Legends for Supplemental Figures

Figure S1. Spontaneous oscillations of $\Delta\Psi_m$ in whole heart with nominally Ca^{2+} free perfusion. Intact hearts were loaded with TMRE, perfused, and imaged by two-photon microscopy under the conditions described in Figure 1 and Materials and Methods. The two panels show a video of the spontaneous $\Delta\Psi_m$ oscillations (left) and a time course of the TMRE fluorescence for three of the cells present in the microscopic field (right). In the video, notice the $\Delta\Psi_m$ oscillations that arise spontaneously in the cell in the center followed later by the immediately adjacent one on top. Bar, 20μm.

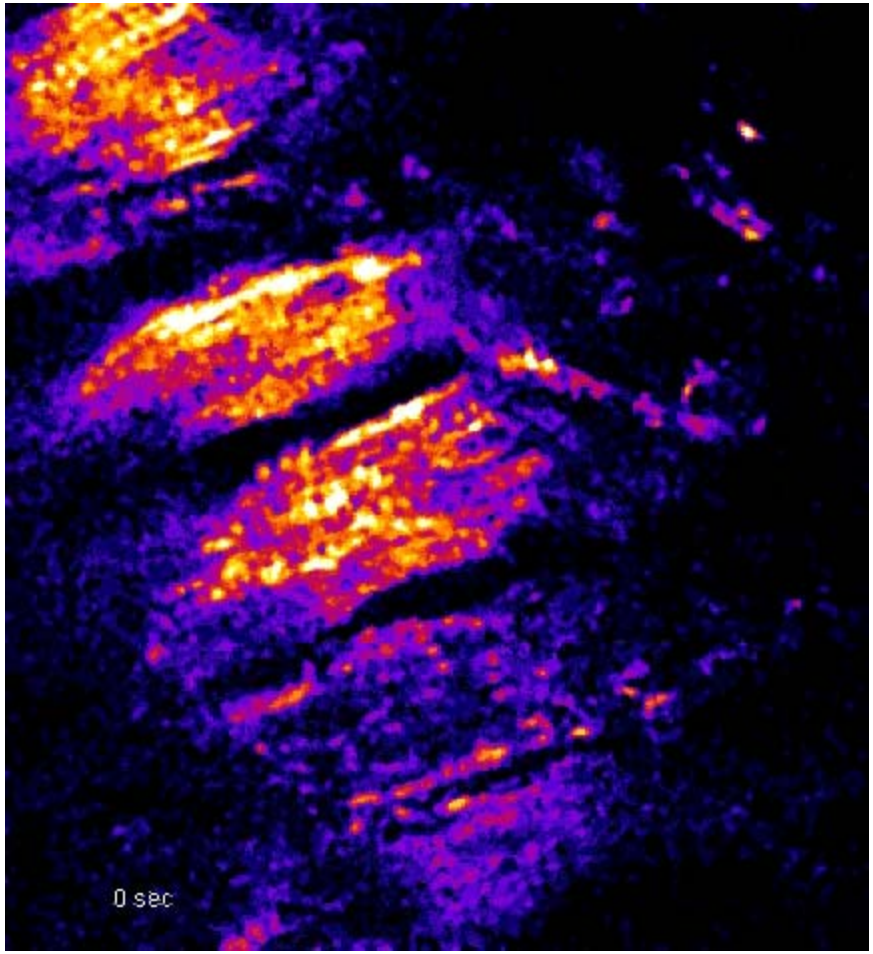
Figure S2. Oscillations in $\Delta\Psi_m$ triggered by a laser flash in heart (nominally Ca^{2+} free). Intact hearts were loaded with TMRE, perfused, and imaged by two-photon microscopy under the conditions described in Figure S1. The top panels show the videos corresponding to the TMRE ($\Delta\Psi_m$, left) and autofluorescence (NADH, right). The time course exhibited by both signals in the video is shown in the bottom panel.
In the video notice that $\Delta\Psi_m$ and NADH oscillations arise after a localized (small box) laser flash in the cell in the center of the field, and followed spontaneously by the adjacent cell on the right. Bar, 20μm.

