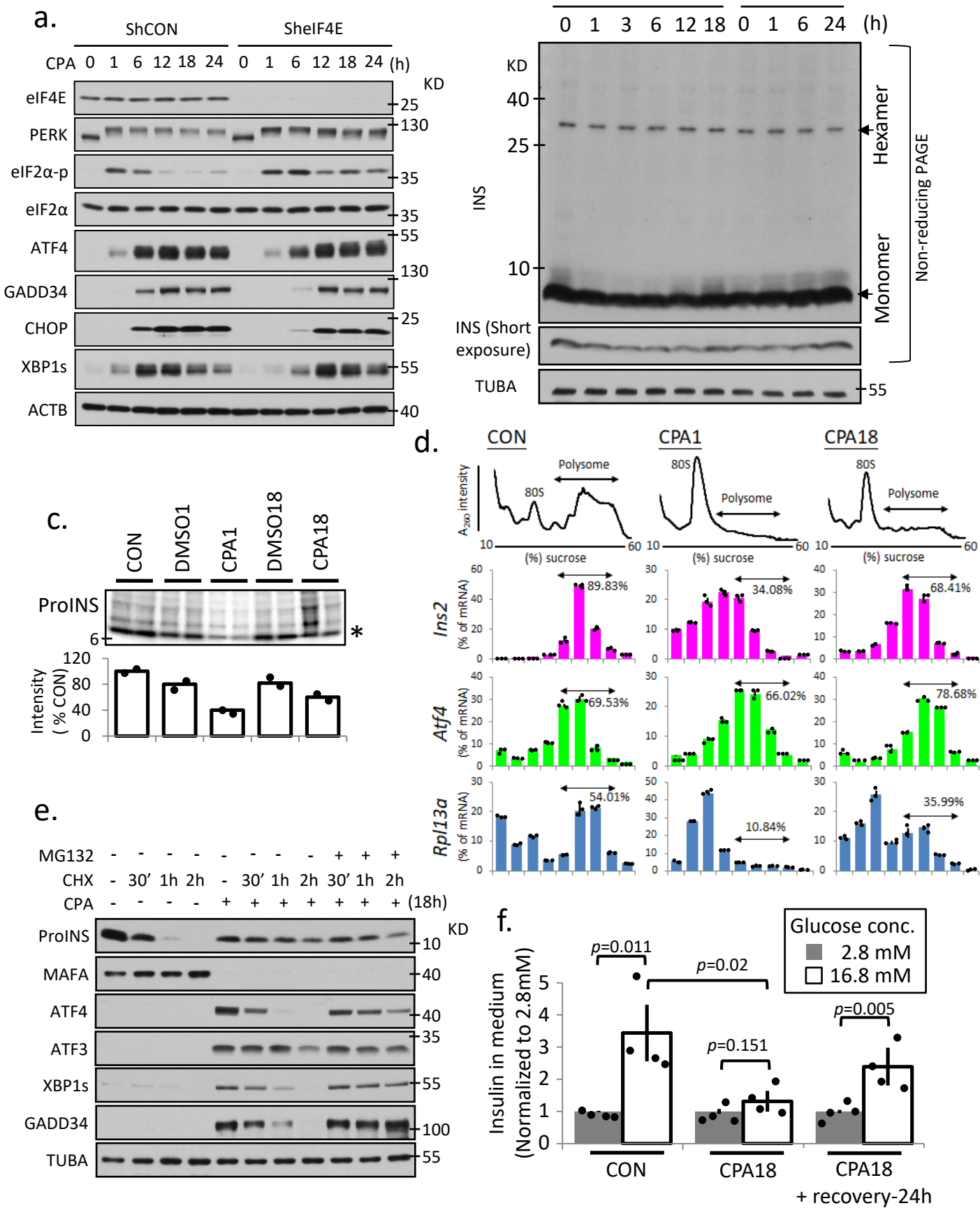Supplementary Information for

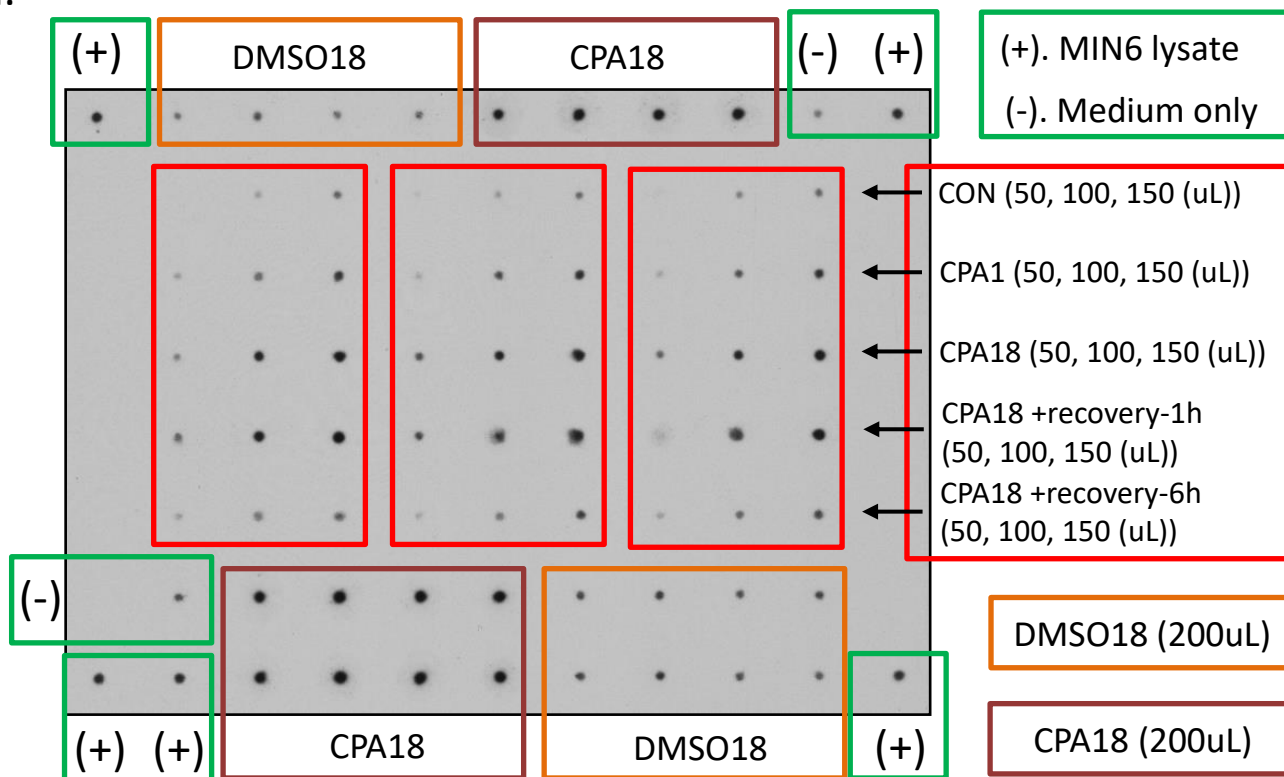**Adaptation to chronic ER stress enforces pancreatic β-cell plasticity**
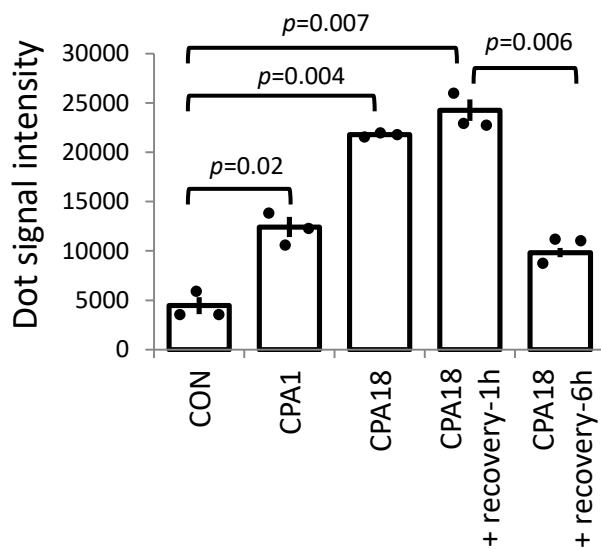
By Chen *et al.* 2022

Supplementary Figure 1.

