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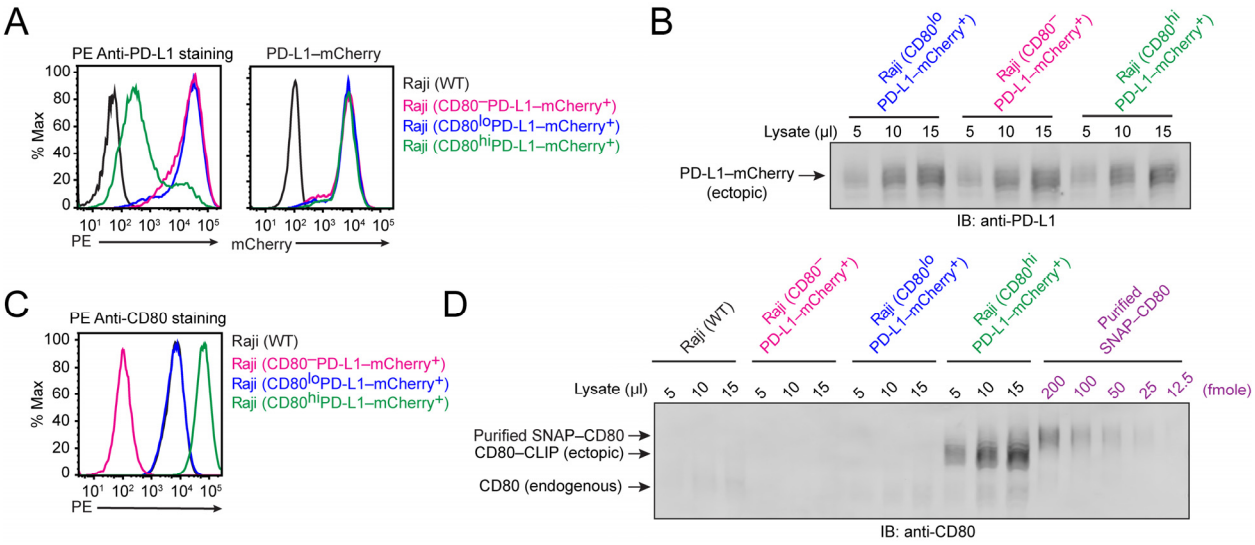
Supplemental Information

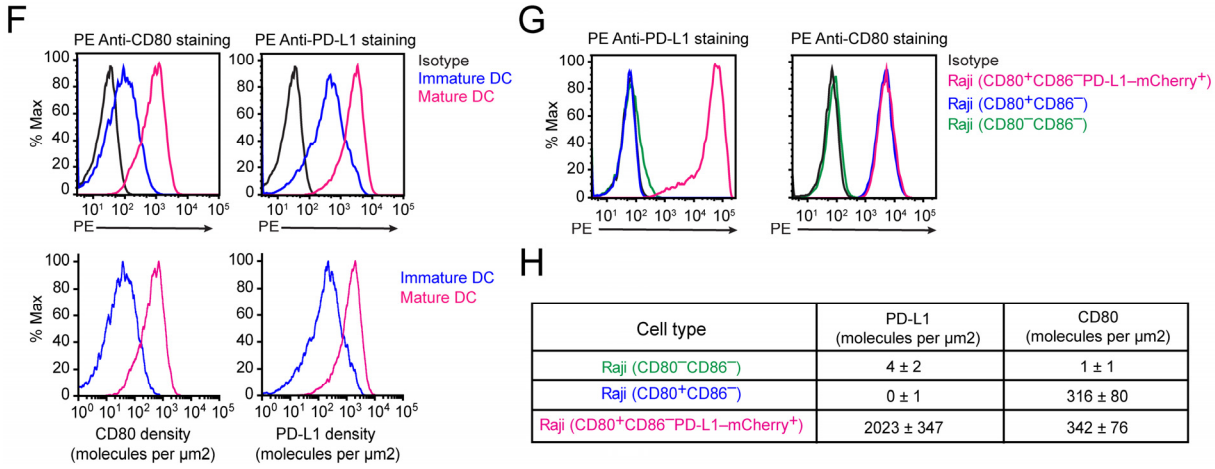
PD-L1:CD80 *Cis*-Heterodimer Triggers the Co-stimulatory Receptor CD28 While Repressing the Inhibitory PD-1 and CTLA-4 Pathways

Yunlong Zhao, Calvin K. Lee, Chia-Hao Lin, Rodrigo B. Gassen, Xiaozheng Xu, Zhe Huang, Changchun Xiao, Cristina Bonorino, Li-Fan Lu, Jack D. Bui, and Enfu Hui



E

Cell type	PD-L1 (molecules per µm2)	CD80 (molecules per µm2)
Raji (WT)	1 ± 1	601 ± 149
Raji (CD80⁻PD-L1–mCherry⁺)	1734 ± 569	6 ± 2
Raji (CD80loPD-L1–mCherry⁺)		587 ± 143
Raji (CD80hiPD-L1–mCherry⁺)		6018 ± 149



H

Cell type	PD-L1 (molecules per µm2)	CD80 (molecules per µm2)
Raji (CD80⁻CD86⁻)	4 ± 2	1 ± 1
Raji (CD80⁺CD86⁻)	0 ± 1	316 ± 80
Raji (CD80⁺CD86⁻PD-L1–mCherry⁺)	2023 ± 347	342 ± 76

Figure S1. Quantification of PD-L1 and CD80 Levels on Raji Cell Lines and Human DCs. Related to Figures 2, 3 & 4.

