Supporting Information

Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia

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Figure S2: Most of the genes in the 5 complexes of the OXPHOS pathway were depressed in sarcopenia. The numbers to the right of the gene symbols are AUC over the time course.

Figure S3: Functional annotation analysis of the genes in cluster #4 (A) and cluster #5 (B) and the overlap between genes in clusters #3, #4 and #5 (C). Quantitative PCR verification of changes in expression of some genes in cluster #5 (D).

Figure S4: Quantitative PCR verification of the changes in expression of transcripts for myosin heavy chain (MyHC) isoforms (A), and Ctgf and MuRF1 in sarcopenia.

Supplementary Methods:

Proteomics Analyses:

Tissue lysis and sample digestion: The gastrocnemius muscle of rats in each of the 6-, 18- or 27-month old groups were pooled proportional to muscle weight. The pooled 6-, 18- and 27-month samples were each homogenized in 8 M urea and 50 mM Tris-HCl (pH 8) using an Omni TH homogeniser. Lysates were reduced with 5 mM dithiothreitol for 30 mins at 56°C and alkylated with 14 mM iodoacetamide at room temperature for 15 mins. Lysates were then acetone precipitated for 4 hours at 4°C with the addition of 0.5% deoxycholate. Samples were centrifuged at 2000 rpm and subsequently washed with ice cold acetone. Protein from each sample was re-suspended in 8 M urea and 50 mM Tris-HCl, and quantified using a BCA assay. Approximately, 500 µg of protein from each sample was diluted 4-fold with 50 mM Tris-HCl (pH 8) and digested using 4 mg of Lys-C at 37°C overnight.

On-column desalting and dimethylation labeling: Digested samples were acidified using formic acid. A 100 mg column was activated and equilibrated using a column volume of the following; 1) 100% methanol, 2) 80% ACN and 0.5% acetic acid, 3) 0.1% formic acid. Sample was then loaded onto the column and subsequently washed with a column volume of 0.1% formic acid. A 0.2 M citrate/ 0.4 M phosphate buffer was passed through the column to prepare it for dimethylation labeling. Peptides were then labeled on-column to produce three labels: 1) Light ($CH_20/NaBH_3CN$), 2) Medium ($CD_20/NaBH_3CN$), 3) heavy ($CD_20/NaBD_3CN$). Rat muscle sample corresponding to the 6 month old rat was labeled with light reagents, 18 month old sample with medium reagents and 27 month old sample was labeled with heavy reagents.

Samples were then washed with 0.1% formic acid and eluted from the column using 80% ACN and 0.5% acetic acid. Samples were then dried down using a vacuum centrifuge.

Strong cation exchange chromatography: Strong cation exchange (SCX) chromatography was performed as described previously with minor changes (5). Briefly, 0.75 mg of peptide from light/medium/heavy rat muscle samples was re-suspended in 500 µl of SCX buffer A (7 mM KH2PO4, pH 2.65, 30% ACN) and separated on a 4.6 mm x 200 mm polysulfethyl aspartamide column (5 µm particles; 200 Å pores; PolyLC), using a 36 min gradient from 0% to 50% buffer B (7 mM KH2PO4, pH 2.65, 30% ACN, 350 mM KCl) at a flow rate of 1 ml/min. Fractions were collected every 1.5 min, dried in a speed-vac evaporator (ThermoFisher), re-suspended in 1% FA, and desalted using self-packed C18 STAGE-tips (4). Peptides were eluted into glass inserts and re-suspended in 100 µl of 5% ACN and 4% FA.

LC−MS/MS Analysis: All LC−MS/MS data were obtained using an LTQ-Orbitrap Discovery hybrid mass spectrometer (Thermo Fisher, San Jose, CA). 2 µL of each SCX fraction was loaded onto a pulled fused silica microcapillary column (125 µm, 18 cm bed volume) packed with C_{18} reverse-phase resin (Magic C18AQ; 5-µm particles; 200-Å pore size; Michrom Bioresources, Auburn, CA) using a Famos autosampler (LC Packings, San Francisco, CA). Once loaded, the peptides were separated using an Agilent 1200 series binary pump across a 95 min linear gradient of 5−27% buffer B (97% ACN, 0.125% FA) with an in-column flow rate of 0.5-1 µL/minute. In each data collection cycle, one full MS scan (375–1800 *m/z*) was acquired in the Orbitrap [6×10^4 resolution setting, automatic gain control (AGC) target of 10^6], followed by 10 data-dependent MS/MS scans in the LTQ using the 10 most abundant ions and collisioninduced dissociation (CID) for fragmentation. The method dynamically excluded previously selected ions for 60 s, as well as rejected singly charged ions and unassigned charge states.

Database Searching: An in-house suite of software tools was used to convert mass spectrometric data from raw file to mzmxl format. Erroneous protein ion charge state and monoisotopic m/z were corrected as per previous publication (3). MS/MS spectra assignments were made with the Sequest algorithm (2) using the entire rat IPI database (version 3.6). Sequest searches were performed using a target-decoy strategy (1) with the rat IPI database in correct orientation (forward database) and the same database but with all sequences in reverse orientation (reverse database). The rat IPI database contained 113,477 entries. The data was searched using a precursor ion tolerance of 50 ppm, considering LysC specificity and allowing two missed cleavages. For dimethyl labels, a static modification of 28.0313 Da was used on the n-terminus and lysine residues. In addition, differential modifications of 4.02310 Da and 8.044336 Da were used for medium and heavy proteins, respectively, on the N-terminus and lysine residues. A protein level false discovery (FDR) rate of less than 1% was used as a threshold for protein identifications using the target decoy strategy. Additional filtering was achieved using a linear discriminant analysis, which combined several parameters into a single probability for each protein and these probabilities were used to achieve a less than 1% FDR. The parameters used for linear discriminant analysis were Xcorr, DCn, protein mass accuracy and charge state, and protein length. After protein level filtering, a 1% protein level false discovery rate was used.

Protein Quantification: Quantification of each protein was determined using the peak heights for light, median and heavy forms for that protein. The criterion for protein quantification was a signal-to-noise ratio of >5 for at least one of the protein species (light, medium, heavy). At the protein level, quantification was determined by calculating the median value of the ratios of light:medium and light:heavy.