Supplementary Figure 1. Dynamic regulation of proinsulin synthesis during in MIN6 cells treated with CPA. a and **e** Western blot analysis of the indicated proteins in MIN6 cells expressing shRNAs for CON or the eIF4E mRNA and treated with CPA for the indicated times (**a**) or treated with the protein synthesis inhibitor CHX or the inhibitor of the proteosomal activity, MG132, in the presence or absence of CPA for the indicated times (**e**). **b** Western blot analysis of insulin by non-reducing SDS-PAGE electrophoresis. Arrows indicated either insulin monomer or hexamer. **c** SDS-Page analysis of metabolically labeled proinsulin in MIN6 cells treated with CPA for the indicated times. *.Quantification of two independent experiments is shown. Source data are provided as a Source Data file. **d** Distribution of the indicated mRNAs on polysome profiles of cell extracts isolated from MIN6 cells treated with CPA for the indicated times. **f** Normalized to each condition of treatment evaluation of GSIS in MIN6 cells treated for the indicated times with CPA, $n=4$ independent experiments. Error bars represent S.E.M. p-value represents the statistical test by two-tailed Student's t-test. Dots in all plots represent independent experiments. Source data are provided as a Source Data file.

Supplementary Figure 2.

a.

(+). MIN6 lysate

(-). Medium only

(+) DMSO18 CPA18 (-) (+)

CON (50, 100, 150 (uL))

CPA1 (50, 100, 150 (uL))

CPA18 (50, 100, 150 (uL))

CPA18 +recovery-1h (50, 100, 150 (uL))

CPA18 +recovery-6h (50, 100, 150 (uL))
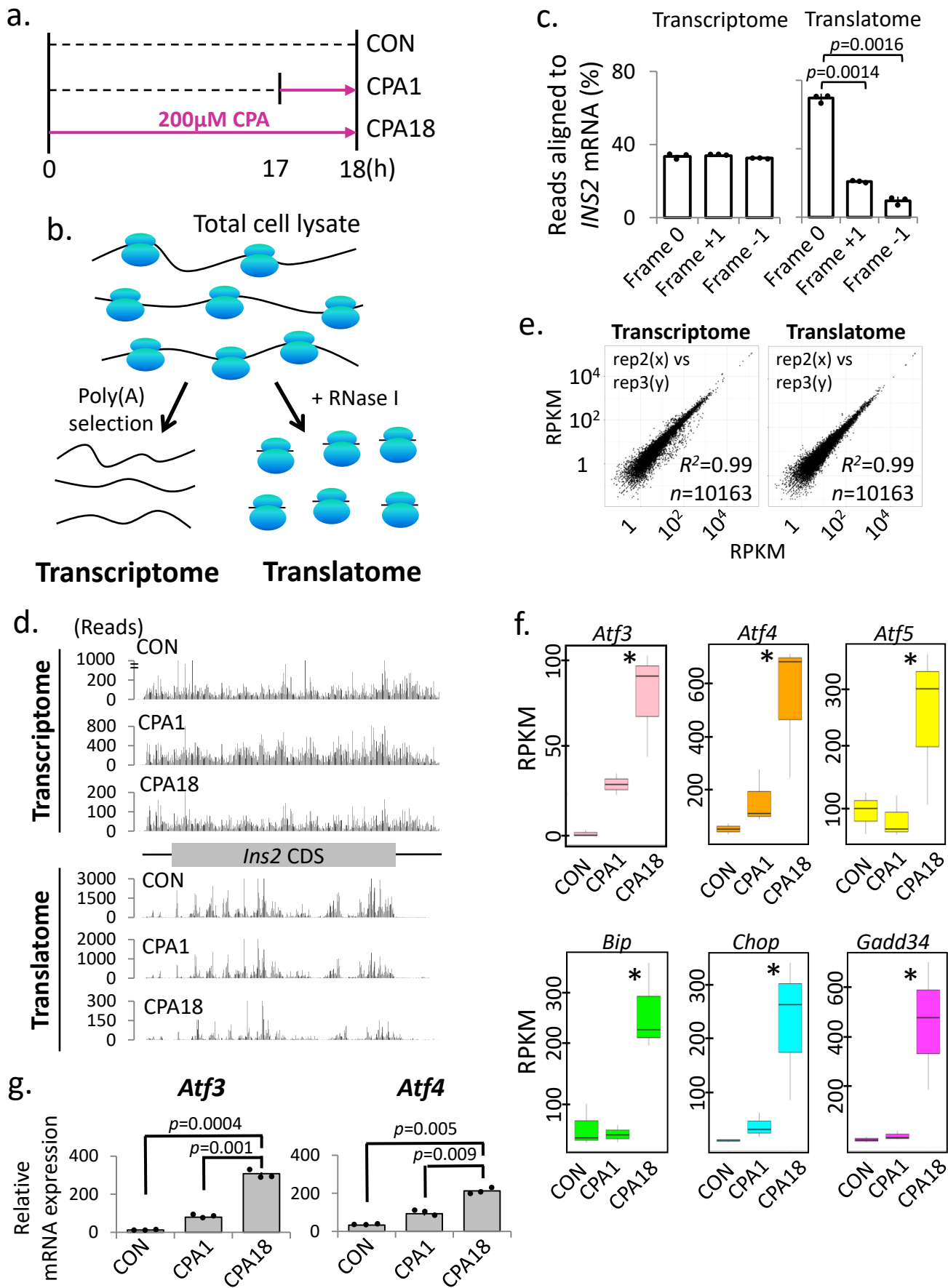
DMSO18 (200uL)

