SUPPLEMENTARY DATA

Mouse models.
Mice were housed in a controlled environment with a 12:12-h light-dark cycle. Male mice were fed a chow (5.5% fat by weight; 5001 Purina Laboratory Rodent Diet) or HF diet (60% kcal from fat; F3282 BioServ) for 16 weeks. Studies were performed at 19 weeks of age. Body composition was assessed using a mq10 nuclear magnetic resonance analyzer (Bruker).

Kinexus Assay. Two hundred and fifty mg of pooled frozen 5h fasted insulin clamped liver tissue from each genotype (3 mice/genotype) was homogenized and needle sheered in 1 mL lysis buffer. The homogenate was centrifuged at 13000 rpm for 30 min at 4C. The supernatant was collected and the protein concentration was determined using the BCA protein assay. Fifty ug of lysate from each genotype was labeled with the Kinex 543 Labeling Dye and the proteins were purified using Microspin G-25 Columns. The full amount of each labeled protein sample was applied to the microarray slide. The slide was incubated in a humidity chamber in the dark on a table top shaker for 2h at room temperature. The slide was washed several times and dried in a swing-bucket bench top centrifuge. The microarray slide was shipped to Kinexus for quantification and data analysis. Signal quantification was performed using ImaGene 9.0 from BioDiscovery. Z scores were calculated by subtracting the overall average intensity of all spots within a sample from the raw intensity for each spot, and dividing it by the standard deviations of all measured intensities within each sample (1). A Z-ratio of ± 1.2 to 1.5 was considered significant.

Mitochondrial oxygen consumption. Mitochondria were isolated from livers of 5h fasted mice and oxygen consumption was measured in air-saturated MiR05 (pH 7.4, 30°C) with a Clark-type oxygen electrode (Oroboros Instruments Corp., Innsbruck, Austria) as previously described (2). State 2 respiration was measured in the presence of 1 mM malate and 50 μM palmitoyl carnitine or 1 mM malate and 10 mM glutamate prior to the addition of ADP. State 3 respiration was measured upon the addition of 0.5 mM ADP. Cytochrome c (10 μM) was added at the end of each measurement to ensure the outer mitochondrial membrane was intact.

SUPPLEMENTARY DATA

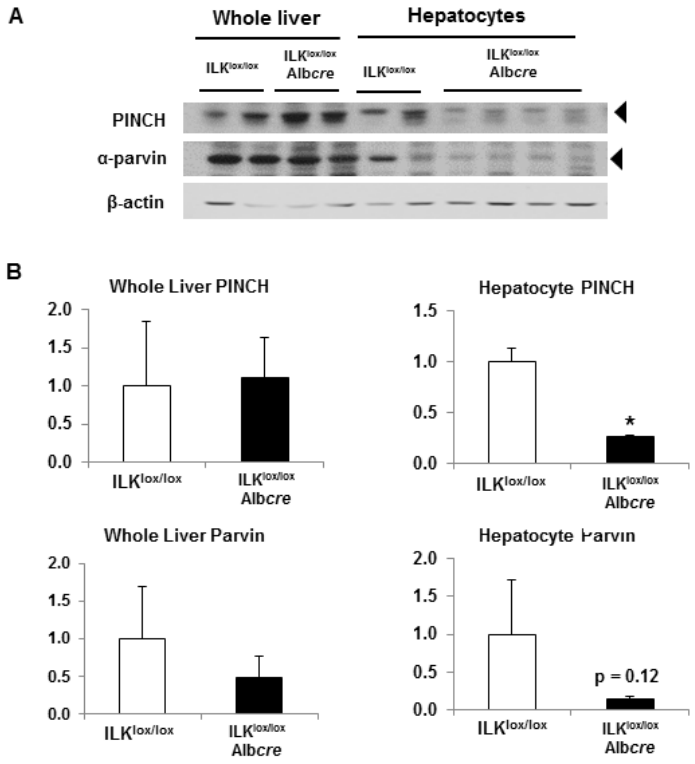
Supplementary Table 1. Protein and phosphoprotein microarray data from 5h fasted livers of high fat (HF) fed ILK$^{lox/lox}$ and ILK$^{lox/lox}$ Alb*cre* mice. Each condition tested represents a pool of frozen liver homogenates from three mice. A Z ratio of ± 1.2 was considered significant. Green represents upregulation and red indicates downregulation compared to ILK$^{lox/lox}$ Alb*cre* mice.

