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Supplemental information

Tetraspanin CD82 restrains phagocyte migration but supports macrophage activation

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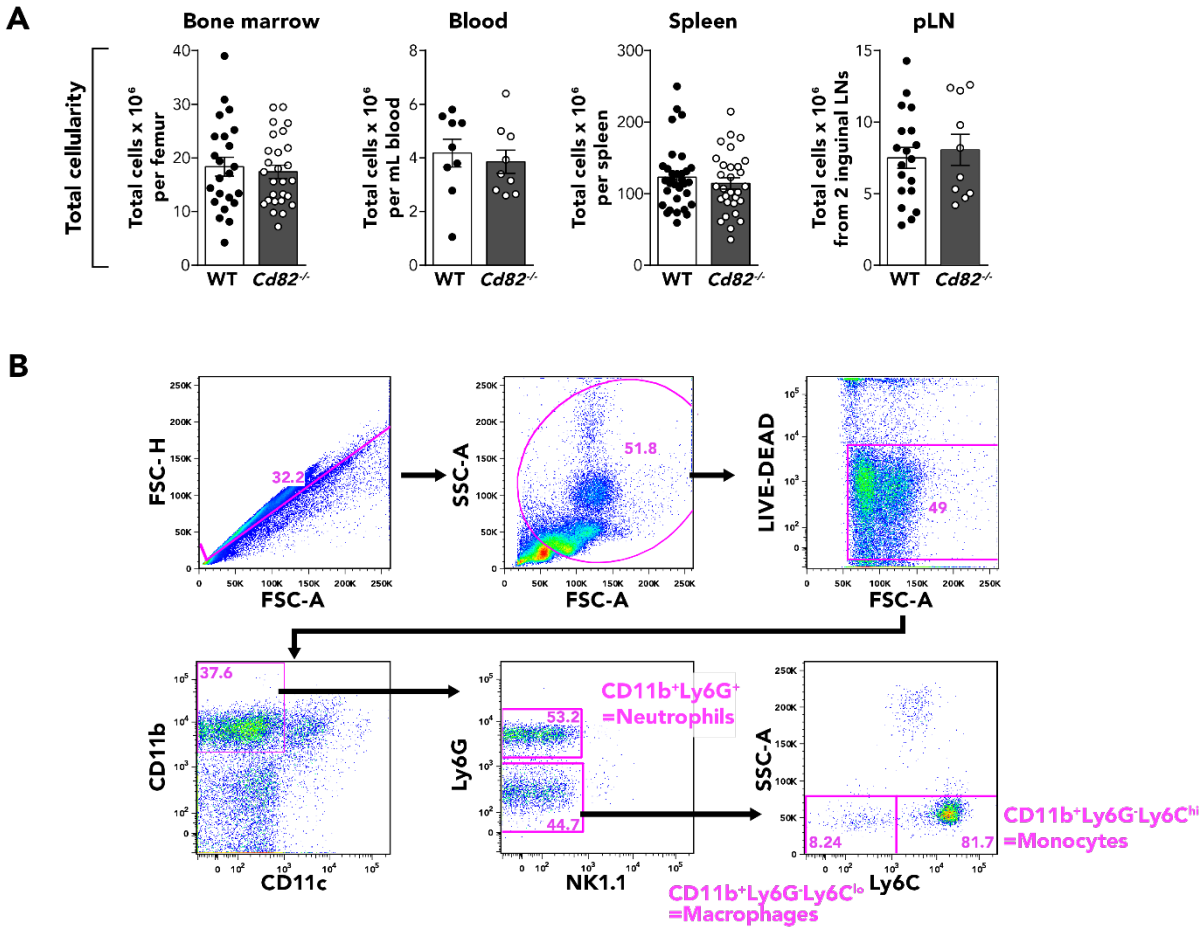
A
Bone marrow
Blood
Spleen
pLN
Total cellularity
Total cells x 10⁶ per femur
Total cells x 10⁶ per mL blood
Total cells x 10⁶ per spleen
Total cells x 10⁶ from 2 inguinal LNs
WT
Cd82-/-
B
FSC-H
FSC-A
32.2
SSC-A
51.8
LIVE-DEAD
49
CD11b
CD11c
37.6
Ly6G
NK1.1
53.2
44.7
CD11b+Ly6G+ =Neutrophils
SSC-A
Ly6C
8.24
81.7
CD11b+Ly6G-Ly6Chi =Monocytes
CD11b+Ly6G-Ly6Clo =Macrophages
C
Bone marrow
Blood
Spleen
pLN
Neutrophils
Monocytes
Macrophages
Cells x 10⁶
Cells x 10⁵
Cells x 10⁴
WT
Cd82-/-

