Supplementary Tables

ESM Table 1: siRNA sequences

siRNA name	Sequences				Reference
siNT	GGAACUAUCGCUUCGUGAA	GGAGCUACUUAAUCGGGAU	UGGCAGUACUUGCGGAUUG	GAGACUUGAGUUCGGGACA	Dharmacon, M-001206-13-20
siNKX2.2	CCGAAGGUCCGGAGGAAGA	GCACCGAGGGCCUUCAGUA	CCGACGAGUCACCGGACAA	CGAGAAAGGUAUGGAGGUG	Dharmacon, M-011341-00-0005
siMNX1	CUAAGAUGCCCGACUUCAA	CGGAUGAAAUGGAAACGCA	CGCAGGAAGCGGAGAAACA	GGACGACGAGGACCAUUUC	Dharmacon, M-010960-01-0005
siHHEX	GGAGAACCCUCAAAGCAAU	GGGCAAACCUCUACUCUGG	UUGGAUAGCUCUCAAUGUU	GCUAAAUGGAGGAGACUAA	Dharmacon, M-011756-01-0005
PERCC1	UUUCAGAGCUCAUCAGCGAUU				Dharmacon-custom

ESM Table 2: List of utilized primers and their sequences

Gene Name	Forward Sequence	Reverse Sequence
HS-*Mnx1*	GCACCAGTTCAAGCTCAACA	CATTTCATCCGCCGGTTCTG
HS-*Cyclophillin A*	GCCGAGGAAAACCGTGTACT	TGCTGTCTTTGGGACCTTGTGT
HS-*SST*	CTGAACCCAACCAGACGGAG	GCCATAGCCGGGTTTGAGTT
HS-*INS*	TTCTACACACCCAAGACCCG	ATTGTTCCACAATGCCACGC
HS-Pre-*INS*	TCTACCTAGTGTGCGGGGAA	AGCAATGGGCAGTTGGCTC
HS-*HHEX*	TGGGCAAACCTCTACTCTGG	GATGGTCTGGTCGTTGGAGA
HS-*KI67*	ACTATCAAAAGGAGCGGGGTC	TGCCTCCTGCTCATGGATTTC
HS-*PERCC1*	CCGAACCCCTAGGACTAAGC	TTGTTTGCTCTCTCGGTCGG
HS-*PERCC1*-exogenous	GAAGTACGGCCACATCACCC	CATCGTATGGGTAGGCCTCG
HS-*IAPP*	AAGCGGAAATGCAACACTGC	GCACCAAAGTTGTTGCTGGA
GFP	GGACGACGGCAACTACAAGA	AAGTCGATGCCCTTCAGCTC
HS-*NKX2.2*	GCTGACCAACACAAAGACGG	TGTCCGGCAGGTCTAAGATG
MM-*Hhex*	AAATACCTCTCCCCACCCGA	TGTTGCTTTGAGGATTCTCCTGT
MM-*Sst*	TCCGTCAGTTTCTGCAGAAGTCTC	GTACTTGGCCAGTTCCTGTTTCCC
MM-*Percc1*	TGT GTA TGT GGG GAC TGC TC	GGG CCC CGG AAT ACA TTA GC

