Supplemental Experimental Procedures

Sciatic nerve transection

Under deep inhaled anesthesia with 2% isoflurane, the right sciatic nerve was exposed at the thigh, just below the sciatic notch. Both the proximal and distal sides were ligated with monocryl 4-0 sutures, and about 2 mm of sciatic nerve was cut between the ligations to prevent axonal regeneration. At 1, 3, 7 and 14 days after surgery, the muscle was harvested and frozen for RNA analysis.

CLAMS

Oxygen consumption (VO2), carbon dioxide production (VCO2), spontaneous motor activity and food intake were measured using the Comprehensive Laboratory Monitoring System (CLAMS, Columbus Instruments), an integrated open-circuit calorimeter equipped with an optical beam activity monitor. For the analysis at resting condition, mice were weighed each time before the measurements and individually placed into sealed chambers (7.9” x 4” x 5”) with free access to food and water. The study was carried out in an experimentation room set at 20-23°C with 12-12 hours (6:00PM~6:00AM) dark-light cycles. Measurements were carried out continuously for 72 hours. During this time, animals were provided with food and water through the equipped feeding and drinking devices located inside the chamber. The amount of food consumed by each animal was monitored through a precision balance. VO2 and VCO2 in each chamber were sampled sequentially for 5 seconds in 10 min intervals, and the motor activity was recorded every second in X and Z dimensions. The airflow rate

through the chamber was adjusted to keep the oxygen differential around 0.3% at resting conditions.

CLAMS analysis during acute running was performed as follows. Before the study, mice were individually placed into treadmill chambers to acclimate them for 15-20 min/day on 3 consecutive days. Mice were weighed prior to and right after running. They were then individually placed into the sealed treadmill chambers (305 x 51 x 44 mm^3). The slope of the treadmill was set at 25° to the horizontal. The study was carried out in an experimentation room set at 20-23°C with 12-12 hours (6:00PM~6:00AM) dark-light cycles. The measurements were made between 9:00 AM – 1:00 PM. During this time, the animals were run on the treadmill one at a time and the treadmill was wiped clean between each test. The system was routinely calibrated before the experiment using a standard gas (20.5% O2 and 0.5% CO2 in N2). VO2 and VCO2 in each chamber were sampled continuously every 5 seconds. The air low rate through the chambers was set at 0.50 LPM. During the analysis mice ran under a treadmill schedule consisting of increasing speeds by increments of 3 m/min every 5 minutes for a total of 30 minutes, starting at 5 m/min. Exhaustion was determined by the mouse sitting on the shocker (1.60mA, 120v, 3Hz) for 5 consecutive seconds, at which point shocker was shut off and the treadmill stopped.

Glucose tolerance test

Age matched AR113Q and wild type males (n=7-8/genotype) were fasted for 5 hours starting at 8:30am. Glucose (50%) was administered at ~1:30 pm via intraperitoneal injection at 1.5g/kg. Blood samples were collected prior to and after the injection at time 0, 15, 30, 60, and 120 minutes via tail vein bleeding. Blood levels of glucose were measured using a

glucometer (Acucheck, Roche) and plasma levels of insulin were determined using Millipore rat/mouse insulin ELISA kit. Animals were restrained repeatedly for less than one minute each time blood samples were collected.

Metabolomics

The mass spectrometer was operated in ESI-mode according to previously published conditions (Lorenz et al., 2011). Data were processed using MassHunter Quantitative analysis version B.07.00. Metabolites in the glycolysis/TCA cycle/pentose phosphate pathways were normalized to the nearest isotope labeled internal standard and quantitated using 2 replicated injections of 5 standards to create a linear calibration curve with accuracy better than 80% for each standard. Other compounds in the analysis were normalized to the nearest internal standard, and the peak areas were used for differential analysis between groups.

