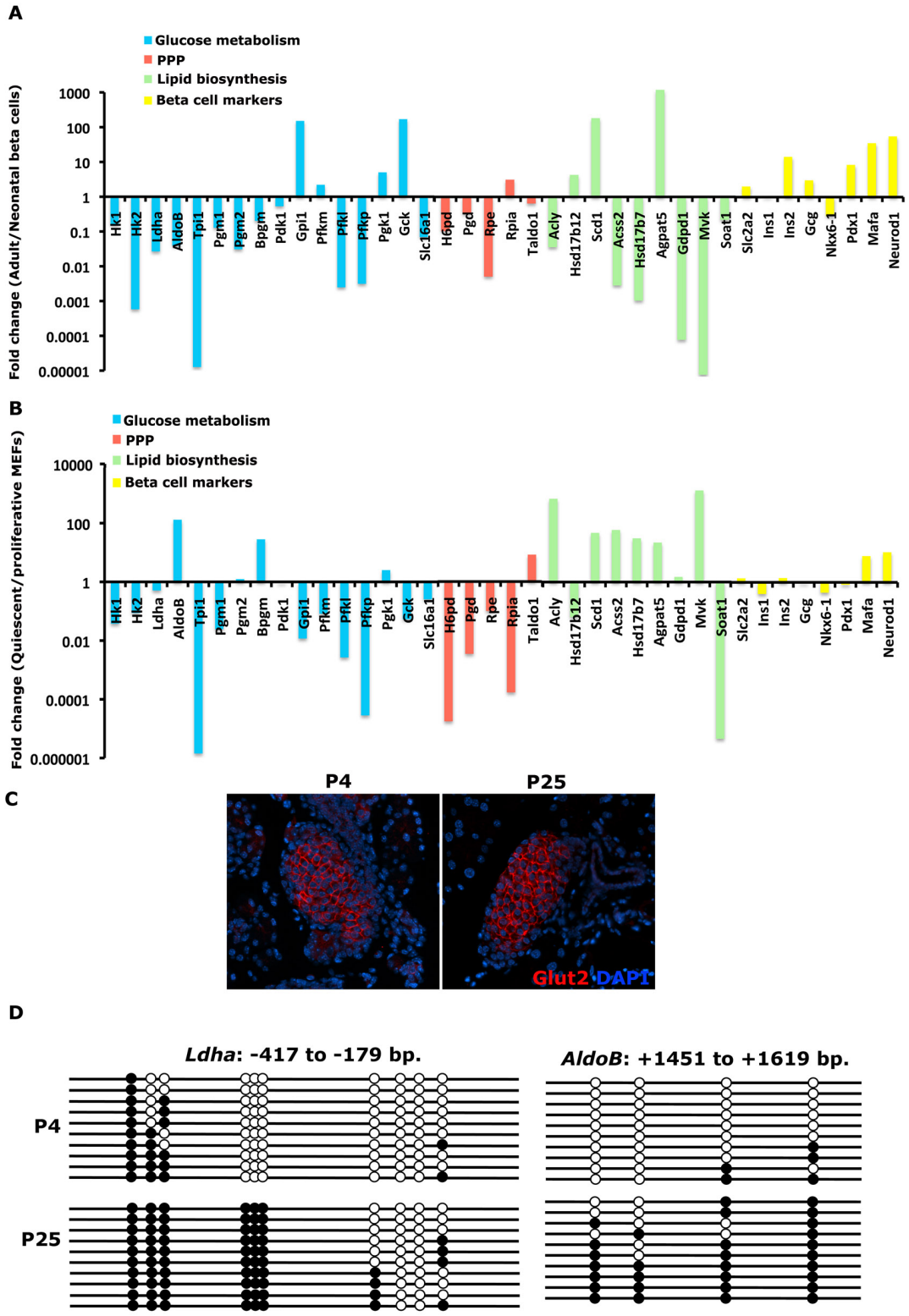
