Supporting Information

Özen et al. 10.1073/pnas.1414063111

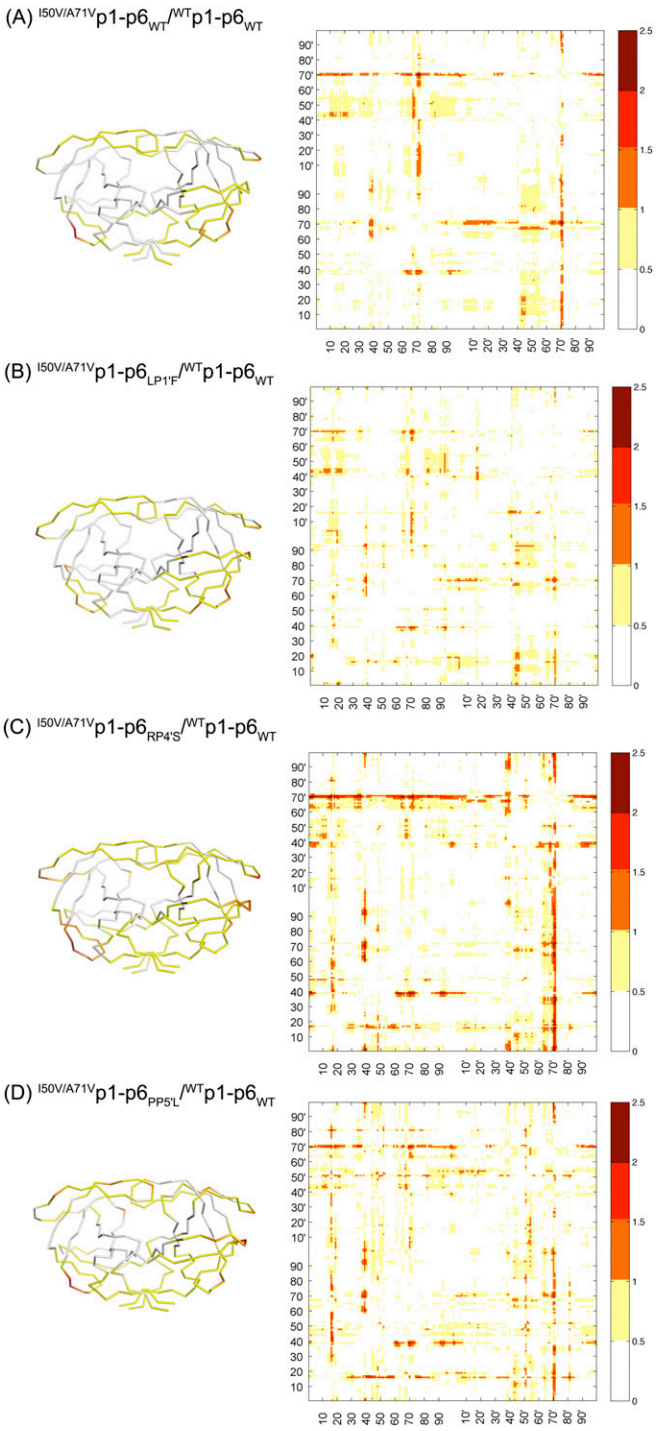

Fig. S1. Distance–difference maps showing the effect of protease–substrate coevolution on internal Cα–Cα distances in I50V/A71V protease with (*A*) WT and (*B–D*) mutant substrate with respect to the WT complex.

