





Supplemental Figure 1. Gene expression changes with bed rest. A) KEGG terms related to up-regulated (red) and down-regulated (blue) transcripts during human bed rest are shown. Transcripts with RPKM>1 and p<0.05 were used in the analysis. B) Levels of the indicated transcripts were measured by RT-qPCR before (PRE) and after (POST) bed rest. Bars represent mean ± standard deviation (N=3/group).



Supplemental Figure 2. Hindlimb unloading results in time-dependent muscle atrophy. A) A representative picture of the hindlimb unloading model is shown. Mice are suspended by the tail but have free movement in the cage during hindlimb unloading. B) Muscle mass for the indicated muscle group is shown for control, 3, 7, and 14 days of hindlimb unloading. Bars represent mean \pm standard deviation (N=5 per group). **p<0.01 versus control by one-way ANOVA with Tukey's multiple comparison test. C) Daily body weight and food intake measurements are shown over the entire course of the study.



Supplemental Figure 3. Hindlimb unloading results in time-dependent muscle atrophy. Levels of the indicated transcript were measured by RT-qPCR in control mice or following 3, 7, or 14 days of hindlimb unloading. *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001 versus control by one-way ANOVA with Tukey's multiple comparison test.

А

6

4

2

0

Muscle weight (mg/g BW)





Soleus

Muscle weight

Muscle weight

(mg/g BW)









Supplemental Figure 4. Muscle mass recovery during reloading. A) Body weight and muscle mass is shown for the indicated muscle group for control, following 10 days of hindlimb unloading, and 10 days of unloading (HU10) followed by 3 or 10 days of reloading (RE3 and RE10). B) Levels of the indicated transcript were measured by RT-qPCR in control mice or following 10 days unloading and 10 days unloading with 3 or 10 days of reloading. Bars represent mean ± standard deviation (N=5 per group). *p<0.05, ***p<0.001 vs. Con; †<0.05 vs. HU10 by one-way ANOVA with Tukey's multiple comparison test. TA, tibialis anterior; EDL, extensor digitorum longus.



Supplemental Figure 5. Efficient and specific deletion of PGC-1 α and PGC-1 β in skeletal muscle. Levels of PGC-1 α (*Ppargc1a*) and PGC-1 β (*Ppargc1b*) transcripts were measured by RT-qPCR in soleus, gastrocnemius, tibialis anterior (TA), and heart. Bars represent mean \pm standard deviation (N=4-5 per group). *p<0.05 vs. WT by Student's T-test.



Supplemental Figure 6. Gene expression changes in the PGC-1 DKO during unloading and reloading. Levels of the indicated transcript were measured by RT-qPCR in wild-type (WT) or muscle-specific PGC-1 α/β knockout mice. Mice were subjected to 10 days of hindlimb unloading (HU-10) or 10 days of hindlimb unloading followed by 21 days of reloading (RE-21). Bars represent mean \pm standard deviation with values to normalized to the WT control. *p<0.05, **p<0.01, ****p<0.001 vs. respective control by one-way ANOVA with Tukey's multiple comparison test.

Supplemental Table. Levels of acylcarnitines, cardiolipin and phospholipid species are shown for control and following hindlimb unloading (HU) for 3, 7, and 14 days Values are nmol/mg protein presented as mean \pm standard deviation (N=4/group). *p<0.05, **p<0.01, ***p<0.001 vs. Control by one-way ANOVA with Tukey's multiple comparison test.

