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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Normalized spectral countsd** | | |  |  |
| **Locus tag** | **GI number** | **Protein / homolog description** | **Mod. Sitec** | **S3** | **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 () 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.

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