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Supplemental information

Parvalbumin affects skeletal muscle trophism through modulation of mitochondrial calcium uptake

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Supplemental Information

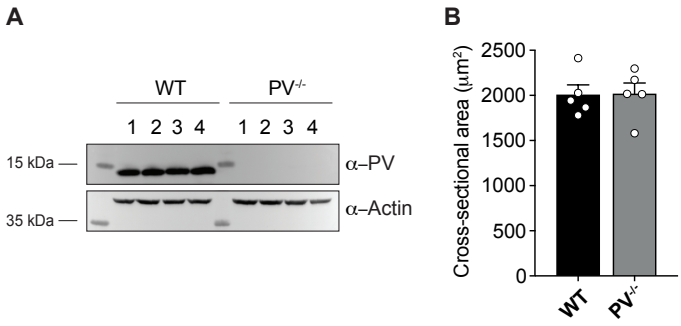

Figure S1. PV protein expression and fiber CSA of TA muscles of WT and PV$^{-/-}$ mice, related to Figure 1.

(**A**) Representative WB of PV expression in WT and PV$^{-/-}$ TA muscles. α-Actin was used as loading control. n=4.

(**B**) CSA of WT and PV$^{-/-}$ TA muscles. The bar diagram represents the mean ± SEM. ≥1500 fibers/muscle, n=5. For data analysis, parametric Student t-test (two-tailed, unpaired) was used.

