Table 5-4. Samp3ylation sites identified via LC-MS/MS proteomic analysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Normalized spectral countsd** |  |  |
| **Locus tag** | **GI number** | **Protein / homolog description** | **Mod. Sitec** | $\overline{x}$ **S3**  | $\overline{x}$ **C** | **R S3/C**$ $ | **COGe** | **SAMP1/2 associated /modifiedf** |
| HVO\_0078 | 292654255 | HemC porphobilinogen deaminase | K233 (3x) | 32.0 | <1.0 | **32.0** | H | - |
| HVO\_0206 | 292654386 | Alanine--tRNA ligase alaS1  | K517 (2x) | 71.7 | 5.5 | **13.0** | J | - |
| HVO\_0337 | 292654517 | Glutaredoxin-like protein | K19 (1x) | 6.3 | <1.0 | **6.3** | O | - |
| HVO\_0359a | 292654537 | Translation elongation factor EF-1 α | K99 (1x) | 125.0 | 31 | **4.0** | J | SAMP1 associated, HVO\_0359 protein accumulates after CLBL treatment |
| HVO\_0455 | 292654632 | CctB thermosome subunit 2 | K280 (1x) | 57.7 | <1.0 | **57.7** | O | - |
| HVO\_0736 | 292654899 | DUF302 superfamily protein | K57 (2x) | 20.3 | <1.0 | **20.3** | S | SAMP2 associated |
| HVO\_0835 | 292654998 | Acetyl-CoA C-acyltransferase acaB1  | K330 (3x) | 55.7 | 4.5 | **12.4** | I | - |
| HVO\_0860 | 292655023 | SufB FeS assembly protein | K115 (3x)K316 (3x) | 408.0 | 17.5 | **23.3** | O | HVO\_0861 (SufB homolog) associated with SAMP2 |
|  HVO\_0966b | 292655126 | Ribose-1,5-bisphosphate isomerase | K210 (3x) | 788.3 | <1.0 | **788.3** | J | K210 - samp2ylatedd |
| HVO\_1081 | 292655239 | Glutaredoxin homolog | K31 (2x) | 10.7 | 5.5 | 1.9 | O | - |
| HVO\_1289 | 292655444 | OsmC-like protein | K59 (2x) | 33.0 | <1.0 | **33.0** | O | K59 - samp2ylated, HVO\_1289 protein accumulates in *∆panA* |
| HVO\_1495 | 292655647 | Fructose PTS enzyme IIB | K83 (2x) | 7.0 | <1.0 | **7.0** | S | - |
| HVO\_1577 | 292655727 | Transcriptional regulator(winged HTH and CBS domains) | K52 (1x) | 25.0 | 16.5 | 1.5 | K | SAMP2 associated |
| HVO\_1611 | 292655761 | Conserved hypothetical protein | K49 (1x) | s.r. | s.r. | s.r. | S | - |
| HVO\_1655 | 292655804 | Thioredoxin-like | K74 (3x) | s.r. | s.r. | s.r. | O | - |
| HVO\_1864 | 292655999 | MoaE-MobB domain protein  | K240 (2x)K247 (2x) | 58.7 | <1.0 | **58.7** | H | K240 and K247 samp1ylated |
| HVO\_1896 | 292656029 | Ribosomal protein S24e | K69 (1x) | s.r. | s.r. | s.r. | J | - |
| HVO\_2011 | 292656139 | Conserved hypothetical protein | K129 (1x) | 60.3 | <1.0 | **60.3** | S | - |
| HVO\_2104 | 292656231 | Arabitol PTS enzyme IIB | K40 (3x)K42 (3x) | 10 | <1.0 | **10** | G | - |
|  HVO\_2177\* | 292656305 | SAMP3 | K18 (3x)K55 (3x)K62 (3x) | 1282 | 1801 | 0.7 | H | - |
| HVO\_2328 | 292656456 | Isochorismatase family protein | K90 (2x) | 68.0 | <1.0 | **68.0** | Q | K90 - samp2ylated |
|  HVO\_B0057 | 292493996 | CobJ precorrin-3B C17-methyltransferase | K341 (1x) | 53.0 | <1.0 | **53.0** | H | encoded in an apparent operon with SAMP2-associated HVO\_B0053 |
|  HVO\_B0324 | 292494257 | Conserved hypothetical protein | K297 (2x) | 34.3 | <1.0 | **34.3** | S | - |

