Supplementary information files

The circular RNA *Cdr1as*, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells

Huanyu Xu, [1]* Sen Guo, [1] Wei Li, [2] Ping Yu, [1]*

[1]Institute of Genomic Medicine, Wenzhou Medical University, Zhejiang, China, [2]Zhejiang Provincial Key Laboratory of Medical Genetics; School of Laboratory Medicine & Life Sciences, Wenzhou Medical University, Zhejiang, China

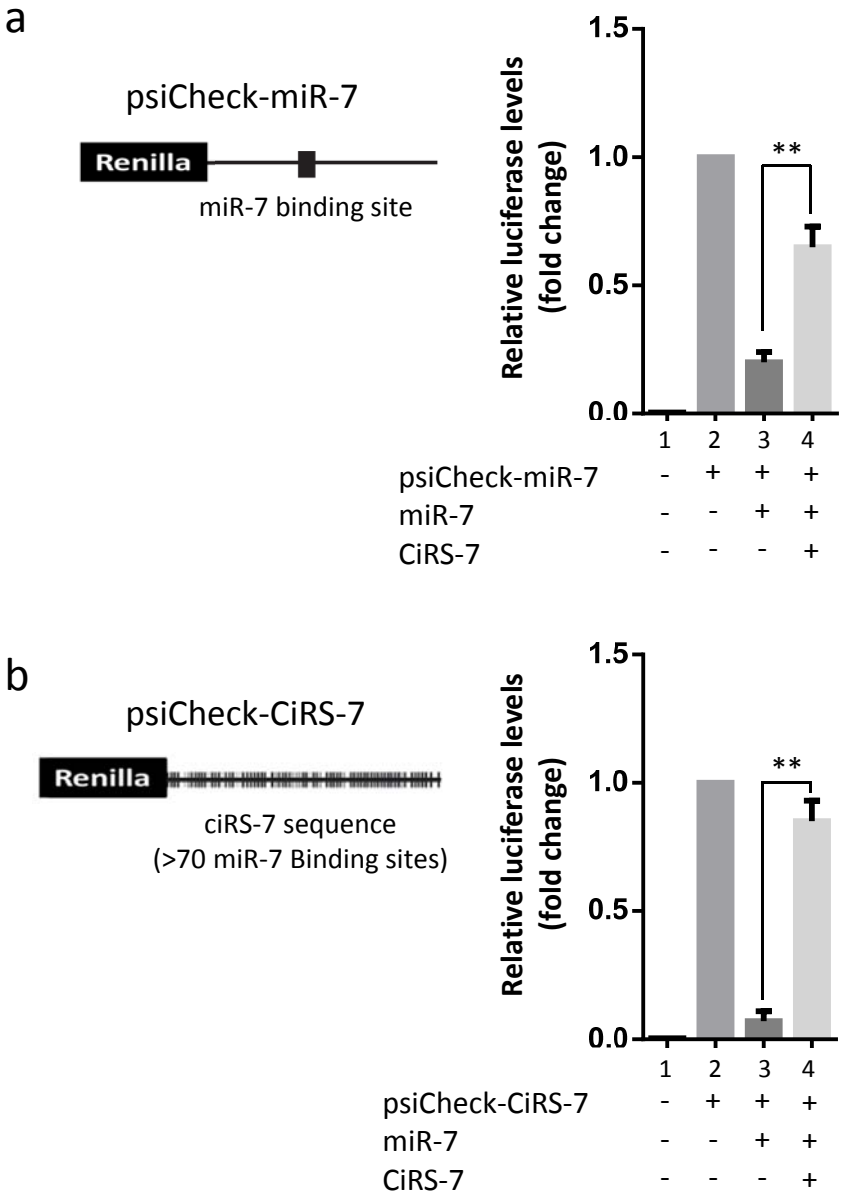
Corresponding author

Ping Yu, and Huanyu Xu;

