SUPPLEMENTARY DATA

Two-hit mechanism of cardiac arrhythmias in diabetic hyperglycemia: reduced repolarization reserve, neurohormonal stimulation and heart failure exacerbate susceptibility

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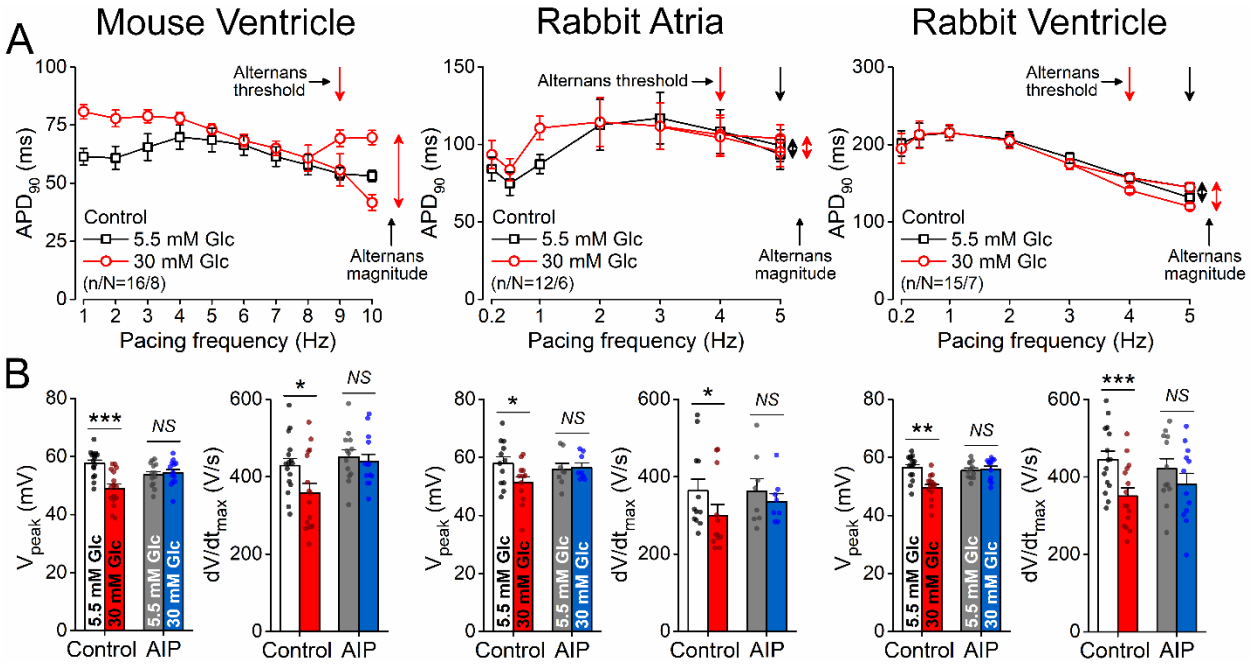
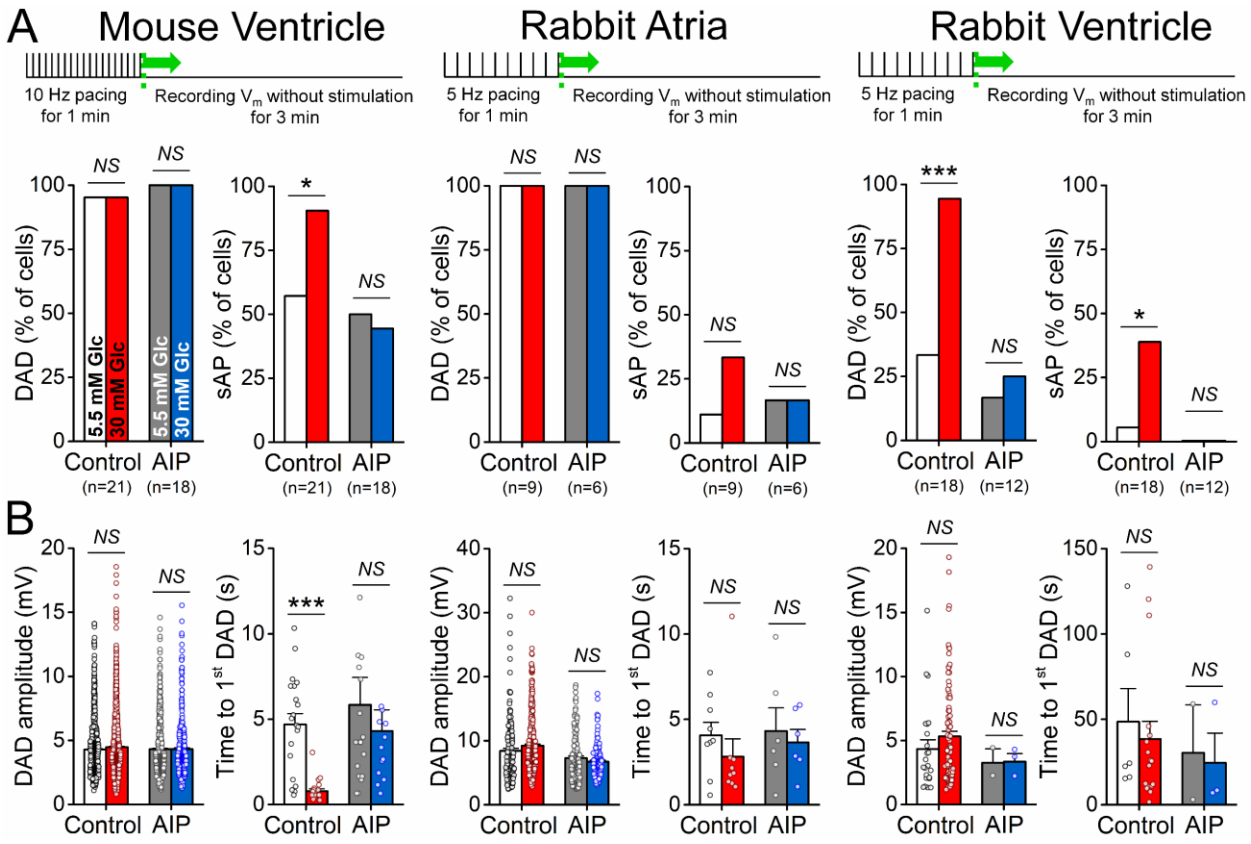
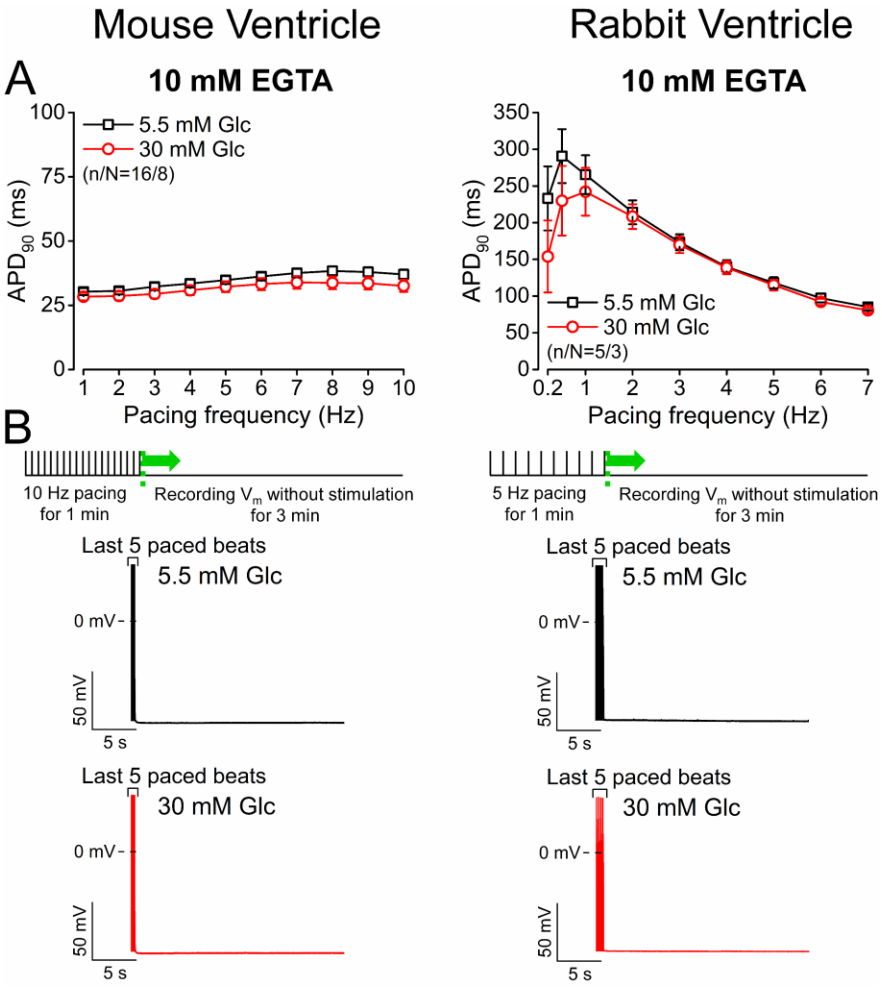


