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Supplemental Information

Keratinocyte-Macrophage Crosstalk by the Nrf2/Ccl2/EGF Signaling Axis Orchestrates Tissue Repair

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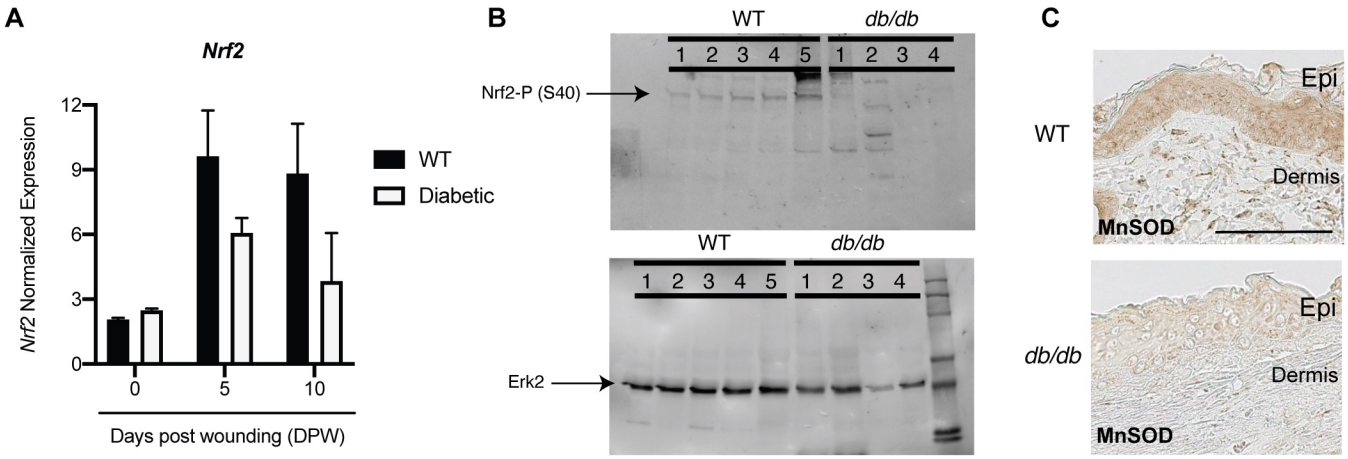
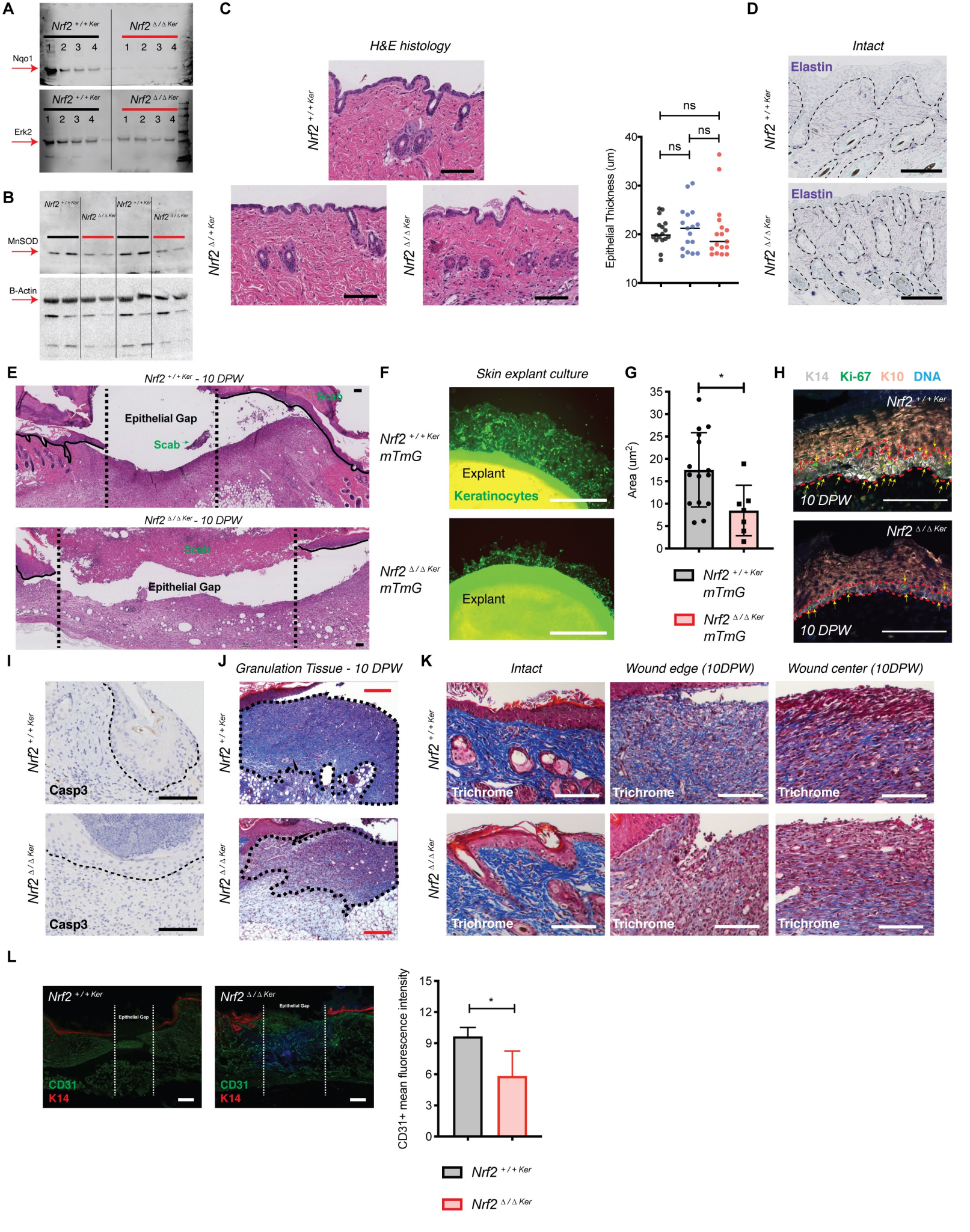
FIGURE S1


