

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Efficient generation of β -like cells from hESCs. (A) Sample of gating strategy used for flow cytometry analyses. Flow cytometric analysis of (B) PDX1 expression in D20 endocrine progenitor cells and (C) INS, GCG and SST expression in D35 β -like cells. 2° Ab = secondary antibody.

Figure S2. Effect of metformin on the growth of pancreatic organoids. (A) Brightfield images of pancreatic organoids with/without various concentrations of metformin treatment *in vitro*. Scale bar = 300 μ m. (B) Diameters of pancreatic organoids treated with different metformin concentrations at the end of 35 days of differentiation. Each dot represents the average readings of two diameter measurements from the same organoid. Measurements taken from 61 untreated organoids and 40, 46, 19 and 35 organoids treated with 0, 10, 25, 50 and μ M of metformin, respectively. One-way ANOVA was performed to determine the significance of the observed size differences. Asterisk (*) indicates P value<0.05 (n=2 independent experiments).

Figure S3. Metformin treatment at the pancreatic progenitor or endocrine progenitor stage also impedes pancreatic β cell development from human embryonic stem cells. Expression of endocrine hormone transcripts *INS*, *GCG* and *SST* in pancreatic β -like cells treated with/without 100 μ M of metformin from the (A) pancreatic progenitor or (B) endocrine progenitor stage (n=3 biological replicates with their own technical triplicates. One

representative experiment is shown in the figure). All error bars indicate standard deviation of three replicates. Asterisk (*) indicates P value<0.05 compared to 0 μ M (One-way ANOVA).

Figure S4. Metformin treatment leads to downregulation of protein expression levels of endocrine hormones INS, GCG and pancreatic β -cell protein CHGA. Flow cytometric analyses of percentage of (A) INS⁺, (B) GCG⁺ and (C) CHGA⁺ cell populations in pancreatic β -like cells pre-treated with various concentrations of metformin at D35 (pancreatic β -like cells stage). Data from one representative experiment is shown on the left, and data from biological replicates summarized on the right). (n=3-4 biological replicates. Asterisk (*) indicates P value<0.05.

Figure S5. Immunostaining of pancreatic β -like cells exposed to increasing concentrations of metformin. Immunostaining for endocrine hormones INS, GCG and SST in pancreatic β -like cells treated with 0, 10, 25, 50 and 100 μ M or 1mM of metformin. Scale bar = 40 μ m.

Figure S6. Metformin treatment at low concentrations does not result in significant changes in transcriptional profile during pancreatic β cell development from human embryonic stem cells. Expression of pancreatic marker and endocrine hormone transcripts *ISL1*, *PCSK2*, *CHGA*, *CHGB*, *INS* and *GCG* during pancreatic β cell differentiation at 0, 10, 25, 50 and 100 μ M or 1mM of metformin (n=3 biological replicates with their own technical triplicates. One representative experiment is shown in the figure). All error bars indicate standard deviation of three replicates. Bars indicated mean expression relative to untreated

samples at D35 of pancreatic β -like cell differentiation. Asterisk (*, ** and ***) indicates P value < 0.05, < 0.01, and < 0.001, respectively, compared to 0 μ M (One-way ANOVA, Dunnett's post-hoc test).

Figure S7. Metformin treatment did not result in significant changes in protein expression levels of pancreatic and endocrine progenitor markers PDX1 and NKX6.1 at D20 of differentiation. Flow cytometric analyses of pancreatic β -like cells pre-treated with various concentrations of metformin at D20 (endocrine progenitor cells). (n=3 biological replicates. One representative experiment is shown in panels A and B).

Figure S8. Metformin perturbs mitochondrial gene expression in differentiated pancreatic β -like cells but not EndoC- β H1 cells or human islets. Expression of mitochondrial electron transport chain transcripts *ATP6*, *ATP8*, *COX1*, *COX2*, *COX3*, *SLC25A4*, *ND1*, *ND2*, *ND3* and *ND4* at (A) D35 of pancreatic beta cell differentiation, (B) in EndoC- β H1 cells or (C) cadaveric pancreatic islets treated with varying concentrations of metformin for 72 hours (n=3 biological replicates with their own technical triplicates). Error bars indicate standard deviation of three replicates. Asterisk (*) indicates P value < 0.05 compared to untreated samples (One-way ANOVA test).

