SUPPORTING INFORMATION TABLE OF CONTENTS

Table S1: Supplemental Parameters for Table 1 (Confirmed Primary hits against KSHV Pr)

Fragment ID	MW	LE (KSHV Pr)	LogD	LipE (pIC_{50} – LogD)	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
1	210.7	0.46	3.12	1.17	33.45	170.55	N=C1NC(=CS1)c2ccc(Cl)cc2
2	212.2	0.34	2.81	0.58	38.91	163.16	Nc1nc(cs1)c2cc(F)c(F)cc2
3	218.2	0.33	2.85	0.46	38.91	157.18	FC(F)(F)c1ccc2nc(N)sc2c1
4	226.7	0.37	3.87	-0.14	24.39	190.69	Cc1ccc(cc1Cl)NC2=NCCS2
5	196.0	0.49	2.84	0.63	15.79	130.91	Brc1ccc2ccnc2c1
6	210.1	0.43	3.35	0.02	15.79	147.47	Brc1cc2c(cc1)ncc2C
7	267.3	n/a	0.96	2.29	62.32	237.22	O=C(O)c1cc2cc(ccc2n1)OCc3ccccc3
8	187.2	0.41	2.52	1.57	39.16	177.26	Nc1cc2c(cc1)oc3CCCCc23
9	233.3	0.35	3.58	0.47	29.02	212.71	CN(C)c2ncnc1sc3CCCCc3c12
10	254.3	0.27	3.08	0.63	72.19	233.67	O=C(Nc1ccccc1C(N)=O)c2ccc(C)cc2
11	212.2	n/a	3.56	-0.56	55.12	198.13	NC(=O)c2ccccc2Nc1ccccc1

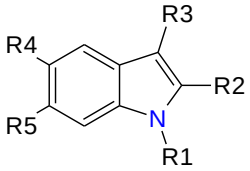
Fragment ID	MW	LE (KSHV Pr)	LogD	LipE (pIC_{50} – LogD)	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
12	290.6	0.25	0.76	2.59	50.44	208.85	OC(=O)c1cc(oc1C(F)(F)F)c2ccc(Cl)cc2
13	277.7	0.26	3.78	-0.31	47.05	248.79	Cc2nc(COC)cc(NCc1ccc(Cl)cc1)n2
14	156.6	0.48	2.10	1.31	20.23	139.20	Clc1ccc(CCO)cc1
15	256.1	0.30	3.75	-0.28	42.35	195.37	Oc1ccc(cc1)Oc2ncc(Cl)cc2Cl
16	221.2	0.30	-0.48	3.92	77.84	190.44	O=C2C(O)C(O)C(=O)N2Cc1ccccc1
17	255.0	0.39	1.96	1.67	52.05	155.80	Nc1cc(cc(Br)c1N)C(F)(F)F
18	179.2	0.37	1.18	2.21	33.45	170.55	O=C(N1CCCCC1)c2ccco2
19 Neg. control	201.1	-----	1.94	1.06	28.68	156.72	FC(F)(F)c1cc2c(cc1)nnc2N

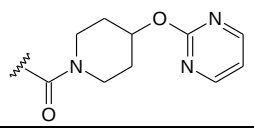
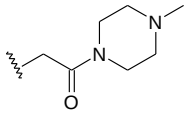
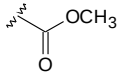
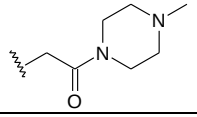
Table S2: Supplemental Parameters for Table 2 (Phenylaminothiazoles)

Fragment ID	MW	LE (KSHV Pr)	LogD	LipE (pIC_{50} – LogD)	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
20	176.24	n/a	2.52	0.40	38.91	153.30	Nc1nc(cs1)c2ccccc2
21	336.05	n/a	3.29	-0.36	38.91	171.18	Br.Nc1nc(cs1)c2ccc(Br)cc2
22	190.26	n/a	3.03	0.08	38.91	169.86	Nc1nc(cs1)c2ccc(C)cc2
23	245.12	0.44	3.73	0.69	38.91	180.37	Nc1nc(cs1)c2cc(Cl)c(Cl)cc2
24	255.13	0.41	3.29	0.53	38.91	171.18	Nc1nc(cs1)c2cc(Br)ccc2
25	326.37	0.28	1.72	2.80	71.45	278.37	O=C(O)c1cc(ccc1)Nc2nc(cs2)c3ccc(OC)cc3
26	456.15	0.30	2.64	2.03	62.22	270.70	Br.O=C(O)c1cc(ccc1)Nc2nc(cs2)c3ccc(Br)cc3
27	330.79	0.27	2.45	1.85	62.22	266.36	O=C(O)c1cc(ccc1)Nc2nc(cs2)c3ccc(Cl)cc3
28	330.79	031	2.36	2.56	62.22	266.36	O=C(O)c3ccc(Nc1nc(cs1)c2ccc(Cl)cc2)cc3
29	375.24	0.30	2.53	2.15	62.22	270.70	O=C(O)c3ccc(Nc1nc(cs1)c2ccc(Br)cc2)cc3
30	365.23	0.31	3.08	2.09	62.22	279.89	O=C(O)c1cc(ccc1)Nc2nc(cs2)c3ccc(Cl)c(Cl)c3

Fragment ID	MW	LE (KSHV Pr)	LogD	LipE (pIC_{50} – LogD)	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
31	365.23	0.31	2.96	2.21	62.22	279.89	O=C(O)c3ccc(Nc1nc(cs1)c2ccc(Cl)c(Cl)c2)cc3
32	296.34	0.24	1.76	1.82	62.22	252.82	O=C(O)c3ccc(Nc1nc(cs1)c2ccccc2)cc3
33	442.17	0.25	5.40	-1.72	34.15	269.25	Br.Brc1ccc(cc1)c3csc(Nc2cc(OC)ccc2)n3
34	456.15	0.22	5.18	-1.72	43.39	267.63	Br.Brc1ccc(cc1)c4csc(Nc2cc3OCOc3cc2)n4

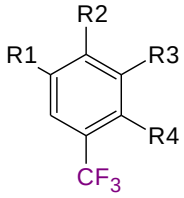
Table S3: Indoles

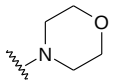
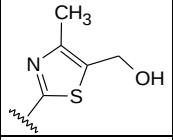
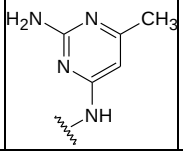
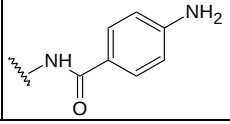


Fragment ID	R1	R2	R3	R4	R5	KSHV Pr IC_{50} (μM)	MW	LE
35	CH_3		H	H	Cl	412.0	370.84	0.18
36		H	H	H	Cl	466.0	291.78	0.23
37	CH_2CO_2H	H	H	H	Br	> 500	254.08	0.33
38	H		H	H	Br	> 500	254.08	0.33
39		H	H	H	Br	> 500	336.23	0.22
40	$CH_2CH_2CO_2H$	H	H	H	Br	> 500	268.11	0.28
41	H	H		Br	H	> 500	224.06	0.36

Fragment ID	LogD	LipE	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
35	2.53	0.86	60.26	320.54	O=C(c2cc1ccc(Cl)cc1n2C)N4CCC(Oc3ncccn3)CC4
36	1.68	1.65	28.48	261.22	O=C(Cn2ccc1ccc(Cl)cc12)N3CCN(C)CC3
37	-0.88	4.15	42.23	175.09	O=C(O)Cn2ccc1ccc(Br)cc12
38	2.76	0.51	42.10	175.44	O=C(OC)c1cc2ccc(Br)cc2n1
39	1.93	1.21	28.48	265.57	O=C(Cn2ccc1ccc(Br)cc12)N3CCN(C)CC3
40	-0.53	3.55	42.23	191.89	O=C(O)CCn2ccc1ccc(Br)cc12
41	2.55	0.58	32.86	149.89	Brc1cc2c(cc1)ncc2C=O

Table S4: Trifluoromethylbenzenes



Fragment ID	R1	R2	R3	R4	KSHV Pr IC_{50} (μM)	MW	LE	LogD	LipE	TPSA ($Å^2$)	Molecular Volume ($Å^3$)	SMILES
42	Br		H	H	221	310.11	0.30	3.51	0.15	12.47	211.36	FC(F)(F)c1cc(Br)c(cc1)N2CCOCC2
43	Br	NH_2	H	H	336	240.02	0.41	2.79	0.68	26.02	144.51	Nc1ccc(cc1Br)C(F)(F)F
44	H		H	H	> 500	273.70	0.25	3.03	0.18	33.12	214.69	FC(F)(F)c1ccc(cc1)c2nc(C)c(CO)s2
45	H	H		H	> 500	268.24	0.24	2.63	0.56	63.83	218.69	Cc2cc(Nc1cc(ccc1)C(F)(F)F)nc(N)n2
46	NO_2	NH_2	H	Cl	> 500	240.57	0.30	3.22	-0.05	71.85	163.50	FC(F)(F)c1cc(c(N)cc1Cl)[N+]([O-])=O
47	H	Br	NH_2	H	> 500	240.02	0.37	2.79	0.37	26.02	144.51	Nc1cc(ccc1Br)C(F)(F)F
48		H	H	H	> 500	280.25	0.21	3.11	-0.11	55.12	229.42	O=C(Nc1cc(ccc1)C(F)(F)F)c2ccc(N)cc2

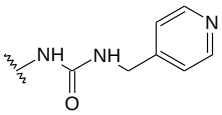
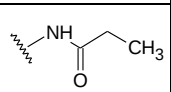
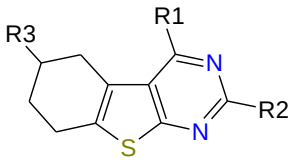
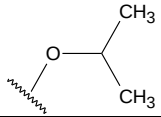
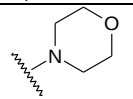
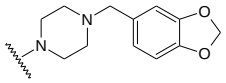
Fragment ID	R1	R2	R3	R4	KSHV Pr IC_{50} (μM)	MW	LE	LogD	LipE	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
49		H	H	H	> 500	295.27	0.20	2.48	0.52	54.02	243.18	FC(F)(F)c1cc(ccc1)NC(=O)NCc2ccncc2
50	Br	H	CH_3	H	> 500	239.04	0.32	4.13	-1.42	0.00	149.79	FC(F)(F)c1cc(C)cc(Br)c1
51	NH_2	NH_2	H	H	> 500	176.14	0.31	1.19	1.47	52.05	137.92	Nc1ccc(cc1N)C(F)(F)F
52	Br	H	H	H	> 500	225.01	0.30	3.62	-1.26	0.00	133.23	FC(F)(F)c1cc(Br)ccc1
53	Br	CH_3	H	H	> 500	239.04	0.27	4.13	-1.86	0.00	149.79	Cc1ccc(cc1Br)C(F)(F)F
54	Cl		H	H	> 500	251.63	0.18	3.39	-1.34	29.10	193.63	Clc1cc(ccc1NC(=O)CC)C(F)(F)F

