# Supplemental Materials

# Redox states of protein cysteines in pathways of protein turnover and cytoskeleton dynamics are changed with aging and reversed by Slc7a11 restoration in mouse lung fibroblasts

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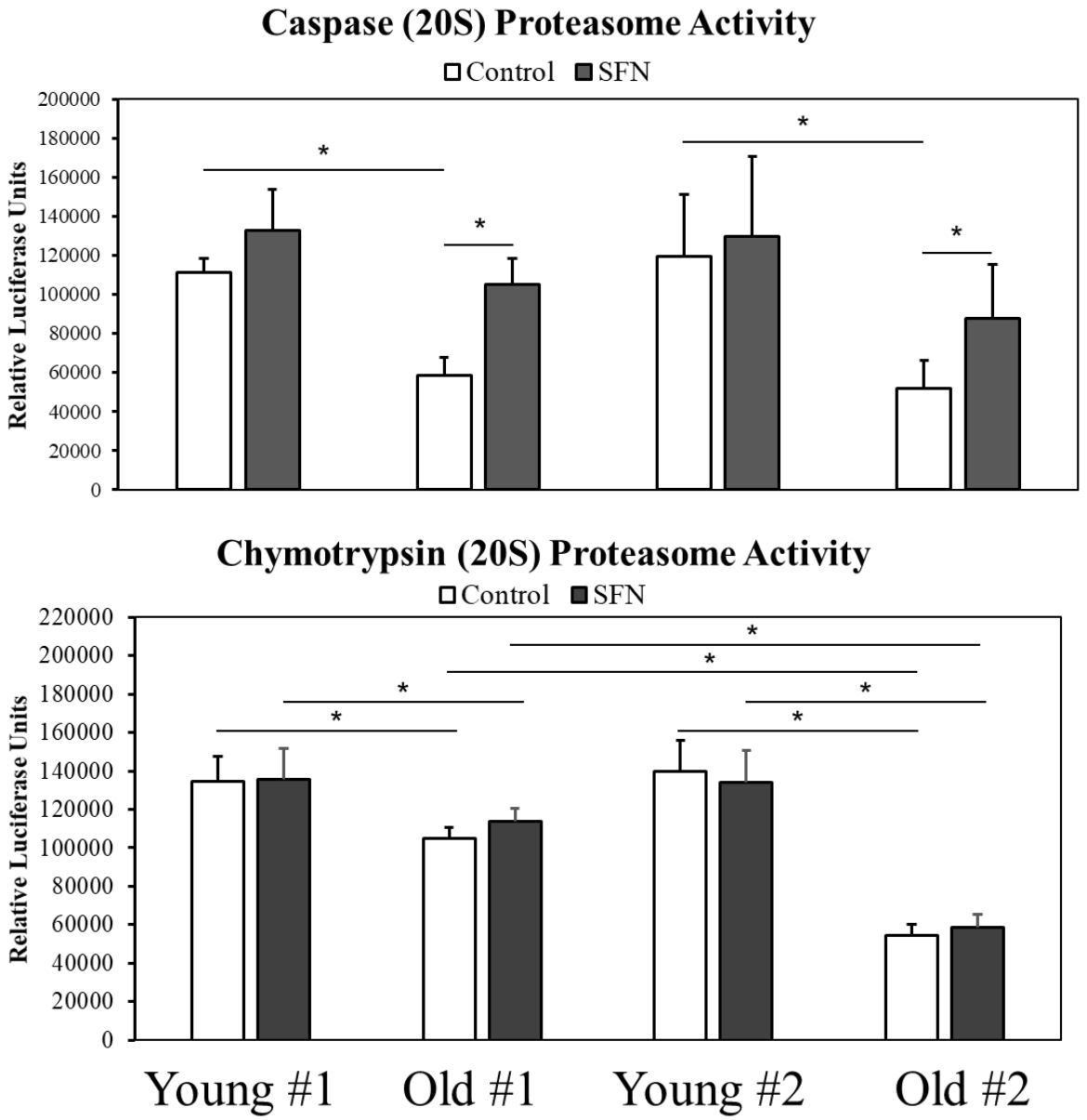
*Proteasomal activity assay*. Proteasomal activity was measured using the Proteasome-Glo Chymotrypsin-Like and Caspase-Like Cell-Based Assays from Promega (#G8660, #G8860, Madison, WI) according to the manufacturer's instructions. Briefly, primary lung fibroblasts (15,000 cells/well) from young or old female C57BL/6 mice were plated onto a 96 well (white walled) tissue culture plate and incubated in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution in a humidified incubator with 5% CO2. After attachment, cells were treated with or without the addition of 5 μM sulforaphane for 6 hours. Afterwards, some fibroblasts were incubated in the presence of a proteasome inhibitor lactacystin (10μM, Cayman Chemical, Ann Arbor, MI) for 2 hours to identify nonspecific protease activity not related to the proteasome. The Proteasome-Glo Cell-Based Reagent (100μl) was then added to each sample and mixed for 2 min at 700 rpm using a plate shaker. Samples were incubated at room temperature for 10 minutes prior to the measurement of luminescence using a Luminoskan Ascent Luminometer (Labsystems, Helsinki, Finland). Samples were normalized to total protein measured using protein assay dye (Bio-Rad, Hercules, CA) and values were reported as relative luciferase units.

