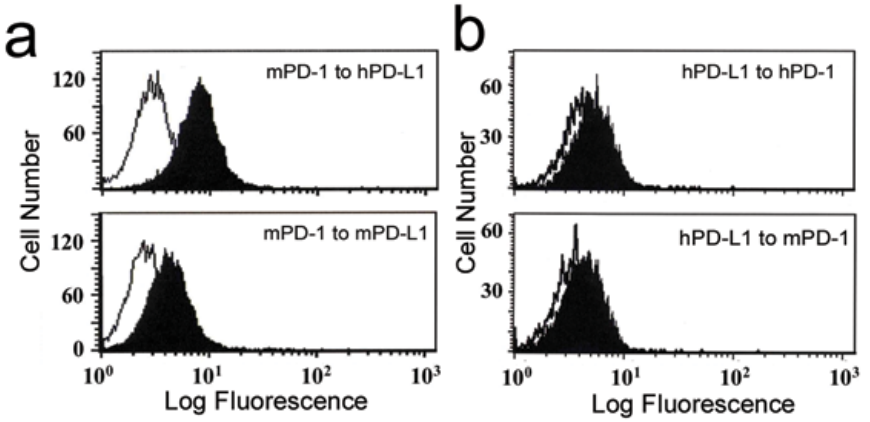
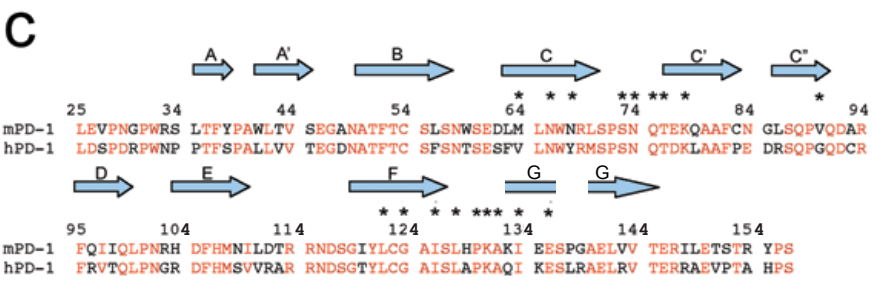
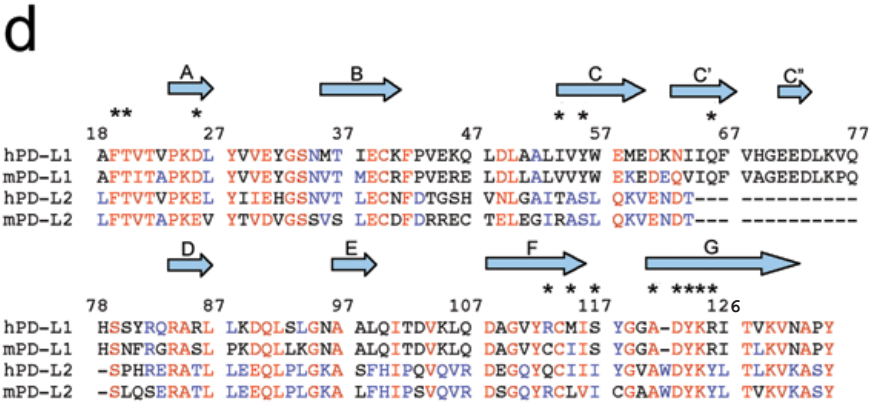
