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Supplemental information

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Pegylated arginine deiminase (ADI-PEG 20), drives arginine turnover and systemic autophagy to dictate energy metabolism

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Figure S1.

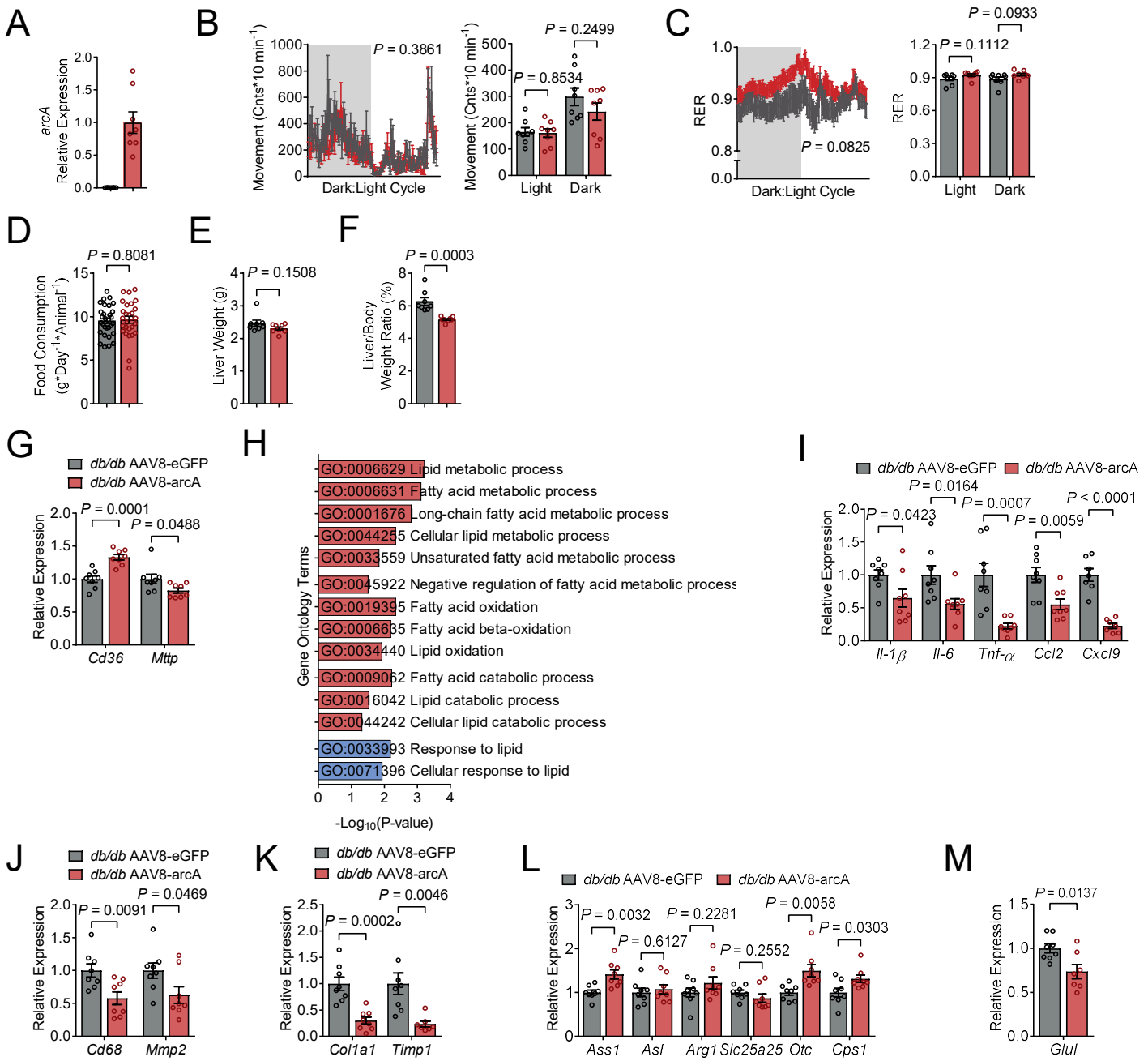


Figure S1. The effect of hepatocyte-specific *arcA* expression in *db/db* mice. Related to Figures 1 and 2.

