
- K5-rtTA;tetO-Cre mice (Sarah Miller)
- Doxycycline food pellets (6gm/kg) for one week or
- Tamoxifen (1mg) daily for 5 days (intraperitoneal injection)

Methods

Cell culture and human skin oragnoids

- Primary human keratinocytes of different ages and gender obtained from University of Pennsylvania
- Cells were grown in supplemented media (50:50, keratinocytes-SFM und Medium 154)
- Nutrient deprivation: media was removed and replaced with un-supplemented media, for 24h
- Primary human keratinocyten were seeded onto acellular human dermis, in growth media
- Organoids maintained at 37°C for 4 days prior to being wounded

Methods

Injury models

- Standard 2mm mechanical punch
- Create a hole in the center of each outer ear
- Ear hole diameter was measured weekly
- Ears were excluded if there were signs of wound infection, tearing of the ear, or abnormal geometric shape

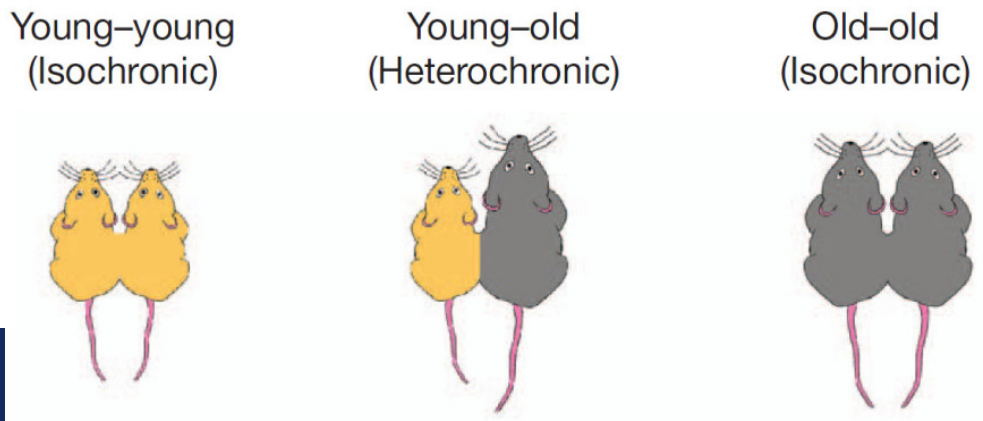
Murine excisional back wound model

- 6mm disposable biopsy punch
- Two circular full thickness wounds on the dorsal back skin of mice
- Silicone wound splints were sutured with 4-0 Nylon to prevent skin contracture
- Borders were monitored by application of permanent marker

Methods

Parabiosis

- Mirror-image incisions at the left and right flanks
- Elbow and knee joints were sutured together
- 1 month after parabiosis surgery – standard ear punch assay



Histology, ChIP, PCR

- Standard histology and immunostaining protocols were performed
- Chromatin Immunoprecipitation (ChIP)
  - Ear tissue 4% formaldehyde, frozen in nitrogen, buffer, antibody (H3K27me3)
- PCR

Methods

Pharmacologic inhibitor experiments

- Mice treated with DZNep received IP injections – three times/week
- Pre-treated for 1 week before ear punch

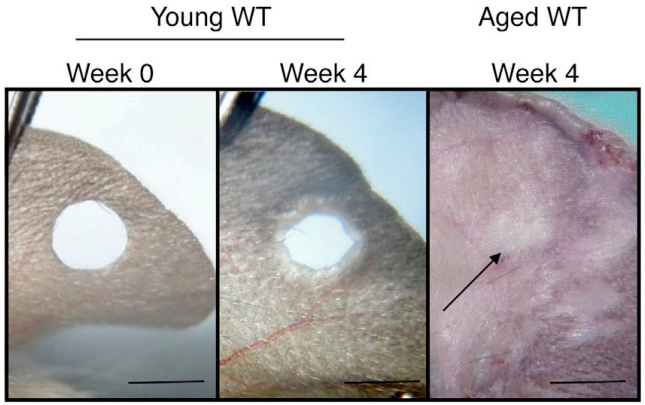
Knockdown experiments

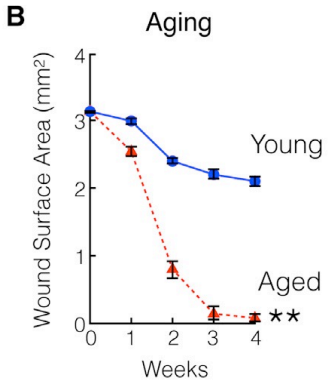
- Obtained Lentiviral knockdown vectors
- Vectors used to create EZH2-knockdown keratinocytes

RESULTS

AGING PROMOTES TISSUE REGENERATION AND DECREASES SCAR FORMATION IN MOUSE EARS

- 1 month old wild-type mice
- 2-mm ear holes
- Closed to significantly larger size compared with 18 month old WT mice



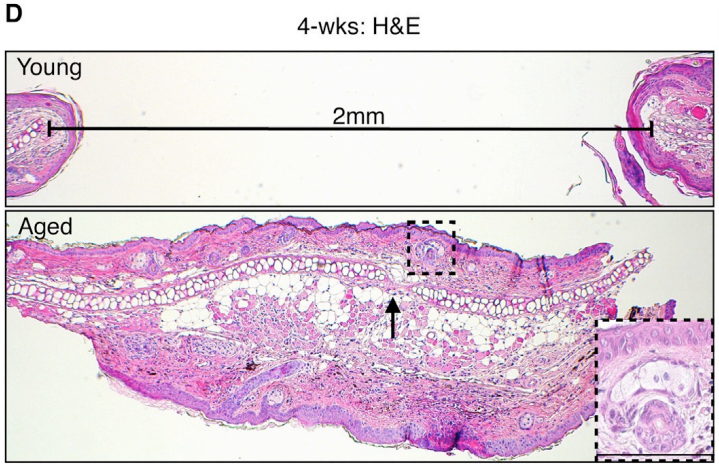


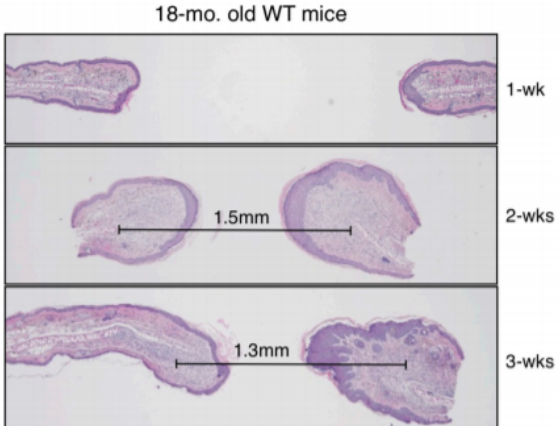
Young mice

- Horizontally oriented fibroblasts and glassy tickenend collagend – consistent with tissue fibrosis and scar formation
- Cartilage end plates 2mm apart – absence of cartilage regeneration

Old mice

- Normal tissue architecture, hair follicels, sebaceous glands, subcutaneous fat
- Opposing cartilage end plates re-anastomosed (black arrow)





