SUPPLEMENTARY INFORMATION FOR

Downregulation of circRNA DMNT3B contributes to diabetic retinal vascular dysfunction through targeting miR-20b-5p and BAMBI

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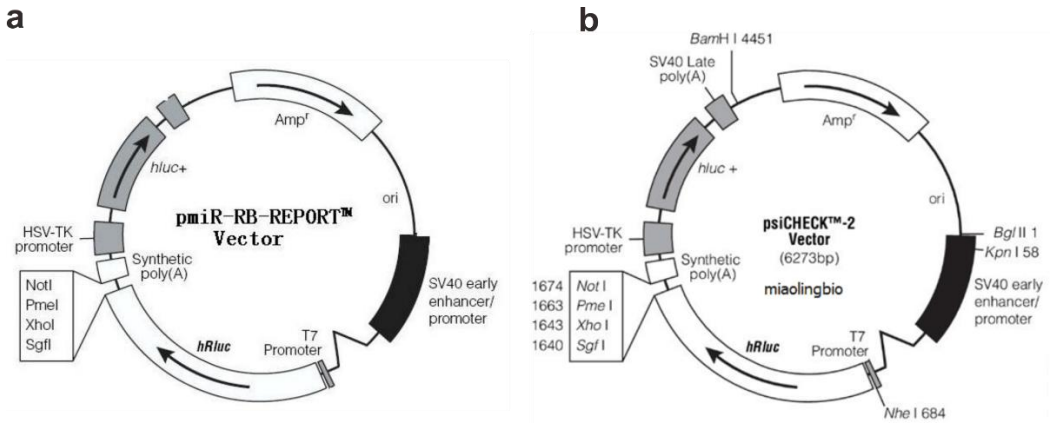
Supplementary Table S1. miRNA inhibitor, miRNA mimic siRNA target and FISH probe sequences used in this study.

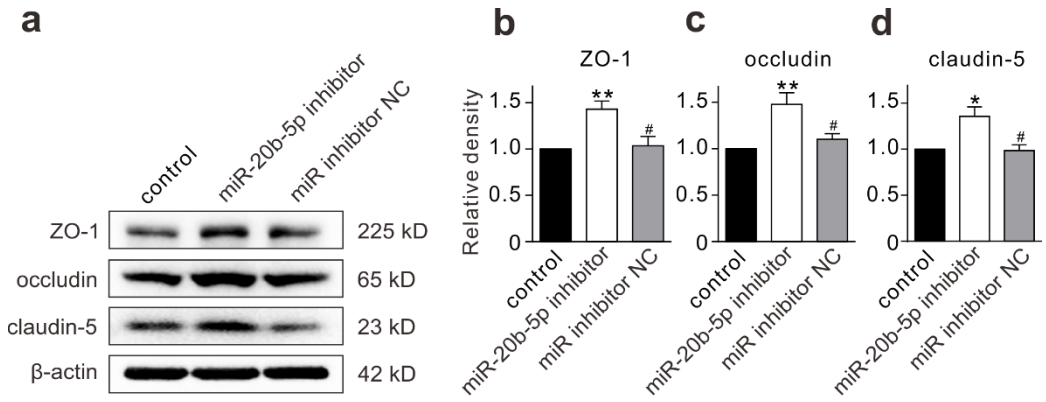
miRNA inhibitor sequences		
hsa-miR-20b-5p inhibitor		CUACCUGCACUAUGAGCACUUUG
miR inhibitor negative control		CAGUACUUUUGUGUAGUACAAA
miRNA mimic sequences		
hsa-miR-20b-5p mimic	Forward	CAAAGUGCUCAUAGUGCAGGUAG
	Reverse	CUACCUGCACUAUGAGCACUUUG
miR mimic negative control	Forward	UUUGUACUACACAAAAGUACUG
	Reverse	CAGUACUUUUGUGUAGUACAAA
siRNA target sequences		
siBAMBI		GAAGACATGTGCAATTACA
siRNA NC		TTCTCCGAACGTGTCACGT
Probes for FISH		
hsa-miR-20b-5p-Cy3		CTACCTGCACTATGAGCACTTTG
hsa-circDNMT3B-FITC		TCTGCAGAGACCTGGTTGCGT

Supplementary Table S2. Primers used in this study.

