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Supplemental Material to:

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Attenuated Listeria monocytogenes reprograms M2-polarized tumor-associated macrophages in ovarian cancer leading to iNOS-mediated tumor cell lysis

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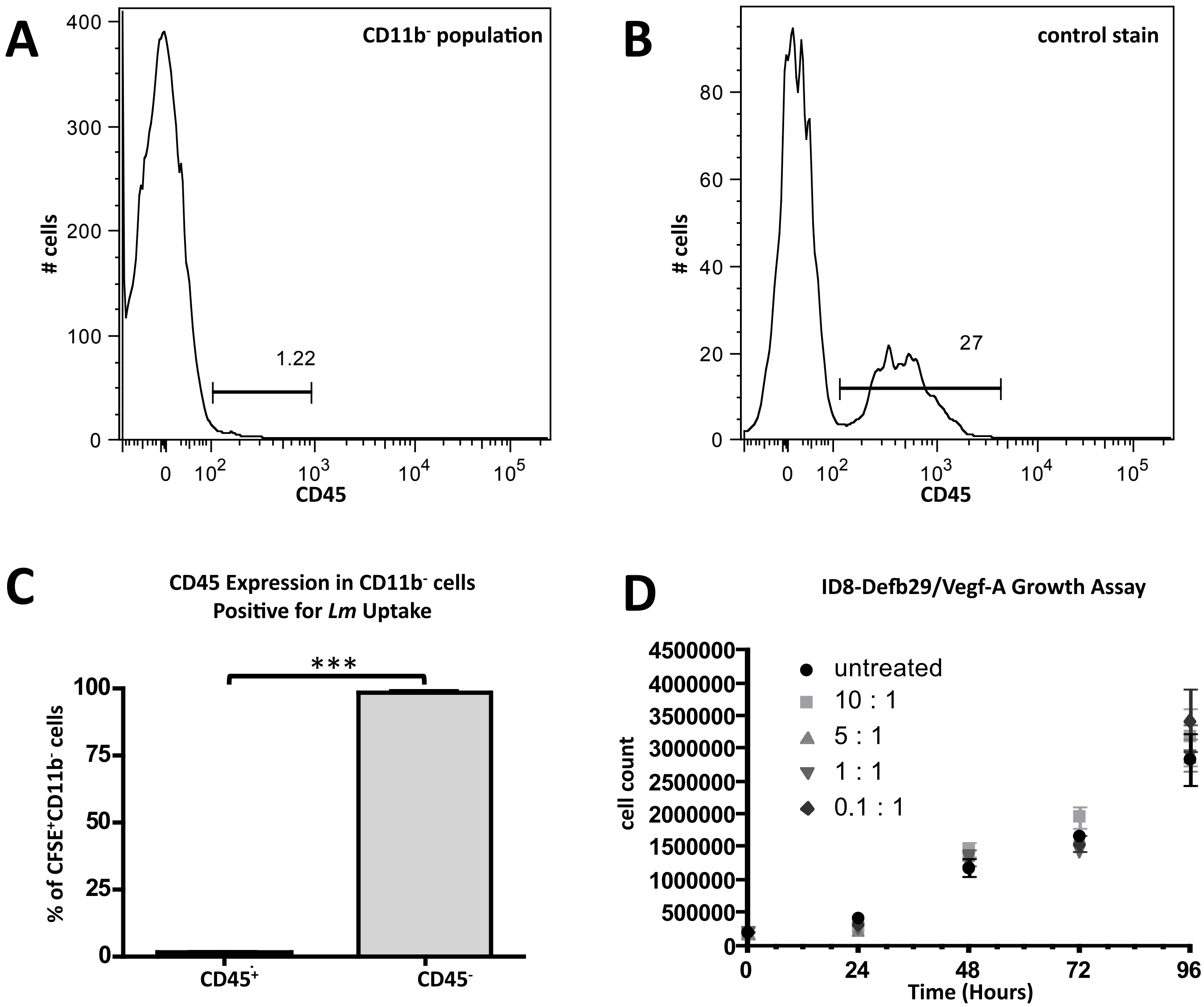
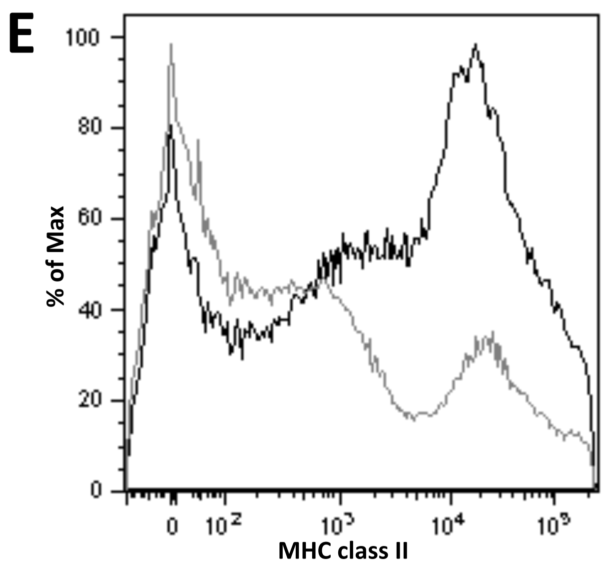
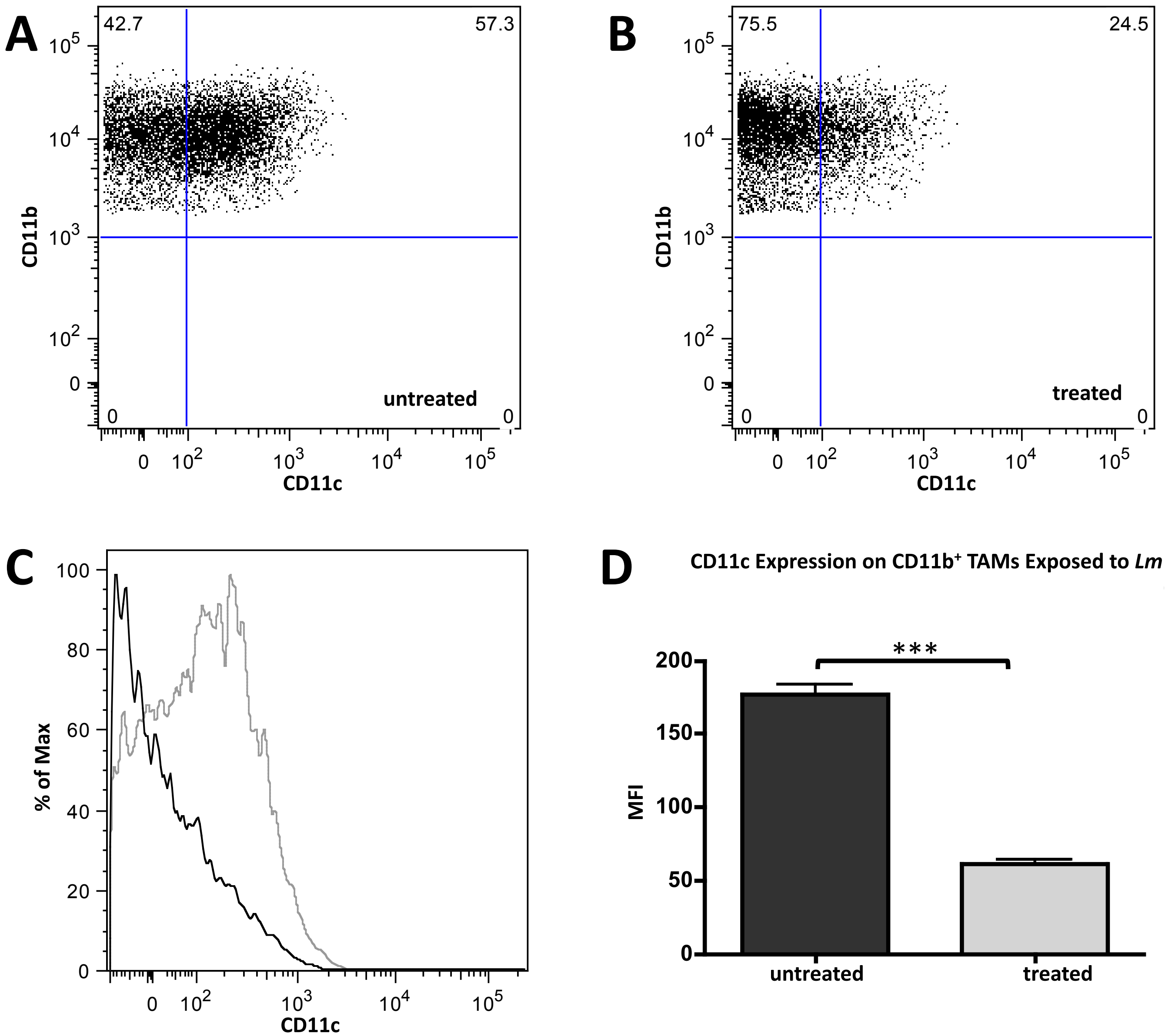
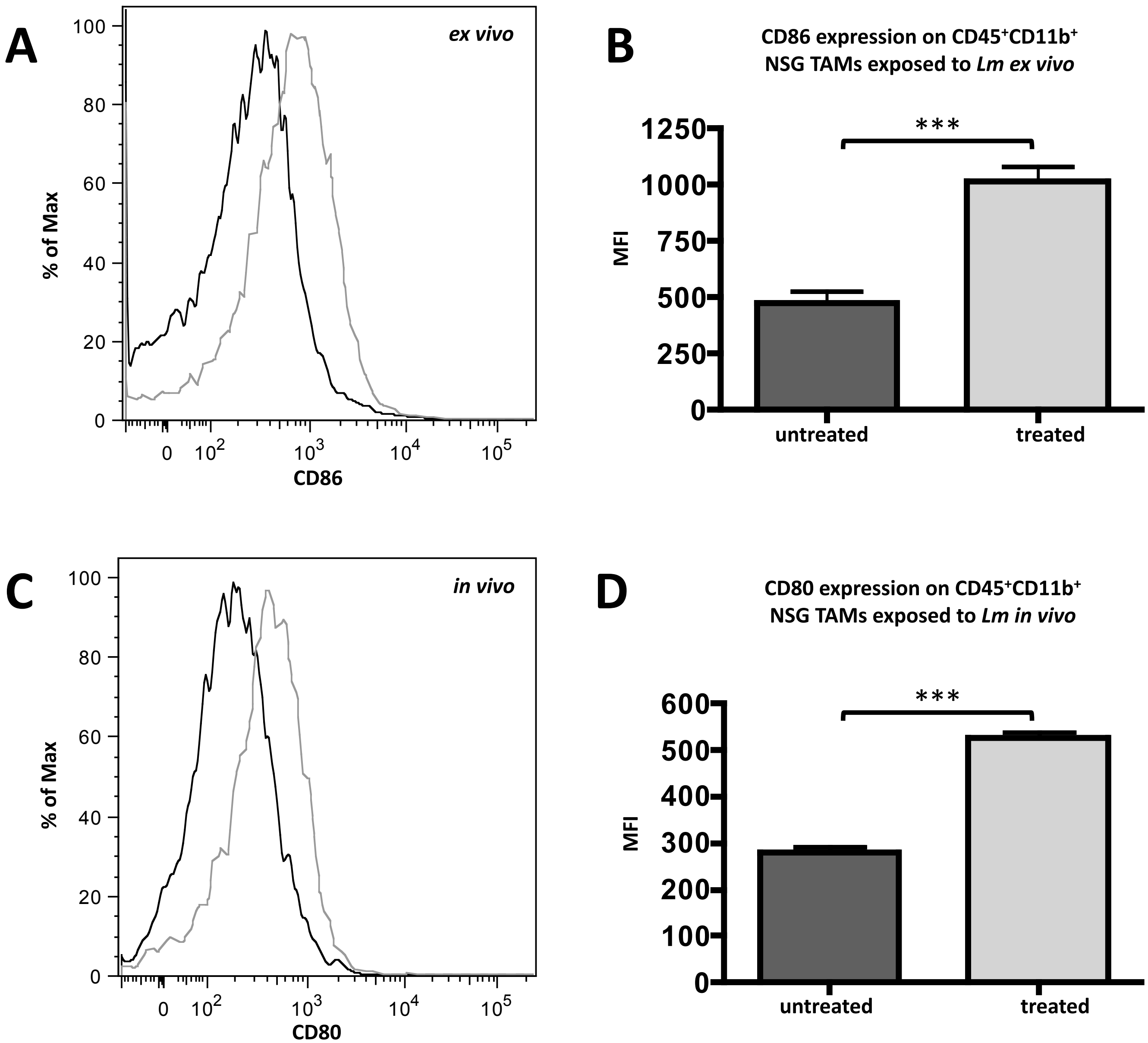




