Supplemental Figures

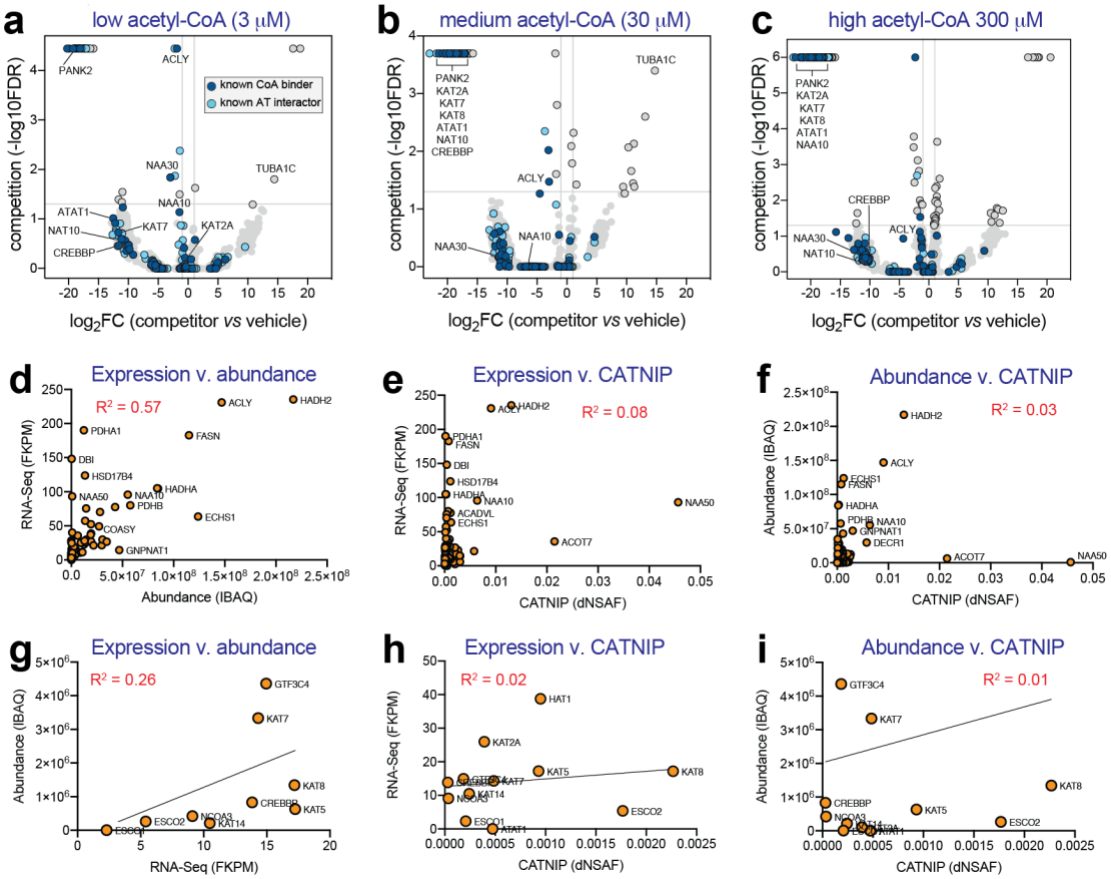

Figure S1, Related to Figure 2. (a-c) Volcano plots depicting competition of proteins captured by Lys-CoA Sepharose by increasing concentrations of acetyl-CoA (0.003, 0.03, and 0.3 μM). Uniprot-annotated CoA-binding proteins ("CoA binder") as well as members of acetyltransferase complexes ("AT interactors") are highlighted in dark blue and light blue, respectively. Fold-enrichments and -log10FDR were calculated by analyzing dNSAF values using the statistical framework QPROT. (d-f) Correlation of CATNIP capture efficiency of CoA-binders with gene expression by RNA-Seq ('expression') or protein abundance by proteomics ('abundance'). (g-i). Correlation of CATNIP capture efficiency of annotated ATs with gene expression by RNA-Seq ('expression') or protein abundance by proteomics ('abundance').