References:

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4. Rappsilber, J., Y. Ishihama, and M. Mann. 2003. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. Anal Chem 75:663-670.
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Table S1: Probes used for real-time PCR quantification of target and house-keeping genes

Gene Symbol	Gene Name	ABI Probe ID
Bad	Bcl2-antagonist of cell death	Rn00575519_m1
Casp3	caspase 3, apoptosis related cysteine protease	Rn00563902_m1
Chrna1	cholinergic receptor, nicotinic, alpha 1 (muscle)	Rn00577938_m1
Chrnd	cholinergic receptor, nicotinic, delta	Rn01401089_m1
Chrne	cholinergic receptor, nicotinic, epsilon	Rn00567899_m1
Chrng	cholinergic receptor, nicotinic, gamma	Rn00569614_m1
Cpt1b	Carnitine palmitoyltransferase 1b	Rn00566242_m1
Ctgf	connective tissue growth factor	Rn00573960_g1
Drp1	dynamin 1-like	Rn00586466_m1
ERRα	Estrogen related receptor, alpha	Rn00433142_m1
Fabp3	Fatty acid binding protein 3	Rn00577366_m1
Fbxo30	F-box only protein 30	Rn01440751_m1
Fis1	Ttc11,fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)	Rn01480911_m1
Fst	Follistatin	Rn00561225_m1
Hdac4	Histone deacetylase 4	Rn01427053_m1
Idh3a	Isocitrate dehydrogenase 3 (NAD+) alpha	Rn00586270_m1
IGF2	insulin-like growth factor 2	Rn01454518_m1
Mfn1	mitofusin 1	Rn00594496_m1
Mfn2	mitofusin 2	Rn00500120_m1
Mstn	Myostatin	Rn00569683_m1
Musk	muscle, skeletal, receptor tyrosine kinase	Rn01422881_m1
Myh1	myosin, heavy polypeptide 1, skeletal muscle, adult; MyHC-2X/D	Rn01751056_m1
Myh2	myosin, heavy polypeptide 2, skeletal muscle, adult; MyHC-2A	Rn01470669_m1
Myh3	myosin, heavy chain 3, skeletal muscle, embryonic	Rn00561539_m1
Myh4	myosin, heavy chain 4, skeletal muscle; MyHC-2B	Rn01496087_g1
Myh7	myosin, heavy chain 7, cardiac muscle, beta; MyHC-slow, MyHC-1,	Rn00691731_m1
Myh8	myosin, heavy chain 8, skeletal muscle, perinatal	Rn01751714_g1
Myog	myogenin	Rn00567418_m1
Opa1	optic atrophy 1 homolog	Rn00592200_m1
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	Rn01453110_m1
Tbp	TATA box binding protein	Rn01455646_m1
Tmem1	transmembrane protein 1	Rn01427961_m1
TRIM63	tripartite motif-containing 63; MuRF1	Rn00590197_M1
Uqcrc1	Ubiquinol-cytochrome c reductase core protein 1	Rn01521194_m1
Utrn	Utrophin	Rn00565137_m1
Vegfa	vascular endothelial growth factor A	Rn01511605_m1
Vps26a	vacuolar protein sorting 26 homolog A	Rn01433543_m1
Znf830	zinc finger protein 830	Rn02748665_s1

Table S2. Peptide score from proteomics analysis. The contribution of single peptide protein species in the dataset was low (<5%) presumably reflecting the high accuracy and sensitivity of the MS methods, the high signal-to-noise cut off used.

No. peptide species	# Genes	% of total peptides	Gene symbols
>2000	1	0.08	Ckm
1000-1999	1	0.08	Mb
500-999	7	0.55	Atp2a2, Atp2a1, Car3, Pgk1, Pkm2, Alb, Ldha
400-499	4	0.32	Acta1, Pgm1, Aldoal1, Aldoa
300-399	7	0.55	Gpi, Pygm, Atp5b, LOC360504, Pvalb, Myl1, Atp5a1
200-299	12	0.95	Gapdh, Hspa8, Aco2, Mdh1, Eno3, Ahnak, Mdh2, Ak1, Park7, Myh2, Tpm1, Tpi1
100-199	40	3.17	Idh2, Hspd1, Mylpf, Srprb, Gpd1, Casq1, Neb, Sucla2, Nnt, Pgam2, Vdac1, MGC72973, Cps1, Anxa6, Cryab, Hspb1, Eef1a2, Acadl, Ldb3, Eef1a1, Myh8, Hadha, Ugp2, Bin1, Tnni2, Myom2, RGD1564958, Etfa, Uqcrc2, Myh6, RGD1565338, RGD1561178, Pygm, Ndufs1, Pfkm, Acadvl
20-100	236	18.69	Pebp1, Got1, Atp5c1, RGD1303003, Got2, Flnc, Ckmt2, Pdhb, Hsp90aa1, LOC297568; Mug1, Hspa9, Pdia3, Hbb, Slc25a4, Map4, Atp5o, Tpm2, Lum, Fh1, Tnnt3, Dcn, Mybpc1, Immt, Slc25a5, Hspa5, Fhl1, Aldh2, Vdac2, Lgals1, Hsp90ab1, Ldhb, Agl, P4hb, Acaa2, Myh3, Akr1b1-s2, Prdx5, Atp2a3, Acat1, Myh1; Myh2, IgG-2a, Sdha, Etfb, Ptrf, Cycs, Ldb3, Apoa4, Trim72, Sdhb, Cisd1, Aldh6a1, LOC299282, Hspe1, Cs, Wdr1, Psmc3, Pdlim3, Fabp4, Pzp, Hibadh, Atp5f1, Acadm, Vapa, Tufm, Tra1, Tpm3, RGD1565368, Echs1, Cacna2d1, Uqcrc1, Tubb6, Ivd, Tuba1a, Prdx1, Myom1, Plec1, Dlat, Vcp, Hspa1b; Hspa1a, Etfdh, Cp, Myl3, Hadh, Stip1, Oxct1, Nme2, Dmd, Ampd1, Myh2, Calr, Serpina3n, Pfn1, Nnt, Mybpc2, Mpz, Hpx, Vdac3, Tln1, Suclg1, Ppp2r1a, Cct8, Uqcrb-ps1; Uqcrb, Tubb5, Col6a3, Anxa2, Actb, Serpina1, Ndufa4, Ndufa10l1, Myh7, Idh3a, Cox5a, Clip1, Atic, Myh2, Dld, Apoa1, Ppp1r3a, Pgm1, Nme2-ps1, Hint1l; Hint1, Gdi2, Aspn, Ywhae, Uba1, Ndufa7, Letm1, Fkbp3, Fga, Eprs, Atp5h, Myh11, Hspa4, Eef2, Cat, Aldh9a1, Ywhag, Ndufa2, Dysf, Cox5b, Cndp2, Sod2, Serpina3k, Eif5a, Cfl1, Cacnb1, Rtn4, Prdx6, Ndufb9, Kif5b, Aldh1l1, Ahnak, Acsl1, Strap, RGD1566161, Mt-atp8, LOC684425, Hspa2, Ckmt2, Ahnak, Tpm2, Stac3, Phkb, Ola1, Nampt, Myh9, Kbtbd10, Hadhb, Gyg1, Gpd1l, Casq2, Postn, Gpt, Glud1, Flna, Dci, Bcap31, Atp5j, RGD1565238, Pdha1, Csda, Anxa1, Ywhab, Gbas, Ttn, Smpx, Gda, Eif3j, Cox4i1, Cct5, Cav1, Akr1b1, Adsl, Serbp1, Rtn2, Psma1, Prdx2, Phb2, Ogdh, Msn, LOC681252, Eef1g, C3, Sncg, Sdpr, RGD1561141, Qdpr, Ndufa9, Mapk12, Ehd1, Atp5l, Sh3bgr, Pdhx, Pdap1, Myoz1, Gys1, Fhl1, Dctn2, Cyc1, Cox6c, Clu, Aifm1, Vim, Prelp, Ndufs4, Ndufs2, Ndufb5, LOC686059, Esd, Dag1, Adss
11-19	235	18.61	Txn1, Slmap, Psme1, Ndufs3, Ndrg2, Mylk2, Mvp, LOC679739; LOC692052, Kng1, Hsdl2, Hsd17b10, Hp, Gart, Des, Cct2, Bag3, Ttn, Synpo2, Slc25a3, Skp1, Rplp2, Prdx3, Peci, Npepps, Nefl, Ndufv1, Hmgb1-ps3, Fbp2, Ehd2, Cdc42, Actn3, Txnl1, Tpm4, Tnnc2, S100a13, RGD1559626, Rdx, Prx, Pgp, Pdlim5, Pdia4, Ogn, Nit2, Ndufa12, Mrps36, LOC678701, LOC497813; Rps7, Lmcd1, Idh3B, Idh1, Hist1h1d, Dlst, Cox7a2; LOC688386, Clic1, Cct7, Atp12a, Arhgdia, Actc1, Acads, Ywhaz, Stim1, Rps8, Rpl28, Reep5, Psma6, Ppp3ca, Nrap, Nefm, Ndufs8, Myl2, Mpi, Map1a, Lrpprc, LOC689064; LOC100134871; MGC72973; Hbb, Itih4, Flot2, Eno1, Ctsd, Csrp1, Crat, Chchd3, Ces3, Asph, Anxa6, Ankrd2, Acaa2, Tuba4a, Ttn,

