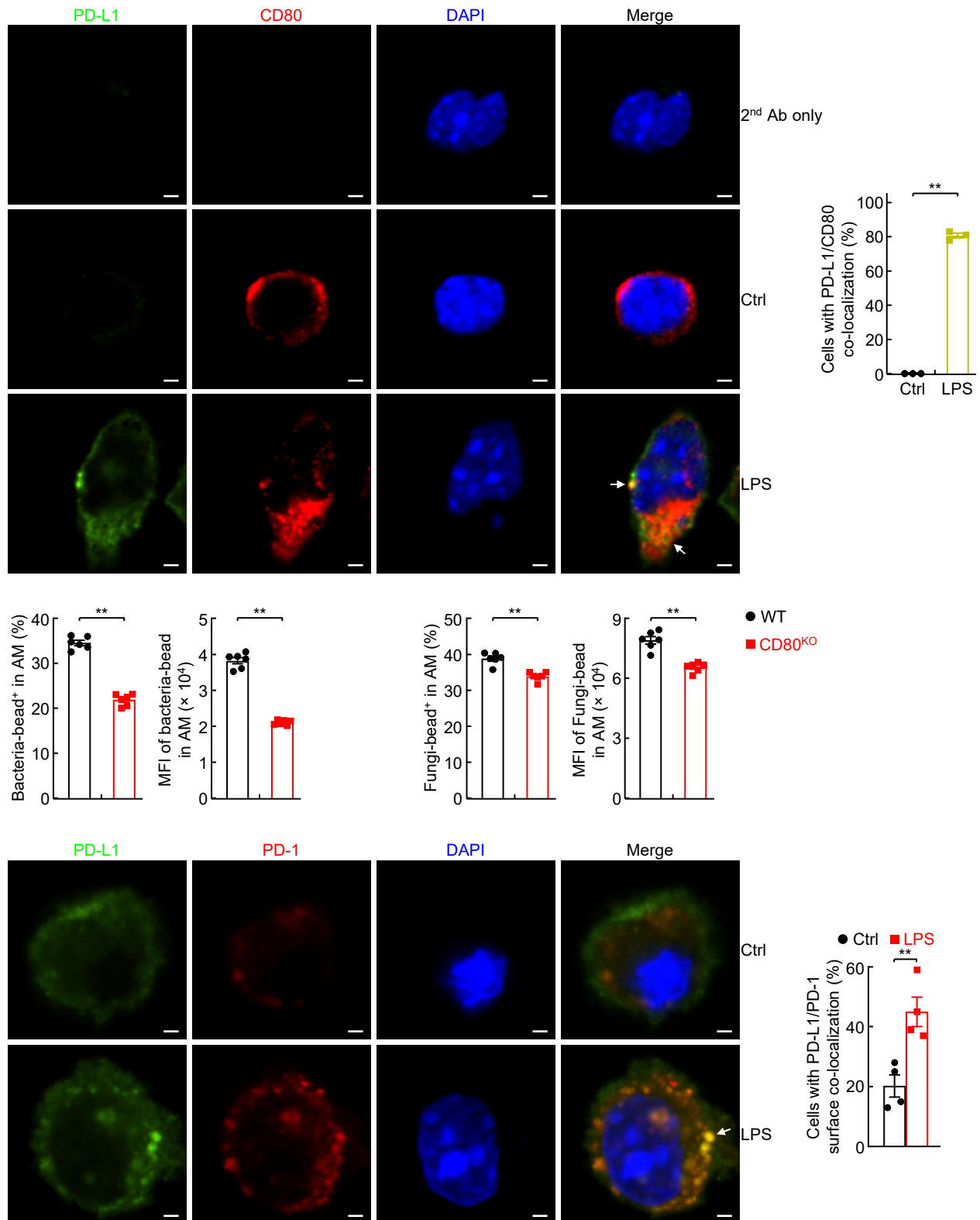
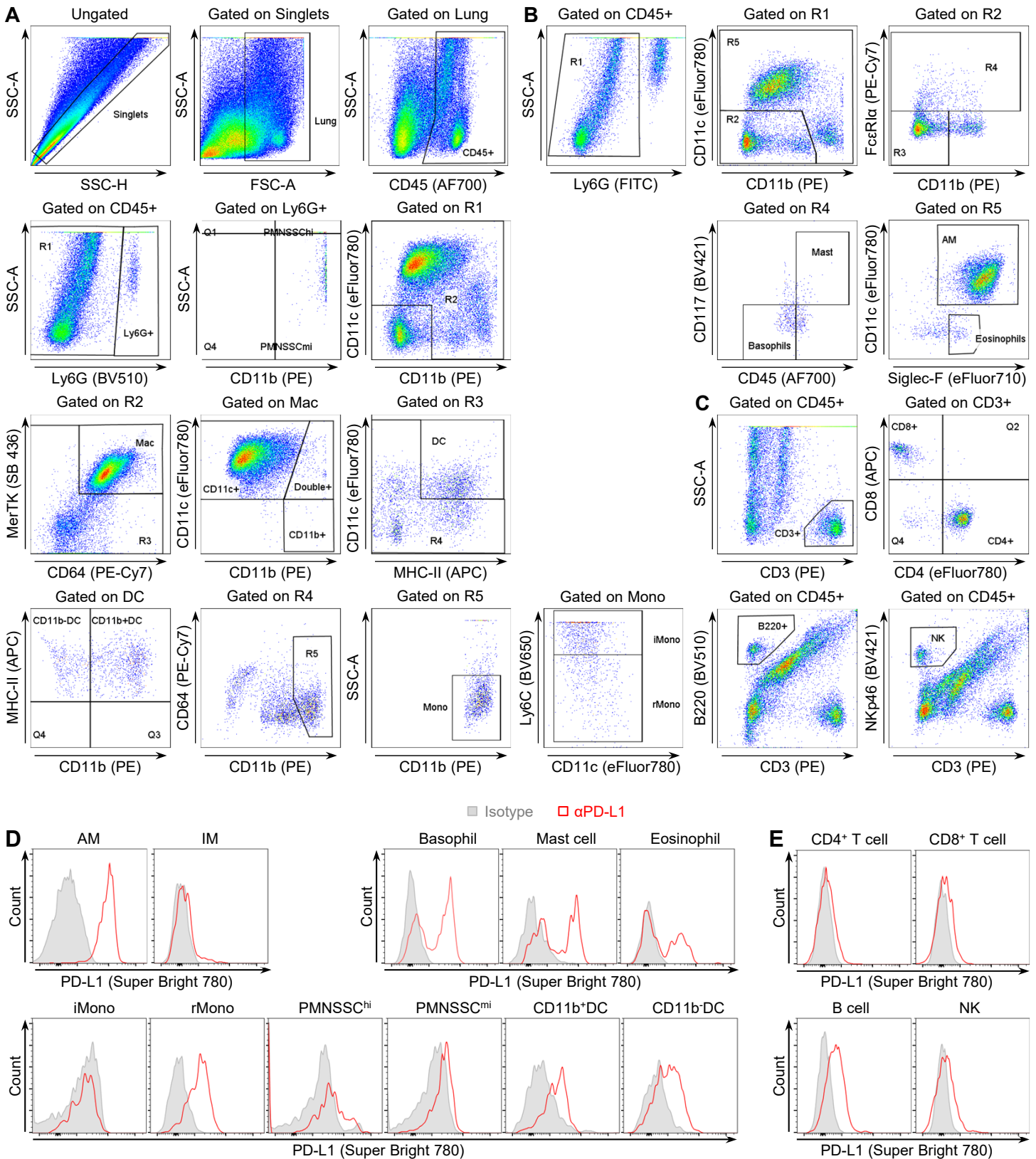


Supplemental Figure 1. FACS gating strategies for macrophages in different tissues of mice. **(A)** Gating strategy for lung macrophages: AM ($CD11c^+CD11b^-$) and IM ($CD11c^-CD11b^+$). **(B)** AM and IM verification by Siglec-F expression. **(C)** Gating strategy for peritoneal macrophage (Mac). **(D)** Gating strategy for splenic macrophage (Mac). **(E)** FACS assays showing neglectable expression of PD-L2 on AMs and IMs.