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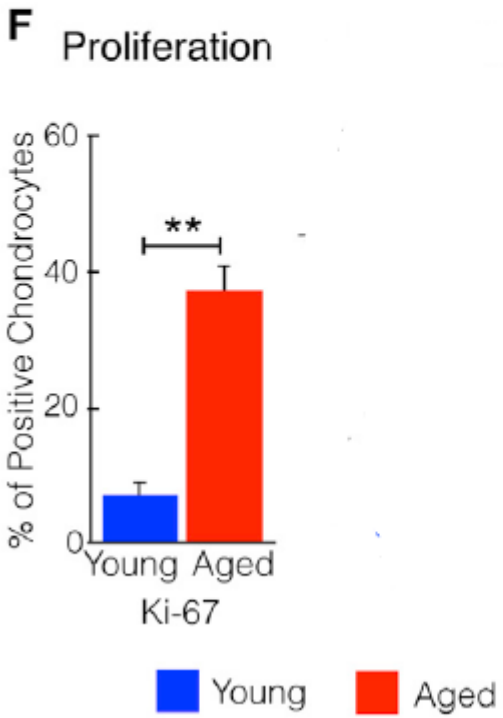

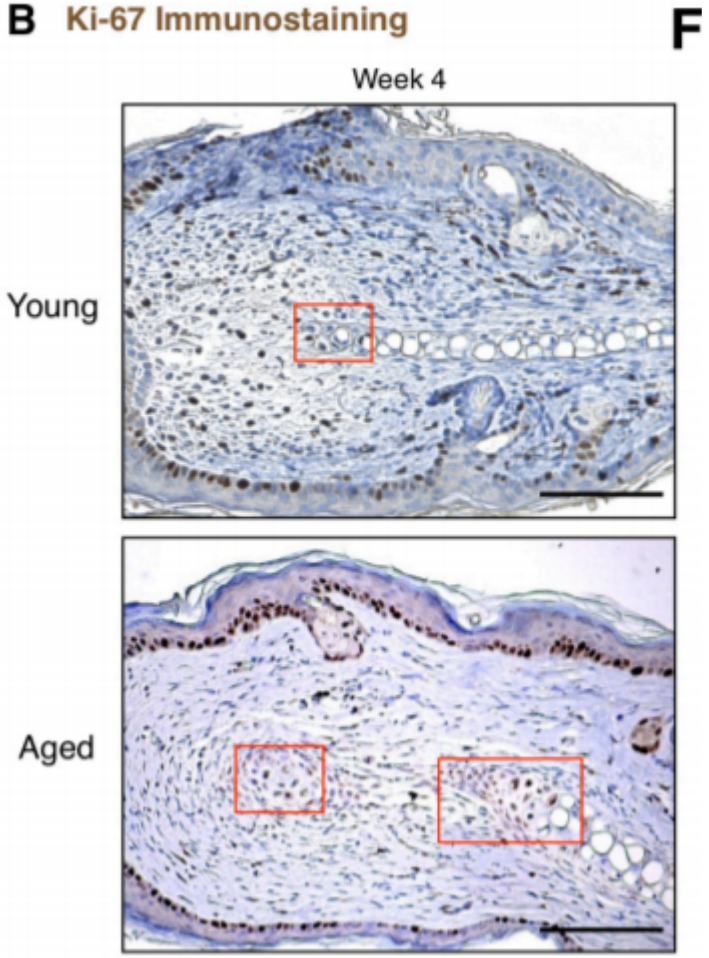

- Significantly more chondrocytes expressed Ki-67

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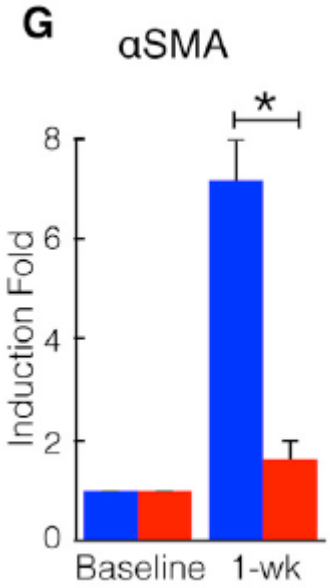
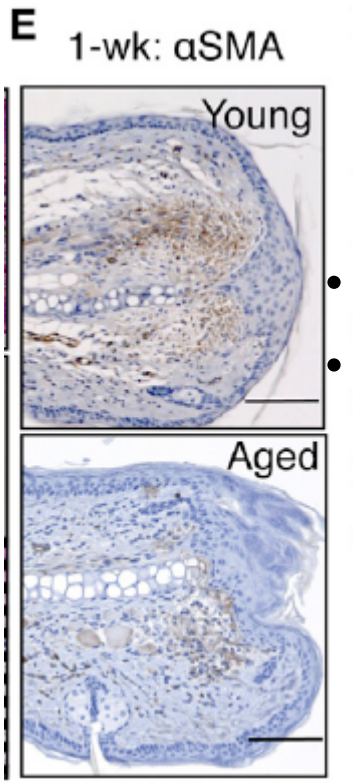
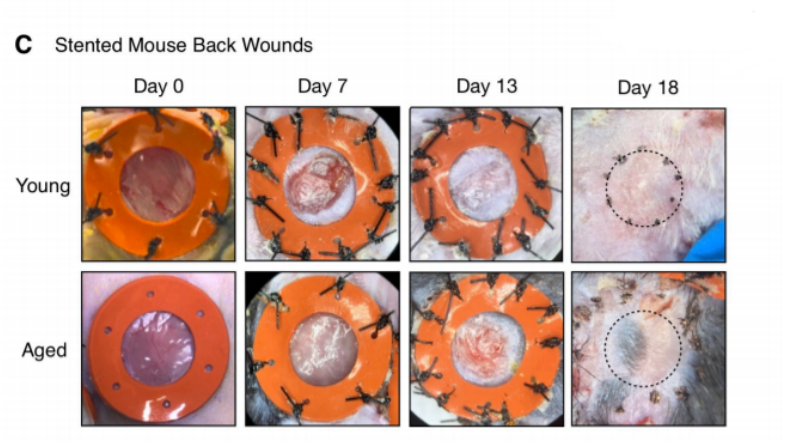


