SUPPLEMENTARY DATA

Supplementary Table 1. Primary antibodies for Western blots

Primary antibody	Residue	Supplier	Reference	Dilution
ACC	-	Cell Signaling	#3662	1:2000
ACC	Ser79	Cell Signaling	#3661	1:2000
AMPKα	-	Cell Signaling	#2532	1:1000
AMPKα1	-	Kinasource	AB-140	1:2000
AMPKα2	-	Kinasource	AB-141	1:2000
AMPKα	Thr172	Cell Signaling	#2535	1:1000
AMPKα	Ser485/491	Cell Signaling	#4185	1:1000
AMPKβ1+2	-	Cell Signaling	#4150	1:1000
AMPKβ	Ser108	Santa Cruz	sc-33535	1:1000
AMPKβ	Ser182	Cell signaling	#4186	1:1000
AMPKγ1	-	AbCam	ab32508	1:1000
AMPKγ2	-	Cell signaling	#2536	1:1000
AMPKγ3	-	Santa Cruz	sc-130687	1:1000
HIF-1α	-	Santa Cruz	sc-10790	1:500
HSP90	-	Santa Cruz	sc-7947	1:1000
IRβ	-	Santa Cruz	sc-711	1:1000
IRS1	Tyr612	Invitrogen	44-816G	1:1000
IRS1	Tyr1222	Cell signaling	#3066	1:1000
IRS1	Ser302	Cell signaling	#2491	1:1000
IRS1	Ser307	Cell signaling	#2381	1:1000
IRS1	Ser612	Cell signaling	#3203	1:1000
IRS1	Ser1101	Cell signaling	#2385	1:1000
pAktSubstrate (PAS)	RXRXXS/T	Cell Signaling	#9614	1:1000
PKBα	-	Upstate	07-416	1:1000
PKBβ	-	Upstate	07-372	1:1000
PKB	Ser473	Cell Signaling	#9271	1:1000
PKB	Thr308	Cell Signaling	#4056	1:1000
PRAS40	-	Cell Signaling	#2640	1:1000
PRAS40	Thr246	Cell Signaling	#2610	1:1000
TBC1D1	Ser237	MRC	S131C	1:1000
TBC1D1	-	MRC	S138C	1:1000

