Analysis of the substrate specificity of the SMYD2 protein lysine methyltransferase and discovery of novel non-histone substrates

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Supplemental figures and figure legends

Supplemental figure 1: Circular dichroism investigation of the purified SMYD2 enzyme.

Supplemental figure 2: Purification of the non-histone substrate candidate protein domains.

Supplemental Table 1: List of potential novel non-histone targets identified in the Scansite search (Fig. 4A).

Supplemental Table 2: List of potential novel non-histone targets identified in the Phosphosite Plus database (Fig. 4B).
Supplemental figure 1: Circular dichroism spectroscopy of the purified SMYD2 enzyme was performed to verify proper protein folding. A) CD spectrum of SMYD2 in the wavelength range of 190 to 240 nm. B) SMYD2 protein stability was investigated by CD melting experiments detected at 210 nm between 20°C to 80°C.
Supplemental figure 2: Purification of the non-histone substrate candidate protein domains. The different candidate non-histone targets were cloned as GST-fusion protein domains, overexpressed and purified by affinity chromatography using glutathione sepharose beads. The 12% and 16% SDS gels show roughly equal protein amounts of all non-histone targets selected from the Scansite search (A) or Phosphosite Plus database (B), which were further used for methylation. Sequencing results of NFKBID revealed mutations and this target was excluded from further analysis. The bands with the expected sizes are labelled with asterisk.
### Supplemental Table 1: List of potential novel non-histone targets identified in the Scansite search (Fig. 4A). The target lysine residue is highlighted by an underscore. Column 1 - Swissprot protein number; column 2 – abbreviation; column 3 – position of the spot in Fig. 4 panel A.

|   |   | Name                                      | Sequence                          |   |
|---|---|-------------------------------------------|-----------------------------------|---|
| 1 | 2 | Artificial peptide                        | RNEPPKLKR5RSQGAF                   | 1A|
| P04637 | p53 | Cellular tumor antigen p53                | RAHSSHLKSKGGQST                   | 3A|
| P04637 | p53 | Cellular tumor antigen p53                | RAHSSHLASKKGQST                   | 4A|
| P04637 | p53 | Cellular tumor antigen p53                | RAHSSHLASKKGQST                   | 6A|
| P51825 | AFF1 | AF4/FMR2 family member 1                 | KPAKPKLKR5RSRREAD                  | 7A|
| Q96D70 | R3HDM4 | R3H domain-containing protein 4        | RRLRAVLR5RSIPME                    | 8A|
| Q6UWZ7 | ABRAAS1 | BRCA1-A complex subunit Abraas 1      | LDDRWQFR5RSRLDLT                   | 9A|
| Q9N0N8 | GNL3L | Guanine nucleotide-binding protein-like 3-like protein | KLSSLINLKR5RSRAC5S | 10A|
| P61978 | HNRNPK | Heterogeneous nuclear ribonucleoprotein K | MEEEEQ5FR5RSRNTE5                  | 11A|
| Q8N38 | NFKBID | NF-kappa-B inhibitor delta               | EGLRQQLKR5RSRPAPP                  | 12A|
| P18615 | NELFE | Negative elongation factor E            | AETKNSS5FGKR5RSRTLE                | 13A|
| Q15772 | SPEG | Striated muscle preferentially expressed protein kinase | AVFEAK5FKR5RSRESPL                 | 14A|
| Q99490 | AGAP2 | Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 2 | EPPAPGL5KR5RGGGR                   | 15A|
| Q12873 | CHD3 | Chromodomain-helicase-DNA-binding protein 3 | PVRTK5KLKR5RGRPRGR                 | 16A|
| Q15650 | TRIP4 | Activating signal cointegrator 1       | QKSGDHL5KR5RGRKKGR                 | 17A|
| Q6PF04 | ZNF613 | Zinc finger protein 613                 | ECGKAFL5K5RSRLVHY                  | 18A|
| Q9GZX5 | ZNF350 | Zinc finger protein 350                | ECGKAFL5K5RSNLNIH                  | 19A|
| Q9NPG3 | UBN1 | Ubinuclein-1                            | SLNPAFL5KR5R5KREEA                 | 20A|
| Q15361 | TTF1 | Transcription termination factor 1      | HLIS5PL5K5KRIC5E                   | 21A|
| P21675 | TAF1 | Transcription initiation factor TFIIH subunit 1 | S5KES5L5K5KSRILLG                 | 1B|
| Q9ULG1 | INO80 | Chromatin-remodeling ATPase INO80      | LYNFSKL5K5SRKW5L                   | 2B|
| Q5EE01 | CENPW | Centromere protein W                   | HVLAAK5V5K5RSRG5                  | 3B|
| Q96565 | CSRNP1 | Cysteine/serine-rich nuclear protein 1 | FTPL5IL5KR5RARRE5P                 | 4B|
| Q965D1 | DCLRE1C | Protein artemis                       | Y5KPL5KLKR5R5ARTVHR                | 5B|
| Q9UBF1 | MAGEC2 | Melanoma-associated antigen C2         | DYFPV5LKR5RAR5E5FM5E               | 6B|
| Q969V6 | MRTFA | Myocardin-related transcription factor A | Q5AKQ5L5KLKR5RALADD                | 7B|
| Q9ULH7 | MRTFB | Myocardin-related transcription factor B | QAT5QM5KLKR5RALADD                 | 8B|
| Q8IZQ8 | MYOCD | Myocardin                             | P5TAQM5KLKR5RALADD                 | 9B|
