supplementary information

For "^{19}F chemical library and ^{19}F-NMR for a weakly bound complex structure"

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PDB ID	Cluster	Cluster size	HADDOCK score
2PPN	1	69	-21.4 ± 0.2
	2	44	-20.4 ± 1.4
	3	28	-22.4 ± 1.0
	4	10	-19.1 ± 1.2
	5	9	-21.1 ± 1.6
	6	7	-19.0 ± 2.3
	7	4	-15.1 ± 2.7
	8	4	-12.5 ± 1.0
1J4I	1	67	-21.1 ± 0.5
	2	49	-20.6 ± 2.2
	3	23	-18.8 ± 1.4
	4	7	-18.3 ± 1.3
	5	7	-19.5 ± 3.9
	6	6	-15.7 ± 1.5
	7	5	-12.5 ±1.0
	8	5	-17.6 ± 4.7
	9	4	-15.1± 2.7
1FKJ	1	57	-25.6 ± 1.2
	2	43	-27.7 ± 2.4
	3	40	-24.7 ± 1.0
	4	9	-23.6 ± 1.0
	5	8	-22.8 ± 2.7
	6	6	-18.8 ± 2.6
2DG3	1	64	-23.8 ± 0.9
	2	47	-19.0 ± 0.8
	3	16	-16.5 ± 2.2
	4	9	-13.4 ± 1.5
	5	8	-12.6 ± 2.6
	6	6	-16.6 ± 3.2
	7	5	-18.7 ± 4.0
	8	5	-11.8 ± 4.4
	9	4	-12.3 ± 2.2
	10	4	-11.8 ±2.5
	11	4	-12.7 ± 3.8
1FKT	1	43	-38.2 ± 2.5
	2	32	-33.4 ± 1.4
	3	28	-31.7 ± 2.7
	4	25	-34.9 ± 1.9
	5	11	-33.4 ± 1.7
	6	9	-29.7 ± 1.2
	7	6	-30.3 ± 1.0
	8	6	-26.5 ± 1.8
	9	5	-30.4 ± 5.4
1F40	1	65	-25.0 ± 2.1
	2	52	-22.0 ± 1.3
	3	17	-22.0 ± 2.8
	4	16	-16.5 ± 1.7
	5	10	-21.2 ± 1.7
	6	9	-18.1 ± 1.7

Table S1. HADDOCK scores for the generated clusters

Table S2. Screening of the eleven cocktails as FKBP12 binder by protein thermal shift assay.[a]

Cocktail	T_m [a] (°C)	ΔT_m [b] (°C)
none	65.53 ± 0.01 [c]	
#1	66.39 ± 0.05	+ 0.86 ± 0.06
#2	71.40 ± 0.32	+ 5.87 ± 0.33
#3	66.18 ± 0.21	+ 0.65 ± 0.22
#4	67.06 ± 0.11	+ 1.53 ± 0.12
#5	65.70 ± 0.13	+ 0.17 ± 0.14
#6	66.52 ± 0.05	+ 0.99 ± 0.06
#7	68.29 ± 0.08	+ 2.76 ± 0.09
#8	67.33 ± 0.11	+ 1.80 ± 0.12
#9	66.44 ± 0.05	+ 0.91 ± 0.06
#10	67.63 ± 0.11	+ 2.10 ± 0.12
#11	66.34 ± 0.19	+ 0.81 ± 0.20
FK506[d]	78.79 ± 0.05	+ 13.3 ± 0.06

[a] T_m values evaluated by protein thermal shift (PST) assay with the standard error of the mean for the T_m values calculated by quintuple experiments

[b] Difference of the average T_m values of FKBP12 in the presence and absence of analyte compounds ($\Delta T_m = T_m{}^b - T_m$)

