Support Information

Molecular Dynamics Reveals Novel Small-Molecule Inhibitors Block PD-1/PD-L1 by Promoting PD-L1 Dimer Stability

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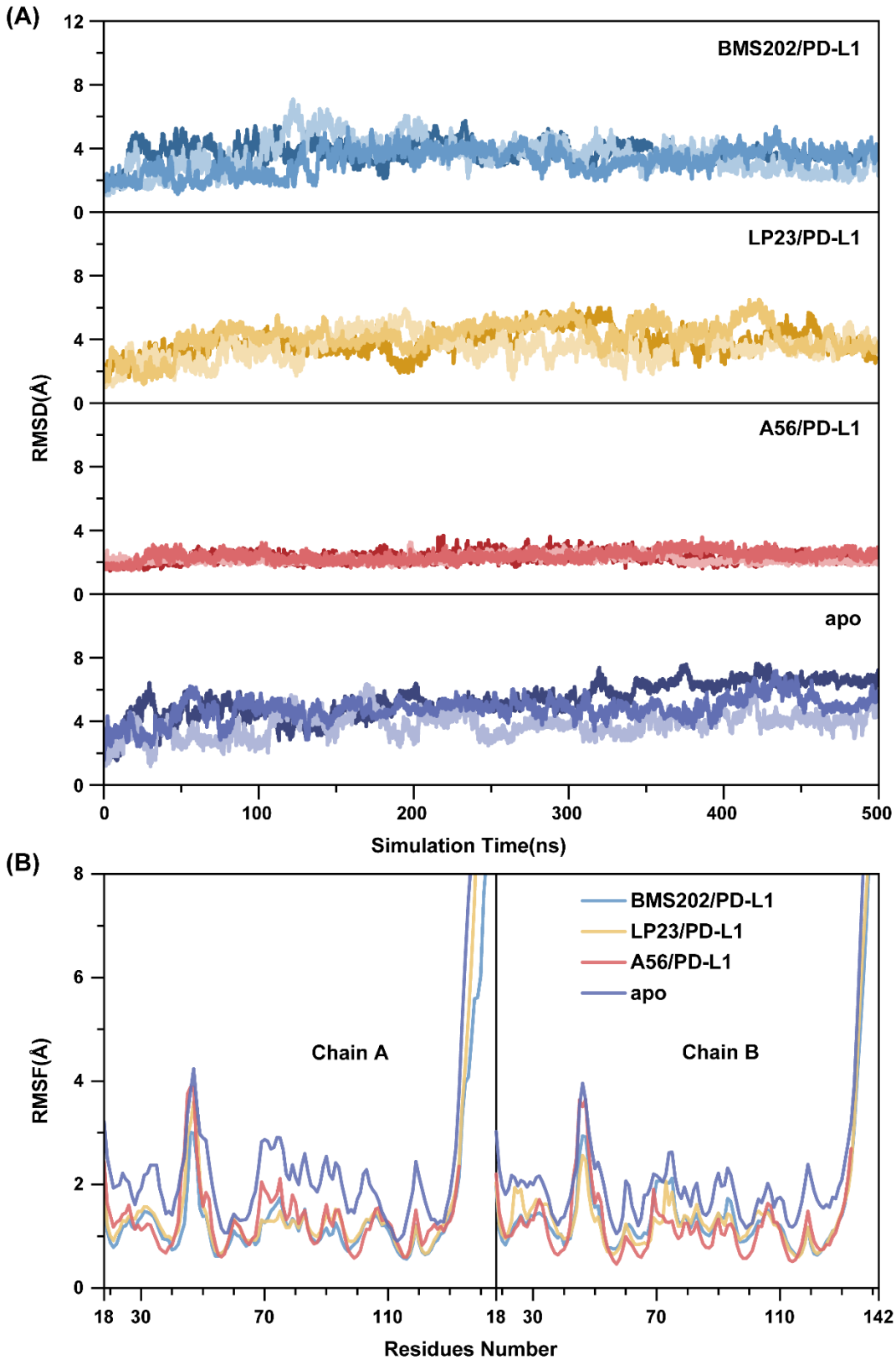

Figure S1. (A) Root mean square deviation (RMSD) (as a function of simulation time) and (B) Root mean square fluctuation (RMSF) of the receptor protein backbone atoms in each system.