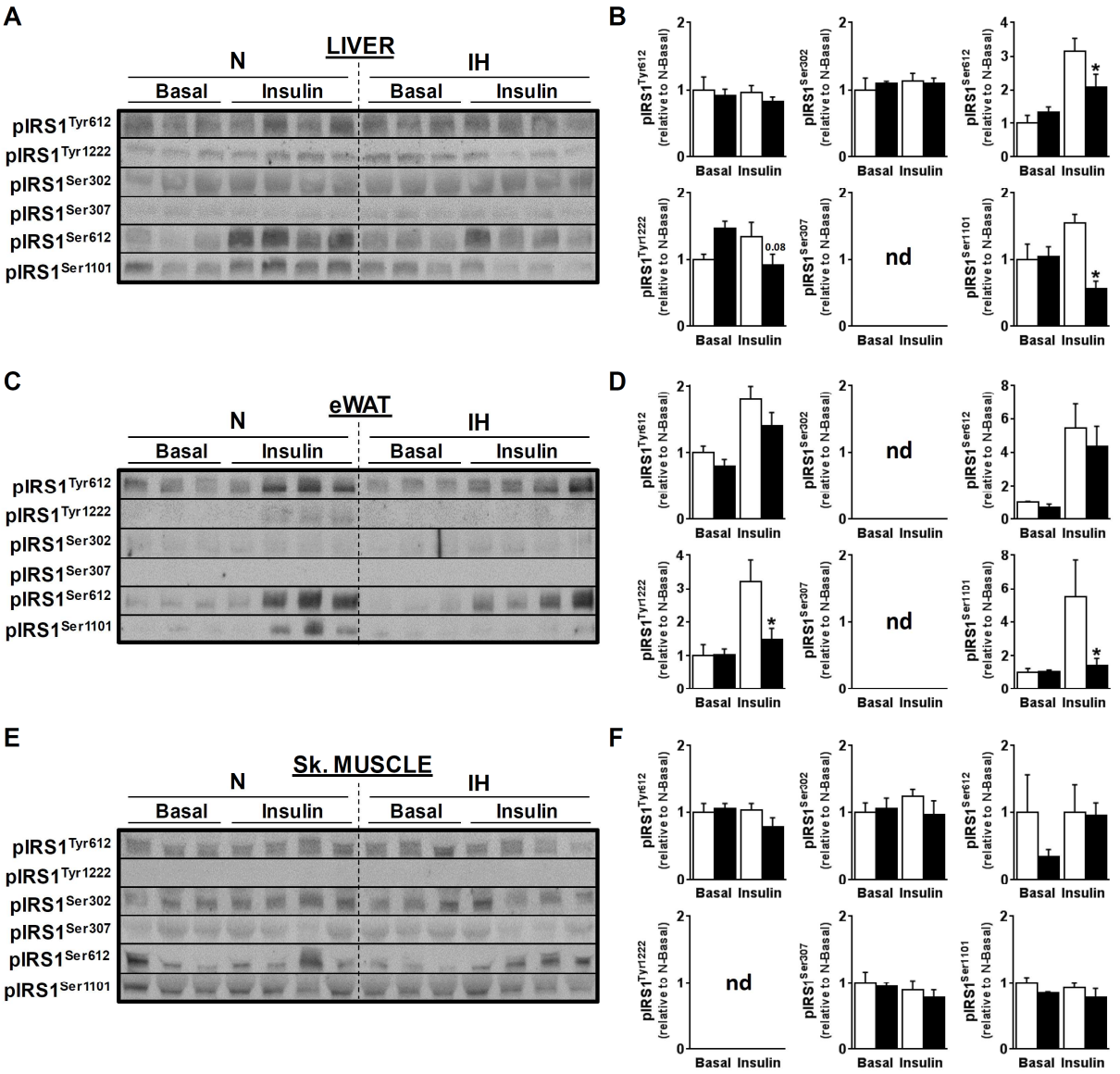
SUPPLEMENTARY DATA

Supplementary Table 2. Primer sequences for qRT-PCR

Gene	Accession number	Forward primer	Reverse primer
Acaca	NM_133360.2	CAGCTGGTGCAGAGGTACCG	TCTACTCGCAGGTACTGCCG
Acacb	NM_133904.2	GCGCCTACTATGAGGCCCAGCA	ACAAACTCGGCTGGGGACGC
Arg1	NM_007482.3	GACCACGGGGACCTGGCCTT	ACTGCCAGACTGTGGTCTCCACC
Cd68	NM_009853.1	CCTCCACCCTCGCCTAGTC	TTGGGTATAGGATTCGGATTTGA
Chil3	NM_009892.2	ACAATTAGTACTGGCCCACCAGGAA	TCCTTGAGCCACTGAGCCTTCA
Cpt1a	NM_013495	AGGAGACAAGAACCCCAACA	AAGGAATGCAGGTCCACATC
Emr1	NM_010130.4	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Fasn	NM_007988	CACAGGCATCAATGTCAACC	TTTGGGAAGTCCTCAGCAAC
Fbp1	NM_019395.2	GCATCGCACAGCTCTATGGT	ACAGGTAGCGTAGGACGACT
Gck	NM_010292.5	TGCGGAGATGCTCTTTGACT	TCTCGGAGAAGTCCCACGAT
G6pc	NM_008061	CCATGGGCGCAGCAGGTGTA	AGCCACGACCTGGTGGGGAA
Gusb	NM_010368.1	CTTCATGACGAACCAGTCAC	GCAATCCTCCAGTATCTCTC
Il1b	NM_008361	GACCCCAAAAGATGAAGGGCT	ATGTGCTGCTGCGAGATTTG
Il6	NM_031168.1	TGTGCAATGGCAATTCTGAT	CTCTGAAGGACTCTGGCTTTG
Itgax	NM_021334.2	GCCACCAACCCTTCCTGGCTG	TTGGACACTCCTGCTGTGCAGTTG
Mlxipl	NM_021455	CCAGCCTCAAGGTGAGCAAA	CATGTCCCGCATCTGGTCA
Pdk4	NM_013743	GATTGACATCCTGCCTGACC	CAGGGCTTTCTGGTCTTCTG
Pck1	NM_011044.2	GGGCCGCTGGATGTCGGAAG	GGTGCGGCCTTTCATGCACC
Pklr	NM_013631	CCTCTGCCTTCTGGATATCGAC	CGATGGTGGCAATGATGCT
Ppargc1a	NM_008904.2	CCCAGAGTCACCAAATGACCCCA	CCTCTTGGTTGGCGGTGGCA
Retnla	NM_020509.3	CCTGCCCTGCTGGGATGACT	GGGCAGTGGTCCAGTCAACGA
Srebf1	NM_011480	GGCCGAGATGTGCGAACT	TTGTTGATGAGCTGGAGCATGT
Slc2a2	NM_031197.2	TCATGTCGGTGGGACTTGTG	CCCAAGGAAGTCCGCAATGT
Tnfa	NM_013693	GTCCCCAAAGGGATGAGAAG	CACTTGGTGGTTTGCTACGA

