Supplemental data

Supplemental experimental procedures

HPLC assay:

Briefly, mice were sacrificed by cervical dislocation and hind limbs were immediately (within 1~2 seconds) dropped into the liquid nitrogen for a snap freezing. Skeletal muscle was quickly dissected on a mechanically refrigerated (-40ºC) thermal plate (Sigma, San Diego, CA). 200μl ice cold acetonitrile was added to frozen tissues and tissues were homogenized with a pellet pestle while adding 100 μl ice-cold ddH_2O. The homogenate was centrifuged at 14,000 rpm, 4°C for 15 min and 150μl supernatant was added with 150μl ddH_2O, centrifuged again and ready for HPLC assay. Standards were prepared exactly in the same way. 50 μl of the preparation was injected to a HPLC system which comprises a Perkin Elmer M-250 binary LC pump (Norwalk CT), a Waters 717 plus autosampler, a Waters 490 programmable multi-wavelength UV detector (Milford, MA) and an ESA 501 chromatography data process system (Chelmsford, MA). The gradient elution was performed on a TSK-GEL HPLC column (Tosoh Biosep, Japan) with a mobile phase rate of 1ml/min by two buffers. Buffer A contained 25mM NaH_2PO_4, 100mg/L tetrabutylammonium with pH5.5, whereas organic buffer B was 10%(v/v) acetonitrile made in a buffer of 200mM NaH_2PO_4, 100mg/L tetrabutylammonium, pH 4.0. The UV detector was programmed 210 nm from 0 to14 min for detecting creatine and phosphocreatine which appeared at about 3.7 and 10 minutes respectively and 260 nm from 10 to 40 min for AMP, ADP and ATP which were eluted out at about 18, 22 and 25 min respectively. The standard curve of each compound was constructed by plotting peak heights (mV) vs. concentrations range from 10~1000 μM for creatine and phosphocreatine and 5~500 μM for ATP and its metabolites. The quantification was carried out using the external standard calibration. Protein concentrations were measured by using BCA protein assay kit (Pierce, USA). Data were expressed as nmol/mg protein.

Transmission Electron Microscopy:

Soleus muscle were fixed for 1h in 2.5% glutaraldehyde, 4% paraformaldehyde, 0.02% picric acid in 0.1M sodium cacodylate buffer, followed by washing with sodium cacodylate buffer for 3 times. Muscles were post-fixed in 1% OsO_4-1.5% aqueous K-ferricyanide for 60 min and

stained with 1.5% aqueous Uranyl acetate for 30 min. Samples were then dehydrated through a graded ethanol series, infiltrated, and embedded in Spurr's resin (Electron Microscopy Sciences, Fort Washington, PA, USA). Sections were cut at 55-60 nm (silver-gold) using a Diatome diamond knife-(Diatome, USA, Hatfield, PA) on an RMC MT-7000 Ultra-microtome (Boeckeler-RMC Instruments, Tucson, AZ). Sections were contrasted with lead citrate and viewed on a JSM 100 CX-II electron microscope (JEOL, USA, Inc., Peabody, MA) operated at 80 kV. Images were recorded on Kodak 4489 Electron Image film (Eastman Kodak Company, Rochester, NY, USA) then digitized on an Epson Expression 1600 Pro scanner (Seiko-Epson Corporation, Suwa, Japan) at 900 dpi for publication. Pictures were taken at a magnification of 4,800 and 19,000X. Density and number of mitochondria was analyzed using ImageJ 1.38x image analysis software (NIH, Bethesda, MD) and expressed as % mitochondrial area.

Western blot:

Tissue samples from the skeletal muscle of WT and HD mouse and myoblast cultures were homogenized in cell extraction buffer containing 50mM Tris-HCl, pH 7.4, 150mM NaCl, 2mM EDTA, 1% SDS, 0.5% NP-40, 0.5% deoxycholate supplemented with protease and phosphatase inhibitors (Sigma). Samples were homogenized, sonicated for 5 seconds and centrifuged at 4000g for 30 min. Protein concentrations of the supernatant were determined using the BCA protein assay, as per the manufacturer's recommended protocol. Equal amounts of protein (45μg) were loaded on to a 4-20% Tricine gel (Invitrogen). Membranes were then blocked for 1h at room temperature in Tris-buffered saline/Tween-20 (TBST) (50mM Tris-HCl, 150mM NaCl, pH 7.4, 1% Tween-20) containing 5% non-fat dried milk. The membranes were incubated overnight at 4^{o}C for PGC-1α (1:2,000, kind gift of Dr. B.M. Spiegelman), AMPK (1:500, Santa Cruz; CA), pAMPK Thr 172 (1:500, Santa Cruz; CA), and β-actin (1:10,000, Chemicon). Membranes were then washed three times with TBST and incubated for 1h with HRP-conjugated secondary antibody and the immunoreactive proteins detected using a chemiluminescent substrate (Pierce, USA) according to the manufacturer's instructions. Films were scanned by film processor (Konica Minolta & Graphic Inc, SRX-101A, USA). Protein expression was quantified using Scion Image for Windows (NIH, USA).

Myosin heavy chain (MHC) immunohistochemistry

In brief, 10μm thin sections of soleus muscle were placed onto slides. Commercial antibodies to fast and slow isoforms of myosin were used, each with a different visualization system for specific identification of each fiber type on the same section. After fixation in acetone for 10 min, sections were incubated with monoclonal antibody to skeletal slow anti-myosin NOQ 7.5.4D (1:2,000, Sigma, St Louis, MO, USA) for 30 min, followed by a peroxidase conjugated rabbit anti-mouse secondary antibody (DAKO, USA) for 1h. Results were visualized as dark immunoreactive black type 1 fibers using the commercial Vector SG substrate kit (Vector Labs, USA). The alkaline phosphatase conjugated monoclonal antibody to skeletal fast anti-myosin MY-32 (1:50, Sigma, St Louis, MO, USA) was then applied for 1h, and red type 2 fibers were visualized using the Vector red alkaline phosphatase substrate kit (Vector Labs, USA).

