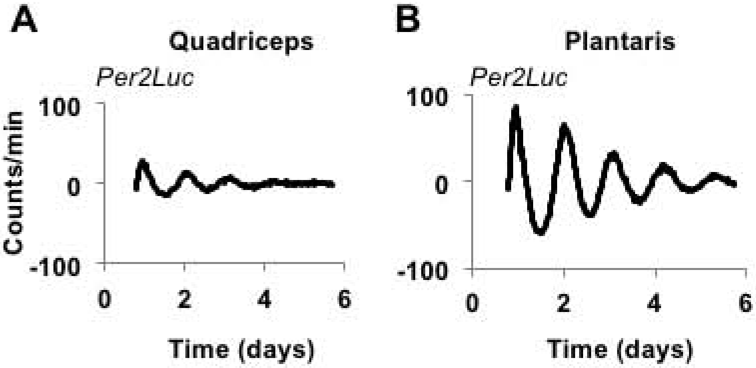

Figure S1. Circadian rhythms in muscle explants. Related to Figure 1.
Continuous recording of luminescence from explants of quadriceps, plantaris or soleus muscles isolated from *PER2::LUC* transgenic mice.

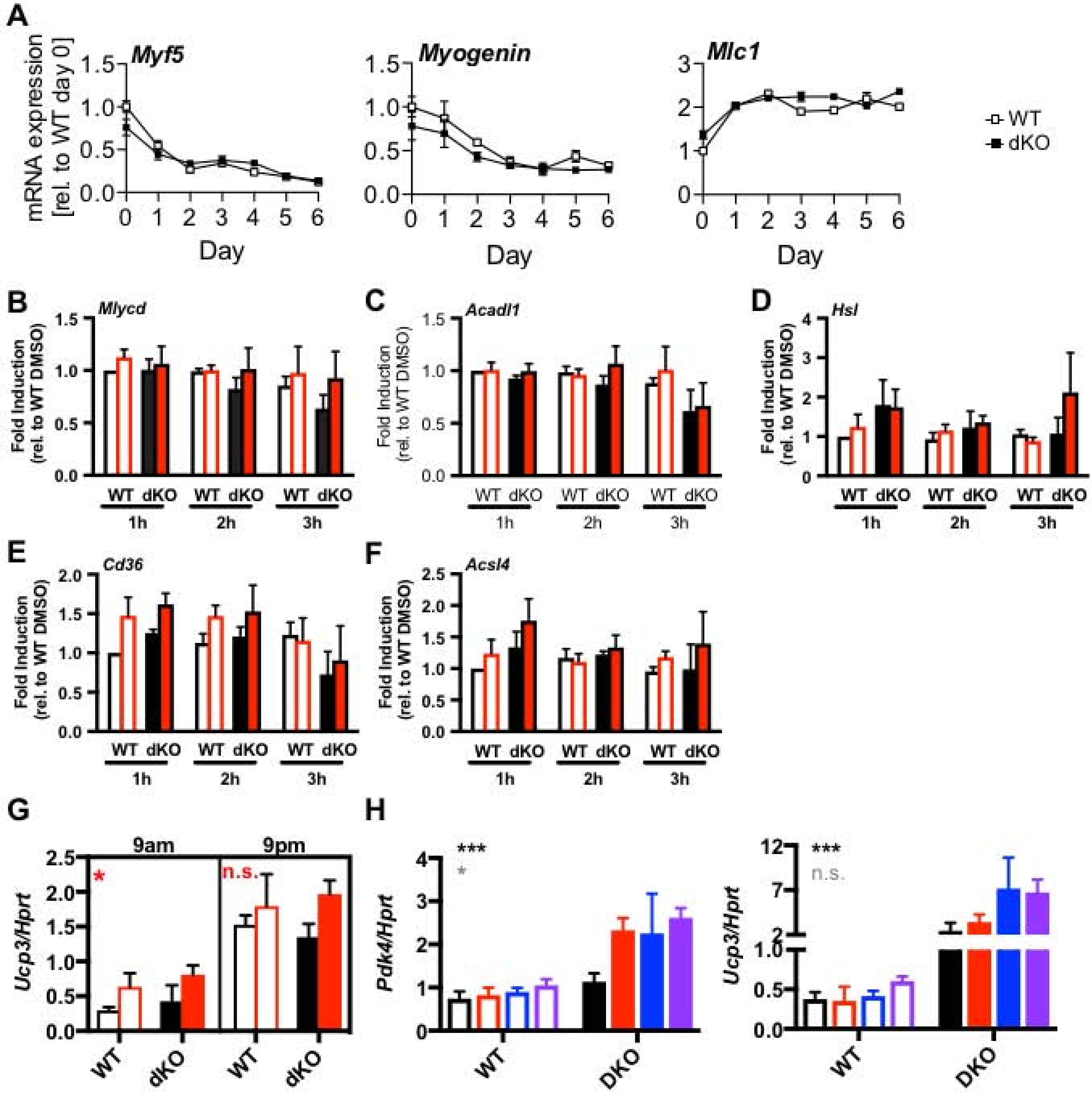

Figure S2. Gene expression in primary myotubes. Related to Figure 3.