Supplemental Figure 1 shows proteasomal activity is lower in old cells, and sulforaphane treatment restores caspase-like, but not chymotrypsin-like, proteasomal activity. This figure is related to Table 1 and described in the 4th paragraph of the Discussion section.




SUPPLEMENTAL FIGURE 1: Caspase-like (top) and chymotrypsin-like (bottom) proteasomal activity. Fibroblasts were isolated from the lungs of 2 Young (#1 and #2) and 2 Old (#1 and #2) female C57BL/6J mice. Caspase-like and chymotrypsin-like proteasomal activity were measured before and after induction of Slc7a11 activity with sulforaphane as described in Supplemental Methods. Both types of proteasomal activity were lower in old cells, but sulforaphane treatment restored only caspase-like proteasomal activity. Fibroblasts from 2 different young mice had similar caspase- and chymotrypsin-like activities. Fibroblasts from both old mice had lower activities than either young mouse, but one biological replicate had even lower chymotrypsin-like activity than the other. Six wells were plated for each condition. * - $p<0.05$ by 2-way ANOVA and Tukey's post-hoc test.

Supplemental Table 1 lists the total 104 proteins with age-dependent Slc7a11-responsive cysteine residues. Those proteins contain at least one cyteine whose redox state becomes more oxidized or reduced with aging and is reversible by Slc7a11 overexpression. This table is related to Figure 4(b) and Table 1 and described in the Results section 3.2. The proteins in this table are used as input for Ingenuity Pathway Analysis.

SUPPLEMENTAL TABLE 1A: Proteins with cysteines whose redox state became more oxidized with aging and restored by Slc7a11 overexpression