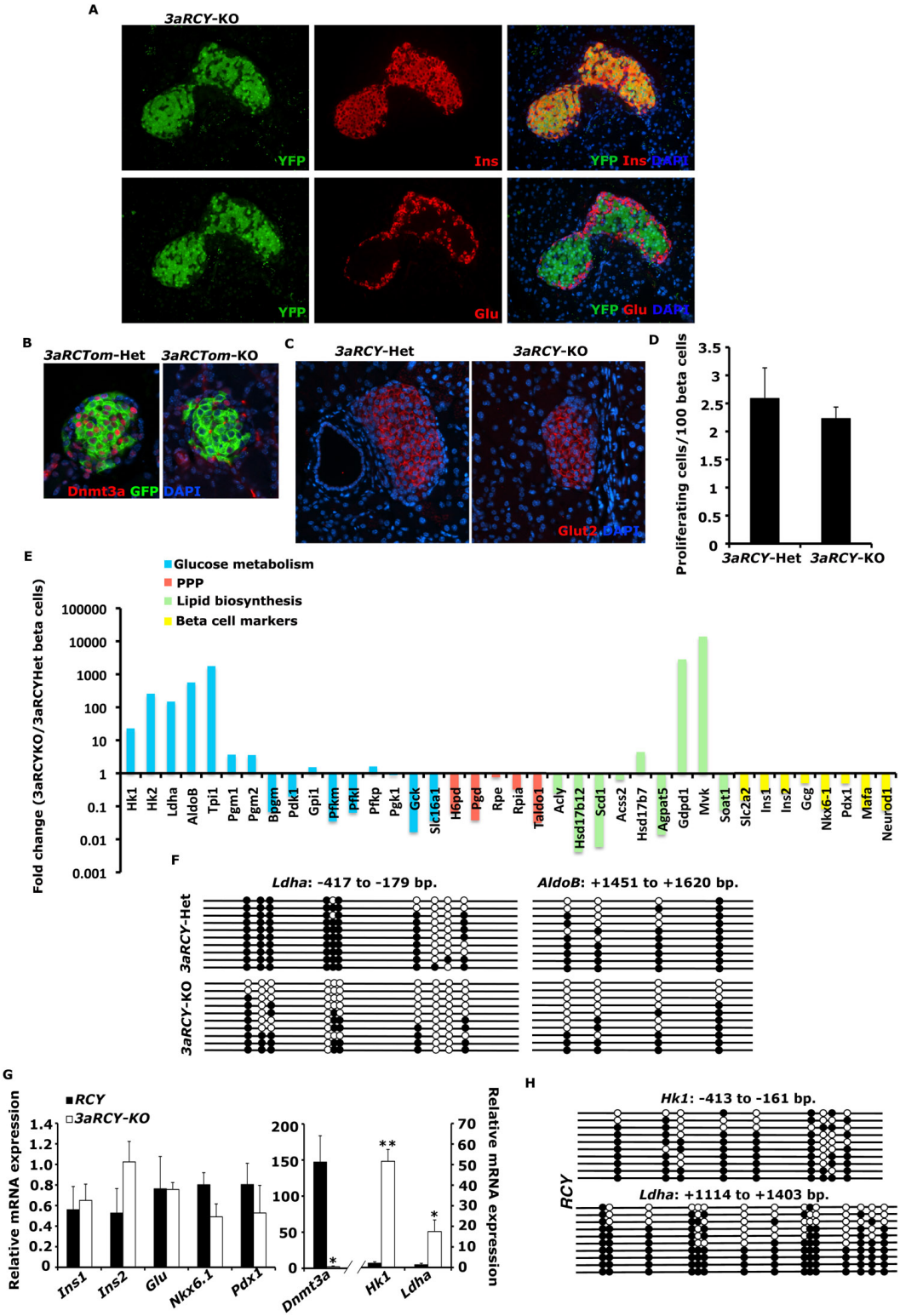
Supplemental Figures

