## Supplementary information

# Autophagic degradation of aquaporin-2 is an early event in hypokalemia-induced nephrogenic diabetes insipidus

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# FULL METHODS

**Experimental animals.** All experiments were approved by the Animal Care and Use Committee of the Faculty of Medicine, Thammasat University. Experiments were conducted on male Sprague-Dawley rats weighing 180-200 g. After 3 days of acclimatization in metabolic cages, rats were randomly assigned into 2 groups: rats fed with a potassium-free diet (TD.88239, Harlan, WI, USA) or control rats fed with standard rat chow. Rats were offered the same amount of food and free access to water. In order to identify the onset of NDI in our setting, rats were monitored for 3 days. Body weight, water intake as well as urine and serum (blood drawn from tail vein) parameters were measured at study onset and daily.

**Sample preparation for proteomic analysis.** IMCD was prepared from renal inner medullas according to Stokes et al.[1] with modifications[2]. IMCD tubules were lysed in 150 μl of lysis buffer (1.5% SDS, 10 mM Tris, pH 6.8) containing protease inhibitors. Protein samples were sonicated and centrifuged at 14,000 *g* for 10 min at 4°C and the supernatants were used for proteomic analysis. Three independent pairs of samples were prepared using four potassium-deprived rats versus four control rats for each pair (IMCD proteins in each group were pooled). Each pair of samples (100 μg per sample) was subjected to one-dimensional SDS-PAGE. The gel was stained with colloidal Coomassie blue, destained, and sliced into 5 blocks per lane (identical slicing on both lanes was performed for each pair). Each block was minced into small pieces (1–1.5 $mm^3$) and in-gel trypsinization was performed as previously described[3]. Tryptic peptides were extracted with 50% acetonitrile (ACN)/0.1% formic acid (FA), desalted, dried under vacuum, and dissolved in 0.1% FA for subsequent LC-MS/MS analysis.

**LC-MS/MS analysis and database searching.** Samples were analyzed using an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a nanoflow

Ultra-high Pressure Liquid Chromatography (nanoACQUITY UPLC, Waters Corp., MA). Mass spectra were searched using two different search algorithms, SEQUEST (Bioworks version 3.31 SP1)[4] and InsPecT (version *20070905*)[5], on rat RefSeq database (National Center for Biotechnology Information, March, 2010, 30734 entries) which included a list of common contaminating proteins from other species. Precursor ion tolerance was 10 ppm, while fragment ion tolerance was 0.8 Da. Three missed trypsin cleavage sites were allowed. Static modification included carbamidomethylation of cysteine (+57.021 Da). Variable modification included oxidation of methionine (+15.995 Da). All datasets were filtered to achieve a false discovery rate of < 1%, estimated based on target-decoy analysis[6]. Peptides matching to multiple protein isoforms were identified using ProMatch software[7].

**Label-free quantification.** Peptide quantification by a label-free technique was performed using QUOIL software[8]. This program calculates relative peptide abundance based on the area under the extracted ion chromatogram of all peptides in both potassium-free (NK) and control conditions. The median peptide abundance of all peptides identified for a given protein was used to infer the protein abundance change. Only proteins with peptides quantified in all three biological replicates were used for statistical significance testing. The $\log_2$ of NK/Control protein abundance ratio was used as the basis for calculation of the average abundance change and statistical significance by unpaired t-test for each protein.

**Bioinformatics.** Automated Bioinformatics Extractor, ABE (http://helixweb.nih.gov/ESBL/ABE/), was used to extract protein names, gene symbols, accession numbers, and Gene Ontology (GO) terms for all data. The DAVID bioinformatics resources (Database for Annotation, Visualization and Integrated Discovery, NIAID, http://david.abcc.ncifcrf.gov/)[9] was used to extract the list of significantly enriched functional annotation clusters, KEGG pathways, and biological process and cellular component GO terms associated with the significantly changed proteins induced by potassium deprivation.

**Targeted LC-MS/MS.** Peptides from candidate proteins were selected based on prior non-targeted LC-MS/MS data. Skyline software[10] was used to create an inclusion list of all selected peptides including three isotopes per peptide. Targeted LC-MS/MS analysis was performed using a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA). Peak areas for each peptide were extracted and integrated using Skyline software.

LC-MS/MS analysis was performed using a standard top 15 method on Q-Exactive orbitrap MS (Thermo Scientific) in conjunction with a Proxeon Easy-nLC II HPLC (Thermo Scientific) and Proxeon nanospray source. The digested peptides were reconstituted in 2% acetonitrile /0.1% trifluoroacetic acid and loaded onto a 100 micron x 25 mm Magic C18 100Å 5U trap reverse phase trap where they were desalted online before being separated using a 75 micron x 150 mm Magic C18 200Å 3U reverse phase column. Data dependent MS/MS data was collected using higher energy collision dissociation (HCD). Peptides were eluted using a gradient of 0.1% formic acid (A) and 100% acetonitrile (B). A 90 minute gradient was ran with 5% to 35% B over 75 minutes, 35% to 80% B over 5 minutes, 80% B for 1 minute, 80% to 5% B over 1 minute, and finally held at 5% B for 8 minutes. The mass spectrometer was operated with a spray voltage of 1.8kV, a capillary temperature of 275°C, and a normalized collision of 27%.

**Quantitative Real-Time PCR.** Total RNA was extracted from whole inner medullas of 7 control and 7 potassium-depleted rats using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. 0.5 µg total RNA were used for first strand cDNA synthesis using an oligo(dT) primer. PCR was performed using 0.5 µl of the first strand cDNA mixture using gene specific primers (see below). PCR was performed with the following conditions: an initial denature at 95°C for 2 min, followed by heating at 95°C for 10 s and 60°C for 10 s repeated for 40 cycles.

Real-time RT-PCR was carried out in a 20 µl reaction volume composed of 2 µl of the first strand cDNA and 18 µl of a real-time PCR master mix containing 10 µl of 2X Power SYBR Green PCR Master Mix (Applied Biosystem, Calsbad, USA), 0.5 µM forward primer, and 0.5 µM reverse primer, using the 7500 Fast System (Applied Biosystems, Calsbad, USA). The PCR amplicon for *18s* rRNA was used for developing standard curves. Quantification between groups was performed using the comparative Ct method.

| Gene name | Primers |
|---|---|
| *Aqp2* | Forward 5'-GAC TGT GCT TAG TGC ATC TCA TTT TAT-3'<br>Reverse 5'-AGG ATC ATG CAA ACT CTG TTC GT-3' |
| *Atp5b* | Forward 5'-CTA TGA CCA TCT CCC GGA ACA-3'<br>Reverse 5'-CAG CTT GTC AGC CTT TGC C-3' |
| *Itgb1* | Forward 5'-CGA TAG GTC CAA CGG CTT AAT CTG TG-3'<br>Reverse 5'-CTT GGA ACT TGG GAT CTG TGC ACT-3' |
| *Vcl* | Forward 5'-TCA GAT GAG GTG ACT CGG TTG G-3'<br>Reverse 5'-GGGTGCTTATGGTTGGGATTCG-3' |
| *Mdh1* | Forward 5'-ATC TCA AAT TGC TCT TAA ACT CG-3'<br>Reverse 5'-TGC TGC ACA GTC GTG ATG AA-3' |
| *18s* | Forward 5'-GTA ACC CGT TGA ACC CCA TT-3'<br>Reverse 5'-CCA TCC AAT CGG TAG TAG CG-3' |
| *Cdh16* | Forward 5'-TCC CGA TGG AAG CAC CAC C-3'<br>Reverse 5'-CCC AGT GCA CCT GGG CAA T-3' |

**Immunoblotting.** Polyclonal antibodies against Aqp2[11], pS256-Aqp2[12], pS261-Aqp2[13], and UT-A1/3[14] were kindly provided by Dr. Mark A. Knepper (NIH). Polyclonal antibodies against Ctnnb1 (PA5-17784) and Cttn (PA5-17730) were purchased from Pierce (Rockford, IL). Monoclonal antibody against RhoA (2117) and polyclonal antibody against Caspase-3 (9662) were purchased from Cell Signaling Technology (Danvers, MA). Immunoblotting was carried out as previously described[15]. To normalize samples for gel loading, samples were first analyzed on a separate gel, stained with Coomassie blue, scanned, and quantified for final justification of protein concentration in each sample prior to immunoblotting experiments[16].

**cAMP measurement.** Intracellular cAMP levels in IMCD were measured by a Direct cAMP ELISA kit (ADI-900-066, Enzo Life Sciences, Farmingdale, NY).

**Electron microscopy.** Fresh renal inner medullas from control and potassium-deprived groups were dissected, minced into 1 $mm^3$ cubes, and fixed in 4% paraformaldehyde with 1% glutaraldehyde 12 hours fixative. Post-fixation was performed in 1% osmium tetroxide in Millonig buffer for 1 h at 4 °C followed by dehydration in a graded ethanol series and propylene oxide. The tissues were embedded in epoxy resin and polymerized at 60 °C for 48 hours. Ultrathin sections were cut and mounted on naked copper grids before staining with 2% uranyl acetate and lead citrate solutions then examined under a transmission electron microscope (FEI Tecnai G2 TWIN 200 kV).

**Immunofluorescence.** Immunofluorescence labeling of kidney sections was done as previously described[17]. Primary antibodies used were against Aqp2 (LL265)[18]; Lamp1 (17768) and LC3 (282666) from Santa Cruz (CA, USA); Itgb1 (4706), Vcl (4650), RhoA (3142), and ERM (3142) from Cell Signaling Technology (Danvers, MA); Ctnnb1 (PA5-17784) and Cttn (PA5-17730) from Pierce (Rockford, IL); and UT-A1 (L179)[14]. Secondary antibodies were conjugated with Alexa Fluor 488 (anti-rabbit: green channel), 555 (anti-chicken: red channel), and 633 (anti-mouse: pink channel) (Invitrogen, CA, USA). Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI, D3571, Invitrogen) and F-actin with rhodamine phalloidin (R415, Life Technologies, Grand Island, NY). Fluorescence images were acquired using an Axio Observer Z1 microscope in conjunction with ApoTome2 (Carl Zeiss, Jena, Germany).

**Immunogold electron microscopy**. All procedures have been described in detail previously[19]. In brief, after anaesthesia, rat kidneys were removed, inner medullas dissected out, fixed in 4% paraformaldehyde solution in 0.1 M $Na^+$cacodylate, pH 7.2, for 3 h, infiltrated with 2.3 M sucrose, and frozen in liquid nitrogen. Ultrathin (70 nm) cryosections were cut on a Reichert Ultracut S cryo-ultramicrotome (Leica) and mounted. Sections were blocked by incubation in PBS containing 0.05 M glycine and 1% BSA before incubation overnight at 4°C with various antibodies diluted in PBS containing 0.1% skim milk powder. Primary antibodies used were a rabbit polyclonal antibody against AQP2[19], and goat polyclonal antibodies against Cathepsin D (Research Diagnostics), MAP LC3β (clone G-2, Santa Cruz) or Lamp1 (C20, Santa Cruz). Primary antibodies were visualized using various combinations (see figure legends) and sequential application of donkey anti-rabbit IgG, goat anti-rabbit IgG or donkey anti-goat IgG conjugated to 5nm, 6nm or 10nm colloidal gold particles (BioCell Research Laboratories) diluted 1:50 in PBS with 0.1% skim milk powder and polyethylene glycol (5 mg/ml). After counterstaining with 0.3% uranyl acetate in 1.8% methylcellulose for 10 min, sections were examined in a FEI Morgagni electron microscope. Immunolabeling controls included incubation without primary antibodies, use of only one secondary antibody (in double labeling experiments) and use of alternatively sized colloidal gold conjugated secondary antibodies.