Target Protein Name	Phospho Site	Full Target Protein Name	Z-ratio
Rb	S807	Retinoblastoma-associated protein 1	3.79
S6K	S424	p70 ribosomal protein-serine S6 kinase	2.46
Fyn	Pan-specific	Fyn proto-oncogene-encoded protein-tyrosine kinase	2.39
PKBb (Akt2)	Pan-specific	Protein-serine kinase B beta	2.37
TBK1	Pan-specific	Serine/threonine-protein kinase TBK1	2.35
Shc1	Y349	SH2 domain-containing transforming protein 1	2.16
PKBb (Akt2)	Pan-specific	Protein-serine kinase B beta	2.15
Syk	Y323	Spleen protein-tyrosine kinase	2.14
S6K	S411	p70 ribosomal protein-serine S6 kinase	2.14
MST1	Pan-specific	Mammalian STE20-like protein-serine kinase 1 (KRS2)	2.12
Myc	S373	Myc proto-oncogene protein	2.08
PKCh	Pan-specific	Protein kinase C eta type	1.97
Gab1	Y627	GRB2-associated binder 1	1.94
Raf1	Pan-specific	Raf1 proto-oncogene-encoded protein-serine kinase	1.92
JNK1/2/3	Pan-specific	Jun N-terminus protein-serine kinase (SAPK) 1/2/3	1.83
HDAC5	S498	Histone deacetylase 5	1.83
GSK3a	Pan-specific	Glycogen synthase-serine kinase 3 alpha	1.80
MEK7 (MAP2K7)	Pan-specific	MAPK/ERK protein-serine kinase 7 (MKK7)	1.77
DDIT3(CHOP)	Pan-specific	DNA damage-inducible transcript 3 protein	1.77
PAK2	Pan-specific	p21-activated kinase 2 (gamma) (serine/threonine-protein kinase PAK 2)	1.75
Smac/DIABLO	Pan-specific	Second mitochondria-derived activator of caspase	1.71
IkBa	Pan-specific	Inhibitor of NF-kappa-B alpha (MAD3)	1.69
GroEL	Pan-specific	GroEL homolog (may correspond to Hsp60)	1.68
FKHR	S256	Forkhead box protein O1	1.64
MEK5 (MAP2K5)	Pan-specific	MAPK/ERK protein-serine kinase 5 (MKK5)	1.60
JAK1	Pan-specific	Janus protein-tyrosine kinase 1	1.57
GATA1	S142	Erythroid transcription factor	1.56
JNK2 (MAPK9)	Pan-specific	Jun N-terminus protein-serine kinase (SAPK) 2	1.53
Tubulin	Pan-specific	Tubulin	-1.51
MEK4 (MAP2K4)	S257+T261	MAPK/ERK protein-serine kinase 4 (MKK4)	-1.52
Bax	Pan-specific	Apoptosis regulator Bcl2-associated X protein	-1.53
4G10	pTyr	4G10	-1.58
Catenin b1	Pan-specific	Catenin (cadherin-associated protein) beta 1	-1.63
PKA Cb	S339	cAMP-dependent protein-serine kinase catalytic subunit beta	-1.65
MLK3	T277+S281	Mixed-lineage protein-serine kinase 3	-1.73
MEK3/6 (MAP2K3/6)	S218/S207	MAPK/ERK protein-serine kinase 3/6 (MKK3/6)	-1.74
BLNK	Y84	B-cell linker protein	-1.85
WIP1	Pan-specific	Protein phosphatase 1D	-1.90
ATF2	T69 + T71	Activating transcription factor 2 (CRE-BP1)	-2.58
Paxillin 1	Y118	Paxillin 1	-3.01

Protein and phosphoprotein expression in livers from 5-h fasted high fat fed ILK$^{lox/lox}$ and ILK$^{lox/lox}$Alb*cre* mice. A Z ratio of ± 1.2 to 1.5 is considered significant.

