Supplementary information

Modeling and therapeutic targeting of inflammation-induced hepatic insulin resistance using human iPSC-derived hepatocytes and macrophages

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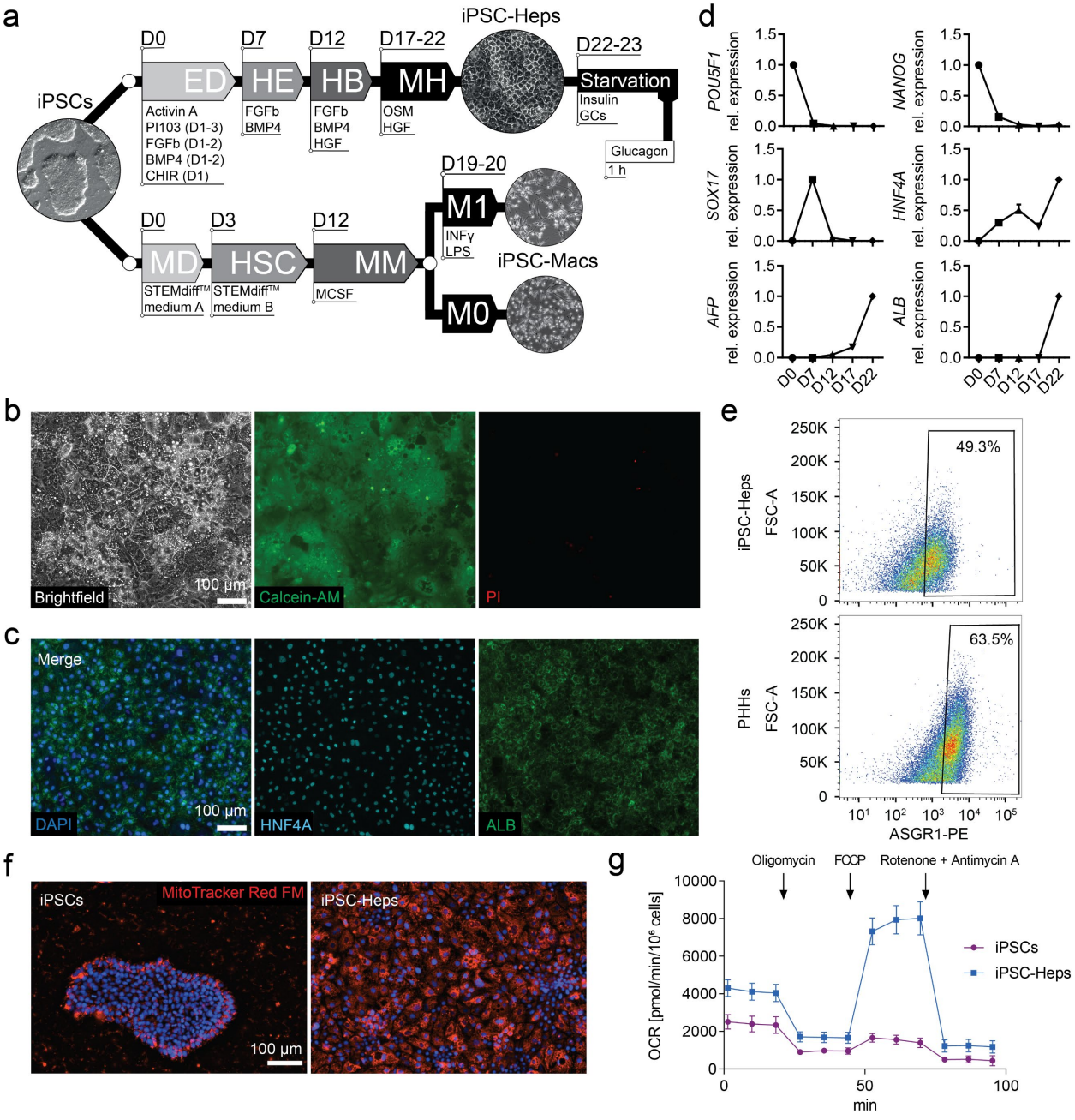
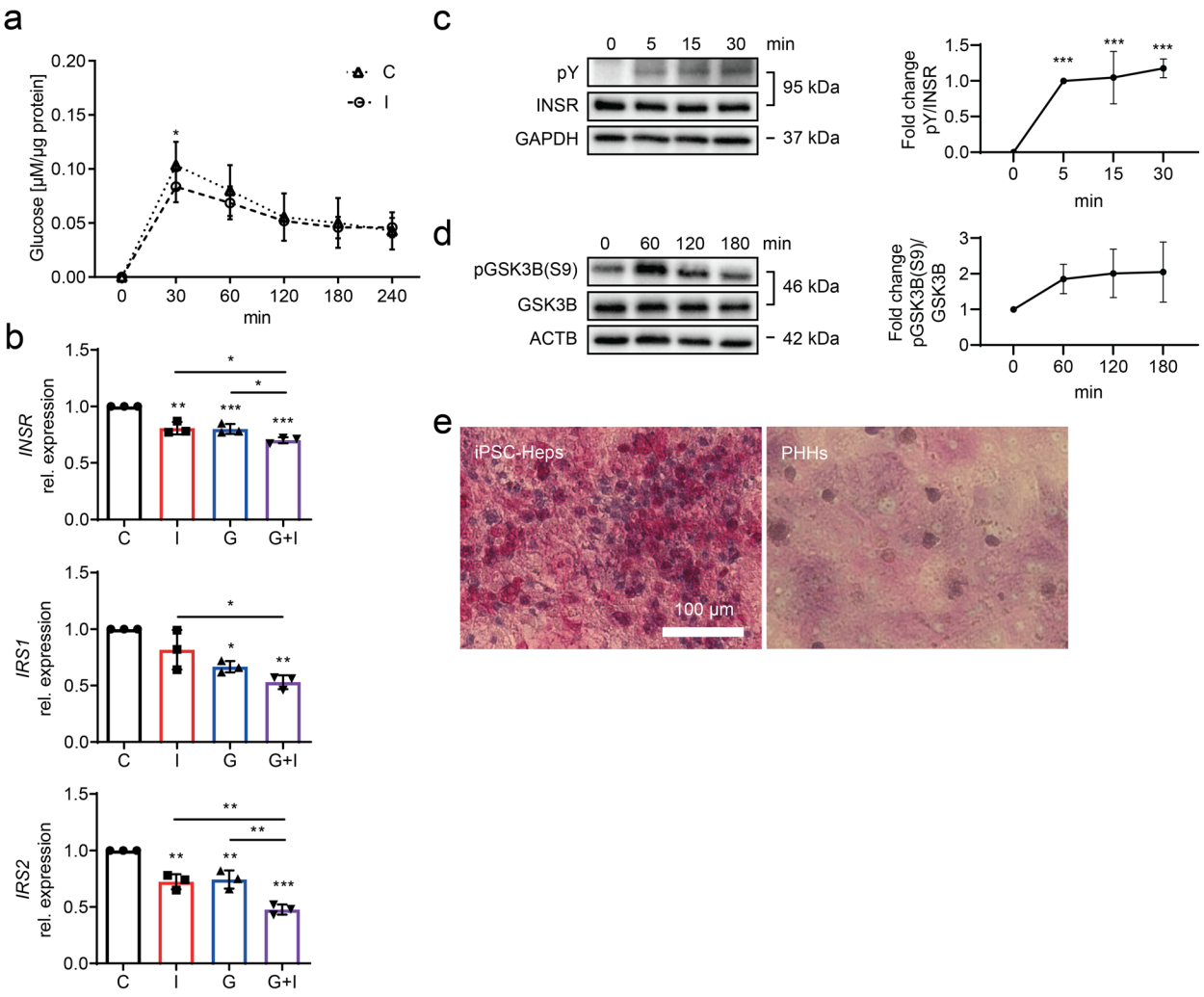
1. Included in this file: Supplementary Figs. 1-8, Supplementary Tables 1-4