[c] T_m value of FKBP12 in the absence of any compound

[d] Positive control

	Cluster 1	9.065	7.359	10.552	10.751	8.799
	Cluster 2	7.688	7.678	8.896	9.341	9.423
Amino acid residue & group		Val55 $C_{\gamma}1$			Ile91 $C_{\delta}1$	
Atom No. of compound		C12	C14	C2	C4	F16
2PPN	Cluster 1	7.430	7.434	7.327	7.475	8.187
	Cluster 2	4.819	6.364	8.237	9.635	6.583
	Cluster 3	8.853	6.987	7.068	7.011	8.216
	Cluster 4 [a]	**3.746**	**4.932**	**4.684**	**6.484**	**4.574**
	Cluster 5	8.295	8.123	9.507	8.629	8.519
	Cluster 6	4.252	4.102	8.141	5.972	8.890
	Cluster 7	10.070	10.093	12.704	14.249	13.000
	Cluster 8	18.363	18.043	13.221	11.330	15.265
1J4I	Cluster 1	7.434	7.430	7.327	7.475	8.187
	Cluster 2	5.310	6.60	8.171	9.466	6.388
	Cluster 3	8.917	7.318	8.444	8.286	6.529
	Cluster 4 [a]	**3.746**	**4.932**	**4.683**	**6.484**	**4.574**
	Cluster 5	9.247	8.602	8.404	6.559	8.811
	Cluster 6	4.809	5.475	8.773	7.508	9.192
	Cluster 7	18.363	18.043	13.221	11.330	15.265
	Cluster 8	4.458	3.912	8.166	9.353	6.074
	Cluster 9	10.070	10.093	12.704	14.249	13.000
1FKJ	Cluster 1	8.429	6.310	7.833	7.602	7.609
	Cluster 2 [a]	**3.225**	**5.374**	**5.747**	**6.047**	**7.048**
	Cluster 3	8.100	7.919	7.855	8.391	5.037
	Cluster 4	3.935	5.822	10.387	10.775	9.635
	Cluster 5	9.345	8.851	10.632	9.552	9.783
	Cluster 6	8.047	5.945	8.220	8.090	8.068
	Cluster 7	6.464	4.724	11.418	11.017	10.187

Table S3. Distances between atom pairs showing STD signals

(Table 3. Continues to the next page.)

	Cluster 3	4.746	6.381	10.022	11.100	8.676
	Cluster 4	5.508	3.504	8.825	8.585	9.105
	Cluster 5	6.376	4.722	9.445	10.130	8.235
	Cluster 6	9.078	7.870	10.330	9.739	9.014
	Cluster 7 [a]	**3.530**	**4.307**	**5.180**	**3.934**	**7.351**
	Cluster 8	12.300	13.256	21.661	21.137	20.821
	Cluster 9	16.997	17.432	16.457	15.223	15.592
	Cluster 10	9.859	9.799	11.180	9.545	11.031
	Cluster 11	4.964	6.303	10.132	11.257	8.647
1FKT	Cluster 1	6.895	7.152	7.363	7.058	8.249
	Cluster 2	7.105	5.104	7.287	7.105	7.285
	Cluster 3	4.622	6.223	6.504	8.418	4.733
	Cluster 4	9.230	10.654	14.517	14.527	12.887
	Cluster 5	6.090	4.409	8.089	8.087	7.365
	Cluster 6	5.469	5.480	11.404	11.564	9.613
	Cluster 7	10.579	11.497	12.820	12.444	11.413
	Cluster 8	12.975	14.006	21.412	19.411	21.992
	Cluster 9	8.434	8.152	7.088	6.038	8.887
1F40	Cluster 1	9.988	8.770	10.513	9.803	9.516
	Cluster 2	5.624	5.438	9.394	11.191	7.224
	Cluster 3	9.964	8.243	9.123	9.986	6.891
	Cluster 4	7.686	7.838	8.480	9.200	9.046
	Cluster 5	4.409	5.226	8.004	10.050	6.031
	Cluster 6 [a]	**4.179**	**5.705**	**4.253**	**6.617**	**3.984**
	Cluster 7	19.240	19.953	13.456	13.165	12.258

[a] Bold clusters possess all carbon-carbon distances of < 7 Å.