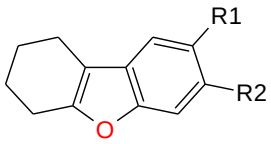
Table S5: Tetrahydrobenzothienopyrimidines



Fragment ID	R1	R2	R3	KSHV Pr IC_{50} (μM)	MW	LE	LogD	LipE	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
55		H	H	71.3	248.34	0.34	4.09	0.06	35.02	225.73	CC(C)Oc2ncnc1sc3CCCCc3c12
56*		CH_3	H	85.1	379.43	0.28	3.85	0.22	38.26	261.50	O=C(O)C(=O)O.Cc1nc(c2c(n1)sc3CCCCc23)N4CCOCC4
57‡		CH_3	H	136.0	459.01	0.18	5.20	-1.33	50.73	377.43	Cl.Cc1nc(c2c(n1)sc3CCCCc23)N6CCN(Cc4ccc5OCOc5c4)CC6
58	OH	H	H	> 500	206.26	0.32	3.17	0.04	46.01	174.82	Oc2ncnc1sc3CCCCc3c12
59	OH	H	CH_3	> 500	220.29	0.19	3.46	-1.41	46.01	191.40	Oc2ncnc1sc3CCC(C)Cc3c12

*** Contains: ethanedioate**
‡ Contains: HCl

Table S6: Tetrahydrodibenzofurans



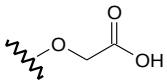
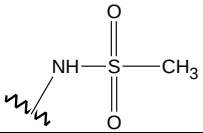
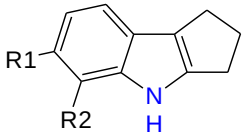
Fragment ID	R1	R2	KSHV Pr IC_{50} (μM)	MW	LE	LogD	LipE	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
60		H	> 500	246.26	0.25	-0.37	3.57	59.67	218.76	O=C(O)COc1cc2c(cc1)oc3CCCCc23
61		CH_3	> 500	279.35	0.23	2.23	0.69	59.31	242.92	CS(=O)(=O)Nc1cc2c(cc1C)oc3CCCCc23

Table S7: Tetrahydrocyclopentaindole



Fragment ID	R1	R2	KSHV Pr IC_{50} (μM)	MW	LE	LogD	LipE	TPSA (Å^2)	Molecular Volume (Å^3)	SMILES
62	Cl	Cl	76.0	226.10	0.41	4.05	0.07	15.79	179.66	Clc3ccc1c(nc2CCCc12)c3Cl

Table S8: Michaelis-Menten parameters, KSHV Pr, P6R substrate + Fragment 30

[Fragment 30], μM	V_{max} (μM/sec)	K_m (μM)
0.00	(32.69 ± 3.08) x 10^{-5}	11.34 ± 2.33
1.56	(26.39 ± 2.88) x 10^{-5}	9.80 ± 2.49
3.13	(23.86 ± 3.32) x 10^{-5}	12.24 ± 3.58
6.25	(14.05 ± 1.82) x 10^{-5}	6.42 ± 2.40
12.5	(8.87 ± 1.84) x 10^{-5}	8.43 ± 4.39
25.0	(5.84 ± 7.53) x 10^{-5}	23.52 ± 49.27

Figure S1

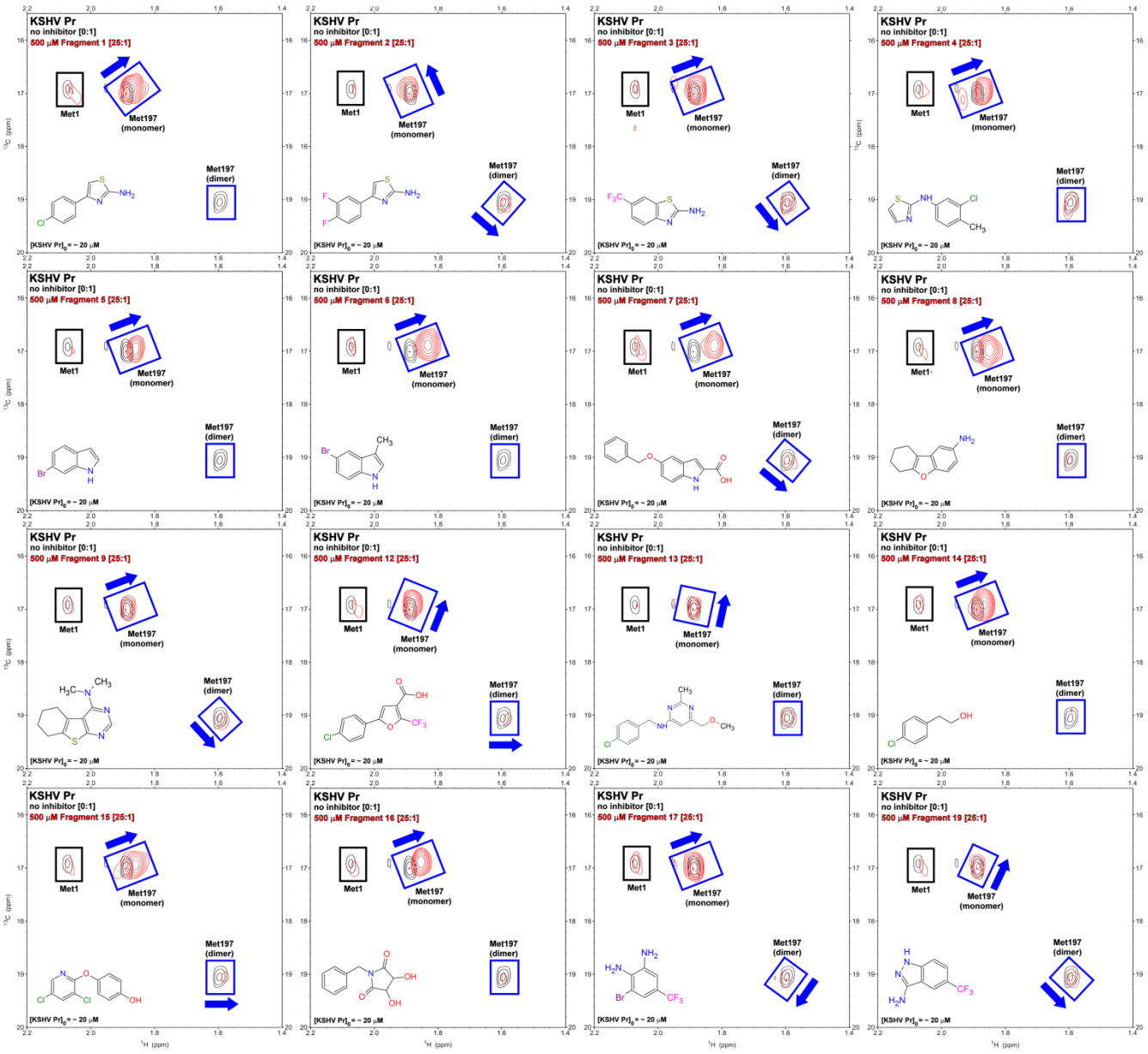


Figure S1:

Overlays of the $^{13}C/^{1}H$-HSQC spectra of selectively ^{13}C-methionine labeled wild-type KSHV Pr in the absence (black) and presence (red) of 25x molar excess of the Table 1 fragments. Loss of intensity of the M197 dimer peak with a concomitant shift of the M197 monomer peak are hallmarks of dimer disruption.

Figure S2

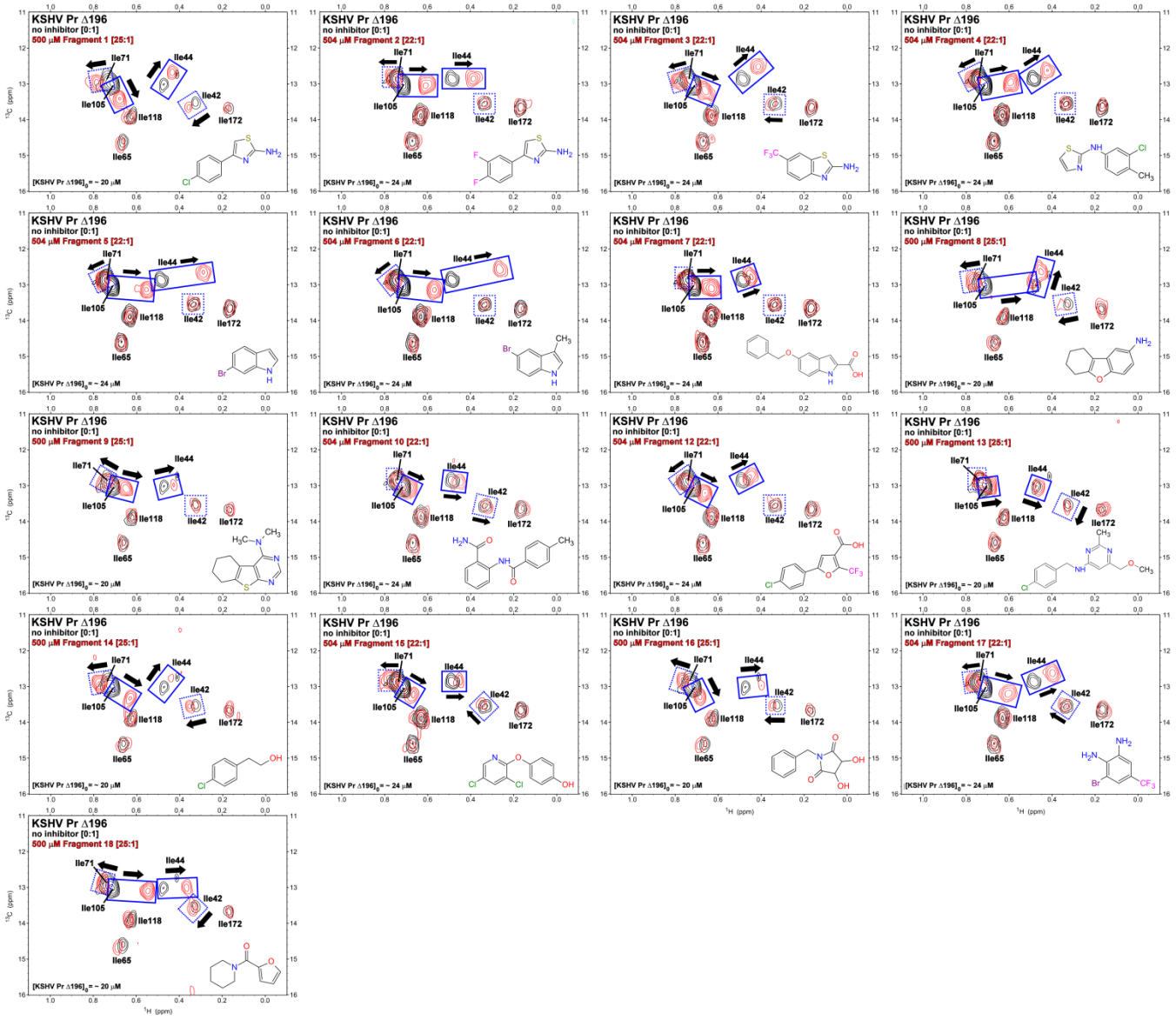


Figure S2:

Overlays of the $^{13}C/^{1}H$-HSQC spectra of selectively ^{13}C-Isoleucine labeled KSHV Pr Δ196 in the absence (black) and presence (red) of 20 - 25x molar excess of the Table 1 fragments. Perturbations of the Ile44 and Ile105 methyl group resonances indicate fragment binding at the dimer interface near the hot spot Trp109.