Supplemental Figure 1.



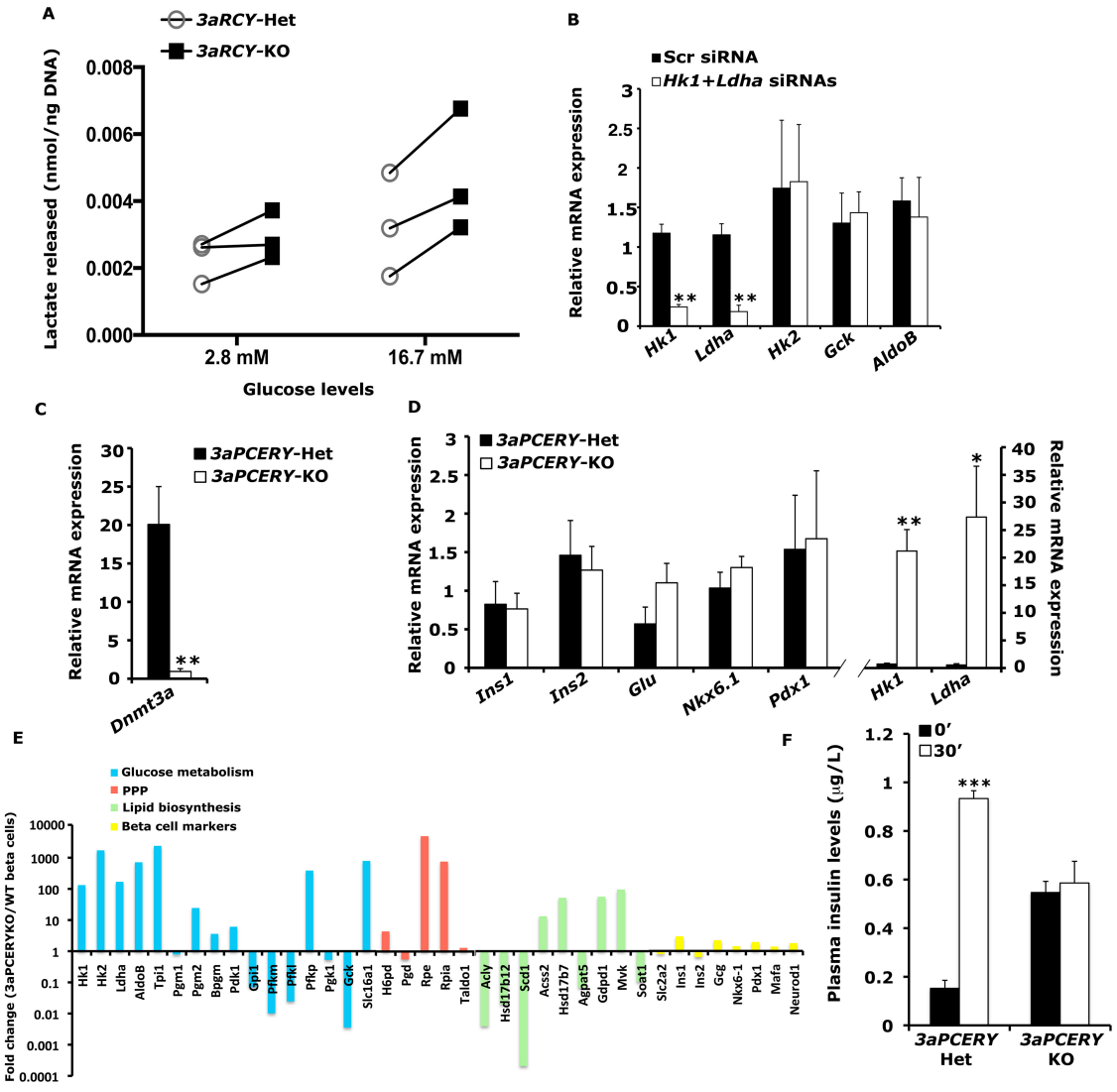
Supplemental Figure 1. (A) Fold changes in the mRNA expression levels of genes involved in glucose metabolism, pentose phosphate pathway (PPP), lipid biosynthesis and beta cell markers, in sorted beta cells from MIP-GFP adult mice (P25) compared to beta cells from neonatal MIP-GFP mice (P4). (N=1; pool of cells sorted from 6-8 mice in each group). **(B)** Fold changes in the mRNA expression levels of genes involved in glucose metabolism, pentose phosphate pathway (PPP), lipid biosynthesis and beta cell markers, in quiescent MEFs compared to proliferating MEFs. (N=1). **(C)** Expression Glut2 (red) in representative pancreatic sections from wildtype mice at indicated ages, by immunostaining. DAPI (blue) counter-stains the nuclei. **(D)** Bisulfite sequencing analysis for the *Ldha* and *AldoB* loci at indicated regions comparing sorted beta cells from P4 and P25 MIP-GFP mice (representative clones from N=3 mice). Each horizontal line with dots is an independent clone and 10 clones are shown here. These regions are almost fully DNA methylated (filled circles) in beta cells from P25 mice, but largely hypomethylated (open circles) in beta cells from P4 mice. For all experiments unless indicated otherwise, N=3 independent experiments.

Supplemental Figure 2.



