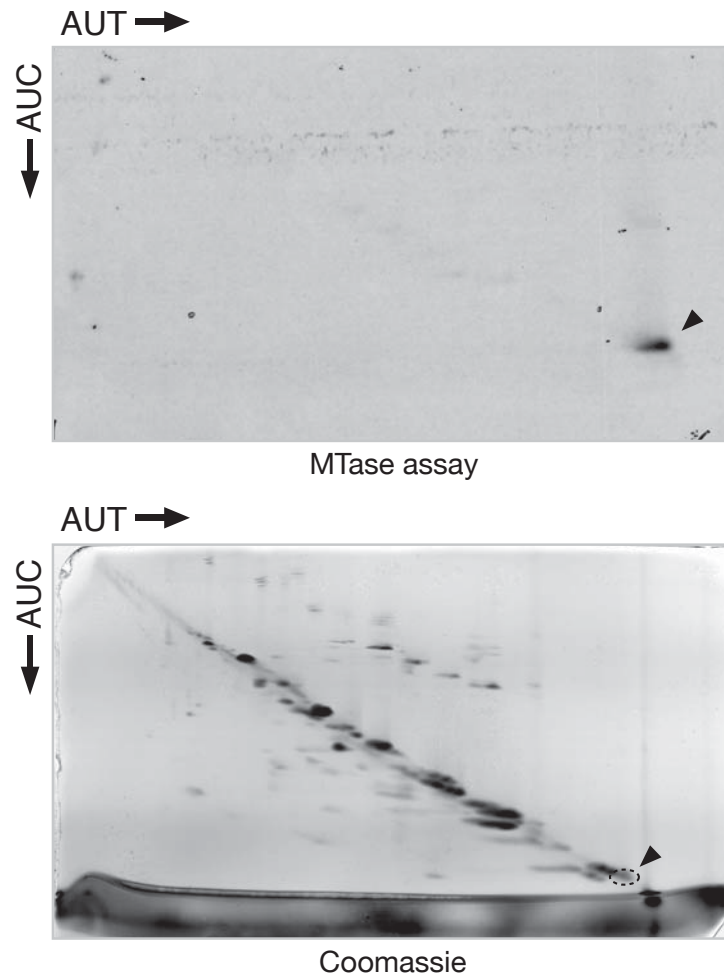
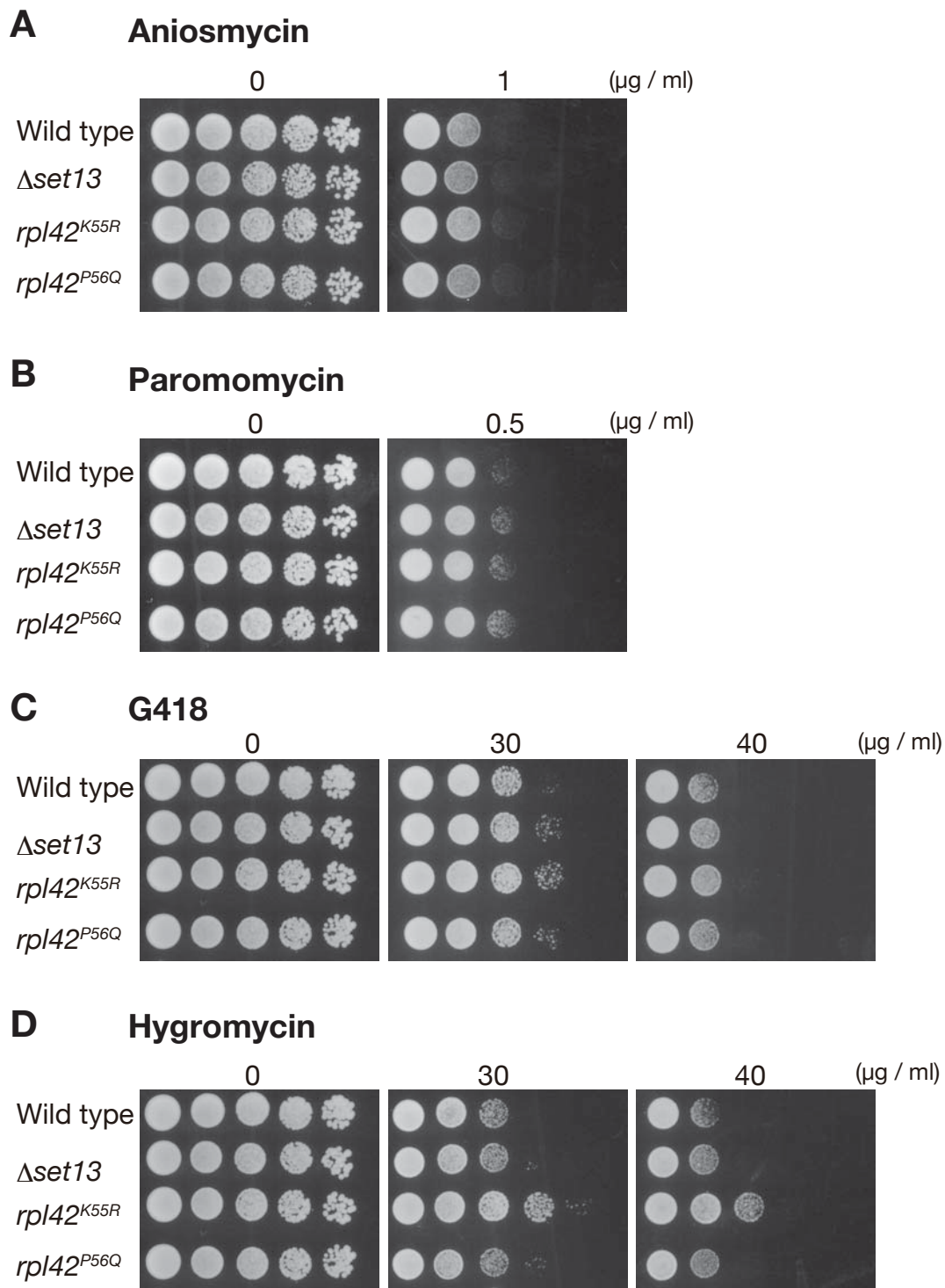