A
Nrf2
Nrf2 Normalized Expression
12
9
6
3
0
0
5
10
Days post wounding (DPW)
WT
Diabetic
B
WT
db/db
1 2 3 4 5 1 2 3 4
Nrf2-P (S40)
WT
db/db
1 2 3 4 5 1 2 3 4
Erk2
C
WT
Epi
Dermis
MnSOD
db/db
Epi
Dermis
MnSOD

Supplemental Figure 1. Dysfunctional activation of Nrf2 in diabetic wounds, Related to Figure 1

A. qPCR of *Nrf2* in whole wounds belonging to WT and *db/db* mice at 10 DPW (n=3). Data was normalized to *18S* and is expressed as mean ± SD.
B. Western Blot (WB) for active Nrf2 (Nrf2 S40-P) on whole wound lysates belonging to WT and *db/db* mice at 10 DPW (n=5,4). Erk2 is a loading control.
C. Representative immunohistochemical labeling of MnSOD expression in wounds belonging to WT and *db/db* mice at 10 DPW. MnSOD (brown). Scale, 100um. (n=3).

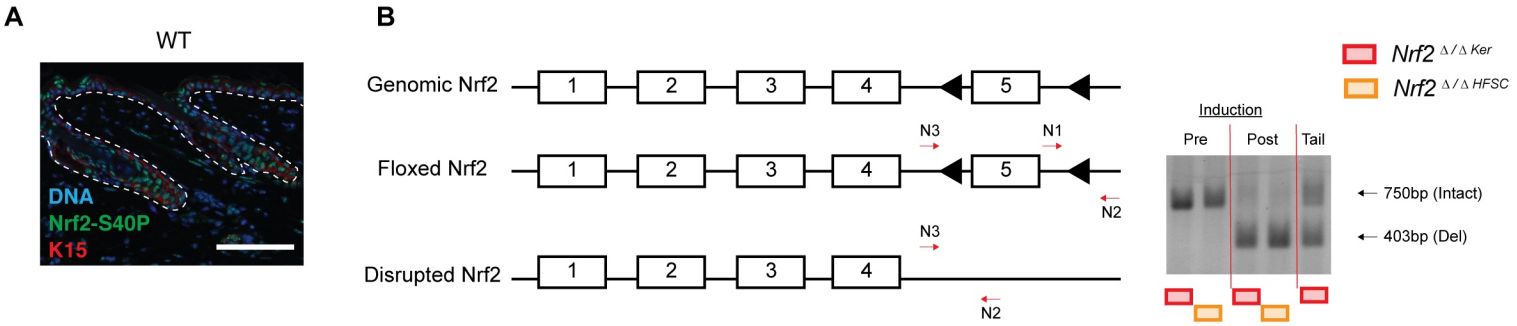
FIGURE S2


A
Nrf2 +/+ Ker
Nrf2 Δ/Δ Ker
1 2 3 4
Nqo1
Erk2
B
MnSOD
B-Actin
C
H&E histology
Nrf2 +/+ Ker
Nrf2 Δ/+ Ker
Nrf2 Δ/Δ Ker
ns
Epithelial Thickness (um)
40
30
20
10
D
Intact
Elastin
E
Nrf2 +/+ Ker - 10 DPW
Epithelial Gap
Scab
Nrf2 Δ/Δ Ker - 10 DPW
F
Skin explant culture
Nrf2 +/+ Ker mTmG
Explant
Keratinocytes
Nrf2 Δ/Δ Ker mTmG
G
Area (um2)
35
30
25
20
15
10
5
0
*
H
K14 Ki-67 K10 DNA
10 DPW
I
Casp3
J
Granulation Tissue - 10 DPW
K
Intact
Wound edge (10DPW)
Wound center (10DPW)
Trichrome
L
CD31
K14
CD31+ mean fluorescence intensity
15
12
9
6
3
0

Supplemental Figure 2. Acute Nrf2 Deletion in the basal epidermis affects keratinocyte and non-keratinocyte autonomous repair defects, Related to Figure 2

A. WB for Nqo1 on whole wound lysates belonging to *Nrf2*$^{\Delta/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW (n=4). Erk2 is a loading control.
B. WB for MnSOD on whole wound lysates belonging to *Nrf2*$^{\Delta/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW (n=4). B-actin is a loading control.
C. Representative H&E images of intact skin belonging to tamoxifen-induced *Nrf2*$^{+/+\ Ker}$, *Nrf2*$^{\Delta/+\ Ker}$, and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice (n=3). Quantification of epithelial thickness (n=3). Scale, 100um.
D. Elastin staining in intact skin belonging to *Nrf2*$^{\Delta/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice (n=3). Scale, 100um.
E. Representative images of epithelial gap in *Nrf2*$^{+/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW. Measurements made on H&E sections are depicted in main figure. (n=13,15)
