SUPPLEMENTAL MATERIAL

Lee et al., 10.1084/jem.20171389

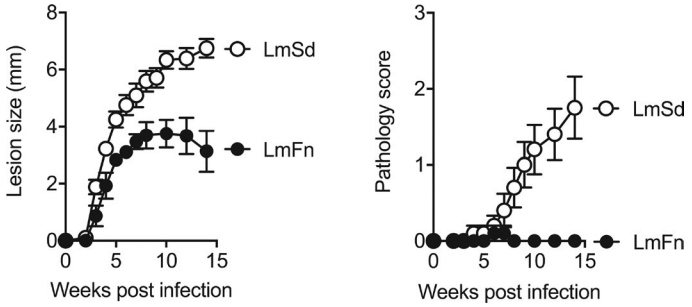

Figure S1. **Nonhealing infection with LmSd in C57BL/6 mice.** Lesion development and pathology scores (0 = no ulceration, 1 = ulcer, 2 = half ear eroded, 3 = ear completely eroded) over the course of infection with 10^3 metacyclic LmFn and LmSd promastigotes in the ear dermis of C57BL/6 mice. Values represent mean ± standard deviation (n = 5 mice per group).

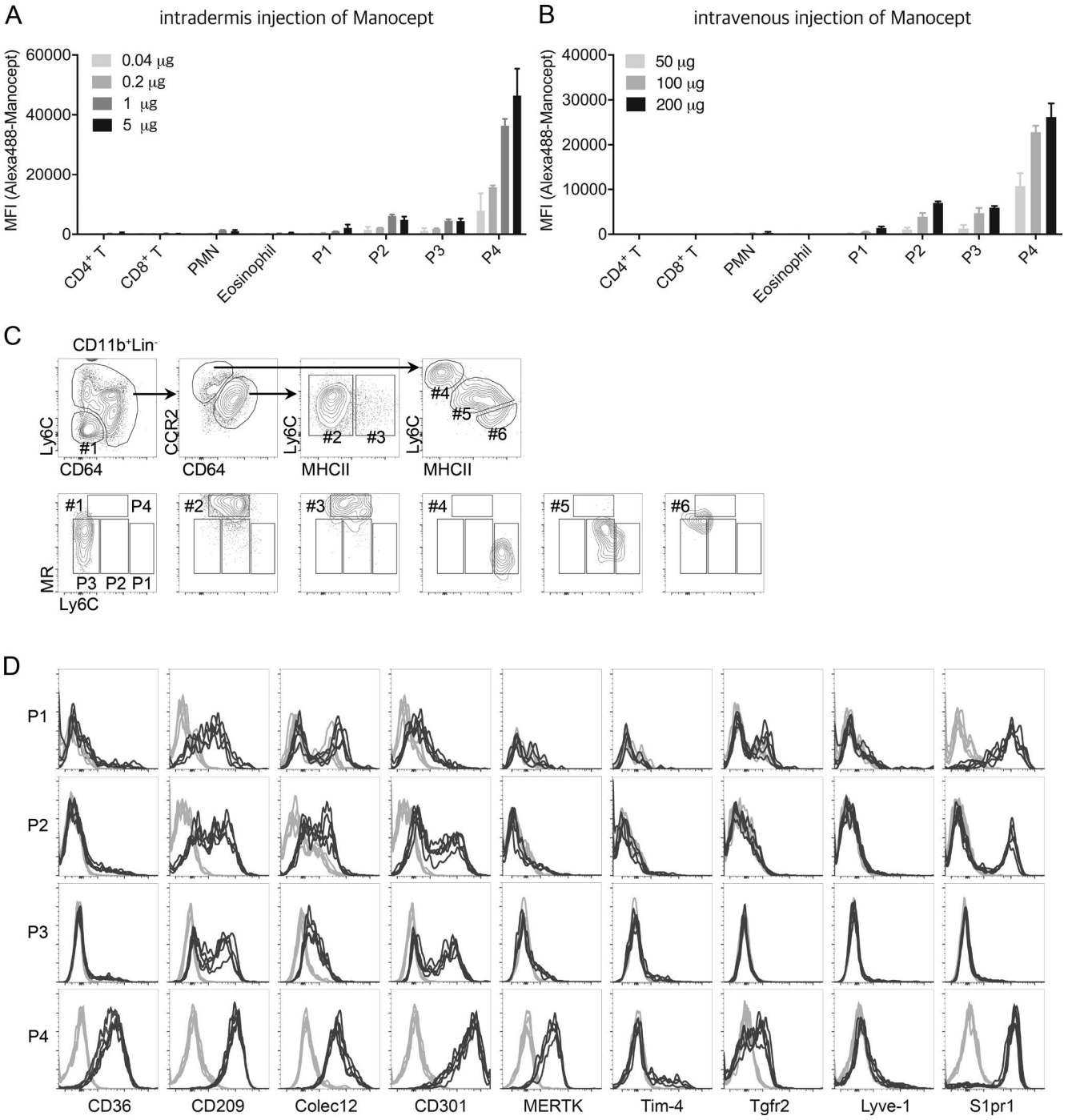

Figure S2. **Phenotype analysis of P1–P4. (A and B)** Flow cytometric analysis of ear isolates from C57BL/6 animals at (A) 1 h after intradermal injection or (B) 2 h after intravenous injection of Manocept–Alexa Fluor 488–containing mannose moieties. PMN, polymorphonuclear leukocyte. Values represent mean ± standard deviation (n = 4 ears per group). **(C)** In the top panels, the dermal populations of $CD11b^+Lin^-$ cells were gated and defined as previously reported (Tamoutounour et al., 2013) as $CD11b^+Ly6C^-CD64^-$ dermal DCs (1), $CD11b^+CCR2^{lo}CD64^{hi}Ly6C^{int}MHCII^-$ and $MHCII^+$ dermal macrophages (2 and 3), $CD11b^+CCR2^{hi}CD64^{lo}Ly6C^{hi}MHCII^-$ dermal monocytes (4), $CD11b^+CCR2^{hi}CD64^{lo}Ly6C^{int}MHCII^{int}$ dermal moDCs (5), and $CD11b^+CCR2^{hi}CD64^{lo}Ly6C^{lo}MHCII^{hi}$ dermal moDCs (6). In the bottom panels, the subsets 1–6 are overlaid on the P1–P4 gates. **(D)** Histograms showing M2 macrophage markers expressed on P1–P4 populations from naive ears.