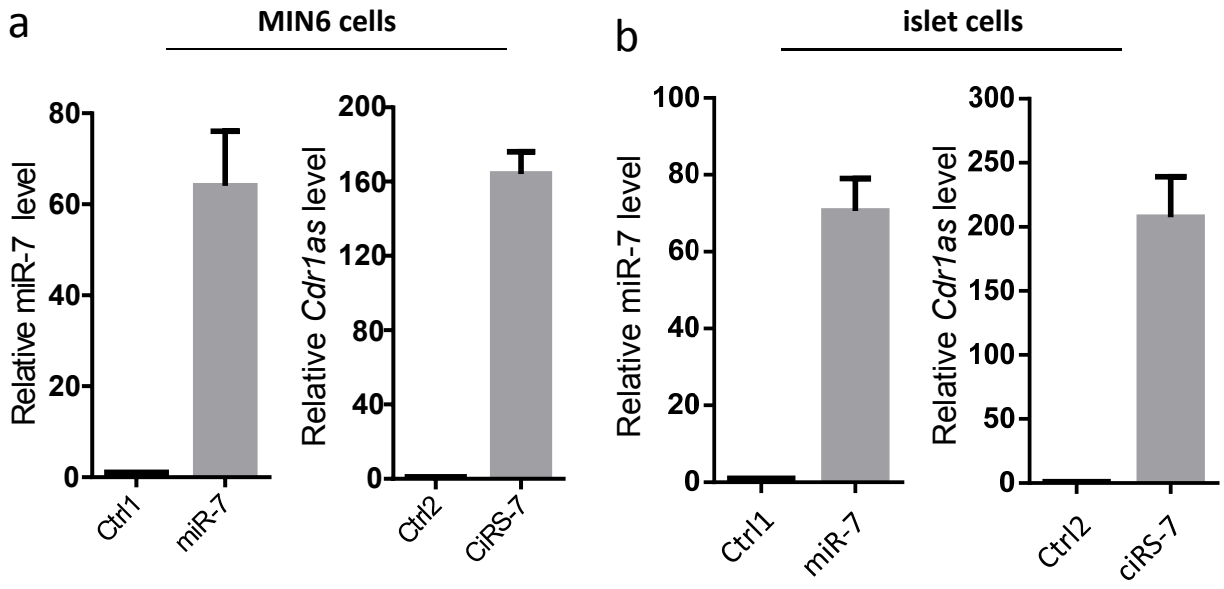
Institute of Genomic Medicine, Wenzhou Medical University, Zhejiang province, China;

Phone: +86 159-8878-1270; Fax: +86-577-86699399

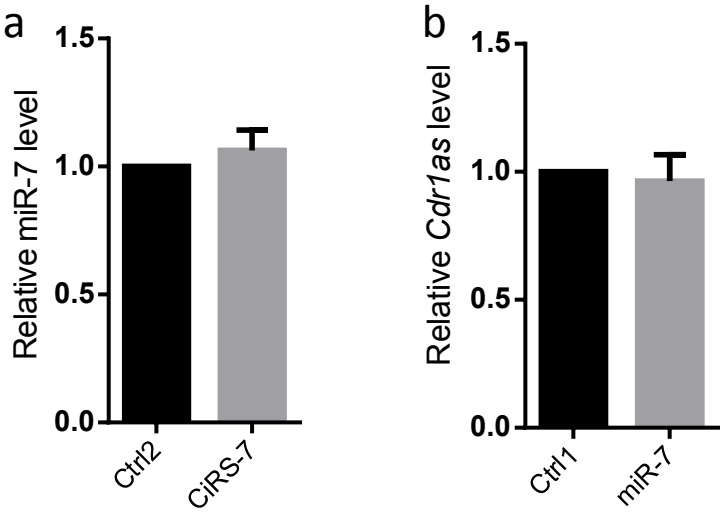
E-mail: jyzwzyu@gmail.com and xuhuanyu1979@gmail.com



