Supplemental Tables

Table S1. Sequencing of enriched sPD-1 library pool from each consecutive sort round. The residue number is listed at the top and the corresponding wild-type amino acid is given.

Clone	bp (Ig1)	AA (Ig1)	33	34	36	37	45	49	50	57	59	70	74	76	77	84	87	88	89	91	92	93	96	99	101	102	103	107	110	112	113	114	116	119	124	125	127	131	132	135	139	140	145	146	147	148
wt PD-1			N	P	T	F	T	N	A	S	T	M	N	T	D	E	S	Q	P	Q	D	S	R	Q	P	N	G	H	V	R	A	R	N	G	G	A	S	K	A	K	R	A	T	E	R	R
S0.1	11	10										A	S				G		L															D		V				V			V			K	G
S0.2	4	3	D																																				V		G					
S0.3	7	5	D									A	D				G																						I			V				
S0.4	2	2										A																											V							
S0.5	---	---																																												
S0.6	4	3											D															R											V							
S0.7	6	4															G		L												D								V							
S0.8	9	9			S								D						L							G										S	S		V						K	G
S0.9	4	4					I						S						L																											
S0.10	8	6										A																									L	R	I	R	G				
S1.1 a	10	6										A					G		L																			R	I				A			
S1.1 b	6	4																	L																		L	R	I							
S1.1 c	9	7										A											R														L	R	I	R		V				
S1.1 d	3	3											D																										V			V				
S1.1 e	6	5										A			A		G																						I			V				
S1.1 g	10	5					I																														F		I			V			K	G
S1.1 h	5	4																																	S	L					V				
S1.1 i	8	7				L						A					G																		S				I				I			
S1.2 a	5	4															G		L																						G					
S1.2 b	6	4																																S		L	R	I							
S1.2 c	8	5																																		L	R	I	R	G					
S1.2 d	5	3												I																									I		G					
S1.2 e	4	4															G		L																				V			V				
S1.2 f	11	7															G																				L	R	V	R						
S1.2 g	9	8											D				G		L					R													L		V			V	I		K	G
S1.2 i	8	7	D										S																								L		V				I		K	G
S2.1	9	6									N		D				G		L																				I			V				
S2.2	6	5									N						G		L																				I			V				
S2.3	8	6							A			A												S													L		I			V				
S2.4	6	5										A																								S			I			V		K		
S2.5	9	7															G		L																	S	L	R	I	R						
S2.6	10	8																	L															T			L	R	I	R		V	I			
S2.7	7	5															G		L																		L	R	I							
S2.8	7	5											D				G		L																	S	S									
S2.9	9	8										A					G		L					R											S			V	R		V				
S2.10	9	7										A				I	G		L																	S			V	R		V				
S2.11	13	10									N		D																			K		S			L	R	I	R					K	G
S2.12	6	5										A	D				G																						I			V				
S2.13	9	8	D									A					G		L																		L	R	I	R		V				
S2.14	12	8										A				I																				S	L				G				K	G
S2.15	8	7												I			G		L																S				I			V				G

