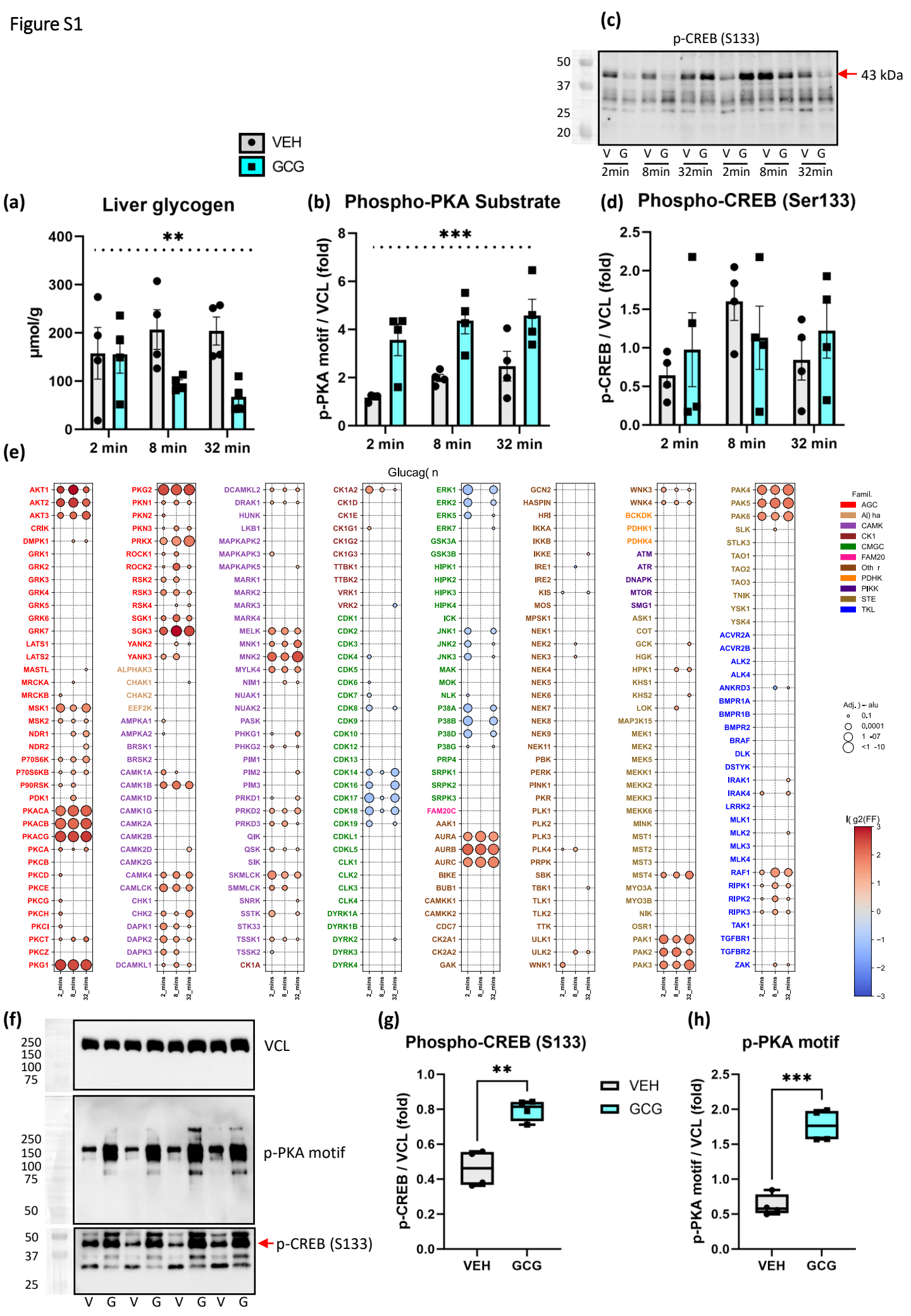
Figure S1


(c)
p-CREB (S133)
50
37
25
20
43 kDa
V G V G V G V G V G V G
2min 8min 32min 2min 8min 32min
VEH
GCG
(a)
Liver glycogen
µmol/g
400
300
200
100
0
**
2 min 8 min 32 min
(b)
Phospho-PKA Substrate
p-PKA motif / VCL (fold)
8
6
4
2
0
***
2 min 8 min 32 min
(d)
Phospho-CREB (Ser133)
p-CREB / VCL (fold)
2.5
2.0
1.5
1.0
0.5
0.0
2 min 8 min 32 min
(e)
Glucag(n
AKT1 AKT2 AKT3 CRIK DMPK1 GRK1 GRK2 GRK3 GRK4 GRK5 GRK6 GRK7 LATS1 LATS2 MASTL MRCKA MRCKB MSK1 MSK2 NDR1 NDR2 P70S6K P70S6KB P90RSK PDK1 PKACA PKACB PKACG PKCA PKCB PKCD PKCE PKCG PKCH PKCI PKCT PKCZ PKG1
PKG2 PKN1 PKN2 PKN3 PRKX ROCK1 ROCK2 RSK2 RSK3 RSK4 SGK1 SGK3 YANK2 YANK3 ALPHAK3 CHAK1 CHAK2 EEF2K AMPKA1 AMPKA2 BRSK1 BRSK2 CAMK1A CAMK1B CAMK1D CAMK1G CAMK2A CAMK2B CAMK2D CAMK2G CAMK4 CAMLCK CHK1 CHK2 DAPK1 DAPK2 DAPK3 DCAMKL1
DCAMKL2 DRAK1 HUNK LKB1 MAPKAPK2 MAPKAPK3 MAPKAPK5 MARK1 MARK2 MARK3 MARK4 MELK MNK1 MNK2 MYLK4 NIM1 NUAK1 NUAK2 PASK PHKG1 PHKG2 PIM1 PIM2 PIM3 PRKD1 PRKD2 PRKD3 QIK QSK SIK SKMLCK SMMLCK SNRK SSTK STK33 TSSK1 TSSK2 CK1A
CK1A2 CK1D CK1E CK1G1 CK1G2 CK1G3 TTBK1 TTBK2 VRK1 VRK2 CDK1 CDK2 CDK3 CDK4 CDK5 CDK6 CDK7 CDK8 CDK9 CDK10 CDK12 CDK13 CDK14 CDK16 CDK17 CDK18 CDK19 CDKL1 CDKL5 CLK1 CLK2 CLK3 CLK4 DYRK1A DYRK1B DYRK2 DYRK3 DYRK4
ERK1 ERK2 ERK5 ERK7 GSK3A GSK3B HIPK1 HIPK2 HIPK3 HIPK4 ICK JNK1 JNK2 JNK3 MAK MOK NLK P38A P38B P38D P38G PRP4 SRPK1 SRPK2 SRPK3 FAM20C AAK1 AURA AURB AURC BIKE BUB1 CAMKK1 CAMKK2 CDC7 CK2A1 CK2A2 GAK
GCN2 HASPIN HRI IKKA IKKB IKKE IRE1 IRE2 KIS MOS MPSK1 NEK1 NEK2 NEK3 NEK4 NEK5 NEK6 NEK7 NEK8 NEK9 NEK11 PBK PERK PINK1 PKR PLK1 PLK2 PLK3 PLK4 PRPK SBK TBK1 TLK1 TLK2 TTK ULK1 ULK2 WNK1
WNK3 WNK4 BCKDK PDHK1 PDHK4 ATM ATR DNAPK MTOR SMG1 ASK1 COT GCK HGK HPK1 KHS1 KHS2 LOK MAP3K15 MEK1 MEK2 MEK5 MEKK1 MEKK2 MEKK3 MEKK6 MINK MST1 MST2 MST3 MST4 MYO3A MYO3B NIK OSR1 PAK1 PAK2 PAK3