(A) Shown on the left are flow cytometry histograms of PE signals of the indicated cell lines (used in Figure 2) stained with PE anti-PD-L1 (eBioscience, 14-5983-82). Shown on the right are flow cytometry histograms of mCherry signals for the same set of cell lines. The overlaid histograms of 3 types of PD-L1–mCherry$^+$ cells indicate that they expressed nearly identical levels of PD-L1–mCherry.

(B) Anti-PD-L1 (eBioscience, 14-5983-82) immunoblot (IB) of the lysates of the indicated cell lines (used in Figure 2). The similar intensities of bands of 3 types of PD-L1–mCherry$^+$ cells confirmed the mCherry flow cytometry result in panel A that 3 types of cell lines expressed similar levels of PD-L1–mCherry. Please note that PD-L1:CD80 *cis*-complexes were disrupted by SDS sample buffer at 95 °C prior to the SDS-PAGE.

(C) Flow cytometry histograms of PE signals of the indicated cell lines (used in Figure 2) stained with PE anti-CD80 (Biolegend, 305208), showing distinct CD80 expression levels.

(D) Anti-CD80 (Novus Biologicals, NBP2-25255) IB of the lysates of indicated cell lines (used in Figure 2), together with decreasing amounts of purified SNAP–CD80, from which a standard curve can be generated to calculate the CD80 levels in each cell line.

(E) Table summarizing the PD-L1 and CD80 expression levels for the indicated cell lines (used in Figure 2). Number of PD-L1 molecules per cell were determined based on the PE anti-PD-L1 staining signal of Raji ($CD80^{-}$PD-L1–mCherry+) cells, using the QUANTUM™ R-PE MESF kit, and the PD-L1 density (molecules per μm^2) further calculated as described in STAR Methods. The lack of CD80 expression ensured that PE anti-PD-L1 bound to PD-L1 with no interference from *cis*-CD80. This PD-L1 density, determined using the $CD80^{-}$ cells, was also assigned to Raji ($CD80^{lo}$PD-L1–mCherry+) cells and Raji ($CD80^{hi}$PD-L1–mCherry+) cells, because 3 types of PD-L1–mCherry+ cells expressed similar levels of PD-L1–mCherry, based on data in panels A and B. Data are presented as mean ± SD, n = 3.

(F) Upper: Flow cytometry histograms of PE signals on immature or mature human DCs stained with either PE anti-CD80 (Biolegend, 305208) or PE anti-PD-L1 (eBioscience, 14-5983-82). The black histograms correspond to signals of isotype control under each condition. Lower: Histograms of CD80 and PD-L1 surface densities on immature and mature DCs calculated from the flow cytometry histograms, using the QUANTUM™ R-PE MESF kit, as described in STAR Methods.

(G) Flow cytometry histograms of PE signals of the indicated Raji cells (used in Figures 3 and 4) stained with PE anti-PD-L1 (Left, eBioscience, 14-5983-82) and PE anti-CD80 (Right, Biolegend, 305208).

(H) Table summarizing the PD-L1 and CD80 expression levels for the indicated cell lines (used in Figures 3 and 4). PD-L1 and CD80 levels were calculated based on PE signals in (**A**) and (**C**) using the QUANTUM™ R-PE MESF kit (Bangs Laboratories Inc, 827). Data are presented as mean ± SD, n = 3.