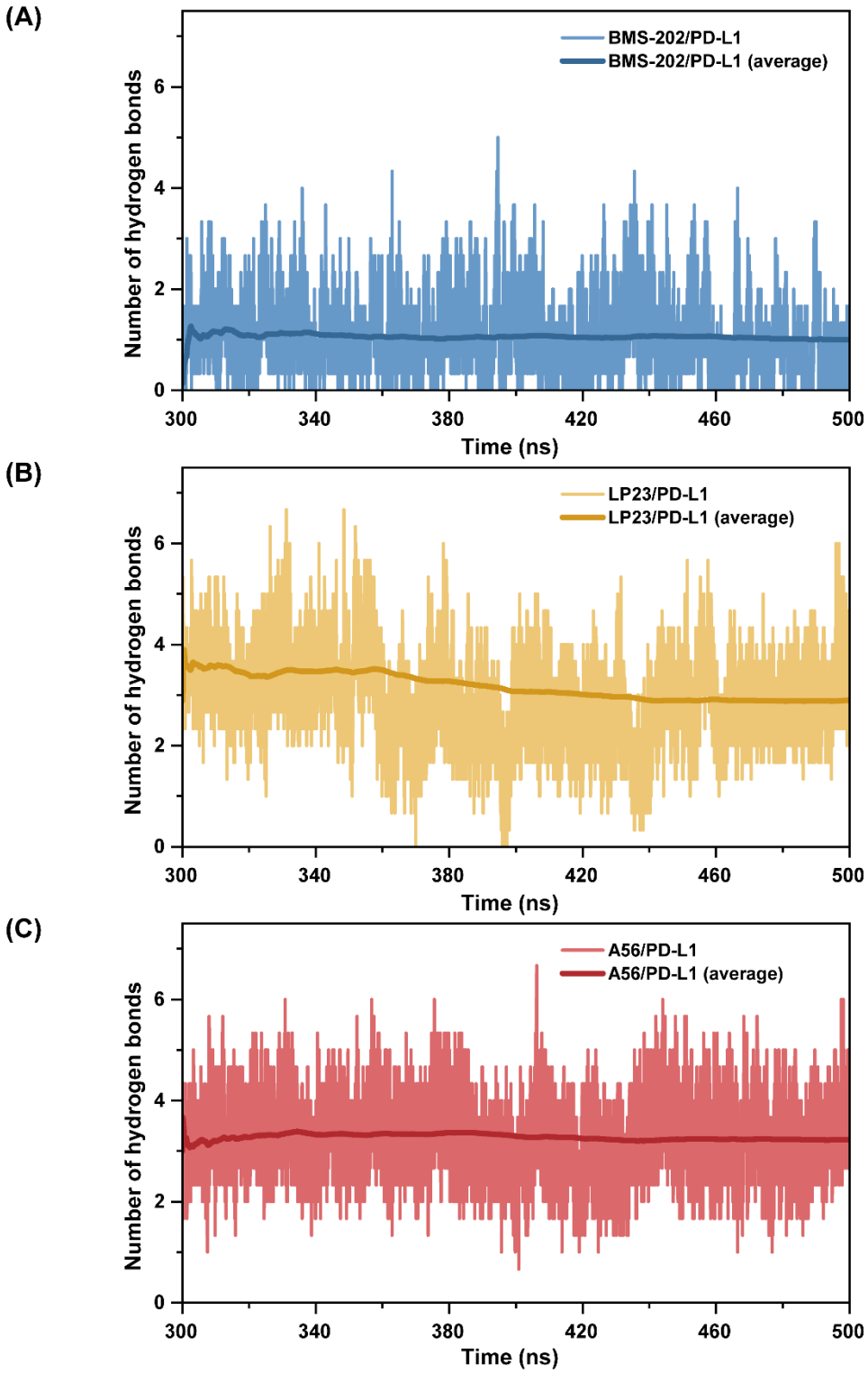

Figure S2. Hydrogen bonding number over time for (A) BMS-202/PD-L1, (B) LP23/PD-L1, and (C) A56/PD-L1 systems during the equilibrium phase of the last 200 ns MD simulation.