			Snta1, Serpinc1, Ppa1, Hprt1, Eef1b2l, Dnm1l, Ddx1, Cdh13, Cand2, C9, Atp1a2, Apoa1bp, Tmsb4x, Selenbp1, RGD1565416, Ppia, Pm20d2, Pdlim5, Myh11, Murc, LOC500183, Hdgf, Gstp1, Cox6b1-ps1; Cox6b1, Cmpk1, Cct6a, Camk2b, C1qbp, Bsg, Basp1, Auh, Alpk3, Aco1, Tmem38a, Serpinf1, Rpl5, RGD1564688, Rcsd1, Rars, Pygl, Psma7, Psma3; Psma3l, Prkar2a, Pdpr, Obscn, Ndufc2, Ndufb11, Nap1l4, Mapt, Lta4h, Hspb6, Hnrnpu, Hmgn2, Hagh, Cnn1, Cfb, Camk2g, Bcat2, Atp5i, Anxa11, Aldh5a1, Ahcy, Vapb, Txlnb, Tpm1, Sgcd, Set, Rpl23, Rpl13, RGD1311863, Psma5, Ppa2, Pdia6, Nid1, Mt-co2, LOC680312, Kera, Itgb1, Homer1, Dync1h1, Dbi, Csrp3, Coq9, Col6a1, Car2, Baz1b, Arpc5l, Aldh7a1, Akap12, Aars, Ybx1, Wars, Visa, Vcl, Usp5, Ttr, Tppp3, Tceb2, Tagln2, Sncg, Rrbp1, Rpn1, RGD1561176, Rab2a, Rab1b-ps1; Rab1b, Psmd2, Psma2, Ppp2r2a, Ppib, Plaa, Pdlim5, Pdcd6ip, Pccb, Naca, Myh13, LOC680097; Hist1h4m; Hist1h4b; LOC679983; RGD1562378; LOC682518; Hist2h4; RGD1565073; LOC684621; LOC680430; LOC684887; LOC684745, Lama2, Idh3g, Gstm4, Gsta2; Gsta3; Gsta1, Gsn, Es1, Erlin2, Ehbp1l1, Ctsb, Crip, Ckap4, Chmp4b; LOC679886, Cald1, Blvra, Atp1a1, Apoe, Actr1b, Acadvl
9-10	68	5.38	Them2, Tcp1, Scp2, Rps23, Rpl4, Rpl17, RGD1561839, Psmc4, Psmb3, Psma4, Prkab2, Otub1, Opa1, Ndufaf4, Ndufa6, Myot, Myl6l, Mbp, Map2k1, LOC297568; Mug1, Hnrnpk, Gsto1, Gstm2, Glod4, Gars, Cpt2, Cdnf, Cbs, Cap2, Atp6v1b2, Arbp, Akap14, Unc45b, Trdn, Tmed10, Tkt, Taldo1, Synpo, Suclg2, Spag9, Serpinh1, Scpep1, Rtn4, RGD1305235, Rcn1, Pfdn5, Pdhx, Pcyox1, Nucks1, Nmt1, Macrod1, Hnrpdl, Hmgn2, Gpx1, Fam114a2, Eea1, Cyb5r1, Cryz, Clpp, Car1, Bpgm, Bin1, Bckdha, Acsf2
7-8	113	8.95	Uso1, Tsc22d1, Tomm34, Tnnc1, Tfam, Tagln, Smtnl1, Rps25, Rpl36a-ps1; Rpl36al; Rpl36a; Rpl36a-ps2, Rad23b, Rab7a, Ptms, Psmd6, Psmc5, Psmc1, Pcmt1, Pc, Pak2, Nsfl1c, Ndufb10, Napa, Mrpl12, Mapt, LOC684270, Kpna3, Kng1l1, Klc1, Hmgb1, Gapdh; LOC685186; LOC682005, F2, Dpysl2, Ddb1, Dctn1, Dci, Cyb5a, Cops3, Clybl, Cct4, Cbr1, Cast, Capg, Canx, Camk2a, C4b, Atp1b1, Apool, Anxa4, 41, 163.00, 41, 154.00, Ywhaq, Ywhah, Yars, Tns1, Tceb1, Synm, Strn3, Spna2, Sod1, Rsu1, Rps3a, Rps11, Rplp1, Rpl3l, Rpl11, Rpl10a, RGD1564560, Rab1, Ptgr2, Psmc2, Plg, Pgd, Pfkfb1, Pdcd5, Osbp, Nme1, Nln, Ncl, Mut, Metap2, Mccc2, Mccc1, Map1b, LOC691933, LOC690131; Hist2h2aa3; LOC682560, LOC686648; LOC680058, Ktn1, Kars, Hmgcl, Gapdhs, Fgb, Epb4.1l2, Eif4b, Dusp3, Dpysl3, Decr1, Cox7a2l, Cand1, Bgn, Bcl2l13, Ass1, Asrgl1, Arhgdib, Arg1, Apoc1, Anxa5, Anxa3, Anp32e, Ak3, Afg3l2, Adh4
5-6	164	12.98	Ttn, Tst, Tpp2, Tpm3, Syap1, Stoml2, Slc16a1, Serpina4, S100a4, Rps6, Rpl23a, RGD1565438, RGD1561425, RGD1561137, RGD1309696, Rab10, Ptma, Psme2, Psmd14, Psmb7, Psmb1, Prepl, Ppp1r2p9, Ppm2c, Pitrm1, Phkg1, Phb, Pdlim7, Pcyt2, Pabpc4, Pa2g4, Nucb1, Nt5c1a, Nploc4, Ndufa5, Myh11, Mthfd1, Me1, LOC680988, LOC362855, Ldb3, Kng1; Kng2, Kat3, Jph2, Itpa, Igh-6, Hmgcs2, Hcca2, Gspt1, Gpx6, Gpx4, Glo1, Fmod, Fech, Eif4g1, Eif3s9, Eif3h, Eif1b, Eef1b2, Dnaja2, Crkl, Cltb, Cdc37, Carkd, Capn2, Calu, Atp5d, Art3, Arpc1b, Arl6ip5, Arl6ip2, Aprt, Aldh3a2, Aldh1a1, Ahsg, Afm, Actr2, Abce1, Zyx, Xirp1, Vps4a, Uqcrfs1, Ubac1, Txndc12, Tubb2c, Tpd52l2, Tomm40, Tnnt1, Timm44, Timm10, Stbd1, Smtnl2, Scye1, Sccpdh, S100b, Rps14, Rpn2, Rpl35, Rpl24, Rnh1, RGD735140, RGD1565192, RGD1565010, RGD1564801, RGD1564318, RGD1309676, Rabggtb, Ptgr2, Psmd7, Prpf19, Prkca, Ppp3cb, Pls3, Picalm, Pfdn1, Pbxip1, Pacsin2, Ociad1, Nqo2, Npepl1, Nap1l1, Mtpn, Mrps35, Marcksl1, Mapre3, Lonp1, LOC287167, Lmnb1, Ipo7, Impdh2, Hspb8, Gsk3b, Gfer, Gc, Fahd2a, Ensa, Eif4a2, Dtna, Dnaja4, Diaph1, Ddx19a, Ddah1, Crip2, Coq3, Cops2, Col1a1, Cnp, Cfi, Capn1, Calu, Atpif1, Arl3, Aldh1a7, Adk, Adh1, Acss2, Acsl6, Acot9, Acad9, A2m