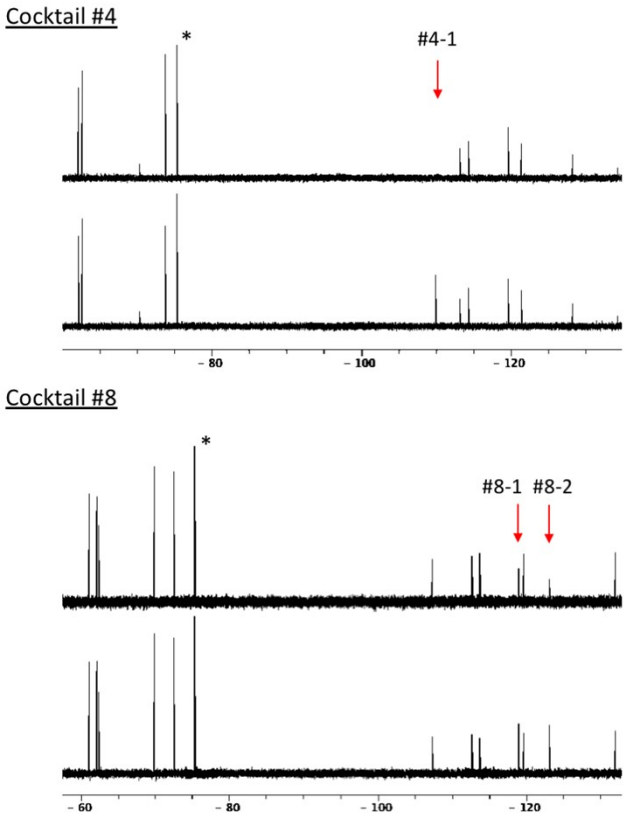

Fig. S1. ^{19}F R_2-filter spectra recorded with proton decoupling for cocktail #4 (A) and #8 (B) without (lower) and with (upper) FKBP12. The FKBP12 and the compound concentrations were 11 μM and 40 μM, respectively. Asterisks are from the signal of sodium trifluoroacetate as a standard. The arrows show the identified hit compounds #4-1, #8-1, and #8-2.

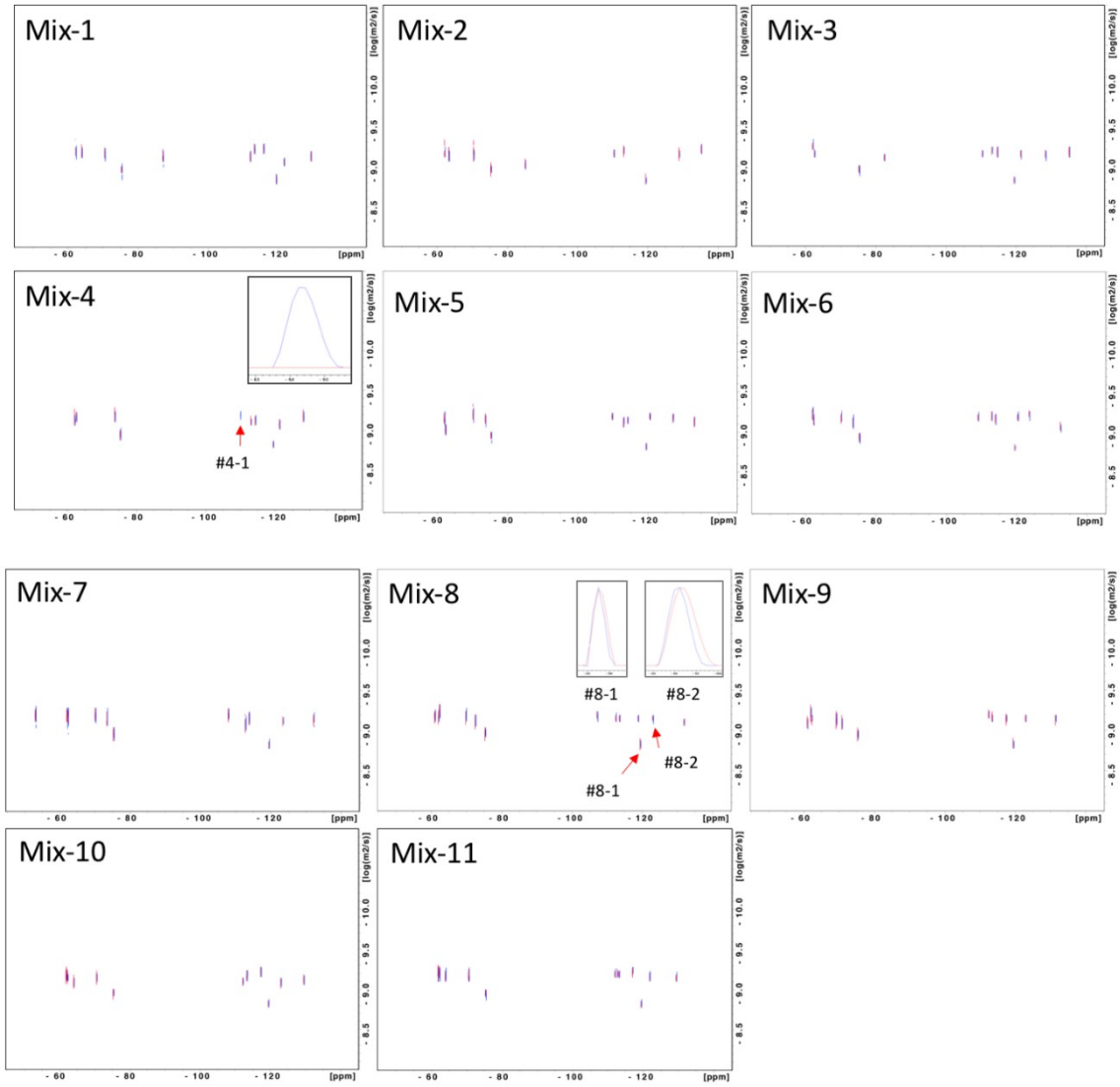

Fig. S2. 2D ^{19}F DOSY spectra recorded for each cocktail without (blue) or with (red) FKBP12. Insets indicate 1D slices of hit compounds, #4-1, #8-1 and #8-2.