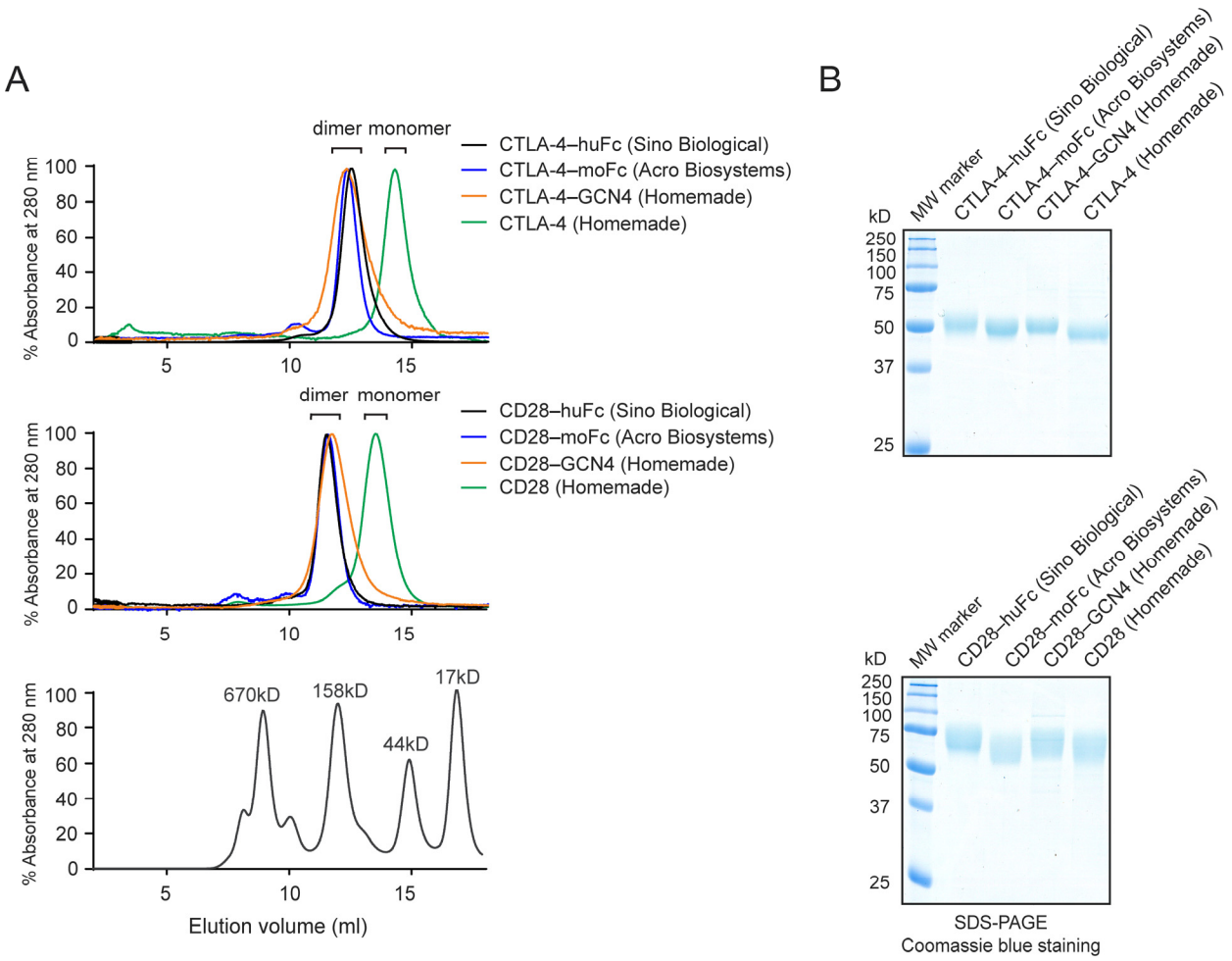


Figure S2 Dimerization States of Soluble Human CTLA-4 and CD28 Proteins Used in This Study. Related to Figures 3 & 4.

(A) Representative size exclusion chromatograms of indicated CTLA-4 proteins (top), CD28 proteins (middle) and protein standards with their molecular weights (MW) indicated in kilo Delton (kD) (bottom). All chromatograms were obtained using a Superdex 200 increase 10/300 column in an AKTA Pure 25L system. CTLA-4–huFc and CD28–huFc proteins obtained from Sino Biological were pre-cleaned by gel filtration chromatography to remove aggregates.

(B) Representative Coomassie blue stained SDS-PAGE of the indicated proteins.

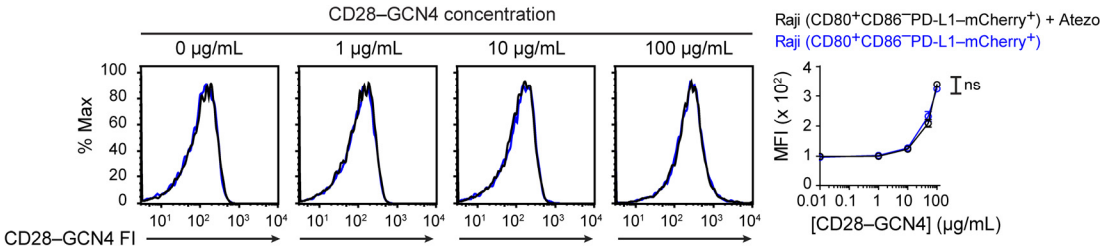


Figure S3 Atezolizumab Does Not Affect CD28–GCN4 Staining of CD80 and PD-L1 Double Positive Cells. Related to Figure 4.

Shown on the left are flow cytometry histograms of CD28–GCN4*SC647 staining of Raji ($CD80^+CD86^-$ PD-L1–$mCherry^+$) cells with or without atezolizumab (Atezo). Shown on the right is the MFI of SC647 plotted against the input concentration at the indicated conditions (means ± SEM, n = 4). Unpaired two-tailed *Student's* t test: ns, $p > 0.05$.

