Supplemental material

Supplementary methods

Materials and Oligonucleotides. Anti-BrdU (ab6326), anti-insulin (guinea pig, ab7842), anti-glucagon (rabbit, ab92517), anti-RFP (ab124754) and IgG (ab37415 ChIP grade for ChIP-seq experiments) antibodies were from Abcam; H3K4me3 (#61379), H3K27ac (#39685) and H3K27me3 (#61017) antibodies were from Active motif; anti-Glut2 (#07-1402-1) was from Merck; anti-cleaved caspase 3 antibody (#9661S) was from Cell Signaling; anti-PC1/3 (AB10553) was from Millipore. Anti-glucagon (mouse, G2654) and anti-FLAG (clone M2) were from Sigma-Aldrich. The HLM006474 compound was first described in (1) and synthetized as previously described (2). The list of primers used in this study is presented in Supplementary Table S2.

Animal Experiments. Mice were maintained according to European Union guidelines for the use of laboratory animals. Mice were housed under a 12-hr light/dark cycle and given a regular chow (A04;Safe).

E2f1-/- (B6; 129S4-*E2f1*tm1Meg/J) mice and *db/+* mice (Janvier Labs) were crossed to obtain *db/db::E2f1⁺/⁺* and *db/db::E2f1⁻/⁻* mice and were previously described in (3). *CMV-CDK4^R24C* (4) and *Cdkn2a-/-* (5) were described elsewhere and were crossed with *E2f1-/-* mice to obtain *E2f1-/-::*CMV-CDK4^R24C and *E2f1-/-::Cdkn2a-/-*.

The congenic mice carrying the floxed *E2f1* allele were thereafter mated with rat insulin II promoter (RIP)-Cre (6) to generate RIPcre^Tg/+::*E2f1^flox/flox* mice. To delete E2f1 in adult β cells, we developed a mouse model allowing targeted recombination of E2f1 in

β cells through the use of a tamoxifen inducible strategy. To this end, $E2f1^{flox/flox}$ were crossed with MIP-CreERT mice (Jax Lab, stock number #024709). $E2f1^{flox/flox}$, Rip-Cre$^{/+}$ and MIP-CreERT were intercrossed to generate pure mutant *Rip-Cre$^{Tg/+}$/E2f1$^{flox/flox}$* and *MIP-CreERT$^{Tg/+}$/E2f1$^{flox/flox}$* mice. A PCR genotyping strategy was subsequently used to identify RIP-cre$^{Tg/+}$::$E2f1^{+/+}$ (*Rip-Cre$^{/+}$*), Rip-cre$^{+/+}$::$E2f1^{flox/flox}$ or *MIP-CreERT$^{+/+}$::E2f1$^{flox/flox}$* (*E2f1$^{fl/fl}$*), RIPcre$^{Tg/+}$::$E2f1^{flox/flox}$ (*E2f1$^{\beta-/-}$*) and *MIP-CreERT$^{Tg/+}$::E2f1$^{flox/flox}$* (*E2f1$^{MIP-CreERT\ \beta-/-}$*) mice. Tamoxifen was dissolved in corn oil (stock solution, 20mg/mL) at 52°C in agitation for 15min and was then administrated to both 2-month-old *E2f1$^{fl/fl}$* control and *E2f1$^{MIP-CreERT\ \beta-/-}$* transgenic animals by gavage once per day for 7 days at the dose of 4mg/day. After gavage, a stock solution of 1 mg/mL (stored at 4°C) was prepared in advance by dissolving 100mg of Tamoxifen Citrate (Biogaran) in 100 mL of drinking water. This solution was then diluted 1:20 in drinking water and provided to the animals for 1 week after the gavage induction in black bottles to prevent any alteration of the drug.

Metabolic phenotyping experiments were performed according to the EMPRESS protocols. For in vivo insulin secretion, *E2f1$^{fl/lf}$* and *E2f1$^{MIP-CreERT\beta-/-}$* mice were starved for 5/6 hours prior Glucose-Stimulated Insulin Secretion (GSIS) to evaluate the basal insulinemia. Blood was collected from mice anesthetized with isoflurane delivered in oxygen at a flow rate of 1l/min. Whole blood samples were collected from the retro-orbital cavity into blood collection tubes containing EDTA, using sterile glass capillaries. To assess insulin secretion level, a second blood sample was collected 20 minutes after an intraperitoneal injection of 2g/kg of body weight of D-(+)-glucose. Plasma was obtained by centrifuging at 2000g for 10 minutes at 4C°. Thereafter, it was transferred into pre-cooled tubes and directly frozen in liquid nitrogen and then stored at -80C°.