Figure S1. Total and phagocyte cellularity is normal in *Cd82*$^{-/-}$ mice, related to Figure 1. (A) Organs and blood from WT and *Cd82*$^{-/-}$ mice were harvested and cells were counted. Each data point represents a single mouse. **(B)** Phagocyte populations were identified by flow cytometry. Single, viable (propidium iodide negative) cells were first gated. Neutrophils were identified as CD11b^{+}, CD11c^{-} and Ly6G^{hi}. Monocytes are CD11b^{+}, CD11c^{-}, Ly6G^{-}, and Ly6C^{hi}. Macrophages are CD11b^{+}, CD11c^{-}, Ly6G^{-}, Ly6C^{lo}. **(C)** The proportion of phagocytes in the organs and blood of WT and *Cd82*$^{-/-}$ mice was determined by flow cytometry. Absolute cell numbers were determined by multiplying proportions by total organ cellularity. Each data point represents a single mouse. All data are presented as mean ±S.E.M. Significance between WT and *Cd82*$^{-/-}$ genotypes was measured by T or U-tests. No significant difference was detected in any organ or phagocyte population.

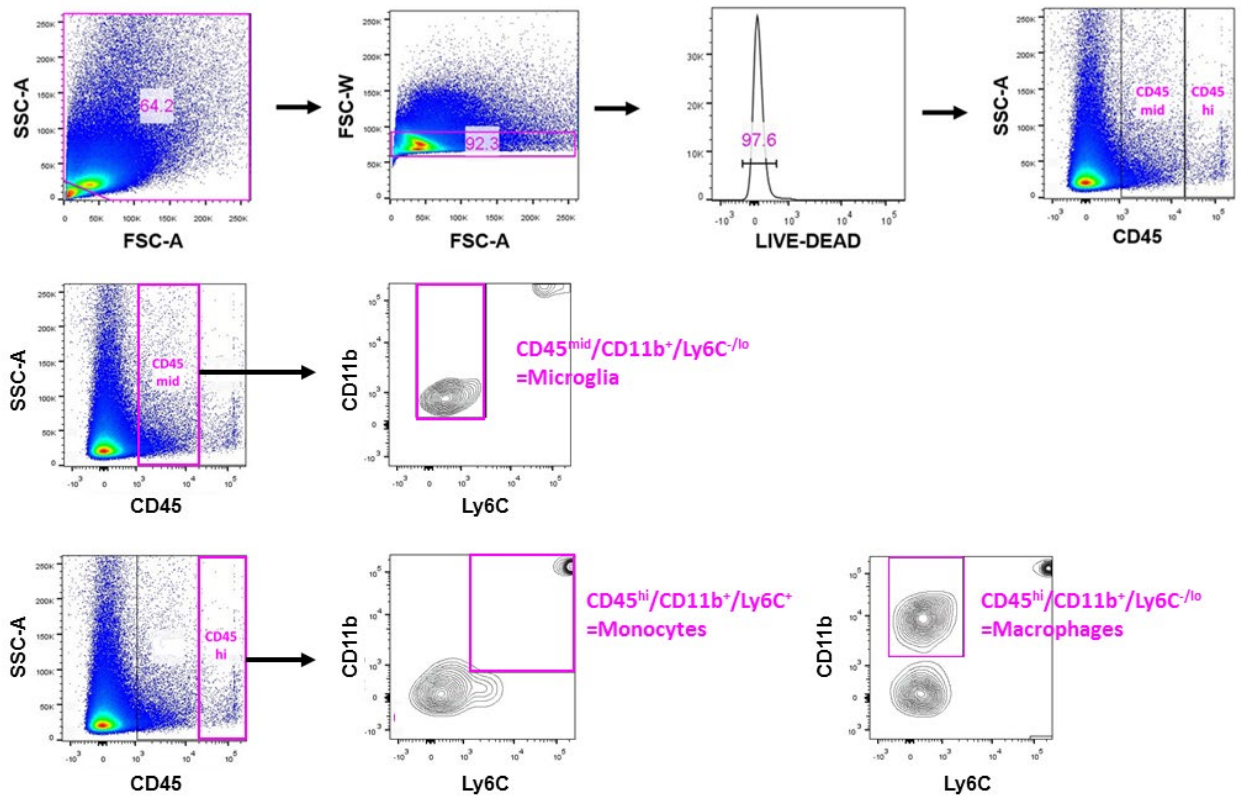

