Supplemental Material (Scharf et al.)

Supplementary Table S1

DESCRIPTION	**LOCUS**	**MK2/3** $^{-/-}$
ANGIOGENESIS		
angiopoietin 1	Angpt1	2.324
angiopoietin-like 7	Angptl7	5.202
CD55 antigen	***Cd55***	***-1.622***
CARBOHYDRATE METABOLISM		
aldolase A, fructose-bisphosphate	***Aldoa***	***-1.649***
enolase 3, beta muscle	***Eno3***	***-1.711***
fructose bisphosphatase 2	Fbp2	1.504
glycogen synthase 1, muscle	Gys1	2.632
lactate dehydrogenase B	Ldhb	1.444
phosphoenolpyruvate carboxykinase 1, cytosolic	Pck1	1.486
phosphoglycerate kinase 1 (Pgk1)	***Pgk1***	***-1.935***
CELL GROWTH		
insulin-like growth factor binding protein 6	Igfbp6	1.555
myostatin	***Mstn***	***-2.542***
myogenin	Myog	1.344
CONTRACTILE APPARATUS		
actin, alpha 1, skeletal muscle	Acta1	1.005
myosin binding protein C, slow-type	Mybpc1	2.363
myosin binding protein C, fast-type	***Mybpc2***	***-3.788***
myosin binding protein H	Mybph	2.251
myosin, heavy polypeptide 1 (d/x), skeletal muscle, adult	***Myh1***	***-2.289***
myosin, heavy polypeptide 2 (IIa), skeletal muscle, adult	Myh2	1.581
myosin, heavy polypeptide 3, skeletal muscle, embryonic	Myh3	3,208
myosin, heavy polypeptide 4 (IIb), skeletal muscle	***Myh4***	***-2.227***
myosin, heavy polypeptide 6, cardiac muscle, alpha	Myh6	1,594
myosin, heavy polypeptide 7 (I/β), cardiac muscle, beta	Myh7	1.718
myosin, light polypeptide 1, MLC1F/MLC3F	***Myl1***	***-2.478***
myosin, light polypeptide 3, MLC1v/MLC1s	Myl3	1.370
myosin light chain, phosphorylatable, fast skeletal muscle	***Mylpf***	***-1.784***
PDZ and LIM domain 3	Pdlim3	1.159
troponin I, skeletal, slow 1	Tnni1	1.329
troponin I, skeletal, fast 2	***Tnni2***	***-1.469***
troponin T1, skeletal, slow	Tnnt1	1.457
FAT METABOLISM		
ATP-binding cassette, sub-family B (MDR/TAP), member 1A	Abcb1a	1.350
ATP-binding cassette, sub-family D (ALD), member 2	Abcd2	2.065
acyl-Coenzyme A dehydrogenase, short chain	Acads	1.495
catalase	Cat	1.810
CD36 antigen	Cd36	1.751
carnitine palmitoyltransferase 1b, muscle	Cpt1b	2.000
fatty acid binding protein 3, muscle and heart	Fabp3	1.760
fatty acid binding protein 5, epidermal	***Fabp5***	***-1.371***
fatty acid synthase	Fasn	2.032
Lipase, hormone sensitive	Lipe	1.576
lipoprotein lipase	Lpl	1.670
pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	1.362
resistin	Retn	2.046
stearoyl-Coenzyme A desaturase 1	Scd1	1.662
solute carrier family 27	Slc27a1	1.893
uncoupling protein 3	Ucp3	1.388
UEV and lactate/malate dehyrogenase domains	Uevld	2.355
HEAT SHOCK RESPONSE		
crystallin, alpha B	Cryab	2.163
heat shock protein 1	Hspb1	1.712