Supplemental Figure 2. (A) Expression profile of Dnmt3a in representative pancreatic sections from wildtyype mice at indicated ages (P1 to 6 weeks) using immunostaining for Dnmt3a (red) and insulin (Ins; green). DAPI (blue) counter-stains the nuclei. **(B)** Representative pancreatic sections from 2 weeks old *3aRCTom*-KO and littermate control *3aRCTom*-Het animals, immunostained for Dnmt3a (red) and GFP (green). DAPI (blue) counter-stains the nuclei. **(C)** Representative pancreatic sections from 2 weeks old *3aRCY*-KO and littermate control *3aRCY*-Het animals, immunostained for Glut2 (red) with DAPI. **(D)** Quantification of proliferating beta cells at 6 weeks in *3aRCY*-KO and littermate control *3aRCY*-Het animals, as percentage of Ki67+ insulin double-positive cells in islets. $p>0.05$, not significant. **(E)** Fold changes in the mRNA expression levels of genes involved in glucose metabolism, pentose phosphate pathway (PPP), lipid biosynthesis and beta cell markers, in sorted YFP+ beta cells from *3aRCY*-KO mice, compared to YFP+ beta cells from *3aRCY*-Het mice, at 6 weeks of age. (N=1; pool of cells sorted from 6-8 mice in each group) **(F)** Bisulfite sequencing analysis for the *Ldha* and *AldoB* loci at indicated regions comparing sorted beta cells from 6 weeks old *3aRCY*-KO and control *3aRCY*-Het littermates (representative clones from N=3 mice). Each horizontal line with dots is an independent clone and 10 clones are shown here. These regions are almost fully DNA methylated (filled circles) in beta cells from *RCY* mice, but largely hypomethylated (open circles) in beta cells from *3aRCY*-KO mice. **(G)** Relative mRNA expression of indicated genes in sorted YFP+ beta cells from *3aRCY*-KO mice, compared to YFP+ beta cells from *RCY* (RIP-Cre heterozygous; YFP homozygous) mice, at 6 weeks of age. *CyclophilinA* was used as a house keeping gene. N=3 independent experiments. **(H)** Bisulfite sequencing analysis for the *Hk1* and *Ldha* loci at indicated regions comparing sorted beta cells from 6 weeks old control *RCY* animals (representative clones from N=3 mice). Each horizontal line with dots is an independent clone and 10 clones are shown here. These regions are largely hypermethylated (filled circles) in beta cells from *RCY* mice. For all experiments unless indicated otherwise, N=3 independent experiments. The error bars represent standard error (SEM) of the mean. *= $P<0.05$; **=$P<0.01$.

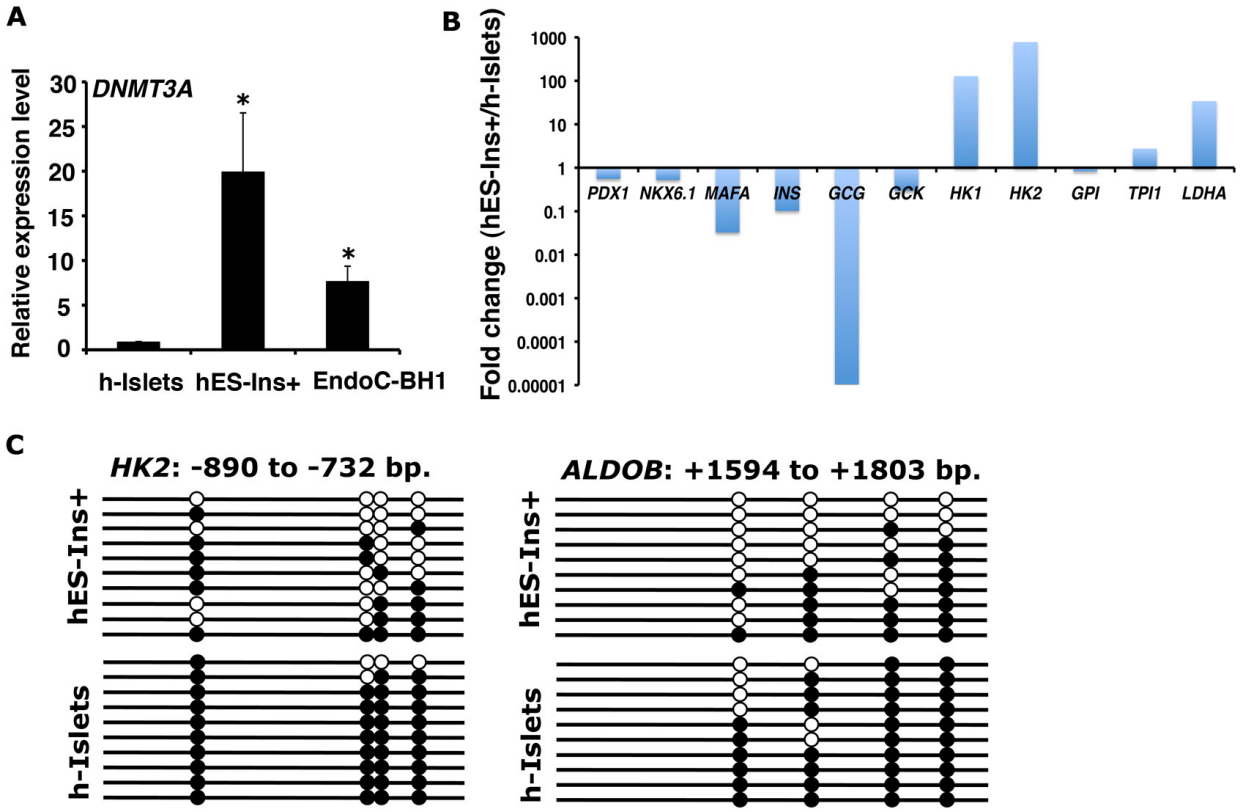
Supplemental Figure 3.



