Supplementary Files

Obesity-induced *miR-455* upregulation promotes adaptive pancreatic β-cell proliferation through the CPEB1/CDKN1B pathway

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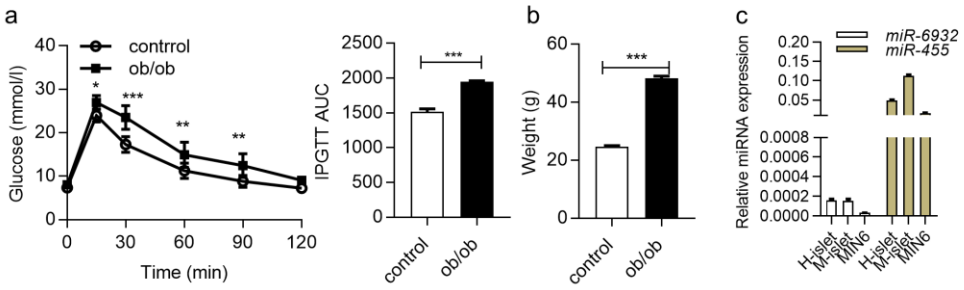
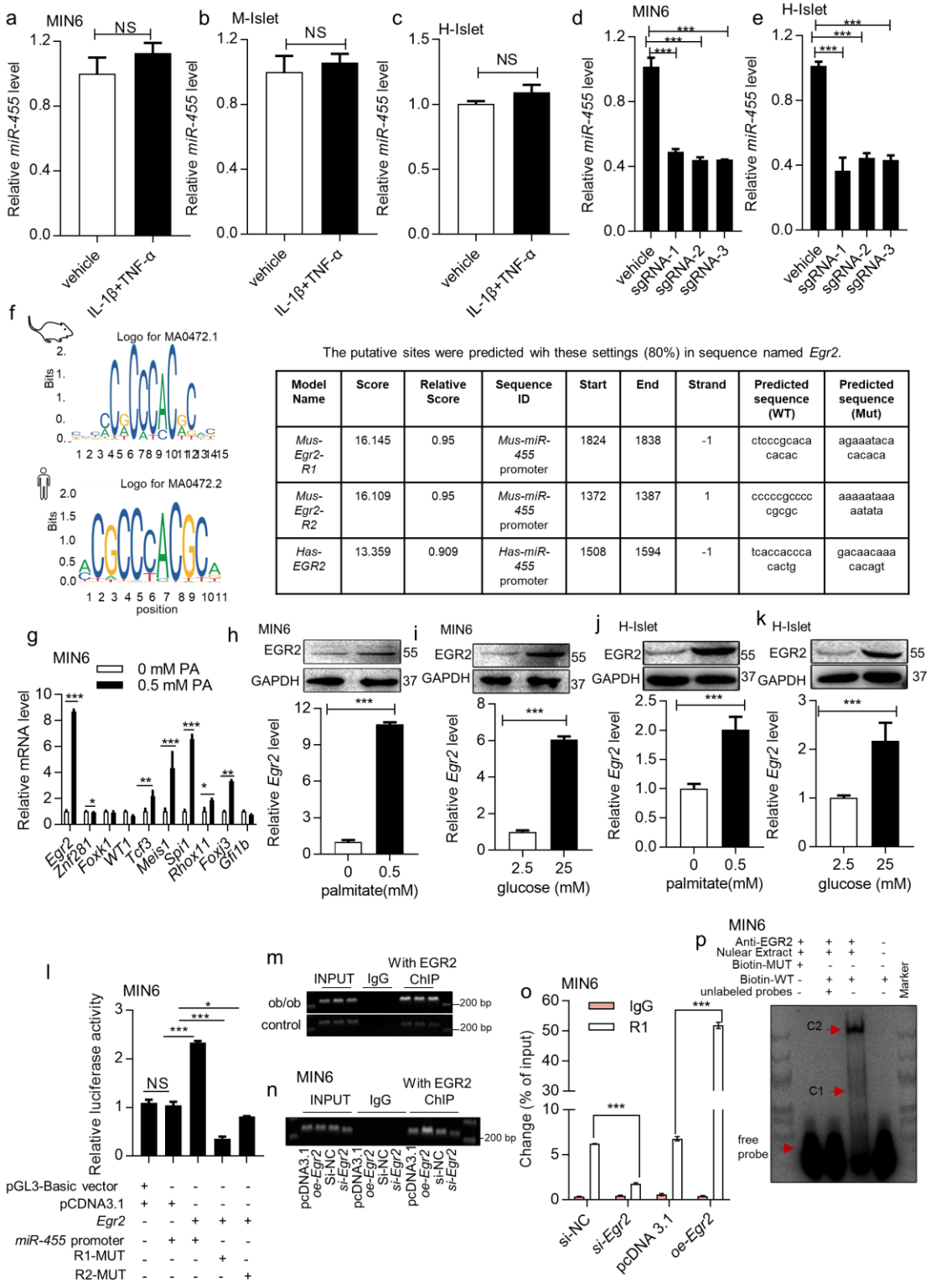
Supplementary Figure 1