CPA18 (200uL)
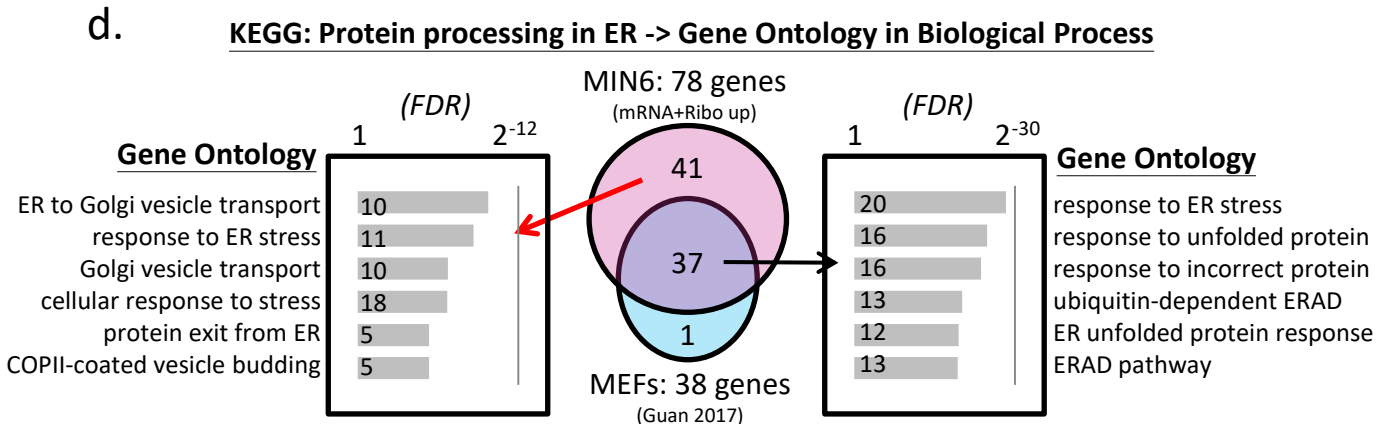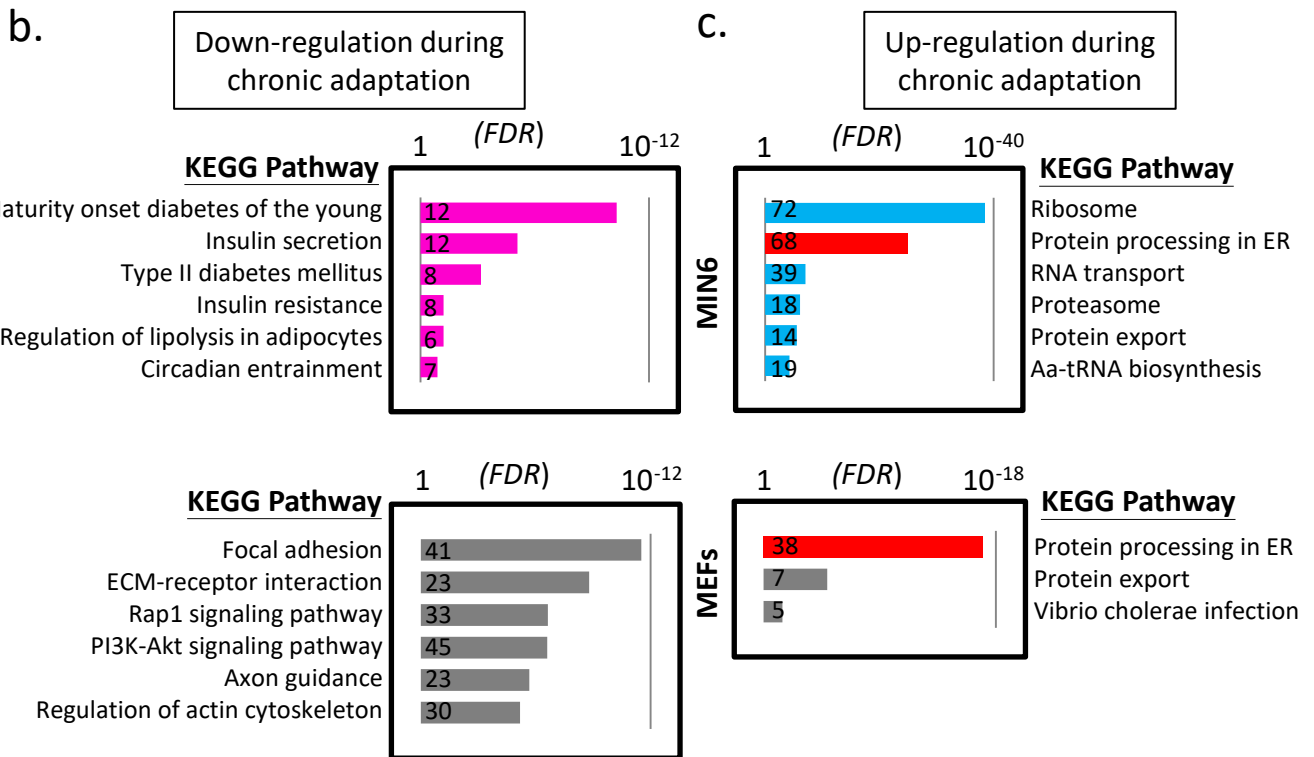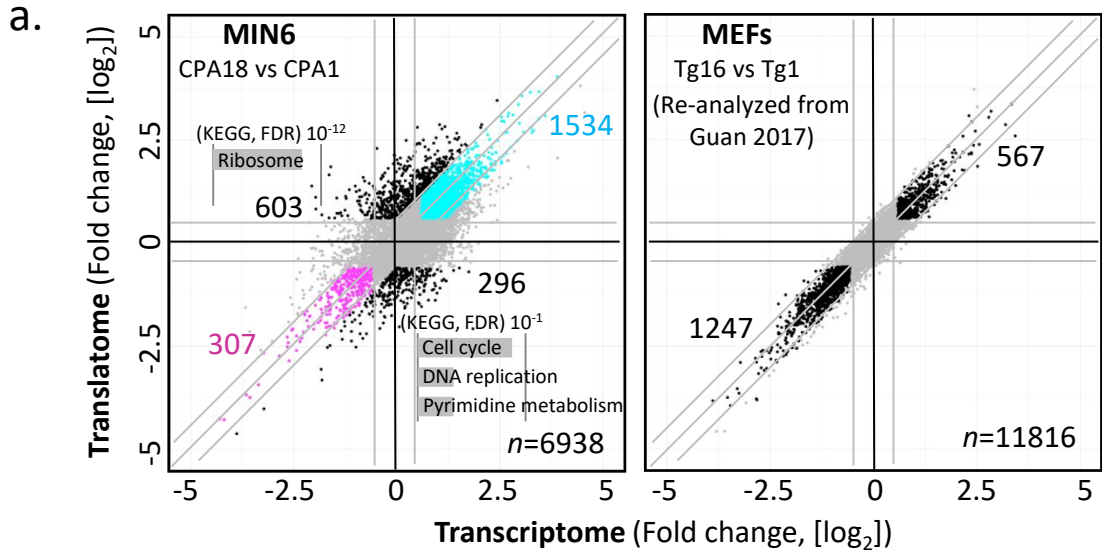
(-)

(+) (+) CPA18 DMSO18 (+)

b.

Supplementary Figure 2. Evaluation of secreted proinsulin from MIN6 cells treated with CPA. **a** Dot blot analysis of proinsulin secreted in the media from MIN6 cells treated with CPA for 1h or 18h, or washout of CPA following 18h treatment, for 1h or 6h. (+): indicates MIN6 cell lysates and serves as a positive control for proinsulin. (-): indicates growth medium not applied to MIN6 cells and serves as a negative control. Red boxes: indicate in a vertical direction the treatment and times of treatment and in a horizontal direction the increasing concentration of the medium volume (50, 100, 150 μl), collected from MIN6 cells incubated at the indicated times. Three independent experiments are shown. Orange boxes: indicate medium collected from MIN6 cells treated with the vehicle of CPA, DMSO, for 18h (200 μl). Purple boxes: indicate the medium collected from MIN6 cells treated with CPA, for 18h (200 μl). Three independent experiments and four technical replicates for each independent experiments are shown. **b** Quantification of the dot-signal intensity in A, from 3 independent experiments of MIN6 cells treated with CPA for the indicated times. Error bars represent S.E.M. *p*-value represents the statistical test by two-tailed paired Student's *t*-test. Dots in all plots represent independent experiments. Source data are provided as a Source Data file.

Supplementary Figure 3.

a.

CON
CPA1
CPA18

200μM CPA

0   17   18(h)

b.

Total cell lysate

Poly(A) selection

+ RNase I

Transcriptome   **Translatome**

c.

Transcriptome   Translatome

Reads aligned to *INS2* mRNA (%)

p=0.0016
p=0.0014

80
40
0

Frame 0 Frame +1 Frame -1   Frame 0 Frame +1 Frame -1

e.

Transcriptome   **Translatome**

rep2(x) vs rep3(y)   rep2(x) vs rep3(y)

R^2=0.99 R^2=0.99
n=10163   *n*=10163

10^4
10^2
1

RPKM

1   10^2 10^4 1 10^2 10^4

RPKM

d.