F. Representative images depicting migration distance traveled by GFP^{+} keratinocytes in skin explants cultured ex vivo. 4mm circular skin biopsies were taken from intact skin belonging to R26$^{mTmG/+}$; *Nrf2*$^{+/+\ Ker}$ and R26$^{mTmG/+}$; *Nrf2*$^{\Delta/\Delta\ Ker}$ mice (n=7).
G. Quantification of the migration distance traveled by GFP^{+} keratinocytes in (F) (n=7). Area measurements (explant base to epithelial sheet edge) were taken from independent high-powered fields (20x) in independent biological replicates.
H. Representative images of Ki67^{+} keratinocytes at the epithelial edge in *Nrf2*$^{+/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ wounds at 10 DPW (n=4). K14 (grey); Ki-67 (green); K10 (orange); DAPI (blue). Measurements made on H&E sections are depicted in main figure.
I. IF labeling of cleaved Caspase3 to demarcate apoptotic cells in basal keratinocytes of the epithelial edge in wounds belonging to *Nrf2*$^{\Delta/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW (n=3). Caspase3 (brown). Scale, 100um.
J. Representative images of the granulation tissue in *Nrf2*$^{+/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ wounds at 10 DPW. Measurements made on H&E sections are depicted in main figure. Scale, 200um. (n=11,8)
K. Trichrome staining of intact and wounded *Nrf2*$^{\Delta/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ skin at 5 and 10 DPW (n =3,3) to assess collagen deposition and organization. Scale, 100um.
L. IF/quantification of CD31^{+} vascular tissue the wounds belonging to *Nrf2*$^{+/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW (n=3). CD31 (green) and K14 (red). Scale, 100px. Quantification of CD31^{+} immunofluorescent labeling in wounds belonging to *Nrf2*$^{+/+\ Ker}$ and *Nrf2*$^{\Delta/\Delta\ Ker}$ mice at 10 DPW (n=3). Labeling was quantified from independent high-powered fields (20x) in independent biological replicates.
All quantified data is represented as mean ± SD.
For (C), two-way ANOVA with multiple comparisons was used to analyze statistical significance of open wounds between groups on respective days post wounding. ns = not significant.
For (G) and (L), unpaired t-tests were used to analyze statistical significance between the two experimental groups. ns = not significant; *p<0.05; ***p<0.001; ****p<0.0001.