2. Additional information: Supplementary Data 1

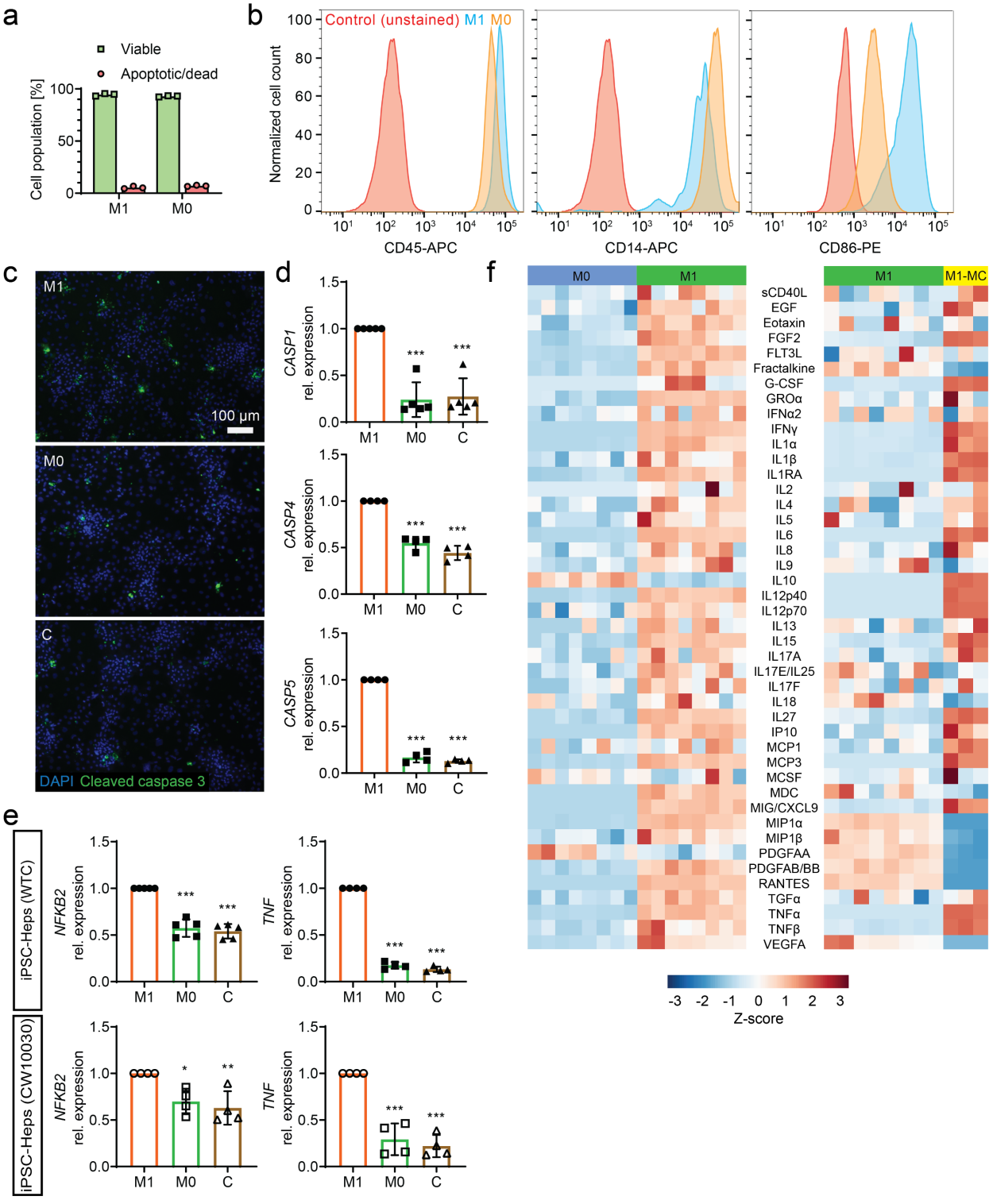


Supplementary Fig. 1 | Generation of iPSC-Heps and iPSC-Macs. a Overview of protocol for generation of iPSC-Heps and iPSC-Macs highlighting developmental stages and differentiation-promoting factors. D, day; ED, endoderm; HE, hepatic endoderm; HB, hepatoblast; MH, mature hepatocyte; MD, mesoderm; HSC, hematopoietic stem cell; MM, mature macrophage; GCs, glucocorticoids. **b** Representative live imaging of iPSC-Heps stained with the viability markers Calcein-AM (viable, green) and propidium iodide (PI; dead, red) at the end of the differentiation protocol. n = 3. **c** Representative immunofluorescence of HNF4A and albumin (ALB) in iPSC-Heps. n = 3. **d** Time course of gene expression analysis of pluripotency (*POU5F1*, *NANOG*), endoderm (*SOX17*) and hepatocyte (*HNF4A*, *AFP*, *ALB*) markers in iPSC-Heps. Data are mean ± SD; n = 3. **e** Representative flow cytometry analysis of the hepatocyte differentiation marker ASGR1 in iPSC-Heps and freshly thawed PHHs. iPSC-Heps: n = 3, PHHs: n = 1. **f** Representative live imaging of mitochondria (MitoTracker Red FM) in iPSCs and iPSC-Heps. n

= 3. **g** Mitochondrial respiration (oxygen consumption rate, OCR) in iPSCs and iPSC-Heps. Data are mean ± SD; iPSCs: n = 4, iPSC-Heps: n = 10. Source data are provided as a Source Data file.

