

**OMTM, Volume 13**

**Supplemental Information**

**AAV9-TAZ Gene Replacement Ameliorates Cardiac**

**TMT Proteomic Profiles in a Mouse Model**

**of Barth Syndrome**

**Silveli Suzuki-Hatano, Madhurima Saha, Meghan S. Soustek, Peter B. Kang, Barry J. Byrne, W. Todd Cade, and Christina A. Pacak**

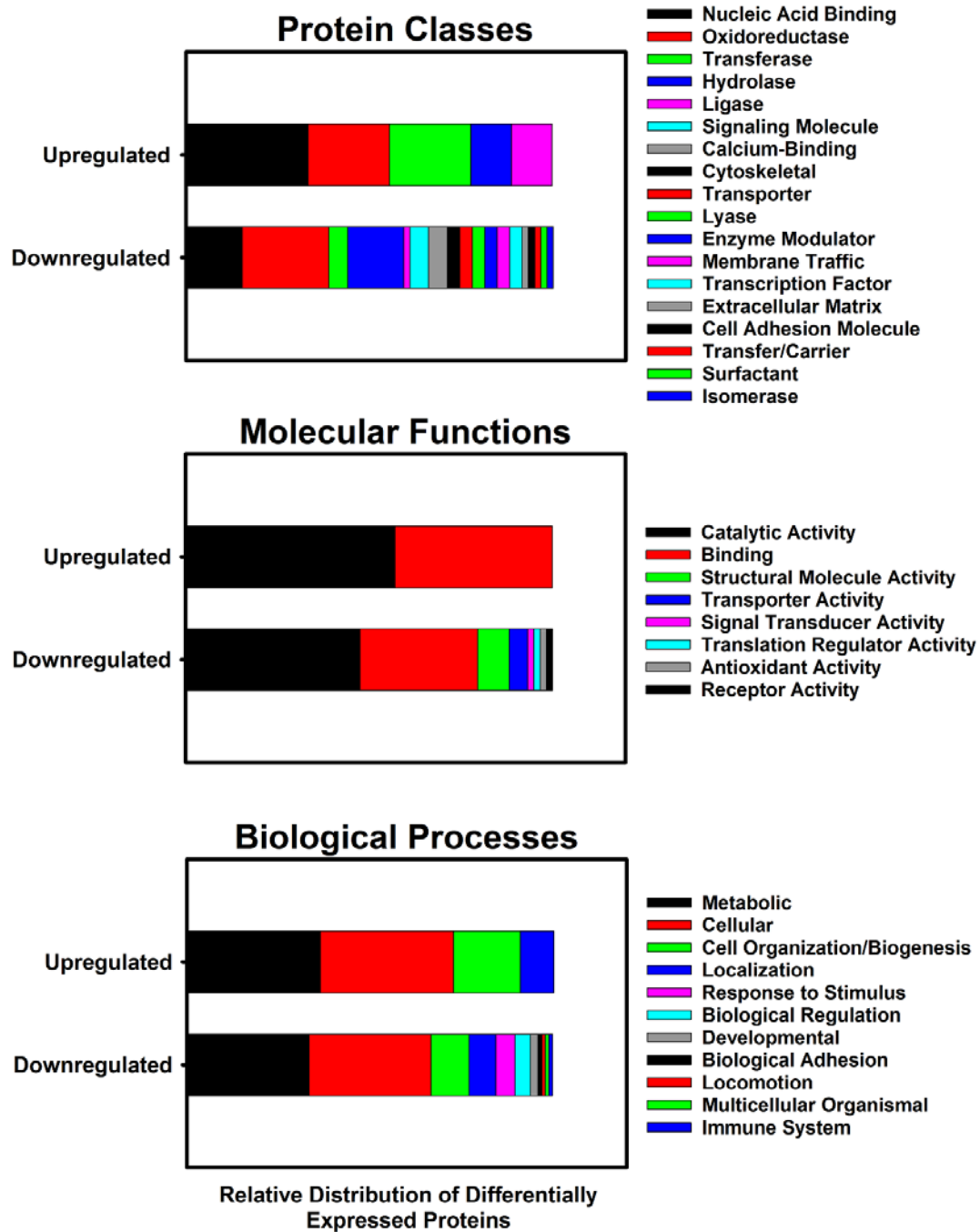
SUPPLEMENTAL DATA

**Supplemental Table 1:** Full list of significantly differentially expressed proteins in BTHS as compared to WT by TMT analysis with 6 pursued hits highlighted in yellow. Fold change values as compared to WT are listed for each group.

