Online Appendix

Supplementary Table 1 The gene primer sequences used in experiments

Gene		Primer sequences	Tm	Product length	GenBank Accession numbers
Primer used in RT-qPCR					
Gapdh	Forward (5'→3')	AGG TCG GTG TGA ACG GAT TTG	60.88	95	XM_036165840.1
	Reverse (3'→5')	GGG GTC GTT GAT GGC AAC A	60.60		
β-catenin	Forward (5'→3')	TCA AGA GAG CAA GCT CAT CAT TCT	60.08	115	NM_001165902.1
	Reverse (3'→5')	CAC CTT CAG CAC TCT GCT TGT G	61.05		
Axin1	Forward (5'→3')	GTTCCAGAGAGGGCTGGT G	59.70	282	NM_001159598.2
	Reverse (3'→5')	GCGCTGCACCCTAATACCT C	60.88		
Small interfering RNA					
Axin1-1	GAACTGGTATCCACTGATT				
Axin1-2	GCCATCTACCGAAAGTACA				
Axin1-3	GCCCACTTTGAATGAAGAT				

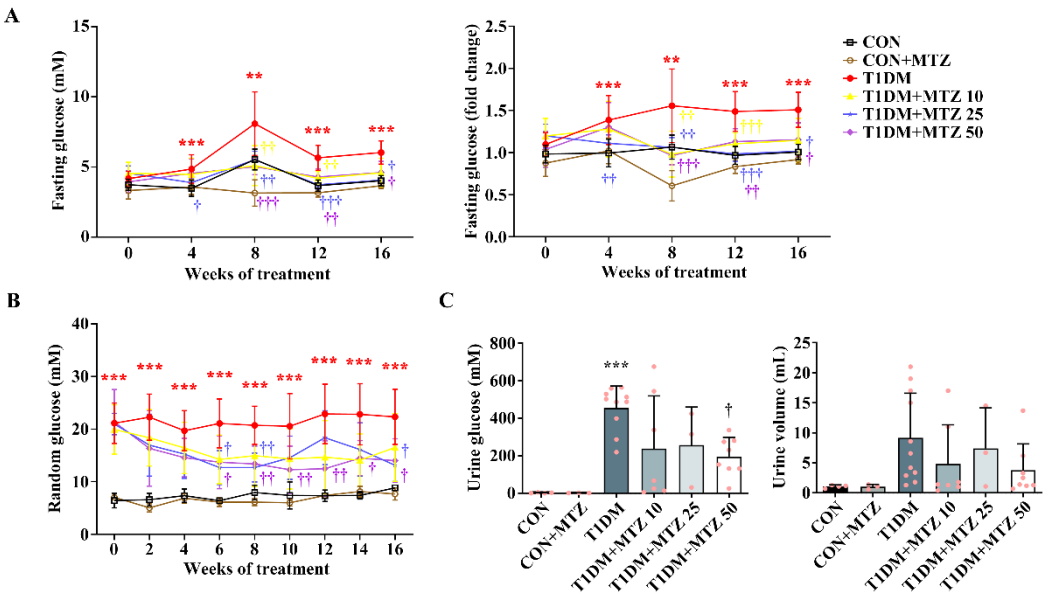
Supplementary Table 2 The antibodies used in experiments