Figure S3A

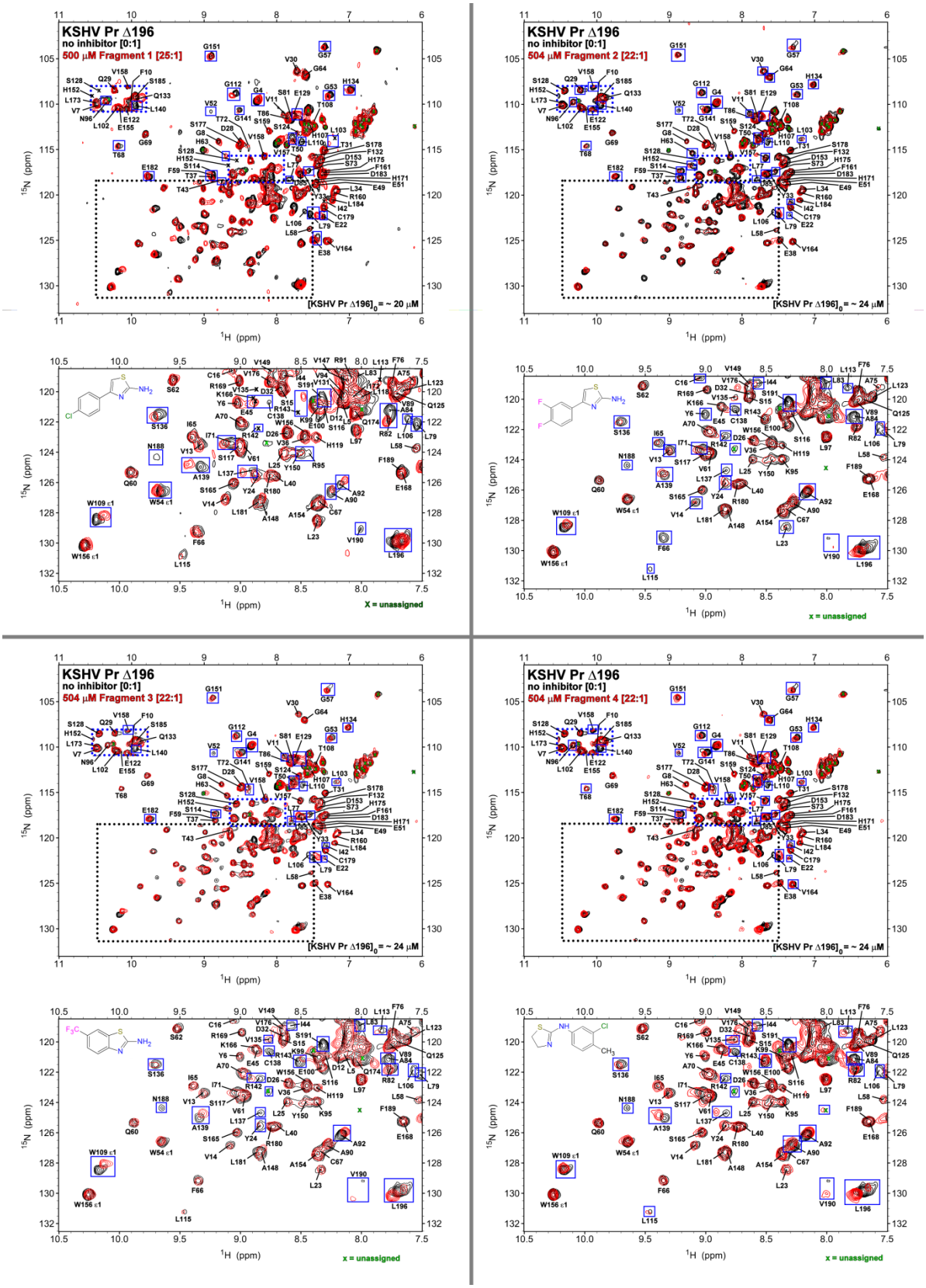

KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 1 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 2 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 3 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 4 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
15N (ppm)
1H (ppm)
X = unassigned
x = unassigned

Figure S3B

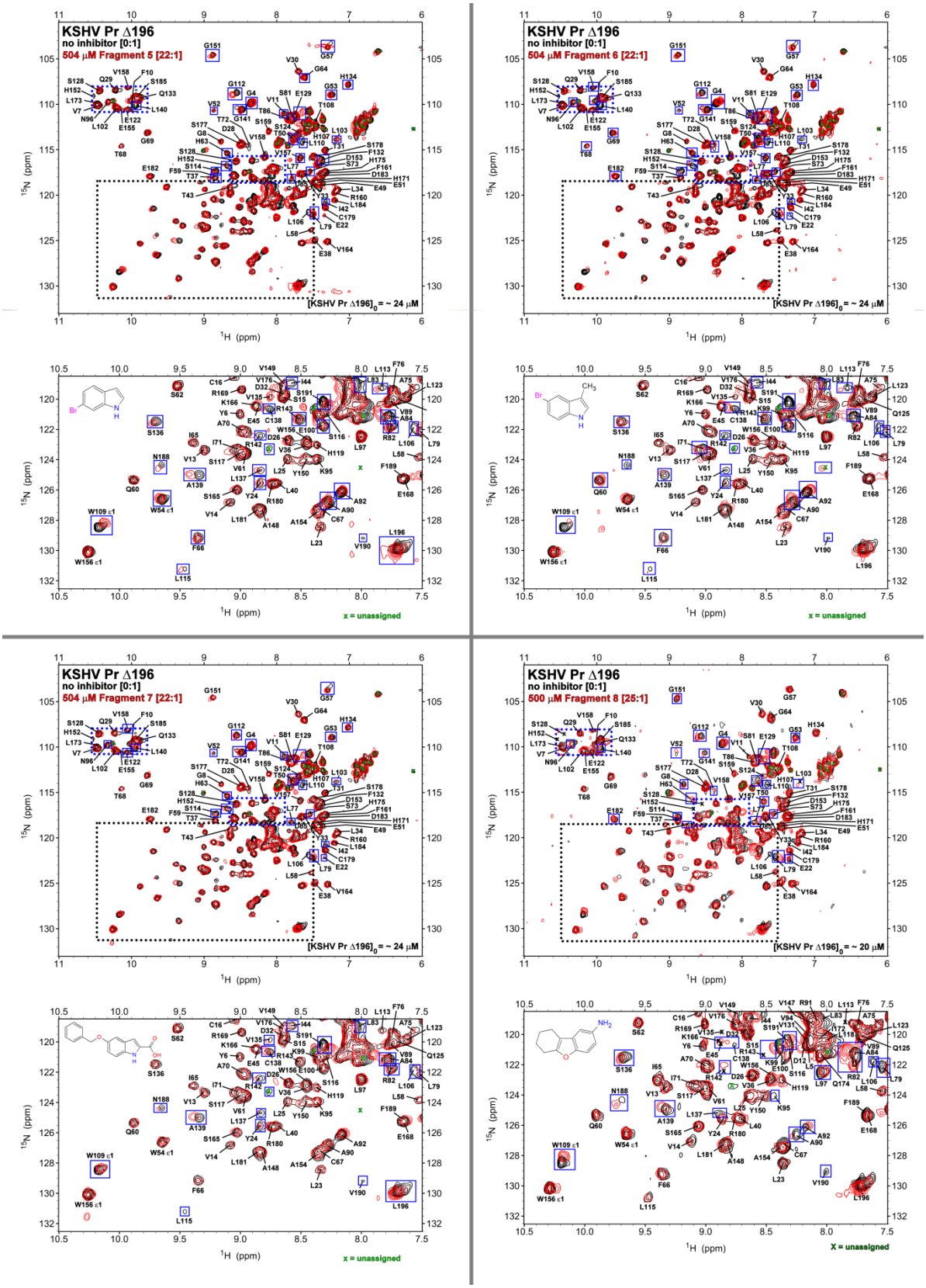

KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 5 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 6 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 7 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 8 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
15N (ppm)
1H (ppm)
x = unassigned
X = unassigned

Figure S3C

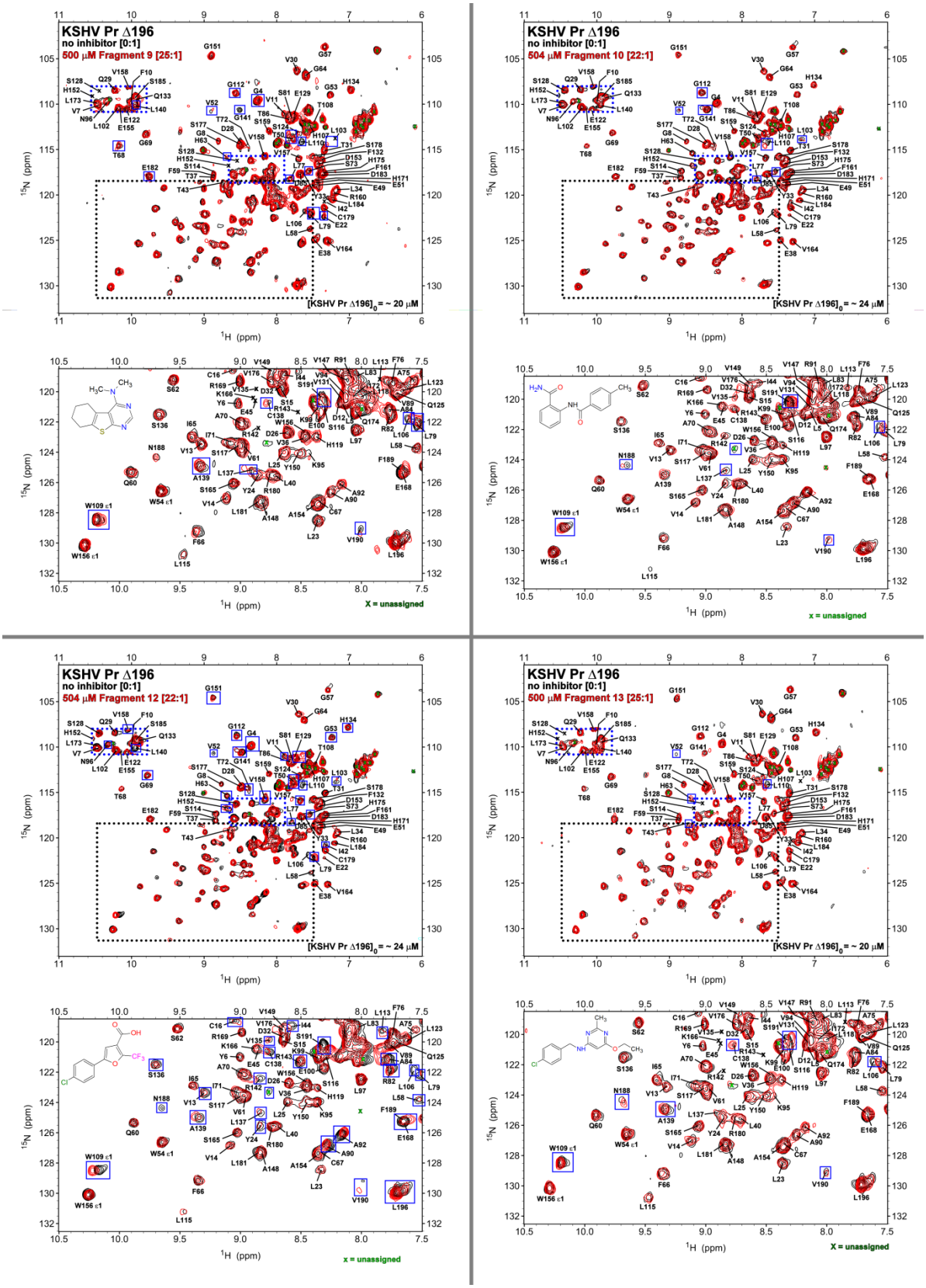

KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 9 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 10 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 12 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 13 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
15N (ppm)
1H (ppm)
X = unassigned
x = unassigned