Table 5-4. aHVO \_0359, homolog of eukaryotic EF-1α with reported ubiquitin isopeptidase activity (Gonen *et al.*, 1996). bHVO\_0966, homolog of the ribose-1,5-bisphosphate isomerase of the proposed novel pathway of AMP metabolism in the hyperthermophilic archaeon *Thermococcus kodakarensis* (Aono *et al.*, 2012; Sato *et al.*, 2007). cModification site with lysine residue covalently attached by an isopeptide bond to SAMP3 indicated by amino acid residue number deduced from genome sequence with the exception of HVO\_2177\* (SAMP3), the latter of which is based on Met22 as the biological start codon. All samp3ylated sites were detected in wild-type (vs. *∆ubaA*) strains expressing Flag-SAMP3A90K. See Methods for details on cell culture and conjugate isolation. The number of biological replicates in which a site was detected in its modified form via high-quality MS/MS spectra is in parentheses. dNormalized spectral count average ($\overline{x}$) and ratio (R) of the averages based on LC-MS/MS analysis of biological replicates of Flag-SAMP3 A90K fractions purified from wild-type (S3) and *ΔubaA* mutant (C, control). eClusters of Orthologous Groups (COGs) based on Wolf *et al.* (Wolf *et al.*, 2012). fProteins/protein homologs found associated with and covalently modified by SAMP1/2, accumulated after treatment with proteasome inhibitor clastolactacystin-β-lactone (CLBL), and enhanced by deletion of the Rpt-like AAA ATPase PAN-A (*∆panA*) were as previously described (Humbard *et al.*, 2010; Hepowit *et al.*, 2012; Kirkland *et al.*, 2008; Kirkland *et al.*, 2007). Proteins not found associated with SAMP1/2 are indicated by (-). Note: s.r., detection of two or more samp3ylated peptides in single replicate.

List of References

Aono, R., Sato, T., Yano, A., Yoshida, S., Nishitani, Y., Miki, K., Imanaka, T., and Atomi, H. (2012) Enzymatic characterization of AMP phosphorylase and ribose-1,5-bisphosphate isomerase functioning in an archaeal AMP metabolic pathway. *J Bacteriol* **194**: 6847-6855.

Gonen, H., Dickman, D., Schwartz, A. L., and Ciechanover, A. (1996) Protein synthesis elongation factor EF-1 a is an isopeptidase essential for ubiquitin-dependent degradation of certain proteolytic substrates. *Adv Exp Med Biol* **389:209-19.**: 209-219.

Hepowit, N. L., Uthandi, S., Miranda, H. V., Toniutti, M., Prunetti, L., Olivarez, O., De Vera, I. M., Fanucci, G. E., Chen, S., and Maupin-Furlow, J. A. (2012) Archaeal JAB1/MPN/MOV34 metalloenzyme (HvJAMM1) cleaves ubiquitin-like small archaeal modifier proteins (SAMPs) from protein-conjugates. *Mol Microbiol* : 10.

Humbard, M. A., Miranda, H. V., Lim, J. M., Krause, D. J., Pritz, J. R., Zhou, G., Chen, S., Wells, L., and Maupin-Furlow, J. A. (2010) Ubiquitin-like small archaeal modifier proteins (SAMPs) in *Haloferax volcanii*. *Nature* **463**: 54-60.

Kirkland, P. A., Gil, M. A., Karadzic, I. M., and Maupin-Furlow, J. A. (2008) Genetic and proteomic analyses of a proteasome-activating nucleotidase A mutant of the haloarchaeon *Haloferax volcanii*. *J Bacteriol* **190**: 193-205.

Kirkland, P. A., Reuter, C. J., and Maupin-Furlow, J. A. (2007) Effect of proteasome inhibitor clasto-lactacystin-b-lactone on the proteome of the haloarchaeon *Haloferax volcanii*. *Microbiology* **153**: 2271-2280.

Sato, T., Atomi, H., and Imanaka, T. (2007) Archaeal type III RuBisCOs function in a pathway for AMP metabolism. *Science* **315**: 1003-1006.

Wolf, Y. I., Makarova, K. S., Yutin, N., and Koonin, E. V. (2012) Updated clusters of orthologous genes for Archaea: a complex ancestor of the Archaea and the byways of horizontal gene transfer. *Biol Direct* **7:** 46-47.