Relative mRNA expression of the indicated genes in WT (open symbols) or dKO (closed symbols) in primary muscle cells at the indicated times during differentiation (A), at the indicated times following treatment with vehicle (DMSO, black) or GW1516 (red) (B-F), or after treatment with vehicle (black), AICAR (daily for 3 days at 9am, red), GW1516 (3 hrs, blue) or AICAR + GW1516 (purple) (G,H). Data represent the mean ± s.e.m of two or three biological replicates, each measured in triplicate. In (G) data were analyzed by 3-way ANOVA for effects of genotype (n.s.), treatment (red symbols) or time of day (P<0.05). In (H) data were analyzed by 2-way ANOVA for effects of genotype (black symbols) or treatment (gray symbols) * P < 0.05, *** P < 0.001.

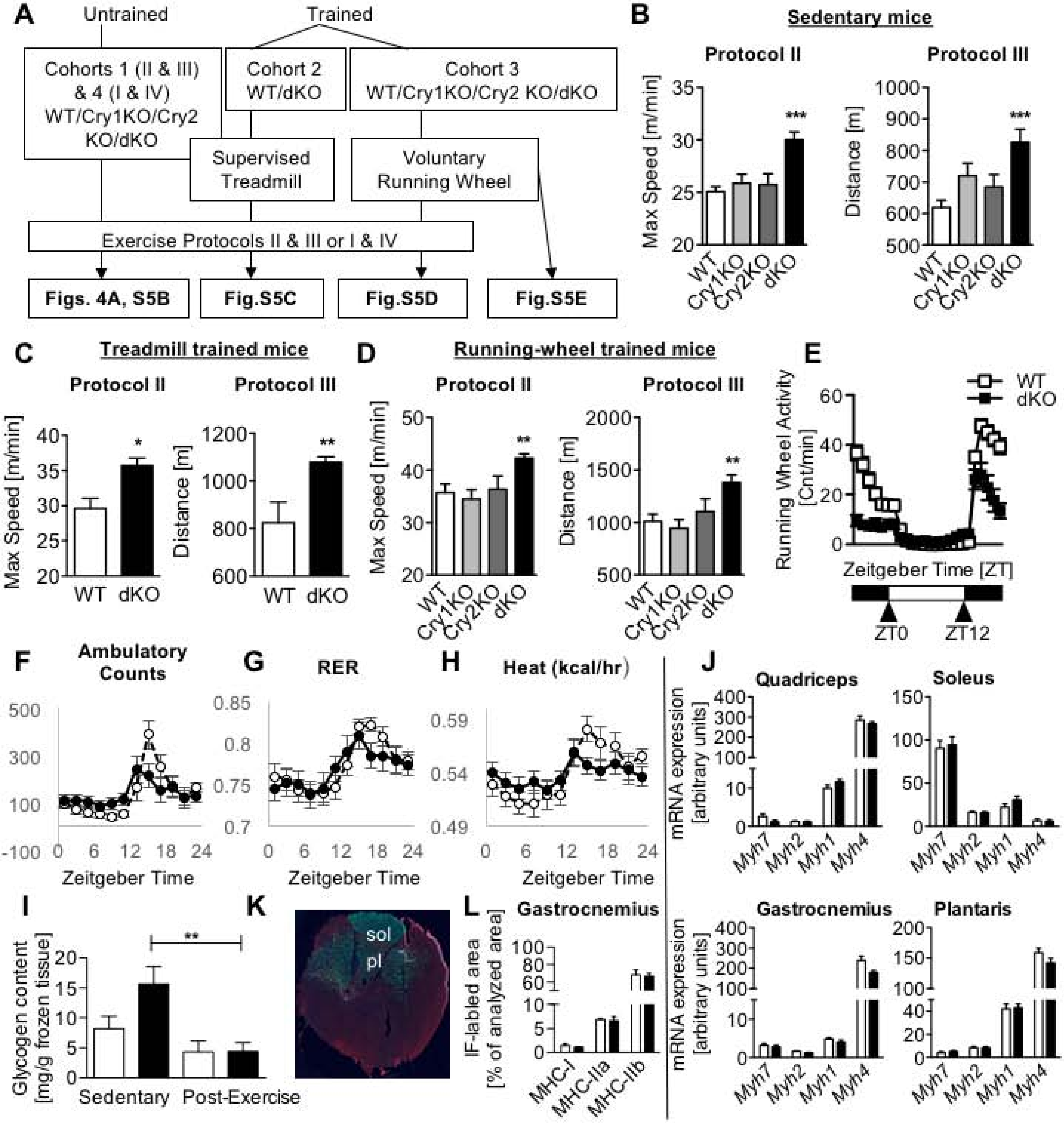

Figure S3. Metabolic analyses of Cry-deficient mice. Related to Figure 4.