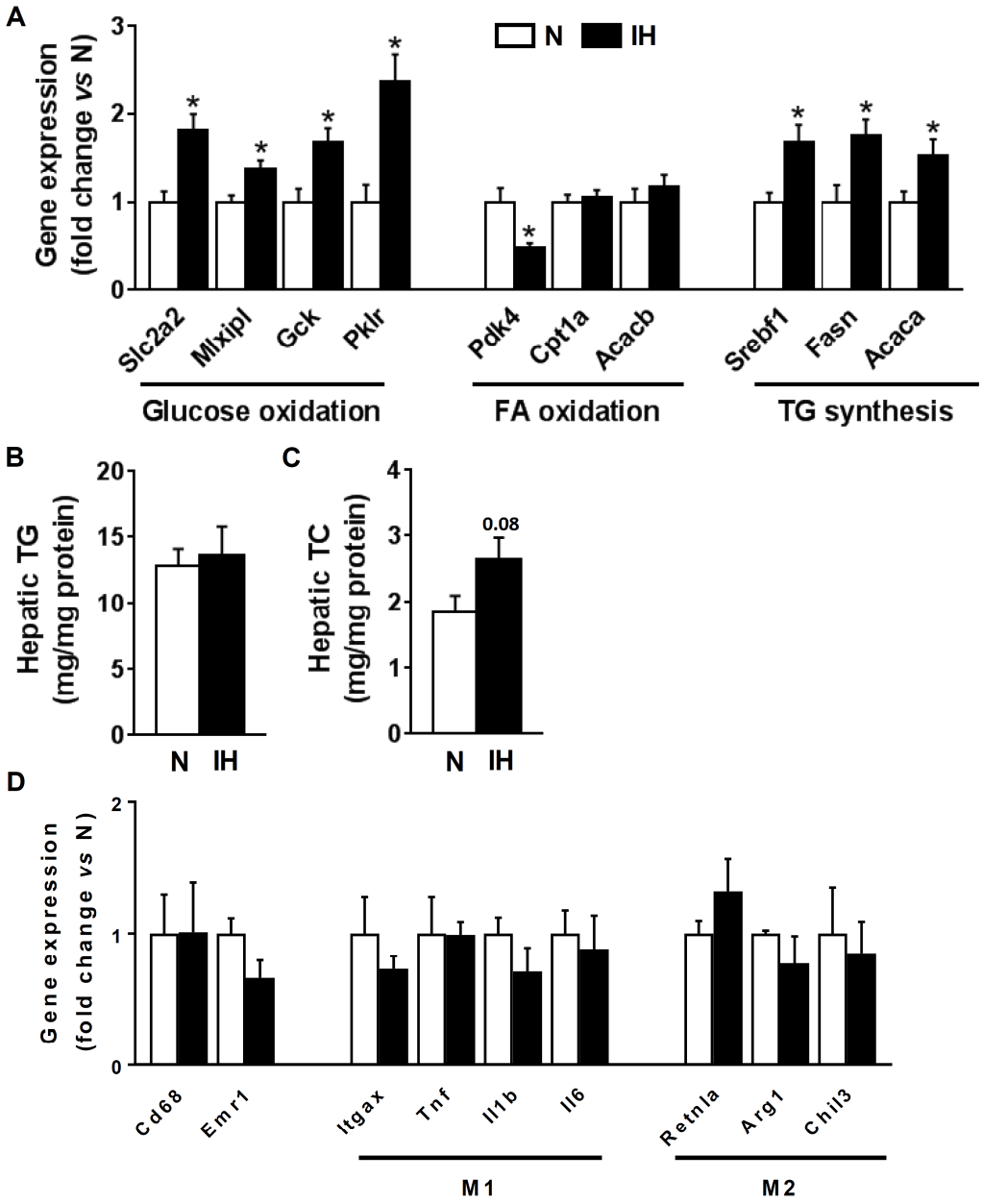
SUPPLEMENTARY DATA

Supplementary Figure 1. Chronic intermittent hypoxia affects IRS1 Ser/Tyr phosphorylation in a tissue-specific manner. The tissue-specific phosphorylation states of IRS1 on various regulatory residues were assessed in liver (A-B), eWAT (C-D) and skeletal muscle (E-F) sampled 10 minutes after PBS (basal) or insulin injection in 6h-fasted mice previously exposed to normoxia (N, open bars) or intermittent hypoxia (IH, closed bars) for 14 days, as described in Figure 1K. The phosphorylation states of Tyr612-IRS1, Tyr1222-IRS1, Ser302-IRS1, Ser307-IRS1, Ser612-IRS1 and Ser1101-IRS1 were determined by Western blot and quantified by densitometric analysis. Data are means ± SEM (n=3 basal, n=5 insulin in each group) and expressed as fold change relative to normoxic mice. * $p<0.05$ *versus* normoxic mice.