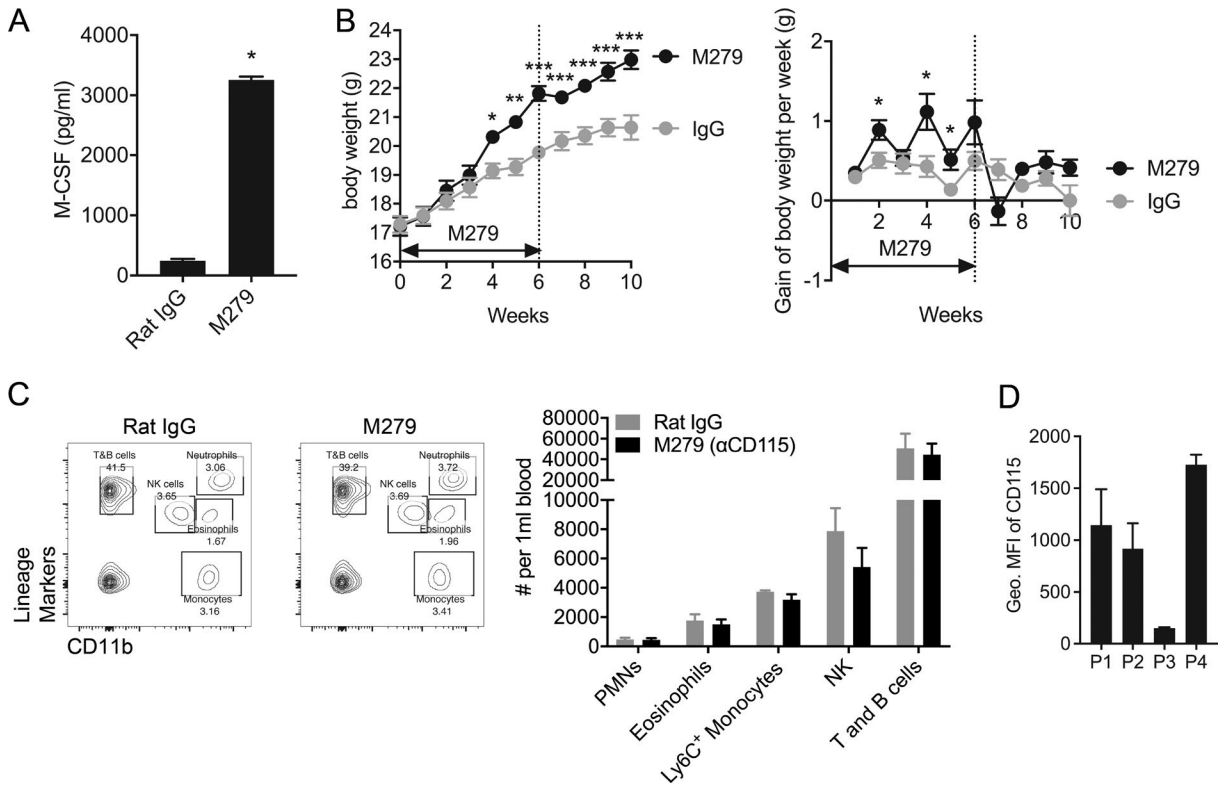

Figure S3. **Effects of prolonged treatment of M279 antibodies in C57BL/6 mice. (A)** The serum concentration of M-CSF in animals treated with 200 µg M279 or control IgG three times a week for 3 wk intraperitoneally. **(B)** Mean body weight changes of animals treated with 200 µg M279 or control IgG three times a week for 6 wk intraperitoneally. **(C)** Flow cytometric analysis of peripheral blood mononuclear cells from mice treated with M279 three times a week for 3 wk. **(D)** MFIs of CSF-1R expression on P1–P4 from naive animals. Values represent mean ± standard deviation (n = 4 mice per group). *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ by nonparametric Mann-Whitney test (B).