Supplemental Figure I. High-glucose effects on action potential parameters in murine ventricular, rabbit atrial and ventricular myocytes.

(*A*) Frequency dependence of action potential duration at 90% repolarization (APD_{90}) in normal (5.5 mM) and high glucose (30 mM) conditions. APD prolongation was induced by high glucose at low pacing frequencies in murine ventricular and rabbit atrial cells. High glucose increased the magnitude of tachypacing-induced APD alternans and lowered the threshold frequency for alternans in both murine and rabbit myocytes. (*B*) AP peak voltage (V_{peak}) and maximal rate of rise (dV/dt_{max}) were reduced by high glucose in both murine and rabbit myocytes at 1 Hz pacing, and CaMKII inhibition using autocamtide-2-related inhibitory peptide (AIP, 1 μM) prevented these effects. (Murine ventricular cells, control: $n = 16$ cells from nine animals; AIP: $n = 13$ cells from six animals. Rabbit atrial cells, control: $n = 12$ cells from six animals; AIP: $n = 8$ cells from three animals. Rabbit ventricular cells, control: $n = 15$ cells from seven animals; AIP: $n = 12$ cells from five animals.) Nested *t*-test; *NS*, non-significant; *$P<0.05$, **$P<0.01$, ***$P<0.001$.

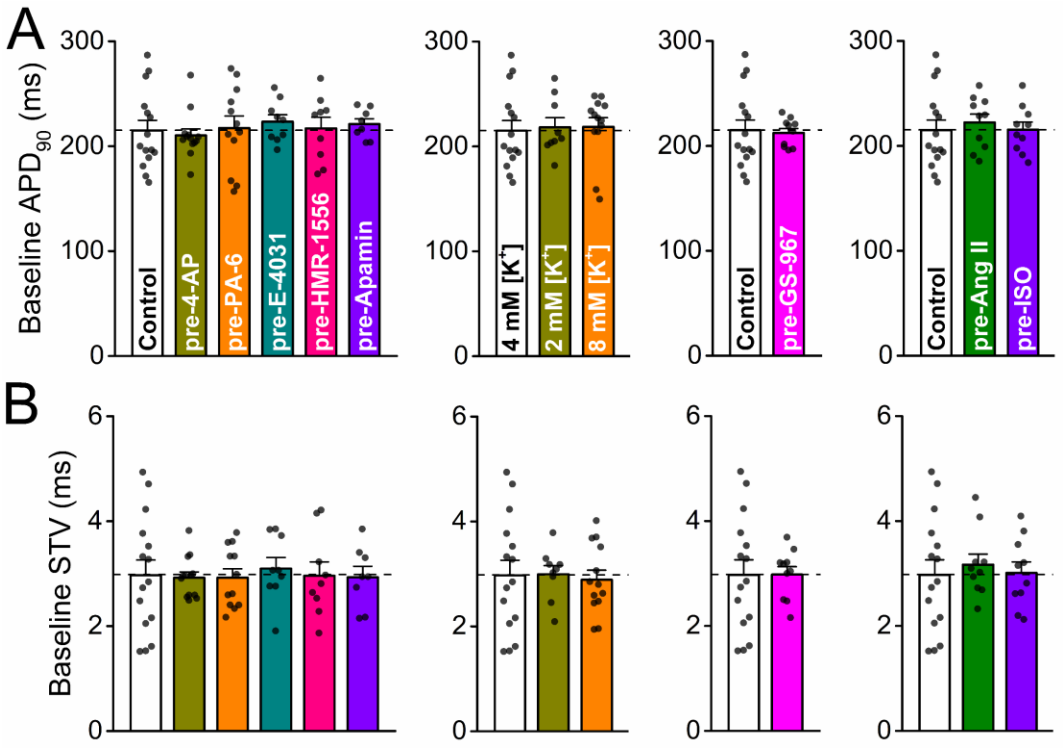


Supplemental Figure II. Arrhythmogenic diastolic activities induced by high-glucose.
(*A*) Percentage of cells exhibiting delayed afterdepolarization (DAD) and spontaneous action potential (sAP) following a tachypacing protocol shown above in control and CaMKII inhibition (AIP, 1 μM). Fisher's exact test; *NS*, non-significant; **P*<0.05, ***P*<0.01, ****P*<0.001. (*B*) DAD amplitude and time to first DAD following cessation of tachypacing. (Murine ventricular cells, control: *n* = 21 cells from nine animals; AIP: *n* = 18 cells from six animals. Rabbit atrial cells, control: *n* = 9 cells from five animals; AIP: *n* = 6 cells from three animals. Rabbit ventricular cells, control: *n* = 18 cells from eight animals; AIP: *n* = 12 cells from five animals.) Nested *t*-test; *NS*, non-significant; ****P*<0.001.



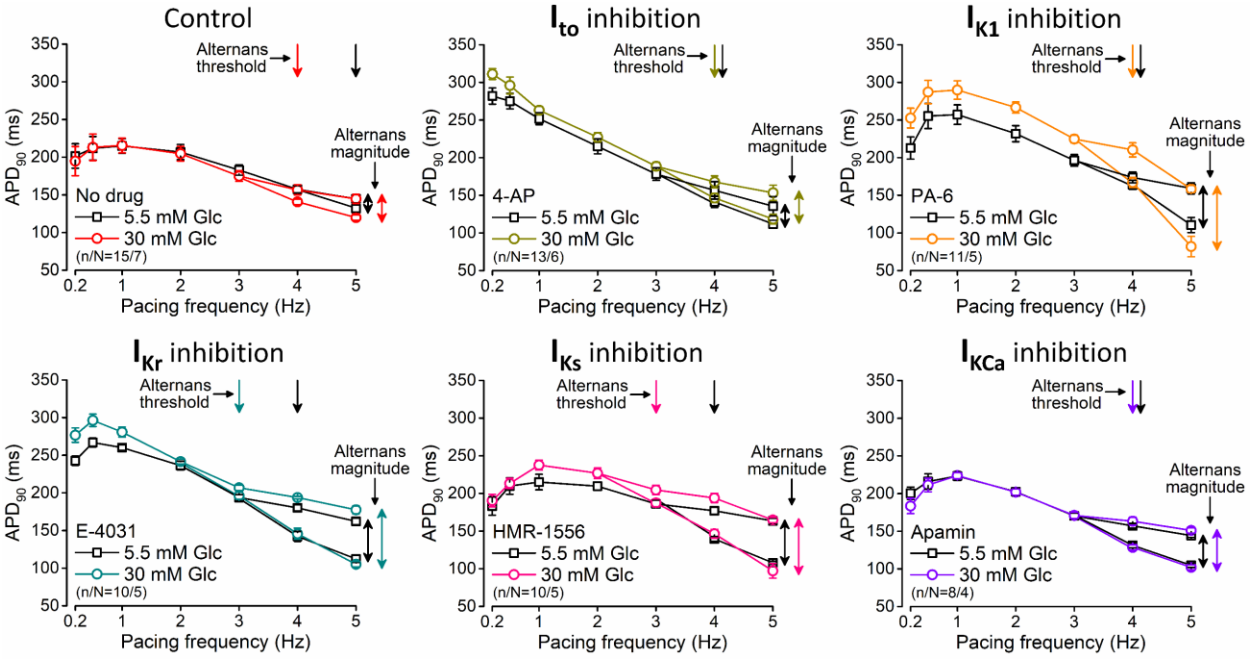
Supplemental Figure III. Arrhythmogenic action potential and diastolic activities induced by high-glucose are calcium-dependent.