ID	N1	N2	pre	GRAY	YELLOW	BLUE	mid1	CREAM	PINK	ORANGE	mid2	GREEN	mid3	RED	post
S3.1	9	8	I A			L	G	S	S			I		V	
S3.2	3	2										V		V	
S3.3	6	5			G	L			S			I		V	
S3.4	10	8	I	D	G	L		S	S			I		V	
S3.5	8	7		D	G	L		S	S			I		V	
S3.6	15	13	L	D	G	L	R	S	S	L		I	R G	V	K G
S3.7	15	9	D	S	G	L		S	S	L	R	I	I	V	K G
S3.8	13	10	D A	D	G			S	S	L	R	V			K G
S3.9	12	10		D	G	L	G	S	S			I		V	
S3.10	8	7	A	D	G	L		S	S			I		V	
S3.11	13	10	A		G	L	S R V	D	S			I	R G	V	
S3.12	6	5							S						
S3.13	6	5		D		L			S					V	
S3.14	9	7	A			L	G		S			V		V	
S3.15	3	2													
S4.1	11	8			G	L		T	S	L		I	V	V	K G
S4.2	8	7	A		G	L		S	S			I		V	
S4.3	11	8			G	L		S	S	L	R	I	R	V	
S4.4	14	11			G	L		S	S	L	R	I	R G	V	K G
S4.5	7	6			G	L	V	S	S			V		V	
S4.6	8	7	N		G	L		S	S			V		V	
S4.7	7	6			G	L		S	S			I		V	
S4.8	10	7			G	L		S	S	L		I		V	K
S4.9	8	7	A		G	L		T	S			I		V	
S4.10	11	9	A N		G		R V		S	L	R	I		V	
S4.11	9	6	K		G	L			S			I		V	
S4.12	7	6			G	L		S	S			I		V	
S4.13	8	6					A	S	S	L	R	I			
S4.14	13	10	A		G	L	A R V	S	D		R	I	R G	V	
S4.15	8	7			G	L		S	S		R	I		V	
S5.1	10	7			G	L		S	S	V		I		V	
S5.2	8	7			G		R M	S	S			I		V	
S5.3	7	6			G		R R	S	S			I			
S5.4	13	10			G		R R	S	S	L			G	V	K G
S5.5	9	8	N		G	L	G	S	S			I			K
S5.7	9	7			G	L	R	S	S	L		I		V	
S5.9	13	11	V	D	G	L	R S	S	S	L		I		V	
S5.10	8	6			G		G								
S5.12	12	10	D N		G		G G R			L		V		V	

Sequence alignment (two groups separated by a horizontal rule). Columns with coloured highlights: gray (unlabeled), yellow (G), blue (L), tan (S/T), pink (S), orange (L/V), green (I/V), red (V).

ID									gray	G	L						S	S	L		I			V			
S6.8.1	13	11				S			■	G	L			R			S	S	L	R	I	R	G	V			
S6.8.2	8	7							■	G				R		M	S	S	L	R	I	R					
S6.8.3	12	10							■	G	L	A					S	S	L	R	I	R		V			
S6.8.6	18	15		S	L		V		■	G	L	G	G				S	S	L	R	I	R	G			K	G
S6.8.9	12	10				S		N	■	G	L			R			T	S	L	R	I			V	A		
S6.8.10	12	9						N	■	G				R			S	S	L		I			V			
S6.8.12	12	10	D						■	G		G	G	R				S	L		I		G		I		
S6.8.13	10	7							■	G	L						S	S	V					V			
S6.12.1	12	9						N	■	G	L					R	T	S	L		I	R	G	V			
S6.12.2	18	15		S	L		V		■	G	L	G	G				S	S	L		I	R	G			K	G
S6.12.3	10	8							■	G	L				V		S	S	L	R				V			
S6.12.4	9	8							■	G	L	G	G	R			S	S	V		V			V			
S6.12.5	13	10							■	G	L	R				R	S	S	L				G			K	G
S6.12.6	13	12						I	■	G	L						S	S	L	R	I	R	G	V		K	
S6.12.7	10	7							■	G	L						S	S	V		V			V			
S6.12.8	12	10	D					N	■	G		G	G	R				S	V		V			V			
S6.12.9	10	8							■	G	L					R	S	S	V	R	I			V			
S6.12.13	13	11				S			■	G	L						S	S	L	R	I	R	G	V			

Table S2. Calculated changes in binding affinity and protein stability (in kcal/mol) between sPD-1 and PD-L1 with mutations within and outside of the binding interface.

Mutations	d Affinity	d Stability (solvated)
8 mutations	-19.03	-69.92
7 mutations (N116S excluded)	-20.08	-66.99
4 outside mutations	-1.61	-30.66
3 interface mutations	-24.78	-30.58
2 mutations on missing loop	1.31	-12.08