SUPPLEMENTAL INFORMATION

Supplemental Figure S1: Development of insulinomas and conditionally immortalized human pancreatic beta cell line.

(A) Schematic representation of the multi-steps procedure developed to derive the EndoC-βH2 cells. Human fetal pancreas was co-transduced with SV40 LT and hTERT excisable lentiviral vectors and grafted under the kidney capsule of immune-incompetent SCID mice. EndoC-βH2 cells were derived following 4 rounds of successive transplantation (first-fourth insulinoma; k: kidney; i: insulinoma) (**B**) Immunohistochemical analysis of part of the fourth insulinoma in comparison to sections of human adult pancreas. Insulinoma cells stained positive for Insulin (INS), SV40 LT, Ki67, PDX1, Chromogranin-A (CHGA). They stained negative for glucagon (GCG) and carboxypeptidase-A (CPA). Rare cells stained positive for somatostatin (SST). Nuclei were stained with Hoechst 33342 fluorescent stain (blue). Scale bar: 50 μm. (**C**) Bright field photograph of EndoC-βH2 cells in culture. Scale bar: 300 μm. (**D**) PCR analysis on genomic DNA from EndoC-βH2 cells indicates that cells have integrated both RIP405-SV40 LT and RIP405-hTERT. Genomic DNA from HEK 293T cells was used as negative control.

Supplemental Figure S2: Determination of the optimal dose of lentiviral vector for efficient CRE expression.

(**A**) EndoC-βH2 cells were transduced with increasing amounts of lentiviral vector expressing CRE recombinase under the control of the CMV ubiquitous promoter. Lentiviral vector amount is expressed as ng of p24 capsid protein. Seven days after transduction, CRE immunostaining was performed to evaluate the percentage of CRE-expressing cells relative to total cell number for each condition of transduction (replicated four times with different lentiviral vector

batches). (**B**) EndoC-βH2 cells were transduced with increasing amounts of either CRE-expressing lentiviral vector (from 15 to 60 ng of p24 capsid protein) or with GFP expressing vector (60 ng of p24 capsid protein). The numbers of living cells were determined every 7 days.

Supplemental Figure S3: Expression of CDK4 and CDK1 in EndoC-βH2 cells compared to human islets.

(**A**) Real-time PCR quantification of *cdk4* and *cdk1* mRNA was performed on non–excised and excised EndoC-**β**H2 cells and human islets. Data are shown as the mean ± SEM of at least three independent experiments. ***P < 0.005 when compared to un-excised cells. (**B**) Immunoblot for CDK4, CDK1 and beta-actin. On the left, comparisons were performed between non–excised and excised EndoC-**β**H2 cells. The hepatoma cell line HepG2, used as a positive control. On the right, comparisons were performed between two human islet cell preparations and non-excised EndoC-**β**H2 cells.