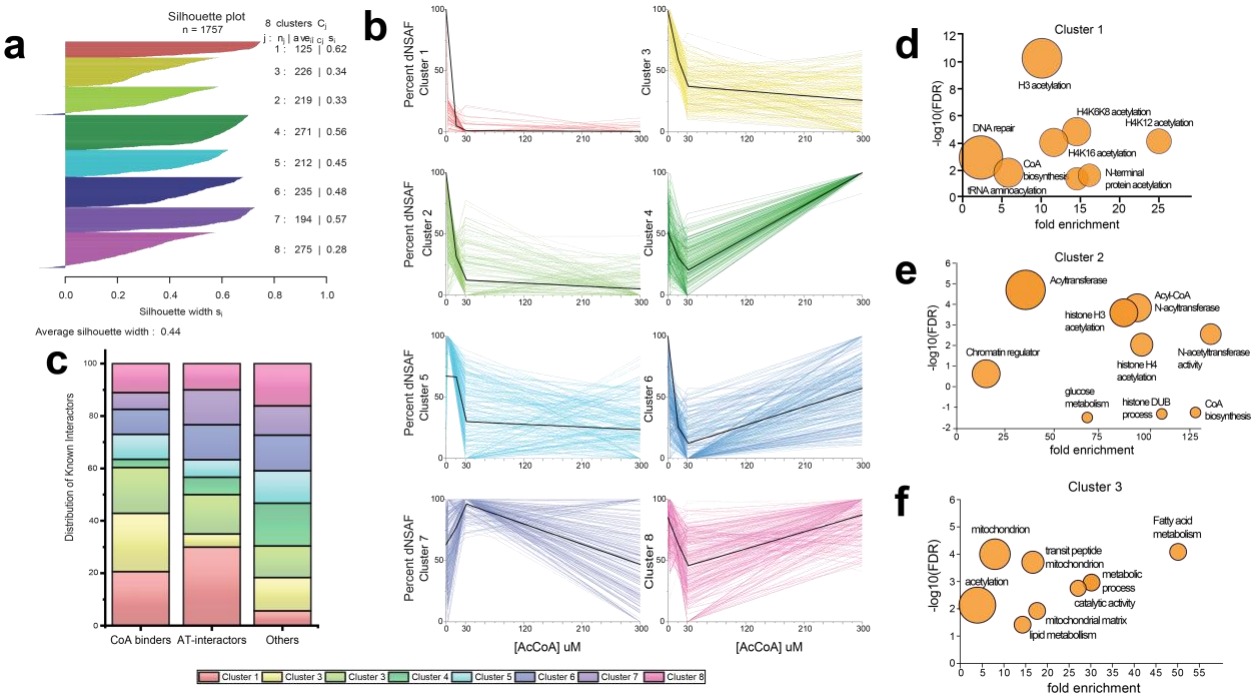

Figure S2, Related to Figure 2. (a) Silhouette plot representative of the 1757 proteins generated in R demonstrating fit of each cluster to the data. (b) Dose-response profiles for all 8 CATNIP clusters (cluster 7 and 8 were not depicted in Fig. 2). Colored lines indicate the capture profiles of individual proteins within each cluster in the presence of increasing concentrations of acetyl-CoA competitor. Black lines indicate the mean capture profile for all proteins in a given cluster. (c) Distribution of CoA-binders, AT-interactors, and non-annotated ('other') proteins in each cluster. Clusters 1-3 contain the majority of annotated CoA-binders and AT-interactors, while non-annotated proteins are distributed fairly evenly between different dose-response profiles. (d-f) Gene ontology analysis of Uniprot annotated CoA-binding and AT interacting proteins lying in CATNIP clusters 1-3. Fold enrichment of a specific functional term is plotted versus statistical significance (-log10[FDR]). The circle size reflects the number of proteins matching a given term. Functional enrichment was performed with the tool DAVID (https://david.ncifcrf.gov) by using GO and Swiss-Prot Protein Information Resource terms.