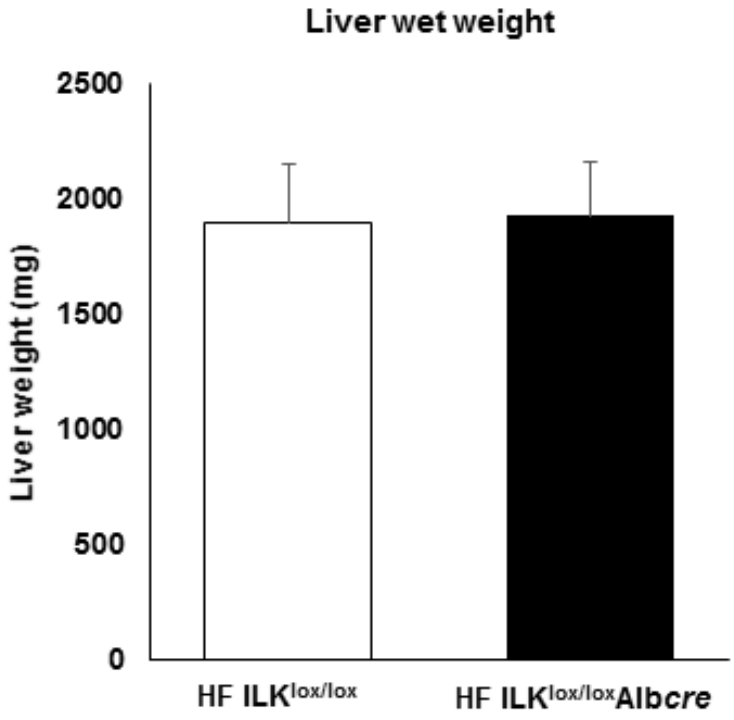
SUPPLEMENTARY DATA

Supplementary Figure 1. The members of the IPP complex PINCH and parvin are downregulated in hepatocytes isolated from $ILK^{lox/lox}$ Alb*cre* mice. (A) Western blot analysis for PINCH and α-parvin in whole liver and hepatocyte homogenates. (B) Quantitative analysis of western blots. Integrated intensities were obtained by the Odyssey and Image J software. Data are represented as means ± SEM; n=5-8/group. **p* < 0.05 compared to $ILK^{lox/lox}$ mice.