| Accession | Gene ID | Description                                                                                                                                 |
|-----------|---------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Q8NG66    | NEK11   | Serine/threonine-protein kinase Nek11                                                                                                         |
| Q15522    | NKX2-8  | Homeobox protein Nkx-2.8                                                                                                                      |
| Q99457    | NAP1L3  | Nucleosome assembly protein 1-like 3                                                                                                           |
| Q9BY77    | POLDIP3 | Polymerase delta-interacting protein 3                                                                                                         |
| Q15543    | TAF13   | Transcription initiation factor TFIIID subunit 13                                                                                              |
| Q9NPG3    | UBN1    | Ubinuclein-1                                                                                                                                  |
| Q6IQ21    | ZNF770  | Zinc finger protein 770                                                                                                                       |
| Q96JM7    | L3MBTL3 | Lethal(3)malignant brain tumor-like protein 3                                                                                                 |
| Q13112    | CHAF1B  | Chromatin assembly factor 1 subunit B                                                                                                         |
| Q9H816    | DCLRE1B | 5’ exonuclease Apollo                                                                                                                         |
| Q93074    | MED12   | Mediator of RNA polymerase II Mediator of RNA polymerase II subunit 12                                                                       |
| Q94913    | PCF11   | Pre-mRNA cleavage complex 2 protein Pcf11                                                                                                      |
| Q98V10    | PHF20   | PHD finger protein 20                                                                                                                         |
| Q9NW9H    | SLTM    | SAFB-like transcription modulator                                                                                                             |
| P11387    | TOP1    | DNA topoisomerase 1                                                                                                                           |
| Q15208    | STK38   | Serine/threonine-protein kinase 38                                                                                                             |
| P43246    | MSH2    | DNA mismatch repair protein Msh2                                                                                                              |
| Q14690    | PDCD11  | Protein RRP5 homolog                                                                                                                          |
| Q8WW01    | TSEN15  | tRNA-splicing endonuclease subunit Sen15                                                                                                       |
| Q95619    | YEATS4  | YEATS domain-containing protein 4                                                                                                             |
| Q9H777    | ELAC1   | Zinc phosphodiesterase ELAC protein 1                                                                                                         |
| Q14739    | LBR     | Delta(14)-sterol reductase LBR                                                                                                                |
| P15923    | TCF3    | Transcription factor E2-alpha                                                                                                                 |
| Q13435    | SF3B2   | Splicing factor 3B subunit 2                                                                                                                  |
| Q12830    | BPTF    | Nucleosome-remodeling factor subunit BPTF                                                                                                      |
| P49916    | LIG3    | DNA ligase 3                                                                                                                                  |
| Q9UQF7    | SMCM    | Structural maintenance of chromosomes protein 3                                                                                               |
| Q14746    | TERT    | Telomerase reverse transcriptase                                                                                                              |
