

Probing protein phosphatase substrate binding: Affinity pull-down of ILKAP phosphatase 2C with phosphopeptides

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Supplementary Information

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Pull-down reactions were performed at 4 °C to prevent dephosphorylation of the phosphopeptides.

Integrin-Linked Kinase-Associated Phosphatase (ILKAP) could potentially dephosphorylate the phosphopeptides and thus prevent the intended pull-down reaction. Thus the pull-downs were performed at two temperatures, 4 °C and 30 °C. We speculated that at 4 °C the enzymatic reaction was slowed down and the pull-down should prevail. While performing the reaction at 30 °C – based on the results by Doehn *et al*¹ – would allow ILKAP to dephosphorylate the targets and thus prevent the pull-down.

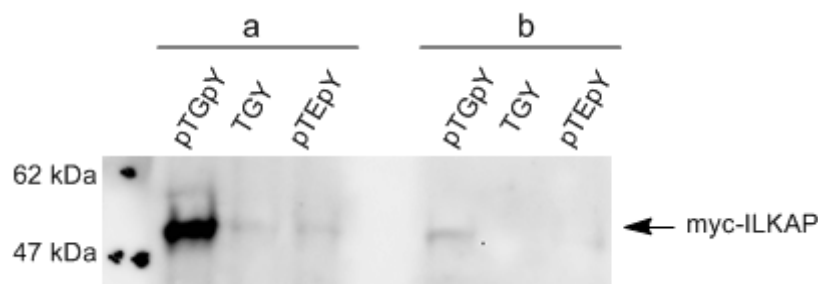


Fig S1: Comparison of pull-down performed at 4 °C (a) and 30 °C (b). pTGpY and TGY are motives derived from the mitogen-activated protein kinase 14 (p38). pTEpY is derived from the extracellular-regulated kinase 5 (ERK5).

As it is shown in figure S1, ILKAP is pulled down by the double phosphorylated pTGpY from the mitogen-activated protein kinase 14 (p38) at 4 °C. Whereas the pull-down reaction performed at 30 °C gave a significantly weaker result. To compare, the corresponding unphosphorylated motive from p38 and pTEpY from the extracellular-regulated kinase 5 (ERK5) could only pull-down ILKAP with limited success both at 4 °C and 30 °C. This is in accordance with kinetic studies from the literature, that these two motives acts not as substrates for ILKAP².

From these experiments it was concluded that ILKAP is indeed active and that it does recognize and dephosphorylate the phosphopeptides attached to the solid support. And that the dephosphorylation can be slowed sufficiently down to allow pull-down of the active phosphatase ILKAP by working at 4 °C. Thus all pull-down experiments were performed at 4 °C.

