

Supplementary materials

Circular RNA *circPPM1F* modulates M1 macrophage activation and pancreatic islet inflammation in type 1 diabetes mellitus

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Figure S1

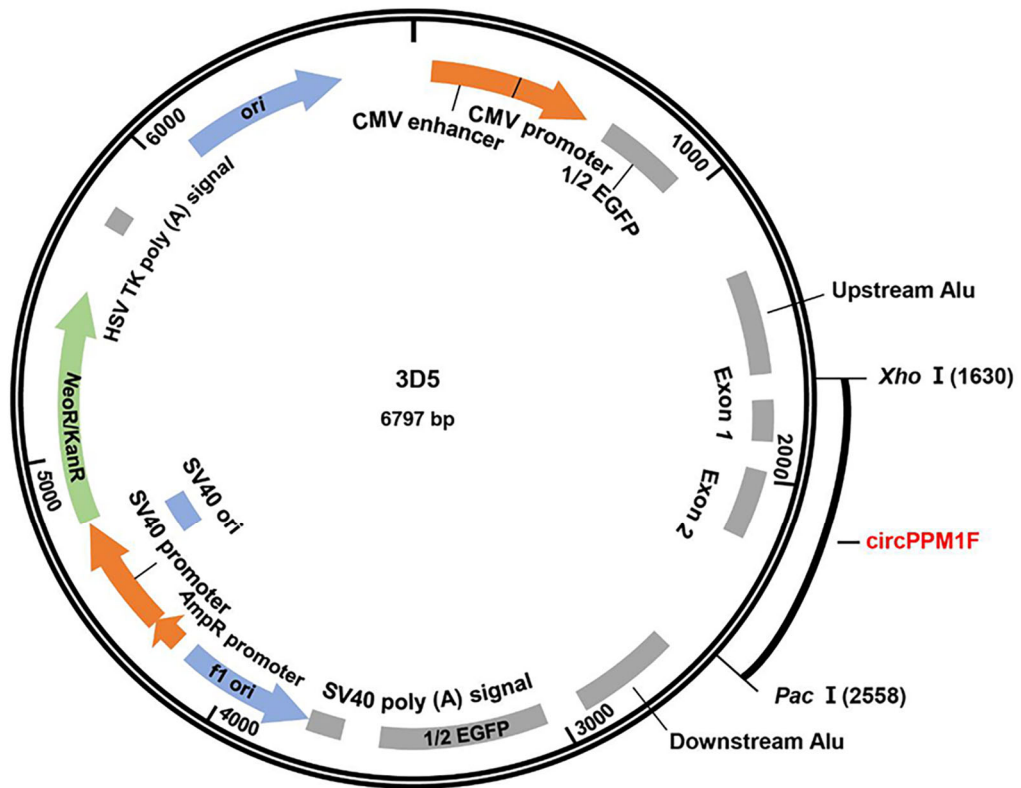


Figure S1. Schematic map of the 3D5-circPPM1F vector.