(A) Chart depicting experimental setups used to obtain data in the indicated figures. (B-D) Maximum speed (protocol II) and distance (protocol III) achieved by sedentary (B), treadmill trained (C) or running-wheel trained (D) male mice. N=7-35. (E) Spontaneous activity measured over 8 days and averaged in 1-hour intervals. N=12-24 animals per genotype. (F-H) Ambulatory movement (F) respiratory exchange ratio (G, RER), and heat production (H) in male littermate mice. Primary data reported in (Lamia et al., 2011). N=6 per genotype. (I) Glycogen content in liver measured in samples isolated from male mice without (Sedentary) or 5 minutes after one hour of intense running on a treadmill (Post-Exercise). (J) mRNA expression of the indicated genes. N=7-8 samples per genotype. (K) Representative immunofluorescence slide scan image of gastrocnemius, plantaris (pl) and soleus (sol) muscles stained for MHCs I (blue), IIa (green) and IIb (red). (L) Semi-automated quantification of images like (K) In all figures, white and black symbols represent WT and dKO respectively. Data represent the mean ± s.e.m. *, **, *** P < 0.05, 0.01, 0.001.

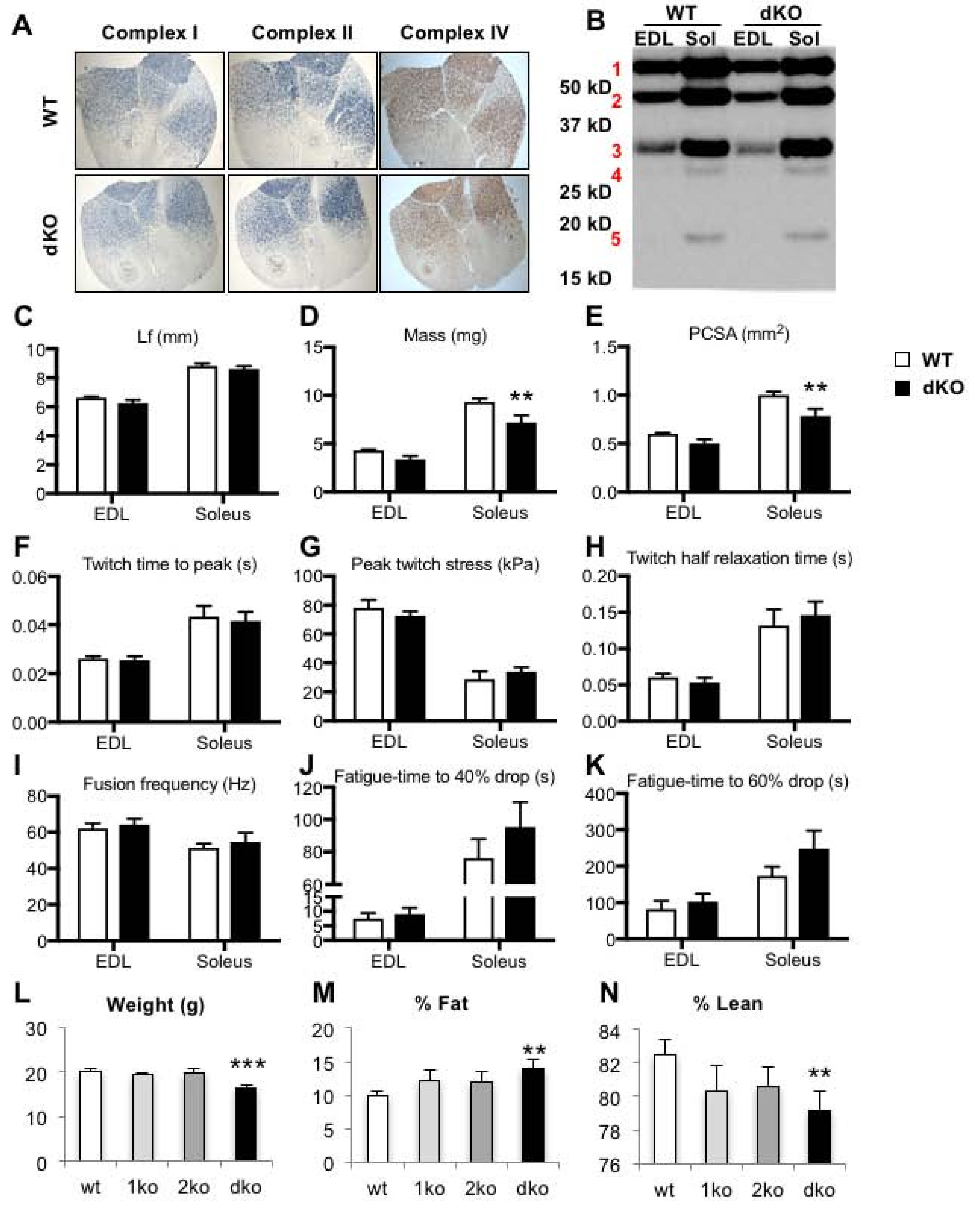

Figure S4. Metabolic analyses of Cry-deficient mice. Related to Figure 4.
(A) Representative images of enzyme activity of mitochondrial respiratory chain complexes in gastrocnemius, plantaris and soleus muscle in WT and dKO mice. (B) Representative Immunoblot of mitochondrial OXPHOS complex composition in extensor digitorum longus (EDL) and soleus of WT and dKO mice. 1 = C-V-α (53 kD), 2 = C-III-Core 2 (47 kD), 3 = C-IV-I (39 kD), 4 = C-II-30 [FeS] (30 kD), 5 = C-I-20 [ND6] (20 kD). (C-K) Extensor digitorum longus (EDL) and soleus muscle fibers