Mitochondria uncoupling

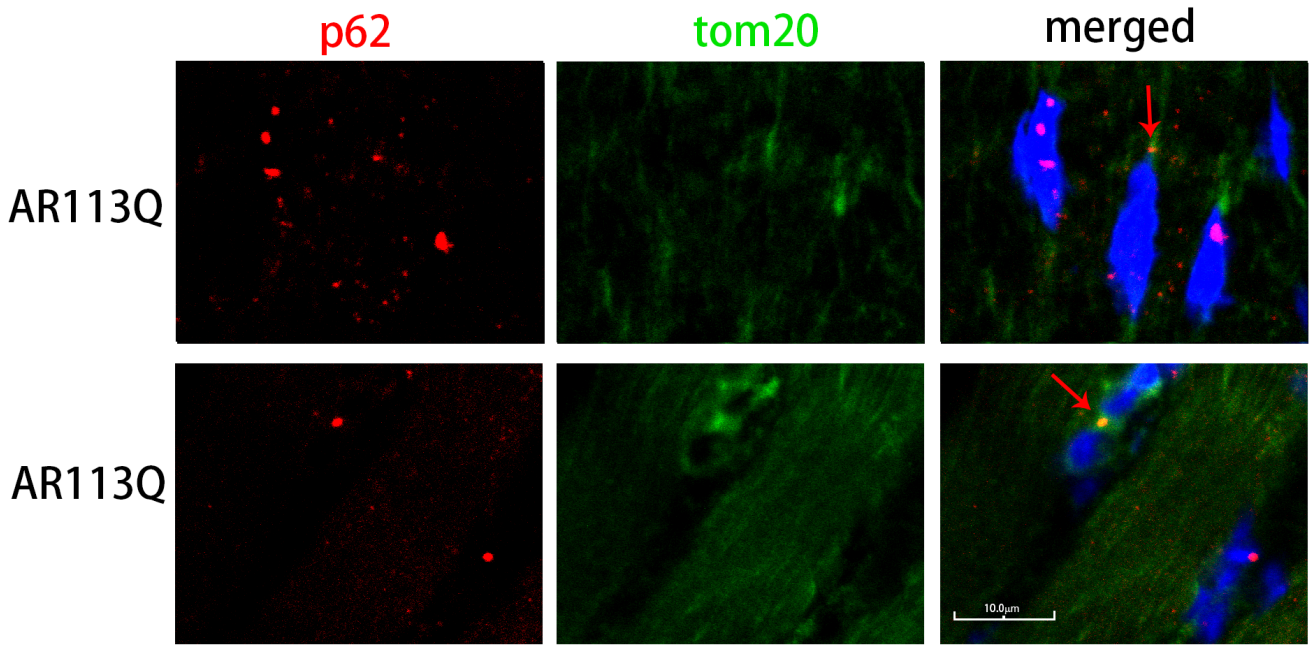
Mitochondria were isolated from quadriceps muscles of WT and AR113Q mice, and uncoupling was assessed as detailed in the Supplemental Experimental Procedures. Briefly, tissues were finely chopped in PBS containing 10 mM EDTA, incubated in 0.25% trypsin in PBS/EDTA for 10 min at 4°C, then homogenized with a pre-cooled dounce homogenizer 10 times with the loose (A) pestle in 5x volume:weight IBM buffer (6.7 mL of 1M sucrose, 5.0 mL of 1 M Tris/HCl, 5 mL of 1 M KCl, 2.0 mL of 0.5 M EDTA, 2 mL of 10% BSA in 100ml final volume, pH 7.4). Unbroken cells and nuclei were pelleted at 900x*g* at 4°C for 5 min.

Supernatant was collected and centrifuged at 6200x*g* at 4°C for 10 min. Pelleted mitochondria were re-suspended in 1 ml IBM buffer, centrifuged again at 6200x*g* at 4°C for 5 min, and finally re-suspended in 150 µl IBM buffer. Protein concentration was measured by BCA assay (Bicinchoninic acid assay, Life Technologies) and mitochondria uncoupling was assessed by Seahorse analysis. Uncoupling was considered as a ratio between basal readings of O_2 consumption in presence of succinate and readings of O_2 consumption after treatment with complex III inhibitor antimycin A, and was calculated as (1- AntiA / Average(Basal1;Basal2)).