Histochemical staining for succinate dehydrogenase (SDH)

For SDH staining GM were excised and dissected immediately and snap frozen in isopentane upon mouse sacrifice. 10μm thin sections were cut in a cryostat and mounted on cover slip. Sections were then incubated at 37^0C for 30 min in 0.1 M Tris buffer containing 0.2g sodium succinate, 0.0005g Phenazine methosulphate and 0.02g Nitroblue tetrazolium, followed by extraction in 30%, 60%, 90%, 60% and 30% acetone in sequence. After 3X wash with PBS, sections were mounted with Vectashield Hard Set Mounting medium (Vector H-1400, USA).

Rotarod performance

A rotarod apparatus (Columbus Instruments, Columbus, OH) was used to measure forelimb and hindlimb motor coordination and balance. The rotarod testing was carried out at last week of GPA treatment (26 weeks of age) in the WT and Tg mice. The mice were trained for 3 days on the rotarod at 5 rpm for up to 300 sec, before being tested for 4 days. WT and Tg mice were then placed on an accelerating rotarod (0.1rpm/sec) from 2 to 20 rpm over 180 sec and maintained at 20 rpm up to 300 sec for 4 days of testing with 3 trials per day. To avoid fatigue, animals were rested for 10 min between each trial and the best time from 3 trials for each day was used in the analysis.

Measurement of myoblast oxygen consumption

For oxygen consumption measurement, GPA treated and untreated myoblast cells were fluid-changed with fresh medium 1 h before performing the assays. Cells were collected by mild trypsinization, pelleted by centrifugation, counted, and resuspended at 2×10^6 cells/ml in Dulbecco's modified Eagle's medium lacking glucose and pyruvate at 37 °C. A 0.5 ml of cell suspension (1×10^6 cells) was transferred into an oxygraph chamber equipped with a Clark-type electrode (Hansatech). Measurements were performed at basal level and in the presence of pyruvate and dinitrophenol (DNP).

Gene Expression Analysis by RT-PCR

After indicated treatments, tissues were harvested and frozen immediately. Total RNA was isolated from frozen WT and HD mouse skeletal muscle and from cultured myoblast using TriZol Reagent. Genomic DNA was removed using RNase free DNase (Ambion). RNA pellets were resuspended in DEPC-treated water (Ambion). Total RNA purity and integrity was confirmed by ND-1000 NanoDrop (NanoDrop Technologies) and 2100 Bioanalyzer (Agilent) respectively with average 260/280 ratios for all study samples ranging from 1.9 to 2.1 and average RIN numbers ranging from 5.0 to 7.5. All qPCR plating was performed on ice. Real-time RT-PCR was performed using the ABI prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA) based on the 5'-nuclease assay. Serial dilutions of 320ng/well, 32 ng, and 3.2 ng of total RNA were plated with two representative genes and housekeeping gene β-actin or 18sRNA. Probe cleavage was consistently observed with 320ng/well, with average Ct value of 30, for the two representative genes. Consistent amplification was observed in all three-dilution points for β-actin or 18sRNA. One-step, singleplex, and triplicates of each sample were plated. Working solution of 160ng/µl was prepared for each sample and 2.5µl of this solution, making 400ng/ qPCR well, was distributed to each triplicate qPCR well. A master-mix comprised of One step RT qPCR MasterMix Plus reagents, (no AmpErase UNG, RTQPRT- 032X, EuroGentec, San Diego, CA), 20X TaqMan Gene Expression Assay (Applied Biosystems) and Molecular Biology Grade H_2O (HyClone) was prepared for each target gene and 5.5µl of this Primer-Probe master-mix was added to the PCR well totaling 8µl qPCR reaction volume. Thermal cycling conditions were 48ºC for 30min, 95ºC for 10 min and then 45 cycles of 95º C for 15 sec and 60º C for 1 min. Relative expression was calculated using the ΔΔCt method. Human specific primer sequences and

mouse specific TaqMan based gene expression assays used for RT-PCR experiments are listed in supplemental data Table S8.

Supplementary Table S1: Gene set enrichment analysis

Groups	Significantly enriched gene pathways
WT+NS	Apoptotic DNA fragmentation and tissue homeostasis pathway [Biocarta], D4-GDI signaling pathway pathway [Biocarta], TGF-beta signaling pathway[KEGG], Olfactory transduction [KEGG], Regulation of p27 phosphorylation during cell cycle progression pathway[Biocarta], Overview of telomerase RNA component gene HTERC transcriptional regulation pathway [Biocarta], Heparan sulfate biosynthesis[KEGG], Focal adhesion [GENMAPP], Monocyte and its surface molecules pathway [Biocarta], Reelin signaling pathway pathway [Biocarta]
HD+NS	Type II diabetes mellitus [KEGG], HIV induced T cell apoptosis pathway [Biocarta], Regulation of EIF4E and P70 S6 kinase pathway [Biocarta], G-secretase mediated ERBB4 signaling pathway pathway [Biocarta], Acetylcholine synthesis [GENEMAPP], Role of EGF receptor transactivation by GPCRS in cardiac hypertrophy pathway [Biocarta] Malate-aspartate shuttle pathway [Biocarta]
WT+GPA	Glycolysis / gluconeogenesis [KEGG] Ribosomal biogenesis pathway [Biocarta] G-secretase mediated ERBB4 signaling pathway pathway [Biocarta] Glycolysis and gluconeogenesis [GENMAPP] Muscle, fat and connective tissue specific genes[GENMAPP] Electron transport chain [GENMAPP] Type-II diabetes mellitus [KEGG] Striated muscle contraction [GENMAPP] Oxidative phosphorylation [KEGG]
HD+GPA	Cell to cell adhesion signaling pathway [Biocarta] ADP-ribosylation factor pathway [Biocarta] Activation of SRC by protein-tyrosine phosphatase alpha pathway [Biocarta] Apoptosis modulation by HSP 70 [GENMAPP] Gap junction [KEGG] Adherens junction [KEGG] Tight junction [KEGG] Cell cycle: G2/M checkpoint pathway [Biocarta] Overview of telomerase protein component gene HTERT transcriptional regulation pathway [Biocarta] HIV-I NEF: negative effector of FAS and TNF pathway [Biocarta] Inositol phosphate metabolism [KEGG]

