Supplementary Material
Solhjoo and O'Rourke. Mitochondrial Instability during Regional Ischemia-Reperfusion Underlies Arrhythmias in Monolayers of Cardiomyocytes JMCC 2014

List of Supplemental Movies:

Movie 1. Loss of mitochondrial inner membrane potential during ischemia in 2 phases. Taken using the 40X objective lens.

Movie 2. Wave of mitochondrial inner membrane potential loss during ischemia. Taken using the 2X objective lens.

Movie 3. Mitochondrial instability upon reperfusion (continuation of experiment shown in Movie 1). Taken using the 40X objective lens.

Movie 4. Wave of mitochondrial inner membrane potential loss initiating 2 hours after the onset of reperfusion. Movie shows 5 hours of reperfusion. Taken using the 2X objective lens.

Movie 5. Propagation of excitation wave through the monolayer prior to coverslip placement.

Movie 6. Ischemia shortens the wavelength and slows conduction. Recorded at 11 min of ischemia.

Movie 7. At 1 hour ischemia, the excitation wave travels around the inexcitable ischemic region.

Movie 8. Wavelets and reentry. Recorded at 1 min reperfusion.

Movie 9. Micro-reentries. Recorded at 3 min reperfusion.

Movie 10. Sustained reentry anchored on the border zone. Recorded at 10 min reperfusion.

Movie 11. Recovery of excitability in the presence of 4′-Cl-DZP. Recorded at 2 min reperfusion.

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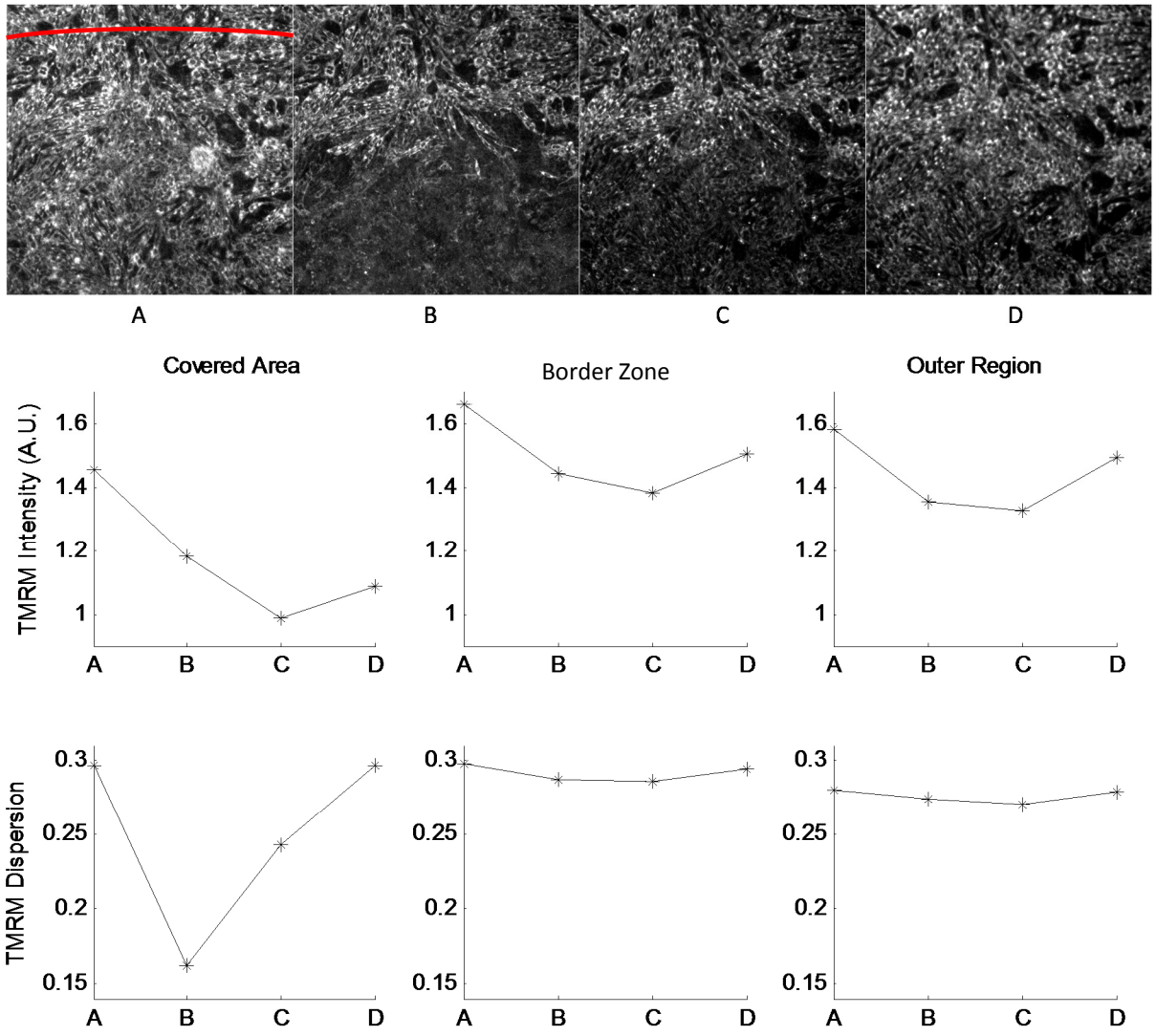