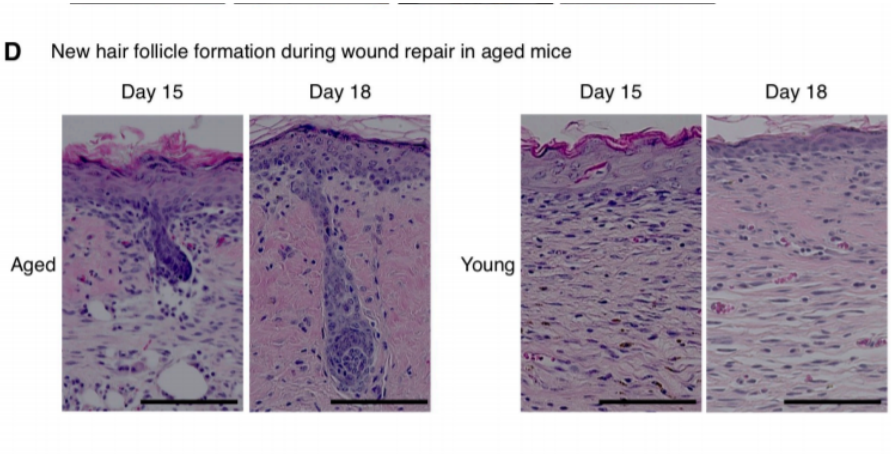


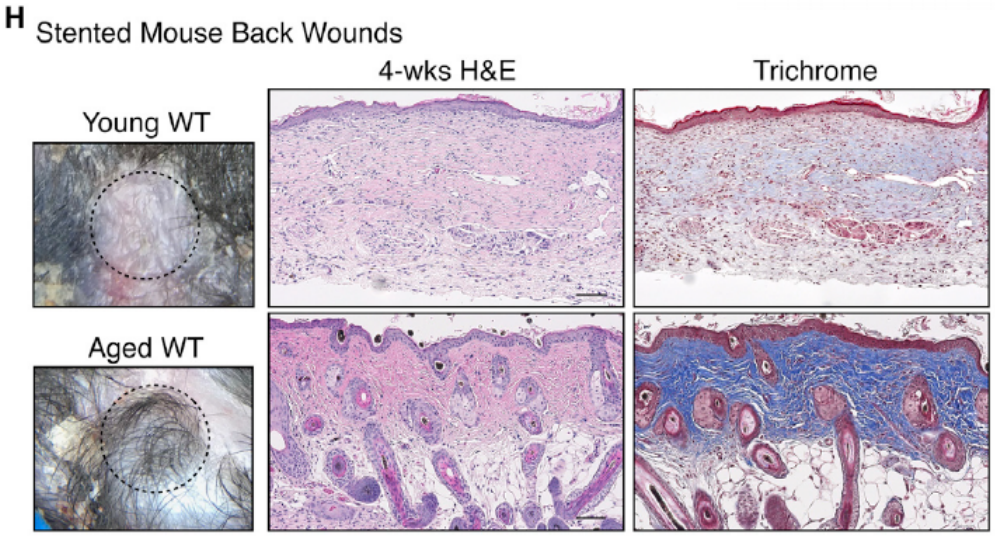
- AlphaSMA brown cells
- Immunostaining of ears fom young and aged mice

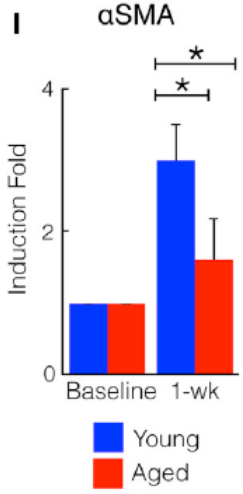
- Injured aged miced expressed significantly lower levels of alpha smooth muscl actin
- Marker of myofibroblasts involved in scar formation

