Site-specific and kinetic characterization of enzymatic and nonenzymatic protein acetylation in bacteria

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Supporting Information

Table S1. The pKa and buried ratio of lysine residues in *Bs*AcsA

Residue	pKa	Buried	Residue	pKa	Buried	Residue	pKa	Buried
Lys32	10.30	23%	**Lys164**	10.85	31%	**Lys410**	11.04	0%
Lys41	10.45	0%	**Lys192**	10.57	0%	**Lys425**	10.26	0%
Lys58	10.46	0%	**Lys205**	9.40	38%	**Lys475**	10.50	50%
Lys60	11.14	13%	**Lys206**	10.59	17%	**Lys490**	10.23	4%
Lys66	8.45	60%	**Lys222**	9.19	24%	**Lys500**	11.74	37%
Lys69	10.47	0%	**Lys239**	11.19	1%	**Lys514**	10.58	0%
Lys73	10.73	3%	**Lys245**	10.53	0%	**Lys516**	10.40	0%
Lys77	10.59	0%	**Lys320**	10.59	0%	**Lys524**	9.86	5%
Lys80	10.98	35%	**Lys346**	10.35	0%	**Lys539**	9.08	26%
Lys98	11.12	18%	**Lys350**	10.31	0%	**Lys541**	10.46	0%
Lys121	7.53	88%	**Lys375**	11.56	0%	**Lys544**	11.29	0%
Lys139	10.49	0%	**Lys381**	10.62	0%	**Lys549**	10.33	0%
Lys148	10.67	0%	**Lys409**	11.45	0%	**Lys556**	6.86	76%

Table S2. Primers for overproduction of proteins and mutations

Primers	Sequence (5'-3')
BSU29680	ATGGGTCGCGGATCCGAATTCATGAACTTGAAAGCGTTACCAGC
	CTCGAGTGCGGCCGCAAGCTTTTAATCCTCCATTGTTGACAGATC
BSU29690 (GST)	CGCGTGGATCCCCAGGAATTCGTGGAACATCATAAAACATACC
	AGTCAGTCACGATGCGGCCGCTAATACATATAACGATGATAAAAA
pET-28-T	TAATACGACTCACTATAGGG
	TGCTAGTTATTGCTCAGCGG
pGEX-4T-T	GGCTGGCAAGCCACGTTTGGTG
	CCGGGAGCTGCATGTGTCAGAGG
K524Q	AAGAGATCCGCCTATTTGTACAGCAGGGTCTTGC
	GTACAAATAGGCGGATCTCTTCTTTCAGTTTATCAG
K524R	AAGAGATCCGCCTATTTGTAAGGCAGGGTCTTGC
	CTTACAAATAGGCGGATCTCTTCTTTCAGTTTATCAG
K524A	AAGAGATCCGCCTATTTGTAGCGCAGGGTCTTGC
	GCTACAAATAGGCGGATCTCTTCTTTCAGTTTATCAG
K549Q	CTTCCGAAAACCAGAAGCGGACAGATCATGAGGCGCGTG
	GTCCGCTTCTGGTTTTCGGAAGCTTATCTTTAAATTCGATCTCAC
K549R	CTTCCGAAAACCAGAAGCGGAAGGATCATGAGGCGCGTG
	CTTCCGCTTCTGGTTTTCGGAAGCTTATCTTTAAATTCGATCTCAC
K549A	CTTCCGAAAACCAGAAGCGGAGCGATCATGAGGCGCGTG
	GCTCCGCTTCTGGTTTTCGGAAGCTTATCTTTAAATTCGATCTCAC

Figure S1. No lysine residue in *Bs*AcsA (BSU29680) corresponding to K611 of *S. erythraea* AcsA2 (SACE_2375) was observed

