

Supplementary Material

Identification of motility-associated progesterone-responsive differentially phosphorylated proteins

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Supplementary Method

Method for measuring total tyrosine kinase activity

Total tyrosine kinase activity was assayed using tyrosine kinase ELISA kit. Capacitated and progesterone treated spermatozoa (10×10^6 /ml) were solubilized in lysis buffer (50 mM HEPES buffer, pH 7.4, containing 0.1 % TRITON X-100, 10% glycerol, 1 mM dithiothreitol, protease inhibitor and cocktail phosphatase inhibitor cocktail) provided in the kit, sonicated and centrifuged and protein content was estimated. Kinase activity was determined in the supernatants using a synthetic peptide polyGlu-Tyr (PGT) as substrate provided in the kit. Standard curves were run along with test samples in each experiment. The assay was done for three different pools of samples per concentration per time point. PTK substrate was reconstituted with PBS and incubated in multiwell plate overnight. After washing the wells were dried at 37°C.

For the PTK assay standard and test samples were incubated with Tyrosine Kinase 0.3 mM ATP at room temperature for 30 minutes. After repeated washing with wash buffer the antibody conjugate (1:100 diluted) was added in each well and incubated for 30 minutes at room temperature. After extensive washing, freshly prepared peroxidase substrate solution was added

to each well and incubate for exactly 7 minutes, in the dark. The reaction was stopped by adding NH_2SO_4 and the plate was read in an ELISA reader set at 492 nm.

Table S1. Mascot score, Sequence coverage, expected and observed pI and molecular weight (mol wt) of the differentially phosphorylated proteins

Spot No	Protein Symbol	Mascot Score	Sequence coverage	Expected pI	Observed pI	Expected mol wt (kDa)	Observed mol wt (kDa)
1	AXDN1	49	11	5.4	5.5	117	116
2	GRP78	59	10	5	6	72	90
3	VINC	52	18	5.5	6	123	120
4	TRI56	55	9	8	5	81	82
5	HSP90A	21	88	4.9	5	84	80
7	KLC4	54	10	5.8	6.2	68.5	70
8	ACCN3	40	8	6.7	6.5	58	60
9	SYDC	58	26	6.1	6	57	55
10	ATPB	77	20	5.2	5	56	55
11	SCOT2	46	11	6.7	5	56	52
12	MFA3L	21	3	5.1	6	45	50
13	PDK3	54	14	8.4	7	46	45
14	No significant hit						
15	CLC4K	62	15	8.4	7	36	35
16	STYL1	31	24	5.7	6	35	30
17	IDLC1	31	19	8.7	5	29	27
18	JPH2	55	4	6.4	5	14	15
19	ALGIL	26	12	4.9	6	21	18
20	IGLL5	38	13	9	6	23	17
21	ARL6	43	15	8.7	7	21	15
22	MUTED	38	33	9.4	5	19	18
23	GPTC4	40	4	8.7	5	15	12
24	DYRL1	49	32	7.7	6.5	21	16

Table S2. Kinases associated with differentially phosphorylated proteins as predicted by scan site analysis