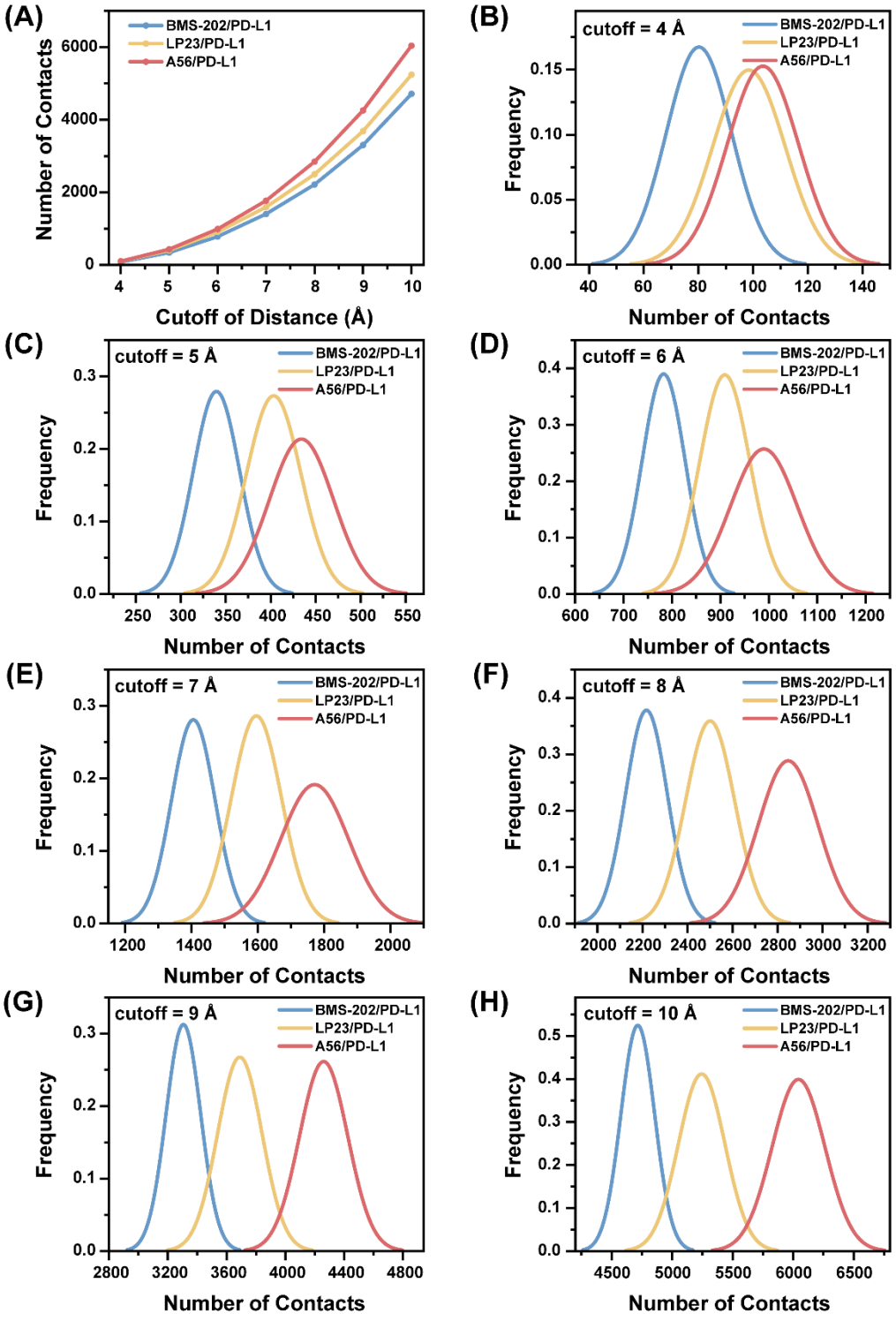

Figure S3. (A) Average number of heavy-atom contacts and (B–H) distributions of heavy-atom contacts between PD-L1 and each of the three inhibitors when the cutoff distance is set to 4–10 Å, respectively.

