

SUPPLEMENTAL INFORMATION

Exercise-like effects by Estrogen-related receptor-gamma in muscle do not prevent obesity and insulin resistance in db/db mice

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Supplemental Table 1. QPCR primer sequences.

FIGURE LEGENDS

Supplemental Figure 1. Overexpression of ERR γ in db/db muscle. (A) ERR γ gene expression measured in gastrocnemius muscle of 6 month-old male mice. (N=4-6). **(B)** Protein expression of ERR γ in gastrocnemius muscle of 6 month-old male mice (N=4-5). * Indicates comparison to db/+ mice; † indicates db/db compared with db/db-ERR γ mice. (*/†p<0.05, ***p<0.001, One-way ANOVA with Tukey's post-hoc test.)

Supplemental Figure 2. Mitochondrial content in db/db ERR γ mice. (A) Quantification of the western blots for mitochondrial complex proteins (N=4). **(B & C)** SDH and NADH-TR staining in medial TA muscles. **(B)** Representative images of the medial TA SDH and NADH-TR staining. **(C)** Percentage of myofibers in the medial TA stained positive for the SDH (N=5-6) and NADH-TR staining (N=4-6). *Indicates comparison to db/+ mice; † indicates db/db compared with db/db-ERR γ mice. (**/††p<0.01, ***/†††p<0.001, One-way ANOVA with Tukey's post-hoc test.)

Supplemental Figure 3. Metabolic and mitochondrial gene expression. (A- B) Metabolic (A) and the mitochondrial (B) gene expression measured by QPCR. The data were obtained on gastrocnemius of 6 month-old fasted male mice (N=6-7). db/+, db/db and db/db-ERR γ mice are represented by hatched, open and black bars, respectively. * Indicates comparison to db/+ mice; † indicates db/db

compared with db/db-ERR γ mice. (*/ \dagger p<0.05, **/ $\dagger\dagger$ p<0.01, ***/ $\dagger\dagger\dagger$ p<0.001, One-way ANOVA with Tukey's post-hoc test).

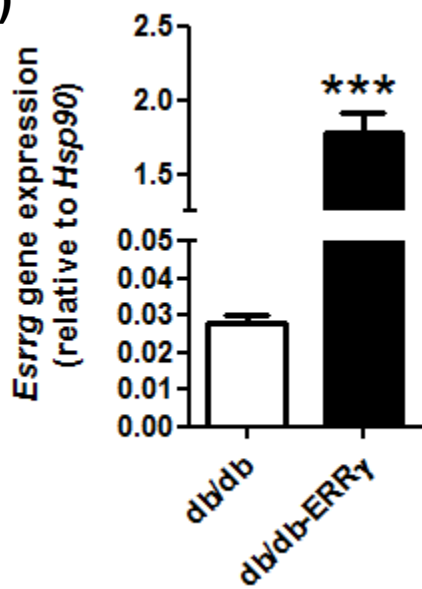
Supplemental Figure 4. Respiratory Exchange Ratio (RER) measured in 5 month-old male db/db (open bars) and db/db-ERR γ (black bars) mice.

Supplemental Figure 5. P-AMPK expression in the muscle. Activating phosphorylation of AMPK on residue Thr-172 measured in gastrocnemius muscles of 6 month old male mice (N=4). dB/+, db/db and db/db-ERR γ mice are represented by hatched, open and black bars, respectively. *Indicates comparison to db/+ mice; \dagger indicates db/db compared with db/db-ERR γ mice. (*/ \dagger p<0.05, One-way ANOVA with Tukey's post-hoc test).

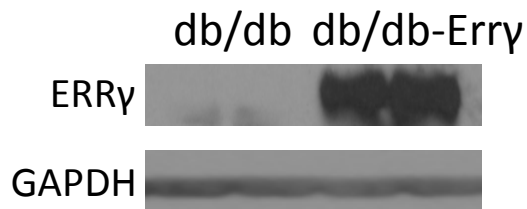
Supplemental Table 1. QPCR primer list.