| Q9H8W5    | TRIM45  | Tripartite motif-containing protein 45                                                                                                          |
| Q15270    | NKX1-1  | NK1 transcription factor-related protein 1                                                                                                   |
| Q8IUF8    | RIOX2   | Ribosomal oxygenase 2                                                                                                                        |
| Accession | Name                  | Function                                                                 | Accession | Name                  | Function                                                                 |
|-----------|-----------------------|--------------------------------------------------------------------------|-----------|-----------------------|--------------------------------------------------------------------------|
| Q9NZC9    | SMARCAL1              | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1 | Q043298   | ZBTB43                | Zinc finger and BTB domain-containing protein 43                          |
| Q9NW38    | FANCL                 | E3 ubiquitin-protein ligase FANCL                                        | Q9HAP2    | MLXIP                 | MLX-interacting protein                                                  |
| Q9BZD4    | NUF2                  | Kinetochore protein Nuf2                                                 | Q13625    | TP53BP2               | Apoptosis-stimulating of p53 protein 2                                   |
| Q92979    | EMG1                  | Ribosomal RNA small subunit methyltransferase NEP1                       | Q96PU4    | UHRF2                | E3 ubiquitin-protein ligase UHRF2                                        |
| Q96019    | ACTL6A                | Actin-like protein 6A                                                    | P54253    | ATXN1                 | Ataxin-1                                                                 |
| O00512    | BCL9                  | B-cell CLL/lymphoma 9 protein                                            | Q14511    | NEDD9                | Enhancer of filamentation 1                                              |
| Q13618    | CUL3                  | Cullin-3                                                                | Q96L91    | EP400                 | E1A-binding protein p400                                                 |
| Q99814    | EPAS1                 | Endothelial PAS domain-containing protein 1                             | Q99814    | EPAS1                 | Endothelial PAS domain-containing protein 1                             |
| Q8TC84    | FANK1                 | Fibronectin type 3 and ankyrin repeat domains protein 1                  | Q9HA82    | CERS4                 | Ceramide synthase 4                                                      |
| Q14693    | LPIN1                 | Phosphatidate phosphatase LPIN1                                          | Q92979    | EMG1                  | Ribosomal RNA small subunit methyltransferase NEP1                      |
| Q13416    | ORC2                  | Origin recognition complex subunit 2                                    | P06400    | RB1                   | Retinoblastoma-associated protein                                        |
| O00268    | TAF4                  | Transcription initiation factor TFIIID subunit 4                         | O00268    | TAF4                  | Transcription initiation factor TFIIID subunit 4                         |
| Q9NYV6    | RRN3                  | RNA polymerase I-specific transcription initiation factor RRN3           | Q95707    | POP4                  | Ribonuclease P protein subunit p29                                       |
| O95707    | POP4                  | Ribonuclease P protein subunit p29                                       | O75150    | RNF40                 | E3 ubiquitin-protein ligase BRE1B                                        |
| O75150    | RNF40                 | E3 ubiquitin-protein ligase BRE1B                                        | O75151    | PHF2                  | Lysine-specific demethylase PHF2                                         |
| O95243    | MBD4                  | Methyl-CpG-binding domain protein 4                                      | Q9H9L7    | AKIRIN1               | Akirin-1                                                                 |
| Q552Q8    | CELF3                 | CUGBP Elav-like family member 3                                          |
| Accession | Gene | Description | Protein Name | Protein Description | Feature | Score |
|-----------|------|-------------|--------------|---------------------|---------|-------|
| Q9GZU7    | CTDSP1 | Carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1 | Carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1 | VHQQVYVLKRPHV | 7E     |
| Q99848    | EBNA1BP2 | Probable rRNA-processing protein EBP2 | Probable rRNA-processing protein EBP2 | PRLHQKLVPTKRP | 8E     |