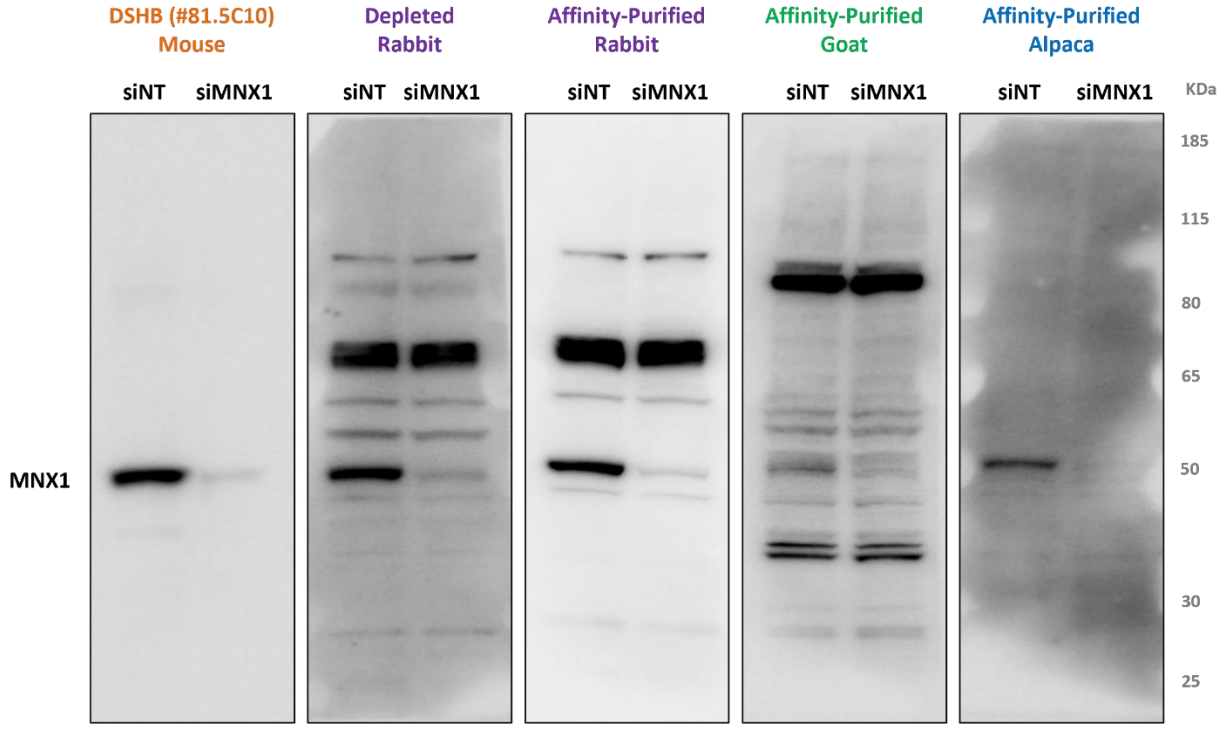
ESM Table 3: List of Antibodies used against surface markers of disaggregates mouse islets

Target	Supplier	Conjugation	Catalog	RRID	Dilution
MmTer119	Biolegend	PerCP/Cy5.5	116228	AB_893636	1/100
Mm/HS CD31	Biolegend	PerCP/Cy5.5	102522	AB_2566761	1/100
MmCD45	Biolegend	PerCP/Cy5.5	103132	AB_893340	1/100
MmCD49f	Biolegend	PE	313612	AB_893373	1/200
MmCD24	Biolegend	APC/Fire750	101840	AB_2650876	1/100
MmCD71	Biolegend	FITC	113806	AB_313567	1/100
MmCD81	Biolegend	BV421	740060	AB_2739825	1/100