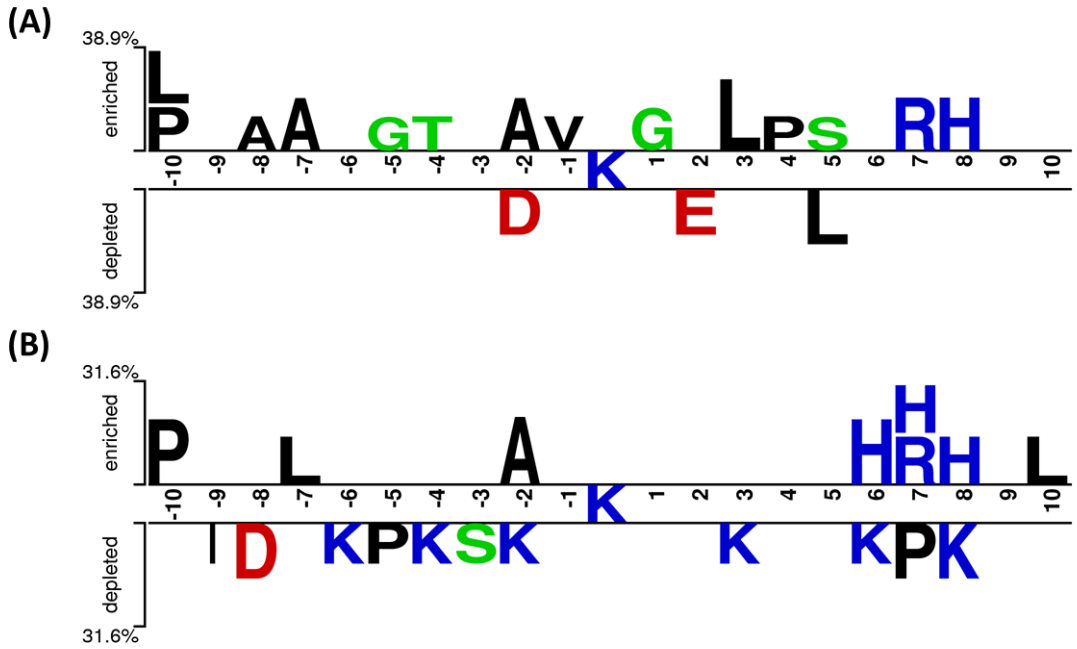

Figure S2. Analysis of the amino acid composition and position relative to acetyllysines. (A) A two sample logo was generated showing the amino acid composition in positions -10 to +10 relative to 14 lysines in Ac-CoA-dependent nonenzymatic acetylation. Only amino acid residues significantly enriched and depleted (0.05< P < 0.1; t-test) are shown. (B) A two sample logo was generated showing the amino acid composition in positions -10 to +10 relative to 19 lysines in AcP-dependent nonenzymatic acetylation. Only amino acid residues significantly enriched and depleted (0.05< P < 0.1; t-test) are shown.