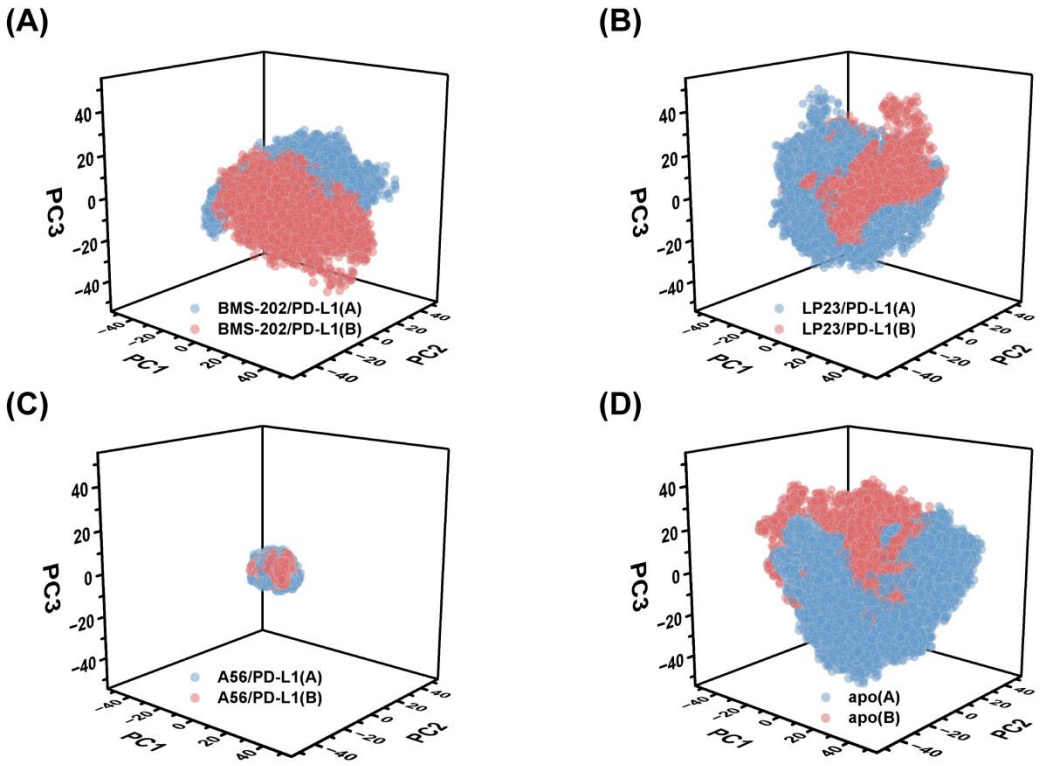

Figure S4. Three-dimensional projections along the first three principal components for each protein chain of the PD-L1 dimer in (A) BMS-202/PD-L1, (B) LP23/PD-L1, (C) A56/PD-L1, and (D) the apo systems , respectively.

Table S1. The average binding free energy for three trajectories of each complex in the last 200 ns MD simulations calculated by the MM/GBSA method, and each value is given with the corresponding standard error of the mean (all values are in kcal/mol).