Table S1. Synthesized peptides

Peptide No.	Target protein and phosphor-residue	Sequence	Elemental composition	Calculated MS	Found MS	Assigned
1	P38 (180pT, 182pY)	Ac-TDDEM p TG p YVATGG-NH ₂	C ₅₅ H ₈₆ N ₁₄ O ₃₀ P ₂ S	1516.48	N. A.	–
2	p38 (180T, 182Y)	Ac-TDDEM T GYVATGG-NH ₂	C ₅₅ H ₈₄ N ₁₄ O ₂₄ S	1356.55	1357.4	[M+H] ⁺
3	p38 (180pT)	Ac-TDDEM p TGYVATGG-NH ₂	C ₅₅ H ₈₅ N ₁₄ O ₂₇ PS	1436.52	1458.7	[M+Na] ⁺
4	p38 (182pY)	Ac-TDDEM T G p YVATGG-NH ₂	C ₅₅ H ₈₅ N ₁₄ O ₂₇ PS	1436.52	1458.6	[M+Na] ⁺
5	p38 (180pS, 182pY)	Ac-TDDEM p S G p YVATGG -NH ₂	C ₅₄ H ₈₄ N ₁₄ O ₃₀ P ₂ S	1502.47	N. A.	–
6	UNG2 (31pT)	Ac-PAVQ G p TGVAGVPGGG-NH ₂	C ₅₄ H ₉₀ N ₁₇ O ₂₁ P	1343.62	1344.8	[M+H] ⁺
7	ERK5 (218pT, 220pY)	Ac-HQYF M p T E p YVATRGGG-NH ₂	C ₇₈ H ₁₁₄ N ₂₂ O ₂₉ P ₂ S	1916.73	1917.2	[M+H] ⁺
8	JNK1 (183pT, 185pY)	Ac-TSFMM p T P p YVVTRGGG-NH ₂	C ₇₂ H ₁₁₅ N ₁₉ O ₂₇ P ₂ S ₂	1803.71	1804.5	[M+H] ⁺
9	ATM, (1981pS)	Ac-AFE E G p SQSTTIGGG-NH ₂	C ₅₇ H ₈₉ N ₁₆ O ₂₇ P	1460.58	1461.7	[M+H] ⁺
10	ATM, (1981S)	Ac-AFE E G SQSTTIGGG-NH ₂	C ₅₇ H ₈₈ N ₁₆ O ₂₄	1380.62	1363.8	[M-H ₂ O+H] ⁺
11	p53 (15pS)	Ac-W E P P L p SQ E TFSGGG-NH ₂	C ₆₉ H ₉₈ N ₁₇ O ₂₆ P	1612.59	1613.5	[M+H] ⁺
12	p53 (15S)	Ac-W E P P L SQ E TFSGGG-NH ₂	C ₆₉ H ₉₇ N ₁₇ O ₂₃	1531.69	1533.2	[M+H] ⁺
13	Chk1 (345pS)	Ac-QG I S F p SQ T CPDGGG-NH ₂	C ₆₂ H ₉₅ N ₁₈ O ₂₆ PS	1570.61	1591.8	[M+Na] ⁺
14	Chk1 (345S)	Ac-QG I S F SQ T CPDGGG-NH ₂	C ₆₂ H ₉₄ N ₁₈ O ₂₃ S	1490.65	1474.0	[M-H ₂ O+H] ⁺
15	Chk2 (68pT)	Ac-LET V S p TQ E LYSIPGGG-NH ₂	C ₇₄ H ₁₁₉ N ₁₈ O ₃₀ P	1770.81	1787.0	[M+O+H] ⁺ a
16	Chk2 (68T)	Ac-LET V S T Q E LYSIPGGG-NH ₂	C ₇₄ H ₁₁₈ N ₁₈ O ₂₇	1690.84	1691.8	[M+H] ⁺
17	Chk2 (387pT)	Ac-RT L C G p T P TYLAPEGGG-NH ₂	C ₇₀ H ₁₁₄ N ₂₁ O ₂₅ PS	1712.76	868.6	[M+Na+H] ²⁺
18	Chk2 (387T)	Ac-RT L C G T P TYLAPEGGG-NH ₂	C ₇₀ H ₁₁₃ N ₂₁ O ₂₂ S	1632.79	1616.0	[M-H ₂ O+H] ⁺
19	Chk2 (516pS)	Ac-Q V LA Q p S T S R K R P GGG-NH ₂	C ₇₀ H ₁₂₃ N ₂₆ O ₂₅ P	1758.89	1759.7	[M+H] ⁺
20	Chk2 (516S)	Ac-Q V LA Q P S T S R K R P GGG-NH ₂	C ₇₀ H ₁₂₂ N ₂₆ O ₂₂	1678.92	1680.2	[M+H] ⁺
21	RSK2 (227pS)	Ac-HE K K A Y p S F C G T V E G GG-NH ₂	C ₇₄ H ₁₁₂ N ₂₁ O ₂₇ PS	1789.75	1772.2	[M-H ₂ O+H] ⁺
22	RSK2 (227S)	Ac-HE K K A Y S F C G T V E G GG-NH ₂	C ₇₄ H ₁₁₁ N ₂₁ O ₂₄ S	1709.78	1692.4	[M-H ₂ O+H] ⁺
23	RSK2 (369pS)	Ac-A K T P K D p S P G I P S GGG-NH ₂	C ₆₅ H ₁₀₈ N ₁₉ O ₂₅ P	1585.75	1570	[M-H ₂ O+H] ⁺
24	RSK2 (369S)	Ac-A K T P K D S P G I P S GGG-NH ₂	C ₆₅ H ₁₀₇ N ₁₉ O ₂₂	1505.78	1490	[M-H ₂ O+H] ⁺
25	RSK2 (386pS)	Ac-QL F R G F p S F VAITSGGG-NH ₂	C ₇₈ H ₁₁₈ N ₂₁ O ₂₄ P	1763.84	1763.7	[M+H] ⁺
26	RSK2 (386S)	Ac-QL F R G F S F VAITSGGG-NH ₂	C ₇₈ H ₁₁₇ N ₂₁ O ₂₁	1683.87	1684.2	[M+H] ⁺
27	RSK2 (577pT)	Ac-EN G L L M p S P C T ANGGG-NH ₂	C ₆₇ H ₁₀₆ N ₁₉ O ₂₇ PS ₂	1703.67	1722.4	[M+H ₂ O+H] ⁺
28	RSK2 (577T)	Ac-EN G L L M S P C T ANGGG-NH ₂	C ₆₇ H ₁₀₅ N ₁₉ O ₂₄ S ₂	1623.70	1623.8	[M+H] ⁺

^a [M+NH₂+H]