3-4	210	16.63	Vat1, Vars, Ube2b, Uba2, Tuba8, Tuba1c, Tsta3, Tspan8, Trip10, Trap1, Tomm70a, Tom1l2, Tiprl, Timm8a1, Tgm2, Tbca, Stat1, Sptbn1, Snx5, Sntb1, Smyd2, Sf3b1, Serpina3m, Rps3, Rps27, Rpl14, Rpl10, RGD1562373, Rabggta, Qars, Pura, Psmd3, Psmc6, Prkaca, Pgm5, Pfdn2, Pccb, Pcca, Paics, Pafah1b1, Oxsm, Nomo1, Ndufv3-ps1; Ndufv3, Napg, Mypn, Mybph, Mrps31, Map7d1, LOC688717, LOC683667, LOC641316, Lmna, Lmna, Lamp1, Itsn1, Itga7, Ide, Hnrnpc, Hmox2, Hk2, Hibch, Hgd, Hars, Gna12, Gmps, Gga1, Gfm1, Gcsh, Fundc2, Fdps, Fam21c, Epb4.1, Eml1, Eif4a1, Eif3e, Eif2b3, Ech1, Dbt, Dak, Ctsl1, Ctsh, Csde1, Copb2, Copb1, Col12a1, Clpx, Cd9, Capza2, Camk2d, Atp2b1, Ate1, Asph, Asah1, Arpc5, Apod, Apoa2, Apex1, Akr1a1, Actn2, Aass, Xpnpep1, Vps35, Vamp2, Uchl3; Uchl3-ps1, Trdn, Tardbp, Syncrip, Sugt1, Sqstm1, Sptb, Spag7, Snx1, Slc25a12, Slc12a2, Set, Serping1, Serpinf2, Serpinb6a, Serpina6, Sephs2, Sbk2, Sars, Sae1, Rps27a, Rnmt, RGD1565045, RGD1563757, RGD1559786, RGD1559475, RGD1359600, RGD1310507, RGD1308874, RGD1306839, RGD1306148, Ran, Rab5b, PVR, Ptges2, Prx, Prune, Prkcsh, Prkacb, Ppp3r1, Ppp1r12b, Postn, Phyh, Phpt1, Pgcp, Pex19, Pcyt1a, Pcnp, Pank4, Nefh, Nedd8, Ndufv3-ps1; Ndufv3, Ndufb4, Ncam1, Myh9, Mt-nd5, Mtdh, Mpst, Mecr, Mapk9, LOC688073, LOC686617, LOC682316, LOC364224, Lcp1, Lamc1, Isoc1, Hspb7, Hsd17b4, Hist1h1b, Hibch, Grpel1, Gpd2, Gls, Glrx3, Ghitm, Fhod1, Fgg, Farsb, Fam120a, Erp29, Eif4ebp1, Eif3s10, Eif3d, Ehd4, Eef1d, Depdc6, Ddx5, Dars, Cox6a1, Coq6, Copa, Col6a2, Col1a2, Ckb, Cct3, Cab39, Bcap29, Asna1, Akr1cl1, Ahnak, Add1, Acot5, Acot2
2	105	8.31	Zadh2, Xdh, Wars2, Vps29, Vac14, Uox, Umps, Ube2m, Trim54, Trim25, Tacc2, Sync, Ssb, Srp72, Setd3, Sec23ip, Scrn3, Rps24, Rock2, RGD1563551, RGD1563180, RGD1561928, RGD1561459, RGD1560289, RGD1560149, Raly, Rab5a, Qk, Psmd1, Prep, Ppp1cc, Pmpcb, Pkia, Pip4k2a, Phka1, Parvb, Padi2, Orm1, Obscn, Nt5e, Np, Nid2, Nes, Nars, Mylk, Msra, Mrps27, Mrps10, Mrpl1, Mrlc2, Mcam, Mapre1, M6prbp1, LOC687565, LOC682245, LOC502730, LOC498793, LOC291863, Lap3, Itih3, Inppl1, Hnrnpl, Hmgn1, Golga4, Glul, Gls2, Gbe1, Fyn, Fn3k, Fkbp4, Fermt2, Fbxo40, Eif3k, Dync1i2, Dync1i2, Dst, Dnajb12, Dffa, Ctnnb1, Ctage5, Copz1, Cops4, Clasp2, Ccdc22, Capza1, Calm-ps2, Cacybp, Btf3, Bag5, Atp8b2, Aldob, Agfg1; Agfg1-s1, Adprh, Acy1, Actr3
1	60	4.75	Tubb3, Timp2, Tfrc, Syncrip, Srp72, Sparcl1, Snap23, Scn4a, Scamp1, Rpl3, Rpl18, Rnaset2, Rilp, RGD1563863, RGD1309537, Prosc, Ppm1e, Parva, Oxsr1, Myeov2, Mrpl48, Mrpl27, mrpl11, Map7d2, Maea, LOC690662, LOC296197, Larp7, Lamb1, IgG-2a, Hdlbp, Hdlbp, Hdac6, H2afx, Gypc, Gucy1a3, Gpt2, Flnb, Fis1, Fam129a, Ehbp1l1, Drg2, Cul3, Csrp2, Copz2, Cnpy3, Cdk5rap3, Bub3, Bat1, Atpbd4, Atp6v1, Atp2b4, Atp1b3, Atg3, Ard1a, Ap3d1, Ak2
Total	**1263**	**100**	

