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Supplemental information

Role of MR1-driven signals and amphiregulin on the recruitment and repair function of MAIT cells during skin wound healing

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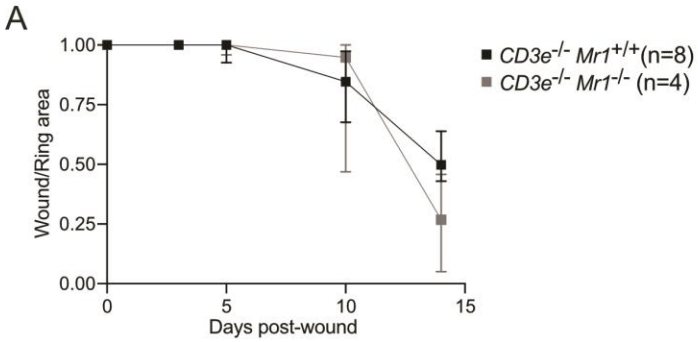

Fig. S1 related to figure 1: MR1 deficiency alone is not sufficient to delay wound closure.
Wound surface follow-up in *Cd3e*$^{-/-}$ *Mr1*$^{+/+}$ (black circle) or *Cd3e*$^{-/-}$ *Mr1*$^{-/-}$ (grey square) mice. *Pooled data from 2 independent (n=8/4) experiments.*