(*A*) Frequency dependence of action potential duration at 90% repolarization (APD_{90}) in normal and high-glucose conditions in murine and rabbit ventricular myocytes. The pipette solution was supplemented with 10 mM EGTA. (*B*) No spontaneous diastolic activity was detected following cessation of tachypacing in the presence of 10 mM EGTA. (Murine ventricular cells: n = 16 cells from eight animals. Rabbit ventricular cells: n = 5 cells from three animals.)



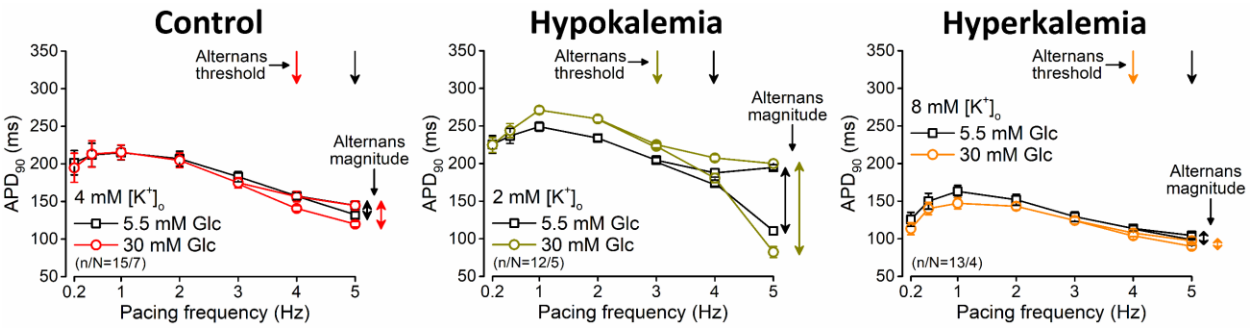
Supplemental Figure IV. Baseline action potential duration and short-term variability among experimental groups before treatment.

(*A*) No difference among experimental groups in action potential duration at 90% repolarization (APD_{90}) at baseline (before any treatment was applied) at 1 Hz steady-state pacing frequency. (*B*) No difference among experimental groups in short-term variability (STV) of APD at baseline. (Control (4 mM $[K^+]_o$): n = 15 cells from seven animals; pre-4-AP: n = 13 cells from six animals; pre-PA-6: n = 12 cells from five animals; pre-E-4031: n = 9 cells from five animals; pre-HMR-1556: n = 9 cells from five animals; apamin: n = 8 cells from four animals; pre-hypokalemia (2 mM $[K^+]_o$): n = 9 cells from five animals; pre-hyperkalemia (8 mM $[K^+]_o$): n = 13 cells from four animals; pre-GS-967: n = 10 cells from five animals; pre-Ang II: n = 10 cells from six animals; pre-ISO: n = 10 cells from five animals.) Nested ANOVA.



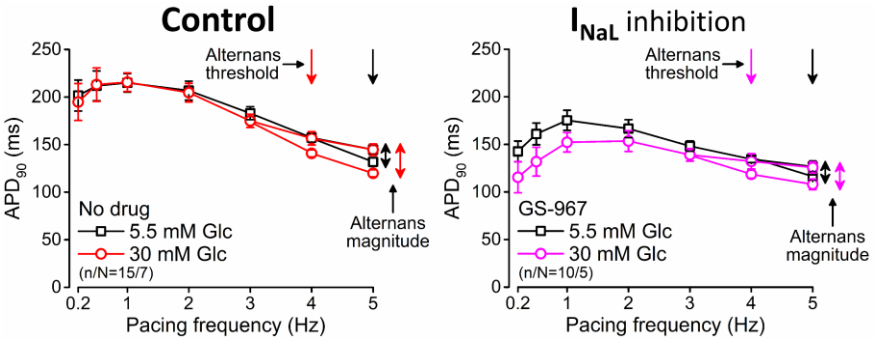
Supplemental Figure V. Frequency dependence of action potential duration in rabbit ventricular myocytes following K^+ channel inhibition.

