Supporting Information

Innate Conformational Dynamics Drive Binding Specificity in Anti-Apoptotic Proteins Mcl-1 and Bcl-2

Esther Wolf[1], Cristina Lento[1], Jinyue Pu[2], Bryan C. Dickinson[2] and Derek J. Wilson[1]*

[1]*Department of Chemistry, York University, Toronto, ON, M3J 1P3;* [2]*Department of Chemistry, University of Chicago, Chicago, IL 60637*

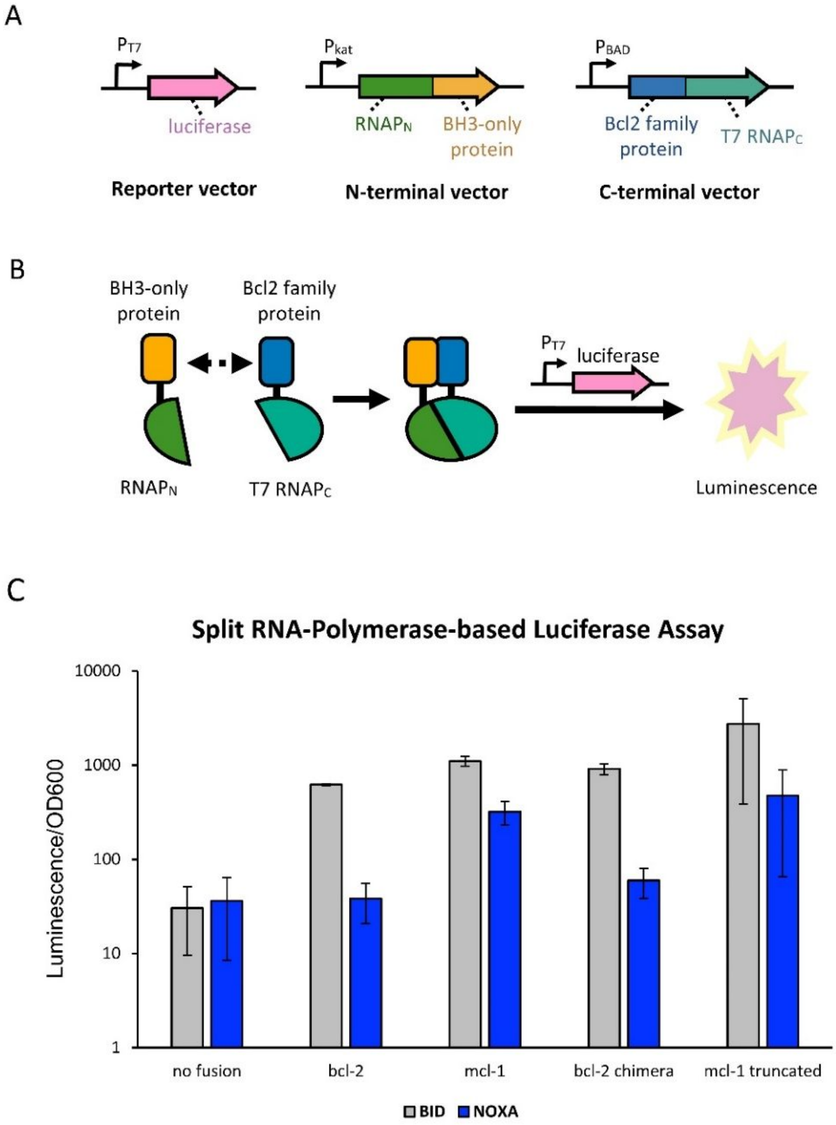


Figure S1. Split RNA polymerase-based luciferase assay comparing WT (∆TM) Bcl-2 and Mcl-1 to Bcl-2 Chimera and Truncated Mcl-1 in *E. coli*.

(A) E. coli vector system to detect the interactions between Bcl-2 family proteins (e.g., Bcl-2, Mcl-1, Bcl-2 Chimera, Truncated Mcl-1) and BH3-only proteins (e.g., BID, NOXA). (B) Schematic of split T7 RNA polymerase-based biosensors to monitor Bcl-2 family PPIs using the vectors shown in (A). A Bcl2-family protein is fused to C-terminal split T7 RNA polymerase (RNAPC), and a BH3-only protein is fused to an evolved proximity-dependent N-terminal T7 RNA polymerase variant(RNAPN). Interaction between the fused proteins lead to the assembly of the RNAP and transcriptional signal of the luminescence reporter in E.coli. (C) Luminescence signal output of split RNAPs with different Bcl2-family proteins interaction with either NOXA or BID assayed in E. coli using the system described in (B). E.coli cells were induced for 3h with arabinose and then analyzed for luminescence. Error bars are ± sem, n = 3. Detailed methods for this experiment are provided in *J. Am. Chem. Soc*. 2017, 139 (34), 11964.

Table S1. Sequences of Bcl-2 and Mcl-1

		amino acid #					amino acid #		
Helix No.	Bcl2 Chimera[a,b]		WT Bcl-2[c]		Helix No.	Mcl1 (Untagged)[d]		WT Mcl-1	GST-Mcl1
		173 total					160aa		
	M	1	1			G	1		225
	A	2	2			S	2		226
	H	3	3			G	3		227
	A	4	4			S	4		228
	G	5	5		α1	D	5	172	229
	R	6	6			E	6	173	230
	T	7	7			L	7	174	231
	G	8	8			Y	8	175	232
	Y	9	9			R	9	176	233
α1	D	10	10			Q	10	177	234
	N	11	11			S	11	178	235
	R	12	12			L	12	179	236
	E	13	13			E	13	180	237
	I	14	14			I	14	181	238
	V	15	15			I	15	182	239
	M	16	16			S	16	183	240
	K	17	17			R	17	184	241
	Y	18	18			Y	18	185	242
	I	19	19			L	19	186	243
	H	20	20			R	20	187	244
	Y	21	21			E	21	188	245
	K	22	22			Q	22	189	246
	L	23	23			A	23	190	247
	S	24	24			T	24	191	248
	Q	25	25			G	25	192	249
	R	26	26			A	26	193	250
	G	27	27			K	27	194	251
	Y	28	28			D	28	195	252
	E	29	29			T	29	196	253
	W	30	30			K	30	197	254
	D	31	31			P	31	198	255
	A	32	32			M	32	199	256
	G	33	33			G	33	200	257
	D	34	34			R	34	201	258
	D	35	35			S	35	202	259
	V	36	36		α2	G	36	203	260
	E	37	37			A	37	204	261
	E	38	38			T	38	205	262
	N	39	39			S	39	206	263
	R	40	40			R	40	207	264

	T	41	41			K	41	208	265
	E	42	42			A	42	209	266
	A	43	43			L	43	210	267
	P	44	44			E	44	211	268
	E	45	45			T	45	212	269
	G	46	46			L	46	213	270
	T	47	47			R	47	214	271
	E	48	48			R	48	215	272
	S	49	49			V	49	216	273
	E	50	50			G	50	217	274
	P	51	91			D	51	218	275
α2	V	52	92			G	52	219	276
	V	53	93			V	53	220	277
	H	54	94			Q	54	221	278
	L	55	95			R	55	222	279
	T	56	96			N	56	223	280
	L	57	97			H	57	224	281
	R	58	98		α3	E	58	225	282
	Q	59	99			T	59	226	283
	A	60	100			A	60	227	284
	G	61	101			F	61	228	285
	D	62	102			Q	62	229	286
	D	63	103			G	63	230	287
	F	64	104			M	64	231	288
	S	65	105			L	65	232	289
	R	66	106			R	66	233	290
	R	67	107			K	67	234	291
	Y	68	108			L	68	235	292
	R	69	109			D	69	236	293
	R	70	110			I	70	237	294
	D	71	111			K	71	238	295
	F	72	112		α4	N	72	239	296
α3	A	73	113			E	73	240	297
	E	74	114			D	74	241	298
	M	75	115			D	75	242	299
	S	76	116			V	76	243	300
	S	77	117			K	77	244	301
	Q	78	118			S	78	245	302
	L	79	119			L	79	246	303
	H	80	120			S	80	247	304
	L	81	121			R	81	248	305
α4	T	82	122			V	82	249	306
	P	83	123			M	83	250	307
	F	84	124			I	84	251	308
	T	85	125			H	85	252	309
	A	86	126			V	86	253	310
	R	87	127			F	87	254	311
	G	88	128			S	88	255	312

	R	89	129			D	89	256	313
	F	90	130			G	90	257	314
	A	91	131			V	91	258	315
	T	92	132			T	92	259	316
	V	93	133		α5	N	93	260	317
	V	94	134			W	94	261	318
	E	95	135			G	95	262	319
	E	96	136			R	96	263	320
	L	97	137			I	97	264	321
	F	98	138			V	98	265	322
	R	99	139			T	99	266	323
	D	100	140			L	100	267	324
	G	101	141			I	101	268	325
	V	102	142			S	102	269	326
α5	N	103	143			F	103	270	327
	W	104	144			G	104	271	328
	G	105	145			A	105	272	329
	R	106	146			F	106	273	330
	I	107	147			V	107	274	331
	V	108	148			A	108	275	332
	A	109	149			K	109	276	333
	F	110	150			H	110	277	334
	F	111	151			L	111	278	335
	E	112	152			K	112	279	336
	F	113	153			T	113	280	337
	G	114	154			I	114	281	338
	G	115	155			N	115	282	339
	V	116	156		α6	Q	116	283	340
	M	117	157			E	117	284	341
	C	118	158			S	118	285	342
	V	119	159			C	119	286	343
	E	120	160			I	120	287	344
	S	121	161			E	121	288	345
	V	122	162			P	122	289	346
	N	123	163			L	123	290	347
	R	124	164			A	124	291	348
	E	125	165			E	125	292	349
α6	M	126	166			S	126	293	350
	S	127	167			I	127	294	351
	P	128	168			T	128	295	352
	L	129	169			D	129	296	353
	V	130	170			V	130	297	354
	D	131	171			L	131	298	355
	N	132	172			V	132	299	356
	I	133	173			R	133	300	357
	A	134	174			T	134	301	358
	L	135	175			K	135	302	359
	W	136	176			R	136	303	360