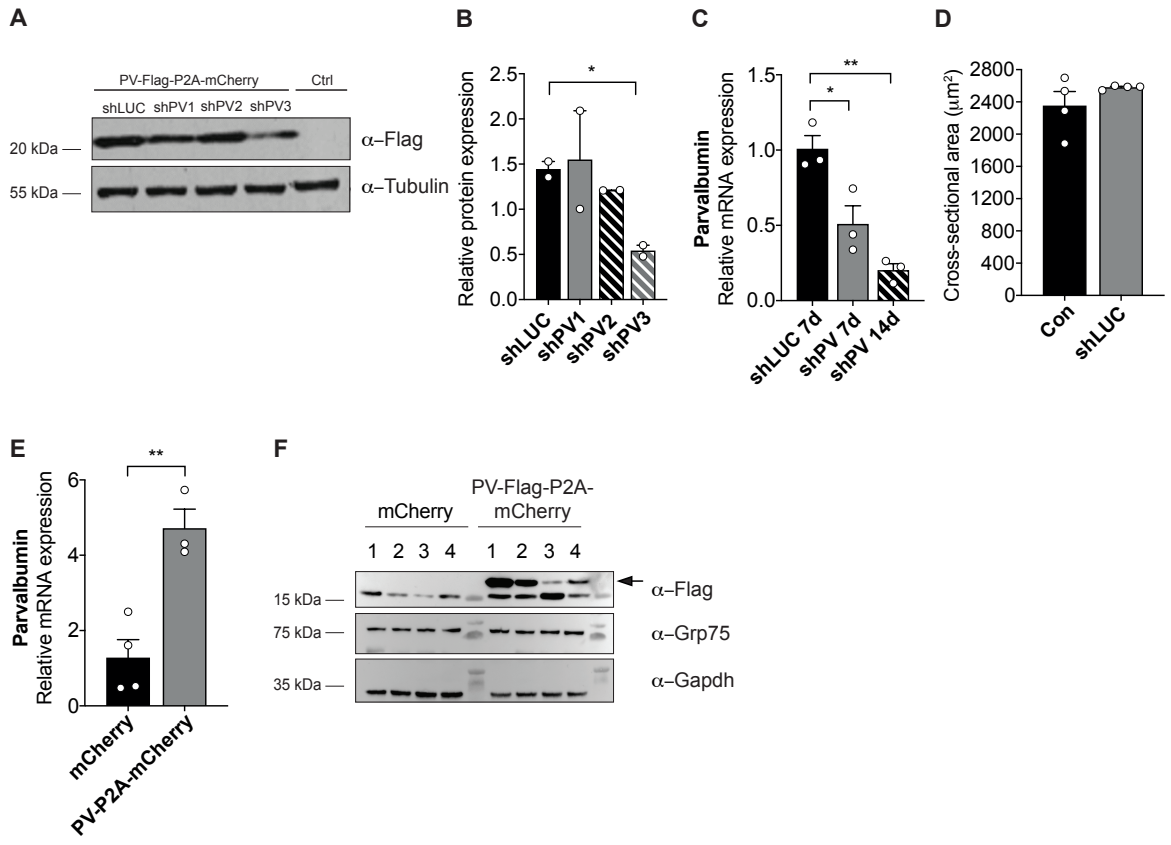

Figure S2. Efficacy of PV silencing and overexpression in *vitro* and *in vivo*, related to Figure 1.

(**A**) and (**B**) WB on HeLa cells transfected for 48 hours with 3 different shRNA plasmids (shPV1-3) or non-targeting shLUC together with pcDNA3.1-PV-Flag plasmid. (A) Representative WB of α-Flag antibody to detect overexpressed PV and α-Tubulin as loading control. (B) Quantification of PV protein levels normalized to Tubulin. The bar diagram represents the mean ± SEM. n=2. For statistical analysis, one-way ANOVA with post hoc Dunnett's multiple comparison test was used. * $p<0.05$.

(**C**) PV mRNA expression levels in FDB muscles electroporated either with shLUC or with the selected shPV for 7 and 14 days. The bar diagram represents the expression levels of PV normalized to shLUC and presented as mean ± SEM. n=3. Expression levels were normalized to POL2. For statistical analysis, one-way ANOVA with post hoc Dunnett's multiple comparisons test was performed. * $p<0.05$; ** $p<0.01$.

(**D**) CSA of TA muscle fibers of WT mice electroporated for 7 days with shLUC compared to the surrounding non-transfected fibers (Con) of the same muscle. The bar diagram represents the mean ± SEM. ≥500 fibers/muscle, n=4. For data analysis, parametric Student t-test (two-tailed, unpaired) was used.

(**E**) PV mRNA expression levels in FDB muscles electroporated for 7 days either with mCherry or with PV-flag-P2A-mCherry. The bar diagram represents the expression levels of PV and presented as mean ± SEM. n=3. Expression levels were normalized to Actin. For statistical analysis, parametric Student t-test (two-tailed, unpaired) was used. ** $p<0.01$.

(**F**) Representative WB of the experiment as in (E). α-Flag antibody was used to detect PV and α-Grp75 and α-Gapdh as loading controls. n=4. The arrow indicates the band relative to overexpressed PV.