(A) mRNA expression of *arcA* in the livers of AAV8-eGFP or AAV8-*arcA* injected *db/db* mice by qPCR. Gene expression was normalized to β -actin mRNA levels. Liver weights.

(B and C) Respiration exchange ratio (RER, B) and locomotion (C) during light and dark cycle (shaded area) in AAV8-eGFP or AAV8-*arcA* injected *db/db* mice.

(D) Food consumption measured in AAV8-eGFP or AAV8-*arcA* injected *db/db* mice.

(E and F) Liver weights (E) and liver weight/body weight ratio (F) of AAV8-eGFP or AAV8-*arcA* injected *db/db* mice.

(G) Hepatic mRNA expression of fatty acid transporters by qPCR.

(H) Gene ontology terms for significant pathways related to DNL and fatty acid oxidation.

(I) Hepatic mRNA expression of cytokines and chemokines by qPCR.

(J) Hepatic mRNA expression of macrophage infiltration by qPCR.

(K) Hepatic mRNA expression of fibrosis development by qPCR.

(L) Hepatic mRNA expression of urea cycle enzymes by qPCR.

(M) Hepatic mRNA expression of glutamine synthetase (*Glul*) by qPCR.

Data represented in mean \pm s.e.m. Each data point represents an individual animal. Exact *P*-value are shown. Statistical significance was determined using unpaired two-tailed Student's *t*-test. Gene expression was normalized to β -actin mRNA levels.

Figure S2.