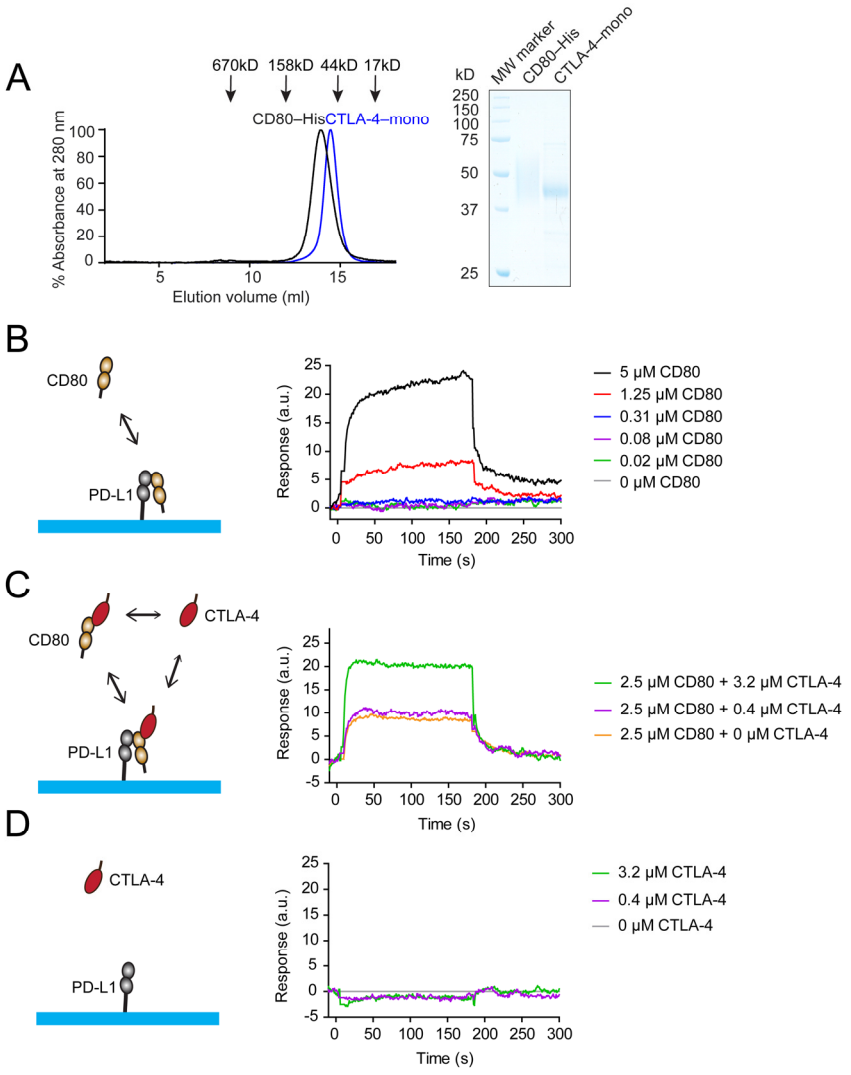


Figure S4 PD-L1 and CTLA-4 Can Simultaneously Bind to Monomeric CD80. Related to Figure 4.

(A) Gel filtration and SDS-PAGE characterization of soluble CTLA-4 monomer and CD80–His monomer used in the SPR assay. Left: gel filtration profiles of CTLA-4 monomer and CD80–His monomer obtained using a Superdex 200 increase 10/300 column. Arrows indicate elution volumes of protein standards. Right: Coomassie blue stained SDS-PAGE of CD80–His monomer and CTLA-4 monomer.

(B) An SPR assay for the interaction between CD80–His monomer in solution and PD-L1 attached to a chip. Shown on the right are the corresponding sensorgrams for the indicated CD80–His concentrations.

(**C**) An SPR assay for measuring the binding of CD80–His monomer and CTLA-4 monomer to a PD-L1 coated chip. Shown on the right are sensorgrams for the indicated CD80:CTLA-4 combinations.

(**D**) An SPR assay for the potential binding of CTLA-4 monomer to a PD-L1 coated chip. Shown on the right are sensorgrams at the indicated CTLA-4 concentrations.

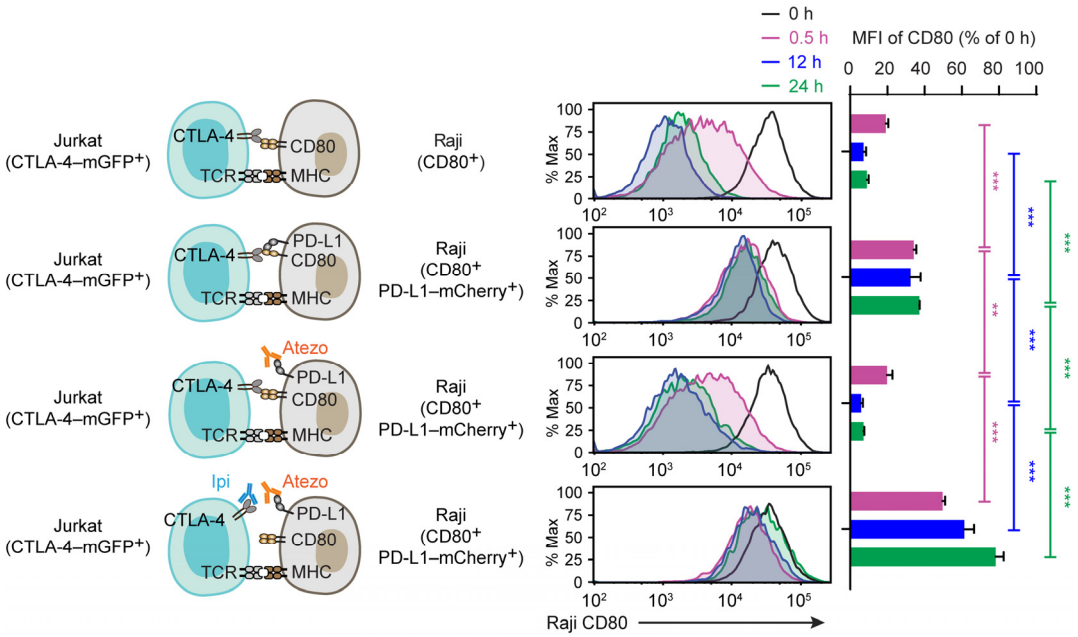


Figure S5 *Cis*-PD-L1 Persistently Protects CD80 from CTLA-4 Mediated Depletion. Related to Figure 5.