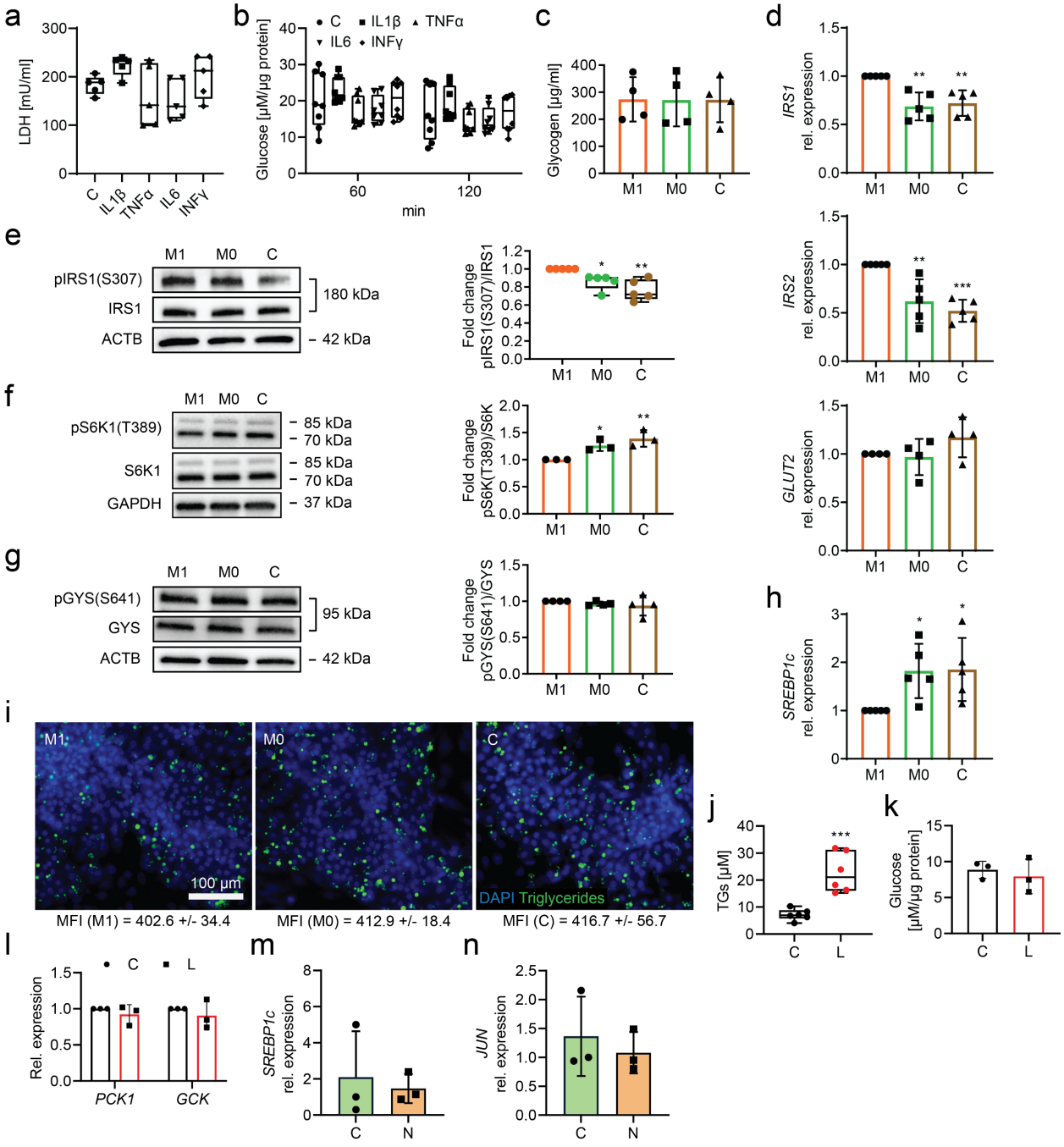


Supplementary Fig. 2 | Insulin and glucagon effects on glucose metabolism in iPSC-Heps. a Time course of analysis of glucose release by iPSC-Heps into glucose-free media after insulin (I) and no insulin (C, control). Data are mean ± SD; n = 6, two-way ANOVA (two-stage step-up method of Benjamini, Krieger and Yekutieli), **P* < 0.05 vs. I at indicated timepoints. **b** Gene expression analysis in iPSC-Heps after 4 h of no hormones (C, control), insulin (I), glucagon (G) or 2 h of glucagon followed by 2 h of insulin (G+I). Data are mean ± SD; n = 3, one-way ANOVA (Tukey's test), **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 vs. C or between indicated conditions. **c** Time course of western blot analysis of INSR autophosphorylation in iPSC-Heps after insulin. Data are mean ± SD; n = 3, one-way ANOVA (Dunnett's test), ****P* < 0.001 vs. 0 min. **d** Time course of western blot analysis of GSK3B phosphorylation in iPSC-Heps after insulin. Data are mean ± SD; n = 3, one-way ANOVA (Dunnett's test). **e** Representative Periodic Acid Schiff staining of iPSC-Heps at the end of the differentiation protocol and PHHs 48 h after plating (glycogen is red/pink, nuclei are purple/unstained). n = 3. Source data are provided as a Source Data file.



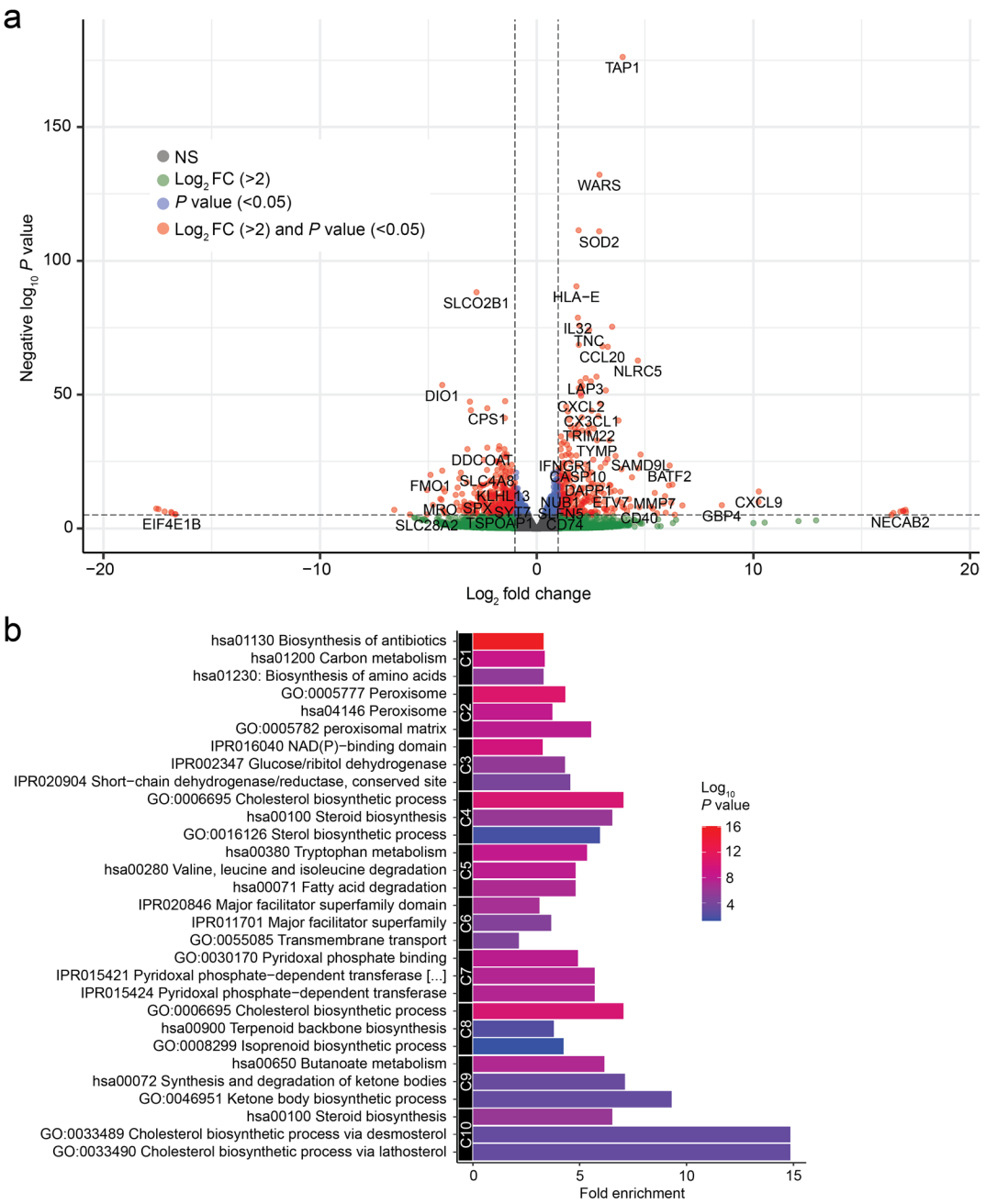
Supplementary Fig. 3 | Inflammation of iPSC-Heps co-cultured with M1 or M0 iPSC-Macs. **a, b** Flow cytometry analysis of viability (**a**) and hematopoietic (CD45) and monocytic (CD14) differentiation and macrophage polarization (CD86) markers (**b**) in M1 or M0 iPSC-Macs. Data are mean ± SD; n = 3. **c-f** Representative immunofluorescence of cleaved caspase 3 particles in iPSC-Heps (**c**), caspase gene expression analysis in iPSC-Heps (*CASP1*: n = 5, *CASP4* and

CASP5: n = 4) (**d**), inflammatory gene expression analysis in WTC and CW10030 iPSC-Heps (WTC *NFKB2*: n = 5, WTC *TNF* and CW10030 *NFKB2* and *TNF*: n = 4) (**e**) and comparative analysis of cytokine and chemokine release into media (M0 and M1: n = 8, M1-MC: n = 3) (**f**) after 24-h co-culture of iPSC-Heps with M1 or M0 iPSC-Macs, in iPSC-Hep mono-culture (C, control) or in M1 iPSC-Mac monoculture (M1-MC). Data are mean ± SD; one-way ANOVA (Dunnett's test), **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. M1. Source data are provided as a Source Data file.

