Supporting Information to:

Targeted Delivery of Antigen to Activated CD169^{+} Macrophages Induces Bias for Expansion of CD8^{+} T cells

Landon J. Edgar, Norihito Kawasaki, Corwin M. Nycholat, James C. Paulson*
*Corresponding Author: jpaulson@scripps.edu

Department of Molecular Medicine, The Scripps Research Institute, California 92037, United States

Table of Contents:

2. Supporting Figures

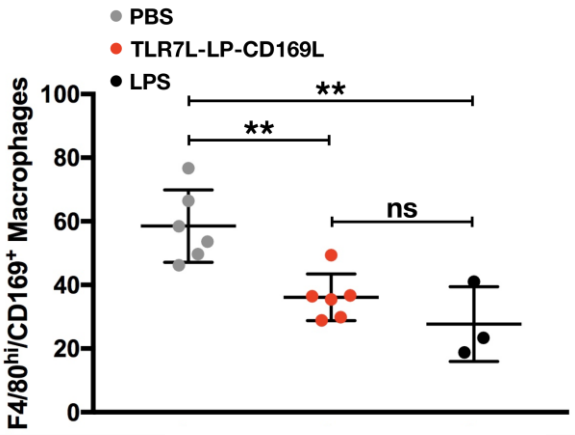

Figure S1. Ratios of splenic F4/80^{hi} vs. CD169^{+} MØs (related to Figure 1).
Values represent the ratio of Lin^{-}CD11b^{+}Ly6C^{-}F4/80^{hi} to Lin^{-}CD11b^{+}Ly6C^{-}CD169^{+} cells from the same spleen. **P<0.01, ns = not statistically significant (P≥0.05).