(Reads)

Transcriptome

CON
1000
200
0

CPA1
800
400
0

CPA18
200
100
0

Ins2 CDS

Translatome

CON
3000
1500
0

CPA1
2000
1000
0

CPA18
300
150
0

f.

Atf3
RPKM
100
50
0
CON CPA1 CPA18
*

Atf4
600
400
200
CON CPA1 CPA18
*

Atf5
300
200
100
CON CPA1 CPA18
*

Bip
RPKM
300
200
100
CON CPA1 CPA18
*

Chop
300
200
100
CON CPA1 CPA18
*

Gadd34
600
400
200
CON CPA1 CPA18
*

g.

Relative mRNA expression

Atf3
p=0.0004
p=0.001
400
200
0
CON CPA1 CPA18

Atf4
p=0.005
p=0.009
400
200
0
CON CPA1 CPA18

Supplementary Figure 3.   Quality controls and validations of the ribosome foot printing and RNA-seq datasets. **a-b** Overview of experimental setup. Transcriptomes were generated from poly(A) selected RNA and translatomes from ribosome protected RNA fragments. **c-d** Reading frame analysis (**c**) and RNA sequencing reads distribution (**d**) across the *Ins*2 mRNA. **e** Scatterplots represent the reproducibility of transcriptomes and translatomes in two independent experiments. **f** Gene expressions changes in the transcriptomics dataset from MIN6 cells treated as in (**a**). The boxes indicate the 25–75th percentiles, the midline indicates the median, whiskers show the maximum/minimum values. *$p<0.0001$, two-sided Wilcoxon–Mann–Whitney test. **g** Relative expression levels for the indicated mRNAs, measured by qRT-PCR. *n*=3 independent experiments. Error bars represent S.E.M. *p*-value represents the statistical test by two-tailed paired Student's *t*-test. Source data are provided as a Source Data file.
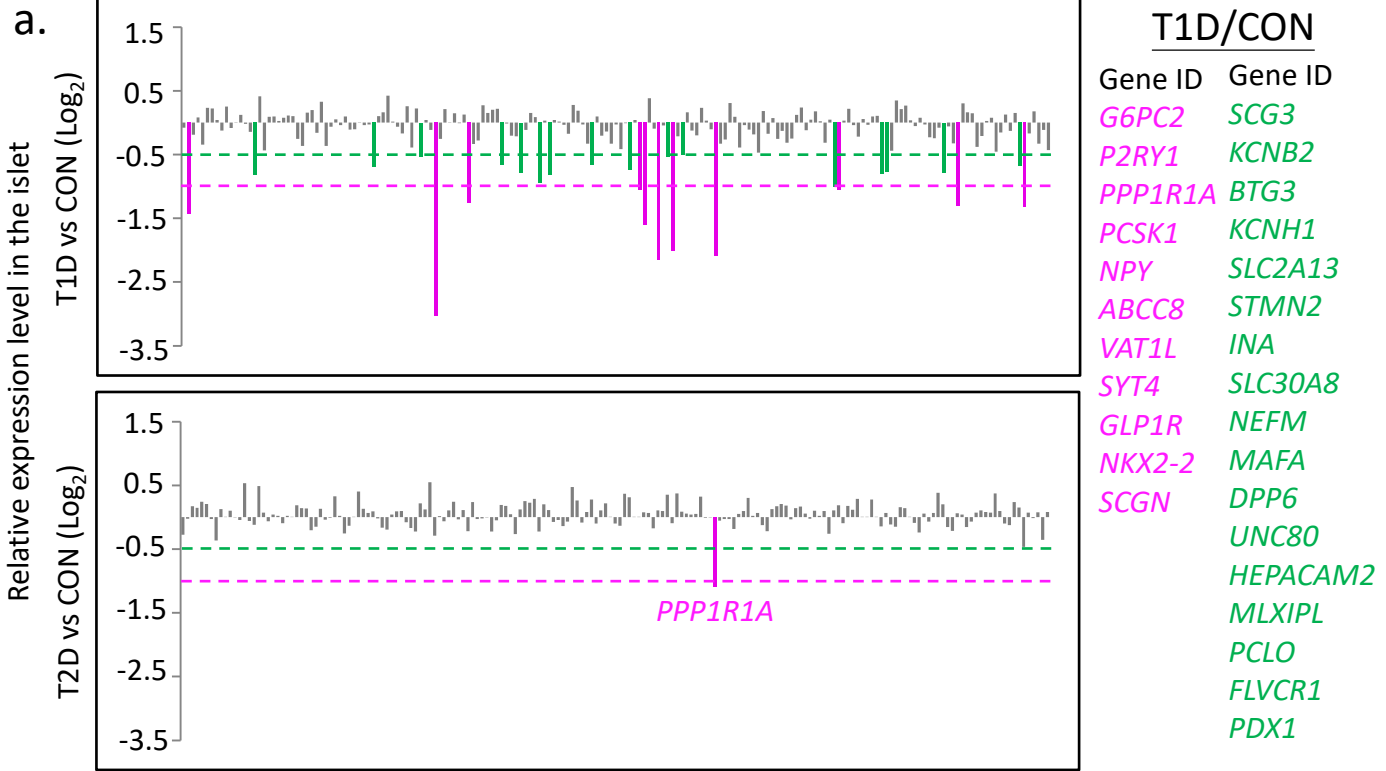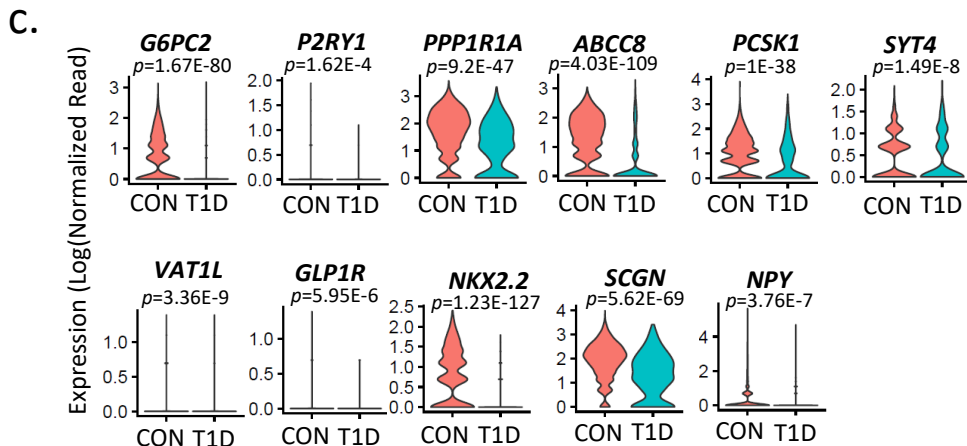
Supplementary Figure 4.

a.

MIN6 CPA18 vs CPA1

(KEGG, FDR) 10^{-12}
Ribosome

603

1534

307

296

(KEGG, FDR) 10^{-1}
Cell cycle
DNA replication
Pyrimidine metabolism

n=6938

MEFs Tg16 vs Tg1 (Re-analyzed from Guan 2017)

567

1247

n=11816

Translatome (Fold change, [log$_2$])

Transcriptome (Fold change, [log$_2$])

b.

Down-regulation during chronic adaptation

(FDR)

KEGG Pathway

	1 — 10^{-12}
Maturity onset diabetes of the young	12
Insulin secretion	12
Type II diabetes mellitus	8
Insulin resistance	8
Regulation of lipolysis in adipocytes	6
Circadian entrainment	7

(FDR)

KEGG Pathway

	1 — 10^{-12}
Focal adhesion	41
ECM-receptor interaction	23
Rap1 signaling pathway	33
PI3K-Akt signaling pathway	45
Axon guidance	23
Regulation of actin cytoskeleton	30

c.

