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online data

SUPPLEMENTARY ONLINE DATA

Discovery of cellular substrates for protein kinase A using a peptide array screening protocol

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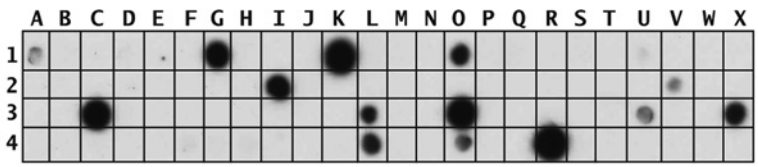

1A: RHRKRRRpSLSFDPSL	mouse MDM2	NP_034916	3C: YDPGRRPpSFRAILRD	JAK3	NP_034719
1B: RHRKRRRSLSFDPSL			3D: YDPGRRPSFRAILRD		
1C: RHRKRRRALSFDPSL			3E: YDPGRRPAFRAILRD		
1G: FPGRRRQpSVLDLMTF	mouse CFTR	NP_066388	3L: GAKLRKVpSKQEEASG	VASP	NP_033525
1H: FPGRRRQSVLDLMTF			3M: GAKLRKVSKQEEASG		
1I: FPGRRRQAVLDLMTF			3N: GAKLRKVAKQEEASG		
1J: NEARRRSpTLFLSTDA	SHANK1A	NP_066388	3O: PPEDRRQpSVSRQPSF	PTPN5	NP_008837
1K: NEARRRpSTLFLSTDA			3P: PPEDRRQSVSRQPSF		
1L: NEARRRSTLFLSTDA			3O: PPEDRRQAVSRQPSF		
1M: NEARRRATLFLSTDA					
1N: NEARRRSALFLSTDA			3U: VRETRRHpSAAGSPSS	TIAM2	NP_036008
			3V: VRETRRHSAAGSPSS		
1O: ARRKRRTpSIGSSSSE	Piccolo	NP_036125	3W: VRETRRHAAAGSPSS		
1P: ARRKRRTSIGSSSSE					
1Q: ARRKRRTAIGSSSSE			3X: KRISRRQpSLELQKCE	BAZ1B	NP_035844
			4A: KRISRRQSLELQKCE		
2I: EELHRRSpSVLENTLP	PIP5K	NP_035216	4B: KRISRRQALELQKCE		
2J: EELHRRSSVLENTLP					
2K: EELHRRSAVLENTLP			4L: PGRPRRGpSLPAGASW	RAP1GAP	EAW94984
2L: EELHRRASVLENTLP			4M: PGRPRRGSLPAGASW		
2M: EELHRRAAVLENTLP			4N: PGRPRRGALPAGASW		
			4O: KMPARRApSLSHPRDI	GRANULIN	NP_002078
2V: PATSRRSpSISGISLE	RIL/PDLIM4	NP_062290	4P: KMPARRASLSHPRDI		
2W: PATSRRSSISGISLE			4Q: KMPARRAALSHPRDI		
2X: PATSRRASISGISLE					
3A: PATSRRSAISGISLE			4R: LGDQRRPpSLPALHFI	ABL1	NP_001106174
3B: PATSRRAAISGISLE			4S: LGDQRRPSLPALHFI		
			4T: LGDQRRPALPALHFI		

Figure S1 Confirmation that phosphorylation is required for peptide recognition by anti-phospho-PKA substrate antibody

Peptide arrays of potential PKA phosphorylation sites were synthesized as described in the main text. Several peptides were arrayed for each potential site. First, phospho-serine or phospho-threonine were incorporated into the potential phospho-acceptor sites during peptide synthesis. In addition, WT and 'alanine mutant' peptides were arrayed. The membrane was not subject to *in vitro* phosphorylation with PKA. Instead, the membrane was incubated with the anti-phospho-PKA substrate antibody, followed by appropriate secondary antisera. Only peptides containing phosphorylated residues are detected by this antibody. Non-phosphorylated peptides were not detected. Furthermore, some peptides that did contain phospho-serine or phospho-threonine were not detected, presumably due to other sequence preferences of the antibody. Finally, some peptides were recognized by the antibody in this experiment, but were not reliably detected in other trials where membranes were phosphorylated with the PKA C-subunit and ATP. This is probably due to the fact that not all peptides containing a consensus motif will serve as good substrates for *in vitro* phosphorylation. ABL1, c-abl oncogene 1, non- receptor tyrosine kinase; BAZ1B, bromodomain adjacent to zinc finger domain, 1B; CFTR, cystic fibrosis transmembrane conductance regulator; IB, immunoblot; JAK3, Janus kinase 3; MDM2, murine double minute 2; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; PTPN5, protein tyrosine phosphatase, non-receptor type 5; RAP1GAP, RAP1 GTPase-activating protein; TIAM2, T-cell lymphoma invasion and metastasis 2; VASP, vasodilator-stimulated phosphoprotein.

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