Supplemental Figure 3. (A) Pairwise comparison of lactate release in islets from 3 pairs of 6 weeks old *3aRCY*-KO and littermate control *3aRCY*-Het mice, at 2.8 mM and 16.7 mM glucose. **(B)** Relative mRNA expression of indicated genes in islets from 6 weeks old *3aRCY*-KO mice, treated with either siRNAs specifically targeting *Hk1*, and *Ldha*, or a control, scrambled siRNA. **=P<0.01. **(C)** Relative mRNA expression of *Dnmt3a* in islets isolated from 3 weeks old *3aPCERY*-KO and control littermate *3aPCERY*-Het mice, **=P<0.01 **(D)** Relative mRNA expression of indicated genes in in YFP+ sorted beta cells from 6 weeks old *3aPCERY*-KO and control littermate *3aPCERY*-Het mice, *=P<0.05; **=P<0.01. **(E)** Fold changes in the mRNA expression levels of genes

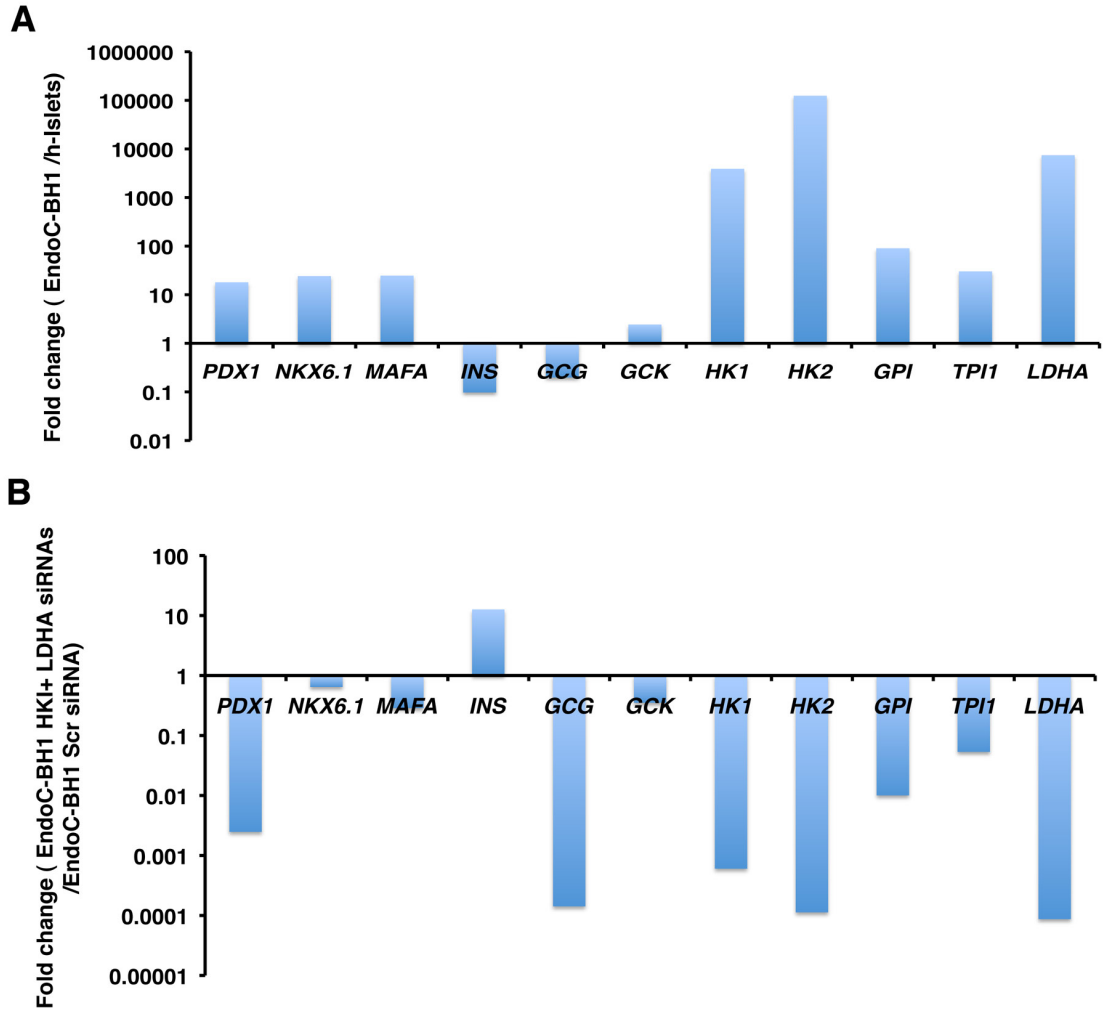
involved in glucose metabolism, pentose phosphate pathway (PPP), lipid biosynthesis and beta cell markers, in sorted YFP+ beta cells from *3aPCERY*-KO mice, compared to YFP+ beta cells from *3aPCERY*-Het mice, at 6 weeks of age. (N=1; pool of cells sorted from 6-8 mice in each group) **(F)** Plasma insulin levels in 6 weeks old *3aPCERY*-KO mice, and littermate control *3aPCERY*-Het mice (fasted 6 hrs.) at 0 and 30 minutes, after and intra-peritoneal injection of 2g/kg glucose (n=3 for each genotype). ***= $P<0.005$. For all experiments unless indicated otherwise, N=3 independent experiments.