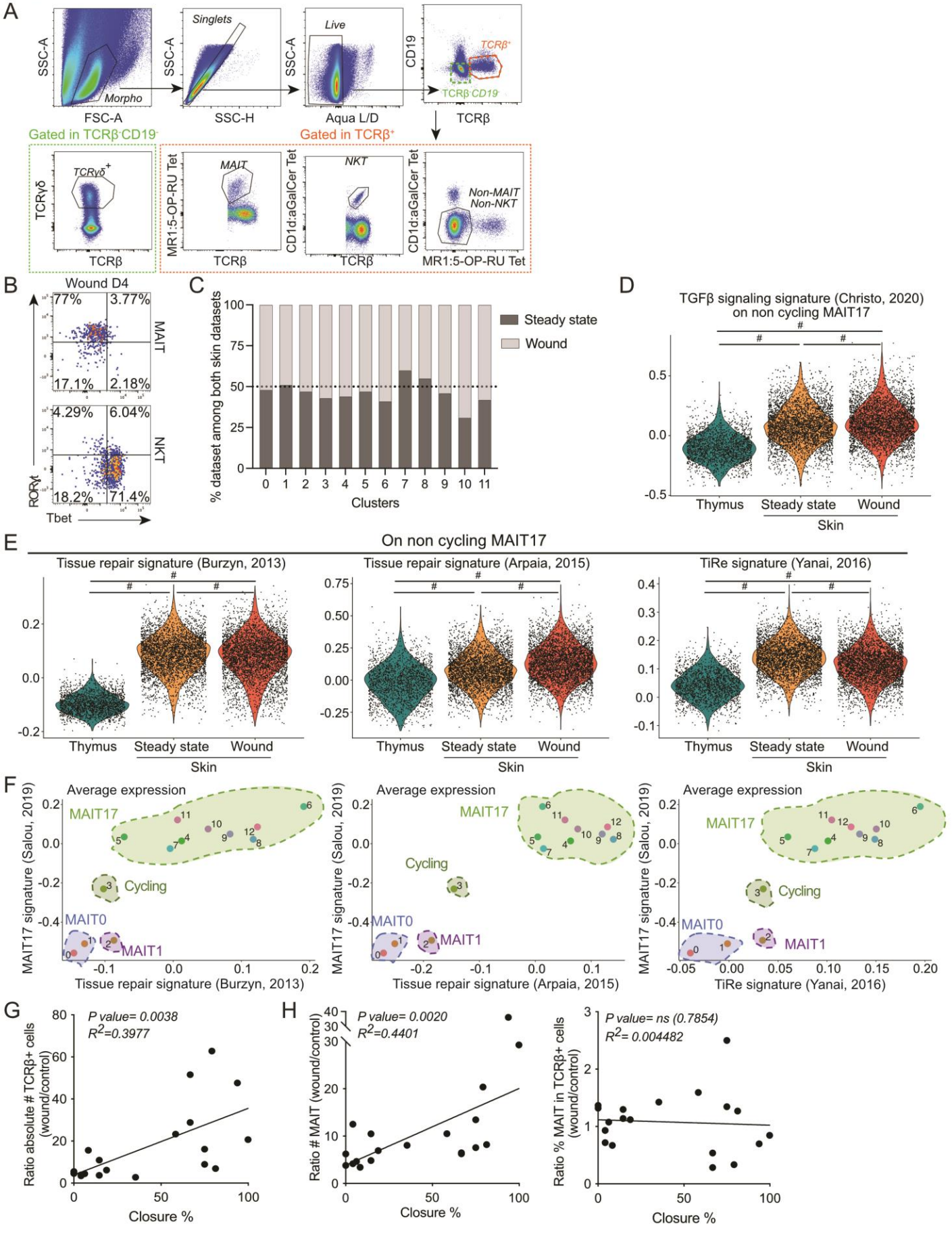
A
SSC-A
FSC-A
Morpho
Singlets
SSC-H
Live
Aqua L/D
CD19
TCRβ
TCRβ+
TCRβ-CD19-
Gated in TCRβ-CD19-
Gated in TCRβ+
TCRγδ
TCRγδ+
MR1:5-OP-RU Tet
MAIT
CD1d:aGalCer Tet
NKT
Non-MAIT
Non-NKT
B
Wound D4
77%
3.77%
17.1%
2.18%
4.29%
6.04%
18.2%
71.4%
MAIT
NKT
RORγt
Tbet
C
% dataset among both skin datasets
Steady state
Wound
Clusters
D
TGFβ signaling signature (Christo, 2020)
on non cycling MAIT17
Thymus
Steady state
Wound
Skin
E
On non cycling MAIT17
Tissue repair signature (Burzyn, 2013)
Tissue repair signature (Arpaia, 2015)
TiRe signature (Yanai, 2016)
F
Average expression
MAIT17
Cycling
MAIT0
MAIT1
MAIT17 signature (Salou, 2019)
G
P value= 0.0038
R2=0.3977
Ratio absolute # TCRβ+ cells (wound/control)
Closure %
H
P value= 0.0020
R2=0.4401
Ratio # MAIT (wound/control)
P value= ns (0.7854)
R2= 0.004482
Ratio % MAIT in TCRβ+ cells (wound/control)

Fig. S2 related to figure 2

(A) Gating strategy used to define MAIT, NKT, non-MAIT non-NKT (mainstream) and γδ T cells.

(B) Flow cytometry example of Tbet and RORγt intracellular staining on MAIT and NKT cells. *Data are representative of 3 independent experiments (n=8).*

(C) Distribution of MAIT cells from the wound and steady-state skin in each single cell cluster, among the total number of skin MAIT cells.

(D) TGFß signaling[27] signature score on non-cycling MAIT17 cells. *Tukey's multiple comparison test.*

(E) Tissue repair signature score as in (D). The signatures are expressed by regulatory T cells producing Areg in the muscle[33] and in the lungs[34]. The 3rd one was extracted from the TiRe database[35]. *Tukey's multiple comparison test.*

(F) Average expression of tissue repair (signatures presented in (E)) and MAIT17[17] signatures in all clusters of the integrated datatset of thymic, wound and steady state skin MAIT cells.