Shown on the left are cartoons depicting 4 types of co-cultured systems: (1st row) Jurkat (CTLA-4–mGFP+) cells co-clutured with WT Raji (CD80+) cells expressing CD80 but not PD-L1; (2nd row): Jurkat (CTLA-4–mGFP+) cells co-clutured with Raji cells co-expressing CD80 and PD-L1–mCherry; (3rd row): Jurkat (CTLA-4–mGFP+) cells co-clutured with Raji cells co-expressing CD80 and PD-L1–mCherry but with the PD-L1 blocked by atezolizumab (Atezo); (4th row): Jurkat (CTLA-4–mGFP+) cells co-cultured with Raji cells co-expressing CD80 and PD-L1–mCherry but with PD-L1 blocked by atezolizumab (Atezo), and CTLA-4 blocked by ipilimumab (Ipi). Shown in the middle are flow cytometry histograms of CD80 surface levels (probed by allophycocyanin anti-CD80) on Raji cells after indicated durations of co-culturing. Shown on the right are CD80 MFI on Raji cells at the indicated time points, normalized to the CD80 MFI at time zero. Data are presented as mean ± SEM from 5 independent replicates.

Unpaired two-tailed *Student's* t test: ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001. See Table S3 for genotypes of cells related to this figure.

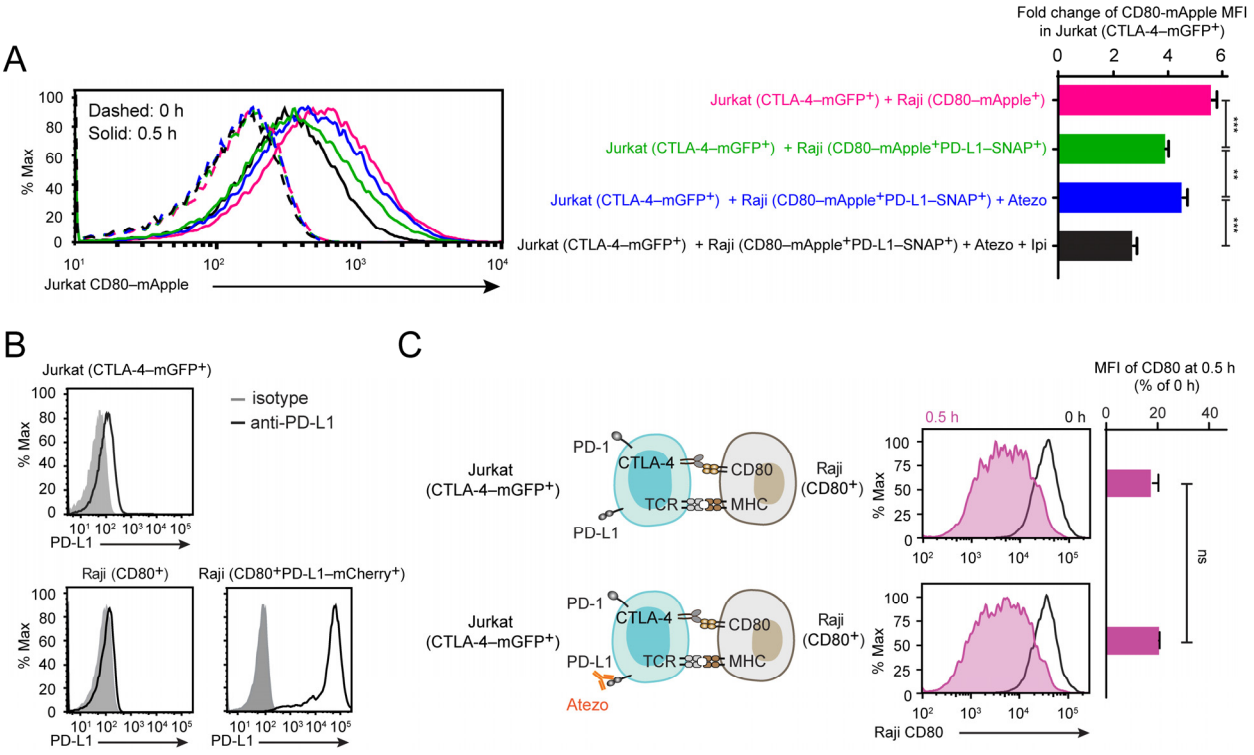


Figure S6. PD-L1 Inhibits the Ability of CTLA-4⁺ Jurkat Cells to Acquire CD80 from Raji APCs. Atezolizumab Mediated CD80 Depletion Does Not Depend on Jurkat-Intrinsic PD-L1. Related to Figure 5.

(A) Shown on the left are raw flow cytometry histograms showing the CD80–mApple fluorescence in Jurkat (CTLA-4–mGFP⁺) cells. *Trans*-endocytosis assay was done as in Figure 5B except measuring CD80–mApple signal in Jurkat cells rather than Raji cells. Shown on the right is a quantification bar graph, with identical color coding to the histograms on the left. CD80–mApple MFI in Jurkat (CTLA-4–mGFP⁺) cells at 0.5 h was divided by the MFI at 0 h to calculate the fold change. Data are shown as mean ± SEM from 4 independent replicates.