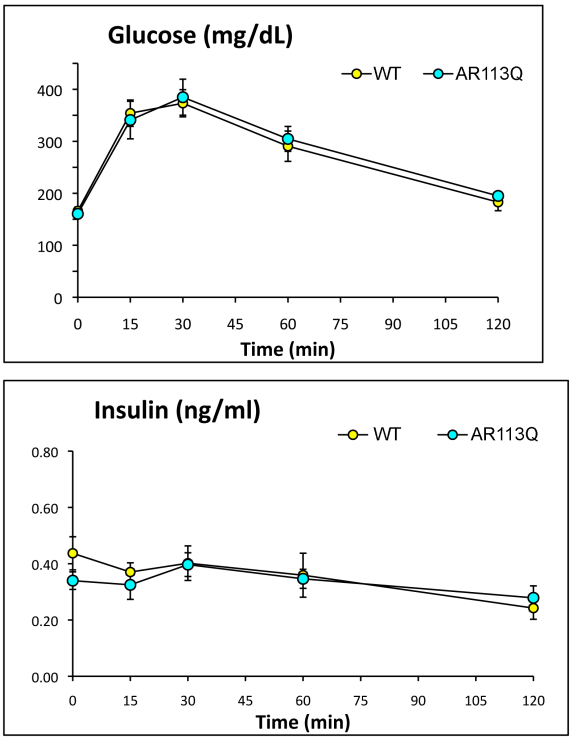
Supplemental Reference

Lorenz, M.A., Burant, C.F., and Kennedy, R.T. (2011). Reducing time and increasing sensitivity in sample preparation for adherent mammalian cell metabolomics. Anal Chem *83*, 3406-3414.

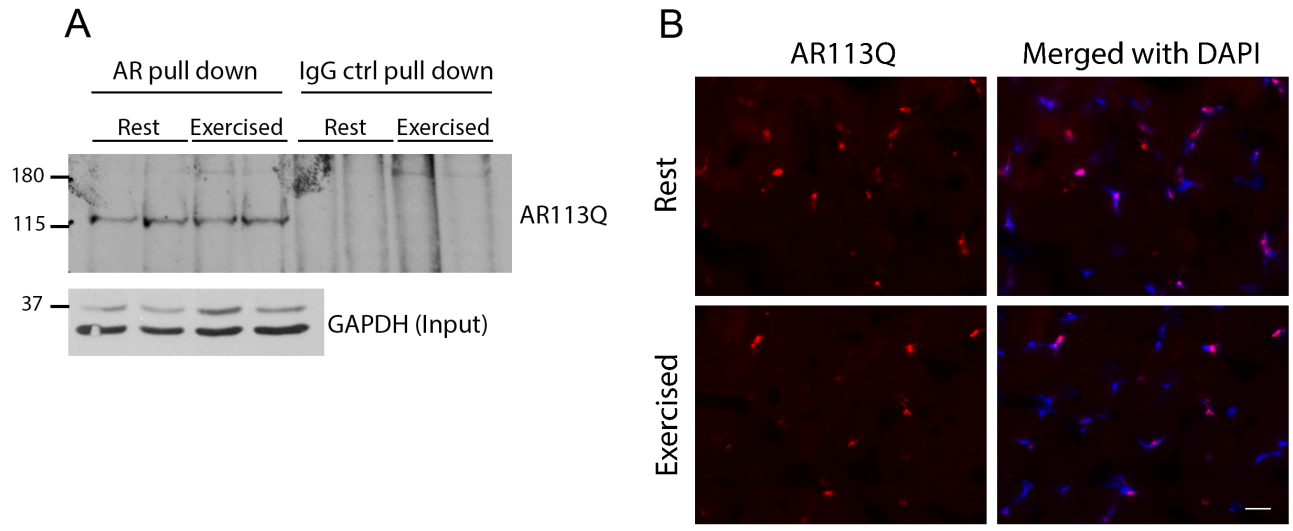
Supplemental Figures and Legends



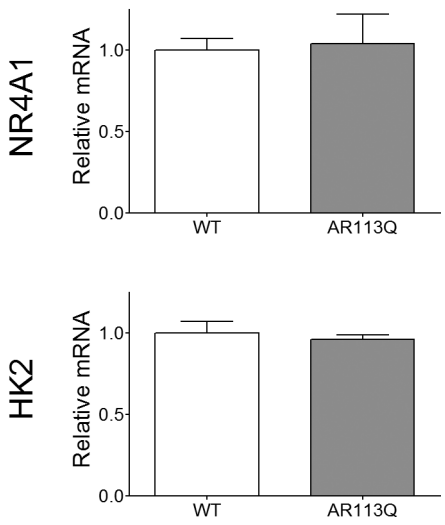
Supplemental Figure 1. Co-localization of p62 and TOM20 in AR113Q skeletal muscle. Related to Figure 3. Punctate p62 staining (red) is observed in nuclei and cytosol of AR113Q muscle. Cytosolic p62 puncta co-localize with the mitochondrial marker TOM20 (green). Scale bar: 10 μm.