Figure S2. Gating strategy used to identify microglia, monocyte and macrophage populations in the retina, related to Figure 2. Phagocyte populations in the retina of RA and ROP mice were identified by flow cytometry. Single, viable cells were first gated. Microglia were identified as $CD45^{mid}CD11b^{+}Ly6C^{-/lo}$, monocytes as $CD45^{hi}CD11b^{+}Ly6C^{+}$ whereas macrophages were identified as $CD45^{hi}CD11b^{+}Ly6C^{-/lo}$.

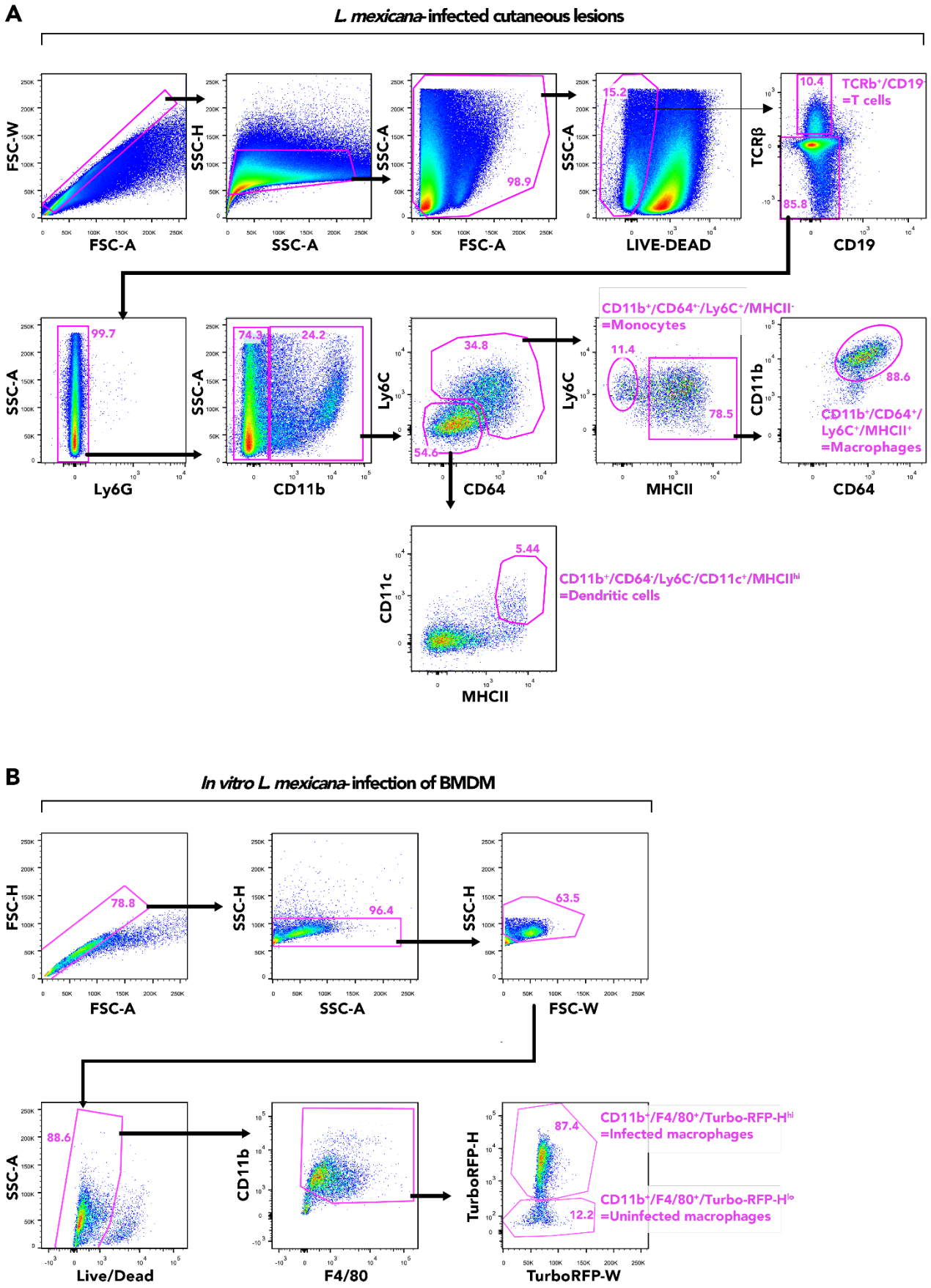
A
L. mexicana-infected cutaneous lesions
FSC-W
FSC-A
SSC-H
SSC-A
SSC-A
FSC-A
98.9
SSC-A
LIVE-DEAD
15.2
TCRβ
CD19
10.4
TCRb+/CD19-
=T cells
85.8
SSC-A
Ly6G
99.7
SSC-A
CD11b
74.3
24.2
Ly6C
CD64
34.8
54.6
Ly6C
MHCII
11.4
78.5
CD11b+/CD64+/Ly6C+/MHCII-
=Monocytes
CD11b
CD64
88.6
CD11b+/CD64+/
Ly6C+/MHCII+
=Macrophages
CD11c
MHCII
5.44
CD11b+/CD64-/Ly6C-/CD11c+/MHCIIhi
=Dendritic cells
B
In vitro L. mexicana-infection of BMDM
FSC-H
FSC-A
78.8
SSC-H
SSC-A
96.4
SSC-H
FSC-W
63.5
SSC-A
Live/Dead
88.6
CD11b
F4/80
TurboRFP-H
TurboRFP-W
87.4
CD11b+/F4/80+/Turbo-RFP-Hhi
=Infected macrophages
12.2
CD11b+/F4/80+/Turbo-RFP-Hlo
=Uninfected macrophages