Supplemental Figure 1. Dispersion of TMRM fluorescence was used, instead of intensity, as a measure of $\Delta\Psi_m$. Loss of mitochondrial $\Delta\Psi_m$, due to ischemia, leads to release of TMRM out of matrix and a decrease in dispersion. Reperfusion redistributes TMRM into the mitochondrial matrix, which is shown by an increase in dispersion. TMRM spatial dispersion is used as $\Delta\Psi$m cannot be shown by the changes in TMRM intensity. Dispersion in border zone and the outer region does not change during the IR protocol. The same experiment as Figure 1; **A:** Beginning of ischemia, **B:** beginning of reperfusion, **C:** 45 minutes of reperfusion, **D:** 2 hours of reperfusion.

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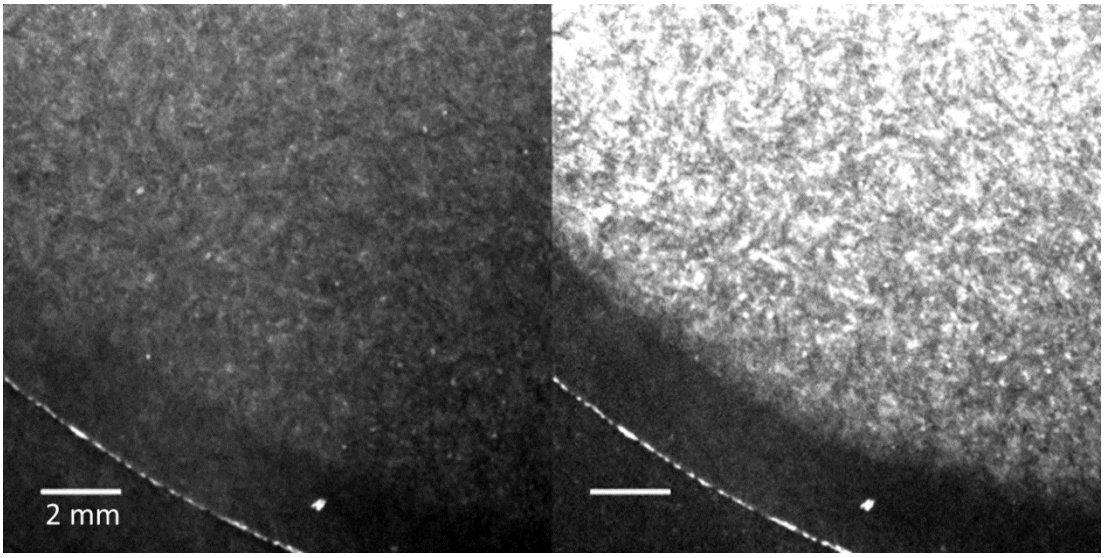