| Q15370    | ELOB   | Elongin-B | Elongin-B | RIVEGKRPDEQR | 9E     |
| P54198    | HIRA   | Protein HIRA | Protein HIRA | VWLTCLKRPLVVHE | 10E    |
| O94913    | PCF11  | Pre-mRNA cleavage complex 2 protein Pcfl1 | Pre-mRNA cleavage complex 2 protein Pcfl1 | PFNDRPFLKRPR | 11E    |
| Q5MJ70    | SPDYA  | Speedy protein A | Speedy protein A | QPKKPIKLKRICKD | 12E    |
| Q6STE5    | SMARCD3 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3 | VDIQEAALKPMKQKR | 14E    |
| Q9NWH9    | SLTM   | SAFB-like transcription modulator | SAFB-like transcription modulator | IDLKSELKRRLNDIT | 15E    |
| Q8NHM5    | KDM2B  | Lysine-specific demethylase 2B | Lysine-specific demethylase 2B | KAPALRFLKRTLSNE | 16E    |
| Q8VBT8    | TMUB1  | Transmembrane and ubiquitin-like domain-containing protein 1 | Transmembrane and ubiquitin-like domain-containing protein 1 | HDTISGLKRTQFPGR | 17E    |
| O75478    | TADA2A | Transcriptional adapter 2-alpha | Transcriptional adapter 2-alpha | TREEEKLKRMLSEV | 18E    |
| Q9UBN7    | HDAC6  | Histone deacetylase 6 | Histone deacetylase 6 | GRFWPHLKASNWSTT | 19E    |
| Q8N1G1    | REXO1  | RNA exonuclease 1 homolog | RNA exonuclease 1 homolog | AQPGBKRLKAPPS | 20E    |
| O75262    | PRDM1  | PR domain zinc finger protein 1 | PR domain zinc finger protein 1 | PLPEDFLKASLAYGI | 21E    |
| P19838    | NFKB1  | Nuclear factor NF-kappa-B p105 subunit | Nuclear factor NF-kappa-B p105 subunit | TRLAALLKAAAGDPL | 1F     |
| Q8WXI9    | GATAD2B | Transcriptional repressor p66-beta | Transcriptional repressor p66-beta | SRMEERLKAANLEMFR | 2F     |
| Q15329    | E2F5   | Transcription factor E2F5 | Transcription factor E2F5 | KDGVLDLKAAADTLA | 3F     |
| Q15003    | NCAPH  | Condensin complex subunit 2 | Condensin complex subunit 2 | KMDDMKKLQSMWSSL | 4F     |
| P05412    | JUN    | Transcription factor AP-1 | Transcription factor AP-1 | YSNPKILKQSMTLNL | 5F     |
| O15151    | MDM4   | Protein Mdm4 | Protein Mdm4 | PSQDQLKQAESST | 6F     |
| P46100    | ATRX   | Transcriptional regulator ATRX | Transcriptional regulator ATRX | SSYVKFLKQATDNSE | 7F     |
| Q76L83    | ASXL2  | Putative Polycomb group protein ASXL2 | Putative Polycomb group protein ASXL2 | HSKKALKQALKQQQ | 8F     |
| O75529    | TAF5L  | TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 5L | TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 5L | VDSGPLEXKQGLRLSQ | 9F     |
| Q96GD3    | SCMH1  | Polycomb protein SCMH1 | Polycomb protein SCMH1 | KTVFSFLKQGHGEV | 10F    |
| Q9NS91    | RAD18  | E3 ubiquitin-protein ligase RAD18 | E3 ubiquitin-protein ligase RAD18 | ASRQSLKQGSRMDN | 11F    |
| Q9UIL8    | PHF11  | PHD finger protein 11 | PHD finger protein 11 | ELLQDLKQTLCSFQE | 12F    |
| Q2TAY7    | SMU1   | WD40 repeat-containing protein SMU1 | WD40 repeat-containing protein SMU1 | DPMIMLKTQTPERYI | 13F    |
| Q8N8U2    | CDYL2  | Chromodomain Y-like protein 2 | Chromodomain Y-like protein 2 | SGLQIMPLKKSQNGM | 14F    |
| P55197   | MLLT10  | Protein AF-10       | KYHFSKLKKSKRGSN | 15F |
|---------|---------|---------------------|------------------|-----|
| Q14005  | IL16    | Pro-interleukin-16  | SHPPRLKKSEILV    | 16F |
| Q02539  | HIST1H1A| Histone H1.1        | TGASKLKKTATGASK  | 17F |
| P16403  | HIST1H1C| Histone H1.2        | GVSLAALKKALAAAG  | 18F |
| P16403  | HIST1H1C| Histone H1.2        | SRIKGLKSLVSKGT   | 19F |
| Q5T653  | PHF19   | PHD finger protein 19| VRKGGALKKGAIART  | 20F |
| Q9P0M6  | H2AFY2  | Core histone macro-H2A.2 | GRLMRYLKKTFKYR | 21F |
| Q96PU4  | UHRF2   | E3 ubiquitin-protein ligase UHRF2 | VVKAGERLKKMSKKKA | 1G |
| P49848  | TAF6    | Transcription initiation factor TFIIID subunit 6 | AEATEPLKSAKPGQE | 2G |
| P15918  | RAG1    | V(D)J recombination- activating protein 1 | MNAHNALKTSGFTMN | 3G |
| Q8TAF3  | WDR48   | WD repeat-containing protein 48 | NWFSVDLKGTMLLTIT | 4G |
| Q6P1X5  | TAF2    | Transcription initiation factor TFIIID subunit 2 | KIVAYELKTGGVLLH | 5G |
|         |         | Artificial peptide | RNEPPKLKRNGAFT   | 7G |
|         |         | Artificial peptide | RNEPPKLARSGAFT   | 8G |
| P04637  | p53     | Cellular tumor antigen p53 | RAHSSHLKSSKGQST | 9G |
| P04637  | p53     | Cellular tumor antigen p53 | RAHSSHLKSSKGQST | 10G |
| P04637  | p53     | Cellular tumor antigen p53 | RAHSSHLKSSKGQST | 11G |
| P04637  | p53     | Cellular tumor antigen p53 | RAHSSHLKSSKGQST | 12G |
| P68431  | HIST1H3A| Histone H3.1        | ARTKQTARKSTGGKA  | 13G |
| P68431  | HIST1H3A| Histone H3.1        | ARTAQTARKSTGGKA  | 14G |
| P68431  | HIST1H3A| Histone H3.1        | PATGGVKPHRYRPG   | 15G |
| P68431  | HIST1H3A| Histone H3.1        | PATGGVAKPHRYRPG  | 16G |
Supplemental Table 2: List of potential novel non-histone targets identified in the Phosphosite Plus database (Fig. 4B). The target lysine residue is highlighted by an underscore. Column 1 - Swissprot protein number; column 2 – abbreviation; column 3 – position of the spot in Fig. 4 panel B.