(G) Correlation between percent of closure and the increase in T cell number in the wound (wound/control site ratio). The line represents the linear regression. *Data are from 6 independent experiments (n=17).*

(H) Correlation between percent of closure and the increase in MAIT cell number or percent in the wound (wound/control ratio). The lines represent linear regressions. *Data are from 6 independent experiments (n=17).*

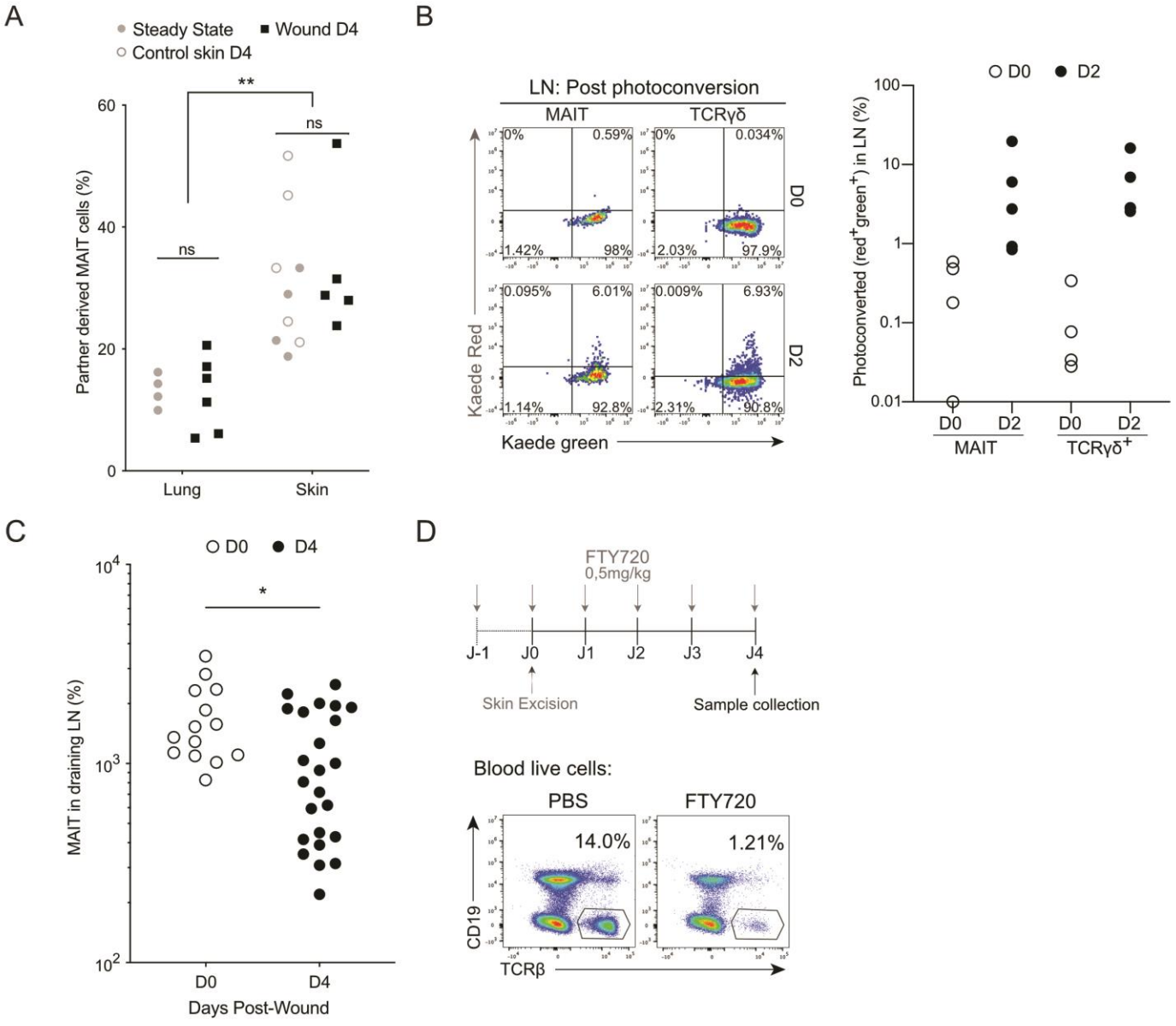

Fig. S3 related to figure 3: MAIT cells exchange are less resident in the skin than in the lungs and exchange between the skin and the LN.