Supplemental Figure 2. $\Delta\Psi_m$ at 5 minutes and 1 hour of ischemia. Loss of $\Delta\Psi_m$ in the ischemic zone during ischemia is shown here. The white curved line on the lower left of the images shows the edge of the coverslip; the part below the line is not covered. Horizontal line shows 2 mm. Mitochondria in a border zone of approximately 2 mm stayed polarized. **Left image:** 5 minutes after coverslip placement. Partial mitochondrial depolarization can be seen in the ischemic region. **Right image:** Mitochondria of the ischemic region are depolarized after 1 hour of ischemia. (TMRM 2 μmol/l; dequench mode: in this mode, the TMRM concentration in the mitochondrial matrix exceeds a critical level above which its fluorescence self-quenches. Consequent depolarization of $\Delta\Psi_m$ is then indicated by an increase in fluorescence due to dequenching.)

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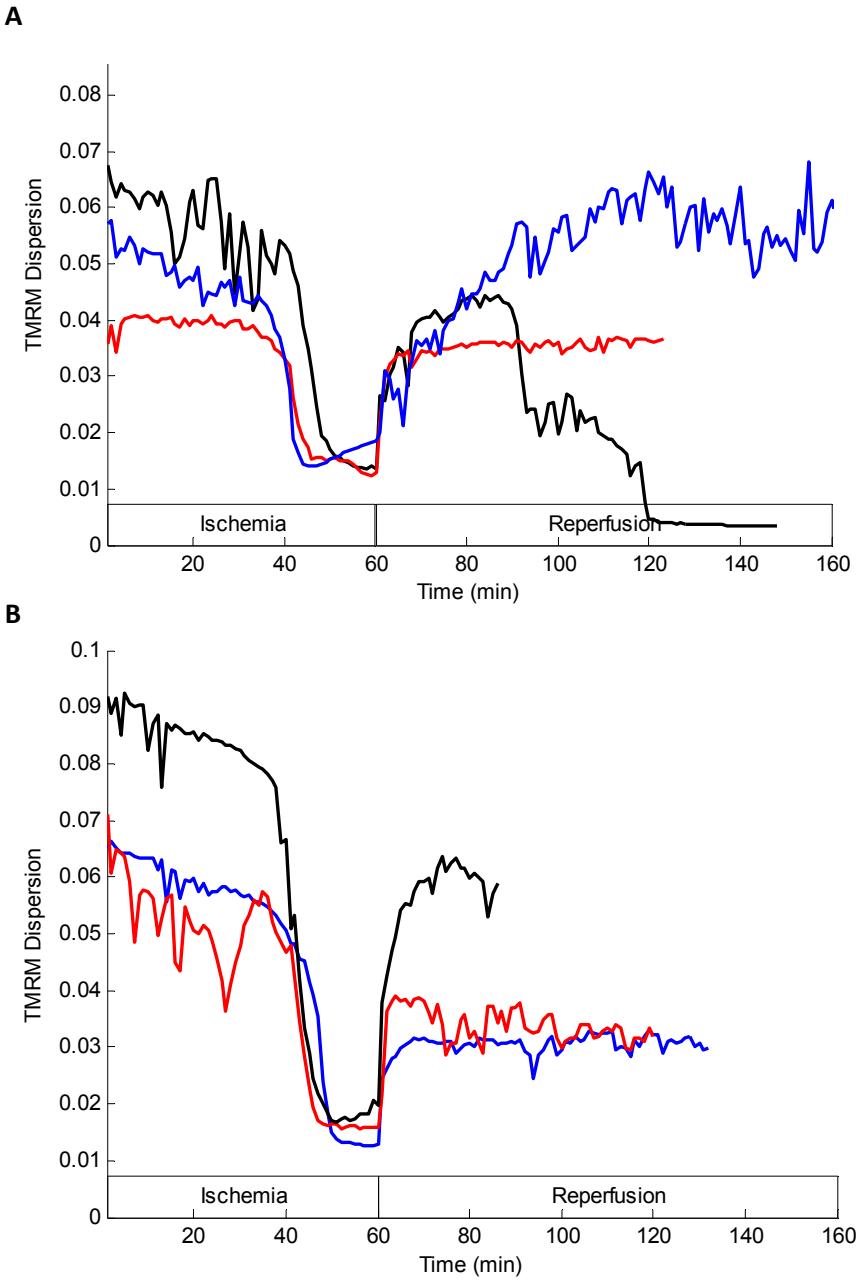
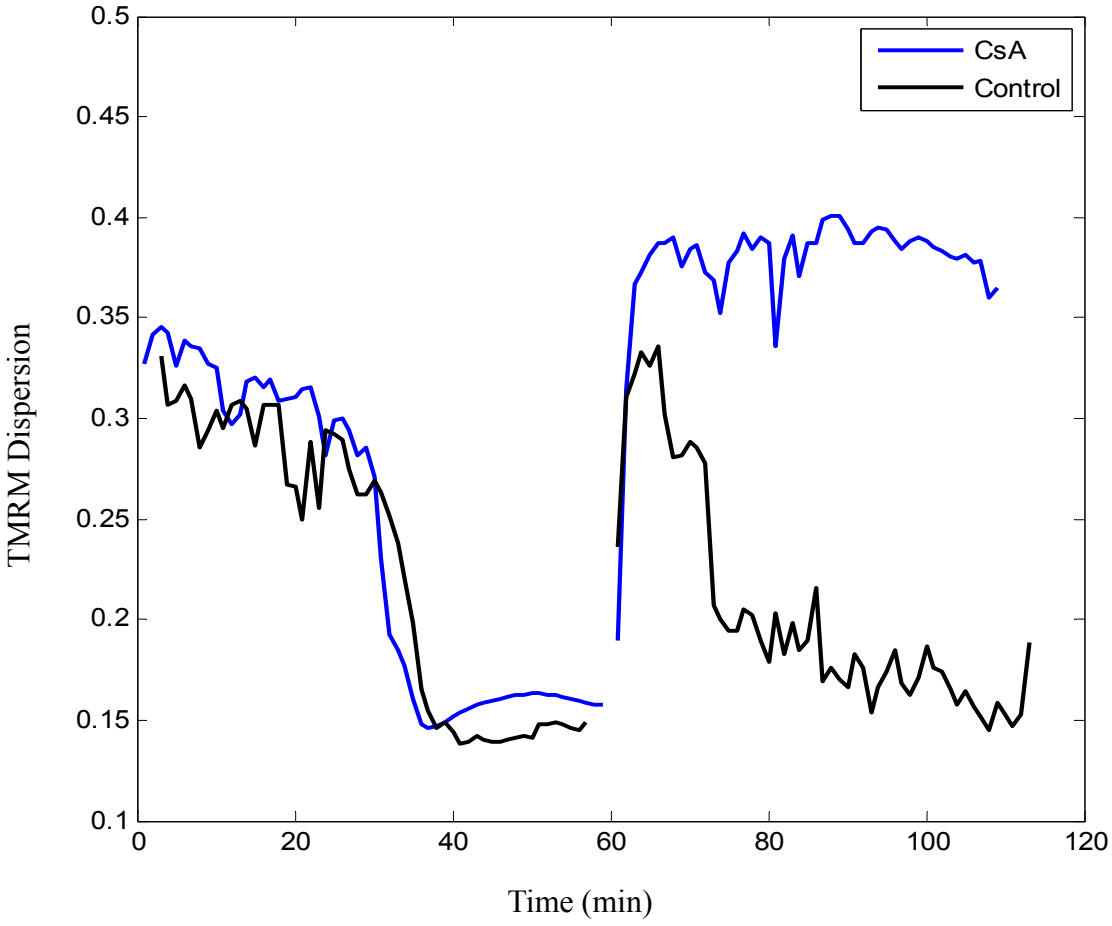
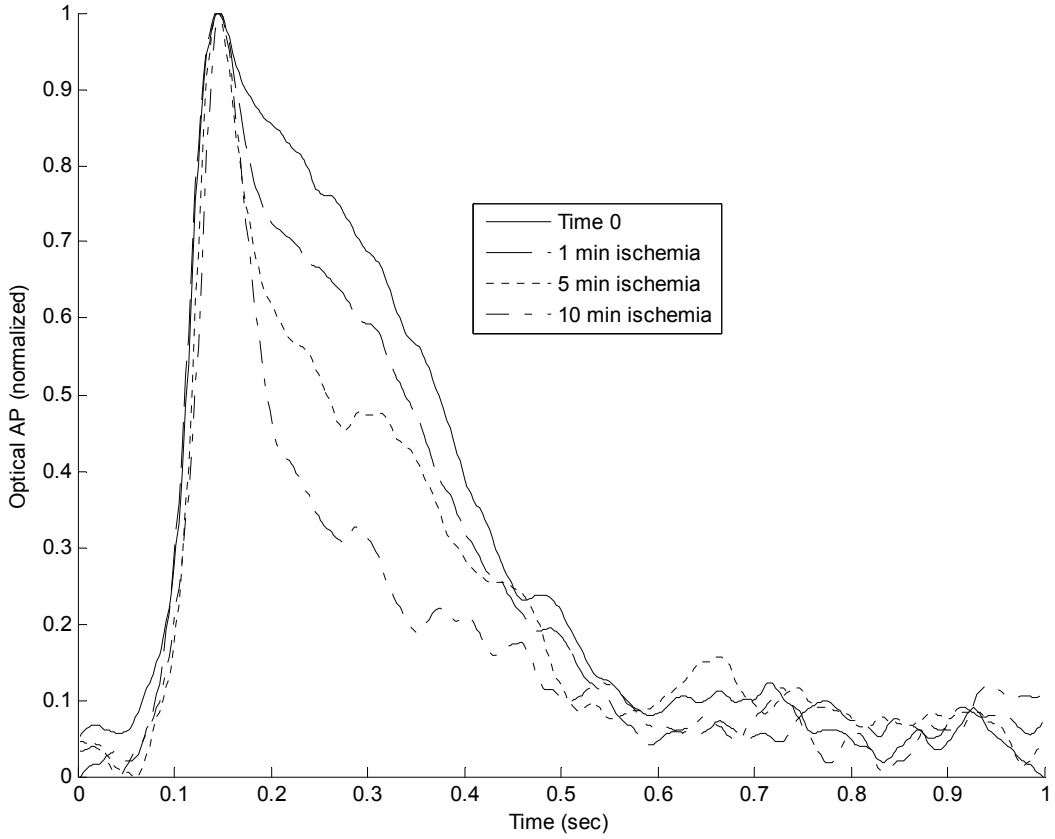