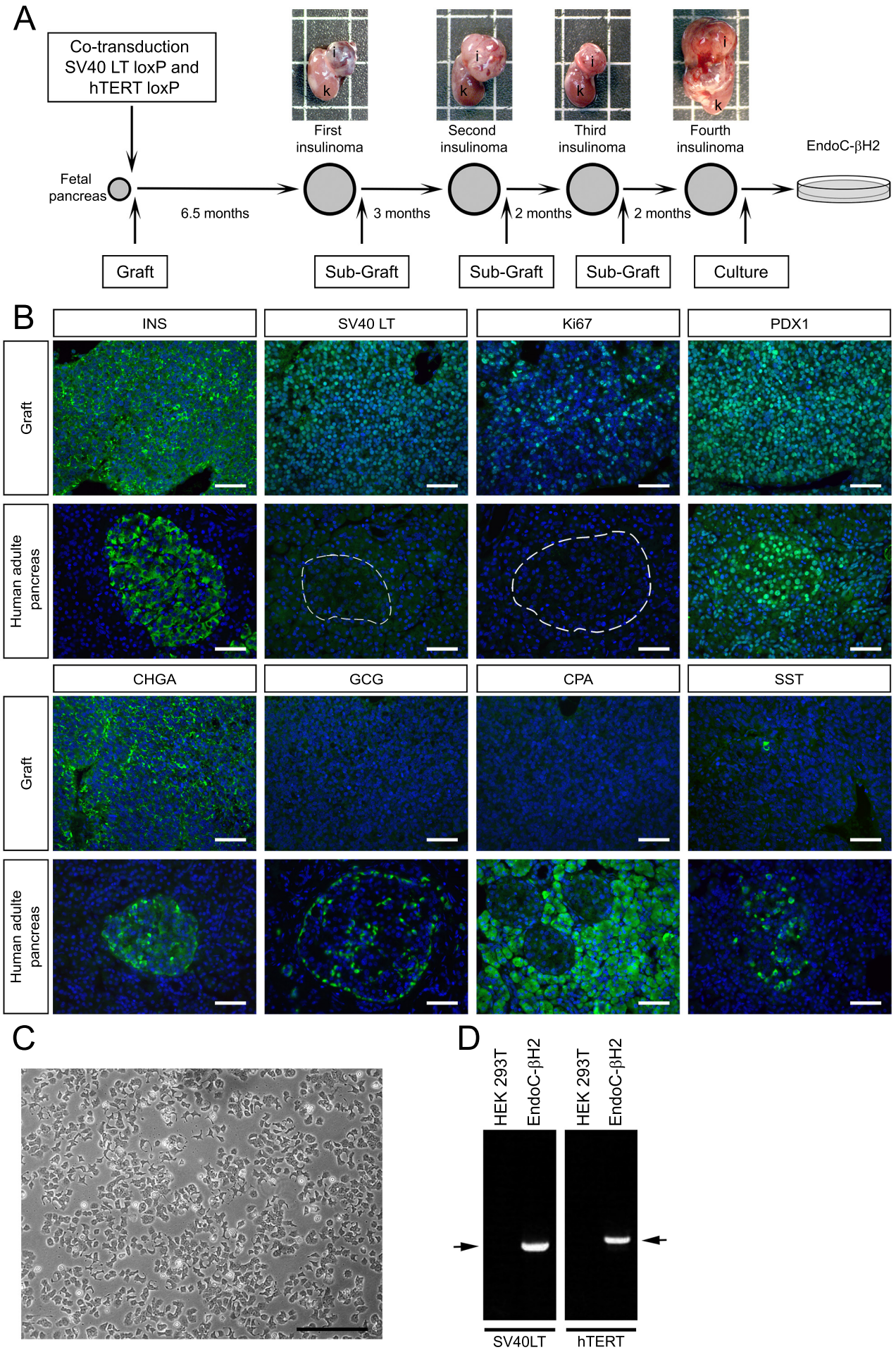
Supplemental Figure S4: Expression of Cyclins in EndoC-βH2 cells compared to human islets.

(**A**) Real-time PCR quantification of *ccnd1, ccnd3, ccne2 and ccnb2* mRNA was performed on non–excised and excised EndoC-**β**H2 cells and human islets. Data are shown as the mean ± SEM of at least three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.005 when compared to un-excised cells. (**B**) Immunoblot for CCND1, CCND3, CCNE2, CCNB2 and beta-actin. On the left, comparisons were performed between non–excised and excised EndoC-**β**H2 cells. The hepatoma cell line HepG2, used as a positive control. On the right, comparisons were performed between two human islet cell preparations and non-excised EndoC-**β**H2 cells.

Supplemental Figure S5: Global Comparative Genomic Hybridization (CGH array) profiles of EndoC-βH2 cells at two different passages

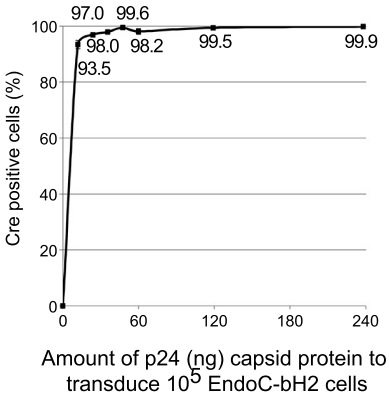
CGH arrays were performed on EndoC-βH2 cells at passages 34 and 85. Male human genomic DNA was used as a reference. Genomic DNA was probed on Agilent SurePrint G3 Human CGH Bundle (4x180K) arrays.