Supplemental Figure 1

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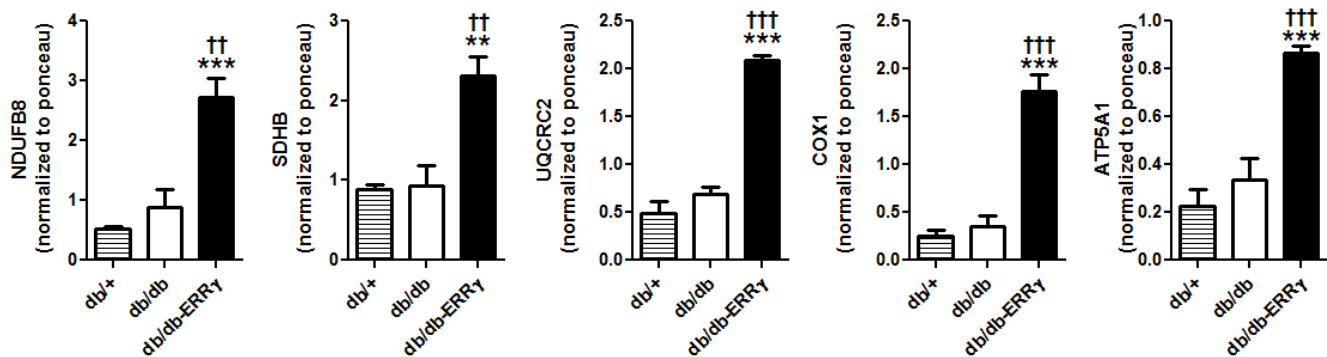


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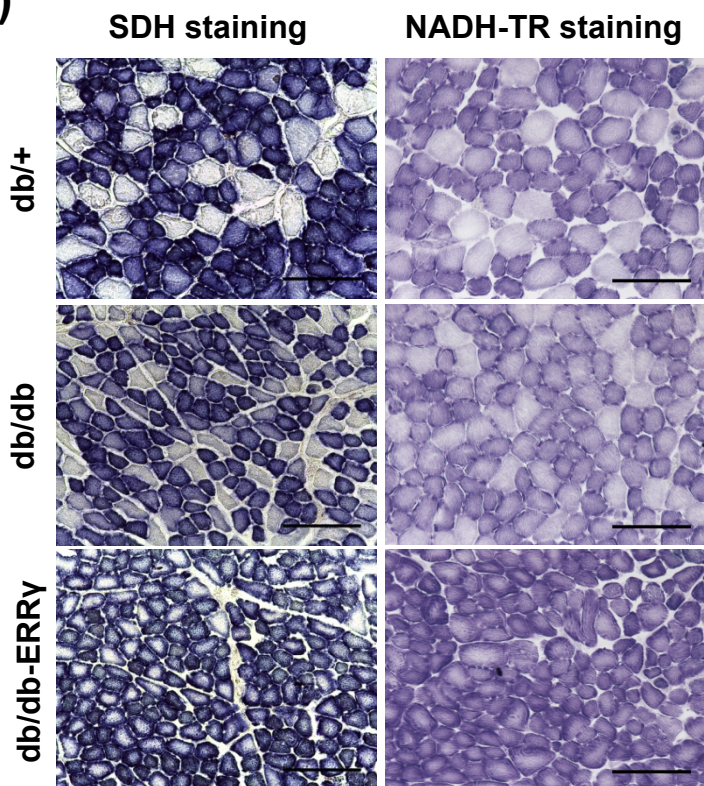