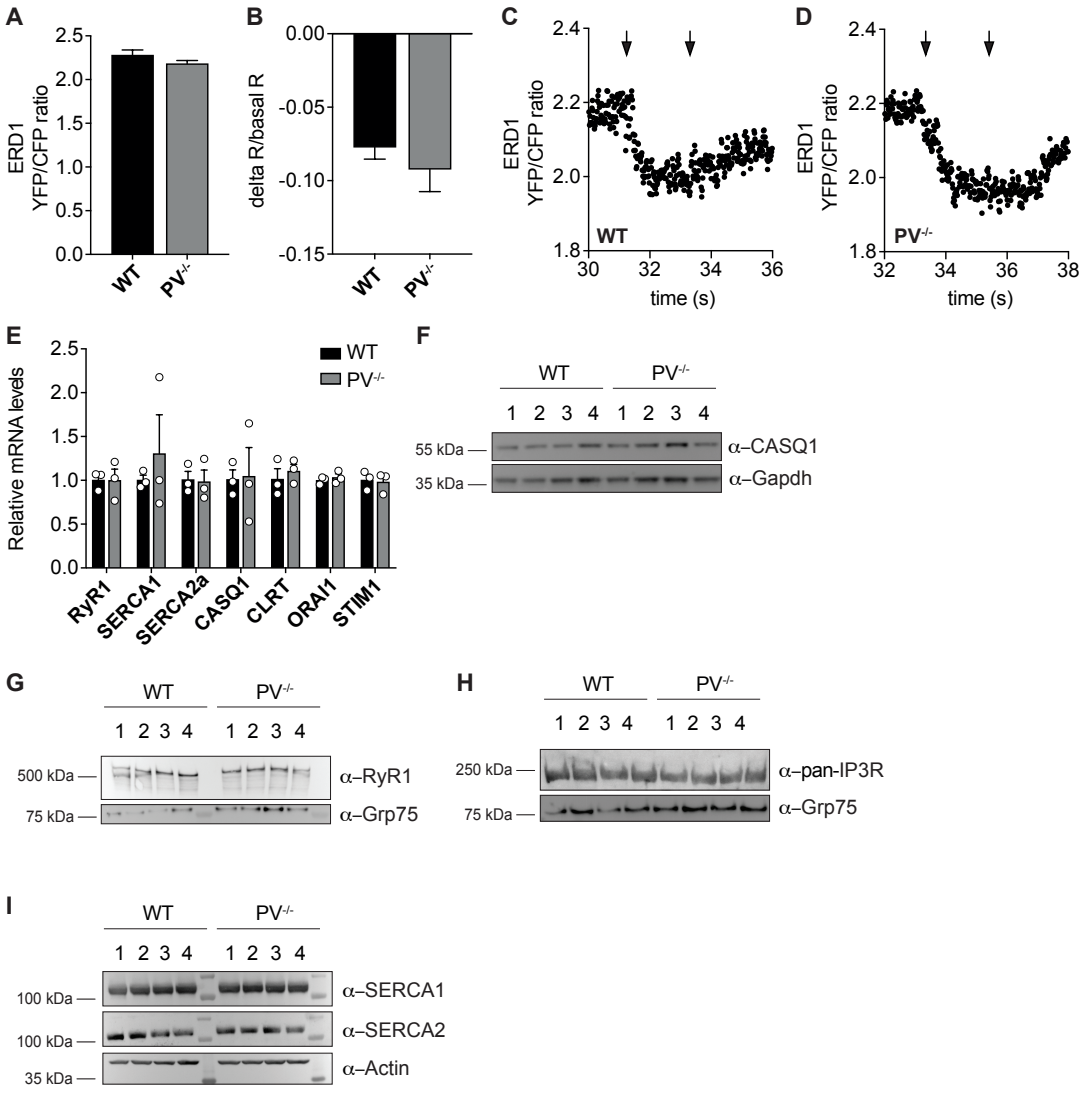

Figure S3. SR Ca^{2+} dynamics and expression of SR Ca^{2+}-related proteins in WT and $PV^{-/-}$ muscle fibers, related to Figure 3.

(**A**) – (**B**) SR free [Ca^{2+}] measured with the FRET-based fluorescent probe D1ER electroporated in FDB muscle of WT and $PV^{-/-}$ mice for 7 days. (A) ERD1 YFP/CFP ratio in resting basal condition of WT and $PV^{-/-}$ fibers. (B) ERD1 YFP/CFP ratio decrease (delta R) after electrical pulses at 60 Hz for 2 seconds at 25°C normalized to the basal ratio (R). For statistical analysis parametric Student t-tests (two tailed, unpaired) were used. Data are expressed as mean ± SEM. n≥14.

(C) – **(D)** Representative traces of the changes in YFP/CFP ratio during a 2 seconds 60 Hz electrical stimulation in WT (C) and $PV^{-/-}$ (D) FDB muscle fibers. Small arrows show the beginning and the end of the stimulation.

(**E**) Expression levels of the indicated genes in TA muscles of WT and $PV^{-/-}$ mice normalized to GAPDH and presented as mean ± SEM. n=3. For statistical analysis, parametric Student t-test (two-tailed, unpaired) was performed.