	M	137	177			D	137	304	361
	T	138	178		α7	W	138	305	362
	E	139	179			L	139	306	363
	Y	140	180			V	140	307	364
	L	141	181			K	141	308	365
	N	142	182			Q	142	309	366
	R	143	183			R	143	310	367
	H	144	184			G	144	311	368
	L	145	185			W	145	312	369
	H	146	186			D	146	313	370
	T	147	187			G	147	314	371
	W	148	188			F	148	315	372
	I	149	189			V	149	316	373
	Q	150	190			E	150	317	374
	D	151	191			F	151	318	375
	N	152	192			F	152	319	376
α7	G	153	193			H	153	320	377
	G	154	194			V	154	321	378
	W	155	195			E	155	322	379
	D	156	196			D	156	323	380
	A	157	197			L	157	324	381
	F	158	198			E	158	325	382
	V	159	199			G	159	326	383
	E	160	200			G	160	327	384
	L	161	201						
	Y	162	202						
	G	163	203						

[a]The starting residue (1) corresponds to the start of WT Bcl2 sequence and continues in Bcl-2 chimera until the loop replacement (34), [b]The sequence corresponding to WT Bcl-X_L is denoted in red (35-50), [c]Note that the sequence corresponding to the loop deletion is omitted which is why the sequence here skips from 51 to 90. [d]GSGS is an artifact after GST tag removal.

Table S2. % Sequence Coverage and Redundancy

State	% Coverage	Redundancy
Bcl2 Only	75	2.28
Bcl2/Bid	69	2.22
Bcl2/Bim	61	1.67
Bcl2/Bad	62	1.76
Bcl2/Noxa	55	1.93
Mcl1 Only	92	2.11
Mcl1/Bid	85	1.54
Mcl1/Bim	84	2.05
Mcl1/Bad	77	1.71
Mcl1/Noxa	92	2.11

Table S3. Mcl1 Peptide List

Mcl-1						
m/z	z	m	Drift Time	Sequence	Start	Stop
676.8288	2	1351.642	3.255	SDELYRQSLEI	^172	181
589.8311	2	1178.655	2.604	EIISRYLRE	180	188
904.4791	1	903.4713	7.161	KPMGRSGAT	197	205
821.4367	3	2461.287	3.038	QATGAKDTKPMGRSGATSRKALET	189	212
644.5959	4	2574.352	2.713	QATGAKDTKPMGRSGATSRKALETL	189	213
581.2994	3	1741.884	2.007	RRVGDGVQRNHETAF	214	228
686.676	3	2057.004	2.333	RRVGDGVQRNHETAFQGM	214	231
447.2531	4	1785.992	2.224	LRKLDIKNEDDVKSL	232	246
710.071	3	2128.201	3.201	LRKLDIKNEDDVKSLSRV	232	249
672.377	3	2015.12	2.93	RKLDIKNEDDVKSLSRV	233	249
703.3295	2	1405.652	3.309	MIHVFSDGVTNW	250	261
637.8085	2	1274.609	2.875	IHVFSDGVTNW	251	261
682.3511	3	2045.036	2.604	MIHVFSDGVTNWGRIVTL	250	267
638.6748	3	1914.01	2.333	IHVFSDGVTNWGRIVTL	251	267
709.9061	2	1417.797	3.689	ISFGAFVAKHLKT	268	280
735.89	2	1470.773	3.58	VAKHLKTINQESC	274	286
861.428	1	861.428	6.835	SCIEPLAE	285	292
667.3611	3	2000.071	2.441	VRTKRDWLVKQRGWDG	299	314
537.5432	4	2147.149	2.17	VRTKRDWLVKQRGWDGF	299	315
1002.437	1	1002.439	8.354	FHVEDLEGG	319	327

Table S4. Bcl2 Peptide List

Bcl-2						
m/z	z	m	Drift Time	Sequence	Start	Stop
449.5257	3	1345.554	2.062	AHAGRTGYDNRE	2	13
507.7625	4	2027.019	2.007	IVMKYIHYKLSQRGYE	14	29
812.7342	3	2435.179	2.984	VEENRTEAPEGTESEPVVHLTL	**91	97
907.7771	3	2720.308	3.309	NRTEAPEGTESEPVVHLTLRQAGDD	*91	103
389.7483	2	777.4809	1.682	PVVHLTL	91	97
511.7341	2	1021.453	2.170	TLRQAGDDF	96	104
434.9001	3	1301.677	1.573	FSRRYRRDF	104	112
573.3018	3	1716.882	2.496	SSQLHLTPFTARGRF	116	130
434.9091	3	1301.704	1.953	HLTPFTARGRF	120	130
583.3159	2	1164.616	2.984	LTPFTARGRF	121	130
503.7474	2	1005.479	2.224	LFRDGVNW	137	144
825.4393	2	1648.863	4.325	LFRDGVNWGRIVAF	137	150
447.2057	2	892.3957	1.736	FRDGVNW	138	144
695.3737	2	1388.732	3.201	FRDGVNWGRIVA	138	149
512.9453	3	1535.812	2.116	FRDGVNWGRIVAF	138	150
789.3725	2	1576.729	3.580	CVESVNREMSPLVD	158	171
829.4379	2	1656.86	3.852	SVNREMSPLVDNIAL	161	175
534.2533	3	1599.736	2.007	WMTEYLNRHLHT	176	187
800.887	2	1599.758	4.120	MTEYLNRHLHTW	177	188
654.7814	2	1307.547	2.930	WIQDNGGWDAF	188	198
469.2087	4	1872.803	2.007	VELYGPSMRHHHHHH	199	207-6His

Table S5. Locations of Hydrophobic and Conserved Residues in BH3 Peptides[a]

Residue Position	Bim	Bid	Bad	Noxa
		S		
	D	Q	N	
	M	E	L	A
	R	D	W	E
	P	I	A	L
	E	I	A	E
	I	R	Q	V
	W	N	R	E
H1 (i)	I	I	Y	C
	A	A	G	A
	Q	R	R	T
	E	H	E	Q
H2 (i+4)	L	L	L	L
	R	A	R	R
	R	Q	R	R
H3 (i+7)	I	V	M	F
	G	G	S	G
i+9	**D**	**D**	**D**	**D**
	E	S	E	K
H4 (i+11)	F	M	F	L
	N	D	V	N
	A	R	D	F
	Y	S	S	R
	Y	I	F	Q
	A	P	K	K
	R	P	K	L
	R	G		L

[a] Hydrophobic residues (H1-H4) of BH3 peptides embed in hydrophobic pockets (P1-P4) of the BH3 binding groove in Bcl-2 and Mcl-1, D (i+9) is bolded to indicate conservation across these BH3 peptides.

