Chemoproteomic study uncovers HemK2/KMT9 as a new target for NTMT1 bisubstrate inhibitors

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Abstract

Understanding the selectivity of methyltransferase inhibitors is important to dissect the functions of each methyltransferase target. From this perspective, here we report a chemoproteomic study to profile the selectivity of a potent protein N-terminal methyltransferase 1 (NTMT1) bisubstrate inhibitor NAH-C3-GPKK ($K_{i, app} = 7 \pm 1$ nM) in endogenous proteomes. First, we describe the rational design, synthesis, and biochemical characterization of a new chemical probe **6**, a biotinylated analogue of NAH-C3-GPKK. Next, we systematically analyze protein networks that may selectively interact with the biotinylated probe **6** in concert with the competitor NAH-C3-GPKK. Besides NTMT1, the designated NTMT1 bisubstrate inhibitor NAH-C3-GPKK was found to also potently inhibit a methyltransferase complex HemK2-Trm112 (also known as KMT9-Trm112), highlighting the importance of systematic selectivity profiling. Furthermore, this is the first potent inhibitor for HemK2/KMT9 reported to date. Thus, our studies lay the foundation for future efforts towards the development of selective inhibitors for NTMT1 and HemK2/KMT9.

INTRODUCTION

Protein methylation regulates numerous biological processes, including transcription, DNA repair, and RNA metabolism.^{1–3} Protein methyltransferases (MTs) are the enzymes that catalyze the transfer of a methyl group from the cofactor S-adenosyl-L-methionine (SAM) onto protein substrates. The substrate preference of MTs determines their respective functions and annotations.^{4–10} For example, protein N-terminal MTs (NTMTs), lysine MTs (PKMTs), and arginine MTs (PRMTs) catalyze the methylation on protein α -N-termini, ϵ -amino group of the lysine, and guanidinium nitrogen atoms of the arginine, respectively.^{4–10} As the dysregulation of MTs has been implicated in various diseases, selectivity of the inhibitors is essential to elucidate the physiological and pharmacological functions of the MTs.^{1–3,11–13}

NTMT1 methylates the protein α -N-terminal amines of a specific motif X-P-K/R, where X represents any amino acid other than D/E.^{5,8,14} Substrates of NTMT1 include centromere H3 variants CENP-A/B, the regulator of chromatin condensation 1 (RCC1), damaged DNA-binding protein 2, poly(ADP-ribose) polymerase 3, and transcription factor-like protein MRG15.^{5,15–18} Recent progress has revealed the important role of NTMT1 in chromosome segregation, mitosis, and DNA damage repair.^{18–20} Knockdown of NTMT1 sensitized the breast cancer cell lines to etoposide and γ irradiation treatment, and knockout of NTMT1 resulted in developmental defects and premature aging in mice.^{20,21} Therefore, such critical cellular processes highlight the need for the development of highly selective chemical probes for NTMT1. Towards this goal, we have previously developed a series of potent NTMT1 bisubstrate inhibitors by covalently linking the SAM mimic with a peptide substrate to allow them to simultaneously occupy neighboring binding sites of SAM and substrate. Two representative examples are NAH-C3-PPKRIA ($K_{i, app} = 39 \pm 10$ nM) and NAH-C4-GPKRIA ($K_{i, app} = 0.13 \pm 0.04$ nM) (Figure 1), where NAH is a SAM mimic by replacing the sulfonium ion with a nitrogen atom. Meanwhile, they displayed selectivity over two representative PKMTs (G9a and SETD7) and three PRMTs (PRMT1, 3, and 7) in the biochemical assays.^{22–25} Furthermore, NAH-C4-GPKRIA exhibits selective

for NTMT1 over its closely related homolog NTMT2, suggesting the unique active site of NTMT1 and high selectivity of NTMT1 bisubstrate inhibitors.²⁵ While NTMT1 bisubstrate inhibitors exhibit decent selectivity among multiple MTs against isolated enzymes in biochemical assays, there is no information on any other targets that those inhibitors may interact within cellular contexts to understand any off-target engagement and delineate the mechanism of action.

Figure 1. Chemical structures of NTMT1 bisubstrate inhibitors.

Chemoproteomic profiling is a powerful approach to provide a global insight into the cellular targets of an inhibitor in a complex biological environment by utilizing immobilized or photoreactive inhibitors. Here, we design and synthesize a biotinylated derivative of NTMT1 bisubstrate inhibitor **1** (NAH-C3-GPKK) to generate probe **6** (NAH-C3-GPKK-biotin), allowing it to be immobilized with streptavidin beads. Pulldown study of probe **6** confirms its ability to efficiently enrich NTMT1 from the whole cell lysates. In addition, probe **6** also binds to another protein methyltransferase, i.e. HemK2-Trm112 complex (also known as KMT9-Trm112), that methylates both lysines of histone H4 and glutamine of the eukaryotic release factor 1 (eRF1).^{4,26} Subsequent validation confirms that compound **1** acts as a dual potent inhibitor for NTMT1 and HemK2/KMT9. To our knowledge, this is the first inhibitor reported for HemK2/KMT9, which offers a new revenue for the use of this compound.

RESULTS AND DISCUSSION

Design and synthesis. To design the optimal probe for the selectivity profiling of NTMT1 bisubstrate inhibitor, we first investigate the structure-activity relationship (SAR) of the NTMT1 bisubstrate inhibitors. Thus, we

synthesized new bisubstrate analogues **1-4** with different peptide sequences and variable linkers to choose the optimal position to install a biotin group. Because of its high potency and selectivity, NAH-C4-GPKRIA was selected as our lead compound. According to the co-crystal structures of NTMT1 in complex with previous NTMT1 bisubstrate inhibitors,^{24,25} we kept the tripeptide GPK intact in our design. The proposed **1-4** were synthesized in a convergent manner by reacting the primary amines (**7-8**) with α -bromo peptide on resin and followed by acid treatment as described before (Schemes 1).^{23,25}

Scheme 1. Synthetic route for compounds **1-4**.^a

^aReagents and conditions: (a) α -bromo peptides on resin, DIPEA, DMF, r.t.; (b) TFA: DODT: H₂O: TIPS = 94: 2.5: 2.5: 1.

Table 1. NTMT1 inhibition evaluation of bisubstrate analogues

Compound ID	Peptide	n	$K_{i, app}$ (nM)
1 (NAH-C3-GPKK)	GPKK	3	7 ± 1
2 (NAH-C4-GPKK)	GPKK	4	5.0 ± 0.3

3 (NAH-C3-GPK)	GPK	3	41 ± 4
4 (NAH-C4-GPK)	GPK	4	44 ± 3

Inhibition Evaluation. The inhibitory activities of compounds **1-4** against NTMT1 were evaluated in the SAH hydrolase (SAHH)-coupled fluorescence assay under the condition that both SAM and the peptide substrate GPKRIA were at their respective K_m values.²⁷ Previous studies demonstrated that NAH-C4-GPKRIA and NAH-C4-GPKR bisubstrate analogs containing a 4-C carbon link showed slightly higher potency than those with a 3-C atom linker.²⁵ However, the NAH-C3-GPKK bisubstrate analogue **1** with the 3-C atom linker ($K_{i, app} = 7 \pm 1$ nM) exhibited similar inhibition as its 4-C linker analogue **2** ($K_{i, app} = 5.0 \pm 0.3$ nM) (Table 1). Likewise, the NAH-C3-GPK bisubstrate analogue **3** with a 3-C carbon linker showed comparable inhibitory activity to compound **4** with a 4-C carbon linker, although the $K_{i, app}$ values of **3** and **4** were increased by 6-8 fold compared to **1** and **2** due to the shortened peptide moiety from a tetrapeptide to a tripeptide.

Figure 2. Methylation inhibition and selectivity study for **1**. (A) Representative MS results of MALDI-MS methylation inhibition assay for **1**; (B) Quantification of methylation progression of GPKRIA by NTMT1 in presence of various concentrations of **1** at 20 min (n=2). (C) Selectivity of **1** against several

methyltransferases and SAH hydrolase (n=2).

MALDI-MS Methylation Inhibition Assay. For further validation, we evaluated the inhibitory effects of compound **1** on N α -methylation progression with an orthogonal MALDI-MS methylation assay (Figure 2A-B).^{24,28} As NTMT1 can trimethylate the N α -amine group of the substrate peptide GPKRIA in which the N α -amine group is a primary amine, there would be mono-, di- and tri-methylation states for the methylated product in the methylation assay. As the concentration of compound **1** increased, the tri-methylated GPKRIA decreased along with the increased level of unmethylated GPKRIA (Figure 2B). For compound **1**, tri-methylation of GPKRIA was almost abolished at 200 nM (Figure 2B). Also, all the methylation products of GPKRIA were reduced to less than 10% at 100 nM of compound **1** (Figure 2A-B). Through calculating the relative reaction rate based on the relative abundance with three species methylated products, quantitative analysis of the data and fitting them with different compound concentrations yielded the $K_{i, app}$ of 15 ± 4 nM for **1** in the MS assay, which is comparable to the inhibitory activity in the fluorescence assay and further validated the potency of **1** for NTMT1.

Selectivity Assessment. Next, we evaluated the selectivity of **1** for NTMT1 against a panel of several SAM-dependent methyltransferases, including two PKMTs (G9a and SETD7), and three PRMTs (PRMT1, PRMT3, and *Tb*PRMT7). We also include the SAHH, a coupling enzyme used in the fluorescence assay, which has a SAH binding site. Based on the results (Figure 2C), compound **1** retained the high selectivity to NTMT1, displaying more than 4,000-fold inhibition over other methyltransferases and SAHH.

Figure 3. X-ray co-crystal structure of NTMT1- **1** (PDB ID: 6PVA). (A) Zoomed view of interactions of **1** (green) complex with NTMT1 (gray). H-bond interactions are shown in yellow dotted lines; (B) Structural alignment of NTMT1-**1** (gray/green) complex with NTMT1-**NAH-C3-PPKRIA** (orange) binary complex (PDB ID: 6DTN); (C) 2D interaction diagram (Schrödinger Maestro) of **1** with NTMT1.