| Sequence | Protein | Description | Protein Group Accessions | Modifications |
|---|---|---|---|---|
| CTTDHISAAGPWLK | Aco2 | Aconitate hydratase, mitochondrial OS=Mus musculus OX=10090 GN=Aco2 PE=1 SV=1 - [ACON_MOUSE] | O99KI0 | C1(iodoTMT6plex) |
| ACLISLGYDVENDR | Actn4 | Alpha-actinin-4 OS=Mus musculus OX=10090 GN=Actn4 PE=1 SV=1 - [ACTN4_MOUSE] | P57780 | C2(iodoTMT6plex) |
| YSYVCPDLVK | Actr3 | Actin-related protein 3 OS=Mus musculus OX=10090 GN=Actr3 PE=1 SV=3 - [ARP3_MOUSE] | O99JY9 | C5(iodoTMT6plex) |
| VCLLGCGISTGYGAAVNTAK | Adh5 | Alcohol dehydrogenase class-3 OS=Mus musculus OX=10090 GN=Adh5 PE=1 SV=3 - [ADHX_MOUSE] | P28474 | C2(iodoTMT6plex); C6(iodoTMT6plex) |
| DCEDPNPLIR | Ap2b1 | AP-2 complex subunit beta OS=Mus musculus OX=10090 GN=Ap2b1 PE=1 SV=1 - [AP2B1_MOUSE] | Q9DBG3 | C2(iodoTMT6plex) |
| EEAPDILCLOETK | Apex1 | DNA-(apurinic or apyrimidinic site) lyase OS=Mus musculus OX=10090 GN=Apex1 PE=1 SV=2 - [APEX1_MOUSE] | P28352 | C8(iodoTMT6plex) |
| NIAFFSTNCVEGTAR | Atp1a1 | Sodium/potassium-transporting ATPase subunit alpha-1 OS=Mus musculus OX=10090 GN=Atp1a1 PE=1 SV=1 - [AT1A1_MOUSE] | O8VDN2 | C9(iodoTMT6plex) |
| CGLOFLGNVASR | Atxn10 | Ataxin-10 OS=Mus musculus OX=10090 GN=Atxn10 PE=1 SV=2 - [ATX10_MOUSE] | P28658 | C1(iodoTMT6plex) |
| INSITVDNCK | Can1 | Adenylyl cyclase-associated protein 1 OS=Mus musculus OX=10090 GN=Can1 PE=1 SV=4 - [CAP1_MOUSE] | P40124 | C9(iodoTMT6plex) |
| TDGFGIDTCR | Capns1 | Calpain small subunit 1 OS=Mus musculus OX=10090 GN=Capns1 PE=1 SV=1 - [CPNS1_MOUSE] | O88456 | C9(iodoTMT6plex) |
| CAOAOTGIDLSTCTK | Ccar2 | Cell cycle and apoptosis regulator protein 2 OS=Mus musculus OX=10090 GN=Ccar2 PE=1 SV=2 - [CCAR2_MOUSE] | O8VDP4 | C1(iodoTMT6plex); C13(iodoTMT6plex) |
| DVEREDIEFICK | Cct4 | T-complex protein 1 subunit delta OS=Mus musculus OX=10090 GN=Cct4 PE=1 SV=3 - [TCPD_MOUSE] | P80315 | C11(iodoTMT6plex) |
| LALEOOOLICK | Cherp | Calcium homeostasis endoplasmic reticulum protein OS=Mus musculus OX=10090 GN=Cherp PE=1 SV=1 - [CHERP_MOUSE] | O8CGZ0 | C10(iodoTMT6plex) |
| AVLDLVDOCPK | Cmpk2 | UMP-CMP kinase 2, mitochondrial OS=Mus musculus OX=10090 GN=Cmpk2 PE=1 SV=2 - [CMPK2_MOUSE] | O3U5O7 | C9(iodoTMT6plex) |
| DGIILCELINK | Cnn3 | Calponin-3 OS=Mus musculus OX=10090 GN=Cnn3 PE=1 SV=1 - [CNN3_MOUSE] | Q9DAW9 | C6(iodoTMT6plex) |
| COOLAAYGILEK | Cops4 | COP9 signalosome complex subunit 4 OS=Mus musculus OX=10090 GN=Cops4 PE=1 SV=1 - [CSN4_MOUSE] | O88544 | C1(iodoTMT6plex) |
| YOOGDFGYCPR | Csnk2b | Casein kinase II subunit beta OS=Mus musculus OX=10090 GN=Csnk2b PE=1 SV=1 - [CSK2B_MOUSE] | P67871 | C9(iodoTMT6plex) |
| LSVACFYGGTPYGGOIER | Ddx21 | Nucleolar RNA helicase 2 OS=Mus musculus OX=10090 GN=Ddx21 PE=1 SV=3 - [DDX21_MOUSE] | O9JIK5 | C5(iodoTMT6plex) |
| HFILDECDK | Ddx39b | Spliceosome RNA helicase Ddx39b OS=Mus musculus OX=10090 GN=Ddx39b PE=1 SV=1 - [DX39B_MOUSE] | O9Z1N5 | C7(iodoTMT6plex) |
| VRPCVVYGGAEIGQQIR | Ddx3x | ATP-dependent RNA helicase DDX3X OS=Mus musculus OX=10090 GN=Ddx3x PE=1 SV=3 - [DDX3X_MOUSE] | Q62167 | C4(iodoTMT6plex) |
| GNEFEDYCLK | Ddx6 | Probable ATP-dependent RNA helicase DDX6 OS=Mus musculus OX=10090 GN=Ddx6 PE=1 SV=1 - [DDX6_MOUSE] | P54823 | C8(iodoTMT6plex) |
| GLYDGPVCEVSVTPK | Dnvsl2 | Dihydropyrimidinase-related protein 2 OS=Mus musculus OX=10090 GN=Dnvsl2 PE=1 SV=2 - [DPYL2_MOUSE] | O08553 | C8(iodoTMT6plex) |
| CYLTMTOALEAR | Dync1h1 | Cytoplasmic dynein 1 heavy chain 1 OS=Mus musculus OX=10090 GN=Dync1h1 PE=1 SV=2 - [DYHC1_MOUSE] | O9JHU4 | C1(iodoTMT6plex); M5(Oxidation) |
