Sours et al. SUPPLEMENTAL MATERIALS

SUPPL. FIGURES

Fig. S1. Peptide coverage in ERK2. (A) The sequence of ERK2, with bars summarizing peptides observed by HX-MS. Peptides colored grey were observed in all six forms of ERK2 (0P-WT, 2P-WT, 0P-LM/GG, 0P-ME/GG, 0P-ET/GG, 0P-TD/GG). Peptides revealing differential proteolysis within the activation lip are colored Orange: dual phosphorylated (2P), or Green: unphosphorylated (0P) and linker mutants. **(B)** Peptides at the hinge show different proteolysis between hinge mutants, as indicated. Only the peptide corresponding to residues 101-110 (WT sequence ^{101}IVQDLMETDL^{110}) was observed in all six ERK2 forms. **(C)** The Gly-Gly mutation increases deuteration at the hinge, shown by peptide GGTDL in ME/GG-ERK2 (blue) compared to peptide METDL in 0P-ERK2 (red) and 2P-ERK2 (black). Curves are shown for apo enzyme (solid lines) and in the presence of 1 mM AMP-PNP (dashed lines). **(D)** The Gly-Gly mutation increases deuteration at the hinge, shown by peptide LMEGGL in TD/GG-ERK2 (purple) compared to peptide METDL in 0P-ERK2 (red) and 2P-ERK2 (black). Curves are shown for apo enzyme (solid lines) and in the presence of 1 mM AMP-PNP (dashed lines).

Fig. S2. Thermodynamics of AMP-PNP binding to wild type and mutant ERK2. Data from isothermal titration calorimetry (ITC) show isotherms for AMP-PNP binding to ERK2 WT and mutant proteins. In each case, upper panels show raw data and lower panels show integrated areas after baseline subtraction. Fitted parameters are indicated in the lower panels (K_d ± s.d. measurements (n=3 for unphosphorylated ERK2 proteins and n=2 for 2P-ERK2) and are summarized in Table 1.

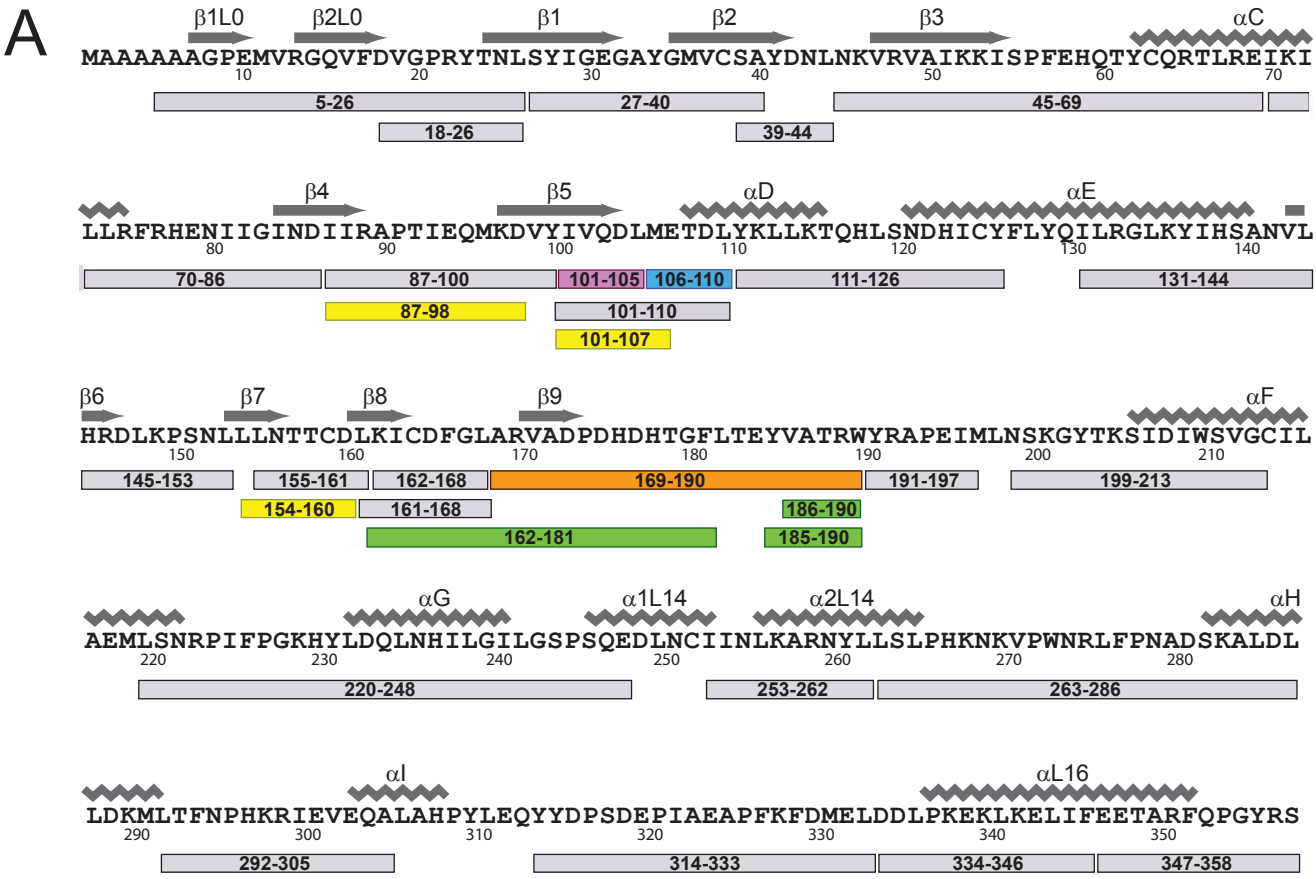
Fig. S3. Specific activities of combinatorial mutants of ERK2. Initial rate measurements (nmol/min/mg) for WT-ERK2 **(A),** ME/GG-ERK2 **(B)**, and ET/GG-ERK2 **(C)** along with eight activation lip mutations are shown, along with a subset of the LM/GG-ERK2 **(B)** and TD/GG-ERK2 **(C)** mutants. Mutants were assayed in their unphosphorylated states or following phosphorylation by active MKK1, as indicated. Bar graphs show average ± s.d. of triplicate measurements.

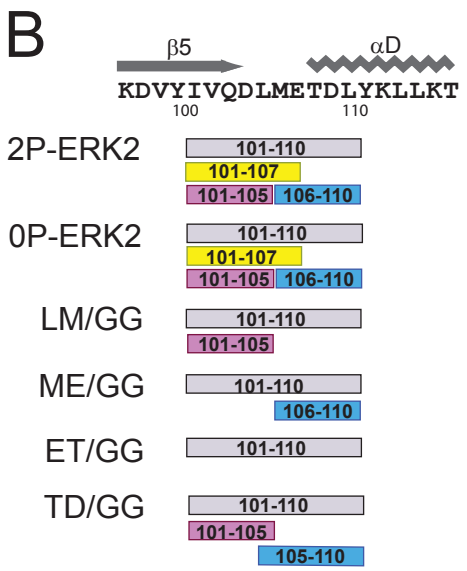
Fig. S4. HX-MS analysis for activation lip mutants of ERK2. Time courses compare HX in unphosphorylated (●) and phosphorylated (o) forms of ERK2, within hinge peptide METDL$_{110}$. Like 2P-ERK2, EEpY-ERK2 shows increased HX in METDL, whereas all other forms show decreased HX. This suggests that only EEpY-ERK2 enhances hinge flexibility, as seen with 2P-ERK2.

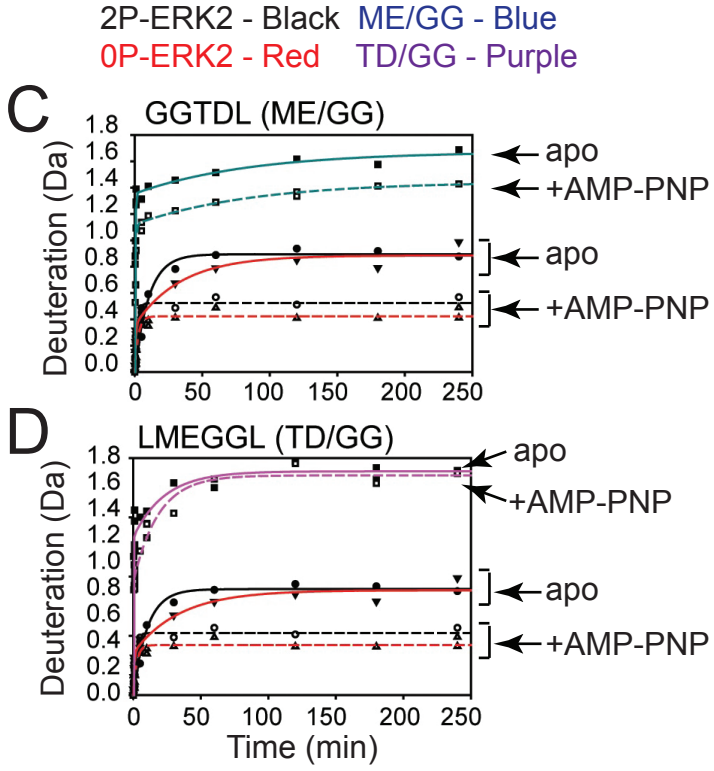
SUPPL. TABLES

Table S1: Observed monoisotopic masses in the HX-MS experiment for 0P- and 2P-WT-ERK2 and hinge mutants.

Table S2: Kinetic parameters for ERK2 in-exchange, fit by non-linear least squares.


