SUPPLEMENTARY MATERIALS, RING ET AL.

Table S1. Peptides observed in pepsin digests of ERK1.

Table S2. Amide hydrogen exchange parameters for 0P-ERK1 and 2P-ERK1.

Figure S1 (1 page). Full length mass measurements of 0P-ERK1 and 2P-ERK1. (**A-C**) 0P-ERK1 and (**D-F**) 2P-ERK1 were analyzed by ESI-MS following separation by RP-HPLC (0.5 mm i.d. x 10 cm column, POROS R1 20 resin). (**A,D**) Charge envelope of each full length protein. (**B,E**) Expanded view of one charge state from each protein. (**C,F**) Deconvoluted mass measurements. Each protein showed the full length mass and several potassium adducts (+38 Da). In each case, the observed full length mass of each protein was consistent with that calculated for demethionylated recombinant protein corresponding to the expected cDNA sequences, which differed by one N-terminal amino acid within the His_6 tag. For 0P-ERK1, the N-terminal His_6 tag sequence was AHHHHHHMAAAAA... and for 2P-ERK1, the N-terminal His_6 tag sequence was AHHHHHHAMAAAAA... Therefore, the mass of 2P-ERK1 was greater than that of the 0P-ERK1 by 231 Da = 71 Da (Ala) + 2 x 80 Da (HPO_3).

Figure S2 (3 pages). HX time courses for peptides observed in ERK1. Peptides are labeled by residue numbers, followed by the corresponding amino acid sequence. HX data on 0P-ERK1 are indicated by closed circles (•), and data on 2P-ERK1 are indicated by open circles (o).

Figure S3 (1 page). Hinge peptides in ERK1 and ERK2. (**A**) Observed peptides corresponding to the hinge region are indicated for ERK1 (this study) and ERK2 (from ref. 11). (**B**) Time courses for each peptide showed no differences in HX between 0P-ERK1 and 2P-ERK1, for peptides 125-145 and 125-141, each containing the hinge sequence, METDL. Overlapping peptides 130-145 and 127-145 also showed no differences in HX, confirming no changes within the METDL region. In contrast, previous studies showed increased HX within the METDL sequence upon phosphorylation and activation of ERK2 (data from ref. 11).

Figure S4 (2 pages). HX time courses for peptides showing protection of ERK1 upon nucleotide binding. HX data on apoenzyme are indicated by closed symbols, and data collected with AMP-PNP are indicated by open symbols. HX data on 0P-ERK1 are shown on page 1 of 2, and HX data on 2P-ERK1 are shown on page 2 of 2.

Figure S5 (1 page). HX protection by AMP-PNP within the DGF motif of ERK1 and ERK1. (**A**) HX measurements show significant protection of peptide 181-187 (KICDFGL) by AMP-PNP in both 0P-ERK1 and 2P-ERK1. In contrast, previous measurements in ERK2 showed higher protection in 2P-ERK2 compared to 0P-ERK2, corresponding to a 10-fold decrease in HX rate in the active form (data from ref. 11). (**B**) Overlay of X-ray structures for ERK1 (PDB: 2ZOQ) and ERK2 (PDB: 1ERK) show little difference in structure between the two enzymes within the DGF motif region.

Figure S6 (1 page). Alignment of ERK1 and ERK2 sequences. Alignment of human ERK1 and rat ERK2 indicate conserved residues (red) and variable residues (black).

Table S1. Peptides observed in pepsin digests of ERK1

Peptide	Sequence	MH^{+1} Calc (mono)[a]	m/z Obs (mono)[b]	z [c]	MH^{+1} obs (mono)[d]	Delta[e]
-7-29	AHHHHHHMAAAAAQGGGGGEPRRTEGVGPGVPGEVE	3557.67	890.20	4	3557.78	-0.11
30-45	MVKGQPFDVGPRYTQL	1835.95	612.65	3	1835.95	0.00
31-45	VKGQPFDVGPRYTQL	1704.91	568.97	3	1704.92	-0.01
46-59	QYIGEGAYGMVSSA	1432.64	716.83	2	1432.66	-0.02
60-88	YDHVRKTRVAIKKISPFEHQTYCQRTLRE	3602.91	901.48	4	3602.92	-0.01
69-90	AIKKISPFEHQTYCQRTLREIQ	2689.42	897.13	3	2689.39	0.03
93-102	LRFRHENVIG	1240.69	414.24	3	1240.72	-0.03
94-102	RFRHENVIG	1127.61	564.32	2	1127.64	-0.03
103-112	IRDILRASTL	1157.70	579.35	2	1157.70	0.00
103-114	IRDILRASTLEA	1357.78	453.26	3	1357.78	0.00
108-119	RASTLEAMRDVY	1411.70	471.25	3	1411.75	-0.05
115-124	MRDVYIVQDL	1251.64	626.32	2	1251.63	0.01
118-124	VYIVQDL	849.47	425.22	2	849.44	0.03
120-126	IVQDLME	847.42	847.43	1	847.43	-0.01
125-141	METDLYKLLKSQQLSND	2026.02	1013.40	2	2025.80	0.22
125-145	METDLYKLLKSQQLSNDHICY	2542.23	1271.59	2	2542.18	0.05
127-145	TDLYKLLKSQQLSNDHICY	2282.15	761.38	3	2282.13	0.02
127-147	TDLYKLLKSQQLSNDHICYFL	2542.30	1271.59	2	2542.18	0.12
130-145	YKLLKSQQLSNDHICY	1952.99	651.67	3	1953.01	-0.02
146-163	FLYQILRGLKYIHSANVL	2148.23	716.74	3	2148.23	0.00
146-172	FLYQILRGLKYIHSANVLHRDLKPSNL	3208.81	642.56	5	3208.80	0.01
147-172	LYQILRGLKYIHSANVLHRDLKPSNL	3061.74	766.18	4	3061.74	0.00
164-172	HRDLKPSNL	1079.60	1079.59	1	1079.59	0.00
173-180	LINTTCDL	892.45	446.73	2	892.45	-0.01
180-187	LKICDFGL	908.49	454.74	2	908.48	0.01
188-203	ARIADPEHDHTGFLTE (0P only) [f]	1808.86	603.63	3	1808.89	-0.03
188-209	ARIADPEHDHTGFLTEYVATRW (2P only) [g]	2745.19	915.73	3	2745.20	-0.01
204-209	YVATRW (0P only) [f]	795.42	795.41	1	795.41	0.01
210-216	YRAPEIM	879.44	440.23	2	879.45	-0.01
210-217	YRAPEIML	992.52	496.76	2	992.53	0.00
217-232	LNSKGYTKSIDIWSVG	1767.93	589.78	3	1767.34	0.59
218-232	NSKGYTKSIDIWSVG	1654.84	552.28	3	1654.84	0.00
236-254	AEMLSNRPIFPGKHYLDQL	2229.15	743.70	3	2229.10	0.05
239-254	LSNRPIFPGKHYLDQL	1898.03	949.50	2	1898.00	0.03
239-267	LSNRPIFPGKHYLDQLNHILGILGSPSQE	3243.73	649.56	5	3243.80	-0.07
239-269	LSNRPIFPGKHYLDQLNHILGILGSPSQEDL	3471.84	868.71	4	3471.84	0.00
255-271	NHILGILGSPSQEDLNC	1809.88	905.43	2	1809.87	0.01
276-292	KARNYLQSLPSKTKVAW	1990.12	995.54	2	1990.09	0.03
280-306	YLQSLPSKTKVAWAKLFPKSDSKALDL	3034.70	759.43	4	3034.72	-0.02
296-306	FPKSDSKALDL	1220.65	407.56	3	1220.68	-0.03
307-323	LDRMLTFNPNKRITVEE	2076.09	692.70	3	2076.10	-0.01
311-323	LTFNPNKRITVEE	1560.84	780.93	2	1560.86	-0.03
311-332	LTFNPNKRITVEEALAHPYLEQ	2583.36	646.60	4	2583.40	-0.04
323-332	EALAHPYLEQ	1170.58	585.80	2	1170.60	-0.02
324-332	ALAHPYLEQ	1041.54	521.28	2	1041.56	-0.02
333-347	YYDPTDEPVAEEPFT	1772.75	886.87	2	1772.74	0.01
333-348	YYDPTDEPVAEEPFTF	1919.82	640.60	3	1919.80	0.02
333-349	YYDPTDEPVAEEPFTFA	1990.86	995.92	2	1990.84	0.02
349-365	AMELDDLPKERLKELIF	2060.11	687.35	3	2060.05	0.06
350-365	MELDDLPKERLKELIF	1989.07	663.69	3	1989.07	0.00
352-365	LDDLPKERLKELIF	1728.99	433.00	4	1728.98	0.01
363-376	LIFQETARFQPGVL	1618.90	809.94	2	1618.88	0.02
366-376	QETARFQPGVL	1245.66	623.34	2	1245.67	-0.01
366-379	QETARFQPGVLEAP	1542.79	771.90	2	1542.79	0.00

[a] Calculated MH^{+1}, monoisotopic mass

[b] Observed mass/charge, monoisotopic mass, from QStar Pulsar datasets.