| ETVSEESNVLCLSK | Eef2 | Elongation factor 2 OS=Mus musculus OX=10090 GN=Eef2 PE=1 SV=2 - [EF2_MOUSE] | P58252 | C11(iodoTMT6plex) |
| GTTEEICQIYLR | Eif3c | Eukaryotic translation initiation factor 3 subunit C OS=Mus musculus OX=10090 GN=Eif3c PE=1 SV=1 - [EIF3C_MOUSE] | Q8R1B4 | C7(iodoTMT6plex) |
| IAIWTTECENR | Eif4e | Eukaryotic translation initiation factor 4E OS=Mus musculus OX=10090 GN=Eif4e PE=1 SV=1 - [IF4E_MOUSE] | P63073 | C8(iodoTMT6plex) |
| CAVVDVPFGGAK | Glud1 | Glutamate dehydrogenase 1, mitochondrial OS=Mus musculus OX=10090 GN=Glud1 PE=1 SV=1 - [DHE3_MOUSE] | P26443 | C1(iodoTMT6plex) |
| ITOSSLTICFPEYTGANK | Gnai2 | Guanine nucleotide-binding protein G(i) subunit alpha-2 OS=Mus musculus OX=10090 GN=Gnai2 PE=1 SV=5 - [GNAI2_MOUSE] | P08752 | C9(iodoTMT6plex) |
| ACADATLSOITNNIDPVGR | Gnb1 | Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 OS=Mus musculus OX=10090 GN=Gnb1 PE=1 SV=3 - [GBB1_MOUSE] | P62874 | C2(iodoTMT6plex) |
| CGDLVFAK | Hdgf | Hepatoma-derived growth factor OS=Mus musculus OX=10090 GN=Hdgf PE=1 SV=2 - [HDGF_MOUSE] | P51859 | C1(iodoTMT6plex) |
| TCNCETEDYGEK | Hnrnpu | Isoform 2 of Heterogeneous nuclear ribonucleoprotein U OS=Mus musculus OX=10090 GN=Hnrnpu - [HNRPU_MOUSE] | O8VEK3-2 | C2(iodoTMT6plex); C4(iodoTMT6plex) |
| AFOVWCVGNEALVGPR | Hsd17b12 | Very-long-chain 3-oxoacyl-CoA reductase OS=Mus musculus OX=10090 GN=Hsd17b12 PE=1 SV=1 - [DHB12_MOUSE] | O70503 | C6(iodoTMT6plex) |
| LECAEMDCEEGWALLK | Ifit1 | Interferon-induced protein with tetratricopeptide repeats 1 OS=Mus musculus OX=10090 GN=Ifit1 PE=1 SV=2 - [IFIT1_MOUSE] | O64282 | C3(iodoTMT6plex); M6(Oxidation); C8(iodoTMT6plex) |
| LASAYGATELODYCGR | Lgals3bp | Galectin-3-binding protein OS=Mus musculus OX=10090 GN=Lgals3bp PE=1 SV=1 - [LG3BP_MOUSE] | O07797 | C14(iodoTMT6plex) |
| CQSLTEDLEFR | Lmnb1 | Lamin-B1 OS=Mus musculus OX=10090 GN=Lmnb1 PE=1 SV=3 - [LMNB1_MOUSE] | P14733 | C1(iodoTMT6plex) |
| LVCGDHSFVK | Mnda | Isoform 2 of Interferon-activable protein 205-B OS=Mus musculus OX=10090 GN=Mnda - [IF15B_MOUSE] | P0DOV1-2 | C3(iodoTMT6plex) |
| HHITPLLSAVYEGHVSCVK | Mtnn1 | Myotrophin OS=Mus musculus OX=10090 GN=Mtnn PE=1 SV=2 - [MTPN_MOUSE] | P62774 | C17(iodoTMT6plex) |
| KOELEEICHDLEAR | Myh9 | Myosin-9 OS=Mus musculus OX=10090 GN=Myh9 PE=1 SV=4 - [MYH9_MOUSE] | O8VDD5 | C8(iodoTMT6plex) |
| LMTDTINEPILLCR | Nars | Asparagine--tRNA ligase, cytoplasmic OS=Mus musculus OX=10090 GN=Nars PE=1 SV=2 - [SYNC_MOUSE] | O8BP47 | M2(Oxidation); C13(iodoTMT6plex) |
| IISDNLTYCK | Nop58 | Nucleolar protein 58 OS=Mus musculus OX=10090 GN=Nop58 PE=1 SV=1 - [NOP58_MOUSE] | Q6DFW4 | C9(iodoTMT6plex) |
| VPFPIPEPDGCK | Npc2 | NPC intracellular cholesterol transporter 2 OS=Mus musculus OX=10090 GN=Npc2 PE=1 SV=1 - [NPC2_MOUSE] | O9Z0J0 | C11(iodoTMT6plex) |
| CGITVSOVSNWFGNK | Pbx1 | Isoform PBX1b of Pre-B-cell leukemia transcription factor 1 OS=Mus musculus OX=10090 GN=Pbx1 - [PBX1_MOUSE] | P41778-2 | C1(iodoTMT6plex) |
| ACTGSLNMTLOR | Pdlim5 | PDZ and LIM domain protein 5 OS=Mus musculus OX=10090 GN=Pdlim5 PE=1 SV=4 - [PDLI5_MOUSE] | O8CI51 | C2(iodoTMT6plex); M8(Oxidation) |
| EVFSSSDTDPDMAFCK | Plod3 | Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3 OS=Mus musculus OX=10090 GN=Plod3 PE=1 SV=1 - [PLOD3_MOUSE] | O9R0E1 | M12(Oxidation); C15(iodoTMT6plex) |
| DHINLPGFCGQNPLR | Pnp | Purine nucleoside phosphorylase OS=Mus musculus OX=10090 GN=Pnp PE=1 SV=2 - [PNPH_MOUSE] | P23492 | C9(iodoTMT6plex) |
| EVGETLLYYGCR | Por | NADPH--cytochrome P450 reductase OS=Mus musculus OX=10090 GN=Por PE=1 SV=2 - [NCPR_MOUSE] | P37040 | C11(iodoTMT6plex) |