PAK4 PAK5 PAK6 SLK STLK3 TAO1 TAO2 TAO3 TNIK YSK1 YSK4 ACVR2A ACVR2B ALK2 ALK4 ANKRD3 BMPR1A BMPR1B BMPR2 BRAF DLK DSTYK IRAK1 IRAK4 LRRK2 MLK1 MLK2 MLK3 MLK4 RAF1 RIPK1 RIPK2 RIPK3 TAK1 TGFBR1 TGFBR2 ZAK
2_mins 8_mins 32_mins
Famil.
AGC
Al) ha
CAMK
CK1
CMGC
FAM20
Oth r
PDHK
PIKK
STE
TKL
Adj.) ← alu
0.1
0.0001
1 -07
<1 -10
I(g2(FF)
3
2
1
0
−1
−2
−3
(f)
250
150
100
75
VCL
250
150
100
75
50
p-PKA motif
50
37
25
p-CREB (S133)
V G V G V G V G
(g)
Phospho-CREB (S133)
p-CREB / VCL (fold)
1.0
0.8
0.6
0.4
0.2
0.0
**
VEH GCG
VEH
GCG
(h)
p-PKA motif
p-PKA motif / VCL (fold)
2.5
2.0
1.5
1.0
0.5
0.0
***
VEH GCG

Supplementary Figure 1 (related to Figure 1).

a: Liver glycogen content of male Sprague Dawley rats treated with either vehicle (VEH) or glucagon (GCG) (1.15 nM) for 2, 8 and 32min (n = 4 rats per group) *in situ*. Data presented as mean ± s.e.m. Statistical tests used were two-way ANOVA with Holm-Sidak post-hoc tests. Dotted line indicates a main effect. Difference in treatment: **P* <0.05, ***P* <0.01, ****P* <0.001; Difference in time: $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001.

b: Western blot quantification of liver phospho-PKA motif protein expression of rat liver as in a.

c: Western blot images of liver phospho-CREB (S133) level of samples as in a.

d: Western blot quantification of c. Samples were obtained from the same experiment, and blots were processed simultaneously.

e: Bubble plot of predicated kinase activation from phosphoproteomics data.

f: Western blot images of phospho-protein kinase A (pPKA) motif protein substrates, phospho-CREB (S133) and loading control vinculin (VCL) of SNU398-GCGR cells treated with either vehicle (VEH) or glucagon (GCG) (1 nM) for 30min (n = 4 per group).

g-h: Western blot quantification of e. Data were analysed by unpaired two-tailed Student's t-test. Difference in treatment: ***P <0.001. Samples were obtained from the same experiment, and blots were processed simultaneously.