Raw mass spectrometry data

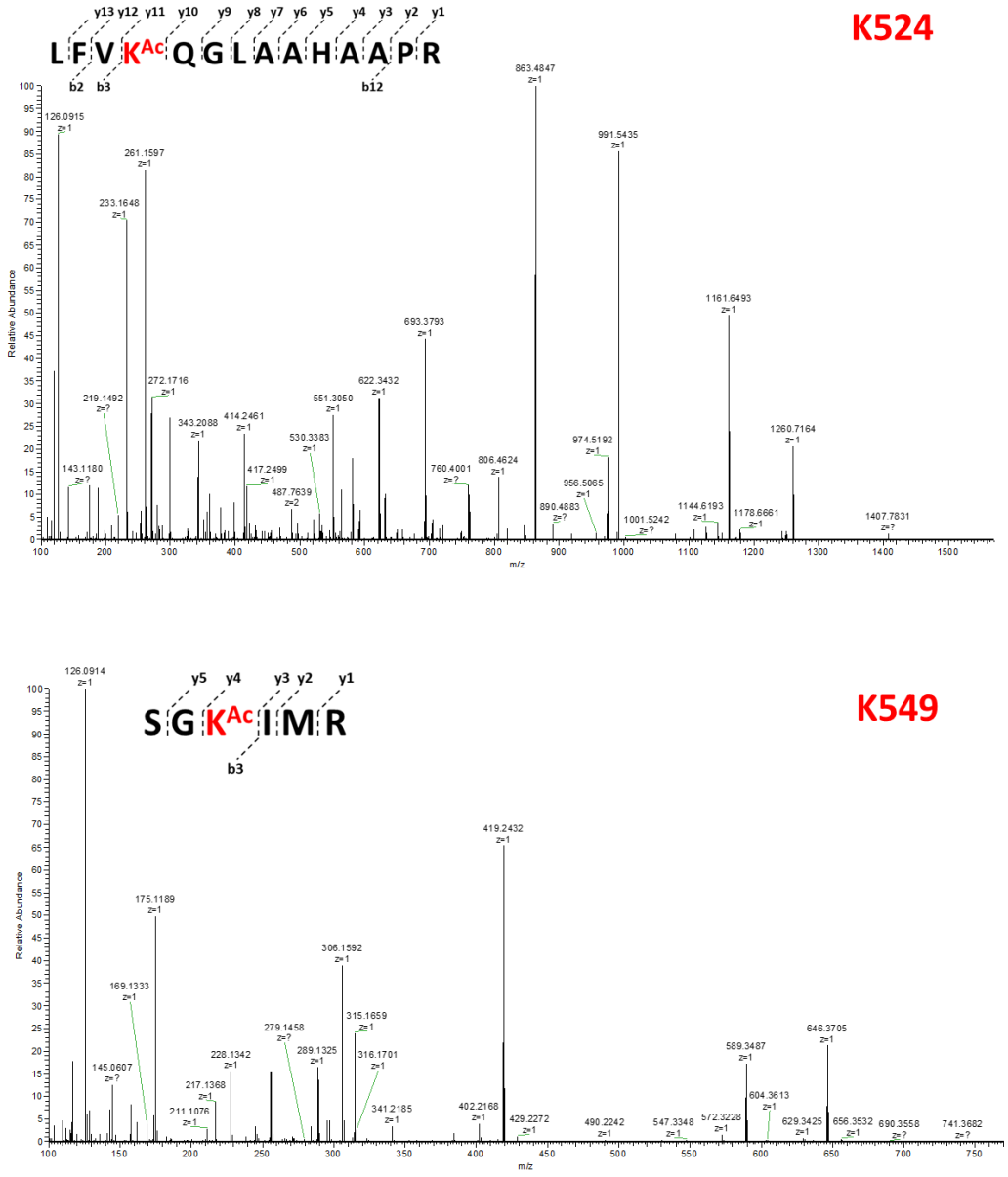

Figure S3. The MS/MS spectra of acetylpeptides containing K524 and K549 from the *Bs*AcsA^{WT} protein isolated from strain grew on acetate.

