

Supplementary files

Table S1. Differentially expressed proteins (DEPs) between KO and WT osteoblasts

Majority protein IDs	Protein name	Gene name	Log ₁₀ (Ratio KO/WT)	-Log ₁₀ Student's T-test <i>p</i> -value	# Peptides	Sequence coverage [%]	Mol. weight [kDa]	Score
Q07113	Cation-independent mannose-6-phosphate receptor	Igf2r	2.376	1.83441	8	4	273.81	24.45
P29268	Connective tissue growth factor	Ctgf	2.06693	1.55464	11	38.8	37.824	13.588
Q99J56	Derlin-1	Derl1	1.42834	1.30125	2	7.6	28.834	3.4049
P49945; P29391	Ferritin light chain 2; Ferritin light chain 1	Ftl2; Ftl1	1.41749	1.88586	1	8.7	20.842	3.3979
O88587	Catechol O-methyltransferase	Comt	0.691961	1.42105	3	18.1	29.486	8.7854
Q9CQV6	Microtubule-associated proteins 1A/1B light chain 3B	Map1lc3b	0.640702	1.43313	1	11.2	14.617	2.7413
P57716	Nicastrin	Ncstn	0.586488	1.55375	8	14.8	78.491	7.9425
P18242	Cathepsin D	Ctsd	0.473705	1.40413	11	33.4	44.953	235.69
O35405	Phospholipase D3	Pld3	0.434557	1.43242	3	6.4	54.388	4.9221
O88653	Regulator complex protein LAMTOR3	Lamtor3	0.430358	1.57383	1	8.9	13.553	2.0685
P03958	Adenosine deaminase	Ada	0.409483	1.89906	2	8	39.991	3.306
Q9WV54	Acid ceramidase;Acid ceramidase subunit alpha;Acid ceramidase subunit beta	Asah1	0.36557	1.46108	11	33.5	44.669	27.28
Q9D281	Protein Noxp20	Fam114a1	0.292821	1.36667	7	16	61.012	19.19
Q8K4Z3	NAD(P)H-hydrate epimerase	Apoa1bp	0.234261	1.6817	4	24.5	30.972	28.783
Q9R0N0	Galactokinase	Galk1	0.146313	1.31582	8	23.5	42.295	23.569
P20108	Thioredoxin-dependent peroxide reductase, mitochondrial	Prdx3	0.132413	1.57848	6	33.5	28.127	36.369
P68254	14-3-3 protein theta	Ywhaq	0.116844	1.44759	12	50.6	27.778	267.1
Q60710	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	Samhd1	0.0739272	1.66242	6	13.6	72.65	12.747
Q61316	Heat shock 70 kDa protein 4	Hspa4	-0.0681311	1.3084	21	34.4	94.132	163.02
P61750	ADP-ribosylation factor 4	Arf4	-0.116745	1.49901	8	54.4	20.396	63.031

P26041	Moesin	Msn	-0.119288	1.51716	26	43.7	67.766	280.42
Q9CZU6	Citrate synthase, mitochondrial	Cs	-0.138243	1.67977	11	27.2	51.736	58.705
O55143	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	Atp2a2	-0.154687	2.32774	18	22	114.86	58.123
P17918	Proliferating cell nuclear antigen	Pcna	-0.156385	1.36056	6	33	28.785	18.645
P00493	Hypoxanthine-guanine phosphoribosyltransferase	Hprt1	-0.171571	2.11466	7	37.6	24.57	26.975
Q9Z2Q6	Septin-5	Sept5	-0.181515	1.67854	4	16.5	42.747	7.3309
Q99MN1	Lysine--tRNA ligase	Kars	-0.188368	1.64474	9	17	67.839	12.438
P62717	60S ribosomal protein L18a	Rpl18a	-0.193097	1.39496	6	34.7	20.732	20.548
Q01768	Nucleoside diphosphate kinase B	Nme2	-0.212598	1.58199	10	78.9	17.363	180.07
Q9CR16	Peptidyl-prolyl cis-trans isomerase D	Ppid	-0.255185	1.69854	3	15.1	40.742	3.0592
Q9WTI7	Unconventional myosin-Ic	Myo1c	-0.260151	1.83578	25	30.3	121.94	70.687
P40142	Transketolase	Tkt	-0.276361	2.5033	19	40.9	67.63	238.94
P14069	Protein S100-A6	S100a6	-0.289586	1.55266	2	16.9	10.051	38.083
Q8R422	CD109 antigen	Cd109	-0.361866	1.5944	8	7.7	161.66	15.029
Q80UM3	N-alpha-acetyltransferase 15, NatA auxiliary subunit	Naa15	-0.36825	1.35176	4	5.5	100.96	4.3588
P35293	Ras-related protein Rab-18	Rab18	-0.708262	1.45724	7	41.7	23.035	11.649
P62878	E3 ubiquitin-protein ligase RBX1; E3 ubiquitin-protein ligase RBX1, N-terminally processed	Rbx1	-1.56738	1.74006	2	24.1	12.274	13.829
Q9CQN3	Mitochondrial import receptor subunit TOM6 homolog	Tomm6	-1.94854	2.94063	1	18.9	7.8648	2.3484