**Re-feeding experiment.** An experiment was conducted on male Sprague-Dawley rats weighing 180-200 g. After 3 days of acclimatization in metabolic cages, fourteen rats were randomly assigned into 2 groups: 1) seven rats fed with a potassium-free diet (TD.88239, Harlan, WI, USA) for 1 day followed by standard rat chow for 3 days; 2) seven control rats fed with standard rat chow for 4 days. Body weight, water intake as well as urine and serum (blood drawn from tail vein) parameters were measured at study onset and daily. Kidneys were harvested and inner medullas were isolated, processed, and analyzed as described above.

## References

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**Supplemental Table 1.** A list of the significantly decreased proteins in response to potassium depletion ($P<0.05$ by two tailed t-test and average $\log_2$(NK/Control) ≤ -0.58).

| RefSeq | Gene Symbol | Protein Name | $\log_2$ (NK/Control) | |
|---|---|---|---|---|
| | | | Mean | SE |
| NP_058889 | Aadat | kynurenine/alpha-aminoadipate aminotransferase, mitochondrial | -4.81 | 1.45 |
| NP_001094010 | Iah1 | isoamyl acetate-hydrolyzing esterase 1 homolog | -2.55 | 0.75 |
| NP_001101788 | Atp6v1a | V-type proton ATPase catalytic subunit A | -2.52 | 0.62 |
| AP_004895 | COX2 | cytochrome c oxidase subunit II (mitochondrion) | -2.32 | 0.42 |
| NP_476473 | Rhoa | transforming protein RhoA precursor | -2.30 | 0.78 |
| NP_001005875 | Psmd12 | 26S proteasome non-ATPase regulatory subunit 12 | -2.25 | 0.50 |
| NP_001011915 | Fermt2 | fermitin family homolog 2 | -2.07 | 0.69 |
| XP_002726232 | Slc25a12 | LOW QUALITY PROTEIN: calcium-binding mitochondrial carrier protein Aralar1 | -1.98 | 0.62 |
| NP_036732 | Me1 | NADP-dependent malic enzyme | -1.91 | 0.45 |
| NP_058977 | Psma4 | proteasome subunit alpha type-4 | -1.70 | 0.29 |
| NP_877966 | Cct4 | T-complex protein 1 subunit delta | -1.67 | 0.43 |
| NP_037042 | Arrb1 | beta-arrestin-1 | -1.64 | 0.42 |
| NP_037322 | Pfkl | 6-phosphofructokinase, liver type | -1.56 | 0.50 |
| NP_114020 | Lgals3 | galectin-3 | -1.54 | 0.13 |
| NP_112330 | Actr3 | actin-related protein 3 | -1.52 | 0.11 |
| NP_062230 | Ezr | ezrin | -1.46 | 0.35 |
| NP_001007005 | Tuba4a | tubulin alpha-4A chain | -1.41 | 0.45 |
| NP_058759 | Kpnb1 | importin subunit beta-1 | -1.39 | 0.17 |
| NP_112262 | Akr1a1 | alcohol dehydrogenase [NADP(+)] | -1.37 | 0.17 |
| NP_001101959 | Iqgap1 | ras GTPase-activating-like protein IQGAP1 | -1.37 | 0.46 |
| NP_058814 | Capns1 | calpain small subunit 1 | -1.26 | 0.33 |
| NP_445809 | Ctnnb1 | catenin beta-1 | -1.21 | 0.09 |
| NP_112646 | Aifm1 | apoptosis-inducing factor 1, mitochondrial precursor | -1.18 | 0.22 |
| NP_001128170 | Aldh4a1 | delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial | -1.18 | 0.33 |
| NP_001102031 | Ahcyl1 | adenosylhomocysteinase-like 1 | -1.15 | 0.24 |
| NP_058895 | Ssr4 | translocon-associated protein subunit delta precursor | -1.14 | 0.39 |
| NP_001100073 | Cct7 | T-complex protein 1 subunit eta | -1.14 | 0.17 |
| NP_001102025 | Lrba | lipopolysaccharide-responsive and beige-like anchor protein | -1.14 | 0.32 |
| NP_445742 | Pgam1 | phosphoglycerate mutase 1 | -1.11 | 0.34 |
| NP_446006 | Picalm | phosphatidylinositol-binding clathrin assembly protein | -1.10 | 0.36 |
| NP_062250 | Ywhab | 14-3-3 protein beta/alpha | -1.10 | 0.22 |
| NP_036663 | Comt | catechol O-methyltransferase | -1.08 | 0.36 |
| NP_001040552 | Eif3a | eukaryotic translation initiation factor 3 subunit A | -1.07 | 0.16 |
| NP_058726 | Pccb | propionyl-CoA carboxylase beta chain, mitochondrial precursor | -1.07 | 0.18 |
| NP_001008888 | Uqcrfs1 | cytochrome b-c1 complex subunit Rieske, mitochondrial | -1.06 | 0.35 |

**Supplemental Table 1.**
*continued*

| RefSeq | Gene Symbol | Protein Name | $\text{Log}_2$ (NK/Control) Mean | SE |
|---|---|---|---|---|
| NP_001009641 | Pepd | xaa-Pro dipeptidase | -1.06 | 0.20 |
| NP_112411 | Psmc5 | 26S protease regulatory subunit 8 | -1.04 | 0.26 |
| NP_001100758 | Flnb | filamin-B | -1.03 | 0.17 |
| NP_001011918 | Anxa11 | annexin A11 | -1.03 | 0.21 |
| NP_942026 | Chdh | choline dehydrogenase, mitochondrial | -1.02 | 0.17 |
| NP_001020050 | Dnpep | aspartyl aminopeptidase | -1.02 | 0.09 |
| NP_001020068 | Ehd2 | EH domain-containing protein 2 | -1.01 | 0.30 |
| NP_058941 | Eef2 | elongation factor 2 | -1.01 | 0.14 |
| NP_001013104 | Capg | macrophage-capping protein | -1.00 | 0.20 |
| NP_599191 | Atp5b | ATP synthase subunit beta, mitochondrial precursor | -0.99 | 0.30 |
| NP_001099188 | Vps35 | maternal embryonic message 3 | -0.99 | 0.33 |
| NP_001004443 | Hexa | beta-hexosaminidase subunit alpha precursor | -0.99 | 0.18 |
| NP_536729 | Atp5i | ATP synthase subunit e, mitochondrial | -0.98 | 0.14 |
| NP_001029298 | Nit2 | omega-amidase NIT2 | -0.97 | 0.25 |
| NP_001008345 | Etf1 | eukaryotic peptide chain release factor subunit 1 | -0.97 | 0.11 |
| NP_705893 | Hspa4 | heat shock 70 kDa protein 4 | -0.96 | 0.15 |
| NP_741984 | Sptan1 | spectrin alpha chain, non-erythrocytic 1 | -0.96 | 0.16 |
| NP_075211 | Tpi1 | triosephosphate isomerase | -0.95 | 0.28 |
| NP_476550 | Kif5b | kinesin-1 heavy chain | -0.95 | 0.29 |
| NP_001041327 | Ndufa8 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 | -0.94 | 0.30 |
| NP_446316 | Vcp | transitional endoplasmic reticulum ATPase | -0.94 | 0.18 |
| NP_001026825 | Manba | beta-mannosidase precursor | -0.94 | 0.17 |
| NP_001011901 | Hsph1 | heat shock protein 105 kDa | -0.91 | 0.14 |
| NP_037036 | Anxa1 | annexin A1 | -0.91 | 0.20 |
| NP_001004223 | Eef1g | elongation factor 1-gamma | -0.91 | 0.00 |
| NP_001120766 | Ndufb9 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 | -0.90 | 0.32 |
| NP_001014102 | Uba1 | ubiquitin-like modifier-activating enzyme 1 | -0.90 | 0.08 |
| NP_742005 | Canx | calnexin precursor | -0.89 | 0.21 |
| NP_112359 | Rnpep | aminopeptidase B | -0.89 | 0.12 |
| NP_072114 | Tkt | transketolase | -0.89 | 0.19 |
| NP_001103355 | Fam129b | niban-like protein 1 | -0.87 | 0.22 |
| NP_058945 | Esyt1 | extended synaptotagmin-1 | -0.86 | 0.02 |
| NP_037184 | Ywhah | 14-3-3 protein eta | -0.86 | 0.15 |
| NP_058710 | Gstm1 | glutathione S-transferase Mu 1 | -0.86 | 0.30 |

**Supplemental Table 1.**
*continued*

| RefSeq | Gene Symbol | Protein Name | $Log_2$ (NK/Control) Mean | SE |
|---|---|---|---|---|
| NP_113698 | Idh1 | isocitrate dehydrogenase [NADP] cytoplasmic | -0.85 | 0.13 |
| NP_596909 | Slc25a10 | mitochondrial dicarboxylate carrier | -0.85 | 0.28 |
| NP_001013902 | Isyna1 | inositol-3-phosphate synthase 1 | -0.84 | 0.20 |
| NP_001098083 | Hnrnpa2b1 | heterogeneous nuclear ribonucleoproteins A2/B1 | -0.84 | 0.26 |
| NP_942040 | Atp6v1e1 | V-type proton ATPase subunit E 1 | -0.83 | 0.19 |
| NP_001099367 | Cct8 | T-complex protein 1 subunit theta | -0.82 | 0.24 |
| NP_598302 | Hadhb | trifunctional enzyme subunit beta, mitochondrial precursor | -0.82 | 0.27 |
| NP_570090 | Gnb2l1 | guanine nucleotide-binding protein subunit beta-2-like 1 | -0.82 | 0.02 |
| NP_059002 | Eci1 | enoyl-CoA delta isomerase 1, mitochondrial precursor | -0.81 | 0.20 |
| NP_001101978 | Eps8l2 | epidermal growth factor receptor kinase substrate 8-like protein 2 | -0.81 | 0.19 |
| NP_112297 | Gnai2 | guanine nucleotide-binding protein G(i) subunit alpha-2 | -0.80 | 0.13 |
| NP_036702 | Glud1 | glutamate dehydrogenase 1, mitochondrial precursor | -0.79 | 0.07 |
| NP_001131115 | Gstt3 | glutathione S-transferase, theta 3 | -0.79 | 0.24 |
| NP_113791 | Ywhae | 14-3-3 protein epsilon | -0.77 | 0.26 |
| NP_001101210 | Ctnnd1 | catenin delta-1 | -0.77 | 0.27 |
| NP_001014183 | Idh2 | isocitrate dehydrogenase [NADP], mitochondrial precursor | -0.77 | 0.18 |
| NP_114039 | Phb | prohibitin | -0.76 | 0.23 |
| NP_058784 | Gdi1 | rab GDP dissociation inhibitor alpha | -0.75 | 0.22 |
| NP_113863 | Actn4 | alpha-actinin-4 | -0.75 | 0.08 |
| NP_446428 | Krt18 | keratin, type I cytoskeletal 18 | -0.75 | 0.17 |
| NP_001094220 | Suclg2 | succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial | -0.75 | 0.11 |
| NP_110463 | Erap1 | endoplasmic reticulum aminopeptidase 1 precursor | -0.75 | 0.21 |
| NP_037023 | Acadvl | very long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor | -0.74 | 0.13 |
| NP_955792 | Krt19 | keratin, type I cytoskeletal 19 | -0.74 | 0.19 |
| NP_001011995 | Tuba1c | tubulin alpha-1C chain | -0.74 | 0.27 |
| NP_647540 | Ehd4 | EH domain-containing protein 4 | -0.74 | 0.20 |
| NP_997475 | Gpi | glucose-6-phosphate isomerase | -0.73 | 0.12 |
| NP_001020868 | Hsdl2 | hydroxysteroid dehydrogenase-like protein 2 | -0.73 | 0.24 |
| NP_112355 | Rala | ras-related protein Ral-A precursor | -0.73 | 0.20 |
| NP_071985 | Prdx3 | thioredoxin-dependent peroxide reductase, mitochondrial precursor | -0.72 | 0.22 |
| NP_037320 | Pygb | glycogen phosphorylase, brain form | -0.71 | 0.12 |
| NP_062040 | Sptbn2 | spectrin beta chain, non-erythrocytic 2 | -0.71 | 0.04 |
| NP_446041 | Rab14 | ras-related protein Rab-14 | -0.69 | 0.04 |
| NP_075237 | Sfxn3 | sideroflexin-3 | -0.69 | 0.08 |

