

Supplementary Information

Supplementary tables

Peptide list obtained from the redox proteomics analysis

Peptide containing cysteine	UniProt, accession number	Master protein name	Fold change	p-value
[K].CLDAFPNLR.[D]	P10649	Glutathione S-transferase Mu 1	9.15	0.027
[K].IIFVVGPGSGKGTQC EK.[I]	Q9R0Y5	Adenylate kinase isoenzyme 1	6.63	0.002
[K].LGRICLDILK.[D]	P61089	Ubiquitin-conjugating enzyme E2 N	5.60	0.000
[R].EKDFIILSCVR.[A]	Q9EPU0	Regulator of nonsense transcripts 1	5.32	0.000
[K].NTEICDELMTR.[H]	Q8VC19	5-aminolevulinate synthase, nonspecific, mitochondrial	4.81	0.008
[R].RVNQAIWLLCTGAR.[E]	P97461	40S ribosomal protein S5	3.98	0.010
[K].EGGPNPENSSLANIL ELCR.[S]	Q8K0Z7	Translational activator of cytochrome c oxidase 1	3.33	0.037
[R].VTWDSSEFCVAVNPR.[F]	Q9WUM4	Coronin-1C	3.32	0.026
[R].VCGAVMETVK.[Q]	Q8VC19	5-aminolevulinate synthase, nonspecific, mitochondrial	3.26	0.050
[K].SLEKVCADLIR.[G]	P60867	40S ribosomal protein S20	3.20	0.038
[K].VTWDSAFCAVNPK.[F]	Q920M5	Coronin-6	3.03	0.026
[R].ITYRELLETCR.[L]	Q99NB1	Acetyl-coenzyme A synthetase 2-like, mitochondrial	2.80	0.032
[K].VVAACAMPVMKGW NILTNSEK.[S]	Q91VD9	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	2.74	0.039
[R].EGVVDIYNCVR.[E]	P28828	Receptor-type tyrosine-protein phosphatase mu	2.73	0.023
[K].VGVGPGSVCTTR.[T]	Q9DCZ1	GMP reductase 1	2.68	0.004
[R].TNGFSLESCR.[S]	O35350	Calpain-1 catalytic subunit	2.65	0.008
[R].VNQAIWLLCTGAR.[E]	P97461	40S ribosomal protein S5	2.62	0.034
[R].VGSVLQEGCEKISQLY GDLKHLK.[T]	Q8K2B3	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	2.60	0.028
[K].GELLRPSPTCEIK.[A]	A2ASS6	Titin	2.52	0.023
[K].LASLTPGFSGADVAVN CNEAALIAAR.[H]	Q8JZQ2	AFG3-like protein 2	2.51	0.029
[K].LTDFGFCAQITPEQSK.[R]	O88643	Serine/threonine-protein kinase PAK 1	2.48	0.033

[R].TTANAIYCPK.[L]	Q8BG32	26S proteasome non-ATPase regulatory subunit 11	2.43	0.037
[K].ADLSNCLYK.[D]	Q0II04	Nebulette	2.38	0.039
[K].DGVVTIGCIGFPNVGK SSLINGLVGR.[K]	P36916	Guanine nucleotide-binding protein-like 1	2.35	0.029
[K].TPPYQIACGISQGLAD NTVVAK.[V]	Q9D0R2	Threonine--tRNA ligase 1, cytoplasmic	2.30	0.033
[R].ASDSSLVCSR.[Y]	Q8BYW9	EGF domain-specific O-linked N-acetylglucosamine transferase	2.24	0.035
[R].VNLVAILCTHK.[H]	Q69ZP3	Probable hydrolase PNKD	2.22	0.005
[R].VAIYMPVSPLAVAAM LACAR.[I]	Q99NB1	Acetyl-coenzyme A synthetase 2-like, mitochondrial	2.18	0.005
[K].ALNVEPDGTGLTCSLA PNILSQL.[-]	P99029	Peroxisome oxidoreductase, mitochondrial	2.11	0.006
[R].VTNDNTFCR.[L]	P29268	CCN family member 2	2.09	0.046
[K].VPTTLAEYCIK.[T]	Q6ZWZ2	Ubiquitin-conjugating enzyme E2 R2	2.09	0.048
[K].TQAIVCQQLDLTHLK.[E]	P61222	ATP-binding cassette sub-family E member 1	2.08	0.014
[R].TIQFVDWCPTGFK.[V]	Q9JJZ2; P68369; P68373	Tubulin alpha-8 chain	2.08	0.049
[R].LIIGQNGILSTPAVSCII R.[K]	Q8BZF8	Phosphoglucosyltransferase-like protein 5	2.08	0.039
[K].RPNKPLFTGLVTQCQK .[M]	Q8K4Z3	NAD(P)H-hydrate epimerase	2.07	0.020
[K].DGSASGTTLEALDCIL PPTRPTDKPLR.[L]	P10126	Elongation factor 1-alpha 1	2.04	0.017
[R].GSLLGCSINISDIR.[D]	O35350	Calpain-1 catalytic subunit	2.02	0.009
[K].EDVKSCAEFVSGSQLR .[I]	Q9DCX2	ATP synthase subunit d, mitochondrial	2.02	0.017
[K].KLPVGFTFSFPCR.[Q]	P17710	Hexokinase-1	1.98	0.014
[R].LEALELKECLAHTPK.[L]	O09131	Glutathione S-transferase omega-1	1.97	0.001
[R].SAVQYAECQSK.[A]	Q8K2I4	Beta-mannosidase	1.95	0.036
[K].ELETQLLEQCTVDTGA AK.[G]	Q9QZB7	Actin-related protein 10	1.95	0.050
[R].LDGALCSYTEK.[D]	P52624	Uridine phosphorylase 1	1.94	0.021
[K].FTCEIQGAPNVR.[F]	A2ASS6	Titin	1.94	0.018
[R].ILALCMGNHELYMR.[R]	P26041	Moesin	1.92	0.029
[K].NMMAACDPR.[H]	Q9ERD7; P68372	Tubulin beta-3 chain	1.91	0.002
[R].YVEPIEDVPCGNIVGL VGVDQFLVK.[T]	P58252	Elongation factor 2	1.89	0.045
[R].LVATDGAFSMDGDIA PLQDICR.[L]	O88986	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial	1.87	0.041
[R].GDSGLYLCK.[A]	A2ASS6	Titin	1.87	0.014
[K].LQAGIDLCETR.[T]	Q8C0M9	Isoaspartyl peptidase/L-asparaginase	1.86	0.049

[R].VFSANSTAACTELAK.[R]	Q9D0M1	Phosphoribosyl pyrophosphate synthase-associated protein 1	1.85	0.002
[R].KNANCSIEESFQR.[F]	P38060	Hydroxymethylglutaryl-CoA lyase, mitochondrial	1.84	0.003
[R].TFVSGACDASIK.[L]	P62880	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	1.84	0.015
[R].SLVANLAAANCYK.[K]	P55264	Adenosine kinase	1.81	0.016
[K].LVEEAIQCAEK.[I]	Q8BH95	Enoyl-CoA hydratase, mitochondrial	1.80	0.002
[K].VVNEINIEDLCLTK.[A]	Q9CQB5	CDGSH iron-sulfur domain-containing protein 2	1.80	0.029
[R].IVVHMAHALKPGEFG LASICNGGGGASALLIEK.[L]	Q8QZT1	Acetyl-CoA acetyltransferase, mitochondrial	1.79	0.037
[R].GTLLSLSEQELLDCDK VDK.[A]	Q9R013	Cathepsin F	1.78	0.041
[K].AVIFCLSADKK.[C]	Q9R0P5	Destrin	1.76	0.026
[R].VGAFVVCKDAEEAK.[R]	P05202	Aspartate aminotransferase, mitochondrial	1.75	0.002
[K].IYQLQVLANC.[A]	P50431	Serine hydroxymethyltransferase, cytosolic	1.74	0.016
[R].NLADCLR.[S]	Q9R1P3	Proteasome subunit beta type-2	1.74	0.041
[K].GSEDLKKHGCTVLTAL GTILK.[K]	P04247	Myoglobin	1.73	0.030
[R].AAQLCGAGMAAVVE K.[I]	P17710	Hexokinase-1	1.73	0.046
[K].SSFATPGVNVGLFCST PAVALGR.[A]	Q9D7J9	Enoyl-CoA hydratase domain-containing protein 3, mitochondrial	1.73	0.013
[R].VVDGIFAVCGVPESK.[L]	Q99KK9	Histidine--tRNA ligase, mitochondrial	1.71	0.012
[R].ILDLTECSAVQFDYSQ ER.[V]	Q9QXT1	Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide	1.71	0.036
[R].MPLGFTFSFPCR.[Q]	P17710	Hexokinase-1	1.70	0.025
[K].TGVGYQLSAVIECAD SAHGLK.[G]	Q9DCZ1	GMP reductase 1	1.70	0.025
[K].LVEEAIQCAEKIASNSK.[I]	Q8BH95	Enoyl-CoA hydratase, mitochondrial	1.69	0.023
[R].DLTTAGAVTQCYR.[D]	P62717	60S ribosomal protein L18a	1.68	0.033
[R].VTNRDIICQIAYAR.[I]	P47962	60S ribosomal protein L5	1.68	0.008
[K].NLDCVVTGLQQGK.[T]	A2ASS6	Titin	1.67	0.002
[K].LPVGFTFSFPCR.[Q]	P17710	Hexokinase-1	1.67	0.036
[R].VGAFVVCKDAEEAK R.[V]	P05202	Aspartate aminotransferase, mitochondrial	1.67	0.012