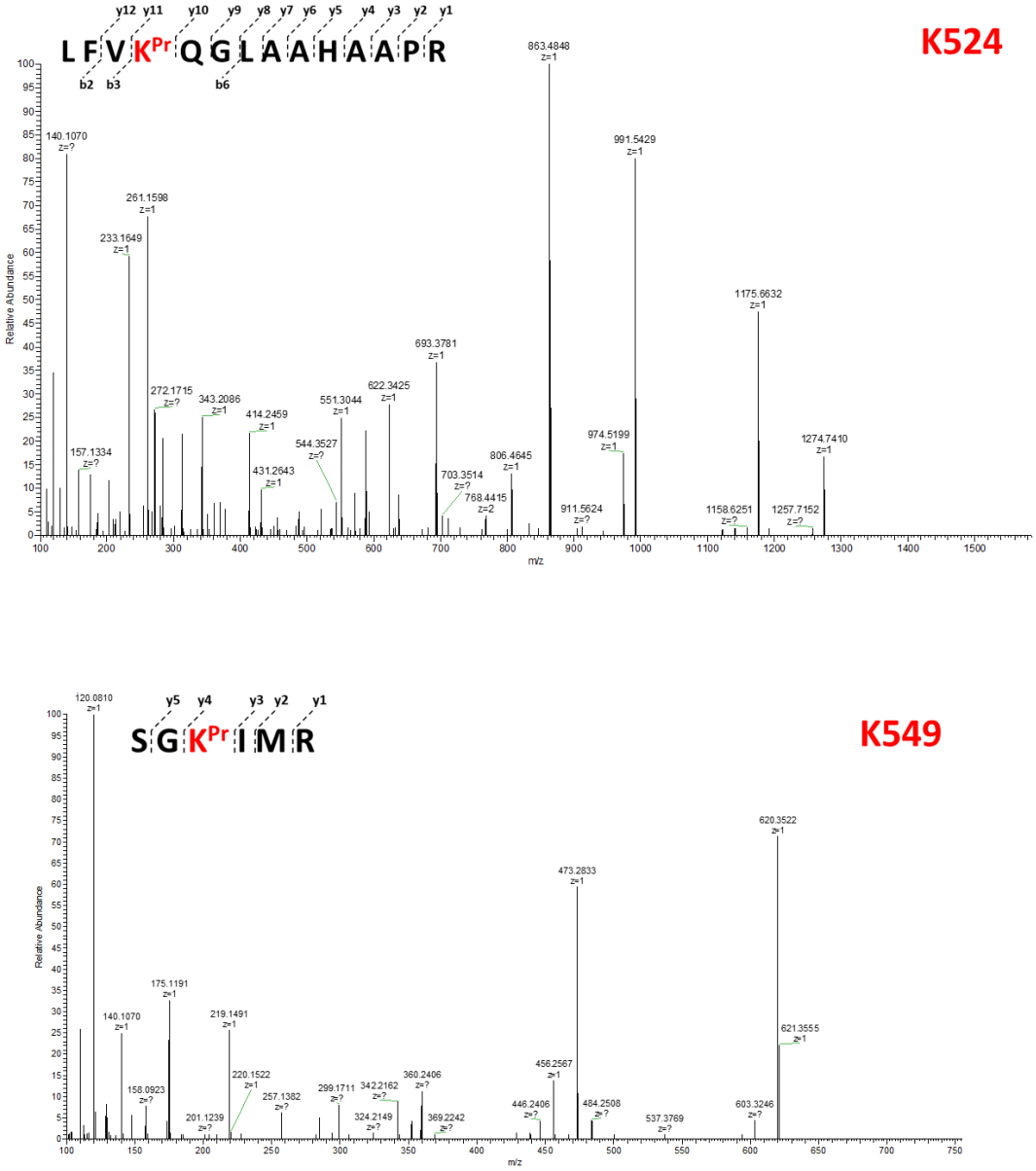

Figure S4. The MS/MS spectra of propionylpeptides containing K524 and K549 from the *Bs*AcsA^{WT} protein isolated from strain grew on propionate.