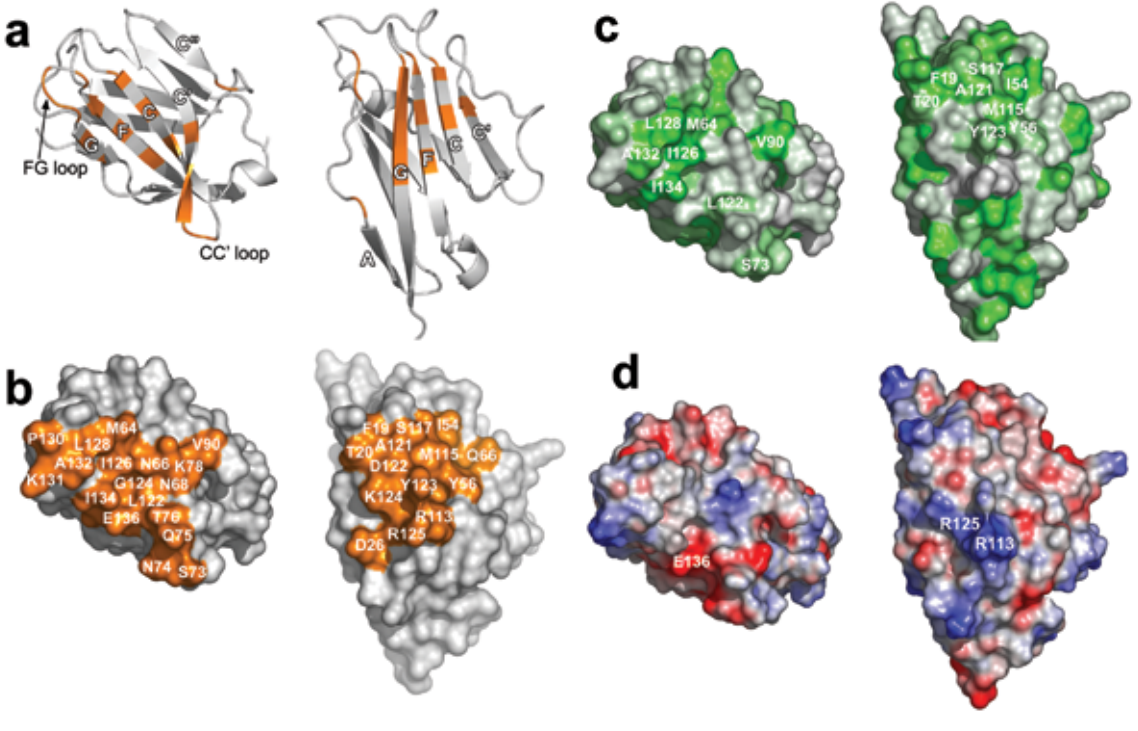
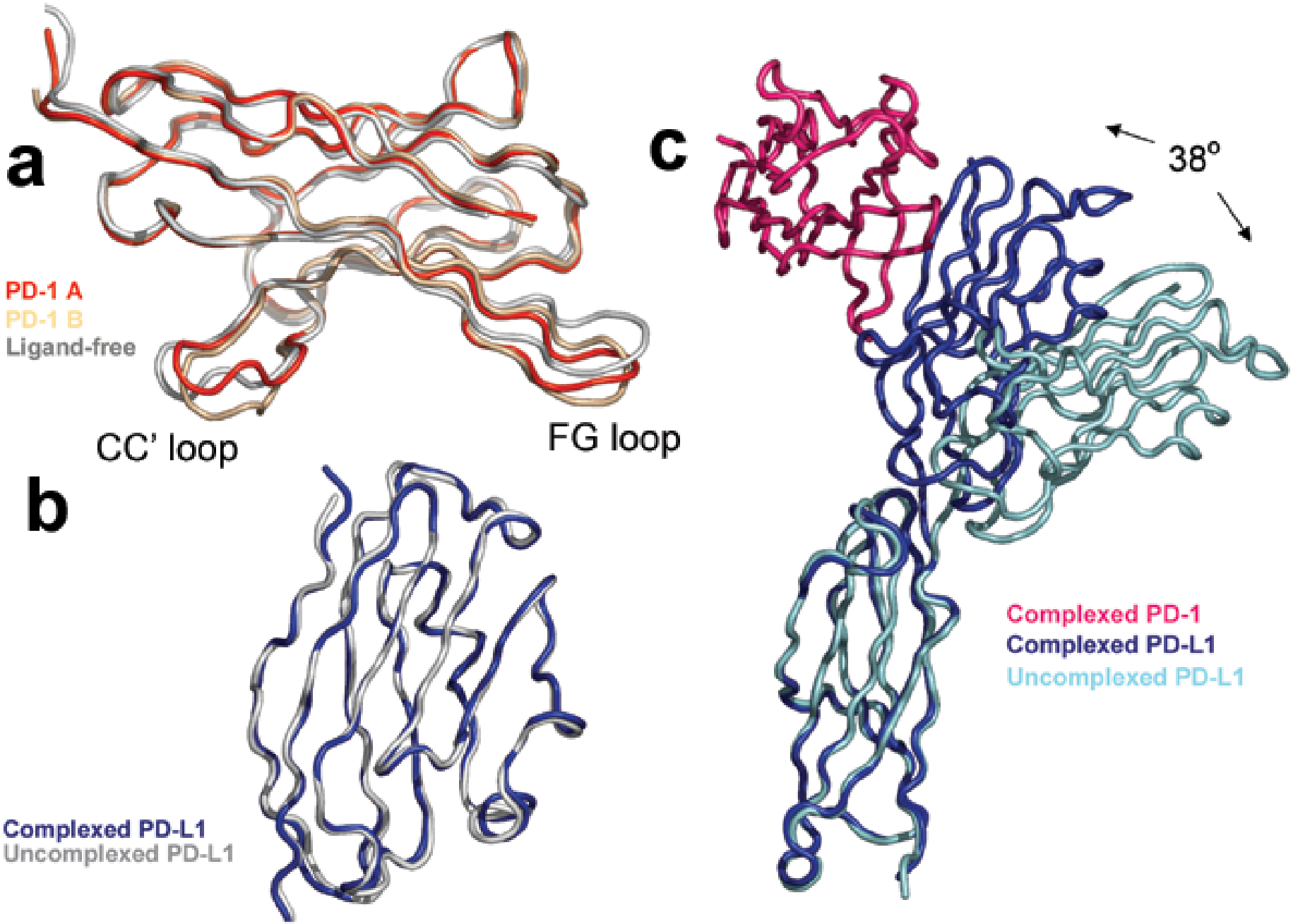