Figure S3D

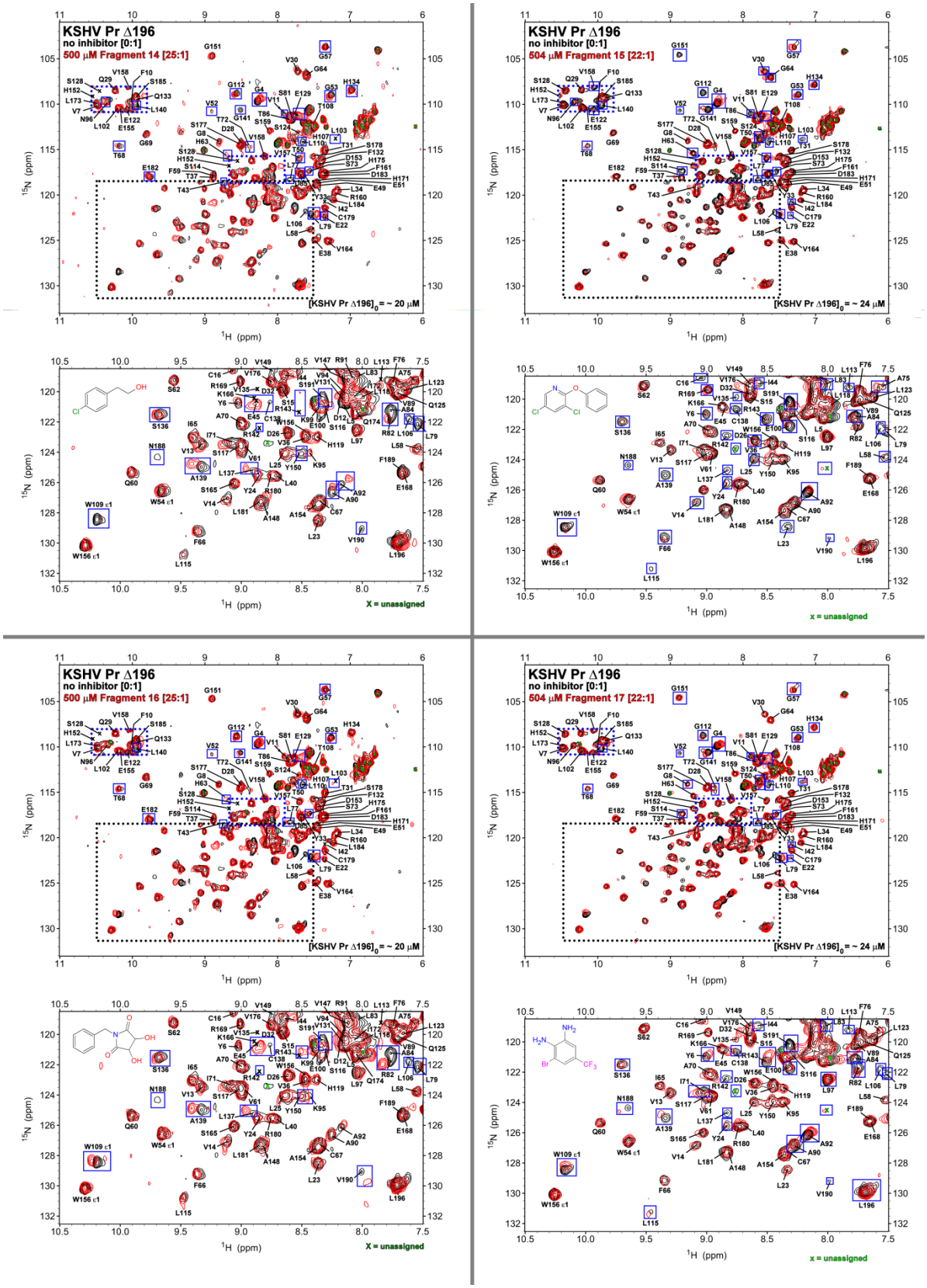
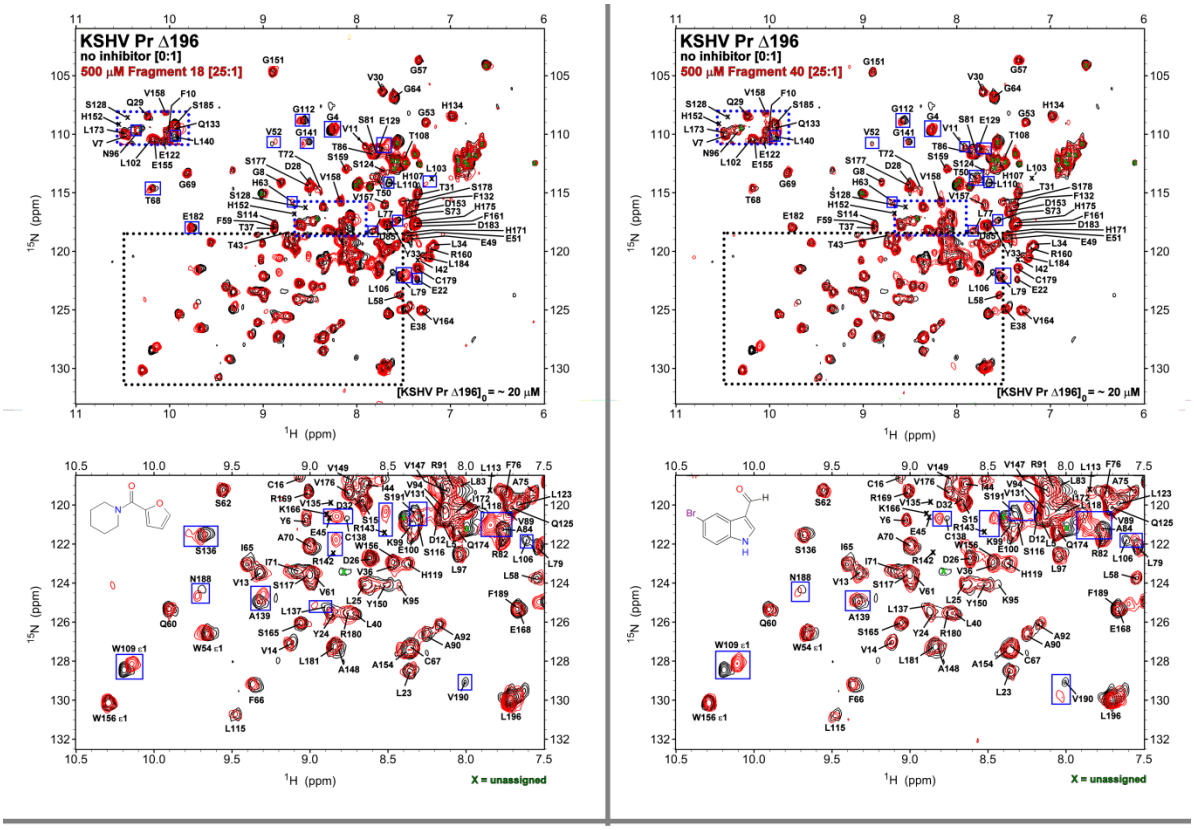

KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 14 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
X = unassigned
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 15 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
x = unassigned
KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 16 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
X = unassigned
KSHV Pr Δ196
no inhibitor [0:1]
504 μM Fragment 17 [22:1]
[KSHV Pr Δ196]0 = ~ 24 μM
x = unassigned
15N (ppm)
1H (ppm)

Figure S3E



Figures S3A-S3E:

Overlays of the $^{15}N/^{1}H$-HSQC spectra of uniformly ^{15}N-labeled KSHV Pr Δ196 in the absence (black) and presence (red) of 20 - 25x molar excess of the Table 1 fragments. Top half of each panel: full $^{15}N/^{1}H$-HSQC spectra with the crowded middle resonances (blue dotted panel) at the inset. The black dotted regions of the spectra are zoomed in the lower half of each panel.