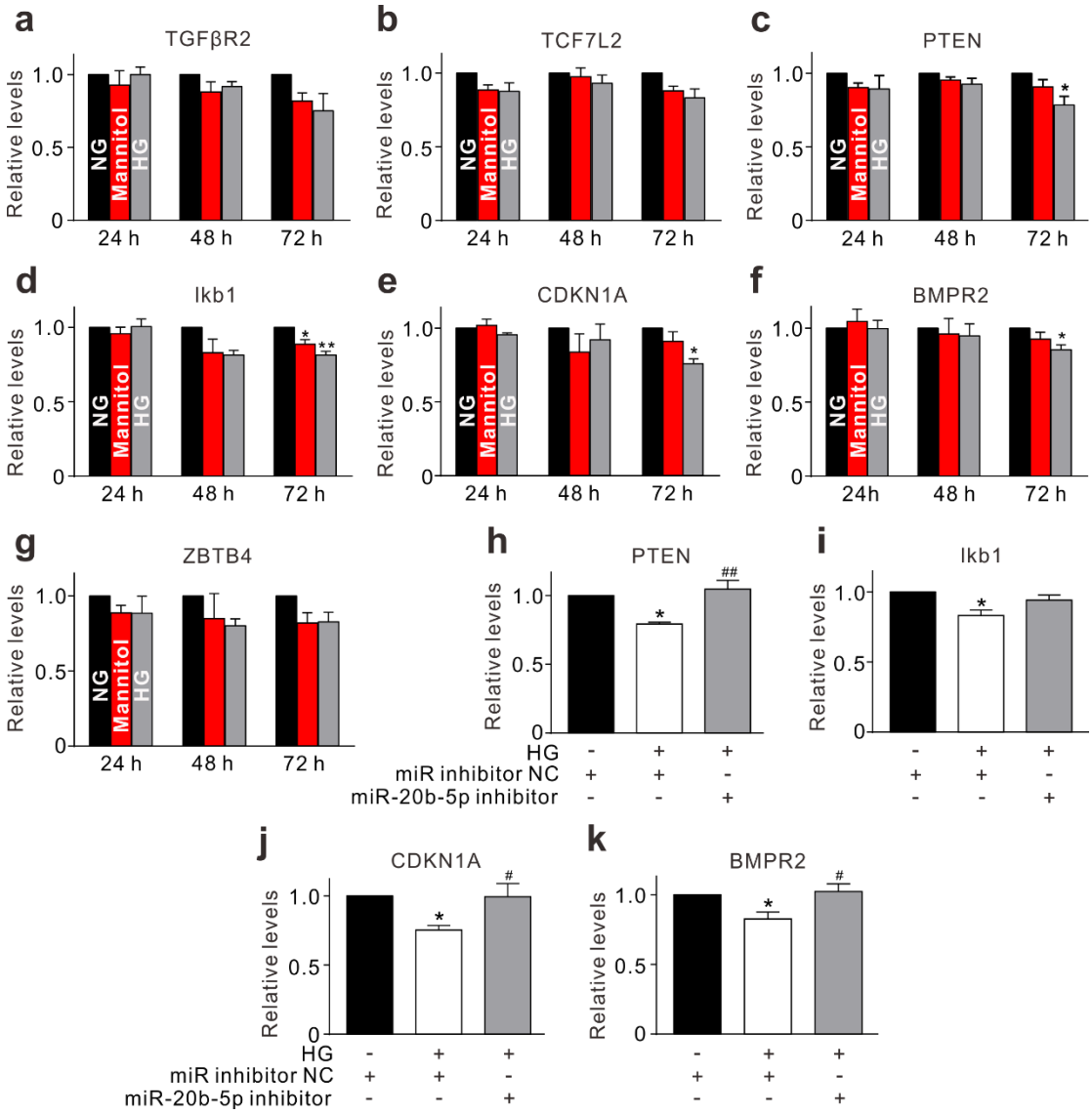
Primer sequences		
rno-miR-20b-5p	Forward	CAAAGTGCTCATAGTGCAGGTA
hsa-miR-20b-5p	Forward	CAAAGTGCTCATAGTGCAGGTA
hsa-BAMBI	Forward	GGCAGCATCACAGTAGCATC
	Reverse	GATCGCCACTCCAGCTACAT
rno-BAMBI	Forward	CTCCTCCCAAGAGTGAAGCC
	Reverse	AAGATCAGTCCGCCAGCAAT
	Reverse	GGGACCTTGGTTAGGTGCAG
hsa-TCF7L2	Forward	CCGACGTAGACCCCAAAACA
	Reverse	ATTTGTCCTACGGTGCCAGG
hsa-PTEN	Forward	CGGTGTCATAATGTCTTTCAGC
	Reverse	TGAAGGCGTATACAGGAACAAT
hsa-lkb1	Forward	CACCGAGGTCATCTACCAGC
	Reverse	ACCTTGCCGTAAGAGCCTTC
hsa-CDKN1A	Forward	CTCAAATCGTCCAGCGACCT
	Reverse	GACTCCTTGTTCCGCTGCTA
hsa-BMPR2	Forward	CCAAGGTCTTGCTGATACGG
	Reverse	CTACCATGGACCATCCTGCT
hsa-ZBTB4	Forward	ACCTTCCTCAGCTTCCATGC
	Reverse	TGCTCAGCCACAGAACAGAG
hsa-circDNMT3B	Forward	GAGACTCATTGGAGGACCAGC
	Reverse	CAGAGACCTGGTTGCGTGTT
hsa-DNMT3B mRNA	Forward	CAGCCCTGGAGACTCATTGG
	Reverse	GGTTGCGTGTTGTTGGGTTT
hsa-circTNFRSF21	Forward	GGTGTTTGTTAGCATGAACTCAAC
	Reverse	CCTGAAGGTTTGGGAGGGTC
hsa-β-actin	Forward	GATGAGATTGGCATGGCTTT
	Reverse	GTCACCTTCACCGTTCCAGT
rno-β-actin	Forward	CTGTCCCTGTATGCCTCTG
	Reverse	ATGTCACGCACGATTTCC
hsa-circDNMT3B vector (pLCDH-ciR)	Forward	CGGAATTCTGAAATATGCTATCTTACAGGTCTCTGCAGACAAACTGGTG
	Reverse	CGGGATCCTCAAGAAAAAATATATTCACCTGGTTGCGTGTTGTTGGGTT
hsa-circDNMT3B vector (pAAV2/DJ-CMV-GFP)	Forward	CGGAATTCTGAAATATGCTATCTTACAGGTCTCTGCAGACAAACTGGTG
	Reverse	CGGGATCCTCAAGAAAAAATATATTCACCTGGTTGCGTGTTGTTGGGTT