PROTEIN	Associated kinase	Protein ID	MOTIF_NAME	MOTIF_GROUP	SCORE	PERCENTILE
ACCN3	GS3K	GSK3A	GSK3 Kinase	Acidophilic serine/threonine kinase	0.3052	1.15E-04
ACCN3	GS3K	GSK3B	GSK3-b	Acidophilic serine/threonine kinase	0.2388	5.40E-05
ACCN3	GS3K	GSK3A	GSK3 Kinase	Acidophilic serine/threonine kinase	0.4302	0.001666
ACCN3	GS3K	GSK3B	GSK3-b	Acidophilic serine/threonine kinase	0.3561	0.001121
ACCN3	GS3K	GSK3A	GSK3 Kinase	Acidophilic serine/threonine kinase	0.4184	0.001285
ACCN3	GS3K	GSK3B	GSK3-b	Acidophilic serine/threonine kinase	0.3262	5.52E-04
ACCN3	MAPK	MAPK3	Erk1 Kinase	Proline-dependent serine/threonine kinase	0.4111	0.001136
ACCN3	MAPK	MAPK3	Erk1 Kinase	Proline-dependent serine/threonine kinase	0.3749	5.11E-04
ACCN3	MAPK	MAPK3	Erk1 Kinase	Proline-dependent serine/threonine kinase	0.4211	0.001391
ACCN3	PTK	SHC1	Shc PTB	Phosphotyrosine binding group	0.3621	1.33E-04
ARL6	PI3K	PDPK1	PDK1 Binding	Kinase binding site	0.5654	0.005243
ARL6	PTK	MAPK1	Erk D-domain	Kinase binding site	0.602	0.008878
AXDN1	CamK	CAMK2G	Calmodulin dependent Kinase 2	Basophilic serine/threonine kinase	0.422	0.001897
AXDN1	Casein	CSNK1G2	Casein Kinase 1	Acidophilic serine/threonine kinase	0.3431	0.001348
AXDN1	PKC	PRKCE	PKC epsilon	Basophilic serine/threonine kinase	0.3423	5.66E-04
AXDN1	PKC	PRKCZ	PKC zeta	Basophilic serine/threonine kinase	0.3829	9.47E-04
AXDN1	SRC PTK	FGR	Fgr SH2	Src homology 2	0.3688	0.001305
AXDN1	SRC PTK	LCK	Lck SH2	Src homology 2	0.3303	0.001878
AXDN1	SRC PTK	SRC	Src SH2	Src homology 2	0.3199	0.001723
DYRL1	PI3K	PDK1	PDK1 Binding	Kinase binding site	0.5774	0.006544
DYRL1	PTK	SORBS1	Cbl-Associated protein C-SH3	Src homology 3	0.684	0.00621
DYRL1	PTK	ITSN	Intersectin SH3A	Src homology 3	0.588	0.003177
DYRL1	PTK	PIK3R1	p85 SH3 mode2	Src homology 3	0.5572	0.004558
DYRL1	PTK	PIK3R1	p85 SH3 mode1	Src homology 3	0.5789	0.007529

GPTC4	PKC	PRKCD	PKC delta	Basophilic serine/threonine kinase	0.3469	0.001219
GPTC4	PKC	PRKCA	PKC alpha/beta/gamma	Basophilic serine/threonine kinase	0.3716	0.001604
GPTC4	PTK	CRK	Crk SH2	Src homology 2	0.3649	0.001689
GRP78	CamK	PRKDC	DNA PK	DNA damage kinase	0.5181	0.009719
GRP78	PI3K	PLK1	PLK1 Kinase	Acidophilic serine/threonine kinase	0.3027	0.004718
GRP78	PI3K	PRKCA	PKC alpha/beta/gamma	Basophilic serine/threonine kinase	0.4495	0.009036
GRP78	PIKK	PRKCA	PKC alpha/beta/gamma	Basophilic serine/threonine kinase	0.4529	0.009774
GRP78	PKC	PDPK1	PDK1 Binding	Kinase binding site	0.6037	0.009464
GRP78	PKC	CAMK2G	Calmodulin dependent Kinase 2	Basophilic serine/threonine kinase	0.4644	0.004771
GRP78	PTK	PDGFRB	PDGFR Kin	Tyrosine kinase	0.3829	0.006587
KLC4	AMPK	PRKAA1	AMP_Kinase	Basophilic serine/threonine kinase	0.4965	9.22E-04
KLC4	PIKK	ATM	ATM Kinase	DNA damage kinase	0.3664	4.62E-04
KLC4	PIKK	PRKDC	DNA PK	DNA damage kinase	0.3697	6.77E-04
KLC4	PIKK	PRKDC	DNA PK	DNA damage kinase	0.4209	0.001862
KLC4	PIKK	YWHAZ	14-3-3 Mode 1	Phosphoserine/threonine binding	0.3215	0.001851
MUTED	Aura	AURKA	Aurora A	Basophilic serine/threonine kinase	0.2875	1.71E-04
MUTED	Aura	AURKB	Aurora B	Basophilic serine/threonine kinase	0.3998	9.47E-04
SCOT2	Casine K	CSNK1G2	Casein Kinase 1	Acidophilic serine/threonine kinase	0.2958	2.95E-04
SCOT2	PI3K	PLK1	PLK1 Kinase	Acidophilic serine/threonine kinase	0.2415	0.001032
TRI56	PKA	PKA	Protein Kinase A	Basophilic serine/threonine kinase	0.316	0.001563
VINC	PI3KK	CDK1	CDK1 motif 1 - [ST]Px[KR]x	Proline-dependent serine/threonine kinase	0.2724	0.001752