a
mPD-1 to hPD-L1
mPD-1 to mPD-L1
Cell Number
Log Fluorescence
b
hPD-L1 to hPD-1
hPD-L1 to mPD-1
Cell Number
Log Fluorescence


c
A
A'
B
C
C'
C"
D
E
F
G
G
mPD-1 LEVPNGPWRS LTFYPAWLTV SEGANATFTC SLSNWSEDLM LNWNRLSPSN QTEKQAAFCN GLSQPVQDAR
hPD-1 LDSPDRPWNP PTFSPALLVV TEGDNATFTC SFSNTSESFV LNWYRMSPSN QTDKLAAFPE DRSQPGQDCR
mPD-1 FQIIQLPNRH DFHMNILDTR RNDSGIYLCG AISLHPKAKI EESPGAELVV TERILETSTR YPS
hPD-1 FRVTQLPNGR DFHMSVVRAR RNDSGTYLCG AISLAPKAQI KESLRAELRV TERRAEVPTA HPS


d
A
B
C
C'
C"
D
E
F
G
hPD-L1 AFTVTVPKDL YVVEYGSNMT IECKFPVEKQ LDLAALIVYW EMEDKNIIQF VHGEEDLKVQ
mPD-L1 AFTITAPKDL YVVEYGSNVT MECRFPVERE LDLLALVVYW EKEDEQVIQF VAGEEDLKPQ
hPD-L2 LFTVTVPKEL YIIEHGSNVT LECNFDTGSH VNLGAITASL QKVENDT--- ----------
mPD-L2 LFTVTAPKEV YTVDVGSSVS LECDFDRREC TELEGIRASL QKVENDT--- ----------
hPD-L1 HSSYRQRARL LKDQLSLGNA ALQITDVKLQ DAGVYRCMIS YGGA-DYKRI TVKVNAPY
mPD-L1 HSNFRGRASL PKDQLLKGNA ALQITDVKLQ DAGVYCCIIS YGGA-DYKRI TLKVNAPY
hPD-L2 -SPHRERATL LEEQLPLGKA SFHIPQVQVR DEGQYQCIII YGVAWDYKYL TLKVKASY
mPD-L2 -SLQSERATL LEEQLPLGKA LFHIPSVQVR DSGQYRCLVI CGAAWDYKYL TVKVKASY


a
C''
C'
C
F
G
FG loop
CC' loop
A
G
F
C
C'
b
P130
M64
L126
V90
A132
I126
N66
K78
K131
G124
N68
I134
L122
E136
T76
Q75
N74
S73
E19
S117
I54
T20
A121
M115
Q66
D122
Y123
Y56
K124
R113
R125
D26
c
L128
M64
V90
A132
I126
I134
L122
S73
F19
S117
A121
I54
T20
M115
Y123
Y56
d
E136
R125
R113


a
PD-1 A
PD-1 B
Ligand-free
CC' loop
FG loop
b
Complexed PD-L1
Uncomplexed PD-L1
c
38°
Complexed PD-1
Complexed PD-L1
Uncomplexed PD-L1