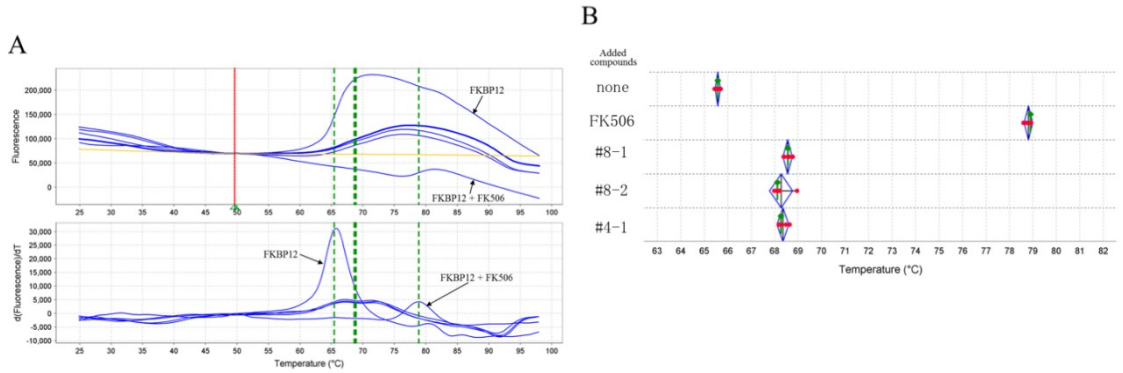

Fig. S3. Confirmation of the interaction between FKBP12 and three hit compounds by protein thermal shift assay. Plot of the fluorescence intensity of the dye (vertical axis) versus the sample temperature (horizontal axis). The solid orange line is the melting curve of the NPC well (A, upper panel). Plot of the fluorescence intensity transition rate per unit change in the sample temperature (vertical axis) versus sample temperature (horizontal axis) (A, lower panel). Diagram of T_m values of the target protein FKBP12 in the presence and absence of analyte compounds. Red dots indicate one T_m value from the five experiments. Green short bars indicate the median T_m value within the five red dots. Blue rhombic diagrams indicate a statistically normal distribution of the T_m values, and the left and right vertexes indicate the 95% lower and upper points of the distribution, respectively. Dashed green lines indicate the T_m values (B).