[c] Observed charge (z), from QStar Pulsar datasets.

[d] Observed MH^{+1}, monoisotopic mass

[e] Delta = Calculated MH^{+1} – Observed MH^{+1}

[f] Unphosphorylated form, peptide observed only in datasets of 0P-ERK1

[g] Diphosphorylated form, peptide observed only in datasets of 2P-ERK1

Table S2. Amide hydrogen exchange parameters for 0P-ERK1 and 2P-ERK1

Residues	Sequence		No. Amide[a]	Exch. Amide[b]	A [c]		B [c]		C [c]		k_1 [c]		k_2 [c]		k_3 [c]		NE[d]	% Exch[e]
					Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.		
-7-29	AHHHHHHMAAAAAQGGGGGEPRRTE GVGPGVPGEVE	0P	36	32	16.5						1.75							
30-45	MVKGQPFDVGPRYTQL	0P	15	13	4.19	0.51	1.54	0.46	2.62	0.20	7.66	1.23	1.08	0.49	0.046	0.008	4.7	64
		2P	15	13	2.51	0.38	3.41	0.34	2.60	0.18	17.9	9.30	1.46	0.25	0.025	0.005	4.5	66
31-45	VKGQPFDVGPRYTQL	0P	14	12	3.55	0.44	1.69	0.41	2.20	0.25	7.34	1.35	0.760	0.355	0.038	0.009	5.9	62
		2P	14	12	4.00	0.32	1.46	0.32	1.96	0.33	4.51	0.55	0.329	0.240	0.016	0.007	4.6	62
46-59	QYIGEGAYGMVSSA	0P	13	13	3.75	0.17	1.56	0.18	3.79	0.19	20.3	5.40	0.576	0.154	0.016	0.007	3.9	70
		2P	13	13	4.18	0.20	3.59	0.21			9.88	1.55	0.026	0.006			5.2	60
60-88	YDHVRKTRVAIKKISPFEHQTYCQRTLRE	0P	28	27	5.61	0.35	4.18	0.35			6.91	1.20	0.034	0.010			17.2	36
		2P	28	27	5.26	0.40	4.85	0.44			8.98	2.32	0.034	0.013			16.9	37
69-90	AIKKISPFEHQTYCQRTLREIQ	0P	21	20	5.36	0.32	3.68	0.33			7.20	1.31	0.045	0.014			11.0	45
		2P	21	20	5.15	0.33	4.12	0.33			7.78	1.44	0.032	0.009			10.7	46
93-102	LRFRHENVIG	0P	9	9	0.58	0.05	1.17	0.04			6.04	1.22	0.130	0.018			7.2	19
		2P	9	9	0.67	0.07	0.96	0.06			5.85	1.56	0.102	0.025			7.4	18
94-102	RFRHENVIG	0P	8	8	0.88	0.29	0.51	0.28			0.51	0.16	0.077	0.058			6.6	17
		2P	8	8	1.03	0.10	0.37	0.10			0.67	0.12	0.021	0.016			6.6	18
103-112	IRDILRASTL	0P	9	9	0.72	0.08	1.88	0.10	1.68	0.09	16.4	7.15	0.729	0.075	0.037	0.004	4.7	48
		2P	9	9	1.50	0.17	2.56	0.16			3.50	0.94	0.126	0.025			4.9	45
103-114	IRDILRASTLEA	0P	11	11	1.09	0.39	3.04	0.36	1.62	0.17	8.17	4.27	1.00	0.19	0.038	0.010	5.2	52
		2P	11	11	2.45	0.19	2.18	0.17	1.09	0.20	3.12	0.30	0.342	0.087	0.028	0.008	5.3	52
108-119	RASTLEAMRDVY	0P	11	11	3.83	0.22	2.25	1.06			12.1	2.91	0.007	0.007				55
		2P	11	11	1.83	1.21	1.98	1.13			23.9	65.1	2.72	1.84				35
115-124	MRDVYIVQDL	0P	9	9	0.19	0.05	1.14	0.05			3.66	1.56	0.329	0.034			7.7	15
		2P	9	9	1.32	0.02					0.25	0.01					7.7	15
118-124	VYIVQDL	0P	6	6	1.10	0.02					0.41	0.03					4.9	18
		2P	6	6	1.08	0.03					0.18	0.02					4.9	18
120-126	IVQDLME	0P	6	6	1.38	0.19	0.97	0.18			6.44	1.60	0.39	0.18			3.6	39
		2P	6	6	1.02	0.12	1.21	0.10			8.90	2.59	0.26	0.072			3.8	37
125-141	METDLYKLLKSQQLSND	0P	16	16	4.49	0.12	2.26	0.13			8.79	0.79	0.039	0.009			9.2	42
		2P	16	16	4.60	0.18	1.99	0.16			8.02	0.90	0.065	0.022			9.4	41
125-145	METDLYKLLKSQQLSNDHICY	0P	20	20	4.47	0.30	1.20	0.32	3.58	0.26	11.6	2.0	0.59	0.36	0.023	0.004	10.7	46
		2P	20	20	3.63	0.16	2.52	0.16	3.41	0.14	23.4	7.5	0.86	0.11	0.012	0.002	10.4	48
127-145	TDLYKLLKSQQLSNDHICY	0P	18	18	4.31	0.27	1.32	0.36	2.38	0.39	10.8	1.7	0.38	0.28	0.023	0.008	10.0	45
		2P	18	18	4.17	0.23	1.30	0.23	2.98	0.21	10.8	1.5	0.46	0.23	0.013	0.003	9.6	47
127-147	TDLYKLLKSQQLSNDHICYFL	0P	20	20	4.28	0.45	1.35	0.44	3.72	0.27	11.7	2.9	0.87	0.52	0.025	0.005	10.7	47
		2P	20	20	3.74	0.19	2.45	0.19	3.51	0.20	16.2	3.1	0.71	0.12	0.011	0.002	10.3	49
130-145	YKLLKSQQLSNDHICY	0P	15	15	4.32	0.15	3.34	0.16			8.75	0.98	0.030	0.006			7.3	51
		2P	15	15	4.08	0.17	3.34	0.16			9.59	1.26	0.036	0.007			7.6	49

Table S2 (cont.)