Supplemental Figure 2

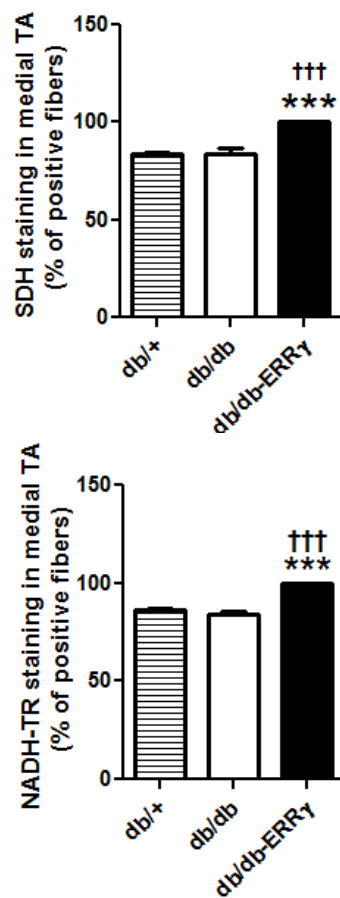
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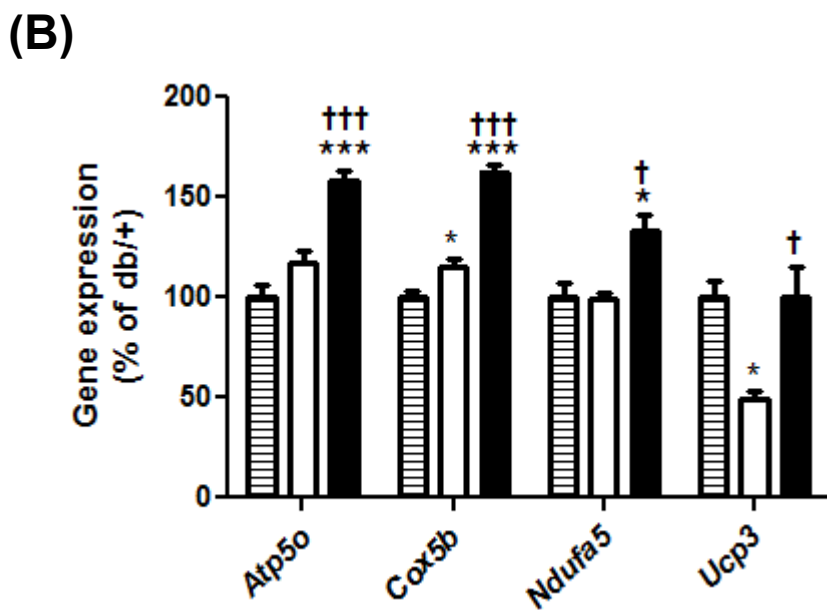
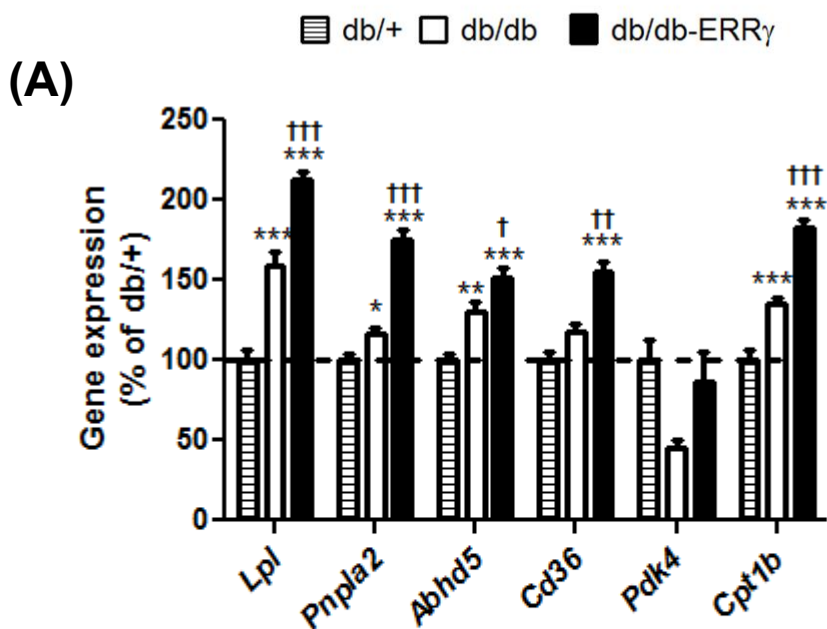
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