[K].ASADLMSYCEEHAR.[S]	Q9DAS9	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	1.67	0.009
[R].YFSLGLPTGSTPLGCK.[K]	Q9CRC9	Glucosamine-6-phosphate isomerase 2	1.66	0.023
[K].AADLVSENCETYEAMR.[D]	Q9JLI6	Selenocysteine lyase	1.66	0.016
[K].ISHTEDCLTFK.[V]	P01027	Complement C3	1.66	0.018
[K].IAYPLGLATLGATVCYP AQSIIAK.[I]	Q78IK4	MICOS complex subunit Mic27	1.65	0.007
[R].AHGSSILACAPLYSWR.[T]	P11688	Integrin alpha-5	1.65	0.001
[K].AVAVEEFCK.[S]	Q6PE15	Palmitoyl-protein thioesterase ABHD10, mitochondrial	1.65	0.001
[K].LAEEESCR.[E]	Q61543	Golgi apparatus protein 1	1.64	0.045
[K].IKNVDCVLLAR.[H]	Q9CQ65	S-methyl-5'-thioadenosine phosphorylase	1.63	0.035
[K].MVAAVACAK.[V]	Q3ULD5	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	1.62	0.006
[K].STLIDALCR.[K]	Q64514	Tripeptidyl-peptidase 2	1.62	0.023
[R].VIATFACSGEK.[E]	Q9JLJ2	4-trimethylaminobutyraldehyde dehydrogenase	1.61	0.008
[R].LSMEQICR.[H]	Q6P4S6	Serine/threonine-protein kinase SIK3	1.61	0.021
[R].SLGLICDECPIGTGPR.[C]	Q60675	Laminin subunit alpha-2	1.59	0.005
[R].LFVSGACDASAK.[L]	P62874	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	1.59	0.007
[R].EGITFHFTPLVCK.[D]	Q8VDL4	ADP-dependent glucokinase	1.59	0.039
[R].KGLIAAICAGPTALLA HEVGFCK.[V]	Q99LX0	Parkinson disease protein 7 homolog	1.59	0.042
[K].LLASCSADMTIK.[L]	P63005	Platelet-activating factor acetylhydrolase IB subunit beta	1.58	0.044
[R].TLSGMESYCVR.[A]	P80315	T-complex protein 1 subunit delta	1.58	0.017
[K].EILGTAQSVGCNVDGR.[H]	P35979	60S ribosomal protein L12	1.56	0.013
[K].LLTQLHYCEK.[A]	Q99KK9	Histidine--tRNA ligase, mitochondrial	1.56	0.027
[K].SAGGIGVAVSCIR.[A]	P07742	Ribonucleoside-diphosphate reductase large subunit	1.56	0.026
[K].VTYCPTEPGTYIINIK.[F]	Q8VHX6	Filamin-C	1.56	0.010
[R].GTFCSFDTPDEAIR.[N]	P61922	4-aminobutyrate aminotransferase, mitochondrial	1.55	0.037
[R].SIQFVDWCPTGFK.[V]	P68368	Tubulin alpha-4A chain	1.55	0.026

[KR].DLVRVCENIPIVLCG NK.[V]	P62827	GTP-binding nuclear protein Ran	1.55	0.043
[K].KGAFDAYVAVGGGSTM	Q8R0N6	Hydroxyacid-oxoacid transhydrogenase, mitochondrial	1.55	0.016
[K].FGANAILGVSLAVCKA GAVEK.[G]	P17182	Alpha-enolase	1.55	0.043
[K].IREIADGLCLEVEGK.[M]	P63028	Translationally-controlled tumor protein	1.55	0.019
[R].AALEALGSCLNNK.[Y]	Q9CZN7	Serine hydroxymethyltransferase, mitochondrial	1.54	0.014
[R].ATNLNCSVIADVR.[H]	Q9D1H8	39S ribosomal protein L53, mitochondrial	1.54	0.030
[K].VFCIGPVFR.[A]	Q922B2	Aspartate--tRNA ligase, cytoplasmic	1.54	0.048
[K].GAVHQLCQSLAGK.[N]	Q8BVI4	Dihydropteridine reductase	1.52	0.010
[R].TSLDLIANVIHCK.[S]	P70404	Isocitrate dehydrogenase [NAD] subunit gamma 1, mitochondrial	1.52	0.016
[K].IEEACEIYAR.[A]	Q9DB05	Alpha-soluble NSF attachment protein	1.52	0.031
[R].TLIQNCGASTIR.[L]	P80318	T-complex protein 1 subunit gamma	1.52	0.039
[K].VFADYEEYIKCQDK.[V]	Q9WUB3	Glycogen phosphorylase, muscle form	1.52	0.006
[K].TFESLVDFCK.[T]	Q61425	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	1.51	0.010
[K].VIYDKDQFMCGETVP APSTNK.[E]	Q8K0W9	DPH3 homolog	1.50	0.046
[R].LISLNMNFCSR.[E]	Q04519	Sphingomyelin phosphodiesterase	1.50	0.049

Supplementary Table 1: List of cysteine containing peptides that were identified in the redox proteomic screen to show an increased total reversible oxidation demonstrated by a fold change of ≥ 1.5 and a p-value of ≤ 0.05 in the left ventricles of HyPer-DAO mice compared to wild type mice after 7 days of D-ala treatment. P values were calculated by two-tailed unpaired t-test.

Peptide list obtained from the redox proteomics analysis

Peptide containing cysteine	UniProt, accession number	Master protein name	Fold change	p-value
[K].TCVADESAANCDKSL HTLFGDK.[L]	P07724	Albumin	0.50	0.027
[R].MAHAMNEYPDSCAV LVR.[R]	Q9WVQ5	Methylthioribulose-1-phosphate dehydratase	0.49	0.010
[R].EVFGSGTACQVCPVH QILYEGK.[Q]	O35855	Branched-chain-amino-acid aminotransferase, mitochondrial	0.49	0.002
[K].QFSYTHICAGASAFGK .[N]	Q99LC5	Electron transfer flavoprotein subunit alpha, mitochondrial	0.49	0.013
[K].TLTPGGHAEHDGQPY CHKPCYGILFGPK.[G]	Q9DCT8	Cysteine-rich protein 2	0.49	0.003
[K].YGHAACFGLQPGCLR.[Q]	Q8C1A5	Thimet oligopeptidase	0.48	0.003
[R].QCLSFPCAGVPIR.[D]	P0CW02	Lymphocyte antigen 6C1	0.48	0.043
[R].EGICGSCAMNINGGN TLACTR.[R]	Q9CQA3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	0.48	0.006
[R].GHIEDCGHWTQIEKP TEVNQILIK.[W]	P34914	Bifunctional epoxide hydrolase 2	0.47	0.001
[R].VDLEISPDFLAVPVGG HENSHCICGNER.[K]	Q61838	Pregnancy zone protein	0.47	0.019
[R].LNQATVTSNRPGIFY GQCSEICGSNHSFMPIVLE MVPLK.[Y]	P00405	Cytochrome c oxidase subunit 2	0.47	0.016
[R].VLCVALGQLDRPLDLA DDGR.[I]	Q6P3E7	Polyamine deacetylase HDAC10	0.47	0.049
[K].TFHETLNCCGSNALTT LTTILR.[N]	P35762	CD81 antigen	0.46	0.047
[R].GDGQTCYDIDECSEQ PSR.[C]	P10493	Nidogen-1	0.46	0.003
[K].QCEAHLGHVFPDGP KPTGQR.[F]	Q78J03	Methionine-R-sulfoxide reductase B2, mitochondrial	0.46	0.031
[K].EKLCYVALDFENEMA TAASSSLEK.[S]	P68033	Actin, alpha cardiac muscle 1	0.46	0.045
[R].ELACDDPEAEQVALLA VDYLNNHLLQGFK.[Q]	P29699	Alpha-2-HS-glycoprotein	0.45	0.048
[R].LDPQLQLHCSDEIANL CAEEAAAQEQTGVVEECL K.[V]	Q61543	Golgi apparatus protein 1	0.45	0.033
[K].MVTMVEIDQMVIDG CK.[K]	P97355	Spermine synthase	0.45	0.036
[R].HYCIVANPVSR.[D]	Q9EQK5	Major vault protein	0.44	0.000
[K].ECCHGDLLECADDDRA ELAK.[Y]	P07724	Albumin	0.44	0.015
[K].LCMCADGTLFK.[V]	P82349	Beta-sarcoglycan	0.44	0.014

[R].TGQCECQPGITGQHCE R.[C]	P02468	Laminin subunit gamma-1	0.44	0.006
[R].FRCPETLFQPSFIGMESAGIHETTYNSIMK.[C]	P68033	Actin, alpha cardiac muscle 1	0.43	0.049
[R].IQPFQNLTLHPACSLGHYSLQLFEGLK.[A]	O35855	Branched-chain-amino-acid aminotransferase, mitochondrial	0.43	0.010
[K].AVLTSQETLFGGSDCTGNFCLFK.[S]	Q921I1	Serotransferrin	0.42	0.050
[R].LAQGICLELSEASSCEEFKK.[E]	Q64314	Hematopoietic progenitor cell antigen CD34	0.42	0.029
[K].SACGNCYLGDAFR.[C]	Q8WTY4	Anamorsin	0.41	0.039
[R].ACLLCSLVK.[T]	P63271	Transcription elongation factor SPT4-A	0.41	0.045
[R].LCGSGFQSIIVSGCQEI CSK.[D]	Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial	0.41	0.047
[R].RCESCAPGYEGNPIQPGGK.[C]	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	0.40	0.023
[K].SLCPVSWVSAWDDR.[I]	P56391	Cytochrome c oxidase subunit 6B1	0.40	0.017
[R].VGTKCCTLPEDQRLPCVEDYLSAILNR.[V]	P07724	Albumin	0.40	0.007
[R].NECFLOQHKDDNPSLP PFERPEAEAMCTSFK.[E]	P07724	Albumin	0.40	0.006
[K].LCYVALDFENEMATAASSSLEK.[S]	P68033	Actin, alpha cardiac muscle 1	0.38	0.027
[R].VALMGSGSHGGFLSCHLIGQYPETYSACIAR.[N]	Q8R146	Acylamino-acid-releasing enzyme	0.38	0.045
[K].NCVILPHIGSATYK.[T]	Q91Z53	Glyoxylate reductase/hydroxypyruvate reductase	0.37	0.001
[K].GDCVECMACSDNTVR.[A]	Q924M7	Mannose-6-phosphate isomerase	0.36	0.001
[K].LYDYCDIPLCASASSFECGKPQVEPK.[K]	P20918	Plasminogen	0.36	0.009
[R].SCAPGSDPDSPLCALCVGGNNPAHMCANNAEGYHGSSGALR.[C]	Q9DBD0	Inhibitor of carbonic anhydrase	0.36	0.047
[K].QDADFPTSLSFQCVNGK.[H]	Q61129	Complement factor I	0.35	0.046
[R].RGTDECAIESIAVAaip IPK.[L]	P97821	Dipeptidyl peptidase 1	0.35	0.046
[R].KPVVPGHVLVCPLRPVER.[F]	O89106	Bis(5'-adenosyl)-triphosphatase	0.35	0.035
[R].LPCVEDYLSAILNRVCLLHEK.[T]	P07724	Albumin	0.34	0.001
[R].MLHSCTSEGSAYR.[K]	Q9R098	Hepatocyte growth factor activator	0.34	0.028
[R].DFNLDGAPYGYTPFCDSR.[R]	Q6P5E4	UDP-glucose:glycoprotein glucosyltransferase 1	0.33	0.029