Supplemental Figure 2. PD-L1 interacts with CD80 in cis and this interaction boosts AM phagocytosis. **(A)** Confocal microscopy showing induction of PD-L1 expression in peritoneal macrophages by LPS and co-localization of PD-L1 and CD80 on their surface ($n = 3$). Scale bar: 1 μ m. **(B)** Decreased ability of CD80^{KO} AMs to phagocytose bacteria and fungi ($n = 6$). Student's *t* test was performed (two tailed, unpaired) and data represent means \pm SEM. ****** $P < 0.01$. **(C)** Confocal microscopy showing increased *cis*-PD-L1/PD-1 interaction on AMs by LPS ($n = 4$). Scale bar: 1 μ m. Student's *t* test was performed (two tailed, unpaired) and data represent means \pm SEM in (A-C). ****** $P < 0.01$.



Supplemental Figure 3. Differential expression of PD-L1 on immune cells of FVB/N mice lung tissues. **(A-C)** FACS gating strategy for SSChi and SSCmi neutrophils (PMN), AM (CD11c⁺) and IM (CD11b⁺) macrophages (Mac), CD11b⁻ and CD11b⁺ dendritic cells (DC), iMono (Ly6C^{hi} classical inflammatory) and rMono (Ly6C^{low} non-classical resident) monocytes (Mono), basophils, mast cells (Mast), eosinophils, CD4⁺ T cells (CD4⁺), CD8⁺ T cells (CD8⁺), B cells (B220⁺) and natural killer (NK) cells. **(D, E)** FACS assays showing differential expression of PD-L1 on major myeloid cells (D) and different lymphocytes (E) in mouse lung ($n = 5$). Representative histograms are shown.

Supplemental Table 1. Antibodies Used

Antibody	Clone	Catalog number	Company	Usage	Purpose
Anti-CD45 Alexa Fluor 700	30-F11, Rat IgG2b, κ	103128	Biolegend, San Diego, CA, USA	0.5 µl per sample	FACS
Anti-CD80 BV510	16-10A1, Armenian Hamster IgG	104741	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-PD-L2 PerCP/Cyanine5.5	TY25, Rat IgG2a, κ	107218	Biolegend, San Diego, CA, USA	1.25 µl per sample	FACS
Anti-CD117 BV421	ACK2, Rat IgG2b, κ	135124	Biolegend, San Diego, CA, USA	5.0 µl per sample	FACS
Anti-NKp46 BV421	29A1.4, Rat IgG2a, κ	137612	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-B220 BV510	RA3-6B2, Rat IgG2a, κ	103248	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-Ly6G BV510	1A8, Rat IgG2a, κ	127633	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-Ly6C BV650	HK1.4, Rat IgG2c, κ	128049	Biolegend, San Diego, CA, USA	1.25 µl per sample	FACS
Anti-CD64 PE-Cy7	X54-5/7.1, Mouse IgG1, κ	139314	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-FcεR1α PE-Cy7	MAR-1, Armenian Hamster IgG	134318	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Anti-CD45 FITC	30-F11, Rat IgG2b, κ	103107	Biolegend, San Diego, CA, USA	0.5 µl per sample	FACS
Anti-MerTK Super Bright 436	DS5MMER, Rat IgG2a, κ	62-5751-82	Thermo Fisher Scientific, Waltham,MA, USA	5.0 µl per sample	FACS
Anti-PD-1 PerCP-eFluor710	J43, Armenian hamster IgG	46-9985-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Anti-MHC-II APC	M5/114.15.2, Rat IgG2b, κ	17-5321-82	Thermo Fisher Scientific, Waltham,MA, USA	0.15 µl per sample	FACS
Anti-CD11c APC-eFluor780	N418, Armenian hamster IgG	47-0114-82	Thermo Fisher Scientific, Waltham,MA, USA	2.5 µl per sample	FACS
Anti-CD3e APC-eFluor780	145-2C11, Armenian hamster IgG	47-0031-82	Thermo Fisher Scientific, Waltham,MA, USA	5 µl per sample	FACS