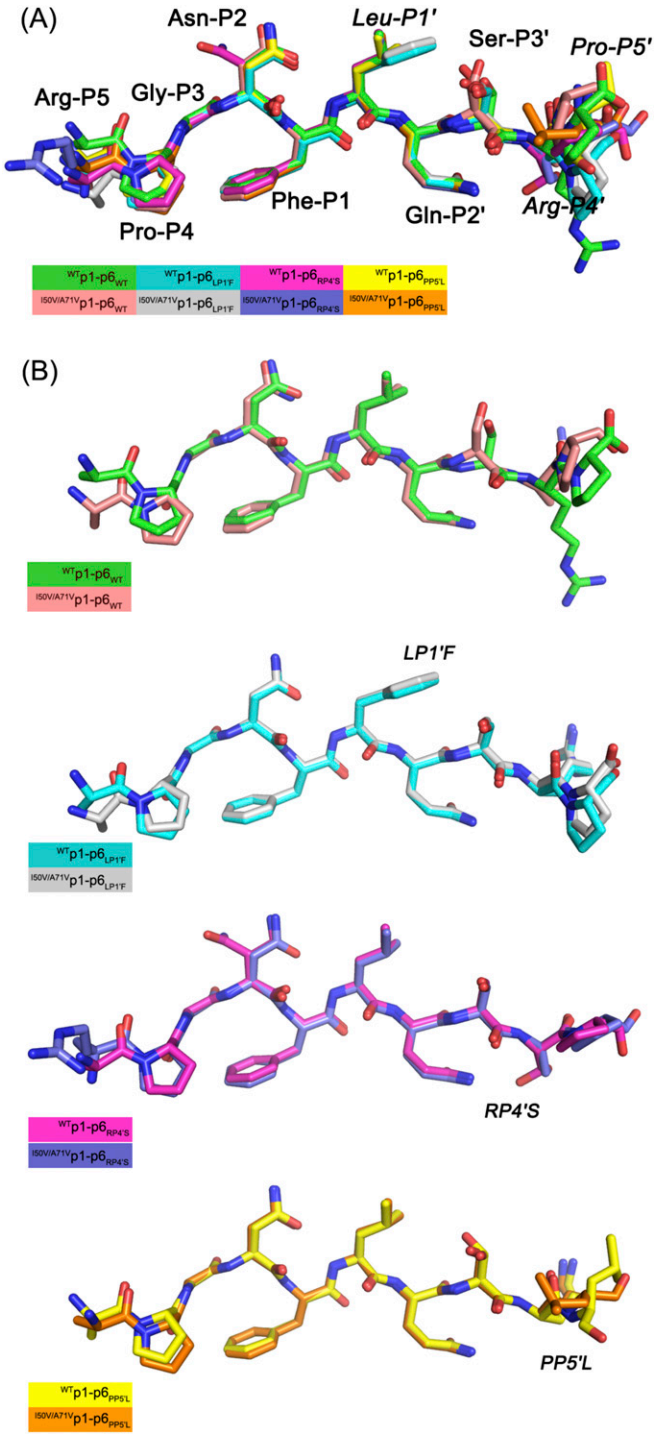

Fig. S2. (*A*) Superposed substrate conformations from eight protease–substrate cocrystal structures. (*B*) Pairwise superposed substrates bound to WT and I50V/A71V protease variants.

(A)