Supplemental Figure S1



Supplemental Figure S2

A



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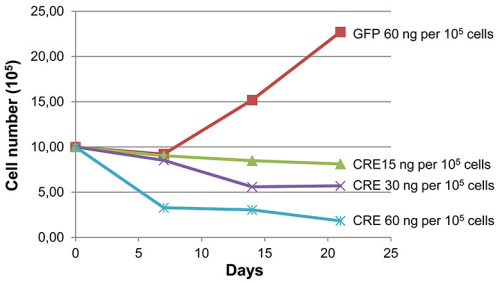
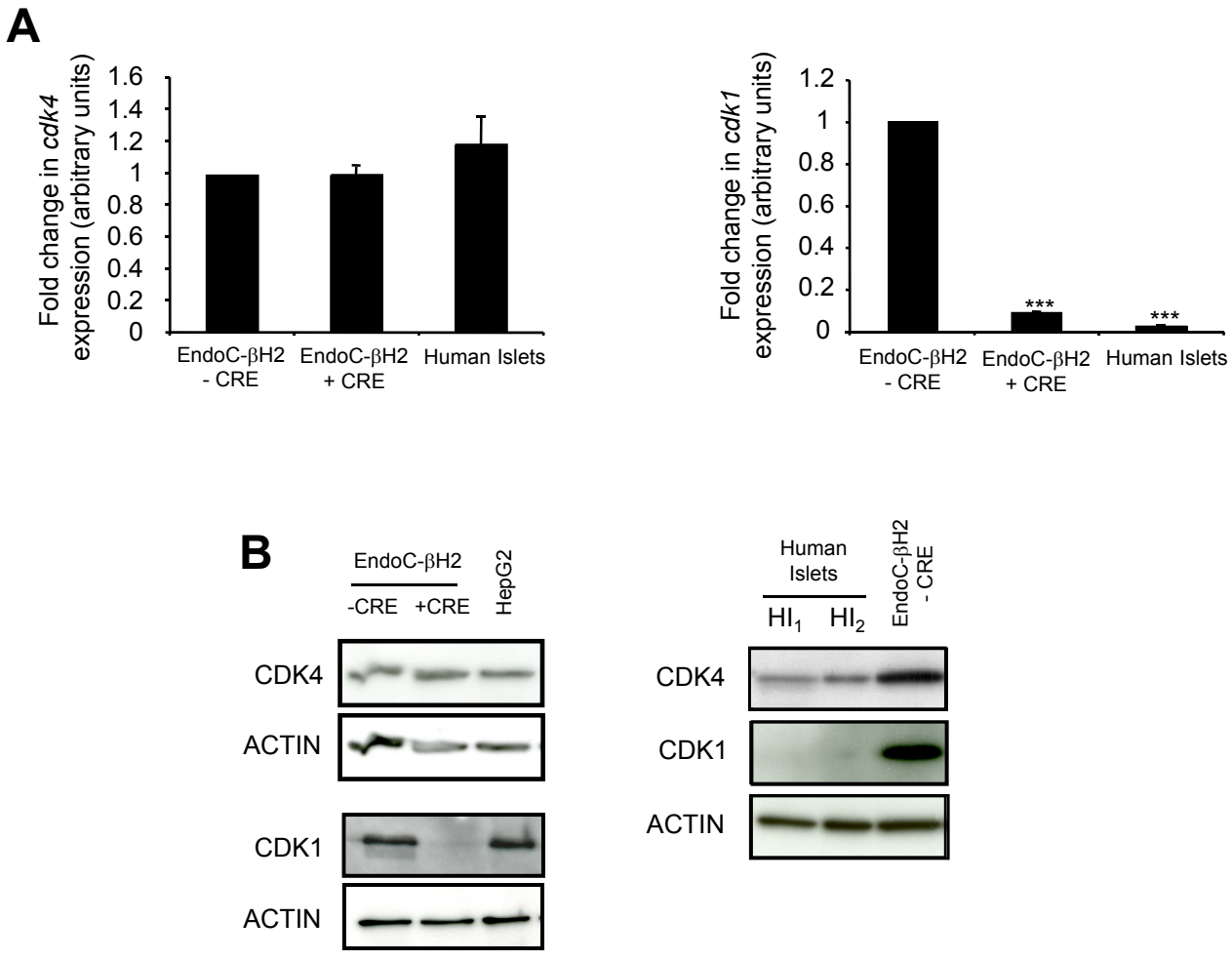
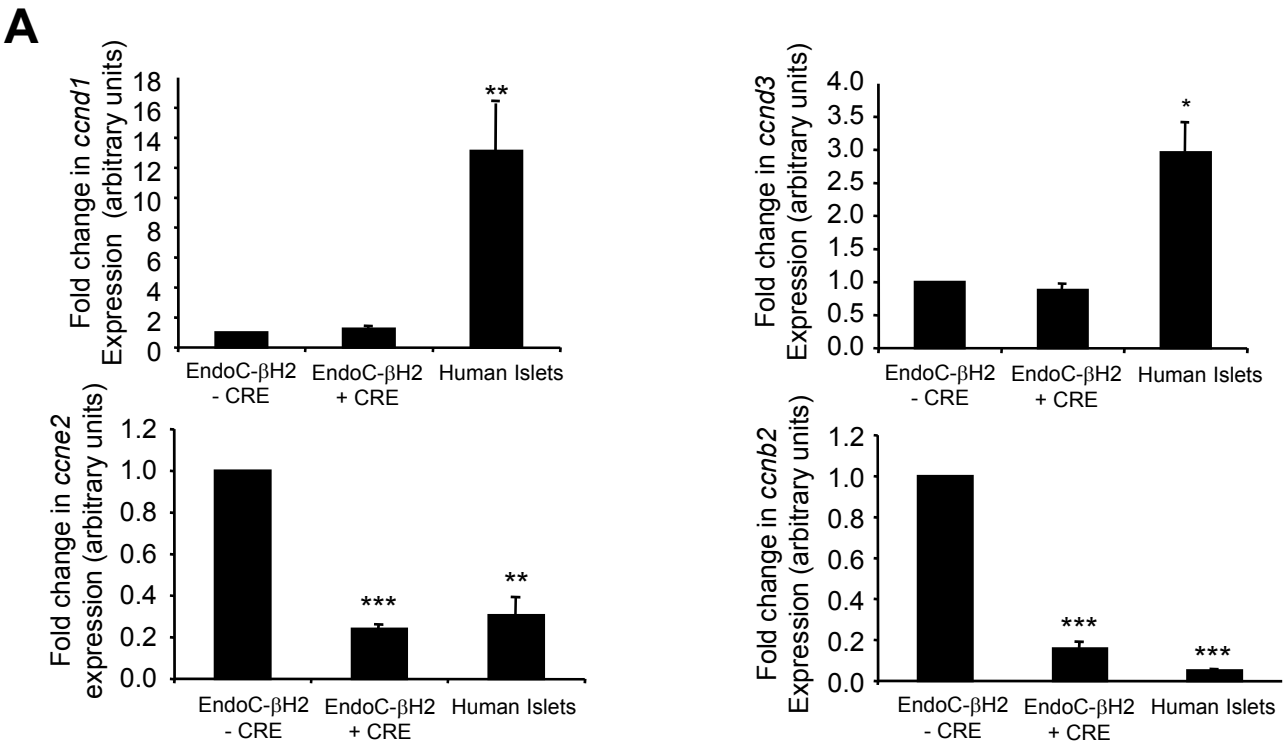
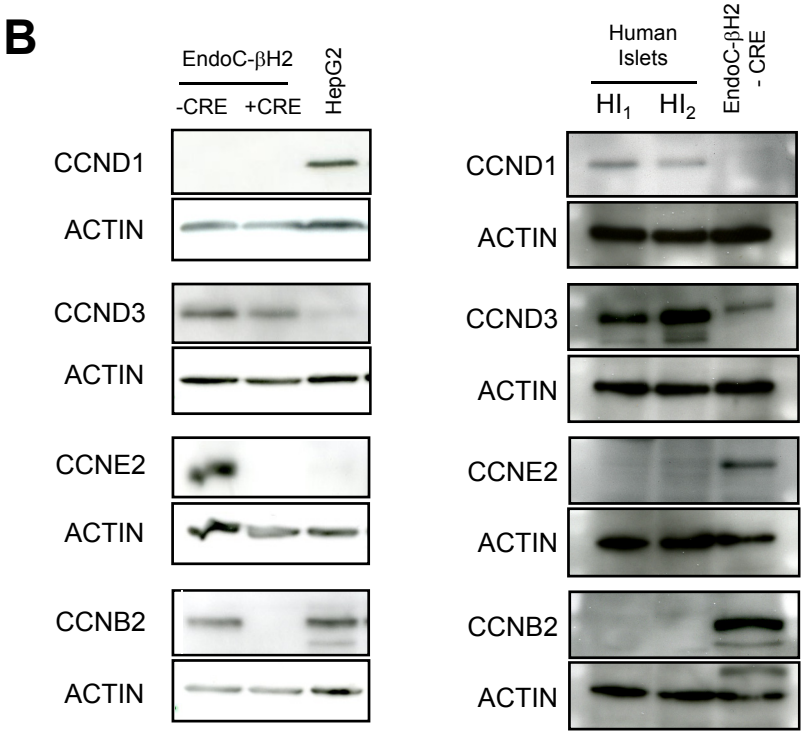


