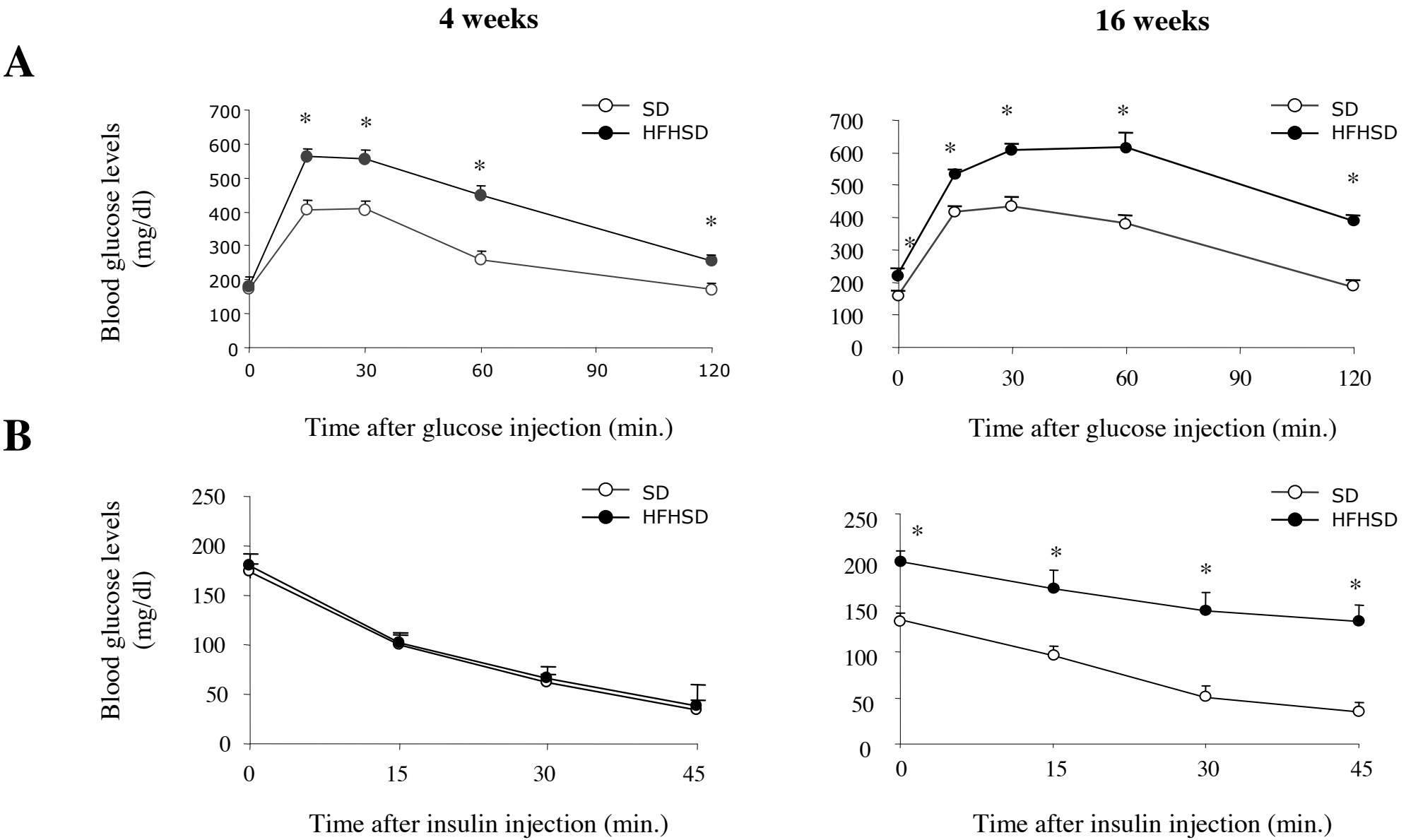

Figure S1: Systemic insulin sensitivity in SD and HFHSD mice. Intraperitoneal glucose (A) and insulin (B) tolerance tests in 6h fasted SD (white circles) and HFHSD (black circles) mice, after 4 (left panel) and 16 (right panel) weeks of diet. Animals were injected intraperitoneally with 2mg/g body weight of glucose or 0.75mU/g body weight of insulin. Data represent the means ± sem of 6-16 mice. * $p<0.01$ vs SD.