Source data are provided as a Source Data file.

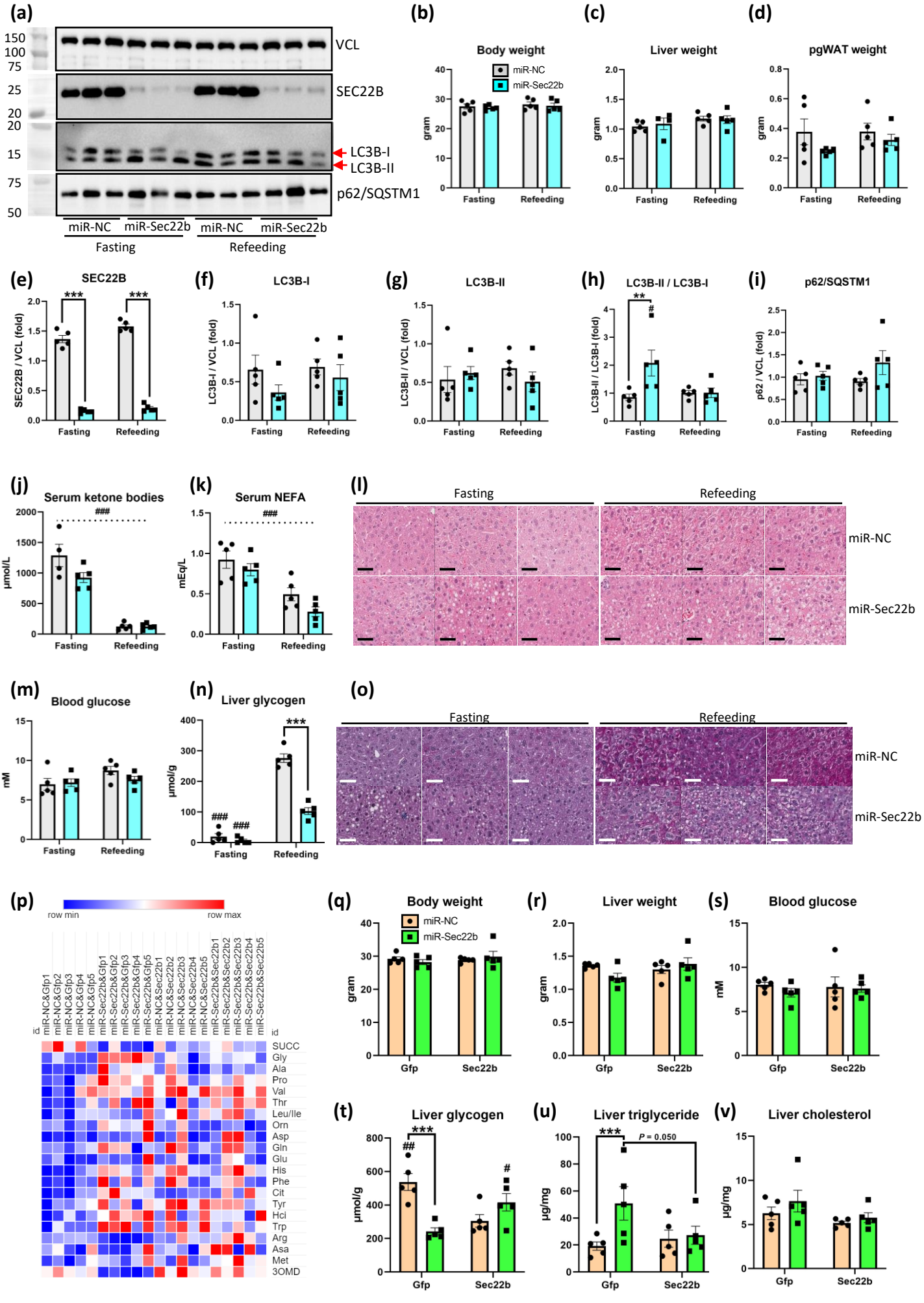
Figure S2

kinase	kinase group	site percentile	percentile rank
MEKK1	STE	97.056 %	1
YANK2	AGC	96.846 %	2
PRPK	Other	96.492 %	3
ZAK	TKL	96.221 %	4
YANK3	AGC	95.983 %	5
NEK11	Other	95.717 %	6
MEKK3	STE	95.708 %	7
BCKDK	PDHK	95.116 %	8
RSK4	AGC	94.904 %	9
COT	STE	93.928 %	10
IKKB	Other	93.480 %	11
MST4	STE	93.365 %	12
DLK	TKL	93.166 %	13
PDHK1	PDHK	93.129 %	14
IKKE	Other	92.714 %	15
TBK1	Other	92.567 %	16
CAMKK2	Other	92.220 %	17
GSK3B	CMGC	92.109 %	18
CAMKK1	Other	91.187 %	19
YSK1	STE	91.137 %	20

Supplementary Figure 2 (related to Figure 2).