|   | 1   | 2                  | Sequence                        | 3  |
|---|-----|--------------------|---------------------------------|----|
| 1 | P04637 | p53              | Cellular tumor antigen p53      | 1A |
|   | P04637 | p53              | Cellular tumor antigen p53      | 2A |
|   |       | Artificial peptide | RNEPPKL5KRSRGAFT               | 3A |
|   |       | Artificial peptide | RNEPPKLARSRGAFT                | 4A |
|   | O14974 | PPP1R12A         | Protein phosphatase 1 regulatory subunit 12A | SEMEEELKMLPDLKA 5A |
|   | Q9NW08 | POLR3B           | DNA-directed RNA polymerase III subunit RPC2 | PVYYQKLKHVMVLDKM 6A |
|   | P30876 | POLR2B           | DNA-directed RNA polymerase II subunit RP2 | PTTYQLRKHVMVDDKI 7A |
|   | P10412 | HIST1H1E         | Histone H1.4                    | TGASGSFKLNNKAAS 8A |
|   | P07305 | H1F0             | Histone H1.0                    | PKKSVAFKKTKKIEK 9A |
|   | Q9Y3A2 | UTP11            | Probable U3 small nucleolar RNA-associated protein 11 | DVKYIEMKRAEAKK 10A |
|   | O94761 | RECQL4           | ATP-dependent DNA helicase Q4    | PDYGQLRKLKMLQQKP 11A |
|   | Q13315 | ATM              | Serine-protein kinase ATM        | AFENAYLKAQEGMR 12A |
|   | O94761 | RECQL4           | ATP-dependent DNA helicase Q4    | QRLKANLKTQLQAGP 13A |
|   | Q13129 | RLF              | Zinc finger protein Rlf          | ALLMHYLLKHNSK 14A |
|   | Q15047 | SETDB1           | Histone-lysine N-methyltransferase SETDB1 | STRGFALKSTHGIAI 15A |
|   | Q09666 | AHNAK            | Neuroblast differentiation-associated protein AHNAK | KLKGPKFKMPFMHFK 16A |
|   | P08319 | ADH4             | All-trans-retinol dehydrogenase [NAD(+)] ADH4 | CGKISNLKSPASDQQ 17A |
|   | Q2M1P5 | KIF7             | Kinesin-like protein KIF7        | MWINVQELQKLVGN 18A |
|   | P68431 | HIST1H3A         | Histone H3.1                    | REIAQDFKTDLRQS 19A |
|   | Q13442 | PDAP1            | 28 kDa heat- and acid-stable phosphoprotein | KAKERYKMMLA 20A |
|   | Q9HAE3 | EFCAB1           | EF-hand calcium-binding domain-containing protein 1 | EEMFHMLKNSLKKQP 21A |
|   | Q6ZNL6 | FGDS             | FYVE, RhoGEF and PH domain-containing protein 5 | DGCFELKRPGRAVP 1B |
|   | Q09666 | AHNAK            | Neuroblast differentiation-associated protein AHNAK | KLKGPKFKMPFMHFK 2B |
|   | Q70E73 | RAPH1            | Ras-associated and pleckstrin homology domains-containing protein 1 | TLKHGSTGKLS 3B |
|   | Q9BTFO | THUMPD2          | THUMP domain-containing protein 2 | QRDDNQLKRRVGE 4B |
|   | O15079-2 | SNPH        | Syntaphilin                     | EVCIRKLARLKTQ 5B |
|   | Q8WXU2 | DNAAF4           | Dynein assembly factor 4, axonemal | YALSVMMKIEEEERK 6B |
| Accession | Description | Protein Name | Description | Accession | Description | Protein Name | Description | Accession | Description | Protein Name | Description |
|-----------|-------------|--------------|-------------|-----------|-------------|--------------|-------------|-----------|-------------|--------------|-------------|
| P06213    | INSR        | Insulin receptor | GGSSLGFKRSYEEHI | 7B        | Q14204      | Dynamin 1 heavy chain | DALLNQLKSPARLR | 8B        | Q9UHQ9      | Cytochrome b5 reductase | SQYLDSLKVGDDVF | 9B        | B011T2      | Unconventional myosin | RVKDVLLKSTCVLEA | 10B       | Q8IWQ1      | TSG2 | HRRGGPLKRRGSSK | 11B       |
| Q9BTFO    | THUMP2      | THUMP domain-containing protein 2 | EIAAKKLIEQMQKI | 12B       | P02533      | Keratin, type II | FTSSSMKGSCGIGG | 13B       | Q2KHR3      | Glutamine and serine-rich protein | RAMKETFKSYMELLV | 14B       |
| P46777    | RPL5        | 60S ribosomal protein L5 | NKVFAGLKAVGDGL | 15B       | Q09666      | Neuroblast differentiation-associated protein | KLGPKFKMPMEMHKF | 16B       | Q9C0C2      | 182 kDa tankyrase-1-binding protein | SLGTKGLKVNLFGL | 17B       |
| Q9UQM7    | CAMK2A      | Calcium/calmodulin-dependent protein kinase | FNARRKLKGAILTM | 19B       | Q9H1E3      | Nuclear ubiquitain casein and cyclin-dependent kinase substrate | KMPKRPKLAVTPS | 20B       | Q7BC6       | Lysine-specific demethylase 3B | KDAVGLKHAESKL | 21B       |
| Q9B229    | DOCK9       | Dedicator of cytokinesis protein 9 | EISQRLLKLYSDKFG | 1C        | Q96666      | Neuroblast differentiation-associated protein | KLGPKFKMPMEMHKF | 2C        | P35749      | Myosin-11 | GREVNALKSLRGN | 3C        |