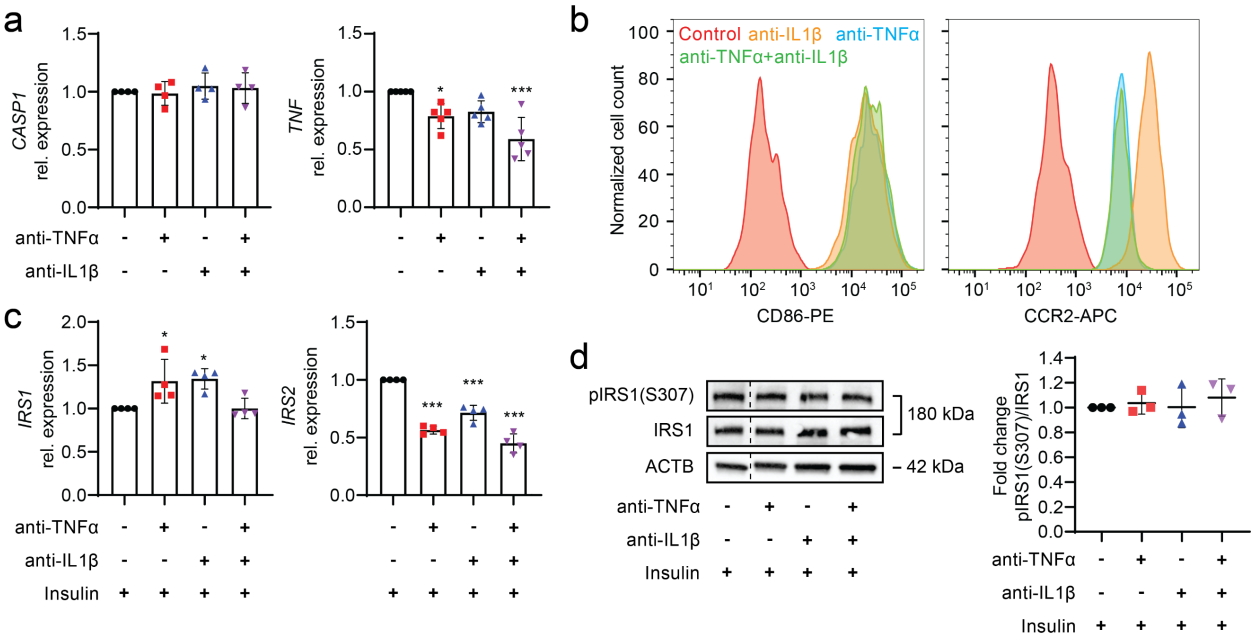


Supplementary Fig. 4 | Glucose metabolism changes in iPSC-Heps co-cultured with M1 or M0 iPSC-Macs or accumulating lipid. a Quantification of LDH release into media by iPSC-Heps treated with the indicated cytokines for 24 h. Data are mean ± SD; n = 5. **b** Quantification of glucose release into 1 mM glucose-containing media 2 h after insulin by iPSC-Heps treated with the indicated cytokines for 24 h. Data are mean ± SD; n = 8. **c** Quantification of glycogen in iPSC-Heps after 24-h co-culture of iPSC-Heps with M1 or M0 iPSC-Macs or in iPSC-Hep mono-culture (C, control). Data are mean ± SD; n = 4, one-way ANOVA (Dunnett's test). **d-g** Gene expression analysis in iPSC-Heps 1 h after insulin (*IRS1* and *IRS2*: n = 5, *GLUT2*: n = 4) (**d**), western blot analysis in iPSC-Heps of IRS1 phosphorylation 5 min after insulin (n = 5) (**e**), western blot analysis in iPSC-Heps of S6K1 phosphorylation 30 min after insulin (n = 3) (**f**) and

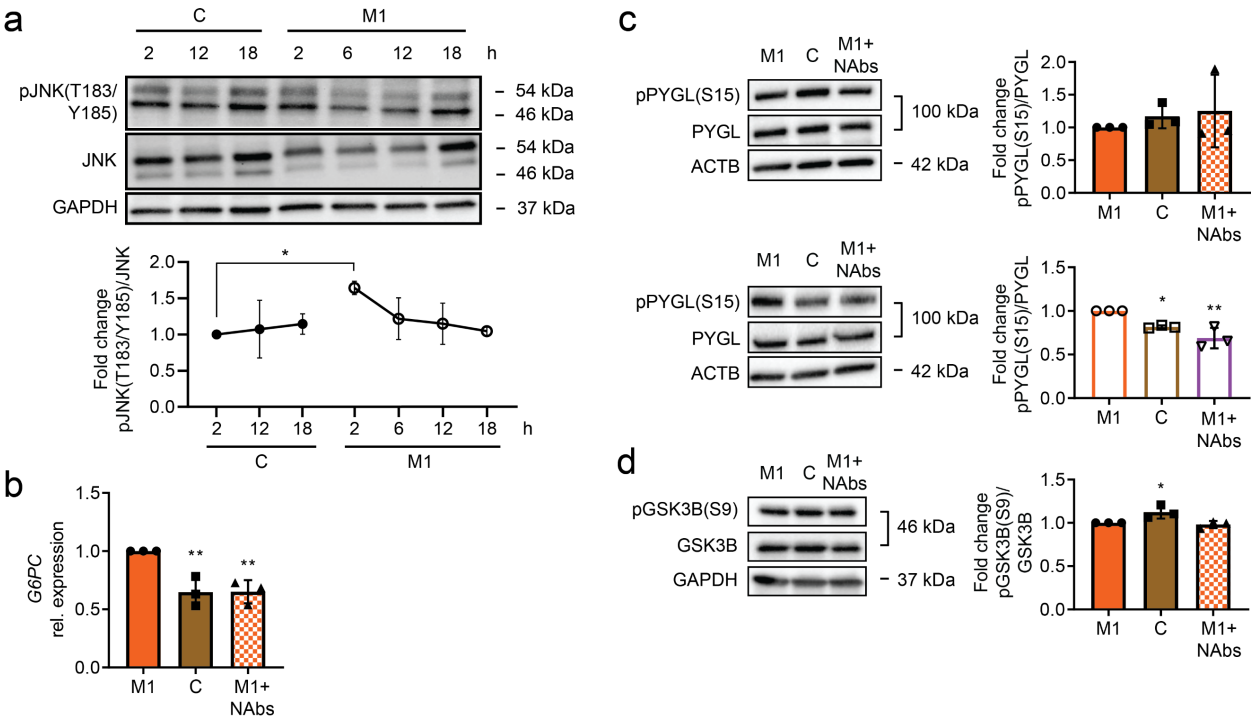
western blot analysis in iPSC-Heps of GYS phosphorylation 2 h after insulin (n = 4) (**g**) after 24-h co-culture of iPSC-Heps with M1 or M0 iPSC-Macs or in iPSC-Hep mono-culture (C, control). Data are mean ± SD; one-way ANOVA (Dunnett's test), **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 vs. M1. **h** Gene expression analysis in iPSC-Heps 1 h after insulin after 24-h co-culture of iPSC-Heps with M1 or M0 iPSC-Macs or in iPSC-Hep mono-culture (C, control). Data are mean ± SD; n = 5, one-way ANOVA (Dunnett's test), **P* < 0.05 vs. M1. **i** Representative triglyceride staining in iPSC-Heps after 24-h co-culture of iPSC-Heps with M1 or M0 iPSC-Macs or in iPSC-Hep mono-culture (C, control). MFI values are mean ± SD; n = 3. MFI, mean fluorescence intensity of BODIPY 493/503. **j-l** Quantification of triglycerides (TGs) (n = 6) (**j**), quantification of glucose release into 1 mM glucose-containing media 2 h after insulin (n = 3) (**k**) and gene expression analysis 1 h after insulin (n = 3) (**l**) in iPSC-Heps after mono-culture without (C, control) or with 100 μM oleate and 100 μM palmitate (L, lipids) for 6 days. Data are mean ± SD; unpaired two-tailed Student's t test, ****P* < 0.001 vs. C. **m, n** Gene expression analysis in iPSC-Heps 1 h after insulin (n = 3) (**m**) and at the end of the differentiation protocol (n = 3) (**n**) comparing iPSC-Heps from 3 healthy (C, control) and 3 NASH (N) patients. Data are mean ± SD; unpaired two-tailed Student's t test. Source data are provided as a Source Data file.