(**F**) – (**I**) Representative WB of WT and $PV^{-/-}$ TA muscles stained with α-CASQ1 (F), α-RyR1 (G), α-IP3R (H), α-SERCA1 and α-SERCA2 (I) antibodies. Where indicated, α-Gadph, α-Grp75 or α-Actin were used as loading controls. n=4.

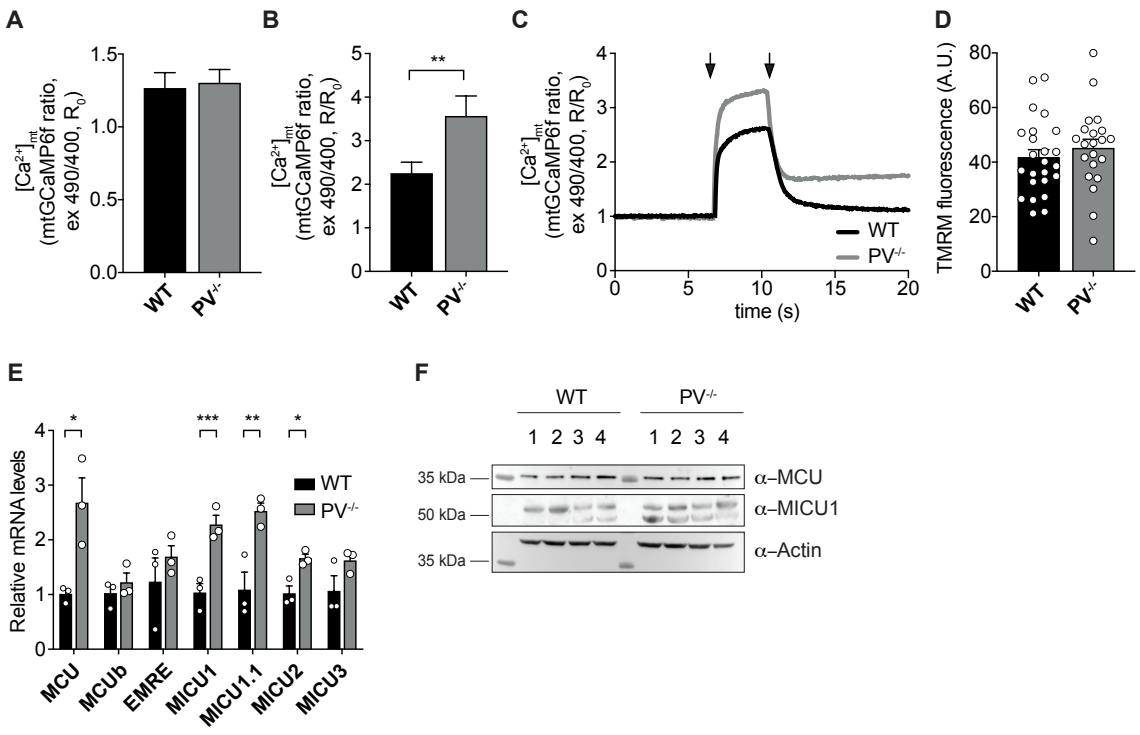

Figure S4. PV ablation enhances mitochondrial Ca^{2+} uptake on single isolated FDB fibers upon electrical stimulation and does not change mitochondrial membrane potential, related to Figure 3.

(**A**) – (**C**) $[Ca^{2+}]_{mt}$ in FDB single fibers of WT and $PV^{-/-}$ mice electroporated 4mtGCaMP6f for 7 days. (A) Resting mitochondrial Ca^{2+} levels. (B) Peak mitochondrial Ca^{2+} transient during tetanic stimulation (5 seconds at 60 Hz). (C) Representative traces of the experiment. The arrows indicate the start and the end of the tetanic stimulation. Data are expressed as mean ± SEM. n≥17. For data analysis, parametric Student t-tests (two tailed, unpaired) was used. ** $p<0.01$.

(**D**) TMRM florescence in FDB fibers of WT and $PV^{-/-}$ mice. The bar diagram represents the mean ± SEM. n=20. For data analysis, parametric Student t-tests (two tailed, unpaired) was used.