Table S3: Clinical chemistry of rats aged 6-27 months

Age (months)	6	12	18	21	24	27
ALT (U/L)	58.1±2.8	74.3±5.0	100.6±10.5	82.5±10.9	73.3±7.8	67.2±11.4
ALP (U/L)	109.5±3.8	105.9±5.4	116.8±9.9	103.6±13.1	86.1±5.2	101.3±28.5
TBIL (mg/dL)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.0
TP (g/dL)	6.1±0.1	6.3±0.0	6.3±0.1	6.2±0.1	6.2±0.1	5.4±0.6
ALB (g/dL)	4.0±0.0	4.0±0.1	3.8±0.1	3.5±0.1	3.6±0.1	2.7±0.4
GLOB (g/dL)	2.2±0.1	2.4±0.1	2.5±0.1	2.6±0.1	2.7±0.1	2.6±0.3
AGR	1.8±0.1	1.7±0.1	1.5±0.0	1.4±0.1	1.4±0.1	0.9±0.1
GLU (mg/dL)	140.7±5.1	137.5±4.8	135.6±2.6	149.2±5.0	122.5±4.3	91.6±17.0
UREA (mg/dL)	18.7±0.8	17.8±0.6	17.6±0.7	15.0±0.7	23.9±6.9	18.3±4.0
CREA (mg/dL)	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.5±0.2	0.3±0.1
Na (mEq/L)	143.4±0.3	144.8±0.2	144.6±0.6	143.0±0.3	145.0±0.7	128.9±16.1
K (mEq/L)	5.0±0.1	4.9±0.1	4.7±0.1	5.0±0.1	4.7±0.1	4.2±0.5
Cl (mEq/L)	102.4±0.3	104.0±0.5	104.0±0.6	103.5±0.4	103.5±0.4	92.0±11.5
CA (mg/dL)	10.4±0.1	10.3±0.3	10.7±0.1	10.5±0.2	10.8±0.3	9.3±1.2
PHOS (mg/dL)	6.3±0.2	5.2±0.1	5.0±0.1	4.6±0.1	5.4±0.7	4.3±0.5
CHOL (mg/dL)	111.1±6.5	155.5±11.4	179.6±8.8	223.6±18.3	247.2±16.3	224.9±36.1
TRIG (mg/dL)	103.7±9.4	131.8±17.1	139.6±16.9	114.2±6.4	149.2±24.2	108.1±15.0
CK (U/L)	593±116	486±82	409±103	513±55	358±53	435±87
GGT (U/L)	ND	3.0	3.5±0.5	4.0±1.0	4.0	ND
MG (mg/dL)	2.0±0.1	2.0±0.1	2.0±0.0	1.9±0.1	2.2±0.1	2.0±0.2
UA (mg/dL)	1.0±0.1	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
IRON (ug/dL)	153.3±6.6	161.5±4.4	188.0±8.6	196.3±11.2	162.2±12.8	117.9±16.0