A
β1L0 β2L0 β1 β2 β3 αC
MAAAAAAGPEMVRGQVFDVGPRYTNLSYIGEGAYGMVCSAYDNLNKVRVAIKKISPFEHQTYCQRTLREIKI
10 20 30 40 50 60 70
5-26
27-40
45-69
18-26
39-44
β4 β5 αD αE
LLRFRHENIIGINDIIRAPTIEQMKDVYIVQDLMETDLYKLLKTQHLSNDHICYFLYQILRGLKYIHSANVL
80 90 100 110 120 130 140
70-86
87-100
101-105
106-110
111-126
131-144
87-98
101-110
101-107
β6 β7 β8 β9 αF
HRDLKPSNLLLNTTCDLKICDFGLARVADPDHDHTGFLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCIL
150 160 170 180 190 200 210
145-153
155-161
162-168
169-190
191-197
199-213
154-160
161-168
186-190
162-181
185-190
αG α1L14 α2L14 αH
AEMLSNRPIFPGKHYLDQLNHILGILGSPSQEDLNCIINLKARNYLLSLPHKNKVPWNRLFPNADSKALDL
220 230 240 250 260 270 280
220-248
253-262
263-286
αI αL16
LDKMLTFNPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFKFDMELDDLPKEKLKELIFEETARFQPGYRS
290 300 310 320 330 340 350
292-305
314-333
334-346
347-358


B
β5 αD
KDVYIVQDLMETDLYKLLKT
100 110
2P-ERK2
101-110
101-107
101-105
106-110
0P-ERK2
101-110
101-107
101-105
106-110
LM/GG
101-110
101-105
ME/GG
101-110
106-110
ET/GG
101-110
TD/GG
101-110
101-105
105-110


2P-ERK2 - Black ME/GG - Blue
0P-ERK2 - Red TD/GG - Purple
C
GGTDL (ME/GG)
Deuteration (Da)
apo
+AMP-PNP
apo
+AMP-PNP
D
LMEGGL (TD/GG)
Deuteration (Da)
apo
+AMP-PNP
apo
+AMP-PNP
Time (min)

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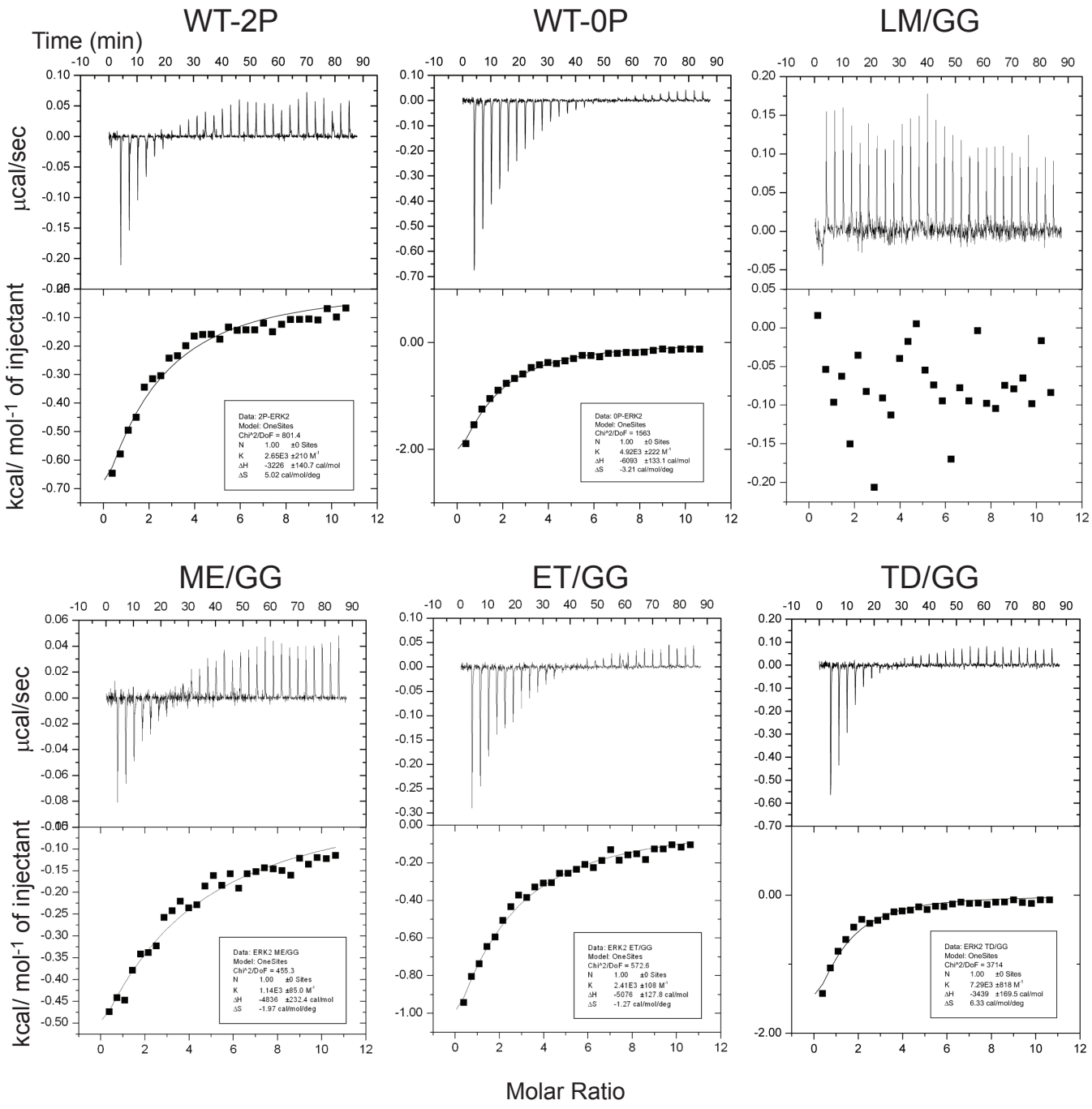

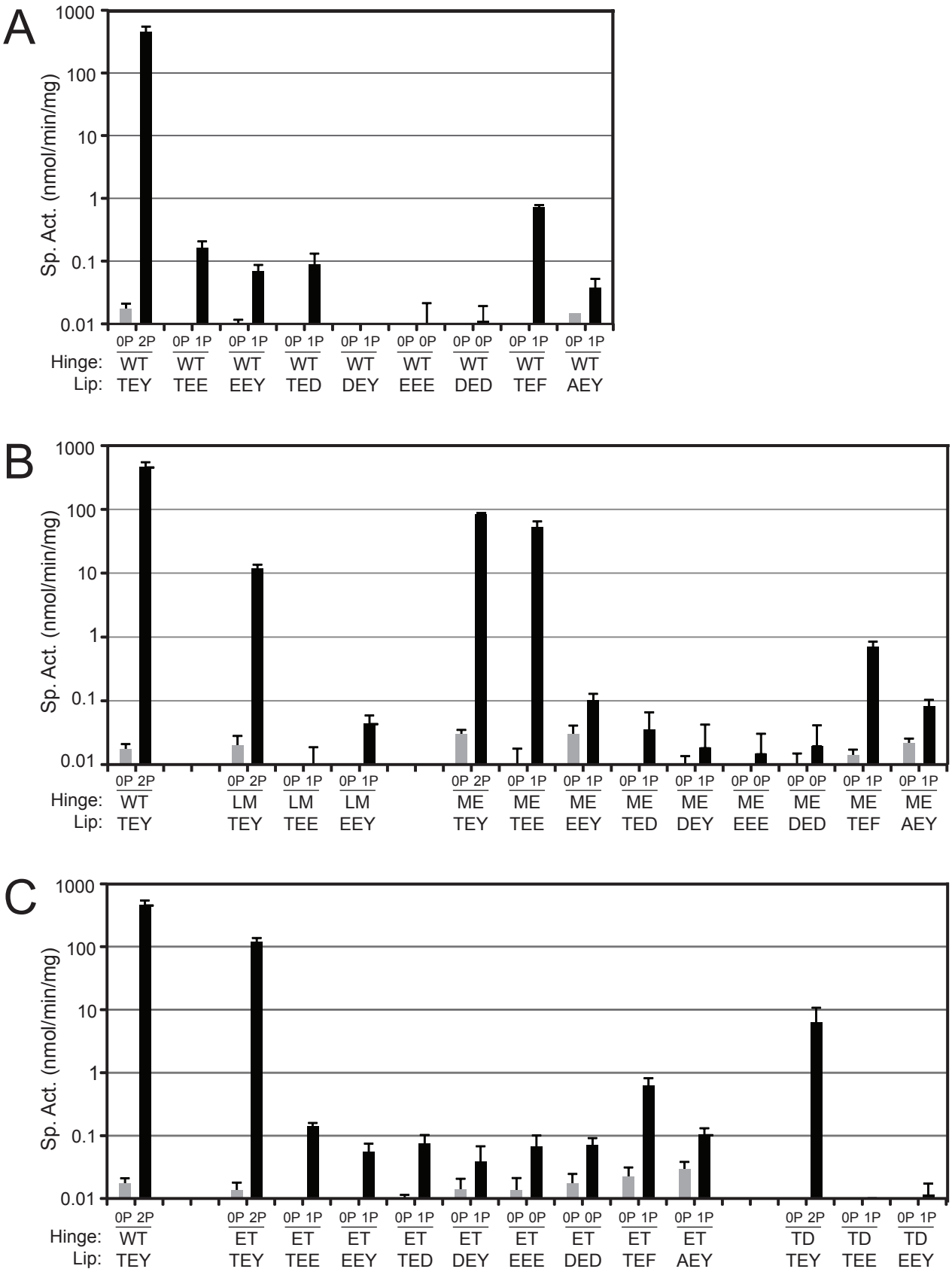
A
B
C
Sp. Act. (nmol/min/mg)
1000
100
10
1
0.1
0.01
Hinge:
Lip:
0P 2P
0P 1P
0P 0P
WT TEY
WT TEE
WT EEY
WT TED
WT DEY
WT EEE
WT DED
WT TEF
WT AEY
LM TEY
LM TEE
LM EEY
ME TEY
ME TEE
ME EEY
ME TED
ME DEY
ME EEE
ME DED
ME TEF
ME AEY
ET TEY
ET TEE
ET EEY
ET TED
ET DEY
ET EEE
ET DED
ET TEF
ET AEY
TD TEY
TD TEE
TD EEY

Sours et al., Figure S4

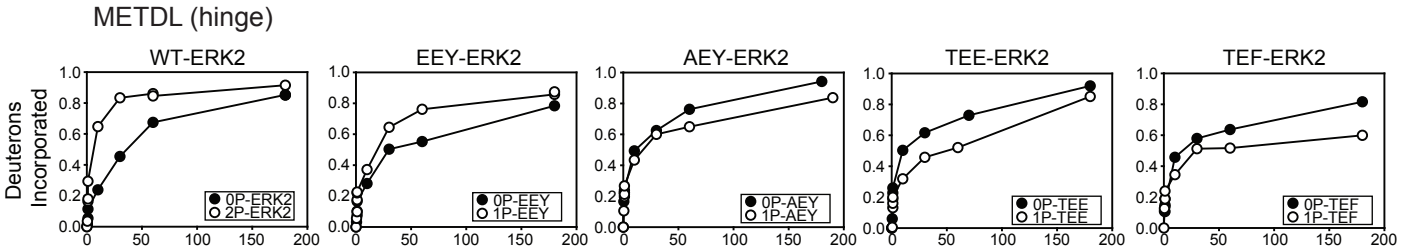

Table S1: Observed peptides in HX-MS experiments of 0P-WT, 2P-WT and hinge mutants of ERK2.