Plasma insulin concentration was evaluated by using Enzyme-Linked Immunosorbent Assay (ELISA) Ultrasensitive immunoassay (Mercodia, Uppsala, Sweden), following manufacturer's instructions. All reagents and samples were pre-warm at room temperature before use. The optical density was read at 450 nm, using a spectrophotometer ELISA reader (Sunrise BasicTecan, Crailsheim, Germany), supported by the Tecan's Magellan data analyses software. Insulin concentration was calculated using the online analyses software (myAssay.com), as suggested from the provider.

Measurment of cell proliferation, apoptosis and β-cell size. To assess cellular proliferation, 5-bromo-2'-deoxyuridine (BrdU, B9285, Sigma Aldrich) was administrated to 4 $E2f1^{β-/-}$ mice and 4 $E2f1^{fl/fl}$ control littermates. BrdU was dissolved in drinking water (1mg/ml) and provided to mice in dark bottles to protect the solution from the light. The solution was prepared fresh and it was changed every two days for 8 days prior to analyses. Pancreata were harvested, embedded in paraffin, and sectioned at 5 µm. The pancreatic sections were co-stained with anti-BrdU and anti-PC1/3, and slides were mounted with DAPI mounting-medium for nuclei-staining. Islet numbers and area were calculated with Axiovision software in squared-Pixel (PC1/3 positive area), and BrdU+/DAPI+ nuclei were counted. To measure apoptosis and β-cell size, pancreatic tissues were fixed with 10% formalin solution, dehydrated and embedded in paraffin. Immunofluorescence analysis were performed on 5 µm tissue sections. The sections were first de-paraffinized and re-hydrated, followed by a heat-induced antigen retrieval in citrate buffer. Sections were then incubated with blocking buffer for 30minutes and primary anti-Glut2 (for measuring β-cell size) or anti-cleaved caspase 3 antibodies (for measuring apoptosis) diluted at 1:100 in blocking buffer solution over night at +4°C. Immunofluorescent stainings were revealed using an

alexa-conjugated anti-rabbit secondary antibody (for Glut-2) or fluorescein-isothiocyanate-conjugated anti-rabbit (for cleaved caspase-3). Nuclei were stained with Dapi (SigmaAldrich, D9542). For cleaved caspase 3 immunostaining, intestinal FFPE sections from murine trinitrobenzene sulphonic acid (TNBS)-induced model of colitis (a kind gift of Dr Laurent Dubuquoy, Lille) was a positive control. Image acquisitions were done using a Spinning disk confocal microscope (Zeiss). 20 islets per mice were analyzed and 5 mice from each genotype were used to analyze β-cell size and apoptosis. Analysis and quantification were performed on ImageJ software using a macro program (developed in the lab and available upon request).

RNA-Sequencing. RNA quality was verified using RNA 6000 nanochips (Agilent, 5067-1511) on the Agilent 2100 bioanalyzer (Agilent, G2939A). Purified RNA (50ng) with RNA integrity number ≥6.5 was subsequently used for library preparation (TruSeq Stranded mRNA Library Preparation Kit, #20020594, Illumina) and sequenced on a HiSeq2500 system (Illumina). 3 biological replicates per condition were sequenced using paired-end mode. The demultiplexing of sequence data (from BCL files generated by Illumina sequencing systems to standard FASTQ file formats) was performed using bcl2fastq Conversion Software (Illumina; version 2.19.1). Trimming of residuals adapters and low-quality reads was performed using Cutadapt software (version 1.7.1). A mean of 54 million paired-end reads of 75 bp were generated for each sample. After initial checks and validation of sequence quality, RNA-seq reads were aligned to the mouse reference genome (mm10) using TopHat2 or STAR Aligner (version 2.5.2b). Subsequently, both quantification and annotation of the reads were performed using Bioconductor package Rsubread. The counting of the different genes

and isoforms was performed using RSEM (version 1.3). Finally, the differential gene expression analyses were performed using Bioconductor package DESeq2.