Systems	Trajectory	ΔE_{vdW}	ΔE_{ele}	ΔG_{gb}	ΔG_{np}	ΔG_{bind}
BMS-202/PD-L1	1	-60.96(±0.22)	-11.16(±0.47)	27.65(±0.40)	-6.80(±0.02)	-51.27(±0.27)
	2	-59.41(±0.24)	-9.91(±0.46)	26.86(±0.36)	-6.54(±0.03)	-49.00(±0.29)
	3	-62.53(±0.23)	-9.27(±0.61)	26.01(±0.51)	-6.81(±0.03)	-52.59(±0.25)
LP23/PD-L1	1	-68.98(±0.26)	-30.25(±2.06)	48.90(±1.78)	-7.47(±0.02)	-57.80(±0.34)
	2	-66.30(±0.28)	-22.88(±1.84)	38.97(±1.52)	-7.33(±0.03)	-57.55(±0.38)
	3	-67.25(±0.27)	-37.42(±1.51)	49.29(±1.28)	-7.54(±0.03)	-62.92(±0.49)
A56/PD-L1	1	-68.16(±0.37)	-27.56(±0.44)	42.39(±0.31)	-8.26(±0.03)	-61.58(±0.35)
	2	-72.95(±0.29)	-22.23(±0.52)	38.74(±0.36)	-8.73(±0.02)	-65.16(±0.29)
	3	-73.43(±0.31)	-27.39(±0.45)	41.70(±0.32)	-8.69(±0.02)	-67.81(±0.41)

Table S2. The average binding free energy between each protein chain of the PD-L1 dimer and the small-molecule inhibitor in the last 200 ns MD simulations calculated by the MM/GBSA method, and each value is given with the corresponding standard error of the mean (all values are in kcal/mol).

Systems	ΔE_{vdW}	ΔE_{ele}	ΔG_{gb}	ΔG_{np}	ΔG_{bind}
BMS202/PD-L1(A)	-28.82(±0.13)	-6.64(±0.28)	16.07(±0.24)	-3.64(±0.02)	-23.03(±0.15)
LP23/PD-L1(A)	-32.46(±0.15)	-34.74(±1.14)	41.55(±0.97)	-3.99(±0.02)	-29.64(±0.23)
A56/PD-L1(A)	-29.76(±0.16)	-18.12(±0.34)	25.95(±0.26)	-3.94(±0.02)	-25.88(±0.17)
BMS202/PD-L1(B)	-32.15(±0.13)	-3.48(±0.15)	13.11(±0.13)	-3.62(±0.02)	-26.13(±0.12)
LP23/PD-L1(B)	-35.05(±0.15)	4.56(±1.15)	8.01(±1.09)	-4.22(±0.02)	-26.70(±0.17)
A56/PD-L1(B)	-41.75(±0.13)	-7.60(±0.19)	18.37(±0.16)	-5.11(±0.02)	-36.09(±0.15)

Table S3. Energy terms of binding free energy for residues within the 5 Å range of the binding pocket obtained by the alanine scanning method, and each value is given with the corresponding standard error of the mean (all values are in kcal/mol).