Antibodies	Manufactures and catalogue numbers	Application and dilution
CA1 (29 KDa)	Abcam #ab108367	WB (1:5000)
CA2 (29 KDa)	Abcam #ab124687	WB (1:3000)
active β-catenin (92 KDa)	CST #8814	WB (1:3000)
β-catenin (92 KDa)	CST #8480	IP (1:50)
β-catenin (92 KDa)	CST #9582	If (1:100)
β-catenin (92 KDa)	Servicebio #GB11015	WB (1:5000)
TCF4/7L2 (58, 79 KDa)	CST #2569	WB (1:2000)
Cyclin D2 (31 KDa)	CST #3741	WB (1:5000)
ANP (17 KDa)	Santa Cruz #sc-515701	WB (1:5000)
Axin1 (110 KDa)	RD #AF3287	IF (1:100)
Axin1 (110 KDa)	CST #2087	WB (1:3000), IP (1:50)
GSK 3β (46 KDa)	CST #9315	WB (1:5000)
phosphor-GSK 3β Ser9 (46 KDa)	CST #9323	WB (1:5000)
CK1α (34 KDa)	Abcam #ab108296	WB (1:5000)
phospho-CK1α Y321 (34 KDa)	Bioworld #BS4602	WB (1:3000)
phospho-CK1α Thr321 (34 KDa)	Invitrogen #PA5-36790	WB (1:3000)
AKT (60 KDa)	CST #4691	WB (1:3000)
phosphor-AKT 473 (60 KDa)	CST #4060	WB (1:3000)
AMPKα (62 KDa)	CST #5831	WB (1:5000)
phospho-AMPKα (62 KDa)	CST #2523	WB (1:3000)
AMPKβ1/2 (30, 38 KDa)	CST #4150	WB (1:5000)
phospho-AMPK β1/2 (30, 38 KDa)	CST #4186	WB (1:3000)
β tubulin (55 KDa)	MilliporeSigma #T4026	WB (1:5000)

Supplementary Table 3 Effects of MTZ on cardiac function in STZ-induced T1DM mice

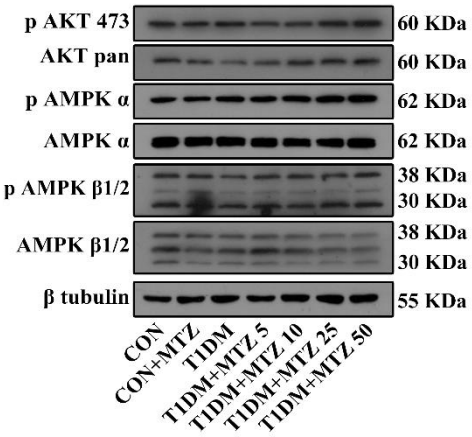
	Control	Control+MTZ 50	T1DM	T1DM+MTZ 10	T1DM+MTZ 25	T1DM+MTZ 50
AET (ms)	52.36 ± 1.84	51.06 ± 2.56	55.93 ± 1.11	57.72 ± 2.37	57.98 ± 3.29	60.04 ± 3.13
IVCT (ms)	10.78 ± 1.08	7.52 ± 1.74	6.75 ± 0.64 *	7.92 ± 1.18	11.90 ± 1.39 †	9.38 ± 1.22
IVRT (ms)	12.18 ± 1.24	11.58 ± 1.81	11.15 ± 1.20	13.11 ± 1.53	12.82 ± 2.74	7.42 ± 1.33
MV ET (ms)	69.91 ± 2.86	64.71 ± 2.58	69.66 ± 1.86	65.81 ± 2.60	72.14 ± 4.674	62.69 ± 1.21 †
MPI	0.52 ± 0.05	0.42 ± 0.09	0.35 ± 0.03 *	0.44 ± 0.06	0.58 ± 0.06 †	0.30 ± 0.03
Stroke volume (μL)	43.07 ± 1.30	40.54 ± 2.51	34.90 ± 1.15 ***	37.73 ± 2.10	31.49 ± 0.95	34.16 ± 1.43
Ejection fraction (%)	60.40 ± 1.32	55.30 ± 3.72	54.47 ± 0.95 *	56.08 ± 2.06	55.20 ± 1.56	57.38 ± 2.57
Fractional shortening (%)	31.94 ± 0.90	28.62 ± 2.34	27.83 ± 0.60 *	29.01 ± 1.31	28.20 ± 1.01	29.87 ± 1.74
Cardiac output (mL/min)	17.88 ± 0.74	19.34 ± 1.58	16.25 ± 0.56	16.38 ± 1.05	12.62 ± 0.78 †	15.06 ± 0.72

*$p < 0.05$ or ***$p < 0.001$ vs. CON group; †$p < 0.05$ vs. T1DM; n = 3-10.