Table of predicted upstream kinases affecting SEC22B S137 phosphorylation.

Figure S3


(a)
VCL
SEC22B
LC3B-I
LC3B-II
p62/SQSTM1
miR-NC
miR-Sec22b
Fasting
Refeeding
(b)
Body weight
(c)
Liver weight
(d)
pgWAT weight
(e)
SEC22B
(f)
LC3B-I
(g)
LC3B-II
(h)
LC3B-II / LC3B-I
(i)
p62/SQSTM1
(j)
Serum ketone bodies
(k)
Serum NEFA
(l)
(m)
Blood glucose
(n)
Liver glycogen
(o)
(p)
row min
row max
(q)
Body weight
(r)
Liver weight
(s)
Blood glucose
(t)
Liver glycogen
(u)
Liver triglyceride
(v)
Liver cholesterol

Supplementary Figure 3 (related to Figure 3).

a: Liver western blot images of SEC22B, LC3, p62/SQSTM1 and loading control vinculin (VCL) levels from male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC) and fasted for 16h (fasting) or fasted and then refed for 5-6h (refeeding).

b: Body weight of mice as in a. Data are mean ± s.e.m.; n = 5 mice per group. Statistical tests used were 2-way ANOVA with Holm-Sidak post-hoc tests. Dotted line shows the main effect. Difference between fasting and refeeding: #*P* <0.05, ##*P* <0.01, ###*P* <0.001; Difference between miR-NC and miR-Sec22b: **P* <0.05, ***P* <0.01, ****P* <0.001.

c: Liver weight of mice (n = 5 mice per group except miR-Sec22b with fasting, n=4).

d: Perigonadal white adipose tissue weight of mice as in a.

e-i: Western blot quantification of a. Samples were obtained from the same experiment, and blots were processed simultaneously.

j: Serum ketone bodies levels of mice as in a.

k: Serum non-esterified fatty acid (NEFA) levels of mice as in a.

l: Liver Hematoxylin and Eosin staining micrographs of mice as in a. Images are representative of three individual mice per group. Scale bar: 50μm.

m: Blood glucose levels of mice as in a.

n: Liver glycogen levels of mice as in a.

o: Mouse liver periodic acid-Schiff (PAS) staining micrographs as in a. Images are representative of three individual mice per group. Scale bar: 50μm.

p: A heatmap of serum amino acid species of male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC), with Flag-tagged Sec22b cDNA (Sec22b) or a control (Gfp), these mice are fasted and then refed for 5-6h. Abbreviations for serum amino acids are provided in Supplementary File 5.

q: Body weight of mice as in p. Data are mean ± s.e.m.; n = 5 mice per group. Statistical tests used were 2-way ANOVA with Holm-sidak post-hoc tests. Difference in AAV-miR (miR-NC and miR-Sec22b): **P* <0.05, ***P* <0.01, ****P* <0.001; Difference in AAV-cDNA (GFP and WT): #*P* <0.05, ##*P* <0.01, ###*P* <0.001.

r: Liver weight of mice as in p.

s: Blood glucose levels of mice as in p.

