SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Structure and Sequence Dependence of XPA DBD Binding of Substrates

(A) Binding of XPA DBD to 8/12 splayed-arm (circle), 8/12 HP splayed-arm with mixed sequence (triangle), and 8/12 HP splayed-arm (square) (substrates 1, 2, and 3 in Figure 1, respectively) as determined by fluorescence anisotropy binding assay. Error bars represent standard deviation from triplicate experiments. (B) Dissociation constants (K_d) determined from each curves in panel A.

Figure S2: Chemical Shift Perturbations (CSPs) Induced by DNA Substrates.

CSPs for each residues observed from the NMR titration of XPA DBD with 8/12 splayed-arm, 8/12 5’ overhang, 8/12 3’ overhang, and 8/4 3’ overhang DNA (substrates 1 without FITC tag, 10, 12, and 13 in Figure 1, respectively). Peaks exchange broadened upon DNA binding are shown as open bars. Only the chemical shifts that were perturbed above the threshold are shown.

Figure S3: V166A Mutant is Less Stable than WT XPA

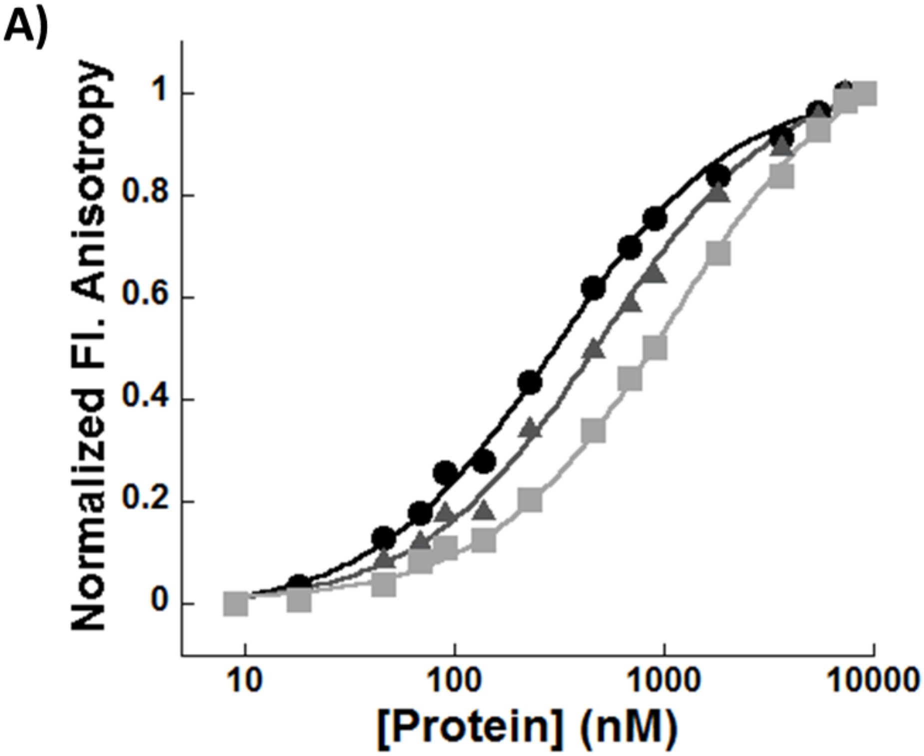
(A) Overlay of ^{1}H 1D NMR spectra of XPA DBD WT (blue) and V166A (red). (B) CD thermal denaturation curves of WT (black) and V166A (gray) XPA DBD. Melting temperatures (Tm) were 45.5 °C (WT) and 41.5 °C (V166A).

Table S1: Backbone Resonance Assignments for XPA DBD

The XPA DBD construct includes N-terminal GPGS sequence from His-tag.