In the table are listed the following DEPs' parameters: the unique protein sequence identifiers (IDs) of those DEPs that have at least half of the peptides that the leading protein has (Majority protein IDs), the Protein name and the Gene Name the protein is associated with, provided by UniProtKB/Swiss-Prot Knowledgebase; the ratio of expression ($\log_{10}$ (Ratio KO/WT)); the negative logarithm of the *p*-value from the Student's T-test comparing the two conditions (KO vs WT) ($-\log_{10}$ Student's T-test *p*-value); the total number of identified peptide sequences matching to the protein (# Peptides); the percentage of the DEP sequence that is covered by the identified peptides (Sequence coverage [%]); the molecular weight mass of the identified protein (Mol. weight [kDa]); MaxQuant's search engine Andromeda Score for the best associated MS/MS spectrum.

Table S2. Gene Ontology (GO) terms and pathways associated with the differentially expressed proteins (DEPs) between KO and WT mice osteoblasts, as obtained by the PANTHER Over-representation Test

	PANTHER Category	<i>Mus musculus</i> reference list (total # 22296)	# Protein input (38 DEPs)	# Protein input (expected)	Over/under expression	Fold enrichment	<i>p</i>-value	FDR (< 0.05)
Molecular function	protein C-terminus binding (GO:0008022)	232	5	0.4	+	12.65	4.87E-05	3.18E-02
	purine nucleoside binding (GO:0001883)	356	6	0.61	+	9.89	3.10E-05	4.72E-02
	nucleoside binding (GO:0001882)	365	6	0.62	+	9.64	3.55E-05	4.06E-02
	identical protein binding (GO:0042802)	1861	12	3.17	+	3.78	4.01E-05	3.66E-02
	small molecule binding (GO:0036094)	2387	15	4.07	+	3.69	3.95E-06	1.80E-02
	nucleotide binding (GO:0000166)	2006	12	3.42	+	3.51	8.32E-05	4.75E-02
	nucleoside phosphate binding (GO:1901265)	2006	12	3.42	+	3.51	8.32E-05	4.23E-02
	catalytic activity (GO:0003824)	5712	23	9.74	+	2.36	5.89E-06	1.35E-02
	protein binding (GO:0005515)	8991	28	15.32	+	1.83	4.06E-05	3.09E-02
Slim Biological Process	organonitrogen compound catabolic process (GO:1901565)	105	4	0.18	+	22.35	3.49E-05	6.22E-02
	organonitrogen compound metabolic process (GO:1901564)	237	5	0.4	+	12.38	5.37E-05	4.79E-02
Cellular component	autolysosome (GO:0044754)	12	2	0.02	+	97.79	2.53E-04	2.54E-02
	secondary lysosome (GO:0005767)	15	2	0.03	+	78.23	3.77E-04	3.61E-02
	ruffle (GO:0001726)	144	4	0.25	+	16.3	1.15E-04	1.44E-02
	myelin sheath (GO:0043209)	214	4	0.36	+	10.97	5.02E-04	4.39E-02
	lysosome (GO:0005764)	419	7	0.71	+	9.8	6.62E-06	1.21E-03
	lytic vacuole (GO:0000323)	420	7	0.72	+	9.78	6.72E-06	1.13E-03
	vacuole (GO:0005773)	511	7	0.87	+	8.04	2.35E-05	3.62E-03
	vesicle (GO:0031982)	1925	12	3.28	+	3.66	5.58E-05	7.47E-03