Co-crystal Structure of Compound 1 (NAH-C3-GPKK) with NTMT1. We obtained the X-ray crystal structure of the NTMT1-**1** complex (PDB ID: 6PVA) (Figure 3A), confirming its engagement with both cofactor and substrate binding sites of NTMT1. The structure of NTMT1-**1** overlaid well with the previously characterized co-crystal structure of bisubstrate inhibitor **NAH-C3-PPKRIA** (orange) with NTMT1 (PDB ID: 6DTN) (Figure 3B).²⁴ The NAH moiety of **1** exhibited almost the same binding mode to NTMT1 as the NAH portion of **NAH-C3-PPKRIA** (Figure 3B), which is nearly identical with SAH in the ternary complex of substrate peptide/SAH with NTMT1 (PDB ID: 5E1M).⁸ Also, the peptide portion GPKK of **1** shows a similar binding mode compared with the PPKR peptide sequence in **NAH-C3-PPKRIA**. Specifically, the carbonyl oxygen of the first Gly forms a hydrogen bond with the side chain of Asn168 (Figure 3C). The second Pro residue inserts into the hydrophobic pocket formed by Leu31, Ile37, and Ile214, while the ϵ -amine group of the third Lys forms three hydrogen bonds with side chains of Asp177, Asp180, and Ser182.

Design of Affinity Probe 6. As the last lysine is exposed to the solvent and its backbone forms interactions

with NTMT1, we proposed the introduction of a clickable propargyl Gly (pG) to the C-terminus of the peptide of **1**, as well as linking the biotin group with pG to obtain the biotinylated probe, would retain the interaction with NTMT1 and allow the derivatization to immobilize the probe on streptavidin beads. Biotin-streptavidin is a powerful non-covalent interaction with a high binding affinity ($K_d = \sim 10^{-14}$ M).²⁹ The biotin-streptavidin system has been widely applied in many biological applications, including proteome profiling, bio-imaging, biotinylated antibodies, and protein purification.²⁹ Thus, probe **6** (NAH-C3-GPKK-biotin) was designed and synthesized for selectivity profiling (Scheme 2). To further confirm our design strategy, we also synthesized compound **5** (NAH-C3-GPKKpG) with a pG as the appendage (Scheme 2). Compound **5** demonstrated a comparable IC_{50} value (120 ± 20 nM) as compound **1** (150 ± 20 nM) in the SAHH-coupled fluorescence assay with a preincubation time of 10 minutes (Figure S1), supporting our design.

Scheme 2. Synthetic route for compounds **5** and **6**.^a

^aReagents and conditions: (a) DIPEA, DMF, r.t.; (b) TFA: DODT: H₂O: TIPS = 94: 2.5: 2.5: 1; (c) Biotin TEG-azide, CuSO₄, sodium ascorbate, DMF, r.t..

Chemical Proteomic Profiling with Immobilized Probe 6. To investigate the optimal condition for the pulldown study, we first preincubated purified recombinant NTMT1 with immobilized probe **6** on streptavidin beads and eluted with a salt gradient in the elution buffer. NTMT1 was eluted off with the probe until the salt

concentration reached 4 M NaCl, confirming strong interaction between **6** and NTMT1 (Figure S2). To remove non-specific and weak binders, we chose the lysis buffer containing 700 mM NaCl to wash the immobilized beads after preincubation with HeLa cell lysates. In parallel, the streptavidin beads alone were used as a negative control to remove any nonspecific binder. NTMT1 was enriched by probe **6** but not in the bead alone (Figure 4A). Specific enrichment of NTMT1 was further confirmed by competition experiments using the free inhibitor **1** (Figure 4A, Probe 6+1), indicating that pre-incubation of the lysate with **1** blocked the interaction between probe **6** and NTMT1 and allowed for the identification of specific interacting partners for probe **6**. NTMT1 was not washed off by the buffer containing 700 mM NaCl (Figure 4A, 700 mM wash lanes in probe **6**), consistent with the tight interaction between NTMT1 and probe **6**.

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Gene name	Protein name	-Log10 (p-value)	Log2 (6/1+6)	Gene name	Protein name	-Log10 (p-value)	Log2 (6/1+6)
CIT	Citron Rho-interacting kinase	3.3	12.1	TRMT112	Multifunctional methyltransferase subunit TRM112-like protein	2.5	6.8
RAB13	Ras-related protein Rab-13	2.6	8.3	ARF3	ADP-ribosylation factor 3	2.5	6.7
HemK2	HemK methyltransferase family member 2	2.8	8.3	SLC25A12	Calcium-binding mitochondrial carrier protein Aralar1	2.5	6.6
DDX3X	ATP-dependent RNA helicase DDX3X	2.5	7.7	RAB7A	Ras-related protein Rab-7a	2.4	6.6
RPN1	Dolichyl-diphospho oligo saccharide protein glycosyl transferase	2.5	7.6	IKBIP	Inhibitor of nuclear factor kappa-B kinase-interacting protein	2.4	6.5
ARF5	ADP-ribosylation factor 5	2.6	7.4	RPL36	60S ribosomal protein L36	2.3	6.4
CANX	Calnexin	2.4	7.4	DDX21	Nucleolar RNA helicase 2	2.1	6.3
NTMT1	N-terminal Xaa-Pro-Lys N-methyltransferase 1	3.2	7.3	SLC25A13	Calcium-binding mitochondrial carrier protein Aralar2	2.5	6.2
EIF4A3	Eukaryotic initiation factor 4A-III	2.4	7.2	RAN	GTP-binding nuclear protein RAN	2.2	6.2
USMG5	Up-regulated during skeletal muscle growth protein 5	2.5	6.9	ATL3	Atlastin-3	2.2	6.2
DHX9	ATP-dependent RNA helicase A	2.5	6.9	MATR3	Matrin-3	2.2	6.1
EIF4A1	Eukaryotic initiation factor 4A-I	2.4	6.8				

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Figure 4. Competitive chemical proteomic profiling with immobilized probe **6**. (A) Probe **6** enriches NTMT1 from HeLa cell lysates. Western blot analysis of NTMT1 in HeLa cells lysates with the presence or absence of NTMT1 inhibitor **1** were incubated with immobilized probe **6**. The NTMT1 immunoblots of the flow-through (FT), 700 mM NaCl wash, and boiled beads with anti-NTMT1 antibody (Cell signaling #13432) were shown. (B) The volcano plot of $-\log_{10}(\text{p-value})$ vs $\log_2(\text{probe/competition})$ for proteins identified via LC-MS/MS using probe **6** and inhibitor **1**. Statistically significant interactors for probe **6** were defined as protein hits with fold change = $\log_2(\text{Intensity (probe 6)}/\text{Intensity (inhibitor 1 + probe 6)}) \geq 6$ and a confidence value = $-\log_{10}(\text{P-value}) \geq 2$. Positions of NTMT1 and HemK2 are indicated in the plot by red circles. (C) The list of proteins that show statistically significant enrichment with probe **6** from HeLa cells. The third column shows confidence values ($-\log_{10}(\text{P-value})$), and the fourth column shows enrichment scores. (D) STRING network of significant interactors for probe **6**. The STRING database was used to generate a network with these interactors. Each circle (node) represents a protein. Each line between the nodes represents a detected connection between two proteins in the STRING database. (E) Reactome pathway analysis of significant interactors for probe **6**. The interactors involved pathways were analyzed by STRING and DAVID databases. Proteins involved in each pathway were shown in the same color (Figure 4D) as the pathway bar. Proteins shown in white were not involved in any pathway from these two databases.

Having confirmed the specific enrichment of NTMT1 by immobilized probe **6**, we performed unbiased proteomic analysis of HeLa cell proteomics enriched by probe **6** through LC-MS/MS. We identified 23 proteins that were preferentially enriched by probe **6** with ≥ 6 -fold change of ($\log_2(\text{probe 6}/\text{probe 6} + 1)$) and ≥ 2 of confidence value ($-\log_{10}(\text{P-value})$) for each identified protein (Figure 4B, Tables S1). Besides NTMT1, HemK methyltransferase family member 2 (HemK2, also known as KMT9^{26,30} or N6AMT1^{4,30}), and its binding partner Trm112, are the only identified methyltransferase subunits among the 23 identified proteins (Figure 4B-C), supporting the relatively high selectivity of the NTMT1 bisubstrate inhibitor. Interestingly, both NTMT1 and HemK2 were identified as differentially expressed genes potentially involved in glioma stem cell DNA hypermethylation (Figure 4D).³¹ Other identified proteins included citron Rho-interacting kinase, small GTPases, RNA helicases, and ADP-ribosylation factors that have been involved in various pathways (Figure 4B-E). Notably, those identified proteins could either directly interact with the bait **6** or indirectly interact through NTMT1 or other possible targets. Validation on those interactions will be pursued through a pulldown study with purified NTMT1 or immunoprecipitation in the future. HemK2 and Trm112 forms a functional methyltransferase complex,^{4,26,30} with HemK2 as the catalytic subunit and Trm112 as an activator. The HemK2-Trm112 methyltransferase complex has a dual methyltransferase activity for either the protein lysine residue of histone H4 (named as KMT9) or glutamine residue of eRF1 (named as HemK2).^{4,26} Although methyltransferase activity on a glutamine peptide (GGQSALR) is better than a lysine peptide (GKGGAKR) with both sequences conform to a similar motif as G-Q/K-XXX-K/R.^{4,26}

HemK2/KMT9 contains the SAM binding site and the substrate-binding site, while probe **6** consisted of a SAM analogue (NAH) and a peptide sequence GPKK containing two lysine residues. Thus, we hypothesized that the HemK2/KMT9 methyltransferase complex may bind with the NAH moiety or/and lysine-rich peptide sequence of the probe **6**. To validate this potential interaction, we tested the inhibitory activity of the parent compound **1** for the HemK2/KMT9 complex with a preferred GGQ substrate peptide.³⁰ In Figure

5, compound **1** exhibited a potent inhibition for HemK2/KMT9 with an IC_{50} value of $0.30 \pm 0.03 \mu M$ at $0.6 \mu M$ of the complex, which is tenfold more potent than a general methyltransferase inhibitor sinefungin (Figure 5A-B). When the concentration of the HemK2/KMT9 complex increased to $10 \mu M$, compound **1** exhibited an IC_{50} value of $6.3 \pm 0.8 \mu M$, while a general methyltransferase inhibitor sinefungin only inhibited about 50% of activity at $100 \mu M$ (Figure 5C-D). As the IC_{50} value was approximately half of the enzyme concentration used in the assay in both cases, compound **1** acted as a tight inhibitor for the HemK2/KMT9 complex.³² To our knowledge, this is the first reported inhibitor for HemK2/KMT9 activity. Through monomethylation of histone H4 lysine 12 (H4K12), HemK2/KMT9 colocalized with H4K12me1 at gene promoters and regulated the gene transcription initiation.²⁶ Overexpression of HemK2/KMT9/N6AMT1 was observed in prostate cancer cells, and knockdown of HemK2/KMT9/N6AMT1 strongly reduced the proliferation of prostate cancer cell lines.²⁶ Besides, knockdown of HemK2/KMT9/N6AMT1 severely inhibited the growth of prostate tumor xenografts.²⁶ Giving those emerging implications of HemK2/KMT9/N6AMT1 in prostate cancers, compound **1** would provide a rational lead for the development of inhibitors targeting the HemK2/KMT9 methyltransferase complex to treat prostate cancer, as well as further improve the selectivity of NTMT1 inhibitors.