Table S2: Primer Sequences for XPA DBD Mutant Construction

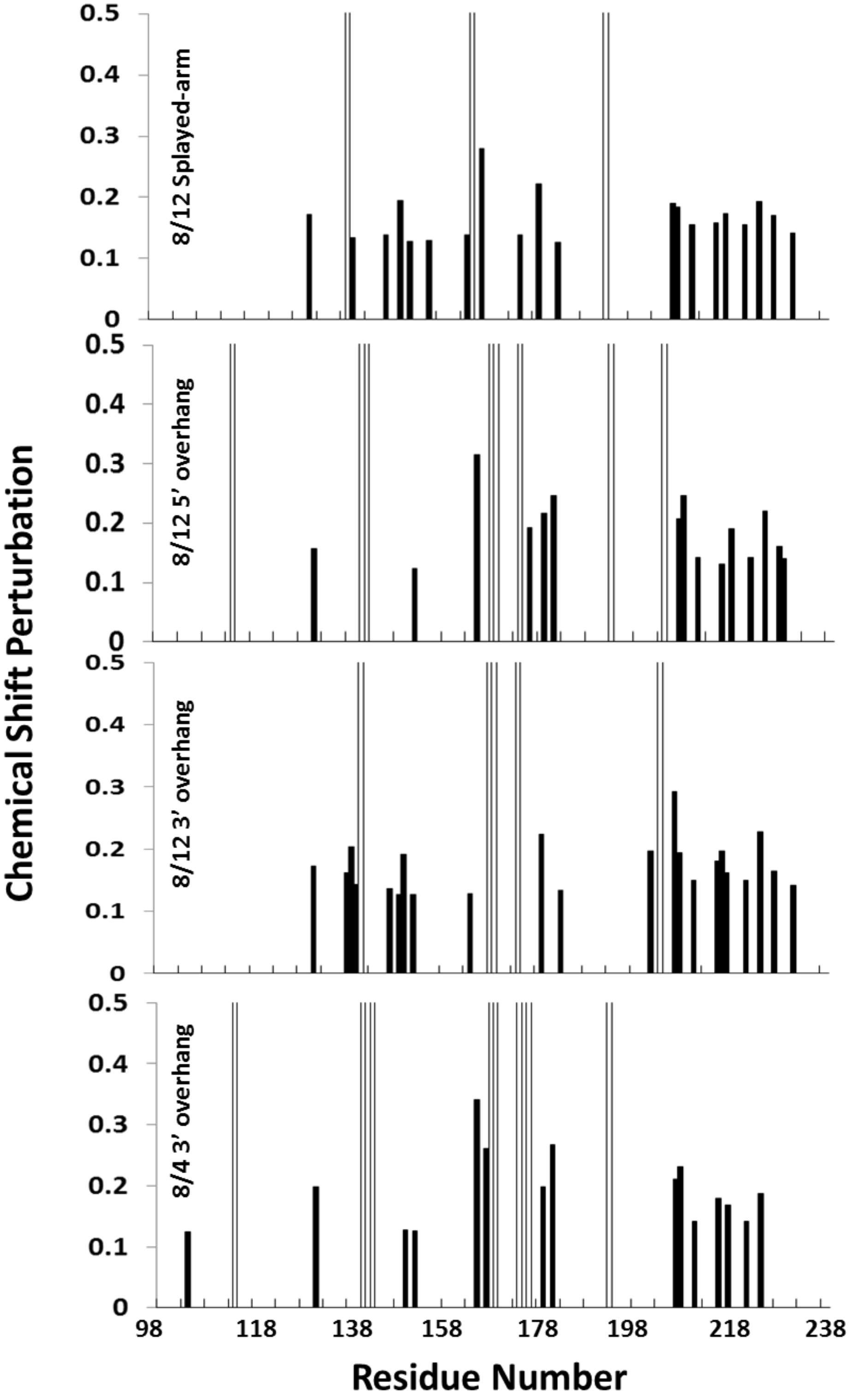
Figure S1



B)

Substrate	Kd (µM)
1 (●)	0.30 ± 0.02
2 (▲)	0.51 ± 0.04
3 (■)	1.10 ± 0.05

Figure S2



Chemical Shift Perturbation
8/12 Splayed-arm
8/12 5' overhang
8/12 3' overhang
8/4 3' overhang
0.5
0.4
0.3
0.2
0.1
0
98
118
138
158
178
198
218
238
Residue Number