Table S4: Hematology of rats aged 6-27 months

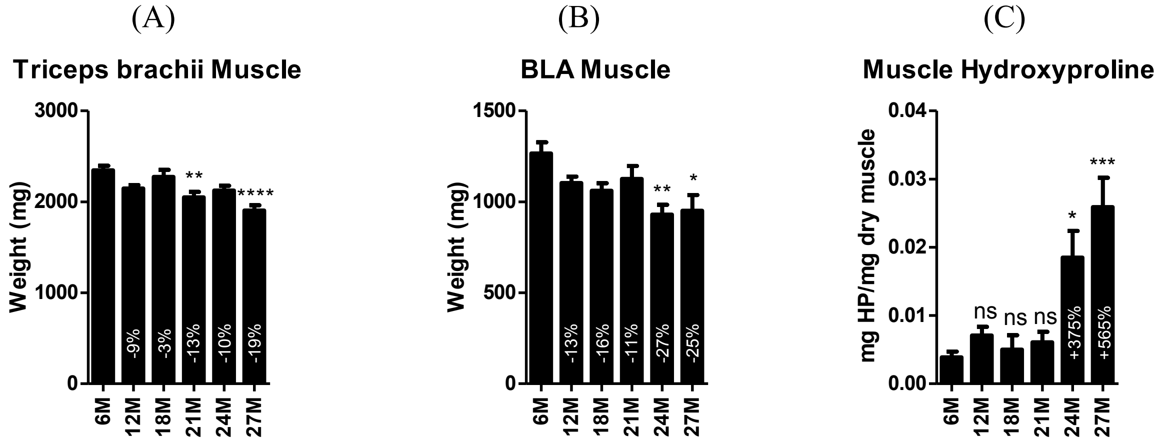
Age (months)	6	12	18	21	24	27
WBC (K/uL)	8.37±0.47	6.81±0.53	6.20±0.69	6.96±0.32	7.02±0.93	9.68±2.64
NEUP (%)	15.31±1.38	20.78±2.86	24.09±2.15	22.37±2.11	26.64±3.29	34.04±5.75
LYMP (%)	77.27±1.36	71.11±2.89	68.36±2.22	70.54±2.24	63.91±3.48	47.68±7.12
MONP (%)	4.20±0.26	5.01±0.22	4.88±0.32	4.87±0.30	6.38±0.47	5.51±0.75
EOSP (%)	1.97±0.23	1.75±0.11	1.39±0.14	1.39±0.21	1.56±0.18	0.92±0.21
BASP (%)	0.15±0.02	0.15±0.02	0.14±0.02	0.23±0.04	0.17±0.03	0.23±0.05
LUCP (%)	1.12±0.12	1.20±0.11	1.14±0.22	0.60±0.10	1.32±0.13	0.60±0.07
NEUA (K/uL)	1.24±0.08	1.47±0.31	1.52±0.24	1.56±0.18	2.06±0.57	4.30±1.87
LYMA (K/uL)	6.50±0.43	4.79±0.36	4.19±0.46	4.90±0.25	4.29±0.35	4.62±0.79
MONA (K/uL)	0.35±0.03	0.34±0.03	0.31±0.04	0.34±0.03	0.46±0.08	0.60±0.12
EOSA (K/uL)	0.17±0.03	0.12±0.01	0.09±0.01	0.10±0.02	0.10±0.02	0.09±0.01
BASA (K/uL)	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.12±0.10
LUCA (K/uL)	0.09±0.01	0.09±0.01	0.07±0.02	0.04±0.01	0.09±0.02	0.17±0.10
RBC (M/uL)	8.28±0.11	7.96±0.07	7.34±0.63	7.65±0.07	7.56±0.25	6.81±0.86
HGB (g/dL)	14.88±0.24	14.52±0.12	13.61±0.99	13.96±0.11	14.08±0.44	12.42±1.55
HCT (%)	41.72±0.68	40.91±0.46	38.77±2.82	39.84±0.39	39.41±1.14	35.35±4.43
MCV (fL)	50.41±0.56	51.39±0.61	54.29±2.21	52.13±0.58	52.23±0.72	46.20±5.78
MCH (pg)	17.98±0.20	18.23±0.17	19.01±0.76	18.28±0.20	18.62±0.21	16.23±2.03
MCHC (g/dL)	35.70±0.17	35.49±0.16	35.09±0.14	35.05±0.15	35.66±0.28	31.31±3.81
PLT (K/uL)	1047±36	944±56	841±109	952±67	1069±81	1002±132
RETP (%)	2.40±0.10	2.32±0.10	3.03±0.75	2.41±0.20	2.77±0.65	2.47±0.37
RETA (K/uL)	198.2±8.3	184.3±7.2	185.5±6.9	183.1±13.9	197.8±32.9	184.0±29.9

Table S5: Expression of genes associated with the neuromuscular junction.

GeneID	Symbol	Cluster#	Name	AUC	P-value
29161	Cav3	1	caveolin 3	-1.68	5.29E-13
24588	Nefm	3	neurofilament, medium polypeptide	1.55	3.12E-10
29220	Thbs4	3	thrombospondin 4	1.55	1.45E-08
24511	Itgb1	4	integrin, beta 1	1.85	6.37E-19
54226	App	4	amyloid beta (A4) precursor protein	2.49	4.21E-21
25745	Myh9	5	myosin, heavy chain 9, non-muscle	1.09	4.45E-09
81725	Musk	5	muscle, skeletal, receptor tyrosine kinase	3.85	1.76E-17
79557	Chrna1	5	cholinergic receptor, nicotinic, alpha 1 (muscle)	11.55	9.43E-23
363287	Hdac4	5	histone deacetylase 4	4.39	1.08E-20
25600	Utrn	5	utrophin	1.15	1.79E-06
25439	F2r	5	coagulation factor II (thrombin) receptor	2.57	1.96E-17
306759	Spock1	N.D			
84358	Efna2	N.D			
140908	Cdk5		cyclin-dependent kinase 5	0.24	3.75E-01
29665	P2rx7	N.D			
29192	Psen1		presenilin 1	0.76	3.25E-11
171287	Epha7	N.D			
112400	Nrg1	N.D			
25636	Prkaca		protein kinase, cAMP-dependent, catalytic, alpha	0.19	5.69E-03
362161	Rapsn		receptor-associated protein of the synapse	0.00	7.32E-02
302975	Syngr3	N.D			
361432	Cdh15		cadherin 15	-0.33	1.64E-02
79433	Myh10		myosin, heavy chain 10, non-muscle	0.59	1.41E-05
114124	Akap1		A kinase (PRKA) anchor protein 1	-0.42	4.09E-07
89843	Cxadr	N.D			
25252	Dlg1		discs, large homolog 1 (Drosophila)	-0.54	2.03E-14
64362	Des		desmin	0.32	1.15E-02
316539	Epha4		Eph receptor A4	0.21	5.41E-03
29366	Serpine2		serine (or cysteine) peptidase inhibitor, clade E, member 2	-1.03	6.42E-04
117559	Sv2a	N.D			
117101	Kcnc3	N.D			
24246	Camk2d		calcium/calmodulin-dependent protein kinase II delta	0.19	4.04E-05
116671	Cdk5r1	N.D			

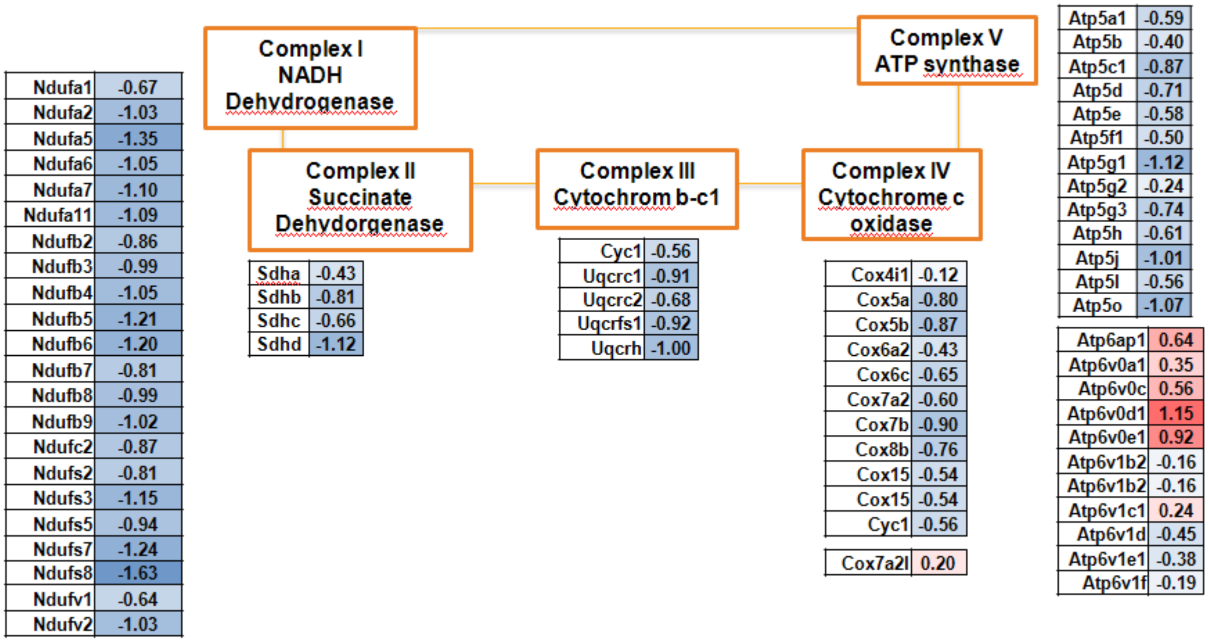
N.D. (not detected).