Figure S3. Gating strategy used for *L. mexicana* infections, related to Figure 3. (A) Phagocyte populations in *L. mexicana*-infected cutaneous lesions and draining lymph nodes (DLN) were identified by flow cytometry. For cutaneous lesions, an 8 mm ø biopsy was taken from the ears of WT and *Cd82*$^{-/-}$ mice. The entire biopsy was then enzymatically digested and analysed by flow cytometry. Because of the large number of cellular debris and non-immune cells, total cell counts were not obtainable and instead populations are presented as cell proportions per whole lesion. Right superficial cervical lymph nodes draining the infected ear were dissected and enzymatically digested. DLN total cell numbers were determined by a hemocytometer. For both cutaneous lesions and DLNs, phagocytes were identified by flow cytometry. Single, viable cells were first gated and then leucocyte populations identified with the following gating strategies: T cells are TCRβ^{+}CD19^{-}, Monocytes are TCRβ^{-}CD19^{-}Ly6G^{-}CD11b^{+}CD64$^{+/-}$Ly6C^{+}MHCII^{-}, macrophages are TCRβ^{-}CD19^{-}Ly6G^{-}CD11b^{+}CD64^{+}Ly6C^{+}MHCII^{+}, and dendritic cells are TCRβ^{-}CD19^{-}Ly6G^{-}CD11b^{+}CD64^{-}Ly6C^{-}CD11c^{+}MHCII^{hi}. (**B**) Gating strategy to determine the proportion of viable, *L. mexicana-TurboRFP* infected macrophages (CD11b^{+}F/80^{+}Turbo-RFP-Hhi) after 3 days. For measurement of parasitic burden, the geometric mean fluorescence intensity (gMFI) of the Turbo-RFP signal in uninfected macrophages was subtracted from the Turbo-RFP gMFI in infected macrophages.