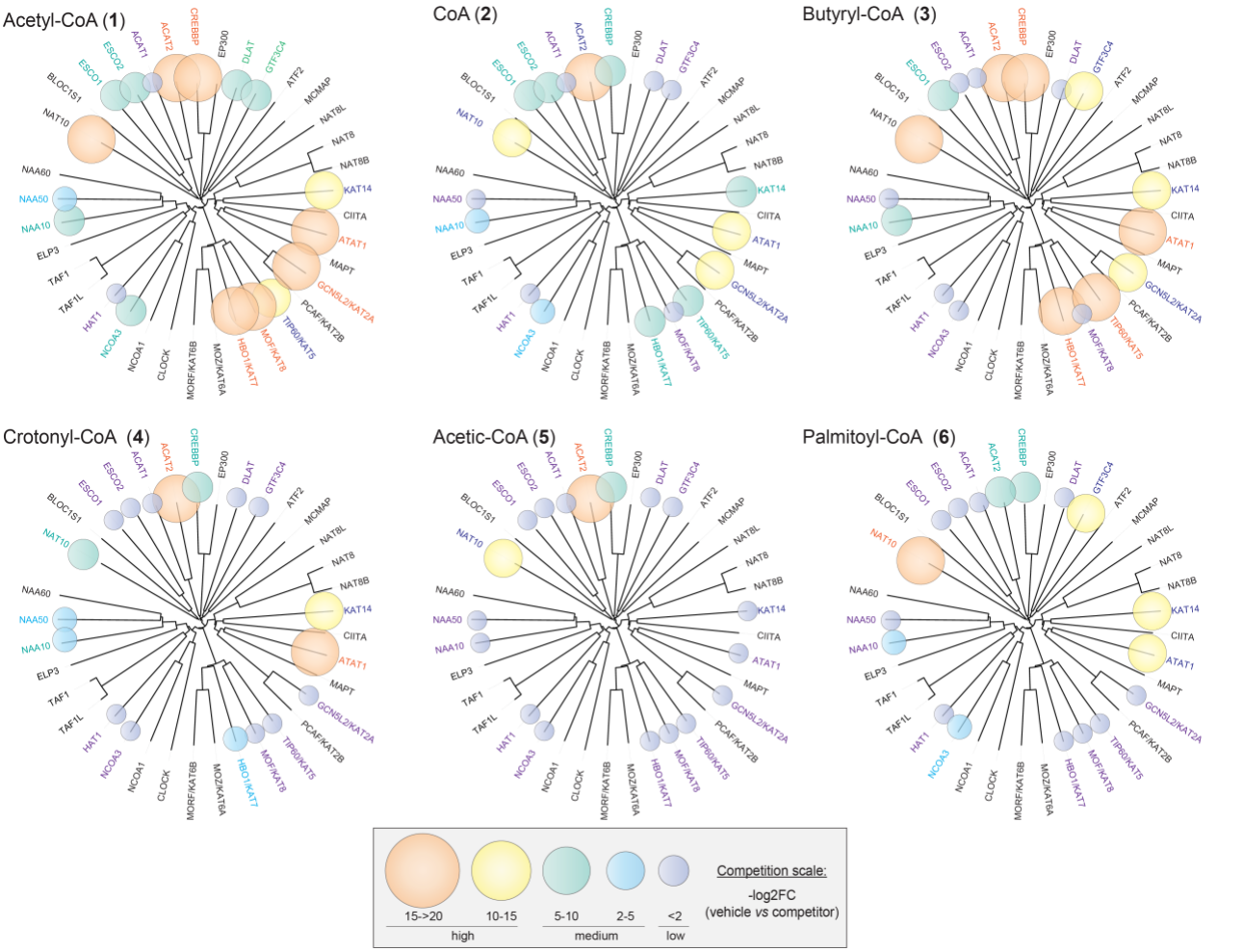

Figure S3, Related to Figure 3. Metabolic acyl-CoA competition profiles of the KAT superfamily. For each protein, metabolic acyl-CoA competition data was overlaid on the unrooted phylogenetic tree of the KAT superfamily. Bubble size and color corresponds to degree of competition observed when proteomes were pre-incubated with CoA metabolites **1**-**6**. All ligands were assessed at 30 μM besides **1**-**2**, which were assessed at both 3 and 30 μM, and **6** whose competition assessed only at 3 μM. In cases where competition was observed at multiple concentrations, the larger log-transformed fold change (-log2FC) value was used for graphical depiction. Log2FC was calculated from dNSAF measurements using QPROT.