Table S2. Summary of ILKAP pull-down specificity for dephosphorylation targets

Peptide No.	ILKAP target	ILKAP pull-down
1	p38 (180pT, 182pY)	Strong
2	p38 (180T, 182Y)	Weak
3	p38 (180pT)	Medium
4	p38 (182pY)	Weak
5	p38 (180pS, 182pY)	Strong
6	UNG2 (31pT)	Weak
7	ERK5 (218pT, 220pY)	Weak
8	JNK1 (183pT, 185pY)	Strong
9	ATM, (1981pS)	Strong
10	ATM, (1981S)	Weak
11	p53 (15pS)	Medium
12	p53 (15S)	Weak
13	Chk1 (345pS)	Strong
14	Chk1 (345S)	Weak
15	Chk2 (68pT)	Medium
16	Chk2 (68T)	Weak
17	Chk2 (387pT)	Weak
18	Chk2 (387T)	Weak
19	Chk2 (516pS)	Weak
20	Chk2 (516S)	Weak
21	RSK2 (227pS)	Medium
22	RSK2 (227S)	Weak
23	RSK2 (369pS)	Weak
24	RSK2 (369S)	Weak
25	RSK2 (386pS)	Strong
26	RSK2 (386S)	Weak
27	RSK2 (577pT)	Medium
28	RSK2 (577S)	Weak

Notes to Tables S3-S7.

Figures 2 to 7 in the paper show quantification of several pull-downs prepared with the same set of phosphopeptides. These were normalized to the highest value in the individual experiment. To show that this is a valid procedure, some of the pull-down experiments were quantified to the pull-down efficiency of one sequence, sequence 7 (ERK5 (218pT, 220pY)).

Comparing the results from the 2 sets of quantifications (table 3A-B; 4A-B; 5A-B and 6A-B), each show the same relative relationship between the pull-down reactions, thus supporting the quantification used in the manuscript.

Table S3A.

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
1	pTGpY	657455	10.35	63491.8	7392.21	1.202476
2	TGY	611152	10.35	59020.22	2920.63	0.475093
3	pTGY	607300	10.35	58648.22	2548.63	0.414581
4	TGpY	621181	10.35	59988.74	3889.15	0.63264
5	pSGpY	603484	10.35	58279.7	2180.11	0.354634
7 (ERK5)	pTEpY	644566	10.35	62247.08	6147.49	1
	BckGrn	580909	10.35	56099.59	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
1	pTGpY	828093	13.56	61088.7	59155.62	2.069861
2	TGY	144359	13.56	10649.41	8716.33	0.304985
3	pTGY	559123	13.56	41246.69	39313.61	1.375587
4	TGpY	637554	13.56	47032.57	45099.5	1.578036
5	pSGpY	1184636	13.56	87391	85457.92	2.990181
7 (ERK5)	pTEpY	413616	13.56	30512.59	28579.51	1
	BckGrn	26204	13.56	1933.08	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
1	pTGpY	214277	5.14	41656.85	1499.46	4.9982
2	TGY	208467	5.14	40527.35	369.96	1.2332
3	pTGY	212604	5.14	41331.61	1174.22	3.914067
4	TGpY	220900	5.14	42944.4	2787.01	9.290033
5	pSGpY	225954	5.14	43926.93	3769.54	12.56513
7 (ERK5)	pTEpY	204139	5.14	39685.96	300	1
	BckGrn	206564	5.14	40157.39	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
1	pTGpY	221440	5.14	43049.38	2855.44	3.345801
2	TGY	209517	5.14	40731.47	537.53	0.629839
3	pTGY	216655	5.14	42119.15	1925.21	2.255823
4	TGpY	238116	5.14	46291.31	6097.37	7.144462
5	pSGpY	237887	4.98	47744.66	7550.72	8.847394
7 (ERK5)	pTEpY	211142	5.14	41047.38	853.44	1
	BckGrn	206752	5.14	40193.94	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
1	pTGpY	385598	16.14	23894.38	23345.97	14.88474
2	TGY	16397	16.14	1016.07	467.67	0.298173
3	pTGY	124646	16.14	7723.95	7175.54	4.574924
4	TGpY	41822	16.14	2591.59	2043.18	1.302675
5	pSGpY	429842	16.14	26636.05	26087.65	16.63276
7 (ERK5)	pTEpY	34161	16.14	2116.86	1568.45	1
	BckGrn	8850	16.14	548.41	-----	

Sum of experiments in table 2A

Peptide No	Sample	Average	SD	SEM
1	pTGpY	5.300216	4.959853	2.218082
2	TGY	0.588258	0.344928	0.154254
3	pTGY	2.506996	1.547744	0.692162
4	TGpY	3.989569	3.531349	1.579245
5	pSGpY	8.27802	5.987428	2.677621
7 (ERK5)	Seq. 7	1	0	0

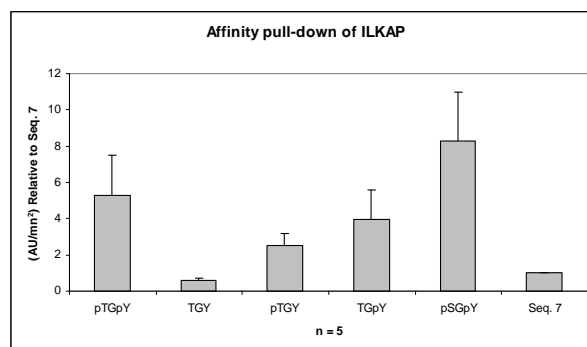


Table S3B.