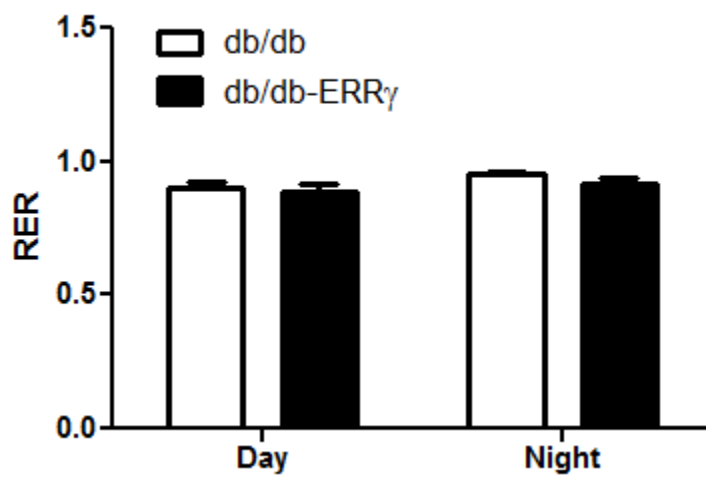
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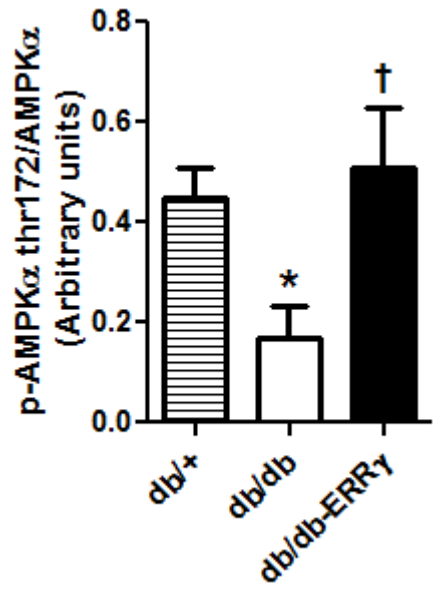
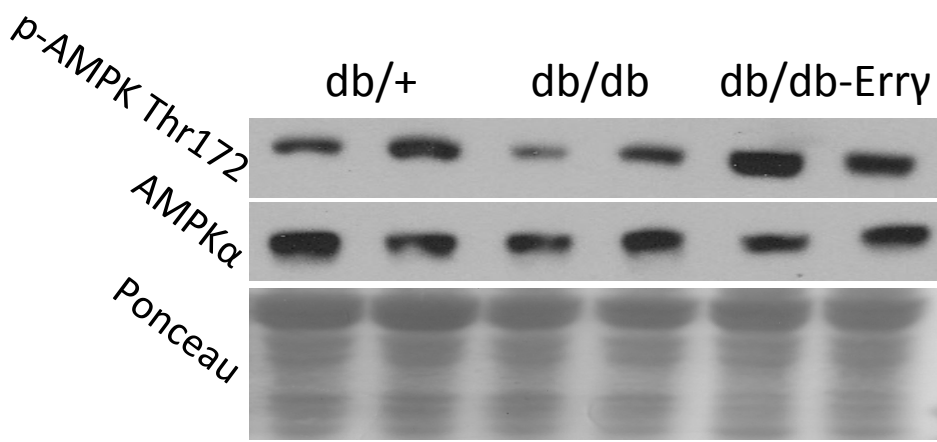
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