Figure S4

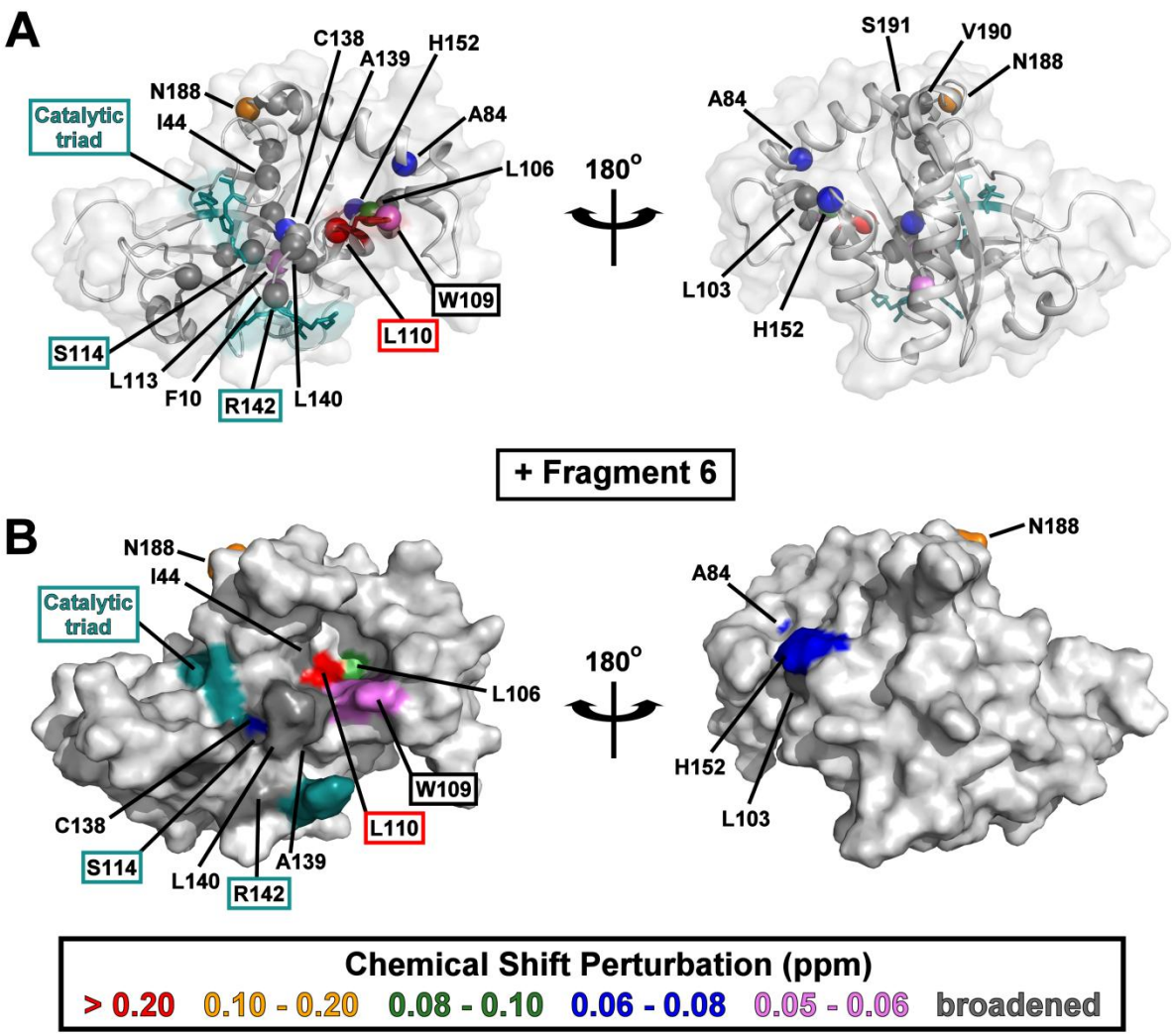


Figure S4:

The structure of monomeric KSHV Pr Δ196 (PDB: 3NJQ) with the $^{15}N/^{1}H^{N}$-HSQC chemical shift perturbations for Fragment **6** indicated by color. Backbone amide resonances which displayed peak broadening upon addition of fragments are indicated in dark gray. Amide backbone nitrogen atoms are shown as colored spheres in (**a**), while surfaces are displayed in (**b**). The catalytic triad (His46, Ser114, and His134) and oxyanion hole (Arg142 and Arg143) residues are highlighted in cyan. Left and right structures are rotated 180° about the vertical axis.

Figure S5

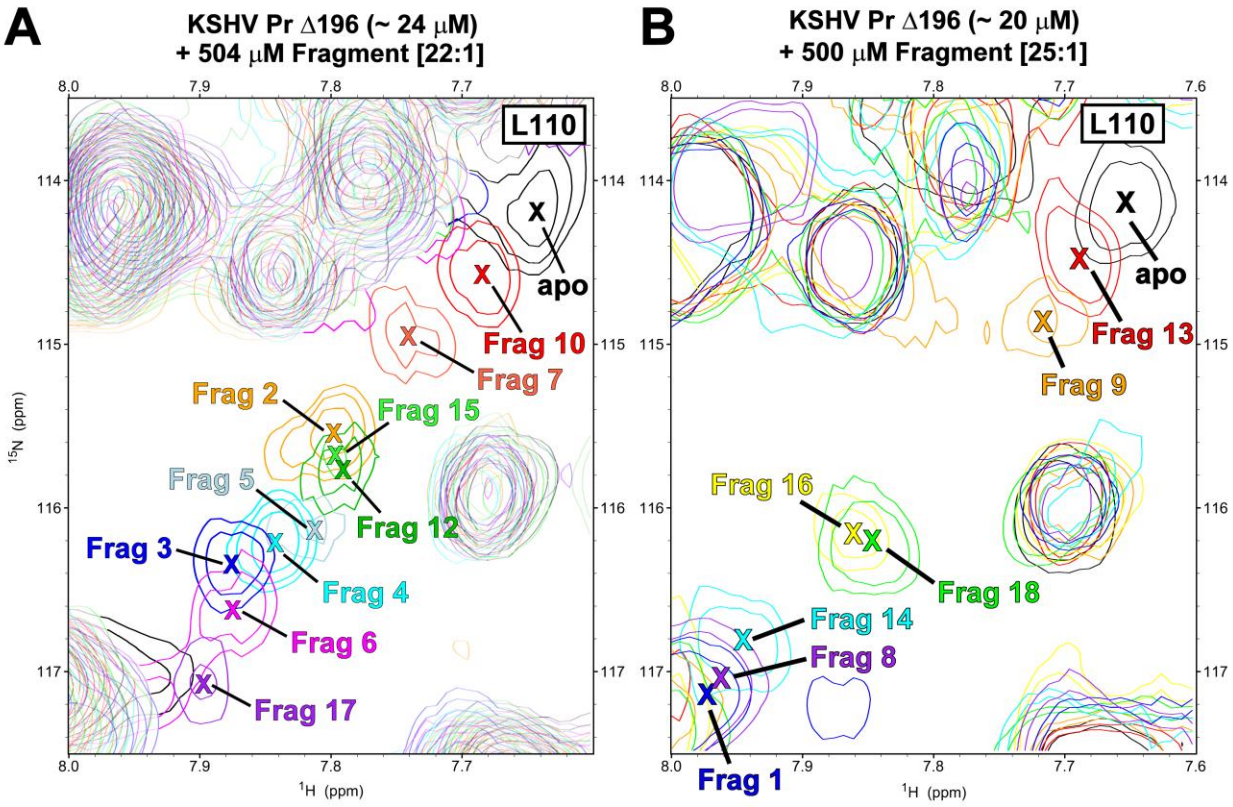


Figure S5:

Backbone ^{15}N/^{1}H-HSQC spectra of KSHV Pr Δ196 in the absence (black) and presence (colors) of 20-25x molar excess Table 1 Fragments, focusing on the Leu110 backbone amide peak. (**A**) Apo vs. Fragments **2** - **7**, **10**, **12**, **15**, and **17**. (**B**) Apo vs. Fragments **1**, **8**, **9**, **13**, **14**, **16**, and **18**. The linear nature of the chemical shift perturbations observed for the Leu110 amide peak are likely influenced more by the rotameric effects of the Trp109 indole conformation than to direct binding interactions with the fragments.

Figure S6

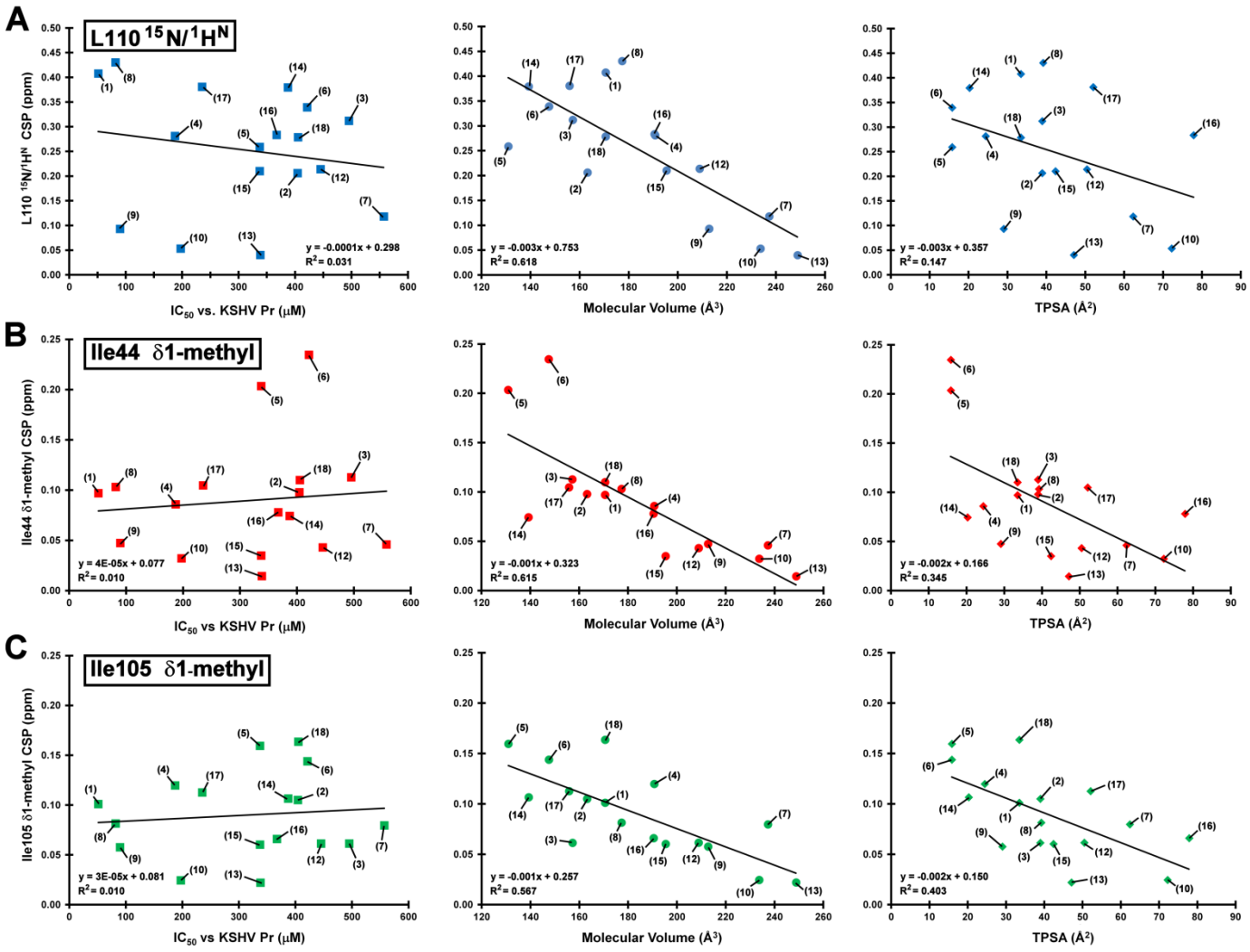


Figure S6:

Chemical shift perturbations (CSP) plotted against (**left**) IC_{50} against KSHV Pr, (**middle**) molecular volume, and (**right**) total polar surface area (TPSA) of the Table 1 Fragments. (**A**) Leu110 backbone amide CSPs. (**B**) Ile44 δ1-methyl CSPs. (**C**) Ile105 δ1-methyl CSPs. Table 1 Fragment numbers are indicated. The best correlations are for the molecular volume, indicating that smaller fragments have a larger effect on the CSP, regardless of its chemical scaffold.