isolated from wildtype and Cry1-/-;Cry2-/- littermate animals (N=5 per genotype) were subjected to the indicated analyses. ** $P < 0.01$ vs. wildtype by 2-way ANOVA. (L-N) Body weight (L) and body composition (fat, M and lean, N) measured by EchoMRI. N=8-12 animals per genotype. **$P<0.01$, ***$P < 0.001$ vs. WT.

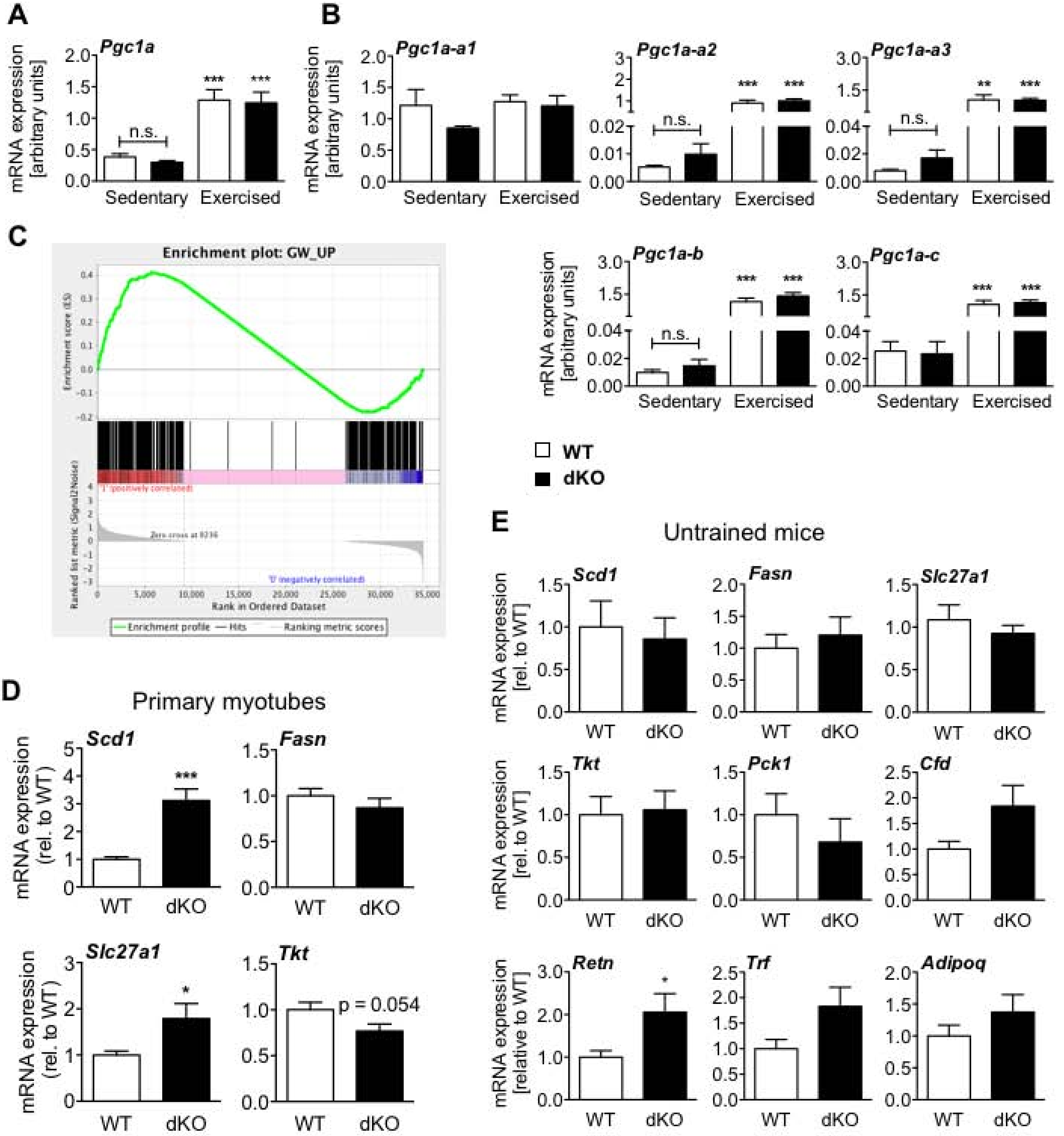

Figure S5. Gene expression in muscle groups in response to exercise *in vivo*. Related to Figure 5.
(A,B) Relative total *Pgc1α* mRNA expression (A) or of the indicated *Pgc1α* transcripts (B) in quadriceps of untrained mice 30 minutes after one hour of either rest (sedentary) or intensive treadmill exercise (exercised). N=7-8 mice per genotype. n.s. not significant, n.s. not significant, **p < 0.01 ***p < 0.001 vs sedentary control or as indicated.
(C) Enrichment plot showing the relative position of each transcript in the gene set within the list of all transcripts detected ranked according to their relative expression change between genotypes. Red – increased expresssion, blue – decreased expression in Cry dKO compared to WT. (D) Relative mRNA expression of the indicated genes in fully differentiated WT (white) or dKO (black) myotubes. Data represent the mean ± s.e.m of three independent experiments each done in triplicates. *p < 0.05, *** p < 0.001 vs. WT. (E) Relative mRNA expression of the indicated genes in quadriceps of untrained WT (white) and dKO (black) mice. Data represent the mean ± s.e.m of 7-8 mice per genotype. *p < 0.05 vs WT.

Table S1 Gene Set Enrichment Analysis Overrepresented Pathways. Related to Figure 5.

Upregulated in Sedentary DKO v. Sedentary WT:	***FDR***
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	0.04