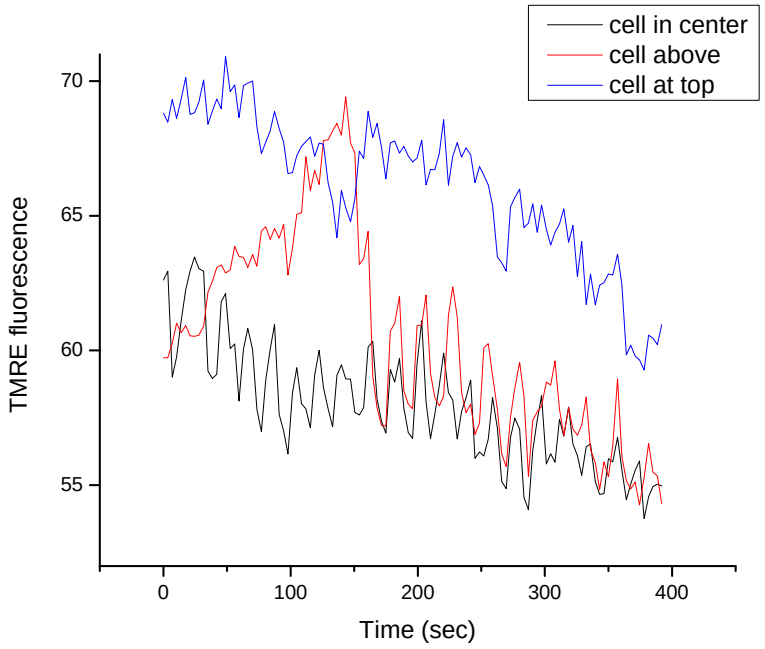
Figure S3. Wave of sustained $\Delta\Psi_m$ depolarization in reperfused heart. Intact hearts loaded with TMRE, perfused, and imaged by two-photon microscopy under the conditions described in Figure S1, were subjected to 30 min of global ischemia followed by reperfusion. The video shows a $\Delta\Psi_m$ depolarization wave occurring spontaneously across the microscopic field (from left to right) after 3 to 5 min of reperfusion.

Figure S4. 4′-chlorodiazepam (4′-Cl DZP) preserves redox status on reperfusion after ischemic injury. Intact hearts were loaded with 4μM CM H_2DCF, perfused, and imaged by two-photon microscopy under the conditions described in Figure S1. After attaining baseline conditions under normoxic perfusion

(T0), 24μM 4′-Cl DZP was added to the perfusate and the heart allowed to equilibrate for 10min (T0+4DZP). After equilibration the heart was subjected to 30 min of global ischemia (I) followed by reperfusion (RPF). Shown are the montages of the NAD(P)H and CM-DCF signals at the indicated times of ischemia and reperfusion. Notice the expected reduction of the NAD(P)H pool at 5min after the onset of ischemia (T5) and its return to baseline (T0) levels after reperfusion without the heterogeneous loss of NAD(P)H in individual cells that was observed during ischemia-reperfusion in untreated hearts.