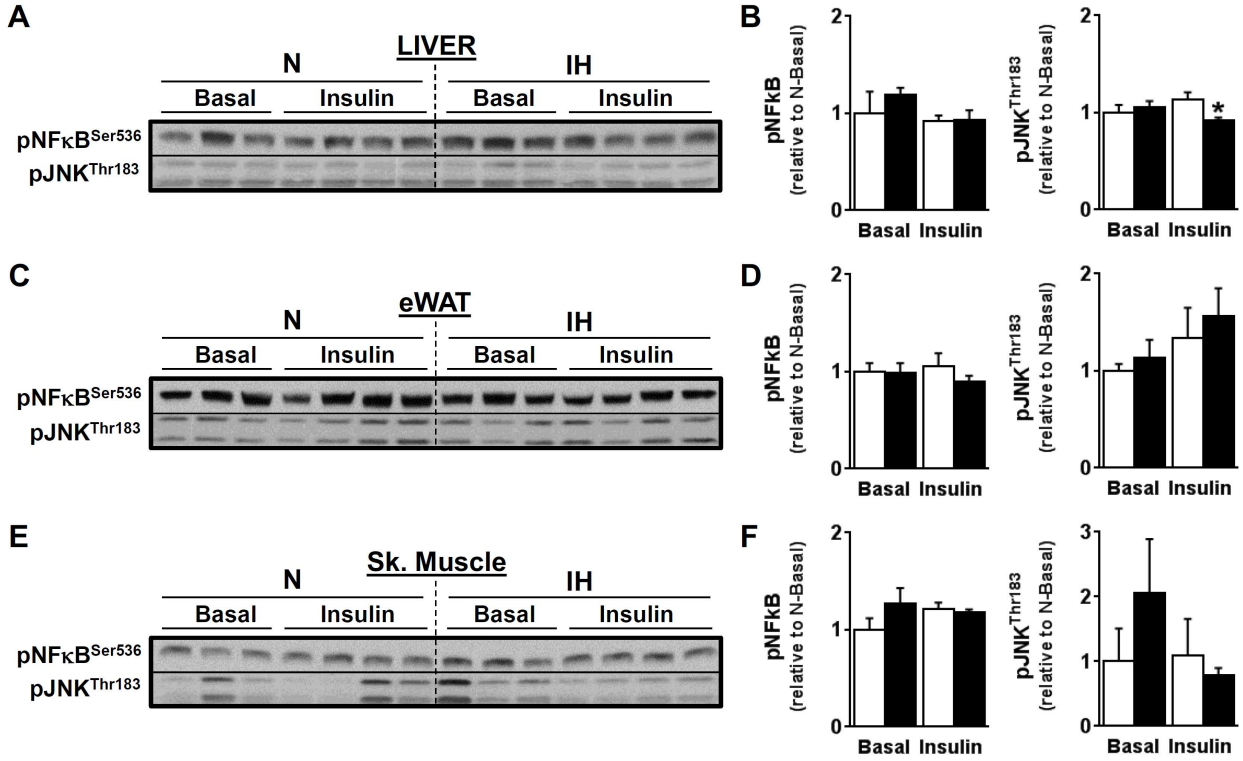
SUPPLEMENTARY DATA

Supplementary Figure 2. Chronic intermittent hypoxia does not affect hepatic steatosis or WAT inflammation. The hepatic mRNA expression of key genes involved in the regulation of glycolysis (Slc2a2: GLUT2; Mlxipl: ChREBP; Gck: GK; Pklr: L-PK), fatty acid (FA) oxidation (Pdk4: PDK4; Cpt1a: CPT-1α; Acacb: ACC2) and triglycerides (TG) synthesis (Srebf1: SREBP-1c; Fasn: FAS; Acaca: ACC1) was measured by RT-qPCR (A) in the livers from 6h-fasted mice previously exposed to normoxia (N, open bars) or intermittent hypoxia (IH, closed bars) for 14 days. Hepatic triglyceride (TG, B) and cholesterol (TC, C) contents were determined. The eWAT mRNA expression of general macrophage markers (Cd68: CD68; Emr1: F4/80) and M1- (Itgax: CD11C; Tnfa: TNF-α; Il1b: IL-1β; Il6: IL-6) or M2-related genes (Retnla: FIZZ1; Arg1: ARG1; Chil3: YM1) was also measured by RT-qPCR (D). The qPCR results are expressed relative to the housekeeping gene RPLP0 as fold change *versus* normoxic mice. Data are means ± SEM (n=6-7 mice/group). * p<0.05 *versus* normoxic mice.