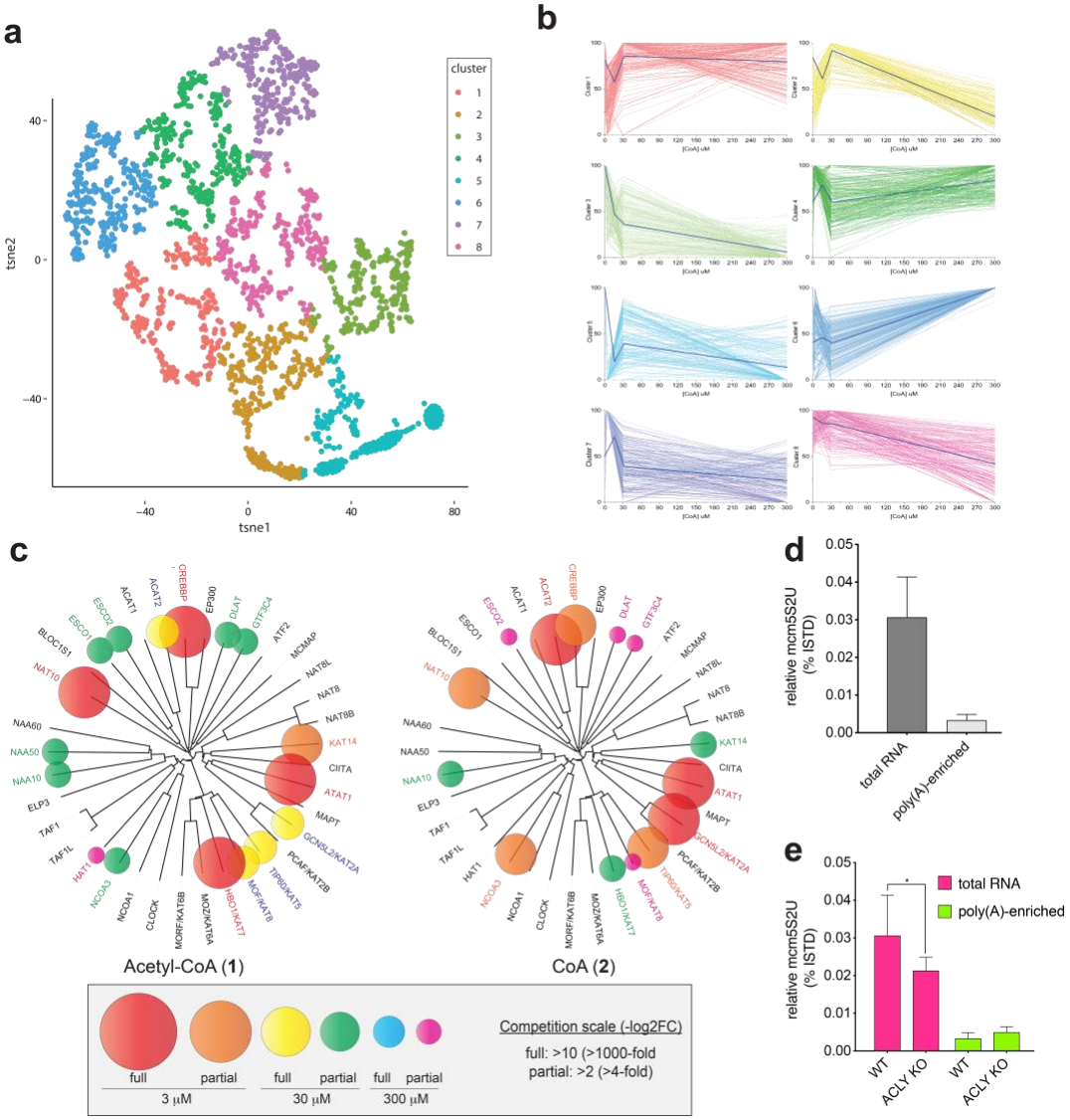

Figure S4, Related to Figure 5. Profiling CoA/protein interactions using CATNIP. (a) t-SNE plot of proteins competed at various concentrations of CoA with proteins colored by the cluster number. Eight clusters were identified as optimal by k-means analysis. Lys-CoA Sepharose was incubated with proteomes in the presence of increasing concentrations of CoA (0, 3, 30, and 300 μM) and protein enrichment was quantified based on distributed normalized spectral abundance factor (dNSAF). Dose-response profiles were transformed into two-dimensional data and used for clustering analysis. n=3 LC-MS/MS runs for each condition. (b) Dose-response profiles of CoA CATNIP clusters. Colored lines indicate the capture profiles of individual proteins within each cluster in the presence of increasing concentrations of CoA competitor. Dark blue lines indicate the mean capture profile for all proteins in a given cluster. (c) Comparing acetyl-CoA and CoA competition of KAT superfamily capture. Circle colors indicate the lowest concentration at which acetyl-CoA (left) or CoA (right) caused a greater than two-fold log-transformed