Published in final edited form as:

Proteomics. 2019 June ; 19(11): e1900001. doi:10.1002/pmic.201900001.

Identification, quantification and systems analysis of protein N-ε lysine methylation in anucleate blood platelets

Anne D. Rocheleau¹, Alexander R. Melrose², Jennifer M. Cunliffe³, John Klimek³, Özgün Babur^{4,5}, Samuel Tassi Yunga^{1,6}, Anh T.P. Ngo¹, Jiaqing Pang¹, Larry L. David^{3,7}, Owen J.T. McCarty¹, Joseph E. Aslan^{1,2,7,*}

¹Department of Biomedical Engineering, Oregon Health & Science University, Portland, Oregon, USA

²Knight Cardiovascular Institute, Oregon Health & Science University, Portland, Oregon, USA

³Proteomics Shared Resource, Oregon Health & Science University, Portland, Oregon, USA

⁴Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon, USA

⁵Computational Biology Program, Oregon Health & Science University, Portland, Oregon, USA

⁶Cancer Early Detection & Advanced Research Center, Oregon Health & Science University, Portland, Oregon, USA

⁷Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon, USA

Abstract

Protein post-translational modifications critically regulate a range of physiological and disease processes. In addition to tyrosine, serine and threonine phosphorylation, reversible N-ε acylation and alkylation of protein lysine residues also modulate diverse aspects of cellular function. Studies of lysine acyl and alkyl modifications have focused on nuclear proteins in epigenetic regulation; however, lysine modifications are also prevalent on cytosolic proteins to serve increasingly apparent, although less understood roles in cell regulation. Here, we characterize the methyl-lysine (meK) proteome of anucleate blood platelets. With high-resolution, multiplex mass spectrometry methods, we identify 190 mono-, di- and tri-meK modifications on 150 different platelet proteins – including 28 meK modifications quantified by tandem mass tag (TMT) labeling. In addition to identifying meK modifications on calmodulin (CaM), GRP78 (HSPA5, BiP) and EF1A1 that have been previously characterized in other cell types, we also uncover more novel modifications on cofilin, drebin-like protein (DBNL, Hip-55), DOCK8, TRIM25 and numerous other cytoplasmic proteins. Together, our results and analyses support roles for lysine methylation in mediating cytoskeletal, translational, secretory and other cellular processes. Mass spectrometry data for this

*Corresponding author: Joseph E. Aslan, Knight Cardiovascular Institute, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Mail code HRC5N, Portland, OR 97239; Tel: (503) 494-6253; Fax: (503) 418-9311; aslanj@ohsu.edu.

Conflict of interest statement

The authors declare no conflicts of interest.

study have been deposited into the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD012217.

Keywords

biomarkers; cardiovascular system; lysine methylation; mass spectrometry – LC-MS/MS; methylation; methyllysine; platelets; post-translational modifications; quantitative labeling; systems biology

Cellular responses underlying physiological homeostasis are mediated, in part, through a dynamic dialog of reversible post-translational modifications (PTMs) amongst intracellular proteins.^[1] While the reversible phosphorylation of serine, threonine and tyrosine residues by protein kinases and phosphatases serves as the most prevalently detailed PTM in cell regulation, other modifications such as the N-ε ubiquitination, acylation or alkylation of lysine residues are also known to regulate cellular function in physiology and disease.^[2] Reversible methylation represents the most abundant alkyl modification of lysine residues, and is catalyzed by several lysine methyltransferase (KMT)^[3] and demethylase enzymes, providing a unique means for precise spatial and temporal regulatory controls of proteins in mediating cellular programs.

The majority of studies of protein lysine alkylation – including lysine methylation – have focused on the roles of these modifications on histones and other nuclear proteins in the epigenetic regulation of transcription and cellular fate. However, biochemical and cell biological studies have revealed that lysine methyltransferases and demethylases also regulate the lysine methylation status of several proteins outside of the nucleus. For instance, the lysine methyltransferase SETD2 – which tri-methylates histone H3 K36 (H3K36me3) – also methylates α-tubulin K40 to regulate microtubule stability.^[4] Similarly, Ezh2, a methyltransferase associated with cancer metastasis, has been reported to methylate talin to regulate F-actin interactions and adhesion turnover in leukocytes.^[5]

We recently detailed acetyl-lysine modifications of cytosolic proteins through a proteomic analysis of human blood platelets.^[6] Platelets are well known as small (~2 μm diameter), anucleate cells that mediate clot formation and vascular repair.^[7] As platelets do not contain nuclei, they also serve as a useful primary cell model for studies of cytosolic protein regulation and function. Here, we provide the first ever description and systematic analysis of the platelet N-ε methyl-lysine (meK) proteome, identifying a number of mono-, di- and tri-meK modifications on cytoplasmic proteins of potential relevance to platelet and cellular function in normal physiology as well as in informing mechanisms and markers of health and disease.