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
pTGpY	828093	13.56	61088.7	59155.62	69.221928
TGY	144359	13.56	10649.41	8716.33	10.199558
pTGY	559123	13.56	41246.69	39313.61	46.003472
TGpY	637554	13.56	47032.57	45099.5	52.773927
PSGpY	1184636	13.56	87391	85457.92	100
BckGrn	26204	13.56	1933.08	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
pTGpY	214277	5.14	41656.85	1499.46	39.778328
TGY	208467	5.14	40527.35	369.96	9.8144601
pTGY	212604	5.14	41331.61	1174.22	31.15022
TGpY	220900	5.14	42944.4	2787.01	73.935016
PSGpY	225954	5.14	43926.93	3769.54	100
BckGrn	206564	5.14	40157.39	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
pTGpY	221440	5.14	43049.38	2855.44	37.816791
TGY	209517	5.14	40731.47	537.53	7.1189238
pTGY	216655	5.14	42119.15	1925.21	25.497039
TGpY	238116	5.14	46291.31	6097.37	80.752167
PSGpY	237887	4.98	47744.66	7550.72	100
BckGrn	206752	5.14	40193.94	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
pTGpY	385598	16.14	23894.38	23345.97	89.490506
TGY	16397	16.14	1016.07	467.67	1.7926873
pTGY	124646	16.14	7723.95	7175.54	27.505505
TGpY	41822	16.14	2591.59	2043.18	7.8319818
PSGpY	429842	16.14	26636.05	26087.65	100
BckGrn	8850	16.14	548.41	-----	

Sum of experiments in table 2B

Sample	Average	SD	SEM
pTGpY	59.07689	21.5193874	10.75969368
TGY	7.231407	3.3568738	1.678436902
pTGY	32.53906	8.03346116	4.016730582
TGpY	53.82327	28.4866088	14.24330438
PSGpY	100	7.1054E-15	3.55271E-15

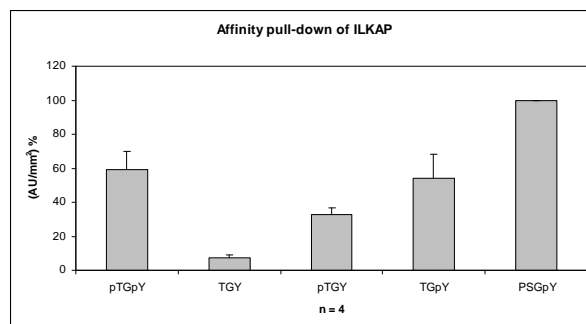


Table S4A.

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
6	UNG2 (31pT)	161188	13.56	11890.89	9957.82	0.348425148
7	ERK5 (218pT, 220pY)	413616	13.56	30512.59	28579.51	1
8	JNK1 (183pT, 185pY)	869982	13.56	64178.87	62245.79	2.177986606
	BckGrn	26204	13.56	1933.08	-----	

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
7	ERK5 (218pT, 220pY)	211142	5.14	41047.38	853.44	1
8	JNK1 (183pT, 185pY)	812878	10.33	78654.54	38460.6	45.06538245
	BckGrn	206752	5.14	40193.94	-----	

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
6	UNG2 (31pT)	33957	16.14	2104.22	1555.81	0.991941088
7	ERK5 (218pT, 220pY)	34161	16.14	2116.86	1568.45	1
8	JNK1 (183pT, 185pY)	574265	16.14	35585.53	35037.12	22.33869106
	BckGrn	8850	16.14	548.41	-----	

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
6	UNG2 (31pT)	459340	9.09	50527.74	7660.34	2.589772543
7	ERK5 (218pT, 220pY)	416591	9.09	45825.32	2957.92	1
8	JNK1 (183pT, 185pY)	693426	9.09	76277.37	33409.97	11.29508912
	BckGrn	389701	9.09	42867.4	-----	

Sum of experiments

Peptide No	Sample	Average	SD	SEM
6	UNG2 (31pT)	1.31004626	0.942268	0.544003
7	Seq. 7	1	0	0
8	JNK1 (183pT, 185pY)	20.21928731	16.02303	8.011516

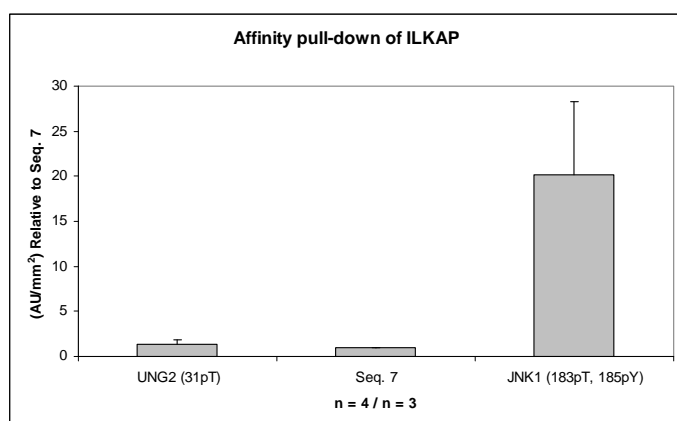


Table S4B.