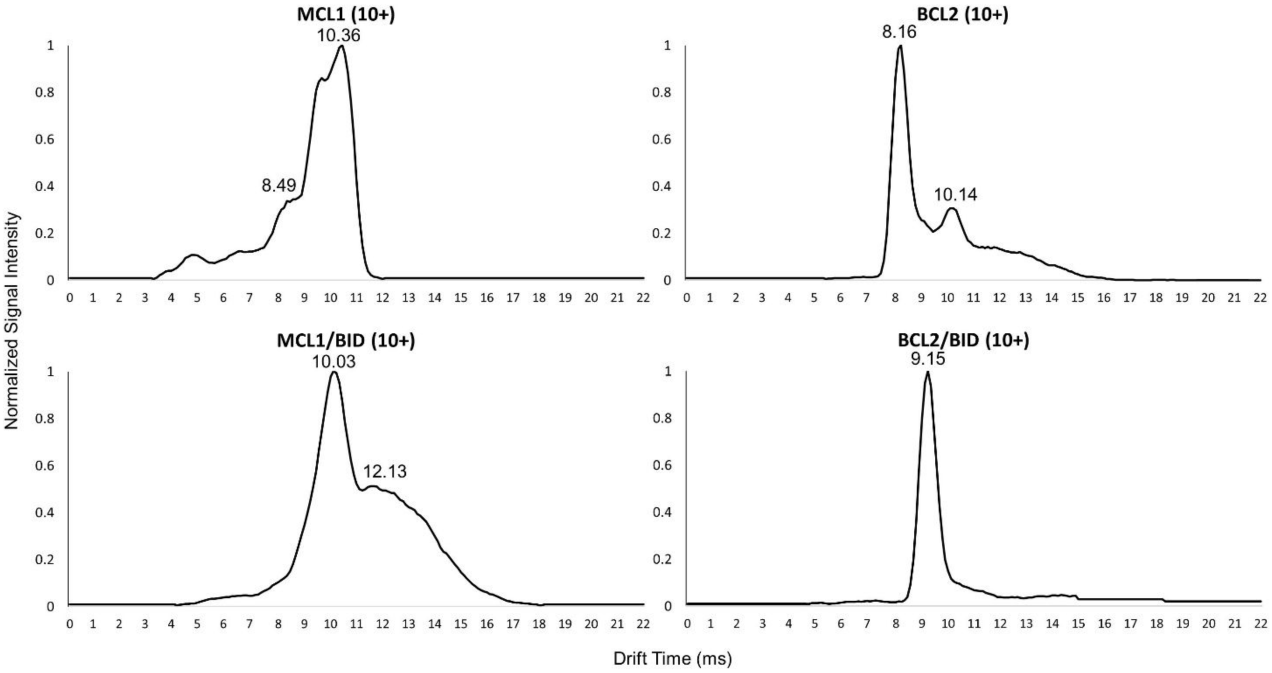


Figure S2. Ion Mobility Chromatograms of Bcl-2 and Mcl-1. The relative signal intensity as a function of drift time (milliseconds) for Mcl-1 (top left), Bcl-2 (top right), Mcl-1 + Bid (bottom left), and Bcl-2 + Bid (bottom right) is shown for their 10+ charge state. These 10+ charge states are less abundant and lower intensity (ion count).

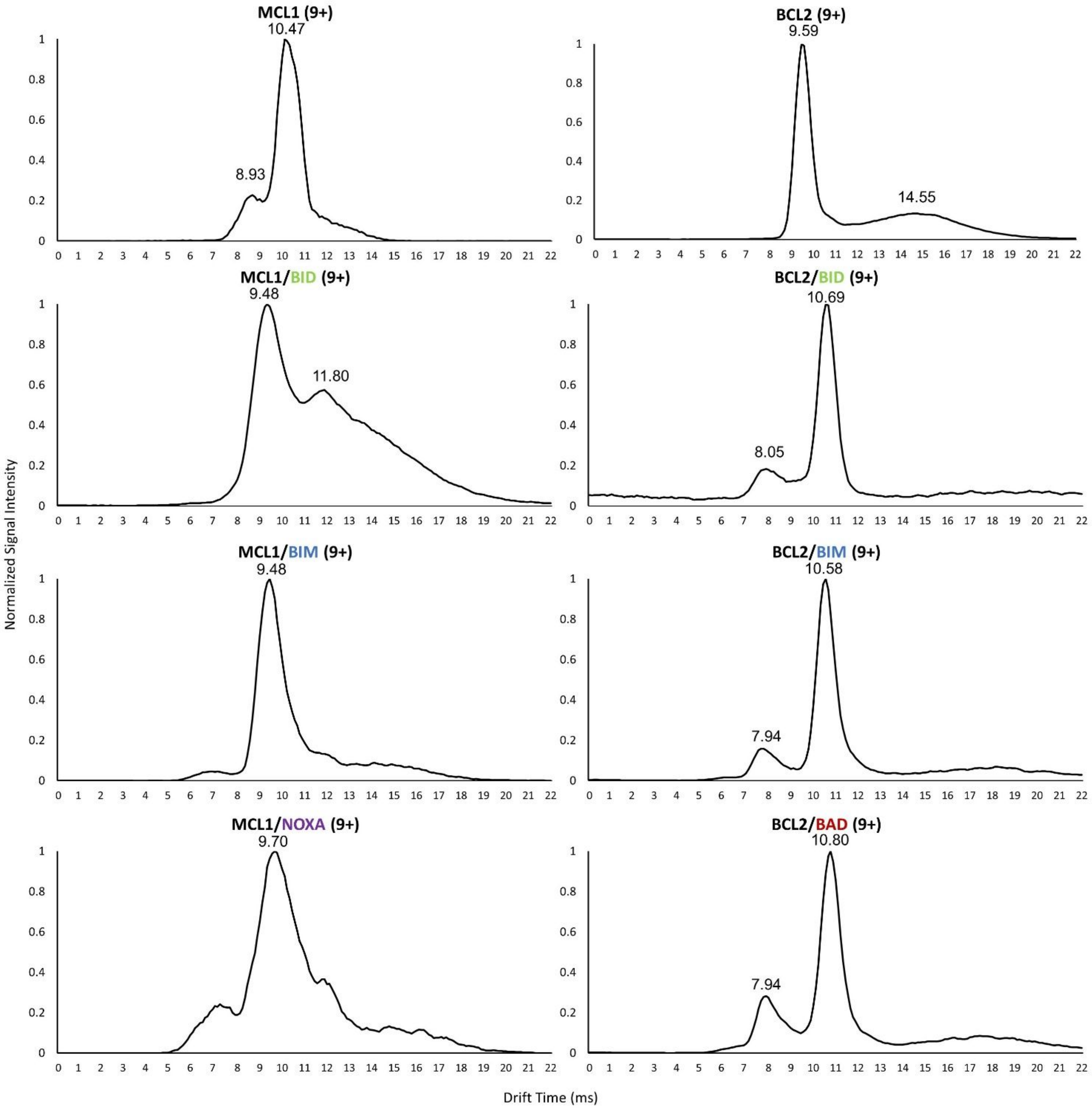


Figure S3. Ion Mobility Chromatograms of Bcl-2 and Mcl-1 with all BH3 peptides. The relative signal intensity as a function of drift time (milliseconds) for the 9+ charge states of Mcl-1 (top left), Bcl-2 (top right), and remainder of bound complexes: Bcl2/Bim, Bcl2/Bad, Mcl1/Bim, and Mcl1/Noxa. Mcl-1 + Bid (bottom left), and Bcl-2 + Bid (bottom right) is shown for their 10+ charge state.

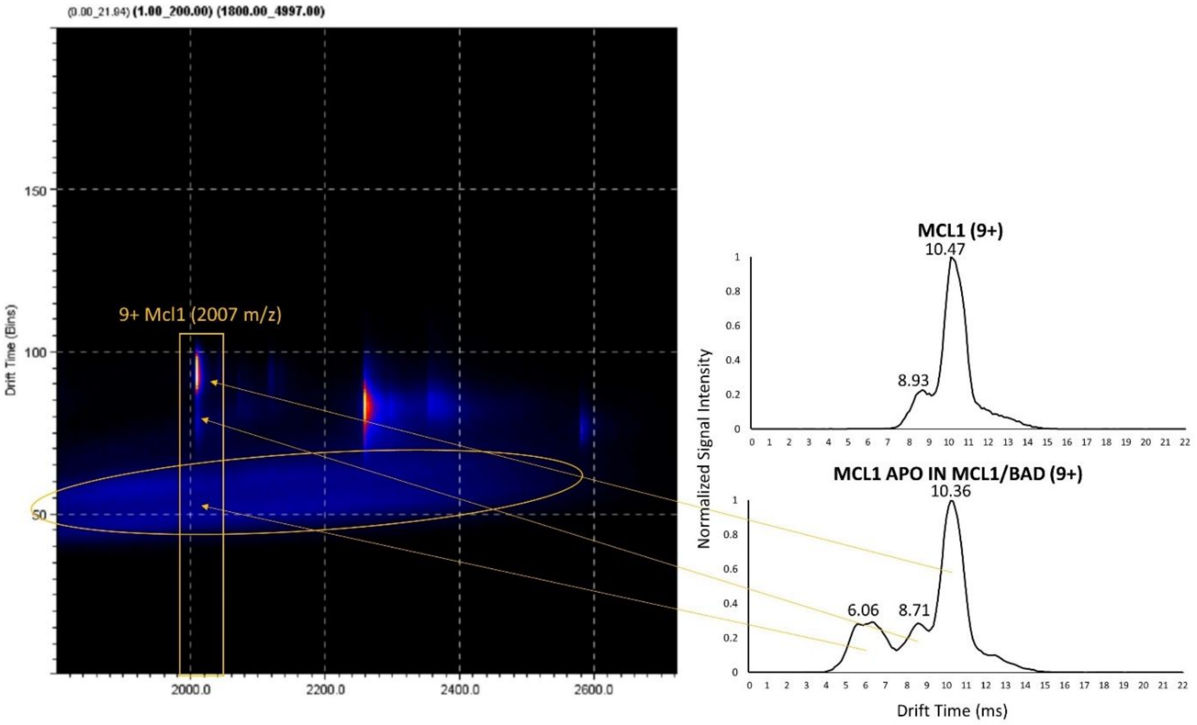


Figure S4. Ion Mobility Chromatograms of unbound Mcl-1 in the “Mcl-1 only” and “Mcl-1/BAD” spectra. The relative signal intensity is plotted as a function of drift time (milliseconds) for the 9+ charge state. The Mcl-1 unbound 9+ peak in Mcl-1/BAD has an artifactual drift time peak at 6.06 which is a result of noise in the sample acquisition (see heat map on the left) to observe noise across a 500 m/z window which does not relate to the species of interest.