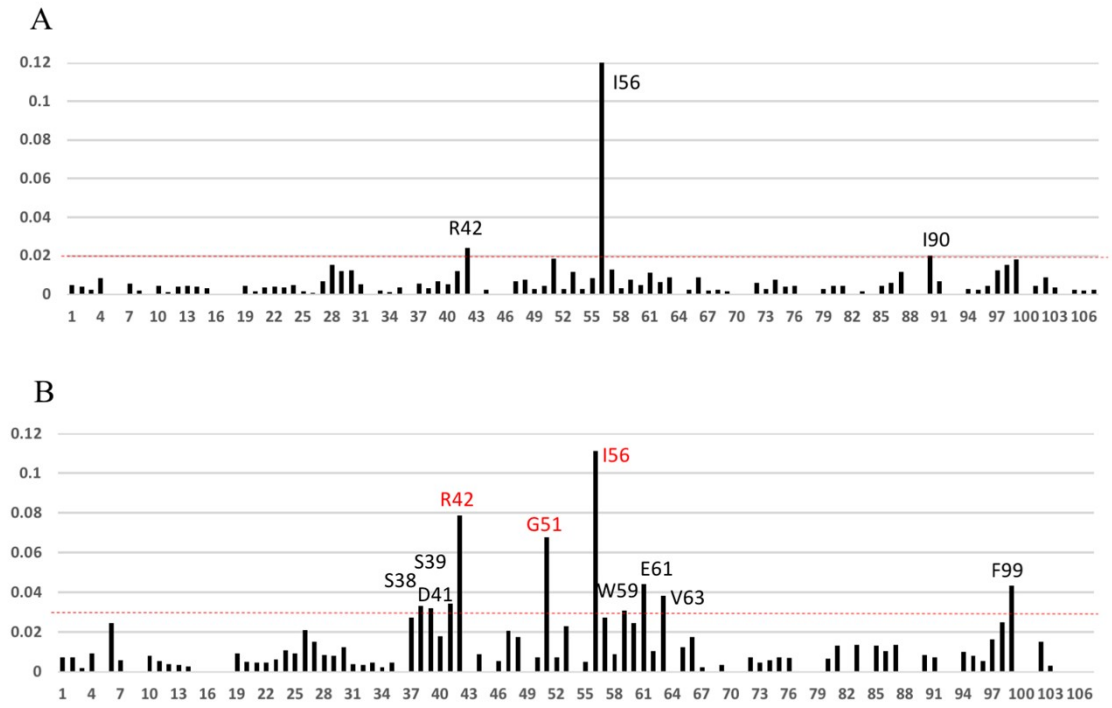

Fig. S4. Chemical shift changes ($\Delta\delta_{obs}$) induced by #4-1 (A) and #8-1 (B) were calculated by Eq. 1,

$$\Delta\delta_{obs} = ((\Delta\delta_H)^2 + (\Delta\delta_N/5)^2)^{1/2} \quad \text{Eq.1}$$

where $\Delta\delta_H$ and $\Delta\delta_N$ are the chemical shift changes of the amide protons and ^{15}N nucleus, respectively. The red dotted lines show the threshold of the selected residues (red bar). The red letters indicate the amino acid residues, which are the top three largest $\Delta\delta$ values.

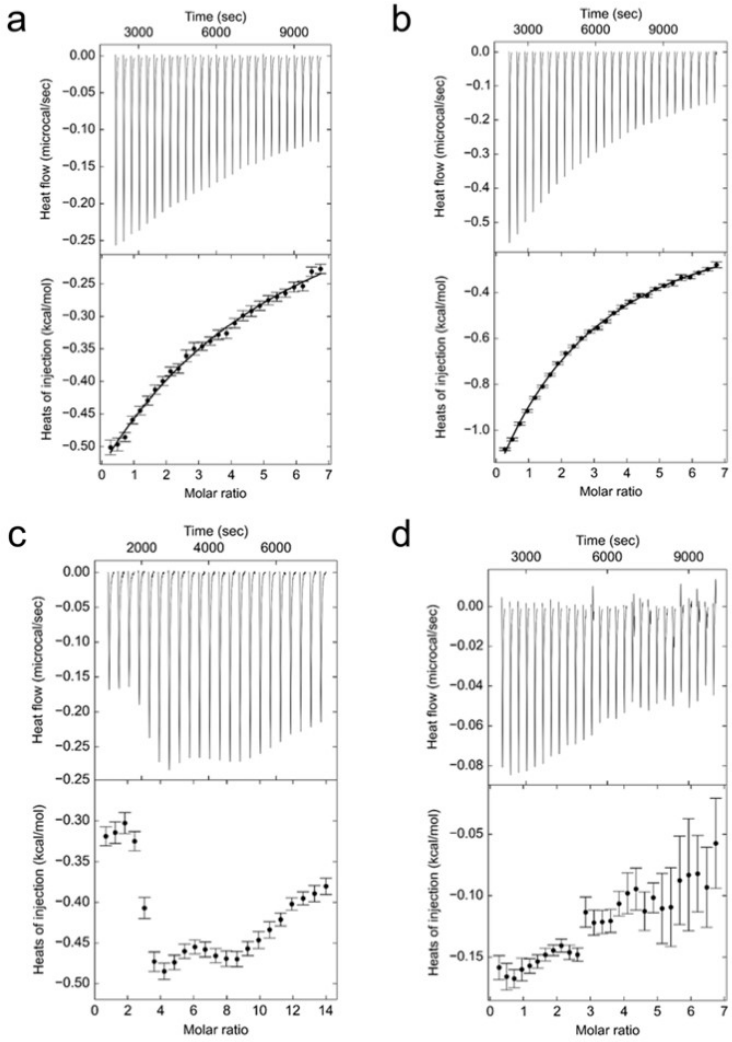

Fig. S5. Representative isothermal titration of FKBP12 to the ligands by using calorimetry. Consecutive titration of FKBP12 to #4-1 (a), #8-1 (b), #8-2 (c), or buffer (d) is shown. Each thermogram (upper panel) and binding isotherm (lower panel) are exhibited. Solid lines in the binding isotherms indicate fitted curves.