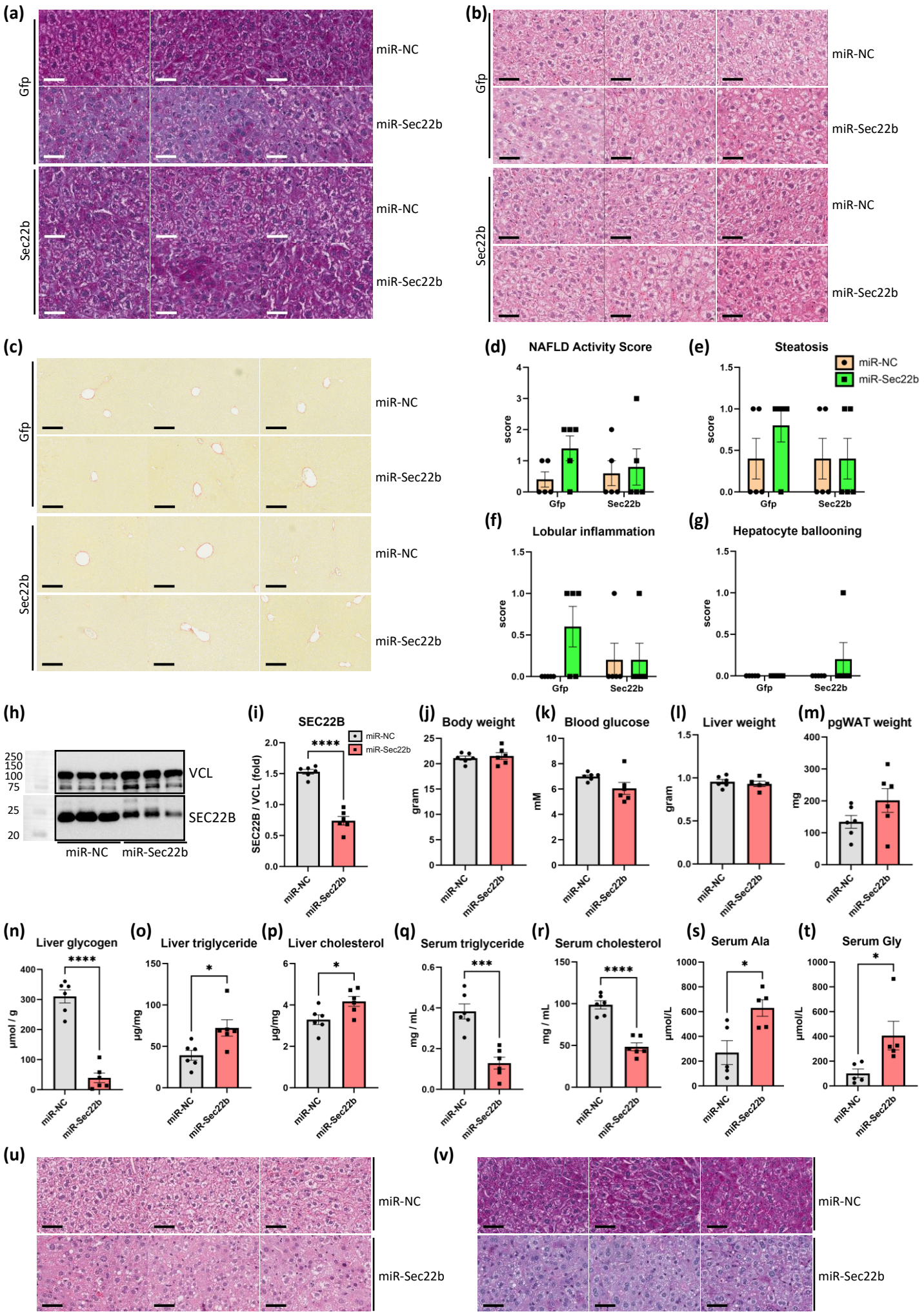
t: Liver glycogen levels of mice as in p.

u: Liver triglyceride levels of mice as in p.

v: Liver cholesterol levels of mice as in p.

Source data are provided as a Source Data file.

Figure S4


(a)
Gfp
Sec22b
miR-NC
miR-Sec22b
(b)
(c)
(d)
NAFLD Activity Score
score
Gfp
Sec22b
(e)
Steatosis
(f)
Lobular inflammation
(g)
Hepatocyte ballooning
(h)
250
150
100
75
25
20
VCL
SEC22B
(i)
SEC22B
SEC22B / VCL (fold)
****
(j)
Body weight
gram
(k)
Blood glucose
mM
(l)
Liver weight
(m)
pgWAT weight
mg
(n)
Liver glycogen
µmol / g
(o)
Liver triglyceride
µg/mg
*
(p)
Liver cholesterol
(q)
Serum triglyceride
***
mg / mL
(r)
Serum cholesterol
(s)
Serum Ala
µmol/L
(t)
Serum Gly
(u)
(v)

Supplementary Figure 4 (related to Figure 3).

a: Liver PAS staining micrographs of male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC), with Flag-tagged Sec22b cDNA (Sec22b) or a control (Gfp), these mice are fasted and then refed for 5-6h. Images are representative of three individual mice per group. Scale bar: 50µm.

b: Liver Hematoxylin and Eosin staining micrographs of mice as in a. Images are representative of three individual mice per group. Scale bar: 50µm.

c: Liver picrosirius red (PSR) staining micrographs of mice as in a. Images are representative of three individual mice per group. Scale bar: 200µm.

d: NAFLD Activity Score of micrographs as in b. Data are mean ± s.e.m.; n = 5 mice per group.

e: Steatosis Score of micrographs as in b.

f: Lobular Inflammation Score of micrographs as in b.

g: Hepatocyte Ballooning Score of micrographs as in b.

h: Liver western blot images of SEC22B and loading control vinculin (VCL) levels of female C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC) and fasted for 16h then refed for 5-6h.

i: Western blot quantification of h. Samples were obtained from the same experiment, and blots were processed simultaneously. Data are mean ± s.e.m.; n = 6 mice per group. Statistical tests used were unpaired two-sided Student's *t*-tests. Difference between miR-NC and miR-Sec22b: **P* <0.05, ***P* <0.01, ****P* <0.001, *****P* <0.0001.

j: Body weight of mice as in h.

k: Blood glucose of mice as in h.

l: Liver weight of mice (n = 6 for miR-NC group and n = 5 for miR-Sec22b group).

m: pgWAT weight of mice as in h.

n: Liver glycogen of mice as in h.

o: Liver triglyceride of mice as in h.

p: Liver cholesterol of mice as in h.

q: Serum triglyceride of mice as in h.

r: Serum cholesterol of mice as in h.