Supplementary table S2: Affymetrix microarray analysis of Tricarboxylic acid cycle (TCA) genes

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Idh3a	1447701_x_at	isocitrate dehydrogenase 3 (NAD+) alpha	1.27	0.218	1.54	0.0988
Idh3g	1416789_at	isocitrate dehydrogenase 3 (NAD+), gamma	1.04	0.766	-1.04	0.598
Idh1	1419821_s_at	isocitrate dehydrogenase 1 (NADP+), soluble	1.12	0.427	-1.11	0.306
Acly	1439459_x_at	ATP citrate lyase	-1.09	0.684	1.49	0.0345
	1439445_x_at		1.75	0.522	-4.79	0.05
Fh1	1424828_a_at	fumarate hydratase 1	1.11	0.448	-1.03	0.658
Mdh1	1438338_at	malate dehydrogenase 1, NAD (soluble)	1.20	0.565	-1.29	0.175
Mdh1b	1429842_at	malate dehydrogenase 1B, NAD (soluble)	-1.33	0.615	1.18	0.732
Pdha2	1450962_at	pyruvate dehydrogenase E1 alpha 2	2.53	0.175	-1.16	0.855
Sdhd	1437489_x_at	succinate dehydrogenase complex, subunit D	1.07	0.562	1.18	0.189
Sdhc	1435986_x_at	succinate dehydrogenase complex, subunit C, iron sulfur (Ip)	1.22	0.416	-1.27	0.121
Sdhb	1418005_at	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	1.11	0.454	-1.22	0.044
Suclg2	1427441_a_at	succinate-Coenzyme A ligase, GDP-forming, beta subunit	1.10	0.52	1.18	0.05
Sucla2	1430402_at	succinate-Coenzyme A ligase, ADP-forming, beta subunit	-1.35	0.64	-1.01	0.97
Sdha	1445317_at	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	-1.15	0.353	-1.10	0.742
Aco1	1423644_at	aconitase 1	1.09	0.623	1.22	0.05
Aco2	1442102_at	aconitase 2, mitochondrial	-1.05	0.845	-1.16	0.48
Csl	1449400_at	citrate synthase like	-1.06	0.852	-1.43	0.0425
Cs	1422578_at	citrate synthase	1.02	0.86	-1.01	0.81

Supplementary table S3: Affymetrix microarray analysis of glycolysis genes

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Pgam2	1418373_at	phosphoglycerate mutase 2	-1.07	0.534	-1.32	0.0147
Pgam1	1426554_a_at	phosphoglycerate mutase 1	-1.29	0.0379	1.45	0.0365
Hk1	1437974_a_at	hexokinase 1	-1.11	0.621	1.27	0.0389
Hk2	1422612_at	hexokinase 2	-1.16	0.174	-1.22	0.247
Aldoa	1423498_at	aldolase 1, A isoform	1.41	0.0138	-1.74	0.0034
	1434799_x_at		1.11	0.418	-1.28	0.05
Ldha	1419737_a_at	lactate dehydrogenase A	1.09	0.478	-1.32	0.0314
Ldhb	1434499_a_at	lactate dehydrogenase B	-1.02	0.955	-1.20	0.682
Gpi1	1450081_x_at	glucose phosphate isomerase 1	1.00	0.975	-1.24	0.0483
Slc25a3	1440302_at	solute carrier family 25 (mitochondrial carrier, phosphate carrier)	1.24	0.309	-1.55	0.0337
Slc2a3	1427770_a_at	solute carrier family 2 (facilitated glucose transporter), member 3	1.16	0.865	4.91	0.0111
Eno3	1417951_at	enolase 3, beta muscle	1.12	0.439	-1.22	0.05
Pkm2	1417308_at	pyruvate kinase, muscle	1.04	0.714	-1.14	0.106
Pfkm	1416780_at	phosphofructokinase, muscle	1.03	0.803	-1.31	0.0332
Ogdh	1445632_at	oxoglutarate dehydrogenase (lipoamide)	-1.07	0.661	-1.07	0.719
Hkdc1	1425900_at	hexokinase domain containing 1	-1.49	0.0434	-1.55	0.106
Pfkl	1453541_at	phosphofructokinase, liver, B-type	1.32	0.455	1.29	0.391
Pdha1	1449137_at	pyruvate dehydrogenase E1 alpha 1	1.01	0.965	-1.07	0.666
Pklr	1438711_at	pyruvate kinase liver and red blood cell	1.33	0.37	-1.54	0.0248
Lrrc16	1446892_at	Leucine rich repeat containing 16	-3.37	0.0494	1.67	0.191
Ldhal6b	1434247_at	lactate dehydrogenase A-like 6B	-1.35	0.561	-1.82	0.118
Tpi1	1452927_x_at	triosephosphate isomerase 1	1.07	0.495	-1.17	0.0416

Supplementary table S4: Affymetrix microarray analysis of fatty acid metabolism genes