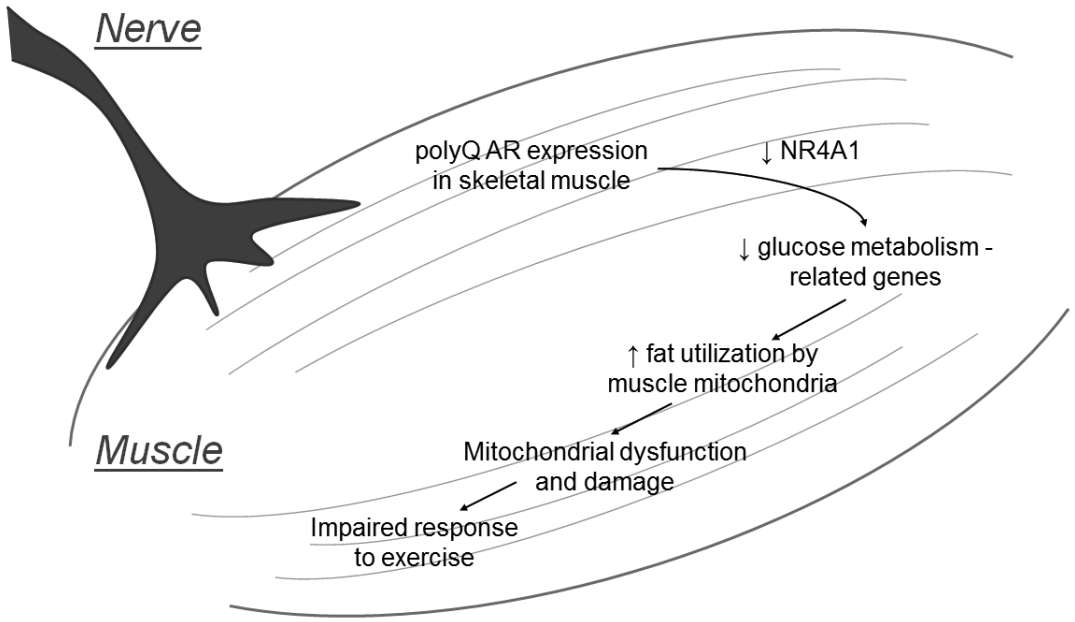
Supplemental Figure 2. Blood glucose and serum insulin levels as measured during a glucose tolerance test. Related to Figure 4. 14 week old wild type (yellow, n=8) or AR113Q males (blue, n=7) were fasted for 5 hours. Glucose (50%) was administered at 1.5g/kg. Blood samples were collected prior to and after the injection at time 0, 15, 30, 60, and 120 minutes. Blood levels of glucose and plasma levels of insulin are reported as mean ± SEM.



Supplemental Figure 3. AR113Q protein expression is unchanged by chronic exercise. Related to Figure 5. AR113Q mice were run on a treadmill 5 days/week for 30 min at 10 meters/minute for 6 weeks, from 8 – 14 weeks of age. Control mice ("rest") were placed on the treadmill for an equivalent time, but the apparatus was not turned on. (**A**) AR protein levels in quadriceps of rest and exercised AR113Q mice were analyzed by immunoprecipitation and western blot. (**B**) Immunofluorescence staining for AR (red) in quadriceps. Nuclei were stained by DAPI (blue). Scale bar: 20 μm.



Supplemental Figure 4. NR4A1 and HK2 gene expression is not altered in spinal cord of AR113Q mice. Related to Figure 7. Relative mRNA levels of NR4A1 (**top**) and HK2 (**bottom**) in spinal cord of WT and AR113Q mice at 14 weeks of age (mean ± SEM; n = 4/group). Values are not statistically different by Student's *t*-test.



Supplemental Figure 5. Model of metabolic consequences of polyQ AR expression in skeletal muscle. Related to Discussion. In AR113Q male mice, peripheral polyQ AR expression is sufficient to impair neuromuscular function (Lieberman et al., 2014). Here we show that this is associated with altered energy homeostasis in skeletal muscle, including diminished expression of genes controlling glucose metabolism and critical upstream regulators such as NR4A1. We propose that this results in a shift towards the utilization of alternative energy sources, such as fat, which damages mitochondria and stimulates their turnover, possibly through mitophagy. This altered energy metabolism lowers respiratory capacity of muscle and contributes to an impaired response to exercise. Changes in the expression of genes that control metabolism in skeletal muscle are rescued by peripheral polyQ AR gene suppression by administration of ASO.

Supplemental Tables uploaded as separate files:

Supplemental Table 1

Differentially expressed genes in quadriceps muscle of AR113Q *versus* WT mice that were rescued by ASO. Related to Figures 1 and 6.