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.20
KEGG_GAP_JUNCTION	0.24
Downregulated in Sedentary DKO v. Sedentary WT: (enriched in WT)	***FDR***
RICKMAN_HEAD_AND_NECK_CANCER_F	0.05
WANG_NFKB_TARGETS	0.05
REACTOME_STRIATED_MUSCLE_CONTRACTION	0.05
CHEMELLO_SOLEUS_VS_EDL_MYOFIBERS_UP	0.05
KEGG_CARDIAC_MUSCLE_CONTRACTION	0.05
KEGG_DILATED_CARDIOMYOPATHY	0.07
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	0.08
REACTOME_PYRUVATE_METABOLISM_AND_CITRIC_ACID_TCA_CYCLE	0.09
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.09
KAAB_HEART_ATRIUM_VS_VENTRICLE_DN	0.09
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	0.10
WONG_MITOCHONDRIA_GENE_MODULE	0.15
REACTOME_PEROXISOMAL_LIPID_METABOLISM	0.15
KEGG_PARKINSONS_DISEASE	0.15
REACTOME_MUSCLE_CONTRACTION	0.16
KEGG_PROPANOATE_METABOLISM	0.17
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	0.18
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	0.18
KEGG_OXIDATIVE_PHOSPHORYLATION	0.20
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	0.21
KEGG_ALPHA_LINOLENIC_ACID_METABOLISM	0.21
MOOTHA_MITOCHONDRIA	0.23
LE_NEURONAL_DIFFERENTIATION_DN	0.23
KEGG_PYRUVATE_METABOLISM	0.23
KEGG_LINOLEIC_ACID_METABOLISM	0.23
REACTOME_CITRIC_ACID_CYCLE_TCA_CYCLE	0.24
STEIN_ESRRA_TARGETS_UP	0.24
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS	0.24
KEGG_FATTY_ACID_METABOLISM	0.24
Upregulated in Exercised DKO v. Exercised WT:	***FDR***
KEGG_O_GLYCAN_BIOSYNTHESIS	0.20
LEE_LIVER_CANCER_MYC_DN	0.21
WEINMANN_ADAPTATION_TO_HYPOXIA_UP	0.22
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_GRANULOCYTE_DN	0.23
Downregulated in Exercised DKO v. Exercised WT:	***FDR***
BIOCARTA_PGC1A_PATHWAY	0.24

Table S2 Transcripts increased by chronic GW1516 treatment *in vivo*. Related to Figure 5.