Figure S7

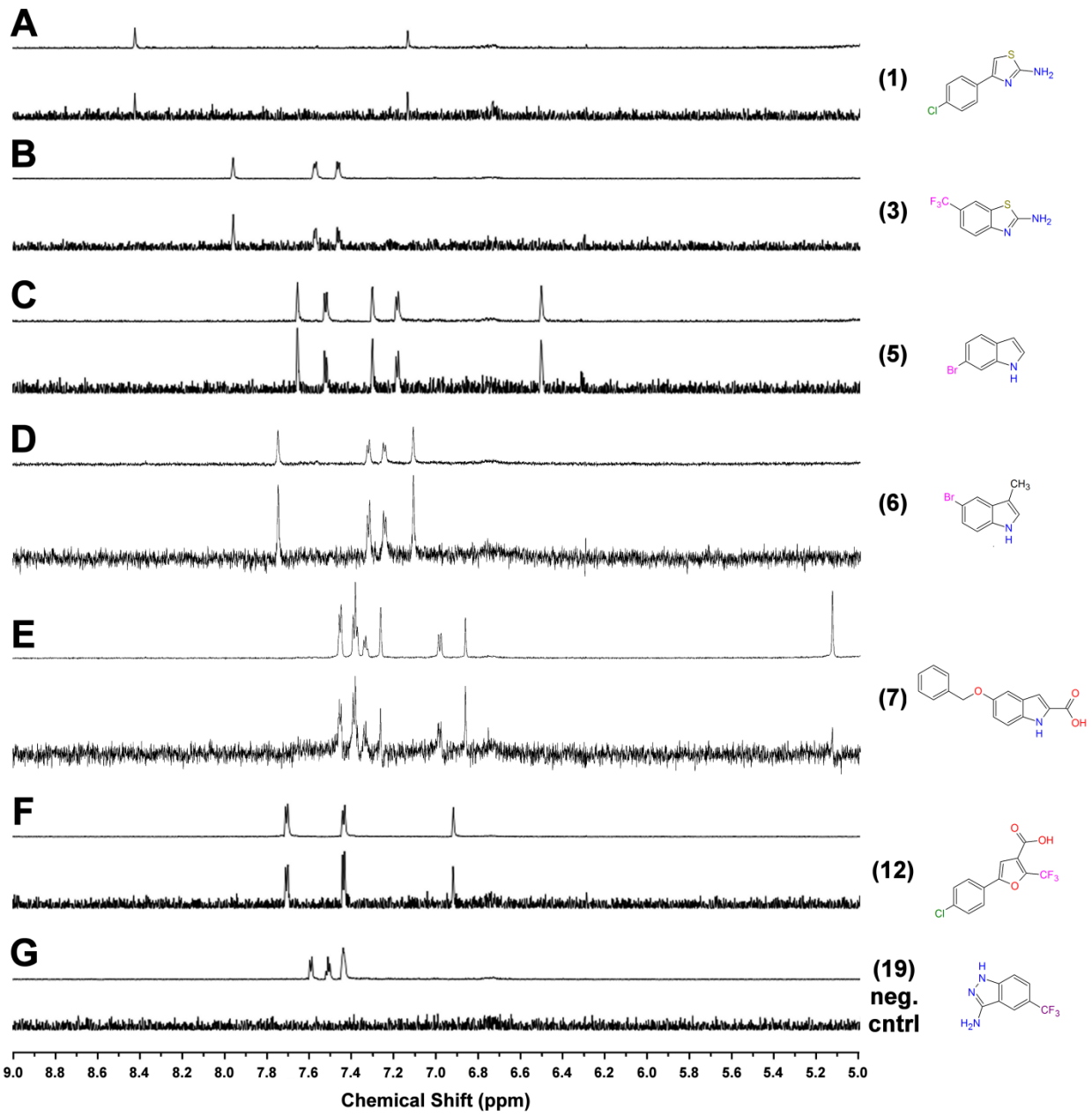


Figure S7:

The 1D-STD NMR spectra of Fragments (**A**) **1**, (**B**) **3**, (**C**) **5**, (**D**) **6**, (**E**) **7**, (**F**) **12**, and (**G**) **19**, focusing on the aromatic region of the ^{1}H spectrum. The top portion of each pair is the off-resonance (control) experiment, while the bottom corresponds to the difference spectrum. The on-resonance pulse was set to 0.9 ppm. Fragment **19** acts as the negative control.

Figure S8

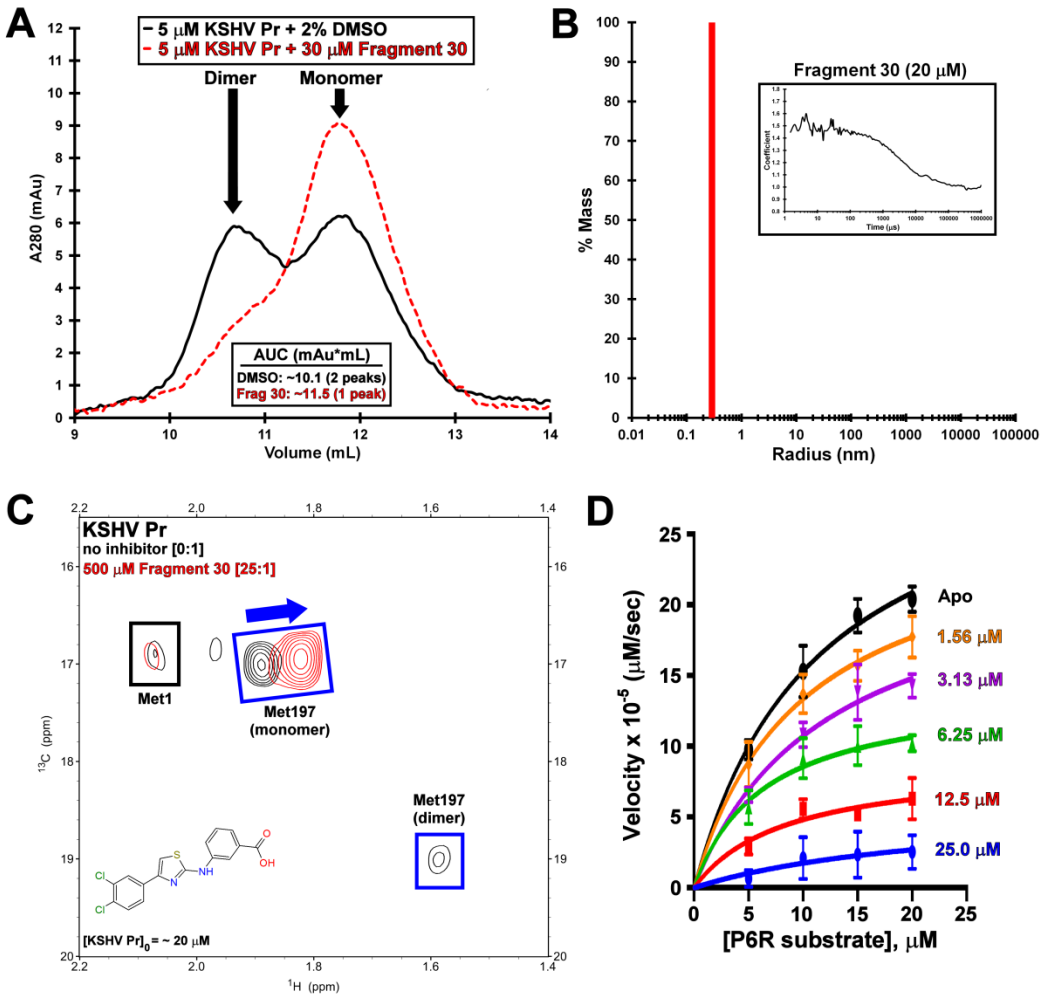


Figure S8:

(**a**) Overlays of analytical size-exclusion chromatograms of 5 μM KSHV Pr with 2% DMSO (black) and 30 μM Fragment 30 (dotted red). The KSHV Pr displays a nearly 1:1 mixture of dimeric and monomeric states under the control conditions. Addition of Fragment 30 shifts the equilibrium to the monomeric state. (**b**) Dynamic light scattering data for 20 μM Fragment **30** in enzyme assay buffer indicates no aggregate formation. In the inset is the raw autocorrelation curve. (**c**) Overlays of the $^{13}C/^{1}H$-HSQC spectra of selectively ^{13}C-methionine labeled KSHV Pr in the absence (black) and presence (red) of ~25x molar excess of Fragment **30** validate the size-exclusion chromatogram results. (**d**) Michaelis-Menten binding curves for KSHV Pr + P6R substrate with varying concentrations of Fragment **30**, as indicated.

Figure S9

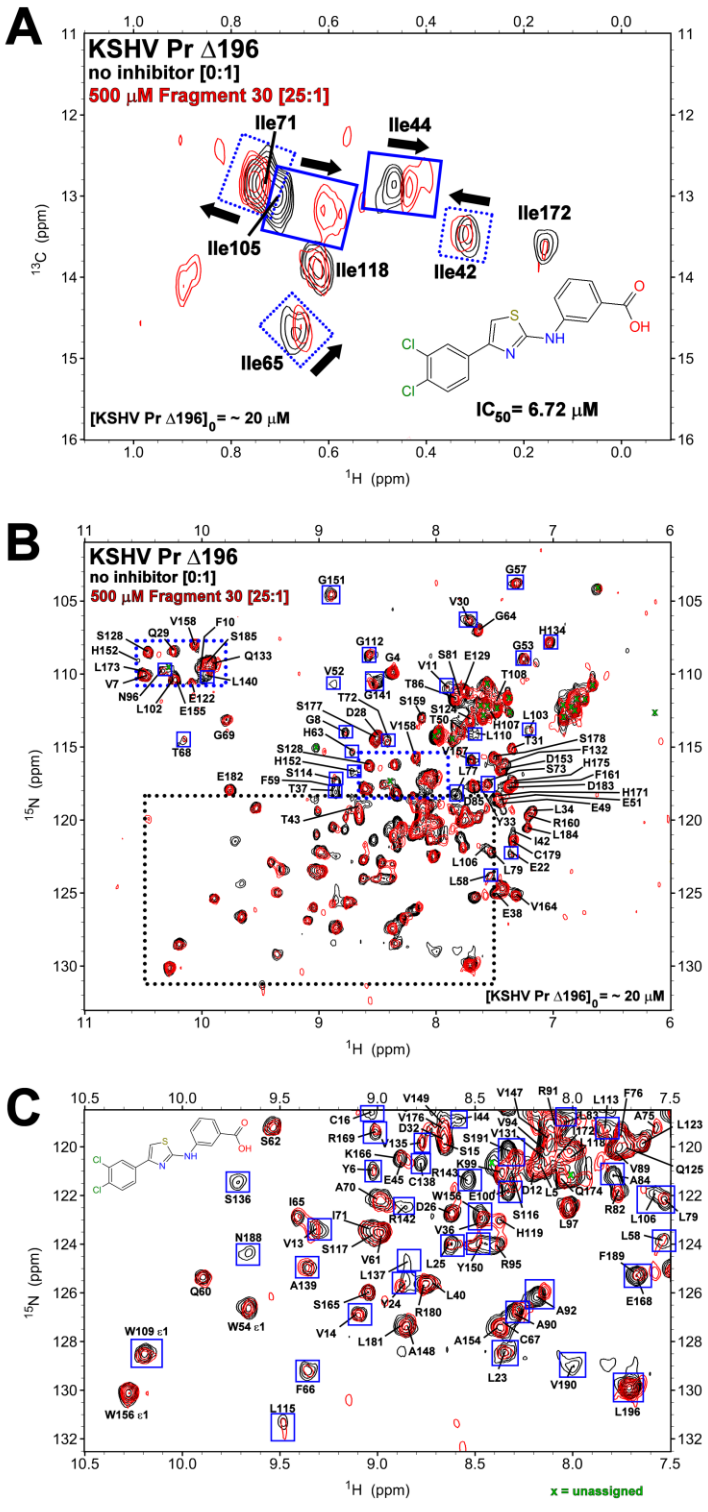


Figure S9:

(**a**) Overlays of the $^{13}C/^{1}H$-HSQC spectra of selectively ^{13}C-Isoleucine labeled KSHV Pr Δ196 in the absence (black) and presence (red) of ~ 25x molar excess of Fragment **30** (Table 2). Perturbations of the Ile44 and Ile105 methyl group resonances indicate fragment binding at the dimer interface near the hot spot Trp109.