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Acot9	1449968_s_at	acyl-CoA thioesterase 9 /// acyl-CoA thioesterase 10	1.34	0.0153	1.47	0.00885
Acot12	1419395_at	acyl-CoA thioesterase 12	-1.09	0.948	-2.59	0.0215
Cpt2	1447820_x_at	carnitine palmitoyltransferase 2	1.23	0.595	-1.38	0.033
Acox2	1420673_a_at	acyl-Coenzyme A oxidase 2, branched chain	1.65	0.591	-1.60	0.597
Acsl1	1447355_at	acyl-CoA synthetase long-chain family member 1	-1.34	0.746	-5.57	0.0193
Adipoq	1447582_x_at	Adiponectin, C1Q and collagen domain containing	3.77	0.0144	1.06	0.849
Slc27a2	1416316_at	solute carrier family 27 (fatty acid transporter), member 2	1.42	0.697	-2.70	0.0309
Slc27a3	1427180_at	solute carrier family 27 (fatty acid transporter), member 3	-1.28	0.666	-1.42	0.0402
Slc27a4	1424441_at	solute carrier family 27 (fatty acid transporter), member 4	-1.12	0.218	1.15	0.288
Slc27a5	1449112_at	solute carrier family 27 (fatty acid transporter), member 5	1.68	0.575	-4.66	0.0216
Cryl1	1459589_at	crystallin, lamda 1	2.22	0.0331	1.04	0.956
Acox3	1437352_at	acyl-Coenzyme A oxidase 3, pristanoyl	-1.06	0.838	-1.49	0.0337
Acsl3	1452771_s_at	acyl-CoA synthetase long-chain family member 3	-1.03	0.931	3.49	0.00148
Acsl4	1433531_at	acyl-CoA synthetase long-chain family member 4	-1.00	0.988	1.39	0.204
Scap	1446568_at	SREBP cleavage activating protein	2.19	0.0402	-2.05	0.0189
Cpt1c	1435281_at	carnitine palmitoyltransferase 1c	-1.56	0.476	1.88	0.0159
Elovl2	1416444_at	elongation of very long chain fatty acids-like 2	2.00	0.445	-3.13	0.0221
Cyp4a10	1424853_s_at	cytochrome P450, family 4, subfamily a, polypeptide 10	2.54	0.334	-2.67	0.0214
Cyp4a12	1424352_at	cytochrome P450, family 4, subfamily a, polypeptide 12	-6.15	0.0128	1.29	0.859
Gpam	1425834_a_at	glycerol-3-phosphate acyltransferase, mitochondrial	-1.15	0.494	1.19	0.0235
Ankrd23	1428183_at	ankyrin repeat domain 23	-1.12	0.302	-1.51	0.024

Supplementary table S5: Affymetrix microarray analysis of fatty acid biosynthesis genes

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Pecr	1439167_at	peroxisomal trans-2-enoyl-CoA reductase	1.45	0.547	1.83	0.275
Prkag2	1451140_s_at	protein kinase, AMP-activated, gamma 2 non-catalytic	1.21	0.221	1.79	0.05
Scd3	1450956_at	stearoyl-coenzyme A desaturase 3	2.94	0.052	1.02	0.956
Olah	1424855_at	oleoyl-ACP hydrolase	-1.35	0.657	-2.46	0.165
Mecr	1417097_at	mitochondrial trans-2-enoyl-CoA reductase	-1.09	0.614	1.20	0.0332
	1417098_s_at	mitochondrial trans-2-enoyl-CoA reductase	1.18	0.18	1.27	0.0285
Fads2	1419031_at	fatty acid desaturase 2	-1.88	0.0457	1.16	0.715
Fads3	1418773_at	fatty acid desaturase 3	1.52	0.55	2.58	0.0278
Fasn	1423828_at	fatty acid synthase	1.30	0.585	1.18	0.535
Acly	1439445_x_at	ATP citrate lyase	1.75	0.522	-4.79	0.05
Pdk3	1426410_at	pyruvate dehydrogenase kinase, isoenzyme 3	-4.36	0.0667	2.25	0.163

Supplementary table S5: Affymetrix microarray analysis of myogenic differentiation and early and late myogenic markers

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Myod1	1418420_at	myogenic differentiation 1	-1.12	0.678	-1.03	0.915
Myog	1419391_at	myogenin	1.36	0.204	-1.17	0.0449
Usmg2	1427781_at	upregulated during skeletal muscle growth 2	-1.19	0.0483	-2.90	0.0151
Des	1426731_at	desmin	1.02	0.887	-1.07	0.421

Supplementary table S7: Affymetrix microarray analysis of muscle specific genes expression