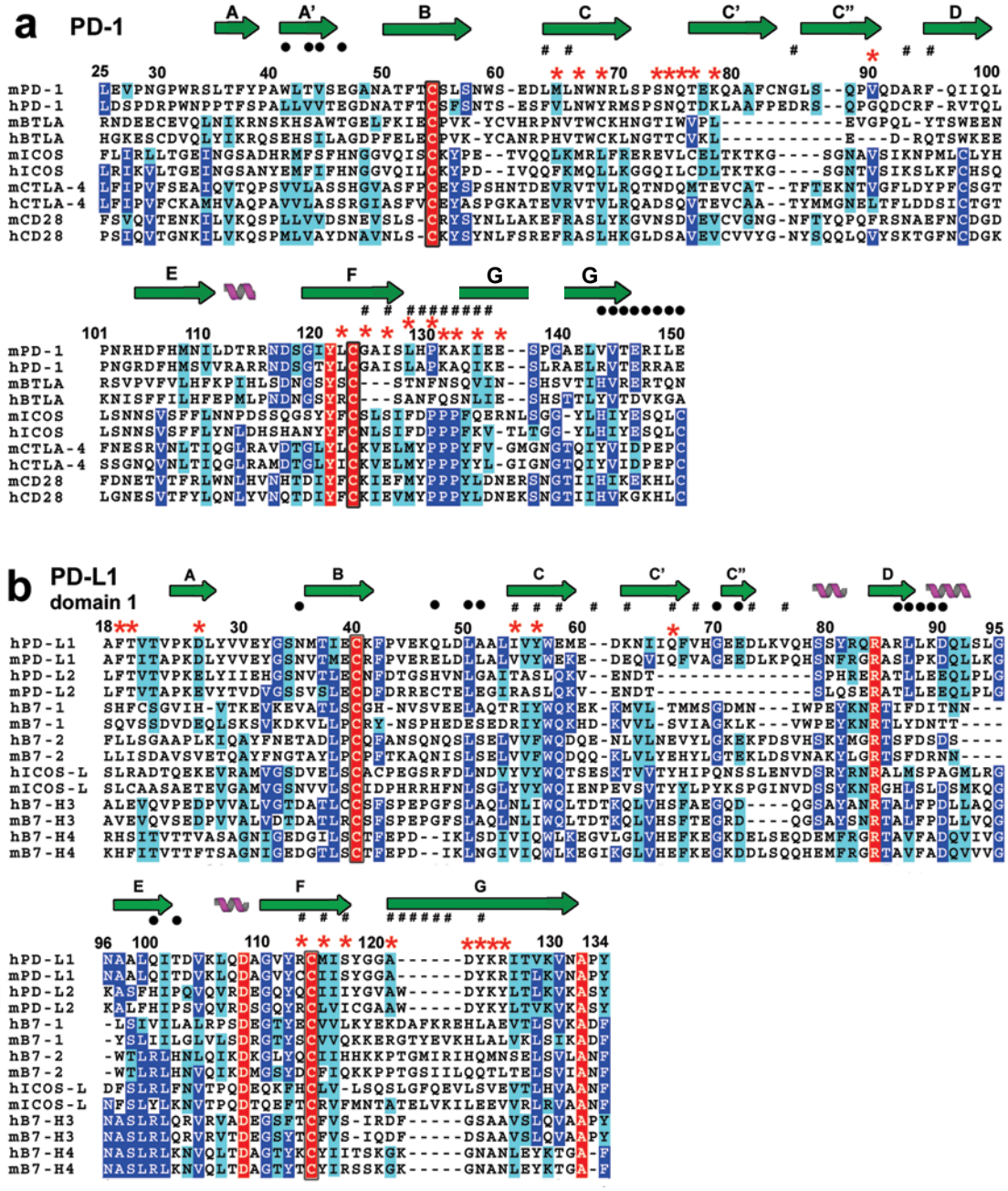

a
PD-1
A
A'
B
C
C'
C"
D
E
F
G
G
mPD-1
hPD-1
mBTLA
hBTLA
mICOS
hICOS
mCTLA-4
hCTLA-4
mCD28
hCD28
b
PD-L1
domain 1
A
B
C
C'
C"
D
E
F
G
hPD-L1
mPD-L1
hPD-L2
mPD-L2
hB7-1
mB7-1
hB7-2
mB7-2
hICOS-L
mICOS-L
hB7-H3
mB7-H3
hB7-H4
mB7-H4