Systems	Protein	Residues	$\Delta\Delta E_{vdW}$	$\Delta\Delta E_{ele}$	$\Delta\Delta G_{gb}$	$\Delta\Delta G_{np}$	$\Delta\Delta G_{bind}$
BMS-202/PD-L1	PD-L1(A)	F19A	-0.20(±0.00)	0.01(±0.00)	-0.06(±0.00)	0.00(±0.00)	-0.25(±0.00)
		T20A	-0.39(±0.02)	0.23(±0.04)	0.07(±0.03)	-0.05(±0.00)	-0.13(±0.02)
		I54A	-0.54(±0.01)	-0.00(±0.00)	0.01(±0.00)	-0.01(±0.00)	-0.53(±0.01)
		Y56A	-1.06(±0.03)	-0.25(±0.01)	0.50(±0.01)	-0.07(±0.00)	-0.88(±0.03)
		M115A	-1.78(±0.02)	-0.41(±0.01)	0.30(±0.01)	-0.16(±0.00)	-2.04(±0.02)
		I116A	-0.14(±0.00)	-0.07(±0.00)	-0.01(±0.00)	-0.00(±0.00)	-0.22(±0.00)
		S117A	-0.22(±0.01)	-0.05(±0.01)	0.11(±0.01)	-0.00(±0.00)	-0.16(±0.01)
		D122A	-1.48(±0.03)	3.40(±0.24)	-2.27(±0.21)	-0.15(±0.00)	-0.50(±0.06)
		Y123A	-1.76(±0.03)	-0.27(±0.02)	0.58(±0.02)	-0.12(±0.00)	-1.56(±0.03)
		K124A	-0.36(±0.03)	-6.51(±0.38)	4.91(±0.30)	-0.11(±0.00)	-2.07(±0.09)
	PD-L1(B)	I54A	-1.06(±0.02)	-0.02(±0.00)	0.15(±0.00)	-0.09(±0.00)	-1.01(±0.02)
		Y56A	-4.35(±0.04)	0.03(±0.04)	0.82(±0.02)	-0.27(±0.00)	-3.77(±0.04)
		Q66A	-1.49(±0.03)	-0.84(±0.07)	1.12(±0.05)	-0.18(±0.00)	-1.39(±0.05)
		M115A	-1.93(±0.02)	-0.03(±0.02)	0.15(±0.01)	-0.05(±0.00)	-1.87(±0.02)
		I116A	-0.15(±0.00)	-0.08(±0.00)	-0.02(±0.00)	0.01(±0.00)	-0.24(±0.00)
		S117A	-0.20(±0.01)	0.04(±0.01)	0.05(±0.01)	-0.00(±0.00)	-0.11(±0.01)
		D122A	-0.13(±0.00)	-1.06(±0.02)	1.10(±0.02)	0.01(±0.00)	-0.07(±0.00)
		Y123A	-0.50(±0.01)	0.16(±0.00)	0.03(±0.00)	0.00(±0.00)	-0.31(±0.01)
LP23/PD-L1	PD-L1(A)	V23A	-0.04(±0.00)	-0.04(±0.00)	0.05(±0.00)	-0.00(±0.00)	-0.04(±0.00)
		D26A	-0.02(±0.00)	24.49(±0.25)	-24.31(±0.24)	-0.00(±0.00)	0.16(±0.01)
		I54A	-0.55(±0.01)	-0.02(±0.00)	0.04(±0.00)	-0.01(±0.00)	-0.54(±0.01)
		V55A	-0.05(±0.00)	-0.03(±0.00)	-0.00(±0.00)	-0.00(±0.00)	-0.07(±0.00)
		Y56A	-0.99(±0.02)	-0.37(±0.01)	0.59(±0.01)	-0.05(±0.00)	-0.81(±0.02)
		M115A	-1.48(±0.02)	-0.20(±0.02)	0.14(±0.02)	-0.10(±0.00)	-1.65(±0.03)
		I116A	-0.15(±0.00)	-0.07(±0.00)	-0.02(±0.00)	-0.00(±0.00)	-0.24(±0.00)