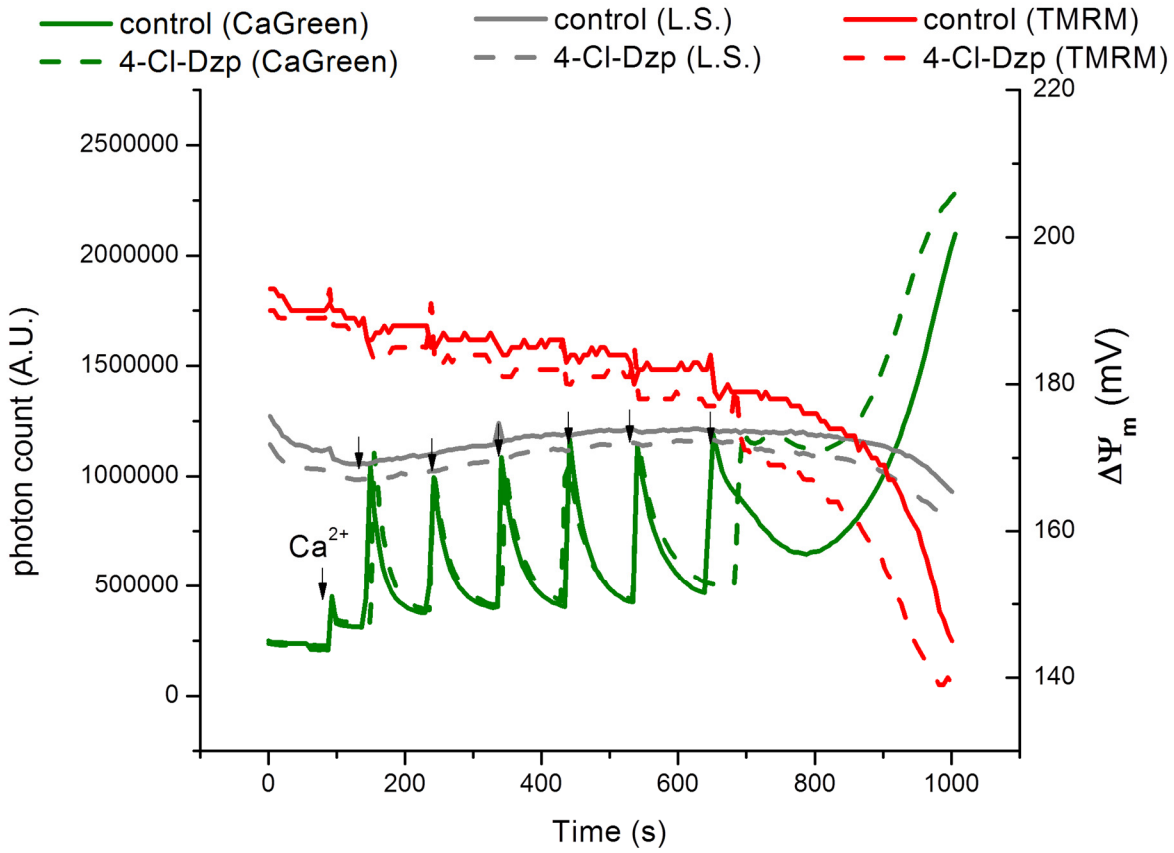
Supplemental Figure 3. Representative traces of IR induced changes in TMRM dispersion in control and in the presence of 4'-Cl-DZP. Changes in TMRM dispersion during IR is shown for 3 control monolayers **(A)**, and 3 monolayers in the presence of 16 μM 4′-Cl-DZP **(B)**.