Residues	Sequence		No. Amide[a]	Exch. Amide[b]	A [c]		B [c]		C [c]		k_1 [c]		k_2 [c]		k_3 [c]		NE[d]	% Exch[e]
					Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.		
146-163	FLYQILRGLKYIHSANVL	0P	17	17	0.37	0.11					0.21	0.23						2
		2P	17	17	0.20	0.12					1.11	1.61						1
146-172	FLYQILRGLKYIHSANVLHRDLKPSNL	0P	26	25	1.16	0.25					0.03	0.03						5
		2P	26	25	0.87	0.25					8.48	7.46						3
147-172	LYQILRGLKYIHSANVLHRDLKPSNL	0P	25	24	0.96	0.20					0.04	0.03						4
		2P	25	24	0.85	0.16					3.36	1.72						4
164-172	HRDLKPSNL	0P	8	7	0.51	0.05					12.5	5.0					6.5	7
		2P	8	7	0.51	0.05					12.5	5.0					6.5	7
173-180	LINTTCDL	0P	7	7	1.38	0.06	0.90	0.06			7.43	0.98	0.077	0.021			4.7	33
		2P	7	7	1.73	0.09	0.86	0.08			6.24	0.87	0.087	0.033			4.4	37
180-187	LKICDFGL	0P	7	7	1.48	0.16	1.21	0.15			0.63	0.10	0.033	0.010			4.3	38
		2P	7	7	2.50	0.08					0.05	0.01					4.5	36
188-203	ARIADPEHDHTGFLTE	0P	15	14	4.68	0.22	1.57	0.15					3.337	0.064			7.7	45
188-209	ARIADPEHDHTGFLTEYVATRW (pTpY)	2P	21	20	5.58	0.28	3.17	0.26			24.0	9.6	0.799	0.117			11.2	44
204-209	YVATRW	0P	5	5	3.81	0.04					14.4	1.1					1.2	76
210-216	YRAPEIM	0P	6	5	0.48	0.15	2.71	0.15			1.07	0.57	0.038	0.005			1.8	64
		2P	6	5	0.90	0.14	2.33	0.71			0.08	0.02	0.004	0.002			1.8	65
210-217	YRAPEIML	0P	7	6	1.57	0.11	2.83	0.11			1.35	0.18	0.036	0.004			1.6	73
		2P	7	6	1.80	0.08	3.74	1.81			0.18	0.02	0.002	0.002			0.5	92
217-232	LNSKGYTKSIDIWSVG	0P	15	15	4.92	0.06	1.72	0.16			17.9	1.52	0.011	0.002			8.4	44
		2P	15	15	4.52	0.14	1.42	0.43			5.22	0.45	0.009	0.006			9.1	40
218-232	NSKGYTKSIDIWSVG	0P	14	14	4.10	0.07	2.19	0.08			14.2	1.17	0.025	0.003			7.7	45
		2P	14	14	3.77	0.11	1.81	0.56			4.08	0.34	0.007	0.004			8.4	40
236-254	AEMLSNRPIFPGKHYLDQL	0P	18	16	3.98	0.35	3.16	0.31	3.47	0.23	15.3	3.60	1.403	0.278	0.056	0.009	5.4	66
		2P	18	16	4.97	0.40	4.14	0.35			5.79	1.12	0.154	0.046			6.9	57
239-254	LSNRPIFPGKHYLDQL	0P	15	13	3.01	0.84	2.57	0.70	3.59	0.24	19.5	15.2	2.363	1.034	0.051	0.009	3.8	71
		2P	15	12	4.36	0.29	3.03	0.25			7.27	1.33	0.072	0.025			4.6	62
239-267	LSNRPIFPGKHYLDQLNHILGILGSPSQE	0P	28	25	4.78	0.86	4.29	0.81	11.1	0.53	17.3	11.0	1.219	0.421	0.032	0.004	4.9	80
		2P	28	25	6.91	0.62	6.56	0.62			7.25	1.83	0.031	0.011			11.5	54
239-269	LSNRPIFPGKHYLDQLNHILGILGSPSQEDL	0P	30	27	5.16	0.71	6.10	0.83	10.0	0.72	21.0	17.8	0.683	0.194	0.026	0.005	5.7	79
		2P	30	27	12.4	1.23					0.98	0.22					14.6	46
255-271	NHILGILGSPSQEDLNC	0P	16	15	1.61	0.28	7.67	0.18	1.76	0.26	21.9	20.7	0.033	0.002	1.345	0.387	4.0	74
		2P	16	15	2.42	0.24	1.36	0.22	2.04	0.15	8.52	1.51	0.627	0.230	0.020	0.005	9.2	39
276-292	KARNYLQSLPSKTKVAW	0P	16	15	5.43	0.18	4.40	0.16			7.83	0.67	0.214	0.027			5.2	66
		2P	16	15	5.18	0.25	4.36	0.21			7.96	1.06	0.114	0.022			5.5	64
280-306	YLQSLPSKTKVAWAKLFPKSDSKALDL	0P	26	24	9.90	0.34	8.70	0.35			6.87	0.71	0.047	0.008			5.4	78
		2P	26	24	9.73	0.75	8.31	1.05			6.65	1.40	0.016	0.006			6.0	75

Table S2 (cont.)

Residues	Sequence		No. Amide[a]	Exch. Amide[b]	A [c]		B [c]		C [c]		k_1 [c]		k_2 [c]		k_3 [c]		NE[d]	% Exch[e]
					Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.	Fit	S.E.		
296-306	FPKSDSKALDL	0P	10	9	2.79	0.10	1.32	0.12			22.7	8.88	0.026	0.009			4.9	46
		2P	10	9	2.91	0.08	3.35	3.85			22.0	5.47	0.002	0.003			2.7	70
307-323	LDRMLTFNPNKRITVEE	0P	16	15	2.11	0.28	2.09	0.32	3.95	0.35	7.03	1.61	0.447	0.186	0.035	0.005	6.8	54
		2P	16	15	2.66	0.17	5.31	0.16			4.45	0.73	0.070	0.008			7.0	53
311-323	LTFNPNKRITVEE	0P	12	11	2.56	0.18	5.03	0.18			4.94	0.96	0.057	0.008			3.4	69
		2P	12	11	2.67	0.21	5.06	0.19			4.49	0.89	0.058	0.008			3.3	70
311-332	LTFNPNKRITVEEALAHPYLEQ	0P	21	19	2.18	0.26	3.27	0.30	4.60	0.36	5.34	0.98	0.374	0.104	0.030	0.004	8.9	53
		2P	21	19	2.73	0.14	4.14	0.57	3.07	0.57	4.38	0.45	0.171	0.036	0.021	0.006	9.1	52
323-332	EALAHPYLEQ	0P	9	8	0.47	0.08	0.86	0.07			10.90	5.94	0.195	0.056			6.7	17
		2P	9	8	1.23	0.07					0.67	0.10					6.8	15
324-332	ALAHPYLEQ	0P	8	7	1.36	0.08					0.65	0.09					5.6	19
		2P	8	7	0.39	0.04	1.01	0.03			21.9	17.3	0.241	0.027			5.6	20
333-347	YYDPTDEPVAEEPFT	0P	14	11	2.74	0.35	2.65	0.76	1.23	0.86	12.4	3.63	0.911	0.400	0.126	0.105	4.4	60
		2P	14	11	3.16	0.11	3.34	0.09			9.47	0.98	0.156	0.016			4.5	59
333-348	YYDPTDEPVAEEPFTF	0P	15	12	3.41	0.24	2.84	0.27	1.22	0.25	26.4	16.9	1.09	0.21	0.051	0.024	4.5	62
		2P	15	12	4.14	0.15	3.13	0.13			8.92	0.94	0.124	0.020			4.7	61
333-349	YYDPTDEPVAEEPFTFA	0P	16	13	3.52	0.53	4.33	0.50			14.9	7.98	0.604	0.141			5.1	60
		2P	16	13	4.12	0.26	3.58	0.21			19.4	8.15	0.136	0.031			5.3	59
349-365	AMELDDLPKERLKELIF	0P	16	15	3.82	0.15	3.11	0.17			9.49	1.30	0.027	0.005			8.1	46
		2P	16	15	3.60	0.19	3.50	0.53			9.52	1.62	0.009	0.003			7.9	47
350-365	MELDDLPKERLKELIF	0P	15	14	3.00	0.26	1.59	0.26	2.19	0.20	18.3	7.09	0.785	0.258	0.016	0.005	7.2	48
		2P	15	14	2.54	0.33	1.52	0.29	2.46	0.14	17.7	7.49	1.55	0.477	0.017	0.003	7.5	47
352-365	LDDLPKERLKELIF	0P	13	12	2.11	0.17	0.94	0.16	2.36	0.12	9.92	1.71	0.570	0.240	0.019	0.003	6.6	45
		2P	13	12	1.64	0.18	1.07	0.17	2.54	0.13	19.4	9.68	1.030	0.294	0.013	0.002	6.7	44
363-376	LIFQETARFQPGVL	0P	13	12	3.18	0.14	1.80	0.42	1.77	0.44	18.8	3.84	0.691	0.212	0.092	0.032	5.3	56
		2P	13	12	3.07	0.13	2.37	0.15	1.29	0.13	22.8	7.15	0.592	0.084	0.018	0.006	5.3	56
366-376	QETARFQPGVL	0P	10	9	3.32	0.11	1.51	0.16	1.58	0.16	18.7	2.71	0.950	0.184	0.072	0.014	2.6	71
		2P	10	9	3.65	0.16	2.47	0.14			20.5	6.38	0.256	0.049			2.9	68
366-379	QETARFQPGVLEAP	0P	13	11	5.05	0.06	2.81	0.06			14.2	0.77	0.199	0.015			3.1	71
		2P	13	11	5.08	0.18	2.68	0.15			17.5	3.29	0.241	0.047			3.2	71

[a] Number of amides = # amino acids – 1

[b] Number of exchangeable amides (N_T) = # amino acids – # prolines – 1

[c] Non-linear least squares fitted values and standard errors of parameters fit to the equation: Deuterons = $N_T - Ae^{-k1t} - Be^{-k2t} - Ce^{-k3t} - NE$

[d] Non-exchanging amides (NE) = $N_T - (A+B+C)$

[e] % Exchange = $(A+B+C)/N_T$

Suppl. Figure 1, Ring et al.