1110014N23Rik	Arl4d	Cox8a	Fabp3	Herpud2	Map3k6	Pcgf3	Ptp4a1	Slc27a1	Tmem120b
1110038D17Rik	Arl6ip1	Cpsf4	Fabp4	Hgs	Mapk1ip1	Pck1	Puf60	Slc35e4	Tmem43
1300010F03Rik	Armcx4	Cpt1b	Fam13a	Hmga2-ps1	Mcam	Pcnt	Pxmp4	Slc36a2	Tmem50a
1700037H04Rik	Arrdc2	Crat	Fasn	Hp	Mcf2l	Pcx	Qsox1	Slc43a1	Tmtc1
2310001A20Rik	Arrdc3	Creld1	Fbp2	Hp1bp3	Mettl7a1	Pde4b	R3hdm1	Slc7a10	Tob2
2310028H24Rik	Art5	Cryab	Fbxo18	Hr	Mgll	Pdia6	Rab11b	Slc7a2	Tor2a
5730469M10Rik	Asb4	Csad	Fbxo21	Hsd11b1	Mgst1	Pdk4	Rab40b	Slc7a8	Trafd1
6430571L13Rik	Atic	Ctnna1	Fbxo31	Hsd17b12	Mid1	Pdlim3	Rbp4	Slc8a3	Trf
8430408G22Rik	Atn1	Ctnnbip1	Fcgrt	Hsd17b4	Mif	Pdpr	Rcan1	Slc9a1	Trim28
9030624J02Rik	Atp13a3	Ctss	Fgfr1	Hsp90ab1	Mll2	Pelp1	Rela	Smarca4	Trim7
9430023L20Rik	Atxn10	Cul9	Fibin	Hspb1	Mlxip	Pepd	Reps1	Smg6	Trp53inp1
9530068E07Rik	B2m	Cyb5	Fkbp5	Hspd1	Mlycd	Per1	Retn	Smtnl2	Trp63
A230051G13Rik	BC031353	Cyp27a1	Fmo1	Hsph1	Mmd	Pex11a	Retsat	Snap29	Tsc22d3
C130074G19Rik	Bcl2l1	Cyp2e1	Fntb	Htra3	Mocs1	Pex16	Rhbdf2	Snapc2	Tspan14
Abhd16a	Bmyc	Cyth2	Furin	Ifi27l1	Mon1a	Pex6	Rhob	Snrnp200	Tspo
Abtb1	Bscl2	D4Wsu53e	Fzd4	Ifi27l2a	Mpdu1	Pfkfb1	Rhobtb1	Snx10	Tssc4
Acaa2	Bsdc1	Dapk3	Gab1	Ifngr1	Mstn	Pfkfb3	Rit1	Sod3	Tuba4a
Acacb	Btg2	Dbi	Gadd45a	Igfbp4	Mtap1s	Pfkfb4	Rnf167	Sorbs2	Tubb2c
Acadl	C3	Dbnl	Gadd45g	Impdh1	Mtap4	Pgd	Rnf31	Sort1	Tubb5
Acads	C4b	Ddit4	Galntl2	Insig1	Mxd4	Pgls	Rpl3	Sparc	Tusc5
Acly	Car3	Ddr1	Gamt	Ipo13	Mxra8	Pik3ip1	Rrp12	Sra1	Tut1
Aco1	Cat	Ddx54	Gatad2b	Ipo4	Mybbp1a	Pik3r1	Samhd1	Srpr	Ube2o
Acot2	Cblb	Ddx56	Gatsl2	Irgq	Mybph	Pik3r2	Scamp4	Srrm2	Ube4a
Acp5	Ccdc80	Dgat1	Gclm	Jak1	Myf6	Pim3	Scand1	Stard3	Ubr4
Acsl1	Ccdc86	Dgat2	Glul	Jun	Myh14	Plbd1	Scarb1	Stk11ip	Uck1
Acsl3	Ccng2	Dgkd	Gm9766	Kctd20	Myo18b	Plcd4	Scd1	Stom	Ucp2
Acss2	Ccrl2	Diap1	Golgb1	Kdm6b	Myo1c	Plec	Sec24b	Sugp1	Ucp3
Actc1	Cdc14a	Dnajb12	Gpam	Klf10	Naca	Plekhh3	Sec31a	Sult1a1	Usp2
Acvr1b	Cdk2ap2	Dpep1	Gprc5c	Klhl38	Nadk	Plin1	Sepp1	Supt3h	Vars
