

Supplementary Table 1. Methylated lysines identified in *S. solfataricus* RNA polymerase

Protein	Enzyme	Method ^a	Peptide sequence	Ion Score	E value	Quality of evidence
RpoA' Rpo1N Sso0225	trypsin	Sol MALDI	KEIYNEIDR	63	2.4e-005	Good MSMS - b ion series covering site of modification and many y ions, specific immonium-17 ion
	ArgC	Sol MALDI	KELASTLAPGYIIER	77	2.1e-007	Good MSMS - b ion series covering site of modification, specific immonium-17 ion
RpoB Rpo2 Sso0227	trypsin	Sol ESI	IVEKTLYEMGVVPVEEVIR	72	9.2e-006	Good MSMS with b ions either side of modification
	ArgC	Sol MALDI	GYKGKEYYR	42	0.0007	Reasonable MSMS spectrum - covers site of modification, specific immonium-17 ion
	ArgC	Sol MALDI	FLQEFKELSPEQAKR	45	0.0005	Reasonable MSMS spectrum - no ions covering site of modification, specific immonium-17 ion
RpoD Rpo3 Sso0071	trypsin	Gel ESI	DIKSEDPSVVPISGDIPVLLGTNQK	82	4.9e-08	No ions covering this part of peptide, so assuming increase in mass due to Me on K, but solid match to peptide sequence
RpoE' Rpo7 Sso0415	trypsin	Sol MALDI	LEWITQTKK	42	0.0028	Good MSMS covering site of modification, specific immonium-17 ion
	trypsin	Sol MALDI	IPPNEFGKPLNEIALNELR	124	1.6e-011	Good MSMS covering site of modification, specific immonium-17 ion
	ArgC	Sol MALDI	QPYLGKLEWITQTKK	100	1.2e-009	Good MSMS covering site of modification, specific immonium-17 ion
	ArgC	Sol MALDI	QPYLGKLEWITQTKK	40	0.0013	Reasonable MSMS, specific immonium-17 ion for 2 monomethylations
	ArgC	Sol MALDI	GIIFGEKSKKVIQKGDKVR	98	1.8e-009	Good MSMS covering sites of modification, specific immonium-17 ion
	ArgC	Sol MALDI	GIIFGEKSKKVIQKGDKVR	55	2.5e-005	can't distinguish which of adjacent K in 2 monomethylated peptide but specific immonium-17 ion for 2 monomethylated species

RpoF Rpo4 Sso0751	trypsin	Sol MALDI	TYTSEDIQKIIDIIR	63	1.7e-005	Good MSMS covering site of modification, specific immonium-17 ion
	trypsin	Sol MALDI	CDAESAQK V IEELSNIVSR	31	0.028	Poor MSMS – not many peaks, specific immonium-17 ion
RpoH Rpo5 Sso5468	trypsin	Sol ESI	HEVLNIDEAYKILK	77	3.9e-006	Good MSMS, site of modification not covered
	ArgC	Sol MALDI	K SQLYGEVVS Y R	101	8.5e-010	Good MSMS covering sites of modification, specific immonium-17 ion
RpoL Rpo11 Sso5577	trypsin	Gel ESI	DALLKAIENIR	58	5.8e-005	Good MSMS covering site of modification
	trypsin	Gel ESI	GMTSHYIDEI K GLTK	40	0.0043	Good MSMS covering site of modification
RpoP Rpo12 Sso5865	trypsin	Sol MALDI	TFTDEQLK V LPGVR	88	6.6e-008	Good MSMS covering site of modification, specific immonium-17 ion
Rpo13 Sso0396	ArgC	Sol MALDI	KAKKAVSKK V KKTKK K KSVEG	110	2.7e-011	Good MSMS covering site of modification, specific immonium-17 ions
	ArgC	Sol MALDI	KAKKAVSKK V KKTKK K KSVEG	63	1.4e-006	Good MSMS covering sites of modification, specific immonium-17 ions
	trypsin	Sol MALDI	K LFEDNY K	30	0.041	Reasonable MSMS - b1 ion covering site of modification plus specific immonium-17 ion

^aMethod abbreviations: Sol: solution digest; Gel: in-gel digest

Bold indicates site of monomethylation, Ion Score is the Mascot Ion Score, E value is the Mascot Expect value, which is a measure of the likelihood that a match is purely random.

Supplementary table 2. Methylated lysines detected in *T. tenax* proteins

	Protein	Accession no.	Peptide seq	Ion Score	E value	Quality of evidence
1	rpoA2	TTX1676	GISLKDIEK	19	0.069	Reasonable MSMS – y ion accounting for site of modification. Unmodified peptide also present
			TLVEAATKGDVEPFR	60	4.5 e-6	Good MSMS – y ion series covering site of modification
2	rpoD -	TTX1672	ASATAVEEGSKAENV	38	0.00064	Good MSMS – y ion series covering site of modification
			TTX0308	QAFETDKYETVEK	37	0.0016
		TTX0308	FYDIKAK	34	0.0042	Good MSMS – y ion series covering site of modification– unmodified peptide also present (Ion Score 19) spectra v similar
			FYDIKAK	38	0.001	Good MSMS – y ion series covering site of modification
3	Ribosomal protein L30	TTX1409	KYGWNSFEELALAFVSGEIER	53	1.3 e-5	Good MSMS – b1 ion covering modification
			IVGDKPLTLDHLKK	23	0.0097	Reasonable MSMS – 1 site of modification covered, coverage of alternative site (K5) refutes modification at K5
4	hypothetical	TTX1876	EYTP EEIAK	41	0.00064	Good MSMS – y ion series covering modification – unmodified peptide also present (Ion Score 43)
			VAIVKAQLTR	21	0.032	Reasonable MSMS - unmodified peptide also present (Ion Score 17 (19=Ident))
5	Conserved	TTX0588	INVEKIVK	32	0.0045	Reasonable MSMS – b ion series covering mod