**Supplemental Table 1.**
*continued*

| RefSeq | Gene Symbol | Protein Name | $\log_2$ (NK/Control) Mean | $\log_2$ (NK/Control) SE |
|---|---|---|---|---|
| NP_001007621 | Pdhb | pyruvate dehydrogenase E1 component subunit beta, mitochondrial precursor | -0.67 | 0.06 |
| NP_059055 | Rab10 | ras-related protein Rab-10 | -0.67 | 0.15 |
| NP_113902 | Hrsp12 | ribonuclease UK114 | -0.66 | 0.23 |
| NP_001012055 | Cdh16 | cadherin-16 precursor | -0.66 | 0.17 |
| NP_062259 | Tgm2 | protein-glutamine gamma-glutamyltransferase 2 | -0.66 | 0.21 |
| NP_445859 | Asah1 | acid ceramidase precursor | -0.66 | 0.22 |
| NP_077374 | Aco2 | aconitate hydratase, mitochondrial precursor | -0.65 | 0.03 |
| NP_071852 | Aldh1a1 | retinal dehydrogenase 1 | -0.65 | 0.07 |
| NP_071794 | Calr | calreticulin precursor | -0.63 | 0.17 |
| NP_001100718 | Vcl | vinculin | -0.63 | 0.15 |
| NP_001099891 | Tpd52 | tumor protein D52 | -0.63 | 0.04 |
| NP_001006982 | Dlst | dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | -0.63 | 0.05 |
| NP_001099959 | Ndufs3 | NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial | -0.62 | 0.22 |
| NP_446450 | Rab8a | ras-related protein Rab-8A | -0.62 | 0.20 |
| NP_062041 | Arg2 | arginase-2, mitochondrial precursor | -0.62 | 0.16 |
| NP_150238 | Mdh1 | malate dehydrogenase, cytoplasmic | -0.61 | 0.04 |
| NP_037035 | Anp32a | acidic leucine-rich nuclear phosphoprotein 32 family member A | -0.61 | 0.16 |
| NP_112643 | Vdac1 | voltage-dependent anion-selective channel protein 1 | -0.61 | 0.15 |
| XP_001059344 | LOC684270 | PREDICTED: isochorismatase domain-containing protein 2A, mitochondrial-like isoform 2 | -0.60 | 0.19 |
| NP_476489 | Sept2 | septin-2 | -0.60 | 0.17 |
| NP_775125 | Tubb5 | tubulin beta-5 chain | -0.59 | 0.18 |
| NP_071795 | Bcat2 | branched-chain-amino-acid aminotransferase, mitochondrial precursor | -0.59 | 0.14 |
| NP_058718 | Itgb1 | integrin beta-1 precursor | -0.58 | 0.17 |
| NP_071785 | Qdpr | dihydropteridine reductase | -0.58 | 0.09 |
| NP_113906 | Rab2a | ras-related protein Rab-2A | -0.58 | 0.07 |

**Supplemental Table 2.** A list of the significantly increased proteins in response to potassium depletion (*P*<0.05 by two tailed t-test and average $\log_2$(NK/Control) $\geq 0.58$).

| RefSeq | Gene Symbol | Protein Name | $\text{Log}_2$ (NK/Control) | |
|---|---|---|---|---|
| | | | **Mean** | **SE** |
| NP_058843 | Cfl1 | cofilin-1 | 1.43 | 0.43 |
| NP_446083 | Banf1 | barrier-to-autointegration factor | 1.30 | 0.42 |
| XP_002727937 | LOC100362689 | PREDICTED: keratin 8-like | 0.99 | 0.23 |

**Supplemental Table 3.** The over-represented GO clusters analyzed by the DAVID bioinformatics resources (using a list of all IMCD-expressed transcripts as a background).

| Category | Term | No. | PValue | Genes | Fold Enrichment | Benjamini | FDR |
|---|---|---|---|---|---|---|---|
| Annotation Cluster 1 | Enrichment Score: 3.0784975303793543 | | | | | | |
| GOTERM_BP_FAT | GO:0006091~generation of precursor metabolites and energy | 17 | 5.19E-07 | Uqcrfs1, Pygb, Atp5b, Dlst, Pfkl, Gpi, Suclg2, Atp6v1e1, Tpi1, Pgam1, Aco2, Mdh1, Pdhb, Atp6v1a, Idh2, Idh1, Atp5i | 4.5831 | 0.0007 | 0.0009 |
| GOTERM_BP_FAT | GO:0006084~acetyl-CoA metabolic process | 7 | 8.83E-05 | Suclg2, Aco2, Mdh1, Pdhb, Dlst, Idh2, Idh1 | 9.0820 | 0.0196 | 0.1446 |
| GOTERM_BP_FAT | GO:0006099~tricarboxylic acid cycle | 6 | 1.06E-04 | Suclg2, Aco2, Mdh1, Dlst, Idh2, Idh1 | 11.8622 | 0.0202 | 0.1740 |
| GOTERM_BP_FAT | GO:0046356~acetyl-CoA catabolic process | 6 | 1.35E-04 | Suclg2, Aco2, Mdh1, Dlst, Idh2, Idh1 | 11.3231 | 0.0224 | 0.2209 |
| GOTERM_BP_FAT | GO:0009109~coenzyme catabolic process | 6 | 1.35E-04 | Suclg2, Aco2, Mdh1, Dlst, Idh2, Idh1 | 11.3231 | 0.0224 | 0.2209 |
| GOTERM_BP_FAT | GO:0006732~coenzyme metabolic process | 11 | 2.02E-04 | Suclg2, Tpi1, Aco2, Tkt, Gstm1, Mdh1, Pdhb, Gstt3, Dlst, Idh2, Idh1 | 4.2682 | 0.0224 | 0.3304 |
| GOTERM_BP_FAT | GO:0009060~aerobic respiration | 6 | 2.57E-04 | Suclg2, Aco2, Mdh1, Dlst, Idh2, Idh1 | 9.9643 | 0.0244 | 0.4204 |
| GOTERM_BP_FAT | GO:0051187~cofactor catabolic process | 6 | 3.12E-04 | Suclg2, Aco2, Mdh1, Dlst, Idh2, Idh1 | 9.5810 | 0.0276 | 0.5105 |
| Annotation Cluster 2 | Enrichment Score: 2.272496263371351 | | | | | | |
| GOTERM_MF_FAT | GO:0019001~guanyl nucleotide binding | 17 | 1.08E-05 | Sept2, Gnai2, Rhoa, Glud1, Rab14, Rab2a, Tuba1c, Suclg2, Rab10, Eef2, Rala, Ehd2, Tgm2, Tubb5, Ehd4, Tuba4a, Rab8a | 3.6442 | 0.0037 | 0.0147 |
| GOTERM_MF_FAT | GO:0032561~guanyl ribonucleotide binding | 17 | 1.08E-05 | Sept2, Gnai2, Rhoa, Glud1, Rab14, Rab2a, Tuba1c, Suclg2, Rab10, Eef2, Rala, Ehd2, Tgm2, Tubb5, Ehd4, Tuba4a, Rab8a | 3.6442 | 0.0037 | 0.0147 |
| GOTERM_MF_FAT | GO:0005525~GTP binding | 16 | 3.10E-05 | Sept2, Gnai2, Rhoa, Glud1, Rab14, Rab2a, Tuba1c, Rab10, Eef2, Ehd2, Rala, Tgm2, Tubb5, Ehd4, Tuba4a, Rab8a | 3.5399 | 0.0053 | 0.0423 |
| GOTERM_MF_FAT | GO:0003924~GTPase activity | 9 | 1.36E-04 | Sept2, Rhoa, Eef2, Ehd2, Rab14, Tubb5, Ehd4, Tuba1c, Tuba4a | 5.7285 | 0.0153 | 0.1848 |
| GOTERM_BP_FAT | GO:0007264~small GTPase mediated signal transduction | 9 | 0.0243 | Iqgap1, Rab10, Ywhab, Rhoa, Rala, Gdi1, Rab14, Rab2a, Rab8a | 2.5247 | 0.5122 | 33.1411 |
| Annotation Cluster 3 | Enrichment Score: 1.0300588015817522 | | | | | | |
| GOTERM_CC_FAT | GO:0005916~fascia adherens | 3 | 0.0063 | Vcl, Sptan1, Ctnnb1 | 22.9568 | 0.0890 | 7.9027 |
| GOTERM_CC_FAT | GO:0014704~intercalated disc | 3 | 0.0168 | Vcl, Sptan1, Ctnnb1 | 14.3480 | 0.1683 | 19.7667 |
| GOTERM_CC_FAT | GO:0005913~cell-cell adherens junction | 4 | 0.0379 | Vcl, Dlst, Sptan1, Ctnnb1 | 5.2774 | 0.2606 | 39.4641 |
| GOTERM_CC_FAT | GO:0005912~adherens junction | 6 | 0.0417 | Flnb, Itgb1, Vcl, Dlst, Sptan1, Ctnnb1 | 3.1023 | 0.2624 | 42.5027 |
| GOTERM_CC_FAT | GO:0070161~anchoring junction | 6 | 0.0459 | Flnb, Itgb1, Vcl, Dlst, Sptan1, Ctnnb1 | 3.0206 | 0.2787 | 45.7232 |
| GOTERM_CC_FAT | GO:0044449~contractile fiber part | 4 | 0.0487 | Vcl, Krt19, Sptan1, Ctnnb1 | 4.7827 | 0.2862 | 47.7020 |
| Annotation Cluster 4 | Enrichment Score: 1.01236038334107 | | | | | | |
| GOTERM_BP_FAT | GO:0030036~actin cytoskeleton organization | 10 | 2.34E-04 | Itgb1, Rhoa, Cfl1, Actn4, Rala, Ehd2, Calr, Krt19, Sptan1, Ezr | 4.6649 | 0.0239 | 0.3832 |
| GOTERM_BP_FAT | GO:0030029~actin filament-based process | 10 | 3.55E-04 | Itgb1, Rhoa, Cfl1, Actn4, Rala, Ehd2, Calr, Krt19, Sptan1, Ezr | 4.4168 | 0.0294 | 0.5800 |
| GOTERM_BP_FAT | GO:0008064~regulation of actin polymerization or depolymerization | 6 | 0.0011 | Itgb1, Rhoa, Cfl1, Actn4, Rala, Ehd2, Calr, Krt19, Sptan1, Ezr | 7.3267 | 0.0813 | 1.8431 |
| GOTERM_BP_FAT | GO:0030832~regulation of actin filament length | 6 | 0.0011 | Rhoa, Cfl1, Actr3, Capg, Sptbn2, Sptan1 | 7.3267 | 0.0813 | 1.8431 |
| GOTERM_BP_FAT | GO:0007010~cytoskeleton organization | 12 | 0.0014 | Itgb1, Rhoa, Cfl1, Actn4, Rala, Ehd2, Calr, Krt19, Tubb5, Sptan1, Ctnnb1, Ezr | 3.1138 | 0.0840 | 2.2210 |
| GOTERM_BP_FAT | GO:0051017~actin filament bundle formation | 3 | 0.0080 | Rhoa, Actn4, Ezr | 20.7589 | 0.2643 | 12.2756 |
| GOTERM_BP_FAT | GO:0007015~actin filament organization | 4 | 0.0231 | Rhoa, Cfl1, Actn4, Ezr | 6.3874 | 0.5025 | 31.8214 |