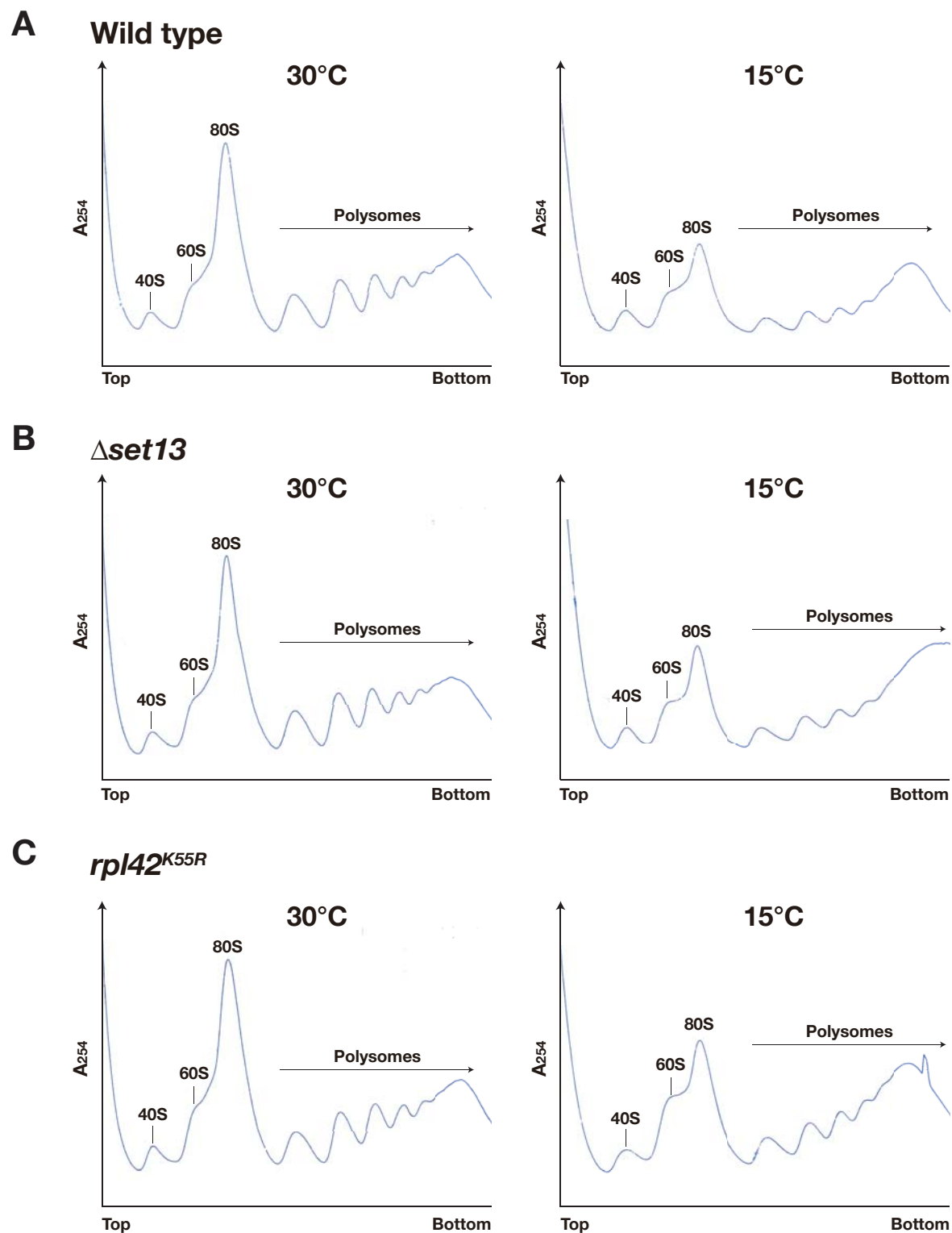
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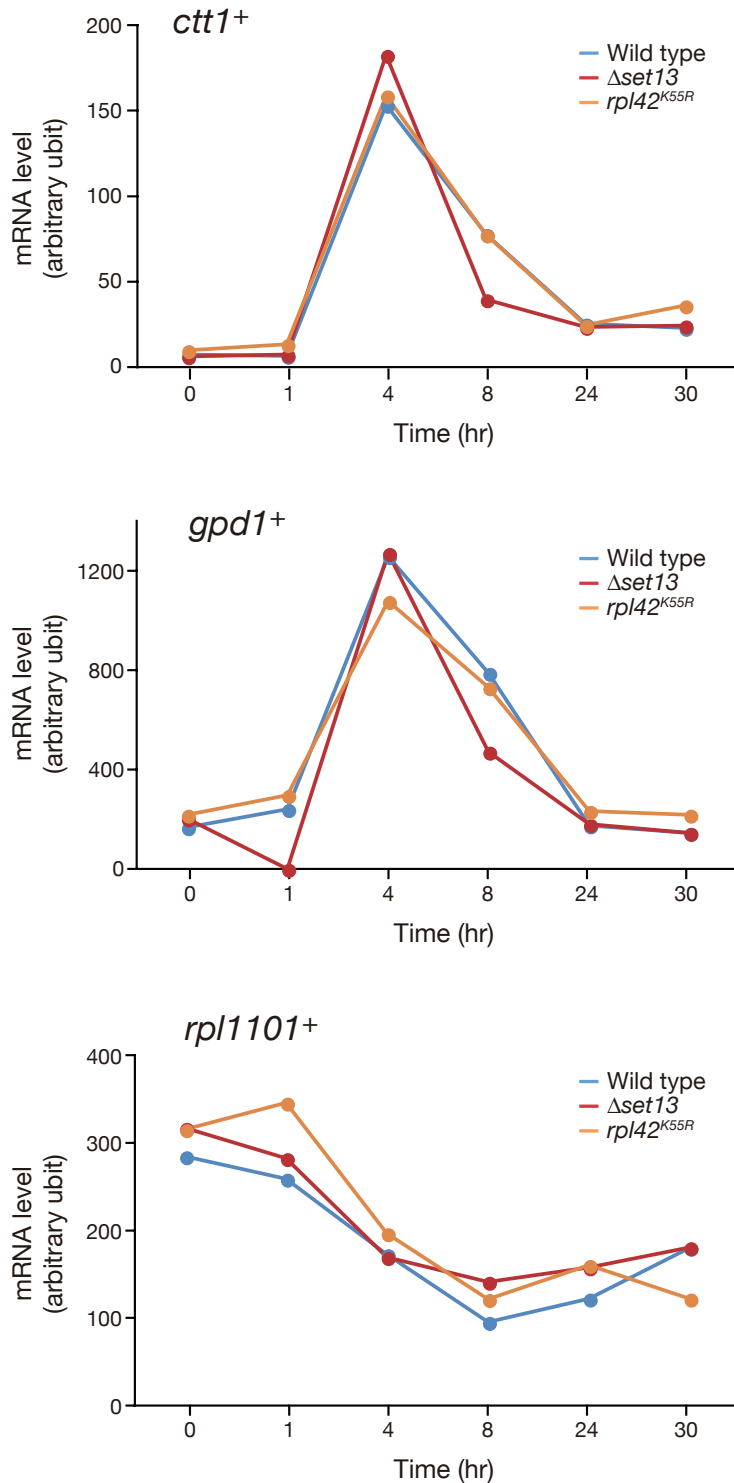
Supplemental Figure S1. Two-dimensional gel analysis of methylated proteins. After incubation with His-Set13 and [^3H] AdoMet, labeled proteins in the $\Delta set13$ nuclear extract were separated first in a gel containing 6% (v/v) acetic acid, 8 M urea, and 0.5% Triton X-100 (AUT) and then on a gel containing 6% (v/v) acetic acid and 1.5 M urea, with 0.15% cetyltrimethylammonium bromide in the upper reservoir buffer. ^3H -labeled proteins were detected by autoradiography (top), and the total proteins were detected by Coomassie staining (bottom). One spot of ^3H -labeled protein in the autoradiograph and the corresponding spot in the Coomassie-stained gel are indicated by arrowheads and a dotted circle.