| GISCNSTTVSESPFK | Ppia1 | Isoform 2 of Peptidyl-prolyl cis-trans isomerase A OS=Mus musculus OX=10090 GN=Ppia1 - [PPIA_MOUSE] | O9D819 | C4(iodoTMT6plex); M5(Oxidation) |
| OLVTNACLIK | Prmt1 | Isoform 3 of Protein arginine N-methyltransferase 1 OS=Mus musculus OX=10090 GN=Prmt1 - [ANM1_MOUSE] | O9JIF0-3 | C7(iodoTMT6plex) |
| YGYEIPVDMLCK | Psma6 | Proteasome subunit alpha type-6 OS=Mus musculus OX=10090 GN=Psma6 PE=1 SV=1 - [PSA6_MOUSE] | O9OUM9 | M9(Oxidation); C11(iodoTMT6plex) |
| TTANAIYCPPK | Psmd11 | 26S proteasome non-ATPase regulatory subunit 11 OS=Mus musculus OX=10090 GN=Psmd11 PE=1 SV=3 - [PSD11_MOUSE] | Q8BG32 | C8(iodoTMT6plex) |
| SSDEAVILCK | Psmd13 | 26S proteasome non-ATPase regulatory subunit 13 OS=Mus musculus OX=10090 GN=Psmd13 PE=1 SV=1 - [PSD13_MOUSE] | O9WVJ2 | C9(iodoTMT6plex) |
| GIGMNEPLVDCEGYPR | Psmd9 | 26S proteasome non-ATPase regulatory subunit 9 OS=Mus musculus OX=10090 GN=Psmd9 PE=1 SV=1 - [PSMD9_MOUSE] | O9CR00 | M4(Oxidation); C11(iodoTMT6plex) |
| VOCHEYVTEGFEK | Ptgr1 | Prostaglandin reductase 1 OS=Mus musculus OX=10090 GN=Ptgr1 PE=1 SV=2 - [PTGR1_MOUSE] | O91YR9 | C3(iodoTMT6plex) |
| OVSTEEAOAWCK | Rab9a | Ras-related protein Rab-9A OS=Mus musculus OX=10090 GN=Rab9a PE=1 SV=1 - [RAB9A_MOUSE] | O9R0M6 | C11(iodoTMT6plex) |
| SPACFTLQELK | Rangap1 | Ran GTPase-activating protein 1 OS=Mus musculus OX=10090 GN=Rangap1 PE=1 SV=2 - [RAGP1_MOUSE] | P46061 | C4(iodoTMT6plex) |
| YGCLSGVR | Rcc2 | Protein RCC2 OS=Mus musculus OX=10090 GN=Rcc2 PE=1 SV=1 - [RCC2_MOUSE] | O8BK67 | C3(iodoTMT6plex) |
| IAVHCTVR | Rpl11 | 60S ribosomal protein L11 OS=Mus musculus OX=10090 GN=Rpl11 PE=1 SV=4 - [RL11_MOUSE] | O9CXW4 | C5(iodoTMT6plex) |
| VACIGAWHPAR | Rpl3 | 60S ribosomal protein L3 OS=Mus musculus OX=10090 GN=Rpl3 PE=1 SV=3 - [RL3_MOUSE] | P27659 | C3(iodoTMT6plex) |
| TGVACSVSOAOK | Rpl9 | 60S ribosomal protein L9 OS=Mus musculus OX=10090 GN=Rpl9 PE=2 SV=2 - [RL9_MOUSE] | P51410 | C5(iodoTMT6plex) |
| GCTATLGNFAK | Rps2 | 40S ribosomal protein S2 OS=Mus musculus OX=10090 GN=Rps2 PE=1 SV=3 - [RS2_MOUSE] | P25444 | C2(iodoTMT6plex) |
| LNISFPATGCOK | Rps6 | 40S ribosomal protein S6 OS=Mus musculus OX=10090 GN=Rps6 PE=1 SV=1 - [RS6_MOUSE] | P62754 | C10(iodoTMT6plex) |
| VGSVLOEGCEK | Sdha | Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Mus musculus OX=10090 GN=Sdha PE=1 SV=1 - [SDHA_MOUSE] | O8K2B3 | C9(iodoTMT6plex) |
| CGGTLEER | SF3A3 | Splicing factor 3A subunit 3 OS=Mus musculus OX=10090 GN=Sf3a3 PE=1 SV=2 - [SF3A3_MOUSE] | O9D554 | C1(iodoTMT6plex) |
| VOENCIDLVGR | SF3B1 | Splicing factor 3B subunit 1 OS=Mus musculus OX=10090 GN=Sf3b1 PE=1 SV=1 - [SF3B1_MOUSE] | O99NB9 | C5(iodoTMT6plex) |
| GLLDVTCK | Skp1 | S-phase kinase-associated protein 1 OS=Mus musculus OX=10090 GN=Skp1 PE=1 SV=3 - [SKP1_MOUSE] | Q9WTX5 | C7(iodoTMT6plex) |
| HCNMVLENVK | Snrnp2 | Small nuclear ribonucleoprotein Sm D2 OS=Mus musculus OX=10090 GN=Snrnp2 PE=1 SV=1 - [SMD2_MOUSE] | P62317 | C2(iodoTMT6plex); M4(Oxidation) |
| CLHMFLOEEAIDR | Snx12 | Sorting nexin-12 OS=Mus musculus OX=10090 GN=Snx12 PE=1 SV=1 - [SNX12_MOUSE] | O70493 | C1(iodoTMT6plex); M4(Oxidation) |
| CNSLEEIK | Sptan1 | Isoform 2 of Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus OX=10090 GN=Sptan1 - [SPTN1_MOUSE] | P16546-2 | C1(iodoTMT6plex) |
| LGLIOEDVASSCIPR | Srrm2 | Isoform 3 of Serine/arginine repetitive matrix protein 2 OS=Mus musculus OX=10090 GN=Srrm2 - [SRRM2_MOUSE] | O8BTR-3 | C12(iodoTMT6plex) |