Supplemental Figure 4.



Supplemental Figure 4. (A) Relative mRNA levels for *DNMT3A* in sorted GFP+ hES-Ins+ cells, and EndoC-BH1 human beta cell line, compared to human islets from cadaveric donors (Average donor age 43 years); (N=3). *CYCLOPHILIN A* was used as a house keeping gene. *= $P<0.05$. **(B)** Fold changes in the mRNA expression levels of indicated genes in sorted GFP+ hES-Ins+ cells, compared to human islets from cadaveric donors (Average donor age 43 years); (N=1). *CYCLOPHILIN A* was used as a house keeping gene. **(C)** Bisulfite sequencing analysis for the *HK2* and *ALDOB* loci at the indicated region comparing sorted beta cells from sorted GFP+ hES-Ins+ cells, compared to human islets from cadaveric donors (representative clones from N=3 samples per group). Each horizontal line with dots is an independent clone and 10 clones are shown here. These regions are hyper-methylated (filled circles) in human islets (h-Islets), but largely hypomethylated (open circles) in sorted Ins+ hES cells (hES-Ins+ cells). For all experiments unless indicated otherwise, N=3 independent experiments.

Supplemental Figure 5.



Supplemental Figure 5. (A) Fold changes in the mRNA expression levels of indicated genes in EndoC-BH1 human beta cell line, compared to human islets from cadaveric donors (Average donor age 43 years); (N=1). *CYCLOPHILIN A* was used as a house keeping gene. **(B)** Fold changes in the mRNA expression levels of indicated genes in EndoC-BH1 human beta cell line transfected with either a mix of specific siRNAs targeting *HK1* and *LDHA*, compared to EndoC-BH1 cells transfected with a control, scrambled siRNA (Scr). (N=1). *CYCLOPHILIN A* was used as a house keeping gene.

Supplemental Table 1
Real-time RT-PCR primers used for mouse mRNA expression analysis.