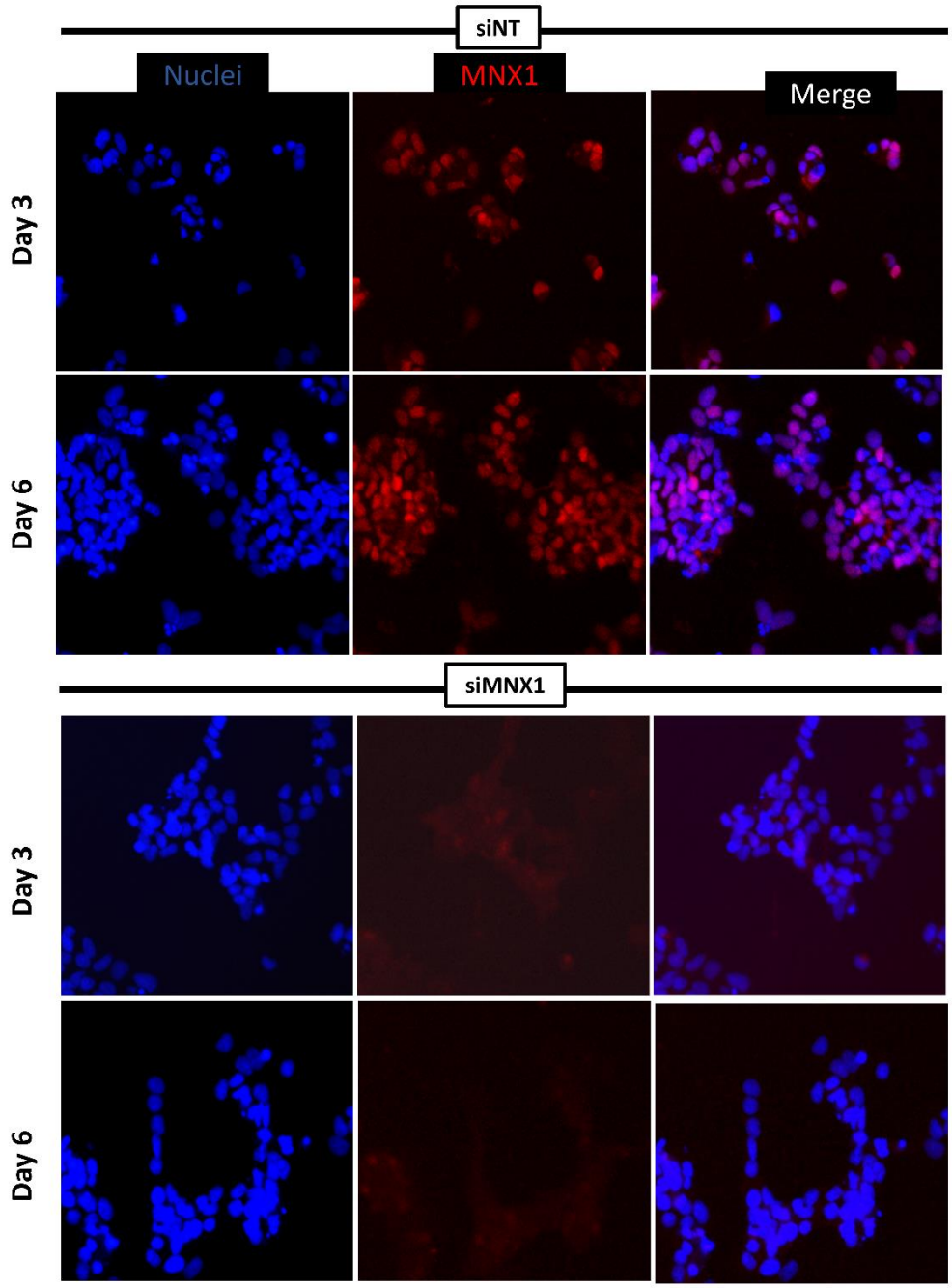
ESM Table 4: Number of reads of main beta cell genes in EndoC-BH1

Gene	Reads
CHGA	152769.9
INS	114591.9
PDX1	8611.236
MNX1	2039.322
NKX2-2	1882.649
NKX6-1	114.4257
HHEX	59.25647
PERCC1	50.23691

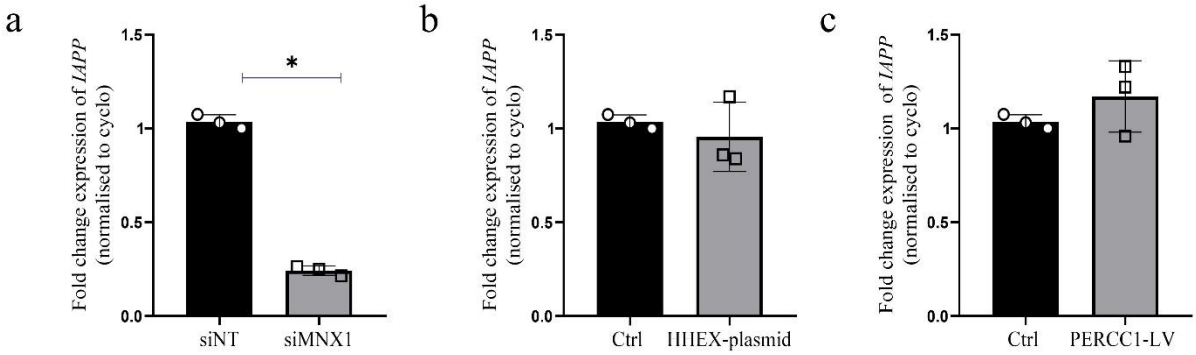
Supplementary Figures



ESM Figure 1: Validation of MNX1 antibodies by western blot
EndoC-BH1 cells were transfected with either siMNX1 or non-targeting siNT for three days followed by protein extraction. Immunoblotting using different MNX1 antibodies: commercial DSHB #81.5C10 or homemade antibodies (a gift from C. Wright) showed great specificity of the DSHB clone for siNT with minimal background while faint band was detected for siMNX1.