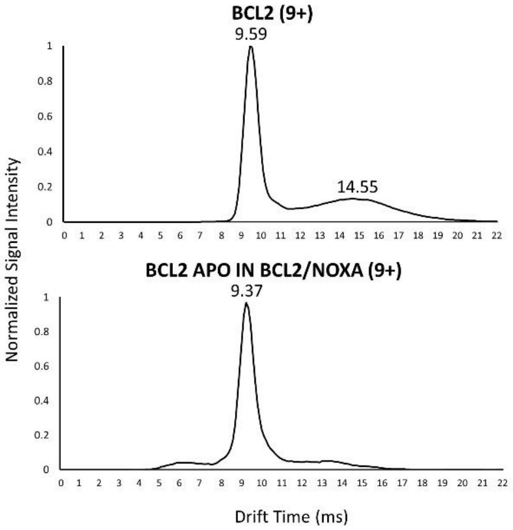


Figure S5. Ion Mobility Chromatograms of unbound Bcl-2 in the “Bcl-2 only” and “Bcl-2/NOXA” spectra. The relative signal intensity is plotted as a function of drift time (milliseconds) for the 9+ charge state. The majority peak of the Bcl2-only 9+ charge state was the same in both states (approximately 9.5 ms).

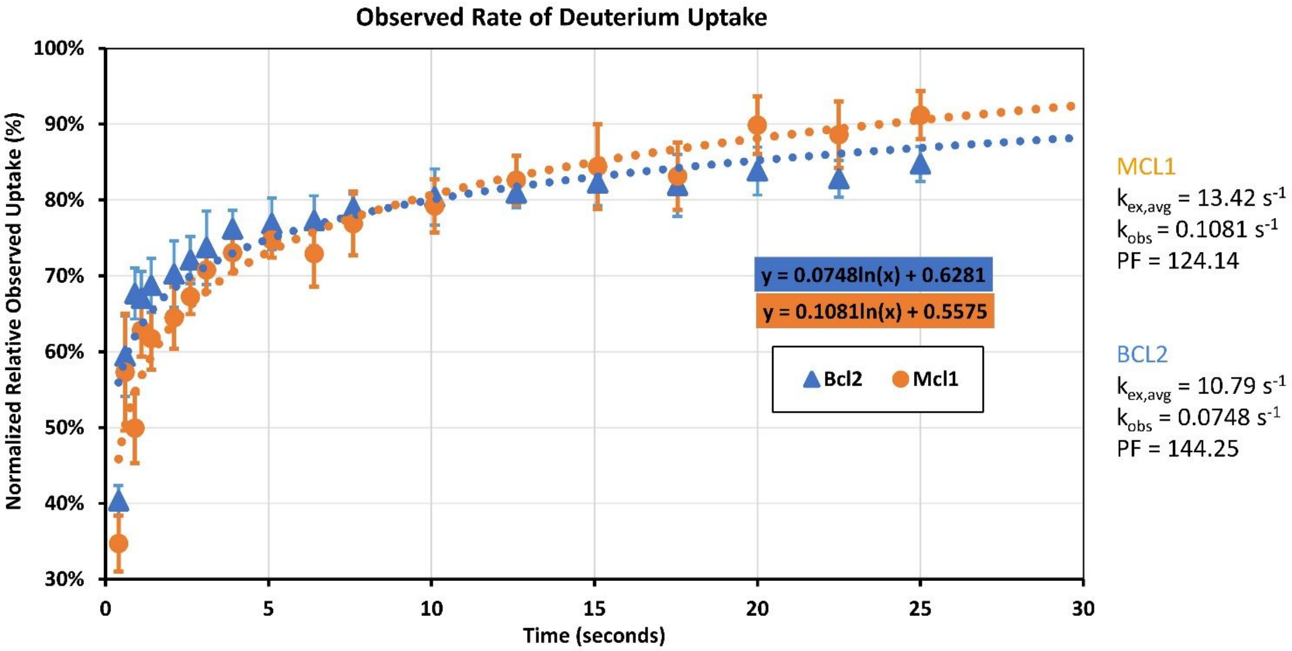


Figure S6. Global TRESI-HDX of intact Bcl-2 and Mcl-1. The normalized relative observed uptake (%) was plotted as a function of time in seconds. Normalized relative uptake was calculated by dividing the deuterium uptake (Da) by the total number of exchangeable amide hydrogens for Bcl-2 and Mcl-1 (165 and 155, respectively). The blue triangles represent Bcl-2 and orange circles represent Mcl-1. The error bars represent 2 sigma which was obtained from a minimum of n=11 charge states. Protection factors (PF) were determined by taking the quotient of the whole protein average intrinsic exchange rate ($k_{ex,avg}$) obtained by averaging the intrinsic rates calculated by Sphere (https://repository.upenn.edu/dissertations/AAI9532311/) at pH 7.0, 20 ºC and the observed rate of HDX (k_{obs}) which were extracted from the exponential trendline. The PF of Mcl-1 is less than Bcl-2, indicating that Bcl-2 is more structured and protected from HDX than Mcl-1.

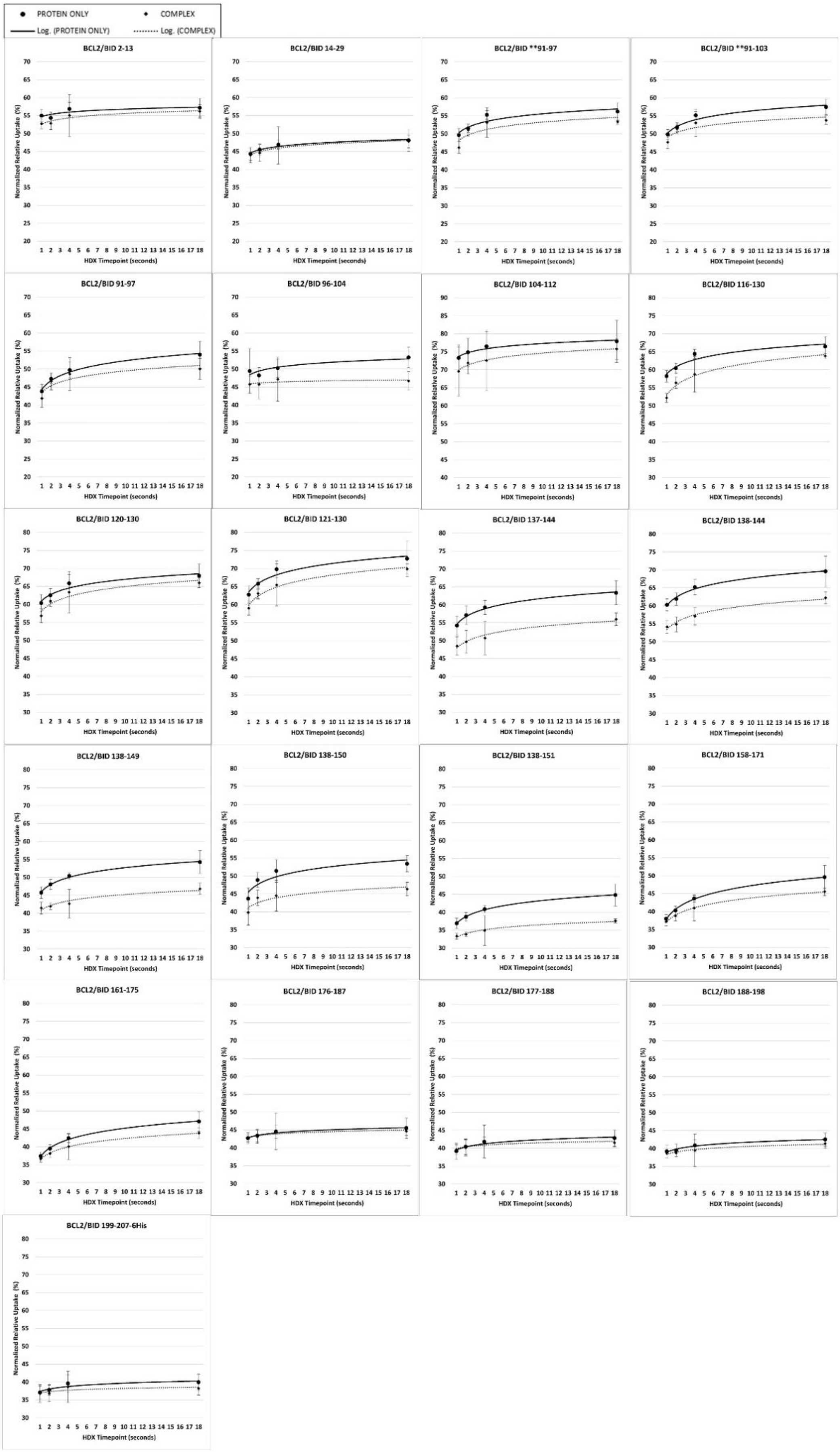


Figure S7. Bcl2 vs. Bcl2/Bid Deuterium Uptake Kinetics Plots.