(A) Quantitation of partner-derived MAIT cells in lung and skin, at steady state and four days after excision. *Pooled data from 3 ($n_{steady\ state+control}$=4/9; $n_{excision}$=6/5) independent experiments. Sídák's multiple comparison test.*

(B) Example of Kaede Green and Red expression (left) and frequency of photoconverted cells (right) in MAIT and γδ T cells from the draining LNs. *Pooled data from 2 independent experiments (n=5).*

(C) Number of MAIT cells in the draining LNs at steady-state (D0) or four days following excision. *Pooled data from 10 independent experiments (n_{D0}=14; n_{D4}=24).* Mann Whitney *test.*

Experimental setup for FTY720 administration (top). Representative T cell staining in the blood after PBS or FTY720 administration (bottom). *Data are representative of 2 independent experiments.*

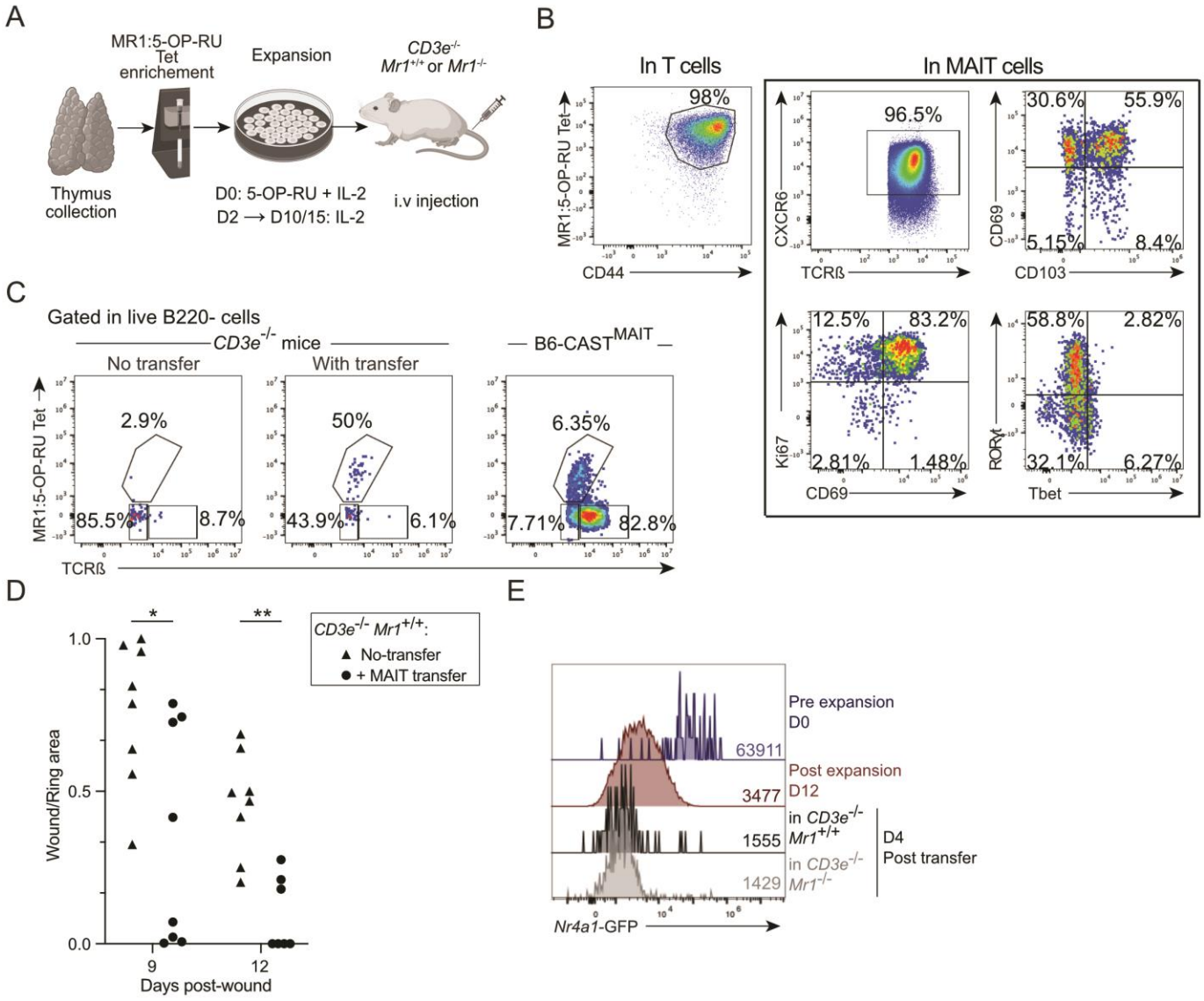

Fig. S4 related to figure 4: MAIT cell phenotype following in vitro expansion and in-vivo transfer.

(A) Experimental cartoon of the thymic MAIT cell expansion protocol (see methods for more details)

(B) Frequency and phenotype of MAIT cells after expansion. *Data are representative of 6 independent experiments.*

(C) Flow cytometry example of MR1:5-OP-RU Tetramer staining in the skin of *Cd3e*$^{-/-}$ mice transferred or not with expanded MAIT cells. B6-CAST^{MAIT} is presented as control. *Data are representative of 2 independent experiments.*

(D) Wound surface (ratio Wound/Ring area) at days 9 and 12 following excision of *Cd3e*$^{-/-}$ *Mr1*$^{+/+}$ mice transferred or not with expanded MAIT cells. *Pooled data from 2 independent experiments (n=7/8). Mann Whitney tests.*

(E) Thymic MAIT cells from *Nr4a1*-GFP were expanded. GFP expression on MAIT cells *ex vivo* in the thymus (blue), after *in vitro* expansion (red) and from the skin of transferred *Cd3e*$^{-/-}$ *Mr1*$^{+/+}$ (black) and *Cd3e*$^{-/-}$ *Mr1*$^{-/-}$ (grey). *Data are representative of 2 independent experiments.*

