

Table S2. Summary of *in vitro* auto- and trans-phosphorylation sites in the EMS1 kinase domain identified by mass spectrometry.

phospho site	phosphopeptide identified	ΔM [ppm]	pRS site probability ^{a)}	pRS Score ^{b)}	ΔCn	Xcorr	Charge	m/z [Da]	RT [min]	Ion matched	TP/PhosF	Activation ^{c)}
Thr 854	WAMtKR	0.06	T(4): 100.0	67	0.00	2.37	2	436.6961	16.16	14/14	6/6	CID
Ser 883	GFVDQNLyFLsGSR	0.52	S(11): 100.0; S(13): 0.0	153	0.00	3.19	2	841.8828	36.92	24/38	33/3	CID
Ser 892	SREPLsINIAMFEQPLLK	1.74	S(1): 0.0; S(6): 100.0	135	0.00	3.39	2	1083.557	46.9	36/50	47/9	CID
Ser 1069	LIsACESHVSTVIAGTFGYI PPEYGQSAR	0.33	S(3): 81.7; S(7): 7.7	36	0.00	3.54	3	1064.169	41	38/166	18/2	CID

Identified transphosphorylated sites

phospho site	phosphopeptide identified	ΔM [ppm]	pRS site probability ^{a)}	pRS Score ^{b)}	ΔCn	Xcorr	Charge	m/z [Da]	RT [min]	Ion matched	TP/PhosF	Activation ^{c)}
Thr 854	WAMtKR	0.83	T(4): 100.0	72	0.00	2.17	2	436.6961	13.13	12/14	4/4	CID
Ser 869	RMEEsRLKGF	0.54	S(5): 92.0	86	0.00	3.12	2	682.4216	31.53	32/46	10/6	CID
Ser 883	GFVDQNLyFLsGSR	0.96	S(11): 99.9; S(13): 0.1	105	0.00	4.41	2	841.8828	36.59	24/38	74/6	CID
Ser 892	REPLsINIAMFEQPLLK	1.88	S(4): 100.0	65	0.00	2.94	2	961.9903	50.53	17/44	15/3	CID
Thr 914, Ser 918	LGDIvEAtDHF ^s KK	0.17	T(8): 100.0; S(12): 100.0	99	0.00	2.68	2	860.3733	21.57	28/50	82/5	CID
Thr 930	KNiIGDGGFGtVYK	0.97	T(11): 98.7; Y(13): 1.3	66	0.00	3.58	2	774.8773	27.23	24/38	22/3	CID
Thr 941	ACLPGEKtVAVK	0.54	T(8): 100.0	208	0.00	2.91	3	451.5599	18.55	18/44	15/15	CID
Ser 1069	LIsACESHVSTVIAGTFGYI PPEYGQSAR	0.33	S(3): 81.7; S(7): 7.7	36	0.00	3.54	3	1064.169	41	38/166	18/2	CID
Ser 1073	LISACE ^s HVSTVIAGTFGYI PPEYGQSAR	0.81	S(10): 81.6; T(11): 13.5	77	0.00	2.98	2	1595.751	41.04	27/83	23/8	CID
Ser 1076, Thr 1077	LISACESHV ^s tVIAGTFGYIP PEYGQSAR	3.1	S(10): 87.4; T(11): 87.4	53	0.00	3.98	2	1635.738	45.76	34/110	72/6	CID

^{a)} pRS site Probabilities: For each phosphorylation site this is an estimation of the probability (0-100%) for the respective site being truly phosphorylated.

pRS Site Probabilities above 75% are good evidence that the respective site is truly phosphorylated.

^{b)} pRS score: This peptide score is based on the cumulative binomial probability that the observed match is a random event.

The value of the pRS score strongly depends on the data scored, but usually scores above 50 give good evidence for a good PSM.

^{c)} CID: collision-induced dissociation