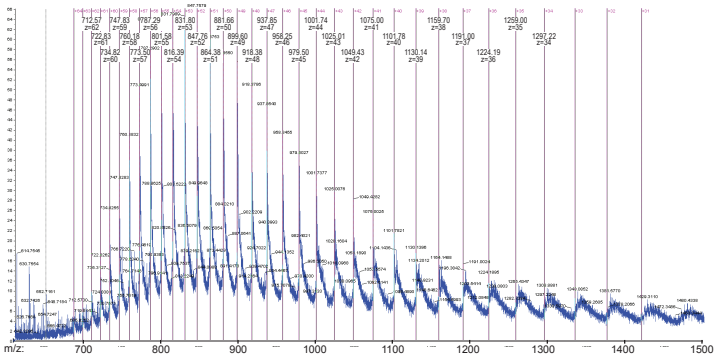
0P-ERK1

N-term Sequence: AHHHHHHMAAAAAQG...
Calc. Mass (av) = 44029.1
Obs. Mass (av) = 44030.8 +/- 2.4
Charge states +31 to +64

2P-ERK1

N-term Sequence: AHHHHHHAMAAAAAQG...
Calc. Mass (av) = 44260.9
Obs. Mass (av) = 44262.6 +/- 1.6
Charge states +29 to +63

A



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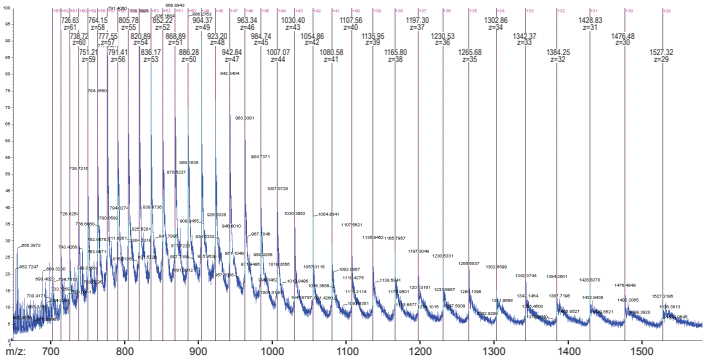
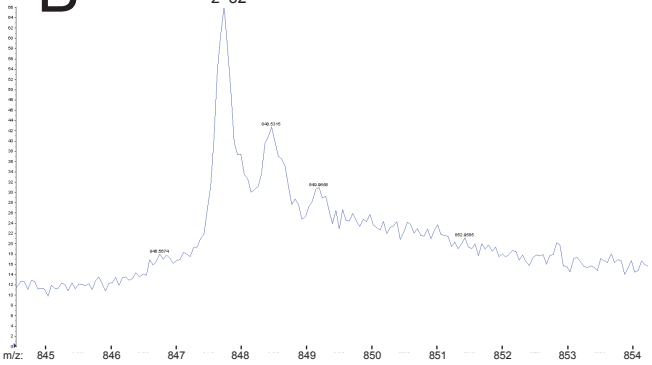


B



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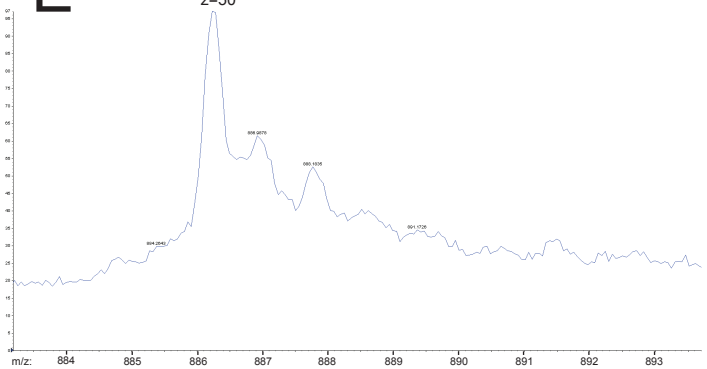
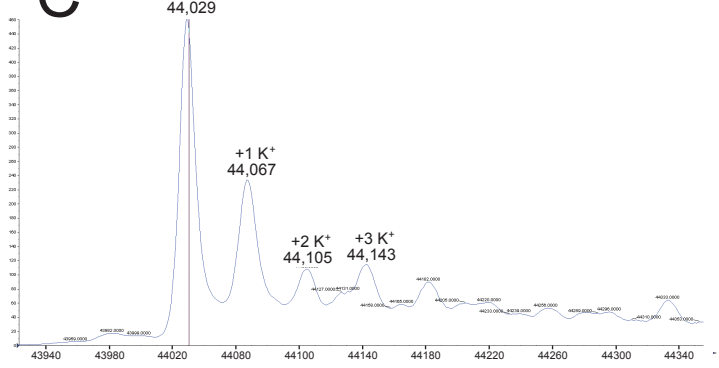