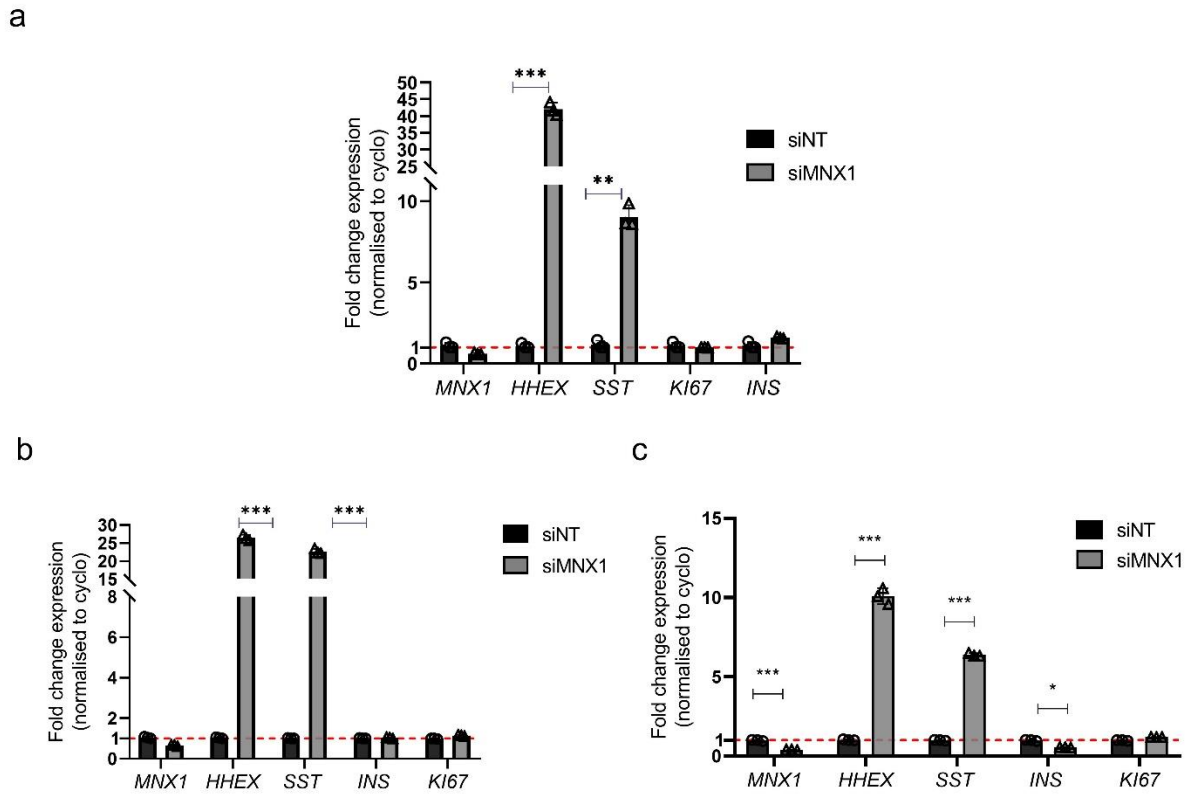


ESM Figure 2: MNX1 staining post siMNX1 transfection
EndoC-BH1 cells were transfected with either siMNX1 or non-targeting siNT for either three or six days, fixed for immunostaining and stained with MNX1 antibody. Nuclei were counter stained with Hoechst.