Supplementary Figure 1 (a) Blood glucose measurements during a glucose tolerance test in 8-week *ob/ob* mice and control mice (n = 6 mice per group). (b) The weight of 8-week-old *ob/ob* mice (n = 6 mice per group). (c) The expression levels of *miR-455* and *miR-6932* in human islets, mouse islets and MIN6 cells. The data are presented as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure 2

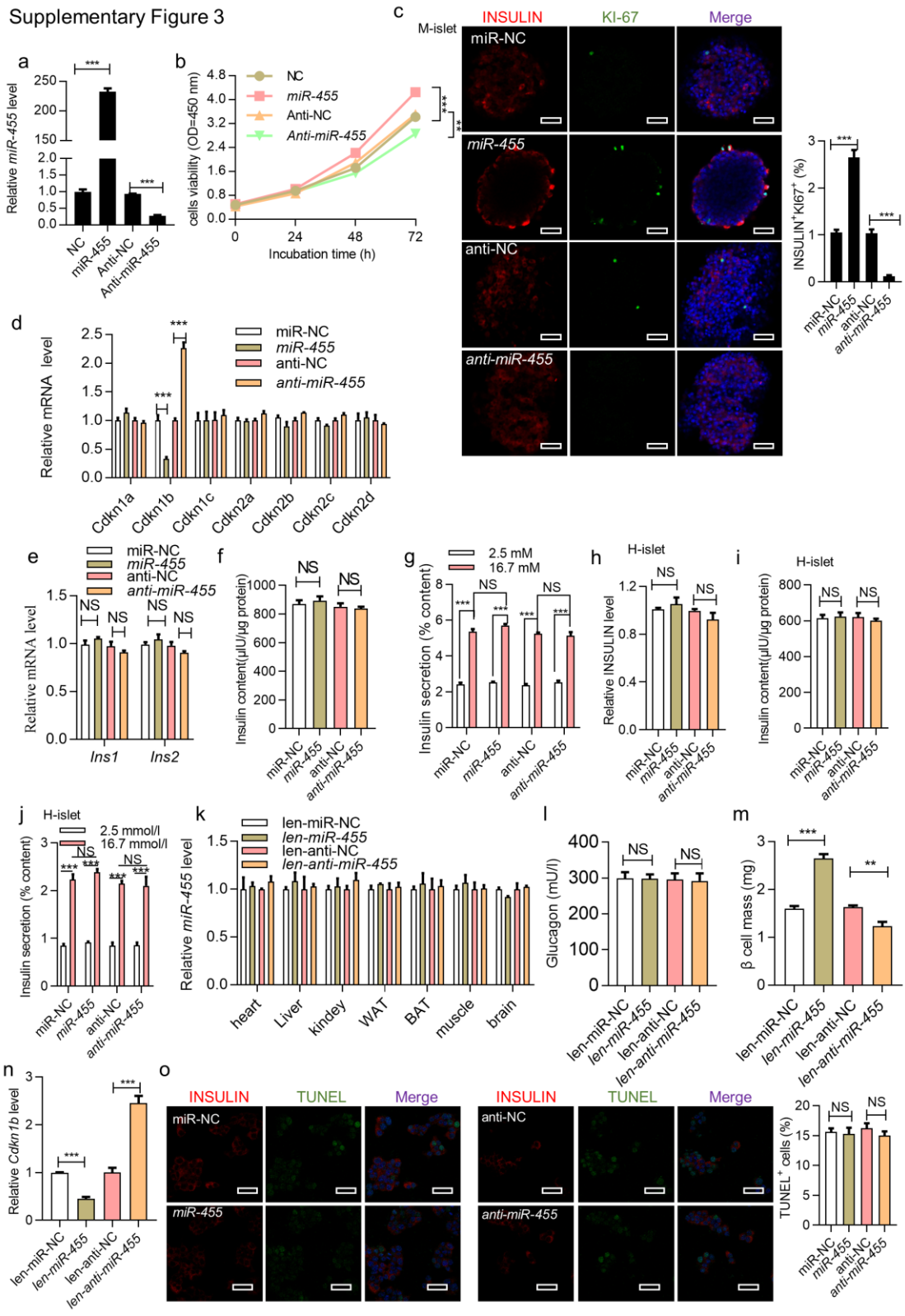


The putative sites were predicted wih these settings (80%) in sequence named *Egr2*.

Model Name	Score	Relative Score	Sequence ID	Start	End	Strand	Predicted sequence (WT)	Predicted sequence (Mut)
Mus-Egr2-R1	16.145	0.95	*Mus-miR-455* promoter	1824	1838	-1	ctcccgcaca cacac	agaaataca cacaca
Mus-Egr2-R2	16.109	0.95	*Mus-miR-455* promoter	1372	1387	1	cccccgcccc cgcgc	aaaaataaa aatata
Has-EGR2	13.359	0.909	*Has-miR-455* promoter	1508	1594	-1	tcaccaccca cactg	gacaacaaa cacagt