Supplemental Figure S2. Rpl42 methylation-defective mutants show no sensitivity to other translational inhibitors. Five-fold dilutions of each strain were plated onto YEA alone or YEA containing anisomycin (A), paromomycin (B), G418 (C), or hygromycin (D) at the indicated concentrations, and further cultured for 3 days.



Supplemental Figure S3. Polysome analysis of wild-type and Rpl42 methylation-defective mutant cells. Wild-type (A), $\Delta set13$ (B), and $rpl42^{K55R}$ (C) cells were grown in YEA medium at 30°C or 15°C. Lysates prepared from these cells were loaded onto 5% to 45% sucrose gradients, and fractionated by centrifugation for 2 h at 36,000 rpm. The gradients were fractionated using a Piston Gradient Fractionator (Biacomp), and monitored by UV at 254 nm. The resultant absorbance profiles are shown, beginning with the top of the gradient on the left. The positions of the 40S, 60S, and 80S ribosomal species are indicated.



Supplemental Figure S5. Transcriptional levels of temperature-responsive genes during cold stress. Real-time RT-PCR was performed to measure the level of mRNA encoding *ctt1* (cytoplasmic catalase), *gpd1* (glycerol-3 phosphate dehydrogenase), or *rpl1101* (60S ribosomal protein L11), after shifting the temperature from 30°C to 15°C. The strain backgrounds were wild-type (blue), $\Delta set13$ (red), or $rpl42^{K55R}$ (orange). The stress-responsive expression of these genes in wild-type cells were previously described (Chen et al. *Mol Biol Cell* 14: 214-229, 2003). The relative amounts of these mRNAs were calculated and normalized to the level of *act1* mRNA.