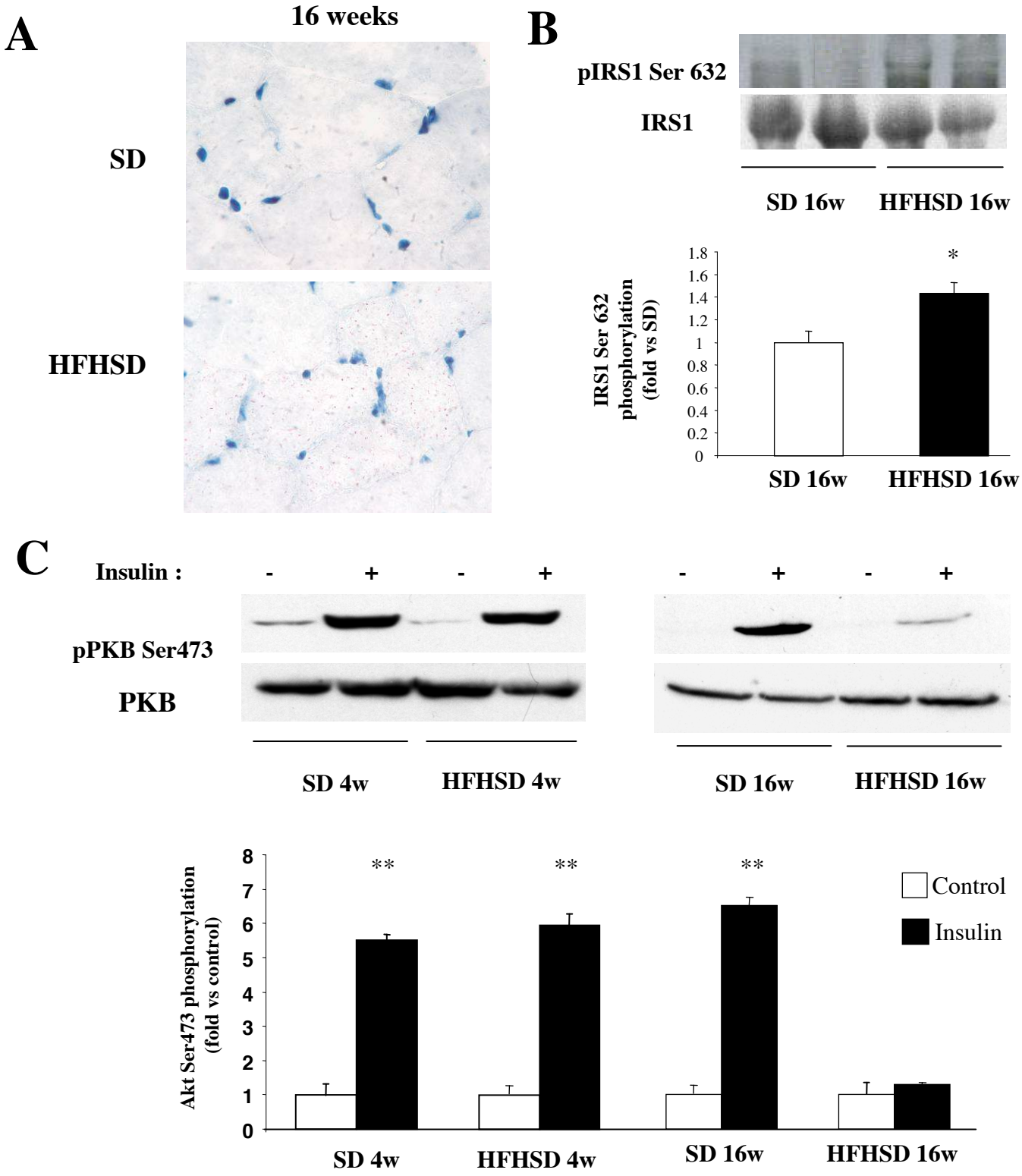

Figure S2: Altered insulin responsiveness in skeletal muscle of 16 week HFHSD mice. A: Oil Red O staining of gastrocnemius muscle from 16 week SD and HFHSD mice. B: Basal IRS1phosphorylation on serine 632 in gastrocnemius muscle of 16 week SD and HFHSD mice. IRS1 phosphorylation was normalized to total IRS1 protein expression. C: Insulin-stimulated Akt phosphorylation on serine 473, measured on muscle fragments incubated ex vivo in the absence or in the presence of insulin (10^{-7} M) for 15 minutes. Akt phosphorylation was normalized to total Akt protein expression. Results are expressed as fold increase over insulin-free basal conditions (n=3). * $p<0.05$, ** $p<0.01$.