C



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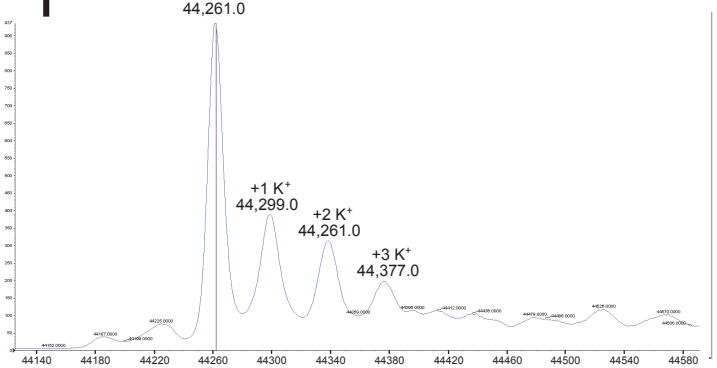
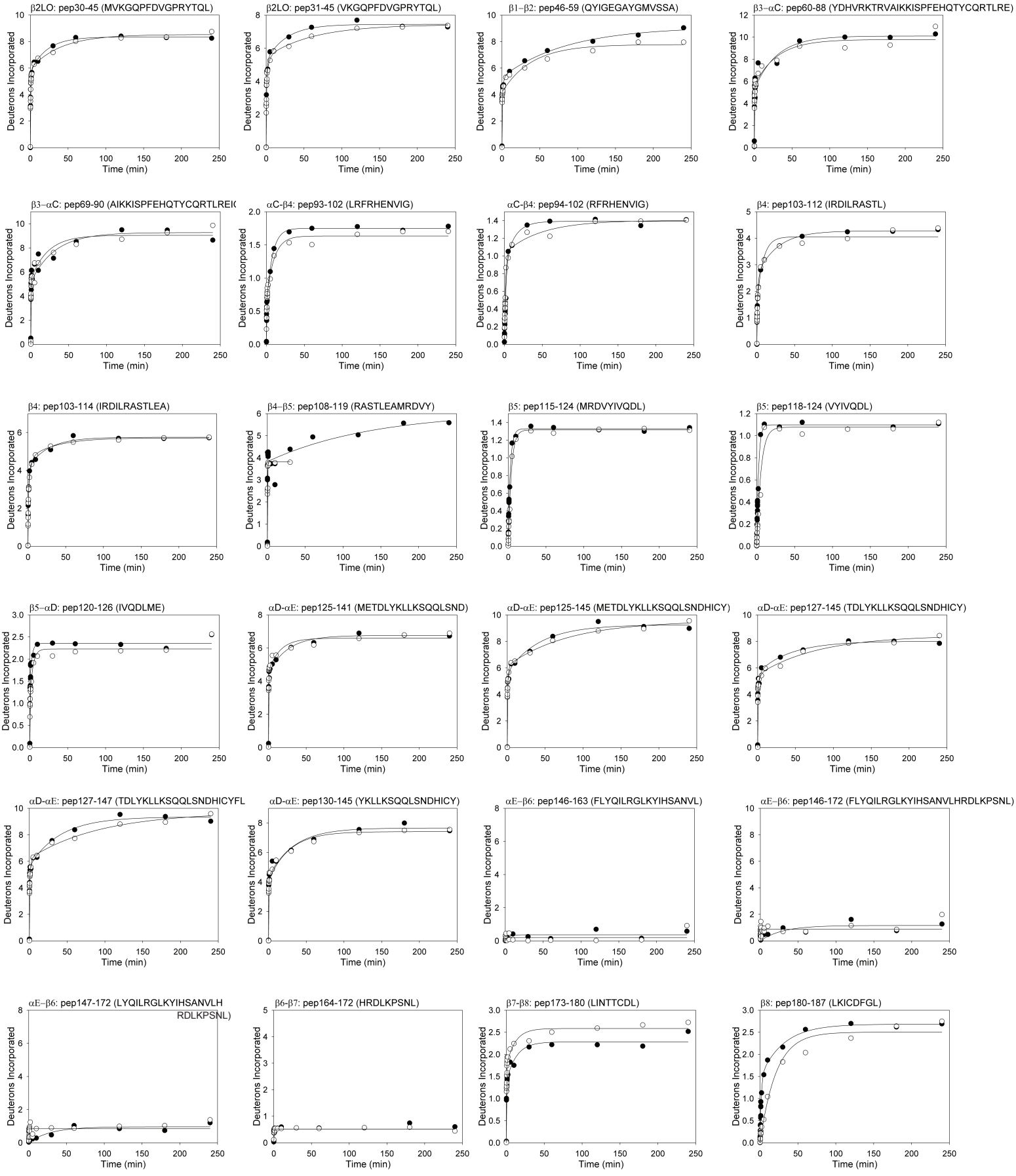
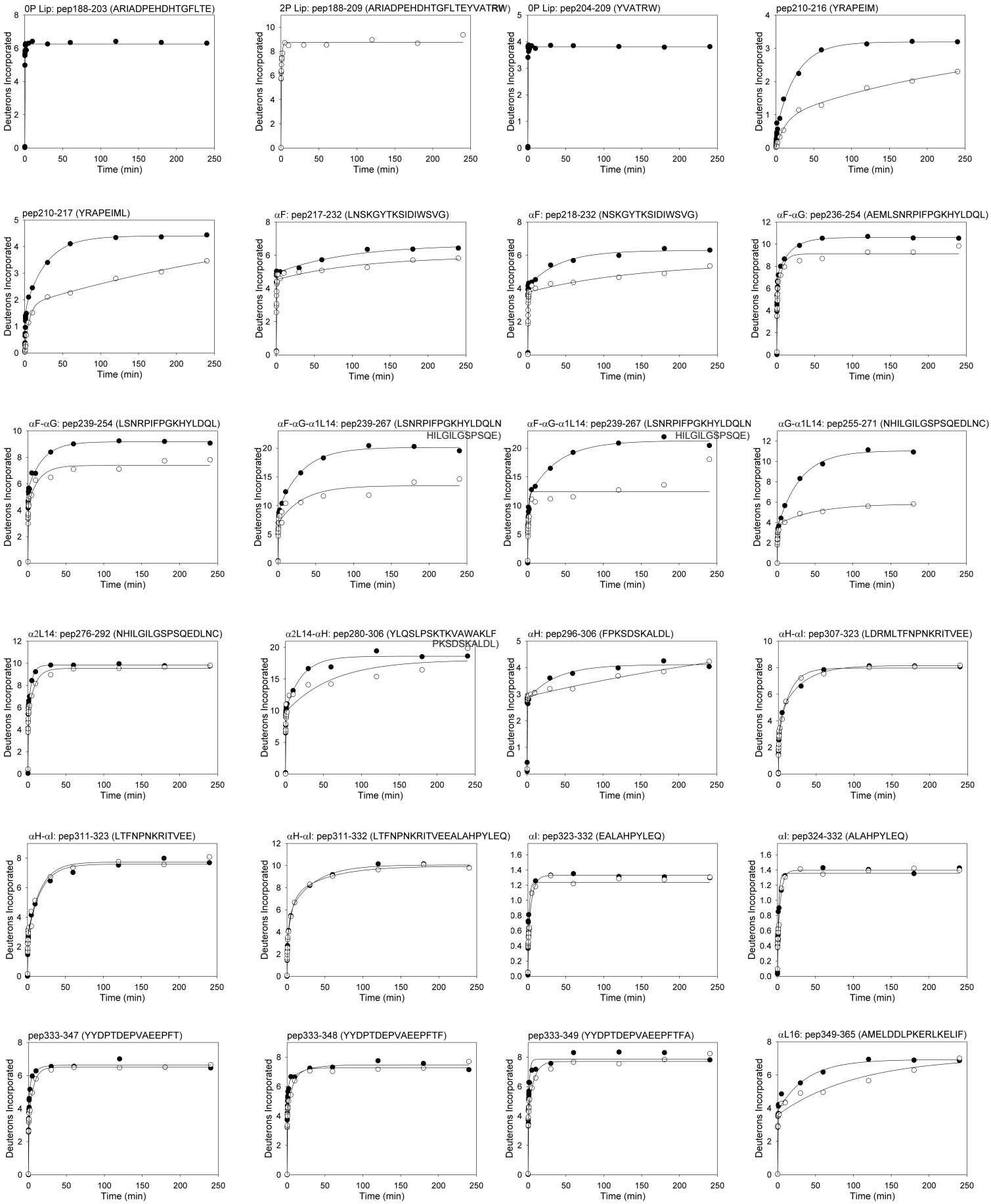
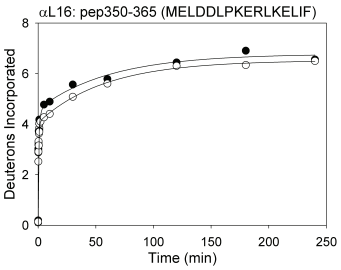
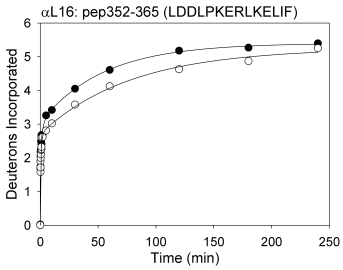
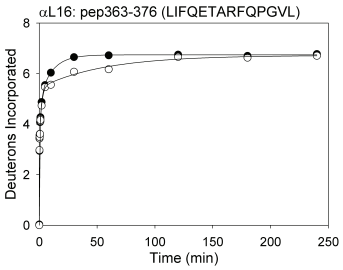


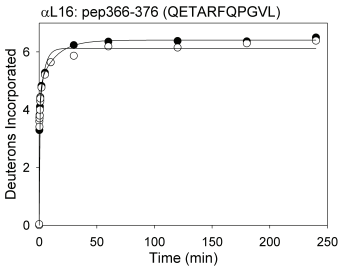

β2LO: pep30-45 (MVKGQPFDVGPRYTQL)
β2LO: pep31-45 (VKGQPFDVGPRYTQL)
β1–β2: pep46-59 (QYIGEGAYGMVSSA)
β3–αC: pep60-88 (YDHVRKTRVAIKKISPFEHQTYCQRTLRE)
β3–αC: pep69-90 (AIKKISPFEHQTYCQRTLREI(
αC-β4: pep93-102 (LRFRHENVIG)
αC-β4: pep94-102 (RFRHENVIG)
β4: pep103-112 (IRDILRASTL)
β4: pep103-114 (IRDILRASTLEA)
β4–β5: pep108-119 (RASTLEAMRDVY)
β5: pep115-124 (MRDVYIVQDL)
β5: pep118-124 (VYIVQDL)
β5–αD: pep120-126 (IVQDLME)
αD-αE: pep125-141 (METDLYKLLKSQQLSND)
αD-αE: pep125-145 (METDLYKLLKSQQLSNDHICY)
αD-αE: pep127-145 (TDLYKLLKSQQLSNDHICY)
αD-αE: pep127-147 (TDLYKLLKSQQLSNDHICYFL
αD-αE: pep130-145 (YKLLKSQQLSNDHICY)
αE–β6: pep146-163 (FLYQILRGLKYIHSANVL)
αE–β6: pep146-172 (FLYQILRGLKYIHSANVLHRDLKPSNL)
αE–β6: pep147-172 (LYQILRGLKYIHSANVLH RDLKPSNL)
β6-β7: pep164-172 (HRDLKPSNL)
β7-β8: pep173-180 (LINTTCDL)
β8: pep180-187 (LKICDFGL)
Deuterons Incorporated
Time (min)