Complete details of materials and methods for this study, including platelet isolation, sample preparation and mass spectrometry analyses can be found in Supporting Information. Briefly, human platelet samples were suspended and probe sonicated in lysis buffer. Approximately 30 mg of protein was reduced, alkylated and digested with trypsin overnight at 37°C before quenching with TFA. Methyl-lysine containing peptides were enriched by immunoaffinity purification and further purified by solid phase extraction. Peptides were

labeled with 10-plex tandem mass tags (TMT), normalized, multiplexed and separated by two-dimensional reverse-phase liquid chromatography. Tandem mass spectrometry data was collected using an Orbitrap Fusion Tribrid instrument configured with an EasySpray NanoSource (Thermo Scientific). LC-MS/MS analysis, TMT data analysis and differential abundance analysis were performed as previously described^[8] and detailed in Supporting Information. Mass spectrometry data for this study have been deposited into the ProteomeXchange Consortium^[9] via the PRIDE partner repository^[10] with the dataset identifier PXD012217.

Previous proteomics-driven studies have identified several lysine methyltransferase and demethylase enzymes in human and mouse platelets, including SETD1A, SETD3, SMYD3, KDM3B and KDM8 (see Supporting Table 1); however, the presence of and roles for lysine methylated proteins in platelets remain unknown. We first examined the lysine mono-, di- and tri-methylation status of proteins in platelets relative to nucleated MDA-MB-231 breast cancer cells by Western blot. As seen in Fig. 1A, MDA-MB-231 total cell lysates contain abundant proteins with a range of molecular weights positive for mono-, di- and tri-meK modifications. Although less pronounced, several platelet proteins were likewise positive for lysine mono-, di- and tri-methylation modifications by Western blot (Fig. 1A). Complementary immunofluorescence microscopy studies of human platelets adherent to fibrinogen demonstrated that lysine mono-, di- and tri-methylated proteins displayed specific patterns throughout the platelet cytosol that redistributed with additional activation by thrombin (Fig. 1B and Supporting Information).

Next, we sought to identify which proteins and specific lysine residues are modified by mono-, di- and tri-methylation in platelets using mass spectrometry (MS) and proteomics tools. As outlined in Fig. 2A, we drew blood from five healthy adult volunteers to prepare five separate samples of quiescent, washed human platelets (>99% CD41⁺ cells by flow cytometry; see Supporting Info) for parallel, multiplexed, quantitative MS analyses of meK modifications in platelets. Washed platelets were separately prepared from each donor prior to lysis in sodium deoxycholate (SDC) buffer, trypsin digestion, meK peptide enrichment, 5-plex tandem mass tag (TMT) labeling and parallel, multiplex MS analysis. As listed in Fig. 2A and Supporting Data, we obtained MS2 spectra for 153 unique peptides containing 55 mono-, 78 di- and 57 tri-meK modifications on 150 different proteins over all 5 human donors. These 190 meK modifications included previously characterized meK modifications on cytosolic proteins such as calmodulin K116 (CaM, encoded by three separate genes CALM1, CALM2 and CALM3), GRP78 (BiP, HSPA5) K585 and the elongation factor EF1A1 K55. Of the meK-containing peptides identified, 34 peptides representing 28 unique meK modifications on 20 different proteins were present at sufficient reporter ion intensities for MS3 quantitation amongst samples from all (n=5) subjects (see Table 1). These modifications included EF1A1 K55 dimethylation (representative MS2 and MS3 spectra shown in Fig. 2B) as well as CALM K116 and GRP78 K585 methylations. In addition, novel methylation sites not yet reported in the literature were found in all 5 platelet samples on drebin-like protein (DBNL, Hip-55), DOCK8, Rab10, desmoplakin (DESP), tensin-3 (TNS3) and other proteins (see Supporting Data). Multiplex quantitation revealed similar reporter ion intensity ranges for most of the 28 meK modifications measurable amongst all five platelet samples (Fig. 2C). However, the total levels and relative abundancies of some

meK-containing peptides (i.e., dimethyl EF1A1 K165) varied widely (>10-fold) between platelet samples from different individuals (Fig. 2D).