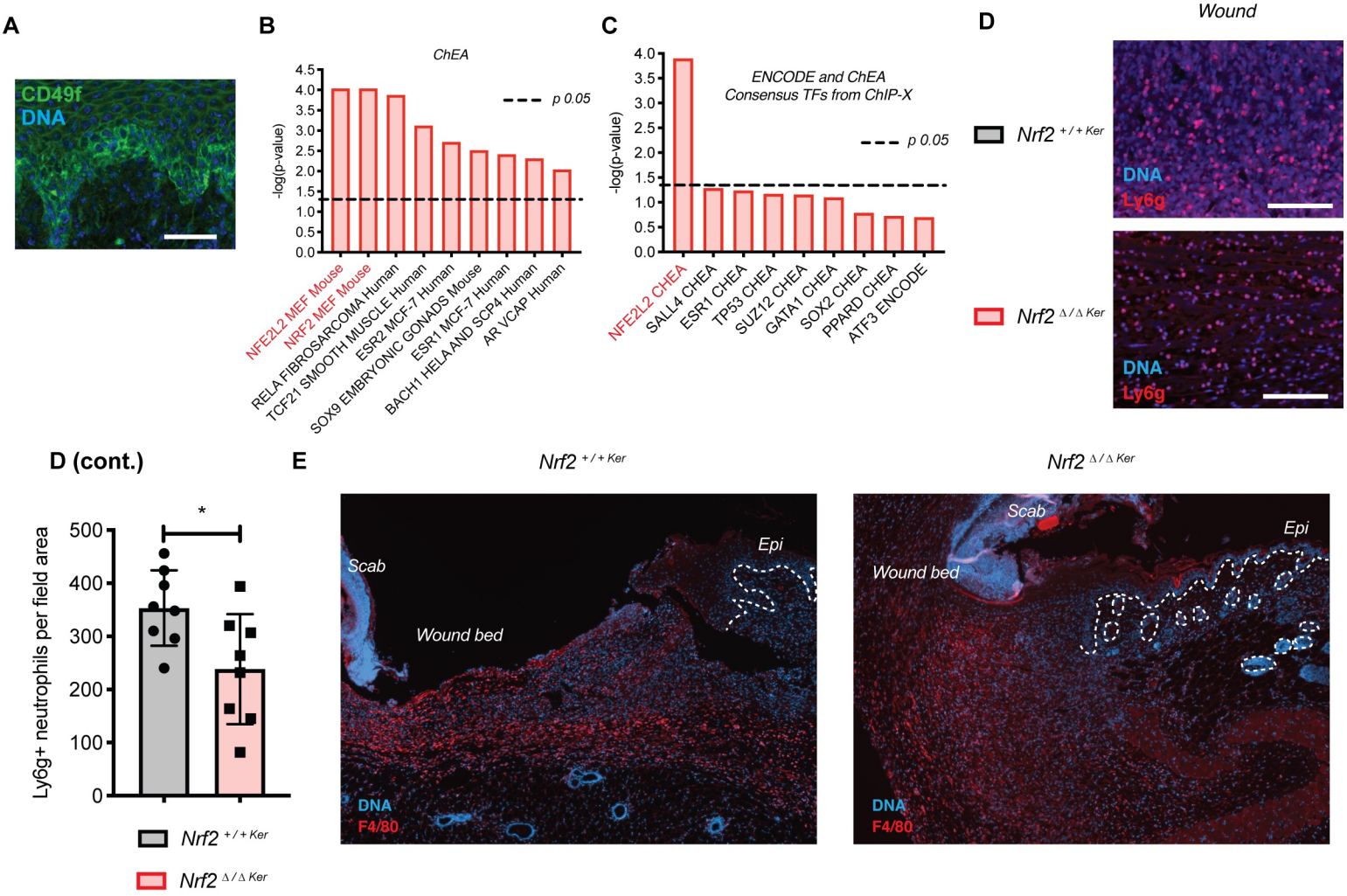
FIGURE S3


A
WT
DNA
Nrf2-S40P
K15
B
Genomic Nrf2
1
2
3
4
5
Floxed Nrf2
N3
N1
N2
Disrupted Nrf2
Nrf2 Δ/Δ Ker
Nrf2 Δ/Δ HFSC
Induction
Pre
Post
Tail
750bp (Intact)
403bp (Del)

Supplemental Figure 3. Nrf2 expression in HFSCs and recombination efficiency in transgenic animals, Related to Figure 2

A. IF labeling of active Nrf2 in intact WT skin demonstrates its expression in Keratin 15 $(K15)^{+}$ hair follicle stem cells (HFSCs) (n=3). Active Nrf2 (Nrf2 S40-P) (green), K15 (red), and DAPI (blue). Scale, 100um.
B. Representative gel image of genotyping PCR showing the Nrf2 floxed locus before and after induced recombination in basal keratinocytes (lanes 1,3) and flow cytometry isolated $Nrf2^{\Delta/\Delta\ HFSC}$ HFSCs (lanes 2,4) (n>3). Lane 5 is of PCR amplification from cell heterogenous tail DNA.