[K].TPVSEHVTKCCSGSLV ERRPCFSALTVEITYVPK.[E]	P07724	Albumin	0.33	0.028
[K].TCVADESAANCDKSL HTLFGDKLCAIPNLR.[E]	P07724	Albumin	0.33	0.014
[K].VDIQTEDLEDGTCK.[V]	Q80X90	Filamin-B	0.32	0.043
[K].RDCGGAAAVLGAFR.[A]	Q6NSR8	Probable aminopeptidase NPEPL1	0.32	0.030
[R].LTASSTCGLHSPQPYCI VSHLQDEK.[K]	Q61292	Laminin subunit beta-2	0.32	0.005
[R].VTVQAACGNSVLQDS R.[L]	Q9EPR5	VPS10 domain-containing receptor SorCS2	0.32	0.024
[K].LCYHVLGTDQSEDILC AEFPDEPK.[W]	Q9QUR6	Prolyl endopeptidase	0.31	0.012
[R].LCRPCQCNDNIDPNA VGNCNR.[L]	P02468	Laminin subunit gamma-1	0.31	0.044
[R].VSVCAETFPDEEEED NDPR.[V]	P12367	cAMP-dependent protein kinase type II-alpha regulatory subunit	0.30	0.006
[K].SDQGEYTCVASSGR.[M]	P35918	Vascular endothelial growth factor receptor 2	0.30	0.018
[K].GEADAMSLDGGFAY VAGHCGLVPVLAENYLST HSSGR.[L]	Q9DBD0	Inhibitor of carbonic anhydrase	0.30	0.031
[K].TNGDQASCENELLK.[F]	P15208	Insulin receptor	0.30	0.045
[K].IDFDLHDLIPSCER.[T]	Q99P30	Peroxisomal coenzyme A diphosphatase NUDT7	0.30	0.023
[K].DWSFYILAHTFTPTTE TDTYACR.[V]	P01887	Beta-2-microglobulin	0.29	0.014
[R].GTVPGSSPCDSSSGTC FCK.[R]	Q61292	Laminin subunit beta-2	0.29	0.049
[R].VFIGKDCIGGCSDLIS MQQTGELMTR.[L]	Q9QUH0	Glutaredoxin-1	0.29	0.016
[R].LQIAACSNQDPLQGT TGLIPLLIGIDVWEHAYYLQ YK.[N]	P09671	Superoxide dismutase [Mn], mitochondrial	0.28	0.019
[R].FQQLVHQMTCLCWE K.[C]	Q9WVA2	Mitochondrial import inner membrane translocase subunit Tim8 A	0.28	0.021
[R].DKLCDLLVANNYFTHF FAPK.[N]	Q61702	Inter-alpha-trypsin inhibitor heavy chain H1	0.27	0.024
[R].AVDVLSELSYAPMTP DHFPTLFCK.[E]	Q7TSQ8	Pyruvate dehydrogenase phosphatase regulatory subunit, mitochondrial	0.27	0.008
[R].LCQTCYPLFQQVAIK.[M]	Q8BGT0	Osteopetrosis-associated transmembrane protein 1	0.27	0.037
[R].SICTTVLELLDK.[Y]	P68254	14-3-3 protein theta	0.26	0.027
[K].FQGPTCETCQTCLGV CAEHK.[E]	P09055	Integrin beta-1	0.25	0.032

[R].FRCPSRHEVVLDL.[H]	Q9ERP3	Tripartite motif-containing protein 54	0.25	0.025
[K].HVTEDCVFIYCQVGD KPYWK.[D]	Q9CQM5	Thioredoxin domain-containing protein 17	0.25	0.016
[R].VGAGGLDGYSVEYCQ EGCSEWTPALQGLTER.[R]	O70468	Myosin-binding protein C, cardiac-type	0.25	0.033
[R].QGTHITVVAHSRPVG HCLEAAAVLSK.[E]	Q9D051	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	0.24	0.010
[K].YHIQVCTTTPCMLRDS DSILETLQR.[K]	Q9D6J6	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	0.24	0.009
[R].SQTEEDCTEELFDLH ARDHCVAHKLFK.[N]	P99028	Cytochrome b-c1 complex subunit 6, mitochondrial	0.24	0.027
[K].LCMAALSHQPQEFT YVEPTNDEICEAFR.[R]	P21614	Vitamin D-binding protein	0.23	0.042
[R].QAVDQISSGFFSPKDP DCFKDVVNMLMYHDR.[F]	Q8CI94	Glycogen phosphorylase, brain form	0.23	0.005
[K].ECCHGDLLECADDR.[A]	P07724	Albumin	0.23	0.013
[K].QYPCTLVGTWNTWY GEQDQAVHLWR.[Y]	O55126	Protein NipSnap homolog 2	0.23	0.022
[K].ISCSFHVTK.[Q]	Q8VE38	Oxidoreductase NAD-binding domain-containing protein 1	0.23	0.019
[R].FSDTCFLDTDGQATC DACAPGYTGR.[R]	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	0.22	0.004
[K].TLTPGGHAEHDGKPF CHKPCYATLFGPK.[G]	Q9DCT8	Cysteine-rich protein 2	0.22	0.004
[K].TVQGAFFGVPVYKDH ENCISGEDITHNGIVYTPK.[H]	Q61838	Pregnancy zone protein	0.22	0.035
[R].FCLEGMEESGSEGLDE LIFAQK.[D]	Q9D1A2	Cytosolic non-specific dipeptidase	0.21	0.044
[K].DLFQCVSFIIPR.[L]	P28665	Murinoglobulin-1	0.20	0.001
[K].LVSEAIAAGIFNDLGS GSNIDLCVISK.[S]	P70195	Proteasome subunit beta type-7	0.20	0.047
[R].EEYQEELEFCEK.[L]	Q99P31	Hsp70-binding protein 1	0.19	0.000
[K].SAGYPGTLIPYRCDLS NEEDILSMFSAVR.[S]	Q3U0B3	Dehydrogenase/reductase SDR family member 11	0.19	0.000
[R].AQCIYMGGSSTCSCSCL PGFSGDGR.[A]	P10493	Nidogen-1	0.19	0.000
[K].ILLCTGAIMEEQAAQL LGVK.[M]	Q9CY45	EEF1A lysine methyltransferase 1	0.19	0.000
[R].VELCAQGSPDLAHLDDGPYEAGGEKEQDPR.[L]	Q61738	Integrin alpha-7	0.19	0.000
[R].ACLISMGYDLGEAEFA R.[I]	Q9JI91	Alpha-actinin-2	0.19	0.001
[K].LVQYLRECEDVMDWI NDK.[E]	P16546	Spectrin alpha chain, non-erythrocytic 1	0.18	0.000

[R].KICALDDNVCMAG LTADAR.[I]	Q9Z2U0	Proteasome subunit alpha type-7	0.18	0.001
[R].QWVLTAAHCFDIPY PDVWR.[I]	P26262	Plasma kallikrein	0.18	0.000
[K].CGPMVLDALIK.[I]	Q9CQA3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	0.18	0.000
[K].KQEGQYYCTASNR.[A]	Q08481	Platelet endothelial cell adhesion molecule	0.18	0.001
[R].VVQCSDLGLDKVPW DFPPDPTLLDLQNNK.[I]	P28654	Decorin	0.18	0.000
[K].QNCFDDFQCAAELYIK .[E]	Q9QUR6	Prolyl endopeptidase	0.17	0.003
[R].QCLSELDACTFHK.[Y]	Q9DBD0	Inhibitor of carbonic anhydrase	0.17	0.023
[R].IEDDMDGGDWSFCD GR.[L]	Q61739	Integrin alpha-6	0.17	0.001
[R].GVQDIVVGEGTHFLIP WVQKPIIFDCR.[S]	P67778	Prohibitin	0.17	0.049
[R].HYCVILDPMPDGGK.[N]	Q9EQK5	Major vault protein	0.17	0.001
[K].HQHDRVCGDVFQL QENVK.[D]	Q61738	Integrin alpha-7	0.17	0.001
[R].VAHMEFCYQELCQLA AER.[R]	Q62261	Spectrin beta chain, non- erythrocytic 1	0.17	0.001
[R].VTGPPIFNVPANEVYL NFESSTHCLADR.[Y]	Q07113	Cation-independent mannose- 6-phosphate receptor	0.17	0.003
[K].LGCGEggGCGACTVMI SK.[Y]	Q00519	Xanthine dehydrogenase/oxidase	0.17	0.002
[R].QAQLQDAGIYECESK. [T]	P29533	Vascular cell adhesion protein 1	0.17	0.001
[R].TEHSPFHEHVLGALCS LVTDFPQGVR.[E]	Q99P31	Hsp70-binding protein 1	0.17	0.005
[R].DKETPSGFTLDDVIQT GVDNPGHPFIMTVGCVA GDEESYTVFK.[D]	P07310	Creatine kinase M-type	0.17	0.001
[K].DGQIFMSACQDQTIR. [L]	Q91VU6	DDB1- and CUL4-associated factor 11	0.17	0.001
[K].TVCTIAAHEGTLAAITF NSSGSK.[L]	Q8R3E3	WD repeat domain phosphoinositide-interacting protein 1	0.16	0.001
[K].AMLFMCSGSIHSLAD EQDIR.[K]	P03921	NADH-ubiquinone oxidoreductase chain 5	0.16	0.039
[K].NCTCGLAEEELER.[E]	Q8WTY4	Anamorsin	0.16	0.001
[K].LQDPFSVYRCHTIMN CTQTCPK.[G]	Q9CQA3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	0.16	0.004
[R].YNFYLYGCTNQGYQL LR.[S]	P42703	Leukemia inhibitory factor receptor	0.16	0.001
[R].GASIAHCPNSNLSLSS GLLNVLEVVK.[H]	Q9R111	Guanine deaminase	0.16	0.003