fold change (-log2FC) in the capture of each of each KAT by Lys-CoA Sepharose, while the size of the oval indicates the degree of competition. Log2FC values were calculated from dNSAF measurements using QPROT. (d) Monitoring levels of the transfer RNA (tRNA) modification 5-methoxycarbonylmethyl-2-thiouridine (mcm5S2U) indicates efficient depletion of tRNAs during sequential poly(A)-enrichment steps using oligo(dT) beads. (e) Levels of the Elp3 AT-dependent RNA modification mcm5S2U are sensitive to ACLY KO in total RNA but not in poly(A)-enriched fraction. Of note, NAT10 AT-dependent ac4C displays the opposite profile (Fig. 4e). Values represent ≥ 3 replicates, analyzed by two-tailed student's t-test (ns = not significant, *P<0.05, **P<0.01, ***P<0.001).

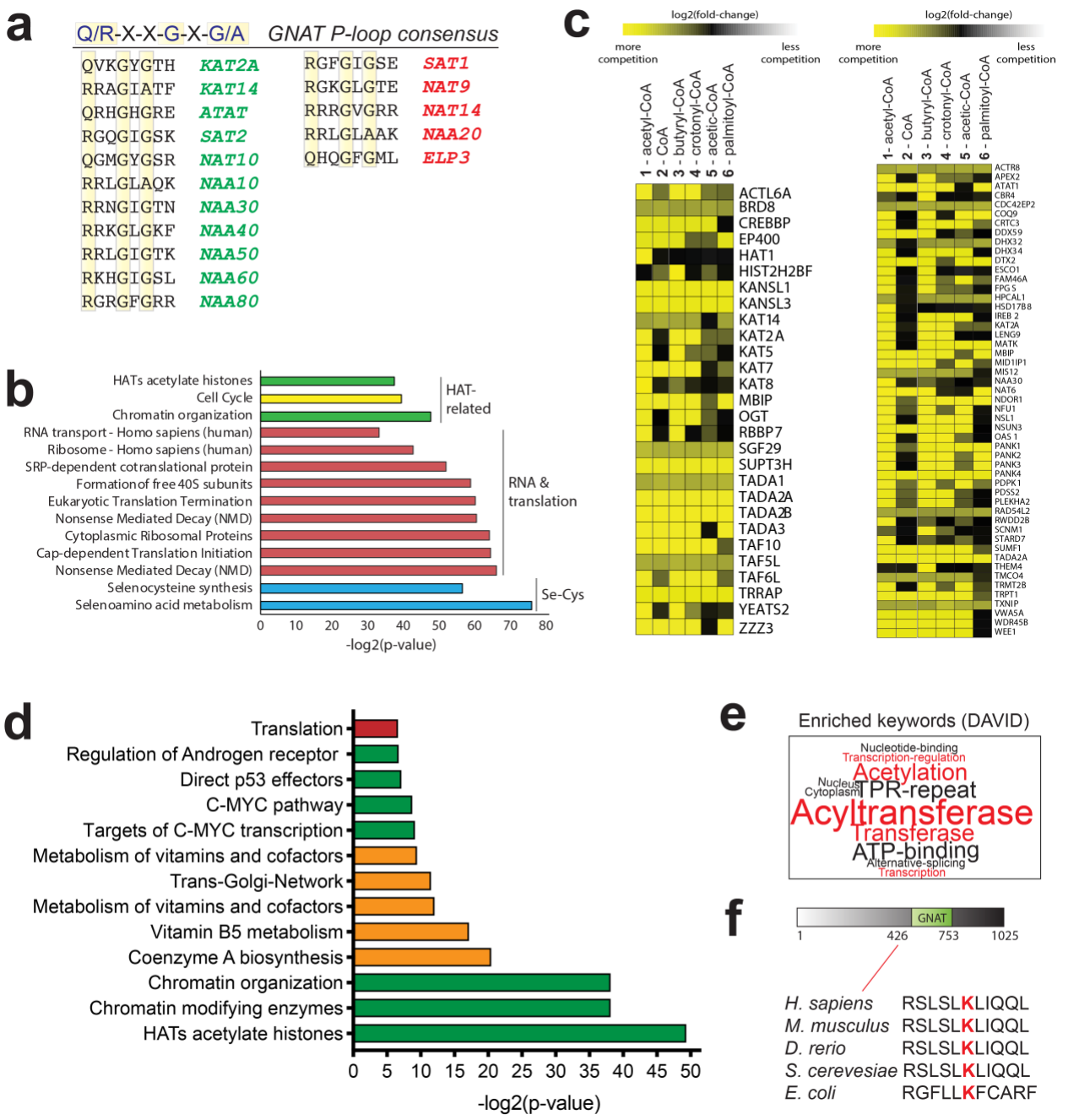

Figure S5, Related to Figure 5. (a) Comparison of GNAT consensus P-loop sequence in proteins captured (left, green) or not captured (right red) by CATNIP. (b) Pathway analysis of proteins identified as potential acyl-CoA interactors by de novo filtering of CATNIP data. Enriched pathways were identified by ConsensusPathDB analysis of 672 proteins displaying multi-ligand (>3) competition and absence of enrichment in common contaminant (CRAPome) database. Terms are grouped according to biological functions. (c) Comparing multi-ligand competition profiles of proteins lying within pathways enriched by de novo CATNIP dataset. Left: the 28 proteins enriched in “HATs acetylate histones” pathway. Right: Potentially novel interactors not detected in the CrapOme database. Yellow = more competition of capture, black = less competition of capture. (d) Pathway analysis of proteins identified as potential acyl-CoA interactors by de novo filtering of CATNIP data and combined with dose-dependent acetyl-CoA CATNIP clustering. Enriched pathways were identified by ConsensusPathDB analysis of 45 proteins displaying multi-ligand (≥3) competition, absence of enrichment in common contaminant (CRAPome) database, and hypersensitivity to dose-dependent acetyl-CoA competition (cluster 1, Fig. 2b-c). (e) Word cloud depicting enriched keywords identified by DAVID analysis of protein subset defined in S5d. Word size corresponds to fold-enrichment of each keyword. (f) NAT10 K426 is conserved from eukaryotes to bacteria.