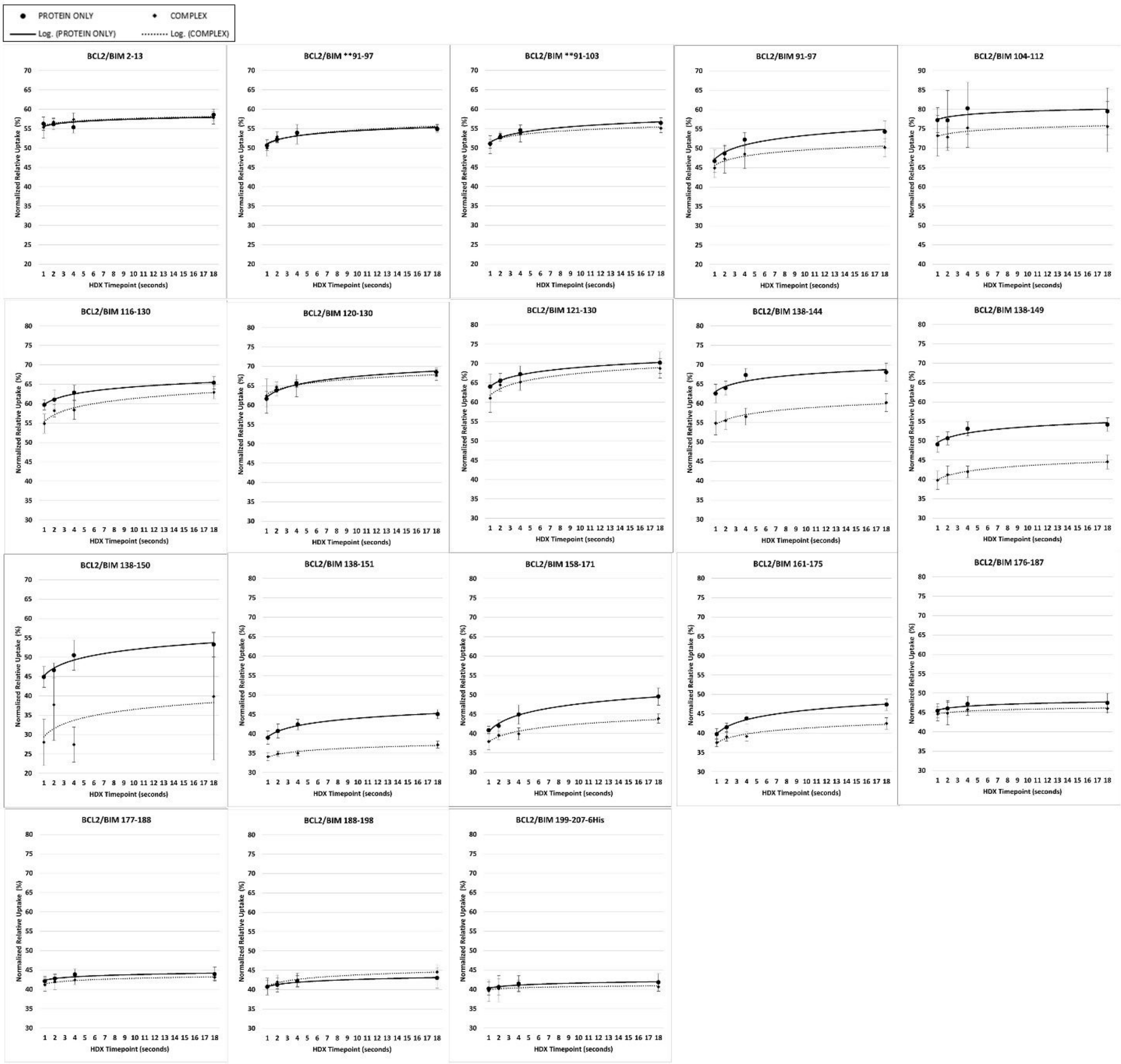
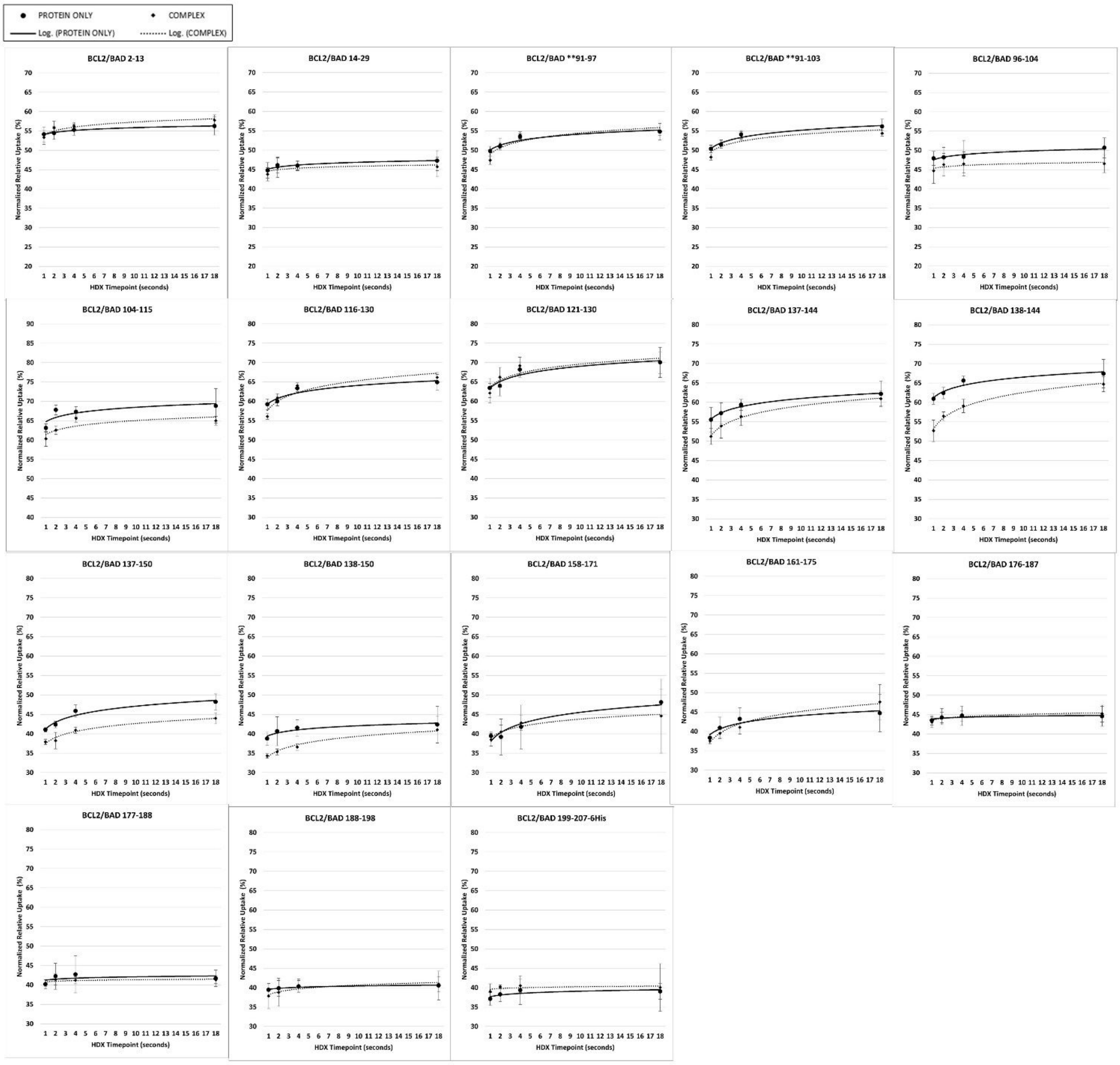


Figure S8. Bcl2 vs. Bcl2/Bim Deuterium Uptake Kinetics Plots.