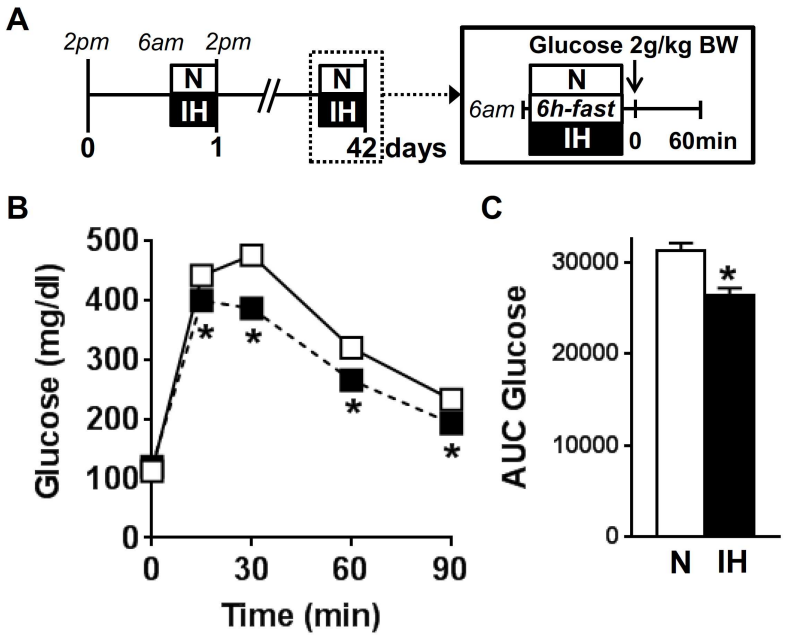
SUPPLEMENTARY DATA

Supplementary Figure 3. Chronic intermittent hypoxia does not affect pro-inflammatory signaling pathways in metabolic tissues. The tissue-specific phosphorylation states of Ser536-NFkB and Thr183-JNK were assessed by Western blot in liver (A-B), eWAT (C-D) and skeletal muscle (E-F) sampled 10 minutes after PBS (basal) or insulin injection in 6h-fasted mice previously exposed to normoxia (N, open bars) or intermittent hypoxia (IH, closed bars) for 14 days, as described in Figure 1K. Data are means ± SEM (n=3 basal, n=5 insulin in each group) and expressed as fold change relative to normoxic mice. * p<0.05 *versus* normoxic mice.