SUPPLEMENTAL TABLE 1

	Forward	Reverse
<i>Abhd5</i>	5'-TGACAGTGATGCGGAAGAAG-3'	5'-AGATCTGGTCGCTCAGGAAA-3'
<i>Angpt1</i>	5'-CATTCTTCGCTGCCATTCTG-3'	5'-GCACATTGCCCATGTTGAATC-3'
<i>Angpt2</i>	5'-GGTTGCTATCCGTAAAGAAGAGC-3'	5'-GGGGAAGGTCAGTGTGTAGATG-3'
<i>Atp5o</i>	5'-TCTCGACAGGTTCCGAGCTT-3'	5'-AGAGTACAGGGCGGTTGCATA-3'
<i>CerS1</i>	5'-GCCACCACACACATCTTTCGG-3'	5'-GGAGCAGGTAAGCGCAGTAG-3'
<i>CerS2</i>	5'-GAAGCCAGCTGGAGATTCAC-3'	5'-GACATCAGAGGCAATGCTGA-3'
<i>CerS3</i>	5'-CTCTGGGAGGTTTGG AATGA-3'	5'-CAGGTGGTGGATGACATGAG-3'
<i>CerS4</i>	5'-CCTGCTGGAGGTTTGTCTTC-3'	5'-GGCAAAGTGATCAGCAGTGA-3'
<i>CerS5</i>	5'-ATTTATTGCCAAGCCCTGTG-3'	5'-AACCAGCATTGGATTTTTTCG-3'
<i>CerS6</i>	5'-CTGAAGAACACGGAGGAAGC-3'	5'-TATGGCACATGGTTTGGCTA-3'
<i>Cd36</i>	5'-CTGGGACCATTGGTGATGAAA-3'	5'-CACCCTCCAATCCCAAGTAAG-3'
<i>Cox5b</i>	5'-GCTGCATCTGTGAAGAGGACAAC-3'	5'-CAGCTTGTAATGGGTTCCACAGT-3'
<i>Cpt1b</i>	5'-GGGCACCCTCTGGGAGTTTGT-3'	5'-TGGCTCACCCACACAGTGT-3'
<i>Esrrg</i>	5'-ACTTGGCTGACCGAGAGTTG-3'	5'-GCCAGGGACAGTGTGGAGAA-3'
<i>Fgf1</i>	5'-GAAGCATGCGGAGAAGAAGT-3'	5'-CGAGGACCGCGCTTACAG-3'

<i>Figf</i>	5'-AAATCGCGCACTCTGAGGA-3'	5'-TGGCAAGACTTTTGAGCTTCAA-3'
<i>Gapdh</i>	5'-TGAAGCAGGCATCTGAGGG-3'	5'-CGAAGGTGGAAGAGTGGGAG-3'
<i>Hsp90</i>	5'-GTCCGCCGTGTGTTTCATCAT-3'	5'-GCACTTCTTGACGATGTTCTTGC-3'
<i>Lpl</i>	5'- GCTGGGCCTAACTTTGAGTATG-3'	5'- CAAAATCAGCGTCATCAGGAGAA-3'
<i>Myh1</i>	5'-CTCCAGGCTGCTTTAGAGGAA -3'	5'-CCTGCTCCTAATCTCAGCATCC-3'
<i>Myh2</i>	5'-AATCTTACAAGAGACAAGCTGAGG-3'	5'-TGCGGAACCTTGGATAGATTTG-3'
<i>Myh4</i>	5'- GAAGAGCCGAGAGGTTACACAC-3'	5'- CAGGACAGTGACAAAGAACGTC-3'
<i>Myh7</i>	5'-ACTGTCAACACTAAGAGGGTCA-3'	5'-TTGGATGATTTGATCTTCCAGGG-3'
<i>Ndufa5</i>	5'-AGCTGGATATGGTCAAGGCG -3'	5'- TGCTAACCAGTGAAGTGGC -3'
<i>Pdk4</i>	5'-AAGCAAAACACAAACACGAGTA-3'	5'- CCCGGGTCATCCAACCA-3'
<i>Pnpla2</i>	5'- CAACGCCACTCACATCTACGG -3'	5'- GGACACCTCAATAATGTTGGCA -3'
<i>Ucp3</i>	5'-GAGATGGTGACCTACGACATCA-3'	5'-GCGTTCATGTATCGGGTCTTTA-3'
<i>Vegfa</i> 121	5'- TGCAGGCTGCTGTAACGATG-3'	5'- CCTTGGCTTGTACATTTTTTCT-3'
<i>Vegfa</i> 165	5'- TGCAGGCTGCTGTAACGATG-3'	5'-GAACAAGGCTCACAGTGATTTTCT-3'
<i>Vegfa</i> 189	5'- TGCAGGCTGCTGTAACGATG-3'	5'- CTCCAGGATTTAAACCGGGATT-3'

<i>Vegfb</i>	5'- TGCCATGGATAGACGTTTATGC-3'	5'- TGCTCAGAGGCACCACCAC-3'
<i>Vegfc</i>	5'- AAGACCGTGTGCGAATCGA-3'	5'- CACAGCGGCATACTTCTTCACT-3'