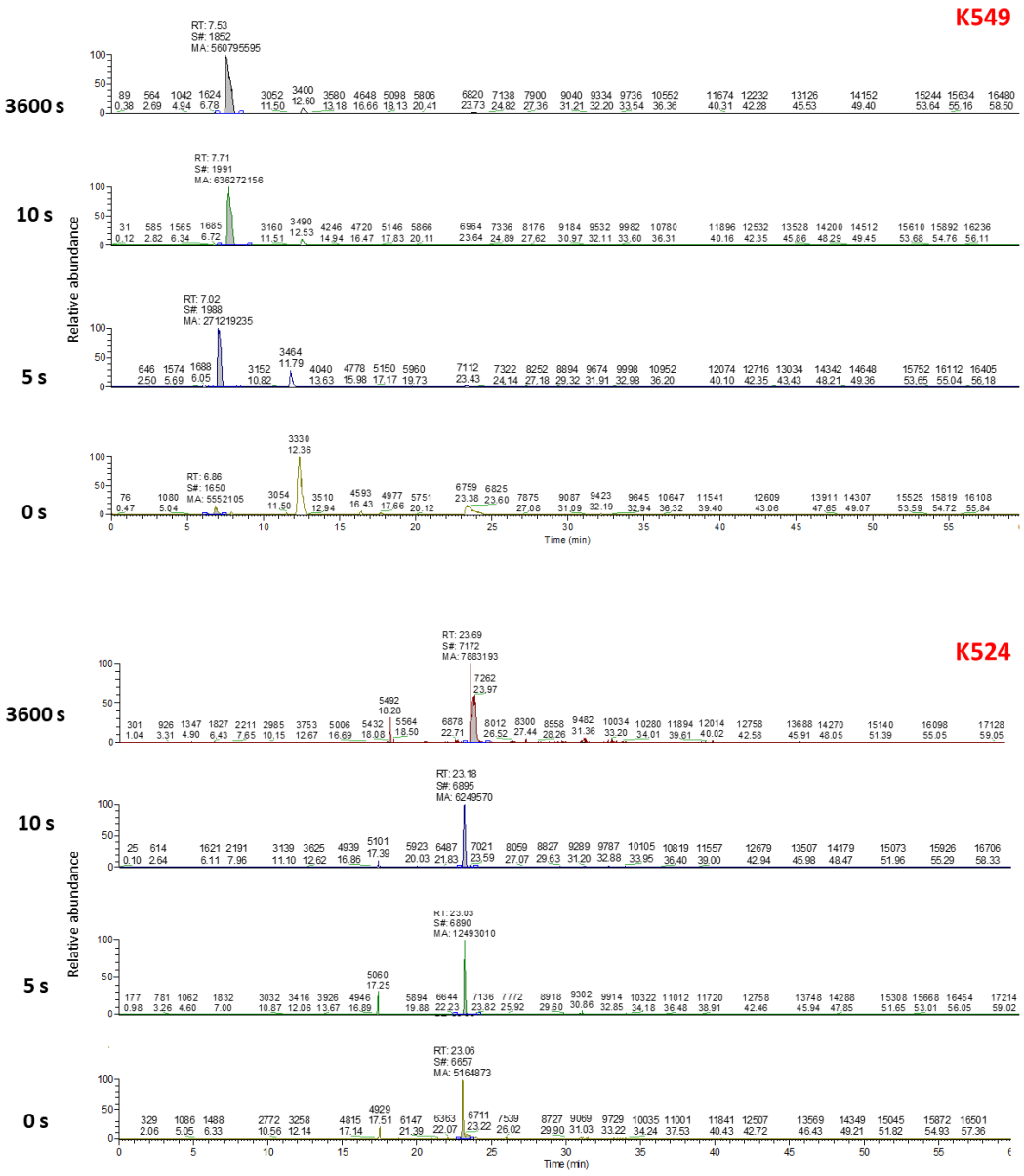

Figure S5. Mass spectrometry quantitative analysis of the AcuA-acetylated peptides containing K524 and K549. The effective AUC (areas under the curves) was in gray.

