**Supplemental Table 1.** TaqMan primer-probe sets for qRT-PCR a.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Protein name** | **Assay product number** |
| **Primary outcome** |
| *Hamp* | Hepcidin | Mm04231240\_s1 |
| **Gluconeogenic regulators of *Hamp*** |
| *Ppargc1a* | PGC1α | Mm01208835\_m1 |
| *Creb3l3* | CREB3L3 | Mm00520279\_m1 |
| **Gluconeogenic and glycogenolytic enzymes** |
| *Pck1* | PEPCK | Mm01247058\_m1 |
| *Pygl* | Liver glycogen phosphorylase | Mm01289790\_m1 |
| **Inflammatory markers** |
| *Il6* | Interleukin-6 | Mm00446190\_m1 |
| *Crp* | C reactive protein | Mm00432680\_g1 |
| *Orm1* | α-1-acid glycoprotein | Mm00435456\_g1 |
| *Saa1* | Serum amyloid A1 | Mm00656927\_g1 |
| **Erythropoietic signals** |
| *Erfe* | Erythroferrone | Mm00557748\_m1 |
| **Housekeeper gene** |
| *Actb* | β-actin | Mm02619580\_g1 |

a CREB3L3, hepatic-specific cAMP response element binding protein-3-like-3; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1α; REDD1, regulated in development and DNA damage response-1; qRT-PCR, quantitative real-time polymerase chain reaction.

**Supplemental Table 2.** *Redd1* primer sequences for qRT-PCR with PowerUp SYBR Green Master Mix a.

|  |  |  |
| --- | --- | --- |
| **Forward primer** (5’-3’) | **Reverse primer** (3’-5’) | **Amplicon size** (bp) |
| TGGTGCCCACCTTTCAGTTG | GTCAGGGACTGGCTGTAACC | 121 |

a *Redd1* primers were previously reported by Gordon et al. [21]. REDD1, regulated in development and DNA damage response-1; qRT-PCR, quantitative real-time polymerase chain reaction.

**Supplemental Table 3.** Power analysis input and output to estimate required sample size for 95% power to detect an effect of treadmill running on liver *Hamp* expression in mice a.

|  |
| --- |
| **F tests - ANOVA: Fixed effects, omnibus, one-way** |
| **Analysis:** | A priori: Compute required sample size |
| **Input:** | Effect size f | = | 1.076453 |
|  | α err prob | = | 0.05 |
|  | Power (1-β err prob) | = | 0.95 |
|  | Number of groups | = | 4 |
| **Output:** | Noncentrality parameter λ | = | 23.175 |
|  | Critical F | = | 3.238872 |
|  | Numerator df | = | 3 |
|  | Denominator df | = | 16 |
|  | Total sample size | = | 20 |
|  | Actual power | = | 0.959038 |

a Power analysis was performed in G\*Power version 3.1 (32). Effect size was determined using an F-statistic derived from estimated means ± SDs from Banzet et al. (30).

**Supplemental Table 4.** Spearman correlations between liver *Hamp* and other outcomes assessed in REDD1 KO and WT mice combined and separately a.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **KO + WT**(*n* = 51) | **WT**(*n =* 26) | **KO**(*n* = 25) |
| **Primary outcome** |
| *Redd1* | -- | -0.083 | -- |
| **Gluconeogenic regulators of *Hamp*** |
| *Ppargc1a* | 0.078 | 0.0024 | –0.17 |
| *Creb3l3* | 0.56\*\*\* | 0.34 | 0.71\*\*\* |
| **Gluconeogenic and glycogenolytic enzymes** |
| *Pck1* | 0.42\*\* | 0.24 | 0.43\* |
| *Pygl* | 0.27 | –0.019 | 0.25 |
| **Inflammatory markers** |
| *Crp* | 0.57\*\*\* | 0.41\* | 0.62\*\* |
| *Il6* | 0.018 | –0.15 | 0.15 |
| *Orm1* | 0.15 | –0.019 | 0.30 |
| **Tissue nonheme iron** |
| Liver nonheme iron | 0.22 | 0.54\*\* | 0.41\* |
| Spleen nonheme iron | –0.14 | –0.40 | 0.12 |

a Correlations were assessed with liver *Hamp* as the dependent variable and are expressed as Spearman’s *ρ*. Samples sizes are *n* = 8 per group for rested mice and *n* = 5-6 per group for exercised mice, except *Crp, Orm1*, and spleen nonheme ironwere not measured in one rested WT animal. KO, knockout; REDD1, regulated in development and DNA response-1; WT, wildtype. \*indicates 0.05 > *P* ≥ 0.01. \*\*indicates 0.01 > *P* ≥ 0.001. \*\*\*indicates *P* < 0.001.