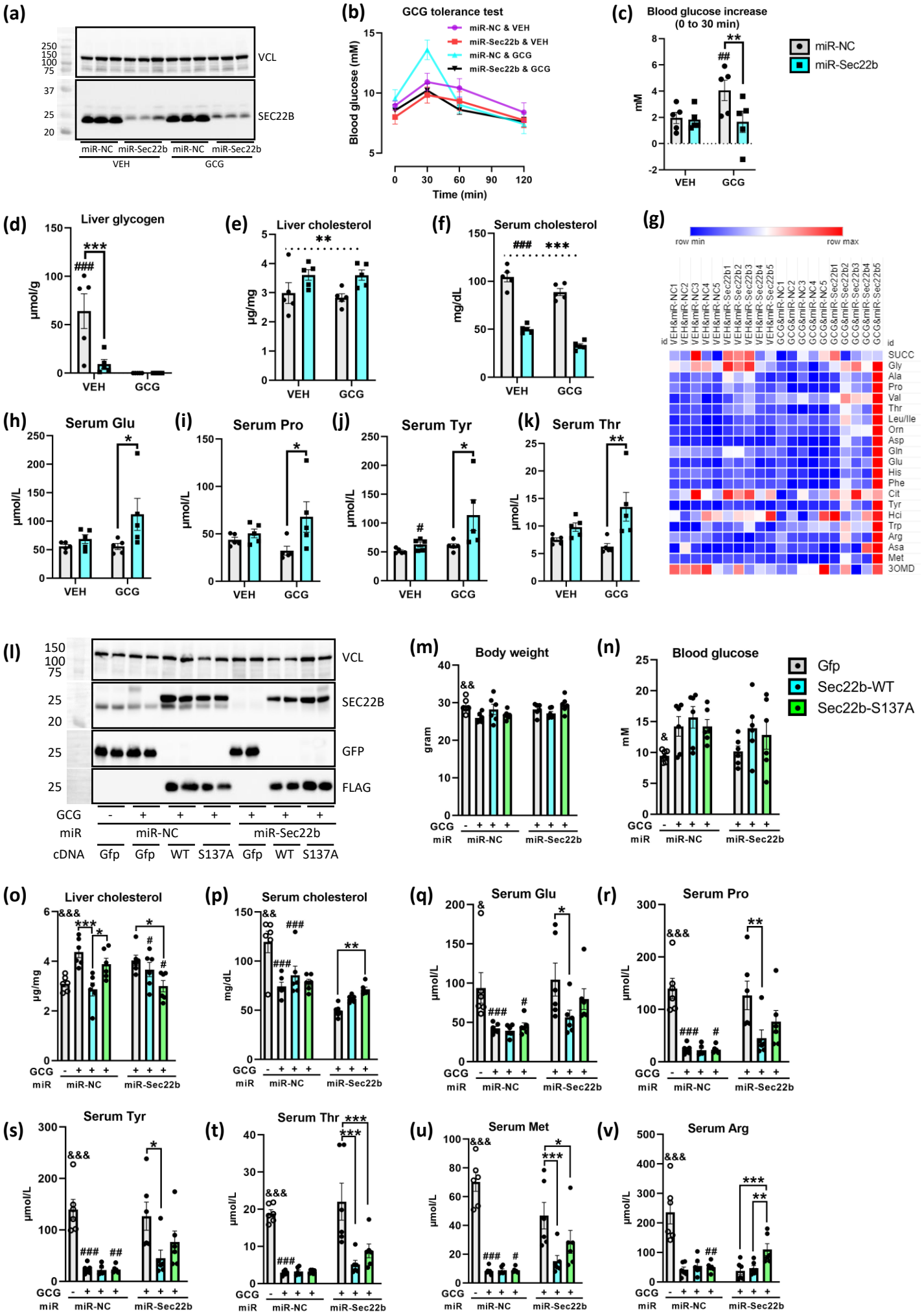
s: Serum alanine of mice as in h.

t: Serum glycine of mice as in h.

u: Liver Hematoxylin and Eosin staining micrographs of mice as in h. Images are representative of three individual mice per group. Scale bar: 50µm.

v: Liver periodic acid-Schiff (PAS) staining micrographs of mice as in h. Images are representative of three individual mice per group. Scale bar: 50µm. Source data are provided as a Source Data file.

Figure S5


(a)
VCL
SEC22B
miR-NC
miR-Sec22b
VEH
GCG
(b)
GCG tolerance test
miR-NC & VEH
miR-Sec22b & VEH
miR-NC & GCG
miR-Sec22b & GCG
Blood glucose (mM)
Time (min)
(c)
Blood glucose increase (0 to 30 min)
mM
(d)
Liver glycogen
µmol/g
(e)
Liver cholesterol
µg/mg
(f)
Serum cholesterol
mg/dL
(g)
row min
row max
SUCC
Gly
Ala
Pro
Val
Thr
Leu/Ile
Orn
Asp
Gln
Glu
His
Phe
Cit
Tyr
Hci
Trp
Arg
Asa
Met
3OMD
(h)
Serum Glu
µmol/L
(i)
Serum Pro
(j)
Serum Tyr
(k)
Serum Thr
(l)
VCL
SEC22B
GFP
FLAG
GCG
miR
cDNA
Gfp
WT
S137A
(m)
Body weight
gram
(n)
Blood glucose
mM
Gfp
Sec22b-WT
Sec22b-S137A
(o)
Liver cholesterol
(p)
Serum cholesterol
(q)
Serum Glu
(r)
Serum Pro
(s)
Serum Tyr
(t)
Serum Thr
(u)
Serum Met
(v)
Serum Arg