| LIGPNCPVPGPGECK | Suclg1 | Succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Mus musculus OX=10090 GN=Suclg1 PE=1 SV=4 - [SUCA_MOUSE] | Q9WUM5 | C6(iodoTMT6plex); C15(iodoTMT6plex) |
| CDFAYETMGTIEVOR | Tomm70 | Mitochondrial import receptor subunit TOM70 OS=Mus musculus OX=10090 GN=Tomm70 PE=1 SV=2 - [TOM70_MOUSE] | O9CZW5 | C1(iodoTMT6plex); M8(Oxidation) |
| CVFEMPNENDK | Vapa | Vesicle-associated membrane protein-associated protein A OS=Mus musculus OX=10090 GN=Vapa PE=1 SV=2 - [VAPA_MOUSE] | O9WV55 | C1(iodoTMT6plex); M5(Oxidation) |
| DCPVPLPGDGDLLVR | Zadh2 | Prostaglandin reductase-3 OS=Mus musculus OX=10090 GN=Zadh2 PE=1 SV=1 - [PTGR3_MOUSE] | O8BGC4 | C2(iodoTMT6plex) |
| IPGGIIEDSCVLR | Cct3 | T-complex protein 1 subunit gamma OS=Mus musculus OX=10090 GN=Cct3 PE=1 SV=1 - [TCPG_MOUSE] | P80318 | C10(iodoTMT6plex) |
| DQGSCGSCWAFGAVEAISDR | Ctsb | Cathepsin B OS=Mus musculus OX=10090 GN=Ctsb PE=1 SV=2 - [CATB_MOUSE] | P10605 | C5(iodoTMT6plex); C8(iodoTMT6plex) |
| TCIHTNGR | Ctsb | Cathepsin B OS=Mus musculus OX=10090 GN=Ctsb PE=1 SV=2 - [CATB_MOUSE] | P10605 | C2(iodoTMT6plex) |
| AEHCVNTPGSFOCYK | Fbln2 | Isoform 2 of Fibulin-2 OS=Mus musculus OX=10090 GN=Fbln2 - [FBLN2_MOUSE] | P37889-2 | C4(iodoTMT6plex); C13(iodoTMT6plex) |
| CSCAAGFLLAADGK | Fbln2 | Isoform 2 of Fibulin-2 OS=Mus musculus OX=10090 GN=Fbln2 - [FBLN2_MOUSE] | P37889-2 | C1(iodoTMT6plex); C3(iodoTMT6plex) |
| CGEGOLCYNLPGSYR | Fbln2 | Isoform 2 of Fibulin-2 OS=Mus musculus OX=10090 GN=Fbln2 - [FBLN2_MOUSE] | P37889-2 | C1(iodoTMT6plex); C7(iodoTMT6plex) |
| CSGPGLSPGMVR | Flna | Filamin-A OS=Mus musculus OX=10090 GN=Flna PE=1 SV=5 - [FLNA_MOUSE] | Q8BTM8 | C1(iodoTMT6plex); M10(Oxidation) |
| SNFTVDCSK | Flna | Filamin-A OS=Mus musculus OX=10090 GN=Flna PE=1 SV=5 - [FLNA_MOUSE] | O8BTM8 | C7(iodoTMT6plex) |
| ACIPOSFTVDSSK | Flnb | Filamin-B OS=Mus musculus OX=10090 GN=Flnb PE=1 SV=3 - [FLNB_MOUSE] | O8UX90 | C2(iodoTMT6plex) |
| IAGPGLSSCVR | Flnb1 | Filamin-B OS=Mus musculus OX=10090 GN=Flnb PE=1 SV=3 - [FLNB_MOUSE] | O80X90 | C9(iodoTMT6plex) |
| CFTDEPOLOSR | Gvin1 | Interferon-induced very large GTPase 1 OS=Mus musculus OX=10090 GN=Gvin1 PE=1 SV=1 - [GVIN1_MOUSE] | O80SU7 | C1(iodoTMT6plex) |
| CWEVSDPEEQLTK | Gvin1 | Interferon-induced very large GTPase 1 OS=Mus musculus OX=10090 GN=Gvin1 PE=1 SV=1 - [GVIN1_MOUSE] | Q80SU7 | C1(iodoTMT6plex) |
| OITOVYGFYDECLR | Ppp2ca | Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform OS=Mus musculus OX=10090 GN=Ppp2ca PE=1 SV=1 - [PP2AA_MOUSE] | P63330 | C12(iodoTMT6plex) |
| AHOLVMEGYNWCHDR | Ppp2ca | Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform OS=Mus musculus OX=10090 GN=Ppp2ca PE=1 SV=1 - [PP2AA_MOUSE] | P63330 | M6(Oxidation); C12(iodoTMT6plex) |
| SEGTYCCGPVSVR | Tgm2 | Protein-glutamine gamma-glutamyltransferase 2 OS=Mus musculus OX=10090 GN=Tgm2 PE=1 SV=4 - [TGM2_MOUSE] | P21981 | C6(iodoTMT6plex); C7(iodoTMT6plex) |
| VVSAMVNCNDDOGVLLGR | Tgm2 | Protein-glutamine gamma-glutamyltransferase 2 OS=Mus musculus OX=10090 GN=Tgm2 PE=1 SV=4 - [TGM2_MOUSE] | P21981 | M5(Oxidation); C8(iodoTMT6plex) |
| VVAPTISSPVCQEQLVEAGR | Tln1 | Talin-1 OS=Mus musculus OX=10090 GN=Tln1 PE=1 SV=2 - [TLN1_MOUSE] | P26039 | C11(iodoTMT6plex) |
| AGALOCSPSDVYTK | Tln1 | Talin-1 OS=Mus musculus OX=10090 GN=Tln1 PE=1 SV=2 - [TLN1_MOUSE] | P26039 | C6(iodoTMT6plex) |
| CVSCLPGOR | Tln1 | Talin-1 OS=Mus musculus OX=10090 GN=Tln1 PE=1 SV=2 - [TLN1_MOUSE] | P26039 | C1(iodoTMT6plex); C4(iodoTMT6plex) |
| OVAASTAOLLVACK | Tln1 | Talin-1 OS=Mus musculus OX=10090 GN=Tln1 PE=1 SV=2 - [TLN1_MOUSE] | P26039 | C13(iodoTMT6plex) |