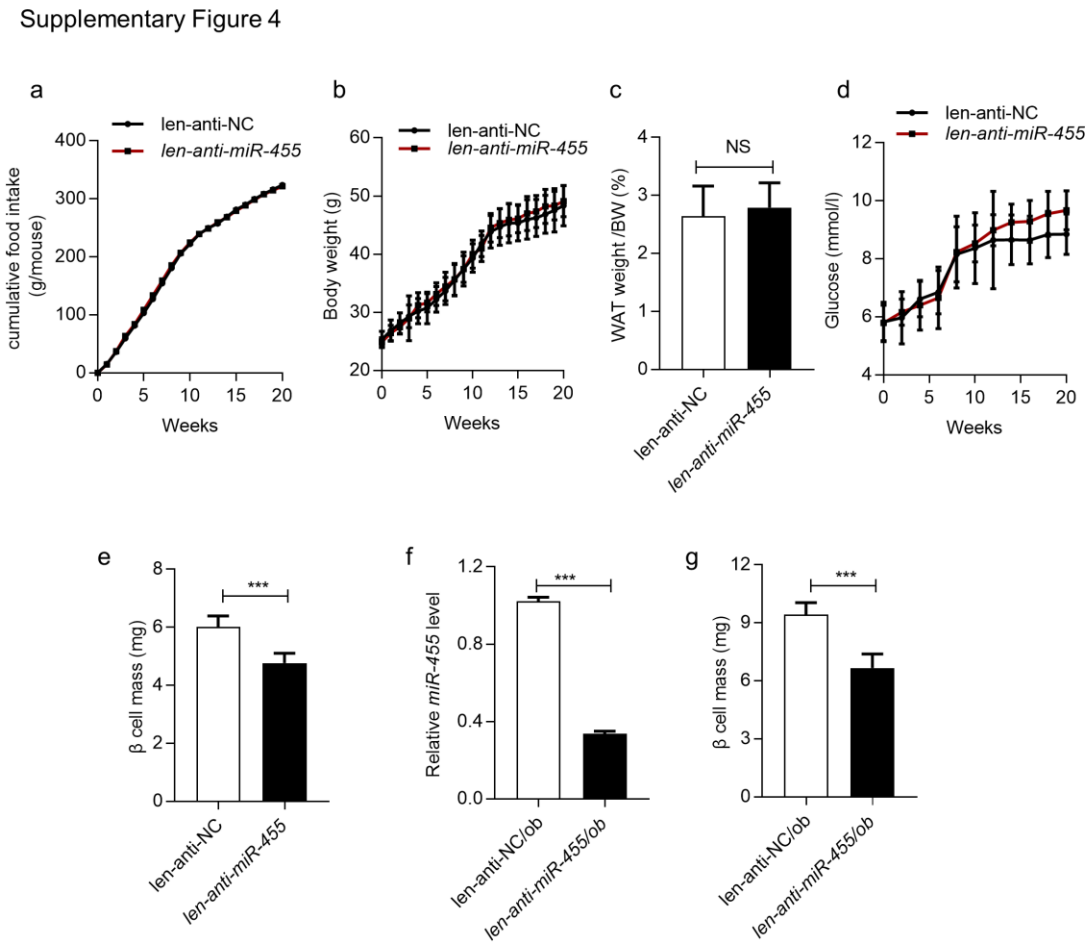
Supplementary Figure 2 MIN6 cells (a), mouse primary islets (b, n = 3), and human islets (c) were incubated with a combination of interleukin-1β (IL-1β, 5 ng/ml) and tumor necrosis factor-α (TNF-α, 30 ng/ml) for 48 h, and qRT-PCR was performed to examine the *miR-455* expression level. (d-e) The expression level of *miR-455* in MIN6 cells (d) and in human islets (e) transfected

with *miR-455* sgRNA. (f) The binding sites of *Egr2* in the *mus-miR-455* and *human-miR-455* promoters predicted by JASPAR. (g) MIN6 cells were incubated with 0.5 mM palmitate for 48 h, and qRT-PCR was performed to examine the expression levels of transcription factors predicted by JASPAR. MIN6 cells and human islets were incubated with 0.5 mM palmitate or 25 mM glucose for 48 h, and the protein and mRNA levels of EGR2 were determined by western blotting and qRT-PCR (h-k), respectively. (l) Relative luciferase activity in MIN6 cells cotransfected with *Egr2* overexpression plasmid and a luciferase reporter containing either *miR-455 promoter* or *miR-455-MUT* (*Egr2*-binding sequence mutated). The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. ChIP experiments were conducted to verify that *Egr2* binds to the promoter of *miR-455* in islets from obese mice (m). ChIP experiments were conducted to verify that *Egr2* binds to the promoter of *miR-455* in MIN6 cells transfected with *oe-Egr2* or *si-Egr2*, followed by RT-PCR (n) and qRT-PCR (o) assays. (p) Based on EMSA results, *Egr2* can directly bind to the *miR-455* promoter in MIN6 cells. C1 and C2 represent nuclear protein-*miR-455* probe complexes and nuclear protein-*miR-455* probe-anti-EGR2 complexes, respectively. The data are presented as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 3 MIN6 cells were transfected with *miR-455* or *anti-miR-455* for 48 h, and qRT-PCR was performed to detect the *miR-455* expression level (a). (b) CCK-8 assays were carried out at 24, 48, and 72 h after transfection. (c) INSULIN^{+}/KI-67^{+} cells were counted via immunofluorescence. Magnification: 20 x; scale bar, 20 μm. (d) The mRNA expression levels of