Supplemental Table S1 SET domain proteins in *Schizosaccharomyces pombe*

Product ^a (ORF ^b)	Substrate	Localization ^c	<i>S. cerevisiae</i> (ORF) ^{d, e}	<i>M. musculus</i> ^f	Other domains	References
Set1 (SPCC306.04c)	Histone H3K4	Nuc	Set1 (YHR119w)	Mll2	RRM	S1
Set2 (SPAC29B12.02c)	Histone H3K36	Nuc	Set2 (YJL168c)	Whsc111	SRI	S2
Set3 (SPAC22E12.11c)	Unknown	Nuc	Set3 (YKR029c) Set4 (YJL105w)	Mll5	PHD	
Clr4 (SPBC428.08c)	Histone H3K9	Nuc	None	Suv39h1/2	chromo	S3
Set5 (SPCC1739.05)	Ef1 α	Nuc/Cyt	Set5 (YHR207c)	Hskm-B		S4
Set6 (SPBP8B7.07c)	Unknown	Nuc/Cyt	None	Zym1	zf-MYND	
Set7 (SPCC297.04c)	Unknown	Cyt	None	G9a, Glp1		
Set8 (SPAC3C7.09)	Unknown	Nuc/Cyt	YHL039w Rkm1 (YPL208w)	XP_134310	similar to Set10	
Set9 (SPCC4B3.12)	Histone H4K20	Nuc	None	Suv4-20h1/2		S5
Set10 (SPBC1709.13c)	Rpl23	Nuc	YHL039w Rkm1 (YPL208w)	NP_082538	similar to Set10	S4, S6
Set11 (SPCC1223.04c)	Rpl12 K3	Nuc	Rkm2 (YDR198c)	C21orf18		S7, S8
Set12 (SPBC16C6.01c)	Unknown	Nuc/Cyt	None	(XP_134310)		
Set13 (SPAC688.14)	Rpl42 K55	Nuc	Set7 (YDR257c)	SETD6		This study, S9

^a The *S. pombe* SET domain proteins are named as reported previously (S1, S10).

^b *S. pombe* geneDB (<http://www.genedb.org/genedb/pombe/>).

^c S11.

^d *Saccharomyces* Genome Database (<http://www.yeastgenome.org/>).

^{e, f} The most related proteins in *S. cerevisiae* and *M. musculus* are listed with reference to S5 and S10.

No apparent ortholog for *S. cerevisiae* Set6 (YPL165c) or YBR030w has been identified in the *S. pombe* genome.

Supplemental References

- S1 Noma, K and Grewal, SI. (2002) *Proc Natl Acad Sci* **99 Suppl 4**: 16438-16445.
- S2 Morris, SA., Shibata, Y, Noma, K, Tsukamoto, Y, Warren, E, Temple, B, Grewal, SI, and Strahl, BD. (2005) *Eukaryot. Cell* **4**: 1446-1454.
- S3 Nakayama, J, Rice, JC, Strahl, BD, Allis, CD, and Grewal, SI. (2001) *Science* **292**: 110-113.
- S4 Shirai, A, Matsuyama, A, Yashiroda, Y, Hashimoto, A, Kawamura, Y, Arai, R, Komatsu, Y, Horinouchi, S, and Yoshida, M. (2008) *J Biol Chem* **283**: 10745-10752.
- S5 Sanders, SL, Portoso, M, Mata, J, Bahler, J, Allshire, RC, and Kouzarides, T. (2004) *Cell* **119**: 603-614.
- S6 Porras-Yakushi, TR, Whitelegge, JP, Miranda, TB, and Clarke, S. (2005) *J Biol Chem* **280**: 34590-34598.
- S7 Sadaie, M, Shinmyozu, K, and Nakayama, J. (2008) *J Biol Chem* **283**: 7185-7195.
- S8 Porras-Yakushi, TR, Whitelegge, JP, and Clarke, S. (2006) *J Biol Chem* **281**: 35835-35845.
- S9 Webb, KJ, Laganowsky, A, Whitelegge, JP, and Clarke, SG. (2008) *J Biol Chem* **283**: 35561-35568.
- S10 Schotta, G, Lachner, M, Sarma, K, Ebert, A, Sengupta, R, Reuter, G, Reinberg, D, and Jenuwein, T. (2004) *Genes Dev* **18**: 1251-1262.
- S11 Matsuyama, A, Arai, R, Yashiroda, Y, Shirai, A, Kamata, A, Sekido, S, Kobayashi, Y, Hashimoto, A, Hamamoto, M, Hiraoka, Y. et al. (2006) *Nat Biotech* **24**: 841-847.