(**E**) Expression levels of genes coding for the MCU complex components in WT and $PV^{-/-}$ mice. Expression is normalized to POL2. The bar diagram represents the genes expression levels normalized to WT and presented as mean ± SEM. n=3. For statistical analysis, parametric Student t-test (two-tailed, unpaired) was performed. * $p<0.05$, ** $p<0.01$, *** $p\leq0.001$.

(**F**) Representative WB of WT and $PV^{-/-}$ TA muscles stained with α-MCU and α-MICU1 antibodies. α-Actin were used as loading controls. n=4.

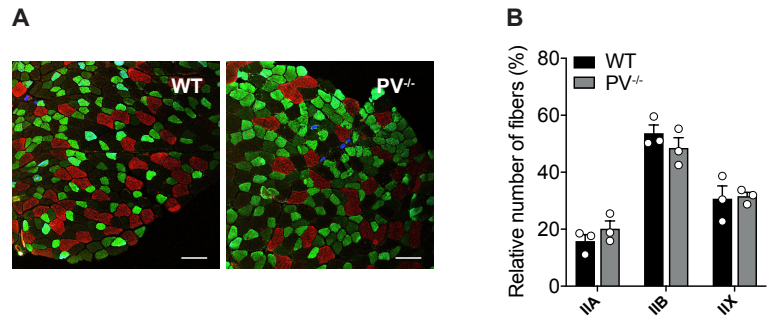

Figure S5. PV ablation does not alter fiber type composition, related to Figure 5.

(**A**) Representative image of 20 µm thick cryosections of WT and $PV^{-/-}$ TA muscles. Scale bar 100 µm. α-SC-71, α-BF-F3 and α-BAD5 primary antibodies were used to label type IIA (green fibers), type IIB (red fibers) and type I (not detected) myosin isoforms, respectively. The unstained fibers correspond to type IIX myosin, typical of fast twitch muscles.

(**B**) Quantitative analysis of fiber type composition in WT and $PV^{-/-}$ TA muscles. mean ± SEM. n=3.

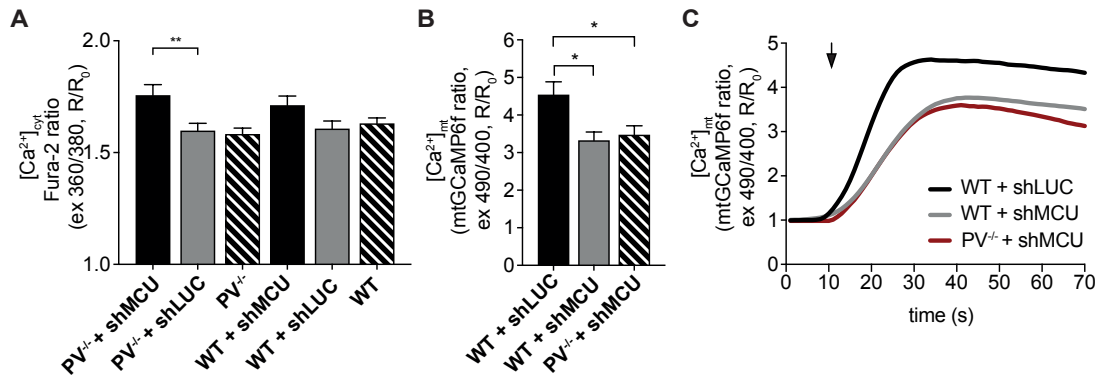

Figure S6. MCU silencing reduces mitochondrial Ca^{2+} uptake in WT and $PV^{-/-}$ fibers and increases peak cytosolic Ca^{2+} transients during 60 Hz stimulation, related to Figure 6.