Supplementary Figure S1. *Lm* infection of ID8-*Defb29*/*Vegf-A* cells does not impact viability. (**A-B**) histogram of CD45 staining on CD11b$^-$ gate from Fig. 2B bottom panel (**A**) compared to control CD45 stain (**B**). (**C**) out of CD11b$^-$ cells that are positive for CFSE-labeled *Lm* uptake, ~98% are CD45$^-$ and are likely tumor cells (n = 4). (**D**) exposure to increasing MOI of *Lm* has no effect on growth of ID8-*Defb29*/*Vegf-A* cells *in vitro*. (**E**) representative histograms of MHC class-II surface expression on CD45$^+$CD11b$^+$ ascites cells from tumor-bearing mice treated with *Lm* (black line) or untreated (gray line) overnight in culture.

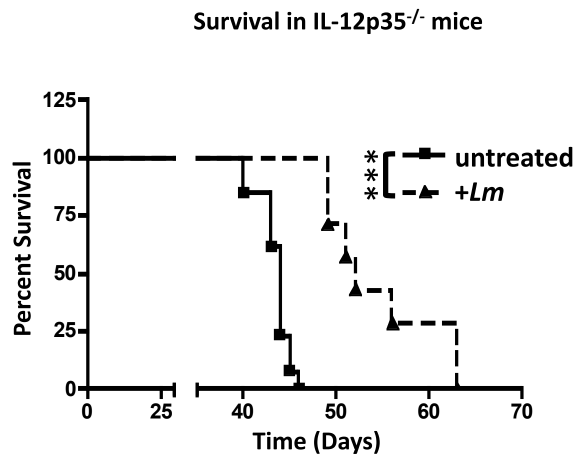


Supplementary Figure S2. *Lm* treatment induces downregulation of CD11c surface expression on $CD45^+CD11b^+$ TAMs. (**A-B**) representative FACs plots of $CD45^+CD11b^+$ TAMs showing marked loss of CD11c expression following treatment with *Lm ex vivo*. (**C**) histogram displaying MFI for CD11c on untreated (dashed gray) and *Lm*-treated (solid black) $CD45^+CD11b^+$ TAMs. (**D**) quantification of multiple replicates of panel (**C**).



Supplementary Figure S3. *Lm* treatment induces upregulation of costimultory surface markers on CD45$^+$CD11b$^+$ TAMs from NOD/scid/Il2R-γ$^{-/-}$ mice both *ex vivo* and *in vivo.* (**A-B**) representative FACs histograms and bar graph of CD45$^+$CD11b$^+$ TAMs from NSG mice showing increase in CD86 expression following treatment of bulk ascites *ex vivo* with *Lm* (light grey) at MOI of 1, relative to untreated (dark gray). (**C-D**) representative FACs histograms and bar graph of CD45$^+$CD11b$^+$ TAMs showing increase in *in vivo* CD80 expression following treatment of NSG mice IP with 10^7 *Lm* (light grey) relative to untreated (dark gray).

A



Supplementary Figure S4. The observed survival from *Lm* treatment in ID8-*Defb29*/*Vegf-A*-challenged C57BL/6J mice is not dependent on the ability of mice to produce Il-12.