Figure S9. Metformin treatment perturbs glycolytic function of human pancreatic β cells.

(A) Seahorse assay measuring glycolysis stress in EndoC- β H1 cells treated with 0 μ M or 1 mM of metformin (n=4). (B) Glucose uptake assay on EndoC- β H1 cells treated with 0, 25, 50,

100 μ M or 1mM of metformin (n=3). All error bars indicate standard deviation of three replicates. Asterisk (*) indicates P value<0.05 compared to untreated samples (Student's t-test).

Figure S10. Metformin inhibits pancreatic β cell function. (A) GSIS assay in EndoC- β H1 cells (data normalized to % total insulin). Data was normalized to total insulin by dividing the total amount of secreted insulin by the total insulin content. (B) GSIS assay in human adult pancreatic islets (n=3) and mouse insulinoma cell line MIN6 (n=4). All error bars indicate standard deviation of three biological replicates. Asterisk (*, ** and ****) indicates P value<0.05, <0.01 and <0.0001, respectively (Student's t-test).

Figure S11. Metformin treatment results in decreased pancreatic β cell viability at high concentration of metformin. (A) Immunostaining for cell proliferation marker Ki-67 and proapoptotic protein cleaved caspase-3 in human immortalized fetal β cell line EndoC- β H1 treated with 0, 10, 25, 50 and 100 μ M or 1mM of metformin. Images from 2 different experiments were quantified, with 3 fields of view used for the quantification per experimental condition (n=2). Scale bar = 40 μ M. Quantification of the percentage of (B) Ki-67⁺ (C) cleaved caspase-3⁺ cells and the total number of DAPI-stained cells. Images from 1 experiment were quantified, with 3 fields of view used for the quantification per experimental condition (n=1). Asterisk (*, **, *** and ****) indicates P value<0.05, <0.01, <0.001 and <0.0001, respectively.

SUPPLEMENTAL TABLE LEGENDS

Table S1: Summary of gene expression in metformin-treated pancreatic β -like cells.

Table S2: Primer sequences.

Genes	Forward sequence	Reverse sequence
<i>β-ACTIN</i>	TTGCCGATCCGCCGCCCGTC	CCCATGCCCACCATCACGCCCTGG
<i>ATP6</i>	CATCCCCGTATGAGCGGGCG	CGGTTAGGCGTACGGCCAGG
<i>ATP8</i>	GTGCTCCAAGCCCCCTCACT	TTAGTTGGGGCATTGCTGTGGT
<i>CHGA</i>	AGCTCCAAGACCTCGCTCTCCA	GATGGCTCTTCCACCGCCTCTTTC
<i>CHGB</i>	GCCAACGCTGCTTCTCAGCC	TGCAGCGAGTCACCATTCCTTCA
<i>COX1</i>	CCCACCCTGGAGCCTCCGTA	CGGATCAGACGAAGAGGGGCG
<i>COX2</i>	GAACCAGGCGACCTGCGACT	TACCCCCGGTCGTGTAGCGG
<i>COX3</i>	CCGACGGCATCTACGGCTCA	AATGCCAGTATCAGGCGGCGG
<i>GCG</i>	ACAGCACACTACCAGAAGACAGCA	TGTGCCCTGTGAATGGCGCT
<i>INS</i>	CCTGCAGGTGGGGCAGGTGGAGC	CGGGTGTGGGGCTGCCTGCG
<i>ISL1</i>	GCGCTGGCGACCCGCTCAGT	GGCCGCGGGTTTGCGGCGTAG
<i>ND1</i>	GCAGGCCCCTTCGCCCTATT	GCGGAATCGGGGGTATGCTGT
<i>ND2</i>	CCTGGCCCAACCCGTCATCT	TGGATGCGGTTGCTTGCGTG
<i>ND3</i>	CACCCCTTACGAGTGCGGCT	TCATGGTAGGGGTAAAAGGAGGCA
<i>ND4</i>	CATGGCAAGCCAACGCCACT	GGCTGGTTGCCTCATCGGGT
<i>PCSK2</i>	TGTGGCTGAAGCCTGGGAGC	CATCGGGTCCCGTGGCTGTT
<i>SLC25A4</i>	TGATACTGCCAAGGGGATGCTGC	GGACACCAGCCCTGCGACTG
<i>SST</i>	GCTGCGCTGTCCATCGTCCT	TTGGCCAGTTCCTGCTTCCCC