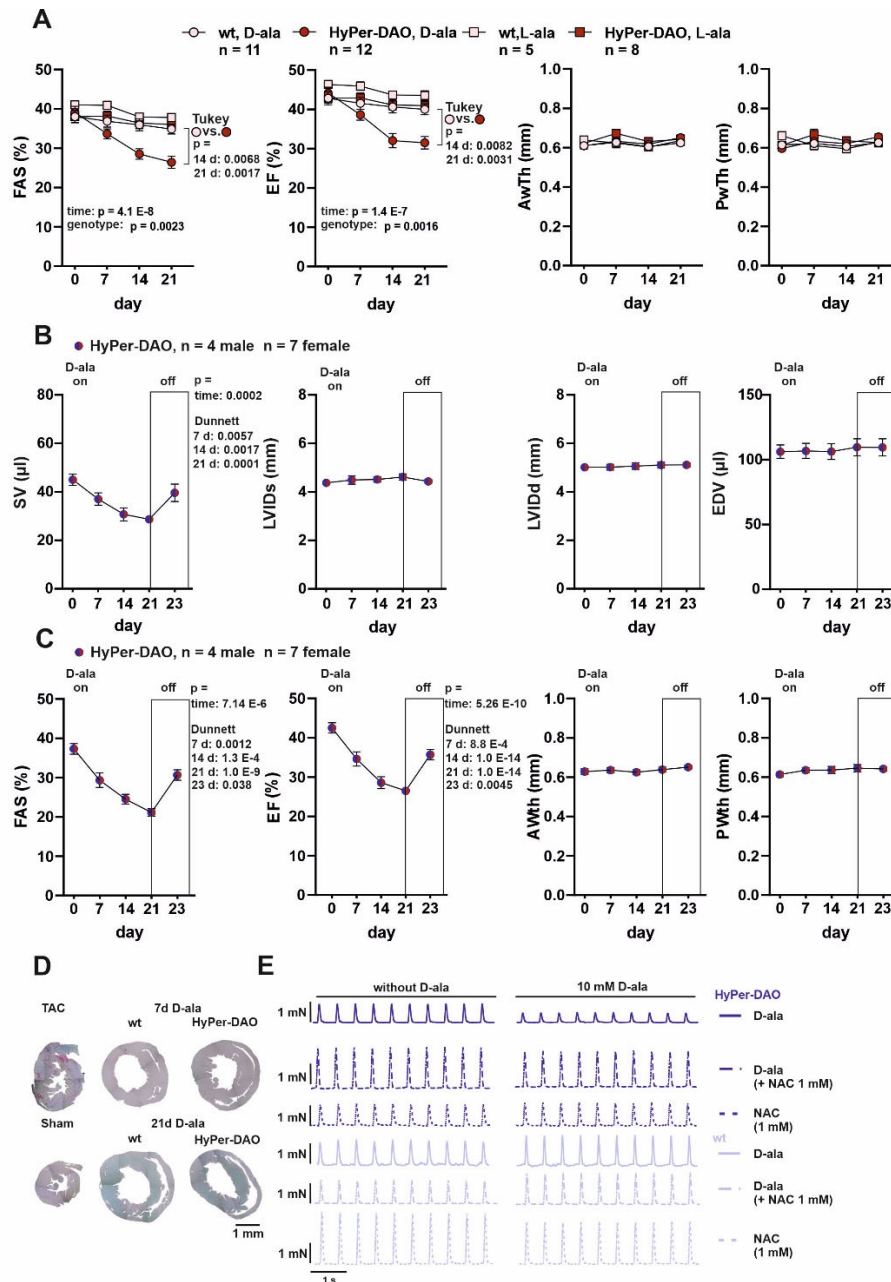
[R].LFGDKTCDLLASFK.[D]	Q60854	Serpin B6	0.16	0.007
[K].KPGVFIPSCDEDEGYR.[K]	Q9ER58	Testican-2	0.16	0.004
[K].FAPVCSICENPIIPR.[D]	Q71FD7	Filamin-binding LIM protein 1	0.16	0.004
[K].NILSHDPDVGTDTMEKEETHHACQEMELK.[V]	Q68FE8	Zinc finger protein 280D	0.16	0.001
[K].ASAFNSWFENAEEDLTDPVRCNSLEEIK.[A]	P16546	Spectrin alpha chain, non-erythrocytic 1	0.16	0.002
[K].AAFDIFVLGAEDGCISTKELGK.[V]	P19123	Troponin C, slow skeletal and cardiac muscles	0.16	0.003
[K].LCYGLNMDFVDPAQITMK.[V]	P07742	Ribonucleoside-diphosphate reductase large subunit	0.16	0.004
[K].ALGFPESLVIQAYFAC EK.[N]	P54726	UV excision repair protein RAD23 homolog A	0.16	0.003
[K].RLDLTYSFLGSQGVGQCYDSSPCER.[Q]	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	0.16	0.004
[K].QCIVAHVPNPPYYVPLVELVPHPETAPATMDR.[T]	Q99KP3	Lambda-crystallin homolog	0.15	0.002
[R].VFIWTCDDASGNMWSPK.[L]	Q9D1M0	Protein SEC13 homolog	0.15	0.002
[K].GASSSSCSETYCGLYPESEPEVK.[A]	Q9JHH6	Carboxypeptidase B2	0.15	0.002
[K].LGRPSLSSEVGVIICDISNPASLDEMAK.[Q]	Q8R127	Saccharopine dehydrogenase-like oxidoreductase	0.15	0.019
[R].VAGEPAADGTVACPCQAPTRPQALSTNLQLSR.[L]	Q1XH17	Tripartite motif-containing protein 72	0.15	0.002
[R].SSNLLGECSPAQR.[E]	Q9QY81	Nuclear pore membrane glycoprotein 210	0.15	0.018
[R].NLHMEQAPVDLDQPTSSFHVGTCTFANAESGTYFDGTGFAK.[A]	Q60675	Laminin subunit alpha-2	0.15	0.002
[R].QLEPGLQGILITCNMNER.[K]	Q99J36	THUMP domain-containing protein 1	0.15	0.015
[K].TCEWIHDSSLSASCK.[E]	Q61207	Prosaposin	0.15	0.006
[K].FFGEFTGFVDMCVQH IPSPK.[V]	O08810	116 kDa U5 small nuclear ribonucleoprotein component	0.15	0.003
[R].WCQSMQDPSASLLER.[Q]	Q9DC50	Peroxisomal carnitine O-octanoyltransferase	0.15	0.003
[K].GVCTEAGMYALR.[E]	P62196	26S proteasome regulatory subunit 8	0.14	0.022
[K].GSFPLDHFGECK.[S]	Q8K0C8	Cytochrome c oxidase assembly protein COX19	0.14	0.019
[R].AFWRELVECFQKISK.[D]	O35459	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	0.14	0.005
[R].LDSSHSCLEVTAATLR.[R]	Q8VCT3	Aminopeptidase B	0.14	0.003

[K].TCNCETEDYGEK.[F]	Q8VEK3	Heterogeneous nuclear ribonucleoprotein U	0.14	0.011
[K].TYAACPQNWIGVENK.[C]	Q91V08	C-type lectin domain family 2 member D	0.14	0.012
[R].EAGTWMDDTCDISK.[Q]	Q61830	Macrophage mannose receptor 1	0.14	0.006
[K].DMFNVNVLALSICTR.[E]	Q3U0B3	Dehydrogenase/reductase SDR family member 11	0.14	0.016
[R].LPLTLGASYEGVAVNDCIESGR.[Q]	P51175	Protoporphyrinogen oxidase	0.14	0.010
[K].VCQCDSNGDCTDVD R.[I]	P15116	Cadherin-2	0.14	0.034
[R].TKCDEWSIIEGKIECE SAETTEDCIEK.[I]	Q92111	Serotransferrin	0.14	0.043
[R].ECSLQVAEDELVSTLK.[H]	P21126	Ubiquitin-like protein 4A	0.13	0.011
[K].FCASGPYGGEDCPQ WMVPITISTEDPNQAK.[L]	Q11011	Puromycin-sensitive aminopeptidase	0.13	0.020
[R].IMRPTDVPDQGLLCD LLWSDPDKDVQGWGEN DR.[G]	P62137	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	0.13	0.001
[R].DSSTCPGDYVLSVSEN SR.[V]	P47941	Crk-like protein	0.13	0.006
[K].DCSIYGEDTVTDEEGK.[F]	Q6GQT9	Nodal modulator 1	0.13	0.013
[R].GADCCVLVFDVTAPN TFK.[T]	P51150	Ras-related protein Rab-7a	0.13	0.007
[R].VGQILHPEECMYAVG QGALAVEVR.[A]	P22907	Porphobilinogen deaminase	0.13	0.013
[K].GEDFYCVTCHETK.[F]	P97447	Four and a half LIM domains protein 1	0.13	0.008
[R].FIAHGDAVMVAGG TDSCISPLSLAGFSR.[A]	Q9D404	3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial	0.13	0.041
[K].LVDGCYSFWQAGLLP LLHR.[A]	Q8K2I1	Protein farnesyltransferase subunit beta	0.13	0.009
[K].LQSLDLSAVAQMTCTG TPPGADCSESECGPNCR.[T]	P02469	Laminin subunit beta-1	0.12	0.020
[K].DVDYVCISDNYWLKG.[N]	Q9D1A2	Cytosolic non-specific dipeptidase	0.12	0.022
[R].VCMVYDLYPTLTPLAV AYAR.[A]	Q9CWJ9	Bifunctional purine biosynthesis proteinATIC	0.12	0.008
[R].LSVSPLPTLTEDDELLC LFGDSPPHPAR.[V]	B2RXS4	Plexin-B2	0.12	0.011
[R].EGQDATAACEVNPLAC LSQTATCAPEIPAFSHGGF AYR.[D]	O70423	Membrane primary amine oxidase	0.12	0.032
[K].ECCHGDLLECADDRA ELAKYMCENQATISSK.[L]	P07724	Albumin	0.12	0.023