**Total 93 oxidized Cys in 82 proteins (Cct3 contains both oxidized and reduced Cys)**

SUPPLEMENTAL TABLE 1B: Proteins with cysteines whose redox state became more reduced with aging and restored by Slc7a11 overexpression

| Sequence | Protein | Description | Protein Group Accessions | Modifications |
|---|---|---|---|---|
| LCYVALDFEOEMATAASSSSLEK | Actb | Actin, cytoplasmic 1 OS=Mus musculus OX=10090 GN=Actb PE=1 SV=1 - [ACTB_MOUSE] | P60710 | C2(iodoTMT6plex); M12(Oxidation) |
| GLGTDEDSLIIEIICSR | Anxa2 | Annexin A2 OS=Mus musculus OX=10090 GN=Anxa2 PE=1 SV=2 - [ANXA2_MOUSE] | P07356 | C14(iodoTMT6plex) |
| ECTIEATA | Ctnb | Isoform 2 of Cellular nucleic acid-binding protein OS=Mus musculus OX=10090 GN=Ctnb - [CNBP_MOUSE] | P53996-2 | C2(iodoTMT6plex) |
| VADPVVTFCETVVETSSLK | Eftud2 | 116 kDa U5 small nuclear ribonucleoprotein component OS=Mus musculus OX=10090 GN=Eftud2 PE=1 SV=1 - [U5S1_MOUSE] | O08810 | C9(iodoTMT6plex) |
| FMCAOLPNPVLDSISIIDTPGILSGEK | Ehd1 | EH domain-containing protein 1 OS=Mus musculus OX=10090 GN=Ehd1 PE=1 SV=1 - [EHD1_MOUSE] | O9WVK4 | M2(Oxidation); C3(iodoTMT6plex) |
| IVLTNPVCTEVGEK | Eif2s3x | Eukaryotic translation initiation factor 2 subunit 3, X-linked OS=Mus musculus OX=10090 GN=Eif2s3x PE=1 SV=2 - [IF2G_MOUSE] | O9Z0N1 | C8(iodoTMT6plex) |
| THGLSDEHVFEVICPSIPGYGFSEASSK | Enhx1 | Epoxide hydrolase 1 OS=Mus musculus OX=10090 GN=Enhx1 PE=1 SV=2 - [HYEP_MOUSE] | O9D379 | C14(iodoTMT6plex) |
| DGSCGVSYVVQEPGDYEVSIK | Flnc | Isoform 2 of Filamin-C OS=Mus musculus OX=10090 GN=Flnc - [FLNC_MOUSE] | Q8VHX6-2 | C4(iodoTMT6plex) |
| ACAETIDPASLWEY | Fscn1 | Fascin OS=Mus musculus OX=10090 GN=Fscn1 PE=1 SV=4 - [FSCN1_MOUSE] | O61553 | C2(iodoTMT6plex) |
| ASADLMSYCEEHAR | Gng12 | Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12 OS=Mus musculus OX=10090 GN=Gng12 PE=1 SV=3 - [GBG12_MOUSE] | O9DAS9 | M6(Oxidation); C9(iodoTMT6plex) |
| TNOPLSTC | Hars | Histidine--tRNA ligase, cytoplasmic OS=Mus musculus OX=10090 GN=Hars PE=1 SV=2 - [SYHC_MOUSE] | O61035 | C8(iodoTMT6plex) |
| CELSSSVOTDINLPYLTMDASGPK | Hspa9 | Stress-70 protein, mitochondrial OS=Mus musculus OX=10090 GN=Hspa9 PE=1 SV=3 - [GRP75_MOUSE] | P38647 | C1(iodoTMT6plex); M18(Oxidation) |
| CATITPDEK | Idh1 | Isocitrate dehydrogenase [NADP] cytoplasmic OS=Mus musculus OX=10090 GN=Idh1 PE=1 SV=2 - [IDHC_MOUSE] | O88844 | C1(iodoTMT6plex) |
| FFOVACDVPELODK | Iqgap1 | Ras GTPase-activating-like protein IQGAP1 OS=Mus musculus OX=10090 GN=Iqgap1 PE=1 SV=2 - [IQGA1_MOUSE] | O9JKF1 | C6(iodoTMT6plex) |
| YNLGAPVAGTCYOAEWDDYVPK | Pebp1 | Phosphatidylethanolamine-binding protein 1 OS=Mus musculus OX=10090 GN=Pebp1 PE=1 SV=3 - [PEBP1_MOUSE] | P70296 | C11(iodoTMT6plex) |
| VCTOLOVLTEAFR | Pef1 | Peflin OS=Mus musculus OX=10090 GN=Pef1 PE=1 SV=1 - [PEF1_MOUSE] | O8BFY6 | C2(iodoTMT6plex) |
| ICIVTK | Psmb1 | Proteasome subunit beta type-1 OS=Mus musculus OX=10090 GN=Psmb1 PE=1 SV=1 - [PSB1_MOUSE] | O09061 | C2(iodoTMT6plex) |
| OLONOLAECK | Rai14 | Ankycorbin OS=Mus musculus OX=10090 GN=Rai14 PE=1 SV=1 - [RAI14_MOUSE] | Q9EP71 | C9(iodoTMT6plex) |
| CAEAGANMIVSGSAIMR | Rpe | Ribulose-phosphate 3-epimerase OS=Mus musculus OX=10090 GN=Rpe PE=1 SV=1 - [RPE_MOUSE] | O8VEE0 | C1(iodoTMT6plex); M8(Oxidation); M16(Oxidation) |
| ALAGCDFLTISPK | Taldo1 | Transaldolase OS=Mus musculus OX=10090 GN=Taldo1 PE=1 SV=2 - [TALDO_MOUSE] | O93092 | C5(iodoTMT6plex) |
| SOLCSOSLEITR | Uso1 | Isoform 4 of General vesicular transport factor p115 OS=Mus musculus OX=10090 GN=Uso1 - [USO1_MOUSE] | O9Z1Z0-4 | C4(iodoTMT6plex) |
| MEDSGVYVCEGVNLIGR | Vcam1 | Vascular cell adhesion protein 1 OS=Mus musculus OX=10090 GN=Vcam1 PE=1 SV=1 - [VCAM1_MOUSE] | P29533 | M1(Oxidation); C9(iodoTMT6plex) |
| IGDEYFTFITDCK | Cct3 | T-complex protein 1 subunit gamma OS=Mus musculus OX=10090 GN=Cct3 PE=1 SV=1 - [TCPG_MOUSE] | P80318 | C12(iodoTMT6plex) |