| | CON | HU 3 Days | HU 7 Days | HU 14 Days |
|--|---------------|----------------|-----------------|----------------|
| CAR-14:2 | 2.72±0.82 | 3.42±1.07 | 3.45±0.10 | 1.04±0.19 |
| CAR-14:1 | 14.00±1.33 | 10.66±1.97 | 11.61±1.04 | 7.59±1.04* |
| CAR-14:0 | 18.6±1.7 | 13.9±1.6 | 14.7±2.5 | 10.7±1.6 |
| CAR-16:2 | 12.01±2.06 | 11.45±2.65 | 9.03±0.75 | 5.20±0.93 |
| CAR-16:1 | 35.3±2.7 | 17.1±3.0** | 18.3±2.6** | 13.5±2.3*** |
| CAR-16:0 | 58.7±6.2 | 29.4±5.5* | 25.7±3.7** | 25.5±5.7** |
| CAR-18:2 | 51.4±8.0 | 28.2±7.6 | 17.2±0.9** | 16.9±3.6** |
| CAR-18:1 | 74.8±10.0 | 34.5±8.7** | 27.8±3.0** | 26.9±5.2** |
| CAR-18:0 | 21.9±3.4 | 16.5±4.0 | 12.8±1.7 | 13.4±2.7 |
| CL-18:2-18:2-16:1-16:1 | 0.0192±0.0039 | 0.0169±0.0013 | 0.0118±0.0017 | 0.0098±0.0024 |
| CL-18:2-18:1-16:1-16:1 | 0.0121±0.0017 | 0.0080±0.0012 | 0.0059±0.0016 | 0.0054±0.0020 |
| CL-18:1-18:1-16:1-16:1 | 0.0049±0.0016 | 0.0017±0.0003 | 0.0023±0.0004 | 0.0028±0.0012 |
| CL-18:1-18:1-16:0-16:1 | 0.0073±0.0018 | 0.0021±0.0006 | 0.0042±0.0006 | 0.0059±0.0022 |
| CL-18:1-18:1-16:0-16:0, 18:2-18:0-16:0-16:0, 18:1- 18:0-16:0-16:1 | 0.0073±0.0029 | 0.0046±0.0011 | 0.0056±0.0008 | 0.0077±0.0034 |
| CL-18:2-18:2-18:2-16:2 | 0.0087±0.0027 | 0.0074±0.0013 | 0.0041±0.0012 | 0.0036±0.0011 |
| CL-18:2-18:2-16:1 | 0.099±0.015 | 0.085±0.012 | 0.060±0.008 | 0.055±0.009 |
| CL-18:2-18:2-18:1-16:1. 18:2-18:2-18:2-16:0 | 0.0910±0.0103 | 0.0635±0.0116 | 0.0489±0.0035* | 0.0487±0.0072* |
| CL-18:2-18:1-18:1-16:1. 18:2-18:2-18:1-16:0 | 0.0567±0.0082 | 0.0324±0.0063 | 0.0321±0.0032 | 0.0363±0.0073 |
| CL-16:1-18:1-18:1-18:1. 18:2-18:2-18:1-16:0 | 0.0340±0.0042 | 0.0190±0.0020 | 0.0187±0.0017 | 0.0269±0.0056 |
| Cl -18:3-18:3-18:2-18:2 | 0.0117+0.0040 | 0.0118+0.0024 | 0.0078+0.0018 | 0.0056+0.0011 |
| CI -18:3-18:2-18:2-18:2 | 0.0423+0.0083 | 0.0469+0.0053 | 0.0319+0.0027 | 0.0254+0.0044 |
| CL -18:2-18:2-18:2 | 0.914+0.086 | 1 004+0 066 | 0.689+0.101 | 0.578+0.113 |
| CI -18:2-18:2-18:2-18:1 | 0.659+0.049 | 0.735+0.058 | 0.551+0.068 | 0.415+0.063 |
| CL -18:2-18:2-18:1-18:1 | 0.210+0.027 | 0.200+0.022 | 0 157+0 014 | 0.127+0.019 |
| CI -18:2-18:2-16:1-22:6 20:4-18:2-18:2-18:3 | 0.0638+0.0095 | 0.0658+0.0093 | 0.0509+0.0041 | 0.0416+0.0045 |