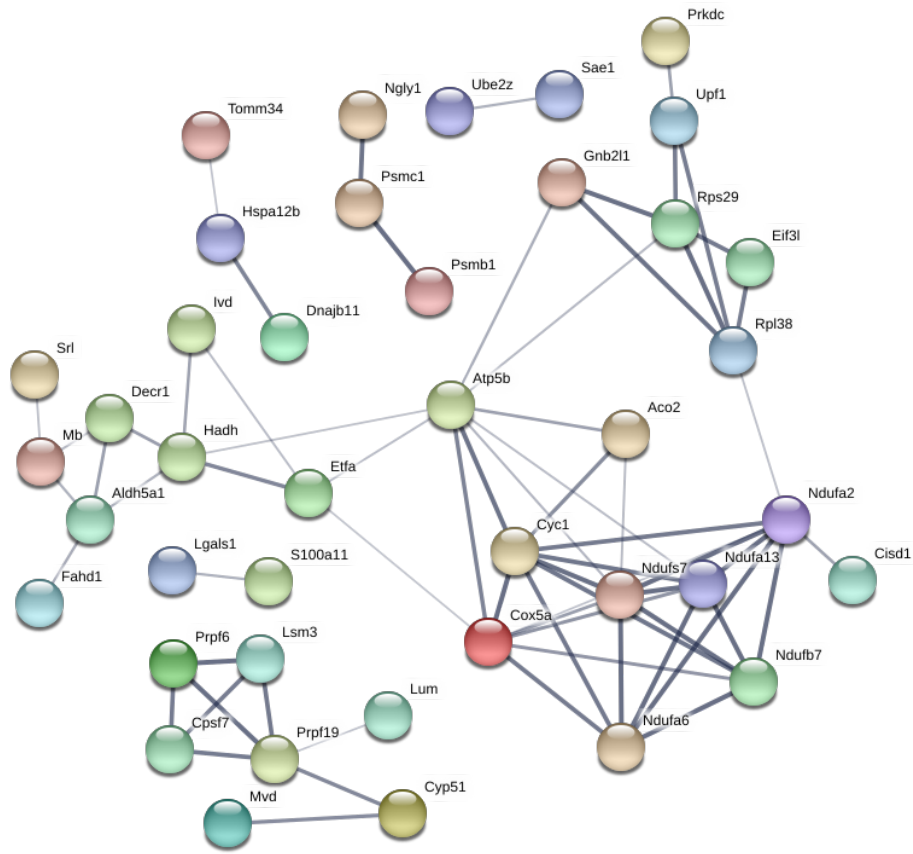
<b>Accession:</b>	<b>BTHS</b>	<b>AAV-TAZAd</b>	<b>AAV-TAZNeo</b>
<b>Q922B1</b>	-1.21424023	-0.529072743	-0.858567209
<b>O70433</b>	-1.19264508	-0.147202107	0.544485386
<b>Q9WV35</b>	-1.14720211	-0.528032212	-0.275786313
<b>Q9D7J9</b>	-1.09387905	-0.437330174	0.010063683
<b>P51885</b>	-0.9686048	-0.53428668	0.12895322
<b>P0DN34</b>	-0.93926084	-0.475936324	0.096261853
<b>Q91ZE0</b>	-0.90914657	0.014355293	0.102993993
<b>P16045</b>	-0.88496676	-0.432454552	-0.006506779
<b>Q8C6I2</b>	-0.81476775	-0.107026185	0.337996464
<b>P97315</b>	-0.78090894	-0.271426397	0.351062899
<b>Q8C547</b>	-0.76366046	-0.066427362	0.096936483
<b>Q4VAE3</b>	-0.75633092	-0.380821784	0.18142064
<b>Q9ERS2</b>	-0.75511294	-0.431481402	0.153156788
<b>Q9CQ75</b>	-0.75511294	-0.650634722	0.029982866
<b>Q78J03</b>	-0.75389599	-0.220109961	0.204766751
<b>P56375</b>	-0.74903843	-0.540568381	0.052415894
<b>P04247</b>	-0.72023158	-0.706041021	-0.007231569
<b>Q8CC86</b>	-0.71904369	-0.526368923	0.005759269
<b>P59266</b>	-0.7095756	0.653518671	0.670840336
<b>Q9CR61</b>	-0.6978272	-0.369594529	0.097610797
<b>Q1XH17</b>	-0.69432126	-0.301337	0.097610797
<b>Q8R0F8</b>	-0.6931545	-0.097733431	0.0614306
<b>Q61425</b>	-0.57346686	-0.446148032	0.182692298
<b>Q9CPU0</b>	-0.55109905	-0.149600603	0.340847077
<b>Q9CQZ5</b>	-0.53742411	-0.277533976	0.21350282
<b>Q9DC70</b>	-0.53742411	-0.242976753	0.197236355
<b>Q8K4Q8</b>	-0.52491512	-0.024736678	0.332278283
<b>Q8BWF0</b>	-0.51766798	-0.312939312	0.182692298
<b>Q9CZJ2</b>	-0.50942987	-0.090802937	0.240619629
<b>Q3U435</b>	-0.49614247	-0.303115909	0.132905812
<b>Q8K0E8</b>	-0.49005085	-0.588248371	0.662661255