AGING PROMOTES TISSUE REGENERATION AND DECREASES SCAR FORMATION IN MOUSE EARS



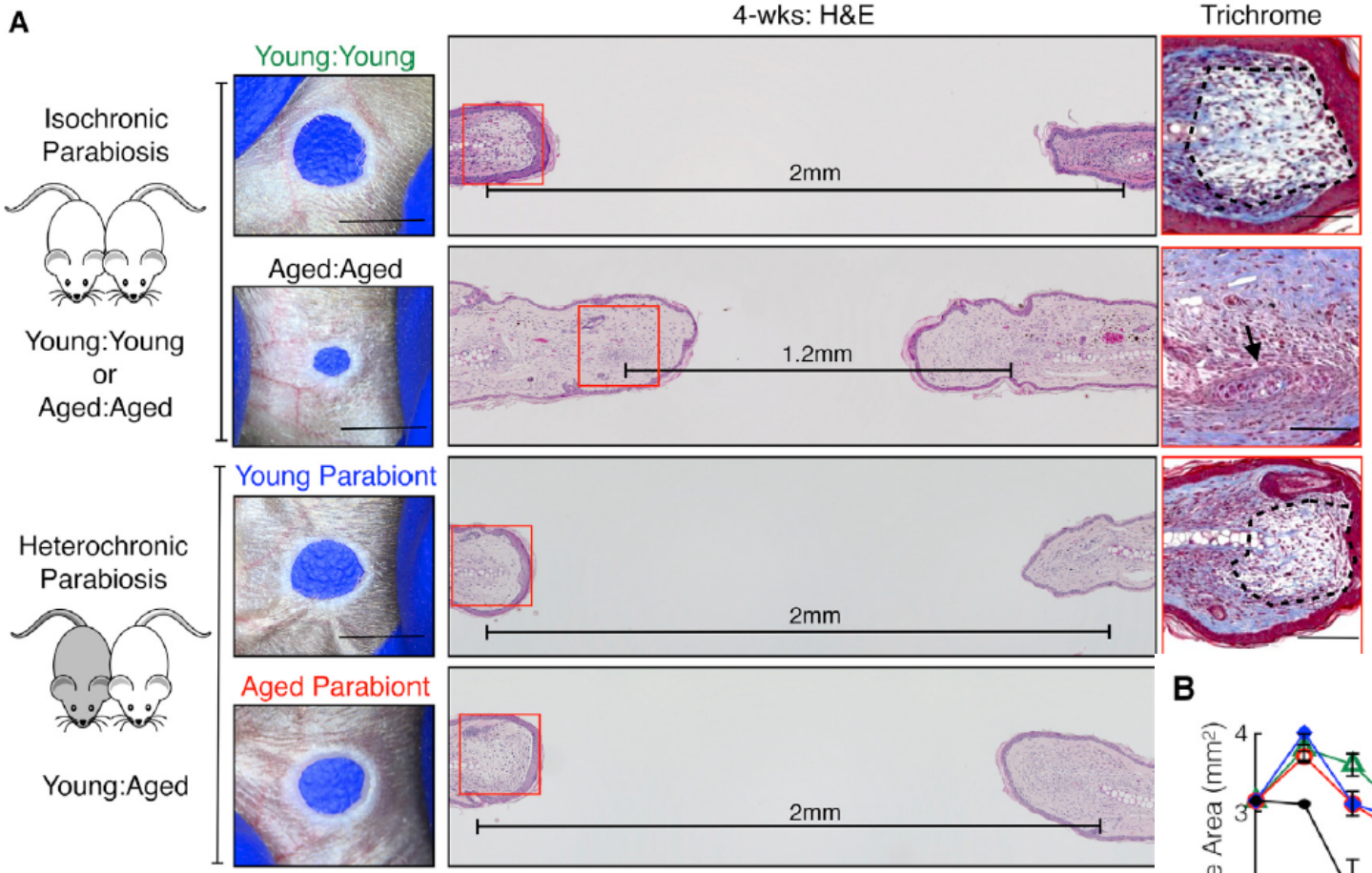


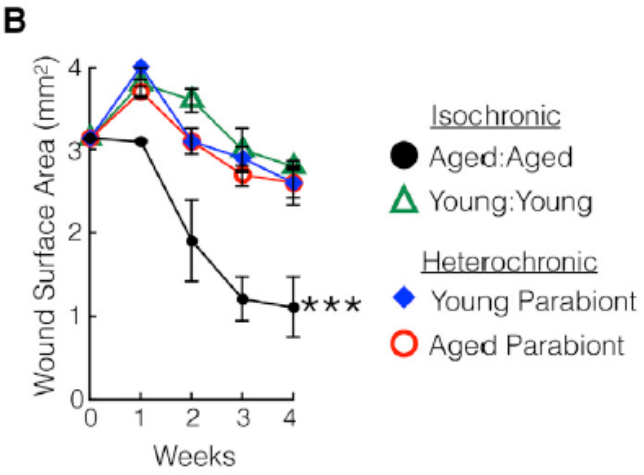


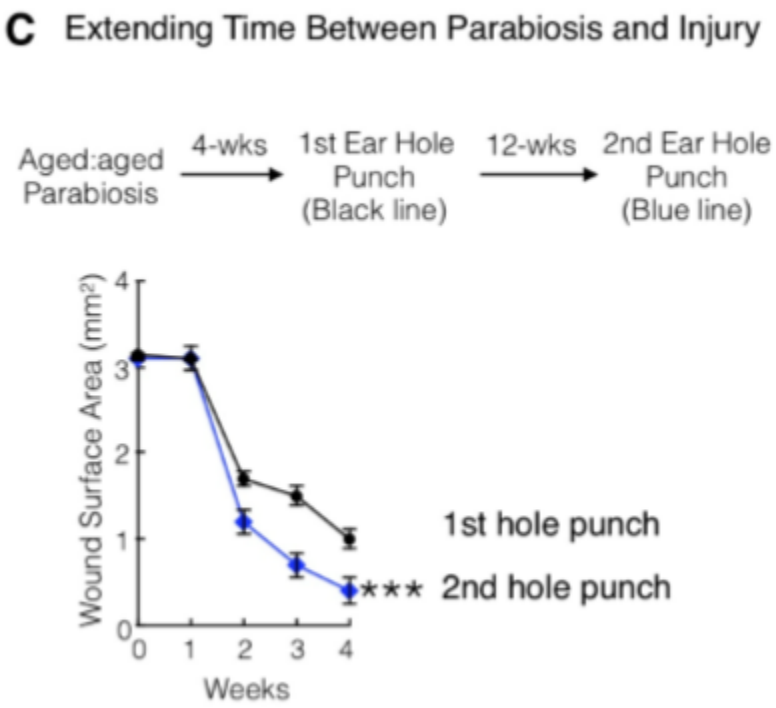


Dark blue collagen represents normal skin, pale light blue collagen represents sacar

CIRCULATINIG FACTOR PROMOTES SCAR FORMATION





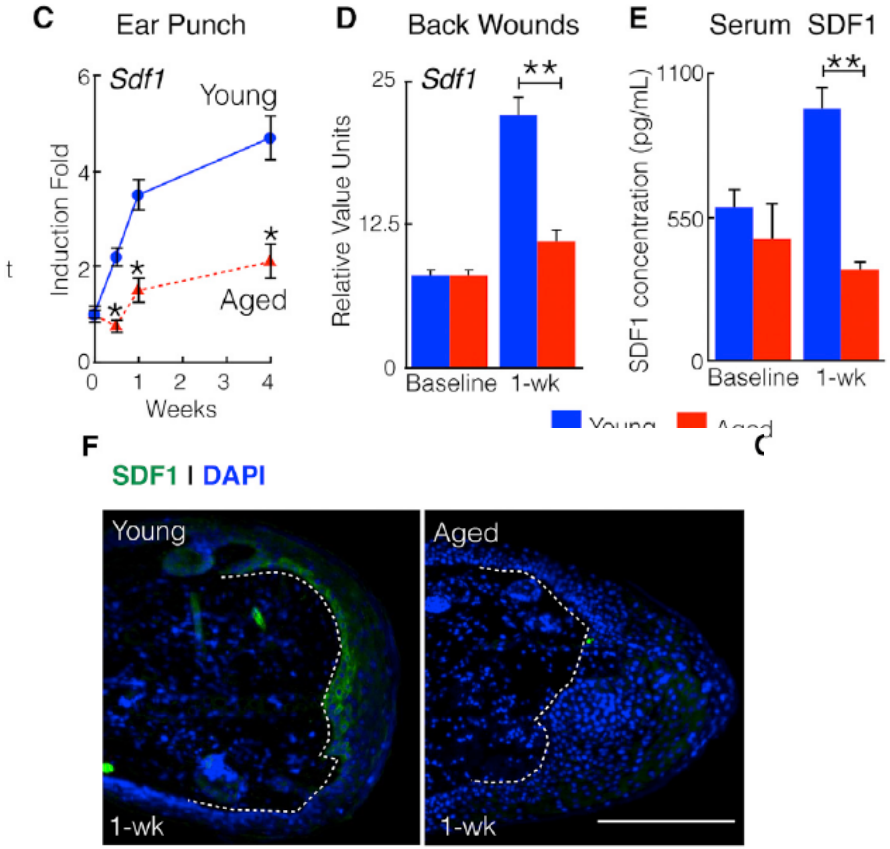


Lengthened time between Parabiosis procedure and ear injury - hole closure improved significantly

Extending the time period between parabiosis procedure and ear injury improves ear hole closure in aged:aged isochronic pairs. After 12-weeks, a second distinct ear hole punch was performed and followed

A circulating factor in young blood promotes scar formation and blocks skin tissue regeneration in aged mice