ChIP sequencing. 1 ng of eluted and purified DNA was used to prepare DNA sequencing library with the Nextflex rapid DNA seq kit 2.0 (Perkin Elmer, NOVA-5188-01) on the NextSeq 500 system (Illumina) using single read 100 base pairs mode. Sequence reads from FASTQ files were mapped to the mouse genome (mm10) using Bowtie2 Aligner (version 2.3.5.1). The demultiplexing of sequence data (from BCL files generated by Illumina sequencing systems to standard FASTQ file formats) was performed using bcl2fastq Conversion Software (Illumina; version 2.20). Trimming of residuals adapters and low-quality reads was performed using TrimGalore (version 0.4.5). Finally, peak-calling was performed with MACS2 software (version 2.2.7.1).

Bioinformatic analysis. Bioinformatic analysis were mainly performed using the open web-based platform Galaxy Europe (https://usegalaxy.eu). Motif search was performed using Pscan Web Interface (7).

Supplementary References

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Supplementary figures and legends

Supplementary Figure S1, related to Figure 1. Validation of the *E2f1$^{\beta-/-}$* mouse model. (A) qPCR-based analysis of *E2f1* mRNA expression in different tissues (pancreatic islets, hypothalamus (Hypo), heart, epididymal white adipose tissue (eWAT), brown adipose tissue (BAT) and muscle) isolated from 3-month-old male *E2f1$^{\beta-/-}$*, RIP-Cre$^{/+}$ and *E2f1*$^{fl/fl}$ control mice (n=3-6). **(B and C)** Body weight (B, n=5-8) and fasting glycaemia (C, n=7-12) of 3-month-old male *E2f1$^{\beta-/-}$*, RIP-Cre$^{/+}$ and control *E2f1*$^{fl/fl}$ mice. Statistical analysis were performed using one-way analysis of variance (ANOVA) with Tukey's test. **p<0.01. Results are represented as mean ± s.e.m.

Supplementary Figure S2, related to Figure 1. Metabolic phenotyping of the *E2f1$^{\beta-/-}$* and *E2f1$^{MIP-CreERT\ \beta-/-}$* mouse models. Body weight (**A, F**, n=5-7), fasting

glycaemia (**B**, **G**, n=5-7), ipGTT (**C**, **H**, n=5-7) and its corresponding AUC (**D**, **I**, n=5-7), and plasma insulin levels at 0 and 30 min after intraperitoneal glucose injection (**E**, **J**, n=5-7) of 6-week-old (**A** to **E**) and 8-week-old (**F** to **J**) male $E2f1^{\beta-/-}$ and control $E2f1^{fl/fl}$ mice. (**K**) Intraperitoneal insulin tolerance test (IPITT) of $E2f1^{\beta-/-}$ (n=8), RIP-Cre$^{/+}$ (n=8) and $E2f1^{fl/fl}$ (n=8) male mice at 12 weeks of age. (**L**) qPCR-based analysis of $E2f1$ mRNA expression in pancreatic islets isolated from 5-month-old male $E2f1^{MIP-CreERT\ \beta-/-}$ and $E2f1^{fl/fl}$ control mice 3 months after tamoxifen administration (n=9-10). (**M-N**) ipGTT (**M**, n=9-10) and its corresponding AUC (**N**, n=9-10) was performed one month after tamoxifen administration in $E2f1^{MIP-CreERT\ \beta-/-}$ and $E2f1^{fl/fl}$ control mice. All values are expressed as mean ± s.e.m. and were analysed by two-tailed unpaired Mann-Whitney test (A, B, D, F, G, I, L, N) or–two-way ANOVA with Bonferroni's multiple comparisons test (C, H, K, M). *$p < 0.05$; **$p<0.01$; ***$p<0.001$.

Supplementary Figure S3, related to Figure 2. The E2f1-CDK4-Cdkn2a pathway is required to maintain normal α-to-β cell ratio. (A) Representative immunofluorescent staining of Glut-2 in pancreatic sections from 3 months old $E2f1^{\beta-/-}$ and control $E2f1^{fl/fl}$ male mice. Quantification of β-cell size was performed as described in the supplemental material using confocal images from 20 islets and 5 mice per genotype and ImageJ macro. **(B-D)** Representative immunofluorescent staining of cleaved caspase 3 (B-C) and BrdU staining (D) in pancreatic sections from 3 months old $E2f1^{\beta-/-}$ and control $E2f1^{fl/fl}$ male mice (B-D). Small intestine FFPE sections from control C57bl6/J mice treated with TNBS were used as a positive control for apoptotic cell detection through cleaved caspase 3 staining (C). Nuclei were stained with Dapi.