<b>Q9CQ00</b>	-0.47593632	-0.032831392	0.549422746
<b>Q99JF5</b>	-0.47493341	0	0.380729449
<b>Q9CQU0</b>	-0.47393119	0.112366523	0.44625623
<b>Q99KP6</b>	-0.46693508	-0.169744676	0.313245852
<b>Q7TQ48</b>	-0.46494241	-0.310253562	0.384602458
<b>Q91ZM8</b>	-0.46295248	-0.097733431	0.01720929
<b>P28667</b>	-0.43733017	0.267835392	0.422771219
<b>Q8CG76</b>	-0.37988284	-0.139235797	0.43349364
<b>Q61102</b>	-0.36215794	-0.064163126	0.367930141
<b>P38585</b>	-0.34831682	-0.06340917	0.43349364
<b>Q9D7N9</b>	-0.33551716	-0.053643428	0.695993813
<b>Q91WS0</b>	-0.33551716	-0.035046947	0.448900951
<b>Q9EP71</b>	-0.33278909	-0.188528969	0.240008965
<b>Q9CYG7</b>	-0.32644358	-0.09157135	0.586884813
<b>Q8VC03</b>	-0.3219281	0.224040274	0.396159489
<b>Q99LC5</b>	-0.32102669	-0.17869796	0.439357178
<b>P62274</b>	-0.31563007	0.006477564	0.3950628
<b>Q9JI39</b>	-0.31563007	-0.015957574	0.364012054
<b>P12787</b>	-0.31383567	-0.070209002	0.502839758
<b>Q3TCN2</b>	-0.30935942	-0.157624512	0.197236355
<b>Q63918</b>	-0.30668032	-0.037265909	0.686164326
<b>Q8VDP4</b>	-0.30400619	0.068326861	0.548436625
<b>P50543</b>	-0.28982725	-0.144010303	0.372394965
<b>P68040</b>	-0.28718415	-0.035786222	0.470927257
<b>Q8K0C4</b>	-0.27928376	0.100977648	0.486971744
<b>Q5FWJ8</b>	-0.24982229	-0.195946441	0.159629186
<b>P97346</b>	-0.2446851	-0.205064337	0.337425664
<b>Q8BGC0</b>	-0.18770716	-0.031356244	0.439357178
<b>P56480</b>	-0.18606493	-0.092340172	0.548929769
<b>Q9CQ62</b>	-0.18114944	-0.087733372	0.50589093
<b>P97313</b>	-0.17055632	-0.017417053	0.40599236
<b>Q99KV1</b>	-0.16326792	-0.03209363	0.617298483
<b>Q9JKY0</b>	-0.16326792	0.053111336	0.420617139
<b>Q62418</b>	-0.16165326	0.121015401	0.562669826
<b>Q9WU84</b>	-0.16004041	0.07244885	0.49978212
<b>Q62422</b>	-0.15440959	0.074505436	0.497740089
<b>Q9R1T2</b>	-0.1496006	0.012211084	0.425459305
<b>Q91YR7</b>	-0.13526908	0.071076162	0.535057595