Supplementary Figure 5 (related to Figure 4).

a: Liver western blot images of SEC22B and loading control vinculin (VCL) levels from male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC) and fasted for 2h acutely treated with saline (VEH) or acyl-glucagon (GCG). Images are of 3 individual mice per group.

b: Blood glucose levels during the 2h intraperitoneal glucagon (GCG) tolerance test of mice as in A. Data are mean ± s.e.m.; n = 5 mice per group.

c: Blood glucose changes from 0 to 30min of mice as in A. Data are mean ± s.e.m.; n = 5 mice per group. Statistical tests used were 2-way ANOVA with Holm-Sidak post-hoc tests. Dotted line shows the main effect. Difference between vehicle and glucagon: #*P* <0.05, ##*P* <0.01, ###*P* <0.001; Difference between miR-NC and miR-Sec22b: **P* <0.05, ***P* <0.01, ****P* <0.001.

d: Liver glycogen levels at 2h of mice as in c.

e: Liver cholesterol levels of mice as in c.

f: Serum cholesterol levels of mice as in c.

g: A heatmap of serum amino acid species at 2h of mice as in c. Abbreviations for serum amino acids are provided in Supplementary File 5.

h-k: Selected serum amino acid levels at 2h of mice as in c.

l: Representative liver western blots for FLAG, GFP, SEC22B and the loading control vinculin (VCL) of male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) or a negative control (miR-NC), and/or flag-tagged AAV-Sec22b wildype (WT) or S137A mutant (S137A) cDNA (Sec22b) or a control (Gfp) and treated with either saline (VEH) or acyl-glucagon (GCG) chronically for 3 wk.

m: Body weight of mice as in M. Data are mean ± s.e.m.; n = 6 mice per group. Statistical tests used include unpaired two-tailed Student T. test (for miR-NC&GFP&VEH and miR-NC&GFP&GCG) and 2-way ANOVA (for all the glucagon treated groups) with Holm-Sidak post-hoc tests. Difference between vehicle and glucagon: &<0.05, &&*P* <0.01, &&&*P* <0.001; Difference between miR-NC and miR-Sec22b: #*P* <0.05, ##*P* <0.01, ###*P* <0.001; Difference in GFP, SEC22B-WT and SEC22B-S137A: **P* <0.05, ***P* <0.01, ****P* <0.001.

n: Blood glucose levels of mice as in m.

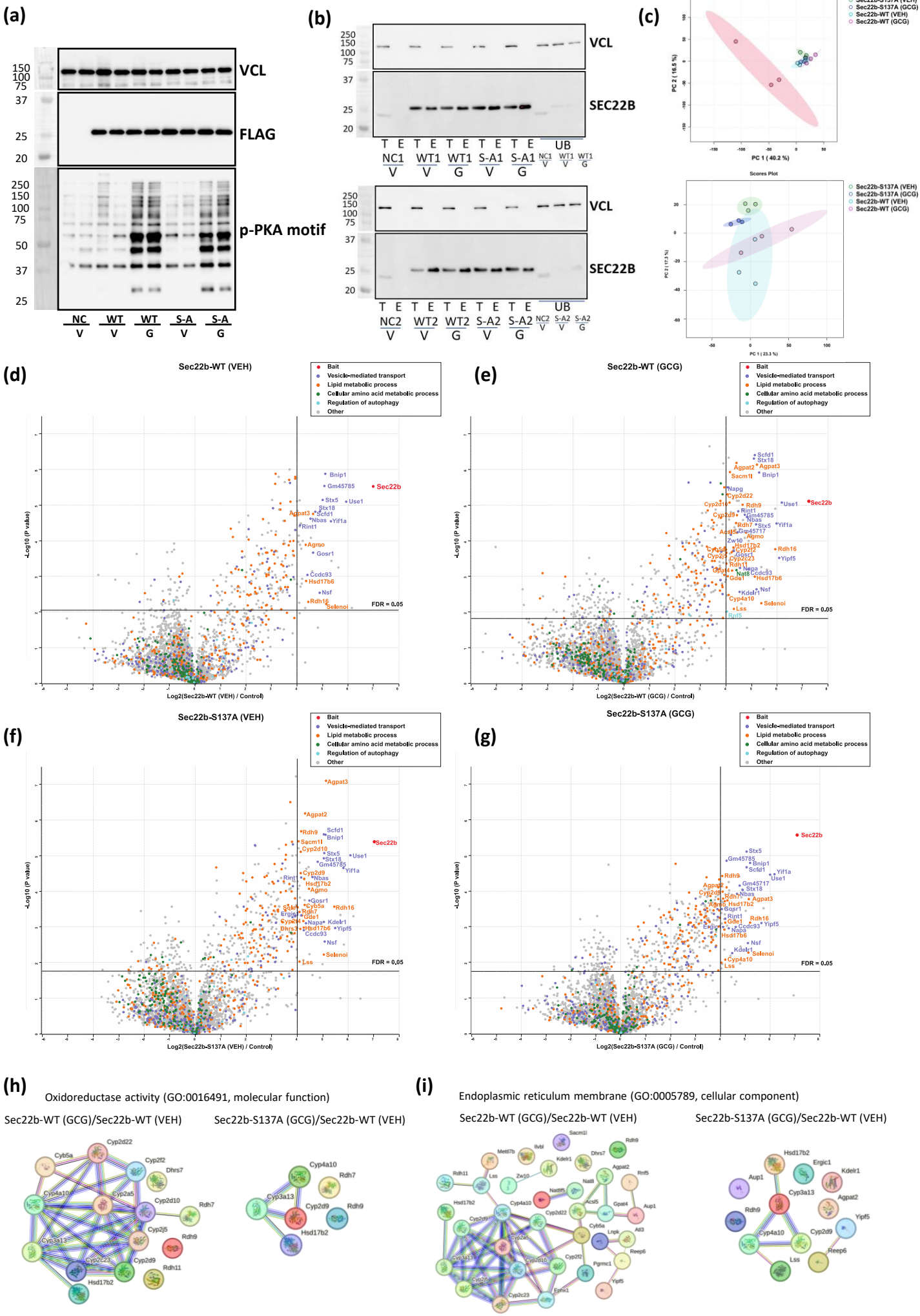
o: Liver cholesterol levels of mice as in m.