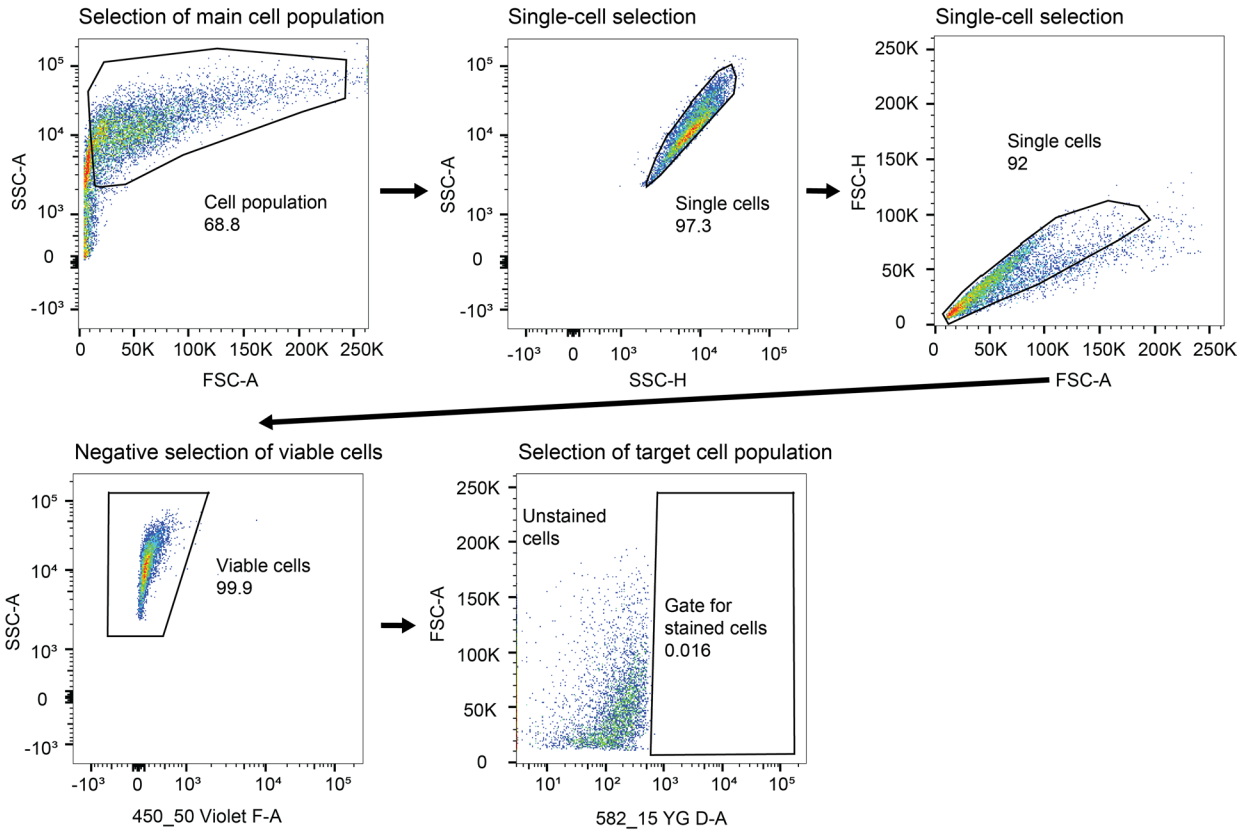
Supplementary Fig. 5 | RNAseq analysis of iPSC-Heps co-cultured with M1 or M0 iPSC-Macs. a Volcano plot of all differentially expressed genes with log2 fold change > 2, *P* value < 0.05 or both in iPSC Heps co-cultured with M1 or M0 iPSC-Macs. Horizontal line indicates *P* value < 0.05 and vertical lines mark log2 fold change of 2.0 and -2.0. n = 3, FDR-adjusted *P* value (*P* < 0.05) by Wald test. FC, fold change; NS, not significant. **b** Top 10 down-regulated pathway clusters enriched (Cluster Enrichment Score > 1.3) in the genes differentially expressed between iPSC Heps co-cultured with M1 or M0 iPSC-Macs identified using DAVID. Vertical axis represents enrichment fold values and horizontal axis shows the names of GO-BP, GO-MF and GO-CC terms and KEGG pathways. Node color indicates the enrichment significance, red represents higher significance. n = 3, FDR-adjusted *P* value (*P* < 0.05) by Wald test.



Supplementary Fig. 6 | Effect of TNFα and/or IL1β neutralization on inflammation and glucose metabolism changes in iPSC-Heps co-cultured with M1 iPSC-Macs. a Gene expression analysis in iPSC-Heps after 24-h co-culture with M1 iPSC-Macs and indicated antibody treatments. Data are mean ± SD; *CASP1*: n = 4, *TNF*: n = 5, one-way ANOVA (Dunnett's test), **P* < 0.05 and ****P* < 0.001 vs. no-antibody condition. **b** Flow cytometry analysis of CD86 and CCR2 in M1 iPSC-Macs after 24-h co-culture with iPSC-Heps and indicated antibody treatments. n = 3. **c**, **d** Gene expression analysis 1 h after insulin (n = 4) (**c**) and western blot analysis of IRS1 phosphorylation 5 min after insulin (n = 3) (**d**) in iPSC-Heps after 24-h co-culture with M1 iPSC-Macs and indicated antibody treatments. Data are mean ± SD; one-way ANOVA (Dunnett's test), **P* < 0.05 and ****P* < 0.001 vs. no-antibody condition. Source data are provided as a Source Data file.



Supplementary Fig. 7 | Effect of TNFα and/or IL1β neutralization on inflammation and glucose metabolism changes in PHHs co-cultured with M1 PHMs. a Time course of western blot analysis of JNK phosphorylation in PHHs after co-culture with M1 PMHs or in PHH mono-culture (C, control). Data are mean ± SD; n = 3, one-way ANOVA (Tukey's test), **P* < 0.05. **b** Gene expression analysis 1 h after insulin after 24-h co-culture of PHHs with M1 or M0 PHMs or in PHH mono-culture (C, control). Data are mean ± SD; n = 3, one-way ANOVA (Dunnett's test), ***P* < 0.01 vs. M1. **c** Western blot analysis of PYGL phosphorylation 30 min after insulin in hepatocytes after 24-h co-culture with M1 macrophages or in hepatocyte mono-culture (C, control) or after 24-h co-culture with M1 macrophages including TNFα and IL1β neutralizing antibodies (M1+NAbs). Primary cells, filled bars/symbols; CW10030 iPSC-derived cells, open bars/symbols. Data are mean ± SD; n = 3, one-way ANOVA (Dunnett's test), **P* < 0.05 and ***P* < 0.01 vs. M1. **d** Western blot analysis of GSK3B phosphorylation 30 min after insulin in PHHs after 24-h co-culture with M1 PMHs or in PHH mono-culture (C, control) or after 24-h co-culture with M1 PHMs including TNFα and IL1β neutralizing antibodies (M1+NAbs). Data are mean ± SD; n = 3, one-way ANOVA (Dunnett's test), **P* < 0.05. Source data are provided as a source Data File.