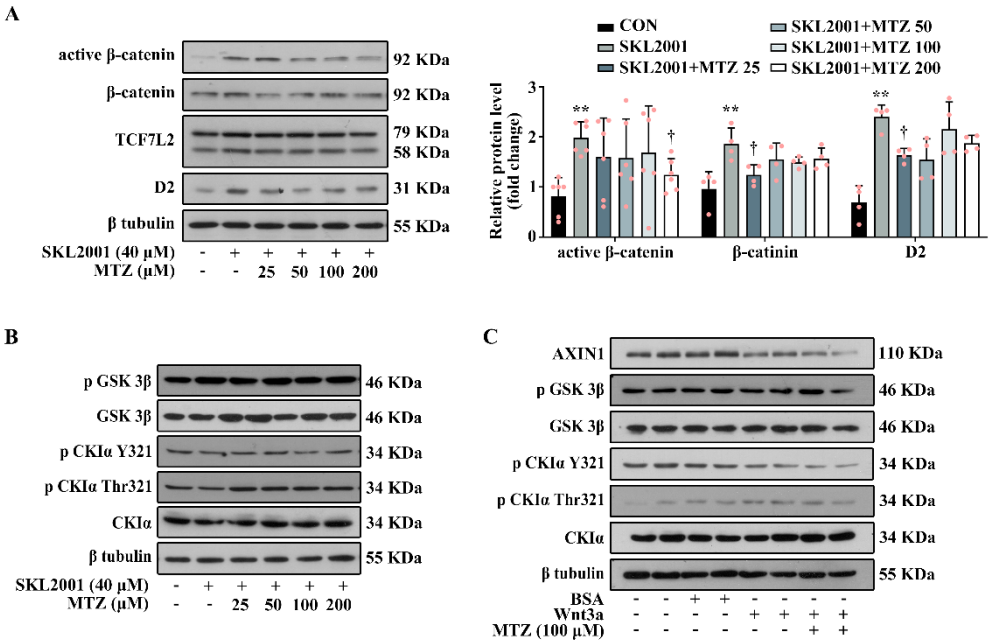
Supplementary Figure 1 Methazolamide showed a hypoglycemic effect and improved glucose tolerance in T1DM mice.

A: Fasting blood glucose levels (**Left**) and its fold change (**Right**) in various groups of mice at the indicated time-points. **B**: The random blood glucose levels of several groups' mice in the indicated times. **C**: The total urine glucose concentration and the urine volume of several groups' mice in the indicated groups in 24 h. $^{**}P < 0.01$ or $^{***}P < 0.001$ vs. Control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, or $^{\dagger\dagger\dagger}P < 0.001$ vs. T1DM; n = 3-10 mice per group.