To gain insights into potential cell physiological roles associated with protein meK modifications in platelet function, we carried out interactive and gene enrichment analyses of the proteins identified with meK modifications in our study using computational tools (see Supporting Information). With ChiBE,^[11] we queried Pathway Commons^[12] to generate a high confidence interactive network based on curated experimental data (Fig. 2E). In the resulting interactome of 91 nodes and 137 relations, a highly interconnected node of 19 “in complex” relations was especially apparent around the E3 ubiquitin ligase TRIM25. Several other effectors associated with protein-protein interactions, organellar organization and actin-spectrin binding were also enriched in our data set, as further detailed and discussed in Supporting Information.

In summary, this study provides the first demonstration that a number of protein lysine residues are specifically modified by N-ε methylation in anucleate blood platelets. While previous biochemical studies have demonstrated generalized roles enzymatic protein alkylation and methylation in platelets, specific roles for reversible protein N-ε meK modifications in platelet function have not yet been described.^[13] As the site specific lysine methylation of some proteins such as EF1A1, calmodulin and GRP78 have already been linked to specific physiological outputs of other cell types,^[14] methylation of these and other proteins may likewise have roles in tuning receptor regulation, intracellular trafficking, secretion protein translation and cytoskeletal assembly processes in platelets (Fig. 2F). Future studies will determine whether and how lysine methylation regulates platelets together with other modifications (i.e., acetylation, ubiquitination, SUMOylation) by modulating protein structures, interactions, stability and cellular function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the the Medical Research Foundation of Oregon and grants from the American Heart Association (17SDG33350075 to J.E.A. and 13EIA12630000 to O.J.T.M.) and the National Institutes of Health (R01HL146549 to J.E.A., R01HL101972 and R01GM116184 to O.J.T.M.).

References

- [1]. Grimes M, Hall B, Foltz L, Levy T, Rikova K, Gaiser J, Cook W, Smirnova E, Wheeler T, Clark NR, Lachmann A, Zhang B, Hornbeck P, Ma'ayan A, Comb M, Sci Signal 2018, 11;Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E, Nucleic Acids Res 2015, 43, D512. [PubMed: 25514926]
- [2]. Lanouette S, Mongeon V, Figeys D, Couture JF, Mol Syst Biol 2014, 10, 724. [PubMed: 24714364]
- [3]. Copeland RA, Solomon ME, Richon VM, Nature reviews. Drug discovery 2009, 8, 724; [PubMed: 19721445] Boriack-Sjodin PA, Swinger KK, Biochemistry 2016, 55, 1557. [PubMed: 26652298]
- [4]. Park IY, Powell RT, Tripathi DN, Dere R, Ho TH, Blasius TL, Chiang YC, Davis IJ, Fahey CC, Hacker KE, Verhey KJ, Bedford MT, Jonasch E, Rathmell WK, Walker CL, Cell 2016, 166, 950. [PubMed: 27518565]