Adh1	Cdk5rap1	Dpp3	Gpt2	Kptn	Napb	Plin2	Serf1	Svil	Wasf2
Adig	Cdo1	Dupd1	Gpx1	Lars2	Net1	Plin4	Serinc2	Synpo2l	Wbp7
Adipoq	Cebpa	Dync1h1	Grb10	Ldhb	Nfkbia	Plin5	Serpina3n	Tacc2	Wdr44
Adrb2	Cebpb	Dyrk1b	Grina	Lgals3bp	Nkain1	Pmepa1	Sesn1	Taldo1	Wdr46
Agpat2	Cebpd	Eci2	Grlf1	Lgals4	Nol3	Pnpla2	Setd1a	Tars2	Wdr8
Agpat3	Ces1d	Eda2r	Grn	Lipe	Nol6	Pnpla7	Sf1	Tbc1d20	Xdh
AI837181	Cfd	Eef1a1	Gse1	Lmo7	Npc2	Pnrc1	Sgk1	Tbc1d4	Xpc
Aifm2	Chac1	Egln3	Gsk3b	Lmtk2	Npdc1	Podn	Sh2b3	Tcap	Yars
Akap13	Chd4	Ehd4	Gstp2	Lpcat3	Nr1h3	Polr3e	Sh3glb2	Tcfcp2l1	Ypel3
Alad	Chpf2	Eif3d	Gstt1	Lpl	Nudt18	Por	Sh3pxd2a	Tcfeb	Ywhab
Aldh2	Chst1	Eif4ebp1	Gstt2	Lrg1	Numa1	Pparg	Shisa2	Tcn2	Ywhag
Amot	Cidec	Elmod3	Gstz1	Lrp1	Nup93	Ppp1r12b	Sik1	Tcp11l2	Zbtb16
Anapc2	Cish	Eml2	H19	Lrrc30	Obsl1	Ppp1r1a	Sik2	Tcta	Zbtb7b
Angptl4	Cited2	Ephx2	H2-Ab1	Lrrc41	Odc1	Ppp2r1b	Sik3	Tecpr1	Zc3h4
Ankrd11	Clec10a	Ercc5	H2-D1	Lrrc58	ORF19	Prkag3	Slc12a2	Tecpr2	Zfp36
Ankrd9	Clip1	Errfi1	H2-Eb1	Lrrc8a	Ormdl3	Prkar2b	Slc1a5	Tenc1	Zfp503
Aoc3	Cnnm3	Esyt1	H2-K1	Lztr1	Osbpl11	Prpf3	Slc25a1	Tgfbr2	Zfp579
Aox1	Cobra1	Etfb	H2afx	Mafb	Otud5	Prpf40b	Slc25a20	Thrsp	Zfp598
Aplp2	Col1a1	Exoc7	H6pd	Maged1	Paf1	Prpf8	Slc25a22	Tirap	Zfp652
Apoc1	Col3a1	Exosc5	Hadh	Maged2	Pak2	Prrc1	Slc25a23	Tkt	Zfp703
Apoe	Coro1b	Eya4	Hba-a1,Hba-a2	Malat1	Palld	Prrc2a	Slc25a25	Tle4	Zfp771
Arid1a	Cox6a1	Ezh1	Herpud1	Mamstr	Pank3	Ptms	Slc25a42	Tmem120a	Zfyve1
									Znfx1

Table S3 Primer sequences for qPCR analysis. Related to Figures 3, 4, and 5.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Acadl	CTC CGC CCG ATG TTC TCA TTC	CTC TAC TCA CTT CTC CAG CTT TC
Acsl4	CAG AAA ACT TGA GCG TTC CTC CAA G	GCA CAT GAG CCA AAG GTA AGT AGC
Adipoq	TGT TCC TCT TAA TCC TGC CCA	CCA ACC TGC ACA AGT TCC CTT
Cd36	GAT TAA TGG CAC AGA CGC AGC C	GAT GCA AAG GCA TTG GCT GGA AG
Cfd	CAT GCT CGG CCC TAC ATG G	CAC AGA GTC GTC ATC CGT CAC
Cox1	TGC TAG CCG CAG GCA TTA CT	CGG GAT CAA AGA AAG TTG TGT TT
Cpt1b	CAC CGG TAC TTG GAT TCT GTG C	GTA GAC ATA TTC TTC CCA CCA GTC