| CL_18:2-18:1-16:1-22:6, 20:4-18:2-18:2-18:2 | 0.0725+0.0066 | 0.0665±0.0093 | 0.0566+0.0035 | 0.0490+0.0029 |
| CL_18:1_18:1_16:1_22:6 | 0 174+0 015 | 0.162+0.015 | 0 122+0 012 | 0.097+0.009** |
| CL_18:1_18:1_16:0_22:6 | 0.129+0.008 | 0.153+0.010 | 0.120+0.008 | 0.087+0.007* |
| CL_18:1-18:0-16:0-22:6 | 0.0486+0.0048 | 0.0478+0.0034 | 0.0412+0.0008 | 0.0334+0.0034* |
| CL_2226_18:2-18:2-18:3 | 0.0195+0.0052 | 0.0288+0.0047 | 0.0215+0.0019 | 0.0123+0.0019 |
| CL-22:6-18:2-18:2-18:2 | 0.675+0.056 | 0.874+0.072 | 0.0213±0.0019 | 0.551+0.049 |
| CL_18-2_18-2_18-2_22-5_18-1_18-2_18-2_22-6 | 0.248+0.022 | 0.310+0.044 | 0.303+0.030 | 0.331±0.043 |
| CL 22:6 22:6 18:2 16:1 | 0.0053±0.0018 | 0.0087±0.0015 | 0.0087±0.0015 | 0.0059+0.0005 |
| CL-22:6-22:6-18:1-16:1 | 0.0092+0.0079 | 0.0144+0.0017 | 0.0161+0.0011 | 0.0093+0.0011 |
| CL_18·2_18·2_20·3_22·6_22·6_22·6_18·1_16·0 | 0.064+0.008 | 0.085+0.006 | 0.077+0.004 | 0.056+0.002 |
| CL_18:2-18:2-20:2-22:6, 22:0-22:0-10:1-10:0 | 0.0396+0.0058 | 0.0684+0.0060* | 0.0669+0.0022** | 0.03610.002 |
| CL 18:2 18:2 20:1 22:6 18:1 18:2 20:2 22:6 | 0.0036±0.0006 | 0.0032±0.0014 | 0.000310.0022 | 0.0016+0.0004 |
| CL-18:2-18:2-22:6-22:6 | 0.146+0.012 | 0.222+0.027 | 0.23/+0.016* | 0.162+0.014 |
| CL 18:1 18:2 22:6 22:6 | 0.0076±0.0014 | 0.0081±0.0008 | 0.0124±0.0018 | 0.0003+0.0010 |
| CL 22:6 22:6 20:4 18:1 | 0.007010.0014 | 0.0001±0.0008 | 0.0124±0.0010 | 0.009310.0010 |
| CL 20.4 20.4 22.6 22.6 | 0.0051±0.0013 | 0.009010.0014 | 0.0134±0.0014 | 0.0113+0.0021 |
| UC0 CL 18:2 18:2 18:2 | 0.000910.0022 | 0.013010.0020 | 0.021110.0022 | 0.0011310.0021 |
| lyso-CL-10.2-10.2-10.2 | 0.0202±0.0041 | 0.0380±0.0150 | 0.0131±0.0004 | 0.0034±0.0013 |
| has CL 19:2 19:2 20:2 | 0.0092±0.0024 | 0.0016+0.0011 | 0.0000±0.0033 | 0.0012±0.0003 |
| lyso-CL-18.2-18.2-20.3 | 0.0008±0.0002 | 0.0016±0.0011 | 0.0005±0.0003 | 0.0002±0.0001 |
| Iyso-CL-16.2-16.2-22.0 | 0.0100±0.0022 | 0.0200±0.0000 | 0.0111±0.0049 | 0.0023±0.0009 |
| | 22.911.2 | 21.3I1.4 | 20.0±1.1 | 24./11.1 |
| | 2.13±0.27 | 2.9/±0.10 | 2.12±0.12 | 2.50±0.17 |
| | 0.430±0.025 | 0.303±0.017 | 0.300±0.022 | 0.309±0.034 |
| SM Total | 23.0±0.0 | 30.0IZ.0 | 29.313.4 | 20.011.1 |
| | 0.240+0.005 | 2.30±0.05 | 2.30±0.23 | 2.19±0.11 |
| CER TOTAL | 0.340±0.035 | 0.286±0.017 | 0.344±0.035 | 0.359±0.018 |