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
UNG2 (31pT)	161188	13.56	11890.89	9957.82	15.99758
ERK5 (218pT, 220pY)	413616	13.56	30512.59	28579.51	45.91396
JNK1 (183pT, 185pY)	869982	13.56	64178.87	62245.79	100
BckGrn	26204	13.56	1933.08	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
ERK5 (218pT, 220pY)	211142	5.14	41047.38	853.44	2.218998
JNK1 (183pT, 185pY)	812878	10.33	78654.54	38460.6	100
BckGrn	206752	5.14	40193.94	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
UNG2 (31pT)	33957	16.14	2104.22	1555.81	4.440462
ERK5 (218pT, 220pY)	34161	16.14	2116.86	1568.45	4.476538
JNK1 (183pT, 185pY)	574265	16.14	35585.53	35037.12	100
BckGrn	8850	16.14	548.41	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
UNG2 (31pT)	459340	9.09	50527.74	7660.34	22.92831
ERK5 (218pT, 220pY)	416591	9.09	45825.32	2957.92	8.853405
JNK1 (183pT, 185pY)	693426	9.09	76277.37	33409.97	100
BckGrn	389701	9.09	42867.4	-----	

Sum of experiments

Sample	Average	SD	SEM
UNG2 (31pT)	14.45545	7.625995676	3.812998
ERK5 (218pT, 220pY)	15.36573	17.79758463	8.898792
JNK1 (183pT, 185pY)	100	7.10543E-15	3.55E-15

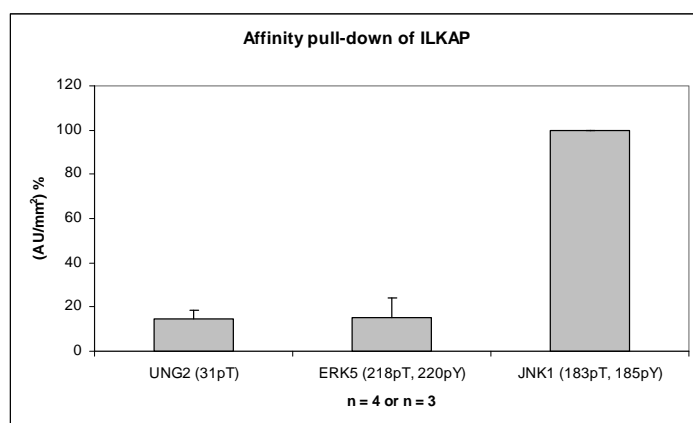


Table S5A.

Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
9	ATM, (1981pS)	661685	9.09	72785.84	29918.44	10.1146887
10	ATM, (1981S)	456180	9.09	50180.14	7312.74	2.472257532
11	p53 (15pS)	485315	9.09	53385.01	10517.61	3.555745253
12	p53 (15S)	551783	9.09	60696.54	17829.14	6.027593715
13	Chk1 (345pS)	550700	9.09	60577.41	17710.01	5.987318792
14	Chk1 (345S)	429887	9.09	47287.89	4420.49	1.494458944
7	ERK5 (pTEpY)	416591	9.09	45825.32	2957.92	1
	BckGrn	389701	9.09	42867.4	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
9	ATM, (1981pS)	1146193	13.56	84555.04	82621.97	2.890951244
10	ATM, (1981S)	901198	13.56	66481.68	64548.6	2.258562166
11	p53 (15pS)	897951	13.56	66242.15	64309.07	2.250180986
12	p53 (15S)	57914	13.56	4272.34	2339.26	0.081850948
7	ERK5 (pTEpY)	413616	13.56	30512.59	28579.51	1
	BckGrn	26204	13.56	1933.08	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
9	ATM, (1981pS)	259986	16.14	16110.57	15562.17	9.922005802
10	ATM, (1981S)	189203	16.14	11724.36	11175.95	7.125474194
11	p53 (15pS)	301914	16.14	18708.73	18160.32	11.57851382
12	p53 (15S)	11039	16.14	684.05	135.65	0.086486659
7	ERK5 (pTEpY)	34161	16.14	2116.86	1568.45	1
	BckGrn	8850	16.14	548.41	-----	
Peptide No	Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
9	ATM, (1981pS)	506123	10.76	47044.45	8153.35	4.977138985
10	ATM, (1981S)	474464	10.76	44101.73	5210.63	3.180782097
11	p53 (15pS)	477651	10.76	44397.96	5506.86	3.361613029
12	p53 (15S)	463371	10.76	43070.62	4179.52	2.551350295
13	Chk1 (345pS)	534004	10.76	49636.01	10744.91	6.559133418
14	Chk1 (345S)	451352	10.76	41953.45	3062.35	1.869383943
7	ERK5 (pTEpY)	436030	10.76	40529.26	1638.16	1
	BckGrn	418406	10.76	38891.1	-----	