Supplemental Figure 4. PTP involvement in large-scale reperfusion-induced mitochondrial $\Delta\Psi_m$ loss. While not able to prevent $\Delta\Psi_m$ oscillations, CsA protected mitochondria from global depolarization. Both monolayers were from the same cell preparation; CsA was applied at 1 μmol/l. Horizontal axis shows the time in minutes from the onset of ischemia. Reperfusion starts at 60 minutes.



Supplemental Figure 5. Optically measured action potential duration (APD) decreases during ischemia. Action potential amplitude is normalized.



Supplemental Figure 6. Lack of effect of 4′-Cl-DZP on Ca^{2+} activation of PTP in isolated mitochondria from the adult rat heart. Extra-mitochondrial Ca^{2+}, measured with Calcium Green-5N (green); $\Delta\Psi_m$, measured ratiometrically with tetramethylrhodamine methyl ester (TMRM; red); and light scattering (L.S.; grey; 540 nm excitation) with 7 sequential additions of 5 μM Ca^{2+} in the absence (solid lines) or presence (dashed lines) of 16 μM 4′-Cl-DZP. Methods were as described previously (Wei, et al, J Gen Physiol. 2012;139(6):465-78).

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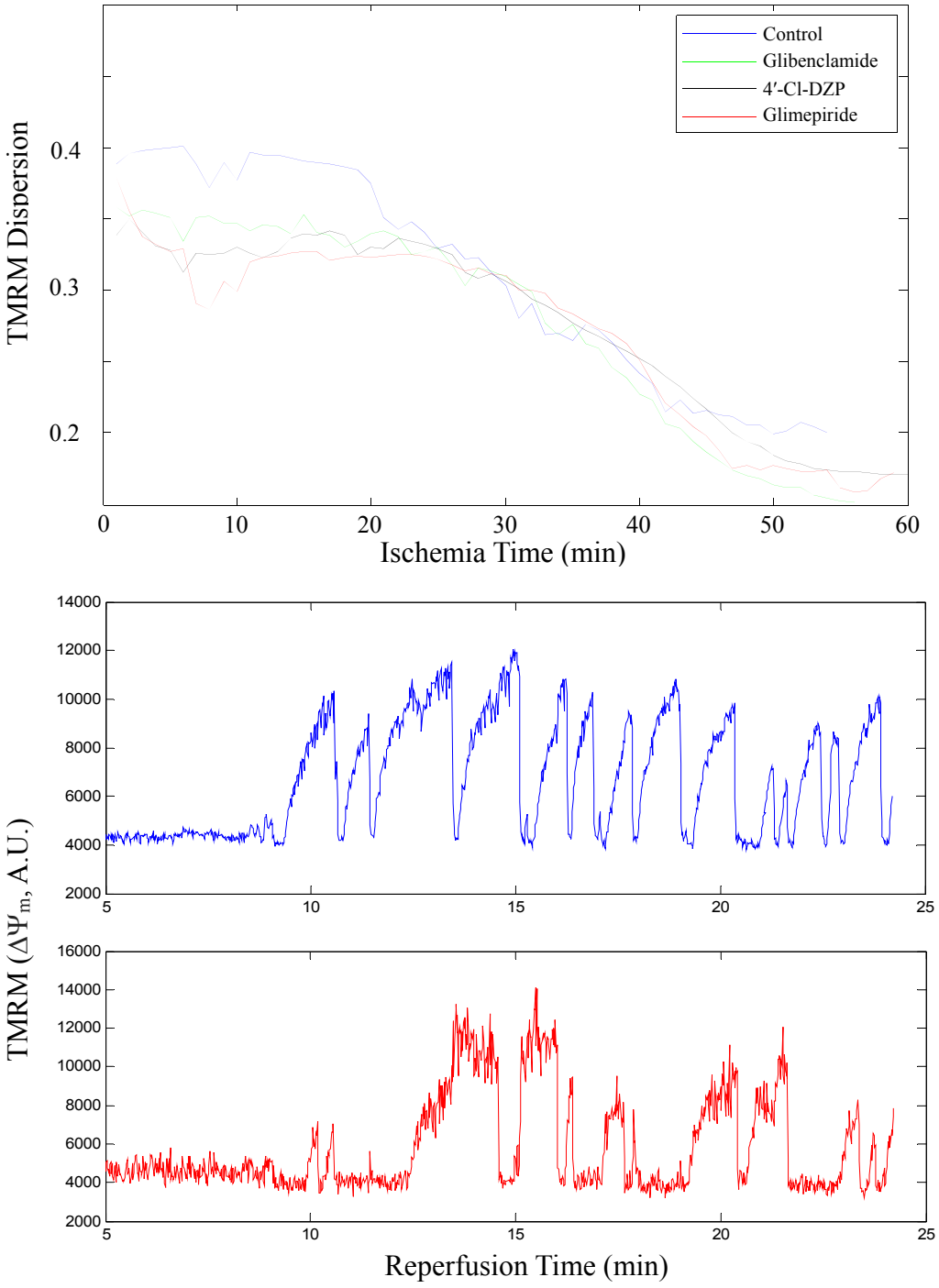
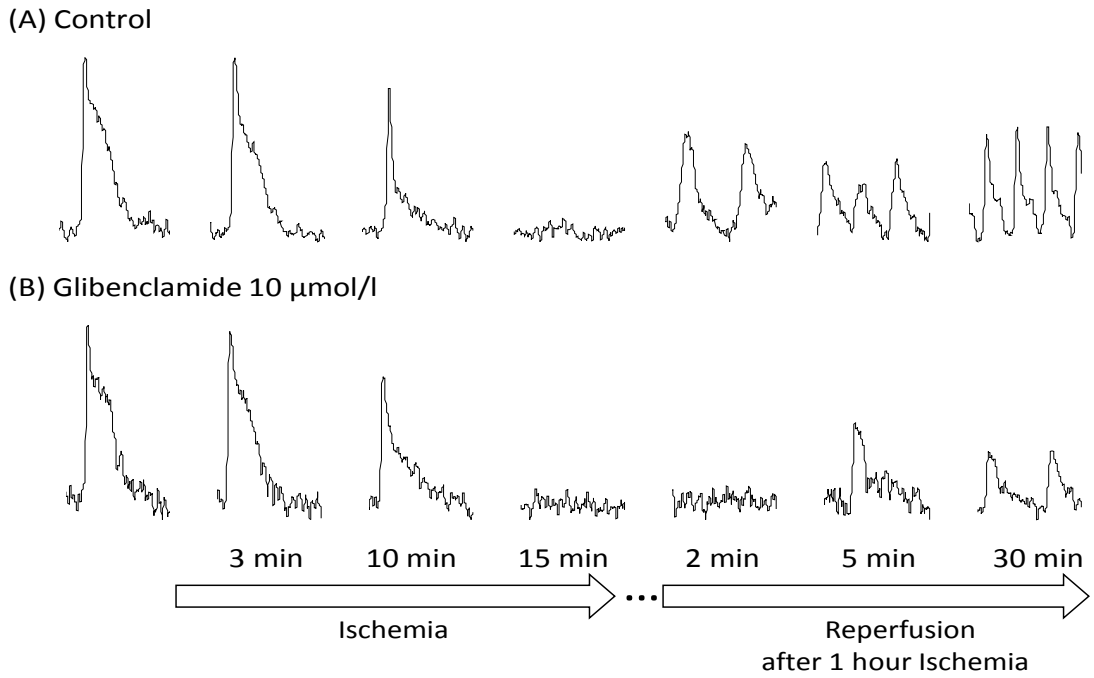