(**A**) FDB muscle fibers of WT and $PV^{-/-}$ mice were electroporated either shLUC or shMCU and 7 days later isolated and cultured. Cytosolic [Ca^{2+}] was determined with Fura-2 AM. Initial peaks of cytosolic Ca^{2+} transients induced by 2 seconds 60 Hz stimulation are shown. The same parameter measured in untrasfected WT and $PV^{-/-}$ fibers are also shown for comparison. Data are presented as mean ± SEM. n>30. ** p<0.01

(**B**) and (**C**) $[Ca^{2+}]_{mt}$ in FDB single fibers of WT and $PV^{-/-}$ mice electroporated with either shLUC or shPV together with 4mtGCaMP6f for 7 days. (B) Peak $[Ca^{2+}]_{mt}$ upon caffeine stimulation. (C) Representative traces of the experiment. The arrow shows the addition of caffeine. Data are presented as mean ± SEM. n≥18. For data analysis, Student t-tests (two tailed, unpaired) was performed. * p<0.05.

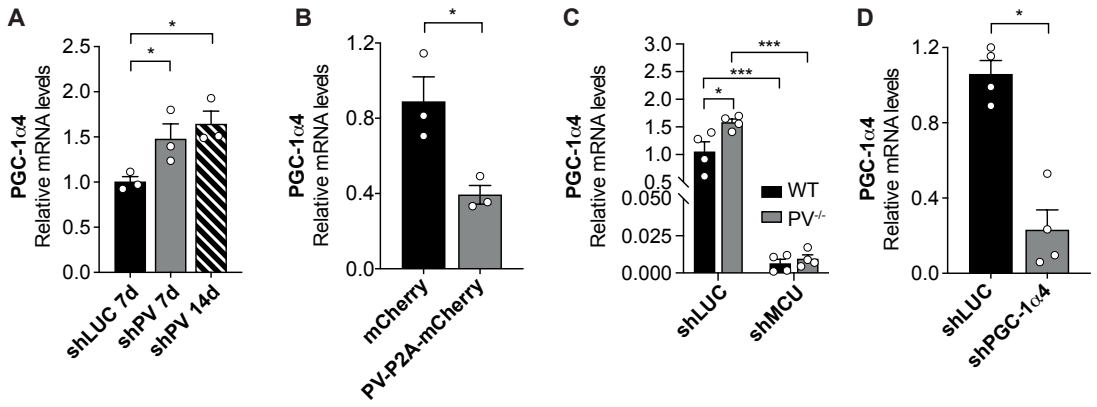

Figure S7. Changes in PGC-1α4 expression following acute PV silencing and overexpression, MCU silencing and efficiency of PGC-1α4 silencing, related to Figure 7.

(**A**) PGC-1α4 expression levels in FDB muscle from WT mice transfected either with shLUC or shPV for 7 or 14 days. The expression levels were normalized to POL2. The bar diagram represents the gene expression levels normalized to WT and presented as mean ± SEM. n=3. For data analysis, two-way ANOVA was used with post hoc Bonferroni's multiple comparison test for each sample. * $p<0.05$.

(**B**) PGC-1α4 expression levels in FDB muscle from WT mice transfected either with mCherry or PV-P2A-mCherry for 7 days. The expression of PGC-1α4 was normalized to Actin. The bar diagram represents the gene expression levels normalized to WT and presented as mean ± SEM. n=3. For data analysis, parametric Student t-tests (two tailed, unpaired) was used. * $p<0.05$.

(**C**) PGC-1α4 expression levels in FDB muscles from of WT and $PV^{-/-}$ mice electroporated either with shLUC or shMCU for 7 days. The expression of PGC-1α4 was assessed and normalized to Actin. The bar diagram represents the gene expression levels normalized to WT and presented as mean ± SEM. n=4. For data analysis, two-way ANOVA was used with post hoc Bonferroni's multiple comparison test for each sample. * $p<0.05$, *** $p<0.001$.

(**D**) PGC-1α4 expression levels in FDB muscle from WT mice electroporated with shLUC or shPGC-1α4 for 7 days. The expression of PGC-1α4 was assessed and normalized to Actin. The bar diagram represents the gene expression levels normalized to shLUC and presented as mean ± SEM. n=4. For data analysis, parametric Student t-tests (two tailed, unpaired) was used. * $p<0.05$.