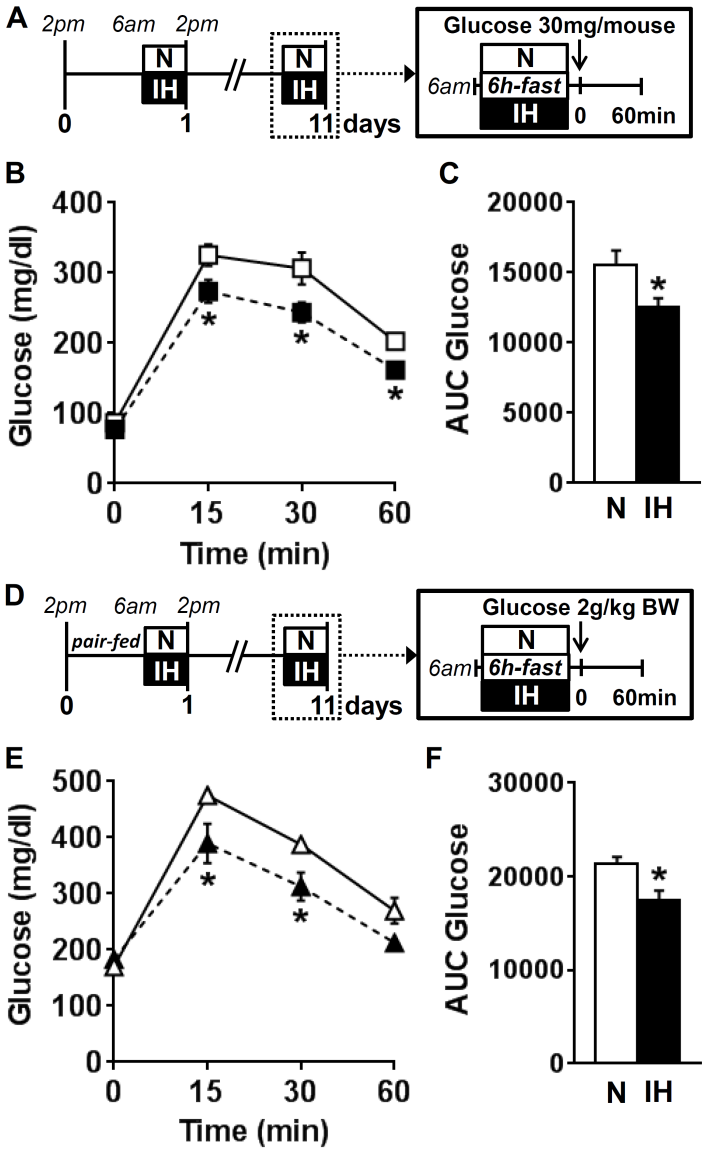
SUPPLEMENTARY DATA

Supplementary Figure 4. Chronic intermittent hypoxia improves whole-body glucose tolerance after prolonged exposure. At day 42, an intraperitoneal GTT (2g/kg of total body weight) was performed (A) in 6h-fasted normoxic (open squares) and cIH mice (closed squares). Blood glucose levels were measured at the indicated time-points (B), and the AUC of the glucose excursion curve was calculated (C). Data are means ± SEM (n=27-32 mice/group). * p<0.05 *versus* normoxic mice.



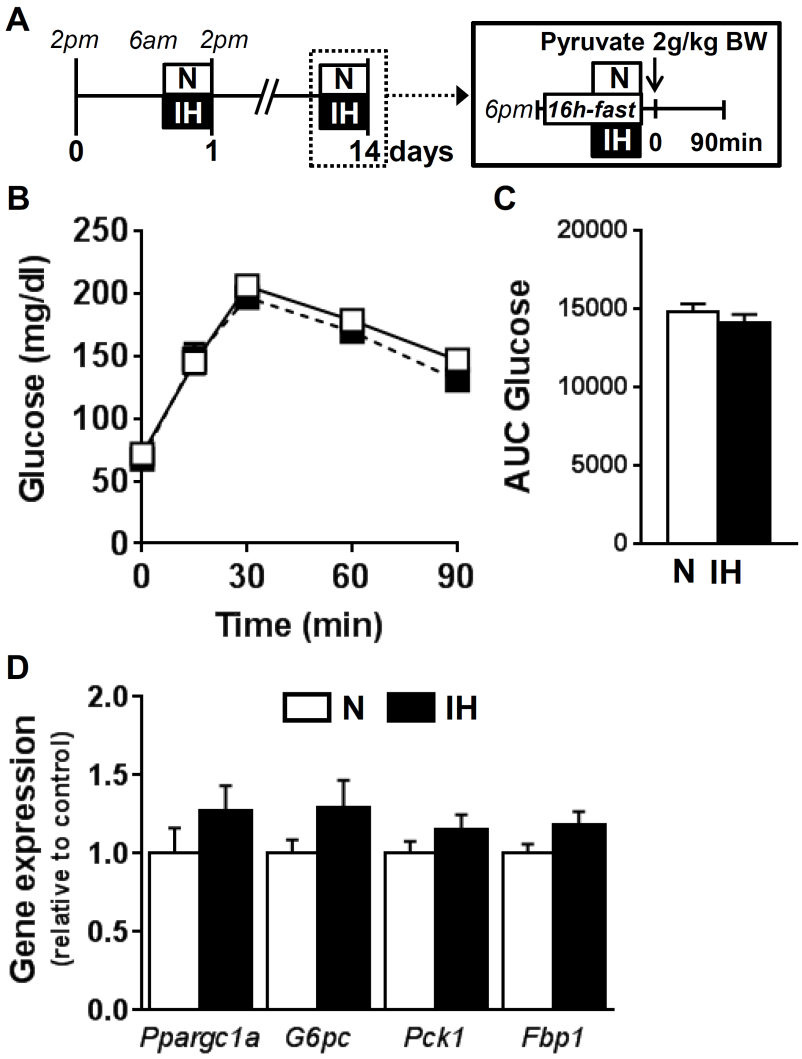
SUPPLEMENTARY DATA

Supplementary Figure 5. Chronic intermittent hypoxia improves whole-body glucose tolerance independently of changes in body weight and food intake. At day 11, an intraperitoneal GTT (30mg/mouse) was performed (A) in 6h-fasted normoxic (open squares) and cIH mice (closed squares). Blood glucose levels were measured at the indicated time-points (B), and the AUC of the glucose excursion curve was calculated (C). An intraperitoneal GTT (2g/kg of total body weight) was performed (D) in 6h-fasted cIH mice (closed triangles) and normoxic mice pair-fed during IH exposure (open triangles). Blood glucose levels were measured at the indicated time-points (E), and the AUC of the glucose excursion curve was calculated (F). Data are means ± SEM (n=6 mice/group). * p<0.05 *versus* normoxic mice.