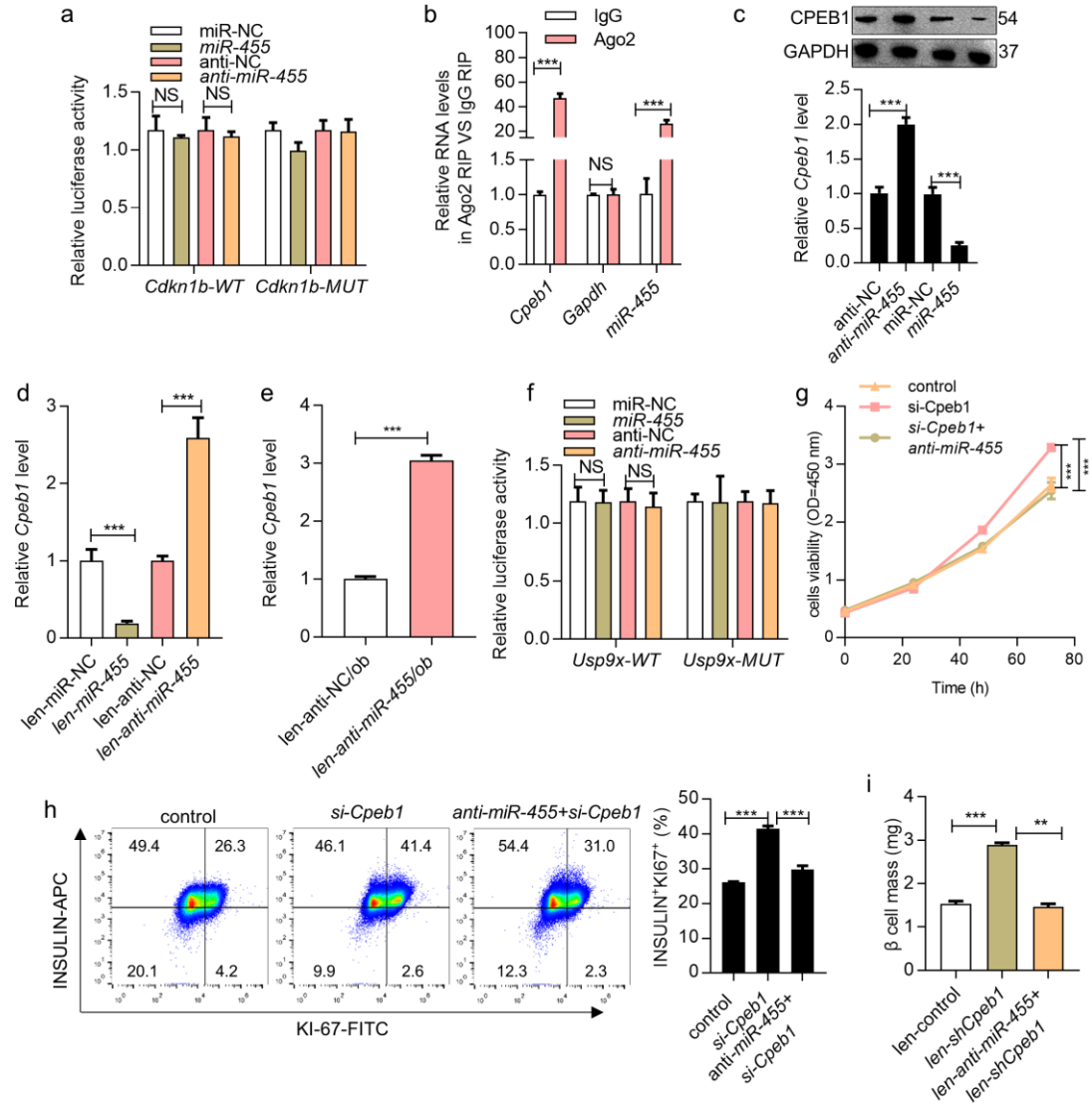
Cdkn1 and *Cdkn2* family genes. The *Ins1* and *Ins2* expression levels (e), insulin content (f) and insulin secretion (g) in MIN6 cells. INSULIN levels (h), insulin content (i) and insulin secretion (j) in human islets. (k) The *miR-455* expression levels in different tissues from *len-miR-455* or *len-anti-miR-455* mice (n = 3). (l) The glucagon level in serum from *len-miR-455* or *len-anti-miR-455* mice (n = 9 mice per group). (m) The β cell mass in *len-miR-455* or *len-anti-miR-455* mice (n = 3). (n) The mRNA levels of *Cdkn1b* in the islets of *len-miR-455* or *len-anti-miR-455* mice (n = 3). (o) $INSULIN^+/TUNEL^+$ cells were counted via immunofluorescence. Magnification: 20 x; scale bar, 20 μm. The data are presented as the mean ± SD. ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 4 Eight-week-old male *len-anti-miR-455* mice and control mice (lentivirus-MIP (mouse insulin2 promoter)-LV3/H1 vector) were exposed to an HFD for 20 weeks.