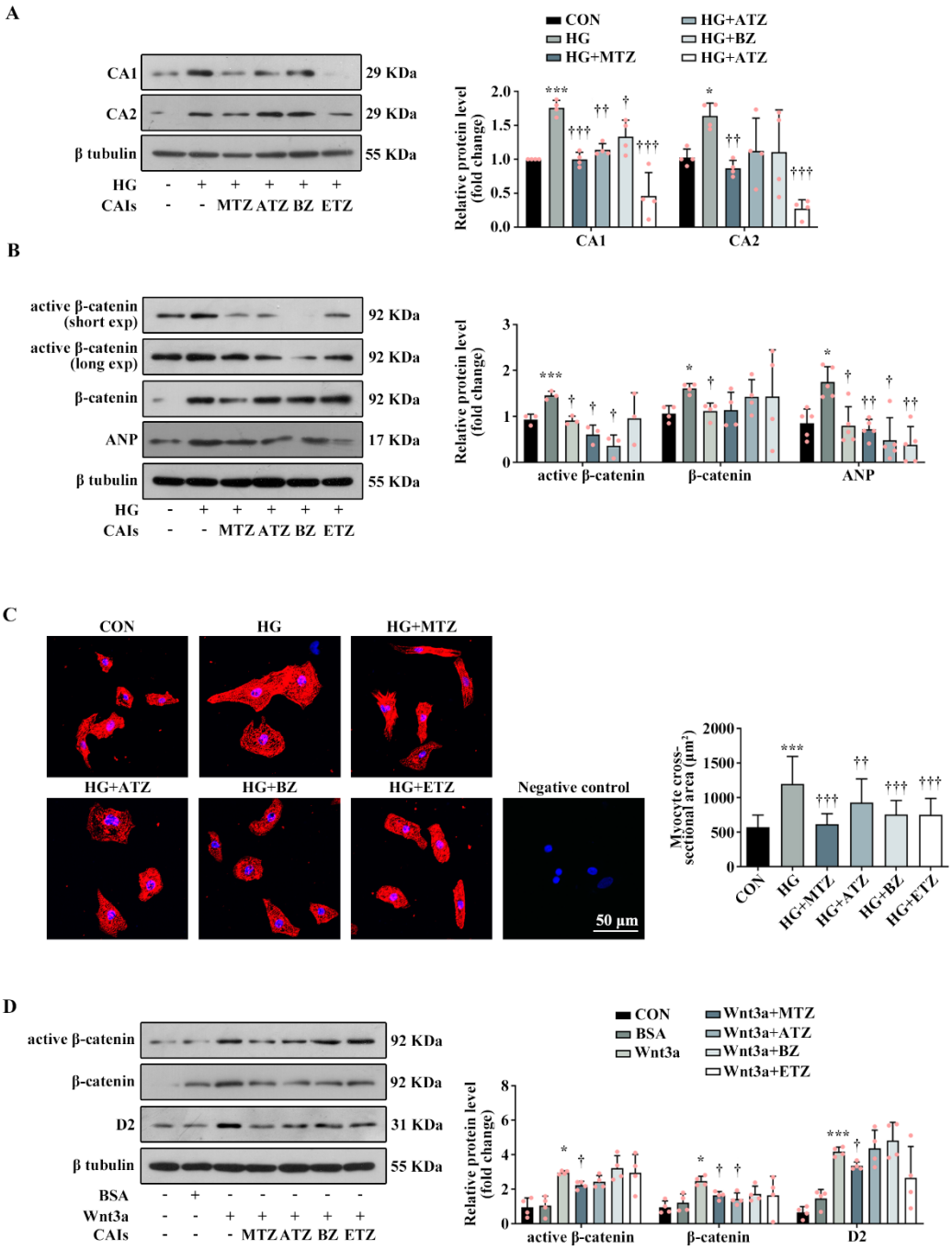
Supplementary Figure 2 Methazolamide did not affect AKT and AMPK pathway in T1DM mice heart. Representative western blot results of the relative protein levels of p AKT473, AKT, p AMPKα, AMPKα, p AMPKβ1/2, and AMPKβ1/2 in hearts from

the indicated groups.



Supplementary Figure 3 Methazolamide attenuated Wnt/β-catenin pathway in SKL2001- or Wnt3a-treated NRCMs.