SUPPLEMENTARY DATA

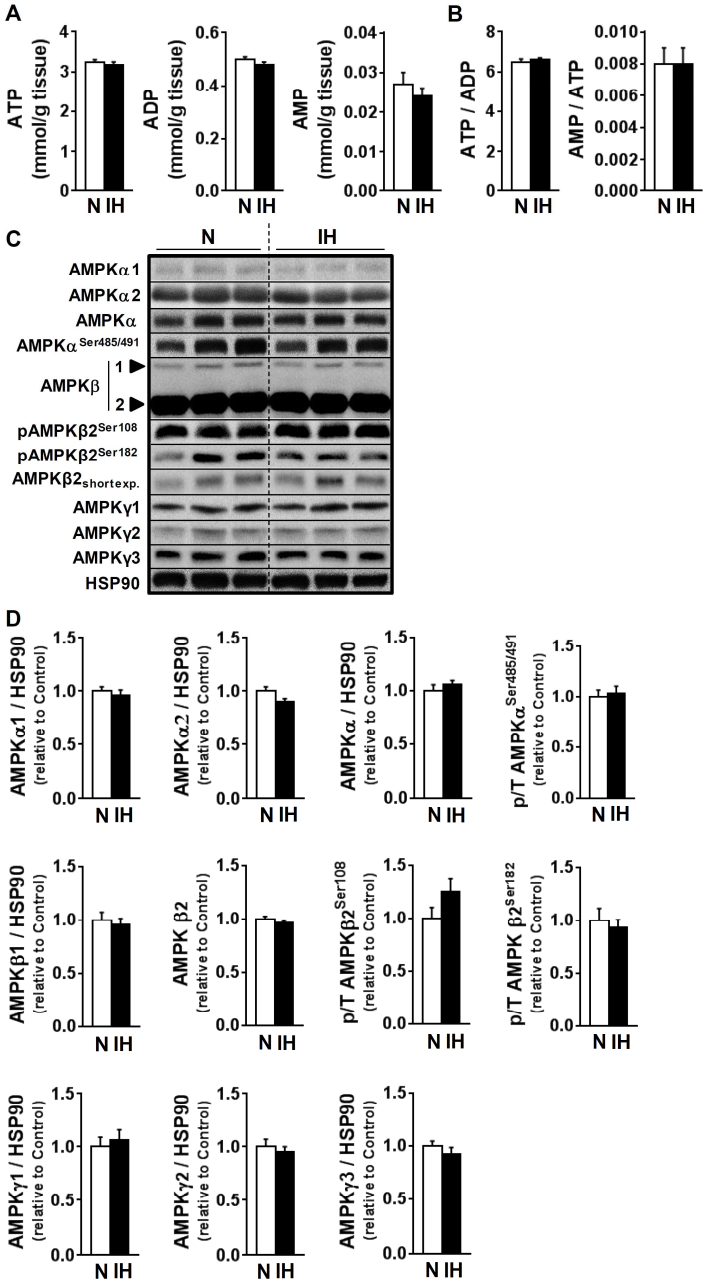
Supplementary Figure 6. Chronic intermittent hypoxia does not affect hepatic gluconeogenesis. At day 14, an intraperitoneal pyruvate tolerance test (2 g/kg total body weight) was performed in mice exposed to normoxia (N, open squares) or intermittent hypoxia (IH, closed squares) fasted for 16 hours (A). Blood glucose levels were measured at the indicated time-points (B), and the AUC of the glucose excursion curve was calculated as a surrogate for hepatic gluconeogenesis (C). The mRNA expression of key genes involved in the regulation of hepatic gluconeogenesis (*Ppargc1a*: PGC1alpha; *G6pc*: G6Pase; *Pck1*: PEPCK; *Fbp1*: FBP1) was measured by RT-qPCR. The results are expressed relative to the housekeeping gene RPLP0 as fold change *versus* normoxic mice. Data are means ± SEM (n=7-8 mice/group in A-C and n=10 in D). * $p<0.05$ *versus* normoxic mice.