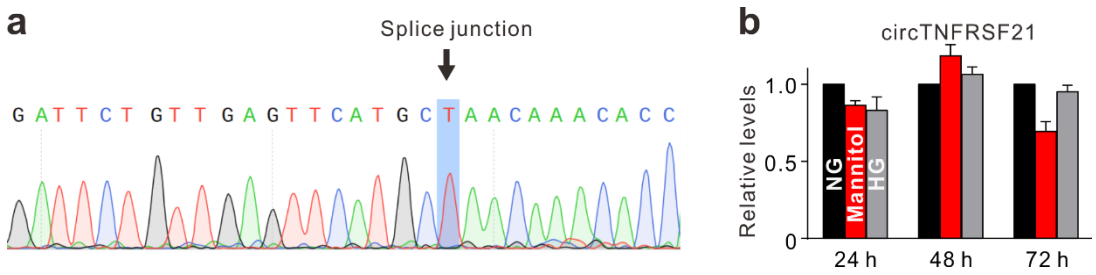
Supplementary Fig. S1. The Maps of luciferase reporter vectors used in this study. (a) Map of pmiR-RB-REPORT vector. **(b)** Map of psiCHECK2 vector.



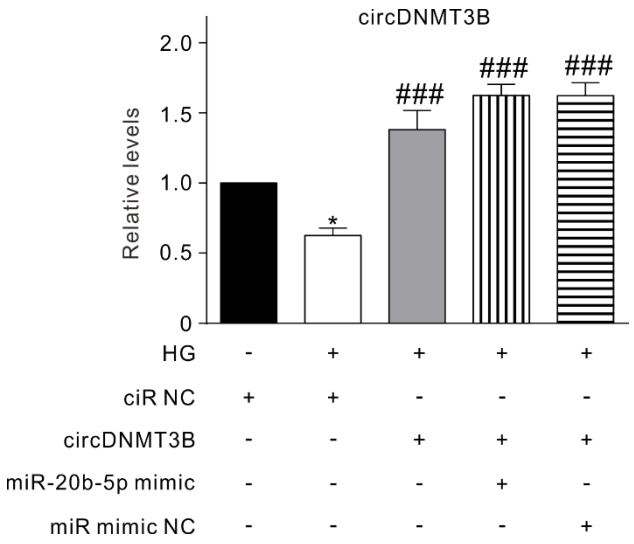
Supplementary Fig. S2. Changes of tight junction-related protein levels in HRMECs under normal glucose conditions. (a) Representative Western blotting results show the changes in protein levels of ZO-1, occludin, and claudin-5 in HRMECs which were transfected with miR-20b-5p inhibitor or miR inhibitor NC in 5 mM glucose (normal glucose, NG) medium. (b-d) Bar chart summarizing the relative density of immunoblot bands of ZO-1 (n = 4) (b), occludin (n = 4) (c), and claudin-5 (n = 3) (d). All data are normalized to control. * $p < 0.05$ and ** $p < 0.01$ vs. control; $^{\#}p < 0.05$ vs. miR-20b-5p inhibitor group. All *in vitro* experiments: n = 3 or 4 biological replicates × 3 technical replicates. Data presented as means with error bars representing standard deviation (SD). Abbreviations: HRMEC = human retinal microvascular endothelial cells, NC = negative control, ZO-1 = zonula occludens-1.