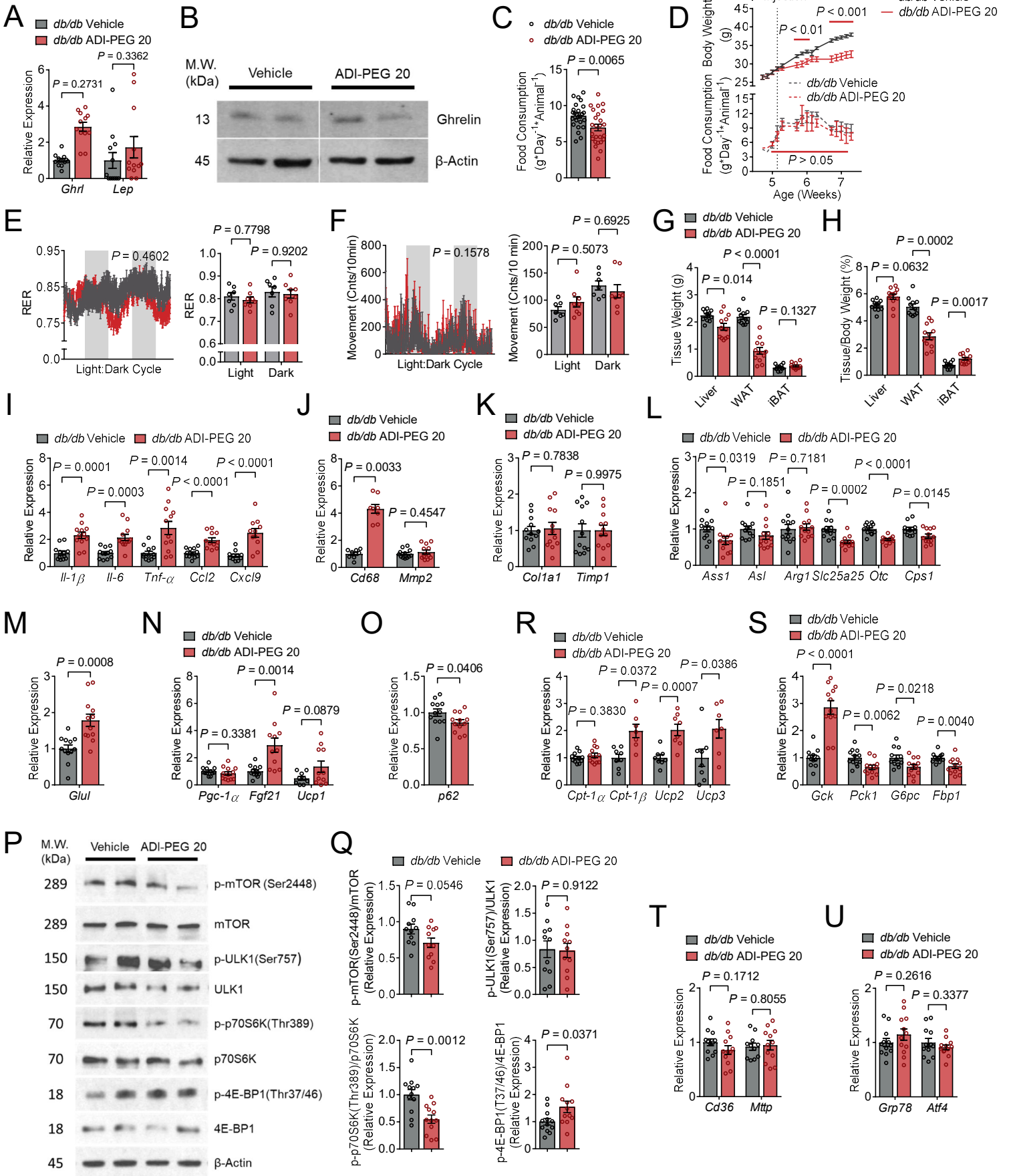


Figure S2. The effect of ADI-PEG 20 treatment in *db/db* mice. Related to Figure 3.

(A) Hepatic mRNA expression of Ghrelin (*Ghrl*) and Leptin (*Lep*) by qPCR.

(B) Western blot analysis of GHRL protein abundance in ADI-PEG 20-treated *db/db* mice.

(C) Food consumption measured in vehicle and ADI-PEG 20 treated *db/db* mice.

(D) Body weight changes superimposed with daily food consumption in vehicle and ADI-PEG 20 treated *db/db* mice.

(E and F) Respiration exchange ratio (RER, C) and locomotion (D) during light and dark cycle (shaded area) in vehicle and ADI-PEG 20 treated *db/db* mice.

(G and H) Liver, white adipose tissue, and brown adipose tissue (iBAT) weights (E). Liver, white, and brown adipose tissue weight/body weight ratio (F) of vehicle and ADI-PEG 20 treated *db/db* mice.

(I) Hepatic mRNA expression of cytokines and chemokines by qPCR.

(J) Hepatic mRNA expression of macrophage infiltration related genes by qPCR.

(K) Hepatic mRNA expression of fibrosis development related genes by qPCR.

(L) Hepatic mRNA expression of urea cycle enzymes by qPCR.

(M) Hepatic mRNA expression of glutamine synthetase (*Glul*) by qPCR.

(N) Hepatic mRNA expression of thermogenic genes, *Pgc-1 α* , *Fgf21*, and *Ucp1* by qPCR.

(O) Hepatic mRNA expression of *p62/Sqstm1* expression by qPCR.

(P) Western blot analysis of mTORC1 signaling substrates in liver samples from vehicle and ADI-PEG20 treated *db/db* mice. β -Actin was used as a loading control.

(Q) Western blot quantifications of (P).

(R) Hepatic mRNA expression of genes related to fatty acid β -oxidation by qPCR.

(S) Hepatic mRNA expression of gluconeogenic genes by qPCR.

(T) Hepatic mRNA expression of genes related in fatty acid intake and export qPCR.

(U) mRNA expression of *Grp78* and *Atf4* in the livers of *db/db* mice treated with or without ADI-PEG 20 by qPCR. Gene expression was normalized to β -actin mRNA levels.

Data represented in mean \pm s.e.m. Each data point represents an individual animal. Exact *P*-value are shown. Statistical significance was determined using unpaired two-tailed Student's *t*-test. Gene expression was normalized to β -actin mRNA levels.

Figure S3.