Figure 5. IC_{50} curves of compound **1** (NAH-C3-GPKK) and sinefungin for HemK2/KMT9-Trm112 activity on a GGQ peptide ($n=3$). (A) and (B) IC_{50} curves of compound **1** (NAH-C3-GPKK) and sinefungin at $0.6 \mu M$ of the complex; (C) and (D) IC_{50} curves of compound **1** (NAH-C3-GPKK) and sinefungin at $10 \mu M$ of the complex.

CONCLUSION

In summary, we have rationally developed an affinity probe **6** based on the parent compound **1** that is a highly potent and relatively selective bisubstrate inhibitor for NTMT1. Chemproteomic studies with probe **6** confirmed the high selectivity of the NTMT1 bisubstrate inhibitor since the HemK2/KMT9-Trm112 methyltransferase complex was the only other methyltransferase that was enriched by probe **6**. Subsequent validation confirmed the parent inhibitor **1** was a tight inhibitor for the HemK2/KMT9 activity. As the first inhibitor for HemK2/KMT9, compound **1** would be a valuable probe and lead to developing potent inhibitors for HemK2/KMT9. Additionally, the future molecular mechanism investigation of **1** for HemK2/KMT9 would afford a solid basis to develop selective inhibitors for either methyltransferase.

METHODS

Chemistry General Procedures. The reagents and solvents were purchased from commercial sources (Fisher) and used directly. Final compounds were purified on preparative high-pressure liquid chromatography (RP-HPLC) was performed on Agilent 1260 Series system. Systems were run with 0-20% methanol/water gradient with 0.1% TFA. Matrix-assisted laser desorption ionization mass spectra (MALDI-MS) data were acquired in positive-ion mode using a Sciex 4800 MALDI TOF/TOF MS. The peptides were synthesized using a CEM Liberty Blue Automated Microwave Peptide Synthesizer with the manufacturer's standard coupling cycles at 0.1 mmol scales. The purity of the final compounds was confirmed by Agilent 1260 Series HPLC system. Systems were run with a 0-40% methanol/water gradient with 0.1% TFA. All the purity of target compounds showed >95%.

*α-bromo peptides (G*PK, G*PKK, G*PKKpG).* To the solution of bromo acetic acid (139 mg, 1 mmol) in DMF(3 mL), DIC (155 μL, 1 mmol) was added and the solution was stirred for 20min at r.t. Then added the

solution into the peptides on the resin (0.1 mmol). The mixture was shaken overnight at r.t. The suspension was filtered, and the resin was washed with DMF (3 ml x 3). Test cleavage (TFA: TIPS: H₂O = 95:2.5:2.5) was performed to monitor the progress of the reaction.

NAH-C3-GPKK (**1**). To a suspension of G*PKK on the resin (0.1 mmol) in DMF (3 mL) was added compound **7** (91 mg, 0.15 mmol) and DIPEA (26 μ L, 0.15 mmol). The mixture was shaken at room temperature overnight. The suspension was filtered, and the resin was subsequently washed with DMF (3 mL x 3), MeOH (3 mL x 3), and CH₂Cl₂ (3 mL x 3). The peptide conjugates were cleaved from the resin in the presence of a cleavage cocktail (10 mL) containing trifluoroacetic acid (TFA)/2,2'-(Ethylenedioxy)-diethanethiol (DODT) /triisopropylsilane (TIPS) /water (94:2.5:1:2.5 v/v) at room temperature for 4-5 h and resin was rinsed with a small amount of TFA. Volatiles were removed under N₂ and the residue was precipitated with 10 vol. cold anhydrous ether, and collected by centrifugation. The supernatant was discarded and the pellet was washed with chilled ether and air-dried. The white precipitate was dissolved in ddH₂O and purified by reverse phase HPLC using a waters system with an XBridge (BEH C18, 5 μ m, 10 X 250 mm) column with 0.1% TFA in water (A) and MeOH (B) as the mobile phase with monitoring at 214 nm and 254 nm. MALDI-MS (positive) m/z: calcd for C₃₆H₆₃N₁₄O₉ [M + H]⁺ m/z 835.4902, found m/z 835.5424.

NAH-C4-GPKK (**2**). The compound was prepared according to the procedure for NAH-C3-GPKK (**1**) and purified by reverse-phase HPLC. MALDI-MS (positive) m/z: calcd for C₃₇H₆₅N₁₄O₉ [M + H]⁺ m/z 849.5059, found m/z 849.7359.

NAH-C3-GPK (**3**). The compound was prepared according to the procedure for NAH-C3-GPKK (**1**) and purified by reverse-phase HPLC. MALDI-MS (positive) m/z: calcd for C₃₀H₅₁N₁₂O₈ [M + H]⁺ m/z 707.3953, found m/z 707.5096.

NAH-C4-GPK (4). The compound was prepared according to the procedure for NAH-C3-GPKK (1) and purified by reverse-phase HPLC. MALDI-MS (positive) m/z: calcd for $C_{31}H_{53}N_{12}O_8$ $[M + H]^+$ m/z 721.4109, found m/z 721.5273.

NAH-C3-GPKKpG (5). The compound was prepared according to the procedure for NAH-C3-GPKK (1) and purified by reverse-phase HPLC. MALDI-MS (positive) m/z: calcd for $C_{41}H_{68}N_{15}O_{10}$ $[M + H]^+$ m/z 930.5274, found m/z 930.6367.

NAH-C3-GPKK-Biotin (6). To a suspension of G*PKK-pG on the resin (0.05 mmol) in DMF (2 mL) was added compound 7 (45 mg, 0.075 mmol) and DIPEA (13 μ L, 0.075 mmol). The mixture was shaken at room temperature overnight. The suspension was filtered, and the resin was subsequently washed with DMF (3 mL x 3), MeOH (3 mL x 3), and CH_2Cl_2 (3 mL x 3). Then $CuSO_4 \cdot 5H_2O$ (25 mg, 0.1 mmol), sodium ascorbate (20 mg, 0.1 mmol), and DMF (2 mL) were mixed with the resin. The mixture was shaken overnight at room temperature. The cleavage and purification of the conjugate were conducted according to the procedure for NAH-C3-GPKK (1). MALDI-MS (positive) m/z: calcd for $C_{59}H_{100}N_{21}O_{15}S$ $[M + H]^+$ m/z 1374.7428, found m/z 1375.1959.

Protein Expression and Purification. Expression and purification of NTMT1, G9a, SETD7, PRMT1, PRMT3, TbPRMT7, and HemK2-Trm112 were performed as previously described.^{8,25,30,33–35}

NTMT1 Biochemical Assays. A fluorescence-based SAHH-coupled assay was applied to study the IC_{50} and $K_{i,app}$ values of compounds with both SAM and the peptide substrate at their K_m values. For 10min pre-incubation, the methylation assay was performed under the following conditions in a final well volume of 40 μ L: 25 mM Tris (pH = 7.5), 50 mM KCl, 0.01% Triton X-100, 5 μ M SAHH, 0.1 μ M NTMT1, 3 μ M SAM, and 10 μ M ThioGlo4. The inhibitors were added at concentrations ranging from 0.15 nM to 10 μ M. After 10 min incubation, reactions were initiated by the addition of 0.5 μ M GPKRIA peptide. For 120min pre-incubation,

NTMT1 and compounds were pre-incubated for 120min. Then the pre-incubation mixture was added into reaction mixture: 25 mM Tris (pH = 7.5), 50 mM KCl, 0.01% Triton X-100, 5 μ M SAHH, 3 μ M SAM, and 10 μ M ThioGlo4. The reactions were initiated by the addition of 0.5 μ M GPKRIA peptide. Fluorescence was monitored on a BMG CLARIOstar microplate reader with excitation of 400 nm and emission of 465 nm. Data were processed by using GraphPad Prism software 7.0.

Selectivity Assays. The selectivity studies of G9a, SETD7, PRMT1, PRMT3, *Tb*PRMT7, and SAHH were performed as previously described.^{24,25,36}

MALDI-MS Methylation Inhibition Assay. The methylation inhibition study of compound **1** with NTMT1 was performed as previously described.²⁵

HemK2 Methylation Inhibition Assay. The methylation inhibition assays of HemK2 was performed in a 20 μ L reaction mixture containing 20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM DTT and 20 μ M SAM at 37°C, using high or low enzyme concentrations (10 μ M or 0.6 μ M), varied concentrations of eRF1 peptide (residues 179-183: KHGRGGQSALRFARL) (10 μ M or 400 μ M) and of compound **1** (NAH-C3-GPKK) or Sinefungin. The SAM-dependent methylation activity was measured by the Promega bioluminescence assay.³⁰

Pulldown Assay. Probe **6** (100 μ L, 100 μ M) was incubated with 50 μ L streptavidin resin overnight in 20mM Tris, 50 mM KCl, pH=7.5. After washing out the nonbounded compound, purified NTMT1 was incubated with the probe-covered resin in 20 mM Tris, 50 mM KCl, 1 mM DTT, pH=7.5 for 4 h. Different concentration of the salt solution (500 mM, 1 M, 2 M, 4 M NaCl) was used to elute NTMT1.