Identification of potential circulating factors



Ear hole injury induces SDF1 expression in injured keratinocytes

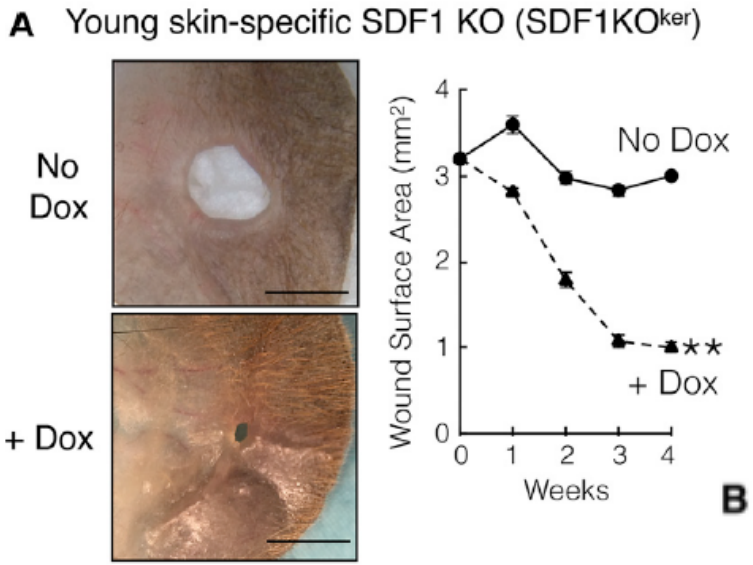
SDF1 = green

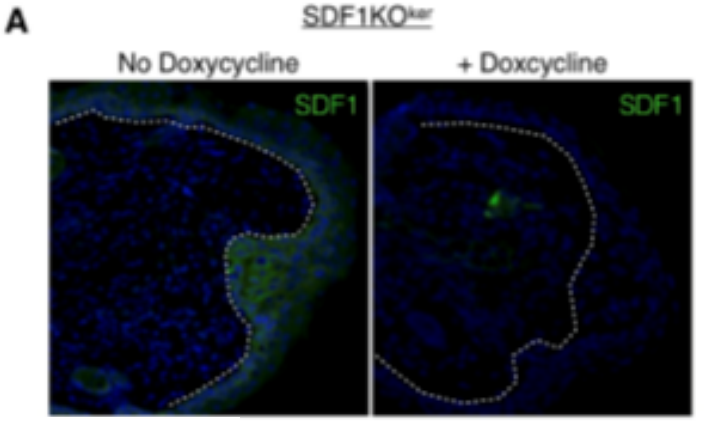
Immunostaining from young and aged mice

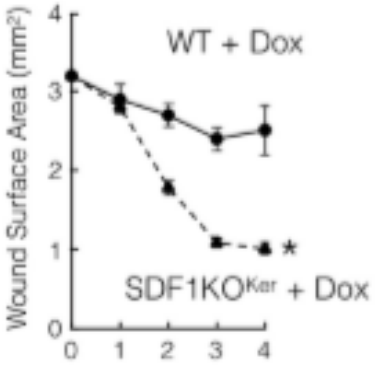
Dotted lines = epidermal border

Hole is located to the right of the section

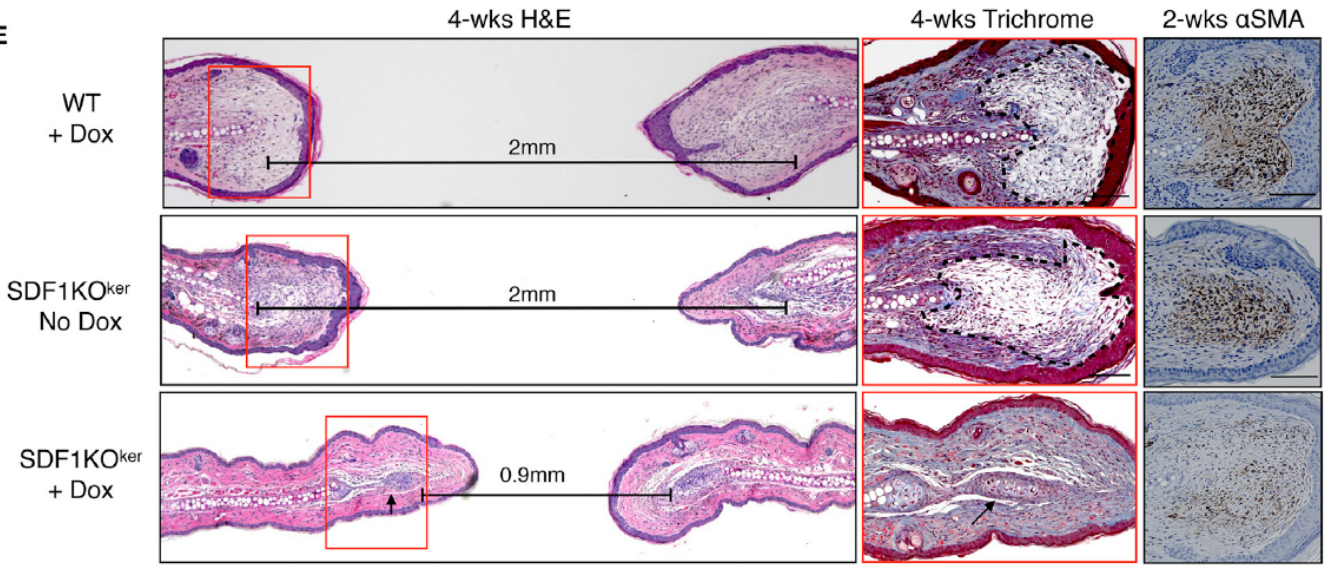
INJURED YOUNG KERATINOCYTES SECRETE SDF1 TO PROMOTE SCAR FORMATION

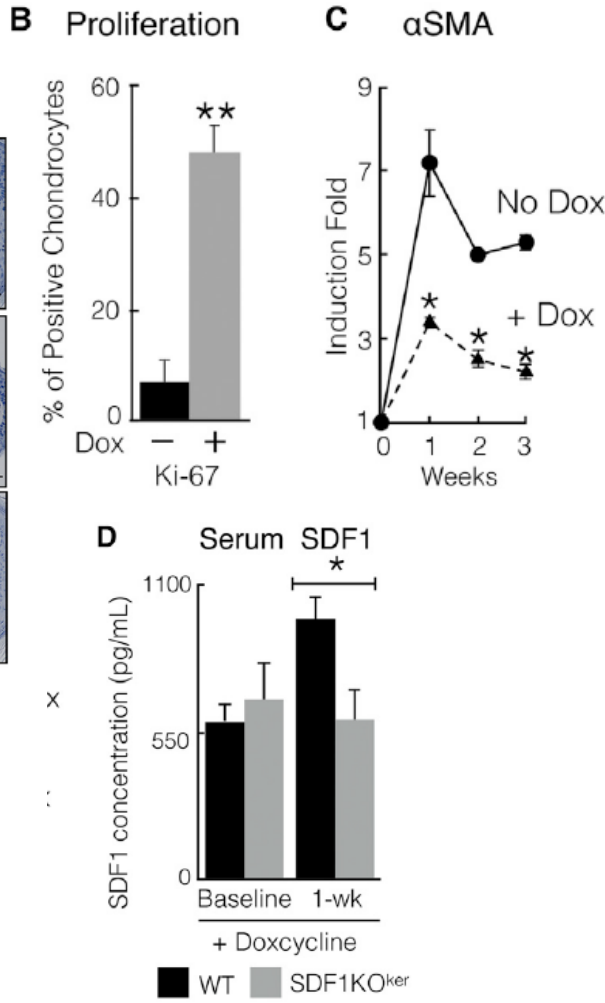






INJURED YOUNG KERATINOCYTES SECRETE SDF1 TO PROMOTE SCAR FORMATION

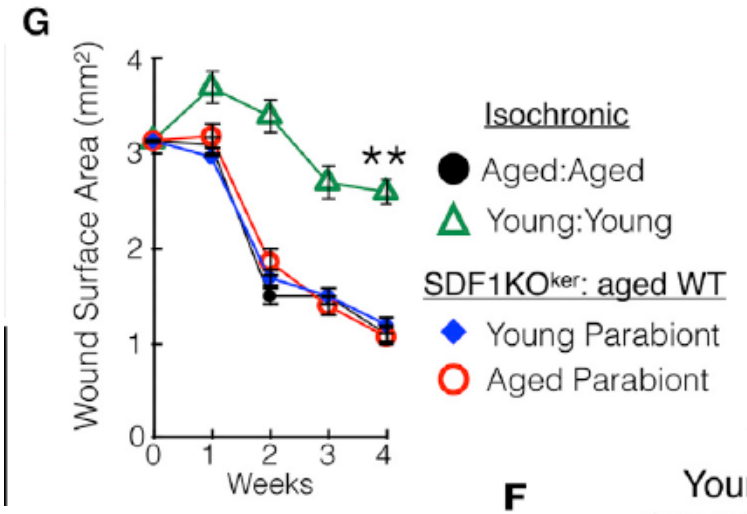


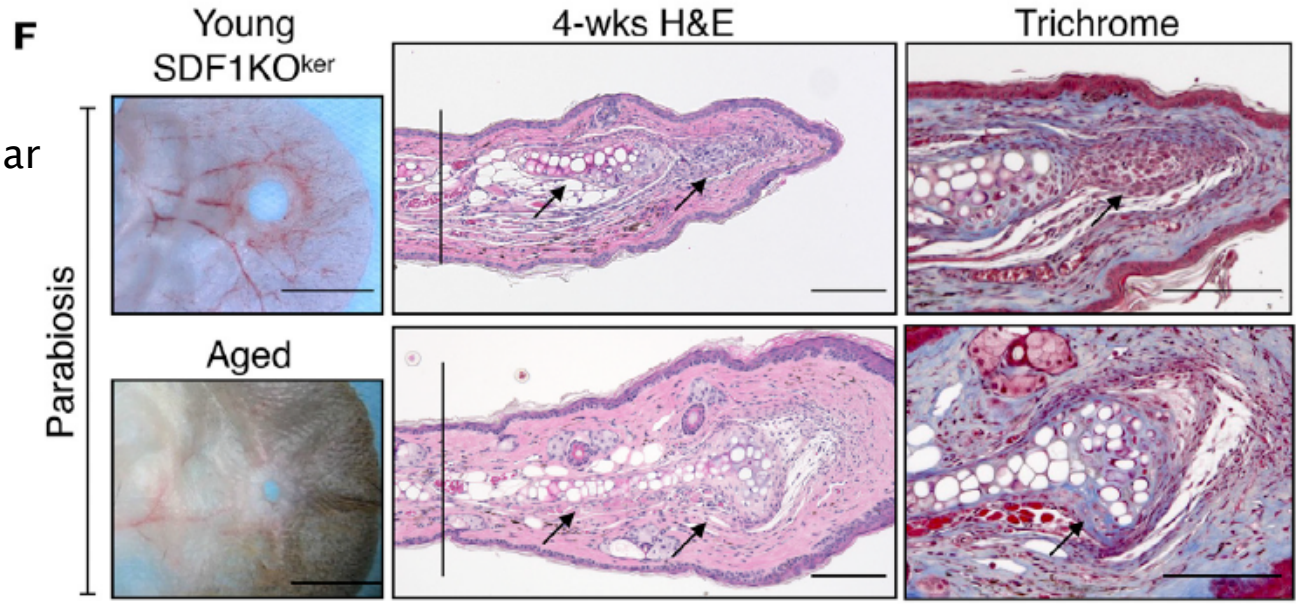


Dotted areas mark scars, black arrow marks chondocyte proliferation, horizontal line indicated the distance between cartilage end plates