Then, cumulative energy intake (a, *n* = 9 mice per group), changes in body weight (b, *n* = 9 mice per group), the white adipose tissue weight per body weight ratio (c, *n* = 9 mice per group) and fasting blood glucose level (FBG) (d, *n* = 9 mice per group) were measured. (e) The β cell mass of *len-anti-miR-455* mice was measured (*n* = 3). (f) *miR-455* expression levels in the islets of *len-anti-miR-455/ob* mice (*n* = 3). (g) The β cell mass in *len-anti-miR-455/ob* mice was measured (*n* = 3). The data are presented as the mean ± SD. *** $p < 0.001$.

Supplementary Figure 5



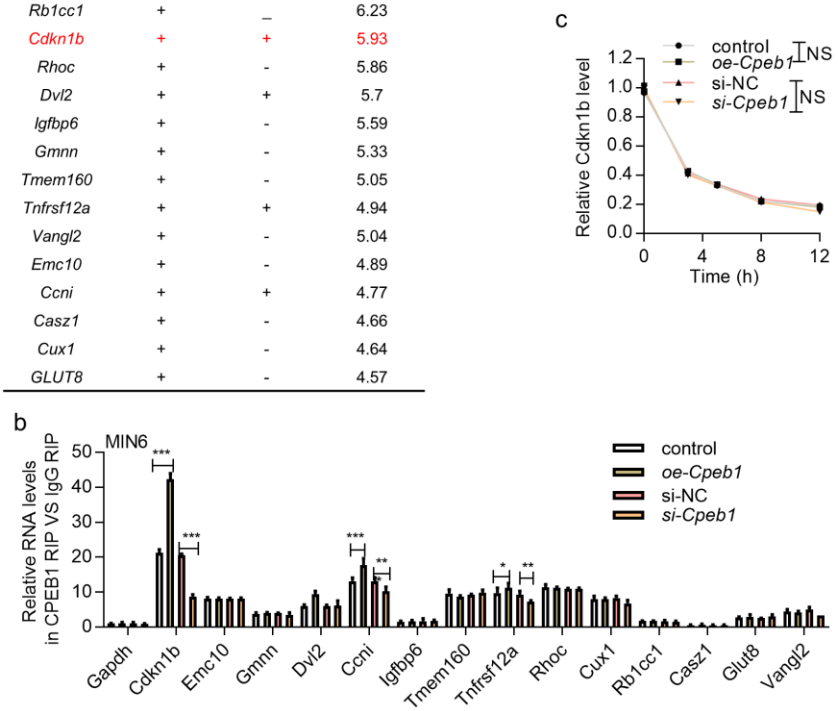
Supplementary Figure 5 (a) Relative luciferase activity of MIN6 cells cotransfected with *miR-455* mimic and a luciferase reporter containing either *Cdkn1b-WT* or *Cdkn1b-MUT*. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (b)

MIN6 cells were transfected with *Cpeb1* overexpression plasmid for 48 h. Then, MIN6 cell lysate was subjected to anti-Ago2 RNA immunoprecipitation (RIP), and the *miR-455* and *Cpeb1* expression levels were examined via qRT-PCR. *Gapdh* served as a control (nonspecific IgG served as a negative control). (c) CPEB1 mRNA and protein levels in MIN6 cells transfected with *miR-455* or *anti-miR-455*. (d-e) The *Cpeb1* mRNA level in islets from *len-miR-455* mice and *len-anti-miR-455/ob* mice (n = 3). (f) Relative luciferase activity of MIN6 cells cotransfected with *miR-455* mimic and a luciferase reporter containing either *Usp9x-WT* or *Usp9x-MUT*. The data are presented as the relative ratio of Renilla luciferase activity to firefly luciferase activity. (g) CCk-8 assays were carried out at 24, 48, and 72 h after transfection with *si-Cpeb1* or *si-Cpeb1* and *anti-miR-455*. (h) The $INSULIN^+/KI\text{-}67^+$ cells were counted via flow cytometry. (i) The β cell mass in *len-shCpeb1* mice was measured (n = 3). The data are presented as the mean ± SD. ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure 6