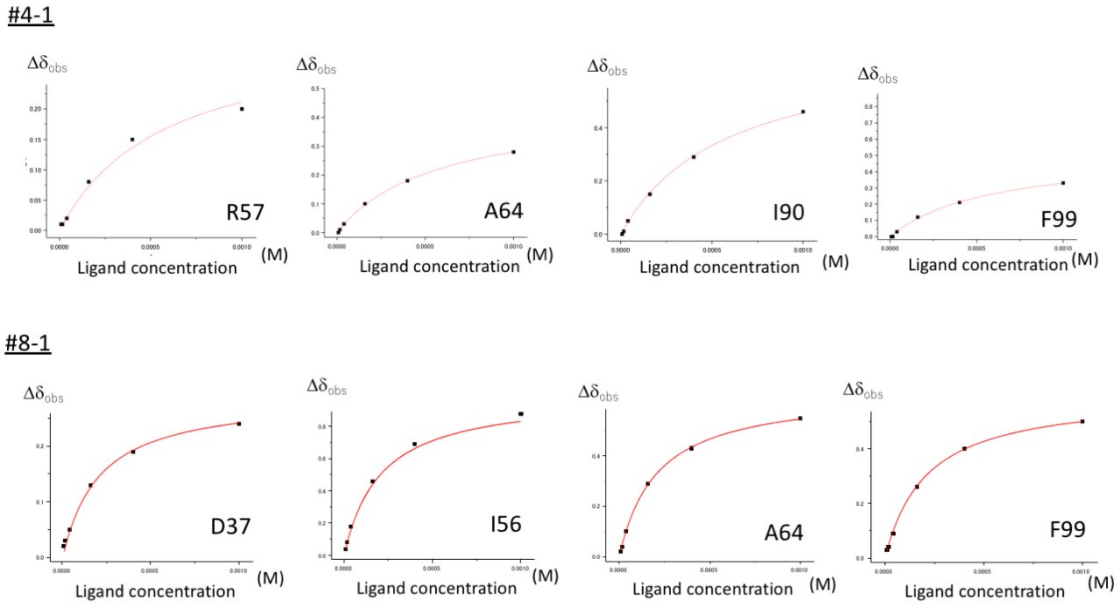

Fig. S6. NMR titration curves of chemical shift changes in ^{1}H-^{15}N HSQC spectra observed for ^{15}N-labeled FKBP12. $\Delta\delta_{obs}$ values were plotted against the free compound concentrations of #4-1 and #8-1. $\Delta\delta_{obs} = ((\Delta\delta_H)^2+(\Delta\delta_N/5)^2)^{1/2}$, where $\Delta\delta_H$ and $\Delta\delta_N$ are the chemical shift changes of the amide protons and ^{15}N nucleus, respectively.

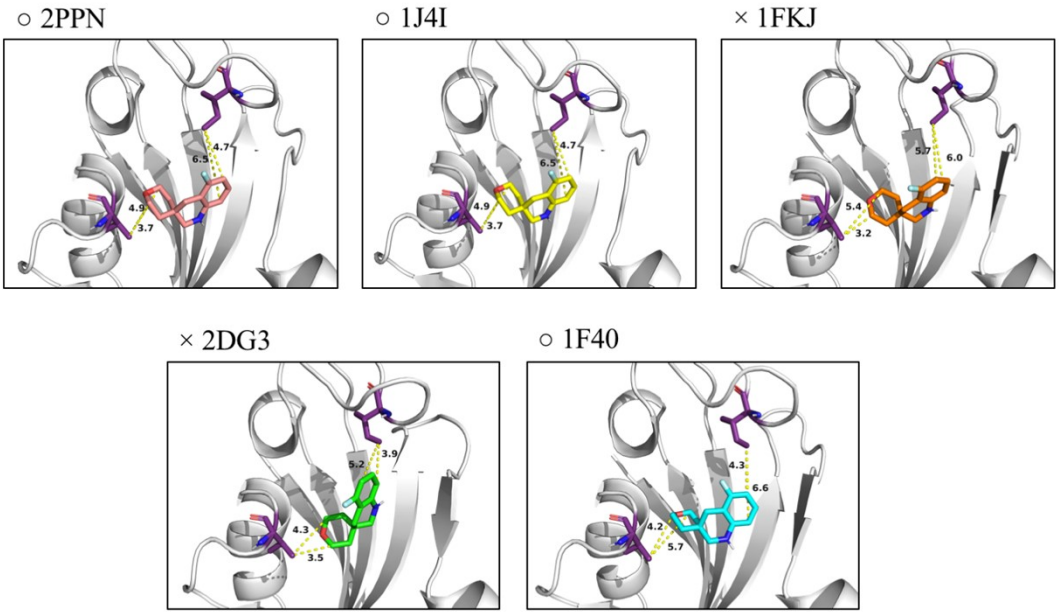

Fig. S7. HADDOCK-derived structural model of the FKBP12 and #8-1 complex structures. The structures displayed were selected based on the 1D ^{1}H STD signals (Table 4) starting from the structures of PDB 2PPN (pink), 1J4I (yellow), 1FKJ (orange), 2DG3 (green), and 1F40 (cyan). The bold letters (2PPN, 1J4I and 1F40) were selected based on the 1D ^{19}F STD signals (Table 4).