Supplemental Figure S3

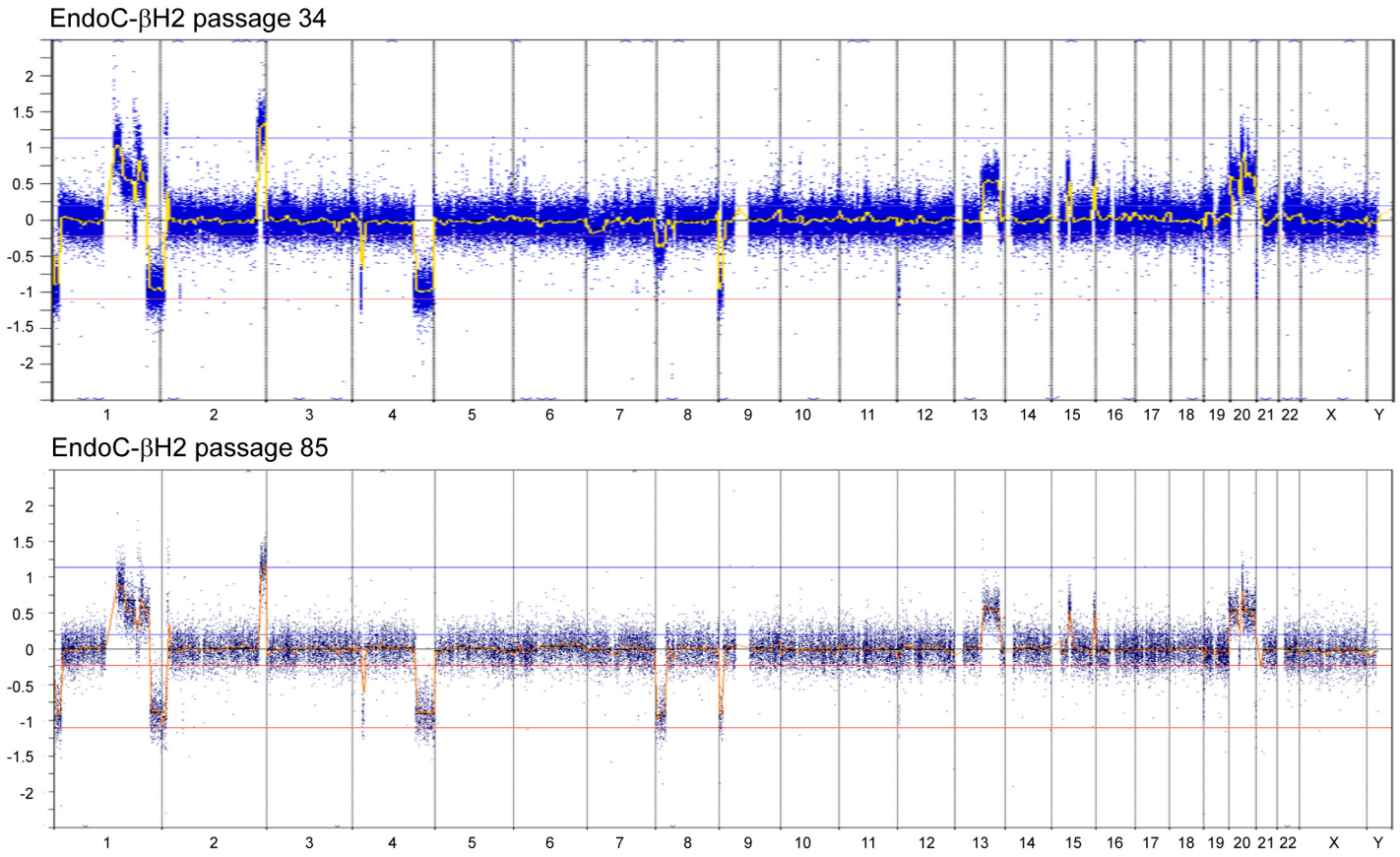


Supplemental Figure S4





Supplemental Figure S5



Supplemental table 1

	EndoC-βH2 control					**EndoC-βH2 + CRE**			
Passage number	46	59	62	77		46	59	62	77
Insulin content (µg per 10^6 cells)	0.066	0.09	0.062	0.064		2.1	2.75	1.98	2.5
Glucose responsiveness in absence of IBMX	NO	NO	NO	NO		YES	YES	YES	YES
Fold induction (0.5 mM vs 15mM glucose)						3.13	2.74	3.8	2.35

SUPPLEMENTAL TABLE S2: Q-PCR probes and primers

Taqman probes

Applied Biosystem Probe reference	Gene
Hs00165861_m1	ABCC8
Hs00265026_s1	KCNJ11
Hs01096908_m1	SLC2A2
Hs00374262_m1	RAB3A
Hs00169095_m1	IAPP
Hs99999904_m1	PPIA

SybGreen primers

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Insulin	AGAGGCCATCAAGCAGATCACTGT	ACAGGTGTTGGTTCACAAAGGCTG
SV40 LT	TGCCTGGAACGCAGTGAGTTTT	AACTCAGCCACAGGTCTGTACCAA
hTERT transgene	TCAGTAACCCCCAGCCCTAA	GCACCTCGCGGTAGTGG
Ki-67	AAGGAACAGCCTCAACCATCAGGA	CCAAGCTTTGTGCCTTCACTTCCA