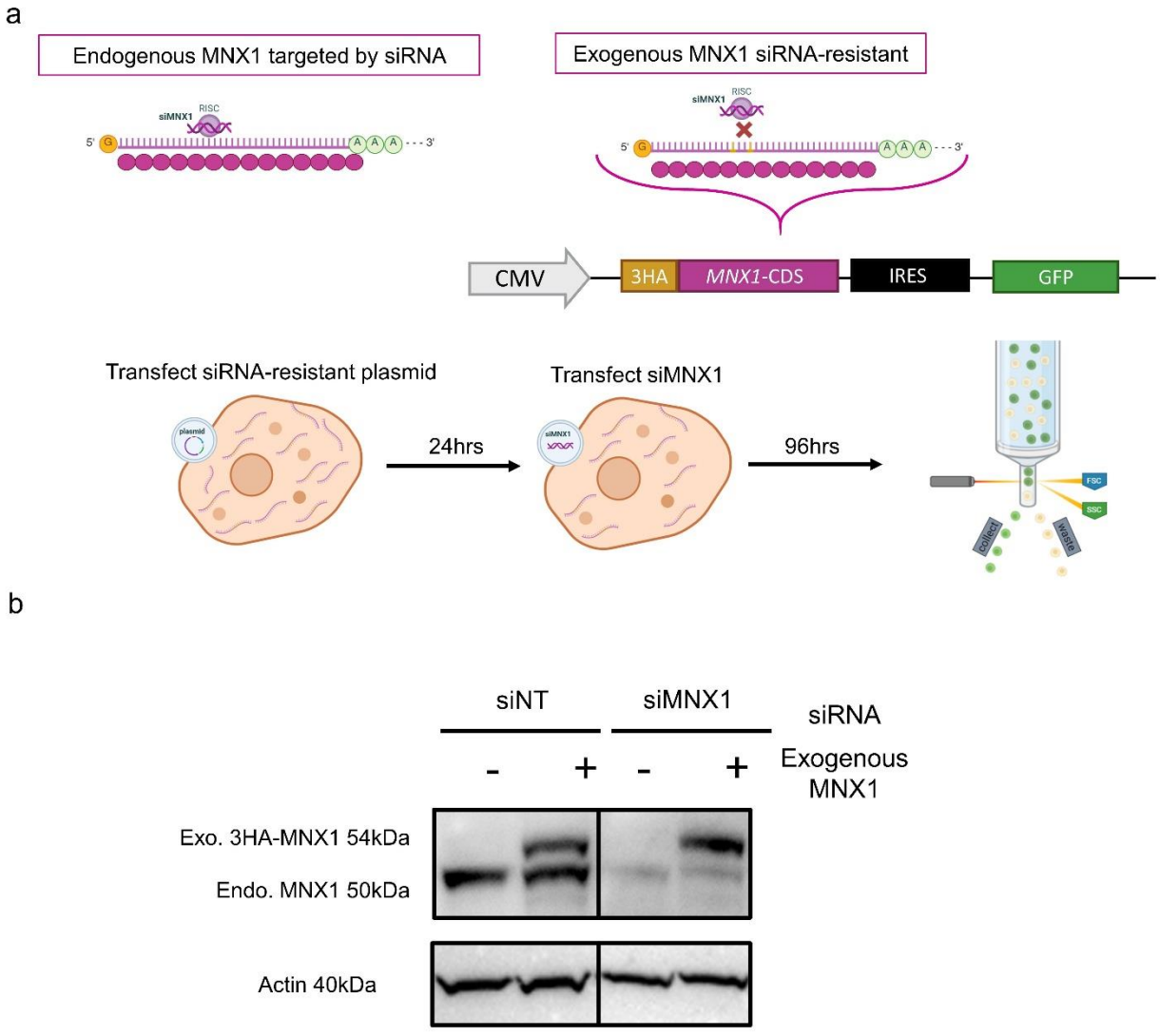


ESM Figure 3: Regulation of *IAPP* expression.