			HD+NS vs WT+NS		HD+GPA vs WT+GPA	
Gene symbol	**Probe set ID**	**Gene name**	**Fold change**	**p value**	**Fold change**	**p value**
Myh1	1427868_x_at	myosin, heavy polypeptide 1, skeletal muscle, adult	-1.36	0.05	-1.25	0.111
	1427520_a_at		1.08	0.551	-1.30	0.0493
	1427867_at		1.02	0.918	-1.17	0.273
Myh4	1458368_at	myosin, heavy polypeptide 4, skeletal muscle	-1.23	0.423	-1.60	0.153
Mb	1451203_at	myoglobin	-1.08	0.572	-1.23	0.0144
Tmod4	1449969_at	tropomodulin 4	-1.02	0.89	-1.28	0.032
Tmod2	1437167_at	tropomodulin 2	1.13	0.722	-1.83	0.446
Ttn	1427446_s_at	titin	1.06	0.675	-1.25	0.0491
	1431928_at		-1.20	0.383	-1.33	0.0028
Mybph	1419487_at	myosin binding protein H	1.21	0.793	-1.61	0.239
Tpm1	1423049_a_at	tropomyosin 1, alpha	1.11	0.491	-1.28	0.0393
Mylk2	1427556_at	myosin, light polypeptide kinase 2, skeletal muscle	-1.23	0.377	-1.82	0.0178
Lmod1	1427485_at	leiomodin 1 (smooth muscle)	1.49	0.19	-2.88	0.0148
Ckm	1417614_at	creatine kinase, muscle	1.11	0.437	-1.25	0.05
Ckmt1	1432418_a_at	creatine kinase, mitochondrial 1, ubiquitous	-1.18	0.637	-1.44	0.628
Actn3	1418677_at	actinin alpha 3	1.09	0.477	-1.30	0.044
Actn4	1423449_a_at	actinin alpha 4	-1.20	0.26	1.31	0.0406
Vim	1450641_at	vimentin	-1.28	0.295	1.14	0.525
Actn4	1423449_a_at	actinin alpha 4	-1.20	0.26	1.31	0.0406
Actn3	1418677_at	actinin alpha 3	1.09	0.477	-1.30	0.044
Myo6	1435559_at	myosin VI	1.16	0.804	2.37	0.0245
Myo5a	1431320_a_at	myosin Va	-1.24	0.402	1.79	0.0395
Mybpc2	1455736_at	myosin binding protein C, fast-type	1.05	0.65	-1.35	0.0389
Myohd1	1459265_at	myosin head domain containing 1	1.13	0.595	-1.49	0.0424
Myo1d	1447006_at	Myosin ID	-1.02	0.94	-1.84	0.05
	1459102_at		1.86	0.217	-3.31	0.0382
	1446620_at		-1.84	0.049	-1.07	0.801
	1444515_at		-2.33	0.044	1.27	0.255
Myo1e	1449941_at	myosin IE	-2.59	0.0465	1.74	0.379
Sepw1	1416521_at	selenoprotein W, muscle 1	1.14	0.326	-1.18	0.0357
Casq1	1422598_at	calsequestrin 1	-1.04	0.742	-1.40	0.0156
Pde6h	1450766_at	phosphodiesterase 6H, cGMP-specific, cone, gamma	1.66	0.473	-2.41	0.0361
Pde4b	1442700_at	phosphodiesterase 4B, cAMP specific	-1.96	0.0048	-1.50	0.132
Lsp1	1417756_a_at	lymphocyte specific 1	-1.57	0.0541	-1.33	0.0527
Cacnb1	1426108_s_at	calcium channel, voltage-dependent, beta 1 subunit	1.03	0.885	-1.24	0.0094
Pdlim3	1449178_at	PDZ and LIM domain 3	-1.08	0.652	-1.24	0.0283
Denr	1449598_at	Density-regulated protein	-2.63	0.179	3.27	0.0166
Dusp9	1433844_a_at	dual specificity phosphatase 9	-1.20	0.674	-2.25	0.042
Dusp6	1415834_at	dual specificity phosphatase 6	-1.44	0.0408	-1.00	0.986
Dusp16	1440651_at	Dual specificity phosphatase 16	-2.80	0.0399	-1.72	0.471
Chrnb1	1420682_at	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)	1.22	0.44	1.53	0.0373
Cfl2	1418067_at	cofilin 2, muscle	1.14	0.332	-1.11	0.0395
Eno3	1417951_at	enolase 3, beta muscle	1.12	0.439	-1.22	0.05
Pfkm	1416780_at	phosphofructokinase, muscle	1.03	0.803	-1.31	0.0332
Mbnl3	1441530_at	Muscleblind-like 3 (Drosophila)	-1.25	0.739	-5.04	0.0092
Capza2	1423058_at	capping protein (actin filament) muscle Z-line, alpha 2	-1.18	0.0463	1.00	0.977
Gem	1426063_a_at	GTP binding protein (gene overexpressed in skeletal muscle)	-1.48	0.015	-1.14	0.653
Tnni1	1450813_a_at	troponin I, skeletal, slow 1	-1.12	0.601	-1.59	0.0398
Tnni2	1438609_x_at	troponin I, skeletal, fast 2	1.11	0.343	1.64	0.0422
Flna	1426677_at	filamin, alpha	-1.68	0.253	1.15	0.415

Supplementary table S8:

Primer sequences used for quantitative Real time PCR

Gene name	GeneSequence	Forward primer	Reverse primer
Primer sequence:			
PGC-1α (H)	NM_013261	tgagagggccaagcaaag	ataaatcacacggcgctctt
PGC-1β (H)	NM_133263	ggcaggcctcagatctaaaa	tcatgggagccttcttgtct
NRF-1 (H)	NM_005011	ccatctggtggcctgaag	gtgcctgggtccatgaaa
TFAM (H)	NM_003201	gaacaactacccatatttaaagctca	gaatcaggaagttccctcca
PPRC1 (H)	NM_015062.3	attttgggagccttggaga	tgagcagcgacacttcattc
PPARA (H)	NM_001001928.2	gcactggaactggatgacag	tttagaaggccaggacgatct
PPARG (H)	NM_138711.3	gacctgaaacttcaagagtaccaaa	tgaggcttattgtagagctgagtc
ALAS1 (H)	NM_000688.4	gaaatgaatgccgtgaggaa	cctccatcggttttcacact
β-actin (H)	NM_001101.2	ccaaccgcgagaagatga	ccagaggcgtacagggatag
CYT C (H)	NM_018947.4	tggagttttgccatgtggt	gagccaaggcaagtggac
COX-IV (H)	BC_021236.2	caccgcgctcgttatcat	tggccacccactctttgt
Myoglobin (H)	NM_005368	cagttggtgctgaacgtctg	ggtgacccttaaagagcctga
MYHC I	NM_030679.1	aatcaaaggtcaaggcctacaa	gaatttggccaggttgacat
MYHC IIA (M)	NM_001039545.1	aacttcaggcaaaagtgaaatctt	gctagattggtgttggattgttc
MYHC IIX/D (M)	DQ021873.1	ggtcgaagttgcatccctaa	ttccggaggtaaggagcag
MYHC IIB (M)	AY963801.1	ctcaaacccttaaagtacttgtctga	ctattggtggcagctcagg
TNNI1 (slow) (H)	NM_003283	ttgactacatgggggaggaa	gacagctcctgggctttct
TNNI2 (fast) (H)	NM_003282.2	gctccaagctcaggacctc	gctatctgcagcatcacactct

H=Human, M= Mouse

Mouse Sequence (ABI TaqMan Gene expression assays)

