

S2 Table. Dissociation constants and thermodynamic parameters from isothermal titration calorimetry assays. In all cases proteins were titrated into the ligand solution (reverse titration).

Comp	Protein	Mutant	K_A (10^6 M ⁻¹)	K_D (nM)	N	ΔH (kcal/mol)	T ΔS (kcal/mol) *	ΔG (kcal/mol)
JQ1	BRD2(1)	wt	20.9 ± 0.7	47.8 ± 1.6	0.875 ± 0.001	-8.826 ± 0.017	0.976	-9.802
	BRD2(1)	D161N	14.3 ± 0.8	69.9 ± 3.9	0.978 ± 0.002	-6.877 ± 0.025	2.673	-9.550
	BRD2(1)	D161Y	57.8 ± 3.0	17.3 ± 0.9	1.100 ± 0.001	-7.356 ± 0.016	2.997	-10.353
	BRD2(1)	Y153H	18.0 ± 0.8	55.5 ± 2.6	0.899 ± 0.002	-9.689 ± 0.031	0.044	-20.09
	BRD2(1)	E140K	26.0 ± 0.9	38.5 ± 1.4	0.933 ± 0.009	-7.574 ± 0.015	2.330	-9.904
	BRD2(1)	R100L	35.8 ± 1.7	27.9 ± 1.3	1.090 ± 0.001	-7.918 ± 0.017	2.175	-10.093
	BRD2(2)	wt	19.7 ± 1.4	50.8 ± 3.7	0.908 ± 0.002	-4.368 ± 0.019	5.331	-9.699
	BRD2(2)	Q443H	42.4 ± 3.8	23.6 ± 2.1	0.913 ± 0.002	-5.169 ± 0.002	4.985	-10.154
	BRD2(2)	R419W	33.4 ± 2.4	29.9 ± 2.2	1.100 ± 0.0019	-3.274 ± 0.011	6.714	-9.988
	BRD3(2)	wt	42.7 ± 2.4	23.4 ± 1.3	0.902 ± 0.001	-6.930 ± 0.017	3.256	-10.186
	BRD3(2)	H395R	142.8 ± 8.2	7.0 ± 0.4	1.100 ± 0.001	-6.606 ± 0.011	4.265	-10.871
	BRD4(1)	wt	55.5 ± 3.7	18.0 ± 1.2	0.912 ± 0.001	-12.020 ± 0.033	-1.605	-10.415
	BRD4(1)	A89V	97.1 ± 8.5	10.3 ± 0.9	0.969 ± 0.001	-11.140 ± 0.033	-0.423	-10.717
	BRD4(2)	wt	55.2 ± 4.6	18.1 ± 1.5	0.931 ± 0.001	-5.844 ± 0.019	4.466	-10.31
BRD4(2)	A420D	22.5 ± 1.9	44.4 ± 3.9	0.990 ± 0.003	-6.530 ± 0.035	3.285	-9.815	

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S2 Table (continued)

Comp	Protein	Mutant	K_A (10^6 M ⁻¹)	K_D (nM)	N	ΔH (kcal/mol)	T ΔS (kcal/mol) *	ΔG (kcal/mol)
PFI-1	BRD2(1)	wt	12.4 ± 0.4	80.6 ± 2.9	0.924 ± 0.001	-19.230 ± 0.048	-9.538	-9.692
	BRD2(1)	D161N	4.90 ± 0.1	204.1 ± 6.6	0.843 ± 0.002	-13.670 ± 0.042	-4.609	-9.061
	BRD2(1)	D161Y	16.6 ± 0.7	60.24 ± 2.6	1.050 ± 0.001	-17.000 ± 0.044	-7.201	-9.799
	BRD2(1)	Y153H	7.4 ± 0.3	135.3 ± 4.9	1.020 ± 0.002	-17.400 ± 0.051	-8.039	-9.361
	BRD2(1)	E140K	10.7 ± 0.5	93.5 ± 4.4	1.060 ± 0.002	-14.390 ± 0.048	-4.870	-9.520
	BRD2(1)	R100L	9.2 ± 0.3	108.3 ± 3.4	0.950 ± 0.001	-14.790 ± 0.036	-5.359	-9.431
	BRD2(2)	wt	5.0 ± 0.2	198.8 ± 6.6	0.985 ± 0.002	-8.998 ± 0.027	-0.008	-8.990
	BRD2(2)	Q443H	4.7 ± 0.2	213.7 ± 7.6	0.978 ± 0.002	-9.901 ± 0.036	-0.936	-8.965
	BRD2(2)	R419W	4.2 ± 0.2	238.7 ± 10.7	1.100 ± 0.003	-6.318 ± 0.025	2.521	-8.839
	BRD3(2)	wt	6.2 ± 0.2	161.0 ± 5.9	0.976 ± 0.002	-10.540 ± 0.034	-1.403	-9.137
	BRD3(2)	H395R	0.70 ± 0.03	1386.0 ± 65.0	1.030 ± 0.007	-3.552 ± 0.032	4.235	-7.787
	BRD4(1)	wt	17.3 ± 0.8	57.8 ± 2.6	0.964 ± 0.002	-19.750 ± 0.056	-9.883	-9.867
	BRD4(1)	A89V	8.5 ± 0.2	117.1 ± 2.8	0.918 ± 0.001	-18.340 ± 0.038	-8.875	-9.465
	BRD4(2)	wt	2.8 ± 0.1	362.3 ± 17.1	1.010 ± 0.004	-10.130 ± 0.062	-1.464	-8.666
BRD4(2)	A420D	3.4 ± 0.2	297.6 ± 14.5	0.974 ± 0.004	-10.260 ± 0.060	-1.484	-8.776	

* Assay temperature = 15^o C, 288.15 K