Sum of experiments

Peptide No	Sample	Average	SD	SEM
9	ATM, (1981pS)	6.976196	3.13103	1.565515
10	ATM, (1981S)	3.759269	1.973227	0.986614
11	p53 (15pS)	5.186513	3.723889	1.861944
12	p53 (15S)	2.18682	2.435504	1.217752
13	Chk1 (345pS)	6.273226	0.285907	0.202169
14	Chk1 (345S)	1.681921	0.187462	0.132557
7	Seq. 7	1	0	0

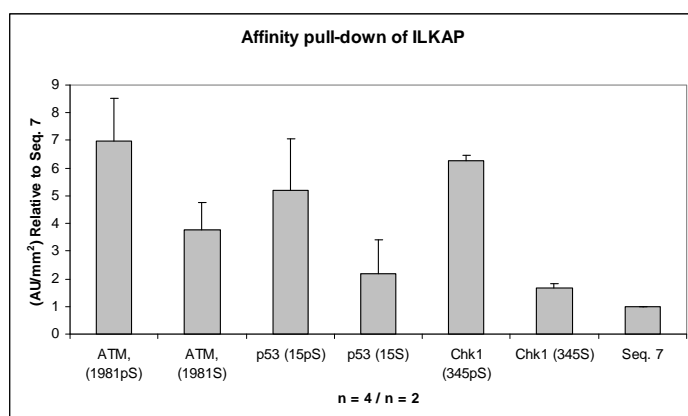


Table S5B.

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
ATM, (1981pS)	661685	9.09	72785.84	29918.44	100
ATM, (1981S)	456180	9.09	50180.14	7312.74	24.44225
p53 (15pS)	485315	9.09	53385.01	10517.61	35.15427
p53 (15S)	551783	9.09	60696.54	17829.14	59.59248
Chk1 (345pS)	550700	9.09	60577.41	17710.01	59.1943
Chk1 (345S)	429887	9.09	47287.89	4420.49	14.77514
BckGrn	389701	9.09	42867.4	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
ATM, (1981pS)	1146193	13.56	84555.04	82621.97	100
ATM, (1981S)	901198	13.56	66481.68	64548.6	78.12523
p53 (15pS)	897951	13.56	66242.15	64309.07	77.83531
p53 (15S)	57914	13.56	4272.34	2339.26	2.831281
BckGrn	26204	13.56	1933.08	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
ATM, (1981pS)	259986	16.14	16110.57	15562.17	85.69326
ATM, (1981S)	189203	16.14	11724.36	11175.95	61.54049
p53 (15pS)	301914	16.14	18708.73	18160.32	100
p53 (15S)	11039	16.14	684.05	135.65	0.746958
BckGrn	8850	16.14	548.41	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
ATM, (1981pS)	506123	10.76	47044.45	8153.35	75.88105
ATM, (1981S)	474464	10.76	44101.73	5210.63	48.49394
p53 (15pS)	477651	10.76	44397.96	5506.86	51.25087
p53 (15S)	463371	10.76	43070.62	4179.52	38.89767
Chk1 (345pS)	534004	10.76	49636.01	10744.91	100
Chk1 (345S)	451352	10.76	41953.45	3062.35	28.50047
BckGrn	418406	10.76	38891.1	-----	

Sum of experiments

Sample	Average	SD	SEM
ATM, (1981pS)	90.39358	10.21363428	5.106817
ATM, (1981S)	53.15048	19.62126397	9.810632
p53 (15pS)	66.06011	24.82466462	12.41233
p53 (15S)	25.5171	24.84138431	12.42069
Chk1 (345pS)	79.59715	20.40285189	10.20143
Chk1 (345S)	21.6378	6.862668026	3.431334

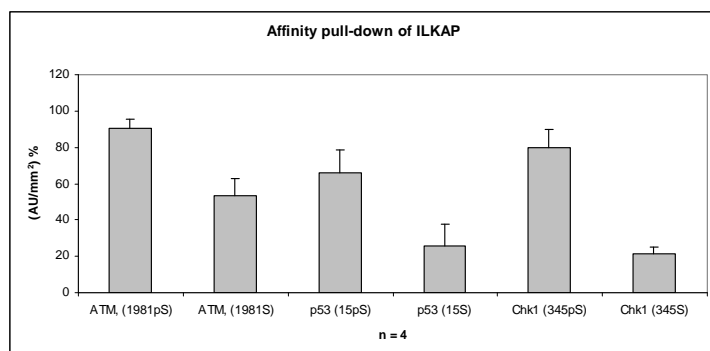


Table S6A.