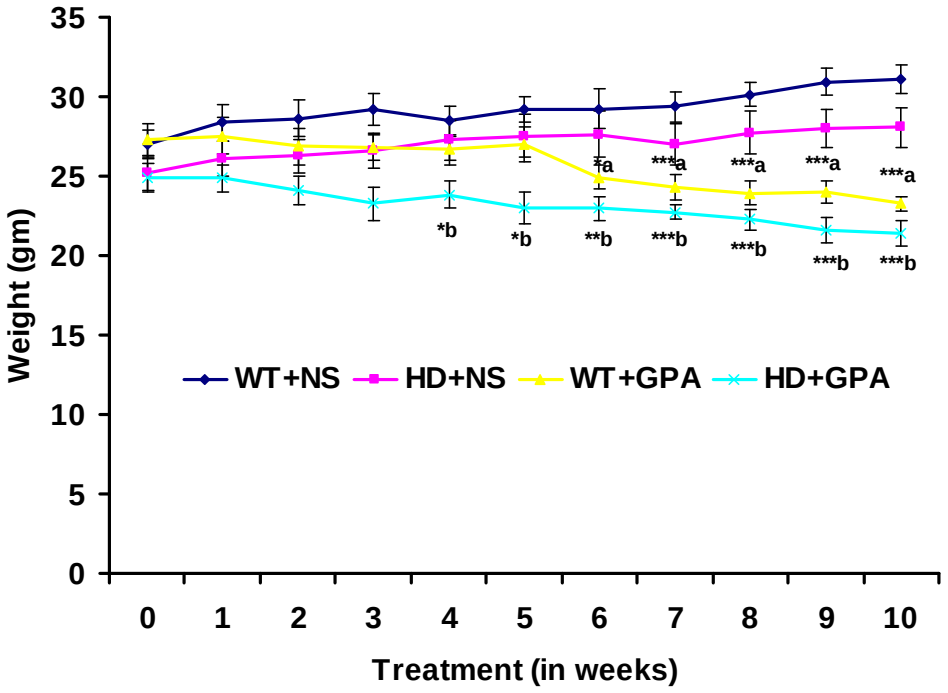
Gene name	TaqMan probe set ID
PGC-1α	Mm00447183_m1
PGC-1β	Mm00504720_m1
NRF-1	Mm00447996_m1
NRF-2	Mm00477784_m1
TFAM	Mm00447485_m1
AMPK	Mm01264787_m1
CREB	Mm00501607_m1
ERR-α	Mm00433143_m1
COX-IV	Mm00446387_m1
Troponin I, skeletal fast	Mm00437157_g1
Troponin I, skeletal slow	Mm00502426_m1
Myoglobin	Mm00442968_m1
PPARα	Mm00440939_m1
PPARδ	Mm00803186_m1
CYTC	Mm01621044_g1

Supplementary table S9:

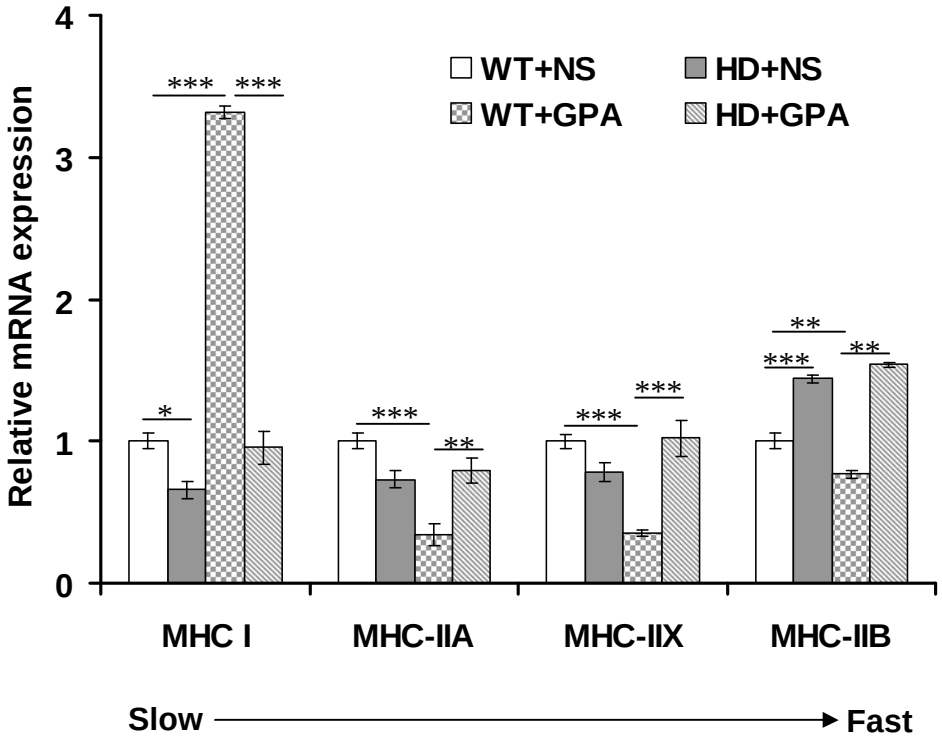
Demographic and genetic data of control subjects and HD patients. Muscle biopsies were obtained from control subjects and HD patients and used for myoblast culture.