- [5]. Gunawan M, Venkatesan N, Loh JT, Wong JF, Berger H, Neo WH, Li LY, La Win MK, Yau YH, Guo T, See PC, Yamazaki S, Chin KC, Gingras AR, Shochat SG, Ng LG, Sze SK, Ginhoux F, Su IH, Nature immunology 2015, 16, 505; [PubMed: 25751747] Su IH, Dobenecker MW, Dickinson E, Oser M, Basavaraj A, Marqueron R, Viale A, Reinberg D, Wulfig C, Tarakhovsky A, Cell 2005, 121, 425. [PubMed: 15882624]
- [6]. Aslan JE, Rigg RA, Nowak MS, Loren CP, Baker-Groberg SM, Pang J, David LL, McCarty OJ, J Thromb Haemost 2015, 13, 1908. [PubMed: 26256950]
- [7]. Aslan JE, in Platelets in Thrombotic and Nonthrombotic Disorders, (Eds: Gresele P, López J, Kleiman N, Page C), Springer, 2017.
- [8]. Plubell DL, Wilmarth PA, Zhao Y, Fenton AM, Minnier J, Reddy AP, Klimek J, Yang X, David LL, Pamir N, Molecular & cellular proteomics : MCP 2017, 16, 873. [PubMed: 28325852]
- [9]. Deutsch EW, Csordas A, Sun Z, Jarnuczak A, Perez-Riverol Y, Ternent T, Campbell DS, Bernal-Llinares M, Okuda S, Kawano S, Moritz RL, Carver JJ, Wang M, Ishihama Y, Bandeira N, Hermjakob H, Vizcaino JA, Nucleic Acids Res 2017, 45, D1100. [PubMed: 27924013]
- [10]. Vizcaino JA, Csordas A, del-Toro N, Dianas JA, Griss J, Lavidas I, Mayer G, Perez-Riverol Y, Reisinger F, Ternent T, Xu QW, Wang R, Hermjakob H, Nucleic Acids Res 2016, 44, D447. [PubMed: 26527722]
- [11]. Babur O, Dogrusoz U, Cakir M, Aksoy BA, Schultz N, Sander C, Demir E, BMC Genomics 2014, 15, 642. [PubMed: 25086704]
- [12]. Cerami EG, Gross BE, Demir E, Rodchenkov I, Babur O, Anwar N, Schultz N, Bader GD, Sander C, Nucleic Acids Res 2011, 39, D685. [PubMed: 21071392]
- [13]. Macfarlane DE, J Biol Chem 1984, 259, 1357. [PubMed: 6198323]
- [14]. Magnani R, Dirk LM, Trievel RC, Houtz RL, Nature communications 2010, 1, 43; Haziza S, Magnani R, Lan D, Keinan O, Saada A, HersHKovitz E, Yanay N, Cohen Y, Nevo Y, Houtz RL, Sheffield VC, Golan H, Parvari R, PLoS genetics 2015, 11, e1005388. [PubMed: 26247364]
- [15]. Molins B, Pena E, Padro T, Casani L, Mendieta C, Badimon L, Arteriosclerosis, thrombosis, and vascular biology 2010, 30, 1246.
- [16]. Cloutier P, Lavalley-Adam M, Faubert D, Blanchette M, Coulombe B, PLoS genetics 2013, 9, e1003210; [PubMed: 23349634] Jakobsson ME, Moen A, Bousset L, Egge-Jacobsen W, Kernstock S, Melki R, Falnes PO, J Biol Chem 2013, 288, 27752; [PubMed: 23921388] Sieber J, Wieder N, Ostrosky-Frid M, Dvela-Levitt M, Aygun O, Udeshi ND, Carr SA, Greka A, J Biol Chem 2017, 292, 18878. [PubMed: 28912266]
- [17]. Dandona P, Thusu K, Khurana U, Love J, Aljada A, Mousa S, Thromb Res 1996, 81, 163; [PubMed: 8822131] Gardiner EE, Arthur JF, Berndt MC, Andrews RK, Current medicinal chemistry. Cardiovascular and hematological agents 2005, 3, 283; [PubMed: 16250859] Luckhoff A, Bohnert M, Busse R, Naunyn Schmiedeberg's Arch Pharmacol 1991, 343, 96. [PubMed: 2030746]
- [18]. Redondo PC, Harper MT, Rosado JA, Sage SO, Blood 2006, 107, 973. [PubMed: 16234361]
- [19]. Gieger C, Radhakrishnan A, Cvejic A, Tang W, Porcu E, Pistis G, Serbanovic-Canic J, Elling U, Goodall AH, Labruno Y, Lopez LM, Magi R, Meacham S, Okada Y, Pirastu N, Sorice R, Teumer A, Voss K, Zhang W, Ramirez-Solis R, Bis JC, Ellinghaus D, Gogele M, Hottenga JJ, Langenberg C, Kovacs P, O'Reilly PF, Shin SY, Esko T, Hartiala J, Kanoni S, Murgie F, Parsa A, Stephens J, van der Harst P, van der Schoot C, Ellen, Allayee H, Attwood A, Balkau B, Bastardot F, Basu S, Baumeister SE, Biino G, Bomba L, Bonnefond A, Cambien F, Chambers JC, Cucca F, D'Adamo P, Davies G, de Boer RA, de Geus EJ, Doring A, Elliott P, Erdmann J, Evans DM, Falchi M, Feng W, Folsom AR, Frazer IH, Gibson QD, Glazer NL, Hammond C, Hartikainen AL, Heckbert SR, Hengstenberg C, Hersch M, Illig T, Loos RJ, Jolley J, Khaw KT, Kuhnel B, Kyrtsonis MC, Lagou V, Lloyd-Jones H, Lumley T, Mangino M, Maschio A, Leach I, Mateo, McKnight B, Memari Y, Mitchell BD, Montgomery GW, Nakamura Y, Nauck M, Navis G, Nothlings U, Nolte IM, Porteous DJ, Pouta A, Pramstaller PP, Pullat J, Ring SM, Rotter JJ, Ruggiero D, Ruokonen A, Sala C, Samani NJ, Sambrook J, Schlessinger D, Schreiber S, Schunkert H, Scott J, Smith NL, Snieder H, Starr JM, Stumvoll M, Takahashi A, Tang WH, Taylor K, Tenesa A, Thein S, Lay, Tonjes A, Uda M, Ulivi S, van Veldhuisen DJ, Visscher PM, Volker U, Wichmann HE, Wiggins KL, Willemsen G, Yang TP, Hua Zhao J, Zitting P, Bradley JR, Dedoussis GV, Gasparini P, Hazen SL, Metspalu A, Pirastu M, Shuldiner AR, van Pelt L.