Supplementary Fig. 8 | Gating strategy for flow cytometry analysis. The main cell population was selected based on pulse area (-A) of forward scatter (FSC) and side scatter (SSC). Pulse height (-H) of FSC and SSC was used to select single cells from the main cell population. SYTOX green/red cell death staining was used to select viable cells. Gates for target cells expressing specific markers detected with fluorophore-conjugated antibodies were defined based on unstained cells. 450_50 Violet F-A and 582_15 YG D-A are flow cytometer-specific laser and filter set combinations for fluorophore detection.

Supplementary Table 1 | **Primers for qRT-PCR.**

Gene	Direction	Sequence
CASP1	Forward	CTCAGGCTCAGAAGGGAATG
	Reverse	ACGCTGTACCCCAGATTTTG
CASP4	Forward	CAAGAGAAGCAACGTATGGCA
	Reverse	AGGCAGATGGTCAAACTCTGTA
CASP5	Forward	TTCAACACCACATAACGTGTCC
	Reverse	GTCAAGGTTGCTCGTTCTATGG
G6PC	Forward	ACTGGCTCAACCTCGTCTTTA
	Reverse	CGGAAGTGTTGCTGTAGTAGTCA
GCK	Forward	CCGCAAGCAGATCTACAACA
	Reverse	AGCTTGTACACGGAGCCATC
GLUT2	Forward	TTGGTGTGATCAATGCACCT
	Reverse	GCCACAGTCTCTTCCTCAGC
INSR	Forward	CATCCGGGGATCACGACTG
	Reverse	ATCAGGTTGTAGAGGCCGAGT
IRS1	Forward	CAAGACCATCAGCTTCGTGA
	Reverse	AGAGTCATCCACCTGCATCC
IRS2	Forward	CGGTGAGTTCTACGGGTACAT
	Reverse	TCAGGGTGTATTCATCCAGCG
NFKB2	Forward	ATGGAGAGTTGCTACAACCCA
	Reverse	CTGTTCCACGATCACCAGGTA
PCK1	Forward	TCATTTAAGGGCCATCAACC
	Reverse	CTCATCAATGCCTTCCCAGT
RPLP0	Forward	GCAGATCCGCATGTCCCTT
	Reverse	TGTTTTCCAGGTGCCCTCG
SREBP1c	Forward	CCATGGATTGCACTTTCGAA
	Reverse	GGCCAGGGAAGTCACTGTCTT
TNF	Forward	CCTCTCTCTAATCAGCCCTCTG
	Reverse	GAGGACCTGGGAGTAGATGAG

Supplementary Table 2 | **Antibodies.**

Antibody	Company	Catalogue #	Dilution
Immunofluorescence			
Goat anti-Albumin	Bethyl Lab.	A80-229A	1:500
Rabbit anti-Cleaved Caspase 3	Cell Signaling	9661s	1:400
Mouse anti-HNF4A	Abcam	ab41898	1:1,000
Donkey anti-Goat IgG (H+L) Secondary Antibody, Alexa Fluor 647	Life Technologies	A-21447	1:500
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 555	Life Technologies	A-31570	1:500
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488	Life Technologies	A-21206	1:500
Flow cytometry			
Mouse anti-ASGR1-PE	SinoBiological	10773-MM02-P	5 μl/test
Mouse anti-CD14-APC	BD Biosciences	561383	5 μl/test
Mouse anti-CD45-APC	BD Biosciences	340943	5 μl/test
Mouse anti-CD86-PE	Biolegend	374205	5 μl/test
Mouse anti-CD192(CCR2)-APC	Biolegend	357207	5 μl/test
Western blotting			
Rabbit anti-AKT	Cell Signaling	9272s	1:1,000
Rabbit anti-Phospho-AKT (Ser473)	Cell Signaling	4060s	1:2,000
Rabbit anti-Phospho-AKT (Thr308)	Cell Signaling	4056s	1:1,000
Rabbit anti-β-Actin (ACTB)	Cell Signaling	4970S	1:5,000
Mouse anti-GAPDH	Santa Cruz Biotechnology	sc-47724	1:2,000
Rabbit anti-Glycogen Synthase	Cell Signaling	3886S	1:1,000
Rabbit anti-Phospho-Glycogen Synthase (Ser641)	Cell Signaling	47043T	1:1,000
Rabbit anti-GSK3B	Cell Signaling	12456T	1:1,000
Rabbit anti-Phospho-GSK3B (S9)	Cell Signaling	5558T	1:1,000
Rabbit anti-Insulin receptor (IR) β	Cell Signaling	23413t	1:1,000
Mouse anti-Phospho-Y	Cell Signaling	96215S	1:1,000
Rabbit anti-IRS-1	Cell Signaling	2382S	1:1,000
Rabbit anti-Phospho-IRS-1 (Ser307)	Cell Signaling	2381T	1:1,000
Rabbit anti-JNK	Cell Signaling	9252s	1:2,000
Rabbit anti-Phospho-JNK (T183/Y185)	Cell Signaling	4668t	1:2,000
Rabbit anti-PYGL	Proteintech	15851-1-AP	1:2,000
Rabbit anti-Phospho-PYGL (Ser15)	Abcam	ab227043	1:2,000
Rabbit anti p70 S6 Kinase (S6K)	Cell Signaling	2708S	1:1,000
Rabbit anti-Phospho-p70 S6 Kinase (S6K) (T389)	Cell Signaling	9205S	1:1,000
Peroxidase AffiniPure Goat Anti-Mouse IgG	Jackson ImmunoResearch	115-035-062	1:10,000
Peroxidase AffiniPure Goat Anti-Rabbit IgG	Jackson ImmunoResearch	111-035-144	1:10,000
Cytokine neutralization			
Infliximab	Selleckchem	A2019	5 μg/ml
Human IL-1 beta/IL-1F2 Antibody	R&D Systems	MAB201-SP	0.2 μg/ml

Supplementary Table 3 | R software information and packages.

Package	Version	Software
annotate	1.68.0	Bioconductor
AnnotationDbi	1.52.0	Bioconductor
AnnotationForge	1.32.0	Bioconductor
ash	1.0-15	CRAN (R 4.0.2)
askpass	1.1	CRAN (R 4.0.2)
assertthat	0.2.1	CRAN (R 4.0.2)
beeswarm	0.4.0	CRAN (R 4.0.2)
Biobase	2.50.0	Bioconductor
BiocFileCache	1.14.0	Bioconductor
BiocGenerics	0.36.1	Bioconductor
BiocManager	1.30.16	CRAN (R 4.0.2)
BiocParallel	1.24.1	Bioconductor
biomaRt	2.46.3	Bioconductor
Biostrings	2.58.0	Bioconductor
bit	4.0.4	CRAN (R 4.0.2)
bit64	4.0.5	CRAN (R 4.0.2)
bitops	1.0-7	CRAN (R 4.0.2)
blob	1.2.2	CRAN (R 4.0.2)
brio	1.1.3	CRAN (R 4.0.2)
cachem	1.0.6	CRAN (R 4.0.2)
calibrate	1.7.7	CRAN (R 4.0.2)
callr	3.7.0	CRAN (R 4.0.2)
Category	2.56.0	Bioconductor
caTools	1.18.2	CRAN (R 4.0.2)
cli	3.2.0	CRAN (R 4.0.2)
cluster	2.1.2	CRAN (R 4.0.2)
clusterProfiler	3.18.1	Bioconductor
colorspace	2.0-3	CRAN (R 4.0.2)
corrplot	0.92	CRAN (R 4.0.2)
cowplot	1.1.1	CRAN (R 4.0.2)
crayon	1.5.0	CRAN (R 4.0.2)
curl	4.3.2	CRAN (R 4.0.2)
data.table	1.14.2	CRAN (R 4.0.2)
DBI	1.1.2	CRAN (R 4.0.2)
dbplyr	2.1.1	CRAN (R 4.0.2)
DelayedArray	0.16.3	Bioconductor
desc	1.4.1	CRAN (R 4.0.2)
DESeq2	1.30.1	Bioconductor
devtools	2.4.3	CRAN (R 4.0.2)
digest	0.6.29	CRAN (R 4.0.2)
DO.db	2.9	Bioconductor
DOSE	3.16.0	Bioconductor
downloader	0.4	CRAN (R 4.0.2)
dplyr	1.0.8	CRAN (R 4.0.2)
ellipsis	0.3.2	CRAN (R 4.0.2)
EnhancedVolcano	1.8.0	Bioconductor