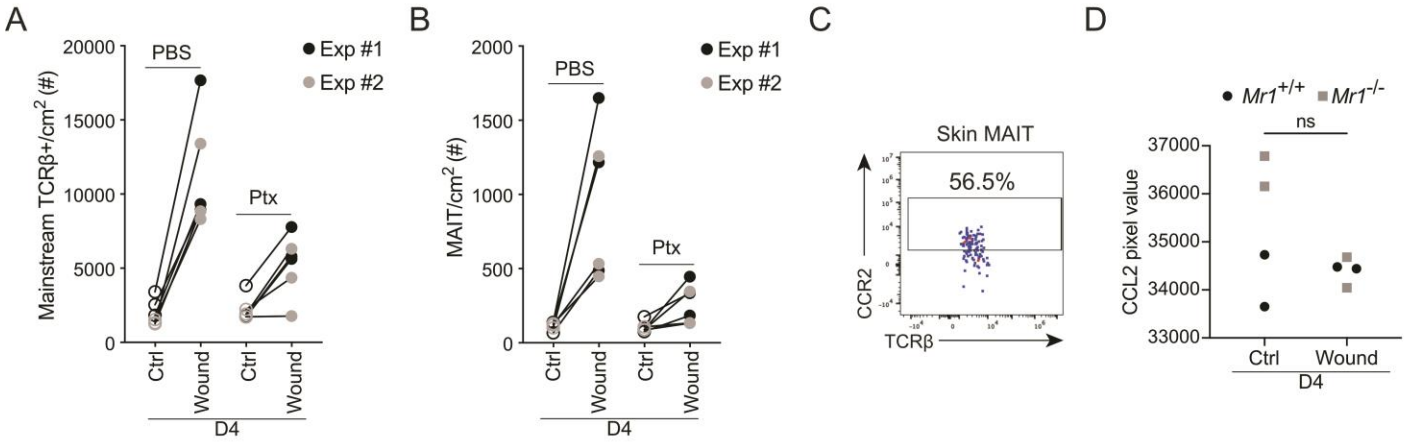

Fig. S5 related to figure 5

(A) Mainstream T cell (TCRß$^{+}$MR1:5-OP-RU-Tet^{-}CD1d:αGalCer-Tet^{-}) numbers (wound/control site) following G protein signaling blocking *in vivo* via Ptx injection. *Pooled data from 2 independent experiments (n=6). Unpaired t-test*

(B) Raw numbers for figure 5A.

(C) Flow cytometry example of CCR2 expression by MAIT cells. *Data are representative of 2 independent experiments.*

(D) Quantitation of CCL2 protein expression in total skin lysates from wound and control skin sites of *Mr1*$^{+/+}$ and *Mr1*$^{-/-}$ mice. *Data are from 2 independent experiments (n=4). Mann Whitney test.*