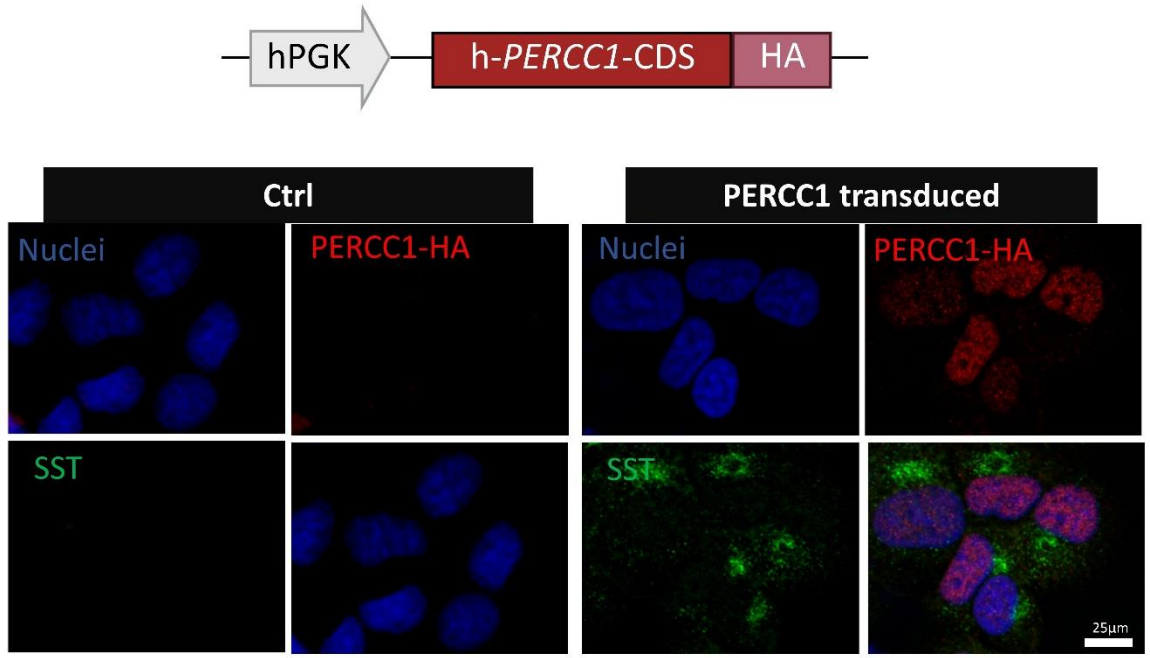
MNX1 knockdown induced the downregulation of *IAPP* (a). This was not reciprocated via the overexpression of either HHEX (b) or PERCC1(c). Data represent mean values of three individual experiments ± SEM. * $p<0.05$



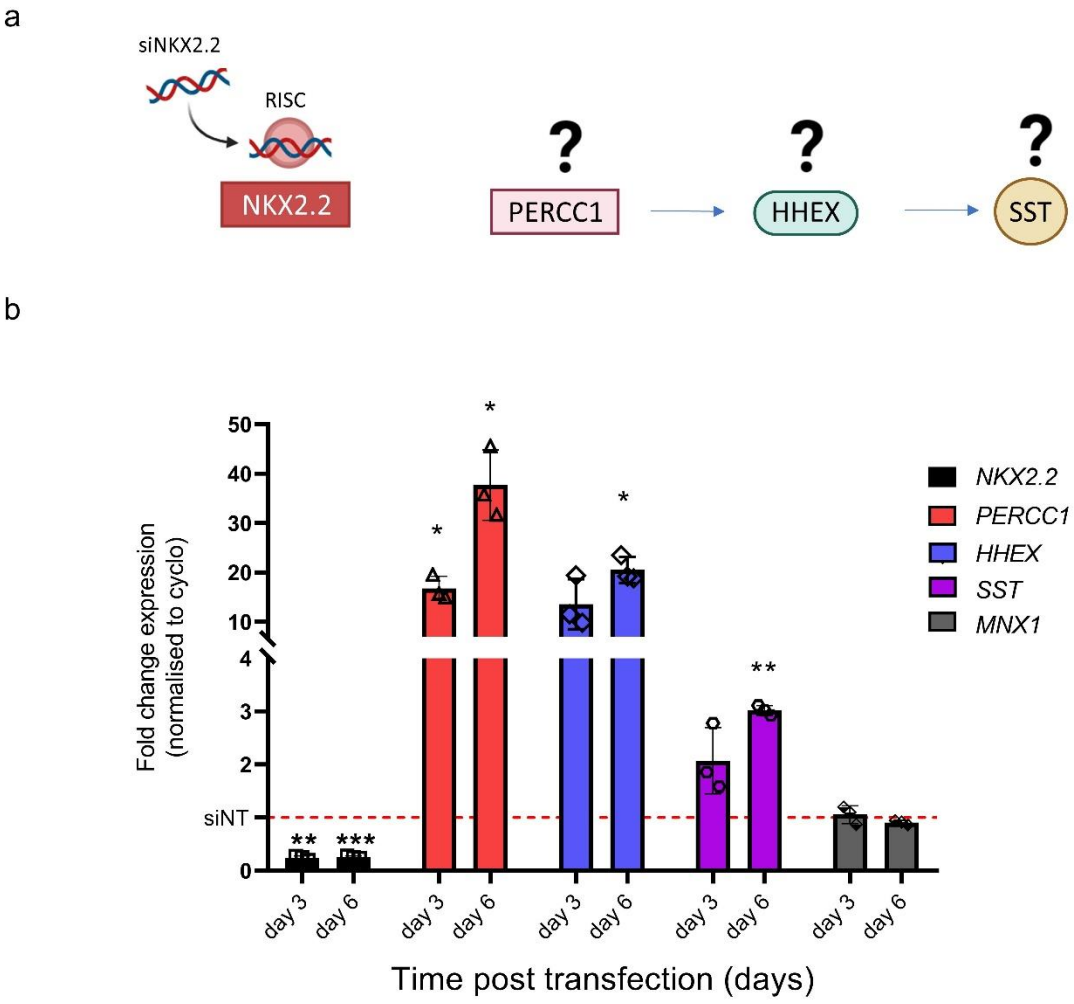
ESM Figure 4: The effect of MNX1 knockdown on different human beta cells models. MNX1 knockdown induced the upregulation of HHEX and SST in (a) 3D cultured aggregates of EndoC-BH1, (b) ECN90 and (c) EndoC-BH2 cells. Data represent mean values of three individual experiments ± SEM. * $p<0.05$, *** $p<0.001$