enrichplot	1.10.2	Bioconductor
extrafont	0.17	CRAN (R 4.0.2)
extrafontdb	1	CRAN (R 4.0.2)
fansi	1.0.2	CRAN (R 4.0.2)
farver	2.1.0	CRAN (R 4.0.2)
fastmap	1.1.0	CRAN (R 4.0.2)
fastmatch	1.1-3	CRAN (R 4.0.2)
fgsea	1.16.0	Bioconductor
fs	1.5.2	CRAN (R 4.0.2)
gage	2.40.2	Bioconductor
gageData	2.28.0	Bioconductor
genefilter	1.72.1	Bioconductor
geneplotter	1.68.0	Bioconductor
generics	0.1.2	CRAN (R 4.0.2)
GenomeInfoDb	1.26.7	Bioconductor
GenomeInfoDbData	1.2.4	Bioconductor
GenomicRanges	1.42.0	Bioconductor
ggalt	0.4.0	CRAN (R 4.0.2)
ggbeeswarm	0.6.0	CRAN (R 4.0.2)
ggforce	0.3.3	CRAN (R 4.0.2)
ggfun	0.0.5	CRAN (R 4.0.2)
ggplot2	3.3.5	CRAN (R 4.0.2)
ggraph	2.0.5	CRAN (R 4.0.2)
ggrastr	1.0.1	CRAN (R 4.0.2)
ggrepel	0.9.1	CRAN (R 4.0.2)
glue	1.6.2	CRAN (R 4.0.2)
GO.db	3.12.1	Bioconductor
GOSemSim	2.16.1	Bioconductor
GOstats	2.56.0	Bioconductor
gplots	3.1.1	CRAN (R 4.0.2)
graph	1.68.0	Bioconductor
graphlayouts	0.8.0	CRAN (R 4.0.2)
gridExtra	2.3	CRAN (R 4.0.2)
GSEABase	1.52.1	Bioconductor
gtable	0.3.0	CRAN (R 4.0.2)
gtools	3.9.2	CRAN (R 4.0.2)
hms	1.1.1	CRAN (R 4.0.2)
httr	1.4.2	CRAN (R 4.0.2)
igraph	1.2.11	CRAN (R 4.0.2)
IRanges	2.24.1	Bioconductor
KEGGgraph	1.50.0	Bioconductor
KEGGREST	1.30.1	Bioconductor
KernSmooth	2.23-20	CRAN (R 4.0.2)
labeling	0.4.2	CRAN (R 4.0.2)
lattice	0.20-45	CRAN (R 4.0.2)
lifecycle	1.0.1	CRAN (R 4.0.2)
limma	3.46.0	Bioconductor
locfit	1.5-9.4	CRAN (R 4.0.2)
magrittr	2.0.2	CRAN (R 4.0.2)

maps	3.4.0	CRAN (R 4.0.2)
marray	1.68.0	Bioconductor
MASS	7.3-55	CRAN (R 4.0.2)
Matrix	1.4-0	CRAN (R 4.0.2)
MatrixGenerics	1.2.1	Bioconductor
matrixStats	0.61.0	Bioconductor
memoise	2.0.1	CRAN (R 4.0.2)
munsell	0.5.0	CRAN (R 4.0.2)
openssl	2.0.0	CRAN (R 4.0.2)
org.Hs.eg.db	3.12.0	Bioconductor
pacman	0.5.1	CRAN (R 4.0.2)
pathview	1.30.1	Bioconductor
pheatmap	1.0.12	CRAN (R 4.0.2)
pillar	1.7.0	CRAN (R 4.0.2)
pkgbuild	1.3.1	CRAN (R 4.0.2)
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pkgload	1.2.4	CRAN (R 4.0.2)
plyr	1.8.6	CRAN (R 4.0.2)
png	0.1-7	CRAN (R 4.0.2)
polyclip	1.10-0	CRAN (R 4.0.2)
prettyunits	1.1.1	CRAN (R 4.0.2)
processx	3.5.2	CRAN (R 4.0.2)
progress	1.2.2	CRAN (R 4.0.2)
proj4	1.0-11	CRAN (R 4.0.2)
ps	1.6.0	CRAN (R 4.0.2)
purrr	0.3.4	CRAN (R 4.0.2)
qvalue	2.22.0	Bioconductor
R.methodsS3	1.8.1	CRAN (R 4.0.2)
R.oo	1.24.0	CRAN (R 4.0.2)
R.utils	2.11.0	CRAN (R 4.0.2)
R6	2.5.1	CRAN (R 4.0.2)
ragg	1.2.2	CRAN (R 4.0.2)
rappdirs	0.3.3	CRAN (R 4.0.2)
RBGL	1.66.0	Bioconductor
RColorBrewer	1.1-2	CRAN (R 4.0.2)
Rcpp	1.0.8.3	CRAN (R 4.0.2)
RCurl	1.98-1.6	CRAN (R 4.0.2)
RDAVIDWebService	1.28.0	Bioconductor
remotes	2.4.2	CRAN (R 4.0.2)
reshape2	1.4.4	CRAN (R 4.0.2)
Rgraphviz	2.34.0	Bioconductor
rJava	1.0-6	CRAN (R 4.0.2)
rlang	1.0.2	CRAN (R 4.0.2)
rprojroot	2.0.2	CRAN (R 4.0.2)
RSQLite	2.2.10	CRAN (R 4.0.2)
rstudioapi	0.13	CRAN (R 4.0.2)
Rtsne	0.15	CRAN (R 4.0.2)
Rttf2pt1	1.3.10	CRAN (R 4.0.2)
rvcheck	0.2.1	CRAN (R 4.0.2)

S4Vectors	0.28.1	Bioconductor
scales	1.1.1	CRAN (R 4.0.2)
scatterpie	0.1.7	CRAN (R 4.0.2)
sessioninfo	1.2.2	CRAN (R 4.0.2)
shadowtext	0.1.1	CRAN (R 4.0.2)
stringi	1.7.6	CRAN (R 4.0.2)
stringr	1.4.0	CRAN (R 4.0.2)
SummarizedExperiment	1.20.0	Bioconductor
survival	3.3-1	CRAN (R 4.0.2)
svglite	2.1.0	CRAN (R 4.0.2)
systemfonts	1.0.4	CRAN (R 4.0.2)
testthat	3.1.2	CRAN (R 4.0.2)
textshaping	0.3.6	CRAN (R 4.0.2)
tibble	3.1.6	CRAN (R 4.0.2)
tidygraph	1.2.0	CRAN (R 4.0.2)
tidyr	1.2.0	CRAN (R 4.0.2)
tidyselect	1.1.2	CRAN (R 4.0.2)
tweenr	1.0.2	CRAN (R 4.0.2)
tximport	1.18.0	Bioconductor
usethis	2.1.5	CRAN (R 4.0.2)
utf8	1.2.2	CRAN (R 4.0.2)
vctrs	0.3.8	CRAN (R 4.0.2)
vipor	0.4.5	CRAN (R 4.0.2)
viridis	0.6.2	CRAN (R 4.0.2)
viridisLite	0.4.0	CRAN (R 4.0.2)
withr	2.5.0	CRAN (R 4.0.2)
XML	3.99-0.9	CRAN (R 4.0.2)
xml2	1.3.3	CRAN (R 4.0.2)
xtable	1.8-4	CRAN (R 4.0.2)
XVector	0.30.0	Bioconductor
yulab.utils	0.0.4	CRAN (R 4.0.2)
zlibbioc	1.36.0	Bioconductor

Supplementary Table 4 | *P* values of statistically significant results.