	mitochondrion (GO:0005739)	1778	11	3.03	+	3.63	1.34E-04	1.58E-02
	cytosol (GO:0005829)	3452	21	5.88	+	3.57	1.94E-08	1.95E-05
	endomembrane system (GO:0012505)	3756	16	6.4	+	2.5	2.20E-04	2.33E-02
	cytoplasmic part (GO:0044444)	8362	35	14.25	+	2.46	2.80E-12	5.62E-09
	intracellular organelle part (GO:0044446)	7217	24	12.3	+	1.95	1.58E-04	1.77E-02
	cytoplasm (GO:0005737)	10822	35	18.44	+	1.9	1.98E-08	1.33E-05
	organelle part (GO:0044422)	7570	24	12.9	+	1.86	4.11E-04	3.76E-02
	membrane-bounded organelle (GO:0043227)	10867	33	18.52	+	1.78	1.65E-06	5.54E-04
	intracellular membrane-bounded organelle (GO:0043231)	10169	30	17.33	+	1.73	4.98E-05	7.14E-03
	intracellular organelle (GO:0043229)	11823	34	20.15	+	1.69	2.13E-06	6.12E-04
	organelle (GO:0043226)	12119	34	20.65	+	1.65	4.73E-06	1.19E-03
	intracellular (GO:0005622)	13544	37	23.08	+	1.6	2.62E-07	1.32E-04
	intracellular part (GO:0044424)	13544	37	23.08	+	1.6	2.62E-07	1.05E-04
	cell part (GO:0044464)	15974	38	27.23	+	1.4	4.90E-06	1.09E-03
	cell (GO:0005623)	15976	38	27.23	+	1.4	4.91E-06	9.86E-04
Reactome pathways	Metabolism of nucleotides (R-MMU-15869)	97	4	0.17	+	24.2	2.58E-05	2.09E-02
	Neutrophil degranulation (R-MMU-6798695)	553	7	0.94	+	7.43	3.86E-05	2.08E-02
	Innate Immune System (R-MMU-168249)	1039	9	1.77	+	5.08	5.07E-05	2.05E-02
	Unclassified (UNCLASSIFIED)	12915	8	22.01	-	0.36	7.43E-06	1.20E-02

The input expected value is the number of proteins it would be expected in the test list for a particular PANTHER category, based on the reference list. Over-representation (+) indicates the presence of more observed proteins in the input list than expected. The fold enrichment is a measure of the frequency of a feature in the input list compared to what would be expected by random chance in a larger background. It is calculated as the ratio of the observed frequency to the expected frequency.

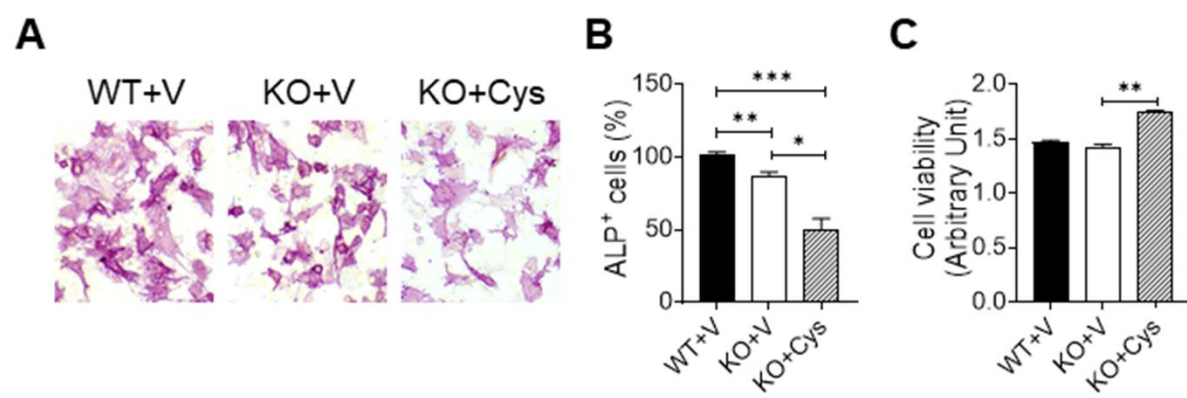
Table S3. Differentially expressed proteins (DEPs) between Pepstatin A (Pep) and vehicle (V) treated- KO osteoblasts.