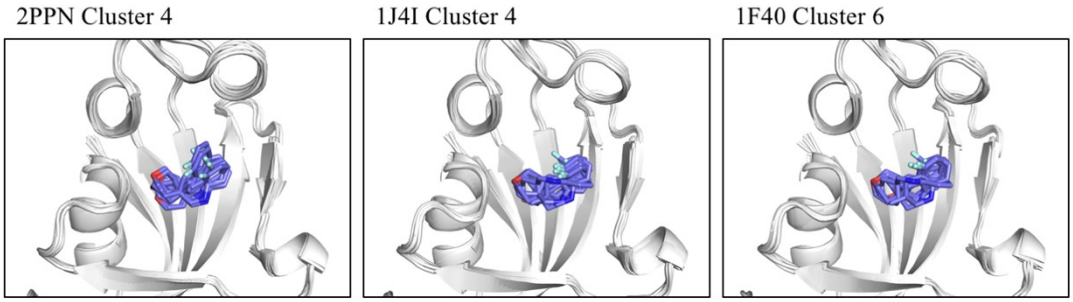

Fig. S8. The overlaid structures included in the HADDOCK clusters were selected based on ^{1}H STD and ^{19}F STD signals. The structures show 2PPN Cluster 4, 1J4I Cluster 4, and 1F40 Cluster 6, which included 10, 7, and 9 structures, respectively. Blue sticks in the structures show the binding conformations of #8-1.

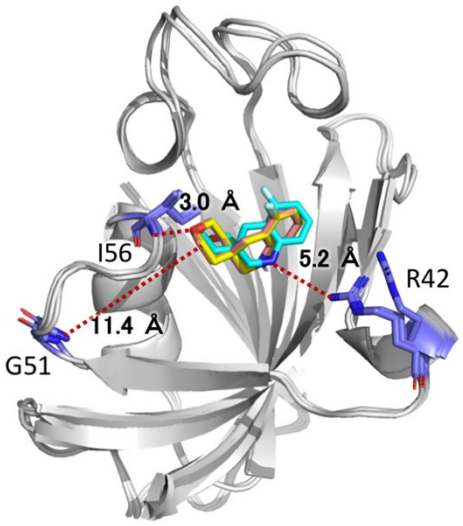

Fig. S9. The displayed modeled structures were selected based on ^{1}H STD and ^{19}F STD signals. The HADDOCK calculations began from the structures of PDB 2PPN (pink), 1J4I (yellow), and 1F40 (cyan). Blue sticks are the residues R42, G51, and I56, whose chemical shifts of the ^{1}H-^{15}N HSQC signals are perturbed by #8-1 titration. Red dotted lines are the distance between the heavy atoms of R42, G51, and I56 and the closest atoms of #8-1 in the modeled structures. Residues R42 and I56 directly interact with #8-1. G51 is located on the loop near the #8-1 binding site.