Up-regulation during chronic adaptation

(FDR)

MIN6

	1 — 10^{-40}	**KEGG Pathway**
	72	Ribosome
	68	Protein processing in ER
	39	RNA transport
	18	Proteasome
	14	Protein export
	19	Aa-tRNA biosynthesis

(FDR)

MEFs

	1 — 10^{-18}	**KEGG Pathway**
	38	Protein processing in ER
	7	Protein export
	5	Vibrio cholerae infection

d.

KEGG: Protein processing in ER -> Gene Ontology in Biological Process

(FDR)

Gene Ontology

	1 — 2^{-12}
ER to Golgi vesicle transport	10
response to ER stress	11
Golgi vesicle transport	10
cellular response to stress	18
protein exit from ER	5
COPII-coated vesicle budding	5

MIN6: 78 genes
(mRNA+Ribo up)

41

37

1

MEFs: 38 genes
(Guan 2017)

(FDR)

Gene Ontology

	1 — 2^{-30}	
20		response to ER stress
16		response to unfolded protein
16		response to incorrect protein
13		ubiquitin-dependent ERAD
12		ER unfolded protein response
13		ERAD pathway

Supplementary Figure 4. Comparative analysis of transcriptomes and translatomes between MIN6 and MEFs in response to ER stress. **a** Scatterplots of fold changes in MIN6 (*left)*, and MEFS (*right*), during progression from acute to chronic ER stress (CPA18 vs CPA1 and Tg16 vs Tg1, respectively). KEGG pathway analysis of genes with either decreased mRNA abundance **b** or increased mRNA abundance (c) in MIN6 (*upper)* and MEFs (*lower)* panels. (d) Gene ontology analysis for genes in the KEEG pathway "protein processing in ER" in MIN6 and MEFs. Notice, MIN6 cells have 68 genes with increased mRNA abundance (panel C, upper) and 10 genes with increased ribo^{ocp} (panel A, included in the 603 genes).

Supplementary Figure 5.

a.

T1D/CON

Gene ID	Gene ID
G6PC2	*SCG3*
P2RY1	*KCNB2*
PPP1R1A	*BTG3*
PCSK1	*KCNH1*
NPY	*SLC2A13*
ABCC8	*STMN2*
VAT1L	*INA*
SYT4	*SLC30A8*
GLP1R	*NEFM*
NKX2-2	*MAFA*
SCGN	*DPP6*
	UNC80
	HEPACAM2
	MLXIPL
	PCLO
	FLVCR1
	PDX1

b.

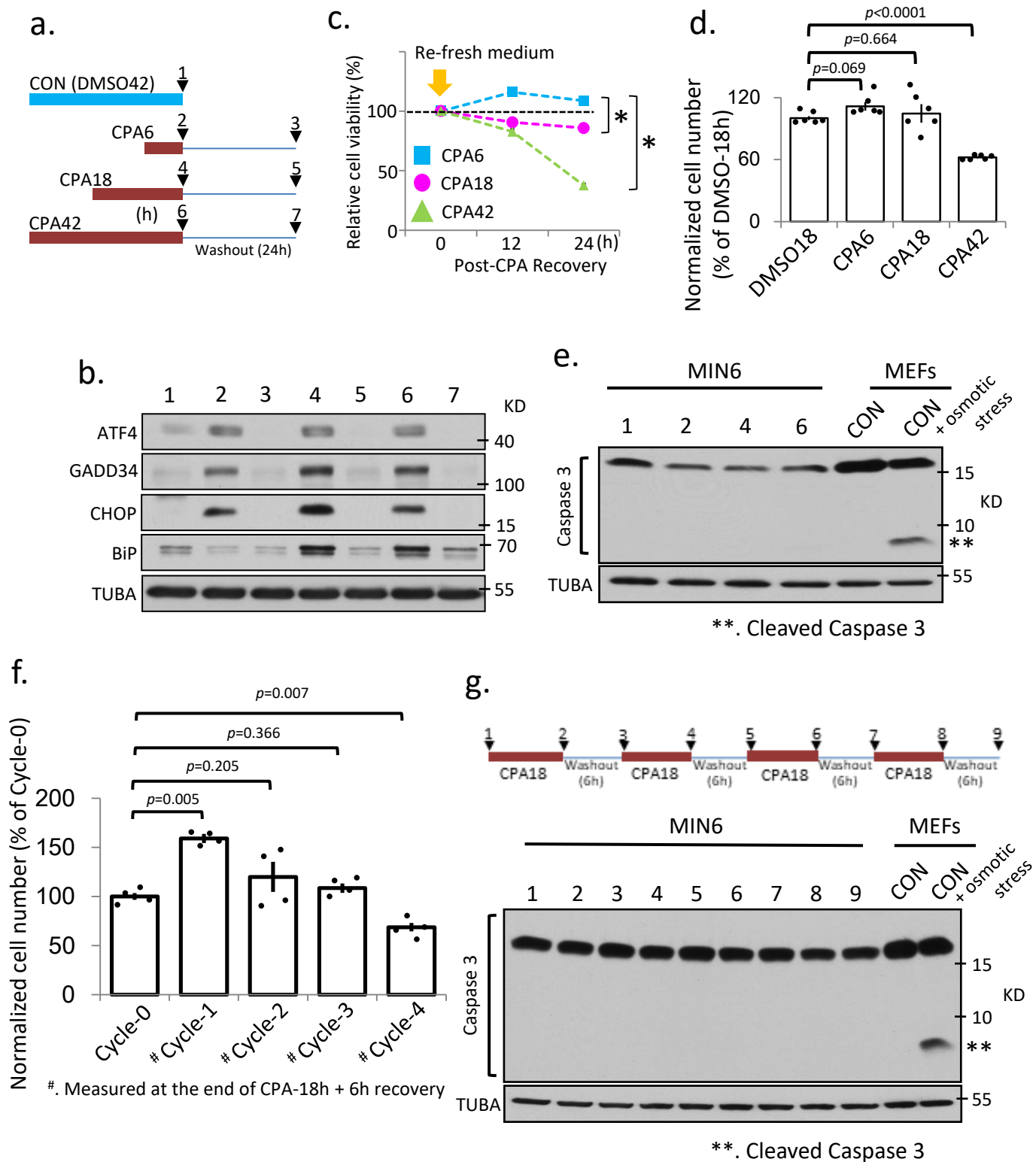| PancDB No.	Age	Sex	T1D duration	α cell (%)	β cell (%)	δ cell (%)	Pp cell (%)	Acinar cell (%)	Duct cell (%)	Others (%)
HPAP019	24	M	ND	874 (21.51)	1278 (31.45)	26 (0.64)	822 (20.23)	613 (15.08)	249 (6.13)	202 (4.97)
HPAP022	37	F	ND	511 (13.74)	1069 (28.75)	70 (1.88)	73 (1.96)	380 (10.22)	1097 (29.51)	518 (13.93)
HPAP024	18	M	ND	126 (6.78)	1106 (59.59)	278 (14.98)	50 (2.69)	165 (8.89)	24 (1.29)	107 (5.77)
HPAP026	24	M	ND	200 (35.53)	94 (16.7)	60 (10.66)	37 (6.57)	66 (11.72)	49 (8.7)	57 (10.12)
HPAP029	23	M	ND	440 (19.51)	423 (18.76)	352 (15.61)	75 (3.33)	192 (8.51)	406 (18)	367 (16.27)
HPAP020	14	M	5 days	4319 (49.98)	1055 (12.21)	116 (1.34)	62 (0.72)	2229 (25.8)	629 (7.28)	231 (2.67)
HPAP021	13	F	7 years	1526 (32.18)	812 (17.12)	44 (0.93)	0 (0)	1780 (37.54)	509 (10.73)	71 (1.5)
HPAP023	17	F	7 years	300 (34.92)	86 (10.01)	10 (1.16)	1 (0.12)	215 (25.03)	171 (19.91)	76 (8.85)
HPAP028	4	M	3 years	861 (20.59)	1407 (33.64)	394 (9.42)	31 (0.74)	365 (8.73)	735 (17.58)	389 (9.3)
HPAP032	10	F	3 years	538 (22.63)	942 (39.67)	468 (19.69)	0 (0)	217 (9.13)	47 (1.98)	165 (6.94)
HPAP055	24	M	7 years	58 (3.25)	77 (4.32)	793 (44.5)	2 (0.11)	77 (4.32)	569 (31.93)	206 (11.56)

c.