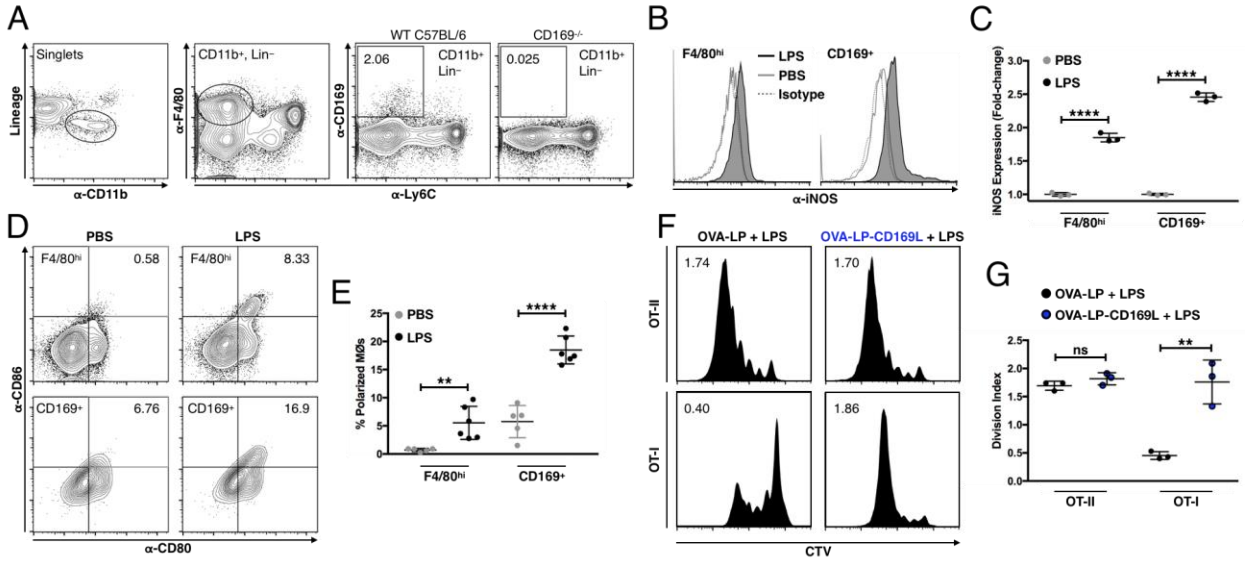

Figure S2. Classical activation of CD169^{+} MØs enables expansion of CD8^{+} T cells (related to Figures 2 and 3).
(A) Gating strategy for analysis of F4/80MØs and CD169MØs in murine spleen by FC. (B) Expression of iNOS in F4/80MØs and CD169MØs upon stimulation with a high dose of LPS *in vivo* as measured by FC. (C) Quantification of data in B. (D) Activation of F4/80MØs and CD169MØs upon stimulation with LPS *in vivo* as measured by CD80 and CD86 expression. Cells populating the upper right quadrant are considered activated. Numbers represent the percentage of activated cells. (E) Quantification of data in D. (F) Proliferation histograms for adoptively transferred CTV stained OT-II or OT-I cells in Ly5b$^{+/+}$ mice injected with both OVA-LP(-CD169L) and LPS. Numbers represent cell division indexes. (G) Quantification of data in F. ***P<0.001, ****P<0.0001, ns = not statistically significant (P≥0.05).

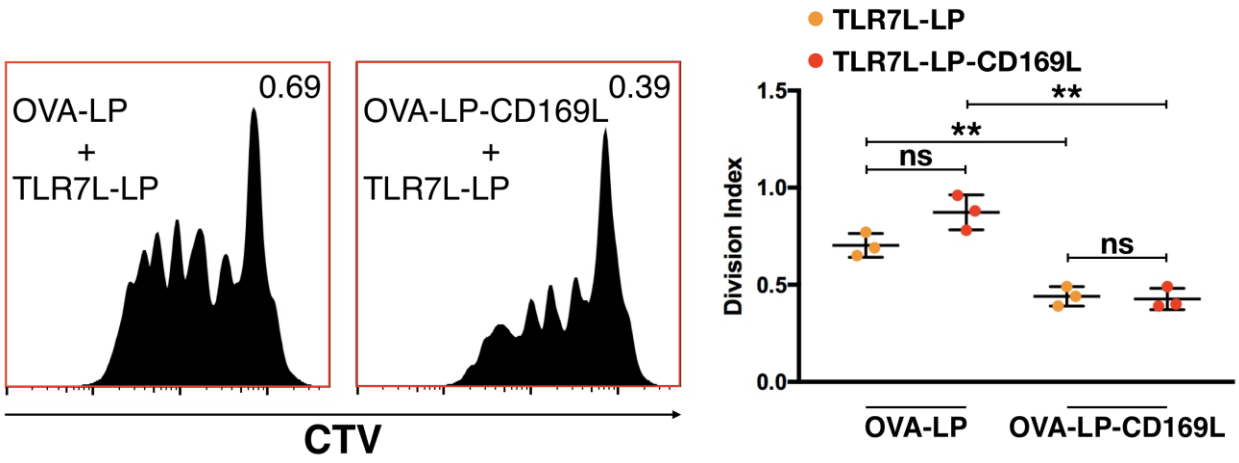

Figure S3. Effect of untargeted TLR7L-bearing liposomes on OT-II cell proliferation (related to Figure 3).
OT-II proliferation was measured as in fig. S2 using WT C56BL/6 mice as hosts. Numbers represent the division index for each representative plot. **P<0.01, ns = not statistically significant (P≥0.05).

3. Table S1: Liposome Formulations (related to Figures 1–3)

Identity	DSPC	Cholesterol	PEG-DSPE	CD169L-PEG-DSPE	AF555-PEG-DSPE	TLR7L-PEG-DSPE
AF-LP-CD169L	57	38	2.75	2	0.25	-
AF-LP	57	38	4.75	-	0.25	-
TLR7L-LP-CD169L	57	38	2.9	2	-	0.1
TLR7L-LP	57	38	4.9	-	-	0.1
OVA-LP-CD169L*	57	38	3	2	-	-
OVA-LP*	57	38	5		-	-

All values in mol %. *Liposomes extruded in 10 mg/mL chicken ovalbumin in PBS.

4. ^{1}H Nuclear Magnetic Resonance Spectrum of TLR7L-PEG-DSPE (related to STAR Methods)

600 MHz, d_6-DMSO