BCL2/BAD 2-13

BCL2/BAD 14-29

BCL2/BAD **91-97

BCL2/BAD **91-103

BCL2/BAD 96-104

BCL2/BAD 104-115

BCL2/BAD 116-130

BCL2/BAD 121-130

BCL2/BAD 137-144

BCL2/BAD 138-144

BCL2/BAD 137-150

BCL2/BAD 138-150

BCL2/BAD 158-171

BCL2/BAD 161-175

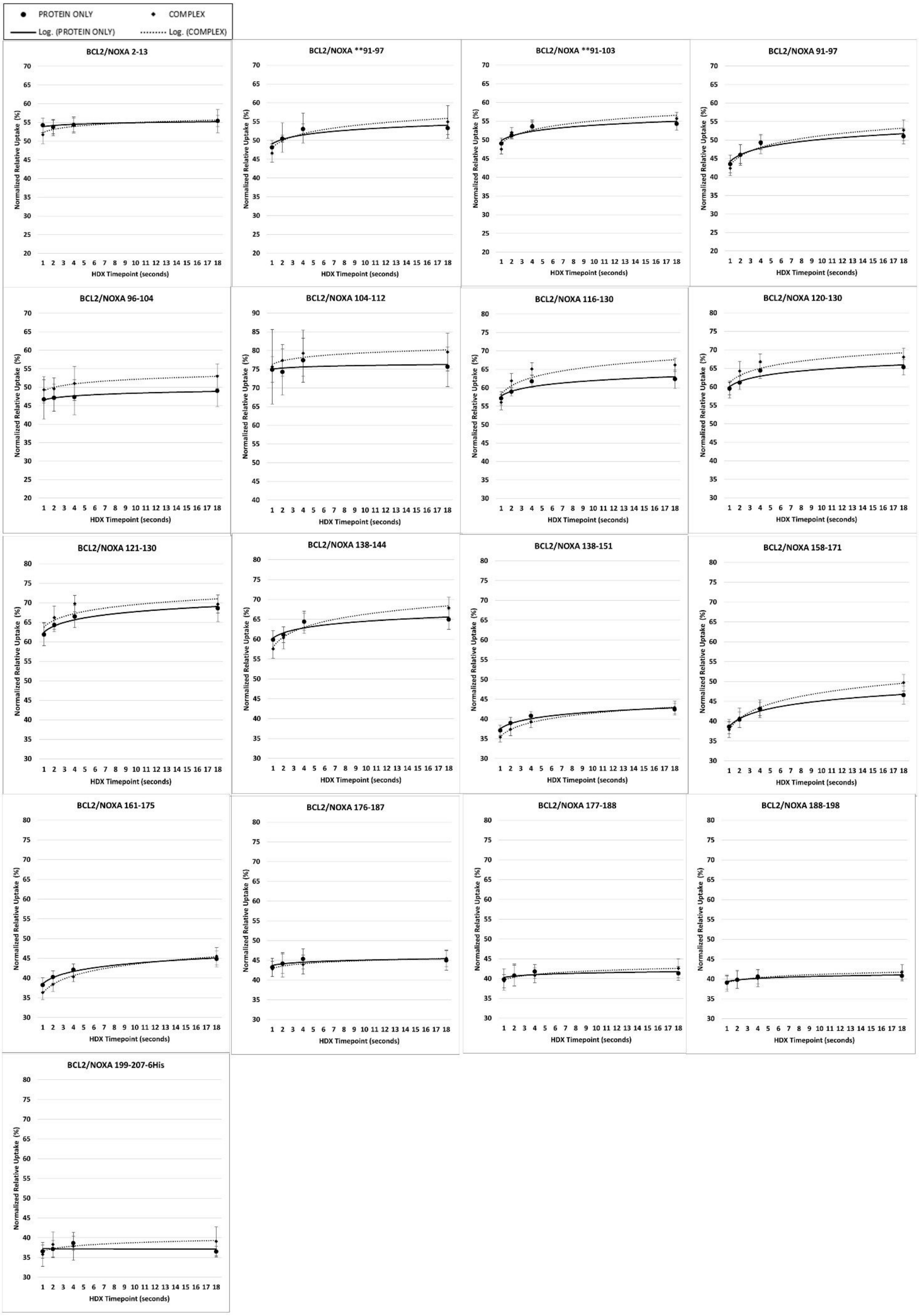
BCL2/BAD 176-187

BCL2/BAD 177-188

BCL2/BAD 188-198

BCL2/BAD 199-207-6His

Figure S9. Bcl2 vs. Bcl2/Bad Deuterium Uptake Kinetics Plots.



BCL2/NOXA 2-13

BCL2/NOXA **91-97

BCL2/NOXA **91-103

BCL2/NOXA 91-97

BCL2/NOXA 96-104

BCL2/NOXA 104-112

BCL2/NOXA 116-130

BCL2/NOXA 120-130

BCL2/NOXA 121-130

BCL2/NOXA 138-144

BCL2/NOXA 138-151

BCL2/NOXA 158-171

BCL2/NOXA 161-175

BCL2/NOXA 176-187

BCL2/NOXA 177-188

BCL2/NOXA 188-198

BCL2/NOXA 199-207-6His

Figure S10. Bcl2 vs. Bcl2/Noxa Deuterium Uptake Kinetics Plots.

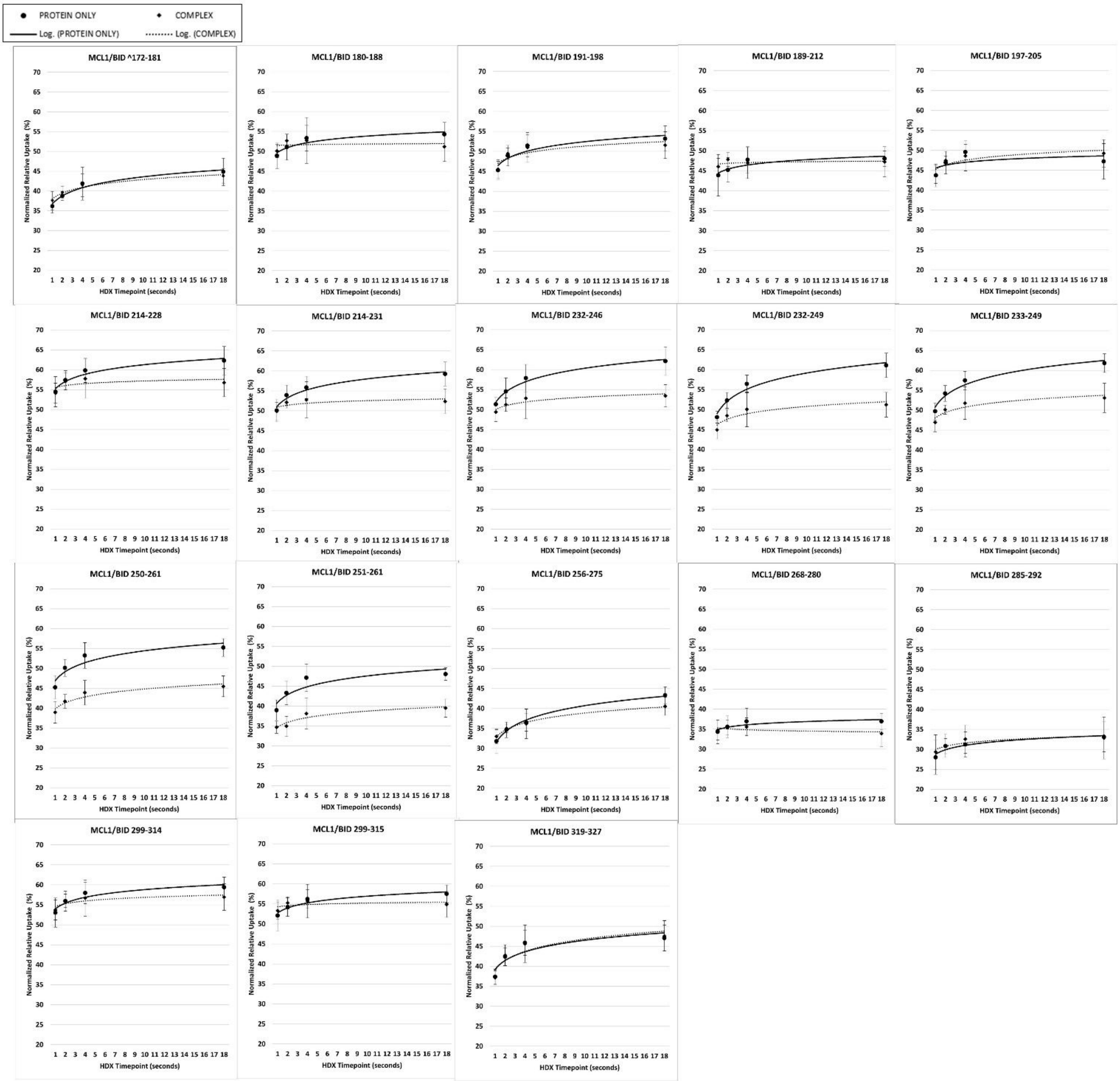


Figure S11. Mcl1 vs. Mcl1/Bid Deuterium Uptake Kinetics Plots.