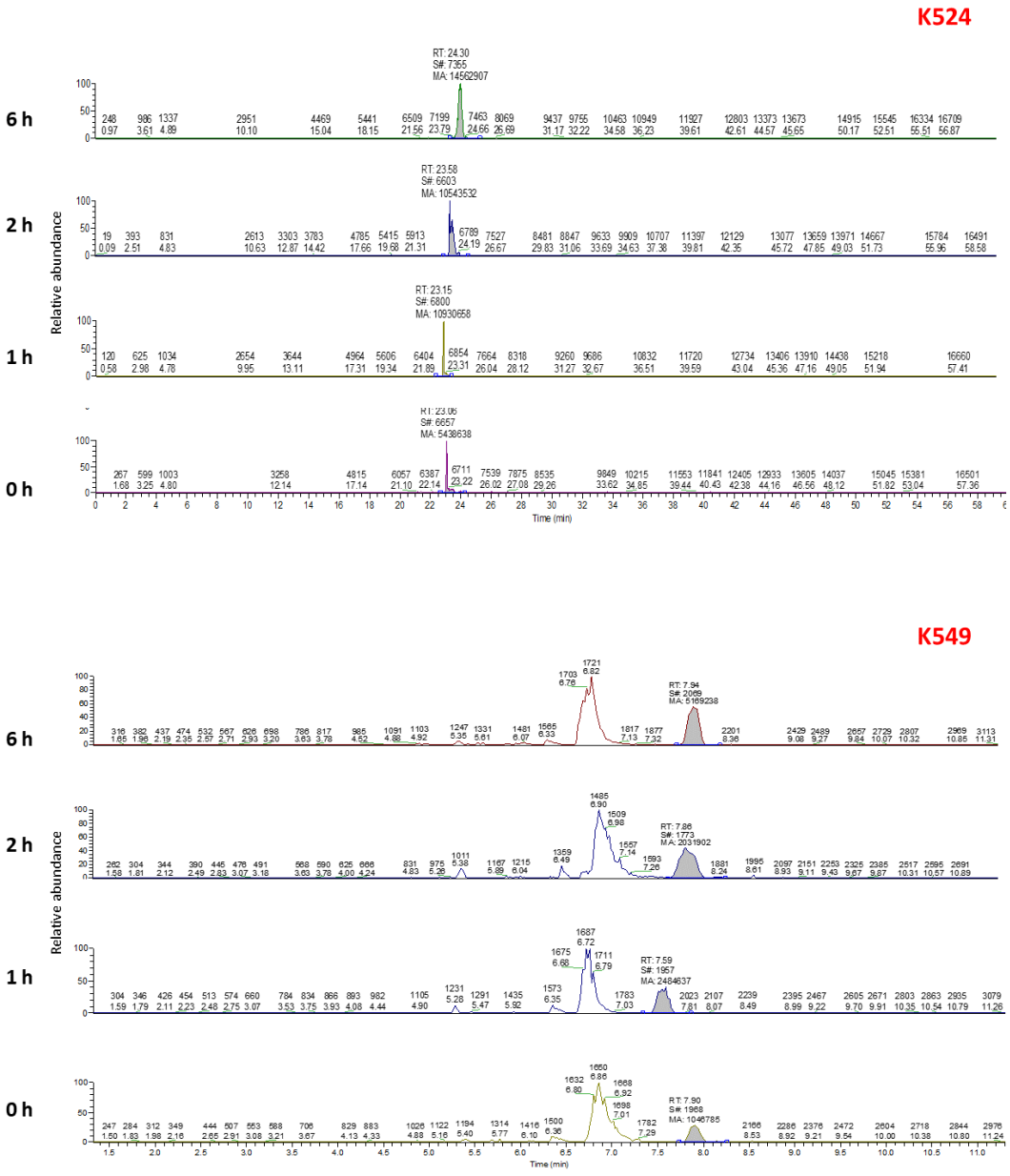

Figure S6. Mass spectrometry quantitative analysis of the Ac-CoA-acetylated peptides containing K524 and K549. The effective AUC (areas under the curves) was in gray.