**Supplemental Table 4.** A list of the significantly changed proteins and their estimated half-lives based on $t_{1/2}$ values at basal conditions of mouse collecting duct proteins as reported by Sandoval et al. (http://helixweb.nih.gov/ESBL/Database/ProteinHalfLives/index.html).

| **Gene Symbol** | **Protein Name** | **Mean $\text{Log}_2$(NK/Control)** | **Estimated Half-Life (Hours)** |
|---|---|---|---|
| Arg2 | arginase-2, mitochondrial precursor | -0.62 | 0.94 |
| Aqp2 | aquaporin-2 | -0.49 | 9.25 |
| Cdh16 | cadherin-16 precursor | -0.66 | 13.86 |
| Picalm | phosphatidylinositol-binding clathrin assembly protein | -1.1 | 14.53 |
| Fermt2 | fermitin family homolog 2 | -2.07 | 18.17 |
| Ehd4 | EH domain-containing protein 4 | -0.74 | 19.71 |
| Gnai2 | guanine nucleotide-binding protein G(i) subunit alpha-2 | -0.8 | 19.99 |
| Itgb1 | integrin beta-1 precursor | -0.58 | 23.03 |
| Fam129b | niban-like protein 1 | -0.87 | 23.96 |
| Atp6v1a | V-type proton ATPase catalytic subunit A | -2.52 | 24.15 |
| Atp6v1e1 | V-type proton ATPase subunit E 1 | -0.83 | 24.2 |
| Ezr | ezrin | -1.46 | 25.54 |
| Rab8a | ras-related protein Rab-8A | -0.62 | 27.17 |
| Anxa11 | annexin A11 | -1.03 | 27.49 |
| Vcp | transitional endoplasmic reticulum ATPase | -0.94 | 27.59 |
| Eps8l2 | epidermal growth factor receptor kinase substrate 8-like protein 2 | -0.81 | 28.66 |
| Hsph1 | heat shock protein 105 kDa | -0.91 | 28.84 |
| Actn4 | alpha-actinin-4 | -0.75 | 30.68 |
| Eif3a | eukaryotic translation initiation factor 3 subunit A | -1.07 | 31.74 |
| Hsdl2 | hydroxysteroid dehydrogenase-like protein 2 | -0.73 | 32.41 |
| Tuba1c | tubulin alpha-1C chain | -0.74 | 33.58 |
| Tgm2 | protein-glutamine gamma-glutamyltransferase 2 | -0.66 | 33.99 |
| Rab10 | ras-related protein Rab-10 | -0.67 | 34.44 |
| Tuba4a | tubulin alpha-4A chain | -1.41 | 35.35 |
| Iqgap1 | ras GTPase-activating-like protein IQGAP1 | -1.37 | 35.4 |
| Vcl | vinculin | -0.63 | 36.23 |
| Vps35 | maternal embryonic message 3 | -0.99 | 37.06 |
| Isyna1 | inositol-3-phosphate synthase 1 | -0.84 | 37.07 |
| Rab14 | ras-related protein Rab-14 | -0.69 | 37.2 |
| Ndufb9 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 | -0.9 | 37.53 |
| Flnb | filamin-B | -1.03 | 37.58 |
| Bcat2 | branched-chain-amino-acid aminotransferase, mitochondrial precursor | -0.59 | 37.67 |
| Kpnb1 | importin subunit beta-1 | -1.39 | 38.08 |
| Pepd | xaa-Pro dipeptidase | -1.06 | 38.63 |
| Gstt3 | glutathione S-transferase, theta 3 | -0.79 | 38.95 |
| Cfl1 | cofilin-1 | 1.43 | 39.05 |
| Kif5b | kinesin-1 heavy chain | -0.95 | 39.07 |
| Eef2 | elongation factor 2 | -1.01 | 39.26 |
| Anxa1 | annexin A1 | -0.91 | 39.81 |
| Ywhab | 14-3-3 protein beta/alpha | -1.1 | 39.98 |
| Rala | ras-related protein Ral-A precursor | -0.73 | 40.22 |
| Rab2a | ras-related protein Rab-2A | -0.58 | 40.72 |
| Etf1 | eukaryotic peptide chain release factor subunit 1 | -0.97 | 41.09 |

**Supplemental Table 4.**
*continued*

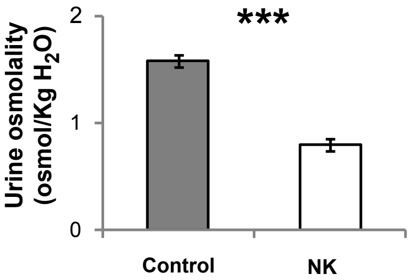
| Gene Symbol | Protein Name | Mean $\text{Log}_2$(NK/Control) | Estimated Half-Life (Hours) |
|---|---|---|---|
| Capns1 | calpain small subunit 1 | -1.26 | 41.36 |
| Calr | calreticulin precursor | -0.63 | 42.23 |
| Hspa4 | heat shock 70 kDa protein 4 | -0.96 | 42.4 |
| Cct7 | T-complex protein 1 subunit eta | -1.14 | 42.74 |
| Hrsp12 | ribonuclease UK114 | -0.66 | 43.09 |
| Cct8 | T-complex protein 1 subunit theta | -0.82 | 43.52 |
| Psmc5 | 26S protease regulatory subunit 8 | -1.04 | 44.18 |
| Suclg2 | succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial | -0.75 | 44.62 |
| Gstm1 | glutathione S-transferase Mu 1 | -0.86 | 44.69 |
| Slc25a10 | mitochondrial dicarboxylate carrier | -0.85 | 45.01 |
| Ndufs3 | NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial | -0.62 | 45.81 |
| Pygb | glycogen phosphorylase, brain form | -0.71 | 46.16 |
| Ywhah | 14-3-3 protein eta | -0.86 | 46.55 |
| Gdi1 | rab GDP dissociation inhibitor alpha | -0.75 | 46.72 |
| Pccb | propionyl-CoA carboxylase beta chain, mitochondrial precursor | -1.07 | 46.92 |
| Pdhb | pyruvate dehydrogenase E1 component subunit beta, mitochondrial precursor | -0.67 | 47.58 |
| Ndufa8 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 | -0.94 | 48.24 |
| Krt18 | keratin, type I cytoskeletal 18 | -0.75 | 48.28 |
| Tubb5 | tubulin beta-5 chain | -0.59 | 48.84 |
| Pfkl | 6-phosphofructokinase, liver type | -1.56 | 49.25 |
| Manba | beta-mannosidase precursor | -0.94 | 50.58 |
| Gnb2l1 | guanine nucleotide-binding protein subunit beta-2-like 1 | -0.82 | 50.84 |
| Uqcrfs1 | cytochrome b-c1 complex subunit Rieske, mitochondrial | -1.06 | 51.11 |
| Mdh1 | malate dehydrogenase, cytoplasmic | -0.61 | 51.67 |
| Cct4 | T-complex protein 1 subunit delta | -1.67 | 51.78 |
| Hexa | beta-hexosaminidase subunit alpha precursor | -0.99 | 52.94 |
| Ywhae | 14-3-3 protein epsilon | -0.77 | 53.02 |
| Krt19 | keratin, type I cytoskeletal 19 | -0.74 | 53.43 |
| Esyt1 | extended synaptotagmin-1 | -0.86 | 53.44 |
| Nit2 | omega-amidase NIT2 | -0.97 | 54.54 |
| Psma4 | proteasome subunit alpha type-4 | -1.7 | 56.34 |
| Eef1g | elongation factor 1-gamma | -0.91 | 57.16 |
| Hadhb | trifunctional enzyme subunit beta, mitochondrial precursor | -0.82 | 58.08 |
| Actr3 | actin-related protein 3 | -1.52 | 58.27 |
| Psmd12 | 26S proteasome non-ATPase regulatory subunit 12 | -2.25 | 59.65 |
| Atp5b | ATP synthase subunit beta, mitochondrial precursor | -0.99 | 60.38 |
| Glud1 | glutamate dehydrogenase 1, mitochondrial precursor | -0.79 | 60.4 |
| Anp32a | acidic leucine-rich nuclear phosphoprotein 32 family member A | -0.61 | 61.15 |
| Aifm1 | apoptosis-inducing factor 1, mitochondrial precursor | -1.18 | 61.72 |
| Pgam1 | phosphoglycerate mutase 1 | -1.11 | 62.58 |
| Aco2 | aconitate hydratase, mitochondrial precursor | -0.65 | 62.74 |
| Eci1 | enoyl-CoA delta isomerase 1, mitochondrial precursor | -0.81 | 63.73 |