Gene	Forward	Reverse
Dnmt3a	5'- AACGGAAACGGGATGAGTG-3'	5'-ACTGCAATTACCTTGGCTTTCT-3'
Hk1	5'- GTGGACGGGACGCTCTAC-3'	5'-TTCACTGTTTGGTGCATGATT-3'
Hk2	5'-CAACTCCGGATGGGACAG-3'	5'-CACACGGAAGTTGGTTCCTC-3'
Ldha	5'-GGCACTGACGCAGACAAG-3'	5'-TGATCACCTCGTAGGCACTG-3'
AldoB	5'-CACACAGCTTCTGATACCTTGG-3'	5'-TGAGCCATGATGACAGGTACA-3'
Mct1	5'-ATGCTGCCCTGTCCTCCT-3'	5'-CCACAAGCCCAGTACGTGTAT-3'
Gck	5'-TATGAAGACCGCCAATGTGA-3'	5'-TTTCCGCCAATGATCTTTTC-3'
Insulin1	5'-CAGCCCTTAGTGACCAGC-3'	5'-TCTCCAGCTGGTAGAGGG-3'
Insulin2	5'-CAGCCCTAAGTGATCCGC-3'	5'-TCTCCAGCTGGTAGAGGG-3' (same reverse primer as *Insulin 1)*
Glut2	5'-TGTGATCCAGTGAGTCTCCAA-3'	5'-GGCGCACATCTATAATGCTCT-3'
Ucn3	5'-GACCTGAGCATTTCCACTCC-3'	5'-CAGAAGTGGCAGCAGGAAGT-3'
Pdx1	5'-GAAATCCACCAAAGCTCACG-3'	5'-CGGGTTCCGCTGTGTAAG-3'
Nkx6.1	5'-GAAGCGTGGTGTTGAGATGA-3'	5'-GGGCCCTTCCAAACAAGT-3'
MafA	5'-CTCCAGAGCCAGGTGGAG-3'	5'-GTACAGGTCCCGCTCCTTG-3'
Glucagon	5'-CATTCACCAGCGACTACAGCAA-3'	5'-TCATCAACCACTGCACAAAATCT-3'
Cyclophilin	5'-GTCTCCTTCGAGCTGTTTGC-3'	5'-AGCCAAATCCTTTCTCTCCA-3'

Supplemental table 2

Real-time RT-PCR primers used for human mRNA expression analysis.

Gene	Forward	Reverse
DNMT3A	5'-ATTCCTTCTCACAACCCGC-3'	5'- TACTTCCAGAGCTTCAGGGC-3'
HK1	5'-GAGAGTTTCATCGGAGAGCC-3'	5'-CAGCGAGAATCGGCTACAG-3'
HK2	5'-GAAGATGCTGCCCACCTTTG-3'	5'-CACCCAAAGCACACGGAAGT-3'
LDHA	5'-GGAGATCCATCATCTCTCCC-3'	5'-GGCCTGTGCCATCAGTATCT-3'
ALDOB	5'-CAATGCTCTGGGCAATTTCT-3'	5'-GGAGGACTCTTCTCTCCCAAA-3'
GCK	5'-CCTTCTTCAGGTCCTCCTCC-3'	5'-GATGGATGTCACAAGGAGCC-3'
INSULIN	5'-CAATGCCACGCTTCTGC-3'	5'-TTCTACACACCCAAGACCCG-3'
PDX1	5'-AAGCTCACGCGTGGAAAG-3'	5'-GGCCGTGAGATGTACTTGTTG-3'
NKX6.1	5'-CCACTTTTTCCGGACAGC-3'	5'-CCCGCCAAGTATTTTGTTTG-3'
MAFA	5'-TTCTCCTTGTACAGGTCCCG-3'	5'-GAGAGCGAGAAGTGCCAACT-3'
TPI1	5'-GCTGAGCGATTTGGGAAG-3'	5'-CCACAGCAATCTTGGGATCT-3'
GPI	5'-CCCTATGACCAGTACCTGCAC-3'	5'-TTCCCATTGGACTCCATGTC-3'
CYCLOPHILIN-A	5'-TTCTGCTGTCTTTGGGACCT-3'	5'-CACCGTGTTCTTCGACATTG-3'

Supplemental table 3

Gene Symbol	Refseq #	Official Full Name
Aldob	NM_144903	Aldolase B, fructose-bisphosphate
Pgm1	NM_025700	Phosphoglucomutase 1
Bpgm	NM_007563	2,3-bisphosphoglycerate mutase
Pdk1	NM_172665	Pyruvate dehydrogenase kinase, isoenzyme 1
Pgm2	NM_028132	Phosphoglucomutase 2
Gpi1	NM_008155	Glucose phosphate isomerase 1
Pfkm	NM_021514	Phosphofructokinase, muscle
Hk1	NM_010438	Hexokinase 1
Pfkp	NM_019703	Phosphofructokinase, platelet
Hk2	NM_013820	Hexokinase 2
Tpi1	NM_009415	Triosephosphate isomerase 1
H6pd	NM_173371	Hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)
Pgd	NM_001081274	Phosphogluconate dehydrogenase
Rpe	NM_025683	Ribulose-5-phosphate-3-epimerase
Rpia	NM_009075	Ribose 5-phosphate isomerase A
Taldo1	NM_011528	Transaldolase 1