HeLa cells (~ 6x10⁸ cells/mL) were lysed with fresh IP buffer containing 25 mM Tris, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 5% glycerol, protease inhibitor (Halt™ protease inhibitor cocktail) pH 7.4. The cell suspension was incubated in rotation for 1 h at 4 °C and centrifuged at 15,000 g for 25 min at 4 °C to obtain

the supernatant. The concentration of protein was determined with a BSA protein assay. The HeLa cell lysate was incubated with probe-covered resin overnight. For the competitor pulldown assay, competitor compound **1** (10 mM, 5 μ L) was treated to the cell lysate first and incubate with compound-covered resin overnight. Resin alone was used as a negative control. Washed the resin five times with 1 mL IP buffer five times to remove the nonbounded proteins. The resin was then washed with 700 mM NaCl five times to remove the weak binding proteins. 1X SDS loading buffer was added and the resin was boiled for 5 min at 95 °C to elute proteins on it. Protein samples (10 μ L) were loaded on 12.5 % SDS-PAGE gels and run at 120 V until the proteins come to the edge of the gel. Coomassie blue was used to stain the gels. Proteins on the gel were transferred to the PVDF membrane. After 5% milk blocking, the membrane was incubated with anti-NTMT1 (1:1000 dilution in 5% BSA in TBST buffer, Cell signaling #13432) at 4 °C overnight. Anti-rabbit IgG, HRP-linked antibody (1:1000 dilution in 5% BSA in TBST buffer, Cell signaling #7074) was incubated at 4 °C for 1 h. The membrane was developed with ECL substrate (BIO-RAD #1705061) and imaged with a Proteinsimple FluorChem R.

Protein samples (10 μ L) were loaded on 12.5 % SDS-PAGE gels and run at 120 V for 10 min. Protein bands were cut and submitted to the Laboratory for Biological Mass Spectrometry at Indiana University for LC-MS/MS.

Co-crystallization and Structure Determination. The co-crystallization and structure determination of compound **1** with NTMT1 were performed as previously described.^{24,25} Purified NTMT1 (30 mg/mL) was mixed with **1** at a 1:2 molar ratio, and the mixture was incubated overnight at 4°C. Using the hanging-drop vapor diffusion method and incubation at 20°C, the crystals of **1** were grown in 1.5 M lithium sulfate, 0.1 M sodium HEPES (pH = 7.5). The crystals were then harvested and flash frozen in liquid nitrogen. Data were collected on a single crystal at 12.0 keV at the GM/CA-CAT ID-D beamline at the Advanced Photon Source,

Argonne National Laboratory. The data were processed (Table S2) and the structure was solved as previously described.^{24,25}

ASSOCIATED CONTENT

Supporting Information

MS and HPLC analysis of compounds **1-6**; Figure S1. IC₅₀ curves for compounds **1** and **5**; Figure S2. Elution of recombinant NTMT1 from probe **6** coupled resin; Table S1. Proteomic analysis of HeLa cell proteomics enriched by probe **6** through LC-MS/MS; Table S2. Crystallography data and refinement statistics (PDB ID: 6PVA).

Accession Codes

The coordinates for the structure of human NTMT1 in complex with compound **NAH-C3-GPKK** (PDB ID: 6PVA) has been deposited in the Protein Data Bank.

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Author Contributions

R.H. conceived and directed the project. R.H. and D.C. designed the compounds. D.C. synthesized, purified, and characterized the compounds. D.C. purified NTMT1 and performed co-crystallization. N.N.

collected X-ray diffraction data and refined the co-crystal structure. Y.M. performed the pulldown study and bioinformatic analysis. X.C. supervised and D.Y. characterized the inhibition study for HemK2-Trm112. D.C. and Y.M. wrote the manuscript. R.H. and X.C. edited the manuscript. All authors provided critical feedback and have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

NTMT, protein N-terminal methyltransferase; SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteines; SAHH, SAH hydrolase; MT, methyltransferase; PKMT, protein lysine methyltransferase;

PRMT, protein arginine methyltransferase; G9a, euchromatic histone-lysine N-methyltransferase 2; SETD7, SET domain-containing protein 7; *TbPRMT7*, *Trypanosoma brucei* protein arginine methyltransferase 7; HemK2, HemK methyltransferase family member 2; rt, room temperature; TFA, trifluoroacetic acid.

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Supporting Information

Chemoproteomic study uncovers a new target HemK2/KMT9 for NTMT1 bisubstrate inhibitors

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Table of Contents

- A. MS and HPLC analysis of compounds **1-6**
- B. Figure S1. IC₅₀ curves for compounds **1** (A) and **5** (B), respectively
- C. Figure S2. Elution of recombinant NTMT1 from probe **6** coupled resin
- D. Table S1. Proteomic analysis of HeLa cell proteomics enriched by probe **6** through LC-MS/MS.
- E. Table S2. Crystallography data and refinement statistics (PDB ID: 6PVA).

MS and HPLC analysis of compounds 1-6
MALDI-MS and HPLC spectra of compound 1

MALDI-MS spectra of compound 1

HPLC spectra of compound 1

MALDI-MS and LC-MS spectra of compound 2

MALDI-MS spectra of compound 2

HPLC spectra of compound 2

MALDI-MS and LC-MS spectra of compound 3

MALDI-MS spectra of compound 3

HPLC spectra of compound 3

MALDI-MS and HPLC spectra of compound 4

MALDI-MS spectra of compound 4

HPLC spectra of compound 4

MALDI-MS and HPLC spectra of compound 5

MALDI-MS spectra of compound 5

HPLC spectra of compound 5

MALDI-MS and HPLC spectra of compound 6

MALDI-MS spectra of compound 6

HPLC spectra of compound 6

Supporting Figure S1. IC_{50} curves for compounds **1** (A) and **5** (B), respectively.

Supporting Figure S2. Elution of recombinant NTMT1 from probe **6** coupled resin.

Probe 6 shows tight binding with NTMT1. Recombinant NTMT1 were incubated with probe 6 coupled streptavidin beads and eluted with different concentration of salt solution (0.15, 0.5, 1, 2, and 4 M).

Supporting Table S1. Proteomic analysis of HeLa cell proteomics enriched by probe 6 through LC-MS/MS.

Accession	Protein name	Fold change = Log2(probe 6/ (inhibitor 1 + probe 6))	Confidence value = $-\log_{10}(\text{P-value})$
O14578-3	Isoform 3 of Citron Rho-interacting kinase [OS=Homo sapiens]	12.1	3.3
Q9Y5N5	HemK methyltransferase family member 2 [OS=Homo sapiens]	8.3	2.8
P51153	Ras-related protein Rab-13 [OS=Homo sapiens]	8.3	2.6
O00571	ATP-dependent RNA helicase DDX3X [OS=Homo sapiens]	7.7	2.5
Q96AG4	Leucine-rich repeat-containing protein 59 [OS=Homo sapiens]	7.6	1.6
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 [OS=Homo sapiens]	7.6	2.5
P84085	ADP-ribosylation factor 5 [OS=Homo sapiens]	7.4	2.6
P27824-2	Isoform 2 of Calnexin [OS=Homo sapiens]	7.4	2.4
Q9BV86-1	N-terminal Xaa-Pro-Lys N-methyltransferase 1 [OS=Homo sapiens]	7.3	3.2
P38919	Eukaryotic initiation factor 4A-III [OS=Homo sapiens]	7.2	2.4
Q08211	Atp-dependent rna helicase a [OS=Homo sapiens]	6.9	2.5
Q96IX5	Up-regulated during skeletal muscle growth protein 5 [OS=Homo sapiens]	6.9	2.5
P60842	Eukaryotic initiation factor 4A-I [OS=Homo sapiens]	6.8	2.4
Q9UI30	Multifunctional methyltransferase subunit TRM112-like protein [OS=Homo sapiens]	6.8	2.5
P61204	ADP-ribosylation factor 3 [OS=Homo sapiens]	6.7	2.5
P51149	ras-related protein Rab-7a [OS=Homo sapiens]	6.6	2.4

Q70UQ0-4	Isoform 4 of Inhibitor of nuclear factor kappa-B kinase-interacting protein [OS=Homo sapiens]	6.5	2.4
Q9Y3U8	60S ribosomal protein L36 [OS=Homo sapiens]	6.4	2.3
P13073	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial [OS=Homo sapiens]	6.4	1.7
Q9NR30-1	Nucleolar RNA helicase 2 [OS=Homo sapiens]	6.3	2.1
P62826	GTP-binding nuclear protein RAN [OS=Homo sapiens]	6.2	2.2
P43243	Matrin-3 [OS=Homo sapiens]	6.2	2.2
O75746	Calcium-binding mitochondrial carrier protein Aralar1 [OS=Homo sapiens]	6.2	2.5
Q9UJS0-2	Isoform 2 of Calcium-binding mitochondrial carrier protein Aralar2 [OS=Homo sapiens]	6.2	2.5
P00403	Cytochrome c oxidase subunit 2 [OS=Homo sapiens]	6.2	1.9
Q6DD88	Atlastin-3 [OS=Homo sapiens]	6.1	2.3
P51148-2	Isoform 2 of Ras-related protein Rab-5C [OS=Homo sapiens]	6.0	2.3
Q96A26	Protein FAM162A [OS=Homo sapiens]	5.9	2.3
O00567	Nucleolar protein 56 [OS=Homo sapiens]	5.9	2.3
Q9NZ45	CDGSH iron-sulfur domain-containing protein 1 [OS=Homo sapiens]	5.7	2.0
P61224-1	Ras-related protein Rap-1b [OS=Homo sapiens]	5.6	2.1
Q14697-2	Isoform 2 of Neutral alpha-glucosidase AB [OS=Homo sapiens]	5.6	2.2
Q9Y277-2	Isoform 2 of Voltage-dependent anion-selective channel protein 3 [OS=Homo sapiens]	5.5	0.9
O75947-1	ATP synthase subunit d, mitochondrial [OS=Homo sapiens]	5.5	1.8
Q9BVP2	Guanine nucleotide-binding protein-like 3 [OS=Homo sapiens]	5.5	2.0
Q16790	Carbonic anhydrase 9 [OS=Homo sapiens]	5.4	1.8