Supplementary Figure 5. β-cell ER stress 334 gene set expression in T1D/T2D datasets. **a** Relative expression of genes in T1D (*upper*), and T2D (*lower*), islet microarray datasets. Genes showing higher decrease in expression (magenta) ($\log_2 < 1$, T1D or T2D vs CON) or moderate decrease in expression (green) ($0.5 < \log_2 \leqq 1$, T1D or T2D vs CON) are highlighted. **b** Description of healthy donors and T1D patients who donated islets for the scRNA-seq analysis. **c** violin-plots of genes in β-cells scRNA-seq datasets. *p*-value represents the statistical test by two-sided Wilcoxon–Mann–Whitney test.

Supplementary Figure 6.
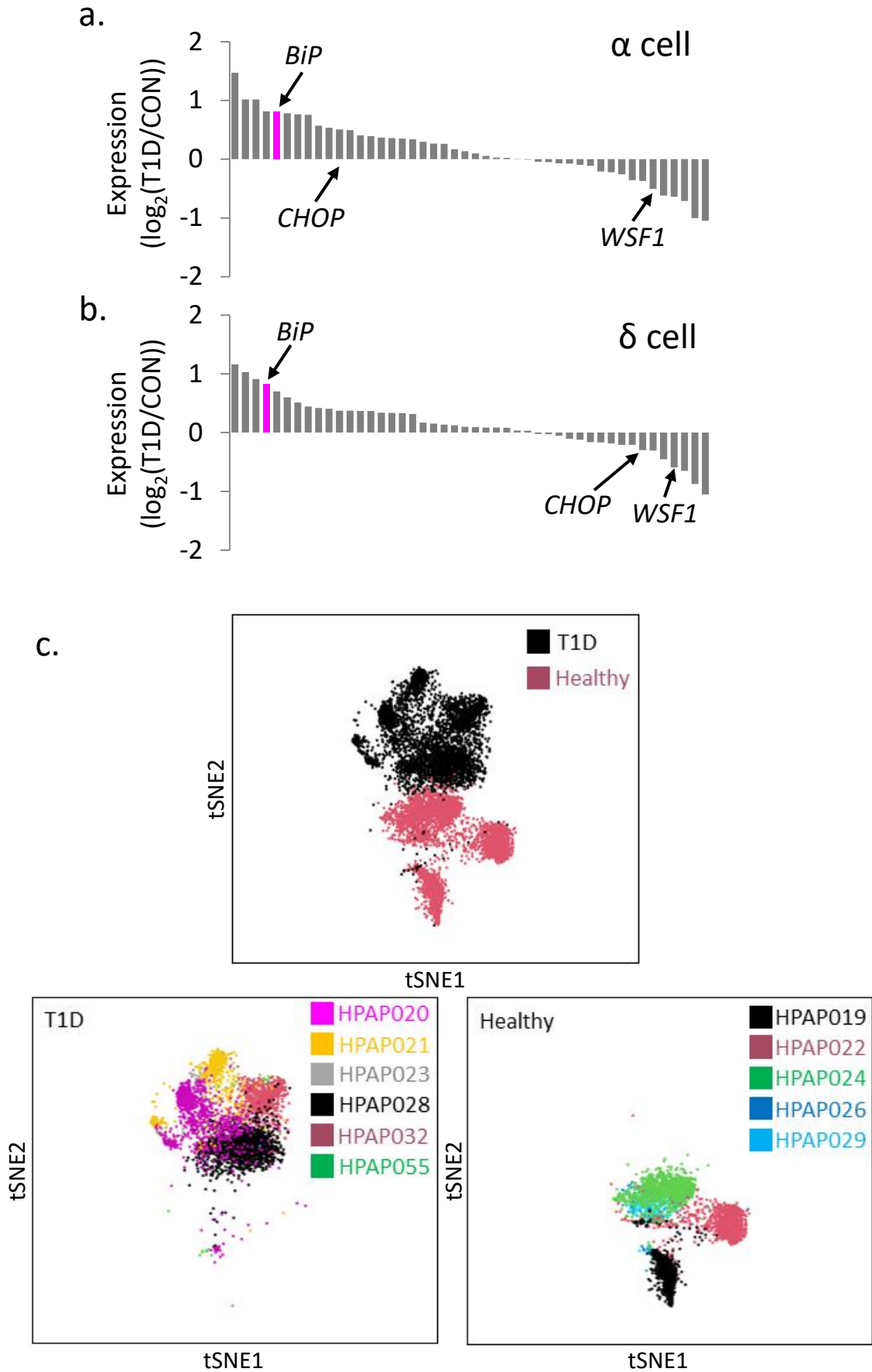
Supplementary Figure 6. Differential gene expression and clustering of scRNA-seq data from healthy and T1D subjects. **a**, **b** Schematic representation of genes as in Fig. 7a (right), in scRNA-seq from α-cells (**a**) and δ-cells (**b**) from healthy and T1D patients. **c** Similar analysis to Fig. 7b, with color-coded individuals from Sup. Fig. 5b. Data shown in Fig. 7b were obtained from scRNA-se data of 11 subjects. In Sup. Fig. 6c each subject is shown with a different color.

Supplementary Figure 7.

a.

CON (DMSO42) 1

CPA6 2 — 3

CPA18 4 — 5

(h)

CPA42 6 — 7

Washout (24h)

c.

Re-fresh medium

Relative cell viability (%)

- CPA6 (blue square)
- CPA18 (magenta circle)
- CPA42 (green triangle)

Post-CPA Recovery

d.

Normalized cell number (% of DMSO-18h)

p<0.0001
p=0.664
p=0.069

DMSO18 CPA6 CPA18 CPA42

b.

	1	2	3	4	5	6	7	KD
ATF4								40
GADD34								100
CHOP								15
BiP								70
TUBA								55

e.

MIN6 — MEFs

1 2 4 6 CON CON + osmotic stress

Caspase 3

15
KD
10
**
TUBA — 55

**. Cleaved Caspase 3

f.

Normalized cell number (% of Cycle-0)

p=0.007
p=0.366
p=0.205
p=0.005

Cycle-0 #Cycle-1 #Cycle-2 #Cycle-3 #Cycle-4

#. Measured at the end of CPA-18h + 6h recovery

g.