Frequency dependence of action potential duration at 90% repolarization (APD_{90}) in normal and high glucose conditions in control and following inhibition of each K^+ channels in rabbit ventricular myocytes. The transient outward K^+ current (I_{to}) was inhibited by 4-aminopyridine (4-AP, 5 mM), the inward rectifier K^+ current (I_{K1}) was inhibited by pentamidine-analogue 6 (PA-6, 200 nM), the rapid delayed rectifier K^+ current (I_{Kr}) was inhibited by E-4031 (1 μM), the slow delayed rectifier K^+ current (I_{Ks}) was inhibited by HMR-1556 (1 μM), and the small-conductance (SK) Ca^{2+}-activated K^+ current (I_{KCa}) was inhibited by apamin (100 nM). Inhibition of K^+ channels exacerbated the effect of high glucose. (Control: n = 15 cells from seven animals; 4-AP: n = 13 cells from six animals; PA-6: n = 11 cells from five animals; E-4031: n = 10 cells from five animals; HMR-1556: n = 10 cells from five animals; apamin: n = 8 cells from four animals.)

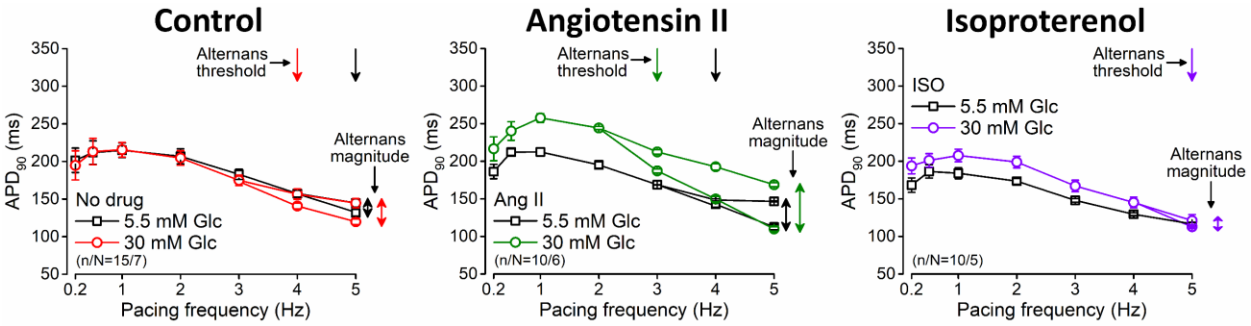


Supplemental Figure VI. Frequency dependence of action potential duration in rabbit ventricular myocytes in hypokalemia and hyperkalemia.

Frequency-dependent effect of high glucose on action potential duration at 90% repolarization (APD_{90}) in control (4 mM $[K^+]_o$), hypokalemia (2 mM $[K^+]_o$) and hyperkalemia (8 mM $[K^+]_o$). Hypokalemia potentiated the effect of high glucose on APD_{90}, whereas hyperkalemia attenuated the glucose effects. (Control: n = 15 cells from seven animals; hypokalemia: n = 12 cells from five animals; hyperkalemia: n = 13 cells from four animals.)



Supplemental Figure VII. Frequency dependence of action potential duration in rabbit ventricular myocytes following late Na^+ current inhibition.
Frequency dependence of action potential duration at 90% repolarization (APD_{90}) in normal and high glucose conditions in control and following inhibition of late Na^+ current (I_{NaL}) using GS-967 (1 μM). High glucose shortened APD_{90} following I_{NaL} inhibition. (Control: n = 15 cells from seven animals; GS-967: n = 10 cells from five animals.)



Supplemental Figure VIII. Frequency dependence of action potential duration in rabbit ventricular myocytes following angiotensin II and isoproterenol stimulation.
Frequency dependence of action potential duration at 90% repolarization (APD_{90}) in normal and high glucose conditions in control and following angiotensin II (100 nM) and isoproterenol (30 nM) pretreatments. High glucose effects were exacerbated following neurohormonal stimulation. (Control: n = 15 cells from seven animals; Ang II: n = 10 cells from six animals; ISO: n = 10 cells from five animals.)