O60762	Dolichol-phosphate mannosyltransferase subunit 1 [OS=Homo sapiens]	5.4	1.7
O60506	Heterogeneous nuclear ribonucleoprotein Q [OS=Homo sapiens]	5.4	0.9
O43169	Cytochrome b5 type B [OS=Homo sapiens]	5.3	1.9
Q9BWF3-1	RNA-binding protein 4 [OS=Homo sapiens]	5.2	2.1
Q9Y2X3	Nucleolar protein 58 [OS=Homo sapiens]	5.2	2.1
P36542-1	ATP synthase subunit gamma, mitochondrial [OS=Homo sapiens]	5.2	1.7
P33993-1	DNA replication licensing factor MCM7 [OS=Homo sapiens]	5.1	2.0
O43809	Cleavage and polyadenylation specificity factor subunit 5 [OS=Homo sapiens]	5.1	1.7
P12956	X-ray repair cross-complementing protein 6 [OS=Homo sapiens]	5.1	2.2
Q07065	Cytoskeleton-associated protein 4 [OS=Homo sapiens]	5.0	2.1
P01891	HLA class I histocompatibility antigen, A-68 alpha chain [OS=Homo sapiens]	5.0	1.9
P51648-2	Isoform 2 of Fatty aldehyde dehydrogenase [OS=Homo sapiens]	5.0	2.2
P22626	heterogeneous nuclear ribonucleoproteins A2/B1 [OS=Homo sapiens]	4.9	1.0
P16615	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 [OS=Homo sapiens]	4.9	0.9
Q9NVI7-2	Isoform 2 of ATPase family AAA domain-containing protein 3A [OS=Homo sapiens]	4.9	1.4
Q9Y4P3	Transducin beta-like protein 2 [OS=Homo sapiens]	4.9	2.0
P20340-1	Ras-related protein Rab-6A [OS=Homo sapiens]	4.8	1.2
P61026	ras-related protein rab-10 [OS=Homo sapiens]	4.7	1.3

P11476-1	Ras-related protein Rab-1A [OS=Homo sapiens]	4.7	1.3
P21796	voltage-dependent anion-selective channel protein 1 [OS=Homo sapiens]	4.7	1.1
P61106	Ras-related protein Rab-14 [OS=Homo sapiens]	4.7	1.3
O00264	Membrane-associated progesterone receptor component 1 [OS=Homo sapiens]	4.7	0.9
P78527	DNA-dependent protein kinase catalytic subunit [OS=Homo sapiens]	4.6	0.9
P34897-1	Serine hydroxymethyltransferase, mitochondrial [OS=Homo sapiens]	4.6	0.9
P69905	Hemoglobin subunit alpha [OS=Homo sapiens]	4.6	1.6
P63173	60s ribosomal protein l38 [OS=Homo sapiens]	4.6	1.4
P62491-1	Ras-related protein Rab-11A [OS=Homo sapiens]	4.5	1.9
P30508	HLA class I histocompatibility antigen, Cw-12 alpha chain [OS=Homo sapiens]	4.5	1.4
Q12905	Interleukin enhancer-binding factor 2 [OS=Homo sapiens]	4.5	0.9
P62273-2	Isoform 2 of 40S ribosomal protein S29 [OS=Homo sapiens]	4.4	0.8
Q8NBJ4	Golgi membrane protein 1 [OS=Homo sapiens]	4.4	1.9
Q9BVK6	Transmembrane emp24 domain-containing protein 9 [OS=Homo sapiens]	4.4	1.6
P16989-1	Y-box-binding protein 3 [OS=Homo sapiens]	4.3	1.8
P05556-1	Integrin beta-1 [OS=Homo sapiens]	4.3	0.9
P46940	Ras GTPase-activating-like protein IQGAP1 [OS=Homo sapiens]	4.2	2.3
O95292	Vesicle-associated membrane protein-associated protein B/C [OS=Homo sapiens]	4.2	1.0
P09669	Cytochrome c oxidase subunit 6C [OS=Homo sapiens]	4.2	0.9

Q9P0L0-2	Isoform 2 of Vesicle-associated membrane protein-associated protein A [OS=Homo sapiens]	4.2	1.5
O00461	Golgi integral membrane protein 4 [OS=Homo sapiens]	4.2	1.6
Q9NZ01-1	Very-long-chain enoyl-CoA reductase [OS=Homo sapiens]	4.1	0.9
P06576	ATP synthase subunit beta, mitochondrial [OS=Homo sapiens]	4.1	3.0
P61978-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein K [OS=Homo sapiens]	4.0	1.2
P22087	rRNA 2'-O-methyltransferase fibrillarin [OS=Homo sapiens]	4.0	1.3
P62910	60S ribosomal protein L32 [OS=Homo sapiens]	3.9	1.6
Q9NXW2-2	Isoform 2 of DnaJ homolog subfamily B member 12 [OS=Homo sapiens]	3.9	1.8
O43390-2	Isoform 2 of Heterogeneous nuclear ribonucleoprotein R [OS=Homo sapiens]	3.8	1.0
P27635	60S ribosomal protein L10 [OS=Homo sapiens]	3.7	1.3
Q8NE86	Calcium uniporter protein, mitochondrial [OS=Homo sapiens]	3.6	1.0
O75367-1	Core histone macro-H2A.1 [OS=Homo sapiens]	3.5	1.0
P62805	histone H4 [OS=Homo sapiens]	3.5	1.4
Q9Y5S2	Serine/threonine-protein kinase MRCK beta [OS=Homo sapiens]	3.4	1.8
P01889	HLA class I histocompatibility antigen, B-7 alpha chain [OS=Homo sapiens]	3.3	0.9
P62753	40S RIBOSOMAL PROTEIN S6 [OS=Homo sapiens]	3.3	1.2
Q14254	Flotillin-2 [OS=Homo sapiens]	3.3	1.3
P42167	Lamina-associated polypeptide 2, isoforms beta/gamma [OS=Homo sapiens]	3.3	2.0
P17301	Integrin alpha-2 [OS=Homo sapiens]	3.3	1.0

Q96CW1	AP-2 complex subunit mu [OS=Homo sapiens]	3.2	1.2
P42677	40S ribosomal protein S27 [OS=Homo sapiens]	3.2	3.5
Q96CS3	FAS-associated factor 2 [OS=Homo sapiens]	3.2	0.9
Q9HDC9	Adipocyte plasma membrane-associated protein [OS=Homo sapiens]	3.1	0.8
P49748-3	Isoform 3 of Very long-chain specific acyl-CoA dehydrogenase, mitochondrial [OS=Homo sapiens]	3.1	1.1
P50914	60S ribosomal protein L14 [OS=Homo sapiens]	3.1	2.0
P27348	14-3-3 protein theta [OS=Homo sapiens]	3.1	0.5
Q9Y265	RuvB-like 1 [OS=Homo sapiens]	3.1	1.0
P62258-1	14-3-3 protein epsilon [OS=Homo sapiens]	3.1	0.5
P17844	probable ATP-dependent RNA helicase DDX5 [OS=Homo sapiens]	3.1	2.8
P61981	14-3-3 protein gamma [OS=Homo sapiens]	3.0	0.5
P19388	DNA-directed RNA polymerases I, II, and III subunit RPABC1 [OS=Homo sapiens]	3.0	0.6
O14929	histone acetyltransferase type B catalytic subunit [OS=Homo sapiens]	3.0	0.5
P60866-2	Isoform 2 of 40S ribosomal protein S20 [OS=Homo sapiens]	3.0	1.0
Q92841	Probable ATP-dependent RNA helicase DDX17 [OS=Homo sapiens]	2.9	1.8
Q71UM5	40S ribosomal protein S27-like [OS=Homo sapiens]	2.9	2.3
P31947-1	14-3-3 protein sigma [OS=Homo sapiens]	2.9	0.4
P14625	Endoplasmic reticulum chaperone [OS=Homo sapiens]	2.8	3.9
P02788	Lactotransferrin [OS=Homo sapiens]	2.7	0.5
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 [OS=Homo sapiens]	2.7	1.7
Q9Y5M8	signal recognition particle receptor subunit beta [OS=Homo sapiens]	2.7	1.3

P45880-1	Isoform 1 of Voltage-dependent anion-selective channel protein 2 [OS=Homo sapiens]	2.7	1.4
P53680	AP-2 complex subunit sigma [OS=Homo sapiens]	2.7	0.5
O75396	Vesicle-trafficking protein SEC22b [OS=Homo sapiens]	2.6	1.6
P48047	ATP synthase subunit O, mitochondrial [OS=Homo sapiens]	2.6	2.0
P63104-1	14-3-3 protein zeta/delta [OS=Homo sapiens]	2.6	0.5
P29401-2	Isoform 2 of Transketolase [OS=Homo sapiens]	2.6	0.9
P14868	Aspartate--tRNA ligase, cytoplasmic [OS=Homo sapiens]	2.5	1.9
O00425	Insulin-like growth factor 2 mRNA-binding protein 3 [OS=Homo sapiens]	2.5	0.6
P31946	14-3-3 protein beta/alpha [OS=Homo sapiens]	2.5	0.4
P08237-3	Isoform 3 of ATP-dependent 6-phosphofructokinase, muscle type [OS=Homo sapiens]	2.4	0.3
Q9Y6M9	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 [OS=Homo sapiens]	2.4	0.8
P25705-1	ATP synthase subunit alpha, mitochondrial [OS=Homo sapiens]	2.4	1.7
P39023	60S ribosomal protein L3 [OS=Homo sapiens]	2.4	1.3
Q9HA64	Ketosamine-3-kinase [OS=Homo sapiens]	2.4	1.8
P32969	60S ribosomal protein L9 [OS=Homo sapiens]	2.3	2.4
Q8N1F7-1	Nuclear pore complex protein Nup93 [OS=Homo sapiens]	2.3	1.4
P62424	60S ribosomal protein L7a [OS=Homo sapiens]	2.3	1.2
Q96A08	Histone H2B type 1-A [OS=Homo sapiens]	2.3	1.0