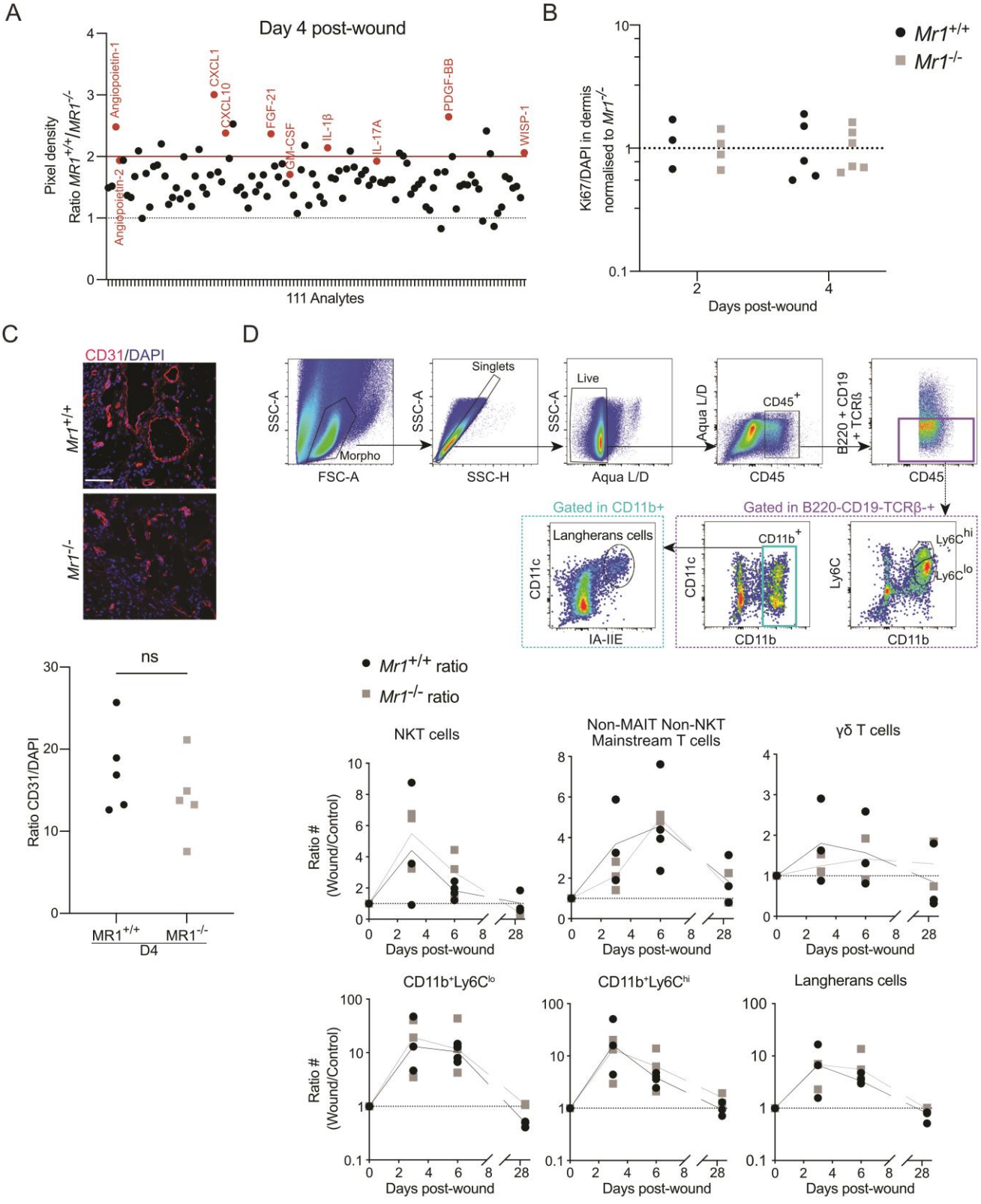
A
Day 4 post-wound
Pixel density
Ratio Mr1+/+/Mr1-/-
111 Analytes
Angiopoietin-1
Angiopoietin-2
CXCL1
CXCL10
FGF-21
GM-CSF
IL-1β
IL-17A
PDGF-BB
WISP-1
B
Mr1+/+
Mr1-/-
Ki67/DAPI in dermis
normalised to Mr1-/-
Days post-wound
C
CD31/DAPI
Mr1+/+
Mr1-/-
ns
Ratio CD31/DAPI
MR1+/+
MR1-/-
D4
D
SSC-A
FSC-A
Morpho
Singlets
SSC-H
Live
Aqua L/D
CD45+
CD45
B220 + CD19 + TCRß
Gated in CD11b+
Langherans cells
CD11c
IA-IIE
Gated in B220-CD19-TCRβ-+
CD11b+
CD11b
Ly6C
Ly6Chi
Ly6Clo
Mr1+/+ ratio
Mr1-/- ratio
NKT cells
Non-MAIT Non-NKT Mainstream T cells
γδ T cells
CD11b+Ly6Clo
CD11b+Ly6Chi
Langherans cells
Ratio # (Wound/Control)
Days post-wound