Joost, Zwaginga JJ, Boomsma DI, Deary IJ, Franke A, Froguel P, Ganesh SK, Jarvelin MR, Martin NG, Meisinger C, Psaty BM, Spector TD, Wareham NJ, Akkerman JW, Ciullo M, Deloukas P, Greinacher A, Jupe S, Kamatani N, Khadake J, Kooner JS, Penninger J, Prokopenko I, Stemple D, Toniolo D, Wernisch L, Sanna S, Hicks AA, Rendon A, Ferreira MA, Ouwehand WH, Soranzo N, Nature 2011, 480, 201. [PubMed: 22139419]

- [20]. Taanman JW, Heiske M, Letellier T, Methods 2010, 51, 374. [PubMed: 20227504]
- [21]. Tucker KL, Kaiser WJ, Bergeron AL, Hu H, Dong JF, Tan TH, Gibbins JM, Proteomics 2009, 9, 4340. [PubMed: 19725075]
- [22]. Malecki J, Ho AY, Moen A, Dahl HA, Falnes PO, J Biol Chem 2015, 290, 423; [PubMed: 25416781] Shimazu T, Furuse T, Balan S, Yamada I, Okuno S, Iwanari H, Suzuki T, Hamakubo T, Dohmae N, Yoshikawa T, Wakana S, Shinkai Y, Sci Rep 2018, 8, 1179. [PubMed: 29352221]
- [23]. Malecki J, Aileni VK, Ho AYY, Schwarz J, Moen A, Sorensen V, Nilges BS, Jakobsson ME, Leidel SA, Falnes PO, Nucleic Acids Res 2017, 45, 4370; [PubMed: 28108655] Jakobsson ME, Malecki J, Nilges BS, Moen A, Leidel SA, Falnes PO, Nucleic Acids Res 2017, 45, 8239. [PubMed: 28520920]
- [24]. Michaux G, Dyer CE, Nightingale TD, Gallaud E, Nurrish S, Cutler DF, J Thromb Haemost 2011, 9, 392. [PubMed: 21070595]

Significance of the Study

In addition to coordinating gene transcription programs in the nucleus, reversible covalent modifications of protein lysine residues also regulate protein homeostasis, cytoskeletal dynamics, metabolic flux and a growing list of intracellular activities underlying physiology and disease. While N-ε lysine alkyl modifications are known to be present on some cytosolic proteins, specific roles for cytosolic protein lysine methylation remain enigmatic. This study uses proteomics and systems biology tools to provide the first-ever description of cytosolic protein N-ε lysine methylation in anucleate blood platelets. The findings of this study are significant, as they provide insights into how protein N-ε lysine mono-, di- and tri-methylation occur in a physiologically relevant, cytosol-specific context independent of nuclear biology. Moreover, as many pathologies associated with platelet dysfunction are the result of aberrant post-translational modification regulation and errors in protein expression and regulation, lysine methylation may serve as a biomarker for cellular health and function in platelets and other cell types. Ultimately, the results of this study will help to identify consensus motifs and specific protein modifications that may serve as therapeutic targets or specific meK-based biomarkers of health and disease in circulating platelets and other readily biopsied, physiologically relevant cell types.

Figure 1. Expression and intracellular localization of lysine methylated proteins in human platelets.