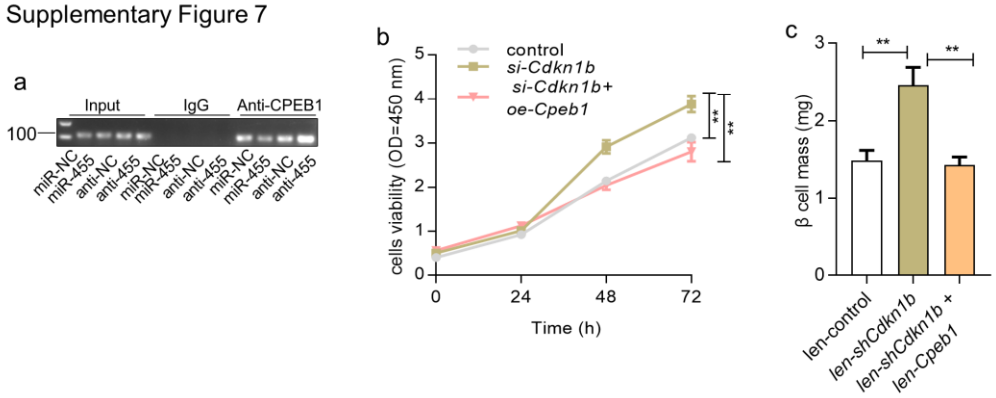
a

Gene name	ctaRAPID	RBPmap	Score
Rb1cc1	+	_	6.23
Cdkn1b	+	+	5.93
Rhoc	+	-	5.86
Dvl2	+	+	5.7
Igfbp6	+	-	5.59
Gmnn	+	-	5.33
Tmem160	+	-	5.05
Tnfrsf12a	+	+	4.94
Vangl2	+	-	5.04
Emc10	+	-	4.89
Ccni	+	+	4.77
Casz1	+	-	4.66
Cux1	+	-	4.64
GLUT8	+	-	4.57



Supplementary Figure 6 (a) ctaRAPID and RBPmap were used to predict mRNAs that can bind to CPEB1. (b) MIN6 cells were transfected with *oe-Cpeb1* and *si-Cpeb1* for 48 h. Then, MIN6 cell lysate was subjected to anti-CPEB1 RNA immunoprecipitation (RIP), and the precipitated RNAs were examined via qRT-PCR. *Gapdh* served as a control (nonspecific IgG served as a negative control). (c) MIN6 cells transfected with *oe-Cpeb1* or *si-Cpeb1* were treated with actinomycin D (10 μg/ml). RNA was isolated at various time points after the treatment and analyzed via RT-qPCR. The data are presented as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure 7



Supplementary Figure 7 (a) RT-PCR-RIP was performed to verify the CPEB1–*Cdkn1b* interaction. (b) CCk-8 assays were carried out at 24, 48, and 72 h after transfection with *si-Cdkn1b* or *si-Cdkn1b and oe-Cpeb1*. (c) The β cell mass in *len-shCpeb1* mice was measured (n = 3). The data are presented as the mean ± SD. ** $p < 0.01$.

Supplementary Spreadsheets:

Table S1 RNA isolated from islets of control mice and *ob/ob* mice, this table shows significantly changed miRNA (Log2 fold change ≥ 2). The mean expression of each group was presented in a log2 scale.

miR_name	control-read	*ob/ob*-read	log2(fold change)	p-value	FDR
mmu-miR-6932-3p	0.00	5.20	12.34	0.000899	0.018575
mmu-miR-455-5p	1075.12	15104.93	3.84	3.60E-08	2.13E-06
mmu-miR-677-3p	1.25	13.57	3.44	6.30E-05	0.002277
mmu-miR-6546-5p	1.29	12.19	3.25	0.000176	0.005328
mmu-miR-212-3p	815.84	7001.88	3.10	9.37E-20	2.44E-17
mmu-miR-132-3p	5648.74	41049.84	2.86	2.43E-21	7.91E-19
mmu-miR-132-5p	589.23	4146.32	2.81	3.84E-16	5.55E-14
mmu-miR-501-5p	91.11	636.67	2.80	2.31E-13	2.31E-11
mmu-miR-212-5p	516.13	3417.69	2.73	4.47E-15	5.81E-13
mmu-miR-1945	2.42	14.32	2.56	0.000368	0.008713
mmu-miR-6546-3p	2.31	11.77	2.35	0.002614	0.038215
mmu-miR-1983	38.48	185.64	2.27	2.38E-08	1.55E-06
mmu-miR-665-5p	5.71	25.86	2.18	9.37E-05	0.003047
mmu-miR-7093-3p	8.78	36.52	2.06	7.71E-05	0.002638
mmu-miR-690	62.28	256.98	2.04	1.63E-07	9.23E-06
mmu-miR-712-5p	3.44	14.07	2.03	0.002991	0.042759
mmu-miR-150-5p	3591.51	867.79	-2.05	1.25E-09	9.00E-08
mmu-miR-142a-3p	6707.08	1492.88	-2.17	4.24E-11	3.67E-09
mmu-miR-669o-5p	18.09	3.74	-2.28	0.000317	0.00792
mmu-miR-592-5p	101.53	18.73	-2.44	3.20E-08	1.98E-06
mmu-miR-216b-5p	5763.70	974.22	-2.56	2.46E-14	2.91E-12
mmu-miR-216a-5p	13489.70	2161.92	-2.64	2.81E-16	4.58E-14
mmu-miR-217-5p	6074.72	865.87	-2.81	1.54E-16	2.86E-14
mmu-miR-216b-3p	741.02	102.69	-2.85	5.42E-14	5.88E-12
mmu-miR-216a-3p	99.84	13.45	-2.89	4.48E-10	3.65E-08
mmu-miR-6969-5p	30.21	4.05	-2.90	1.22E-06	6.10E-05
mmu-miR-142a-5p	12379.31	1612.28	-2.94	3.99E-19	8.66E-17
mmu-miR-217-3p	297.30	38.14	-2.96	3.63E-13	3.37E-11
mmu-miR-150-3p	10.44	1.27	-3.03	0.000203	0.005812
mmu-miR-6969-3p	69.65	8.49	-3.04	2.76E-09	1.89E-07
mmu-miR-196b-5p	13.90	1.26	-3.46	1.28E-05	0.000522
mmu-miR-184-3p	4929.72	257.98	-4.26	1.16E-30	7.54E-28
mmu-miR-122-3p	191.54	3.09	-5.95	8.33E-28	3.61E-25
mmu-miR-122-5p	5805.91	84.06	-6.11	4.47E-52	5.81E-49

Table S2 The primers used in PCR (5'-3').