Supplemental Table S2. LC-MS analysis of Rpl42

Peptide	Strain	Time (min)	Observed mass	Experimental mass	Theoretical mass ^c	Difference	Peptide#	
Finnigan LTQ (Thermo Fisher Scientific)								
QSGFGGQTKPVFHK (47~60 aa)	Wild type	7.28	511.77 (MH ³⁺) ^a	1533.30 (MH ⁺)	1517.79 (MH ⁺)	+15.51	23	
	$\Delta set13$	7.23	507.19 (MH ³⁺) ^a	1519.56 (MH ⁺)		+1.77	34	
E*SGFGGQTKPVFHK (47~60 aa) E*: pyroE	Wild type	8.12	506.24 (MH ³⁺) ^a	1516.72 (MH ⁺)	1500.75 (MH ⁺)	+15.97	32	
	$\Delta set13$	8.12	501.45 (MH ³⁺) ^a	1502.35 (MH ⁺)		+1.60	45	
NanoFrontierLD (HITACHI)								
QSGFGGQTKPVFHK (47~60 aa)	Wild type	23.0	1532.04 (MH ⁺) ^b	1532.04 (MH ⁺)	1517.79 (MH ⁺)	+14.25	n.d.	
			766.53 (MH ²⁺) ^b	1532.06 (MH ⁺)		+14.27	n.d.	
			511.36 (MH ³⁺) ^b	1532.08 (MH ⁺)		+14.29	n.d.	
			383.78 (MH ⁴⁺) ^b	1532.10 (MH ⁺)		+14.31	n.d.	
	$\Delta set13$	23.2	1517.99 (MH ⁺) ^b	1517.99 (MH ⁺)		+0.20	n.d.	
			759.51 (MH ²⁺) ^b	1518.02 (MH ⁺)		+0.23	n.d.	
			506.68 (MH ³⁺) ^b	1518.04 (MH ⁺)		+0.25	n.d.	
			380.27 (MH ⁴⁺) ^b	1518.06 (MH ⁺)		+0.27	n.d.	
	E*SGFGGQTKPVFHK (47~60 aa) E*: pyroE	Wild type	24.9	1515.00 (MH ⁺) ^b	1515.00 (MH ⁺)	1500.75 (MH ⁺)	+14.25	n.d.
				758.01 (MH ²⁺) ^b	1515.03 (MH ⁺)		+14.28	n.d.
				505.68 (MH ³⁺) ^b	1515.05 (MH ⁺)		+14.30	n.d.
$\Delta set13$		25.2	1500.97 (MH ⁺) ^b	1500.97 (MH ⁺)		+0.22	n.d.	
			751.00 (MH ²⁺) ^b	1501.00 (MH ⁺)		+0.25	n.d.	
			501.01 (MH ³⁺) ^b	1501.02 (MH ⁺)		+0.27	n.d.	

^a Average of at least three independently observed masses.

^b Representative masses at the indicated elution time.