Fabp3	CAC AGA GAT CAA CTT TCA GCT GGG	CCA TGA GTG AGA GTC AGG ATG AG
Fasn	GGA GGT GGT GAT AGC CGG TAT	TGG GTA ATC CAT AGA GCC CAG
Hprt	TGC TCG AGA TGT CAT GAA GG	TAT GTC CCC CGT TGA CTG AT
Hsl	CTT CCA GTT CAC ACC TGC CAT C	CCA CGC AAC TCT GGG TCT ATG
Lpl	CAT GGA TGG ACG GTA ACG GGA ATG	CCC TAA GAG GTG GAC GTT GTC TAG
Mlc1	GCA ACA GGA GGA CTT CAA GGA GGC	ATT GGT GCC CAG AGC CCG GA
Mlycd	GCA CGT CCG GGA AAT GAA CG	GAG AAG AAG TAA CAT CTC CGG TAG
Myf5	AGC TGC TGA GGG AAC AGG TGG A	ATT CAG GCA TGC CGT CAG AGC A
Myh1	TCT GCA GAC GGA GTC AGG T	TTG AGT GAA TGC CTG TTT GC
Myh2	GTC AAA GGA GGA GGA ACA GCA GC	GTT GAG TGA ATG CTT GCT TCC CC
Myh4	TGG CCG AGC AAG AGC TAC	TTG ATG AGG CTG GTG TTC TG
Myh7	CCA AGG GCC TGA ATG AGG AG	GCA AAG GCT CCA GGT CTG AG
MyoD	TGG CAT CCG GTG CAC TGC AG	CCC TCC AGA AGG GTA GCA CGC T
Myogenin	GTC CCA ACC CAG GAG ATC ATT	AGT TGG GCA TGG TTT CGT CT
Pax7	TCT CCA AGA TTC TGT GCC GAT	CGG GGT TCT CTC TCT TAT ACTC C
Pck1	CTG CAT AAC GGT CTG GAC TTC	CAG CAA CTG CCC GTA CTC C
Pdk4	AGG GAG GTC GAG CTG TTC TC	GGA GTG TTC ACT AAG CGG TCA
Pgc1a (total)	AAC CAG TAC AAC AAT GAG CCT G	AAT GAG GGC AAT CCG TCT TCA
Pgc1a-a1	GGA CAT GTG CAG CCA AGA CTC T	CAC TTC AAT CCA CCC AGA AAG CT
Pgc1a-a2	CCA CCA GAA TGA GTG ACA TGG A	GTT CAG CAA GAT CTG GGC AAA
Pgc1a-a3	AAG TGA GTA ACC GGA GGC ATT C	TTC AGG AAG ATC TGG GCA AAG A
Pgc1a-b	GAC ATG GAT GTT GGG ATT GTC A	ACC AAC CAG AGC AGC ACA TTT
Pgc1a-c	AGT GAC ATG GAT GTT GGG ATT G	GAA TGC CTC CGG TTA CTC ACT T
Retn	AAG AAC CTT TCA TTT CCC CTC CT	GTC CAG CAA TTT AAG CCA ATG TT
RNaseP	GCC TAC ACT GGA GTC GTG CTA CT	CTG ACC ACA CGA GCT GGT AGA A
Scd1	TTC TTG CGA TAC ACT CTG GTG C	CGG GAT TGA ATG TTC TTG TCG T
Slc27a1	CGC TTT CTG CGT ATC GTC TG	GAT GCA CGG GAT CGT GTC T
Tkt	ATG GAA GGT TAC CAT AAG CCA GA	TGC AGC ATG ATG TGG GGT G
Trf	TGG GGG TTG GGT GTA CGA T	AGC GTA GTA GTA GGT CTG TGG
Ucp3	CTG CAC CGC CAG ATG AGT TT	ATC ATG GCT TGA AAT CGG ACC