Figure S1: Weights of the triceps brachii (A) and bulbocavernosus levator anii muscle (B), and the hydroxyproline content of gastrocnemius muscles of 6-, 12-, 18-, 21-, 24- and 27-month old rats. *, **, ***, **** indicate $p < 0.05, 0.01, 0.001, 0.0001$ vs. 6M, respectively ($n = 7\text{-}10$).



(A) (B) (C)

Supplemental Figure S2: Most of the genes in the 5 complexes of the OXPHOS pathway were depressed in sarcopenia. The numbers to the right of the gene symbols are AUC over the time course.



Ndufa1	-0.67
Ndufa2	-1.03
Ndufa5	-1.35
Ndufa6	-1.05
Ndufa7	-1.10
Ndufa11	-1.09
Ndufb2	-0.86
Ndufb3	-0.99
Ndufb4	-1.05
Ndufb5	-1.21
Ndufb6	-1.20
Ndufb7	-0.81
Ndufb8	-0.99
Ndufb9	-1.02
Ndufc2	-0.87
Ndufs2	-0.81
Ndufs3	-1.15
Ndufs5	-0.94
Ndufs7	-1.24
Ndufs8	-1.63
Ndufv1	-0.64
Ndufv2	-1.03

Sdha	-0.43
Sdhb	-0.81
Sdhc	-0.66
Sdhd	-1.12

Cyc1	-0.56
Uqcrc1	-0.91
Uqcrc2	-0.68
Uqcrfs1	-0.92
Uqcrh	-1.00

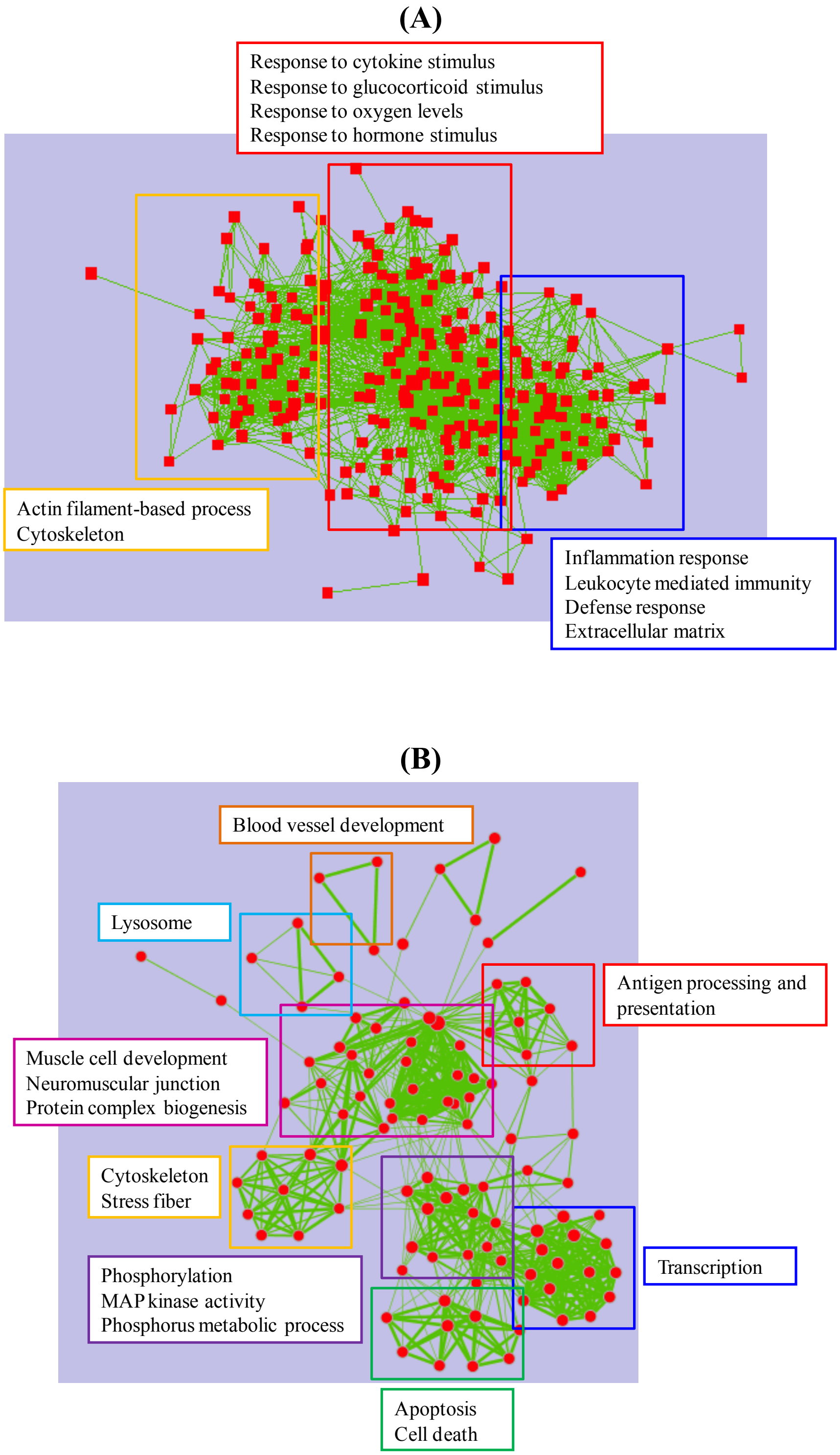
Cox4i1	-0.12
Cox5a	-0.80
Cox5b	-0.87
Cox6a2	-0.43
Cox6c	-0.65
Cox7a2	-0.60
Cox7b	-0.90
Cox8b	-0.76
Cox15	-0.54
Cox15	-0.54
Cyc1	-0.56