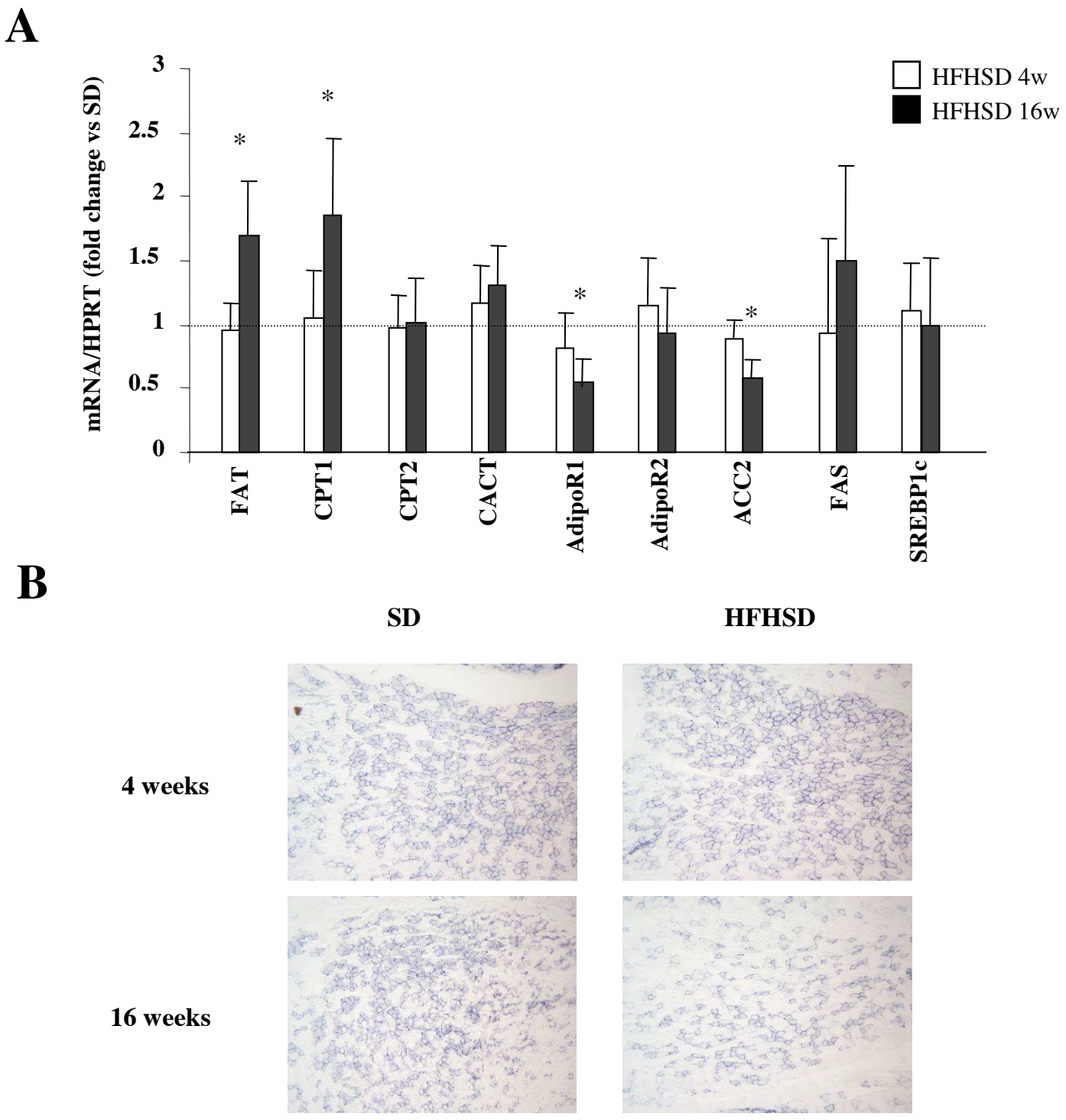

Figure S3: Oxidative and lipid metabolisms in muscle of SD and HFHSD mice. A: mRNA levels of lipid metabolism genes, determined by real-time RT-PCR, in gastrocnemius muscle of SD and HFHSD mice, After 4 and 16 weeks of diet (n=6). Results are expressed relative to SD condition (dotted line). * $p< 0.05$. B: Succinate dehydrogenase staining of gastrocnemius muscle from 4 and 16 week SD and HFHSD mice. Images have been taken in the deep gastrocnemius muscle of mice, which has a higher proportion of slow twitch fibers.