Young doxy treated SDF1KOker mice

Aged WT mice



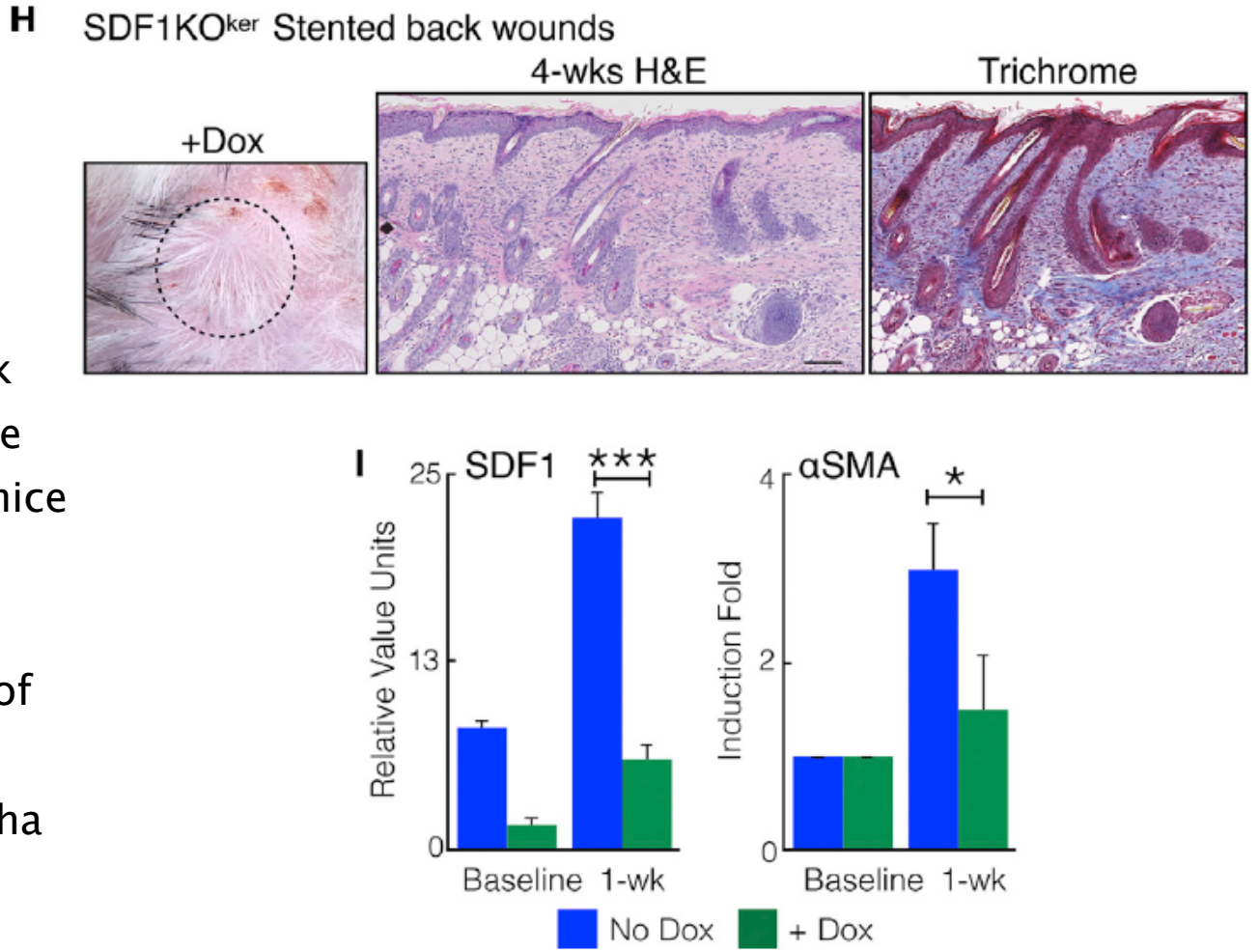


- Both parabiont closed ear holes to a significantly smaller size
- Cartilage regeneration and decreased scar formation

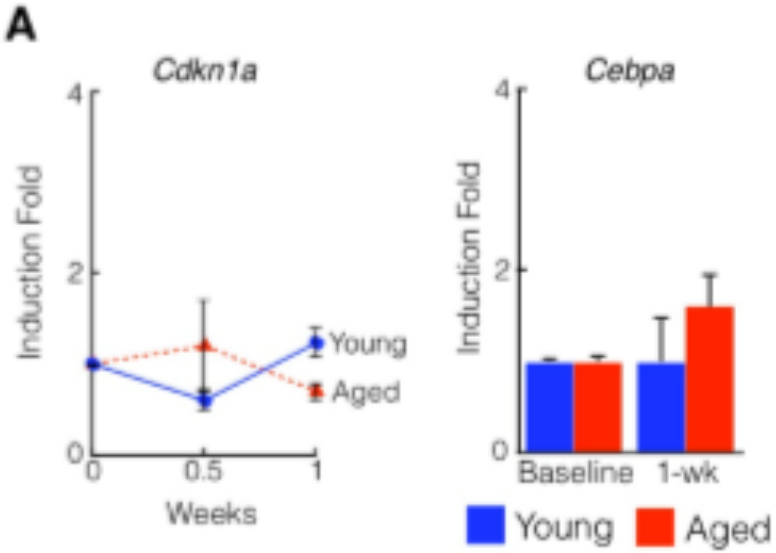
Non-doxycycline treated SDF1Koker mice

Silicone-stented back wounds on doxycycline-treated SDF1Koker mice

- Compared with non doxycycline treated SDF1KOker mice, silicone-stented back wound on docycycline treated SDF1KOker mice exhibited diminished scar formation, evidenced by return of hair follicles and reduced levels of alpha SMA