					Observed m/z of analyzed peptide ion						
Residues	Peptide sequence	# Exch. Amides	Calc. Mass	Calc. m/z of ion	2P-WT	0P-WT	LM/GG	ME/GG	ET/GG	TD/GG	LC elution time
5.26	AAAGPEMVRGQVFDVGPRYTNL	19	2347.17	783.39	783.44	783.40	783.42	783.41	783.42	783.47	9.6
18.26	DVGPRYTNL	7	1033.52	517.76	517.79	517.81	517.82		517.81	517.80	7.5
27.40	SYIGEGAYGMVCSA	13	1406.58	704.29	704.29	704.31	704.35	704.33	704.35	704.37	9.5
39.44	SAYDNL	5	681.30	682.30	682.32	682.33	682.34	682.31	682.34	682.37	6.7
45.69	NKVRVAIKKISPFEHQTYCQRTLRE	23	3043.65	1015.55	1015.55	1015.62	1015.54	1015.57	1015.62	1015.64	8.2
70.86	IKILLRFRHENIIGIND	16	2063.20	688.73	688.74	688.77	788.74	788.75	688.78	688.80	9.3
87.98	IIRAPTIEQMKD	10	1413.76	707.88	707.87	707.88					7.9
87.100	IIRAPTIEQMKDVY	12	1675.90	838.95	838.94	838.99	839.00	839.00	839.00	839.01	9.2
101.105	IVQDL	4	586.33	586.33	587.32	587.35				587.38	5.8
101.107	IVQDLME	6	846.42	847.42	847.44	847.42					7.3
101.110	IVQDLMETDL	9	1175.57	1176.57	1176.56	1176.56					9.0
101.110	IVQDGGETDL	9	1045.49	1046.49			1046.54				6.0
101.110	IVQDLGGTDL	9	1029.53	515.77				515.81			7.7
101.110	IVQDLMGGDL	9	1059.53	1060.53					1060.67		9.0
101.110	IVQDLMEGGL	9	1073.54	1074.54						1074.66	6.0
105.110	LMEGGL	5	618.30	619.30						619.39	7.0
106.110	METDL	4	607.25	608.26	608.25	608.28					6.4
106.110	GGTDL	4	461.21	462.21				462.22			7.0
111.126	YKLLKTQHLSNDHICY	15	1975.00	659.33	659.35	659.39	659.35	659.36	659.39	659.41	8.2
131.144	ILRGLKYIHSANVL	13	1595.95	532.98	532.99	532.97	532.97	532.99	532.97	533.00	8.7
145.153	HRDLKPSNL	7	1078.59	540.29	540.30	540.33	540.30	540.31	540.32	540.35	5.6
154.160	LLNTTCD	6	778.35	779.35	779.36	779.37	779.36	779.38	779.43	779.43	5.0
155.161	LNTTCDL	6	778.35	779.35	779.36	779.39					7.1
161.168	LKICDFGL	7	907.48	454.74	454.76	454.78	454.76	454.76	454.76	454.77	10.0
162.168	KICDFGL	6	794.40	795.40	795.42	795.43	795.42	795.43	795.44	795.49	9.0
162.181	KICDFGLARVADPDHDHTGF	18	2213.03	1107.52		1107.56	1107.56	1107.6	1107.61	1107.62	9.1
169.190	ARVADPDHDHTGFLpTEpYVATRW	20	2716.15	680.04	680.07						10.0
185.190	YVATRW	5	794.41	795.41		795.44	795.44	795.43	795.44	795.49	8.1
186.190	VATRW	4	631.34	632.34		632.41					6.8
191.197	YRAPEIM	5	878.43	440.23	440.21	440.23	440.24	440.25	440.25	440.25	7.7
199.213	NSKGYTKSIDIWSVG	14	1653.84	827.92	827.92	827.94	827.92	827.94	827.99	828.00	9.4
220.248	LSNRPIFPGKHYLDQLNHILGILGSPSQE	25	3242.72	1081.91	1081.97	1082.90	1081.96	1081.95	1081.95	1081.98	10.8
253.262	IINLKARNYL	9	1216.73	609.36	609.38	609.40	609.38	609.40	609.39	609.44	8.3
263.286	LSLPHKNKVPWNRLFPNADSKALDL	21	2872.57	958.52	958.6	958.50	958.53	958.57	958.58	958.58	9.9
292.305	LTFNPHKRIEVEQA	12	1680.89	841.45	841.47	841.42	841.47	841.49	841.52	841.54	7.9
314.333	YYDPSDEPIAEAPFKFDMEL	16	2376.05	1189.03	1189.01	1189.06	1189.01	1189.07	1189.08	1189.11	11.0
334.346	DDLPKEKLKELIF	11	1586.89	529.96	529.99	530.01	529.99	529.99	530.01	530.00	9.4
347.358	EETARFQPGYRS	10	1439.68	720.84	720.84	720.89	720.87	720.89	720.91	720.89	6.8

Table S2: Fitted kinetic parameters for hydrogen-deuterium exchange time courses of WT and mutant ERK2.

Sequence [1]	ERK2	%BE	Condition	Avg StDev at 1 min	RSS	A[2] (s.d.)	B (s.d.)	C (s.d.)	N (s.d.)	k_1 (s.d.)	k_2 (s.d.)	k_3 (s.d.)
AAAGPEMVRGQVFDVGPRYTNL	2P-WT	20.5%	Control	0.0987	2.499	7.290(0.768)	4.471(0.568)		11.761(0.215)	8.800(5.072)	0.345(0.104)	
			AMP-PNP	0.2301	1.790	10.598(0.637)	1.858(0.551)		12.456(0.213)	1.805(0.218)	0.048(0.036)	
	0P-WT	19.4%	Control	0.1955	0.743	6.247(0.938)	5.384(0.895)		11.632(0.136)	4.705(1.272)	0.456(0.148)	
			AMP-PNP	0.1538	2.758	10.074(0.567)	2.500(0.509)		12.573(0.356)	3.146(0.557)	0.029(0.018)	
	LM/GG	20.8%	Control	0.1001	2.001	9.518(0.475)	3.457(0.435)		12.975(0.194)	4.552(0.815)	0.153(0.053)	
			AMP-PNP	0.2488	1.132	9.590(0.458)	3.416(0.330)		13.006(0.113)	8.598(2.242)	0.244(0.070)	
	ME/GG	20.5%	Control	0.3247	2.410	9.077(0.888)	3.187(0.678)		12.263(0.289)	5.816(1.804)	0.245(0.148)	
			AMP-PNP	0.3453	3.128	9.202(0.871)	3.441(0.750)		12.643(0.305)	2.598(0.643)	0.066(0.037)	
	ET/GG	20.5%	Control	0.1233	2.929	7.854(0.860)	4.472(0.699)		12.325(0.181)	4.709(1.076)	0.298(0.114)	
			AMP-PNP	0.1927	2.838	8.146(0.709)	4.367(0.498)		12.512(0.218)	6.288(1.610)	0.178(0.060)	
	TD/GG	20.1%	Control	0.0853	1.038	7.696(0.487)	4.840(0.376)		12.536(0.152)	5.250(0.185)	0.185(0.033)	
			AMP-PNP	0.2761	1.145	7.188(0.417)	5.395(0.266)		12.583(0.153)	8.201(2.564)	0.172(0.026	
DVGPRYTNL	2P-WT	37.3%	Control	0.1300	0.066	1.511(0.094)			1.511(0.035)	0.716(.103)		
			AMP-PNP	0.0682	0.117	1.224(0.151)	0.948(0.110)		2.172(0.065)	3.800(1.360)		
	0P-WT	30.9%	Control	0.2465	0.451	0.997(0.301)	1.195(0.273)		2.192(0.078)	4.058(3.324)	0.270(0.122)	
			AMP-PNP	0.1068	0.264	1.360(0.236)	0.978(0.200)		2.338(0.064)	7.958(6.992)	0.293(0.162)	
	LM/GG	21.7%	Control	0.0500	0.108	2.382(0.252)	0.236(0.228)		2.618(0.080)	1.657(0.349)	0.059(0.128)	
			AMP-PNP	0.1006	0.171	1.857(0.174)	0.879(0.114)		2.736(0.057)	8.441(4.583)	0.105(0.043)	
	ME/GG		Control									
			AMP-PNP									
	ET/GG	..3686	Control	0.0475	0.523	0.984(0.473)	0.990(0.462)		1.974(0.099)	1.513(1.307)	0.073(0.062)	
			AMP-PNP	0.0680	0.073	0.792(0.101)	1.085(0.063)		1.877(0.037)	10.263(9.044)	0.089(0.018)	
	TD/GG	38.3%	Control	0.1245	0.214	1.835(0.120)			1.835(0.057)	0.458(0.087)		
			AMP-PNP	0.1239	0.552	2.306(.217)			2.306(0.086)	0.945(0.165)		
SYIGEGAYGMVCSA	2P-WT	25.0%	Control	0.1388	0.558	2.826(0.312)	2.992(0.207)		5.818(0.109)	7.677(4.214)	0.089(0.019)	
			AMP-PNP	0.2795	0.247	2.910(0.192)	2.427(0.128)		5.337(0.087)	4.051(0.761)	0.042(0.007)	
	0P-WT	20.0%	Control	0.3011	0.707	3.354(0.245)	2.450(0.295)		5.804(0.101)	6.465(2.303)	0.135(0.031)	
			AMP-PNP	0.0717	0.606	2.853(.204)	2.257(0.163)		5.110(0.117)	4.822(1.372)	0.033(0.008)	
	LM/GG	20.1%	Control	0.0051	0.542	3.757(0.196)	2.645(0.175)		5.402(0.112)	5.387(1.305)	0.074(0.017)	
			AMP-PNP	0.0072	0.108	3.489(0.154)	1.997(0.183)	1.248(0.199)	6.735(0.300)	6.664(1.115)	0.202(0.047)	0.008(0.006)
	ME/GG	19.8%	Control	0.0709	0.989	3.777(0.354)	2.444(0.233)		6.220(0.184)	5.082(1.225)	0.038(0.013)	
			AMP-PNP	0.0065	0.064	2.858(0.124)	1.194(0.240)	1.780(0.200)	5.831(0.299)	5.708(0.838)	0.149(0.057)	0.009(0.004)
	ET/GG	19.9%	Control	0.0961	0.225	3.673(0.257)	1.161(0.243)	1.662(0.263)	6.496(0.148)	5.521(0.853)	0.273(0.213)	0.016(0.007)
			AMP-PNP	0.0668	0.171	2.772(0.193)	1.397(0.322)	1.682(0.324)	5.851(0.457)	6.626(1.439)	0.156(0.074)	0.008(0.006)
	TD/GG	19.8%	Control	0.2459	0.788	4.033(0.295)	2.141(0.178)		6.174(0.115)	6.476(1.479)	0.073(0.019)	
			AMP-PNP	0.0281	0.242	3.060(0.176)	2.248(0.115)		5.308(0.089)	5.078(0.970)	0.040(0.007)	
SAYDNL	2P-WT	21.2%	Control	0.1470	0.104	0.316(0.150)	0.720(0.118)		1.035(0.054)	1.749(1.648)	0.040(0.020)	
			AMP-PNP	0.0323	0.010	0.409(0.045)		0.752(0.106)	1.161(0.117)	3.868(2.201)		0.007(0.002)