Muscle biopsy code	Genotype	Age in years (at biopsy)	Gender	CAG repeats
HD21401	HD	68	M	18/40
HD25401	HD	62	F	10/42
HD17801	HD	36	M	16/49
HD21102	HD	32	M	17/58
HD17803	HD	46	M	17/51
HD7411	HD	58	M	10/44
HD16101	HD	67	M	17/42
Ravera	HD	40	M	18/48
Control 8022	Control	36	M	-
Control 7931	Control	50	M	-
Control 7865	Control	48	M	-
Control 8413	Control	68	F	-
Control 7843	Control	61	F	-
Sebastiano	Control	27	M	-
Valentino	Control	48	M	-
Cardio1	Control	51	F	-

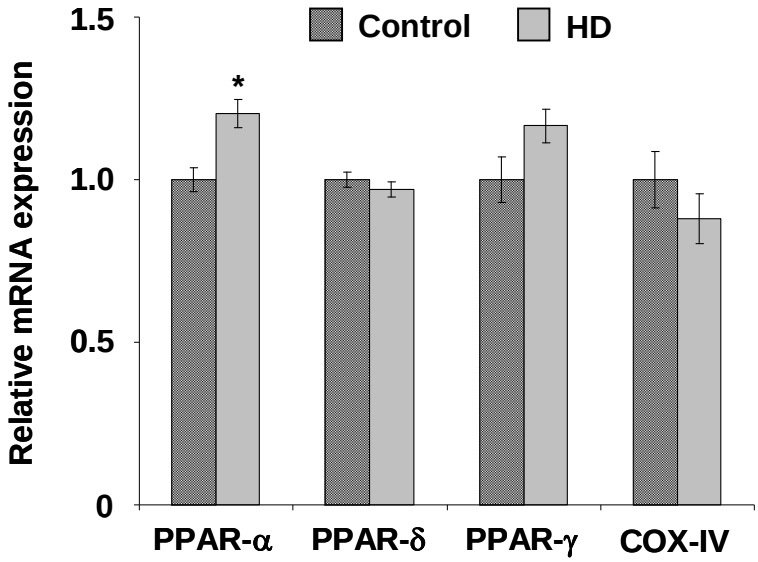
Supplementary Figure S1:



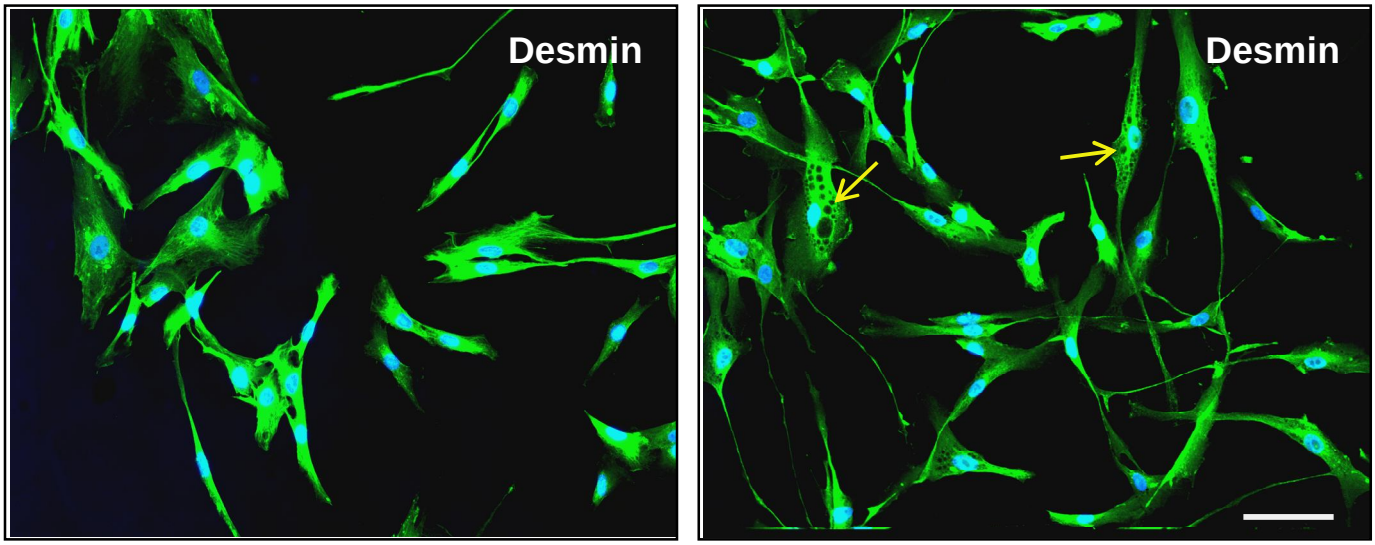
Supplementary Figure S2:



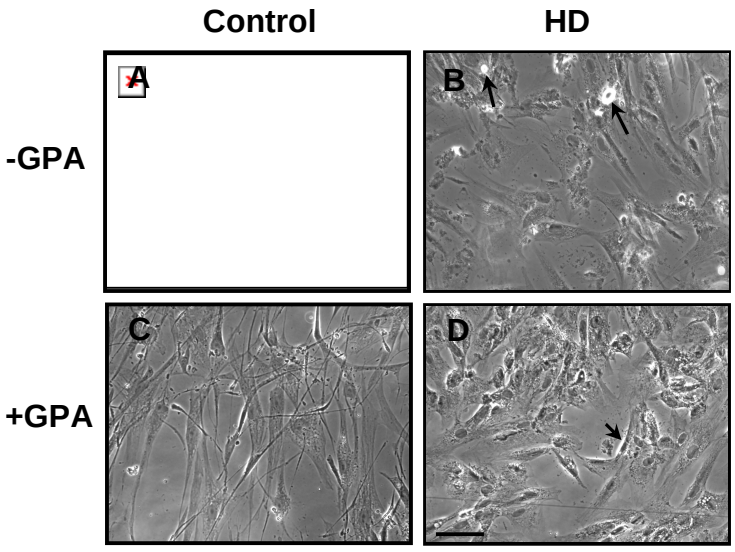
Supplementary Figure S3:



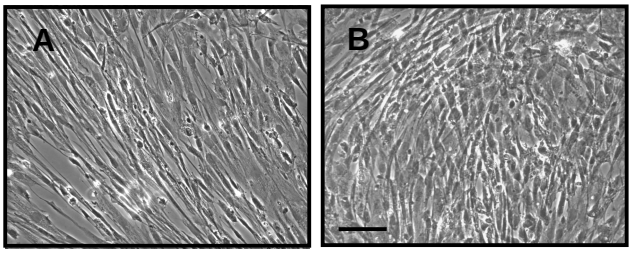
Supplementary Figure S4:



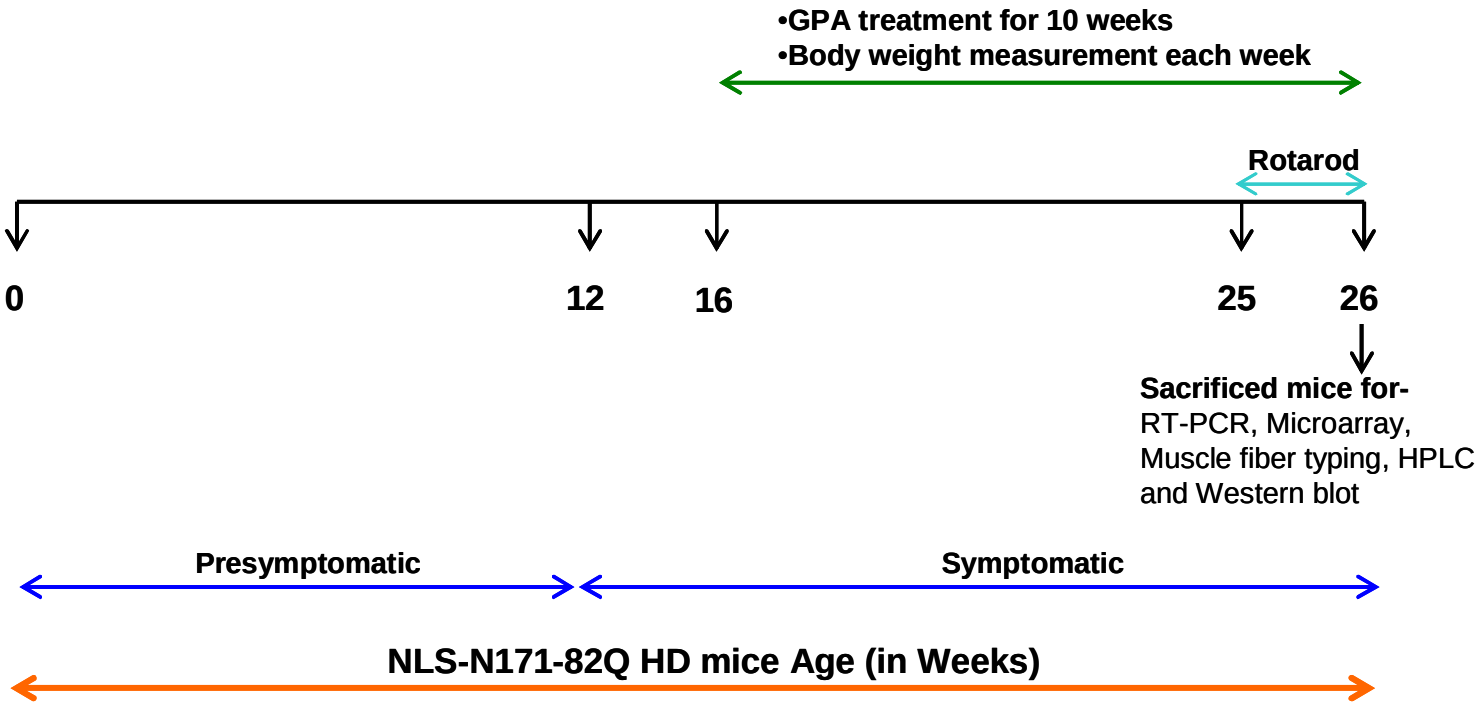
Supplementary Figure S5:



Supplementary Figure S6:



Supplementary Figure S7: Time points for experiments



Supplemental figures

Figure S1: Body weight of WT and NLS-N171-82Q HD mice treated with either normal saline (NS) or catabolic stressor β-guanidinopropionic acid (GPA). GPA treatment caused significant reduction in body weight in WT and HD mice. *p<0.05, **p<0.01, ***p<0.001. a=versus WT+NS and b=versus HD+NS mice. (n=9-11 mice /group)

Figure S2: Relative mRNA expression of different isoforms of Myosin heavy chain (MHC) in soleus muscles from WT and NLS-N171-82Q HD mice treated with NS or GPA. mRNA levels were normalized to β–actin. Data are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 (n=5 mice in each group).

Figure S3: Quantitative real-time PCR analysis in RNA isolated from muscle biopsies from symptomatic human HD patients and matched control subjects. Relative mRNA expression of PPAR-α, δ, γ and COXIV was measured by normalizing values to β-actin. (n=9 control subjects and n=13 HD patients), Data are expressed as mean ± SEM. *p<0.05.

Figure S4: Characterization of myoblast culture derived from human muscle biopsies of control subjects and HD patients. Myoblast cultures were immunostained with muscle specific marker desmin. Arrows indicate the presence of apoptotic vacuoles in HD myoblast. (Scale bar=50μm)

Figure S5: Morphological analysis of proliferating myoblasts cultures derived from muscle biopsies from control subjects and symptomatic HD patients. Proliferating myoblasts were grown in presence of 15% fetal bovine serum. Morphological characterization of control (A) and HD (B) myoblasts revealed an irregular morphology and presence of vacuoles in HD myoblast as compared to control myoblasts. GPA treatment of HD myoblasts (C) further increased the impairment of morphology and number of vacuoles as compared to GPA treated control myoblasts (D). (Scale bar=50μm)

Figure S6: Myoblasts cultures derived from muscle biopsies from control subjects and symptomatic HD patients were differentiated into myotubes by culturing in presence of 5% FBS. An irregular morphology and alignment was observed in HD myotubes (A), while control myotubes were typical myotubes morphology (B). (Scale bar=50μm)

Figure S7: Time line for GPA treatment and complete experiment. GPA treatment was started at 16th week of NLS-N171-82Q age and continued till 26th week. Rotarod was started at 25th week. Mice were sacrificed at 26th week of age for measurement of different biochemical parameters.