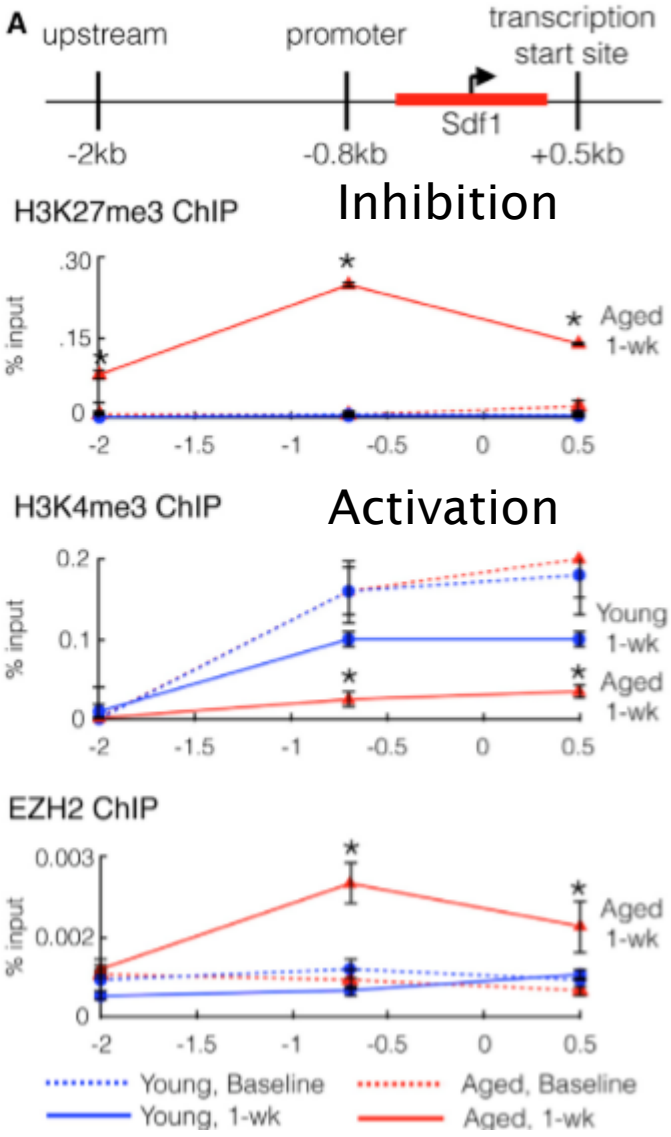
Fazit: circulating SDF1 in young blood originates from wounded keratinocytes to drive scar formation

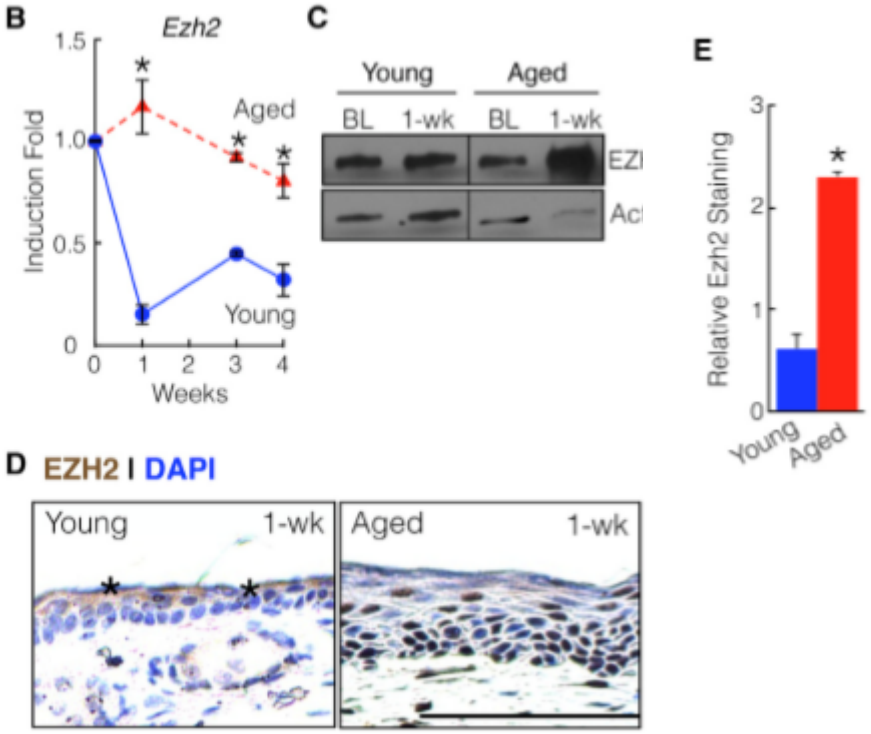


Known transcriptional regulators of SDF1 are unchanged with age. Relative mRNA levels of Cdknla and Cebpa in wound edge tissue from young or aged WT mice at baseline and 1 week post-injury

AGING SUPRESSES SDF1 ACTIVATION VIA INCREASED RECRUITMENT OF EZH2 AND H3K27m3 TO THE SDF1 GENE

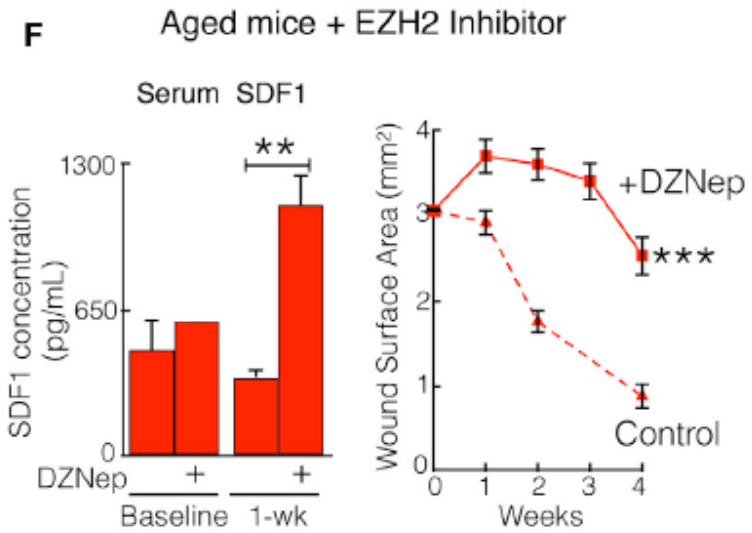
- Wound edge tissue injured aged mice:
  - increased enrichment of histone H3 lysine 27 trimethylation (H3K27me) = epigenetic marker of gene inhibition at the SDF1 promoter
  - Decreased histone H3 lysine 4 trimethylation (HeK4me3) enrichment = epigenetic marker of gene activation
  - Increased levels of EZH2 transcript and protein and increased EZH2 enrichment at the SDF1 promoter and transcription start
    - EZH2 catalyzes the addition of methyl groups to H3K27