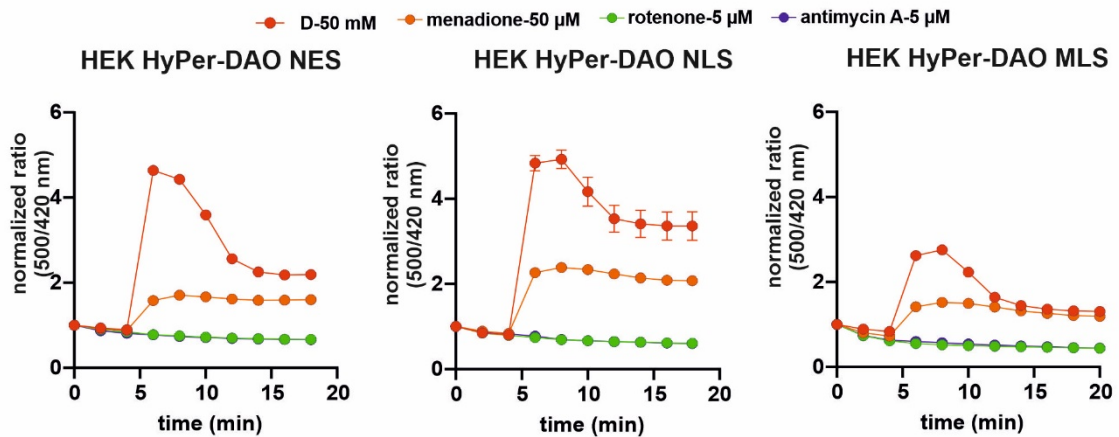
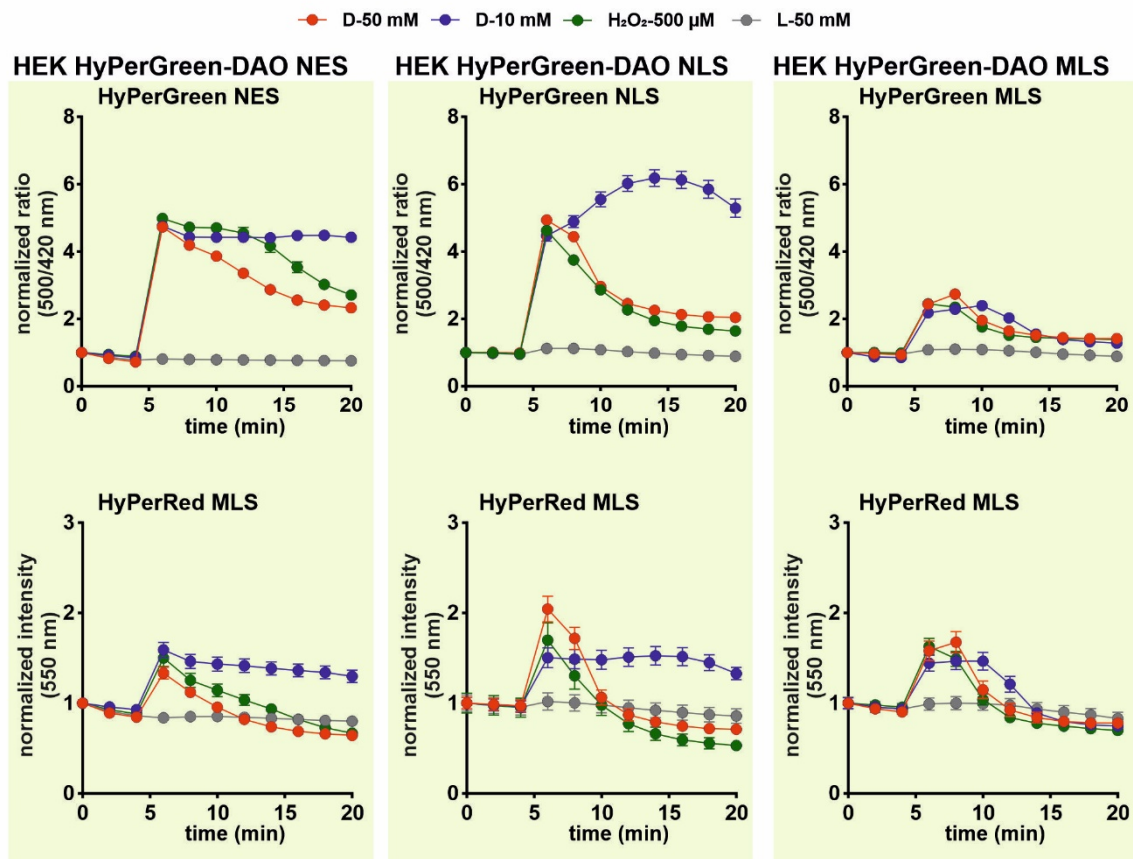
[R].FSFCFSPEPEAEQAAGP GPCER.[L]	Q64337	Sequestosome-1	0.12	0.024
[K].FPVFNMSYNPAENAV LLCTR.[A]	Q8CIE6	Coatomer subunit alpha	0.12	0.028
[K].QFLECAQNQSDVKLC EGFNEVLR.[Q]	Q9D1L0	Coiled-coil-helix-coiled-coil- helix domain-containing protein 2	0.11	0.005
[R].IIPLEQCQSYFDMK.[T]	Q9Z319	Atrial natriuretic peptide- converting enzyme	0.11	0.029
[K].DFAPGKPLKCVIKHPN GTQETILLNHTFNETQIE WFR.[A]	Q99KI0	Aconitate hydratase, mitochondrial	0.11	0.017
[K].ILVEGIGEVQEYVDVC DYAAGLSR.[M]	Q9DBF1	Alpha-aminoadipic semialdehyde dehydrogenase	0.11	0.033
[R].DDILCPDCGKDI.[-]	O70433	Four and a half LIM domains protein 2	0.10	0.007
[K].TLTSGGHAHEGKPY CNHPCYSAMFGPK.[G]	P63254	Cysteine-rich protein 1	0.10	0.030
[K].LCLSEIHK.[M]	P15208	Insulin receptor	0.10	0.031
[R].WLLLCNPGLAEIIVER. [I]	Q8CI94	Glycogen phosphorylase, brain form	0.09	0.040
[K].FVTAVATGGPDNQV HFEGYQVSNQCMALVR.[D]	P60670	Nuclear protein localization protein 4 homolog	0.09	0.048
[K].GCEECFCSGVSNR.[C]	Q60675	Laminin subunit alpha-2	0.09	0.000
[-].MEESEYESVLCVKPEVH VYR.[I]	Q9D1J1	Adaptin ear-binding coat- associated protein 2	0.09	0.033
[R].NSDVMDCVILDDGGF LLMANHDDYTNIQGR.[F]	O08532	Voltage-dependent calcium channel subunit alpha-2/delta- 1	0.09	0.040
[K].SHCAEPFTEYWTCLDY SNMQLFR.[H]	Q9DCJ5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	0.08	0.009
[R].KNGLMDHEDFRACLI SMGYDLGAEFAR.[I]	Q9JI91	Alpha-actinin-2	0.08	0.040
[R].SQSVRPGADVTFICTA K.[S]	Q05793	Basement membrane-specific heparan sulfate proteoglycan core protein	0.07	0.039
[K].SEPDKADPFSFEGPEI VDCDGCTIDWK.[K]	Q78ZA7	Nucleosome assembly protein 1-like 4	0.07	0.040
[R].VVYLQYPSLAPHHQC SQLFLYDWYTK.[V]	Q8R146	Acylamino-acid-releasing enzyme	0.07	0.008
[K].TCGFDFSGALEDISKIP EQSVLLHACAHNPTGVD PRPEQWK.[E]	P05202	Aspartate aminotransferase, mitochondrial	0.06	0.009
[K].LFQEEFPGIPYPPDAA VECHRGECQSEGVLFQGG NR.[K]	Q91X72	Hemopexin	0.06	0.003

Supplementary Table 2: List of cysteine containing peptides that were identified in the redox proteomic screen to show a decreased total reversible oxidation demonstrated by a fold change of $\leq$

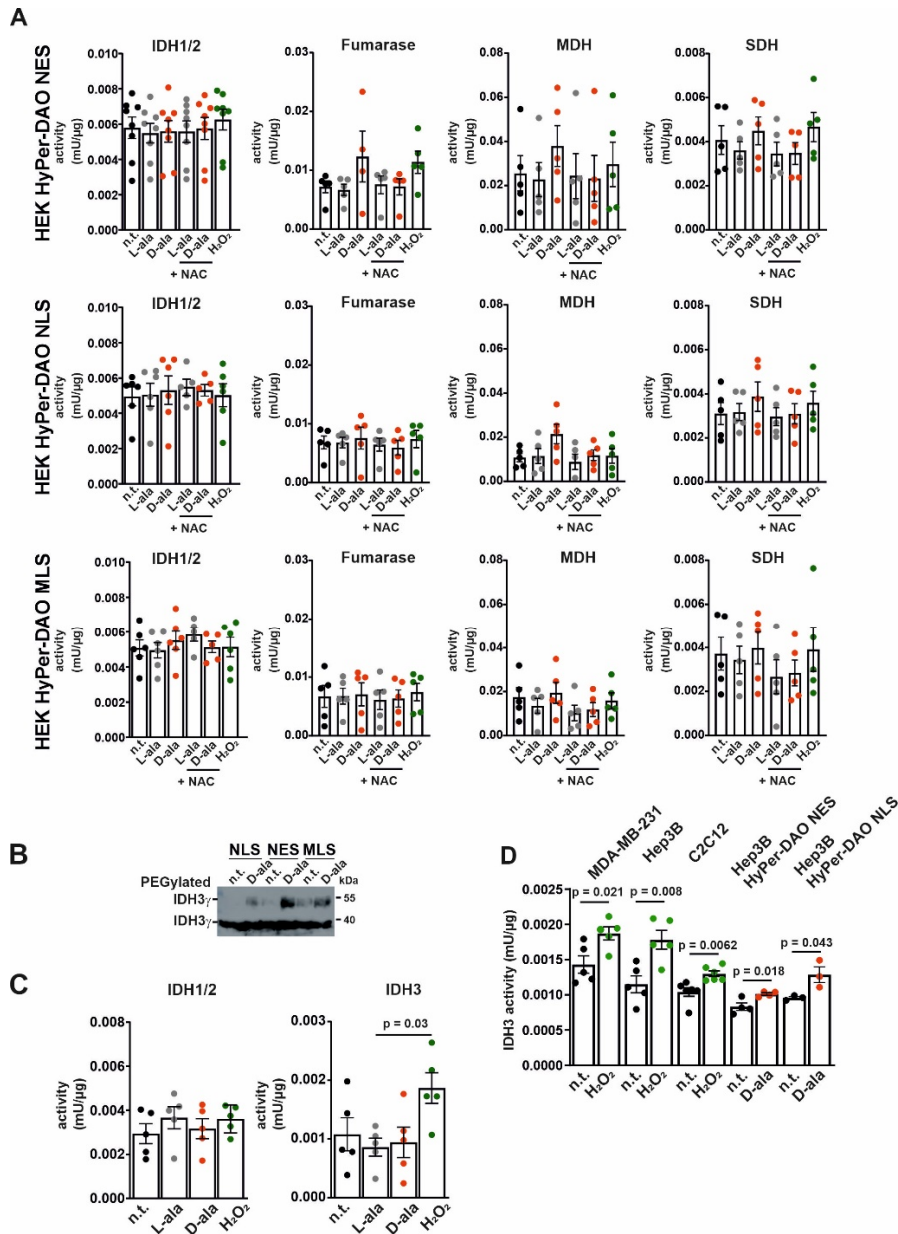
0.5 and a p-value of ≤ 0.05 in the left ventricles of HyPer-DAO mice compared to wild type mice after 7 days of D-ala treatment. P values were calculated by two-tailed unpaired t-test.