	0P-WT	21.1%	Control	0.1428	0.110	0.486(0.168)	0.757(0.158)		1.243(0.099)	1.477(1.114)	0.021(0.011)	
			AMP-PNP	0.1232	0.102	0.469(0.120)	0.559(0.103)		1.028(0.080)	2.496(1.859)	0.017(0.010)	
	LM/GG	20.5%	Control	0.0261	0.006	0.405(.0.036)	0.772(0.024)		1.177(0.021)	7.171(2.640)	0.021(0.002)	
			AMP-PNP	0.1400	0.058	0.584(0.108)		0.881(0.222)	1.462(0.251)	1.165)0.375)		0.007(0.004)
	ME/GG	20.7%	Control	0.0569	0.056	0.522(0.095)	0.811(0.077)		1.333(0.073)	6.987(4.400)	0.018(0.005)	
			AMP-PNP	0.1650	0.025	0.530(0.085)	0.528(0.086)		1.057(0.085)	5.457(2.976)	0.015(.007)	
	ET/GG	21.9%	Control	0.0430	0.116	0.574(0.161)		0.815(0.206)	1.389(0.226)	2.659(1.070)		0.009(0.005)
			AMP-PNP	0.0447	0.010	0.433(0.058)	0.578(0.043)		1.010(0.025)	4.019(3.073)	0.034(0.007)	
	TD/GG	21.8%	Control	0.0189	0.045	0.476(0.080)		1.577(0.965)	2.052(0.994)	2.076(0.752)		0.003(0.003)
			AMP-PNP	0.0158	0.063	0.525(0.100)	0.695(0.105)		1.220(0.108)	4.321(2.462)	0.013(0.006)	
NKVRVAIKKISPFEHQTYCQRTLRE	2P-WT	21.4%	Control	0.1898	2.422	4.054(0.655)	2.574(0.445)		6.628(0.237)	5.172(2.727)	0.067(0.036)	
			AMP-PNP	0.2548	1.955	5.036(0.646)	1.409(0.546)		6.445(0.284)	0.846(0.208)	0.035(0.033)	
	0P-WT	21.0%	Control	0.3271	0.697	5.481(0.260)	1.729(0.255)		7.210(0.228)	1.865(0.194)	0.017(0.008)	
			AMP-PNP	0.1989	1.743	5.228(0.366)		2.437(0.728)	7.664(0.822)	1.357(0.186)		0.008(0.007)
	LM/GG	21.4%	Control	0.0573	0.833	4.525(0.283)	3.129(0.261)		7.654(0.144)	3.670(0.788)	0.061(0.016)	
			AMP-PNP	0.1926	3.519	5.782(0.856)	1.831(0.707)		7.612(0.295)	1.396(0.387)	0.027(0.027)	
	ME/GG	21.2%	Control	0.0533	0.043	3.178(0.151)	3.373(0.137)	1.749(0.454)	8.299(0.548)	5.162(0.643)	0.268(0.036)	0.006(0.004)
			AMP-PNP	0.1004	0.219	5.261(0.214)	2.180(0.292)		7.441(0.315)	1.843(0.156)	0.011(0.004)	
	ET/GG	20.4%	Control	0.0466	0.727	3.790(0.360)	3.203(0.262)		6.993(0.104)	5.237(1.235)	0.212(0.045)	
			AMP-PNP	0.0767	1.137	3.874(0.505)	2.822(0.415)		6.696(0.278)	2.596(0.781)	0.021(0.009)	
	TD/GG	21.1%	Control	0.1030	0.948	4.052(0.355)	3.114(0.227)		7.166(0.126)	6.805(1.980)	0.102(0.021)	
			AMP-PNP	0.2186	1.359	3.925(0.558)	3.230(0.480)		7.155(0.170)	3.105(1.183)	0.138(0.040)	
IKILLRFRHENIIGIND	2P-WT	20.6%	Control	0.1039	0.324	1.588(0.190)	1.884(0.150)		3.443(0.142)	1.203(0.272)	0.015(0.004)	
			AMP-PNP	0.1087	0.305	1.359(0.203)	1.400(0.154)		2.759(0.111)	1.124(0.304)	0.027(0.009)	
	0P-WT	20.8%	Control	0.1471	0.262	1.844(0.135)		3.289(1.043)	5.133(1.094)	0.674(0.119)		0.004(0.002)
			AMP-PNP	0.1224	0.242	1.322(0.142)	2.146(0.194)		3.468(0.225)	0.638(0.154)	0.010(0.003)	
	LM/GG	20.9%	Control	0.1001	0.261	1.723(0.167)	2.420(0.172)		4.143(0.162)	1.171(0.218)	0.015(0.004)	
			AMP-PNP	0.0608	0.133	1.928(0.123)		2.227(0.161)	4.154(0.194)	0.621(0.091)		0.009(0.002)
	ME/GG	21.1%	Control	0.1262	0.110	1.637(0.121)	2.427(0.175)		4.064(0.197)	1.359(0.192)	0.010(0.002)	
			AMP-PNP	0.1173	0.139	1.498(0.148)		2.826(0.396)	4.324(0.444)	0.741(0.146)		0.007(0.002)
	ET/GG	20.6%	Control	0.0362	0.222	1.720(0.145)	2.307(0.145)		4.037(0.160)	0.917(0.148)	0.013(0.003)	
			AMP-PNP	0.0962	0.189	1.517(0.156)		2.587(0.320)	4.104(0.361)	0.739(0.166)		0.008(0.003)
	TD/GG	20.3%	Control	0.0921	0.392	1.661(0.207)	2.281(0.173)		3.942(0.172)	1.353(0.316)	0.015(0.004)	
			AMP-PNP	0.0889	0.156	1.720(0.145)	2.379(0.170)		4.098(0.192)	0.742(0.124)	0.011(0.003)	
IIRAPTIEQMKD	2P-WT	19.9%	Control	0.2996	0.902	4.380(0.510)	2.452(0.460)		6.832(0.148)	0.773(0.154)	0.047(0.021)	
			AMP-PNP	0.0129	0.138	4.494(0.209)	2.469(0.183)		6.963(0.081)	0.669(0.061)	0.034(0.006)	
	0P-WT	20.9%	Control	0.1636	0.650	5.342(0.251)	1.856(0.239)		7.198(0.208)	0.673(0.070)	0.018(0.008)	
			AMP-PNP	0.1804	0.621	5.033(0.256)	2.189(0.221)		7.222(0.157)	0.788(0.077)	0.019(0.006)	
IIRAPTIEQMKDVY	2P-WT	18.7%	Control	0.2807	0.893	4.687(0.407)	2.316(0.345)		7.003(0.143)	0.833(0.132)	0.043(0.017)	
			AMP-PNP	0.0959	0.508	4.629(0.272)	2.565(0.224)		7.194(0.137)	0.805(0.093)	0.029(0.007)	
	0P-WT	20.2%	Control	0.2679	0.064	1.879(0.362)	4.267(0.340)	2.699(1.439)	8.844(1.516)	2.355(0.668)	0.320(0.036)	0.003(0.003)
			AMP-PNP	0.1551	0.722	5.018(0.282)	2.285(0.243)		7.304(0.159)	0.816(0.086)	0.021(0.007)	