Anti-CD4 APC-eFluor780	RM4-5, Rat IgG2a, κ	47-0042-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Anti-CD11b PE	M1/70, Rat IgG2b, κ	12-0112-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Anti-CD11c PE-Cy7	N418, Armenian hamster IgG	25-0114-82	Thermo Fisher Scientific, Waltham,MA, USA	2.5 µl per sample	FACS
Anti-CD16/CD32	93, Rat IgG2a, λ	14-0161-85	Thermo Fisher Scientific, Waltham,MA, USA	1.0 µl per sample	FACS
Anti-CD3 PE	145-2C11, Armenian hamster IgG	12-0031-83	Thermo Fisher Scientific, Waltham,MA, USA	2.5 µl per sample	FACS
Anti-CD4 PE-Cy7	RM4-5, Rat IgG2a, κ	25-0042-81	Thermo Fisher Scientific, Waltham,MA, USA	1.25 µl per sample	FACS
Anti-CD8 APC	53-6.7, Rat IgG2a, κ	17-0081-83	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Anti-F4/80 APC	BM8, Rat IgG2a, κ	17-4801-82	Thermo Fisher Scientific, Waltham,MA, USA	2.0 µl per sample	FACS
Anti-GranzB FITC	NGZB, Rat IgG2a, κ	11-8898-80	Thermo Fisher Scientific, Waltham,MA, USA	0.25 µl per sample	FACS
Anti-IFNγ FITC	XMG1.2, Rat IgG1, κ	11-7311-81	Thermo Fisher Scientific, Waltham,MA, USA	1.0 µl per sample	FACS
Anti-PD-1 eFluor450	J43, Armenian hamster IgG	48-9985-82	Thermo Fisher Scientific, Waltham,MA, USA	2.5 µl per sample	FACS
Anti-PD-L1 PE-Cy7	MIH5, Rat IgG2a, λ	25-5982-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Anti-PD-L1 Super Bright 780	MIH5, Rat IgG2a, λ	78-5982-82	Thermo Fisher Scientific, Waltham,MA, USA	0.3 µl per sample	FACS
Anti-Siglec-F PerCP-eFluor710	1RNM44N, Rat IgG2a, κ	46-1702-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Rat IgG2a kappa Isotype Control, PE-Cyanine7	eBR2a	25-4321-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Rat IgG2a kappa Isotype Control, Super Bright 780	eBR2a	78-4321-82	Thermo Fisher Scientific, Waltham,MA, USA	0.3 µl per sample	FACS
Armenian Hamster IgG Isotype Control, PerCP-eFluor 710	eBio299Arm	46-4888-82	Thermo Fisher Scientific, Waltham,MA, USA	0.625 µl per sample	FACS
Armenian Hamster IgG Isotype Control, eFluor 450	eBio299Arm	48-4888-82	Thermo Fisher Scientific, Waltham,MA, USA	2.5 µl per sample	FACS
Armenian Hamster IgG Isotype Control, BV510	HTK888	400942	Biolegend, San Diego, CA, USA	2.5 µl per sample	FACS
Rat IgG2a, κ Isotype Control, PerCP/Cyanine5.5	RTK2758	400531	Biolegend, San Diego, CA, USA	1.25 µl per sample	FACS
Latex Beads-Rabbit IgG-FITC Complex		500290	Cayman Chemical, Ann Arbor, MI, USA	1:100	FACS
pHrodo Green <i>S. aureus</i> Bioparticles Conjugate		P35367	Thermo Fisher Scientific, Waltham,MA, USA	1 mg/ml	FACS
Zymosan A (<i>S. cerevisiae</i>) BioParticles AF488 conjugate		Z23373	Thermo Fisher Scientific, Waltham,MA, USA	0.25 mg/ml	FACS
Anti-PD-1	29F.1A12, Rat IgG2a, κ	BE0273	BioXcell, West Lebanon, NH, USA	20 µg/ml	Bloackade
Anti-PD-L1	10F.9G2, Rat IgG2b, κ	BE0101	BioXcell, West Lebanon, NH, USA	20 µg/ml	Bloackade
Anti-CD80	Rabbit polyclonal	ab254579	Abcam, Cambridge, UK	20 µg/ml	IF
Anti-PD-L1	10F.9G2, Rat IgG2b, κ	BE0101	BioXcell, West Lebanon, NH, USA	20 µg/ml	IF
Anti-PD-1	D7D5W, Rabbit IgG	84651	Cell Signaling Technology, Danvers, MA, USA	1:100	IF
Donkey anti-Rat FITC		DKXRT-003-DFITC	ImmunoReagents Inc., Raleigh, NC, USA	1:200	IF
Donkey anti-Rabbit IgG-R		sc-2095	Santa Cruz Biotechnology, Dallas, TX,USA	1:200	IF
Donkey anti-Rat AF488		A21208	Thermo Fisher Scientific, Waltham,MA, USA	1:400	IF
Donkey anti-Rabbit AF568		A10042	Thermo Fisher Scientific, Waltham,MA, USA	1:400	IF