Cdk4	TCAAGGTAACCCTGGTGTTTGAGC	GGCGCATCAGATCCTTGATCGTTT
Cdk1	CAGAGCTTTGGGCACTCCCAATAA	GCTAGGCTTCCTGGTTTCCATTTG
Ccnd1	AACACGGCTCACGCTTACCTCA	ACGACAGACAAAGCGTCCCTCAA
Ccnb2	TCAGCATGATCCCTCAGCTGAAC	ACGGCAGCCTAGGACCTTCCTAT
Ccnd3	TGGCTTACTGGATGCTGGAGGTAT	ACAGGTAGCGATCCAGGTAGTTCA
Ccne2	GCTGCCTTGTGCCATTTTACCTC	TCCCACTCCAAACCTGAGGCTTTC
PPIA (cyclo)	ATGGCAAATGCTGGACCCAACA	ACATGCTTGCCATCCAACCACT
PDX1	TACTGGATTGGCGTTGTTTGTGGC	AGGGAGCCTTCCAATGTGTATGGT
MAFA	GAGTTGGCACTTCTCGCTCT	TTCAGCAAGGAGGAGGTCAT
GLIS3	ACAACCCCTCCTCCCAGTTA	TGATGTGGTGAGGAGATGGA
RFX6	CATGTCGAACTCCAGTCCTAGCTT	AGTCCAGGGTTTCTTGAGCTGGAT
NKX2.2	GGTGGCAGATTTCGCTTTTCGTTG	CGGTCCTGAAGGTCATTTTGGCA
NKX6.1	ATTCGTTGGGGATGACAGAG	CGAGTCCTGCTTCTTCTTGG
NEUROD	ATTGCACCAGCCCTTCCTTTGATG	TCGCTGCAGGATAGTGCATGGTAA
MYT1	AGTGTCTGCCCAGGTGTCTT	GACAGACAATAGCTGTGGGGA
MNX1	AGAAGGCGGAAACCCACAGTGTT	CAGCAGTTTGAACGCTCGTGACA
PAX6	GCCCAGCTTCACCATGGCAAATAA	ATCATAACTCCGCCCATTCACCGA
hTBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA