

Supplementary Table 1. Antibodies

anti-human TCR V β 13.1 APC	BioLegend	Cat#362407; RRID: AB_2728348
anti-human CD8 α PE	BioLegend	Cat#300907; RRID: AB_314111
anti-human PD-1 PE	BioLegend	Cat#329905; RRID: AB_940481
anti-human PD-1 Alexa Fluor 488	BioLegend	Cat#367407; RRID: AB_2566677
Rabbit monoclonal anti-human SHP2 Alexa Fluor 647	Abcam	Cat#ab310059; Clone: Y478
anti-Myc PE	Santa Cruz Biotechnology	Cat#sc-40 PE; RRID: AB_627268
anti-Flag PE	Biolegend	Cat#637309; RRID: AB_2563147
Rabbit monoclonal anti-human SHP2	Santa Cruz Biotechnology	Cat#sc-280; RRID: 632401
Goat anti-Rabbit IgG IRDye 800CW	LI-COR	Cat#926-32211; RRID: AB_621843
anti-human PD-1 Alexa Fluor 647	BioLegend	Cat#367420; RRID: AB_2721354
anti-human CD69 Alexa Fluor 647	BioLegend	Cat#310906; RRID: AB_314841
Rabbit monoclonal anti-human phosphorylated CD28 (Tyr191)	Cell Signaling Technology	Cat#16399T; Clone: E5B9Z
F(ab)-Goat anti-rabbit IgG Fc Alexa Fluor 647	Thermo Fisher Scientific	Cat#A66788
anti-human CD3 ϵ	BioXCell	Cat#BE0231; RRID: AB_2687713; Clone: UCHT-1

Supplementary Table 2. Bacterial and viral strains

DH5 alpha chemocompetent cells	Vazyme	Cat#C502-02
BL21 (DE3) chemocompetent cells	Vazyme	Cat#C504-02
Lentivirus expressing human 1G4 TCR $\alpha\beta$	This study	N/A
Lentivirus expressing human CD8 $\alpha\beta$	This study	N/A
Lentivirus expressing human PD-1 WT	This study	N/A
Lentivirus expressing human PD-1 Y223F	This study	N/A
Lentivirus expressing human PD-1 Y248F	This study	N/A
Lentivirus expressing human PD-1 Y223F Y248F	This study	N/A
Lentivirus expressing human SHP2 t-SH2–GFP	This study	N/A
Lentivirus expressing human SHP2 C459S–GFP	This study	N/A

Supplementary Table 3. Chemicals, motifs, and recombinant proteins

Chemical: SHP099	MedChemExpress	Cat#HY-100388
Chemical: Dimethylsulfoxide (DMSO)	Thermo Fisher Scientific	Cat#20688
Chemical: 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP)	Thermo Fisher Scientific	Cat#D6567
Chemical: (3-Aminopropyl)triethoxysilane (APTES)	Sigma–Aldrich	Cat#440140
Chemical: Glutaraldehyde solution	Sigma–Aldrich	Cat#354400
Chemical: Alexa Fluor 405 dye	Thermo Fisher Scientific	Cat#A30000
Chemical: DOPC lipid	Avanti Polar Lipids	Cat#850375
Chemical: Biotin-PE lipids	Avanti Polar Lipids	Cat#860511
Chemical: Ni–NTA lipid	Avanti Polar Lipids	Cat#790404
Chemical: biotin–PEG3500–NHS	JenKem Technology	Cat#A5026
Chemical: RPMI 1640 medium	Gibco,	Cat#31870074
Chemical: DMEM medium	ThermoFisher	Cat#11965092
Chemical: Opti-MEM medium	Gibco	Cat#11058021
Chemical: Streptavidin-coated magnetic bead	Thermo Fisher Scientific	Cat#65305
Chemical: polystyrene bead	Polyscience	Cat#17145-5
Chemical: Epoxy-labelled beads powder	Thermo Fisher Scientific	Cat#14301
Chemical: Quantum R-PE MESF bead	Bangs Labs	Cat#827A
Chemical: CRISPR–Cas9 enhancer	Integrated DNA Technology	Cat#1075916
Peptide: pITIM–pITSM	QYAOBIO	N/A
Peptide: pITIM–ITSM	QYAOBIO	N/A
Peptide: ITIM–pITSM	QYAOBIO	N/A
Peptide: ITIM–ITSM	QYAOBIO	N/A
Peptide: pITIM	QYAOBIO	N/A
Peptide: pITSM	QYAOBIO	N/A
Peptide: pCD3ε	GL Biochem	N/A
Peptide: pCD28	GL Biochem	N/A
Protein: Avi–10×His–SHP2–Flag–Spy	This study, for MT experiment	N/A
Protein: Avi–10×His–SHP2 E76A–Flag–Spy	This study, for MT experiment	N/A
Protein: Avi–10×His–N-SH2 C-SH2–Flag–Spy	This study, for MT experiment	N/A
Protein: Avi–10×His–PTP–Flag–Spy	This study, for MT experiment	N/A
Protein: 10×His–spy-catcher	This study, for MT experiment	N/A
Protein: Avi–Myc–PD-1	This study, for micropipette adhesion experiment	N/A
Protein: Avi–Myc–PD-1 Y223F	This study, for micropipette adhesion experiment	N/A

Protein: Avi-Myc-PD-1 Y248F	This study, for micropipette adhesion experiment	N/A
Protein: Avi-Myc-PD-1 Y223F Y248F	This study, for micropipette adhesion experiment	N/A
Protein: SHP2-Flag-Spy	This study, for micropipette adhesion experiment	N/A
Protein: t-SH2-Flag-Spy	This study, for micropipette adhesion experiment	N/A
Protein: Lck	This study, for in vitro PD-1 phosphorylation experiment	N/A
Protein: Lck kinase domain	This study, for in vitro PD-1 phosphorylation experiment	N/A
Protein: 10×His-PD-1	This study, for LLPS experiment	N/A
Protein: 10×His-PD-1 Y223F	This study, for LLPS experiment	N/A
Protein: 10×His-PD-1 Y248F	This study, for LLPS experiment	N/A
Protein: 10×His-PD-1 Y223F Y248F	This study, for LLPS experiment	N/A
Protein: SHP2	This study, for enzymatic activity, and LLPS experiments	N/A
Protein: SHP2 C459S	This study, for crystal structure, ITC, MST, enzymatic activity, and LLPS experiments	N/A
Protein: N-SH2	This study, for ITC and MST experiments	N/A
Protein: C-SH2	This study, for ITC and MST experiments	N/A
Protein: t-SH2	This study, for ITC, MST, and LLPS experiments	N/A
Protein: 10×His-UD-SH3-SH2	This study, for LLPS experiment	N/A
Protein: 9C-HLA-A2-10×His-Avi	Wu et al., 2019, for SLB experiment	https://www.cell.com/molecular-cell/fulltext/S1097-2765(18)31072-4
Protein: ICAM-1 ECD-Avi-12×His	This study, for SLB experiment	N/A
Protein: CD86 ECD-Avi-12×His	This study, for SLB experiment	N/A
Protein: PD-L1 ECD-Avi-12×His	This study, for SLB experiment	N/A
Protein: UCHT-1 Fab-12×His AF568	Fei et al., 2026, for SLB experiment	https://www.biorxiv.org/content/10.64898/2026.03.28.715001v1
Protein: PD-L1 ECD-12×His AF405	Fei et al., 2026, for SLB experiment	https://www.biorxiv.org/content/10.64898/2026.03.28.715001v1
Protein: Cas9	Thermo Fisher Scientific	Cat#A36496

Supplementary Table 4. Critical commercial assays

ClonExpress II One Step Cloning Kit	Vazyme	Cat#C113
Gel DNA Extraction Mini Kit	Axygen	Cat#AP-MN-P-250
Plasmid Extraction Mini Kit	Axygen	Cat#AP-GX-250
JCSG Core I-IV	QIAGEN	Cat#130920
Crystal Screen	Hampton Research	Cat#HR2-110
Index Crystallization Screen	Hampton Research	Cat#HR2-144
Quantum™ MESF beads	Bangs Labs	Cat#827A

Supplementary Table 5. Cell lines

HEK293T cells	American Type Culture Collection (ATCC)	Cat#CRL-3216
Jurkat E6-1 cells	American Type Culture Collection (ATCC)	Cat#TIB-152
Jurkat 76 cells	Kindly provided by Dr. Jie Sun's lab at Zhejiang University	N/A
SF9 cells	National Biomedical Cell Resource (BMCR)	Cat#1101INS-PUMC000117
1G4 TCR, CD8, PD-1-expressing J76 cells	This study	N/A
1G4 TCR, CD8, PD-1 Y223F-expressing J76 cells	This study	N/A
1G4 TCR, CD8, PD-1 Y248F-expressing J76 cells	This study	N/A
1G4 TCR, CD8, PD-1 Y223F Y248F-expressing J76 cells	This study	N/A
SHP2 KO Jurkat cells	This study	N/A
SHP2 KO, SHP2 t-SH2-expressing Jurkat cells	This study	N/A
SHP2 KO, SHP2 C459S-expressing Jurkat cells	This study	N/A

Supplementary Table 6. Oligonucleotides

5' GCGCACTGGTGATGACAAAG 3'	Integrated DNA Technology	SHP2 KO CRISPR–Cas9 sgRNA
5' CATTGGGATCACCATCGTGT 3'	Integrated DNA Technology	SHP2 KO CRISPR–Cas9 sgRNA