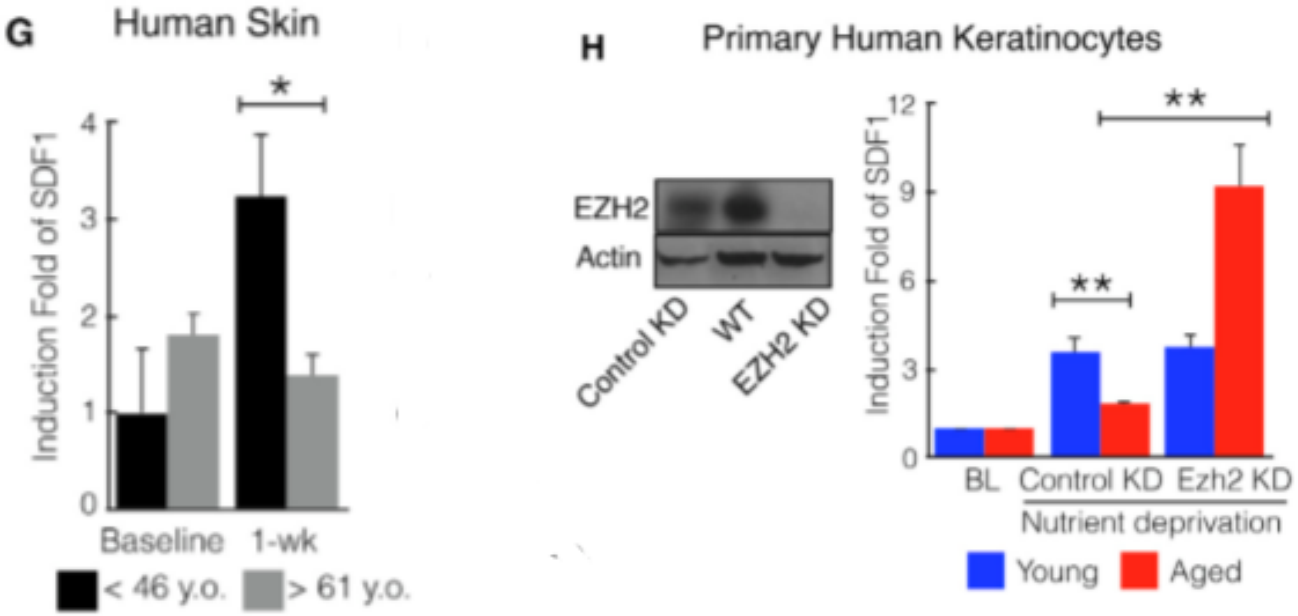
Shown are H3K27 me3, H3K4me3 and EZH2 chromatin immunoprecipitation of ear wound edge tissue at baseline and 1 week post-injury at 3 different locations of the SDF1 gene

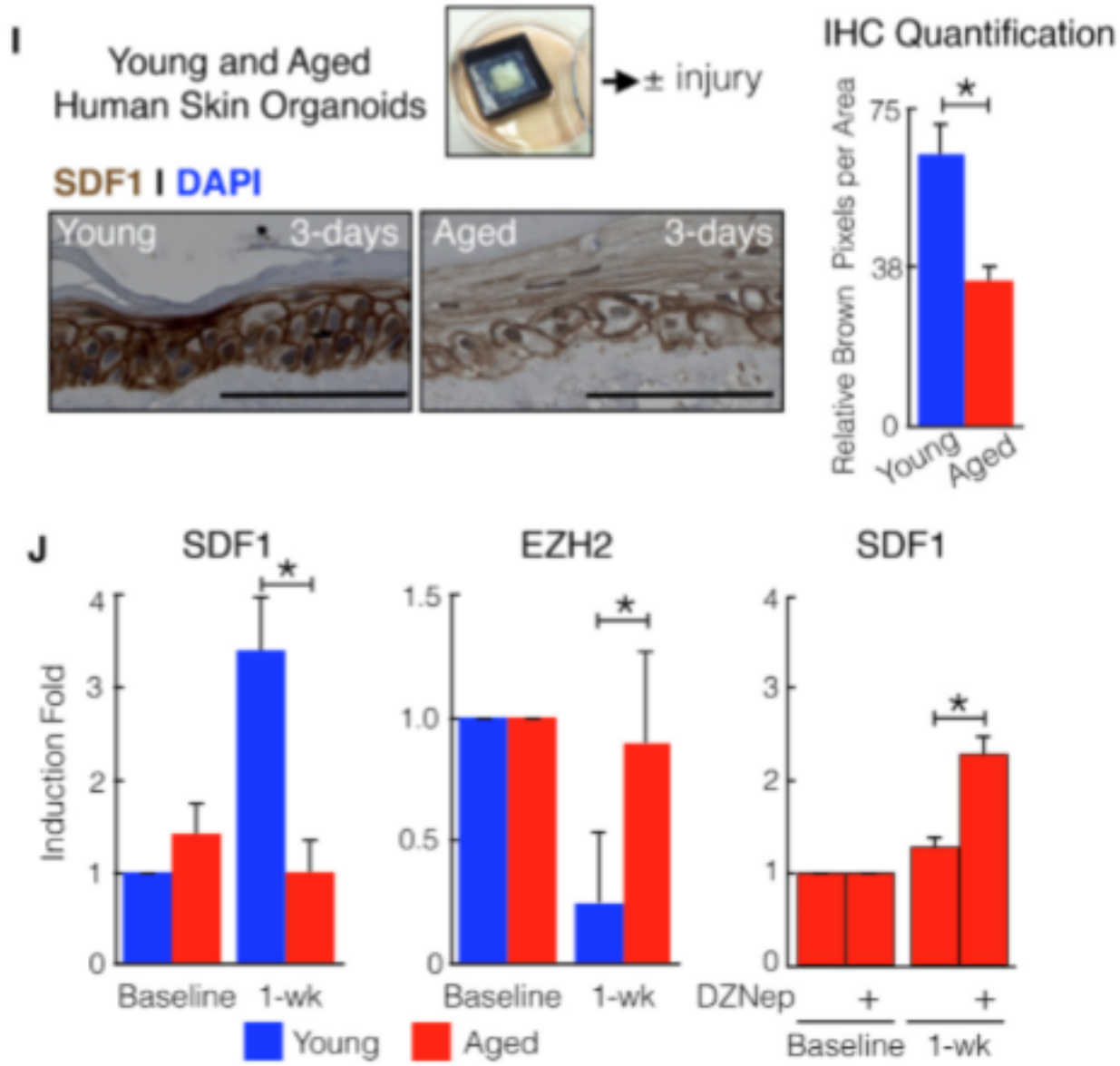
- Aged mice treated with 3-Deazaneplanocin = pharmacologic inhibitor of EZH2→ restored SDF1 induction, and ear holes closed with larger sizes



Aging supresses SDF1 induction through increased recruitment of EZH2 to the SDF1 promoter

Human skin exhibits age-dependet EZH2 mediated SDF1 induction

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Young and Aged
Human Skin Organoids
± injury
SDF1 I DAPI
Young
3-days
Aged
3-days
IHC Quantification
Relative Brown Pixels per Area
75
38
0
Young
Aged
J
SDF1
Induction Fold
4
3
2
1
0
Baseline
1-wk
Young
Aged
EZH2
1.5
1.0
0.5
0
Baseline
1-wk
SDF1
4
3
2
1
0
DZNep
+
+
Baseline
1-wk

Inhibition of SDF1 or EZH2 may be used to decrease scar formation in humans in potential future clinial trials

Discussion

- Results counter current dogma that tissue function inevitably worsens with age and uncovers potential mechanisms to explain the paradoxical effect of againg on skin tissue regeneration
- Aging slows the speed of skin re-epithelialization
- Young ears repair faster but a scar develops
- Aged ears repair slower but to a better resolution
- Overexpression of SDF1 speeds up skin re-epithelialization
- Alternative interpretation is: regenerative healing is the “default” program and young age inhibits this process
- Scar formation is the dominant form of wound repair in mammals at any age
- Ear and back wound models, represent different systems with different cell types involved
- Keratinocyte secreted SDF1 regulates the choice between tissue regeneration and scar formation
- Increased SDF1 also drives scar formation in other organs (mouse lung, zebra fish fin)
- Future studies needed to elucidate wether the precise cellular and molecular mechanisms are conserved in other organs
- Although skin specific loss of SDF1 significantly improves skin tissue regeneration, knockout mice do not fully close injured ears holes → suggests that other factors also likely participate in tissue regeneration

Highlights

CXC motif chemokine 12 CXCL 12

- Full-thickness skin wounds in aged but not young mice fully regenerate
- Genetic deletion of SDF1 in young skin enhanced tissue regeneration
- Aging remodels chromatin accessibility at the SDF1 gene to inhibit SDF1 transcription
- Human skin also exhibits age-dependent SDF1 suppression

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