	hypothetical					
6	nusA	TTX1674	VTKSPSGNLVAITTVAPSDK	66	3.1 e-5	Good MSMS – unmodified peptide also present (Ion Score 51) and dimethylated - see below
			VTKSPSGNLVAITTVAPSDK	49	0.00056	Good MSMS
			SQAALAVGKGGNNVR	33	0.0042	Reasonable MSMS – covering site of modification - unmodified peptide also present (Ion Score 60)
7	Malate dehydrogenase	TTX1427	FQQSVEAISK	52	6.7 e-7	Good MSMS
			IVLIDIIK	28	0.0068	Reasonable MSMS
8	sdhB/frdB	TTX0863	ISVLDVLLK	25	0.018	Reasonable MSMS
9	ferredoxin	TTX0985	AKYGIQVDPLPPS	24	0.019	Reasonable MSMS
10	Conserved hypothetical	TTX1408	VSTGIKELDEALEGGIPK	60	2.9 e-6	Good MSMS - unmodified peptide also present (Ion Score 72)
			KEPEAMPQIVVIDIFGLLK	53	7.7 e-7	Good MSMS - unmodified peptide also present (Ion Score 65)
			QLGMDFEK	28	0.008	So-so MSMS - unmodified peptide also present (Ion Score 43)
			KYSYQLK	28	0.017	So-so MSMS – immonium ion -17 present -unmodified peptide also present (Ion Score 28)
11	hypothetical	TTX1576	KLQQITSLLK	47	8.4 e-5	Good MSMS b ion series covering modification 2Me spectrum similar
			KLQQITSLLK	25	0.011	
			KWFFEEAVPR	44	0.00019	Good MSMS
			SELITKGEK	26	0.15	Reasonable MSMS

			LAKEEVELLK (mono & di)	27	0.015	Mono and diMe – similar reasonable spectra
12	Putative dehydrogenase	TTX0535	AAALINYIDSIFVK	62	3 e-1.1	Good MSMS
			KVVESITQYLEGR (mono & di)	92 (m) 35 (di)	4.9 e-9 0.0016	Good MSMS Reasonable MSMS
13	hypothetical	TTX1565	DVEEELSKLPVDVSR	34	0.0014	Good MSMS – y ion series covering modification
14	Conserved hypothetical	TTX2090	ALGAEVEQLK	34	0.0025	Reasonable MSMS - unmodified peptide also present (Ion Score 14)
			EVAGAVIKEGDLSEAPVAVQFK	27	0.0029	Reasonable MSMS
15	Conserved hypothetical	TTX1155	TGAPIYVADTLGSLTEDASQYIQ PPSGK	73	7.2 e-8	Good MSMS
16	porA	TTX1785	GIIGAIDAEYGK	45	0.00022	Good MSMS
			AIKGEYGDWIVLL	25	0.011	Reasonable MSMS
17	Gsp putative type II secretion system protein	TTX0896	AELLQALADKGVDAEAVAEAVR	59	1.7 e-6	Good MSMS
18	Rfc small subunit	TTX1851	IKLTEDGIDAIYEISQGD MR	30	0.002	Reasonable MSMS
19	Eif-1A	TTX0636	GYGEVIDNLDKLA	46	0.00011	Good MSMS – y ion series covering modification
20	Conserved hypothetical	TTX2059	LSAILVK	24	0.033	So-so MSMS
21	aEF-1 alpha subunit	TTX0013	TGQTIEQKPQFIK	42	0.0005	Good MSMS – covering site of modification
			TGQTIEQKPQFIK	19	0.068	Reasonable MSMS

			TIAAGQIVEIK PAK VEIK	35	0.00093	reasonable MSMS – covering site of modification
			VETGVIKPGDK VIV MPPAK	32	0.0026	reasonable MSMS – covering site of modification
22	Conserved hypothetical	TTX0298	K AVEYGLSLGAK	23	0.038	Reasonable MSMS
23	lysS	TTX2056	L K ELQVPLK	35	0.0015	Reasonable MSMS
24	Conserved hypothetical	TTX0105	VLD M DELEKYR	33	0.0037	Good MSMS – covering site of modification
25	Protein-L-isoaspartate O-methyltransferase	TTX0958	E K LAYVSFVK	23	0.02	Reasonable MSMS- unmodified peptide also present (Ion Score 27)
26	NAD dependent dehydrogenase	TTX1281	SLG K NELETATGER	38	0.00061	Reasonable MSMS- unmodified peptide also present (Ion Score 42)
27	Hypothetical protein	TTX1885	QDAADQ V VKMLDQPVER	59	3.6 e-6	Good MSMS – covering site of modification
28	Putative DNA helicase	TTX0530	EIA K LPGFGEK	31	0.0073	Good MSMS – covering site of modification - unmodified peptide also present (Ion Score 36)
			LIDASSAA K LAAEIESTSASR	50	3.3 e-5	Good y ion series up to site of modification, doubly charged y ions N-term to modification
29	Conserved hypothetical	TTX1840	LED V LKELA	30	0.007	Reasonable MSMS – covering site of modification - unmodified peptide also present (Ion Score 36)
			QEEIL K SVADLNR	30	0.0045	Reasonable MSMS
30	Conserved hypothetical	TTX1724	LVPSE K IGTPGVDGSIAIYETK	26	0.0046	Reasonable MSMS

Bold indicates monomethylation of lysine, also (m), Underlining indicates dimethylation of lysine, also (di), Ion Score is the Mascot Ion Score, E value is the Mascot Expect value, which is a measure of the likelihood that a match is purely random.