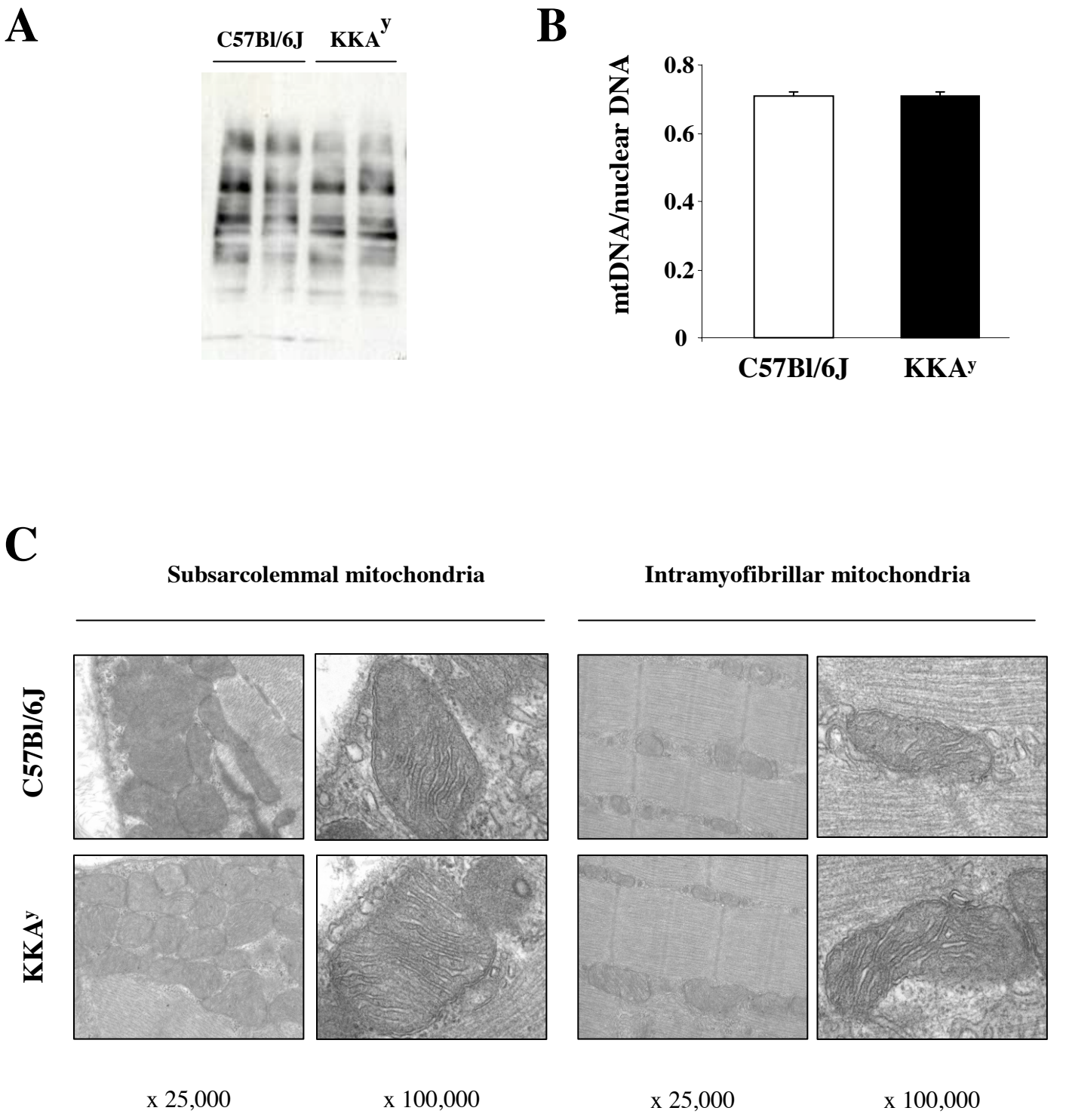

Figure S4: Lack of muscle oxidative stress and mitochondrial alterations in KKA^y mice. A- Immunoblots showing total protein carbonylation in gastrocnemius muscle of C57Bl/6J and KKA^y mice. B- mtDNA levels, determined by real time PCR, in skeletal muscle of C57Bl/6J and KKA^y mice (n=6). mtDNA copy number was calculated as the ratio of COX1 to cyclophilin A. C- Transmission electronic microscopy images (magnification x25,000 and x100,000) of subsarcolemmal and intermyofibrillar mitochondria in gastrocnemius muscle of C57Bl/6J and KKA^y mice.