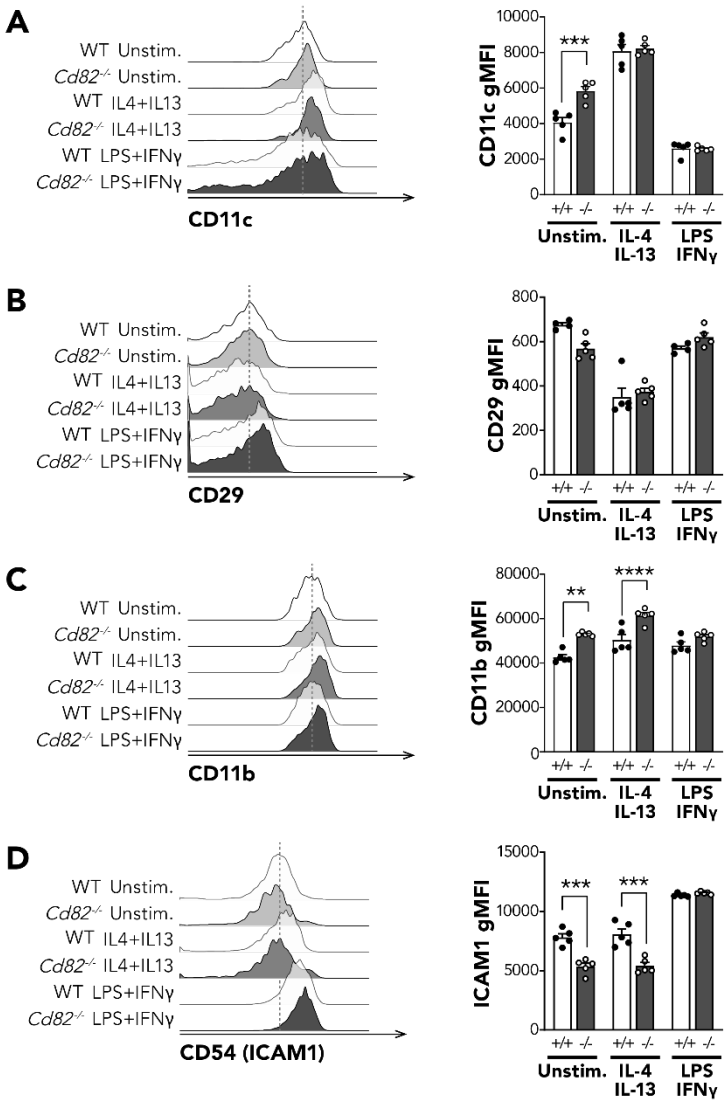

Figure S4. Expression of surface proteins is altered in *in vitro* differentiated Cd82$^{-/-}$ macrophages, related to Figure 4. Macrophages were differentiated *in vitro* from the bone marrow of WT and *Cd82*$^{-/-}$ mice. After 7 days differentiation, macrophages were either left unstimulated ('Unstim.') or activated to an anti-inflammatory 'M2' phenotype with IL-4 and IL-13, or a pro-inflammatory 'M1' phenotype with LPS and IFNγ, for a further 24 hrs before analysis by flow cytometry. Single, viable, F4/80^{+}CD11b^{+} macrophages were first gated and then the analysis of the surface proteins CD11c (A), CD29 (B), CD11b (C) and CD54 (D) was assessed by flow cytometry. Histograms of expression levels are shown with quantification of the geometric mean fluorescence intensity (bars). Each dot represents technical replicates which were repeated in two independent experiments, where BMDM were differentiated from separate mice. All data are presented as mean ±S.E.M. *P* values for the effect of CD82 deficiency and interaction with macrophage activation were calculated by 2-way ANOVA and is indicated in the top left corner of each graph. Additionally, Sidak post-hoc multiple comparison analysis of individual time points is indicated by *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.