		S117A	-0.23(±0.01)	-0.07(±0.02)	0.14(±0.02)	-0.00(±0.00)	-0.16(±0.01)
		D122A	-1.35(±0.01)	33.38(±0.15)	-32.61(±0.15)	-0.08(±0.00)	-0.66(±0.04)
		Y123A	-3.99(±0.04)	0.14(±0.03)	0.47(±0.03)	-0.15(±0.00)	-3.54(±0.04)
		K124A	-1.03(±0.03)	-33.20(±0.27)	32.61(±0.26)	-0.12(±0.00)	-1.74(±0.05)
		R125A	-0.71(±0.06)	-60.32(±1.19)	55.58(±0.96)	-0.32(±0.01)	-5.76(±0.22)
		I126A	-0.02(±0.00)	0.11(±0.00)	-0.12(±0.00)	0.00(±0.00)	-0.03(±0.00)
	PD-L1(B)	I54A	-0.85(±0.02)	-0.08(±0.00)	0.16(±0.00)	-0.07(±0.00)	-0.84(±0.02)
		Y56A	-4.80(±0.03)	-1.08(±0.03)	1.04(±0.03)	-0.31(±0.00)	-5.16(±0.04)
		D61A	-0.36(±0.01)	38.28(±0.37)	-37.70(±0.37)	0.01(±0.00)	0.23(±0.01)
		Q66A	-0.97(±0.02)	-0.22(±0.09)	0.52(±0.06)	-0.12(±0.00)	-0.79(±0.03)
		M115A	-2.56(±0.03)	-0.00(±0.02)	0.16(±0.01)	-0.09(±0.00)	-2.49(±0.03)
		I116A	-0.14(±0.00)	-0.03(±0.00)	-0.06(±0.00)	0.00(±0.00)	-0.23(±0.00)
		S117A	-0.18(±0.01)	-0.16(±0.02)	0.22(±0.02)	-0.00(±0.00)	-0.13(±0.01)
A56/PD-L1	PD-L1(A)	T20A	-0.14(±0.01)	0.25(±0.04)	-0.18(±0.03)	-0.01(±0.00)	-0.08(±0.01)
		V21A	-0.07(±0.00)	0.00(±0.00)	-0.07(±0.00)	0.00(±0.00)	-0.14(±0.00)
		I54A	-0.38(±0.01)	0.01(±0.00)	0.00(±0.00)	-0.04(±0.00)	-0.41(±0.01)
		Y56A	-2.70(±0.02)	0.35(±0.03)	0.68(±0.02)	-0.28(±0.00)	-1.95(±0.02)
		M115A	-2.51(±0.04)	-0.56(±0.02)	0.60(±0.01)	-0.35(±0.00)	-2.82(±0.04)
		I116A	-0.04(±0.00)	-0.01(±0.00)	-0.02(±0.00)	-0.00(±0.00)	-0.06(±0.00)
		S117A	-0.03(±0.00)	0.00(±0.00)	0.03(±0.00)	-0.00(±0.00)	0.00(±0.00)
		D122A	-0.66(±0.05)	-6.71(±0.38)	5.80(±0.28)	-0.10(±0.00)	-1.66(±0.10)
		Y123A	-3.43(±0.05)	-0.66(±0.03)	1.02(±0.02)	-0.09(±0.00)	-3.16(±0.05)
		K124A	-0.76(±0.03)	-2.31(±0.14)	0.58(±0.12)	-0.08(±0.00)	-2.56(±0.08)
		R125A	-0.81(±0.03)	-4.14(±0.15)	3.27(±0.12)	-0.17(±0.01)	-1.84(±0.06)
	PD-L1(B)	I54A	-1.36(±0.02)	0.02(±0.00)	0.17(±0.00)	-0.12(±0.00)	-1.29(±0.02)
		V55A	-0.11(±0.00)	-0.09(±0.00)	-0.00(±0.00)	-0.00(±0.00)	-0.21(±0.00)
		Y56A	-4.09(±0.03)	-2.33(±0.03)	1.97(±0.02)	-0.20(±0.00)	-4.65(±0.03)
		N63A	-1.17(±0.02)	-0.92(±0.04)	0.85(±0.04)	-0.03(±0.00)	-1.27(±0.02)
		Q66A	-1.77(±0.03)	-1.39(±0.09)	0.69(±0.03)	-0.15(±0.00)	-2.62(±0.08)

		V68A	-0.92(±0.02)	0.01(±0.00)	0.13(±0.01)	-0.12(±0.00)	-0.89(±0.02)
		M115A	-2.28(±0.02)	-0.90(±0.01)	0.68(±0.01)	-0.13(±0.00)	-2.62(±0.02)
		I116A	-0.18(±0.00)	-0.14(±0.00)	-0.02(±0.00)	0.01(±0.00)	-0.32(±0.00)
		S117A	-0.25(±0.01)	0.19(±0.01)	0.02(±0.01)	-0.01(±0.00)	-0.04(±0.01)
		D122A	-0.09(±0.00)	-0.66(±0.02)	0.79(±0.02)	0.00(±0.00)	0.05(±0.00)
		Y123A	-0.66(±0.02)	-0.13(±0.01)	0.30(±0.01)	-0.05(±0.00)	-0.54(±0.02)