(B) Flow cytometry histograms showing low levels of PD-L1 expression on Jurkat (CTLA-4–mGFP⁺), WT Raji (CD80⁺), and Raji (CD80⁺PD-L1–mCherry⁺) cells.

(C) A *trans*-endocytosis assay using WT Raji (CD80⁺) cells lacking PD-L1 and Jurkat (CTLA-4–mGFP⁺) cells. Because the lack of PD-L1 on WT Raji (CD80⁺) cells, atezolizumab treatment would block the PD-L1 on Jurkat cells. Shown on the right is a flow cytometry histogram showing CD80 expression level on the Raji cells, +/-atezolizumab (Atezo), at indicated time points. Bar graph showing CD80 MFI on Raji at 0.5 h, normalized to CD80 MFI at 0 h (mean ± SEM, n = 5).

Unpaired two-tailed *Student's* t test: ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p < 0.001. See Table S3 for genotypes of cells related to this figure.

Table S1 List of oligos. Related to STAR Methods.

Name	Sequence	Note
EH-98	cctttccatgggtcttttctgcag atggacgctatgaagagag	clone signal peptide of HIV envelope glycoprotein into pPPI4, pair with EH-99
EH-99	ggtggctccatcggaatctagcatggat	clone signal peptide of HIV envelope glycoprotein into pPPI4, pair with EH-98
EH-100	tagattccgatggagccacccgcagttc	clone Strep-SNAP tag into pPPI4, pair with EH-101
EH-101	gtgatggaattcagattggaatcggacagtctcgctaccgctaccgctacctc	clone Strep-SNAP tag into pPPI4, pair with EH-100
EH-102	ttccaatctgaattccatcaccatcaccatcaccatcaccatcactgagcggc	clone His_{10} tag into pPPI4, pair with EH-103
EH-103	agctctagatgcatgctcgagcggccgctcagtgatggtgatg	clone His_{10} tag into pPPI4, pair with EH-102
EH-104	gactgtccgattccaatctggcgttatccacgtgaccaag	clone CD80 extracellular domain CDS into pPPI4, pair with EH-105
EH-105	gtgatggtgatggtgatggccgttatcaggaaaatgctc	clone CD80 extracellular domain CDS into pPPI4, pair with EH-104
EH-1069	ctctagatgcatgctcgagctcagttatcaggaaaatgctcttg	clone CD80 extracellular domain CDS without His_{10} tag into pPPI4, pair with EH-104
EH-1081	gaaccggacccgctttgatatcactaataacctc	clone CD80 with I92R mutation into pPPI4 or pHR, pair with EH-105 or EH439
EH-1082	tgatatcaaagcgggtccggttcttgtactc	clone CD80 with I92R mutation into pPPI4 or pHR, pair with EH-104 or EH438
EH-112	gactgtccgattccaatctggcttattcacagtgacagtc	clone PD-L2 extracellular domain CDS into pPPI4, pair with EH-113
EH-113	gtgatggtgatggtgatggcctggatgggtcctgggttc	clone PD-L2 extracellular domain CDS into pPPI4, pair with EH-112
EH-489	gactgtccgattccaatctgaattcggtggttctggtggttctatg	clone GCN4–His6 extracellular domain into pPPI4, pair with EH-490
EH-490	tcagtgatggtgatggtgatgtctctcgcccacaagct	clone GCN4–His6 extracellular domain into pPPI4, pair with EH-489
EH-491	ctctagatgcatgctcgagcggccgctcagtgatggtgatggtgatgtc	clone GCN4–His6 extracellular domain into pPPI4, pair with EH-489
EH-493	gactgtccgattccaatctgaaaacaagattttggtgaagcagtc	clone CD28 extracellular domain CDS into pPPI4-GCN4–His_6, pair with EH-494
EH-494	tagaaccaccagaaccaccgaagggcttagaaggtccgggaa	clone CD28 extracellular domain CDS into pPPI4-GCN4–His_6, pair with EH-493
EH-495	gactgtccgattccaatctgaaaaagcaatgcacgtggcccag	clone CTLA-4 extracellular domain CDS into pPPI4-GCN4–His_6, pair with EH-496
EH-496	tagaaccaccagaaccaccgaagtcagaatctgggcacggt	clone CTLA-4 extracellular domain CDS into pPPI4-GCN4–His_6, pair with EH-495
EH-676	tagatgcatgctcgagctcagtgatggtgatggtgatggccgggcttagaaggtccggga	clone CD28–His_6 extracellular domain CDS into pPPI4, pair with EH-493
EH-677	ctagatgcatgctcgagctcagtgatggtgatggtgatggccgtcagaatctggcac	clone CTLA-4–His_6 extracellular domain CDS into pPPI4, pair with EH-495
EH-1431	atttcgcaatctttgtccattcggaatctagcatggatttc	clone signal peptide of HIV envelope glycoprotein fused with SNAP into pPPI4, pair with EH-98
EH-433	atggacaaagattgcgaaatgaaac	clone SNAP–CTLA-4 into pPPI4, pair with EH-103
EH-460	tggagctctcgagaattctcatgatcttcctcctgctaatg	clone PD-L2 signal peptide into pHR, pair with EH-461
EH-461	atttcgcagtctttgtccatagctgctatctggtgaagc	clone PD-L2 signal peptide into pHR, pair with EH-460
EH-428	atggacaaagactgcgaaatg	clone CLIP tag into pHR, pair with EH-429