Supplementary Figure 1: Echocardiographic analysis and cardiac fibrosis in HyPer-DAO mice, related to Figure 2 (A, B and C) Echocardiographic analysis of fractional area shortening (FAS), ejection fraction (EF), anterior wall thickness (AwTh) and posterior wall thickness (PwTh), stroke volume (SV), left ventricular inner diameter in systole (LVIDs), left ventricular inner diameter in diastole (LVIDd), and enddiastolic volume (EDV) in female (in A) and a mixed group of female and male (in B and C) HyPer-DAO or wild type (wt) mice after treatment with D-ala or L-ala in the drinking water as indicated. **(D)** Representative figures of Sirius red/Fast green stained cardiac slices of HyPer-DAO mice after 7 or 21 days of D-ala treatment and mice after TAC or sham surgery. For each mouse the staining was performed once. **(E)** Original tracings of force development measured in heart slices from HyPer-DAO and wt mice after treatment 10 mM D-ala +/- 1 mM NAC. mean $\pm$ SEM, one-way ANOVA (B and C) or two-way ANOVA (A). Source data are provided as a Source Data file.

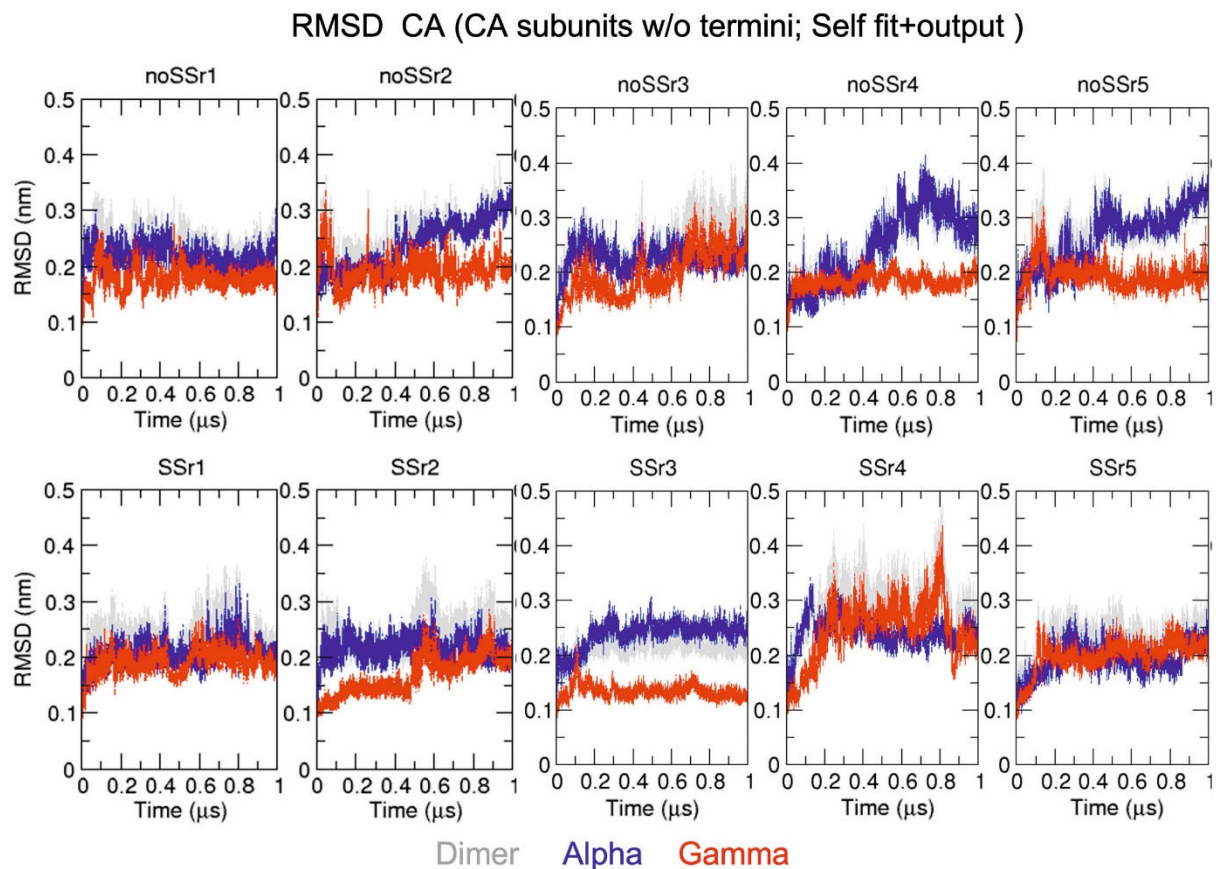
A**B****Supplementary Figure 2: Intracellular distribution of H_2O_2 in HEK cells, related to Figure 4A-C**

(A) HyPer-Green fluorescence responses were recorded after treating HyPer-DAO-NLS cells with D-alal ($n = 42$ independent cells), menadione ($n = 44$ independent cells), rotenone ($n = 42$ independent cells) or antimycin A ($n = 40$ independent cells), which were added at time point 4 min. **(B)** HEK cells overexpressing HyPer-DAO fusion protein in the cytoplasm (HyPer-DAO-NES), the nucleus (HyPer-DAO-NLS) and the mitochondrial matrix (HyPer-DAO-MLS) were transiently transfected to overexpress the H_2O_2 sensor HyPerRed localized to the mitochondrial matrix (HPerRed-MLS). The HyPer Green and HyPer Red fluorescence responses were recorded after stimulation with D-alal, L-alal or H_2O_2 , which were added at time point 4 min. Ratios are normalized to the HyPer ratio prior treatment, $n = 25$ independent cells per condition were analyzed. Data are presented as mean values $\pm$ SEM. Source data are provided as a Source Data file.



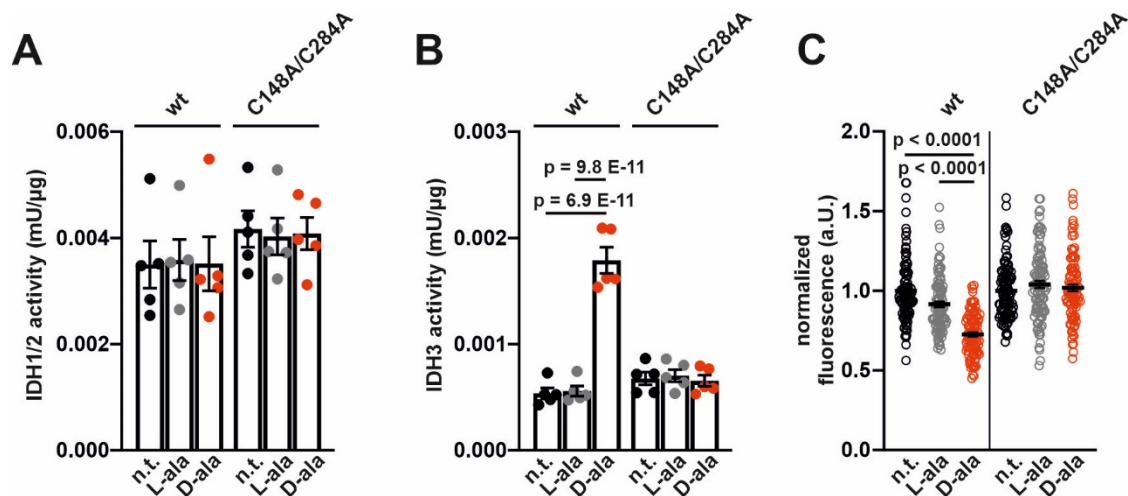
Supplementary Figure 3: TCA cycle enzyme activities in HyPer-DAO overexpressing HEK cells, related to Figure 4 D-G

(A) IDH1/2, fumarase, malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) activities in cell extracts obtained from HEK HyPer-DAO-NES, HEK HyPer-DAO-NLS and HEK HyPer-DAO-MLS cells either non-treated (n.t.) or after treatment with 50 mM D-ala, 50 mM L-ala $\pm$ 8 mM NAC or 500 μ M H₂O₂ for 20 min. IDH1/2 activity: $n = 8$ independent experiments for HyPer-DAO NES, $n = 6$ for HyPer-DAO NLS and MLS except for $n = 5$ for D-ala and L-ala + NAC, $n = 5$ independent experiments for all other conditions. **(B)** Representative immunoblot for IDH3 γ oxidation analyzed by PEG switch assay in HEK HyPer-DAO-NLS, HEK HyPer-DAO-NES and HyPer-DAO-MLS cells n.t. and after treatment with 50 mM D-ala for 20 min. The experiment has been performed three times. **(C)** IDH1/2 and IDH3 activity in cell extracts obtained from HEK wt cells either non-treated (n.t.) or after treatment with 50 mM D-ala, 50 mM L-ala or 500 μ M H₂O₂ μ M for 20 min, $n = 5$ independent experiments. **(D)** IDH3 activity in cell extracts obtained from MDA-MB-231, Hep3B, C2C12, Hep3B HyPer-DAO-NES and Hep3B HyPer-DAO-NLS cells either non-treated (n.t.) or 20 min treatment with 500 μ M H₂O₂ for MDA-MB-231, 250 μ M H₂O₂ for Hep3B, 5 μ M H₂O₂ for C2C12 and 5 mM D-ala for Hep3B HyPer-DAO-NES and -NLS, respectively. 5, 5, 6, 4 and 3 independent experiments for MDA-MB-231, Hep3B, C2C12, Hep3B HyPer-DAO-NES and HyPer-DAO-NLS cells, respectively. Data are presented as mean values $\pm$ SEM, one-way ANOVA (A and C) or two tailed unpaired t-test (D). Source data are provided as a Source Data file.