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
Chk2 (68pT)	459136	9.09	50505.3	7637.9	2.582186131
Chk2 (68T)	416604	9.09	45826.75	2959.35	1.000483448
Chk2 (387pT)	419705	9.09	46167.86	3300.46	1.115804349
Chk2 (387T)	408614	9.09	44947.84	2080.44	0.703345594
Chk2 (516S)	462715	8	57828.1	14960.7	5.057844702
ERK5 (218pT, 220pY)	416591	9.09	45825.32	2957.92	1
BckGrn	389701	9.09	42867.4	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	Relative to 7
Chk2 (68pT)	454071	10.76	42206.18	3315.08	2.023660692
Chk2 (68T)	443335	10.76	41208.27	2317.17	1.414495532
Chk2 (387pT)	420823	10.76	39115.76	224.66	0.137141671
Chk2 (387T)	477208	10.76	44356.78	5465.68	3.33647507
Chk2 (516pS)	457609	10.76	42535.04	3643.94	2.224410314
Chk2 (516S)	566259	11.38	49772.22	10881.13	6.642287689
ERK5 (218pT, 220pY)	436030	10.76	40529.26	1638.16	1
BckGrn	418406	10.76	38891.1	-----	

Sum of experiments

Peptide No	Sample	Average	SD	SEM
15	Chk2 (68pT)	2.302923	0.279263	0.161228
16	Chk2 (68T)	1.207489	0.207006	0.119512
17	Chk2 (387pT)	0.626473	0.489331	0.282508
18	Chk2 (387T)	2.01991	1.316565	0.760097
19	Chk2 (516pS)	2.22441	0	0
20	Chk2 (516S)	5.850066	3.054673	1.763566
7	Seq. 7	1	0	0

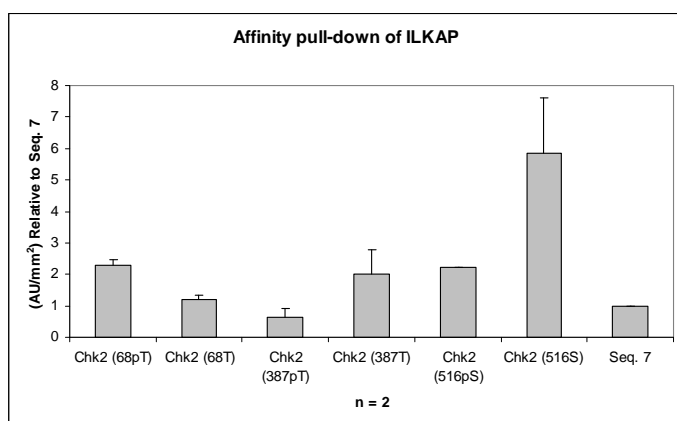


Table S6B.

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
Chk2 (68pT)	459136	9.09	50505.3	7637.9	51.05309
Chk2 (68T)	416604	9.09	45826.75	2959.35	19.78083
Chk2 (387pT)	419705	9.09	46167.86	3300.46	22.06087
Chk2 (516pS)	408614	9.09	44947.84	2080.44	13.90603
Chk2 (516S)	462715	8	57828.1	14960.7	100
BckGrn	389701	9.09	42867.4	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
Chk2 (68pT)	454071	10.76	42206.18	3315.08	30.46632
Chk2 (68T)	443335	10.76	41208.27	2317.17	21.29531
Chk2 (387pT)	420823	10.76	39115.76	224.66	2.064675
Chk2 (387T)	477208	10.76	44356.78	5465.68	50.23081
Chk2 (516pS)	457609	10.76	42535.04	3643.94	33.48862
Chk2 (516S)	566259	11.38	49772.22	10881.13	100
BckGrn	418406	10.76	38891.1	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
Chk2 (68pT)	92776	16.95	5472.85	5078.62	100
Chk2 (68T)	16902	16.95	997.05	602.82	11.86976
Chk2 (387pT)	9305	16.95	548.9	154.67	3.045512
Chk2 (387T)	16480	16.95	972.15	577.92	11.37947
Chk2 (516pS)	34206	16.95	2017.81	1623.58	31.96892
Chk2 (516S)	42611	16.95	2513.62	2119.39	41.73161
BckGrn	6683	16.95	394.23	-----	

Sum of experiments

Sample	Average	SD	SEM
Chk2 (68pT)	60.50647	29.16342348	16.83703
Chk2 (68T)	17.64863	4.132789575	2.385999
Chk2 (387pT)	9.057018	9.203823902	5.313679
Chk2 (387T)	30.80514	19.4256716	11.2151
Chk2 (516pS)	26.45452	8.894785881	5.135261
Chk2 (516S)	80.5772	27.46798146	15.8582