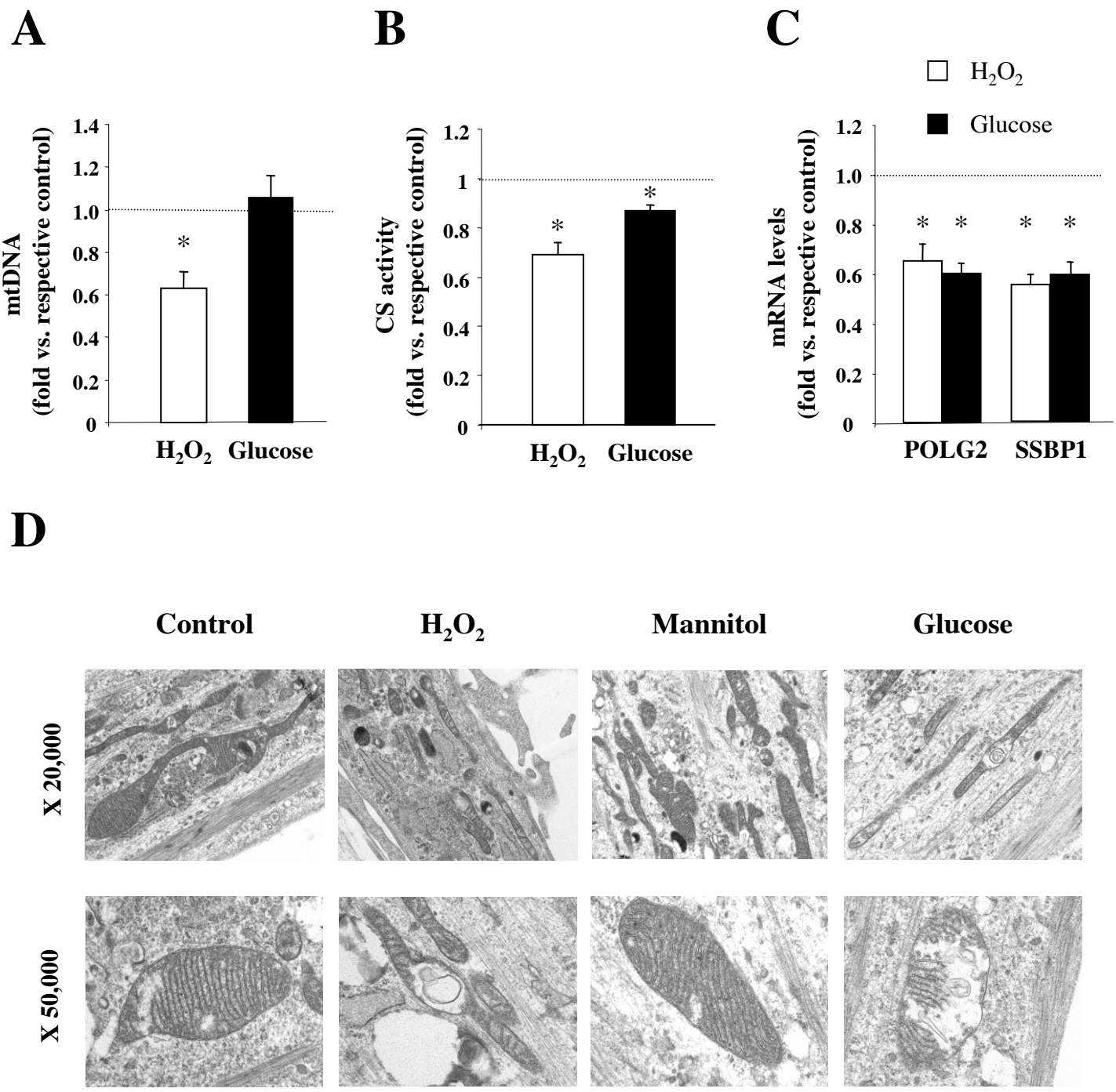

Figure S5: Effects of ROS on mitochondria density, structure and function in human myotubes. A: mtDNA copy number from myotubes treated for 96 hours with H_2O_2 (0.1mM) and glucose (25mM). B: Citrate synthase (CS) activity measured in total lysates of myotubes treated with H_2O_2 (0.1mM) and glucose (25mM) for 96 hours. C: mRNA levels of POLG2 and SSBP1 genes, determined by real-time RT-PCR, in H_2O_2 and glucose-treated myotubes for 96 hours. D- Transmission electronic microscopy images (magnification x 20,000 and x 50,000) of mitochondria in human myotubes treated or not with H_2O_2 and glucose for 96 hours. Mannitol (25mM) is added as control for glucose treatment. All results are expressed relative to untreated cells (dotted line) (n=3 in triplicate). * $p<0.05$.

Table S1 : Metabolic characteristics of age-matched C57Bl/6J control and KKA^{y} mice.

	C57Bl/6J	**KKA^{y}**
Body weight (g)	22.7 ± 0.5	28 ± 0.8**
Fat weight (g)	0.39 ± 0.02	0.81 ± 0.07**
Glucose (mg/dl)	166.8 ± 6.3	304.3 ± 46**
Insulin (ng/ml)	0.51 ± 0.04	3.83 ± 1.2*
TG (g/l)	0.85 ± 0.05	3.29 ± 0.4**
FFA (mM)	0.1 ± 0.02	0.14 ± 0.01
H_2O_2 (μM)	61.9 ± 7	85.4 ± 4.8*

Data represent the means ± sem of 10 mice per group.

* $p<0.05$, ** $p<0.001$ vs the control mice.

Supplemental Table 2 : Sequences of primers used for real-time PCR.