**Supplemental Table 4.**
*continued*

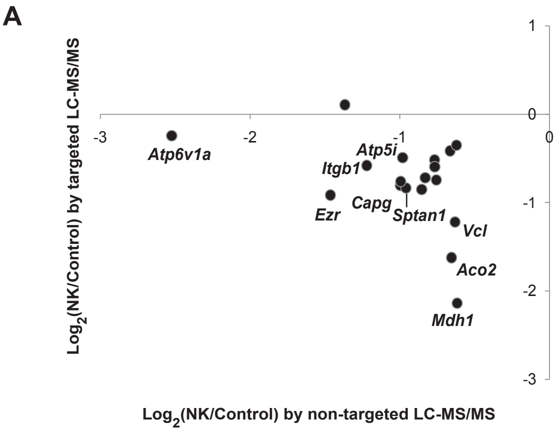
| Gene Symbol | Protein Name | Mean $Log_2$(NK/Control) | Estimated Half-Life (Hours) |
|---|---|---|---|
| Dlst | dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | -0.63 | 64.59 |
| Prdx3 | thioredoxin-dependent peroxide reductase, mitochondrial precursor | -0.72 | 68.84 |
| Qdpr | dihydropteridine reductase | -0.58 | 69.98 |
| Tpi1 | triosephosphate isomerase | -0.95 | 70.78 |
| Aldh4a1 | delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial | -1.18 | 75.14 |
| Phb | prohibitin | -0.76 | 75.47 |
| Vdac1 | voltage-dependent anion-selective channel protein 1 | -0.61 | 76.69 |
| Slc25a12 | LOW QUALITY PROTEIN: calcium-binding mitochondrial carrier protein Aralar1 | -1.98 | 77.48 |
| Acadvl | very long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor | -0.74 | 77.51 |
| Tkt | transketolase | -0.89 | 81.32 |
| Rnpep | aminopeptidase B | -0.89 | 85.54 |
| Asah1 | acid ceramidase precursor | -0.66 | 88.6 |
| Iah1 | isoamyl acetate-hydrolyzing esterase 1 homolog | -2.55 | 94.11 |
| Idh2 | isocitrate dehydrogenase [NADP], mitochondrial precursor | -0.77 | 97.41 |
| Lrba | lipopolysaccharide-responsive and beige-like anchor protein | -1.14 | 113.71 |
| Erap1 | endoplasmic reticulum aminopeptidase 1 precursor | -0.75 | 337.24 |
| Aadat | kynurenine/alpha-aminoadipate aminotransferase, mitochondrial | -4.81 | N/A |
| COX2 | cytochrome c oxidase subunit II (mitochondrion) | -2.32 | N/A |
| Rhoa | transforming protein RhoA precursor | -2.3 | N/A |
| Me1 | NADP-dependent malic enzyme | -1.91 | N/A |
| Arrb1 | beta-arrestin-1 | -1.64 | N/A |
| Lgals3 | galectin-3 | -1.54 | N/A |
| Akr1a1 | alcohol dehydrogenase [NADP(+)] | -1.37 | N/A |
| Ctnnb1 | catenin beta-1 | -1.21 | N/A |
| Ahcyl1 | adenosylhomocysteinase-like 1 | -1.15 | N/A |
| Ssr4 | translocon-associated protein subunit delta precursor | -1.14 | N/A |
| Comt | catechol O-methyltransferase | -1.08 | N/A |
| Chdh | choline dehydrogenase, mitochondrial | -1.02 | N/A |
| Dnpep | aspartyl aminopeptidase | -1.02 | N/A |
| Ehd2 | EH domain-containing protein 2 | -1.01 | N/A |
| Capg | macrophage-capping protein | -1 | N/A |
| Atp5i | ATP synthase subunit e, mitochondrial | -0.98 | N/A |
| Sptan1 | spectrin alpha chain, non-erythrocytic 1 | -0.96 | N/A |
| Uba1 | ubiquitin-like modifier-activating enzyme 1 | -0.9 | N/A |
| Canx | calnexin precursor | -0.89 | N/A |
| Idh1 | isocitrate dehydrogenase [NADP] cytoplasmic | -0.85 | N/A |
| Hnrnpa2b1 | heterogeneous nuclear ribonucleoproteins A2/B1 | -0.84 | N/A |
| Ctnnd1 | catenin delta-1 | -0.77 | N/A |
| Gpi | glucose-6-phosphate isomerase | -0.73 | N/A |
| Sptbn2 | spectrin beta chain, non-erythrocytic 2 | -0.71 | N/A |

**Supplemental Table 4.**
*continued*

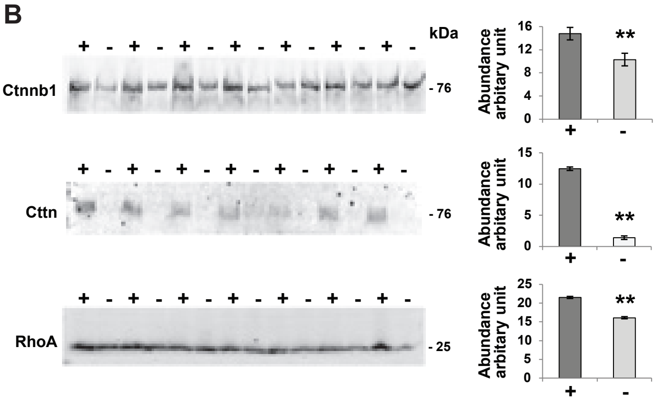
| Gene Symbol | Protein Name | Mean $\mathrm{Log_2}$(NK/Control) | Estimated Half-Life (Hours) |
|---|---|---|---|
| Sfxn3 | sideroflexin-3 | -0.69 | N/A |
| Aldh1a1 | retinal dehydrogenase 1 | -0.65 | N/A |
| Tpd52 | tumor protein D52 | -0.63 | N/A |
| LOC684270 | PREDICTED: isochorismatase domain-containing protein 2A, mitochondrial-like isoform 2 | -0.6 | N/A |
| Sept2 | septin-2 | -0.6 | N/A |
| Banf1 | barrier-to-autointegration factor | 1.3 | N/A |
| LOC100362689 | PREDICTED: keratin 8-like | 0.99 | N/A |



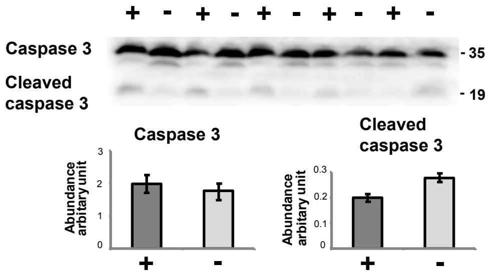
Supplementary Figure 1. Urine osmolality of urine recovered from bladders at 24 hours after potassium deprivation. Rats received a potassium-free diet (NK; □) or normal chow diet (control; ■) for 1 day. Upon sacrificing to harvest kidney tissues, urine was recovered from rats that had full bladders (n = 4 per each group). Bladder urine osmolality of potassium-deprived rats was significantly lower than that of control rats after 24 hours (0.8±0.1 vs. 1.6±0.06 osmol/kg H2O, respectively, $P<0.01$).



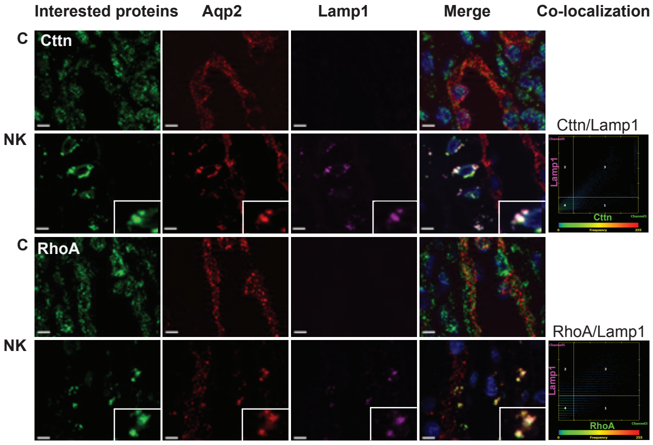
| Protein Name | Gene Symbol | Log$_2$(NK/Control) targeted | Log$_2$(NK/Control) non-targeted |
|---|---|---|---|
| Integrin beta 1 | *Itgb1* | -1.22 | -0.58 |
| Vinculin | *Vcl* | -1.22 | -0.63 |
| Ezrin | *Ezr* | -0.92 | -1.46 |
| Spectrin alpha chain, non-erythrocytic 1 | *Sptan1* | -0.84 | -0.96 |
| Macrophage-capping protein | *Capg* | -0.81 | -1 |
| Alpha-actinin-4 | *Actn4* | -0.74 | -0.75 |
| Catenin delta-1 | *Ctnnd1* | -0.52 | -0.77 |
| Ras GTPase-activating-like protein IQGAP1 | *Iqgap1* | 0.11 | -1.37 |
| Malate dehydrogenase, cytoplasmic | *Mdh1* | -2.14 | -0.61 |
| Aconitate hydratase, mitochondrial precursor | *Aco2* | -1.62 | -0.65 |
| Isocitrate dehydrogenase [NADP] cytoplasmic | *Idh1* | -0.85 | -0.85 |
| Isocitrate dehydrogenase [NADP], mitochondrial precursor | *Idh2* | -0.59 | -0.77 |
| ATP synthase subunit beta, mitochondrial precursor | *Atp5b* | -0.76 | -0.99 |
| ATP synthase subunit e, mitochondrial | *Atp5i* | -0.49 | -0.98 |
| V-type proton ATPase subunit E 1 | *Atp6v1e1* | -0.72 | -0.83 |
| V-type proton ATPase catalytic subunit A | *Atp6v1a* | -0.24 | -2.52 |
| NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial | *Ndufs7* | -0.41 | -0.66 |
| NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial | *Ndufs3* | -0.35 | -0.62 |



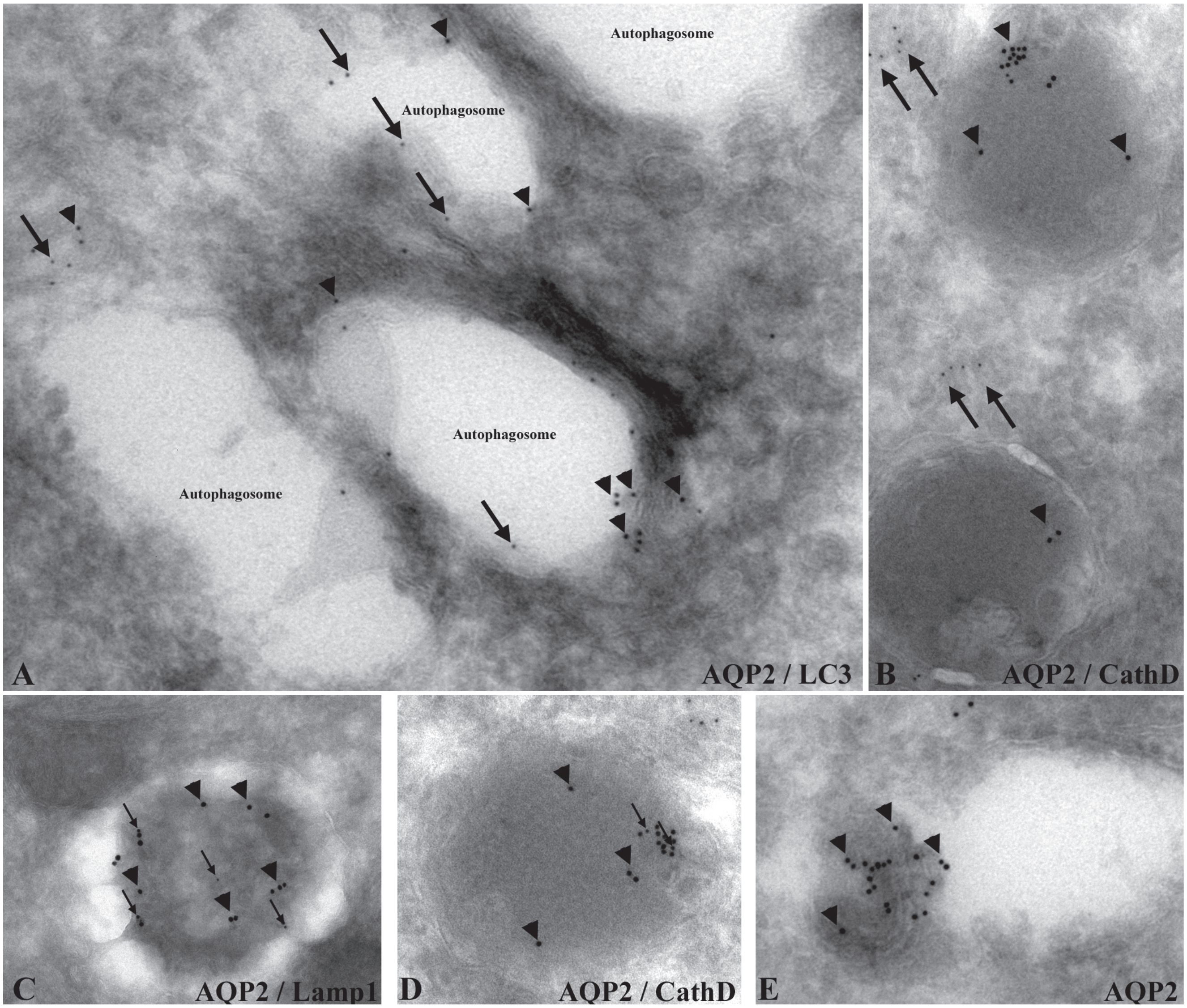
Supplementary Figure 2. Data verification by targeted LC-MS/MS and immunoblotting. (A) X and Y axes of the scatter plot represent protein abundance ratios [log2(NK/Control)] quantified by non-targeted and targeted LC-MS/MS techniques, respectively. (B) Immunoblotting of whole inner medullary proteins from rats fed with a potassium-free diet (-, n=7) and control rats (+, n=7) revealed significant down-regulation of Ctnnb1 (**, P<0.01), Cttn (***, P<0.005), and RhoA (*, P<0.05) in potassium-deprived (NK) rats (□) compared with control rats (■).