Supplementary Figure 4: Root mean square deviation (RMSD) computed over the C α -atoms for all MD simulations, related to Figure 6

Curves show results separately for the whole dimer (without disordered termini, grey), the α subunit (blue), and the γ subunit (red).



Supplementary Figure 5: Point mutation of IDH3γ Cys148 and Cys284 prevents H₂O₂ induced IDH3 activity and impaired ATP production in the mitochondria, related to Figure 7.

IDH1/2 (**A**) and IDH3 (**B**) activity in cell extracts obtained from HEK HyPer-DAO-NLS wild type (wt) or C148A/C284A cells non-treated (n.t.) and after treatment with 50 mM D-ala or L-ala for 20 min, $n = 5$ independent experiments per condition. (**C**) Mitochondrial ATP levels determined with the fluorescence sensor ATP-red in HEK HyPer-DAO-NLS wt and C148A/284A cells n.t. and after treatment with 50 mM D-ala or L-ala for 20 min. $n = 100$ cells per condition were analyzed. Data are presented as mean values $\pm$ SEM, one-way ANOVA (B and C). Source data are provided as a Source Data file.

Non-cropped blots

Figure 1B

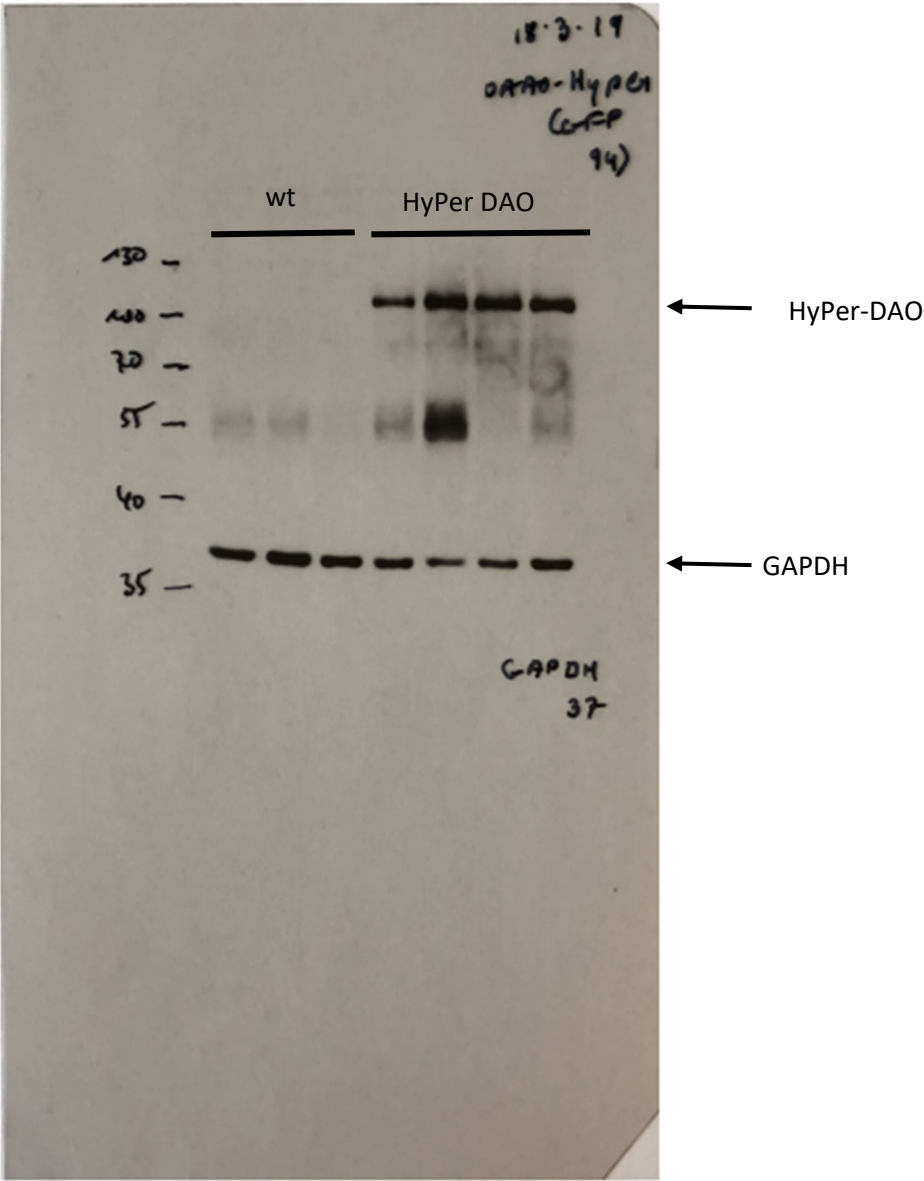


Figure 1C

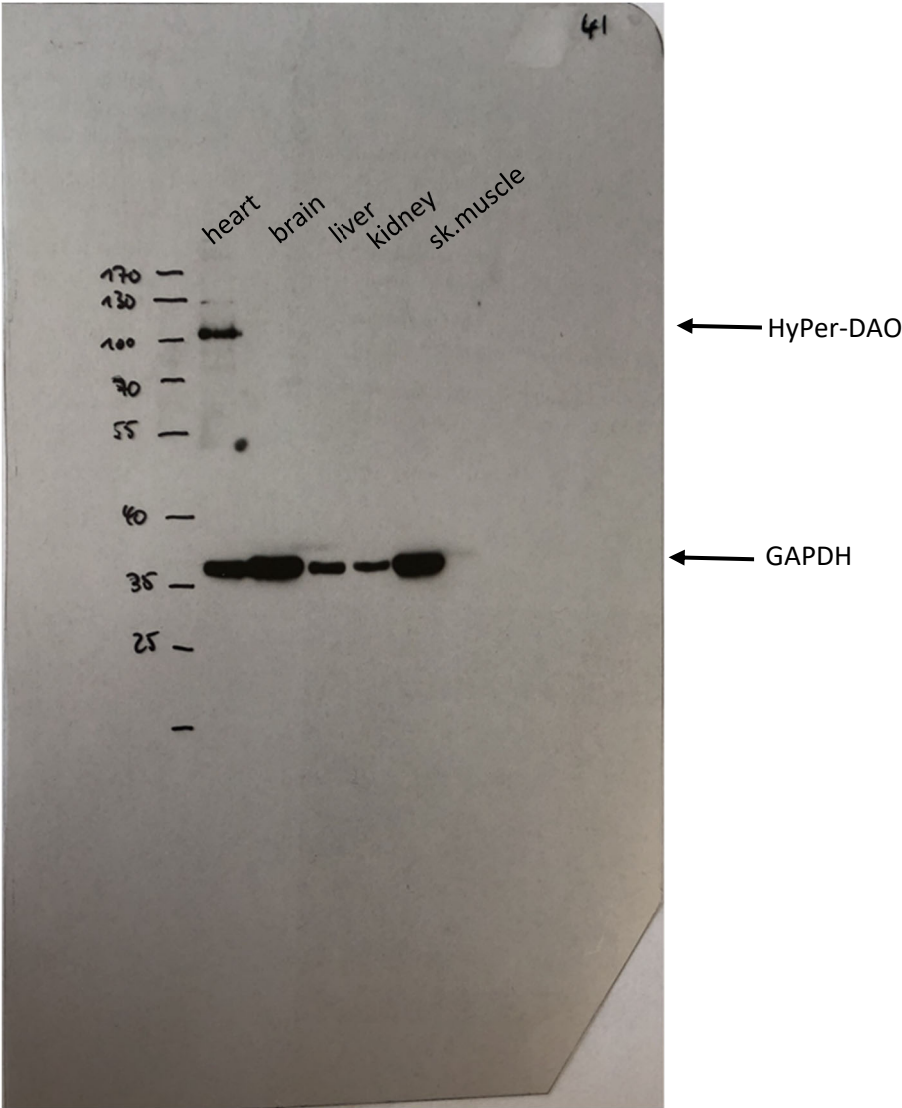


Figure 3G

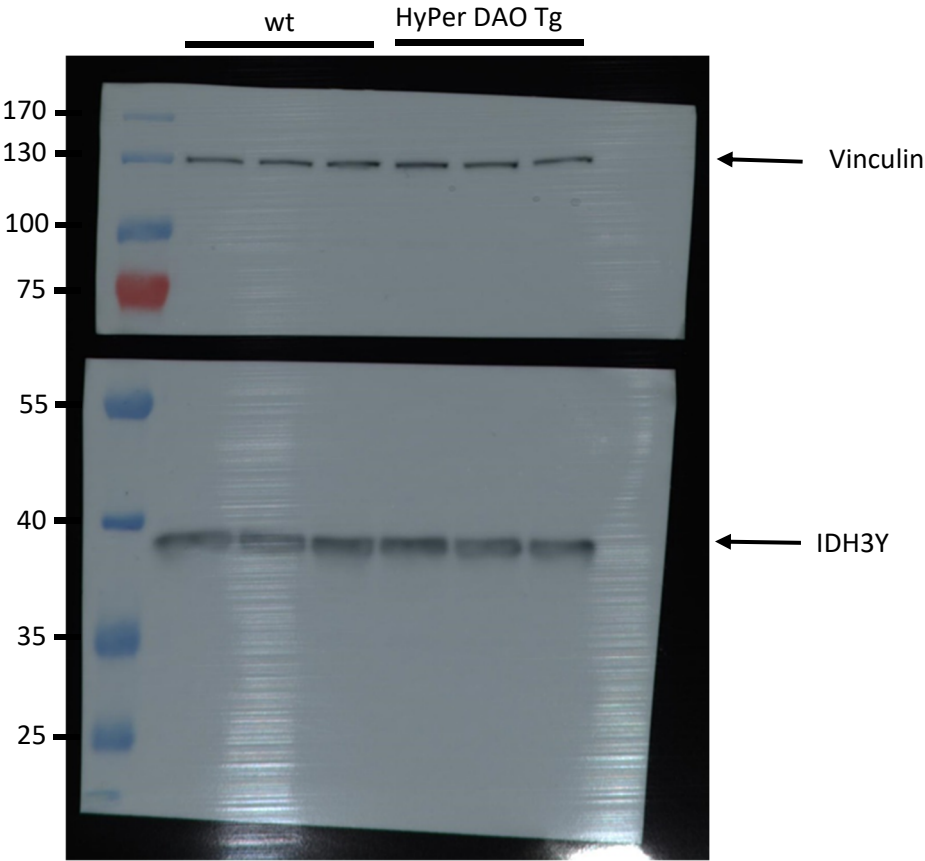


Figure 3K

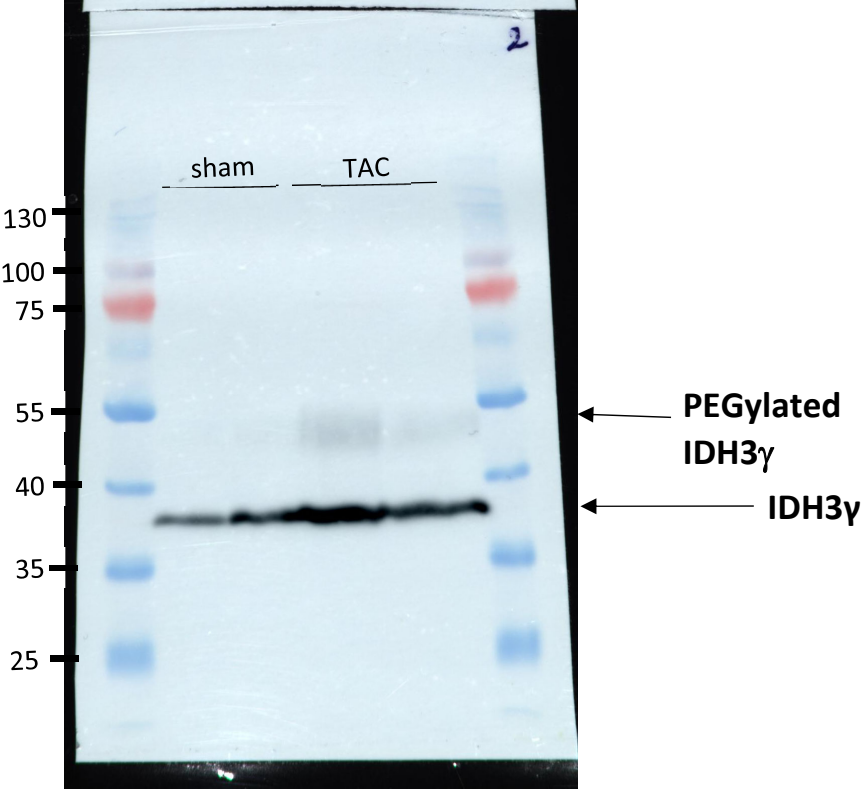


Figure 4E

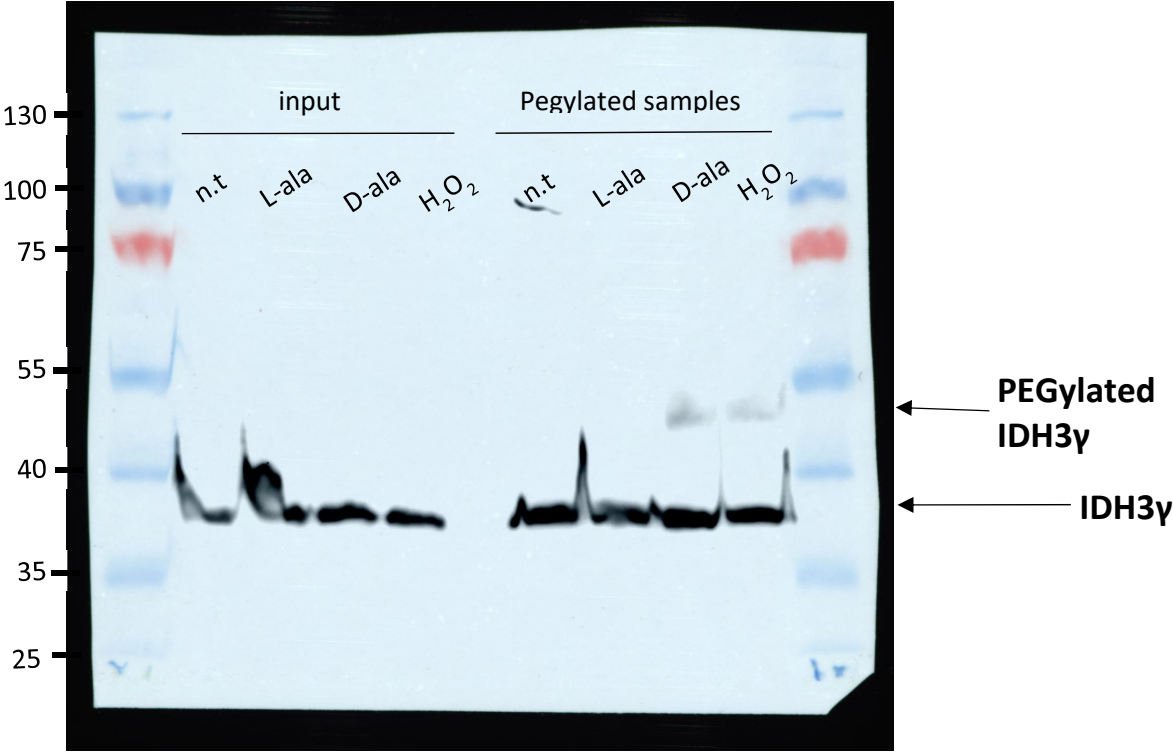


Figure 4G

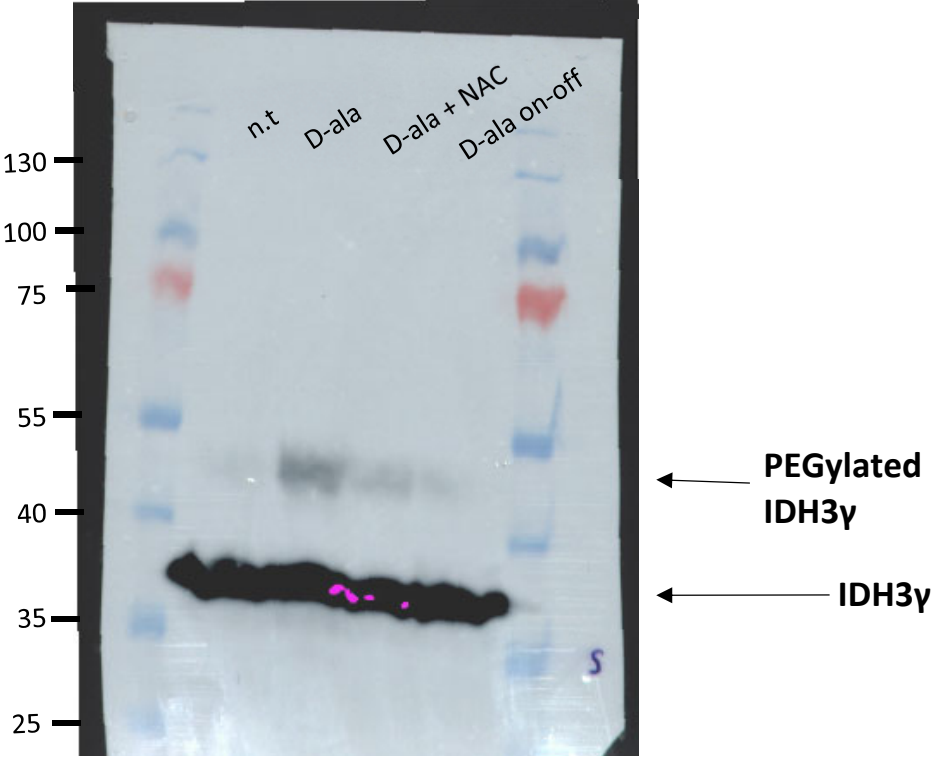
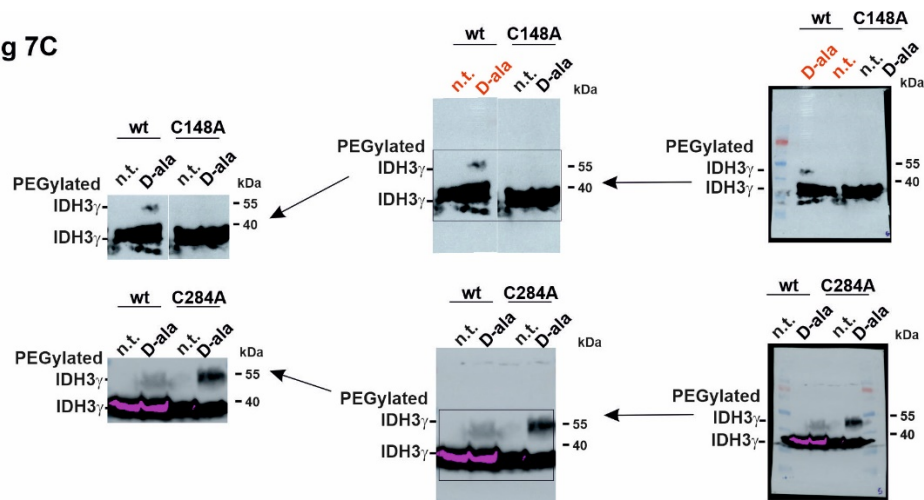


Figure 7C

Fig 7C



Supplementary Figure 3B