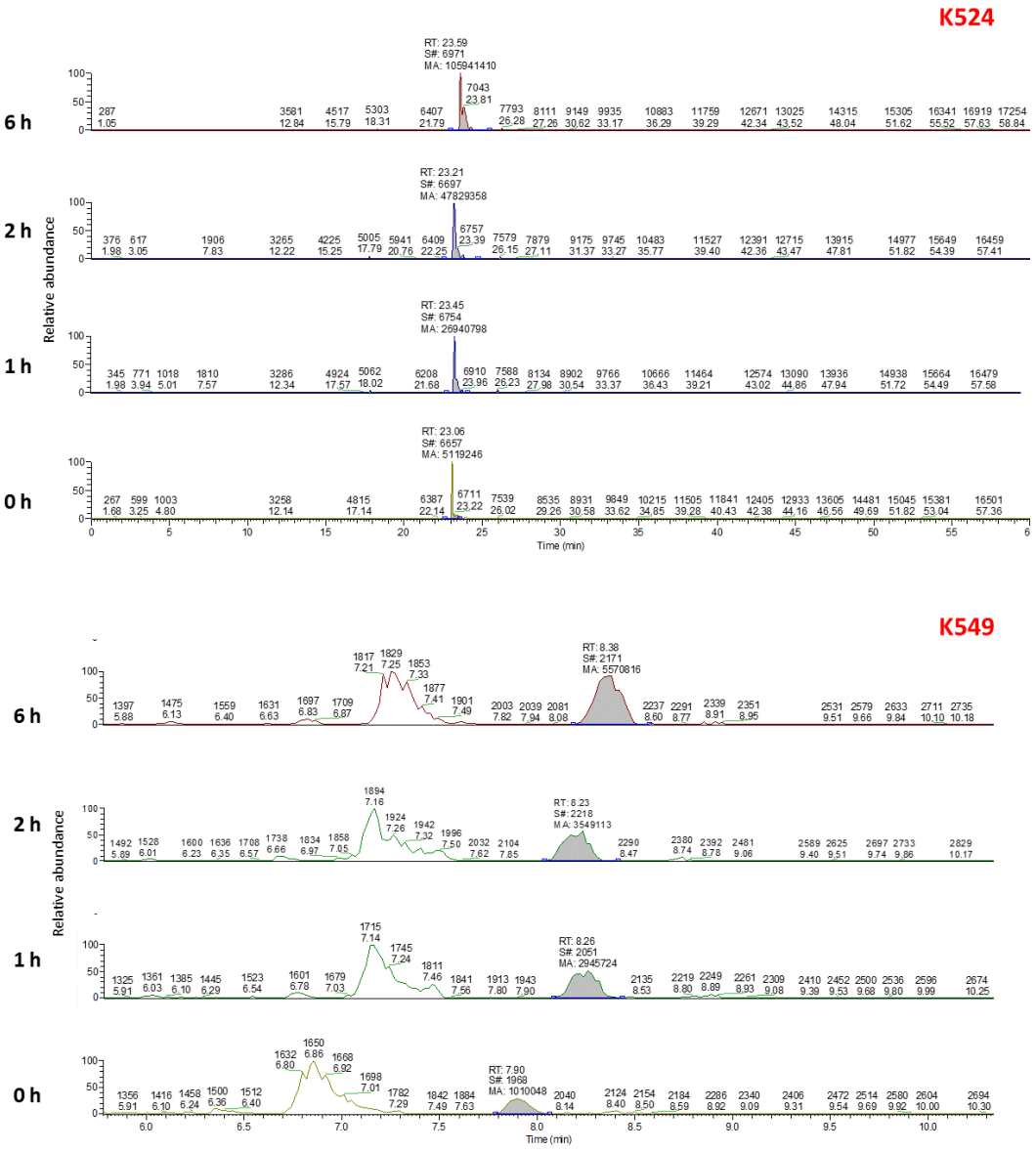

Figure S7. Mass spectrometry quantitative analysis of the AcP-acetylated peptides containing K524 and K549. The effective AUC (areas under the curves) was in gray.