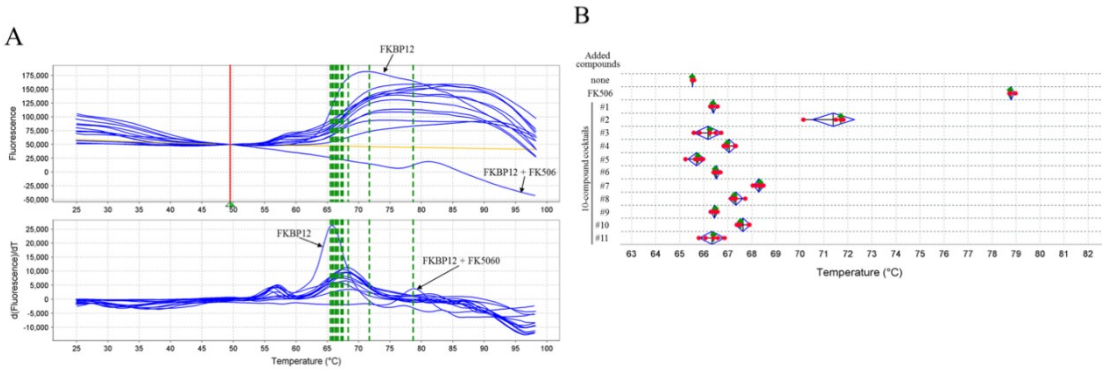

Fig. S10. Screening of hit compounds for FKBP12 using eleven chemical compound cocktails by performing a protein thermal shift assay. Plot of fluorescence intensity of the dye (vertical axis) versus the sample temperature (horizontal axis). The solid orange line is a melting curve of an NPC well (A, upper panel). Plot of fluorescence intensity transition rate per unit charge in sample temperature (vertical axis) versus the sample temperature (horizontal axis) (A, lower panel). Diagram of T_m values of the target protein FKBP12 in the presence and absence of analyte compounds. Red dots indicate one T_m value for five experiments. Green short bars indicate the median T_m value within the five red dots. Blue rhombic diagrams indicate a statistically normal distribution of the T_m values, and the left and right vertexes indicate the 95% lower and upper points of the distribution, respectively. Dashed green lines indicate T_m values (B).

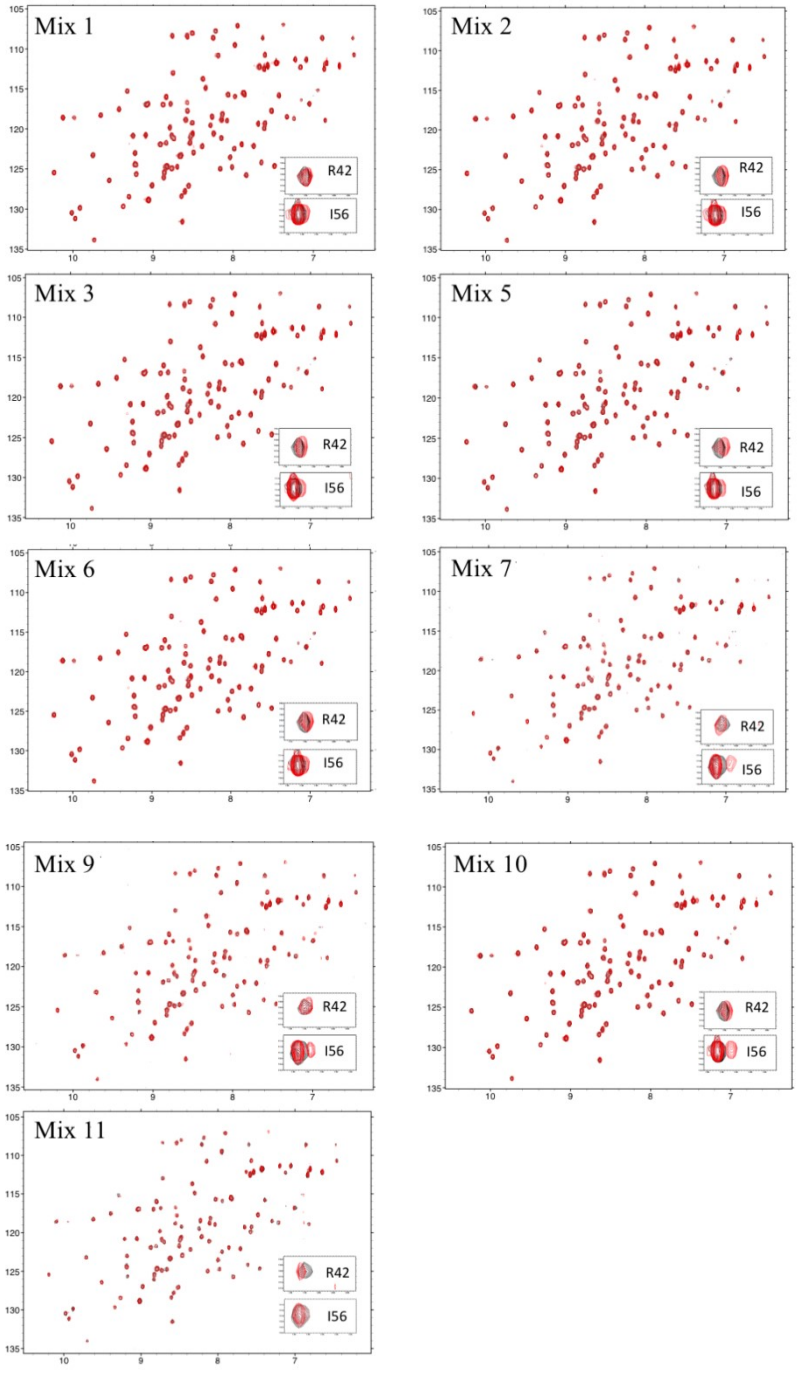

Fig. S11. NMR titrations between FKBP12 and each cocktail. Overlay of the ^{15}N HSQC spectra of FKBP12 in the absence (black) or presence (red) of the 11 cocktails. Insets indicate expanded views of the Arg42, and Ile56 resonances.