(A) Replicate samples of washed human platelets ($1 \times 10^9/\text{ml}$) and whole MDA-MB-231 cell lysates were separated by SDS-PAGE and examined by Western blot for the presence of lysine methylated proteins with antisera reactive to mono-, di- and tri-methyl lysine moieties. Relative positions of molecular mass markers are indicated. (B) Replicate samples of washed human platelets ($2 \times 10^7/\text{ml}$) were prepared and allowed to adhere to fibrinogen-coated cover glass (45 min, 37°C) in the absence or presence of thrombin (1 U/ml). Following fixation and permeabilization, platelets were stained with antisera reactive to mono-, di- and tri-methyl lysine moieties and AlexaFluor 488 secondary antibodies (green) together with TRITC-phalloidin (red) as a marker of the platelet actin cytoskeleton. Scale bar = 10 μm .

Figure 2. MS identification, quantification and analysis of protein meK modifications in human platelets.

(A) Study workflow. Washed platelet samples were prepared from five individual blood donors. Following lysis and trypsin digestion, meK-containing peptides were enriched, further purified and labeled with 10-plex TMT reagent. Labeled samples were combined and analyzed by LC-MS/MS to ultimately identify 190 meK modifications on 150 proteins and to quantify 34 peptides representing 28 meK modifications. (B) Representative MS2 and MS3 spectra identifying and quantifying EF1A1 K55 dimethylation in five platelet samples. (C) Plots of measured reporter ion intensities and (D) deviations from inter-subject mean reporter ion intensity for 25 peptides (numbered #1–25, as listed in panel 2D and Table 1) representing 28 meK modifications (n=5). See Supporting Data for individual reporter ion intensity measurements, coefficients of variation (CV) and other values. (E) A protein-protein interaction (PPI) network of 150 lysine methylated proteins identified in platelets was generated from a query of Pathway Commons using ChiBE. Gray connecting lines represent “in complex” relations. (F) Preliminary model putatively placing selected lysine methylated proteins identified in this study (CALM, BiP, ADCY10, EF2, etc.) into

well-established receptor signaling, secretory, trafficking, protein translation and cytoskeletal remodeling processes in platelet regulation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1.

Lysine methylation modifications present and quantified amongst platelet samples from all individuals (n=5). References to previously identified roles for proteins in platelet function as well as meK modifications in other cellular systems are shown in *Protein name* and *Site mods* columns, respectively. See Supporting Data for additional details.

	UniProt	Protein name	Gene ID	Site mods
1	P11021	GRP78, BiP ^[15]	HSPA5	K585 (tri) ^[16]
2	P11021	GRP78, BiP	HSPA5	K585 (di); K591 (m)
3	Q96PN6	Adenylate cyclase type 10	ADCY10	K522 (di)
4	Q7Z5J8	Ankyrin+armadillo rpt. protein	ANKAR	K943 (tri); K948 (m)
5	Q13634	Cadherin-18	CDH18	K686 (di)
6	P0DP23	Calmodulin-1 ^[17]	CALM1	K116 (di)
7	P0DP23	Calmodulin-1	CALM1	K116 (tri) ^[14]
8	P27482	Calmodulin-like protein 3	CALML3	K116 (di)
9	P23528	Cofilin-1 ^[18]	COF1	K112 (m)
10	P23528	Cofilin-1	COF1	K114 (m)
11	Q8NF50	Dedicator of cytkns. pro. 8 ^[19]	DOCK8	K1180 (tri)
12	P15924	Desmoplakin	DSP	K1374 (tri); K1384 (m)
13	P54098	DNA poly. subunit gamma-1 ^[20]	POLG	K768 (tri)
14	Q9UJU6	Drebrin-like protein (Hip-55) ^[21]	DBNL	K164 (m)
15	P38117	Electron trans. flavopro. beta	ETFB	K200 (di) ^[22]
16	P68104	Elongation factor 1-alpha 1	EEF1A1	K55 (di) ^[23]
17	P68104	Elongation factor 1-alpha 1	EEF1A1	K79 (tri) ^[23]
18	P68104	Elongation factor 1-alpha 1	EEF1A1	K165 (di) ^[23]
19	P13639	Elongation factor 2	EEF2	K407 (di)
20	Q5T1M5	FK506-binding protein 15	FKB15	K736 (m)
21	P61026	Ras-related protein Rab-10 ^[24]	RAB10	K198 (m)
22	O75818	Ribonuclease P subunit p40	RPP40	K160 (di)
23	Q12872	Supp. white-apricot homolog	SFSWAP	K666 (m)
24	Q68CZ2	Tensin-3	TNS3	K1187 (di)
25	Q5VVX9	Ubiquitin-conj. enzyme E2 U	UBE2U	K226 (di)