Protein ID	Gene name	Protein name	Gene ID	Log ₂ (FC Pep/V)	q-value
P46062	Sipa1	Signal-induced proliferation-associated protein 1	20469	5.038635	0.037601785
P09450	Junb	Transcription factor JunB	16477	1.590511	0.016116659
Q5DTK1	Chss3	Chondroitin sulfate synthase 3	78923	1.384066	0.043477807
Q6ZQ88	Kdm1a	Lysine-specific histone demethylase 1A	99982	1.368524	0.034605251
P60521	Gbrl2	Gamma-aminobutyric acid receptor-associated protein-like 2	93739	1.274071	0.024688524
Q8VEE1	Lmcd1	LIM and cysteine-rich domains protein 1	30937	1.219759	0.044616176
Q8VC65	Nrm	Nurim	106582	1.213827	0.022053735
Q8VD76	Tf2h3	General transcription factor IIH subunit 3	209357	1.213007	0.013221426
Q3U2C5	Rn149	E3 ubiquitin-protein ligase RNF149	67702	1.180321	0.002609583
E9Q5G3	Kif23	Kinesin-like protein KIF23	71819	1.158557	0.032635521
Q91XC9	Pex16	Peroxisomal membrane protein PEX16	18633	1.116526	0.04580868
O08609	Max	Max-like protein X	21428	1.057887	0.033884702
Q91W52	Tmm19	Transmembrane protein 19	67226	1.041509	0.005820667
Q9QWH1	Phc2	Polyhomeotic-like protein 2	54383	1.031098	0.026504006
P59110	Senp1	Sentrin-specific protease 1		1.020518	0.019472084
Q80WV3	Chst2	Carbohydrate sulfotransferase 2	54371	-1.013907	0.042579732
Q3ULJ0	Gpd1l	Glycerol-3-phosphate dehydrogenase 1-like protein	333433	-1.028411	0.031375259
Q8R060	Zwilc	Protein zwilch homolog	68014	-1.041875	0.009508835
Q6P9J9	Ano6	Anoctamin-6	105722	-1.12918	0.037178
Q8CGC4	Ls14b	Protein LSM14 homolog B	241846	-1.147407	0.041468128
O70201	Birc5	Baculoviral IAP repeat-containing protein 5	11799	-1.17025	0.028857874
Q6PAL8	Den5a	DENN domain-containing protein 5A	19347	-1.177381	0.018427412
Q60969	Styx	Serine/threonine/tyrosine-interacting protein	56291	-1.310415	0.037274055
Q8BJL1	Fbxo30	F-box only protein 30	71865	-1.335167	0.045912647
Q8VCM7	Fgg	Fibrinogen gamma chain	99571	-1.460121	0.011425654
Q80YA3	Ddhd1	Phospholipase DDHD1	114874	-1.513901	0.020552961