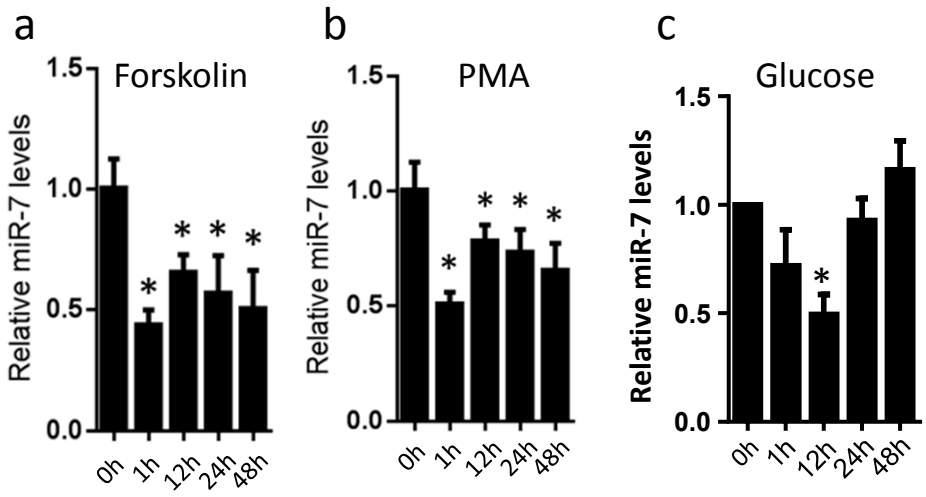
Supplementary Fig. 1 Physical interaction of Cdr1as and miR-7 in islet cells. (**a**) The renilla activity of the psiCheck-miR-7 (column 2) was suppressed about 80% by transfection of miR-7 (column 3), but not scrambled miR-7 (not shown). However, transfection of *Cdr1as* (column 4) significantly rescued the inhibitory effect of miR-7 on renilla activity. (**b**) Likewise, the renilla activity of the psiCheck-CiRS-7 (column 2) was also suppressed about 90% by transfection of miR-7 (column 3), but not scrambled miR-7 (not shown). However, transfection of *Cdr1as* (column 4) significantly rescued the inhibitory effect of miR-7 on renilla activity. Luciferase activity, as the corresponding gene is included in the same vector, was served as an inner control and measured by using a Microplate Luminometer (Berthold Technologies, Germany). *n* =3, Mean ± SEM, representing three independent experiments In triplicates. **, $P < 0.01$



Supplementary Fig. 2 – Validation of expression miR-7 and *Cdr1as* expression in MIN6 cells (**a**) and islet cells (**b**) by transfecting each corresponding plasmid (miRVec-miR-7 or pCDNA3-ciRS-7) for 48, and compared with controls (see details in Materials and Methods). Experiments were independently performed for three times in triplicates.



Supplementary Fig. 3 Quantification of miR-7 (**a**) or *Cdr1as* level (**b**) levels derived from transfections with pCDNA3-ciRS-7 along with plasmids expressing miR-7 (miRVec-miR-7). Experiments were independently performed three time in triplicates.



Supplementary Fig. 4 miR-7 expression responding to forskolin, PMA and glucose. (**a**) miR-7 expression in islets treated by forskolin (10 μM). (**b**) PMA (1 μM) stimulated miR-7 expression in islets. (**c**) Effect of glucose on miR-7 expression in islets. $n = 3$, *, $P < 0.05$; **, $P < 0.01$.

Supplementary Table 1. Primers used for quantitative real-time PCR

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
miR-7	TGGAAGACTAGTGATTTTGTTGT	Universal primer(Qiagen)
mus-ciRS-7	GTGTCTCCAGTGTATCGGCG	TACTGGCACCACTGGAAACC
Mus-cdr1	TGGAAGATCACGATTGTCTGGA	GGTGCCAGTACCAAGGTCTTC
Pax6	GCGGAGTTATGATACCTACACC	GAAATGAGTCCTGTTGAAGTGG
WT-Myrip	GCaagcttCAGGGCAGGGGCTGAAATAA	ACACTGGTAGCACAAAGGCAtgatcaGC
Mut-Myrip	TGTGaCaagCTATGATGCTCCT	ATAGcttGtCACAAGGACCCCC
Q-Myrip	TCGAAGCATCTTCCGTGACC	GGTCAAGGCACTGTCGTGTA
GAPDH	GTCGGTGTGAACGGATTTG	GAATTTGCCGTGAGTGGAG
Insulin1	AAGCAGGTCATTGTTTCAACA	TTGGGTGTGTAGAAGAAGCC
Insulin2	GCGTGGCTTCTTCTACACACC	CCAGTGCCAAGGTCTGAAGG