	LM/GG	20.2%	Control	0.1575	0.231	1.889(0.572)	3.747(0.439)	2.252(0.282)	7.888(0.167)	3.707(1.692)	0.402(0.144)	0.016(0.005)
			AMP-PNP	0.0596	0.142	1.978(0.421)	4.042(0.377)	1.998(0.235)	8.018(0.331)	2.757(0.955)	0.326(0.063)	0.008(0.004)
	ME/GG	20.1%	Control	0.1254	0.151	1.537(0.367)	4.330(0.280)	2.327(0.653)	8.194(0.771)	4.538(1.734)	0.379(0.080)	0.006(0.004)
			AMP-PNP	0.1212	0.207	2.049(0.627)	3.612(0.527)	2.296(0.581)	7.966(0.726)	2.897(1.312)	0.364(0.124)	0.007(0.005)
	ET/GG	20.0%	Control	0.0314	0.095	1.509(0.221)	4.304(0.159)	1.933(0.176)	7.746(0.222)	4.506(1.113)	0.346(0.048)	0.009(0.003)
			AMP-PNP	0.1156	0.167	1.409(0.368)	4.091(0.275)	2.094(0.279)	7.593(0.345)	6.285(3.614)	0.391(0.093)	0.009(0.004)
	TD/GG	19.5%	Control	0.0704	0.884	5.365(0.323)	1.947(0.259)		7.312(0.217)	0.826(0.095)	0.018(0.009)	
			AMP-PNP	0.0851	0.634	5.213(0.325)	2.051(0.265)		7.264(0.193)	0.758(0.088)	0.021(0.009)	
IVQDL	2P-WT	14.2%	Control	0.0739	0.102		1.086(0.057)		1.086(0.046)		0.037(0.007)	
			AMP-PNP	0.0263	0.126		1.032(0.071)		1.032(0.065)		0.018(0.004)	
	0P-WT	13.9%	Control	0.0530	0.009	0.306(0.063)	0.774(0.066)		1.080(0.028)	0.580(0.235)	0.034(0.007)	
			AMP-PNP	0.0671	0.062			1.263(0.240)	1.263(0.247)			0.007(0.003)
IVQDG	LM/GG		Control									
			AMP-PNP									
	ME/GG		Control									
			AMP-PNP									
	ET/GG		Control									
			AMP-PNP									
	TD/GG	19.6%	Control	0.0330	0.026	0.141(0.017)			1.122(0.028)	1.122(0.038)		
			AMP-PNP	0.0054	0.005	0.187(0.045)	0.947(0.076)		1.134(0.089)	1.191(0.512)	0.183(0.005)	
IVQDLME	2P-WT	21.27	Control	0.2180	0.153	0.888(0.158)	1.100(0.103)		1.988(0.616)	5.718(3.626)	0.054(0.017)	
			AMP-PNP	0.1092	0.082	0.509(0.099)	1.288(0.074)		1.797(0.063)	2.678(1.250)	0.022(0.004)	
	0P-WT	25.4%	Control	0.0797	0.141	0.751(0.397)	1.166(0.393)		1.917(0.048)	1.583(1.222)	0.128(0.062)	
			AMP-PNP	0.1322	0.201		0.685(0.174)	1.078(0.167)	1.767(0.098)		0.366(0.233)	0.017(0.008)
METDL	2P-WT	19.3%	Control	0.0951	0.038		0.895(0.041)		0.895(0.029)		0.097(0.015)	
			AMP-PNP	0.0312	0.021	0.524(0.046)			0.524(0.024)		0.564(0.145)	
	0P-WT	14.2%	Control	0.0521	0.034	0.412(0.069)	0.523(0.062)		0.935(0.038)	2.353(0.955)	0.033(0.012)	
			AMP-PNP	0.0319	0.050	0.532(0.044)			0.532(0.02)	0.837(0.160)		
	LM/GG		Control									
			AMP-PNP									
GGTDL [(4)]	ME/GG	24.7%	Control	0.0586	0.048	1.355(0.077)	0.318(0.093)		1.673(0.099)	2.794(0.362)	0.012(0.010)	
			AMP-PNP	0.0895	0.046	1.121(0.082)	0.324(0.098)		1.445(0.106)	1.810(0.269)	0.011(0.010)	
	ET/GG		Control									
			AMP-PNP									
LMEGGL [(4)]	TD/GG	23.5%	Control	0.1615	0.165	1.332(0.139)	0.557(0.085)		1.888(0.060)	5.253(1.854)	0.043(0.022)	
			AMP-PNP	0.0757	0.071	0.951(0.095)	0.903(0.059)		1.854(0.045)	7.070(3.198)	0.054(0.012)	
IVQDLMETDL	2P-WT	20.5%	Control	0.2001	0.187	2.041(0.171)	1.703(0.131)		3.744(0.076)	1.211(0.182)	0.028(0.008)	
			AMP-PNP	0.1333	0.526	1.090(0.230)		3.214(1.259)	4.304(1.345)	0.627(0.365)		0.005(0.004)
	0P-WT	20.5%	Control	0.1881	0.424	2.150(0.261)	1.547(0.239)		3.698(0.120)	0.781(0.170)	0.034(0.014)	
			AMP-PNP	0.1285	0.540	1.058(0.225)	1.898(0.204)		2.955(0.191)	0.738(0.319)	0.015(0.006)	
IVQDGGETDL	LM/GG	21.0%	Control	0.0358	0.319	2.196(0.220)	1.133(0.200)		3.329(0.145)	1.824(0.391)	0.021(0.011)	
			AMP-PNP	0.0998	0.115	1.360(0.306)	1.136(0.235)	0.727(0.157)	3.222(0.116)	4.631(1.988)	0.372(0.259)	0.014(0.010)

IVQDLGGTDL	ME/GG	20.1%	Control	0.0273	0.115	1.322(0.142)	1.848(0.090)		3.170(0.059)	10.155(9.329)	0.081(0.014)	
			AMP-PNP	0.0371	0.222	1.054(0.183)	2.026(0.132)		3.080(0.094)	3.603(1.812)	0.035(0.007)	
IVQDLMGGDL	ET/GG	18.7%	Control	0.1108	0.247	1.842(0.165)	1.238(0.102)		3.081(0.066)	5.202(1.219)	0.059(0.016)	
			AMP-PNP	0.0779	0.103	1.594(0.114)	1.392(0.096)		2.986(0.093)	3.733(0.618)	0.017(0.004)	
IVQDLMEGGL	TD/GG	16.7%	Control	0.2113	0.356	2.417(0.210)	1.435(0.154)		3.853(0.079)	3.390(0.704)	0.063(0.019)	
			AMP-PNP	0.0788	0.261	1.676(0.200)	2.341(0.160)		4.016(0.138)	1.363(0.294)	0.019(0.004)	
YKLLKTQHLSNDHICY	2P-WT	23.3%	Control	0.3159	0.817	1.747(0.333)	1.160(0.215)		2.907(0.116)	7.246(6.296)	0.105(0.055)	
			AMP-PNP	0.3110	1.222	1.896(0.371)	0.973(0.227)		2.869(0.160)	5.387(3.596)	0.054(0.038)	
	0P-WT	23.4%	Control	0.0605	0.119	2.412(0.111)		1.280(0.461)	3.691(0.493)	2.109(0.226)		0.006(0.004)
			AMP-PNP	0.2519	0.824	1.877(0.270)	1.158(0.254)		3.035(0.238)	3.092(1.342)	0.015(0.011)	
	LM/GG	22.9%	Control	0.2031	0.213	2.298(0.122)	1.736(0.172)		4.036(0.176)	4.070(0.833)	0.013(0.004)	
			AMP-PNP	0.1522	0.397	2.505(0.209)		1.334(0.409)	3.840(0.463)	2.332(0.443)		0.007(0.006)
	ME/GG	23.3%	Control	0.1107	0.046	2.301(0.112)		2.625(1.624)	4.926(1.673)	1.829(0.190)		0.003(0.003)
			AMP-PNP	0.0735	0.184	1.940(0.`56)		2.679(1.393)	4.619(1.423)	3.851(0.884)		0.004(0.003)
	ET/GG	23.7%	Control	0.1036	0.088	2.406(0.105)		1.922(0.309)	4.328(0.334)	1.878(0.181)		0.007(0.002)
			AMP-PNP	0.2231	0.329	2.267(0.200)		1.866(0.650)	4.133(0.682)	3.428(0.678)		0.006(0.004)
	TD/GG	23.7%	Control	0.0985	0.916	2.714(0.317)	3.375(0.226)		6.089(0.210)	5.779(1.875)	0.022(0.005)	
			AMP-PNP	0.2716	1.014	2.828(0.382)	3.177(0.318)		6.005(0.289)	3.561(1.358)	0.024(0.007)	
ILRGLKYIHSANVL	2P-WT	20.8%	Control	0.1343	0.356	1.541(0.203)	0.994(0.149)		2.525(0.123)	1.188(0.289)	0.019(0.010)	
			AMP-PNP	0.1029	0.419	1.317(0.254)	0.881(0.197)		2.198(0.116)	1.632(0.604)	0.037(0.022)	
	0P-WT	20.8%	Control	0.1961	0.129	1.700(0.099)		1.198(0.197)	2.898(0.219)	0.776(0.099)		0.009(0.004)
			AMP-PNP	0.0993	0.212	1.168(0.136)	1.459(0.148)		2.627(0.162)	0.711(0.175)	0.012(0.004)	
	LM/GG	21.1%	Control	0.1159	0.216	1.500(0.156)	1.812(0.170)		3.312(0.172)	0.755(0.153)	0.014(0.004)	
			AMP-PNP	0.0380	0.091	1.570(0.112)	1.400(0.099)		2.970(0.115)	0.677(0.104)	0.011(0.003)	
	ME/GG	20.8%	Control	0.1219	0.118	1.553(0.124)		1.837(0.310)	3.390(0.345)	1.145(0.171)		0.007(0.003)
			AMP-PNP	0.0427	0.060	1.269(0.094)		1.738(0.131)	3.007(0.155)	0.600(0.098)		0.009(0.002)
	ET/GG	20.9%	Control	0.1316	0.279	1.589(0.160)		1.733(0.289)	3.322(0.328)	0.607(0.144)		0.009(0.004)
			AMP-PNP	0.0968	0.145	1.380(0.142)		1.596(0.216)	2.977(0.252)	0.482(0.133)		0.009(0.004)
	TD/GG	21.0%	Control	0.1309	0.239	1.426(0.157)	1.362(0.178)		2.788(0.203)	1.093(0.223)	0.012(0.005)	
			AMP-PNP	0.1113	0.096	1.574(0.109)		1.473(0.228)	3.046(0.258)	0.657(0.098)		0.008(0.003)
HRDLKPSNL	2P-WT	22.2%	Control	0.1473	0.152	0.507(0.123)			0.507(0.040)	1.164(0.485)		
			AMP-PNP	0.0329	0.056	0.585(0.071)			0.585(0.020)	5.771(2.452)		
	0P-WT	22.0%	Control	0.1243	0.073	0.544(0.057)			0.544(0.026)	1.692(0.366)		
			AMP-PNP	0.0461	0.153	0.535(0.080)			0.535(0.034)	2.056(0.664)		
	LM/GG	21.0%	Control	0.0339	0.112	0.755(0.083)			0.755(0.038)	3.066(1.010)		
			AMP-PNP	0.0460	0.135	0.838(0.113)			0.838(0.038)	2.573(0.722)		
	ME/GG	21.4%	Control	0.1808	0.067	0.835(0.097)			0.835(0.035)	1.452(0.319)		
			AMP-PNP	0.0086	0.013	0.735(0.044)			0.735(0.018)	1.682(0.223)		
	ET/GG	22.2%	Control	0.0402	0.096	0.509(0.087)			0.509(0.027)	2.678(0.862)		
			AMP-PNP	0.0396	0.081	0.525(0.083)			0.525(0.032)	1.494(0.433)		
	TD/GG	23.6%	Control	0.0930	0.429	0.619(.186)			0.619(0.063)	1.426(0.764)		
			AMP-PNP	0.0473	0.283	0.641(0.157)			0.641(0.051)	2.915(1.565)		