Q16891-2	Isoform 2 of MICOS complex subunit MIC60 [OS=Homo sapiens]	2.3	1.9
Q16891-4	Isoform 4 of MICOS complex subunit MIC60 [OS=Homo sapiens]	2.3	1.9
Q96S19-4	Isoform 4 of UPF0585 protein C16orf13 [OS=Homo sapiens]	2.2	0.9
Q9Y3D9	28S ribosomal protein S23, mitochondrial [OS=Homo sapiens]	2.2	1.1
P39656	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit [OS=Homo sapiens]	2.2	0.4
Q07020	60S ribosomal protein L18 [OS=Homo sapiens]	2.2	3.0
P83731	60S ribosomal protein L24 [OS=Homo sapiens]	2.1	1.3
P40939	Trifunctional enzyme subunit alpha, mitochondrial [OS=Homo sapiens]	2.1	1.5
P52597	Heterogeneous nuclear ribonucleoprotein F [OS=Homo sapiens]	2.1	1.6
P42765	3-ketoacyl-CoA thiolase, mitochondrial [OS=Homo sapiens]	2.0	0.4
P31689-1	DnaJ homolog subfamily A member 1 [OS=Homo sapiens]	2.0	1.8
P24539	ATP synthase F(0) complex subunit B1, mitochondrial [OS=Homo sapiens]	1.9	1.5
P23396-1	40S ribosomal protein S3 [OS=Homo sapiens]	1.9	1.3
P18085	ADP-ribosylation factor 4 [OS=Homo sapiens]	1.8	1.6
Q12797	Aspartyl/Asparaginyl beta-hydroxylase [OS=Homo sapiens]	1.8	0.4
Q86UE4	protein LYRIC [OS=Homo sapiens]	1.7	0.9
P62241	40S ribosomal protein S8 [OS=Homo sapiens]	1.7	1.3
Q9Y241	HIG1 domain family member 1A, mitochondrial [OS=Homo sapiens]	1.7	0.5
P50454	Serpin H1 [OS=Homo sapiens]	1.7	0.9
P18124	60S ribosomal protein L7 [OS=Homo sapiens]	1.6	1.1

P08574	Cytochrome c1, heme protein, mitochondrial [OS=Homo sapiens]	1.6	0.4
P10809	60 kDa heat shock protein, mitochondrial [OS=Homo sapiens]	1.6	1.5
P07195	L-lactate dehydrogenase B chain [OS=Homo sapiens]	1.6	0.3
Q13492-1	Phosphatidylinositol-binding clathrin assembly protein [OS=Homo sapiens]	1.6	0.3
Q92769	Histone deacetylase 2 [OS=Homo sapiens]	1.5	0.3
P46781	40S ribosomal protein S9 [OS=Homo sapiens]	1.5	1.1
P04075	fructose-bisphosphate aldolase A [OS=Homo sapiens]	1.5	0.3
Q9Y230	RuvB-like 2 [OS=Homo sapiens]	1.5	2.0
P35268	60S ribosomal protein L22 [OS=Homo sapiens]	1.5	0.9
P08195-4	Isoform 4 of 4F2 cell-surface antigen heavy chain [OS=Homo sapiens]	1.5	0.7
P61353	60S ribosomal protein L27 [OS=Homo sapiens]	1.4	0.7
Q15233	Non-POU domain-containing octamer- binding protein [OS=Homo sapiens]	1.4	0.6
Q12931	heat shock protein 75 kDa, mitochondrial [OS=Homo sapiens]	1.4	0.2
Q15365	Poly(RC)-binding protein 1 [OS=Homo sapiens]	1.4	0.9
P62244	40S ribosomal protein S15a [OS=Homo sapiens]	1.4	1.6
P02786	Transferrin receptor protein 1 [OS=Homo sapiens]	1.3	1.5
Q7Z2W4	zinc finger CCCH-type antiviral protein 1 [OS=Homo sapiens]	1.2	0.7
Q14684	Ribosomal RNA processing protein 1 homolog B [OS=Homo sapiens]	1.2	1.0
O95782	AP-2 complex subunit alpha-1 [OS=Homo sapiens]	1.2	0.6
Q99567	Nuclear pore complex protein Nup88 [OS=Homo sapiens]	1.2	0.2

P46776	60S ribosomal protein L27a [OS=Homo sapiens]	1.2	1.0
P57721-1	Poly(rC)-binding protein 3 [OS=Homo sapiens]	1.2	1.1
Q00839	Heterogeneous nuclear ribonucleoprotein U [OS=Homo sapiens]	1.1	1.2
P31943	Heterogeneous nuclear ribonucleoprotein H [OS=Homo sapiens]	1.1	0.8
P63010-2	Isoform 2 of AP-2 complex subunit beta [OS=Homo sapiens]	1.1	0.8
P08238	Heat shock protein HSP 90-beta [OS=Homo sapiens]	1.1	2.1
P67809	Nuclease-sensitive element-binding protein 1 [OS=Homo sapiens]	1.1	0.2
P54136-1	arginine--tRNA ligase, cytoplasmic [OS=Homo sapiens]	1.1	1.3
O43175	D-3-phosphoglycerate dehydrogenase [OS=Homo sapiens]	1.1	3.1
Q9NX63	MICOS complex subunit MIC19 [OS=Homo sapiens]	1.0	1.4
P35232	Prohibitin [OS=Homo sapiens]	0.9	0.5
P62829	60S ribosomal protein L23 [OS=Homo sapiens]	0.9	0.4
Q01813	ATP-dependent 6-phosphofructokinase, platelet type [OS=Homo sapiens]	0.9	1.3
P05388	60S acidic ribosomal protein P0 [OS=Homo sapiens]	0.9	1.5
P52272	Heterogeneous nuclear ribonucleoprotein M [OS=Homo sapiens]	0.9	0.5
Q9UHX1-1	poly(U)-binding-splicing factor PUF60 [OS=Homo sapiens]	0.9	0.1
P18077	60S ribosomal protein L35a [OS=Homo sapiens]	0.9	1.7
P39019	40S ribosomal protein S19 [OS=Homo sapiens]	0.9	0.8
P28066-1	Proteasome subunit alpha type-5 [OS=Homo sapiens]	0.9	0.1

Q5QNW6-2	Isoform 2 of Histone H2B type 2-F [OS=Homo sapiens]	0.8	0.5
P62277	40S ribosomal protein S13 [OS=Homo sapiens]	0.8	0.7
P62701	40S ribosomal protein S4, X isoform [OS=Homo sapiens]	0.8	1.4
Q14980-1	nuclear mitotic apparatus protein 1 [OS=Homo sapiens]	0.8	0.4
Q09666-1	Neuroblast differentiation-associated protein AHNAK [OS=Homo sapiens]	0.8	0.9
P46783	40S ribosomal protein S10 [OS=Homo sapiens]	0.7	1.1
Q99714-1	3-hydroxyacyl-CoA dehydrogenase type-2 [OS=Homo sapiens]	0.7	0.9
P06899	Histone H2B type 1-J [OS=Homo sapiens]	0.7	0.4
P04406-1	glyceraldehyde-3-phosphate dehydrogenase [OS=Homo sapiens]	0.7	0.2
P11940-1	Polyadenylate-binding protein 1 [OS=Homo sapiens]	0.7	1.0
P46778	60S ribosomal protein L21 [OS=Homo sapiens]	0.6	0.6
P62847-4	Isoform 4 of 40S ribosomal protein S24 [OS=Homo sapiens]	0.6	0.3
P62266	40S ribosomal protein S23 [OS=Homo sapiens]	0.6	0.6
Q99623	Prohibitin-2 [OS=Homo sapiens]	0.5	0.4
P18621-3	Isoform 3 of 60S ribosomal protein L17 [OS=Homo sapiens]	0.5	0.3
P68366	Tubulin alpha-4A chain [OS=Homo sapiens]	0.5	0.3
P68104	Elongation factor 1-alpha 1 [OS=Homo sapiens]	0.5	0.4
Q96PK6-1	RNA-binding protein 14 [OS=Homo sapiens]	0.5	0.5
P35613	Basigin [OS=Homo sapiens]	0.5	0.7
Q13573	SNW domain-containing protein 1 [OS=Homo sapiens]	0.4	0.1
P26641	elongation factor 1-gamma [OS=Homo sapiens]	0.4	0.6

P84098	60S ribosomal protein L19 [OS=Homo sapiens]	0.4	0.2
P04083	annexin A1 [OS=Homo sapiens]	0.4	0.1
Q9BWM7	Sideroflexin-3 [OS=Homo sapiens]	0.4	0.1
P06493	Cyclin-dependent kinase 1 [OS=Homo sapiens]	0.3	0.3
P06733-1	alpha-enolase [OS=Homo sapiens]	0.3	0.0
P60900	Proteasome subunit alpha type-6 [OS=Homo sapiens]	0.3	0.1
Q13310-3	Isoform 3 of Polyadenylate-binding protein 4 [OS=Homo sapiens]	0.3	0.5
P62888	60S ribosomal protein L30 [OS=Homo sapiens]	0.2	0.1
P26373-1	60S ribosomal protein L13 [OS=Homo sapiens]	0.2	0.3
P07900-2	Isoform 2 of Heat shock protein HSP 90-alpha [OS=Homo sapiens]	0.2	0.4
P62913	60S ribosomal protein L11 [OS=Homo sapiens]	0.2	0.6
P62750	60S ribosomal protein L23a [OS=Homo sapiens]	0.2	0.0
Q9BUF5	Tubulin beta-6 chain [OS=Homo sapiens]	0.2	0.4
P04908	histone H2A type 1-B/E [OS=Homo sapiens]	0.2	0.1
O43795	Unconventional myosin-Ib [OS=Homo sapiens]	0.2	0.1
P27105	erythrocyte band 7 integral membrane protein [OS=Homo sapiens]	0.2	0.1
Q12965	unconventional myosin-Ie [OS=Homo sapiens]	0.1	0.1
P22695	Cytochrome b-c1 complex subunit 2, mitochondrial [OS=Homo sapiens]	0.1	0.0
P83881	60S ribosomal protein L36a [OS=Homo sapiens]	0.1	0.0
Q13509	tubulin beta-3 chain [OS=Homo sapiens]	0.1	0.1
Q92614-1	Unconventional myosin-XVIIIa [OS=Homo sapiens]	0.1	0.0
P00338-3	Isoform 3 of L-lactate dehydrogenase A chain [OS=Homo sapiens]	0.0	0.0