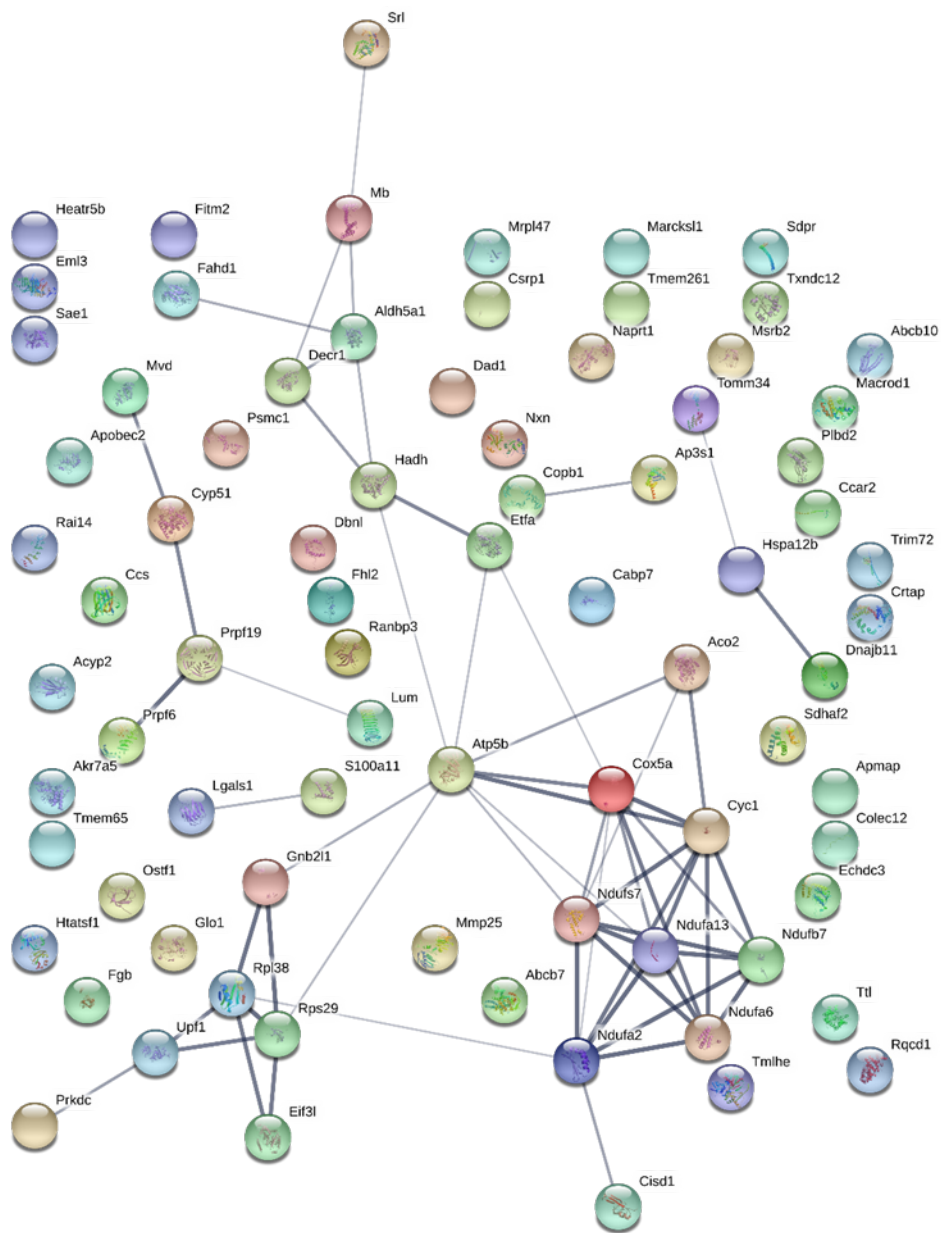
<b>Q9D0M3</b>	-0.12579321	-0.051399153	0.492109553
<b>Q9JIF7</b>	-0.12500636	-0.040229845	0.570462931
<b>P62192</b>	-0.12343394	0.055195654	0.701327257
<b>Q9CYD3</b>	-0.11536301	0.073820233	0.364572432
<b>Q8K2Y7</b>	-0.10314693	-0.160846613	0.443076151
<b>Q8QZY1</b>	-0.09234017	0.074505436	0.530570204
<b>Q9JJI8</b>	-0.08543548	-0.028410464	0.479230561
<b>Q9CT10</b>	-0.08008791	0.118359726	0.591200673
<b>Q99KI0</b>	-0.06945188	0.007195501	0.635986504
<b>Q9EPU0</b>	-0.0671829	0.085424656	0.478713002
<b>Q91YP0</b>	0.039840265	0.209141398	0.50589093
<b>O09061</b>	0.053806444	0.13553487	0.641546029
<b>Q8R059</b>	0.141432791	0.201633861	0.644317778
<b>Q3UE20</b>	0.14208663	0.10701825	0.54399072
<b>Q3UE37</b>	0.224657734	-0.015228388	0.525066592
<b>Q8BP47</b>	0.22650853	0.250961574	0.862748926
<b>Q9JHI5</b>	0.239398042	0.668119125	1.520799374
<b>B7ZNG4</b>	0.360083296	0.096261853	0.403813062
<b>Q921W0</b>	0.456806149	0.432424977	0.699551633
<b>P62311</b>	0.911499849	0.536550296	1.13422094
<b>Q8BTV2</b>	0.998195503	0.536550296	1.246408087
<b>Q61239</b>	1.033863452	0.566084491	1.181102551