EH-429	cacctccaccgctaccgctaccgctaccacccagcccaggcttgc	clone CLIP tag into pHR, pair with EH-428
EH-462	tagcggtagcggtggaggtggaagcagcttattcacagtgacagtcc	clone PD-L2 without signal peptide into pHR, pair with EH-463
EH-463	gtcgactctagagtcgcttagatagcactgttcacttcc	clone PD-L2 without signal peptide into pHR, pair with EH-462
EH-398	ggagctctcgagaattctcatgggccacacacggaggc	clone CD80 signal peptide into pHR, pair with EH-437; clone CD80 CDS into pHR-mGFP, pair with EH-688
EH-437	atttcgcaatctttgtccatacctgaacagaagtgagaaag	clone CD80 signal peptide into pHR, pair with EH-398
EH-433	atggacaaagattgcgaaatgaaac	clone SNAP tag into pHR, pair with EH-434
EH-434	cacctccaccgctaccgctaccgctacctcccagacccggtttacc	clone SNAP tag into pHR, pair with EH-433
EH-438	tagcggtagcggtggaggtggaagcagcgttatccacgtgaccaagg	clone CD80 or CD80 (I92R) without signal peptide into pHR, pair with EH-439 or EH-1082
EH-439	caggtcgactctagagtcgcttatacagggcgtacactttcc	clone CD80 or CD80 (I92R) without signal peptide into pHR, pair with EH-438 or EH-1081
EH-410	caccggaaattgaggtatggacact	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD80_1, pair with EH-411
EH-411	aaacagtgtccatacctcaatttc	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD80_1, pair with EH-410
EH-412	caccggcccatggcttcagatgctt	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD80_2, pair with EH-413
EH-413	aaacaagcatctgaagccatgggc	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD80_2, pair with EH-412
EH-563	caccggagtaacattctctttgtga	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD86_1, pair with EH-564
EH-564	aaactcacaaagagaatgttactc	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD86_1, pair with EH-563
EH-565	caccggtgatggccttcctgctctc	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD86_2, pair with EH-566
EH-566	aaacgagagcaggaaggccatcac	synthesis DNA sequence of CD80 sgRNA to generate pX330GFP-CD86_2, pair with EH-565
EH-688	cgcaagcttgatatcctgcagacgtacagggcgtacactttc	clone CD80 into pHR-mGFP, pair with EH-398
EH-747	cttgcggtaccgcgggcccgggatccaatggacaaagactgcgaaatg	clone CLIP to replace mGFP in pHR-CD80–mGFP, pair with EH-748
EH-748	caggtcgactctagagtcgcggccgctttaacccagcccaggcttgc	clone CLIP to replace mGFP in pHR-CD80–mGFP, pair with EH-747
EH-59	ggagctctcgagaattctcacgatgctcaggctgctcttggc	clone CD28 into pHR-mCherry, pair with EH-60
EH-60	caagcttgatatcctgcagacgggagcgataggctgcgaagt	clone CD28 into pHR-mCherry, pair with EH-59
EH-751	ttttttggaggcctaggctgaattctcatgggccacacacggaggc	clone CD80 to replace PD-1 in pHR-dSV40-PD-1–mGFP (Zhao et al., 2018), pair with EH-688
EH-68	ctctcgagaattctcaccatggcttgccttggatttc	clone CTLA-4 fused with mGFP into pHR-dSV40, pair with EH-690
EH-690	tggtggcgaccggtggatcacgattgatgggaataaaataaggc	clone CTLA-4 fused with mGFP into pHR-dSV40, pair with EH-68
EH-749	cgtgatccaccggtcgccaccatggtgagcaagggcgag	clone mGFP fused with CTLA-4 into pHR-dSV40, pair with EH-73
EH-73	aggtcgactctagagtcgcggccgct	clone mGFP fused with CTLA-4 into pHR-dSV40, pair with EH-749

Table S2 List of Recombinant DNAs. Related to STAR Methods.