Supplementary Figure 3. Immunoblotting of whole inner medulla for caspase 3 and cleaved caspase 3. Rats received a potassium-free diet (-, n = 6) or normal chow diet (+, n = 6) for 1 day. The abundances of caspase 3 and cleaved caspase 3 in whole inner medulla were not significantly different between the 2 groups.



Supplementary Figure 4. Co-localization of other down-regulated proteins with autophagy markers. The inner medulla sections of control rats (C) and rats fed with a potassium-free diet for 1 day (NK) were triple-labeled against AQP2 (red), Lamp1 (pink), and down-regulated proteins including Cttn and RhoA (green). Insets demonstrate a magnified view of the areas where significant co-localization was observed as a group of puncta. These co-localized puncta were not observed in control sections. Scale bar = 4 µm. Scatter plots demonstrate the degree of colocalization between Cttn and Lamp1 (Pearson correlation coefficient, R = 0.80) as well as RhoA and Lamp1 (R = 0.74) in potassium-free condition.



Supplementary Figure 5. Immunogold electron microscopy of AQP2 in autophagosomes. (A) In kidney inner medulla collecting ducts cells of potassium-deprived rats, multiple membrane-bound structures morphologically resembling autophagosomes were observed that co-labeled for AQP2 (small gold particles, arrows) and LC3β (large gold particles, arrow heads). B-E) Additional examples of AQP2 (small gold particles, arrows) in autophagosomes, where in the majority of instances it co-labeled with cathepsin-D or Lamp1 (large gold particles, arrow heads).

**Supplementary Data 3.** A list of protein names, peptide sequences, precursor m/z ratios, retention times, and integrated peak areas of the regulated proteins analyzed by the targeted LC-MS/MS.

| Protein Name | Peptide Sequence | Sample | Charge | Precursor Mz | Retention Time | Area |
|---|---|---|---|---|---|---|
| sp\|O88989\|MDHC_RAT | EVGVYEALKDDSWLK | Control | 2 | 876.45 | 37.19 | 27629933 |
| sp\|O88989\|MDHC_RAT | EVGVYEALKDDSWLK | NK | 2 | 876.45 | 37.12 | 8174179 |
| sp\|O88989\|MDHC_RAT | GEFITTVQQR | Control | 2 | 589.81 | 20.66 | 1.86E+09 |
| sp\|O88989\|MDHC_RAT | GEFITTVQQR | NK | 2 | 589.81 | 20.17 | 1.18E+09 |
| sp\|O88989\|MDHC_RAT | NVIIWGNHSSTQYPDVNHAK | Control | 3 | 760.71 | 23.25 | 2.68E+09 |
| sp\|O88989\|MDHC_RAT | NVIIWGNHSSTQYPDVNHAK | NK | 3 | 760.71 | 22.7 | 4.37E+08 |
| sp\|O88989\|MDHC_RAT | VIVVGNPANTNCLTASK | Control | 2 | 879.46 | 23.87 | 2.51E+09 |
| sp\|O88989\|MDHC_RAT | VIVVGNPANTNCLTASK | NK | 2 | 879.46 | 23.5 | 4.39E+08 |
| sp\|P10719\|ATPB_RAT | AIAELGIYPAVDPLDSTSR | Control | 2 | 994.52 | 40.91 | 53664217 |
| sp\|P10719\|ATPB_RAT | AIAELGIYPAVDPLDSTSR | NK | 2 | 994.52 | 40.87 | 2737559 |
| sp\|P10719\|ATPB_RAT | DQEGQDVLLFIDNIFR | Control | 2 | 961.49 | 73.66 | 80938848 |
| sp\|P10719\|ATPB_RAT | DQEGQDVLLFIDNIFR | NK | 2 | 961.49 | 73.79 | 85205822 |
| sp\|P10719\|ATPB_RAT | FTQAGSEVSALLGR | Control | 2 | 718.38 | 32.24 | 1.09E+08 |
| sp\|P10719\|ATPB_RAT | FTQAGSEVSALLGR | NK | 2 | 718.38 | 31.88 | 2.31E+08 |
| sp\|P10719\|ATPB_RAT | VALTGLTVAEYFR | Control | 2 | 720.40 | 41.92 | 66015491 |
| sp\|P10719\|ATPB_RAT | VALTGLTVAEYFR | NK | 2 | 720.40 | 42.1 | 34701128 |
| sp\|P10719\|ATPB_RAT | VLDSGAPIKIPVGPETLGR | Control | 3 | 640.37 | 32.59 | 3.67E+08 |
| sp\|P10719\|ATPB_RAT | VLDSGAPIKIPVGPETLGR | NK | 3 | 640.37 | 32.31 | 2.43E+08 |
| sp\|P10719\|ATPB_RAT | VVDLLAPYAK | Control | 2 | 544.82 | 29.96 | 2.44E+08 |
| sp\|P10719\|ATPB_RAT | VVDLLAPYAK | NK | 2 | 544.82 | 29.63 | 1.16E+08 |
| sp\|P15800\|LAMB2_RAT | ALVEGGGILSR | Control | 2 | 536.31 | 23.84 | 1.09E+09 |
| sp\|P15800\|LAMB2_RAT | ALVEGGGILSR | NK | 2 | 536.31 | 23.4 | 50355880 |
| sp\|P15800\|LAMB2_RAT | DGFFGLSASNPR | Control | 2 | 634.31 | 32 | 1.03E+08 |
| sp\|P15800\|LAMB2_RAT | DGFFGLSASNPR | NK | 2 | 634.31 | 31.67 | 67645938 |
| sp\|P15800\|LAMB2_RAT | ETPSWTGVGFVR | Control | 2 | 668.34 | 31.17 | 71017264 |
| sp\|P15800\|LAMB2_RAT | ETPSWTGVGFVR | NK | 2 | 668.34 | 30.94 | 39066516 |
| sp\|P15800\|LAMB2_RAT | GFSGVFPACHPCHACFGDWDR | Control | 3 | 827.34 | 30.77 | 26946637 |
| sp\|P15800\|LAMB2_RAT | GFSGVFPACHPCHACFGDWDR | NK | 3 | 827.34 | 30.46 | 13605183 |
| sp\|P15800\|LAMB2_RAT | GSCYPATGDLLVGR | Control | 2 | 733.36 | 26.7 | 2.01E+08 |
| sp\|P15800\|LAMB2_RAT | GSCYPATGDLLVGR | NK | 2 | 733.36 | 26.35 | 80190312 |
| sp\|P15800\|LAMB2_RAT | KAEGVLAAQAR | Control | 3 | 371.88 | 13.3 | 2.6E+08 |
| sp\|P15800\|LAMB2_RAT | KAEGVLAAQAR | NK | 3 | 371.88 | 12.85 | 67198128 |
| sp\|P15800\|LAMB2_RAT | LGMVQAIVAAR | Control | 2 | 564.83 | 29.19 | 1.2E+08 |
| sp\|P15800\|LAMB2_RAT | LGMVQAIVAAR | NK | 2 | 564.83 | 28.83 | 24492035 |
| sp\|P15800\|LAMB2_RAT | LQELEGTYEENERELEVK | Control | 3 | 736.69 | 25.94 | 1.48E+08 |
| sp\|P15800\|LAMB2_RAT | LQELEGTYEENERELEVK | NK | 3 | 736.69 | 25.39 | 42837940 |
| sp\|P15800\|LAMB2_RAT | LREGQEVEFLVTSLPR | Control | 3 | 625.01 | 37.43 | 47677949 |
| sp\|P15800\|LAMB2_RAT | LREGQEVEFLVTSLPR | NK | 3 | 625.01 | 37.41 | 12003665 |
| sp\|P15800\|LAMB2_RAT | QKAETVQAALEEAQR | Control | 3 | 557.96 | 22.04 | 1.43E+09 |
| sp\|P15800\|LAMB2_RAT | QKAETVQAALEEAQR | NK | 3 | 557.96 | 21.54 | 3.09E+08 |
| sp\|P15800\|LAMB2_RAT | RLEQWAQELQQTGVLGAFESSFLNLQGK | Control | 3 | 1,059.88 | 65.62 | 50843800 |
| sp\|P15800\|LAMB2_RAT | RLEQWAQELQQTGVLGAFESSFLNLQGK | NK | 3 | 1,059.88 | 65.93 | 45568353 |
| sp\|P15800\|LAMB2_RAT | VLDISIPASPEQIQR | Control | 2 | 833.46 | 33.73 | 1.13E+08 |
| sp\|P15800\|LAMB2_RAT | VLDISIPASPEQIQR | NK | 2 | 833.46 | 33.52 | 67180630 |
| sp\|P15800\|LAMB2_RAT | YSEIEPSTEGEVIYR | Control | 2 | 886.42 | 25.48 | 4.73E+08 |
| sp\|P15800\|LAMB2_RAT | YSEIEPSTEGEVIYR | NK | 2 | 886.42 | 25.07 | 75281209 |
| sp\|P15999\|ATPA_RAT | AVDSLVPIGR | Control | 2 | 513.80 | 25.05 | 2.06E+08 |
| sp\|P15999\|ATPA_RAT | AVDSLVPIGR | NK | 2 | 513.80 | 24.59 | 64808605 |
| sp\|P15999\|ATPA_RAT | EVAAFAQFGSDLDAATQQLLSR | Control | 3 | 780.06 | 62.07 | 2279068 |
| sp\|P15999\|ATPA_RAT | EVAAFAQFGSDLDAATQQLLSR | NK | 3 | 780.06 | 61.63 | 1634993 |
| sp\|P15999\|ATPA_RAT | EVAAFAQFGSDLDAATQQLLSR | Control | 2 | 1,169.59 | 62.06 | 2410507 |
| sp\|P15999\|ATPA_RAT | EVAAFAQFGSDLDAATQQLLSR | NK | 2 | 1,169.59 | 61.63 | 1502781 |
| sp\|P15999\|ATPA_RAT | LKEIVTNFLAGFEP | Control | 2 | 789.43 | 49.95 | 55017158 |
| sp\|P15999\|ATPA_RAT | LKEIVTNFLAGFEP | NK | 2 | 789.43 | 49.92 | 11788256 |
| sp\|P15999\|ATPA_RAT | TGAIVDVPVGDELLGR | Control | 2 | 805.94 | 37.62 | 4.02E+08 |
| sp\|P15999\|ATPA_RAT | TGAIVDVPVGDELLGR | NK | 2 | 805.94 | 37.63 | 3.58E+08 |
| sp\|P15999\|ATPA_RAT | VVDALGNAIDGK | Control | 2 | 586.32 | 22.9 | 2.72E+08 |
| sp\|P15999\|ATPA_RAT | VVDALGNAIDGK | NK | 2 | 586.32 | 22.38 | 1.21E+08 |