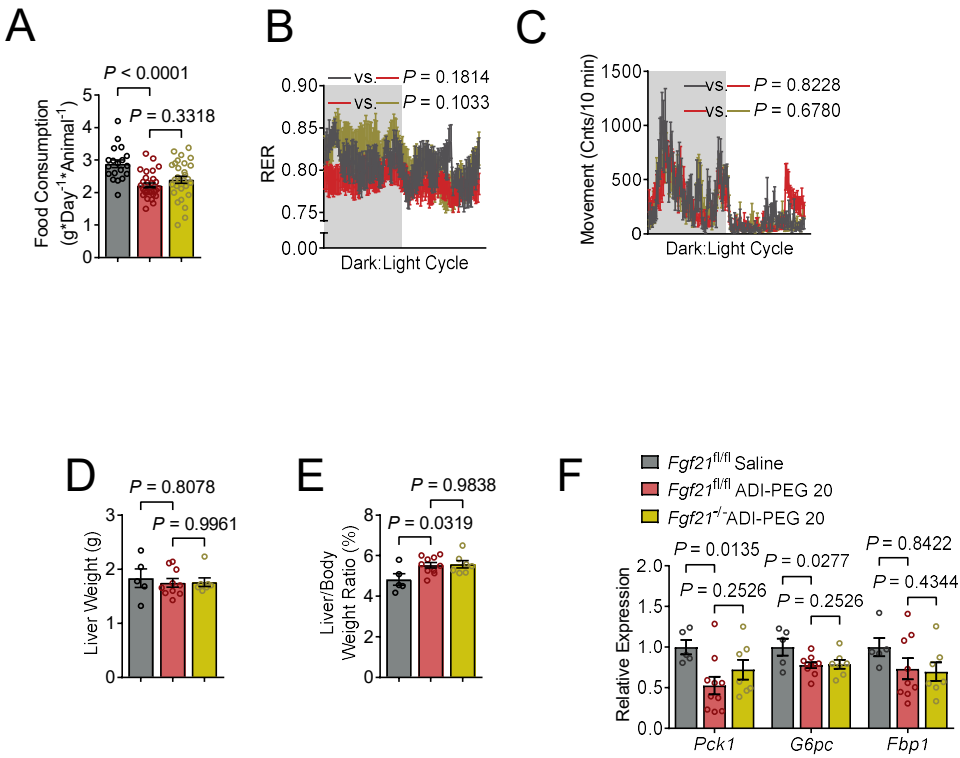


Figure S3. Supplemental information on ADI-PEG 20-treated *Fgf21^{fl/fl}* and *Fgf21^{-/-}* LKO mice. Related to Figure 4.

(A) Food consumption measured in vehicle and ADI-PEG 20 treated *Fgf21* LKO mice.

(B and C) Respiration exchange ratio (RER, C) and locomotion (D) during light and dark cycle (shaded area) in vehicle and ADI-PEG 20 treated *Fgf21* LKO mice.

(D and E) Liver weights (D). Liver weight/body weight ratio (E) of vehicle and ADI-PEG 20 treated *Fgf21* LKO mice.

(F) Hepatic mRNA expression of gluconeogenic genes, *Pck1*, *G6pc*, and *Fbp1* by qPCR. Gene expression was normalized to β -actin mRNA levels.

Data represented in mean \pm s.e.m. Each data point represents an individual animal. Exact *P*-value are shown. Statistical significance was determined using unpaired two-tailed Student's *t*-test.

Figure S4.