| Q9UQM3    | BARX2       | Homeobox protein BarH-like 2 | KWKKMVLKGGQEAAP | 4C        | Q96666      | Neuroblast differentiation-associated protein | KLGPKFKMPMEMHKF | 5C        | P38117      | Electron transfer flavoprotein subunit beta | ATLPNIMKAKKKIE | 6C        |
| P0CI25    | TRIM49      | Tripartite motif-containing protein 49 | KHNLEMLKKKGKEIF | 7C        | A6ND10      | Putative tripartite motif-containing protein 49B | KHNLEMLKKKGDIF | 8C        | Q09161      | Nuclear cap-binding protein subunit | ANTESYLKRQKTHV | 9C        |
| Q9NX65    | ZSCAN32     | Zinc finger and SCAN domain-containing protein 32 | NRLGFHKKNEIKKEN | 10C       | O43482      | Protein Mis18-beta | NRLKSMKLSEVTP | 11C       | P46777      | 60S ribosomal protein L5 | NKVFAGLKAVGDGL | 12C       |
| Q96666    | AHNAK       | Neuroblast differentiation-associated protein | KLGPKFKMPMEMHKF | 13C       | Q8N7X4      | Melanoma-associated antigen B6 | EKESILKADMLKCV | 14C       |
| Protein ID | Name                   | Description                                                                 | Peptide Sequence | C.D. |
|------------|------------------------|------------------------------------------------------------------------------|------------------|------|
| Q09666     | AHNAK                  | Neuroblast differentiation-associated protein AHNAK                           | PDVNLNLKGPVKGD  | 15C  |
| Q9NQS1     | AVEN                   | Cell death regulator Aven                                                    | KGLGMQLKGPLPGG  | 16C  |
| P60174     | TPI1                   | Triosephosphate isomerase                                                    | EKLRGWLKSNVSDAV | 17C  |
| P42566     | EPS15                  | Epidermal growth factor receptor substrate 15                               | KYDEIFLKTSDKMDG | 18C  |
| Q8WZ42     | TTN                    | Titin                                                                        | DAGKYILKLENSSGS | 19C  |
| Q9UNZ2     | NSFL1C                 | NSFL1 cofactor p47                                                           | VKPKGAFKAFTGEGQ | 20C  |
| Q9Y2W1     | THRAP3                 | Thyroid hormone receptor-associated protein3                                | RERSPALKSPLOSVV | 21C  |
| P10636-8   | MAPT                   | Microtubule-associated protein tau                                          | PVPMPLKKNVSKIG | 1D   |
| Q86UE4     | MTDH                   | Protein LYRIC                                                                | NVQVSNFKSGKDST  | 2D   |
| P10636-8   | MAPT                   | Microtubule-associated protein tau                                          | IGSTENLHKPGGGK  | 3D   |
| Q96G03     | PGM2                   | Phosphoglucomutase-2                                                         | SFVQAFAFDLPP    | 4D   |
| Q96666     | AHNAK                  | Neuroblast differentiation-associated protein AHNAK                           | KLKPFPFKAEMNIK | 5D   |
| Q55WX8     | ODR4                   | Protein odr-4 homolog                                                       | CGSVNLKGAHKRA   | 6D   |
| Q96666     | AHNAK                  | Neuroblast differentiation-associated protein AHNAK                           | KWKPFPFKAEMNK  | 7D   |
| Q01518     | CAP1                   | Adenyl cyclase-associated protein 1                                          | THKNPALKAQSPVR  | 8D   |
| Q01518     | CAP1                   | Adenyl cyclase-associated protein 1                                          | THKNPALKAQSPVR  | 9D   |
| Q9UBF8     | PI4KB                  | Phosphatidylinositol 4-kinase beta                                           | ISLSSNLKRTASNK | 10D  |
| P10809     | HSPD1                  | 60 kDa heat shock protein, mitochondrial                                      | TLVNLKVKYGLQV  | 11D  |
| Q96666     | AHNAK                  | Neuroblast differentiation-associated protein AHNAK                           | KLKPFPFKAEMNIK | 12D  |
| Q5TAP6     | UTP14C                 | U3 small nuclear RNA-associated protein 14 homolog C                         | MMERMSLKHQNSGW  | 13D  |
| Q8IUL5     | ZBTB8A                 | Zinc finger and BTB domain-containing protein 8A                            | VKRKDLKRLRCHT  | 14D  |
| Q13557     | CAMK2D                 | Calcium/calmodulin-dependent protein kinase type II subunit delta            | FNARRKLKGAILTTM | 15D  |