p: Serum cholesterol levels of mice as in m.

q-v: Selected serum amino acid levels of mice as in m.

Source data are provided as a Source Data file.

Figure S6


(a)
VCL
FLAG
p-PKA motif
NC V
WT V
WT G
S-A V
S-A G
(b)
VCL
SEC22B
NC1 V
WT1 V
WT1 G
S-A1 V
S-A1 G
NC2 V
WT2 V
WT2 G
S-A2 V
S-A2 G
UB
(c)
Scores Plot
Control
Sec22b-S137A (VEH)
Sec22b-S137A (GCG)
Sec22b-WT (VEH)
Sec22b-WT (GCG)
PC 1 (40.2 %)
PC 2 (16.5 %)
PC 1 (23.3 %)
PC 2 (17.3 %)
(d)
Sec22b-WT (VEH)
(e)
Sec22b-WT (GCG)
(f)
Sec22b-S137A (VEH)
(g)
Sec22b-S137A (GCG)
Bait
Vesicle-mediated transport
Lipid metabolic process
Cellular amino acid metabolic process
Regulation of autophagy
Other
FDR = 0.05
-Log10 (P value)
Log2(Sec22b-WT (VEH) / Control)
Log2(Sec22b-WT (GCG) / Control)
Log2(Sec22b-S137A (VEH) / Control)
Log2(Sec22b-S137A (GCG) / Control)
(h)
Oxidoreductase activity (GO:0016491, molecular function)
Sec22b-WT (GCG)/Sec22b-WT (VEH)
Sec22b-S137A (GCG)/Sec22b-WT (VEH)
(i)
Endoplasmic reticulum membrane (GO:0005789, cellular component)
Sec22b-WT (GCG)/Sec22b-WT (VEH)
Sec22b-S137A (GCG)/Sec22b-WT (VEH)

Supplementary Figure 6 (related to Figure 5).

a: Male C57Bl/6N mice administered with adeno-associated viruses expressing a microRNA to silence Sec22b (miR-Sec22b) with re-expressing of Sec22b cDNA (Sec22b-WT or Sec22b-S137A) or a AAV control (miR-NC & Gfp). The mice were acutely treated with either saline (VEH) or acyl-glucagon (GCG) (n=3/group). Co-immunoprecipitation proteomics analysis of the liver lysates was then conducted. Western blot images of liver phospho-protein kinase A (pPKA) motif protein substrates, FLAG and loading control Vinculin (VCL) NC: control (GFP); WT: Flag-tagged Sec22b; S-A: Flag-tagged Sec22b S137A mutant. V: saline injection; G: acyl-GCG injection.

b: Western blot images of immunoprecipitation of FLAG-tagged SEC22B. T: total input; UB: unbounded fraction; E: elution; of samples prepared as in A.

c: Principal component analysis (PCA) among Control (red), Sec22b-S137A with VEH (green), Sec22b-S137 with GCG (purple), Sec22b-WT with VEH (blue) and Sec22b-WT with GCG (pink) co-immunoprecipitation samples.

d-g: Volcano plots of proteins bounded with Sec22b-WT and Sec22b-S137A mutant treated with VEH or GCG (Log2>4, FDR<0.05).

h: STRING visualization of proteins involved in GO pathway Oxidoreductase activity (GO:0016491, molecular function).

i: STRING visualization of proteins involved in GO pathway Endoplasmic reticulum membrane (GO:0005789, cellular component).