Table 2. Data collection and refinement of the PD-1/PD-L1 complex

Data collection	
X-ray source	19-BM (Argonne)
Wavelength, Å	0.97943
Resolution limits, Å	50-2.65 (2.74-2.65)
Space group	P212121
Unit cell dimensions, Å	56.5 x 63.9 x 159.9
Completeness, %	98.9 (91.4)
Average redundancy	6.0 (3.8)
R_{sym}, %	7.5 (43.4)
I/σ	19.4 (2.9)
No. of observations/unique reflections	103,838/17,320
Refinement statistics	
Resolution limits, Å	20-2.65 (2.72-2.65)
No. of reflections in working set	16,327 (1050)
No. of reflections in (5%) test set	898 (52)
R_{work}, %	21.4
R_{free}, %	26.8
No. of atoms: Protein; glycerol; water	 3,562; 6; 78
Bond lengths, rmsd, Å	0.01
Bond angles, rmsd, °	1.40
Average B factor, $Å^2$	68
Ramachandran, % (favored, allowed, generous, forbidden)	 89.8, 9.2, 1.0, 0.0

Highest resolution shell is shown in parentheses.

Table 3. Interactions between PD-1 and PD-L1 in the complex

Hydrogen bonds and salt bridges					
PD-1 contact atom	PD-1 residue location	PD-L1 contact atom	PD-L1 residue location	Distance, Å	*** < 3.3 Å * > 3.3 Å
N 66 Nδ2	C strand	A 121 O	G strand	3.5	*
N 68 Nδ2	C strand	Y 123 OH	G strand	2.8	***
S 73 Oγ	CC' loop	D 26 Oδ2	A strand	3.1	***
N 74 Oδ1	CC' loop	R 125 NH1	G strand	3.2	***
Q 75 Oε1	CC' loop	R 125 N	G strand	2.8	***
Q 75 Nε2	CC' loop	R 125 O	G strand	2.7	***
Q 75 Nε2	CC' loop	D 26 Oδ1	A strand	3.0	***
T 76 Oγ1	C' strand	Y 123 O	G strand	3.0	***
T 76 O	C' strand	K 124 Nζ	G strand	3.0	***
K 78 Nζ	C' strand	A 121 O	G strand	3.2	***
K 78 Nζ	C' strand	F 19 O	N-terminus	2.9	***
P 130 O	FG loop	Q 66 Nε2	C' strand	3.5	*
A 132 N	FG loop	Q 66 Oε1	C' strand	3.2	***
E 136 Oε1	G strand	Y 123 OH	G strand	2.6	***
E 136 Oε1	G strand	R 125 NH2	G strand	3.0	***
E 136 Oε2	G strand	R 113 Nε	F strand	3.3	*
E 136 Oε2	G strand	R 113 NH2	F strand	3.2	***
E 136 Oε2	G strand	R 125 NH2	G strand	3.0	***
Water-mediated hydrogen bonds					
I 134 O	G strand	Y 56 OH	C strand	3.0 / 3.0	*** / ***
I 134 N	G strand	Y 56 OH	C strand	3.0	***

Table 4. Murine PD-1 mutations and binding data from Zhang *et al.* (1) reconciled with the PD-1/PD-L1 complex structure

mPD-1 residue (number-ing from this work)	Residue number in ref. 1	Human equivalent	Muta-tion from ref. 1	Location from crystal structures [ref. 1 and this work]	Is any atom within 4 Å of PD-L1?	Binding BIAcore, %	Side chain located in interface or solvent	mPD-1 residue (number-ing from this work)
D 62	29	S	A	BC loop	no	115	solvent	D 62
D 62	29	S	S	BC loop	no	125	solvent	D 62
M 64*	31	V	A	C strand	yes	67	interface	M 64*
N 66*	33	same	A	C strand	yes	52	interface	N 66*
N 68*	35	Y	none	C strand	yes	not done	interface	N 68*
S 73*	40	same	none	CC' loop	yes	not done	interface	S 73*
N 74*	41	same	none	CC' loop	yes	not done	interface	N 74*
Q 75*	42	same	none	CC' loop	yes	not done	interface	Q 75*
T 76*	43	same	none	C' strand	yes	not done	interface	T 76*
K 78*	45	same	A	C' strand	yes	2	interface	K 78*
N 84	51	E	A	C'C" loop	no	101	solvent	N 84
L 86	53	R	A	C" strand	no	102	solvent	L 86
Q 88	55	same	S	C" strand	no	88	solvent	Q 88
V 90*	57	G	A	C"D loop	yes	87	interface	V 90*
H 107	74	same	S	E strand	no	96	solvent	H 107
R 114	81	same	S	EF loop	no	89	solvent	R 114
L 122*	89	same	none	F strand	yes	not done	interface	L 122*
G 124*	91	same	none	F strand	yes	not done	interface	G 124*
I 126*	93	same	A	F strand	yes	0	interface	I 126*
L 128*	95	same	A	F strand/FG loop	yes	3	interface	L 128*
H 129	96	A	A	FG loop	no	60	solvent	H 129
P 130*	97	same	A	FG loop	yes	83	interface	P 130*
K 131*	98	same	A	FG loop	yes	42	interface	K 131*
A 132*	99	same	L	FG loop	yes	121	interface	A 132*
I 134*	101	same	A	G strand	yes	4	interface	I 134*
E 136*	103	same	A	G strand	yes	9	interface	E 136*
D 62/ A 132	99/ 29	same/ S	L/ A	FG loop/ BC loop	yes/ no	135	solvent/ interface	D 62/ A 132