Supplemental Table 2

Differentially expressed genes in quadriceps muscle of AR113Q *versus* WT mice that were not rescued by ASO. Related to Figures 1 and 6.

Supplemental Table 3

Differentially expressed genes in AR113Q *versus* WT quadriceps muscles associated with Protein Kinase A signaling. Related to Figure 7.

gene symbol	NCBI ID	Description	log_2(FC)	p-value	q-value
Adcy2	210044	adenylate cyclase 2	0.61	0.00005	0.00095
Calm1	12313	calmodulin 1	-0.65	0.00005	0.00095
Camk2a	12322	calcium/calmodulin-dependent protein kinase II alpha	-0.95	0.00005	0.00095
Cnga3	12790	cyclic nucleotide gated channel alpha 3	1.22	0.00020	0.00317
Crem	12916	cAMP responsive element modulator	0.74	0.00010	0.00175
Dusp10	63953	dual specificity phosphatase 10	-1.06	0.00005	0.00095
Flnc	68794	filamin C, gamma	0.65	0.00010	0.00175
Gng10	14700	guanine nucleotide binding protein (G protein), gamma 10	0.60	0.00200	0.02128
Itpr1	16438	inositol 1,4,5-trisphosphate receptor 1	-1.16	0.00005	0.00095
Myl2	17906	myosin, light polypeptide 2, regulatory, cardiac, slow	-1.34	0.00005	0.00095
Myl3	17897	myosin, light polypeptide 3	-1.57	0.00005	0.00095
Myl9	98932	myosin, light polypeptide 9, regulatory	-0.65	0.00005	0.00095
Mylk2	228785	myosin, light polypeptide kinase 2, skeletal muscle	-1.38	0.00005	0.00095
Mylpf	17907	myosin light chain, phosphorylatable, fast skeletal muscle	-1.32	0.00005	0.00095
Pde7a	18583	phosphodiesterase 7A	-0.84	0.00005	0.00095
Phkg1	18682	phosphorylase kinase gamma 1	-0.65	0.00005	0.00095
Plcd1	18799	phospholipase C, delta 1	0.61	0.00020	0.00317
Plcl1	227120	phospholipase C-like 1	0.59	0.00140	0.01598
Ppp1r3a	140491	protein phosphatase 1, regulatory (inhibitor) subunit 3A	-0.64	0.00005	0.00095
Ppp1r3d	228966	protein phosphatase 1, regulatory subunit 3D	0.74	0.00310	0.03003
Prkar2a	19087	protein kinase, cAMP dependent regulatory, type II alpha	-0.61	0.00005	0.00095
Ptprd	19266	protein tyrosine phosphatase,	1.10	0.00005	0.00095

		receptor type, D			
Pygm	19309	muscle glycogen phosphorylase	-0.96	0.00105	0.01267
Smad3	17127	SMAD family member 3	-0.59	0.00005	0.00095
Tgfb3	21809	transforming growth factor, beta 3	0.69	0.00005	0.00095
Tnni1	21952	troponin I, skeletal, slow 1	-1.59	0.00005	0.00095
Ttn	22138	titin	-1.07	0.00020	0.00317
Vasp	22323	vasodilator-stimulated phosphoprotein	-1.01	0.00005	0.00095

Supplemental Table 4

gene symbol	Description	AR113Q vs WT log2(FC)
Atf3	activating transcription factor 3	2.94
Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	0.79
Ucp3	uncoupling protein 3 (mitochondrial, proton carrier)	0.64
Pgk1	phosphoglycerate kinase 1	-0.61
Phkg1	phosphorylase kinase gamma 1	-0.65
Ppp1r3c	protein phosphatase 1, regulatory (inhibitor) subunit 3C	-0.70
Bpgm	2,3-bisphosphoglycerate mutase	-0.71
Lpin1	lipin 1	-0.75
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	-0.80
Pfkm	phosphofructokinase, muscle	-0.93
Pygm	muscle glycogen phosphorylase	-0.96
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	-1.05
Eno3	enolase 3, beta muscle	-1.13
Aldoa	aldolase A, fructose-bisphosphate	-1.15
Pgam2	phosphoglycerate mutase 2	-1.20
Phka1	phosphorylase kinase alpha 1	-1.24
Prkag3	protein kinase, AMP-activated, gamma 3 non-catatlytic subunit	-1.27

Genes altered in quadriceps muscle of AR113Q mice responsive to NR4A1 expression. Related to Figure 7.