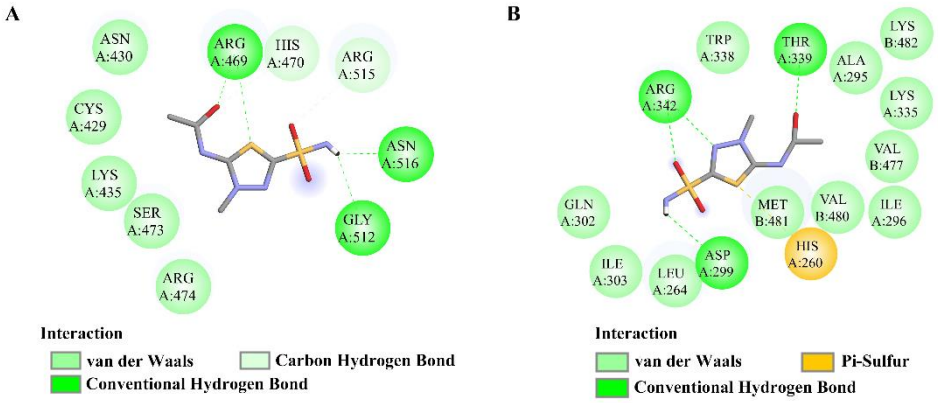
A: Representative Western blot and quantitative results of the relative protein levels of active β-catenin, β-catenin, TCF7L2, and D2 in NRCMs subject to SKL2001 with or without MTZ (48 h). $^{**}P < 0.01$ vs. Control; $^{\dagger}P < 0.05$ vs. SKL2001. The above results from four to six independent experiments. **B**: Representative Western blot results of the relative protein levels of β-catenin degradation complex members (p GSK3β, GSK3β, p CKIα, and CKIα) in NRCMs treated as indicated in **A**. **C**: Representative western blot results of the relative protein levels of β-catenin degradation complex members (p GSK3β, GSK3β, p CKIα, and CKIα) in NRCMs subject to Wnt3a with or without MTZ (48 h).



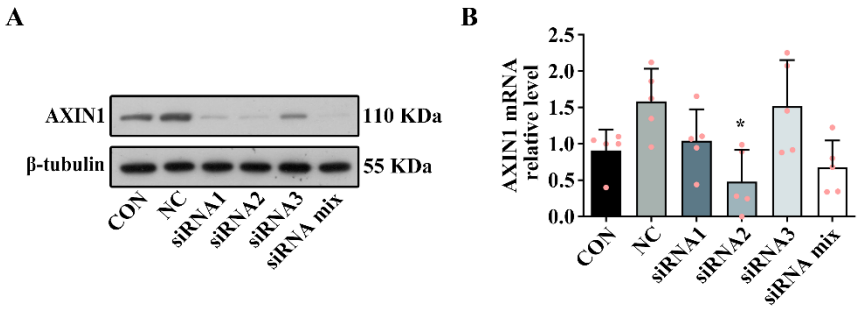
Supplementary Figure 4 CA inhibitors decreased CAs and β-catenin in high glucose- or Wnt3a-treated NRCMs.

A and **B**: Representative Western blot and quantitative results of the relative protein levels of CAs, active β-catenin, β-catenin and ANP in NRCMs subject to high glucose (HG) without or with CA inhibitors (48 h). $^{*}P < 0.05$ or $^{***}P < 0.001$ vs. Control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, or $^{\dagger\dagger\dagger}P < 0.001$ vs. HG. The above results from three to five

independent experiments. **C**: Representative images of α-actinin staining and quantification of cell size were shown in the indicated groups (red: α-actinin, blue: DAPI; n = 60 NRCMs per group). ***$P < 0.001$ vs. Control; $^{\dagger\dagger}P < 0.01$ or $^{\dagger\dagger\dagger}P < 0.001$ vs. HG. **D**: Representative Western blot and quantitative results of the relative protein levels of active β-catenin, β-catenin, and cyclin D2 in NRCMs subject to Wnt3a with or without CA inhibitors (48 h). *$P < 0.05$ or ***$P < 0.001$ vs. Control; $^{\dagger}P < 0.05$ vs. Wnt3a. The above results from four independent experiments.



Supplementary Figure 5 The 2D diagram of ligand-receptor interaction in molecular docking was performed. **A**: MTZ-β-catenin interaction. **B**: MTZ—β-catenin-AXIN1 interaction; A chain: β-catenin; B chain: AXIN1.



Supplementary Figure 6 AXIN1 was inhibited in AXIN1 siRNA -treated NRCMs. **A**: Representative western blot results of AXIN1 in NRCMs subject to negative control (NC) or AXIN1 siRNA. **B**: Quantitative results of the relative mRNA levels of AXIN1 in NRCMs subject to NC or AXIN1 siRNA. *$P < 0.05$ vs. NC. The above results from

five independent experiments.