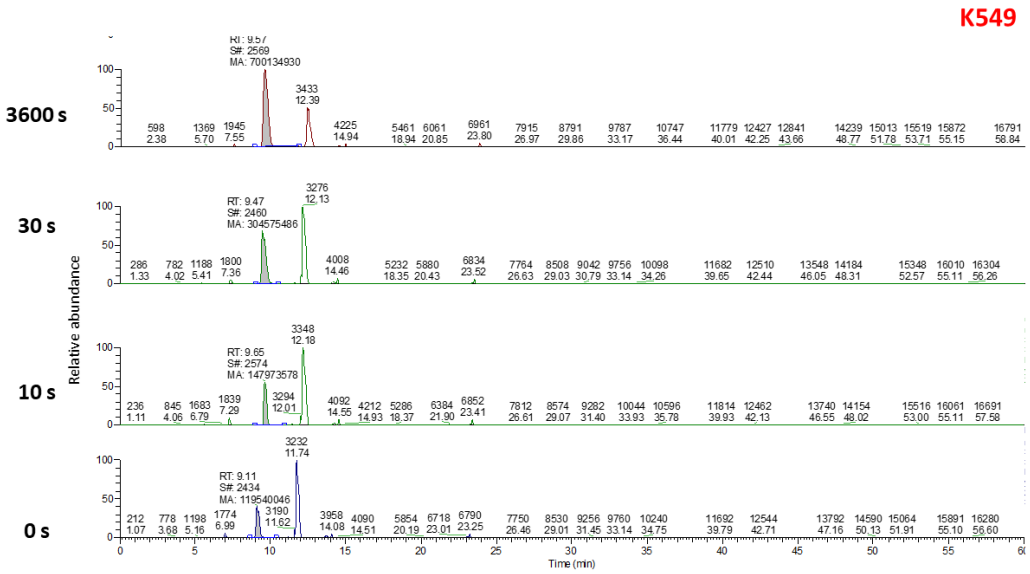

Figure S8. Mass spectrometry quantitative analysis of the AcuA-propionylated peptides containing K549. The effective AUC (areas under the curves) was in gray.

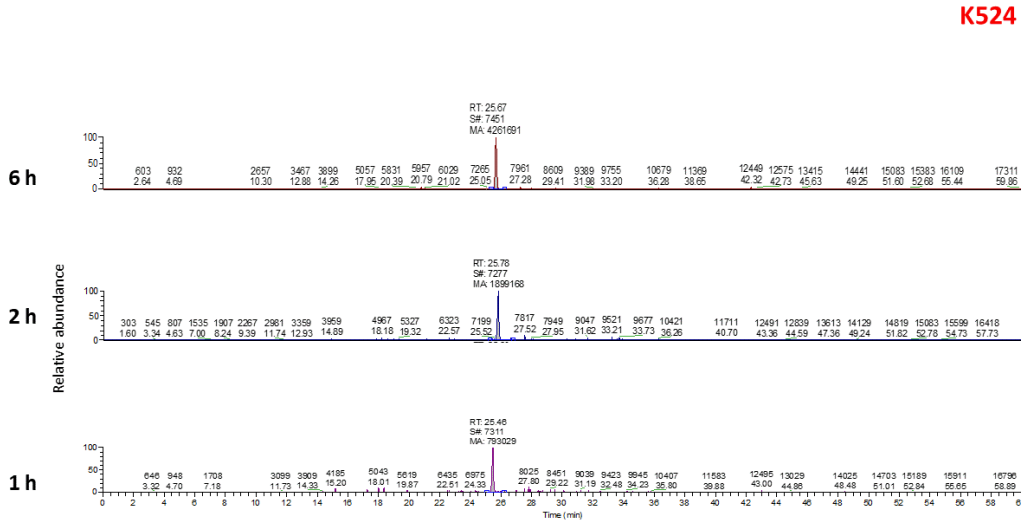

Figure S9. Mass spectrometry quantitative analysis of the Pr-CoA-propionylated peptides containing K524. The effective AUC (areas under the curves) was in gray.