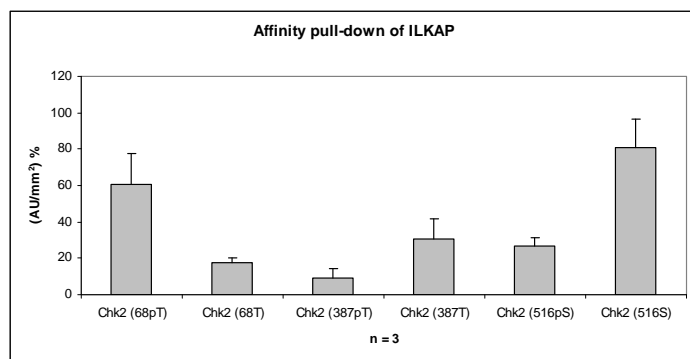


Table S7.

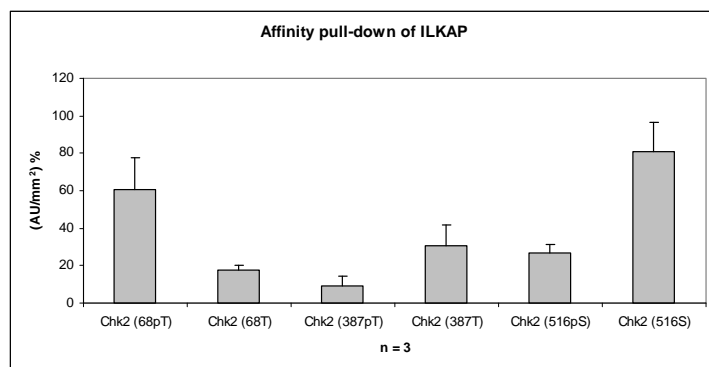
Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
RSK2 (227pS)	624400	14.17	44051.89	3394.69	12.68885
RSK2 (227S)	599622	14.17	42303.79	1646.58	6.154671
RSK2 (369pS)	594202	14.17	41921.4	1264.2	4.725391
RSK2 (369S)	594691	14.17	41955.9	1298.7	4.854347
RSK2 (386pS)	955490	14.17	67410.54	26753.34	100
RSK2 (386S)	615107	14.17	43396.27	2739.06	10.2382
RSK2 (577pT)	620405	14.17	43770.04	3112.84	11.63533
RSK2 (577T)	584772	14.17	41256.11	598.91	2.238636
BckGrn	576283	14.17	40657.2	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
RSK2 (227pS)	13038	16.95	769.11	374.88	15.4075
RSK2 (227S)	9294	16.95	548.25	154.02	6.330196
RSK2 (369pS)	12917	16.95	761.97	367.74	15.11405
RSK2 (369S)	9687	16.95	571.44	177.21	7.283301
RSK2 (386pS)	38501	16.95	2271.17	1876.94	77.14192
RSK2 (386S)	17950	16.95	1058.87	664.64	27.31659
RSK2 (577pT)	47929	16.95	2827.33	2433.1	100
RSK2 (577T)	22021	16.95	1299.02	904.79	37.18672
BckGrn	6683	16.95	394.23	-----	

Sample	AU	Area (mm ²)	AU/mm ²	(A-B)/mm ²	%
RSK2 (227pS)	374541	14.55	25734.18	1222.46	35.55265
RSK2 (227S)	364493	14.55	25043.79	532.08	15.47441
RSK2 (369pS)	365359	14.55	25103.29	591.58	17.20485
RSK2 (369S)	364634	14.55	25053.48	541.77	15.75623
RSK2 (386pS)	402873	14.55	27680.83	3169.11	92.16682
RSK2 (386S)	355982	14.55	24459.01	1	0.029083
RSK2 (577pT)	406793	14.55	27950.17	3438.45	100
RSK2 (577T)	378650	14.55	26016.5	1504.79	43.76361
BckGrn	356749	14.55	24511.71	-----	

Sum of experiments

Sample	Average	SD	SEM
Chk2 (68pT)	60.50647	29.16342348	16.83703
Chk2 (68T)	17.64863	4.132789575	2.385999
Chk2 (387pT)	9.057018	9.203823902	5.313679
Chk2 (387T)	30.80514	19.4256716	11.2151
Chk2 (516pS)	26.45452	8.894785881	5.135261
Chk2 (516S)	80.5772	27.46798146	15.8582



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2. H. Yamaguchi, G. Minopoli, O. N. Demidov, D. K. Chatterjee, C. W. Anderson, S. R. Durell and E. Appella, (2005), *Substrate specificity of the human protein phosphatase 2C delta Wip1*, *Biochemistry*, **44**, 5285-5294.