INTERSTITIAL TISSUE COMPONENT / EXTRACELLULAR MATRIX		
collagen, type XVI, alpha 1	Col16a1	1.652
collagen, type I, alpha 1	Col1a1	1.597
matrix metallopeptidase 2	Mmp2	1.658
tissue inhibitor of metalloproteinase 2	Timp2	1.855
tenascin C	Tnc	4.979
Tenomodulin	Tnmd	5.219
MITOCHONDRIAL PROTEINS		
ATP synthase, H$^+$ transporting, mitochondrial F1 complex, alpha subunit 1	Atp5a1	1.725
ATP synthase, H$^+$ transporting, mitochondrial F1 complex, beta subunit	Atp5b	1.844
mitochondrially encoded cytochrome c oxidase I	COX1	2.153
mitochondrially encoded cytochrome c oxidase II	COX2	1.610
mitochondrially encoded cytochrome c oxidase III	COX3	1.368
microsomal glutathione S-transferase 1	***Mgst1***	***-1.575***
microsomal glutathione S-transferase 2	Ndufv1	1.761
NADH dehydrogenase (ubiquinone) flavoprotein 1	Ndufv2	1.592
NADH dehydrogenase (ubiquinone) flavoprotein 2	Ndufv3	1.975
NADH dehydrogenase (ubiquinone) flavoprotein 3	Mgst2	1.753
pyruvate dehydrogenase complex, component X	Pdhx	1.627
solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20	Slc25a20	1.602
solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Slc25a25	2.876
superoxide dismutase 2, mitochondrial	Sod2	2.060
OXYGEN CARRIERS		
hemoglobin, beta adult major chain	Hbb-b2	1.819
myoglobin	Mb	1.479
solute carrier family 38, member 4	***Slc38a4***	***-1.824***
TRANSCRIPTION		
ankyrin repeat domain 2	Ankrd2	3.631
calcium channel, voltage-dependent, L type, skeletal muscle, Cav1.1	***Cacna1s***	***-1.030***
CCAAT/enhancer binding protein (C/EBP), delta	Cebpd	2.010
cAMP responsive element binding protein 1	Creb1	1.830
E2F transcription factor 2	***E2f2***	***-1.096***
early growth response 1	***Egr1***	***-1.926***
HOP homeobox	Hopx	1.026
Jun oncogene	Jun	1.568
myocyte enhancer factor 2C	Mef2c	1.702
nuclear factor of activated T-cells, cytoplasmic	Nfatc1	2.712
nuclear receptor subfamily 4, group A, member 2	Nr4a2	3.079
nuclear respiratory factor 1	Nrf1	2.040
paired box gene 7	Pax7	2.627
Plexin A1	Plxna1	1.130
peroxisome proliferative activated receptor, gamma, coactivator 1 alpha, PGC-1α	Ppargc1a	2.456
retinol binding protein 1, cellular	***Rbp1***	***-2.282***
serum response factor	***Srf***	***-1.025***
TRANSPORT		
ATPase, Ca^{2+} transporting (SERCA1), fast twitch 1	***Atp2a1***	***-1.570***
ATPase, Ca^{2+} transporting (SERCA2), slow twitch 2	Atp2a2	3.148
Phospholamban	Pln/Plb	1.204
ryanodine receptor 1, skeletal muscle (Ryr1)	RyR1	1.110
ryanodine receptor 2, cardiac (Ryr2)	RyR2	1.106
sodium channel, voltage-gated, type V, alpha	Scn5a	1.556
solute carrier family 2 (facilitated glucose transporter), member 1	Slc2a1	1.002
solute carrier family 1 (neutral amino acid transporter), member 5	Slc1a5	4.620
sodium/calcium exchanger, member 1, NCX1	Slc8a1	1.300
sodium/calcium exchanger, member 2, NCX2	***Slc8a2***	***-1.062***
sodium/calcium exchanger, member 3, NCX3	***Slc8a3***	***-1.352***
transporter 1, ATP-binding cassette	***Tap1***	***-2.736***
Transferrin	Trf	1.539

OTHERS		
adrenergic receptor, beta 3	Adrb3	2.435
annexin A6	Anxa6	2.105
aldehyde dehydrogenase family 3, subfamily A1	Aldh3a1	1.977
arrestin domain containing 2	Arrdc2	3.173
cDNA sequence BC053393	***BC053393***	***-5.625***
calsequestrin 1, fast type skeletal muscle isoform	***Casq1***	***-1.362***
calsequestrin 2, cardiac muscle isoform	Casq2	1.812
calcium/calmodulin-dependent protein kinase II, alpha	Camk2a	1.158
calcium/calmodulin-dependent protein kinase II, beta	Camk2b	1.047
calcium/calmodulin-dependent protein kinase II, gamma	Camk2d	1.250
carbonic anhydrase 3	Car3	1.295
carbonic anhydrase 4	Car4	1.231
cartilage intermediate layer protein 2	Cilp2	3.021
cadherin 15	Cdh15	2.090
creatine kinase, muscle	***Ckm***	***-1.572***
cartilage oligomeric matrix protein	Comp	4.296
cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	1.274
gelsolin	Gsn	2.610
metallothionein 2	Mt2	4.452
myomesin family, member 3	Myom3	1.810
Ngfi-A binding protein 1	***Nab1***	***-1.033***
Ngfi-A binding protein 2	Nab2	1.047
protein tyrosine phosphatase-like, member b	Ptplb	1.415
parvalbumin	***Pvalb***	***-3.504***
suprabasin	Sbsn	2.139
sarcolipin	Sln	***-1.185***
tropomodulin 3	***Tmod3***	***-2.081***
xin actin-binding repeat containing 1	Xirp1	2.169

Table S1 Effects of MK2/3-deficiency on the expression of functional groups of fiber type-specific genes identified by DNA microarray analysis. Shown is a set of transcripts filtered for genes characteristic of fast and slow muscle fiber types in addition to other classified genes. Changes in mRNA expression in MK2/3$^{-/-}$ compared with WT soleus, as revealed by DNA microarray analysis, with down-regulated genes shown in bold. Each sample was pooled from soleus of three mice. Data are the average of n=3 samples in each group.