^c MH⁺¹(mono)

Supplemental Table S3. Additional methylated peptides identified in LC-MS/MS analysis of Rpl42

Site	Peptide sequence	Methyl#	Observed mass	Experimental mass	Theoretical mass ^b	Difference	wild-type# ^c	<i>Δset13</i> # ^c
K15	TYCPG <u>K</u> NCR	Tri	599.60 (MH ²⁺) ^a	1198.19 (MH ⁺)	1155.50 (MH ⁺)	+42.69	2 (1)	2 (2)
K78	LECVS <u>C</u> K	Mono	455.33 (MH ²⁺) ^a	909.65 (MH ⁺)	895.39 (MH ⁺)	+14.26	1 (2)	1 (1)
	LECVS <u>C</u> YK	Di	608.18 (MH ²⁺) ^a	1215.35 (MH ⁺)	1187.41 (MH ⁺)	+27.94	1 (0)	
		Tri	614.92 (MH ²⁺) ^a	1228.82 (MH ⁺)		+41.41	2	2 (0)
K80	Y <u>K</u> NQLVLK	Di	517.69 (MH ²⁺) ^a	1034.36 (MH ⁺)	1005.60 (MH ⁺)	+28.76	1 (3)	1 (7)
		Tri	524.03 (MH ²⁺) ^a	1047.04 (MH ⁺)		+41.44	1	4
K86	NQLVL <u>K</u> R	Di	450.38 (MH ²⁺) ^a	899.74 (MH ⁺)	871.06 (MH ⁺)	+28.68	1 (4)	2 (6)
		Tri	456.91 (MH ²⁺) ^a	912.81 (MH ⁺)		+41.75	2	3
K97	HFELGGE <u>K</u>	Mono	466.24 (MH ²⁺) ^a	931.46 (MH ⁺)	916.45 (MH ⁺)	+15.01	1 (91)	
	HFELGGE <u>K</u> K	Tri	544.11 (MH ²⁺) ^a	1087.21 (MH ⁺)	1044.54 (MH ⁺)	+42.67	1 (0)	1 (23)
K100	T <u>K</u> GAAIQF.-	Tri	877.44 (MH ⁺) ^a	877.44 (MH ⁺)	835.46 (MH ⁺)	+41.97	2 (61)	1 (81)

^a Representative masses.

^b MH⁺¹(mono).

^c Number of methylated (unmodified) peptide

Supplemental Table S4 *S. pombe* strains used in this study

Strain	Genotype	Source
SP976	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18</i>	Nakayama et al. 2002a
JY741	<i>hⁱ ade6-M216 leu1-32 ura4-D18</i>	From M. Yoshida
SPYB1259	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 set13::kan^r</i>	This study
SPYB1257	<i>h⁹⁰ ade6-M216 leu1-32 ura4-D18 pREP1-EGFP-set13</i>	This study
AS2142	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 Δset13::rep(T)</i>	This study
AS2122	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 rpl42^{K55R}</i>	This study
AS2120	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 rpl42^{P56Q}</i>	This study
AS2151	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 Δgcn5::rep(T)</i>	This study
AS2127	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 leu1⁺<<pDUAL-GFHI-rpl42</i>	This study
AS2130	<i>h⁹⁰ ade6-M210 leu1-32 ura4-D18 Δset13::kan^r leu1⁺<<pDUAL-GFHI-rpl42</i>	This study
AS2183	<i>hⁱ ade6-M210 leu1-32 ura4-D18 Δset13::rep(T)</i>	This study
AS2135	<i>hⁱ ade6-M210 leu1-32 ura4-D18 rpl42^{K55R}</i>	This study
AS2134	<i>hⁱ ade6-M210 leu1-32 ura4-D18 rpl42^{P56Q}</i>	This study
AS2155	<i>hⁱ ade6-M216 leu1-32 ura4-D18 Δgcn5::rep(T)</i>	This study
AS2200	<i>hⁱ ade6-M216 leu1-32 ura4-D18 Δgcn2::rep(T)</i>	This study
AS2212	<i>hⁱ ade6-M210 leu1-32 ura4-D18 Δgcn2::rep(T) Δset13::rep(T)</i>	This study
AS2202	<i>hⁱ ade6-M210 leu1-32 ura4-D18 Δgcn2::rep(T) rpl42^{K55R}</i>	This study
AS2208	<i>hⁱ ade6-M210 leu1-32 ura4-D18 Δgcn2::rep(T) rpl42^{P56Q}</i>	This study
AS2210	<i>hⁱ ade6-M216 leu1-32 ura4-D18 Δgcn2::rep(T) Δgcn5::rep(T)</i>	This study