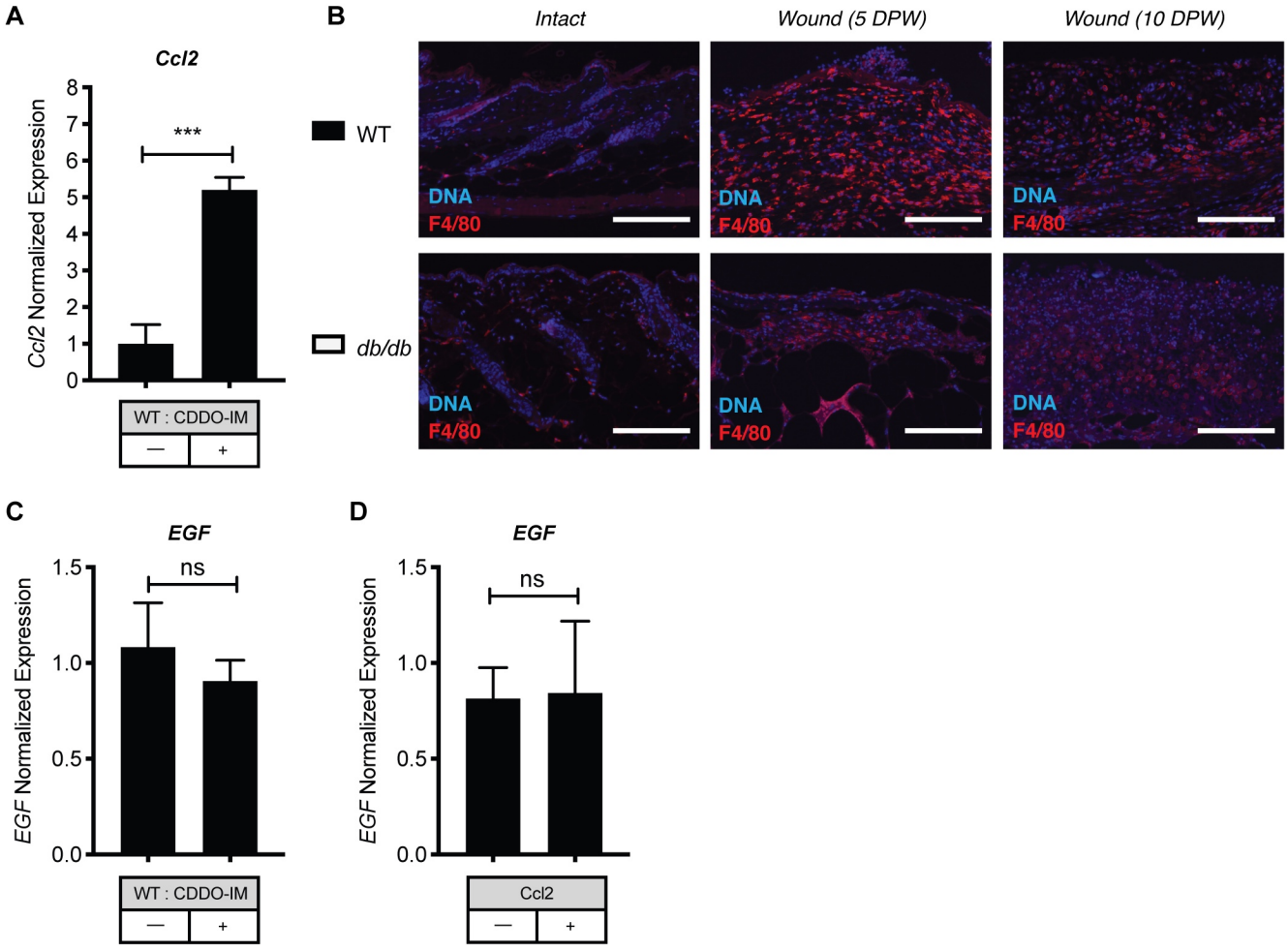
FIGURE S4


A
CD49f
DNA
B
ChEA
-log(p-value)
p 0.05
NFE2L2 MEF Mouse
NRF2 MEF Mouse
RELA FIBROSARCOMA Human
TCF21 SMOOTH MUSCLE Human
ESR2 MCF-7 Human
SOX9 EMBRYONIC GONADS Mouse
ESR1 MCF-7 Human
BACH1 HELA AND SCP4 Human
AR VCAP Human
C
ENCODE and ChEA
Consensus TFs from ChIP-X
-log(p-value)
p 0.05
NFE2L2 CHEA
SALL4 CHEA
ESR1 CHEA
TP53 CHEA
SUZ12 CHEA
GATA1 CHEA
SOX2 CHEA
PPARD CHEA
ATF3 ENCODE
D
Wound
Nrf2 +/+ Ker
Nrf2 Δ/Δ Ker
DNA
Ly6g
D (cont.)
Ly6g+ neutrophils per field area
*
Nrf2 +/+ Ker
Nrf2 Δ/Δ Ker
E
Nrf2 +/+ Ker
Nrf2 Δ/Δ Ker
Scab
Epi
Wound bed
DNA
F4/80

Supplemental Figure 4. Epidermal Nrf2 deletion impairs keratinocyte-mediated signaling for immune cell trafficking during wound repair, Related to Figure 3