(**b**) Overlays of the $^{15}N/^{1}H$-HSQC spectra of of uniformly ^{15}N-labeled KSHV Pr Δ196 in the absence (black) and presence (red) of ~ 25x molar excess of Fragment **30** (Table 2). The crowded middle resonances (blue dotted panel) are at the inset.

(**c**) Zoomed view of the black dotted regions of the spectra from panel (**b**).

Figure S10

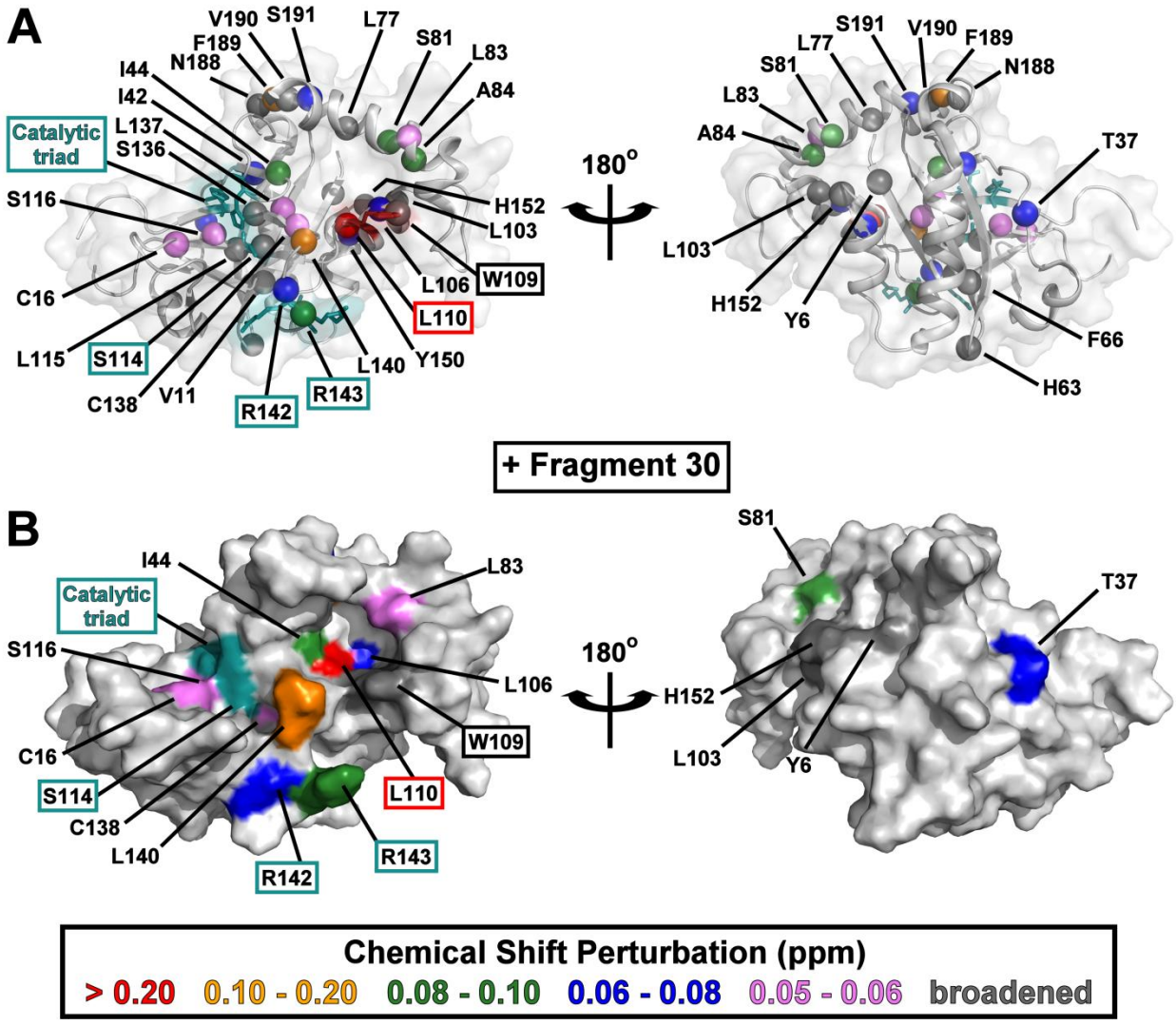


Figure S10:

The structure of monomeric KSHV Pr Δ196 (PDB: 3NJQ) with the $^{15}N/^{1}H^{N}$-HSQC chemical shift perturbations for Fragment **30** indicated by color. Backbone amide resonances which displayed peak broadening upon addition of fragments are indicated in dark gray. Amide backbone nitrogen atoms are shown as colored spheres in (**a**), while surfaces are displayed in (**b**). The catalytic triad (His46, Ser114, and His134) and oxyanion hole (Arg142 and Arg143) residues are highlighted in cyan. Left and right structures are rotated 180° about the vertical axis.

Figure S11

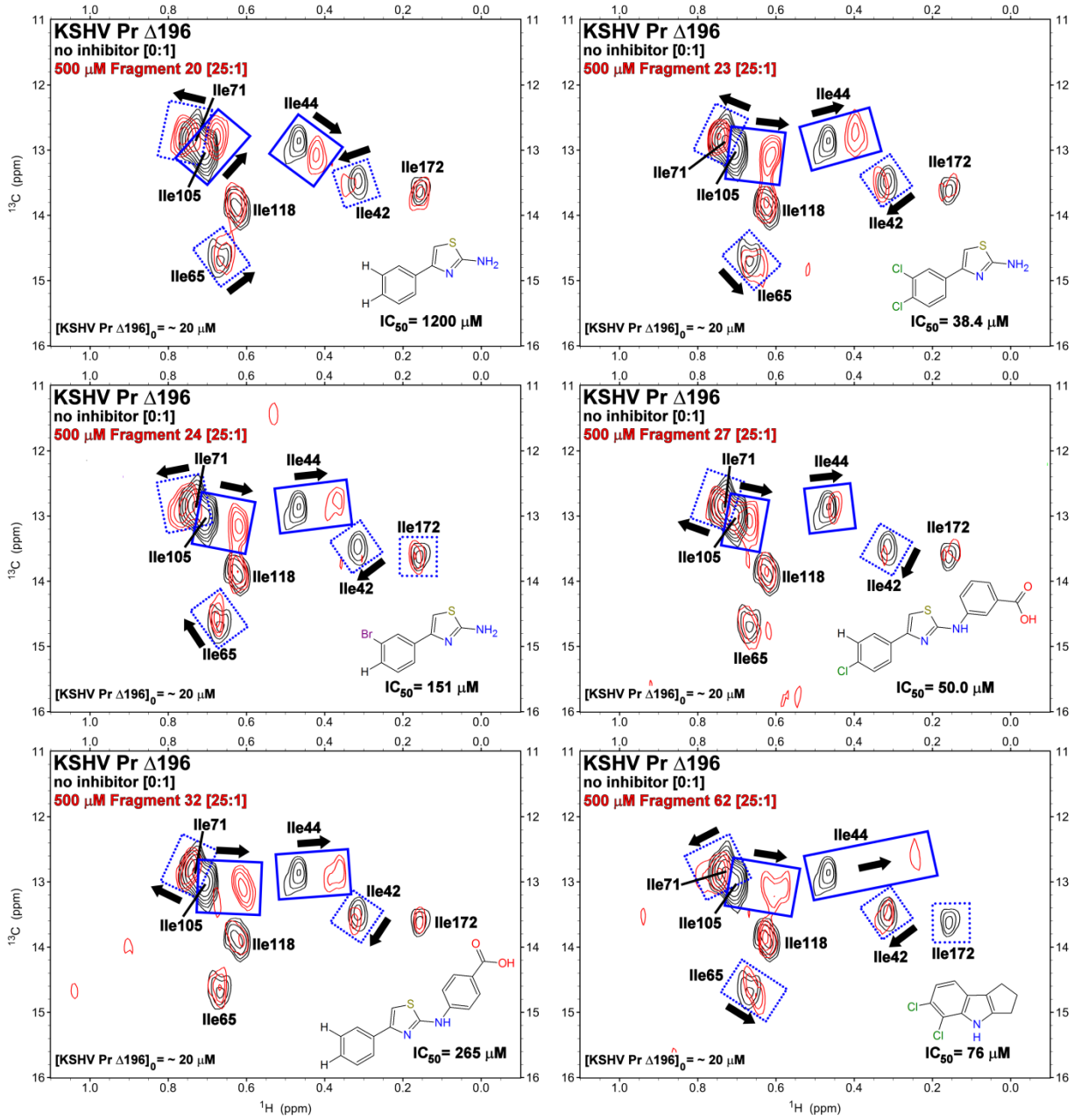


Figure S11:

Overlays of the $^{13}C/^{1}H$-HSQC spectra of selectively ^{13}C-Isoleucine labeled KSHV Pr Δ196 in the absence (black) and presence (red) of ~ 25x molar excess of selected aminothiazole fragments (Table 2) and Fragment **62** (Table S7). Perturbations of the Ile44 and Ile105 methyl group resonances indicate fragment binding at the dimer interface near the hot spot Trp109.