ESM Figure 5: A MNX1 construct resistant to siRNA.
(a) A MNX1 plasmid resistant to MNX1 siRNA was constructed by mutating the siRNA binding sites. This plasmid or an empty equivalent were introduced in EndoC-BH1 cells followed by the knockdown of endogenous MNX1 and FACS sorted for GFP. (b) The expression of the exogenous 3HA-MNX1 and its resistance to MNX1 siRNA were analysed by Western blot.

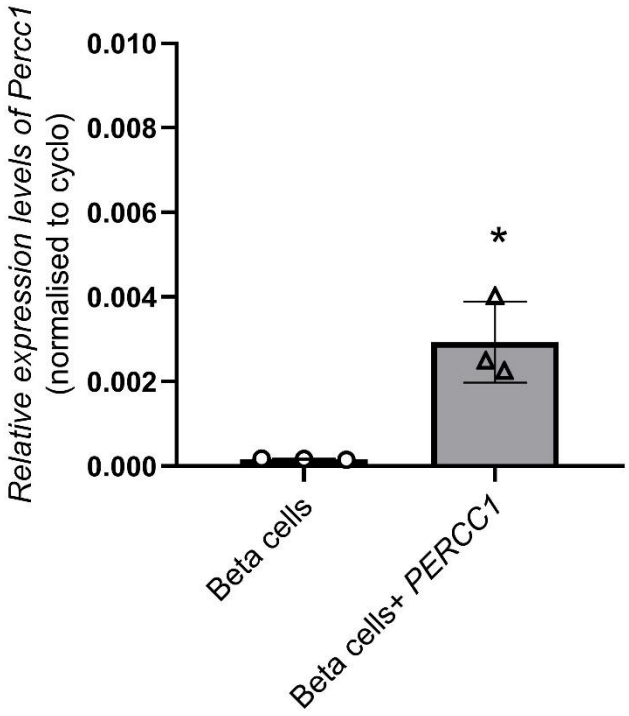


ESM Figure 6: PERCC1 is a nuclear protein.
EndoC-BH1 cells were transduced with a lentiviral vector expressing PERCC1-HA. The cells were next fixed and stained with anti-HA antibody that revealed a nuclear localization of the protein within the transduced cells. Some of the positively transduced cells show SST positive staining in their cytoplasm.



ESM Figure 7: NKX2.2 knockdown upregulated *HHEX*, *PERCC1* and *SST* expression in EndoCBH1

(a) EndoC-BH1 cells were transfected with siRNAs targeting NKX2.2 (b) that efficiently reduces its expression. (b) This reduction in NKX2.2 expression was paralleled by the induction of *PERCC1*, *HHEX* and *SST* similar to what is observed upon MNX1 knockdown. Data represent mean values of three individual experiments ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. RISC, RNA-induced silencing complex.



ESM Figure 8: Expression of mouse *Percc1* in mouse beta cells infected with PERCC1-LV. Mouse beta cells that overexpress exogenous PERCC1 manifest and increase in the endogenous mouse *Percc1* compared to controls. However, it should be noted that these levels are still marginal (Ct 38 to Ct34). Data represent mean values of three individual experiments ± SEM. * $p<0.05$