Q8CE33	Klhl11	Kelch-like protein 11	217194	-1.892887	0.006976772
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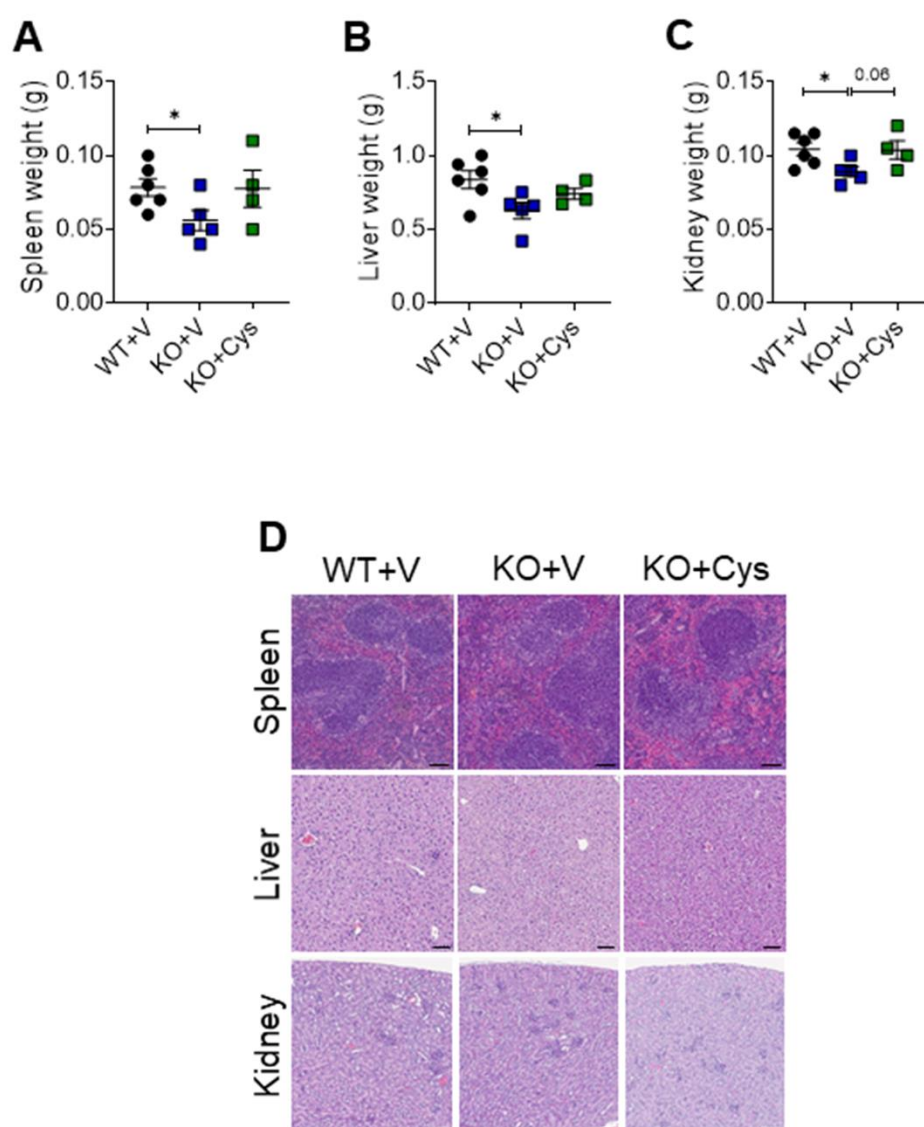
In the table are listed the following DEPs' parameters: the unique protein sequence identifiers (Protein IDs), the Gene Name the protein is associated with, the protein name provided by UniProtKB/Swiss-Prot Knowledgebase; the unique gene identifier (Gene ID) assigned by the NCBI Entrez Gene database; the ratio of expression [$\log_2(\text{Fold Change})$]; and the p-values adjusted for multiple comparisons using the Benjamini-Hochberg False Discovery Rate procedure (q-value).

Supplementary Figure 1: *In vitro* effect of cysteamine on *ctns*^{-/-} osteoblasts isolated from long bones. Osteoblasts were isolated from femurs and tibias of neonatal WT and KO mice and treated with cysteamine (Cys, 100μM) or Vehicle (V). **A)** Representative pictures (Original magnification 10X) and **B)** densitometric analysis of ALP staining. **C)** Cell viability assessed by XTT assay. Data are reported as mean±SEM of at least 3 independent cell cultures. *p<0.05, **p<0.01, ***p<0.001.



Supplementary Figure 1

Supplementary Figure 2: Effect of cysteamine treatment *in vivo*. WT and KO mice treated with vehicle (V) or cysteamine (Cys, 120 µg/g/day). **A-C)** Weight of **A)** spleen, **B)** liver and **C)** kidney of wild-type and *ctns*^{-/-} mice treated with vehicle or with cysteamine. Data are reported as mean±SEM. *p<0.05. **D)** Representative pictures of Hematoxylin/eosin staining of spleen, liver and kidney. Original Magnification 10X. Scale bar 500 µm.



Supplementary Figure 2