P07910-1	Heterogeneous nuclear ribonucleoproteins C1/C2 [OS=Homo sapiens]	0.0	0.0
P14618	Pyruvate kinase PKM [OS=Homo sapiens]	0.0	0.0
Q9UKS6	Protein kinase C and casein kinase substrate in neurons protein 3 [OS=Homo sapiens]	0.0	0.0
P68371	Tubulin beta-4B chain [OS=Homo sapiens]	0.0	0.0
P36578	60S ribosomal protein L4 [OS=Homo sapiens]	0.0	0.0
P11021	78 kDa glucose-regulated protein [OS=Homo sapiens]	0.0	0.0
P19338	Nucleolin [OS=Homo sapiens]	-0.1	0.0
P05387	60S acidic ribosomal protein P2 [OS=Homo sapiens]	-0.1	0.1
P61254	60S ribosomal protein L26 [OS=Homo sapiens]	-0.2	0.1
Q01105	Protein SET [OS=Homo sapiens]	-0.2	0.0
P21333	Filamin-A [OS=Homo sapiens]	-0.2	0.1
P07814	Bifunctional glutamate/proline--tRNA ligase [OS=Homo sapiens]	-0.2	0.1
Q02878	60S ribosomal protein L6 [OS=Homo sapiens]	-0.2	0.1
Q14244-7	Isoform 7 of Ensconsin [OS=Homo sapiens]	-0.2	0.3
P0DMV8	heat shock 70 kDa protein 1A [OS=Homo sapiens]	-0.3	0.3
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial [OS=Homo sapiens]	-0.3	0.1
P62851	40S ribosomal protein S25 [OS=Homo sapiens]	-0.3	0.2
P61513	60S ribosomal protein L37a [OS=Homo sapiens]	-0.3	0.0
P62906	60S ribosomal protein L10A [OS=Homo sapiens]	-0.3	0.5

P63096-1	Guanine nucleotide-binding protein G(i) subunit alpha-1 [OS=Homo sapiens]	-0.3	0.3
P05141	ADP/ATP translocase 2 [OS=Homo sapiens]	-0.3	0.2
P62249	40S ribosomal protein S16 [OS=Homo sapiens]	-0.3	0.3
P07437	tubulin beta chain [OS=Homo sapiens]	-0.3	0.4
Q15046	Lysine--tRNA ligase [OS=Homo sapiens]	-0.4	0.9
P38646	Stress-70 protein, mitochondrial [OS=Homo sapiens]	-0.4	0.9
P35579-1	Myosin-9 [OS=Homo sapiens]	-0.4	0.2
P49411	elongation factor Tu, mitochondrial [OS=Homo sapiens]	-0.5	0.5
Q02543	60S ribosomal protein L18a [OS=Homo sapiens]	-0.5	0.2
Q8WV41	Sorting nexin-33 [OS=Homo sapiens]	-0.5	0.1
P0C0S5	Histone H2A.Z [OS=Homo sapiens]	-0.5	0.2
Q13155	aminoacyl tRNA synthase complex-interacting multifunctional protein 2 [OS=Homo sapiens]	-0.5	0.1
Q69YQ0-1	Cytospin-A [OS=Homo sapiens]	-0.5	0.3
Q9BQE3	Tubulin alpha-1C chain [OS=Homo sapiens]	-0.6	0.5
Q4KMQ1	Taperin [OS=Homo sapiens]	-0.6	0.2
P06702	Protein S100-A9 [OS=Homo sapiens]	-0.6	0.1
Q9BVA1	Tubulin beta-2B chain [OS=Homo sapiens]	-0.6	0.7
P11142-1	Heat shock cognate 71 kDa protein [OS=Homo sapiens]	-0.6	0.7
P09651-1	Heterogeneous nuclear ribonucleoprotein A1 [OS=Homo sapiens]	-0.6	0.4
P55084	Trifunctional enzyme subunit beta, mitochondrial [OS=Homo sapiens]	-0.7	0.1
Q92522	Histone H1x [OS=Homo sapiens]	-0.7	0.1
P08754	Guanine nucleotide-binding protein G(k) subunit alpha [OS=Homo sapiens]	-0.7	0.5
Q96EY1-1	DnaJ homolog subfamily A member 3, mitochondrial [OS=Homo sapiens]	-0.7	0.2

P61247	40S ribosomal protein S3a [OS=Homo sapiens]	-0.7	0.6
P35237	serpin B6 [OS=Homo sapiens]	-0.8	0.2
P12236	ADP/ATP translocase 3 [OS=Homo sapiens]	-0.8	0.6
Q562R1	Beta-actin-like protein 2 [OS=Homo sapiens]	-0.8	0.4
P17858-1	ATP-dependent 6-phosphofructokinase, liver type [OS=Homo sapiens]	-0.8	0.7
Q8IVT2	Mitotic interactor and substrate of PLK1 [OS=Homo sapiens]	-0.8	0.3
Q9HAV0	Guanine nucleotide-binding protein subunit beta-4 [OS=Homo sapiens]	-0.8	0.1
O00763	Acetyl-CoA carboxylase 2 [OS=Homo sapiens]	-0.9	1.1
P49790	Nuclear pore complex protein Nup153 [OS=Homo sapiens]	-0.9	0.2
P51571	translocon-associated protein subunit delta [OS=Homo sapiens]	-0.9	0.2
P04899-4	Isoform sGi2 of Guanine nucleotide-binding protein G(i) subunit alpha-2 [OS=Homo sapiens]	-0.9	1.1
P46777	60S ribosomal protein L5 [OS=Homo sapiens]	-1.0	0.1
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 [OS=Homo sapiens]	-1.0	0.1
Q9Y512	sorting and assembly machinery component 50 homolog [OS=Homo sapiens]	-1.1	0.2
Q6WCQ1-2	Isoform 2 of Myosin phosphatase Rho-interacting protein [OS=Homo sapiens]	-1.1	0.2
Q9Y6I3-1	Isoform 2 of Epsin-1 [OS=Homo sapiens]	-1.1	1.6
P23284	peptidyl-prolyl cis-trans isomerase B [OS=Homo sapiens]	-1.1	0.5
Q5T750	Skin-specific protein 32 [OS=Homo sapiens]	-1.1	0.2
P62263	40S ribosomal protein S14 [OS=Homo sapiens]	-1.2	0.8

P05166-2	Isoform 2 of Propionyl-CoA carboxylase beta chain, mitochondrial [OS=Homo sapiens]	-1.2	0.9
Q9NZT1	Calmodulin-like protein 5 [OS=Homo sapiens]	-1.2	0.6
P05165-1	Propionyl-CoA carboxylase alpha chain, mitochondrial [OS=Homo sapiens]	-1.2	1.9
P23528	Cofilin-1 [OS=Homo sapiens]	-1.2	0.1
P02545	Prelamin-A/C [OS=Homo sapiens]	-1.2	0.2
P40429	60S ribosomal protein L13a [OS=Homo sapiens]	-1.2	0.2
P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 [OS=Homo sapiens]	-1.4	0.2
P62269	40S ribosomal protein S18 [OS=Homo sapiens]	-1.4	3.4
Q13085-1	Acetyl-CoA carboxylase 1 [OS=Homo sapiens]	-1.4	1.8
P31327-3	Isoform 3 of Carbamoyl-phosphate synthase [ammonia], mitochondrial [OS=Homo sapiens]	-1.4	1.9
P27708	CAD protein [OS=Homo sapiens]	-1.4	1.9
P62917	60S ribosomal protein L8 [OS=Homo sapiens]	-1.4	0.6
P62987	Ubiquitin-60S ribosomal protein L40 [OS=Homo sapiens]	-1.4	0.7
P62979	Ubiquitin-40S ribosomal protein S27a [OS=Homo sapiens]	-1.4	0.7
P30050-1	60S ribosomal protein L12 [OS=Homo sapiens]	-1.4	1.6
O94905-1	Erlin-2 [OS=Homo sapiens]	-1.5	0.4
Q00325-1	Phosphate carrier protein, mitochondrial [OS=Homo sapiens]	-1.5	0.8
P63000-2	Isoform B of Ras-related C3 botulinum toxin substrate 1 [OS=Homo sapiens]	-1.5	0.3
P06748	Nucleophosmin [OS=Homo sapiens]	-1.5	0.2
P62280	40S ribosomal protein S11 [OS=Homo sapiens]	-1.6	1.4
P12004	proliferating cell nuclear antigen [OS=Homo sapiens]	-1.6	1.3

Q96HS1-1	Serine/threonine-protein phosphatase Pgam5, mitochondrial [OS=Homo sapiens]	-1.7	0.9
Q06830	peroxiredoxin-1 [OS=Homo sapiens]	-1.7	0.7
Q8N3V7-1	synaptopodin [OS=Homo sapiens]	-1.9	0.4
B0I1T2-1	unconventional myosin-Ig [OS=Homo sapiens]	-1.9	0.5
Q8NFJ5	Retinoic acid-induced protein 3 [OS=Homo sapiens]	-1.9	1.1
P81605-2	Isoform 2 of Dermcidin [OS=Homo sapiens]	-1.9	0.5
P35658-3	Isoform 3 of Nuclear pore complex protein Nup214 [OS=Homo sapiens]	-1.9	1.0
P61626	lysozyme c [OS=Homo sapiens]	-1.9	0.8
P47756-1	F-actin-capping protein subunit beta [OS=Homo sapiens]	-1.9	0.4
P07355	Annexin A2 [OS=Homo sapiens]	-2.0	0.6
P61313-1	60S ribosomal protein L15 [OS=Homo sapiens]	-2.0	0.3
Q86V48-1	Leucine zipper protein 1 [OS=Homo sapiens]	-2.0	1.2
P29508	Serpin B3 [OS=Homo sapiens]	-2.1	0.4
O43707	Alpha-actinin-4 [OS=Homo sapiens]	-2.1	1.5
Q14978-2	Isoform Beta of Nucleolar and coiled-body phosphoprotein 1 [OS=Homo sapiens]	-2.1	1.2
Q15149-1	plectin [OS=Homo sapiens]	-2.2	0.8
Q12904-2	Isoform 2 of Aminoacyl tRNA synthase complex-interacting multifunctional protein 1 [OS=Homo sapiens]	-2.3	0.8
P51572-2	Isoform 2 of B-cell receptor-associated protein 31 [OS=Homo sapiens]	-2.3	1.4
Q13263	Transcription intermediary factor 1-beta [OS=Homo sapiens]	-2.3	1.2
O95425	Supervillin [OS=Homo sapiens]	-2.3	0.6
P09493-5	Isoform 5 of Tropomyosin alpha-1 chain [OS=Homo sapiens]	-2.4	0.4
P31153	S-adenosylmethionine synthase isoform type-2 [OS=Homo sapiens]	-2.4	0.6
O75477	erlin-1 [OS=Homo sapiens]	-2.4	0.7