A. Immunofluorescent labeling of CD49f in WT skin belonging demonstrate its specificity to the basal epidermis. CD49f (green), DAPI (blue). Scale, 50um.
B. EnrichR Chromatin immunoprecipitation enrichment analysis (ChEA) of bulk RNA-seq comparing DEGs in wound-associated keratinocytes from *Nrf2* $^{+/+\ Ker}$ and *Nrf2* $^{\Delta/\Delta\ Ker}$ wounds at 5 DPW.
C. EnrichR ChIP-X analysis of bulk RNA-seq comparing DEGs in wound-associated keratinocytes from *Nrf2* $^{+/+\ Ker}$ and *Nrf2* $^{\Delta/\Delta\ Ker}$ wounds at 5 DPW.
D. IF labeling of neutrophils trafficked into the wounds of *Nrf2* $^{+/+\ Ker}$ and *Nrf2* $^{\Delta/\Delta\ Ker}$ mice at 2 DPW (n=10). Ly6g (red) and DAPI (blue). Scale, 100um. Quantification of $Ly6g^{+}$ immunofluorescent labeling in wounds belonging to *Nrf2* $^{+/+\ Ker}$ and *Nrf2* $^{\Delta/\Delta\ Ker}$ mice at 2 DPW. Labeling was quantified from independent high-powered fields (20x) in independent biological replicates. The data is represented as mean ± SD and unpaired t-tests were used to analyze statistical significance between the two experimental groups. ns=not significant; *$p<0.05$.
E. IF of macrophages trafficked into the wounds of *Nrf2* $^{+/+\ Ker}$ and *Nrf2* $^{\Delta/\Delta\ Ker}$ mice at 5 DPW (n=10). F4/80 (red) and DAPI (blue). Scale, 100um.

FIGURE S5


A
Ccl2
Ccl2 Normalized Expression
***
WT : CDDO-IM
—
+
B
Intact
Wound (5 DPW)
Wound (10 DPW)
WT
db/db
DNA
F4/80
C
EGF
EGF Normalized Expression
ns
WT : CDDO-IM
—
+
D
EGF
EGF Normalized Expression
ns
Ccl2
—
+

Supplemental Figure 5. Macrophage trafficking dysfunction in diabetic mice and the effects of CDDO-IM, Ccl2 treatment in keratinocytes, Related to Figure 4 and 5

A. qPCR of *Ccl2* in CDDO-IM treated WT keratinocytes. Data normalized to *18S* (n=3).
B. Immunofluorescent labeling of macrophages trafficked into the wounds of from WT and *db/db* mice at 0, 5, and 10 DPW (WT, *db/db*: n=4,3 (0 DPW), n=9,10 (5 DPW), n=7,7 (10 DPW). Paraffin sections (5um) were stained with antibodies against F4/80 (red) and DAPI (blue) as a nuclear counterstain. Scale bar, 100um.
C. qPCR of *EGF* in CDDO-IM treated WT keratinocytes. Data normalized to *18S* (n=3).
D. qPCR of *EGF* in WT keratinocytes after treatment with exogenous Ccl2. Data normalized to *18S* (n=3).

Oligonucleotides (cont.)		
EGF F 5’-GGAAGCCACGCTTACATTCAT-3’	Life Technologies	N/A
EGF R 5’-ACTGAGTAGAAGATCCGATCACC-3’	Life Technologies	N/A
Nqo1 F 5’-AGGATGGGAGGTACTCGAATC-3’	Life Technologies	N/A
Nqo1 R 5’-AGGCGTCCTTCCTTATATGCTA-3’	Life Technologies	N/A
Nrf2 F 5’-CACATTGGGATTCACGCATA-3’	Life Technologies	N/A
Nrf2 R 5’-CCCCTGGAAGTGTCAAACAG-3’	Life Technologies	N/A
Sod2 F 5’-GTGAAACCTCACTCACGGC-3’	Life Technologies	N/A
Sod2 R 5’-ACAGCACCCCAGTCATAGTG-3’	Life Technologies	N/A
Cre F 5’-TAAAGATATCTCACGTACTGACGGTG-3’	Life Technologies	N/A
Cre R 5’-TCTCTGACCAGAGTCATCCTTAGC-3’	Life Technologies	N/A
Nrf2 flox N1 5’-TCTTAGGCACCATTTGGGAGAG-3’	Life Technologies	N/A
Nrf2 flox N2 5’-TACAGCAGGCATACCATTGTGG-3’	Life Technologies	N/A
Nrf2 flox N3 5’-TGAGAGCTTCCCAGACTCACTT-3’	Life Technologies	N/A
Rosa F 5’-CGAGGCGGATCACAAGCAATA-3’	Life Technologies	N/A
Rosa R 5’-TCAATGGGCGGGGGTCGTT-3’	Life Technologies	N/A

Table S1. Oligonucleotides used for qPCR and genotyping, Related to STAR Methods