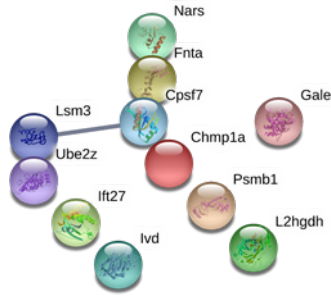
**Supplemental Figure 1: PANTHER ontology classification distributions of proteins differentially expressed in BTHS - A) Distribution of protein classifications in up (top) or down (bottom) regulated proteins. B) Distribution of molecular functions in up (top) or down (bottom) regulated proteins. C) Distribution of biological processes in up (top) or down (bottom) regulated proteins.**



**Supplemental Figure 2: STRING interaction diagram.** STRING 10.2 analysis of all proteins found to be dysregulated in BTHS revealed multiple interactive groups. Line thickness indicates the strength of data support.



**Supplemental Figure 3: STRING interaction diagram.** STRING 10.2 analysis of all proteins down-regulated in BTHS. Line thickness indicates the strength of data support.



**Supplemental Figure 4: STRING interaction diagram.** STRING 10.2 analysis of all proteins up-regulated in BTHS. Line thickness indicates the strength of data support.

**Supplemental Table 2: STRING Identified Biological Processes from proteins dysregulated in BTHS.**

#pathway ID	pathway description	observed count	gene
GO.0055114	oxidation-reduction process	22	
GO.0044710	single-organism metabolic process	34	
GO.0008152	metabolic process	51	

**Supplemental Table 3: STRING Identified Molecular Functions from proteins dysregulated in BTHS.**

#pathway ID	pathway description	observed count	gene
GO.0016491	oxidoreductase activity	18	
GO.0003824	catalytic activity	42	
GO.0008137	NADH dehydrogenase (ubiquinone) activity	4	
GO.0043167	ion binding	38	
GO.0016651	oxidoreductase activity, acting on NAD(P)H	5	
GO.0003674	Molecular function	59	
GO.0048037	cofactor binding	7	
GO.0050662	coenzyme binding	6	
GO.0046914	transition metal ion binding	15	
GO.0036094	small molecule binding	21	



**Supplemental Table 4:** STRING Identified KEGG Pathways from proteins dysregulated in BTHS.

#pathway ID	pathway description	observed gene count
190	Oxidative phosphorylation	8
5012	Parkinson s disease	8
5010	Alzheimer s disease	8
5016	Huntington s disease	8
4932	Non-alcoholic fatty liver disease (NAFLD)	7
1100	Metabolic pathways	17
650	Butanoate metabolism	3
1120	Microbial metabolism in diverse environments	5