Supplementary Figure S4, related to Figure 2. The E2f1-CDK4-Cdkn2a pathway is required to maintain normal α-to-β cell ratio. (A, B). Representative immunofluorescent staining of insulin and glucagon in pancreatic sections from 16-week-old global *E2f1* knockout male mice (*E2f1⁻ᐟ⁻*, B) compared to littermate controls (*E2f1⁺ᐟ⁺*, A). **(C)** Quantification of glucagon (Glucagon +) and insulin (Insulin +) labelled cells from the different mouse models (n=3 per gentotype). **(D)** Scheme representing the different genetically-engineered mouse models used in this study. **(E to H)** Representative immunofluorescent staining of insulin and glucagon in pancreatic sections from male CMV-CDK4^{R24C} (E), *E2f1⁻ᐟ⁻*::CMV-CDK4^{R24C} (F), *Cdkn2a⁻ᐟ⁻* (G), *E2f1⁻ᐟ⁻*::*Cdkn2a⁻ᐟ⁻* (H) mice. Values in C are expressed as mean ± s.e.m. and were analysed by one-way ANOVA with Tukey's test. ***$p<0.001$ and ****$p<0.0001$ compared to *E2f1⁺ᐟ⁺*; ####$p<0.0001$ compared to *E2f1⁻ᐟ⁻*.

Supplementary Figure S5, related to Figure 2. Altered α-to-β cell ratio in germline *E2f1*-deficient mice. (A) Representative immunofluorescent staining of insulin and glucagon in pancreatic sections from 14 to 15 week old global *E2f1* knockout male mice in a *Db/Db* background (*Db/Db::E2f1⁻ᐟ⁻*) compared to littermate control (*Db/Db::E2f1⁺ᐟ⁺*). **(B)** Quantification of glucagon (Glucagon +) and insulin (Insulin +) labelled cells in *Db/Db::E2f1⁻ᐟ⁻* and *Db/Db::E2f1⁺ᐟ⁺* mice calculated from C (n=4 per genotype). Values in B are expressed as mean ± s.e.m. and were analysed by two-tailed unpaired *t*-test. *$p < 0.05$; **$p<0.01$.

Supplementary Figure S6, related to Figure 4. Gene expression analysis of pancreatic islets isolated from β cell-specific *E2f1* knockout mouse (E2f1$^{β-ᐟ-}$) compared to littermate controls (*E2f1*$^{fl/fl}$). (A) Aligned reads and sequencing

coverage of *E2f1* gene exons in *E2f1^fl/fl* and *E2f1^β-/-* isolated islets in RNA-sequencing experiments. **(B and C)** Heatmap from gene set enrichment analysis (GSEA) displaying differentially expressed β-cell specific genes **(B)** and α-cell specific genes **(C)** in islets isolated from *E2f1^β-/-* and *E2f1^fl/fl* mice. **(D)** qPCR-based analysis of *E2f1, Pcsk9, Foxo1* and some β-cell specific genes (*Pdx1, Mafa, Ins2, Glp1r*) in islets isolated from 6-month-old *E2f1^β-/-* and *E2f1^fl/fl* control male mice (n=5-11). **(E)** qPCR-based analysis of *Arx* (α-cell specific gene) in islets isolated from 6-month-old E2f1^β-/- and E2f1^fl/fl control male mice (n=3-5). Results are represented as mean ± s.e.m. Statistical analysis for D and E were performed using one-way ANOVA with Tukey's test. *p < 0.05; **p<0.01; ***p<0.001.

Supplementary Figure S7, related to Figure 6. Chromatin state of specific examples of *E2f1^β-/-* up- and down- regulated genes in pancreatic islets and Min6 cells. (A-B) Integrated Genome Browser (IGB, (8)) was used to visualize H3K4m3, H3K27ac and H3K27me3 ChIP-seq signal intensities from Min6 cells and mouse pancreatic islets (9) within a series of **(A)** *E2f1^β-/-* down-regulated genes and **(B)** *E2f1^β-/-* up-regulated genes.