Gene name	Forward Primer	Reverse Primer
oe-*Cpeb1*	ctatagggagacccaagctgATGGCTTTCTCTCTGGAAGAAGC	gctgatcagcgggtttaaacTCAGTTCTTCTGGTTCCTCATTAGG
oe-Egr2	ctatagggagacccaagctgATGATGACCGCCAAGGCC	gctgatcagcgggtttaaacTCACGGTGTCCTGGTTCGAG
Has-EGR2	ctatagggagacccaagctgtATGATGACCGCCAAGGCC	gctgatcagcgggtttaaacTCAAGGTGTCCGGGTCCG
oe-Cdkn1b	ctatagggagacccaagctgATGTCAAACGTGAGAGTGTCTAACG	gctgatcagcgggtttaaacTTACGTCTGGCGTCGAAGGC
promoter-*miR-455*	gagctcttacgcgtgctagcATTGGCAGAGGACAAGAACAGG	cagtaccggaatgccaagctGGCAGGCCCCTACTTCATG
promoter-*miR-455-MUT*	gatctttatgtgtgtgtTGTGGTATGAATCGCCTTGTAGA	acacacacataaagaTCCCGCAGGCACACTCCA
Has-miR-455 promoter	agaacatttctctatcgataGCTCGACTTGCCTGCCAGG	agcttacttagatcgcagatGATGACATAGGCCTTGAGGCA
MUS-EMSA-WT	CGGGAGTGTGTGTGCGGGAGTGTG	CACACTCCCGCACACACACTCCCG
MUS-EMSA-MUT	CGGGATGTGTGTGTATTTCTTGTG	CACAAGAAATACACACACACCCCG
HAS-EMSA-WT	CTGTCCAGTGTGGGTGGTGACAATC	GATTGTCACCACCCACACTGGACAG
HAS-EMSA-MUT	CTGTCACTGTGTTTGTTGTCCAATC	GATTGGACAACAAACACAGTGACAG
Cpeb1-WT	TCGAGtgacatgtct gccaattttg ttctgggtgg gcacatttgg taatcctgtc tgT	CTAGAcagacaggattaccaaatgtgcccacccagaacaaaattggcagacatgtcaC
Cpeb1-MUT	TCGAGtgacatgtct gccaatttCACtcCgggtgTTACACCtttgg taatcctgtc tgT	CTAGAcagacaggattaccaaaGGTGTAAcacccGgaGTGaaattggcagacatgtcaC
Cdkn1b-WT	TCGAGttgtactacctgtgtatatagtttttatcttttactct gtagcacataaT	CTAGAttatgtgctacagagtaaaagataaaaactatatacacaggtagtacaaC
Cdkn1b-MUT	TCGAG TtgtactacctgtgtatatagtGGCCGCcttttactctgt agcacataa T	CTAGAttatgtgctacagagtaaaagGCGGCCactatatacacaggtagtacaaC

Table S3 The sequence of siRNA or sgRNA.

SiRNA/sgRNA name	Forward promer	
si-Cpeb1	GCAGCACACAGUCAGUAUUTT	AAUACUGACUGUGUGCUGCTT
si-Cdkn1b	CCCGGUCAAUCAUGAAGAATT	UUCUUCAUGAUUGACCGGGTT
si-Egr2	AAGGTATCATCAATATTGTGAGT	ACTCACAATATTGATGATACCTT
miR-455-sgRNA-1	CACCGGTGGCTCACTTCCCAAGCAG	AAACCTGCTTGGGAAGTGAGCCACC
miR-455-sgRNA-2	CACCGGGTCGCCCGCGCCAGGCCCA	AAACTGGGCCTGGCGCGGGCGACCC
miR-455-sgRNA-3	CACCGGCTGCATCCCTTGCTGCGTC	AAACGACGCAGCAAGGGATGCAGCC
Has-si-EGR2	AGCUGUCUGACAACAUCUACC	UAGAUGUUGUCAGACAGCUGG
Has-miR-455 sgRNA-1	CACCGACTAACAGCGGCTGCGAACC	AAACGGTTCGCAGCCGCTGTTAGTC
Has-miR-455 sgRNA-1	CACCGAAGGGCAGTAACCTCGCCCG	AAACCGGGCGAGGTTACTGCCCTC
Has-miR-455 sgRNA-1	CACCGGCAGAAGGAAGCTCGCGGCT	AAACAGCCGCGAGCTTCCTTCTGCC

Table S4 The primers used in Real-time PCR (5'-3').