**Supplemental Table 5:** List of up-regulated proteins that were rescued following neonatal (blue) or both ages of (light green) treatment and their respective functions. (Function Adapted from UniProtKB Data Bank<sup>1</sup>)

Uniprot ID	Protein	Expression level in BTHS	Gene	Function
Q8R059	UDP-glucose 4-epimerase	Upregulated	Gale	UDP-Gal plays a critical role in the pathway of galactose catabolism in which galactose is converted to the glycolytic intermediate glucose 6-phosphate. UDP-sugar interconversions are important in the synthesis of glycoproteins and glycolipids.
Q3UE20	Amine oxidase	Upregulated	N/A	Catalyzes the oxidative deamination and formation of corresponding aldehyde, hydrogen peroxide an ammonia.
Q921W0	Charged multivesicular body protein 1a	Upregulated	Chmp1a	Associated component of the endosomal sorting required for transport complex III that is involved in multivesicular

				body (MVB) formation and sorting.
Q91YP0	L-2-hydroxyglutarate dehydrogenase, mitochondrial	Upregulated	L2hgdh	Converts L-2-hydroxyglutarate to 2-ketoglutarate that after subsequent process produces energy.
O09061	Proteasome subunit beta type-1	Upregulated	Psmb1	Component of the 20S core proteasome complex that is involved in the proteolytic degradation of intracellular proteins. Associated with 19S regulatory particles, forms the 26S proteasome. Participates in the ATP-dependent degradation of ubiquitinated proteins.
Q3UE37	Ubiquitin-conjugating enzyme E2 Z	Upregulated	Ube2z	Catalyzes the covalent attachment of ubiquitin to other proteins. May be involved in apoptosis regulation.
Q8BP47	Asparagine-tRNA ligase, cytoplasmic	Upregulated	Nars	Catalyzes the ligation of an amino acid to its cognate transfer RNA molecule in a highly specific reaction.
Q9JH15	Isovaleryl-CoA dehydrogenase, mitochondrial	Upregulated	Ivd	Mitochondrial matrix enzyme that catalyzes the third step in leucine metabolism.

**Supplemental Table 6:** List of proteins that are not rescued after treatment and respective function. (Function Adapted from UniProtKB Data Bank)

<b>Uniprot ID</b>	<b>Protein</b>	<b>Expression level in BTHS</b>	<b>Gene</b>	<b>Function</b>
Q91ZE0	Trimethyllysine dioxygenase, mitochondrial	Downregulated	Tmlhe	Converts trimethyllysine (TML) into hydroxytrimethyllysine (HTML).
P61804	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1	Downregulated	Dad1	Subunit of oligosaccharyl transferase and participates in the first step in protein N-glycosylation.
Q9JI39	ATP-binding cassette sub-family B member 10, mitochondrial	Downregulated	Abcb10	May mediate critical mitochondrial transport functions related to heme biosynthesis.
Q9DCR2	AP-3 complex subunit sigma-1	Downregulated	Ap3s1	It facilitates the budding of vesicles from the Golgi membrane and may be directly involved in trafficking to lysosomes.
Q91YR7	Pre-mRNA-processing factor 6	Downregulated	Prpf6	Involved in pre-mRNA splicing. Enhances dihydrotestosterone-induced transactivation activity of AR, as well as dexamethasone-induced transactivation activity of NR3C1, but does not affect estrogen-induced transactivation.
Q9D0P8	Intraflagellar transport protein 27 homolog	Upregulated	Ift27	Essential for male fertility, spermiogenesis and sperm flagella formation. Plays a role in the early development of the kidney. May be involved in the regulation of ureteric bud initiation.
P62311	U6 snRNA-associated Sm-like protein LSm3	Upregulated	Lsm3	Plays role in pre-mRNA splicing as component of the U4/U6-U5 tri-snRNP complex that is involved in spliceosome assembly, and as component of the

				precatalytic spliceosome (spliceosome B complex).
Q8BTV2	Cleavage and polyadenylation specificity factor subunit 7	Upregulated	Cpsf7	Component of the cleavage factor Im (CFIm) complex that functions as an activator of the pre-mRNA 3'-end cleavage and polyadenylation processing required for the maturation of pre-mRNA into functional mRNAs.
Q61239	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	Upregulated	Fnta	Contributes either a farnesyl group or a geranylgeranyl group in thioether linkage to the cysteine residue of proteins with a C-terminal CAAX box.