Acly	NM_134037	ATP citrate lyase
Hsd17b12	NM_019657	Hydroxysteroid (17-beta) dehydrogenase 12
Scd1	NM_009127	Stearoyl-Coenzyme A desaturase 1
Acss2	NM_019811	Acyl-CoA synthetase short-chain family member 2
Hsd17b7	NM_010476	Hydroxysteroid (17-beta) dehydrogenase 7
Agpat5	NM_026792	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)
Gdpd1	NM_025638	Glycerophosphodiester phosphodiesterase domain containing 1
Mvk	NM_023556	Mevalonate kinase
Soat1	NM_009230	Sterol O-acyltransferase 1
Ak4	NM_009647	Adenylate kinase 4
Cdkn1a	NM_007669	Cyclin-dependent kinase inhibitor 1A (P21)
Pfkl	NM_008826	Phosphofructokinase, liver, B-type
Vegfa	NM_009505	Vascular endothelial growth factor A
Slc16a1	NM_009196	Solute carrier family 16 (monocarboxylic acid transporters), member 1
Slc16a3	NM_030696	Solute carrier family 16 (monocarboxylic acid transporters), member 3
Gck	NM_010292	Glucokinase
Slc2a2	NM_031197	Solute carrier family 2 (facilitated glucose transporter), member 2

Ins1	NM_008386	Insulin I
Ins2	NM_008387	Insulin II
Nkx6-1	NM_144955	NK6 homeobox 1
Pdx1	NM_008814	Pancreatic and duodenal homeobox 1
Gcg	NM_008100	Glucagon
Mafa	NM_194350	V-maf musculoaponeurotic fibrosarcoma oncogene family, protein A (avian)
Neurod1	NM_010894	Neurogenic differentiation 1
Dlk1	NM_010052	Delta-like 1 homolog (Drosophila)
Ppia	NM_008907	Peptidylprolyl isomerase A
Ldha	NM_010699	Lactate dehydrogenase A
Pgk1	NM_008828	Phosphoglycerate kinase 1
Actb	NM_007393	Actin, beta
MGDC	SA_00106	Mouse Genomic DNA Contamination
RTC	SA_00104	Reverse Transcription Control
PPC	SA_00103	Positive PCR Control

Supplemental table 4

Primers used for Bisulfite sequencing analysis in mice

	Location	Forward	Reverse
Ldha Set 1	+1114 to +1403 bp.	5'- GGTTTTAGTAAGGGTTAAATTTTGA-3'	5'-ATACCAAAAATCAAAAACAAAAAAC-3'
Ldha Set 2	-417 to -179 bp.	5'- AAGTGGGGTAGGAGAAAGGTTTAT-3'	5'-ATACTCCTCAACCTCAAAAACAAAA -3'
Hk1	-413 to -161 bp.	5'-GGATAGGTTGGGAATAGGTTAAAAT-3'	5'-CATCCTCCCTATTAAAAAAACACAC–3'
AldoB	+1451 to +1619 bp.	5'- TGTATTAGAGTAGTAGAGTGGAAGT-3'	5'-TAATAAAACAAAACACAAATACCTC-3'

Supplemental table 5

Primers used for Bisulfite sequencing analysis in humans

	Location	Forward	Reverse
LDHA	-348 to -159 bp.	5'- TAAGGTTTTATTGTGAGTGGGAGTT-3'	5'- CCCTATTTCTCAATATCCAAAACAA-3'
HK1	-4389 to -4234 bp.	5'- GAGGATTTTGTAGTAGTTGGGTTTG-3'	5'-ACTCTAACCCCTAATCTTCTCTCCTT-3'
HK2	-890 to -732 bp.	5'– ATTTTAGGAGGTGTTTAAGGGTATG -3'	5'-TTTCCCAATTATCTCAACAAATAAC-3'
ALDOB	+1594 to +1803 bp.	5'- TGTTTTTTTGTGGTTATTTTTTGTT-3'	5'-CCCAAACTTATCTTAAACTCCTAAATTC -3'

Supplemental table 6

Primers used for ChIP analysis in mice

	Location	Forward	Reverse
Ldha	+1114 to +1220 bp.	5'-GGGCTCTAGTAAGGGCCAAA-3'	5'-GCGCAATTTCAGAATCAGGT-3'
Hk1	-324 to -215 bp.	5'-TGAACTCTGACAGGCTGTGG-3'	5'-AAACTAGGCGGCTTCACAGA-3'
C (Arx) Negative control	+1348 to +1470 bp.	5'-AGTGCCCCTCTTGCTACCTT-3'	5'-TAGGGTGGGGCAAATTTTTA-3'