Supplementary Figure S8, related to Figure 7. E2f1 ChIP-seq validation. (A) Western blot assay showing efficacy of pCMV-hE2F1-Flag plasmid transfection using a Flag antibody in Min6 cells transfected during 24h with the empty pCMV plasmid (Empty plasmid) and pCMV-hE2F1-Flag (E2f1 Flag) plasmid during 24h and 48h. **(B)** Pie-chart showing localization of E2F1 binding in the genome divided in proximal promotor (<1kb), distal promotor (between 1 and 3 kb), gene body, downstream and distal intergenic region. **(C)** Transcription factor binding motifs enriched in ChIP-Seq

from Min6 cells transfected with pCMV-hE2F1-Flag plasmid. **(D)** Integrated genome browser (IGB) representation of hE2F1-Flag fixation site in the promoter region of *Ezh2* in Min6 cells transfected with pCMV plasmid (Empty-Flag) or pCMV-hE2F1-Flag (E2f1-Flag) plasmid (n=2). **(E)** Representation of hE2F1-Flag fixation site in the promotor of *Ccne1* in Min6 cells transfected with pCMV plasmid or pCMV-hE2F1-Flag plasmid realized using IGB software (n=2). **(F, G, H)** ChIP-qPCR showing fixation of hE2F1-Flag in *Ezh2* (F), *Ccne1* (G) and an intergenic region in Min6 cells transfected with pCMV plasmid or pCMV-hE2F1-Flag after an immunoprecipitation with IgG or Flag antibodies (n=2).

Supplementary Figure S9, related to Figure 7. Pathway analysis of direct and indirect E2f1 target genes. (A-D) IGB profiles of E2f1 bound (A-B) and unbound (C-D) genes identified through E2f1-Flag Chip-seq analysis. (E-H) Ingenuity Pathway Analysis (IPA) of E2f1 bound and unbound downregulated (E-G) and upregulated (F-H) genes in *E2f1$^{\beta-/-}$* compared to *E2f1$^{fl/fl}$*. Histogram of the 10 top enriched canonical pathways across input gene lists are shown.

Supplementary Figure S10, related to Figure 7. Pathway analysis of E2f1 direct and indirect target genes. (A) Genomic localisation of 16-state ChromHMM model. Heatmap displays the fold enrichment for each state for each 200-bp bin position within 2 kb around a set of transcription start sites (TSSs). **(B to G)** Ingenuity pathway analysis identified 10 top canonical pathways of *E2f1$^{\beta-/-}$* up- and down-regulated genes belonging to segment 6 (B-C), 14 (D-E) and 15 (F-G).

Supplementary Table S1, related to Figure 5. Donor information.

Supplementary Table S2, related to Figures 5, S1, S2, S6 and S8. List of oligonucleotides used in this study.

Supplementary Table S3, related to Figures 4, 6, 7, S6, S7, S8, S9 and S10. List of ChIP-seq and RNA-seq data sets used in this study.

Supplementary Table S4, related to Figures 4, 6 and S6. List of transcripts differentially regulated in $E2f1^{\beta-/-}$ and $E2f1^{fl/fl}$ pancreatic islets identified by RNA-sequencing and their chromatin state.

Supplementary Table S5, related to figure 4. Pathway analysis of RNA-sequencing data in pancreatic islets isolated from control $E2f1^{fl/fl}$ and β-cell specific $E2f1^{\beta-/-}$ deficient mice identifies potential upstream regulators associated to $E2f1$ deficiency.

Supplementary Table S6, related to figure 7, S9 and S10. E2f1-flag ChIP-seq in Min6 cells reveals direct and indirect E2f1 target genes involved in beta cell function. Download the excel file at : https://nextcloud.univ-lille.fr/index.php/s/ATYtizjgHkQCEpb

Supplementary Table S7, related to figure 7 and S10. List of the up- and downregulated genes that are bound by E2f1 at the chromatin level identified through ChromHMM 16-state segmentation (10). Chromatin state of E2f1 target genes were identified through intersecting E2f1-flag ChIP sequencing and RNA-sequencing data in pancreatic islets isolated from control $E2f1^{fl/fl}$ and β-cell specific $E2f1^{\beta-/-}$ deficient mice. Genes belonging to segment 6, 14 and 15 are listed with their corresponding

adjPval and Log2FC. Download the excel file at: https://nextcloud.univ-lille.fr/index.php/s/cgxD5gQjqKbwZpQ