Fig. S6 related to figure 6: Investigating the potential impact of MAIT cells in the wounds.

(A) Protein expression in *Mr1*$^{+/+}$ as compared to *Mr1*$^{-/-}$ wound (D4) lysates (calculated from pixel density: each *Mr1*$^{+/+}$ duplicate was divided by the average of *Mr1*$^{-/-}$ duplicates in each independent experiment. Geometric mean is represented). *Pooled data from 2 independent experiments (4* Mr1$^{+/+}$ *blots and 3* Mr1$^{-/-}$ *blots).*

(B) Quantitation of proliferation in the dermis by immunofluorescence, given by the Ki67/DAPI ratio and normalized to the average expression in *Mr1*$^{-/-}$ wounds for each experiment. *Pooled data from 1 (D2, n=3/4) and 2 (n=5/6) independent experiments.*

(C) Representative immunofluorescence images of vessels given by CD31 staining (red) in wounds of *Mr1*$^{+/+}$ and *Mr1*$^{-/-}$ animals four days post-excision (top). Vessel density was quantified by the CD31/DAPI ratio under the two epidermal tongues and in the granulation site in the center, the average of all three measurements for each individual mouse is represented (right). *Pooled data from 2 independent experiments (n=5) analyzed blindly. Mann Whitney test.*

Gating strategy used to define Langherans cells, CD11b^{+}Ly6C^{Lo} and CD11b^{+}Ly6C^{hi} CD11b^{+} cells (top) and ratio of absolute numbers of NKT, γδ T, Langherans, CD11b^{+}Ly6C^{lo} and CD11b^{+}Ly6C^{hi} cells (wound/control skin site) for each individual mouse. *Data are representative of 2 independent experiments (n=3).*