| A6NCV1     | OR6C74                 | Olfactory receptor 6C74                                                      | KQVKDFKHTVKKIE | 16D  |
| Q71F23     | CENPU                  | Centromere protein U                                                         | SQMLTNLKRKNKMI | 17D  |
| Q6ZV50     | RFX8                   | DNA-binding protein RFX8                                                     | KTMRMVLKSSRVS  | 18D  |
| Q09666     | AHNAK                  | Neuroblast differentiation-associated protein AHNAK                           | KLKPFPFKAEMNIK | 19D  |
| Q9C066     | DNAH6                  | Dynein heavy chain 6, axonemal                                               | KESKSLKIQKLDTS | 20D  |
| Q04206     | RELA                   | Transcription factor p65                                                     | KRTYETFKSIMKKSP | 21D  |
| Q04206     | RELA                   | Transcription factor p65                                                     | ETFKSMJKSPFGP   | 1E   |
| Uniprot ID | Description | Accession Number | Protein Name | Description |
|-----------|-------------|------------------|--------------|-------------|
| Q5TIE3    | von Willebrand factor A domain-containing protein SB1 | TDFIKGMKKKSRAER | Q5TIE3 | 2E |
| O75366    | Advillin | ALGFIKMSYSPSSTN | O75366 | 3E |
| O75541    | Zinc finger protein 821 | DREAMRLKRANETPE | O75541 | 4E |
| Q96AY2    | Crossover junction endonuclease EME1 | GSDLSTMGKETLQG | Q96AY2 | 5E |
| Q16186    | Proteasomal ubiquitin receptor ADRM1 | RAGKMSLGGTTVPD | Q16186 | 6E |
| Q96966    | Neuroblast differentiation-associated protein AHNAK | KVKGSKFKMPLSIS | Q96966 | 7E |
| O88737    | Protein bassoon | DEITYGLKKNVEQQ | O88737 | 8E |
| Q8BH00    | 2-aminomuconic semialdehyde dehydrogenase | KVRSYVLKAQTEGAR | Q8BH00 | 9E |
| Q969V6    | Myocardin-related transcription factor A | PANLDDMKVAELKQE | Q969V6 | 10E |
| P10860    | Glutamate dehydrogenase 1, mitochondrial | KHRSSVMKQFLETAI | P10860 | 11E |
| Q6P3S1-5  | DENN domain-containing protein 1B | RAHSSHLKSKGQST | Q6P3S1-5 | 12E |
| P04637    | Oxysterol-binding protein-related protein 1 | REISNFLMIKEDDF | P04637 | 13E |
| Q98XW6    | Glutamate dehydrogenase 1, mitochondrial | KHRSSVMKQFLETAI | Q98XW6 | 14E |
| P04637    | Cellular tumor antigen p53 | RAHSSHLKSKGQST | P04637 | 15E |
| Q02318    | Sterol 26-hydroxylase, mitochondrial | FAHMPPLKAVLKETL | Q02318 | 16E |
| Q05516    | Zinc finger and BTB-domain-containing protein 16 | GELAVGMKSESRTIG | Q05516 | 17E |
| O75072    | Fukutin | WTEFVDMKTHPCET | O75072 | 18E |
| Q9UPS6    | Histone-lysine N-methyltransferase SETD1B | LMPMLLKGKHKLY | Q9UPS6 | 19E |
| Q07889    | Son of sevenless homolog 1 | RFYSQMGKQLAIK | Q07889 | 20E |
| P11142    | Heat shock cognate 71 kDa protein | AVVQSDMKHWPFMVV | P11142 | 21E |
| Q9NYF8    | Bcl-2-associated transcription factor 1 | YKSKVSLLKGNRESDG | Q9NYF8 | 22E |
| O95373    | Importin-7 | SLAEILLKKIYKDQ | O95373 | 23E |
| Q3KQV3    | Zinc finger protein 792 | SRSSDLMKHQRVHTG | Q3KQV3 | 24E |
| P34897    | Serine hydroxymethyltransferase, mitochondrial | TAKLQDFKSSLKDS | P34897 | 25E |
| P33778    | Histone H2B type 1-B | IYYVKLQVHPDGT | P33778 | 26E |
| P11387    | DNA topoisomerase 1 | DWKSKEMKVQRAVA | P11387 | 27E |
| Q72624    | Calmodulin-lysine N-methyltransferase | KLLRQVLKQKHLDDC | Q72624 | 28E |
| Q8IX06    | Putative exonuclease GOR | DGFVETFKKELSRA | Q8IX06 | 29E |
| Q2Q1W2    | E3 ubiquitin-protein ligase TRIM71 | KATGDGLKRALQGKV | Q2Q1W2 | 30E |
| Q63HN8    | E3 ubiquitin-protein ligase RNF213 | GKPRSLKGIPTLYV | Q63HN8 | 31E |
| Q96M93  | ADAD1     | Adenosine deaminase domain-containing protein 1 | ITESPFPKSGMSMAS | 11F   |
|---------|-----------|------------------------------------------------|----------------|------|
| Q14117  | DPYS      | Dihydropyrimidinase                             | KGEVATLKSRVTKEK | 12F  |
| Q15910  | EZH2      | Histone-lysine N-methyltransferase EZH2         | HCRKIQLKDGGSSNH | 13F  |
| J3KP02  | LEKR1     | Leucine-, glutamate- and lysine-rich protein 1  | AEFESRLKEIDSND  | 14F  |
| Q8TEW8  | PARD3B    | Partitioning defective 3 homolog B              | AGLGVSLKGNKSRET | 15F  |
| Q99909  | SSX3      | Protein SSX3                                    | KIVVYMKRKYEAMT  | 16F  |
| Q7RTT5  | SSX7      | Protein SSX7                                    | KISYYMKRKYEAMT  | 17F  |