**Total 23 reduced Cys in 23 proteins (Cct3 contains both oxidized and reduced Cys)**

Total 104 proteins with age-dependent Slc7a11-responsive Cys

Supplemental Table 2 provides IPA analysis of proteins whose redox state changes with Slc7a11 overexpression in old fibroblasts regardless of their redox states in young fibroblasts. Cysteine redox states were compared between old fibroblasts and old Slc7a11-overexpressing fibroblasts. This table is described in the Results section 3.2.

SUPPLEMENTAL TABLE 2: IPA analysis of proteins whose redox states were changed by Slc7a11 overexpression in old fibroblasts. Fisher's exact test *p*-values for all the items shown were less than 0.05.

| Name | p-value | Overlap |
|---|---|---|
| **Regulation of eIF4 and p70S6K Signaling** | 2.09E-09 | 6.4 % 10/157 |
| **EIF2 Signaling** | 6.26E-08 | 4.5 % 10/224 |
| **mTOR Signaling** | 4.53E-06 | 3.8 % 8/210 |
| **Antiproliferative Role of Somatostatin Receptor 2** | 2.41E-05 | 6.5 % 5/77 |
| **Relaxin Signaling** | 5.68E-05 | 4.0 % 6/150 |

Supplemental Table 3 provides IPA analysis of age-dependent proteins whose redox states were not rescued by Slc7a11 overexpression in old fibroblasts. Those age-dependent Slc7a11-nonresponsive protein cysteines indicate there are Slc7a11-independent regulation of protein redox signaling. This table is described in the Results section 3.3.

SUPPLEMENTAL TABLE 3: IPA analysis of age-dependent proteins whose redox states were not rescued by Slc7a11 overexpression in old fibroblasts. Fisher's exact test *p*-values for all the items shown were less than 0.05.

| Name | p-value | Overlap |
|---|---|---|
| **Hepatic Fibrosis / Hepatic Stellate Cell Activation** | 3.27E-05 | 2.7 % 5/186 |
| **Protein Ubiquitination Pathway** | 2.00E-04 | 1.8 % 5/273 |
| **Epithelial Adherens Junction Signaling** | 2.32E-04 | 2.6 % 4/152 |
| **Actin Cytoskeleton Signaling** | 9.04E-04 | 1.8 % 4/218 |
| **EIF2 Signaling** | 1.00E-03 | 1.8 % 4/224 |

Supplemental Table 4 provides GO-based biological process analysis of proteins whose redox states were not rescued by Slc7a11 overexpression in old fibroblasts. Those age-dependent Slc7a11-nonresponsive protein cysteines indicate there are Slc7a11-independent regulation of protein redox signaling. This table is described in the Results section 3.3.

SUPPLEMENTAL TABLE 4: GO-based biological process analysis of proteins whose redox states were not rescued by Slc7a11 overexpression in old fibroblasts. Fisher's exact test *p*-values for all the items shown were less than 0.05.

| Term | Count | p-value | -log10 (p-value) |
|---|---|---|---|
| protein folding | 6 | <0.001 | 4.845 |
| wound healing | 4 | 0.002 | 2.811 |
| actomyosin structure organization | 3 | 0.002 | 2.709 |
| mitotic cytokinesis | 3 | 0.002 | 2.618 |
| translation | 6 | 0.003 | 2.554 |
| skin development | 3 | 0.009 | 2.057 |
| IRES-dependent viral translational initiation | 2 | 0.017 | 1.772 |
| platelet degranulation | 2 | 0.019 | 1.714 |
| heart development | 4 | 0.025 | 1.594 |
| cytoskeleton organization | 3 | 0.026 | 1.577 |
| single organismal cell-cell adhesion | 3 | 0.028 | 1.547 |
| cerebellar Purkinje cell layer development | 2 | 0.038 | 1.417 |
| actin filament-based movement | 2 | 0.041 | 1.392 |
| actin filament capping | 2 | 0.043 | 1.367 |