Figure S3

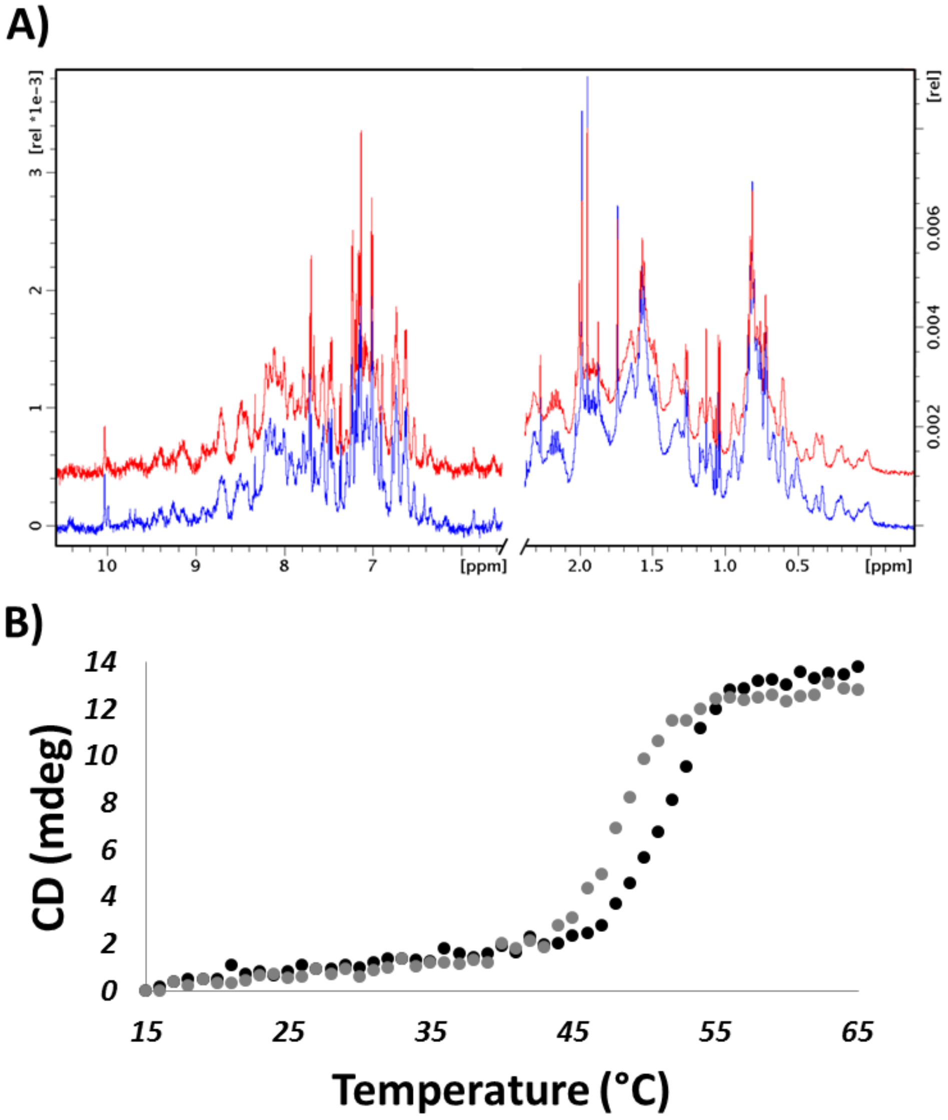

A)
[rel *1e-3]
0
1
2
3
10
9
8
7
[ppm]
2.0
1.5
1.0
0.5
[ppm]
[rel]
0.002
0.004
0.006
B)
CD (mdeg)
0
2
4
6
8
10
12
14
15
25
35
45
55
65
Temperature (°C)