*Denotes a residue in the interface of the PD-1/PD-L1 complex.

Binding by wildtype mPD-1 was 100% in Zhang *et al.* (1).

1. Zhang X, *et al.* (2004) Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity* 20:337–347.

Table 5. Murine PD-L1 mutations and binding data from Wang *et al.* (1) reconciled with murine PD-1/ human PD-L1 complex and human PD-L1 structures

hPD-L1 interface residues (*) and mutated residues	Murine equiv-alent	Muta-tion in ref. 1	Residue location from modeling in ref. 1	Location from crystal structure (this work)	Is any atom within 4 Å of PD-1?	Binding to mPD-1 by FACS	Binding to mPD-1 by ELISA, %	Side chain located in interface, solvent, or buried.	hPD-L1 interface residues (*) and mutated residues
F 19*	same	none	n.r.	N-term	yes	not done	not done	interface	F 19*
T 20*	same	none	n.r.	N-term	yes	not done	not done	interface	T 20*
D 26*	same	none	n.r.	A strand	yes	not done	not done	interface	D 26*
L 27	same	A	A ' strand	A ' strand	no	++++	100	solvent	L 27
E 31	same	S	A ' strand	A ' strand	no	++	50	solvent	E 31
S 34	same	Y	B strand	B strand	no	++++	60	solvent	S 34
T 37	same	Y	B strand	B strand	no	++	5	solvent	T 37
D 49	same	S	B/C loop	B/C loop	no	++++	30	solvent	D 49
I 54*	same	none	n.r.	C strand	yes	not done	not done	interface	I 54*
Y 56*	same	S	C strand	C strand	yes	++++	100	interface	Y 56*
E 58	same	S	C strand	C strand	no	+++++	300	solvent	E 58
K 62	E	S	C/C ' loop	C/C' loop	no	++++	50	solvent	K 62
Q 66*	same	none	n.r.	C ' strand	yes	not done	not done	interface	Q 66*
F 67	same	A	C ' strand	C ' strand	no	+/-	2	buried	F 67
H 69	A	F	C ' strand	C'/C" loop	no	+++++	300	solvent	H 69
E 72	same	S	C ' strand	C" "strand"	no	++++	60	solvent	E 72
K 75	same	S	C ' '/D loop	C" "strand"	no	++++	100	solvent	K 75
K 89	same	S	D strand	D strand	no	++++	60	solvent	K 89
A 98	same	F	E strand	E strand	no	++++	40	solvent	A 98
Q 100	same	S	E strand	E strand	no	++++	100	solvent	Q 100
R 113*	C	Y	F strand	F strand	yes	+++++	300	interface	R 113*
M 115*	I	A	F strand	F strand	yes	+/-	3	interface	M 115*
S 117*	same	Y	F strand	F strand	yes	++++	100	interface	S 117*
A 121*	same	none	n.r.	G strand	yes	not done	not done	interface	A 121*
D 122*	same	none	n.r.	G strand	yes	not done	not done	interface	D 122*
Y 123*	same	none	n.r.	G strand	yes	not done	not done	interface	Y 123*
K 124*	same	S	G strand	G strand	yes	+	3	interface	K 124*
R 125*	same	none	n.r.	G strand	yes	not done	not done	interface	R 125*
I 126	same	A	G strand	G strand	no	-	1.4	buried	I 126
K 129	same	S	G strand	G strand	no	++	35	solvent	K 129

*Denotes a residue in the interface of the PD-1/PD-L1 complex.

n.r., not reported.

Binding by wildtype mPD-L1 was ++++ by FACS and 100 % by ELISA in Wang *et al.* (1).

1. Wang S, *et al.* (2003) Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. *J Exp Med* 197:1083–1091.