Figure S12A

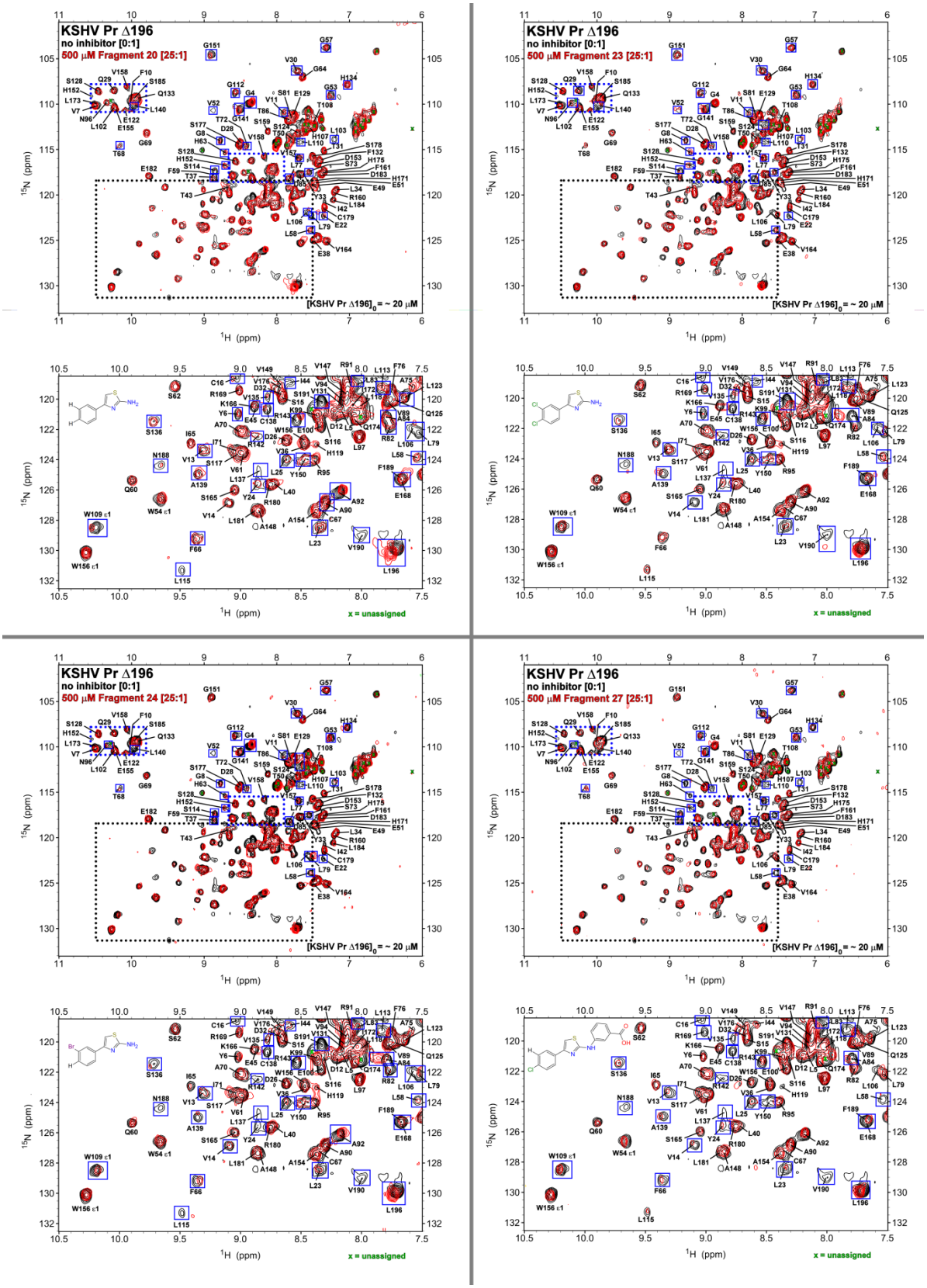
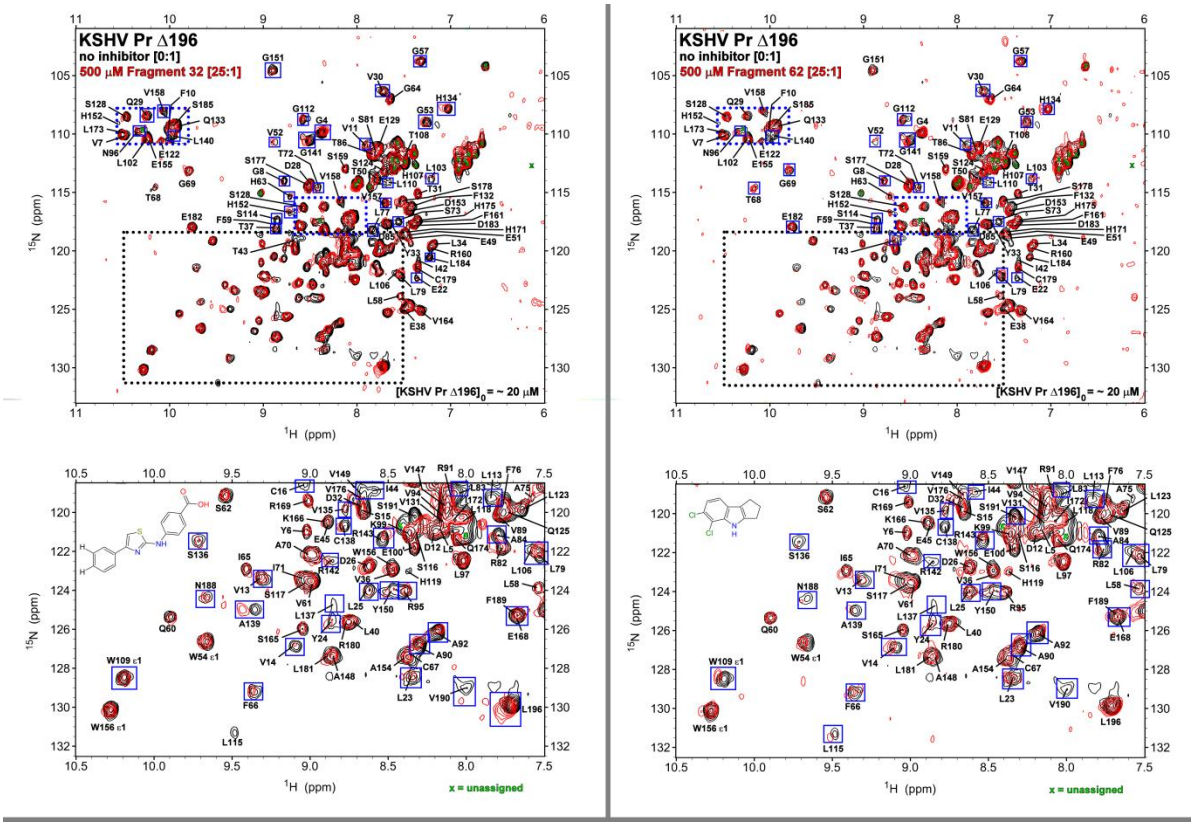

KSHV Pr Δ196
no inhibitor [0:1]
500 μM Fragment 20 [25:1]
500 μM Fragment 23 [25:1]
500 μM Fragment 24 [25:1]
500 μM Fragment 27 [25:1]
[KSHV Pr Δ196]0 = ~ 20 μM
15N (ppm)
1H (ppm)
x = unassigned

Figure S12B



Figures S12A-S12B:

Overlays of the $^{15}N/^{1}H$-HSQC spectra of uniformly ^{15}N-labeled KSHV Pr Δ196 in the absence (black) and presence (red) of ~ 25x molar excess of selected aminothiazole fragments (Table 2) and Fragment **62** (Table S7). Top half of each panel: full $^{15}N/^{1}H$-HSQC spectra with the crowded middle resonances (blue dotted panel) at the inset. The black dotted regions of the spectra are zoomed in the lower half of each panel.

Figure S13

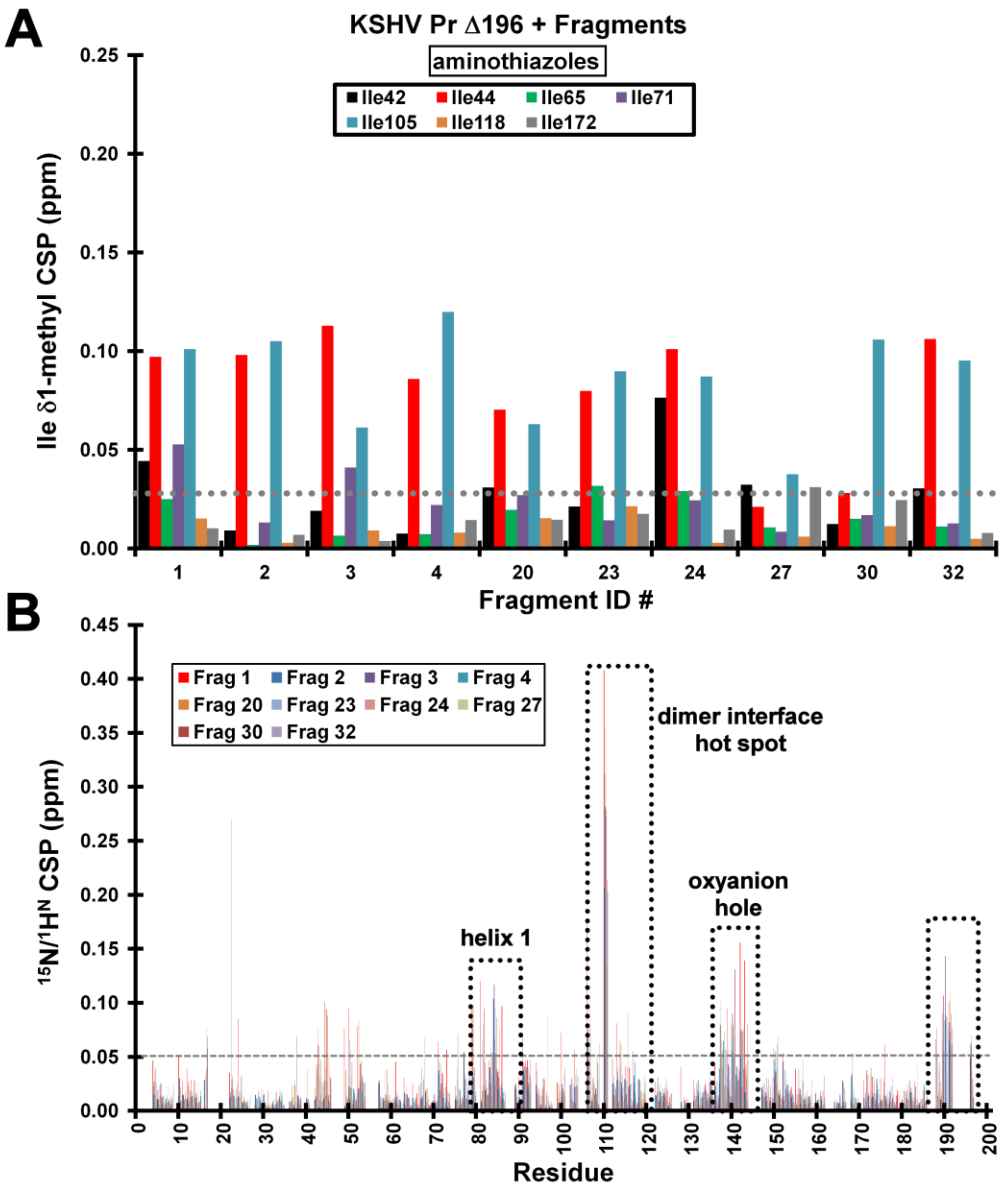


Figure S13:

(**A**) Isoleucine δ1-methyl and (**B**) backbone $^{15}N/^{1}H^{N}$ amide CSPs of KSHV Pr Δ196 in the presence of 25x molar excess of selected aminothiazole fragments (Table 2). CSPs were calculated from the $^{13}C/^{1}H$- and $^{15}N/^{1}H$-HSQC spectra appearing in Supporting Figures S9, S11 and S12. The most perturbed backbone amides are highlighted in dotted boxes, and include residues at dimer interface near the hot spot W109, the oxyanion hole, helix 1, and the C-terminus. The largest CSP values for those fragments which demonstrate binding to KSHV Pr are consistently observed for the Leu110 backbone amide, as well as the Ile44 and Ile105 δ1-methyl groups. Dotted gray lines represent lower CSP thresholds.