Figure S2

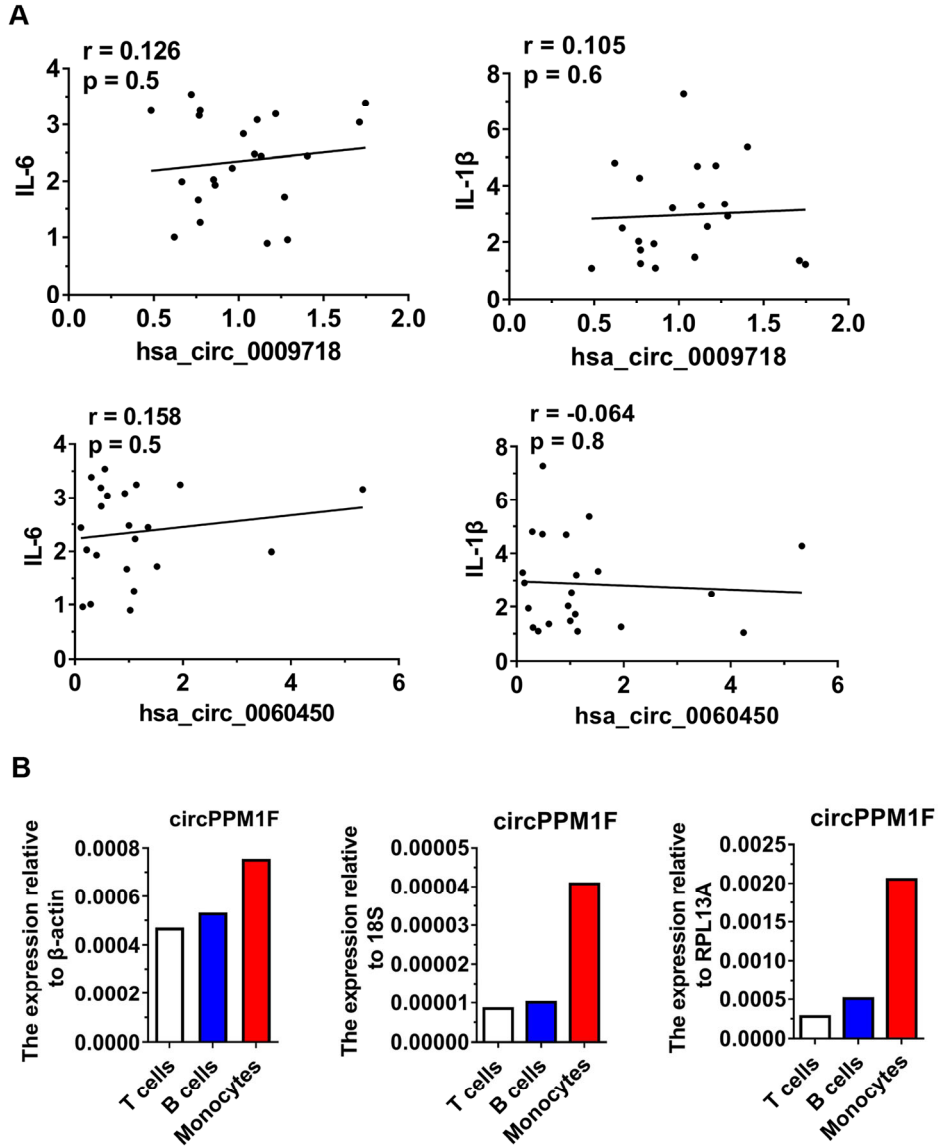


Figure S2. Expression of circRNAs in peripheral blood mononuclear cells (PBMCs). **A.** Correlation analysis of the expression of *hsa_circ_0009718* or *hsa_circ_0060450* and *IL-6*, or *IL-1 β* levels in PBMCs from patients with type 1 diabetes mellitus (T1DM) ($n = 22$, Pearson's correlation). **B.** qRT-PCR assay was used to measure *circPPM1F* expression in monocytes, T cells, and B cells in PBMCs.