Table S1

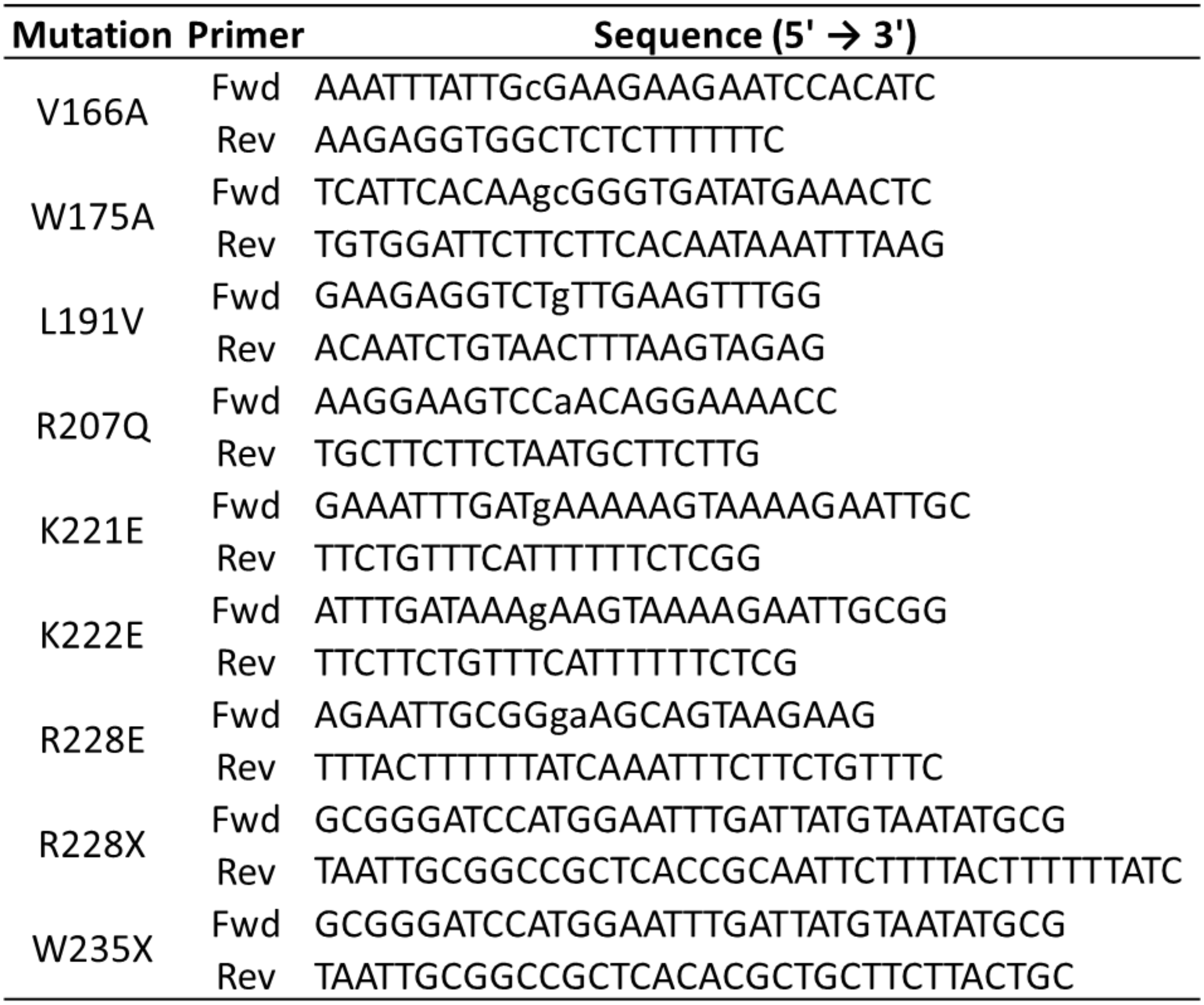
Residue #	Assignment #	Atom	^{15}N (ppm)	^{1}H (ppm)	CO (ppm)	C_α (ppm)	C_β (ppm)
His-1	1	G					
His-2	2	P			177.527	63.515	32.073
His-3	3	G	110.291	8.652	174.258	45.285	
His-4	4	S	115.584	8.162	174.631	58.374	63.753
98	5	M	121.954	8.419	177.6	55.5	32.673
99	6	E	121.508	8.274	175.65	56.671	30.243
100	7	F	119.388	7.895	174.709	55.441	40.05
101	8	D	121.26	8.203	175.022	53.934	41.271
102	9	Y	118.015	7.683	174.985	57.471	39.749
103	10	V	121.986	8.609	173.652	60.501	33.058
104	11	I	123.33	7.919	175.919	58.932	37.709
105	12	C	128.224	8.854	178.312	59.909	30.88
106	13	E	131.505	9.51	176.128	58.342	29.931
107	14	E	121.174	9.236	177.55	57.969	30.687
108	15	C	117.383	8.388	177.421	59.049	32.948
109	16	G	112.793	8.092	173.548	46.314	
110	17	K	122.083	8.62	176.321	56.311	33.35
111	18	E	121.376	8.525	177.049	55.899	30.448
112	19	F	121.057	9.576	172.18	56.277	41.501
113	20	M	118.486	8.819	175.026	56.524	34.155
114	21	D	116.943	8.018	174.776	53.333	44.136
115	22	S	115.757	8.248	172.442	56.382	66.346
116	23	Y	125.942	10.549	178.576	62.577	39.506
117	24	L	118.712	9.235	179.621	57.829	40.69
118	25	M	123.928	8.339	178.733	57.921	32.524
119	26	N	117.912	8.645	177.425	56.156	38.36
120	27	H	110.585	7.698	175.319	57.892	29.539
121	28	F	111.862	7.205	174.34	57.17	41.073
122	29	D	116.425	7.901	174.54	55.891	39.4
123	30	L	123.66	7.745		51.589	44.619
124	31	P			173.514	62.546	28.005
125	32	T	123.292	8.82	174.631	61.324	71.856
126	33	C	135.299	9.797	174.917	58.571	30.687
127	34	D	116.918	8.989	178.12	57.246	40.243
128	35	N	120.702	8.547	176.661	56.096	38.921
129	36	C	121.521	7.759	174.848	61.345	30.592
130	37	R	120.614	6.863	176.714	56.698	30.157
131	38	D	123.469	8.225	176.486	51.635	41.368
132	39	A	126.093	8.903	177.172	53.914	18.866