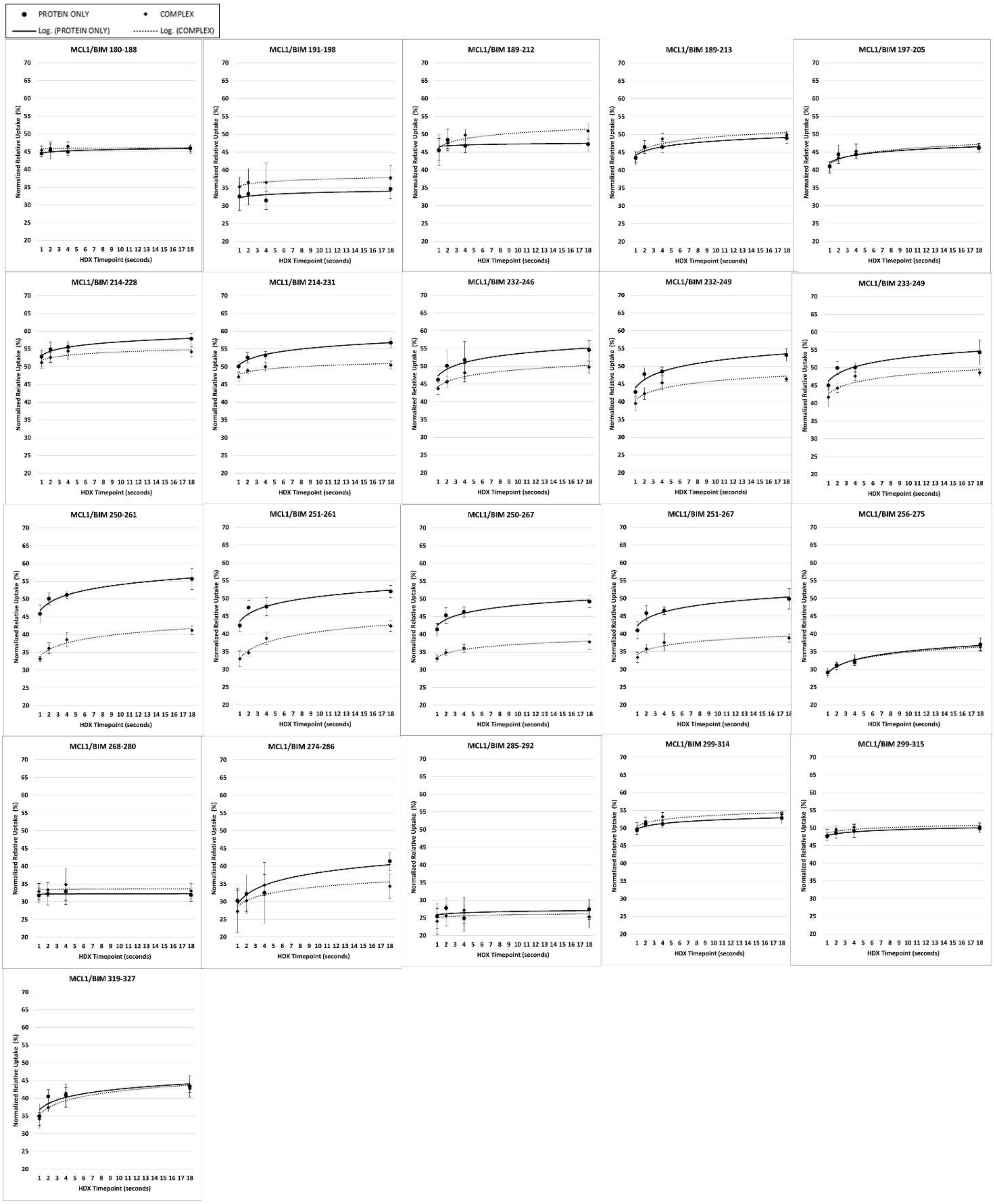


Figure S12. Mcl1 vs. Mcl1/Bid Deuterium Uptake Kinetics Plots.

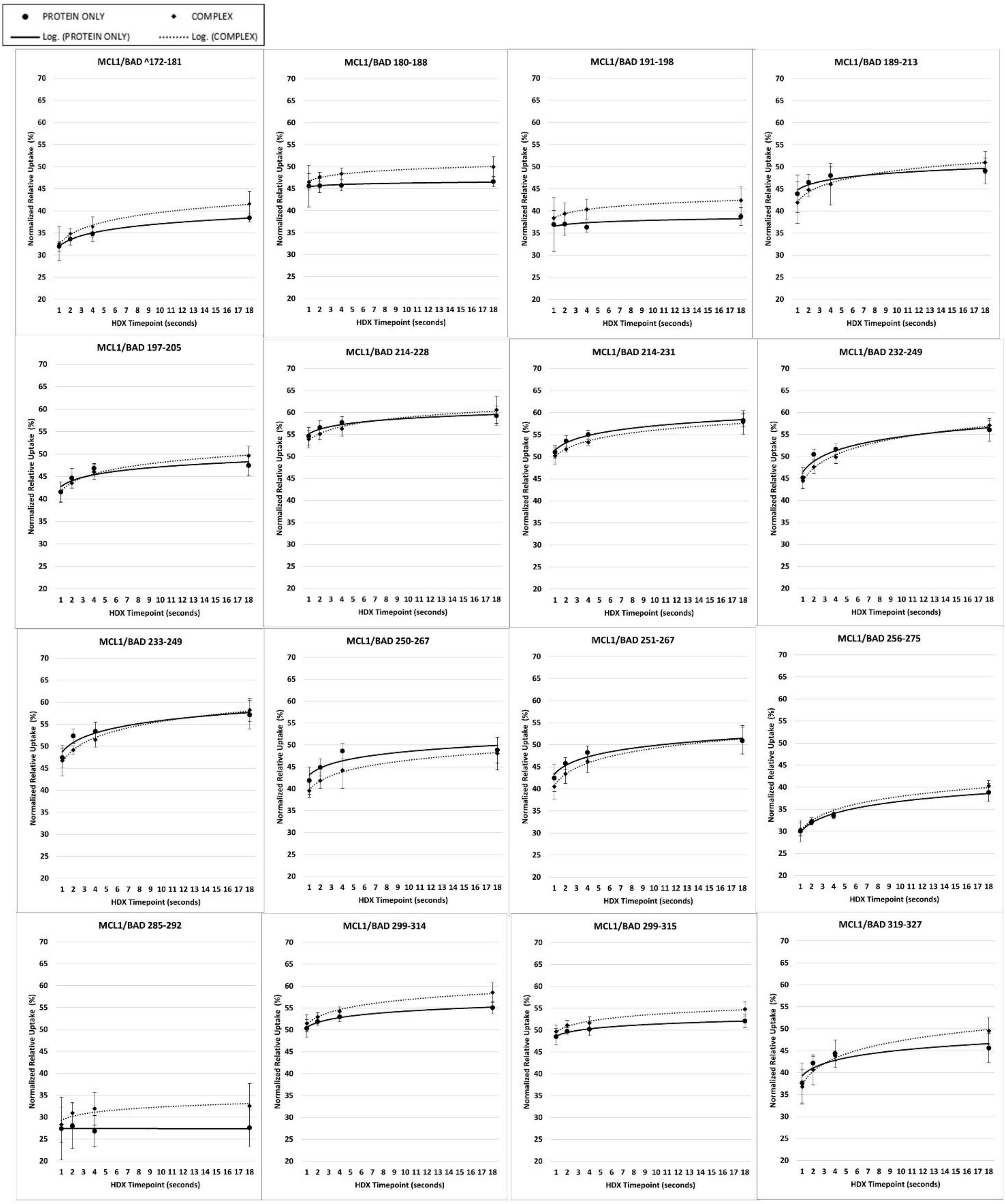


Figure S13. Mcl1 vs. Mcl1/Bad Deuterium Uptake Kinetics Plots.

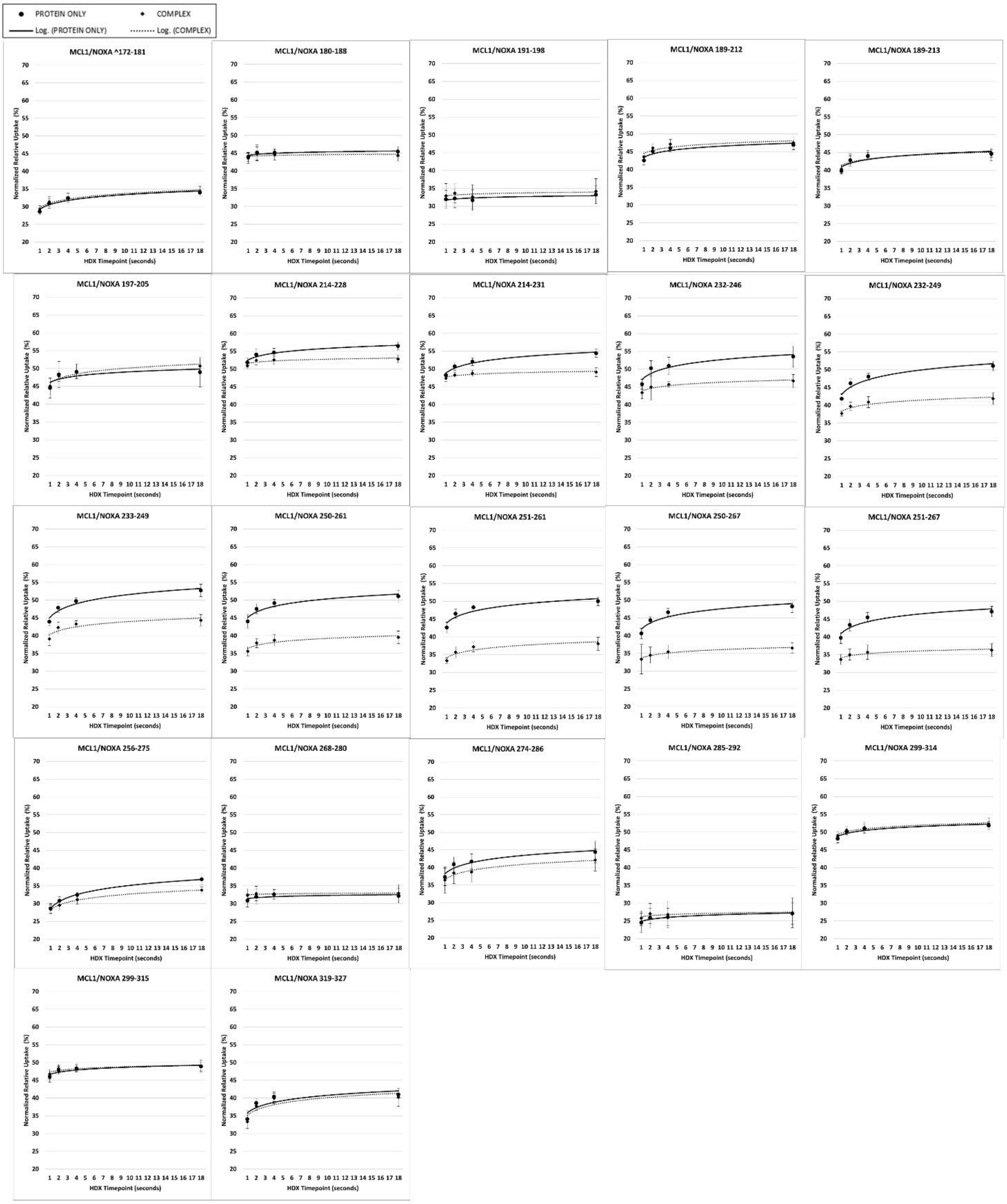


Figure S14. Mcl1 vs. Mcl1/Noxa Deuterium Uptake Kinetics Plots.