LLNTTCD [5]	2P-WT	17.6%	Control	0.0874	0.077	1.874(0.110)	0.532(0.070)	2.405(0.042)	5.776(1.225)	0.065(0.030)
			AMP-PNP	0.1253	0.127	1.338(0.157)	0.707(0.109)	2.046(0.046)	10.172(9.595)	0.158(0.095)
	0P-WT	19.4%	Control	0.1506	0.099	1.913(0.179)	0.492(0.147)	2.405(0.077)	2.468(0.512)	0.056(0.054)
			AMP-PNP	0.1464	0.104	1.638(0.197))	0.618(0.160)	2.255(0.057)	1.645(0.387)	0.043(0.027)
	LM/GG	19.2%	Control	0.0075						
			AMP-PNP	0.1212	0.486	1.861(0.264)	0.938(0.199)	2.798(0.103)	2.571(0.915)	0.028(0.015)
	ME/GG	19.1%	Control	0.0059	0.112	1.795(0.234)	1.018(0.183)	2.813(0.074)	9.946(9.638)	0.238(0.111)
			AMP-PNP		0.120	1.792(0.206)	0.953(0.157)	2.745(0.089)	5.579(2.219)	0.054(0.025)
	ET/GG	19.7%	Control	0.0871	0.047	1.870(0.076)	0.974(0.054)	2.844(0.050)	5.627(0.811)	0.019(0.004)
			AMP-PNP		0.146	1.596(0.170)	0.679(0.120)	2.275(0.080)	4.362(1.108)	0.029(0.016)
	TD/GG	19.3%	Control	0.1400	0.218	1.989(0.260)	0.805(0.197)	2.794(0.091)	4.296(3.721)	0.035(0.022)
			AMP-PNP	0.2012	0.337	1.690(0.232)	0.806(0.197)	2.495(0.184)	4.156(1.716)	0.025(0.019)
LNTTCDL	2P-WT	20.6%	Control	0.0617	0.058	1.906(0.090)	0.537(0.058)	2.444(0.048)	5.113(0.794)	0.023(0.011)
			AMP-PNP	0.0815	0.082	1.511(0.099)	0.684(0.063)	2.195(0.048)	5.613(1.295)	0.046(0.013)
	0P-WT	22.3%	Control	0.1263	0.200	1.849(0.112)	0.804(0.180)	2.652(0.191)	3.547(0.690)	0.011(0.007)
			AMP-PNP	0.0335	0.117	1.704(0.112)	0.636(0.093)	2.340(0.076)	1.711(0.239)	0.017(0.009)
LKICDFGL	2P-WT	22.3%	Control	0.1150	0.128	0.376(0.122)	1.739(0.091)	2.115(0.073)	1.057(0.647)	0.019(0.004
			AMP-PNP	0.1006	0.285	0.899(0.103)		0.899(0.094)	0.028(0.010)	
	0P-WT	23.0%	Control	0.0944	0.214	0.894(0.142)	1.376(0.143)	2.270(0.135)	0.632(0.231)	0.016(0.006)
			AMP-PNP	0.0388	0.084	0.362(0.078)	1.438(0.180)	1.800(0.201)	1.937(0.933)	0.008(0.003)
	LM/GG	22.4%	Control	0.0626	0.571	1.020(0.288)	1.519(0.272)	2.536(0.187)	0.684(0.361)	0.020(0.010)
			AMP-PNP	0.0416	0.108	0.880(0.119)	1.531(0.088)	2.412(0.058)	1.152(0.275)	0.018(0.003)
	ME/GG	22.4%	Control	0.1133	0.12	0.676(0.132)	1.865(0.110)	2.540(0.077)	2.128(0.930)	0.024(0.004)
			AMP-PNP	0.0122	0.764	1.573(0.199)		1.573(0.179)	0.027(0.012)	
	ET/GG	23.2%	Control	0.1511	0.247	0.861(0.141)	1.480(0.115)	2.341(0.110)	0.676(0.239)	0.013(0.004)
			AMP-PNP	0.0725	0.056	0.374(0.088)	1.415(0.072)	1.788(0.070)	5.962(4.027)	0.017(0.003)
	TD/GG	23.1%	Control	0.1517	0.09	0.911(0.124)	1.582(0.103)	2.493(0.049)	1.120(0.275)	0.031(0.006)
			AMP-PNP	0.0286	0.071	0.633(0.099)	1.587(0.077)	2.219(0.064)	2.584(1.005)	0.021(0.003)
KICDFGL	2P-WT	24.4%	Control	0.0467	0.055		1.203 (.050)	1.203 (.048)		0.023(.003)
			AMP-PNP	0.0415	0.146		0.534 (.061)	0.534 (.061)		0.033 (.013)
	0P-WT	20.5%	Control	0.1171	0.093	0.689 (.086)	0.774 (.118)	1.463 (.129)	0.903 (.228)	0.012 (.005)
			AMP-PNP	0.0993	0.057	0.404 (.065)	0.083 (.082)	1.239 (.091)	2.317 (.916)	0.011 (.004)
	LM/GG	25.9%	Control	0.0223	0.02	0748 (.080)	0.498 (.070)	1.246 (.072)	0.261 (.058)	0.012 (.006)
			AMP-PNP	0.0432	0.151	0.551 (.135)	0.787 (.105)	1.338 (.075)	0.971 (.443)	0.017 (.007)
	ME/GG	23.8%	Control	0.0880	0.07	0.488 (.113)	0.897 (.097)	1.385 (.055)	1.408 (.623)	0.028 (.008)
			AMP-PNP	0.0390	0.035		0.886 (.067)	0.886 (.068)		0.013 (.003)
	ET/GG	21.8%	Control	0.1087	0.137	0.428 (.120)	1.009 (.080)	1.436 (.046)	3.556 (2.363)	0.044 (.011)
			AMP-PNP	0.0211	0.032	0.254 (.064)	0.841 (.056)	1.095 (.056)	4.631 (2.828)	0.016 (.003)
	TD/GG	21.2%	Control	0.0622	0.08	0.840 (.106)	0.763 (.087)	1.603 (.060)	1.475 (.356)	0.025 (.009)
			AMP-PNP	0.0668	0.031	0.477 (.076)	0.833 (.064)	1.310 (.055)	1.353 (.394)	0,017 (.004)
KICDFGLARVADPDHDHTGF [3]	2P-WT		Control							
			AMP-PNP							

	0P-WT	20.0%	Control	0.0334	1.628	4.107(0.560)	2.662(0.536)		6.770(0.202)	0.586(0.153)	0.036(0.017)	
			AMP-PNP	0.0864	1.429	2.239(0.485)	3.584(0.430)		5.822(0.223)	1.237(0.470)	0.033(0.011)	
	LM/GG	20.1%	Control	0.0863	0.177	1.342(0.272)	3.744(0.276)	2.476(0.336)	7.452(0.113)	7.775(6.311)	0.345(0.096)	0.022(0.006)
			AMP-PNP	0.0737	0.1	1.202(0.166)	4.716(0.154)	2.850(1.609)	8.768(1.718)	6.904(3.640)	0.235(0.023)	0.004(0.003)
	ME/GG	20.1%	Control	0.0753	1.113	3.613(0.493)	3.600(0.441)		7.213(0.221)	0.766(0.189)	0.028(0.009)	
			AMP-PNP	0.0731	0.885	2.017(0.418)	4.163(0.343)		6.180(0.234)	1.478(0.581)	0.022(0.005)	
	ET/GG	19.8%	Control	0.1476	0.95	4.228(0.375)	2.879(0.316)		7.107(0.146)	0.699(0.116)	0.028(0.008)	
			AMP-PNP	0.1139	1.297	2.680(0.506)	3.715(0.423)		6.395(0.233)	1.025(0.353)	0.028(0.009)	
	TD/GG	19.9%	Control	0.1023	1.482	4.479(0.683)	2.602(0.634)		7.080(0.172)	0.803(0.188)	0.049(0.025)	
			AMP-PNP	0.1861	0.981	3.106(0.490)	3.666(0.415)		6.772(0.191)	0.859(0.223)	0.035(0.010)	
ARVADPDHDTGFLpTEpYVATRW [3]	2P-WT	20.5%	Control	0.1376	1.323	6.313(0.457)	1.498(0.298)		7.811(0.185)	3.342(0.973)	0.026(0.021)	
			AMP-PNP	0.0234	1.903	6.088(0.473)	1.814(0.297)		7.902(0.229)	6.597(0.473)	1.814(0.297)	
YVATRW [3]	2P-WT		Control									
			AMP-PNP									
	0P-WT	26.5%	Control	0.2188	0.356	3.513(0.121)			3.513(0.044)	7.662(1.810)		
			AMP-PNP	0.2393	1.426	3.720(0.243)			3.720(0.092)	5.189(1.421)		
	LM/GG	21.1%	Control	0.0394	0.593	3.814(0.147)			3.814(0.072)	5.755(1.211)		
			AMP-PNP	0.0557	1.186	3.941(0.311)			3.941(0.088)	4.029(0.841)		
	ME/GG	20.1%	Control	0.0587	0.226	3.400(0.150)			3.400(0.043)	10.792(3.551)		
			AMP-PNP	0.1558	0.698	3.551(0.262)			3.551(0.077)	5.616(1.433)		
	ET/GG	19.6%	Control	0.2528	1.185	3.494(0.313)			3.494(0.088)	5.358(1.193)		
			AMP-PNP	0.0173	0.291	3.556(0.190)			3.556(0.060)	8.340(1.902)		
	TD/GG	19.8%	Control	0.1760	0.945	3.432(0.269)			3.432(0.073)	5.430(1.063)		
			AMP-PNP	0.1664	0.856	3.264(0.277)			3.264(0.079)	4.923(1.285)		
VATRW [3]	2P-WT		Control									
			AMP-PNP									
	0P-WT	29.0%	Control	0.1272	0.094	3.021(0.101)			3.021(0.030)	7.130(1.084)		
			AMP-PNP	0.1794	0.213	3.062(0.133)			3.062(0.035)	7.838(1.774)		
YRAPEIM	2P-WT	22.2%	Control	0.0791	0.051	0.296(0.088)	1.413(0.067)		1.710(0.042)	1.165(0.631)	0.024(0.004)	
			AMP-PNP	0.0651	0.061	0.269(0.089)	1.545(0.063)		1.814(0.055)	3.890(3.551)	0.023(0.003)	
	0P-WT	24.8%	Control	0.2004	0.095	0.646(0.116)	1.807(0.109)		2.453(0.070)	0.674(0.267)	0.024(0.004)	
			AMP-PNP	0.1182	0.148	0.685(0.132)	1.842(0.113)		2.528(0.067)	0.954(0.329)	0.023(0.004)	
	LM/GG	21.2%	Control	0.0532	0.061	0.520(0.080)	1.734(0.069)		2.254(0.054)	7.058(6.187)	0.031(0.004)	
			AMP-PNP	0.0869	0.084	0.815(0.122)	1.557(0.094)		2.372(0.063)	0.955(0.262)	0.017(0.003)	
	ME/GG	24.8%	Control	0.1804	0.204	0.578(0.185)	1.766(0.159)		2.344(0.110)	1.123(0.657)	0.021(0.006)	
			AMP-PNP	0.0872	0.183	0.738(0.191)	1.594(0.162)		2.332(0.124)	0.826(0.389)	0.018(0.006)	
	ET/GG	25.7%	Control	0.0855	0.15	0.691(0.127)	1.737(0.104)		2.428(0.085)	0.612(0.246)	0.018(0.004)	
			AMP-PNP	0.0613	0.063	0.728(0.125)	1.748(0.120)		2.476(0.959)	0.384(0.169)	0.015(0.003)	
	TD/GG	25.6%	Control	0.0792	0.258	0.704(0.186)	1.725(0.152)		2.428(0.095)	0.760(0.382)	0.023(0.006)	
			AMP-PNP	0.0794	0.069	0.796(0.119)	1.797(0.107)		2.593(0.079)	0.616(0.184)	0.024(0.004)	
NSKGYTKSIDIWSVG	2P-WT	21.1%	Control	0.1536	0.368	2.906(0.306)	1.308(0.251)		4.214(0.088)	3.504(0.977)	0.096(0.043)	
			AMP-PNP	0.3429	0.754	3.352(0.344)	0.981(0.251)		4.334(0.203)	2.684(0.651)	0.024(0.021)	