Cox7a2l	0.20

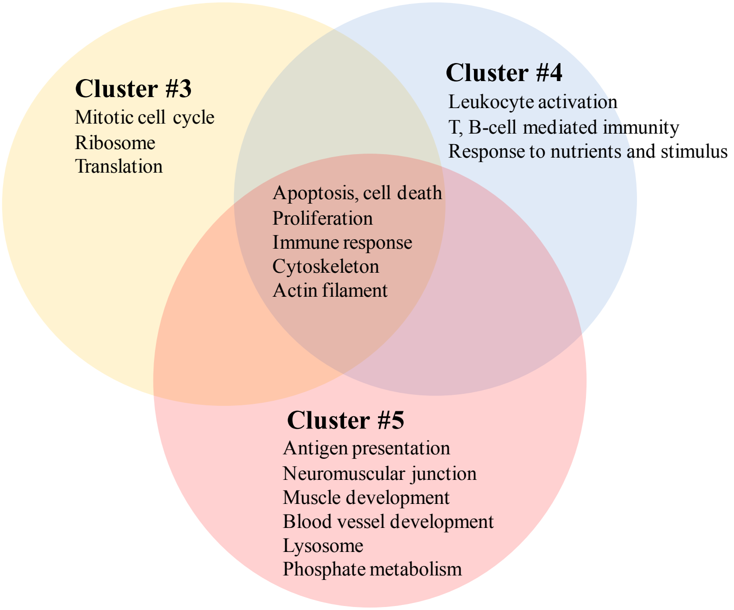
Atp5a1	-0.59
Atp5b	-0.40
Atp5c1	-0.87
Atp5d	-0.71
Atp5e	-0.58
Atp5f1	-0.50
Atp5g1	-1.12
Atp5g2	-0.24
Atp5g3	-0.74
Atp5h	-0.61
Atp5j	-1.01
Atp5l	-0.56
Atp5o	-1.07

Atp6ap1	0.64
Atp6v0a1	0.35
Atp6v0c	0.56
Atp6v0d1	1.15
Atp6v0e1	0.92
Atp6v1b2	-0.16
Atp6v1b2	-0.16
Atp6v1c1	0.24
Atp6v1d	-0.45
Atp6v1e1	-0.38
Atp6v1f	-0.19

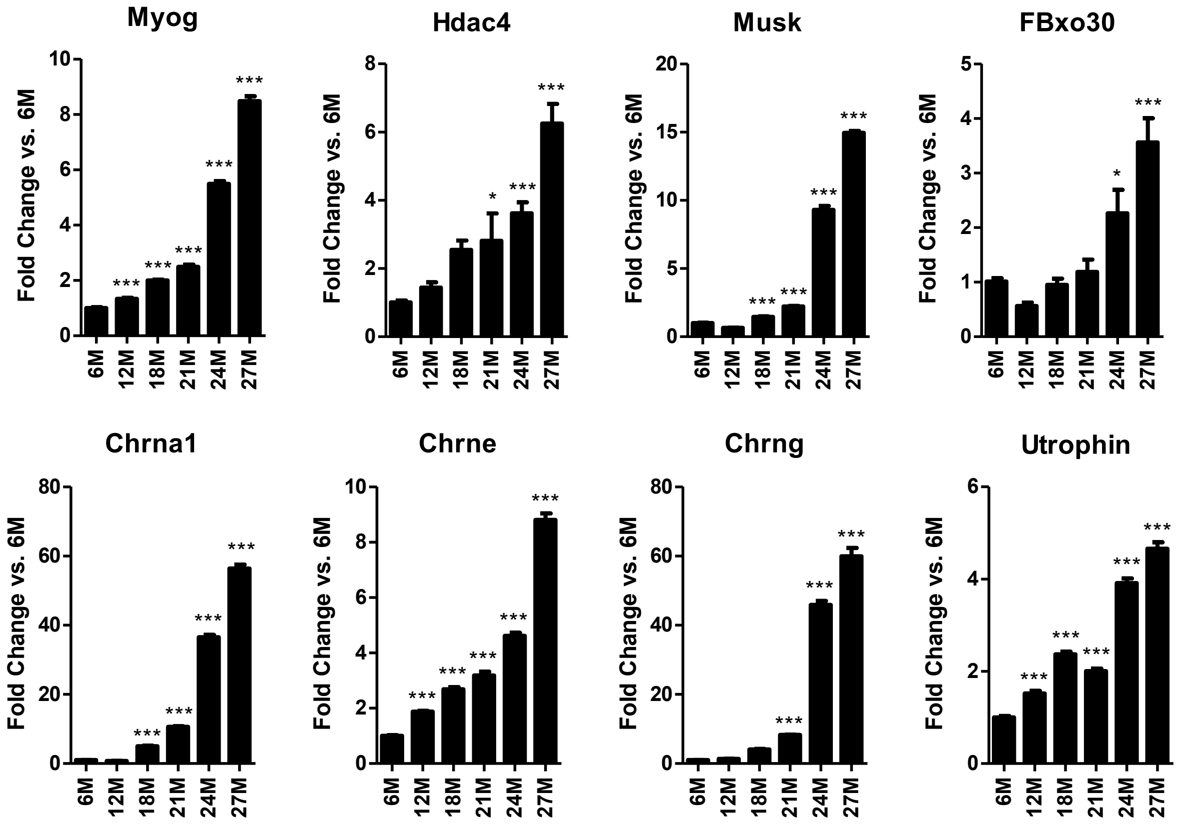
Supplemental Figure S3: Functional annotation analysis of the genes in cluster #4 (A) and cluster #5 (B) and the overlap between genes in clusters #3, #4 and #5 (C). Quantitative PCR verification of changes in expression of select genes in cluster #5 (D). *, **, ***, **** indicate $p < 0.05, 0.01, 0.001, 0.0001$ vs. 6M, respectively (n = 7-10).



(C)


Cluster #3
Mitotic cell cycle
Ribosome
Translation
Cluster #4
Leukocyte activation
T, B-cell mediated immunity
Response to nutrients and stimulus
Apoptosis, cell death
Proliferation
Immune response
Cytoskeleton
Actin filament
Cluster #5
Antigen presentation
Neuromuscular junction
Muscle development
Blood vessel development
Lysosome
Phosphate metabolism

(D)


Myog
Hdac4
Musk
FBxo30
Chrna1
Chrne
Chrng
Utrophin
Fold Change vs. 6M
6M
12M
18M
21M
24M
27M
0
2
4
6
8
10
5
15
20
1
3
20
40
60
80
*
***

Supplemental Figure S4: Quantitative PCR verification of the changes in expression of transcripts for myosin heavy chain (MyHC) isoforms (A) and Ctgf and the E3 ligase, MuRF1 (B) in sarcopenia. *, **, ***, **** indicate $p < 0.05, 0.01, 0.001, 0.0001$ vs. 6M, respectively (n = 7-10).