SUPPLEMENTARY DATA

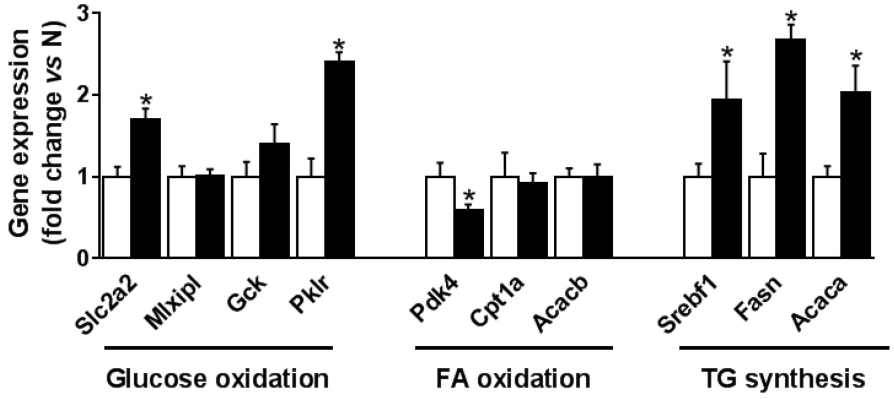
Supplementary Figure 7. Chronic intermittent hypoxia does not affect skeletal muscle adenine nucleotide content or AMPK subunits expression and phosphorylation

AMP, ADP and ATP contents (A) were measured in skeletal muscle from mice exposed to normoxia (N, open bars) or intermittent hypoxia (IH, closed bars). ATP-to-ADP and AMP-to-ATP ratios were calculated from nucleotide concentrations (B). The protein expression of AMPKα, β and γ isoforms and the phosphorylation state of Ser485/491-AMPKα1, Ser108-AMPKβ2 and Ser182-AMPKβ2 were assessed by Western blot (C) in skeletal muscle and quantified by densitometric analysis (D). The results were calculated and expressed as fold change relative to normoxic mice. Data are means ± SEM (n=7 animals/group). * $p<0.05$ *versus* control.



SUPPLEMENTARY DATA

Supplementary Figure 8. Effects of chronic intermittent hypoxia on hepatic mRNA expression of glycolytic and lipogenic genes in muscle-specific AMPKα1α2$^{-/-}$ double knockout mice. The mRNA expression of key genes involved in the regulation of glycolysis, fatty acid (FA) oxidation and triglycerides (TG) synthesis was measured by RT-qPCR in 6h-fasted muscle-specific AMPKα1α2$^{-/-}$ mice previously exposed to normoxia (open bars) or intermittent hypoxia (closed bars) for 14 days. The qPCR results are expressed relative to the housekeeping gene RPLP0 as fold change *versus* normoxic mice. Data are means ± SEM (n=4-5 mice/group). * $p<0.05$ *versus* normoxic mice.



SUPPLEMENTARY DATA

Supplementary Figure 9. Improvement of whole-body glucose tolerance by chronic intermittent hypoxia is abolished in whole-body AMPKα2 knockout mice. Whole-body AMPKα2$^{-/-}$ male mice were subjected to normoxia (N, open bars) or intermittent hypoxia (IH, closed bars) for 14 days (A). Blood hematocrit (B), mean daily food intake (C), body weight (D) and HOMA-IR (E) were measured at the end of the experimental period. At day 11, an intraperitoneal glucose tolerance test (ipGTT, 2 g/kg total body weight) was performed in 6h-fasted mice fasted, as described in Figure 4A. Blood glucose levels were measured at the indicated time-points (F), and the AUC of the glucose excursion curve was calculated (G). The plasma insulin levels were measured immediately before glucose injection (t=0min) and after 15 minutes (H). The t15-on-t0 ratio was calculated as a surrogate for glucose-induced insulin secretion (I). The protein expression of AMPKα1, AMPKα2, pan-AMPKα, ACC, TBC1D1 and HSP90, and phosphorylation states of Thr172-AMPK, Ser79-ACC and Ser237-TBC1D1, were assessed by Western blot (J) in skeletal muscle sampled 15 minutes after glucose injection. After densitometric analysis, the phospho-to-total ratios were calculated and expressed as fold change relative to normoxic mice (K). The mRNA expression of key genes involved in the regulation of glycolysis, fatty acid (FA) oxidation and triglycerides (TG) synthesis was measured by RT-qPCR (L) in 6h-fasted whole-body AMPKα2$^{-/-}$ mice previously exposed to normoxia (open bars) or intermittent hypoxia (IH, closed bars) for 14 days. The qPCR results are expressed relative to the housekeeping gene RPLP0 as fold change *versus* normoxic mice. Data are means ± SEM (n=4-5 mice/group). * $p<0.05$ *versus* normoxic mice.

SUPPLEMENTARY DATA