**Supplemental Table 7:** List of proteins rescued following either adult (green) or neonatal (blue) treatment and their respective functions. (Function Adapted from UniProtKB Data Bank<sup>1</sup>)

Uniprot ID	Protein	Expression level in BTHS	Gene	Function
Q9CPU0	Lactoylglutathione lyase	Downregulated	Glo1	Catalyzes the conversion of hemimercaptal, formed from methylglyoxal and glutathione, to S-lactoylglutathione. Involved in the regulation of TNF-induced transcriptional activity of NF-kappa-B. Required for normal osteoclastogenesis.
Q9CYD3	Cartilage-associated protein	Downregulated	Crtap	Necessary for efficient 3-hydroxylation of fibrillar collagen prolyl residues.
Q8K2Y7	39S ribosomal protein L47, mitochondrial	Downregulated	Mrpl47	Structural constituent of ribosome. Mitochondrial translation.
Q62422	Osteoclast-stimulating factor 1	Downregulated	Ostf1	Induces bone resorption, acting probably through a signaling cascade which results in the secretion of factor(s) enhancing osteoclast formation and activity.
Q3TCN2	Putative phospholipase B-like 2	Downregulated	Plbd2	Hydrolase activity. Lipid catabolic process.
Q61425	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	Downregulated	Hadh	Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA.
Q8CC86	Nicotinate phosphoribosyltransferase	Downregulated	Naprt	Catalyzes the first step in the biosynthesis of NAD from nicotinic acid, the ATP-dependent synthesis of beta-nicotinate D-ribonucleotide from nicotinate and 5-phospho-D-ribose 1-

				phosphate. Helps prevent cellular oxidative stress via its role in NAD biosynthesis.
P38585	Tubulin--tyrosine ligase	Downregulated	Ttl	Catalyzes the post-translational addition of a tyrosine to the C-terminal end of detyrosinated alpha-tubulin.
Q8R059	UDP-glucose 4-epimerase	Upregulated	Gale	UDP-Gal plays a critical role in the pathway of galactose catabolism in which galactose is converted to the glycolytic intermediate glucose 6-phosphate. UDP-sugar interconversions are important in the synthesis of glycoproteins and glycolipids.
Q3UE20	Amine oxidase	Upregulated	N/A	Catalyzes the oxidative deamination and formation of corresponding aldehyde, hydrogen peroxide and ammonia.
Q921W0	Charged multivesicular body protein 1a	Upregulated	Chmp1a	Associated component of the endosomal sorting required for transport complex III that is involved in multivesicular body (MVB) formation and sorting.

**Supplemental Table 8:** Primers used in quantitative RT-PCR

Gene		Catalog	Company
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	Mm99999915_g1	Thermo Scientific
4 ½ LIM 2 protein	Fhl2	Mm00515781_m1	Thermo Scientific
Lumican	Lum	Mm01248292_m1	Thermo Scientific
Fat storage-inducing transmembrane protein	Fitm2	Mm04212060_m1	Thermo Scientific
Trimethyllysine dioxygenase	Tmlhe	Mm00454748_m1	Thermo Scientific
Heat Shock 70 KDa Protein 12B	Hspa12b	Mm01329626_m1	Thermo Scientific
Transmembrane protein 65	Tmem65	Mm01207874_m1	Thermo Scientific

**Supplemental Table 9:** Antibodies used in western blotting (WB) and immunohistochemistry (I).

Protein		Dilution (WB / I)	Company
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1:5000 / -	Cell Signaling #2118
Cytochrome C oxidase subunit II	MTCO2	- / 1:200	Abcam #ab110258
Transmembrane protein 65	Tmem65	1:250 / -	Sigma Aldrich #HPA025020
Connexin-43	Cx43	1:500 / 1:400	Sigma Aldrich #C6219

1. The UniProt, C (2017). UniProt: the universal protein knowledgebase. *Nucleic acids research* **45**: D158-D169.