Supplemental Figure 7. Effect of K_{ATP} inhibition on mitochondria during coverslip ischemia/reperfusion. Upper panel: K_{ATP} inhibition did not affect the rate of coverslip ischemia-induced mitochondrial depolarization. A trend towards delayed phase 2 $\Delta\Psi_m$ loss during ischemia was observed with 4′-Cl-DZP treatment (see manuscript text for summary data) but did not reach statistical significance. **Lower panels:** Examples of reperfusion-induced $\Delta\Psi_m$ oscillation in 2 adjacent mitochondrial clusters in presence of glimepiride 1 μmol/l.



Supplemental Figure 8. Effect of K_{ATP} inhibition on AP during IR. During ischemia, AP is lost similar to control. However, APD shortening is slightly inhibited. Recovery is slowed but reentrant waves still form on reperfusion.

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Supplementary Table. Effect of coverslip-induced ischemia on electrophysiology of NRVM monolayers. The data is shown as mean ± standard deviation.

	Control	4' Cl-DZP
APD Before Ischemia (msec)	138 ± 54	152 ± 58
CV Before Ischemia (cm/sec)	15.5 ± 6.1	14.38 ± 3.32
APA Decrease after 5 min of Ischemia (%)	77 ± 25	73 ± 13
APD Decrease after 5 min of Ischemia (%)	57.9 ± 30.8	59.7 ± 20.5
CV after 5 min of Ischemia(cm/sec)	8.3 ± 4.4†	14.16 ± 6.74*
Time to Inexcitability (min)	15.4 ± 5.4	13.1 ± 5

*$P < 0.05$ versus control, † $P < 0.05$ CV at 5min ischemia vs. before ischemia