Supplementary Table 7. Recombinant DNA

pGEX-6p-1 Avi-10×His-SHP2-Flag-Spy	This study, for MT experiment	N/A
pGEX-6p-1 Avi-10×His-SHP2 E76A-Flag-Spy	This study, for MT experiment	N/A
pGEX-6p-1 Avi-10×His-N-SH2 C-SH2-Flag-Spy	This study, for MT experiment	N/A
pGEX-6p-1 Avi-10×His-PTP-Flag-Spy	This study, for MT experiment	N/A
pGEX-6p-1 10×His-spy-catcher	This study, for MT experiment	N/A
pET28a CD86-10×His-Avi	This study, for TIRF experiment	N/A
pET28a PD-L1-10×His-Avi	This study, for TIRF experiment	N/A
pGEX-6p-1 GST-TEV-Avi-Myc-PD-1	This study, for micropipette adhesion experiment	N/A
pGEX-6p-1 GST-TEV-Avi-Myc-PD-1 Y223F	This study, for micropipette adhesion experiment	N/A
pGEX-6p-1 GST-TEV-Avi-Myc-PD-1 Y248F	This study, for micropipette adhesion experiment	N/A
pGEX-6p-1 GST-TEV-Avi-Myc-PD-1 Y223F Y248F	This study, for micropipette adhesion experiment	N/A
pGEX-6p-1 SHP2-GST-TEV-Flag-Spy	This study, for micropipette adhesion experiment	N/A
pGEX-6p-1 N-SH2 C-SH2-GST-TEV-Flag-Spy	This study, for micropipette adhesion experiment	N/A
pFastBac 1 6×His-MBP-3C-Lck	This study, for in vitro PD-1 phosphorylation experiment	N/A
pFastBac 1 6×His-MBP-3C-Lck kinase domain	This study, for in vitro PD-1 phosphorylation experiment	N/A
pET28a 10×His-PD-1	This study, for LLPS experiment	N/A
pET28a 10×His-PD-1 Y223F	This study, for LLPS experiment	N/A
pET28a 10×His-PD-1 Y248F	This study, for LLPS experiment	N/A
pET28a 10×His-PD-1 Y223F Y248F	This study, for LLPS experiment	N/A
KS 6×His-SUMO-C-SH2	This study, for ITC and MST experiments	N/A
KS 6×His-SUMO-N-SH2 C-SH2	This study, for ITC, MST, and LLPS experiments	N/A
pET28a 10×His-Lck UD-SH3-SH2	This study, for LLPS experiment	N/A
pMD2.G	Addgene	Cat#12259
psPAX2	Addgene	Cat#12260

pHAGE CD8αβ	This study, for lentivirus infection	N/A
pHAGE 1G4 TCRαβ	This study, for lentivirus infection	N/A
pHAGE PD-1	This study, for lentivirus infection	N/A
pHAGE PD-1 Y223F	This study, for lentivirus infection	N/A
pHAGE PD-1 Y248R	This study, for lentivirus infection	N/A
pHAGE PD-1 Y223F Y248F	This study, for lentivirus infection	N/A
pHAGE t-SH2–GFP	This study, for lentivirus infection	N/A
pHAGE C459S–GFP	This study, for lentivirus infection	N/A

Supplementary Table 8. Softwares

ImageJ	Schneider et al. 2012	https://fiji.sc
Prism 10	GraphPad Software	https://www.graphpad.com
Labview 2020	National Instruments (NI)	https://www.ni.com/labview
Adobe Illustrator 2025	Adobe Inc.	https://www.adobe.com/products/illustrator.html
PyMOL	The PyMOL Molecular Graphics System	https://www.pymol.org
Chimera	UCSF	https://www.cgl.ucsf.edu/chimera/
NanoTemper Analysis	NanoTemper Technologies	https://nanotempertech.com/
XDS	Kabsch	https://xds.mr.mpg.de/
Pointless	CCP4	https://www.ccp4.ac.uk/html/pointless.html
Aimless	CCP4	https://www.ccp4.ac.uk/html/aimless.html
Phaser	CCP4	https://phaser.io/
Coot	Emsley et al., 2004	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
PHENIX	Adams et al., 2010	https://www.phenix-online.org/
Hidden Markov Model analysis	Kindly provided by Dr. Jie Yan's Lab at University of Singapore	N/A
QI algorithm (modified)	van Loenhout et al., 2012	https://www.sciencedirect.com/science/article/pii/S006349512004535
Origin 7.0	OriginLab Corporation	https://www.originlab.com/index.aspx?go=PRODUCTS/Origin