Q86YZ3	Hornerin [OS=Homo sapiens]	-2.4	1.2
P25311	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	-2.5	1.1
Q9UHB6-4	Isoform 4 of LIM domain and actin-binding protein 1 [OS=Homo sapiens]	-2.5	0.8
Q9UHB6	LIM domain and actin-binding protein 1 [OS=Homo sapiens]	-2.5	0.8
P04040	catalase [OS=Homo sapiens]	-2.5	0.8
P05109	Protein S100-A8 [OS=Homo sapiens]	-2.6	0.4
P50402	Emerin [OS=Homo sapiens]	-2.6	0.7
P60660	Myosin light polypeptide 6 [OS=Homo sapiens]	-2.6	0.5
Q96P63-2	Isoform 2 of Serpin B12 [OS=Homo sapiens]	-2.7	1.3
Q08554-2	Isoform 1B of Desmocollin-1 [OS=Homo sapiens]	-2.7	0.6
P68032	Actin, alpha cardiac muscle 1 [OS=Homo sapiens]	-2.7	1.0
Q08188	Protein-glutamine gamma-glutamyltransferase E [OS=Homo sapiens]	-2.8	0.7
P60953	Cell division control protein 42 homolog [OS=Homo sapiens]	-2.8	2.7
Q13501-1	sequestosome-1 [OS=Homo sapiens]	-2.9	2.6
P48594	Serpin B4 [OS=Homo sapiens]	-2.9	0.5
O75955	Flotillin-1 [OS=Homo sapiens]	-2.9	0.9
Q96C19	EF-hand domain-containing protein D2 [OS=Homo sapiens]	-3.0	1.1
O14974	Protein phosphatase 1 regulatory subunit 12A [OS=Homo sapiens]	-3.0	1.2
Q02978	Mitochondrial 2-oxoglutarate/malate carrier protein [OS=Homo sapiens]	-3.0	0.8
Q02218	2-oxoglutarate dehydrogenase, mitochondrial [OS=Homo sapiens]	-3.0	1.0
O14639-5	Isoform 5 of Actin-binding LIM protein 1 [OS=Homo sapiens]	-3.0	0.9
P60174	Triosephosphate isomerase [OS=Homo sapiens]	-3.1	1.2
Q8WWI1-3	Isoform 3 of LIM domain only protein 7 [OS=Homo sapiens]	-3.1	1.8

Q02413	Desmoglein-1 [OS=Homo sapiens]	-3.1	0.7
O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial [OS=Homo sapiens]	-3.1	4.0
P52907	F-actin-capping protein subunit alpha-1 [OS=Homo sapiens]	-3.2	0.6
P60468	protein transport protein Sec61 subunit beta [OS=Homo sapiens]	-3.2	1.1
P32119	Peroxiredoxin-2 [OS=Homo sapiens]	-3.3	0.6
Q15517	corneodesmosin [OS=Homo sapiens]	-3.4	0.5
P11498	pyruvate carboxylase, mitochondrial [OS=Homo sapiens]	-3.5	3.3
P04792	Heat shock protein beta-1 [OS=Homo sapiens]	-3.5	1.5
Q01650	large neutral amino acids transporter small subunit 1 [OS=Homo sapiens]	-3.6	3.0
P15924-1	Desmoplakin [OS=Homo sapiens]	-3.6	0.6
P31944	Caspase-14 [OS=Homo sapiens]	-3.6	2.3
P22735	Protein-glutamine gamma- glutamyltransferase K [OS=Homo sapiens]	-3.7	1.3
Q14847-2	Isoform 2 of LIM and SH3 domain protein 1 [OS=Homo sapiens]	-3.7	4.1
Q5SRD1	Putative mitochondrial import inner membrane translocase subunit Tim23B [OS=Homo sapiens]	-3.7	0.8
P05787-2	Isoform 2 of Keratin, type II cytoskeletal 8 [OS=Homo sapiens]	-3.8	0.8
Q13813	Spectrin alpha chain, non-erythrocytic 1 [OS=Homo sapiens]	-4.0	1.8
Q13813-2	Isoform 2 of Spectrin alpha chain, non- erythrocytic 1 [OS=Homo sapiens]	-4.0	1.8
Q9ULV4-3	Isoform 3 of Coronin-1C [OS=Homo sapiens]	-4.0	0.9
P52943	Cysteine-rich protein 2 [OS=Homo sapiens]	-4.0	3.1
Q01082-3	Isoform 2 of Spectrin beta chain, non- erythrocytic 1 [OS=Homo sapiens]	-4.0	2.2
Q6NYC8	Phostensin [OS=Homo sapiens]	-4.2	2.4

P50416	Carnitine O-palmitoyltransferase 1, liver isoform [OS=Homo sapiens]	-4.3	3.9
Q01082-1	Spectrin beta chain, non-erythrocytic 1 [OS=Homo sapiens]	-4.3	2.4
O95816	BAG family molecular chaperone regulator 2 [OS=Homo sapiens]	-4.4	2.9
P16403	Histone H1.2 [OS=Homo sapiens]	-4.5	2.0
P10412	Histone H1.4 [OS=Homo sapiens]	-4.5	2.0
P49207	60S ribosomal protein L34 [OS=Homo sapiens]	-4.5	0.7
Q9NQT5	exosome complex component RRP40 [OS=Homo sapiens]	-4.5	2.6
P06753-2	Isoform 2 of Tropomyosin alpha-3 chain [OS=Homo sapiens]	-4.6	1.7
P50238	Cysteine-rich protein 1 [OS=Homo sapiens]	-4.6	3.7
Q16643-3	Isoform 3 of Drebrin [OS=Homo sapiens]	-4.7	1.3
Q9UDY2-7	Isoform 7 of Tight junction protein ZO-2 [OS=Homo sapiens]	-4.7	3.3
P14923	Junction plakoglobin [OS=Homo sapiens]	-4.7	0.7
P62081	40S ribosomal protein S7 [OS=Homo sapiens]	-4.8	1.0
P05089-2	Isoform 2 of Arginase-1 [OS=Homo sapiens]	-5.0	1.2
O14950	Myosin regulatory light chain 12B [OS=Homo sapiens]	-5.3	4.1
Q9NYL9	tropomodulin-3 [OS=Homo sapiens]	-5.4	1.4
P46779-3	Isoform 3 of 60S ribosomal protein L28 [OS=Homo sapiens]	-5.5	3.3
P68431	Histone H3.1 [OS=Homo sapiens]	-5.5	1.5
P16401	Histone H1.5 [OS=Homo sapiens]	-5.7	1.8
Q5D862	Filaggrin-2 [OS=Homo sapiens]	-5.9	1.6
O43324-1	Eukaryotic translation elongation factor 1 epsilon-1 [OS=Homo sapiens]	-6.2	3.7
P08670	Vimentin [OS=Homo sapiens]	-6.4	1.2
Q13835	Plakophilin-1 [OS=Homo sapiens]	-6.6	1.3
P62899	60S ribosomal protein L31 [OS=Homo sapiens]	-6.7	3.8

P62854	40S ribosomal protein S26 [OS=Homo sapiens]	-6.7	2.2
P08708	40S ribosomal protein S17 [OS=Homo sapiens]	-7.1	3.7
P46782	40S ribosomal protein S5 [OS=Homo sapiens]	-7.3	5.0
Q07157	Tight junction protein ZO-1 [OS=Homo sapiens]	-7.8	3.3
Q07157-2	Isoform Short of Tight junction protein ZO-1 [OS=Homo sapiens]	-7.8	3.3
P62841	40S ribosomal protein S15 [OS=Homo sapiens]	-8.2	5.7
P62633-1	Cellular nucleic acid-binding protein [OS=Homo sapiens]	-8.5	3.4
P62633-4	Isoform 4 of Cellular nucleic acid-binding protein [OS=Homo sapiens]	-8.5	3.4

Supporting Table S2. Crystallography data and refinement statistics (PDB ID: 6PVA).

Data Collection	NTMT1/1
λ (Å)	1.0332
Space group	P3 ₁ 21
a, b, c (Å)	72.95, 72.95, 82.09
α , β , γ (°)	90, 90, 120
Resolution (Å)*	30 – 1.84 (1.89 – 1.84)
Completeness (%)*	99.8 (97.8)
Redundancy*	6.5 (5.3)
R_{sym} †*	0.12 (3.3)
I / σ (I)*	9.9 (0.4)
CC _{1/2}	1.0 (0.14)
Refinement	
Resolution (Å)	30 – 1.84
No. reflections	22,330
$R^{\S}/R_{\text{free}}^{\P}$	0.20/0.24
r.m.s. deviations	
Bonds (Å)	0.003
Angles (°)	0.728
No. Protein atoms	1858
No. Ligand atoms	59
No. Waters	170
B-factors (Å²)	
Wilson B	35.45
Protein	37.80
Ligands	34.4
Waters	42.7
Ramachandran Analysis[‡]	
Favored (%)	99.1
Allowed (%)	0.9
Outliers (%)	0
PDB code	6PVA

† $R_{\text{sym}} = \sum_{hkl,j} (|I_{hkl} - \langle I_{hkl} \rangle|) / \sum_{hkl,j} I_{hkl}$, where $\langle I_{hkl} \rangle$ is the average intensity for a set of j symmetry related reflections and I_{hkl} is the value of the intensity for a single reflection within a set of symmetry-related reflections.

§ R factor = $\sum_{hkl} (||F_o| - |F_c||) / \sum_{hkl} |F_o|$ where F_o is the observed structure factor amplitude and F_c is the calculated

structure factor amplitude.

[¶] $R_{\text{free}} = \sum_{hkl, T} (||F_o| - |F_c||) / \sum_{hkl, T} |F_o|$, where a test set, T (5% of the data), is omitted from the refinement.

[‡] Performed using Molprobity within PHENIX.

* Indicates statistics for last resolution shell shown in parenthesis.