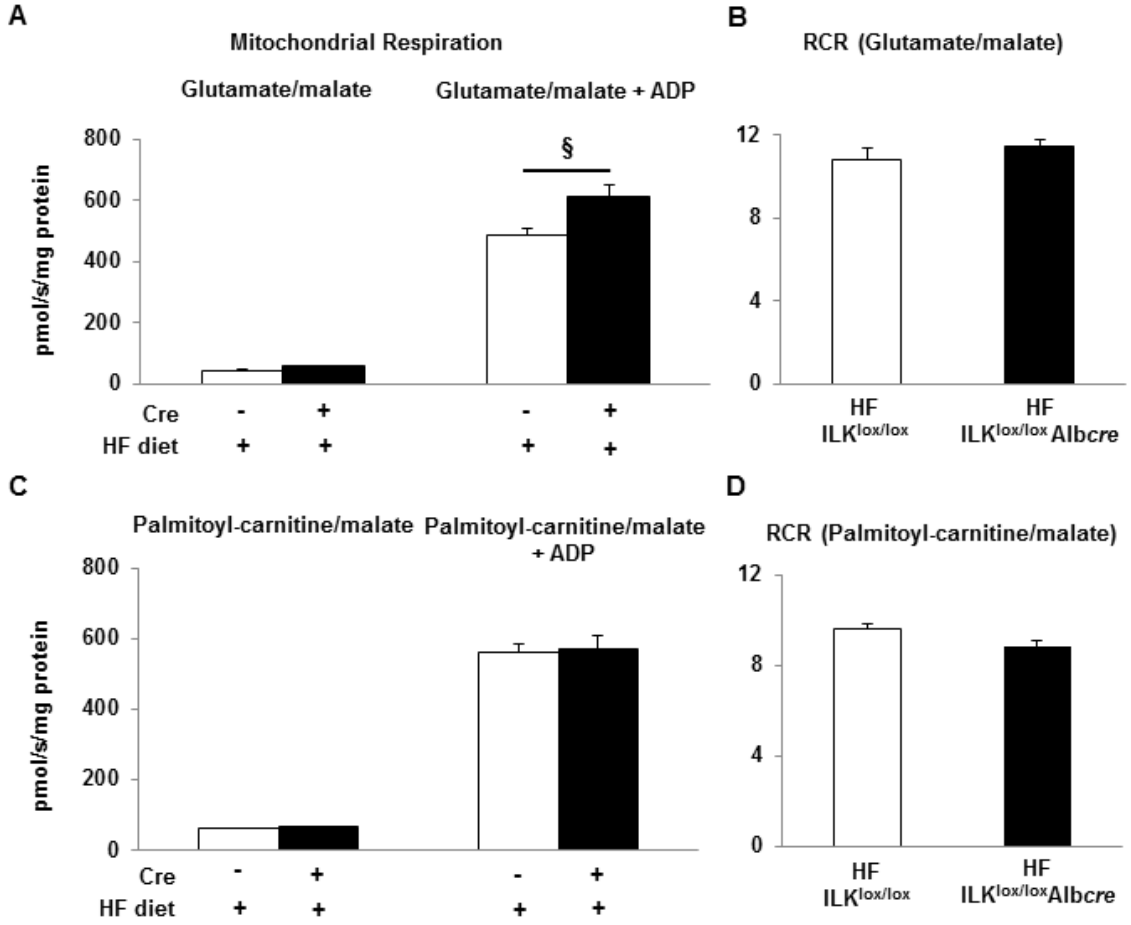
SUPPLEMENTARY DATA

Supplementary Figure 2. Liver wet weight is not different between high fat (HF) fed ILK$^{lox/lox}$ and ILK$^{lox/lox}$ Alb*cre* mice. Data are represented as means ± SEM; n=5-8/group.



SUPPLEMENTARY DATA

Supplementary Figure 3. Complex I supported mitochondrial respiration is higher in high fat (HF) fed ILK$^{lox/lox}$ Alb*cre* mice. High resolution respirometry was performed on mitochondria isolated from livers of 5h fasted mice and assessed with the substrates glutamate and malate (A) and palmitoyl-carnitine and malate (C). The respiratory control ratio (RCR) (B and D) was determined as state 3/state 3 respiration. Data are represented as means ± SEM; n=7-8/group. *$p < 0.05$ compared to HF fed ILK$^{lox/lox}$ Alb*cre* mice.



SUPPLEMENTARY DATA

References:

1. Cheadle C, Vawter MP, Freed WJ, Becker KG: Analysis of microarray data using Z score transformation. The Journal of molecular diagnostics : JMD 2003;5:73-81

2. Hasenour CM, Ridley DE, Hughey CC, James FD, Donahue EP, Shearer J, Viollet B, Foretz M, Wasserman DH: 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) effect on glucose production, but not energy metabolism, is independent of hepatic AMPK in vivo. J Biol Chem 2014;289:5950-5959