| Protein Name | Peptide Sequence | Sample | Charge | Precursor Mz | Retention Time | Area |
|---|---|---|---|---|---|---|
| sp\|P16086\|SPTN1_RAT | ALINADELANDVAGAEALLDR | Control | 2 | 1,077.56 | 59.87 | 11944585 |
| sp\|P16086\|SPTN1_RAT | ALINADELANDVAGAEALLDR | NK | 2 | 1,077.56 | 60.1 | 2228942 |
| sp\|P16086\|SPTN1_RAT | DLASVQALLR | Control | 2 | 543.32 | 36.68 | 82889968 |
| sp\|P16086\|SPTN1_RAT | DLASVQALLR | NK | 2 | 543.32 | 36.63 | 52267455 |
| sp\|P16086\|SPTN1_RAT | DLSSVQTLLTK | Control | 2 | 602.84 | 34.06 | 38216655 |
| sp\|P16086\|SPTN1_RAT | DLSSVQTLLTK | NK | 2 | 602.84 | 33.79 | 85398047 |
| sp\|P16086\|SPTN1_RAT | KVEDLFLTFAK | Control | 2 | 655.87 | 35.6 | 40655748 |
| sp\|P16086\|SPTN1_RAT | KVEDLFLTFAK | NK | 2 | 655.87 | 35.45 | 33799298 |
| sp\|P16086\|SPTN1_RAT | LFGAAEVQR | Control | 2 | 495.77 | 20.49 | 1.34E+09 |
| sp\|P16086\|SPTN1_RAT | LFGAAEVQR | NK | 2 | 495.77 | 19.98 | 2.18E+08 |
| sp\|P16086\|SPTN1_RAT | LGESQTLQQFSR | Control | 2 | 697.36 | 23 | 4.23E+08 |
| sp\|P16086\|SPTN1_RAT | LGESQTLQQFSR | NK | 2 | 697.36 | 22.47 | 79519088 |
| sp\|P16086\|SPTN1_RAT | LQQSHPLSANQIQVK | Control | 3 | 564.31 | 18.97 | 5.46E+08 |
| sp\|P16086\|SPTN1_RAT | LQQSHPLSANQIQVK | NK | 3 | 564.31 | 18.48 | 2.72E+08 |
| sp\|P16086\|SPTN1_RAT | SSLSSAQADFNQLAELDR | Control | 2 | 976.47 | 38.4 | 65671603 |
| sp\|P16086\|SPTN1_RAT | SSLSSAQADFNQLAELDR | NK | 2 | 976.47 | 38.32 | 53829417 |
| sp\|P25304\|AGRIN_RAT | DQCPETCQFNSVCLSR | Control | 2 | 1,000.92 | 25.45 | 2.96E+08 |
| sp\|P25304\|AGRIN_RAT | DQCPETCQFNSVCLSR | NK | 2 | 1,000.92 | 25.03 | 55283370 |
| sp\|P25304\|AGRIN_RAT | SIESTLDDLFR | Control | 2 | 648.33 | 49.28 | 8823450 |
| sp\|P25304\|AGRIN_RAT | SIESTLDDLFR | NK | 2 | 648.33 | 49.36 | 1085476 |
| sp\|P25304\|AGRIN_RAT | SPVCGSDGVTYGTECDLKK | Control | 3 | 691.65 | 19.22 | 5.93E+08 |
| sp\|P25304\|AGRIN_RAT | SPVCGSDGVTYGTECDLKK | NK | 3 | 691.65 | 18.8 | 1.4E+08 |
| sp\|P25304\|AGRIN_RAT | VCTFGAVCSAGQCVCPR | Control | 3 | 643.61 | 24.25 | 1.74E+08 |
| sp\|P25304\|AGRIN_RAT | VCTFGAVCSAGQCVCPR | NK | 3 | 643.61 | 23.92 | 22662303 |
| sp\|P25304\|AGRIN_RAT | VCTFGAVCSAGQCVCPR | Control | 2 | 964.92 | 24.25 | 5.65E+08 |
| sp\|P25304\|AGRIN_RAT | VCTFGAVCSAGQCVCPR | NK | 2 | 964.92 | 23.92 | 75280040 |
| sp\|P25304\|AGRIN_RAT | VTCDGSYRPVCAQDGHTYNNDCWR | Control | 3 | 977.74 | 20.58 | 1.82E+09 |
| sp\|P25304\|AGRIN_RAT | VTCDGSYRPVCAQDGHTYNNDCWR | NK | 3 | 977.74 | 20.04 | 7.93E+08 |
| sp\|P29419\|ATP5I_RAT | ELAEAEDVSIFK | Control | 2 | 675.84 | 30.58 | 34248784 |
| sp\|P29419\|ATP5I_RAT | ELAEAEDVSIFK | NK | 2 | 675.84 | 30.26 | 24347147 |
| sp\|P31977\|EZRI_RAT | AKFYPEDVADELIQDITQK | Control | 3 | 741.71 | 55.87 | 21598975 |
| sp\|P31977\|EZRI_RAT | AKFYPEDVADELIQDITQK | NK | 3 | 741.71 | 56.13 | 15579260 |
| sp\|P31977\|EZRI_RAT | AQEEAERLEADR | Control | 2 | 708.84 | 21.89 | 5.14E+08 |
| sp\|P31977\|EZRI_RAT | AQEEAERLEADR | NK | 2 | 708.84 | 21.35 | 2E+08 |
| sp\|P49134\|ITB1_RAT | FCECDNFNCDR | Control | 2 | 768.77 | 18.93 | 7.21E+08 |
| sp\|P49134\|ITB1_RAT | FCECDNFNCDR | NK | 2 | 768.77 | 18.49 | 3.44E+08 |
| sp\|P49134\|ITB1_RAT | FQGPTCETCQTCLGVCAEHK | Control | 3 | 795.00 | 21.18 | 1.36E+09 |
| sp\|P49134\|ITB1_RAT | FQGPTCETCQTCLGVCAEHK | NK | 3 | 795.00 | 20.74 | 5.24E+08 |
| sp\|P49134\|ITB1_RAT | LRPEDITQIQPQQLLLK | Control | 3 | 678.40 | 38.59 | 64676780 |
| sp\|P49134\|ITB1_RAT | LRPEDITQIQPQQLLLK | NK | 3 | 678.40 | 38.55 | 45004470 |
| sp\|P49134\|ITB1_RAT | LRPEDITQIQPQQLLLK | Control | 2 | 1,017.09 | 38.59 | 2143072 |
| sp\|P49134\|ITB1_RAT | LRPEDITQIQPQQLLLK | NK | 2 | 1,017.09 | 38.46 | 2955661 |
| sp\|P49134\|ITB1_RAT | NVLSLTDRGEFFNELVGQQR | Control | 3 | 774.73 | 41.69 | 29387689 |
| sp\|P49134\|ITB1_RAT | NVLSLTDRGEFFNELVGQQR | NK | 3 | 774.73 | 41.75 | 11240184 |
| sp\|P49134\|ITB1_RAT | SAVTTVVNPK | Control | 2 | 508.29 | 19.58 | 1.07E+09 |
| sp\|P49134\|ITB1_RAT | SAVTTVVNPK | NK | 2 | 508.29 | 22.9 | 1.79E+08 |
| sp\|P56574\|IDHP_RAT | DLAGCIHGLSNVK | Control | 2 | 692.36 | 26.47 | 1.5E+08 |
| sp\|P56574\|IDHP_RAT | DLAGCIHGLSNVK | NK | 2 | 692.36 | 26.02 | 16523431 |
| sp\|P56574\|IDHP_RAT | GRPTSTNPIASIFAWTR | Control | 3 | 625.67 | 20.87 | 1.98E+09 |
| sp\|P56574\|IDHP_RAT | GRPTSTNPIASIFAWTR | NK | 3 | 625.67 | 19.71 | 1.76E+09 |
| sp\|P56574\|IDHP_RAT | LNEHFLNTTDFLDTIK | Control | 3 | 640.99 | 39.82 | 1.12E+08 |
| sp\|P56574\|IDHP_RAT | LNEHFLNTTDFLDTIK | NK | 3 | 640.99 | 39.81 | 57352310 |
| sp\|P56574\|IDHP_RAT | LNEHFLNTTDFLDTIK | Control | 2 | 960.99 | 39.82 | 18298301 |
| sp\|P56574\|IDHP_RAT | LNEHFLNTTDFLDTIK | NK | 2 | 960.99 | 39.86 | 12129029 |
| sp\|P56574\|IDHP_RAT | TIEAEAAHGTVTR | Control | 2 | 678.35 | 15.17 | 5.53E+08 |
| sp\|P56574\|IDHP_RAT | TIEAEAAHGTVTR | NK | 2 | 678.35 | 14.64 | 4.85E+08 |
| sp\|P85972\|VINC_RAT | AIPDLTAPVAAVQAAVSNLVR | Control | 2 | 1,038.59 | 68.37 | 15602311 |
| sp\|P85972\|VINC_RAT | AIPDLTAPVAAVQAAVSNLVR | NK | 2 | 1,038.59 | 69 | 12632743 |
| sp\|P85972\|VINC_RAT | AQQVSQGLDVLTAK | Control | 2 | 729.40 | 25.3 | 58228975 |
| sp\|P85972\|VINC_RAT | AQQVSQGLDVLTAK | NK | 2 | 729.40 | 24.82 | 13247948 |
| sp\|Q6AYC4\|CAPG_RAT | EGNPEEDITADQTNAQAAALYK | Control | 3 | 783.70 | 27.26 | 59606240 |
| sp\|Q6AYC4\|CAPG_RAT | EGNPEEDITADQTNAQAAALYK | NK | 3 | 783.70 | 26.87 | 12313392 |
| sp\|Q6AYC4\|CAPG_RAT | QAALQVADGFISR | Control | 2 | 688.37 | 30.82 | 32302780 |
| sp\|Q6AYC4\|CAPG_RAT | QAALQVADGFISR | NK | 2 | 688.37 | 30.55 | 16019944 |