Gene	Forward Primer	Reverse Primer
Mus-miR-455	TAATCTGACTATGTGCCTTTGGACT	TATGGTTTTGACGACTGTGTGAT
Has-miR-455	TAATCTGACTATGTGCCTTTGGACT	TATGGTTTTGACGACTGTGTGAT
Cpeb1	AAGGATTGCTGGGACAACCAA	GGCCACGGGGAGATTCTTG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Cdkn1b	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
ChIP-R1	AGTACAGTGCGTGGCTGTGTCTGCG	AACTCACGAGCACGGCTCAGCCCTG
ChIP-R2	GCTCCTCCCGGCGCCGTCG	TGGGTCCCCAAGTGTGACGCTG
HAS-ChIP	GGCCCTGGGTGACCCTTGAACTGAT	GCTGCGAACCCGGAGTGCCAACATT
Ins1	CACTTCCTACCCCTGCTGG	ACCACAAAGATGCTGTTTGACA
Ins2	GCTTCTTCTACACACCCATGTC	AGCACTGATCTACAATGCCAC
Zfp281	CCTTCCCCCAGAGTATGGTTA	TCCTTCTTGAAAGTCATGTCCG
Foxl1	GAGCAGAGGGTCACACTGAAC	CTTCCTGCGCCGATAATTGC
Tcf3	CGCACCAGCAGTACAGATGAG	CAGCTTGGTCTGCGCCTTA
Meis1	GCAAAGTATGCCAGGGGAGTA	TCCTGTGTTAAGAACCGAGGG
Spi1	ATGTTACAGGCGTGCAAAATGG	TGATCGCTATGGCTTTCTCCA
Rhox11	TGAGCTTCTGTGAGGAAGAAATC	CCTCTTTCGGGATGCCTGTTA
Foxj3	AGCCTAACATCTATGGACTGGT	GGTCAAGGAGTGCATTCTTCTTA
Gfi1b	ATGCCACGGTCCTTTCTAGTG	GGAAGGCTCTGGTTCAGCAA
Wt1	GAGAGCCAGCCTACCATCC	GGGTCCTCGTGTTTGAAGGAA
Ecm10	TCAATGGCCTCTACAGGGTCC	AGCTTCTGAACCATCAAGTGTC
Igfbp6	GCTGCTAATGCTGTTGTTCGC	GCACTTAGGGCTGTAGACCC
Tmem160	CTCCGCAAGGCACATGAGA	AGACCCACCGCATAGGAGG
Casz1	CCTCGCCTTTCTTGGGCAA	CACTGGGCATCACAGAAGTCC
Gmnn	GGGAGCCCAAGAGAATGTGAA	CAAGCCTTTTGGCAACTCATTT
Vangl2	ACTCGGGCTATTCCTACAAGT	TGATTTATCTCCACGACTCCCAT
Cux1	GGGGGAACAGGTTTCCAATAC	AAGGACTGCATTAGCTGTCGC
Dvl2	GGTGTAGGCGAGACGAAGG	GCTGCAAAACGCTCTTGAAATC
GLUT8	CCCTTCGTGACTGGCTTTG	TGGGTAGGCGATTTCCGAGAT
Ccni	CAGGACCTTTGGAAAACCAGAG	TTGGATGGGCTTTTACTGTAGC
Rb1cc1	GACACTGAGCTAACTGTGCAA	GCGCTGTAAGTACACACTCTTC
Tnfrsf12a	GTGTTGGGATTCGGCTTGGT	GTCCATGCACTTGTCGAGGTC
Rhoc	ATGGCTGCGATCCGAAAGAAG	GCACGTAGACCTCTGGAAACT
Cdkn1a	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Cdkn1c	CGAGGAGCAGGACGAGAATC	GAAGAAGTCGTTCGCATTGGC
Cdkn2a	CGCAGGTTCTTGGTCACTGT	TGTTCACGAAAGCCAGAGCG
Cdkn2b	CCCTGCCACCCTTACCAGA	CAGATACCTCGCAATGTCACG
Cdkn2c	CCTTGGGGGAACGAGTTGG	AAATTGGGATTAGCACCTCTGAG
Cdkn2d	CTGAACCGCTTTGGCAAGAC	GCCCTCTCTTATCGCCAGAT
Has-CPEB1	GATGCAAATGACTTGTGCCTTG	GGCTGAGGAATCTGAGTCCTG

Has-EGR2	TCAACATTGACATGACTGGAGAG	AGTGAAGGTCTGGTTTCTAGGT
Has-Cdkn1b	AACGTGCGAGTGTCTAACGG	CCCTCTAGGGGTTTGTGATTCT
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Table S5 The antibodies used in Western blot, IHC and IF.

Protein	Catalog	Dilution for WB	Dilution for IHC and IF	FACS
INSULIN-647	Cell Signaling (c27c9)			1:50
KI-67 FITC	Biolegend (652409)			1:50
EGR2	Abcam (ab108399)	1:1000		
CPEB1	Abcam (ab73287)	1:1000		
CDKN1B	Abcam (193379)	1:1000		
INSULIN	Servicebio (GB13121)		1:500	
GLUCAGON	CST (2760)		1:500	
Argonaute 2	CST (2897s)	1:1000		
KI-67	Abcam (ab1667)		1:500	
GAPDH	Abcam (ab181602)	1:1000		