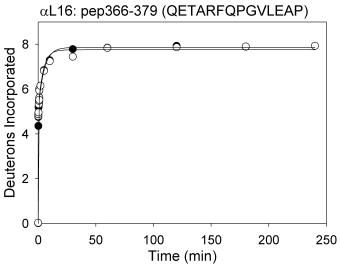

0P Lip: pep188-203 (ARIADPEHDHTGFLTE)
2P Lip: pep188-209 (ARIADPEHDHTGFLTEYVATRW)
0P Lip: pep204-209 (YVATRW)
pep210-216 (YRAPEIM)
pep210-217 (YRAPEIML)
αF: pep217-232 (LNSKGYTKSIDIWSVG)
αF: pep218-232 (NSKGYTKSIDIWSVG)
αF-αG: pep236-254 (AEMLSNRPIFPGKHYLDQL)
αF-αG: pep239-254 (LSNRPIFPGKHYLDQL)
αF-αG-α1L14: pep239-267 (LSNRPIFPGKHYLDQLN HILGILGSPSQE)
αF-αG-α1L14: pep239-267 (LSNRPIFPGKHYLDQLN HILGILGSPSQE)
αG-α1L14: pep255-271 (NHILGILGSPSQEDLNC)
α2L14: pep276-292 (NHILGILGSPSQEDLNC)
α2L14-αH: pep280-306 (YLQSLPSKTKVAWAKLF PKSDSKALDL)
αH: pep296-306 (FPKSDSKALDL)
αH-αI: pep307-323 (LDRMLTFNPNKRITVEE)
αH-αI: pep311-323 (LTFNPNKRITVEE)
αH-αI: pep311-332 (LTFNPNKRITVEEALAHPYLEQ)
αI: pep323-332 (EALAHPYLEQ)
αI: pep324-332 (ALAHPYLEQ)
pep333-347 (YYDPTDEPVAEEPFT)
pep333-348 (YYDPTDEPVAEEPFTF)
pep333-349 (YYDPTDEPVAEEPFTFA)
αL16: pep349-365 (AMELDDLPKERLKELIF)
Deuterons Incorporated
Time (min)

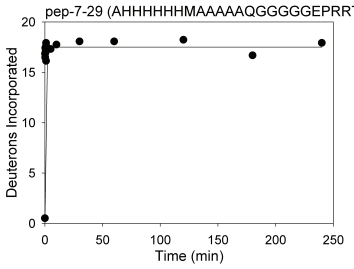

αL16: pep350-365 (MELDDLPKERLKELIF)
Deuterons Incorporated
Time (min)


αL16: pep352-365 (LDDLPKERLKELIF)
Deuterons Incorporated
Time (min)

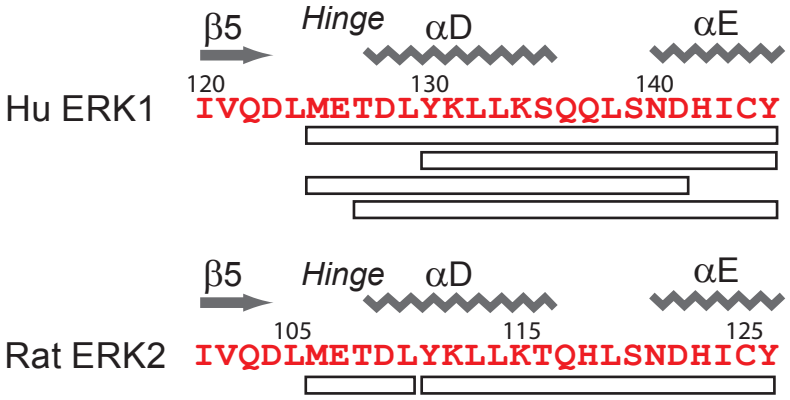

αL16: pep363-376 (LIFQETARFQPGVL)
Deuterons Incorporated
Time (min)


αL16: pep366-376 (QETARFQPGVL)
Deuterons Incorporated
Time (min)

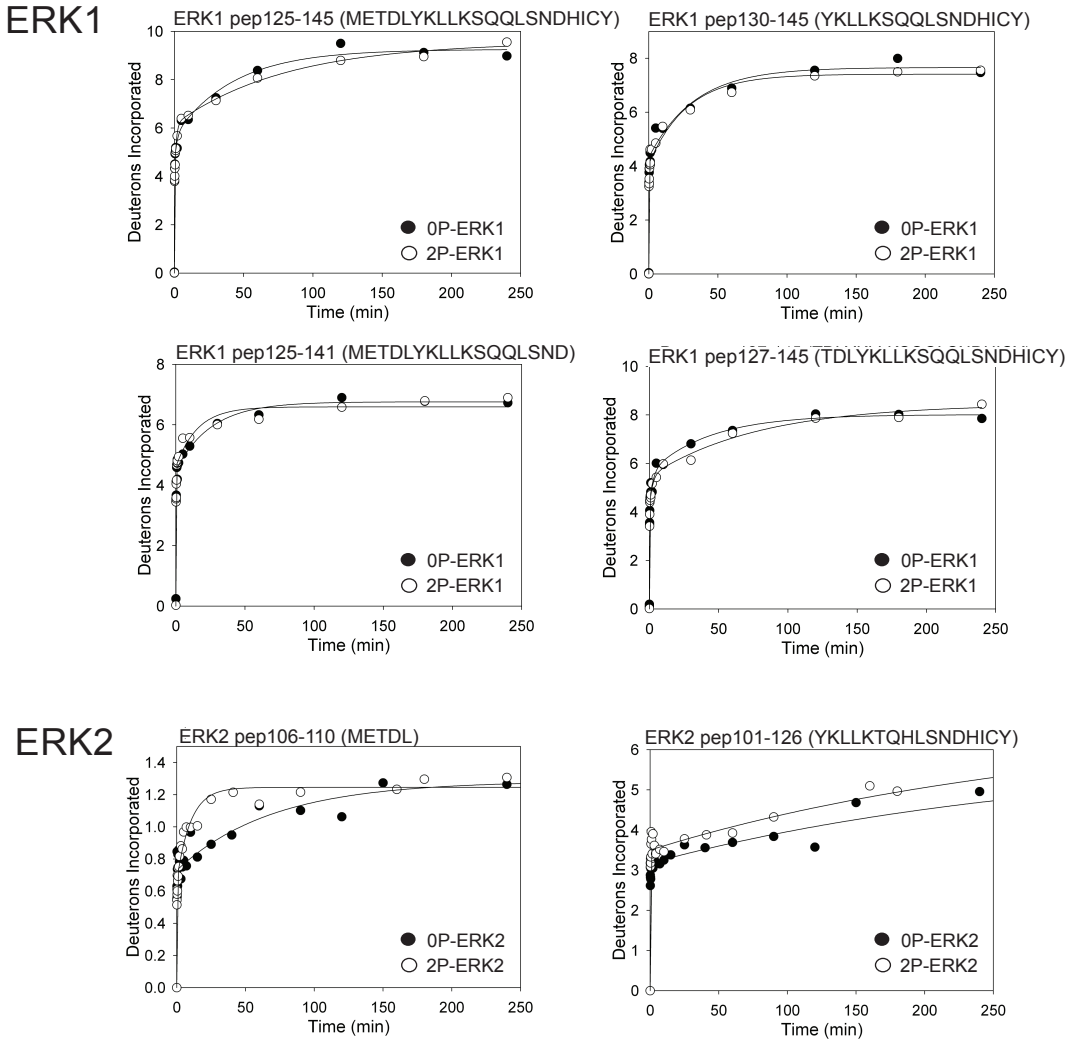

αL16: pep366-379 (QETARFQPGVLEAP)
Deuterons Incorporated
Time (min)