1 CPA18 2 Washout (6h) 3 CPA18 4 Washout (6h) 5 CPA18 6 Washout (6h) 7 CPA18 8 Washout (6h) 9

MIN6 — MEFs

1 2 3 4 5 6 7 8 9 CON CON + osmotic stress

Caspase 3

15
KD
10
**
TUBA — 55

**. Cleaved Caspase 3

Supplementary Figure 7. MIN6 cells can recover even after prolonged ER stress exposure. **a** Experimental setup. **b**, **e**, **g** Western blot analysis for the indicated proteins and treatment of MIN6 cells as shown in **a** (**b**, **e**) or in the schematic of **g**. **c** cell viability during recovery from short (6h), chronic (18h) and prolonged (42h), ER stress exposure. **d, f** Evaluation of the number of MIN6 cells remaining attached on the culture plates following the indicated treatment with CPA. *n*=6/4 in d/f, respectively. Asterisks (**) indicate cleaved Caspase 3. As a positive control for Caspase 3 cleavage, we used cell extracts from MEFs treated with hypertonic stress 600 mOsm (Farabaugh *et al.*, 2020). Error bars represent S.E.M. **p*<0.05. *p*-value represents the statistical test by two-tailed Student's *t*-test. Dots in all plots represent independent experiments. Source data are provided as a Source Data file.

Supplementary Figure 8.

a.

b.

c.

PA: Palmitic acid

OA: Oleic acid
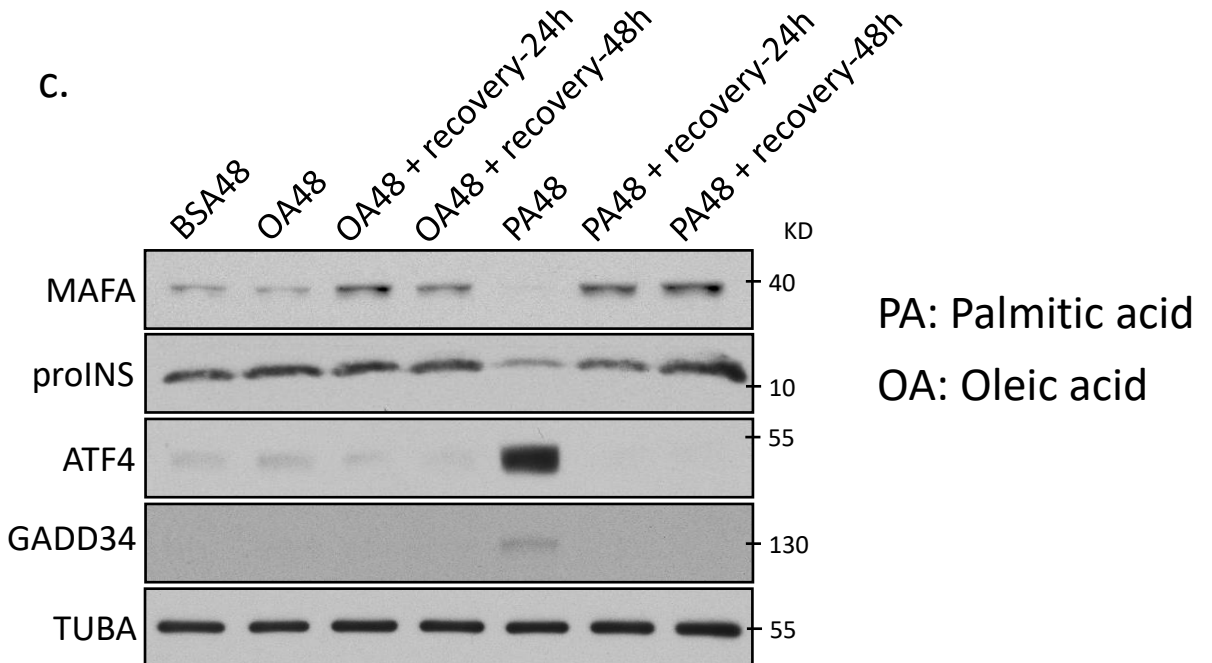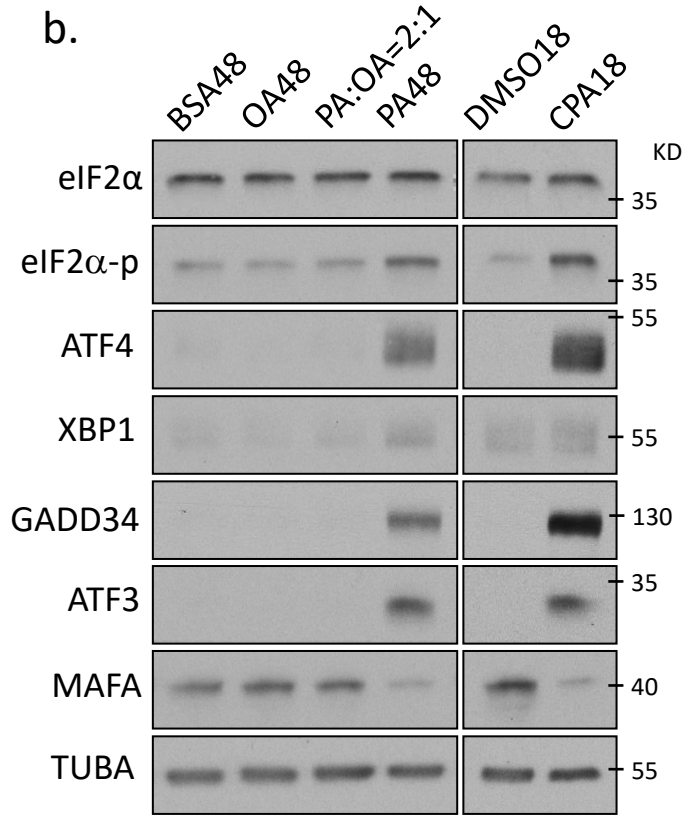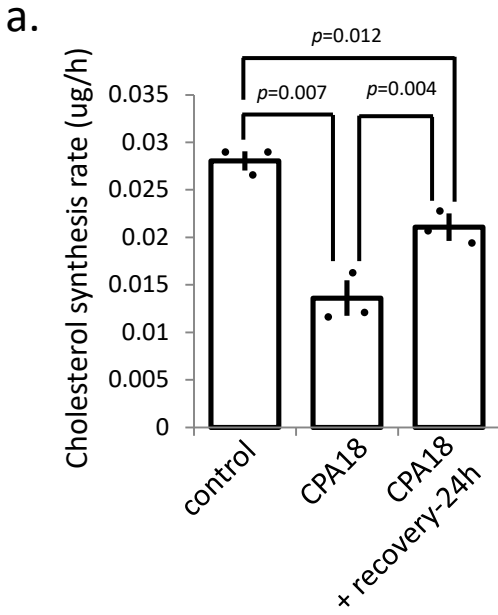
Supplementary Figure 8. Cholesterol biosynthesis rates in MIN6 cells treated with CPA and reversible UPR gene expression in palmitic acid-treated MIN6 cells. a Cholesterol biosynthesis was measure by metabolic labeling of the cells with deuterium and analysis of labelled cholesterol by Gas Chromatography-Tandem Mass Spectrometry. *n*=3 independent experiments. Dots in all plots represent independent experiments. Source data are provided as a Source Data file. Error bars represent S.E.M. *p*-value represents the statistical test by two-tailed paired Students' *t*-test. Treatments with CPA are indicated. **b**, **c** Western blot analysis for the indicated proteins in MIN6 cells treated with oleic acid (400 mM), palmitic acid (400 mM) or a 2:1 ration of palmitic acid:oleic acid (400 mM) for 48 h, or washout after 48h treatment with palmitic acid. Analysis of extracts isolated from CPA-treated MIN6 cells is also shown.

Supplementary Figure 9.

a.

| MAFA Nucleus | MAFA | Nucleus |

CON

CPA18

CPA18 + recovery-24h

b.

Relative intensity (MAFA/Nucleus)

CON   CPA18   CPA18 + recovery-24h

c.