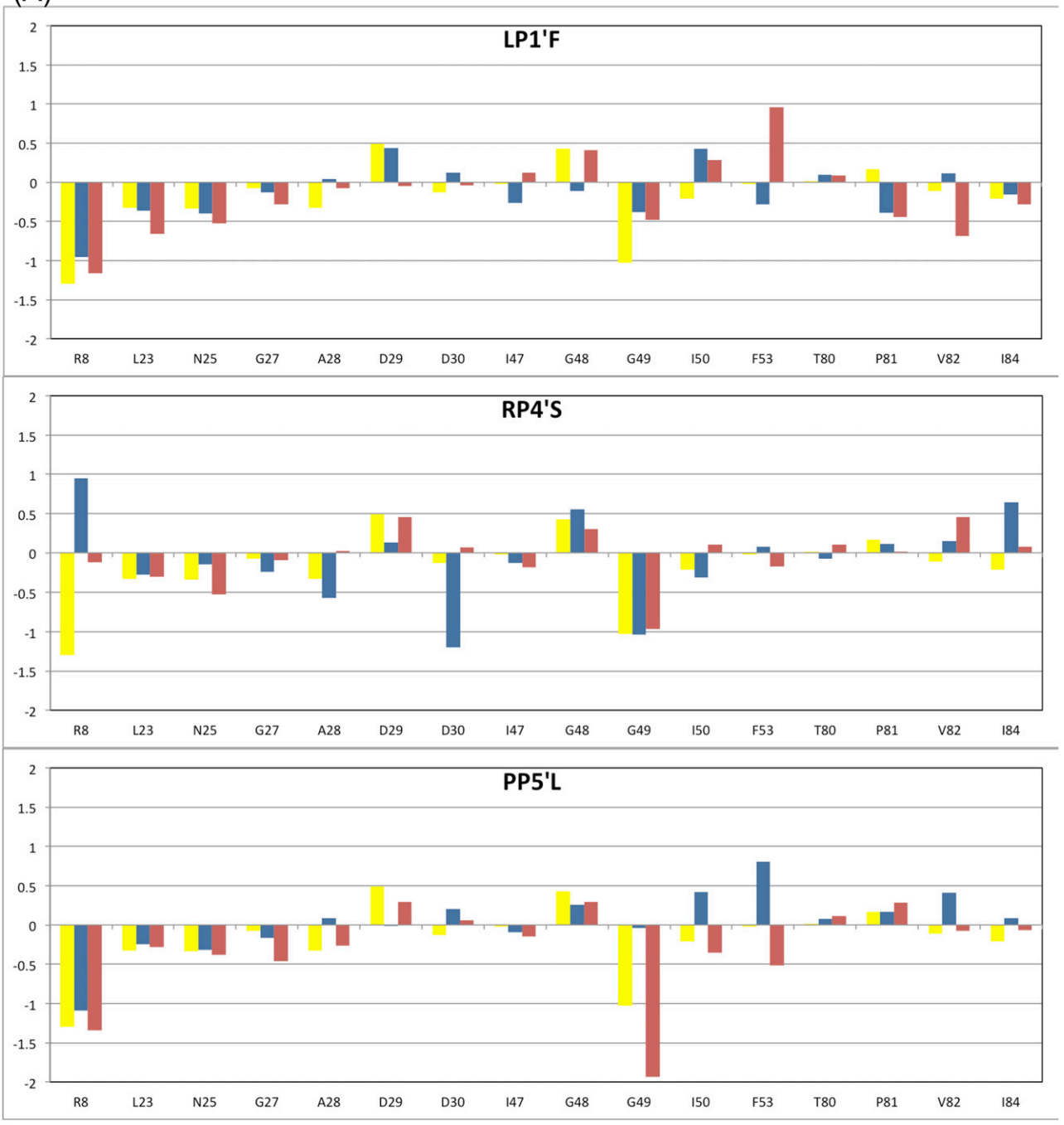

Fig. S3. (Continued)

Fig. S3. The changes in vdW contacts of protease residues (*A*) in monomer and (*B*) relative to WT complex. Negative values indicate enhanced interactions.

Table S1. Crystallographic statistics for HIV-1 protease with p1-p6 substrate cocrystal structures