Genes	Forward primer	Reverse primer
COX1*	5'-TGC TTA CAC CAC ATG AAA CA-3'	5'- TTTTTTTTTTTTTTTTTTTTTTTTTTTATTTT-3'
COX3*	5'-GAA GCC GCA GCA TGA TAC TG-3'	5'-TTTTTTTTTTTTTTTTTTTTTTTTTTAAGATC-3'
PGC1α	5'-TCC TCT GAC CCC AGA GTC AC-3'	5'-CTT GGT TGG CTT TAT GAG GAG G-3'
PGC1β	5'-GGA AGC GGC GGG AAA AGG CC-3'	5'-GCT GTC AAA ATC CAT GGC TTC-3'
NRF1	5'-GCT GCT GC GTG GCA ACA GG-3'	5'-TTG GGT TTG GAG GGT GAG AT-3'
NRF2	5'-CCA AGT CCT GCA TTG GGT GG-3'	5'-GCA AAA ACT GCC ATA GTT GG-3'
mtTFA	5'-GCT TGG AAA ACC AAA AAG AC-3'	5'-CCC AAG ACT TCA TTT CAT T-3'
ERRα	5'- GGT GTG GCA TCC TGT GAG GC-3'	5'-AGG CAC TTG GTG AAG CGG CA-3'
Mfn2	5'-AAG CAC TTT GTC ACT GCC AAG-3'	5'-TTG TCC CAG AGC ATG GCA TTG-3'
POLG1	5'-GCG GCT GGT GGA AGA GCG TT-3'	5'-GAG ATG GCC ATG TGC ATG CTC-3'
POLG2	5'-CAC TAT GTT AAT TGC CTG G-3'	5'-CCA GAA ATC AAG CCA CTG G-3'
SSBP1	5'-CGT CAG TTT GTA AGA CAT GAG-3'	5'-CCC CTG ATC GCC ACA TCT C-3'
FAT/CD36	5'-TGA TGA TGA ACA GCA GCA ACA T 3'	5'-AGA CTG TGT TGT CCT CAG CG-3'
CPT1	5'-TAT AAC AGG TGG TTT GAC A-3'	5'-CAG AGG TGC CCA ATG ATG-3'
UCP2	5' - CTA CTG TCG AGG AGA TCG AG -3'	5' –GCA GCA GTT TGG GTT GTT TC-3'
UCP3	5' – CCT ACA GAA CCA TCG CCA GG -3'	5'–ACC GGG GAG GCC ACC ACT GT-3'
Gp91	5'- TTG GGT CAG CAC TGG CTC TG -3'	5'- TGG CGG TGT GCA GTG CTA TC -3'
p22	5'- GTC CAC CAT GGA GCG ATG TG-3'	5'- CAA TGG CCA AGC AGA CGG TC -3'
p67	5'- CTG GCT GAG GCC ATC AGA CT -3'	5'- AGG CCA CTG CAG AGT GCT TG -3'
p40	5'- GCC GCT ATC GCC AGT TCT AC -3'	5'- GCA GGC TCA GGA GGT TCT TC -3'
p47	5'- GAT GTT CCC CAT TGA GGC CG -3'	5'- GTT TCA GGT CAT CAG GCC GC -3'
GSR	5'-GGG TGG CAC TTG CGT GAA TG -3'	5'-TTC AGG CGG CTC ACA TAG GC -3'
GPx	5' GTG AGC CTG GGC TCC CTG CG 3'	5' ACT TGA GGG AAT TCA GAA TC 3'
CAT	5'- TCA GGA TGT GGT TTT CAC TG -3'	5'- GTG TAA AAT TTC ACT GCA AAC -3'
SOD2	5' TCA TGC AGC TGC ACC ACA GC-3'	5' CCA TTG AAC TTC AGT GCA GG-3'
Prdx3	5'-GAC ATA CTG TGG TCT GCC TCT GC-3'	5'-CCT TTA AAA TAG GGC GCG TG -3'
Prdx5	5'- ACA CCT GGC TGT TCT AAG ACC C-3'	5'- TCA TTA ACG CTC AGA CAG GCC-3'
CS	5'-CCA TCC ATA GTG ACC ATG AG-3'	5'-CTT TGC CAA CTT CCT TCT GT-3'

* Since mitochondria-encoded genes have not intron, and to ensure that we amplify only mRNA, we have used a reverse primer which is localized on poly A RNA.