DNA	SOURCE	IDENTIFIER
pPPI4	Lee et al., 2015	N/A
pPPI4-Strep–SNAP–PD-L1–His_{10}	Zhao et al., 2018	N/A
pPPI4-Strep–SNAP–PD-L2–His_{10}	This study	N/A
pPPI4-Strep–SNAP–CD80–His_{10}	This study	N/A
pPPI4-Strep–SNAP–CD80 (I92R)–His_{10}	This study	N/A
pPPI4-Strep–SNAP–CD80	This study	N/A
pPPI4-Strep–SNAP–CTLA-4–GCN4–His_6	This study	N/A
pPPI4-Strep–SNAP–CTLA-4–His_6	This study	N/A
pPPI4-SNAP–CTLA-4–His_6	This study	N/A
pPPI4-Strep–SNAP–CD28–GCN4–His_6	This study	N/A
pPPI4-Strep–SNAP–CD28–His_6	This study	N/A
pHR-CLIP–PD-L1	Zhao et al., 2018	N/A
pHR-CLIP–PD-L2	This study	N/A
pHR-SNAP–CD80	This study	N/A
pHR-SNAP–CD80 (I92R)	This study	N/A
pHR-SNAP–CD86	Zhao et al., 2018	N/A
pX330GFP-CD80_1	This study	N/A
pX330GFP-CD80_2	This study	N/A
pX330GFP-CD86_1	This study	N/A
pX330GFP-CD86_2	This study	N/A
pMD2.G	Addgene	12259
psPAX2	Addgene	12260
pHR-PD-1–mGFP	Hui et al., 2017	N/A
pHR-PD-L1–mCherry	Hui et al., 2017	N/A
pHR-PD-L1–SNAP	Zhao et al., 2018	N/A
pHR-CD80–CLIP	This study	N/A
pHR-CD80–mGFP	This study	N/A
pHR-dSV40-CD80–mGFP	This study	N/A
pHR-CD28–mCherry	This study	N/A
pHR-dSV40-CTLA-4–mGFP	This study	N/A
pHR-dSV40-CD80–mApple	This study	N/A

Table S3 List of cell lines genotype. Related to STAR Methods.

Cell line	Genotype	Figures related
Jurkat (WT)	*CD28*$^{+/+}$	Figure 5
Jurkat (PD-1–mGFP^{+})	*CD28*$^{+/+}$*PD-1–mGFP*$^{+}$	Figure 2
Jurkat (CD28–mCherry^{+})	*CD28*$^{+/+}$*CD28–mCherry*$^{+}$	Figure 3
Jurkat (CD28^{+})	*CD28*$^{+/+}$	Figure 3
Jurkat (CTLA-4–mGFP^{+})	*CTLA-4–mGFP*$^{+}$	Figures 5, S5, S6
Raji (WT)	*CD80*$^{+/+}$*CD86*$^{+/+}$	Figure S1
Raji (CD80^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$	Figures 5, S5, S6
Raji (CD80^{-}PD-L1–mCherry^{+})	*CD80*$^{-/-}$*CD86*$^{+/+}$*PD-L1–mCherry*$^{+}$	Figures 2, S1
Raji (CD80^{lo}PD-L1–mCherry^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$*PD-L1–mCherry*$^{+}$	Figures 2, S1
Raji (CD80^{hi}PD-L1–mCherry^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$*CD80–CLIP*$^{+}$*PD-L1–mCherry*$^{+}$	Figures 2, S1
Raji (CD80–mGFP^{+}CD86^{+})	*CD80*$^{-/-}$*CD86*$^{+/+}$*CD80–mGFP*$^{+}$	Figure 3
Raji (CD80–mGFP^{+}CD86^{+}CLIP–PD-L1^{+})	*CD80*$^{-/-}$*CD86*$^{+/+}$*CD80–mGFP*$^{+}$*CLIP–PD-L1*$^{+}$	Figure 3
Raji (CD86^{+}CLIP–PD-L1^{+})	*CD80*$^{-/-}$*CD86*$^{+/+}$*CLIP–PD-L1*$^{+}$	Figure 3
Raji (CD80^{-}CD86^{+})	*CD80*$^{-/-}$*CD86*$^{+/+}$	Figure 3
Raji (CD80^{+}CD86^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$	Figure 3
Raji (CD80^{+}CD86^{+}PD-L1–mCherry^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$*PD-L1–mCherry*$^{+}$	Figure 3
Raji (CD80^{-}CD86^{-})	*CD80*$^{-/-}$*CD86*$^{-/-}$	Figures 3, 4, S1
Raji (CD80^{+}CD86^{-})	*CD80*$^{+/+}$*CD86*$^{-/-}$	Figures 3, 4, S1
Raji (CD80^{+}CD86^{-}PD-L1–mCherry^{+})	*CD80*$^{+/+}$*CD86*$^{-/-}$*PD-L1–mCherry*$^{+}$	Figures 3, 4, S1, S3
Raji (CD80–mGFP^{wd}CD86^{-}PD-L1–mCherry^{+})	*CD80*$^{-/-}$*CD86*$^{-/-}$*CD80–mGFP*$^{+}$*PD-L1–mCherry*$^{+}$	Figure 4
Raji (CD80–mGFP^{+}CD86^{-})	*CD80*$^{-/-}$*CD86*$^{-/-}$*CD80–mGFP*$^{+}$	Figure 4
Raji (CD80–mGFP^{+}CD86^{-}PD-L1–SNAP^{+})	*CD80*$^{-/-}$*CD86*$^{-/-}$*CD80–mGFP*$^{+}$*PD-L1–SNAP*$^{+}$	Figure 4
Raji (CD80^{+}PD-L1–mCherry^{+})	*CD80*$^{+/+}$*CD86*$^{+/+}$*PD-L1–mCherry*$^{+}$	Figures 5, S5, S6
Raji (CD80–mApple^{+})	*CD80*$^{-/-}$*CD86*$^{-/-}$*CD80–mApple*$^{+}$	Figures 5, S6
Raji (CD80–mApple^{+}PD-L1–SNAP^{+})	*CD80*$^{-/-}$*CD86*$^{-/-}$*CD80–mApple*$^{+}$*PD-L1–SNAP*$^{+}$	Figures 5, S6