| Q7RTT3  | SSX9P     | Putative protein SSX9                           | KIIYYMKRKYEAMT  | 18F  |
| Q00973  | B4GALNT1  | Beta-1,4 N-acetylgalactosaminyltransferase 1    | KHRLLFFKHLQCMTC | 19F  |
| P13639  | EEF2      | Elongation factor 2                             | PKLVEGLRLAKSDP  | 20F  |
| Q9Y222  | MTO1      | Protein MTO1 homolog, mitochondrial             | YERACWMKSSLIEGI | 21F  |
| P10619  | CTSA      | Lysosomal protective protein                     | RQYSYLGKGSGBKHL | 1G   |
| Q07065  | CKAP4     | Cytoskeleton-associated protein 4                | SQVEADLKLMLRTAVD | 2G   |
| Q9Y2K6  | USP20     | Ubiquitin carboxyl-terminal hydrolase 20        | FFADELKGDNMYS  | 3G   |
| Q6ZN66  | GBP6      | Guanylate-binding protein 6                     | DWLHEGFKKKYEEMN | 4G   |
| Q9UK7   | MYO15A    | Unconventional myosin-XV                       | TSLARFLKJTLSEK  | 5G   |
| Q02880  | TOP2B     | DNA topoisomerase 2-beta                        | HNWPSLLKHFLEEF | 6G   |
| Q94830  | DDHD2     | Phospholipase DDHD2                             | TRSMSMDKNLLGSL | 7G   |
| P35580  | MYH10     | Myosin-10                                       | KADEWLMKNMDPLND | 8G   |
| Q8NB5   | ATAD1     | ATPase family AAA domain-containing protein 1   | KQAEKLMKQIGVKNV | 9G   |
| P62805  | HIST1H4A  | Histone H4                                      | EETRGVLKVFLENVI | 10G  |
| Q8NBQ5  | HSD17B11  | Estradiol 17-beta-dehydrogenase 11              | AYEFAKLKSKLVLWD | 11G  |
| P07900  | HSP90AA1  | Heat shock protein HSP 90-alpha                 | ANMERIMKAQALRDN | 12G  |
| P61081  | UBE2M     | NEDD8-conjugating enzyme Ubc12                  | PDDLLNFKLVICPDE | 13G  |
| Q8WVM7  | STAG1     | Cohesin subunit SA-1                           | KHLDDLKIQIKFVE  | 14G  |
| Q03164  | KMT2A     | Histone-lysine N-methyltransferase 2A           | KHLDDLKIQIKFVE  | 15G  |
| P2769S  | APEX1     | DNA-(apurinic or apyrimidinic site) lyase        | SGKPATLKICSWNVD | 16G  |
| O14862  | AIM2      | Interferon-inducible protein AIM2               | GAVSAVMKTIRIFQK | 17G  |
| P78524  | ST5       | Suppression of tumorigenicity 5 protein         | VHIQSMKLKAPSYRT | 18G  |
| Q6PJ6   | FBXO38    | F-box only protein 38                           | EQIKADMKAARDIPE | 19G  |
| Q9Y3M9  | ZNF337    | Zinc finger protein 337                         | SYYSHKLKRLHREKR | 20G  |
| Q14847  | LASP1     | LIM and SH3 domain protein 1                    | TPENLRKQQLQELQS | 21G  |
| Q12768  | WASHC5    | WASH complex subunit 5                         | YVNIYGLKIQWCEEVS | 1H   |
| Accession | Description                                | Peptide Sequence                        | Length |
|-----------|--------------------------------------------|----------------------------------------|--------|
| P68433    | Histone H3.1                               | REIAQDFKTDLRFQS                        | 2H     |
| P33991    | DNA replication licensing factor MCM4       | EELAEALKKLILSKG                        | 3H     |
| P07305    | Histone H1.0                               | LVTTGVLQKQTKGVGA                       | 4H     |
| Q9Y6Q9    | Nuclear receptor coactivator 3             | GTNLGLKSSQSVQS                         | 5H     |
| O75420    | GRB10-interacting GYF protein 1            | LVRGLGLKNSRSSPS                       | 6H     |
| P11142    | Heat shock cognate 71 kDa protein          | AVVQSDMKHWPFMVV                        | 7H     |
| Q8WXG6-3  | MAP kinase-activating death domain protein | ATPFPSLKGNRRALV                        | 8H     |
| Q5MNZ9    | WD repeat domain phosphoinositide-interacting protein 1 | QMNVYHFKKGTEICN | 9H     |
| A5PKW4    | PH and SEC7 domain-containing protein 1    | RTHEAKLKAMASELR                       | 10H    |
| Q6KC79    | Nipped-B-like protein                      | GGRSGALKNFVIPKI                       | 11H    |
| P53396    | ATP-citrate synthase                       | MEFVNMMKEKGLIM                         | 12H    |
| P04637    | Cellular tumor antigen p53                 | RAHSSHLKSKKGQST                       | 13H    |
| P04637    | Cellular tumor antigen p53                 | RAHSSHLASKKGQST                       | 14H    |
| A5PKW4    | Artificial peptide                        | RNEPPKLRSRGAF                        | 15H    |
| A5PKW4    | Artificial peptide                        | RNEPPKLARSRGAF                       | 16H    |