	WT_{WT}	$WT_{LP1'F}$	$WT_{RP4'S}$	$WT_{PP5'L}$	$I50V_{WT}$	$I50V_{LP1'F}$	$I50V_{RP4'S}$	$I50V_{PP5'L}$
PDB code	1KJF (ref. 1)	4OBH (ref. 2)	4QJ9	4QJA	4QJ2	4QJ6	4QJ7	4QJ8
Resolution (A°)	2.00	1.85	1.84	1.54	2.14	1.50	1.67	2.00
Space group	P212121	P21	P212121	P212121	P21	P21	P21	P21
a (A°)	51.3	51.1	50.9	51.0	52.2	51.4	51.6	51.3
b (A°)	59.1	62.8	57.9	58.2	63.3	59.8	58.2	62.7
c (A°)	61.8	61.5	61.8	61.8	58.8	60.6	61.8	57.5
R_{merge} (%)	6.7	7.3	10.4	4.6	3.3	5.6	4.7	7.6
Completeness (%)	93.4	99.7	98.4	97.1	94.5	97.5	94.6	94
Total no. of reflections	41,786	113,050	127,633	195,712	69,773	220,012	119,749	64,360
No. of unique reflections	12,376	28,931	16,417	27,160	20,171	56,731	40,242	23,065
No. of molecules	1	2	1	1	2	2	2	2
R_{free} (%)	25.1	21.28	20.81	17.78	23.84	21.41	22.97	23.53
R_{factor} (%)	20.3	16.3	16.01	15.17	18.35	17.40	17.87	17.03
RMS in bond length (A°)	0.006	0.009	0.0093	0.0088	0.0097	0.0090	0.0089	0.0094
RMS angle (degrees)	1.3	1.423	1.492	1.362	1.462	1.341	1.402	1.463
No. of waters	101	215	161	198	279	421	404	214

1. Prabu-Jeyabalan M, Nalivaika E, Schiffer CA (2002) Substrate shape determines specificity of recognition for HIV-1 protease: Analysis of crystal structures of six substrate complexes. *Structure* 10(3):369–381.
2. Kolli M, Ozen A, Kurt-Yilmaz N, Schiffer CA (2014) HIV-1 protease-substrate coevolution in nelfinavir resistance. *J Virol* 88(13):7145–7154.

Table S2. Hydrogen bonds between the p1-p6 substrate and HIV-1 protease in cocrystal structures

Protease	Substrate	WT_{WT}	$I50V_{WT}$	$I50V_{LP1'F}$	$WT_{LP1'F}$	$I50V_{RP4'S}$	$WT_{RP4'S}$	$I50V_{PP5'L}$	$WT_{PP5'L}$
Arg8′ NH2	P4 Pro O	4.0	3.01	2.72	2.73	2.94	2.97	2.89	2.94
Gly48 O	P3 Gly N	3.01	2.97	3.03	2.77	2.90	3.03	2.88	3.03
Asp29 N	P3 Gly O	2.79	2.87	2.82	2.86	2.86	2.92	2.82	2.80
Gly48 O	P2 Asn N	3.05	2.86	2.88	2.74	2.85	2.88	2.96	2.95
Gly27 O	P1 Leu/Phe N	2.93	2.90	2.81	2.88	2.90	2.80	2.78	2.88
Asn-25′ ND2	P1 Leu/Phe O	2.7	4.0/2.7	2.72	2.74	3.9/2.9	3.7/2.7	2.70	2.76
Gly27 O	P2′ Gln N	2.95	2.94	2.97	3.07	2.91	2.83	2.95	2.98
Asp-30′ N	P2′ Gln OE1	2.74	2.72	2.89	2.86	2.81	2.82	2.83	2.77
Asp-30′ OD2	P2′ Gln NE2	2.95	2.73	2.79	3.02	2.81	4.6/2.8	2.98	2.88
Asp-29 N	P2′ Gln O	3.07	3.07	3.12	3.15	3.04	3.01	3.06	3.10
Gly-48′ O	P3′ Ser N	3.00	2.58	2.83	2.89	2.81	2.89	2.84	2.85
Gly-48′ N	P3′ Ser O	2.78	2.91	3.07	3.26	2.87	3.01	3.02	3.02
Asp-29′ OD2	P4′ Arg/Ser N	3.8	2.95	2.85	2.95	2.96	3.06	2.84	2.94
Asp-30′ OD2	P4′ Arg NE/Ser OG	3.02				2.59	2.6/2.6		

Bonds with acceptor–donor distances less and more than 3 Å are indicated by red and yellow, respectively. Distances greater than 3.8 Å are not considered bonded but are listed for comparison in green.