133	40	D	112.937	7.763	176.313	55.27	41.972
134	41	D	117.241	7.143		55.499	
135	42	K			178.229	60.822	32.915
136	43	H	113.655	8.579	173.435	55.629	29.925
137	44	K	117.423	6.739	176.176	56.48	33.604
138	45	L	122.922	8.366	176.94	53.338	43.144
139	46	I	118.119	9.492	174.075	58.992	42.525
140	47	T	115.453	8.81	175.879	61.88	71.174
141	48	K	121.959	8.851	177.87	60.497	33.472
142	49	T	112.162	7.95	176.324	66.802	68.89
143	50	E	122.942	7.956	178.529	59.704	29.712
144	51	A	121.126	8.843	179.393	55.32	18.399
145	52	K	114.541	8.257	176.836	60.633	32.324
146	53	Q	116.53	7.944	177.964	58.605	29.472
147	54	E	115.589	8.801	177.323	57.512	29.823
148	55	Y	113.217	7.29	172.449	58.039	37.103
149	56	L	115.58	7.036	176.154	55.753	39.059
150	57	L	117.263	7.464	176.313	52.886	45
151	58	K	121.374	9.495	178.551	54.418	35.581
152	59	D	122.7	8.979	179.52	59.113	39.691
153	60	C	115.176	8.619	176.364	60.095	26.868
154	61	D	123.273	7.406	176.144	57.179	42.76
155	62	L	116.254	7.305	177.633	56.747	42.637
156	63	E	113.903	8.047	179.545	57.326	32.397
157	64	K	116.532	7.946	176.684	56.012	33.27
158	65	R	120.052	6.948	174.543	56.478	30.377
159	66	E	122.316	8.254		54.391	32.641
160	67	P					
161	68	P			177.481	63.018	31.932
162	69	L	122.373	8.215	178.059	55.205	42.375
163	70	K	125.279	9.724	173.94	56.206	34.386
164	71	F	111.656	7.041	175.617	54.773	41.21
165	72	I	118.013	9.231	174.925	59.47	41.572
166	73	V	123.285	8.522	176.014	61.5	33.284
167	74	K	128.395	8.913	175.156	54.789	35.306
168	75	K	124.706	8.54	176.332	56.026	32.611
169	76	N	122.673	8.88		51.253	39.472
170	77	P					
171	78	H				58.09	
172	79	H	120.334	8.129			29.188
173	80	S			175.256	59.561	63.578
174	81	Q	121.074	8.731	176.088	56.621	28.667
175	82	W	119.852	7.98	176.799	56.918	29.868