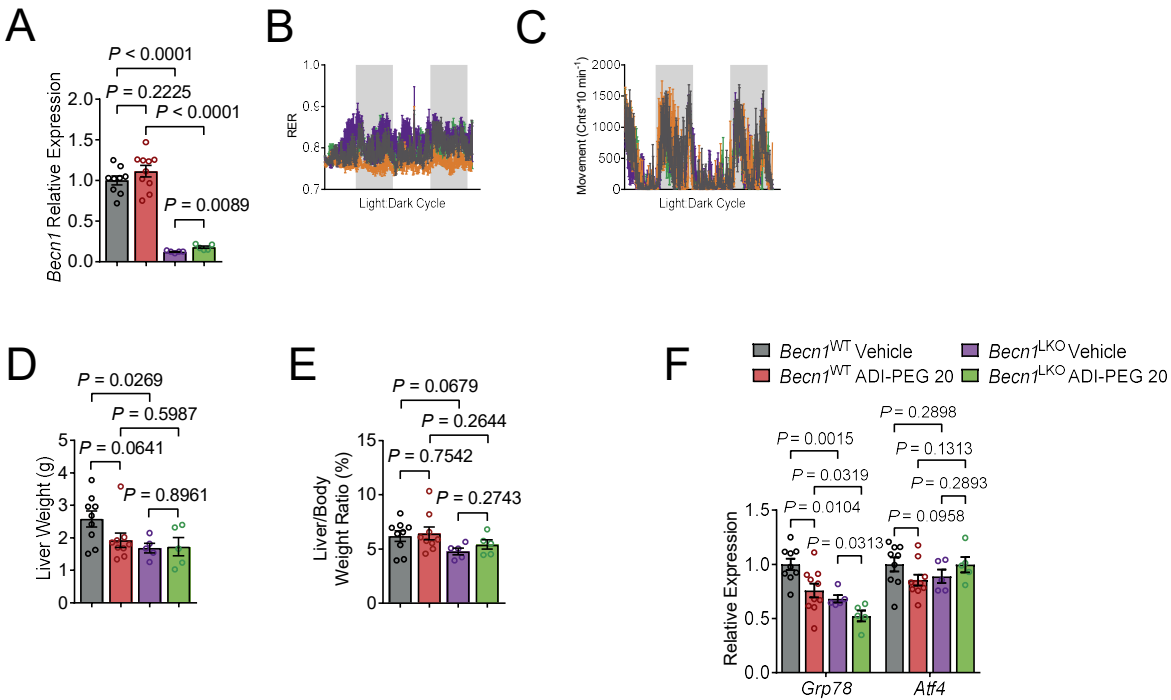


Figure S4. Supplemental information on ADI-PEG 20-treated *Becl1*^{fl/fl} and *Becl1*^{-/-} LKO mice. Related to Figure 5.

(A) mRNA expression of *Becl1* in the livers of *Becl1* LKO mice treated with or without ADI-PEG 20 by qPCR. Gene expression was normalized to β -actin mRNA levels.

(B and C) Respiration exchange ratio (RER, C) and locomotion (D) during light and dark cycle (shaded area) in vehicle and ADI-PEG 20 treated *Becl1* LKO mice.

(D and E) Liver weights (D). Liver weight/body weight ratio (E) of vehicle and ADI-PEG 20 treated *Becl1* LKO mice.

(F) mRNA expression of *Grp78* and *Atf4* in the livers of *Becl1* LKO mice treated with or without ADI-PEG 20 by qPCR. Gene expression was normalized to β -actin mRNA levels.

Data represented in mean \pm s.e.m. Each data point represents an individual animal. Exact *P*-value are shown. Statistical significance was determined using unpaired two-tailed Student's *t*-test.

Figure S5.

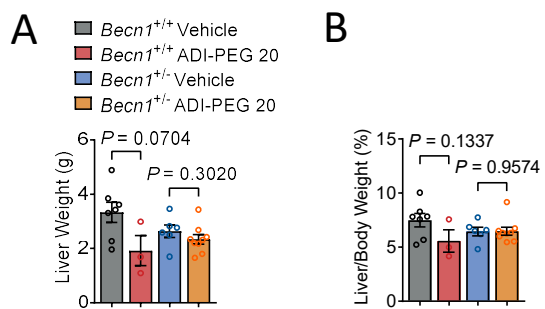


Figure S5. Supplemental information on ADI-PEG 20-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice. Related to Figure 6.

(A) Liver weights.

(B) Liver weight/body weight ratio of vehicle and ADI-PEG 20 treated *Becn1*^{+/+} and *Becn1*^{+/-} mice.

Data represented in mean \pm s.e.m. Each data point represents an individual animal. Exact *P*-value are shown. Statistical significance was determined using unpaired two-tailed Student's *t*-test.

Table S1.