	0P-WT	24.2%	Control	0.0832	0.784	3.958(0.331)	2.289(0.419)		6.247(0.447)	3.685(0.801)	0.011(0.006)	
			AMP-PNP	0.1908	0.346	3.636(0.217)	2.188(0.162)		5.823(0.154)	7.719(2.364)	0.020(0.005)	
	LM/GG	24.5%	Control	0.1397	0.238	3.967(0.130)	2.231(0.128)		6.198(0.109)	5.792(0.977)	0.027(0.007)	
			AMP-PNP	0.1631	0.473	3.874(0.232)	2.291(0.176)		6.165(0175)	4.719(0.887)	0.014(0.004)	
	ME/GG	24.0%	Control	0.1398	0.45	3.845(0.230)		2.790(0.520)	6.635(0.541)	5.922(1.002)		0.008(0.003)
			AMP-PNP	0.1042	0.208	3.840(0.165)		3.238(0.510)	7.079(0.530)	4.890(0.677)		0.007(0.002)
	ET/GG	24.7%	Control	0.0363	0.576	3.868(0.306)	2.648(0.233)		6.516(0.225)	4.391(1.050)	0.019(0.007)	
			AMP-PNP	0.0954	0.275	3.726(0.196)		3.593(0.520)	7.318(0.545)	5.987(0.904)		0.007(0.002)
	TD/GG	24.3%	Control	0.1055	0.349	3.928(0.254)	1.840(0.183)		5.768(0.161)	11.358(8.529)	0.023(0.007)	
			AMP-PNP	0.1503	0.581	4.527(0.370)		1.847(0.559)	6.374(0.615)	1.968(0.349)		0.009(0.008)
LSNRPIFPGKHYLDQLNHILGILGSPSQE	2P-WT	24.5%	Control	0.2266	1.816	5.647(0.692)	3.512(0.534)		9.159(0.324)	2.907(0.931)	0.053(0.025)	
			AMP-PNP	0.1988	1.593	5.989(0.628)	2.836(0.470)		8.825(0.241)	1.359(0.260)	0.042(0.017)	
	0P-WT	24.4%	Control	0.1827	2.839	5.008(0.643)	4.523(0.450)		9.531(0.218)	4.689(1.877)	0.103(0.029)	
			AMP-PNP	0.1789	2.378	6.636(0.456)	4.520(0.523)		11.156(0.552)	1.840(0.274)	0.012(0.005)	
	LM/GG	24.0%	Control	0.0689	0.215	4.635(0.312)	3.216(0.255)	4.749(0.354)	12.601(0.462)	7.592(1.977)	0.355(0.111)	0.009(0.002)
			AMP-PNP	0.1336	0.408	5.163(0.336)	3.261(0.552)	3.100(0.357)	11.524(0.403)	4.865(0.906)	0.189(0.067)	0.012(0.007)
	ME/GG	24.0%	Control	0.1169	0.078	4.864(0.153)	3.838(0.194)	6.867(4.892)	15.568(5.050)	6.106(0.684)	0.164(0.020)	0.002(0.002)
			AMP-PNP	0.0543	2.625	5.774(0.603)	5.818(0.423)		11.592(0.360)	4.888(1.654)	0.026(0.006)	
	ET/GG	24.6%	Control	0.0546	1.279	4.541(0.484)	2.833(1.153)	2.948(1.210)	10.322(0.256)	9.361(5.142)	0.205(0.169)	0.022(0.014)
			AMP-PNP	0.3628	0.658	4.591(0.519)	2.459(1.027)	3.246(1.119)	10.296(0.257)	5.510(1.446)	0.232(0.216)	0.022(0.012)
	TD/GG	25.2%	Control	0.0893	1.46	5.338(0.490)	5.212(0.349)		10.550(0.211)	3.834(1.038)	0.061(0.013)	
			AMP-PNP	0.2293	2.366	5.646(0.570)	5.406(0.370)		11.051(0.243)	5.295(1.813)	0.075(0.015)	
IINLKARNYL	2P-WT	19.8%	Control	0.1557	0.719	3.826(0.390)	2.532(0.325)		6.358(0.106)	3.609(0.981)	0.158(0.038)	
			AMP-PNP	0.2149	0.849	4.728(0.384)	1.654(0.297)		6.382(0.174)	1.847(0.305)	0.047(0.023)	
	0P-WT	20.9%	Control	0.2754	1.42	5.120(0.404)	1.443(0.343)		6.563(0.198)	1.288(0.185)	0.031(0.023)	
			AMP-PNP	0.2744	0.871	3.582(0.384)	2.801(0.357)		6.382(0.115)	3.043(0.893)	0.108(0.027)	
	LM/GG	20.8%	Control	0.1273	0.172	2.683(0.281)	2.853(0.258)	1.299(0.299)	6.835(0.122)	6.810(2.308)	0.349(0.124)	0.019(0.010)
			AMP-PNP	0.0496	1.482	4.810(0.576)	1.729(0.495)		6.539(0.164)	1.426(0.317)	0.042(0.024)	
	ME/GG	21.1%	Control	0.0520	0.037	2.271(0.189)	3.235(0.142)	1.328(0.105)	6.834(0.120)	12.482(9.357)	0.449(0.067)	0.011(0.003)
			AMP-PNP	0.0959	0.122	2.857(0.271)	2.508(0.254)	1.389(0.282)	6.755(0.139)	5.614(1.331)	0.307(0.114)	0.017(0.008)
	ET/GG	21.1%	Control	0.0200	0.118	2.549(0.191)	3.140(0.135)	1.132(0.254)	6.821(0.319)	7.710(1.882)	0.315(0.058)	0.008(0.005)
			AMP-PNP	0.2337	0.208	2.654(0.332)	2.833(0.255)	1.474(0.410)	6.961(0.516)	7.206(2.579)	0.341(0.116)	0.008(0.006)
	TD/GG	20.3%	Control	0.1018	0.105	2.438(0.277)	2.939(0.202)	1.249(0.161)	6.626(0.080)	11.882(11.814)	0.420(0.108)	0.020(0.007)
			AMP-PNP	0.1659	0.206	2.595(0.420)	2.868(0.307)	1.171(0.316)	6.634(0.197)	7.976(4.842)	0.329(0.147)	0.016(0.012)
LSLPHKNKVPWNRLFPNADSKALDL	2P-WT	23.2%	Control	0.0590	2.34	5.628(0.514)	3.398(0.324)		9.025(0.261)	4.439(1.212)	0.026(0.009)	
			AMP-PNP	0.4213	1.699	5.901(0.464)	3.455(0.337)		9.357(0.283)	4.208(0.970)	0.023(0.007)	
	0P-WT	22.9%	Control	0.2494	0.688	5.209(0.391)	1.487(0.564)	4.281(1.495)	10.977(1.846)	5.557(1.507)	0.225(0.181)	0.006(0.005)
			AMP-PNP	0.2633	2.229	5.927(0.390)	3.944(0.423)		9.871(0.432)	4.572(1.167)	0.013(0.005)	
	LM/GG	24.3%	Control	0.1344	0.808	5.862(0.219)	4.667(0.268)		10.528(0.260)	4.113(0.611)	0.016(0.003)	
			AMP-PNP	0.0311	0.406	5.244(0.313)	1.948(0.317)	4.179(1.509)	11.371(1.726)	5.131(0.906)	0.212(0.088)	0.005(0.003)
	ME/GG	24.3%	Control	0.1214	1.744	5.326(0.456)	4.743(0.592)		10.069(0.616)	5.109(1.115)	0.011(0.004)	
			AMP-PNP	0.1956	0.927	5.675(0.351)		5.433(0.976)	11.108(1.020)	4.530(0.862)		0.007(0.003)