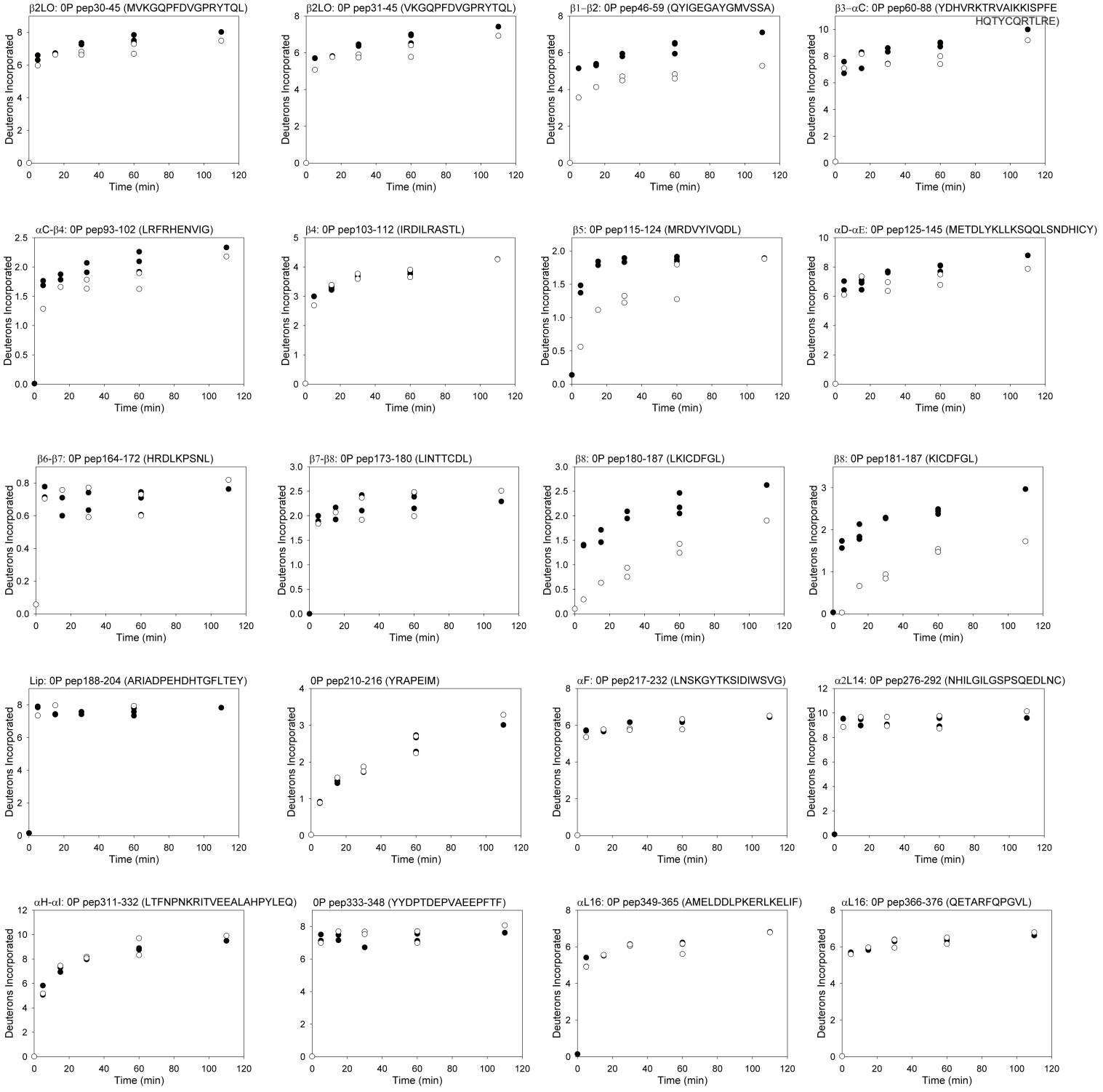

pep-7-29 (AHHHHHHMAAAAAQGGGGGEPRRTEGVGPGVPGEVE)
Deuterons Incorporated
Time (min)

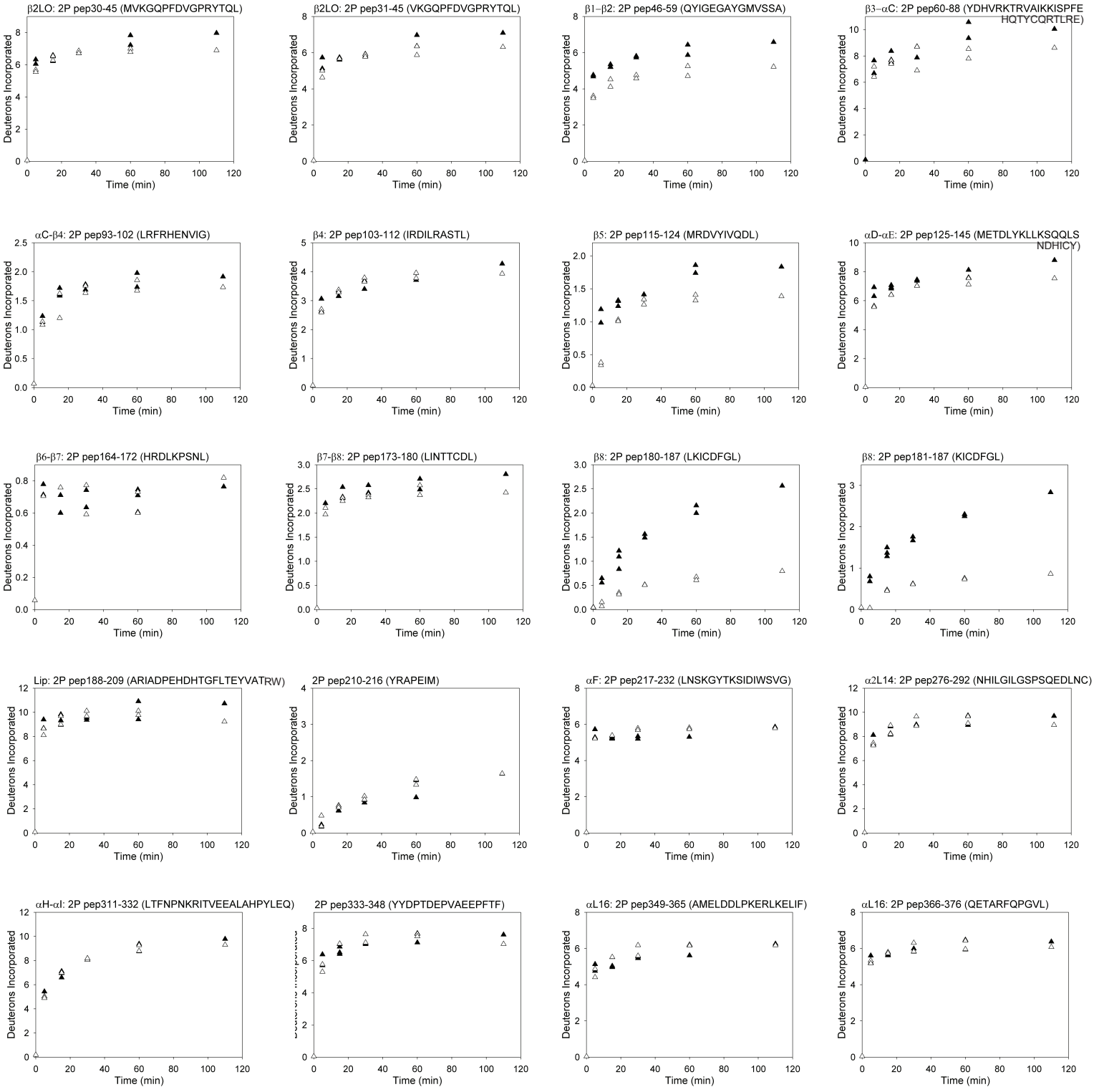
A


β5
Hinge
αD
αE
120
130
140
Hu ERK1
IVQDLMETDLYKLLKSQQLSNDHICY
β5
Hinge
αD
αE
105
115
125
Rat ERK2
IVQDLMETDLYKLLKTQHLSNDHICY