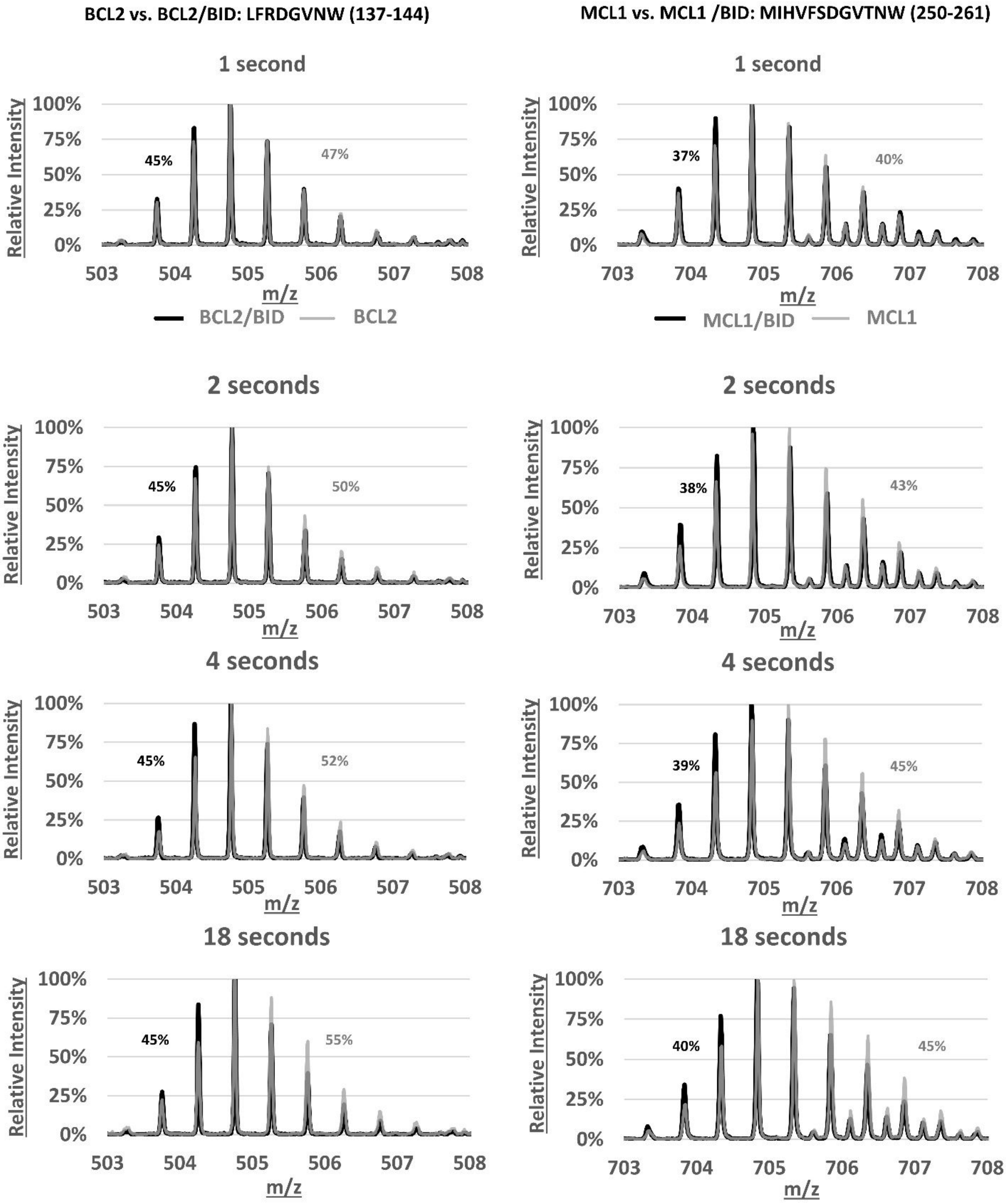
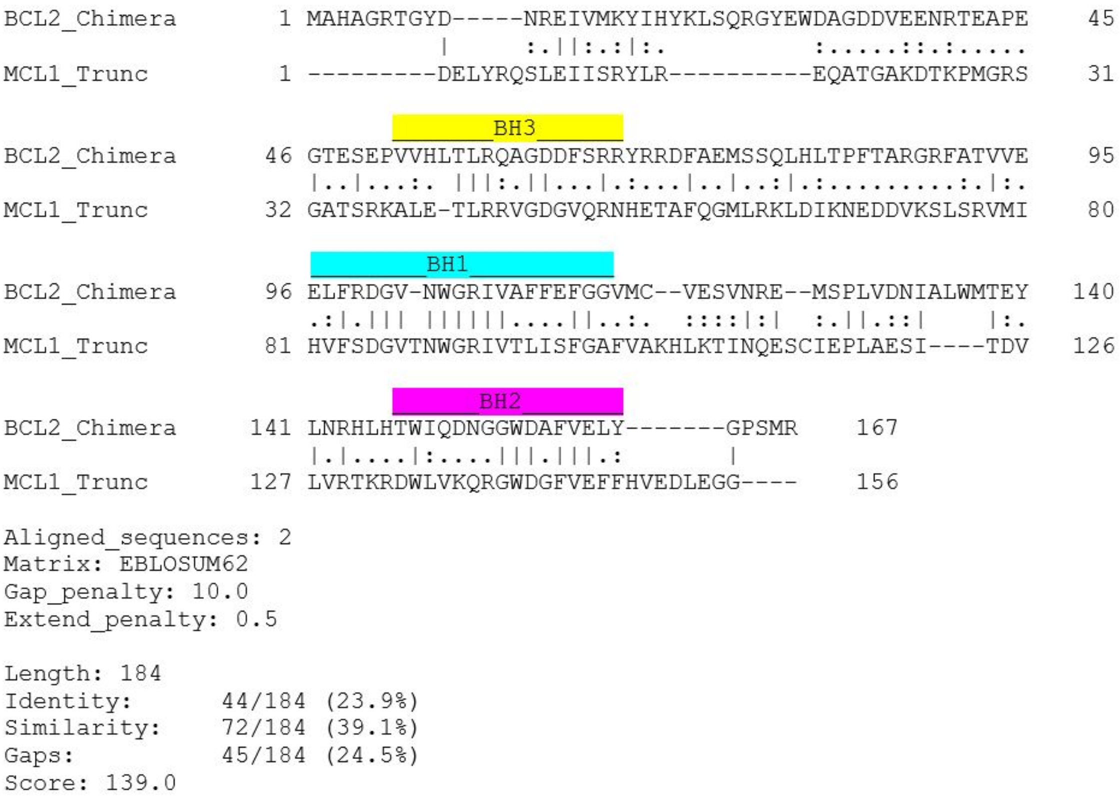


Figure S15. Raw Mass Spectra of Bcl-2 (left) and Mcl-1 (right) experiencing protection from HDX in the presence of BID BH3 peptide. In each case, the peptide corresponding to the protein alone is grey, and the bound protein is black. From top to bottom, the timepoints shown are 1, 2, 4, and 18 seconds. Note that during analysis in MS Studio, spectra were fit using peaks not experiencing spectral overlap.



```
                            ______BH3_______
BCL2_Chimera       1 MAHAGRTGYD-----NREIVMKYIHYKLSQRGYEWDAGDDVEENRTEAPE     45
                              |     :.||:.:|:.          :.....::.:.....
MCL1_Trunc         1 ---------DELYRQSLEIISRYLR----------EQATGAKDTKPMGRS     31

                           ______BH3_______
BCL2_Chimera      46 GTESEPVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVE     95
                     |..|...:. |||:.||...|.:...|..|..:|.:..........:.|:.
MCL1_Trunc        32 GATSRKALE-TLRRVGDGVQRNHETAFQGMLRKLDIKNEDDVKSLSRVMI     80

                     ________BH1__________
BCL2_Chimera      96 ELFRDGV-NWGRIVAFFEFGGVMC--VESVNRE--MSPLVDNIALWMTEY    140
                     .:|.||| ||||||....||..:.  ::::|:|  :.||.::|    |:.
MCL1_Trunc        81 HVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESI----TDV    126

                           ______BH2_______
BCL2_Chimera     141 LNRHLHTWIQDNGGWDAFVELY-------GPSMR    167
                     |.|....|:....|||.|||.:       |
MCL1_Trunc       127 LVRTKRDWLVKQRGWDGFVEFFHVEDLEGG----    156

Aligned_sequences: 2
Matrix: EBLOSUM62
Gap_penalty: 10.0
Extend_penalty: 0.5

Length: 184
Identity:      44/184 (23.9%)
Similarity:    72/184 (39.1%)
Gaps:          45/184 (24.5%)
Score: 139.0
```

Figure S16. Sequence Alignment of Bcl-2 Chimera and Truncated Mcl-1. Sequences shown here are those used in this work with cleavage artifacts and purification tags omitted. Sequence alignment completed using EMBOSS Needle. Lines indicate sequence identity whereas dots indicate high (:) or moderate sequence (.) similarity. Bcl-2 Homology domains colour-coded as follows: BH1 (cyan), BH2 (magenta), BH3 (yellow).