	ET/GG	24.4%	Control	0.2261	0.974	5.717(0.299)	3.907(0.233)		9.623(0.235)	4.331(0.536)	0.016(0.003)	
			AMP-PNP	0.2244	1.607	5.352(0.447)	4.471(0.430)		9.822(0.436)	4.702(0.962)	0.014(0.004)	
	TD/GG	23.9%	Control	0.0822	2.312	5.585(0.512)	3.897(0.330)		9.482(0.268)	4.437(1.249)	0.025(0.008)	
			AMP-PNP	0.1988	2.215	5.524(0.531)	3.795(0.334)		9.319(0.257)	6.449(2.509)	0.047(0.014)	
LTFNPHKRIEVEQA	2P-WT	30.5%	Control	0.1828	0.929	2.506(0.607)	1.881(0.538)		4.386(0.194)	0.948(0.403)	0.033(0.024)	
			AMP-PNP	0.0703	1.828	2.051(0.465)	3.457(0.310)		5.508(0.241)	4.489(3.093)	0.035(0.009)	
	0P-WT	27.5%	Control	0.0617	1.648	2.643(0.528)	2.213(0.475)		4.856(0.180)	1.828(0.733)	0.053(0.030)	
			AMP-PNP	0.1710	3.755	2.454(0.820)	2.759(0.743)		5.214(0.280)	1.512(0.938)	0.042(0.028)	
	LM/GG	23.4%	Control	0.2700	0.887	2.150(0.359)	2.979(0.326)		5.129(0.160)	7.649(7.566)	0.096(0.030)	
			AMP-PNP	0.1435	0.325	2.722(0.336)	2.767(0.276)		5.490(0.101)	1.013(0.211)	0.036(0.007)	
	ME/GG	26.1%	Control	0.2625	1.357	2.328(0.455)	2.649(0.293)		4.976(0.203)	6.635(4.192)	0.060(0.024)	
			AMP-PNP	0.1328	0.513	1.993(0.293)	2.824(0.185)		4.817(0.134)	9.007(8.822)	0.058(0.014)	
	ET/GG	30.2%	Control	0.2974	1.096	2.356(0.424)	2.526(0.341)		4.872(0.187)	1.267(0.421)	0.030(0.012)	
			AMP-PNP	0.1659	0.977	2.920(0.382)	2.326(0.331)		5.247(0.302)	1.313(0.330)	0.016(0.008)	
	TD/GG	31.5%	Control	0.2489	3.237	1.876(0.689)	3.105(0.502)		4.981(0.315)	3.541(3.048)	0.046(0.022)	
			AMP-PNP	0.0668	0.718	1.733(0.310)	3.332(0.198)		5.065(0.136)	5.708(3.669)	0.068(0.013)	
YYDPSDEPIAEAPFKFDMEL	2P-WT	22.4%	Control	0.0875	2.247	6.948(0.54)	2.561(0.361)		9.509(0.165)	9.048(4.619)	0.177(0.061)	
			AMP-PNP	0.4553	0.523	7.144(0.401)	2.533(0.195)		9.677(0.151)	6.754(1.530)	0.152(0.050)	
	0P-WT	22.0%	Control	0.0434	0.901	7.089(0.331)	2.769(0.276)		9.858(0.117)	7.701(1.986)	0.246(0.066	
			AMP-PNP	0.0707	0.757	8.948(0.382)	1.134(0.341)		10.083(0.255)	2.111(0.242)	0.027(0.025)	
	LM/GG	22.2%	Control	0.2549	0.42	7.402(0.261)	2.992(0.233)		10.394(0.095)	13.013(8.691)	0.246(0.056)	
			AMP-PNP	0.2381	0.479	7.052(0.350)	3.100(0.277)		10.152(0.077)	8.542(2.145)	0.282(0.073)	
	ME/GG	21.4%	Control	0.1089	0.114	7.460(0.150)	2.230(0.099)		9.689(0.055)	7.215(0.636)	0.147(0.028)	
			AMP-PNP	0.0983	0.35	7.317(0.236)	2.397(0.150)		9.714(0.092)	7.730(1.242)	0.118(0.026)	
	ET/GG	21.3%	Control	0.0915	0.644	7.374(0.295)	2.555(0.186)		9.929(0.088)	8.780(1.648)	0.185(0.049)	
			AMP-PNP	0.3386	0.824	7.082(0.395)	2.608(0.273)		9.690(0.126)	11.350(5.526)	0.144(0.061)	
	TD/GG	21.0%	Control	0.1269	0.196	7.538(0.204)	2.356(0.134)		9.894(0.088)	9.265(1.701)	0.132(0.032)	
			AMP-PNP	0.3056	0.46	7.203(0.297)	2.816(0.192)		10.019(0.110)	12.328(7.092)	0.149(0.044)	
DDLPKEKLKELIF	2P-WT	19.1%	Control	0.2048	0.223	1.978(0.175)	1.697(0.127)		3.675(0.075)	1.801(0.313)	0.033(0.008)	
			AMP-PNP	0.0402	0.187	1.925(0.147)	1.607(0.101)		3.531(0.072)	3.215(0.648)	0.036(0.007)	
	0P-WT	19.2%	Control	0.1240	0.315	2.607(0.169)	1.334(0.147)		3.941(0.119)	1.483(0.189)	0.021(0.008)	
			AMP-PNP	0.0222	0.43	1.650(0.173)	1.894(0132)		3.544(0.085)	7.176(4.418)	0.073(0.016)	
	LM/GG	19.5%	Control	0.0367	0.034	1.637(0.063)	1.36(0.126)	1.313(0.127)	4.309(0.182)	11.390(6.046)	0.198(0.043)	0.009(0.004)
			AMP-PNP	0.0339	0.316	2.475(0.254)	1.592(0.181)		4.067(0.156)	2.066(0.523)	0.015(0.006)	
	ME/GG	19.2%	Control	0.0347	0.064	1.619(0.126)	1.380(0.140)	2.246(1.280)	5.245(1.393)	6.715(1.569)	0.212(0.062)	0.004(0.004)
			AMP-PNP	0.0294	0.289	2.195(0.211)	1.627(0.172)		3.822(0.133)	2.362(0.552)	0.021(0.007)	
	ET/GG	19.4%	Control	0.1242	0.135	1.496(0.201)	1.371(0.150)	1.426(0.198)	4.293(0.255)	5.900(1.841)	0.293(0.134)	0.009(0.005)
			AMP-PNP	0.0491	0.033	1.696(0.082)	1.306(0.130)	1.189(0.330)	4.191(0.419)	8.374(1.785)	0.143(0.030)	0.006(0.004)
	TD/GG	19.0%	Control	0.0380	0.721	2.496(0.287)	1.545(0.221)		4.041(0.168)	1.854(0.441)	0.021(0.010)	
			AMP-PNP	0.0459	0.386	2.554(0.238)	1.495(0.195)		4.050(0.179)	1.623(0.291)	0.017(0.008)	
EETARFQPGYRS	2P-WT	19.5%	Control	0.1002	0.631	4.011(0.299)	2.396(0.185)		6.408(0.110)	8.669(3.955)	0.085(0.022)	
			AMP-PNP	0.2146	0.705	4.038(0.297)	2.190(0.188)		6.228(0.100)	6.182(1.723)	0.107(0.025)	

0P-WT	19.0%	Control	0.0345	0.761	3.931(0.257)	2.479(0.207)	6.410(0.111)	5.662(1.604)	0.082(0.019)
		AMP-PNP	0.0854	0.241	3.949(0.152)	2.634(0.120)	6.583(0.069)	13.189(11.349)	0.078(0.010)
LM/GG	19.2%	Control	0.0472	0.313	3.904(0.133)	2.727(0.118)	6.631(0.087)	9.233(3.210)	0.064(0.010)
		AMP-PNP	0.1468	0.315	3.794(0.215)	2.650(0.133)	6.444(0.072)	9.142(3.540)	0.074(0.012)
ME/GG	19.2%	Control	0.1274	0.161	3.895(0.147)	2.542(0.091)	6.436(0.062)	9.322(1.930)	0.087(0.012)
		AMP-PNP	0.1934	0.238	4.138(0.183)	2.358(0.115)	6.497(0.089)	6.680(1.246)	0.048(0.009)
ET/GG	19.7%	Control	0.1160	0.181	3.715(0.135)	2.744(0.078)	6.460(0.048)	9.731(2.133)	0.825(0.008)
		AMP-PNP	0.2473	0.317	4.108(0.204)	2.425(0.124)	6.533(0.087)	7.925(1.622)	0.048(0.009)
TD/GG	19.8%	Control	0.0841	0.361	3.999(0.227)	2.449(0.142)	6.449(0.083)	7.486(2.046)	0.088(0.015)
		AMP-PNP	0.1592	0.338	3.955(0.232)	2.604(0.148)	6.559(0.091)	11.702(8.467)	0.111(0.018)

0P-ERK2 Ctl Avg. St. Dev.	0.1539
2P-ERK2 Ctl Avg. St. Dev.	0.1447
Avg. across samples	0.1222

Footnotes:

(1) Information for each peptide includes amino acid sequence, protein designation, % back exchange (BE), experimental condition (± AMP-PNP), the standard deviation of the # of deuterons incorporated at 1 min, and the residual sum of squares (RSS).

(2) A, B, and C equal the number of amides exchanging with rates k_1, k_2, and k_3 respectively. N equals A+B+C. Values are corrected for artifactual in-exchange, back-exchange, and percentage D2O during the incubation.

(3) Four peptides around the activation lip were differentially recovered in datasets of 0P-WT and hinge mutants *vs* 2P-WT-ERK2 (see Fig. S1). Three peptides were not observed in 2P-WT datasets. ARVADPDHDTGFLpTEpYVATRW was observed only in 2P-WT datasets.

(4) Peptides were not sequenced by LC-MS/MS but were identified by peptide mass fingerprinting. Peptide m/z were within 0.1 Da from calculated, and observed only in the hinge mutant

(5) LLNTTCD showed individual variation between ERK2 forms. In LM/GG, the late time points showed low intensity and could not be fit, although error at 1 min could be calculated. ME/GG & ET/GG showed only one measurable time point in +AMP-PNP datasets, therefore errors could not be measured.

(6) Fitted parameters were omitted when a peptide was not observed or not quantifiable