Figure S3

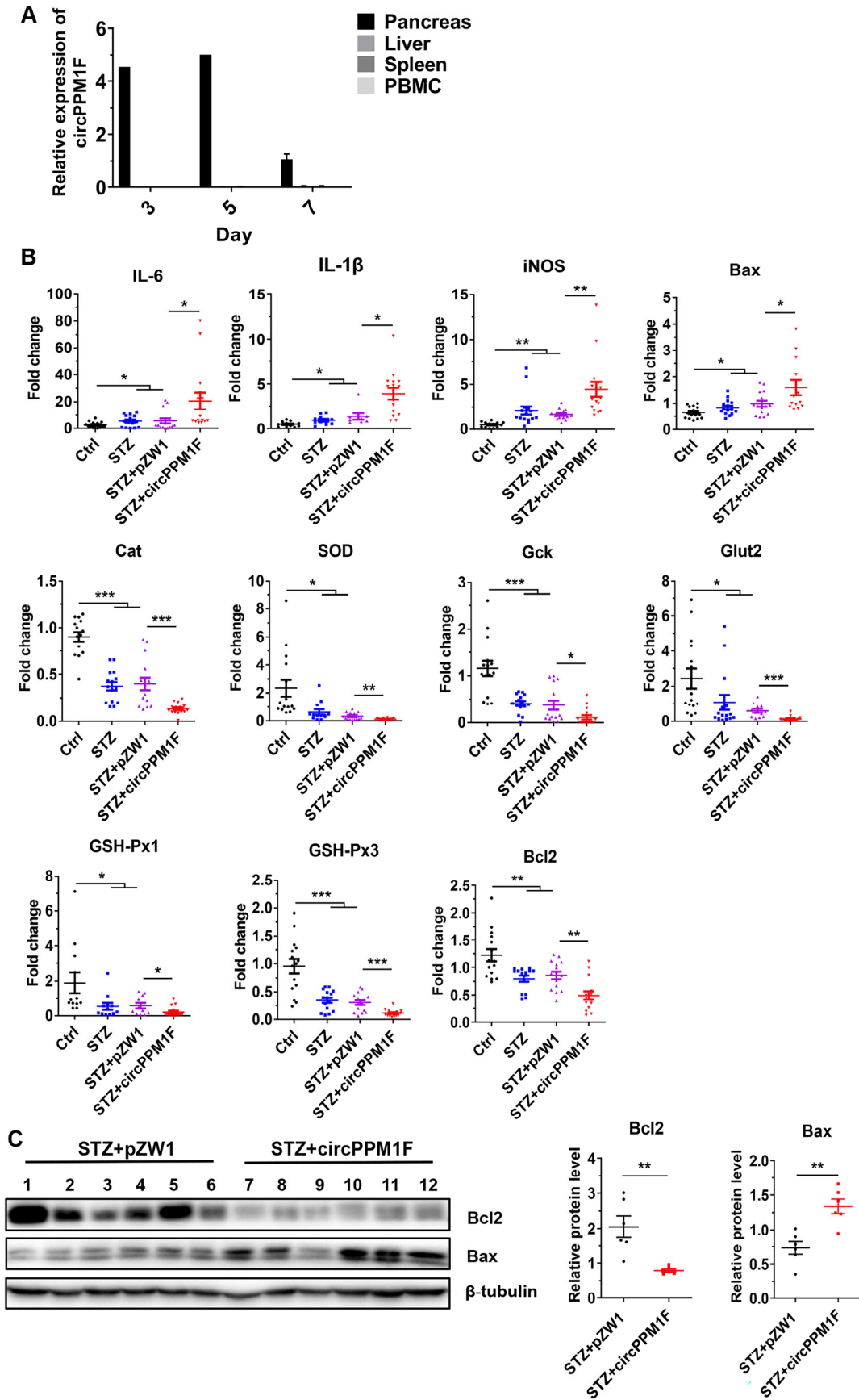


Figure S3. The evaluation of pancreatic injury in STZ-induced diabetic mouse model. **A.** Dynamic expression profiles of *circPPM1F* in pancreas, liver, spleen, and PBMCs from mice with injection of *circPPM1F* plasmid for 3, 5, or 7 days. **B.** qRT-PCR analyses of *IL-6*, *IL-1 β* , *iNOS*, *Bax*, *Sod2*, *Cat*, *GSH-Px-1*, *GSH-Px-3*, *Glut2*, *Gck*, and *Bcl2* expression in pancreas tissues from experimental model mice. **C.** The levels of Bcl2 and Bax protein in 6 random pancreas tissue samples from STZ-treated mice with *circPPM1F* or pZW1 injection (left). The levels of Bcl2 and Bax were normalized to those of β -tubulin and quantified using Image J software (right). Data are presented as means \pm SEM from three independent experiments. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table S1 Clinical characteristics of T1DM patients and healthy controls

Characteristic	Patients (n = 47)	Healthy controls (n = 49)
Sex, no. (M/F)	22/25	21/28
Age (y)	8.5 ± 0.6	8.0 ± 1.2
Duration (y)	1.8 ± 0.4	0
BMI (Kg/m ²)	16.7 ± 0.6	N/A
cGLU (mmoL/L)	18.0 ± 1.7	N/A
HbA1C (%)	11.0 ± 0.5 (ref. 4 ~ 6)	N/A
c peptide (ng/mL)	0.5 ± 0.1 (ref. 1.1 ~ 4.4)	N/A
GADA, no. (+/-)	8/39	0/49
IA-2A, no. (+/-)	21/26	0/49
IAA, no. (+/-)	7/40	0/49
ICA, no. (+/-)	9/38	0/49
Urine ketone, no. (+/-)	14/33	0/49

Data for age, BMI, cGLU, HbA1C and c peptide are expressed as means ± SEM.

Table S2 Primer sequences used for amplification

Name	Usage	Sequence (5'- 3')
β -actin	qPCR forward	CACCATTGGCAATGAGCGGTTC
	qPCR reverse	AGGTCTTTGCGGATGTCCACGT
GAPDH	qPCR forward	GTCTCCTCTGACTTCAACAGCG
	qPCR reverse	ACCACCCTGTTGCTGTAGCCAA
U1	qPCR forward	GGGAGATACCATGATCACGAAGGT
	qPCR reverse	CCACAAATTATGCAGTCGAGTTTCCC
MALAT1	qPCR forward	CTTAAGCGCAGCGCCATTTT
	qPCR reverse	CCTCAAACCCCAAGACCAA
<i>circPPM</i>	qPCR forward	TTGTGTTCTGTGCAAGGGG
<i>IF</i>	qPCR reverse	TGCAGCCGACAGAGTTCAGA
hsa_circ_0009718	qPCR forward	TCACACCTAGCGAGAGCAGT
	qPCR reverse	TGCATACCTTGGTAGCCAGC
hsa_circ_0060450	qPCR forward	GGGAAGCAGTGCCGTGAAC
	qPCR reverse	GTCCTGCTGTCCAAACTGCCT
PPM1F	qPCR forward	GCTGCTACAGACAGACCTTTCC
	qPCR reverse	GGCGGTAAAGAACTCTGTGCC
HuR	qPCR forward	TGTTCTCTCGGTTTGGGCGGAT
	qPCR reverse	TCTTCTGCCTCCGACCGTTTGT
TNF- α	qPCR forward	CTCTTCTGCCTGCTGCACTTTG
	qPCR reverse	ATGGGCTACAGGCTTGTCACTC
IL-6	qPCR forward	AGACAGCCACTCACCTCTTCAG
	qPCR reverse	TTCTGCCAGTGCCTCTTTGCTG
IL-1 β	qPCR forward	CCACAGACCTTCCAGGAGAATG
	qPCR reverse	GTGCAGTTCAGTGATCGTACAGG
CXCL10	qPCR forward	GGTGAGAAGAGATGTCTGAATCC
	qPCR reverse	GTCCATCCTTGGAAGCACTGCA
EIF4A3	qPCR forward	GGCACAGGAAAAACAGCCACCT
	qPCR reverse	TGTAGTCACCGAGAGCAAGCAG
FUS	qPCR forward	CAGACAGGGAAACTGGCAAGCT
	qPCR reverse	GGCGAGTAGCAAATGAGACCTTG
Primer1	Forward	TGAGATTTTCATCAGGCTCGAGGTACGCCGCTGTCCACGT
	Reverse	TAGGGCTTCTGGAAGACATCCCCGATGGCTCTGGAG
Primer2	Forward	CTCCAGAGCCATCGGGGATGTCTTCCAGAAGCCCTA
	Reverse	GGCCAAAAACTTTTAATTAATCCTGTCACCTTCTGCTG