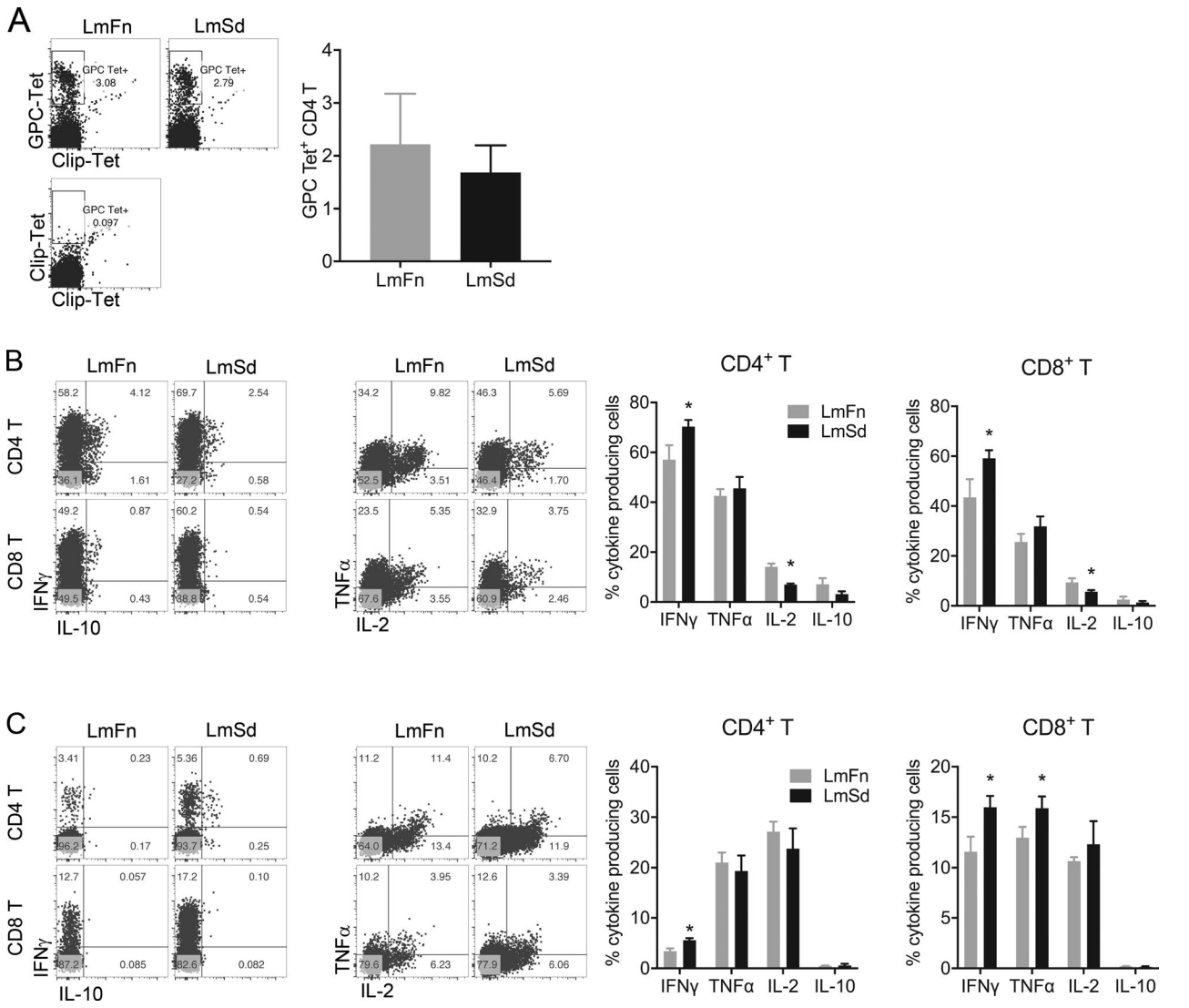

Figure S4. **Th1 polarization in LmFn- and LmSd-infected C57BL/6 mice. (A)** The detection of antigen-specific T cell development (GPC 335–351 NDAFGVMPPVARLTPEQ) in ear lesions of mice infected with 10^6 metacyclic promastigotes at 8 d p.i. **(B and C)** The frequency of PMA/ionomycin-restimulated $CD4^+$ T and $CD8^+$ T cells in ear lesions (B) and dLNs stained positive for IFN-γ, TNF-α, IL-2, and IL-10 at 8 d p.i. with 10^6 metacyclic promastigotes (C). Values represent mean ± standard deviation (n = 4 mice per group). *, $P < 0.05$ by nonparametric Mann-Whitney test (B and C).

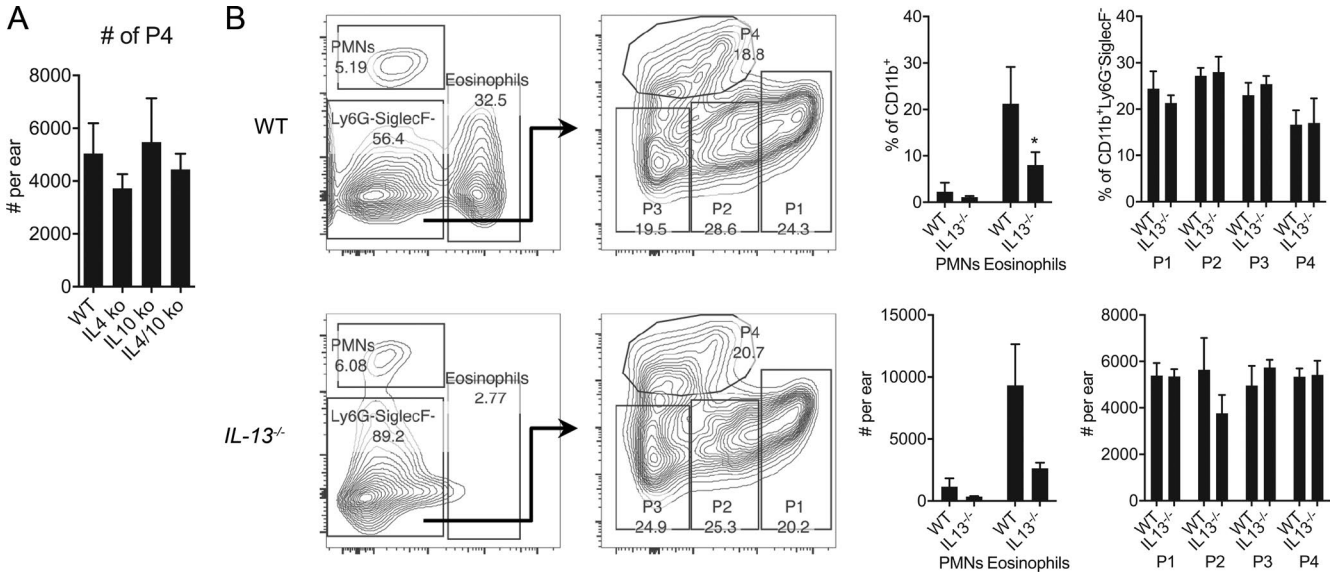

Figure S5. **IL-13 is not required to maintain the P4 dermal macrophages in LmSd-infected mice. (A)** The frequency of P1–P4 populations in naive C57BL/6, *il4*$^{-/-}$, *il10*$^{-/-}$, and *il4/10*$^{-/-}$ mice. ko, knockout. **(B)** Representative plots (left) showing the frequency of polymorphonuclear leukocytes (PMNs), eosinophils, and P1–P4 populations in C57BL/6 and *il13*$^{-/-}$ mice at day 12 p.i. with 2×10^5 LmSd. Bar graphs show the frequency and absolute number of these populations. Values represent mean ± standard deviation (n = 5 mice per group). *, $P < 0.05$ by nonparametric Mann-Whitney test (B).

REFERENCE

Tamoutounour, S., M. Guilliams, F. Montanana Sanchis, H. Liu, D. Terhorst, C. Malosse, E. Pollet, L. Ardouin, H. Luche, C. Sanchez, et al. 2013. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity.* 39:925–938. https://doi.org/10.1016/j.immuni.2013.10.004