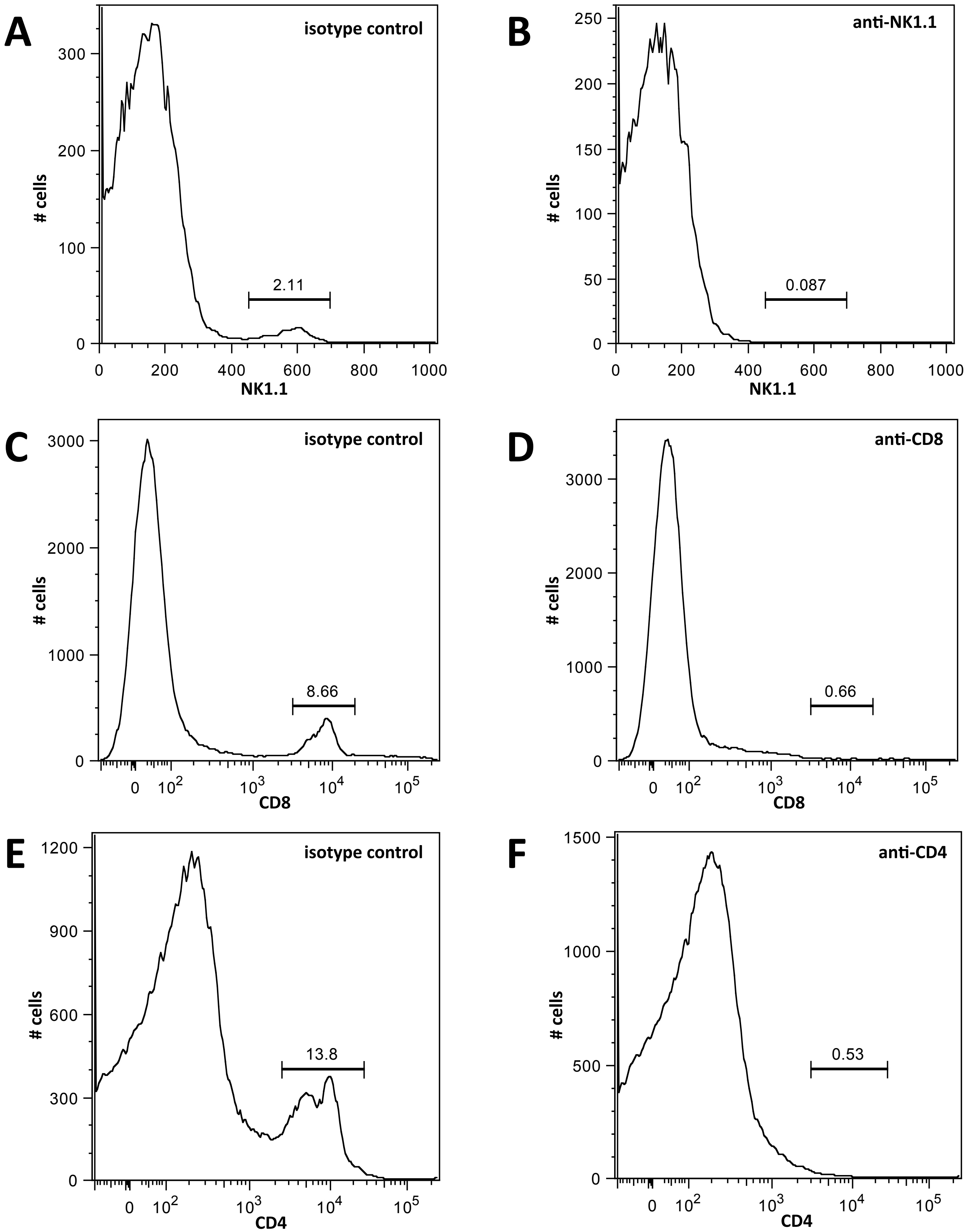
A

95°C	3:00	initial melting
95°C	0:10	repeat 35X
55°C	0:10	
72°C	0:30	
95°C	0:10	final extension

B

Gene	**Forward Primer**	**Reverse Primer**
Gapdh	TGAAGCAGGCATCTGAGGG	CGAAGGTGGAAGAGTGGGAG
Tgf-β	GGACTCTCCACCTGCAAGAC	GACTGGCGAGCCTTAGTTTG
Ido	AATCAAAGCAATCCCCACTG	AAAAACGTGTCTGGGTCCAC
Vista	AGGACAGTGACAGCATCACG	AGGCCACCTGTCTCTGCTTA
Arg-1	AAAGCTGGTCTGCTGGAAAA	ACAGACCGTGGGTTCTTCAC
RANKL	GCAGAAGGAACTGCAACACA	GATGGTGAGGTGTGCAAATG
Mmp7	AGCTATGCAGCTCACCCTGT	GAGCCTGTTCCCACTGATGT
Tnf-α	TAGCCAGGAGGGAGAACAGA	TTTTCTGGAGGGAGATGTGG
Il-1β	CCCAAGCAATACCCAAAGAA	GCTTGTGCTCTGCTTGTGAG
Il-6	CCGGAGAGGAGACTTCACAG	TCCACGATTTCCCAGAGAAC
Il-12	CTCCTGTGGGAGAAGCAGAC	CAGATAGCCCATCACCCTGT
Cxcl10	TCCTTGTCCTCCCTAGCTCA	ATAACCCCTTGGGAAGATGG
Nos2	TGGTGGTGACAAGCACATTT	AAGGCCAAACACAGCATACC

Supplementary Figure S5. qRT-PCR information. (**A**) cycling parameters for q-PCR machine utilized for mRNA expression analysis. (**B**) primer sequences used for q-PCR. Primers predicted using consensus mRNA sequences as presented on UCSC Genome Browser and Primer3 v.0.4.0 software from the Whitehead Institute.[32]



Supplementary Figure S6. Lymphocyte depletion validation. (**A-B**) histograms of splenocytes from isotype treated mice (**A**) compared to anti-NK1.1 depleted (**B**). (**C-D**) histograms of splenocytes from isotype treated mice (**C**) compared to anti-CD8b depleted (**D**). (**E-F**) histograms of splenocytes from isotype treated mice (**E**) compared to anti-CD4 depleted (**F**). C57BL/6 splenocytes harvested 24hr post-IP injection.