| Protein Name | Peptide Sequence | Sample | Charge | Precursor Mz | Retention Time | Area |
|---|---|---|---|---|---|---|
| sp\|Q6AYC4\|CAPG_RAT | TTSGTTPAAIR | Control | 2 | 538.29 | 17.7 | 1.31E+10 |
| sp\|Q6AYC4\|CAPG_RAT | TTSGTTPAAIR | NK | 2 | 538.29 | 17.29 | 9.43E+09 |
| sp\|Q6AYC4\|CAPG_RAT | YREGGVESAFHK | Control | 3 | 460.56 | 16.07 | 1.69E+08 |
| sp\|Q6AYC4\|CAPG_RAT | YREGGVESAFHK | NK | 3 | 460.56 | 15.49 | 1.11E+08 |
| sp\|Q6PCU2\|VATE1_RAT | ARDDLITDLLNEAK | Control | 3 | 529.62 | 42.43 | 24613327 |
| sp\|Q6PCU2\|VATE1_RAT | ARDDLITDLLNEAK | NK | 3 | 529.62 | 42.48 | 14961577 |
| sp\|Q99KI0\|ACON_MOUSE | AKDINQEVYNFLATAGAK | Control | 3 | 651.67 | 40.2 | 31959489 |
| sp\|Q99KI0\|ACON_MOUSE | AKDINQEVYNFLATAGAK | NK | 3 | 651.67 | 40.26 | 15263689 |
| sp\|Q99KI0\|ACON_MOUSE | FKLEAPDADELPR | Control | 3 | 500.93 | 27.79 | 23448224 |
| sp\|Q99KI0\|ACON_MOUSE | FKLEAPDADELPR | NK | 3 | 500.93 | 27.57 | 23901528 |
| sp\|Q99KI0\|ACON_MOUSE | IVYGHLDDPANQEIER | Control | 3 | 623.64 | 22.79 | 4.03E+09 |
| sp\|Q99KI0\|ACON_MOUSE | IVYGHLDDPANQEIER | NK | 3 | 623.64 | 22.22 | 8.89E+08 |
| sp\|Q99KI0\|ACON_MOUSE | NAVTQEFGPVPDTAR | Control | 2 | 801.40 | 25.13 | 7.64E+08 |
| sp\|Q99KI0\|ACON_MOUSE | NAVTQEFGPVPDTAR | NK | 2 | 801.40 | 24.66 | 1.62E+08 |
| sp\|Q9JKF1\|IQGA1_MOUSE | EQLWLANEGLITK | Control | 2 | 757.91 | 35.59 | 22087791 |
| sp\|Q9JKF1\|IQGA1_MOUSE | EQLWLANEGLITK | NK | 2 | 757.91 | 35.46 | 20840005 |
| sp\|Q9JKF1\|IQGA1_MOUSE | ILAIGLINEALDEGDAQK | Control | 2 | 942.01 | 48.9 | 5836516 |
| sp\|Q9JKF1\|IQGA1_MOUSE | ILAIGLINEALDEGDAQK | NK | 2 | 942.01 | 48.99 | 7172014 |
| sp\|Q9QXQ0\|ACTN4_RAT | KAGTQIENIDEDFRDGLK | Control | 3 | 683.68 | 27.13 | 12965927 |
| sp\|Q9QXQ0\|ACTN4_RAT | KAGTQIENIDEDFRDGLK | NK | 3 | 683.68 | 26.74 | 14027889 |
| sp\|Q9QXQ0\|ACTN4_RAT | KDDPVTNLNNAFEVAEK | Control | 3 | 635.32 | 30.06 | 22813940 |
| sp\|Q9QXQ0\|ACTN4_RAT | KDDPVTNLNNAFEVAEK | NK | 3 | 635.32 | 29.79 | 13642678 |
| sp\|Q9QXQ0\|ACTN4_RAT | TIPWLEDRVPQK | Control | 2 | 741.41 | 22.55 | 1.32E+09 |
| sp\|Q9QXQ0\|ACTN4_RAT | TIPWLEDRVPQK | NK | 2 | 741.41 | 22.06 | 3.61E+08 |
| tr\|D3ZG43\|D3ZG43_RAT | FDLNSPWEAFPAYR | Control | 2 | 856.91 | 45.78 | 8293906 |
| tr\|D3ZG43\|D3ZG43_RAT | FDLNSPWEAFPAYR | NK | 2 | 856.91 | 45.91 | 7244166 |
| tr\|D3ZG43\|D3ZG43_RAT | SLADLTAVDVPTR | Control | 2 | 679.37 | 30.64 | 73297380 |
| tr\|D3ZG43\|D3ZG43_RAT | SLADLTAVDVPTR | NK | 2 | 679.37 | 30.26 | 20075558 |
| tr\|D3ZG43\|D3ZG43_RAT | VVAEPVELAQEFR | Control | 2 | 743.90 | 34.06 | 45662024 |
| tr\|D3ZG43\|D3ZG43_RAT | VVAEPVELAQEFR | NK | 2 | 743.90 | 33.79 | 35823927 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | ALSAIADLLTSEHER | Control | 3 | 542.62 | 34.89 | 43370824 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | ALSAIADLLTSEHER | NK | 3 | 542.62 | 34.73 | 30304991 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | ALSAIADLLTSEHER | Control | 2 | 813.43 | 34.89 | 13136138 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | ALSAIADLLTSEHER | NK | 2 | 813.43 | 34.68 | 11156517 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | GYELLFQPEVVR | Control | 2 | 725.39 | 37.62 | 33706956 |
| tr\|D3ZZZ9\|D3ZZZ9_RAT | GYELLFQPEVVR | NK | 2 | 725.39 | 37.5 | 7987965 |
| tr\|D4A133\|D4A133_RAT | AKEILQEEEDLAEIVQLVGK | Control | 3 | 752.08 | 52.34 | 13329593 |
| tr\|D4A133\|D4A133_RAT | AKEILQEEEDLAEIVQLVGK | NK | 3 | 752.08 | 52.85 | 11274484 |
| tr\|F1LRD5\|F1LRD5_RAT | FKDIFQEIYDK | Control | 2 | 723.37 | 34.08 | 23763188 |
| tr\|F1LRD5\|F1LRD5_RAT | FKDIFQEIYDK | NK | 2 | 723.37 | 33.89 | 12406194 |
| tr\|F1LRD5\|F1LRD5_RAT | LVTGWVKPIIIGR | Control | 3 | 484.64 | 32.29 | 1.43E+08 |
| tr\|F1LRD5\|F1LRD5_RAT | LVTGWVKPIIIGR | NK | 3 | 484.64 | 32.02 | 75259744 |
| tr\|F1LRD5\|F1LRD5_RAT | LVTGWVKPIIIGR | Control | 2 | 726.46 | 32.29 | 54160062 |
| tr\|F1LRD5\|F1LRD5_RAT | LVTGWVKPIIIGR | NK | 2 | 726.46 | 32.02 | 31654584 |
| tr\|F1LRD5\|F1LRD5_RAT | TVEAEAAHGTVTR | Control | 2 | 671.34 | 12.74 | 3.82E+08 |
| tr\|F1LRD5\|F1LRD5_RAT | TVEAEAAHGTVTR | NK | 2 | 671.34 | 12.31 | 2.58E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | EAQLALGNAAADATEAK | Control | 2 | 822.42 | 23.9 | 9.59E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | EAQLALGNAAADATEAK | NK | 2 | 822.42 | 23.49 | 1.35E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | EGFFGNPLAPNPADK | Control | 2 | 787.39 | 33.31 | 15994331 |
| tr\|F1MAA7\|F1MAA7_RAT | EGFFGNPLAPNPADK | NK | 2 | 787.39 | 33.1 | 15449792 |
| tr\|F1MAA7\|F1MAA7_RAT | GKAEQQTADQLLAR | Control | 3 | 510.27 | 19.12 | 8.23E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | GKAEQQTADQLLAR | NK | 3 | 510.27 | 18.63 | 4.94E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | GKAEQQTADQLLAR | Control | 2 | 764.91 | 19.08 | 6.06E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | GKAEQQTADQLLAR | NK | 2 | 764.91 | 18.61 | 3.89E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | LCRPCQCNDNIDPNAVGNCNR | Control | 3 | 849.69 | 18.47 | 8.05E+09 |
| tr\|F1MAA7\|F1MAA7_RAT | LCRPCQCNDNIDPNAVGNCNR | NK | 3 | 849.69 | 17.94 | 6.23E+09 |
| tr\|F1MAA7\|F1MAA7_RAT | LGNTEACSPCHCSPVGSLSTQCDSYGR | Control | 3 | 1,000.75 | 22.24 | 3.72E+09 |
| tr\|F1MAA7\|F1MAA7_RAT | LGNTEACSPCHCSPVGSLSTQCDSYGR | NK | 3 | 1,000.75 | 21.79 | 8.33E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | LNTFGDEVFNDPK | Control | 2 | 748.36 | 28.36 | 1.17E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | LNTFGDEVFNDPK | NK | 2 | 748.36 | 27.92 | 46524809 |
| tr\|F1MAA7\|F1MAA7_RAT | LSAEDLVLEGAGLR | Control | 2 | 721.90 | 41.37 | 2.54E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | LSAEDLVLEGAGLR | NK | 2 | 721.90 | 41.37 | 94478398 |
| tr\|F1MAA7\|F1MAA7_RAT | NTLQEANDILNNLKDFDR | Control | 3 | 711.69 | 46.64 | 84195614 |
| tr\|F1MAA7\|F1MAA7_RAT | NTLQEANDILNNLKDFDR | NK | 3 | 711.69 | 46.67 | 50473072 |

| **Protein Name** | **Peptide Sequence** | **Sample** | **Charge** | **Precursor Mz** | **Retention Time** | **Area** |
|---|---|---|---|---|---|---|
| tr\|F1MAA7\|F1MAA7_RAT | NTLQEANDILNNLKDFDR | Control | 2 | 1,067.03 | 46.64 | 13721834 |
| tr\|F1MAA7\|F1MAA7_RAT | NTLQEANDILNNLKDFDR | NK | 2 | 1,067.03 | 46.67 | 9503503 |
| tr\|F1MAA7\|F1MAA7_RAT | PSAYNFDNSPVLQEWVTATDIR | Control | 3 | 841.74 | 51.44 | 27358551 |
| tr\|F1MAA7\|F1MAA7_RAT | PSAYNFDNSPVLQEWVTATDIR | NK | 3 | 841.74 | 51.66 | 27664903 |
| tr\|F1MAA7\|F1MAA7_RAT | QDIAVISDTYFPR | Control | 2 | 762.89 | 34.75 | 19701153 |
| tr\|F1MAA7\|F1MAA7_RAT | QDIAVISDTYFPR | NK | 2 | 762.89 | 34.54 | 18522501 |
| tr\|F1MAA7\|F1MAA7_RAT | SAGYLDDVTLQSAR | Control | 2 | 748.37 | 27.41 | 1.5E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | SAGYLDDVTLQSAR | NK | 2 | 748.37 | 27.04 | 68896850 |
| tr\|F1MAA7\|F1MAA7_RAT | SQECYFDPELYR | Control | 2 | 803.85 | 30.93 | 76211584 |
| tr\|F1MAA7\|F1MAA7_RAT | SQECYFDPELYR | NK | 2 | 803.85 | 30.59 | 20130136 |
| tr\|F1MAA7\|F1MAA7_RAT | SRVESTEQLIEIASR | Control | 3 | 573.31 | 27.93 | 68400214 |
| tr\|F1MAA7\|F1MAA7_RAT | SRVESTEQLIEIASR | NK | 3 | 573.31 | 27.45 | 15200854 |
| tr\|F1MAA7\|F1MAA7_RAT | SRVESTEQLIEIASR | Control | 2 | 859.46 | 27.93 | 68054988 |
| tr\|F1MAA7\|F1MAA7_RAT | SRVESTEQLIEIASR | NK | 2 | 859.46 | 27.45 | 14960844 |
| tr\|F1MAA7\|F1MAA7_RAT | TLPTGCFNTPSIEKP | Control | 2 | 831.41 | 28.65 | 2.25E+08 |
| tr\|F1MAA7\|F1MAA7_RAT | TLPTGCFNTPSIEKP | NK | 2 | 831.41 | 28.4 | 1.09E+08 |
| tr\|Q5RJN0\|Q5RJN0_RAT | AHQSVAATGSPSSTQSAVSK | Control | 3 | 634.32 | 11.84 | 1.55E+08 |
| tr\|Q5RJN0\|Q5RJN0_RAT | AHQSVAATGSPSSTQSAVSK | NK | 3 | 634.32 | 11.38 | 1.13E+08 |
| tr\|Q5RJN0\|Q5RJN0_RAT | LDDLINWAR | Control | 2 | 558.30 | 33.36 | 12215269 |
| tr\|Q5RJN0\|Q5RJN0_RAT | LDDLINWAR | NK | 2 | 558.30 | 33.13 | 9494062 |