Supplemental Fig. S3. Expression levels of some candidate mRNAs. (a-g) Bar charts showing the changes of TGFβR2 (a), TCF7L2 (b), PTEN (c), lkb1 (d), CDKN1A (e), BMPR2 (f) and ZBTB4 (g) levels, assayed by qRT-PCR, in HRMECs cultured in 5 mM glucose (normal glucose, NG), 5 mM glucose plus 25 mM mannitol (Mannitol), or 30 mM glucose (HG) medium for 24 h, 48 h, and 72 h, respectively. Data are normalized to corresponding NG groups. n = 3 for each group, * $p < 0.05$ and ** $p < 0.01$ vs. NG. (h-k) Bar charts showing the changes of PTEN (h), lkb1 (i), CDKN1A (j) and BMPR2 (k) levels in HRMECs under different conditions. HRMECs were transfected with miR NC (control) or miR-20b-5p inhibitor (miR-20b-5p inhibitor group) with or without HG treatment. Data are normalized to control. n = 4 for each group, * $p < 0.05$ vs. control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. HG group. All *in vitro* experiments: n = 3 or 4 biological replicates × 3 technical replicates. All data presented as means with error bars representing standard deviation (SD). Abbreviations: BMPR2 = bone morphogenetic protein receptor type 2, CDKN1A = cyclin-dependent kinase inhibitor 1A, HG = high glucose, HRMEC = human retinal microvascular endothelial cells, miR = microRNA, NC = negative control, NG = normal glucose, PTEN = phosphatase and tensin homolog, qRT-PCR = quantitative reverse-transcription polymerase chain reaction. TCF7L2 = transcription factor 7 like 2, TGFβR2 = transforming growth factor beta receptor 2, ZBTB4 = zinc finger and BTB domain containing 4.



Supplementary Fig. S4. Expression levels of circTNFRSF21. (a) The Sanger sequencing showing that the amplified product of divergent primers was consistent with circTNFRSF21 sequence in circBase. (b) qRT-PCR results reveal the changes of circTNFRSF21 levels in HRMECs cultured in 5 mM glucose (normal glucose, NG), 5 mM glucose plus 25 mM mannitol (Mannitol), or 30 mM glucose (HG) medium for 24 h, 48 h, and 72 h, respectively. Data are normalized to corresponding NG groups. n = 3 for each group. All *in vitro* experiments: n = 3 biological replicates × 3 technical replicates. Data presented as means with error bars representing standard deviation (SD). Abbreviations: HG = high glucose, HRMEC = human retinal microvascular endothelial cells, NG = normal glucose, qRT-PCR = quantitative reverse-transcription polymerase chain reaction, TNFRSF21 = TNF receptor superfamily member 21.



Supplementary Fig. S5. Expression levels of circDNMT3B. Summarized data showing the changes of circDNMT3B levels in HRMECs, assayed with qRT-PCR, under different conditions. All data are normalized to ciR NC group. n = 6 for each group,* $p < 0.05$ vs. ciR NC group, and $^{###}p < 0.001$ vs. HG. All *in vitro* experiments: n = 3 or 4 biological replicates × 3 technical replicates. Data presented as means with error bars representing standard deviation (SD). Abbreviations: ciR = circular RNA, DNMT3B = DNA methyltransferase 3 beta, HG = high glucose, HRMEC = human retinal microvascular endothelial cells, miR = micro RNA, NC = negative control, qRT-PCR = quantitative reverse-transcription polymerase chain reaction.