B

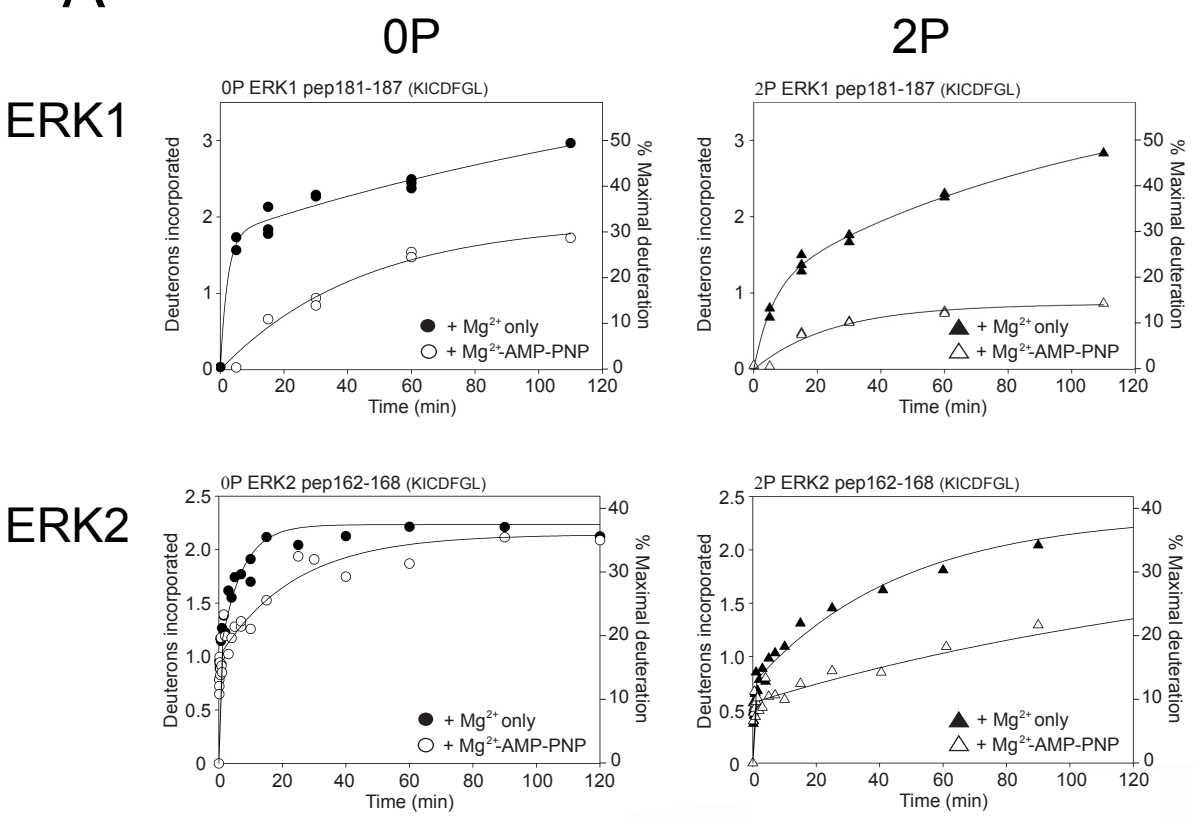

ERK1
ERK1 pep125-145 (METDLYKLLKSQQLSNDHICY)
ERK1 pep130-145 (YKLLKSQQLSNDHICY)
ERK1 pep125-141 (METDLYKLLKSQQLSND)
ERK1 pep127-145 (TDLYKLLKSQQLSNDHICY)
ERK2
ERK2 pep106-110 (METDL)
ERK2 pep101-126 (YKLLKTQHLSNDHICY)
Deuterons Incorporated
Time (min)
0P-ERK1
2P-ERK1
0P-ERK2
2P-ERK2


β2LO: 0P pep30-45 (MVKGQPFDVGPRYTQL)
β2LO: 0P pep31-45 (VKGQPFDVGPRYTQL)
β1–β2: 0P pep46-59 (QYIGEGAYGMVSSA)
β3–αC: 0P pep60-88 (YDHVRKTRVAIKKISPFE HQTYCQRTLRE)
αC-β4: 0P pep93-102 (LRFRHENVIG)
β4: 0P pep103-112 (IRDILRASTL)
β5: 0P pep115-124 (MRDVYIVQDL)
αD-αE: 0P pep125-145 (METDLYKLLKSQQLSNDHICY)
β6-β7: 0P pep164-172 (HRDLKPSNL)
β7-β8: 0P pep173-180 (LINTTCDL)
β8: 0P pep180-187 (LKICDFGL)
β8: 0P pep181-187 (KICDFGL)
Lip: 0P pep188-204 (ARIADPEHDHTGFLTEY)
0P pep210-216 (YRAPEIM)
αF: 0P pep217-232 (LNSKGYTKSIDIWSVG)
α2L14: 0P pep276-292 (NHILGILGSPSQEDLNC)
αH-αI: 0P pep311-332 (LTFNPNKRITVEEALAHPYLEQ)
0P pep333-348 (YYDPTDEPVAEEPFTF)
αL16: 0P pep349-365 (AMELDDLPKERLKELIF)
αL16: 0P pep366-376 (QETARFQPGVL)
Deuterons Incorporated
Time (min)


β2LO: 2P pep30-45 (MVKGQPFDVGPRYTQL)
β2LO: 2P pep31-45 (VKGQPFDVGPRYTQL)
β1–β2: 2P pep46-59 (QYIGEGAYGMVSSA)
β3–αC: 2P pep60-88 (YDHVRKTRVAIKKISPFE HQTYCQRTLRE)
αC-β4: 2P pep93-102 (LRFRHENVIG)
β4: 2P pep103-112 (IRDILRASTL)
β5: 2P pep115-124 (MRDVYIVQDL)
αD-αE: 2P pep125-145 (METDLYKLLKSQQLS NDHICY)
β6-β7: 2P pep164-172 (HRDLKPSNL)
β7-β8: 2P pep173-180 (LINTTCDL)
β8: 2P pep180-187 (LKICDFGL)
β8: 2P pep181-187 (KICDFGL)
Lip: 2P pep188-209 (ARIADPEHDHTGFLTEYVATRW)
2P pep210-216 (YRAPEIM)
αF: 2P pep217-232 (LNSKGYTKSIDIWSVG)
α2L14: 2P pep276-292 (NHILGILGSPSQEDLNC)
αH-αI: 2P pep311-332 (LTFNPNKRITVEEALAHPYLEQ)
2P pep333-348 (YYDPTDEPVAEEPFTF)
αL16: 2P pep349-365 (AMELDDLPKERLKELIF)
αL16: 2P pep366-376 (QETARFQPGVL)
Deuterons Incorporated
Time (min)

Suppl. Figure 5, Ring et al.

A



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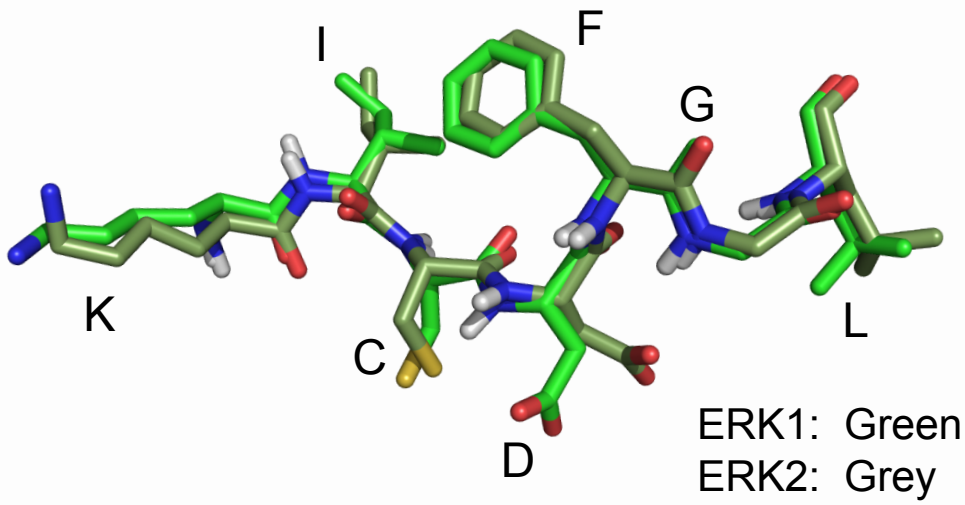

Suppl. Figure 6, Ring et al