176	83	G	108.66	8.252	173.663	45.483	
177	84	D	120.647	8.26	176.076	54.677	41.498
178	85	M	120.55	8.604	174.424	54.786	34.883
179	86	K	123.599	8.251	174.968	56.246	34.933
180	87	L	122.71	9.3	175.046	53.953	44.46
181	88	Y	119.886	9.291	174.999	56.535	42.713
182	89	L	123.099	9.387	178.243	54.662	41.362
183	90	K	131.865	8.778	177.686	61.795	32.065
184	91	L	117.448	9.398	179.859	58.741	41.936
185	92	Q	116.783	7.191	178.552	58.377	27.618
186	93	I	123.884	7.688	177.298	60.897	34.353
187	94	V	120.709	8.6	179.147	66.781	31.745
188	95	K	118.677	7.211	178.723	59.976	32.447
189	96	R	119.433	8.038	177.954	56.443	27.825
190	97	S	116.021	8.956	176.179	62.71	63.087
191	98	L	121.949	7.855	180.738	57.779	41.167
192	99	E	121.164	7.926	178.823	59.101	29.555
193	100	V	118.517	7.954	178.033	65.938	32.419
194	101	W	117.447	8.639	177.696	58.856	30.126
195	102	G	111.575	8.129	174.109	46.746	
196	103	S	109.937	7.539	173.65	57.178	65.566
197	104	Q	122.67	9.263	177.975	58.481	28.021
198	105	E	118.995	9.001	178.869	60.519	28.609
199	106	A	122.802	8.107	180.71	54.924	19.004
200	107	L	121.846	7.35	177.668	58.019	41.033
201	108	E	117.69	8.313	179.887	59.386	28.454
202	109	E	120.037	8.099	178.352	59.338	29.434
203	110	A	122.35	7.552	180.727	54.756	18.502
204	111	K	118.696	8.044	178.883	59.778	32.26
205	112	E	120.272	7.769	178.711	58.871	29.25
206	113	V	119.246	7.91	177.882	64.892	31.973
207	114	R	120.452	7.843	178.032	58.144	30.267
208	115	Q	120.202	8.28	178.041	58.055	29.594
209	116	E	120.266	8.098	176.91	57.971	32.692
210	117	N	122.063	8.274	176.864	54.486	41.34
211	118	R	121.605	8.139	175.267	56.12	30.483
212	119	E	118.26	7.767	175.387	55.886	29.742
213	120	K	117.835	8.403	176.714	58.256	32.806
214	121	M	121.962	8.19	177.952	57.72	32.393
215	122	K	119.026	8.039	177.178	56.561	32.325
216	123	Q	121.021	8.008	177.234	57.384	32.599
217	124	K	120.081	8.134	177.296	57.584	28.659
218	125	K	120.778	8.377	177.809	58.182	29.555

219	126	F	118.434	8.332	176.633	54.643	38.427
220	127	D	121.022	8.184	175.555	58.003	39.51
221	128	K	122.054	8.281	176.351	56.901	32.749
222	129	K	121.562	8.135	176.91	56.923	32.851
223	130	V	121.723	8.276	177.178	56.863	32.43
224	131	K	120.948	8.031	176.585	63.135	32.649
225	132	E	124.102	8.246	176.148	56.794	32.862
226	133	L	120.44	8.198	177.621	58.008	39.549
227	134	R	120.325	8.129	176.43	56.392	29.132
228	135	R	121.95	8.199	176.122	56.315	30.754
229	136	A	125.167	8.271	177.776	52.576	19.172
230	137	V	119.468	8.075	176.391	62.432	32.664
231	138	R	124.603	8.38	176.335	56.218	30.786
232	139	S	117.066	8.33	174.703	58.45	63.888
233	140	S	117.899	8.373	174.617	58.581	63.739
234	141	V	120.64	7.989	175.852	62.576	32.437
235	142	W	124.233	8.064	175.744	57.276	29.528
236	143	K	123.779	7.885	175.47	55.857	33.37
237	144	R	122.728	8.12	176.145	56.104	30.611
238	145	E	123.013	8.483	175.845	56.7	30.375
239	146	T	120.159	7.813		63.196	70.675

Table S2



Mutation	Primer	Sequence (5' → 3')
V166A	Fwd	AAATTTATTGcGAAGAAGAATCCACATC
	Rev	AAGAGGTGGCTCTCTTTTTTC
W175A	Fwd	TCATTCACAAgcGGGTGATATGAAACTC
	Rev	TGTGGATTCTTCTTCACAATAAATTTAAG
L191V	Fwd	GAAGAGGTCTgTTGAAGTTTGG
	Rev	ACAATCTGTAACTTTAAGTAGAG
R207Q	Fwd	AAGGAAGTCCaACAGGAAAACC
	Rev	TGCTTCTTCTAATGCTTCTTG
K221E	Fwd	GAAATTTGATgAAAAAGTAAAAGAATTGC
	Rev	TTCTGTTTCATTTTTTTCTCGG
K222E	Fwd	ATTTGATAAAgAAGTAAAAGAATTGCGG
	Rev	TTCTTCTGTTTCATTTTTTTCTCG
R228E	Fwd	AGAATTGCGGgaAGCAGTAAGAAG
	Rev	TTTACTTTTTTATCAAATTTCTTCTGTTTC
R228X	Fwd	GCGGGATCCATGGAATTTGATTATGTAATATGCG
	Rev	TAATTGCGGCCGCTCACCGCAATTCTTTTACTTTTTTATC
W235X	Fwd	GCGGGATCCATGGAATTTGATTATGTAATATGCG
	Rev	TAATTGCGGCCGCTCACACGCTGCTTCTTACTGC