Figure	Panel	Condition	*P* value
Main Figures			
1a		I vs. G (60 min) I vs. G (120 min) I vs. G (180 min)	0.0004 <0.0001 0.0208
1c	*PCK1*	C vs. G I vs. G G vs. G+I	0.0076 0.0027 0.0255
	G6PC	C vs. G C vs. G+I I vs. G G vs. G+I	<0.0001 0.0282 <0.0001 0.0003
	GCK	C vs. I C vs. G+I I vs. G G vs. G+I	0.0480 0.0248 0.0170 0.0091
1d	pAKT(T308)/AKT	10 min 20 min 30 min	0.0454 0.0327 0.0051
	pAKT(S473)/AKT	20 min 30 min	0.0350 0.0295
	pS6K1(T389)/S6K1	15 min 30 min	0.0075 0.0210
	pPYGL(S15)/PYGL	30 min	0.0355
1e		G vs. I	0.0239
2b	LDH	M1 vs. M0 M1 vs. C	0.0203 0.0052
	Cl. caspase 3	M1 vs. M0 M1 vs. C	0.0007 0.0004
2c	WTC pJNK(T183/Y185)	M1 vs. M0 M1 vs. C	0.0010 0.0005
	CW10030 pJNK(T183/Y185)	M1 vs. M0 M1 vs. C	0.0245 0.0035
2d	WTC IL1β	M1 vs. M0 M1 vs. C	0.0005 0.0001
	WTC TNFα	M1 vs. M0 M1 vs. C	0.0049 0.0046
	CW10030 IL1β	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	CW10030 TNFα	M1 vs. M0 M1 vs. C	0.0026 0.0021
2e		M1 v. M0 (30 min) M1 vs. C (30 min) M1 v. M0 (60 min) M1 vs. C (60 min) M1 v. M0 (120 min) M1 vs. C (120 min)	0.0034 <0.0001 0.0037 <0.0001 0.0050 <0.0001
2f	pAKT(T308)/AKT	M1 vs. M0	0.0009

		M1 vs. C	0.0134
	pAKT(S473)/AKT	M1 vs. M0 M1 vs. C	0.0017 0.0148
	pPYGL(S15)/PYGL	M1 vs. M0 M1 vs. C	0.0122 0.0475
2g	*G6PC*	M1 vs. M0 M1 vs. C	0.0440 0.0281
	PCK1	M1 vs. M0 M1 vs. C	0.0009 0.0009
	GCK	M1 vs. M0 M1 vs. C	0.0136 0.0008
2h		C vs. N	0.0006
4b		M1 vs. M1+anti-TNFα M1 vs. M1+anti-TNFα+anti-IL1β	0.0024 0.0003
4c		M1 vs. M1+anti-IL1β M1 vs. M1+anti-TNFα+anti-IL1β	0.0187 0.0282
4d		M1 vs. M1+anti-IL1β (30 min) M1 vs. M1+anti-TNFα+anti-IL1β (30 min) M1 vs. M1+anti-IL1β (60 min) M1 vs. M1+anti-TNFα+anti-IL1β (60 min) M1 vs. M1+anti-TNFα (120 min) M1 vs. M1+anti-IL1β (120 min) M1 vs. M1+anti-TNFα+anti-IL1β (120 min)	0.0039 0.0002 0.0009 0.0027 0.0017 0.0179 <0.0001
4e	pAKT(T308)/AKT	M1 vs. M1+anti-TNFα+anti-IL1β	0.0073
	pAKT(S473)/AKT	M1 vs. M1+anti-TNFα+anti-IL1β	0.0273
4f	*PCK1*	M1 vs. M1+anti-TNFα+anti-IL1β	0.0284
	G6PC	M1 vs. M1+anti-TNFα M1 vs. M1+anti-TNFα+anti-IL1β	0.0070 0.0125
	GCK	M1 vs. M1+anti-TNFα M1 vs. M1+anti-IL1β M1 vs. M1+anti-TNFα+anti-IL1β	0.0361 0.0037 0.0055
4g	pPYGL(S15)/PYGL	M1 vs. M1+anti-TNFα+anti-IL1β	0.0091
5a	IL1β	M1 vs. M0 M1 vs. C	0.0261 0.0069
	TNFα	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
5c	*JUN*	M1 vs. M0 M1 vs. C M1 vs. M1+NAbs	<0.0001 <0.0001 0.0142
	CASP1	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	NFKB2	M1 vs. M0 M1 vs. C M1 vs. M1+NAbs	0.0001 0.0002 0.0005
	TNF	M1 vs. M0 M1 vs. C M1 vs. M1+NAbs	<0.0001 <0.0001 0.0235
5d	Primary cells	M1 vs. C M1 vs. M1+NAbs	0.0279 0.0020

	CW10030 iPSC-derived cells	M1 vs. C M1 vs. M1+NAbs	0.0016 0.0066
5e	Primary cells pAKT(S473)/AKT	M1 vs. C M1 vs. M1+NAbs	0.0063 0.0020
	CW10030 iPSC-derived cells pAKT(S473)/AKT	M1 vs. C M1 vs. M1+NAbs	0.0435 0.0085
5f	Primary cells *PCK1*	M1 vs. C M1 vs. M1+NAbs	0.0042 0.0057
	Primary cells *GCK*	M1 vs. C M1 vs. M1+NAbs	0.0458 0.0164
	CW10030 iPSC-derived cells *PCK1*	M1 vs. C M1 vs. M1+NAbs	0.0389 0.0096
	CW10030 iPSC-derived cells *GCK*	M1 vs. C M1 vs. M1+NAbs	0.0306 0.0232
Supplementary Figures			
2a		C vs. I (30 min)	0.0336
2b	*INSR*	C vs. I C vs. G C vs. G+I I vs. G+I G vs. G+I	0.0010 0.0008 <0.0001 0.0338 0.0459
	IRS1	C vs. G C vs. G+I I vs. G+I	0.0119 0.0015 0.0265
	IRS2	C vs. I C vs. G C vs. G+I I vs. G+I G vs. G+I	0.0015 0.0025 <0.0001 0.0032 0.0019
2c	pY/INSR	5 min 15 min 30 min	0.0006 0.0005 0.0002
3d	*CASP1*	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	CASP4	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	CASP5	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
3e	WTC *NFKB2*	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	WTC *TNF*	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
	CW10030 *NFKB2*	M1 vs. M0 M1 vs. C	0.0176 0.0055
	CW10030 *TNF*	M1 vs. M0 M1 vs. C	<0.0001 <0.0001
4d	*IRS1*	M1 vs. M0 M1 vs. C	0.0017 0.0039
	IRS2	M1 vs. M0	0.0028

		M1 vs. C	0.0005
4e		M1 vs. M0 M1 vs. C	0.0340 0.0014
4f		M1 vs. M0 M1 vs. C	0.0416 0.0072
4h		M1 vs. M0 M1 vs. C	0.0411 0.0349
4j		L vs. C	0.0005
6a	*TNF*	M1 vs. M1+anti-TNFα M1 vs. M1+anti-TNFα+anti-IL1β	0.0309 0.0002
6c	*IRS1*	M1 vs. M1+anti-TNFα M1 vs. M1+anti-IL1β	0.0315 0.0199
	IRS2	M1 vs. M1+anti-TNFα M1 vs. M1+anti-IL1β M1 vs. M1+anti-TNFα+anti-IL1β	<0.0001 <0.0001 <0.0001
7a		C 2h vs. M1 2h	0.0151
7b		M1 vs. C M1 vs. M1+NAbs	0.0064 0.0067
7c	CW10030 iPSC-derived cells pPYGL(S15)/PYGL	M1 vs. C M1 vs. M1+NAbs	0.0304 0.0026
7d		M1 vs. C	0.0376