Table S1. Primer and shRNA sequences. Related to STAR Methods.

Gene	Forward	Reverse
CALRETICULIN	AGGCTCCTTGGAGGATGATT	TCCCACTCTCCATCCATCTC
CALSEQUESTRIN 1	GAGCCTATGACCATCCCAGA	GTCGGGGTTCTCAGTGTTGT
DRP1	GTTCCACGCCAACAGAATAC	CCTAACCCCCTGAATGAAGT
EMRE	GGACTCTGGGCTCTTGTCAC	AGAACTTCGCTGCTCTGCTT
FIS1	AAGTATGTGCGAGGGCTGT	TGCCTACCAGTCCATCTTTC
GAPDH	CACCATCTTCCAGGAGCGAG	CCTTT ATGGTGGTGAAGAC
MCU	AAAGGAGCCAAAAAGTCACG	AACGGCGTGAGTTACAAACA
MCUb	CTGGCTTACTTGGTGGGTGT	CGCTGCGATTTCTTGTGGAA
MICU1	AAGGCAGCATCTTCTACAGCC	CCTGCTCAAACTCCTCCATGT
MICU1.1	CTTTGATGGAAAGGAGTTCTGGC	CCTCCATGTCTACCTCTCCGT
MICU2	TGGAGCACGACGGAGAGTAT	GCCAGCTTCTTGACCAGTGT
MICU3	CGACCTTCAAATCCTGCCTG	TCTGCGTGCTCTGACCTTAC
MITOFUSIN 1	GACCCGTGCGAAAGAGAGAG	TCGAGCAAAAGTAGTGGCCA
MITOFUSIN 2	ATGTTACCACGGAGCTGGAC	AACTGCTTCTCCGTCTGCAT
OPA1	ATACTGGGATCTGCTGTTGG	AAGTCAGGCACAATCCACTT
ORAI1	AAGCCGCGCCAAGCTCAAAG	GGTGGGTAGTCATGGTCTGTGTCCAG
PGC-1α	CGCTGCTCTTGAGAATGGAT	CGCAAGCTTCTCTGAGCTTC
PGC-1α4	TCACACCAAACCCACAGAAA	CTGGAAGATATGGCACAT
POL2	CGACGACTTTGATGACGTTG	GCTCACCAGATGGGAGAATC
PV	GATAGGAGCCTTTGCTGCTG	GCCAGAAGCGTCTTTGTTTC
RYR1	GGTCTGATTATTGATGCTTTTGGGG	TGGTCTCCATGTCTTCCTTCACTTG
SERCA1	GGCCTCATGTCAGCTACCATCAGC	TCGGGGGCCTCAAAGACCTC
SERCA2a	ATGGGGCTCCAACGAATTGC	GCCAAAACGAAAGATATACATGCTGC
SIRT1	TTTTAAGGCTGTTGGTTCCAG	TACCTCAGCACCGTGGAATA
VEGF	ATCCTGGAGCGTTCACTGTG	TTAACTCAAGCTGCCTCGCC
Pik3r1	AAACTCCGAGACACTGCTGA	AGACTCATTCCGGTAGTGGT
shMCU	GATCGGATCCGAGATGACCGTGAATCTTCA AGAGAGATTCACGGTCATCTCGGATCTTTTTG	AATTCAAAAAGATCCGAGATGACCGTGAAT CTCTCTTGAAGATTCACGGTCATCTCGGATCC
shPV	GATCGAGAACCCGGATGAGGTGAAGATTC AAGAGATCTTCACCTCATCCGGGTTCTTTTTTG	AATTCAAAAAAGAACCCGGATGAGGTGAA GATCTCTTGAATCTTCACCTCATCCGGGTTCTC
shPGC-1α4	GATCGATAAATGTGCCATATCTTCCATTCA AGAGATGGAAGATATGGCACATTTATTTTTTG	AATTCAAAAAATAAATGTGCCATATCTTCCA TCTCTTGAATGGAAGATATGGCACATTTATC