Beside the altered expression of genes implicated in fiber type-specific gene expression, some of the genes found to be up-regulated in MK2/3-deficient soleus encode for proteins involved in regeneration/repair processes, such as embryonic MyHC (Myh3; see also Fig. 3B) and α-cardiac MyHC (Myh6), both markers of newly forming myofibers in regenerating muscle (1). Further up-regulated genes are involved in satellite cells activation (11), such as cadherin 15 (Cdh15) and paired box gene 7 (Pax7; see also Fig. 3B). Regeneration requires proliferation of myogenic and non-myogenic cells, often accompanied by increased amounts of connective tissue formation (1). Together with increased expression of components of the extracellular matrix as found in the endo- and epimysium of muscle fibers, like tenomodulin (Tnmd) and collagen type I (Col1a1), and of a marker of collagen processing, matrix metalloproteinase 2 (Mmp2), the data imply a regeneration/repair process in soleus muscle from MK2/3$^{-/-}$ mice.

These process might be caused by down-regulation of myostatin (Mstn), a negative regulator of satellite activation (9), together with increased p38γ signaling which positively regulates the expansion of amplifying satellite cells (7). Since regeneration and repair can result from intense, but also from continuous contractile activity (10), slow fiber-rich muscles contain more satellite cells than fast muscles (6), and are suggested to have a higher turnover rate during normal physiological maintenance of the muscle. Therefore, data indicating a regeneration/repair process are in line with the increased content of slow and decreased number of fast fibers in MK2/3-deficient soleus. Alternatively, given that p38 and MK2/3 are involved in the cellular response to a variety of stress stimuli including oxidative stress (5), the cellular response to oxidative or even mechanical stress stimuli might be somewhat hampered by MK2/3 ablation thus inducing the regeneration processes. Accordingly, the mRNA for matrix metalloproteinase 2, important for elevated collagen processing and biosynthesis following mechanical stress (8), and tenascin C of the endomysium, essential for full-scale muscle damage repair (2, 4), are elevated in MK2/3-deficient soleus. In addition, heat shock proteins as markers for fiber transformation resulting from enhanced neuromuscular activity are enhanced (3).

Supplementary Table S2

DESCRIPTION	**LOCUS**	**$MK2/3^{-/-}$**
TRANSCRIPTION		
early growth response 1	***Egr1***	***-2.034***
myocyte enhancer factor 2C	Mef2c	1.172
peroxisome proliferative activated receptor, gamma, coactivator 1 alpha, PGC-1α	Ppargc1a	2.156
serum response factor	***Srf***	***-1.015***
TRANSPORT		
ATPase, Ca^{2+} transporting (SERCA2), slow twitch 2	Atp2a2	3.148
calcium channel, voltage-dependent, L type, cardiac, Cav1.2a	***Cacna1c***	***-1.015***
Phospholamban	Pln/Plb	1.014
ryanodine receptor 2, cardiac (Ryr2)	RyR2	1.106
sodium/calcium exchanger, member 1, NCX1	Slc8a1	1.150
sodium/calcium exchanger, member 3, NCX3	Slc8a3	1.103
OTHERS		
actin, alpha 1, skeletal muscle	Acta1	1.032
calsequestrin 2, cardiac muscle isoform	Casq2	1.012
calcium/calmodulin-dependent protein kinase II, alpha	Camk2a	1.880
calcium/calmodulin-dependent protein kinase II, beta	Camk2b	1.971
calcium/calmodulin-dependent protein kinase II, gamma	Camk2d	1.750
myosin, heavy polypeptide 7 (I/β), cardiac muscle, beta	Myh7	1.103
natriuretic peptide precursor typeA, atrial natriuretic factor (ANF)	Nppa	1.057
natriuretic peptide precursor typeB, brain natriuretic peptide (BNP)	Nppb	1.098

Table S2 Effects of MK2/3-deficiency on the expression of selected genes in the heart determined by DNA microarray analysis. Changes in transcript levels are shown for a filtered set of genes encoding for transcription factors, transporters, Ca^{2+}-handling proteins, and markers for hypertrophy in MK2/3$^{-/-}$ vs. WT heart. Down-regulated genes are indicated in bold. Each sample was pooled from the heart of three mice.

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