Insulin in medium (Normalized to 2.8mM)

Glucose conc.
■ 2.8 mM □ 16.8 mM

p=0.029

p=0.243

p<0.0001

p<0.0001

CON   CPA-18h   CPA18 + recovery-72h   CPA18 + recovery-96h
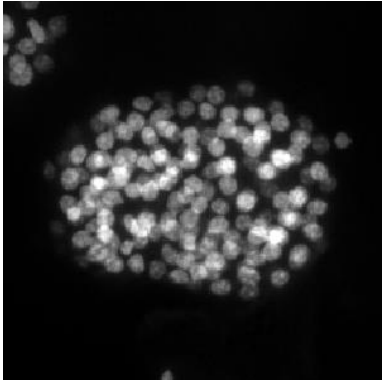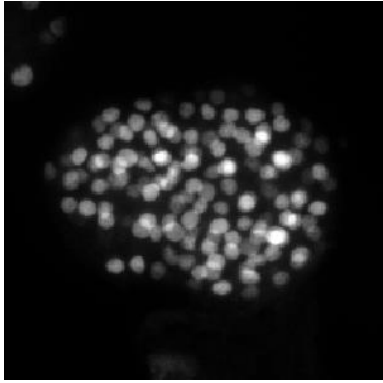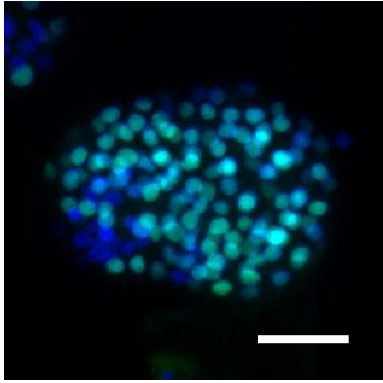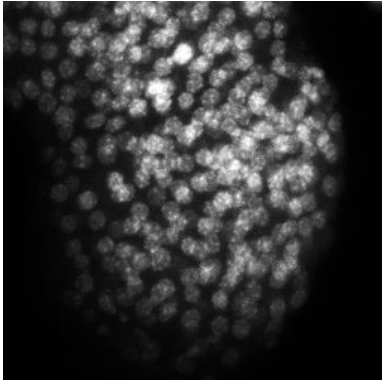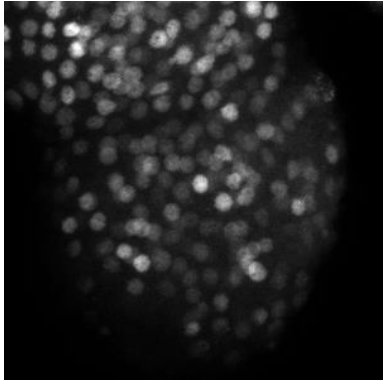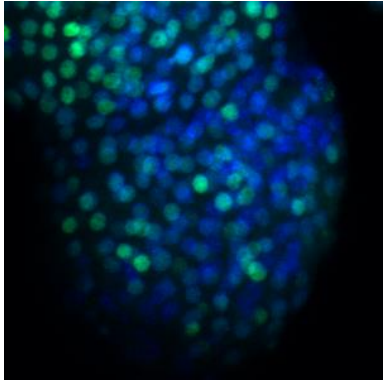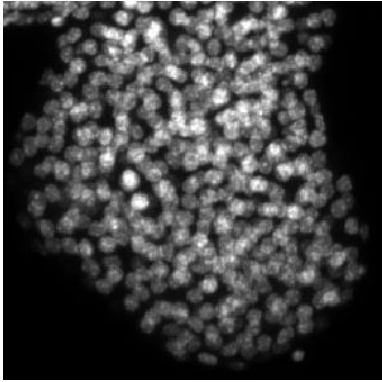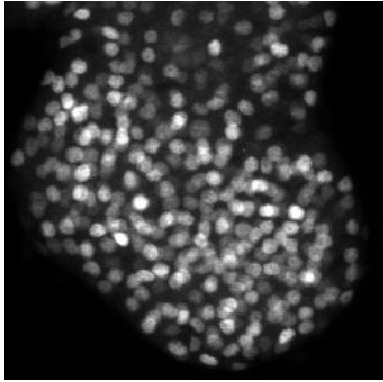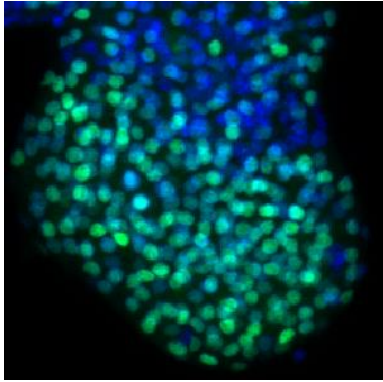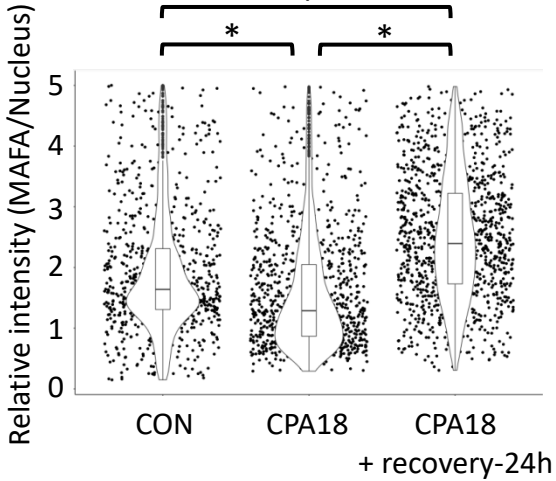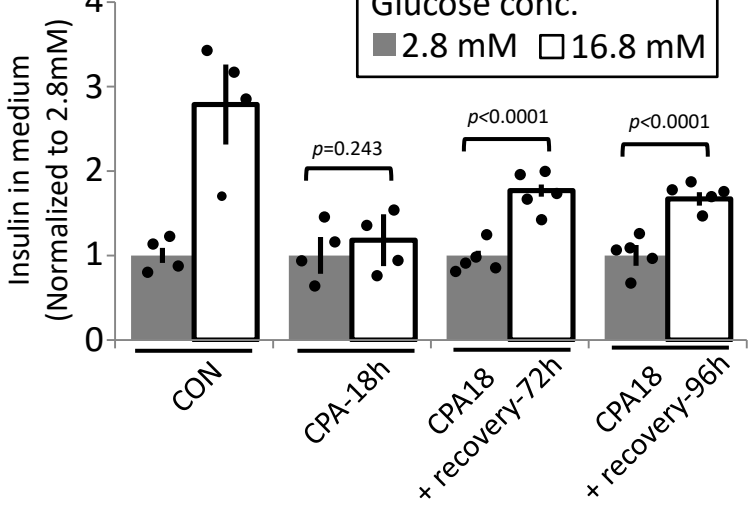
Supplementary Figure 9. Reversible CPA-induced inhibition of MAFA and GSIS in mouse islets. a Fluorescence immunocytochemistry for MAFA of mouse islets treated with CPA (200uM) for 18h and a 24h washout after 18h CPA-treatment. Scale bar, 50μm. Representative images are shown, **b** Quantification of the relative fluorescent intensity of CON (n=810), CPA18 (n=1890) and CPA18 with a 24h washout of CPA (n=808). *p<0.0001, two-sided Wilcoxon–Mann–Whitney test. The boxes indicate the 25–75th percentiles, the midline indicates the median, whiskers show the maximum/minimum values. **c** Normalized to each condition of treatment evaluation of GSIS in mouse islets treated with CPA for 18h (n=4 mice), or CPA-treatment for 18h followed by washout for 72h (n=5 mice) or 96h (n=5 mice). Error bars represent S.E.M. p-value represents the statistical test by two-tailed Student's t-test. Source data are provided as a Source Data file.