Gene	Forward (5' - 3')	Reverse (5' - 3')
<i>β-Actin</i>	GAT TAC TGC TCT GGC TCC TAG	GAC TCA TCG TAC TCC TGC TTG
<i>arcA</i>	ATT CAC CCA TCG CAA ACG AC	GCG ATT TCC ATC TCG GTA GCT
<i>Arg1</i>	CTC CAA GCC AAA GTC CTT AGA G	AGG AGC TGT CAT TAG GGA CAT C
<i>Asl</i>	CTA TGA CCG GCA TCT GTG GAA	AGC AAC CTT GTC CAA CCC TTG
<i>Ass1</i>	ACA CCT CCT GCA TCC TCG T	GCT CAC ATC CTC AAT GAA CAC CT
<i>Atf4</i>	AGC AAA ACA AGA CAG CAG CC	ACT CTC TTC TTC CCC CTT GC
<i>Becn1</i>	ATG GAG GGG TCT AAG GCG TC	TGG GCT GTG GTA AGT AAT GGA
<i>Ccl2</i>	TTA AAA ACC TGG ATC GGA ACC AA	GCA TTA GCT TCA GAT TTA CGG GT
<i>Cd36</i>	GGA ACT GTG GGC TCA TTG C	CAT GAG AAT GCC TCC AAA CAC
<i>Cd68</i>	TGT CTG ATC TTG CTA GGA CCG	GAG AGT AAC GGC CTT TTT GTG A
<i>Col1a1</i>	GCT CCT CTT AGG GGC CAC T	CCA CGT CTC ACC ATT GGG G
<i>Cps1</i>	ACA TGG TGA CCA AGA TTC CTC G	TTC CTC AAA GGT GCG ACC AAT
<i>Cpt-1α</i>	AGT GGC CTC ACA GAC TCC AG	GCC CAT GTT GTA CAG CTT CC
<i>Cpt-1β</i>	GCA CAC CAG CAG GCA GTA GCT TT	CAG GAG TTG ATT CCA GAC AGG TA
<i>Cxcl9</i>	GGA GTT CGA GGA ACC CTA GTG	GGG ATT TGT AGT GGA TCG TGC
<i>Fbp1</i>	CAC CGC GAT CAA AGC CAT CT	AGG TAG CGT AGG ACG ACT TCA
<i>Fgf21</i>	CTG CTG GGG GTC TAC CAA G	CTG CGC CTA CCA CTG TTC C
<i>G6pc</i>	TCT GTC CCG GAT CTA CCT TG	GCT GGC AAA GGG TGT AGT GT
<i>Gck</i>	CAA CTG GAC CAA GGG CTT CAA	TGT GGC CAC CGT GTC ATT C
<i>Ghrl</i>	TCA AGC TGT CAG GAG CTC AGT A	TTG TCA GCT GGC GCC TCT T
<i>Glul</i>	TGA ACA AAG GCA TCA AGC AAA TG	CAG TCC AGG GTA CGG GTC TT
<i>Grp78</i>	GAA AGG ATG GTT AAT GAT GCT GAG	GTC TTC AAT GTC CGC ATC CTG
<i>Il-1β</i>	GCA ACT GTT CCT GAA CTC AAC T	ATC TTT TGG GGT CCG TCA ACT
<i>Il-6</i>	CTG CAA GAG ACT TCC ATC CAG	AGT GGT ATA GAC AGG TCT GTT GG
<i>Lep</i>	GAG ACC CCT GTG TCG GTT C	CTG CGT GTG TGA AAT GTC ATT G
<i>Mmp2</i>	CAA GTT CCC CGG CGA TGT C	TTC TGG TCA AGG TCA CCT GTC
<i>Mttp</i>	ATG ATC CTC TTG GCA GTG CTT	TGA GAG GCC AGT TGT GTG AC
<i>Otc</i>	ACA CTG TTT GCC TAG AAA GCC	CCA TGA CAG CCA TGA TTG TCC
<i>p62/Sqstm1</i>	AGG ATG GGG ACT TGG TTG C	TCA CAG ATC ACA TTG GGG TGC
<i>Pck1</i>	GAT GGG CAT ATC TGT GCT GG	CAG CCA CCC TTC CTC CTT AG
<i>Pgc1α</i>	ACA CCG CAA TTC TCC CTT GT	CGG CGC TCT TCA ATT GCT TT
<i>Slc25a15</i>	GCT GCC TCA AGA CCT ACT CC	CCG TAA CAC ATG AAC AGC ACC
<i>Timp1</i>	GCA ACT CGG ACC TGG TCA TAA	CGG CCC GTG ATG AGA AAC T
<i>Tnfa</i>	CAG GCG GTG CCT ATG TCT C	CGA TCA CCC CGA AGT TCA GTA G
<i>Ucp1</i>	AGG CTT CCA GTA CCA TTA GGT	CTG AGT GAG GCA AAG CTG ATT T
<i>Ucp2</i>	ATG GTT GGT TTC AAG GCC ACA	CGG TAT CCA GAG GGA AAG TGA T
<i>Ucp3</i>	CTG CAC CGC CAG ATG AGT TT	ATC ATG GCT TGA AAT CGG ACC

Table S1. Mouse primer sequences used for quantitative RT-PCR. Related to STAR Methods.