Table S3 Sequences of NC and siRNAs

Name	Target mRNA	Sense sequence (5'- 3')
si-circPPM1F (-1)	<i>circPPM1F</i>	CUGAACUCUGUCGGCUGCATT
si-circPPM1F (-2)	<i>circPPM1F</i>	CUCUGUCGGCUGCAGAGCGTT
si-PPM1F (-1)	PPM1F	GACAGACCUUUCGAAUUCTT
si-PPM1F (-2)	PPM1F	AGCCUGGCACAGAGUUUCUTT
si-PPM1F (-3)	PPM1F	ACCGACCAGAUGUUUCUCATT
si-HuR (-1)	HuR	GAACGAAUUUGAUCGUCAATT
si-HuR (-2)	HuR	GCAGAUGUUUGGGCCGUUUTT
si-HuR (-3)	HuR	GGCUUUGUGACCAUGACAATT
si-EIF4A3 (-1)	EIF4A3	GCAUCUUGGUGAAACGUGATT
si-EIF4A3 (-2)	EIF4A3	CCGAGUGCUUAUUUCUACATT
si-EIF4A3 (-3)	EIF4A3	CGAUGAACGUUGCUGAUCUTT
si-FUS (-1)	FUS	CAGAGUUACAGUGGUUAUATT
si-FUS (-2)	FUS	GCCAAGAUCAAUCCUCAUTT
si-FUS (-3)	FUS	GUGAGAAUGUUACAAUUGATT
NC	--	UUCUCCGAACGUGUCACGUTT

Table S4 The details of all antibodies

Description (Anti-)	Sources	Cat./clone	Dilutions /WB	Dilutions /IHC/IF	Flow cytometry
PPM1F	Abcam, Cambridge, UK	ab200394	1 : 1000		
p65	CST, USA	#4764; C22B4	1 : 1000		
p-p65 (Ser536)	CST, USA	3033S; 93H1	1 : 1000		
p38	CST, USA	9212S	1 : 1000		
p-p38 (T180/Y182)	CST, USA	9216S; 28B10	1 : 1000		
JNK	CST, USA	9258S; 56G8	1 : 1000		
p-JNK (T183/Y185)	CST, USA	9251S	1 : 1000		
ERK1/2	CST, USA	4695S;137F5	1 : 1000		
p-ERK1/2 (T202/Y204)	CST, USA	4376S; 20G11	1 : 1000		
mTOR	CST, USA	2983S;7C16	1 : 1000		
p-mTOR (Ser2448)	CST, USA	5536S; D9C2	1 : 1000		
Stat3	CST, USA	9139S; 124H6	1 : 1000		
p-Stat3 (Y705)	CST, USA	9131S	1 : 1000		
HuR	CST, USA	#12582; D9W7E	1 : 1000		
Bcl2	CST, USA	15071S; 124	1 : 1000		
Bax, NT	EMD Millipore, USA	#06-499	1 : 1000		
β-Tubulin	Abcam, Cambridge, UK	ab6046	1 : 5000		
Ki-67	Signalway Texas, USA	Ab, #48871; 9N16		1 : 500	
Insulin	Proteintech, Chicago, USA	66198-1-Ig		1 : 1000	
F4/80 for IHC	CST, USA	#70076S		1 : 500	
F4/80 for IF	Servicebio	GB11027		1:8000	
F4/80	eBioscience	12-4801-80; BM8,			0.25 µg/T

iNOS	eBioscience	53-5920-80; CXNFT	0.06 µg/T
iNOS for IF	Servicebio	GB11119	1:500