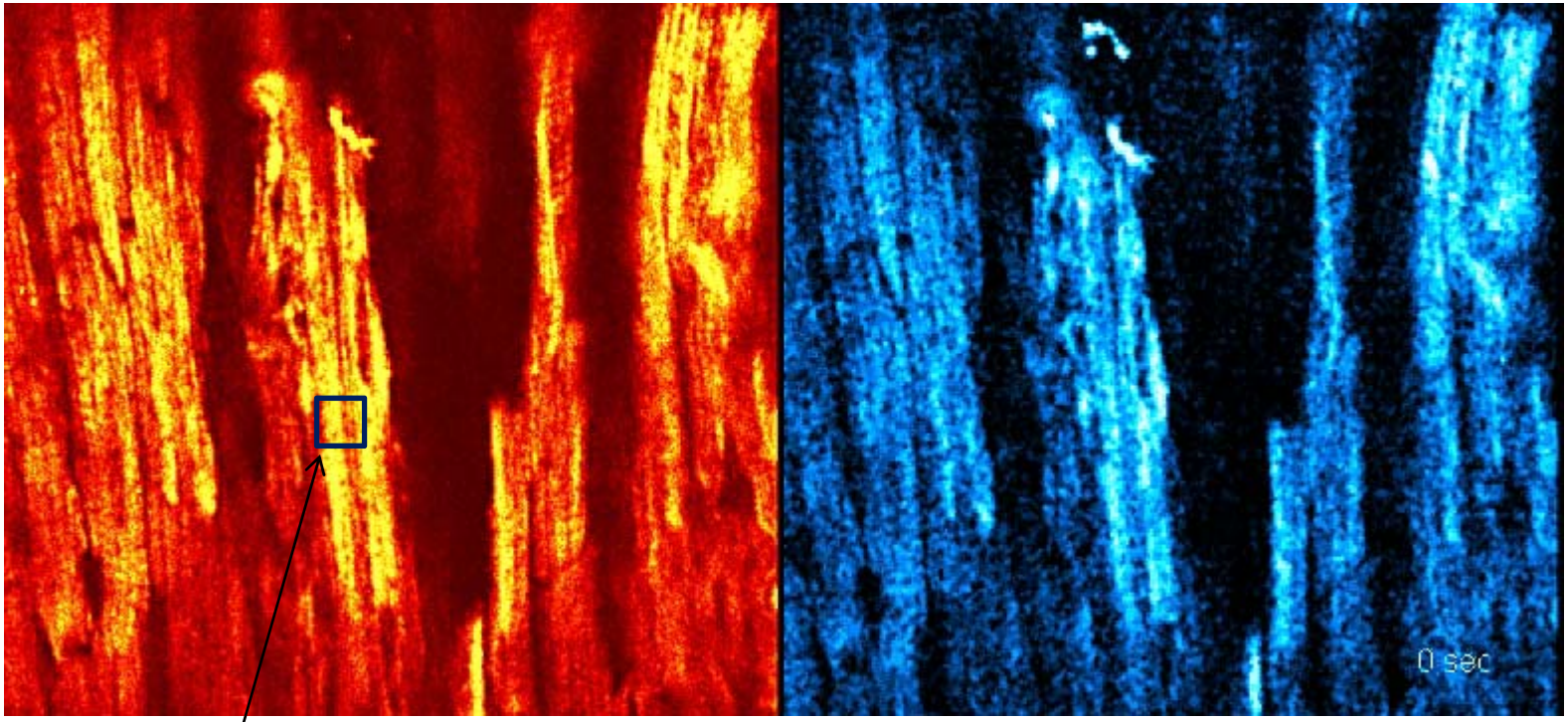
Spontaneous Oscillations of $\Delta\Psi_m$ in whole heart with nominally Ca-free perfusion



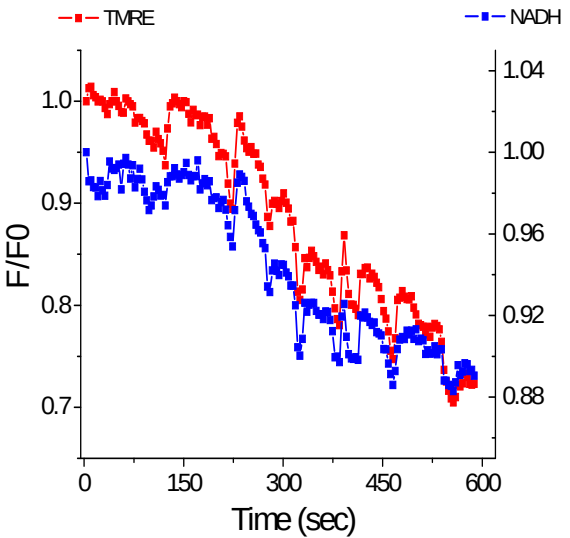


Slodzinski et al Figure S1

Oscillations in $\Delta\Psi_m$ triggered by a laser flash in heart (nominally Ca-free)



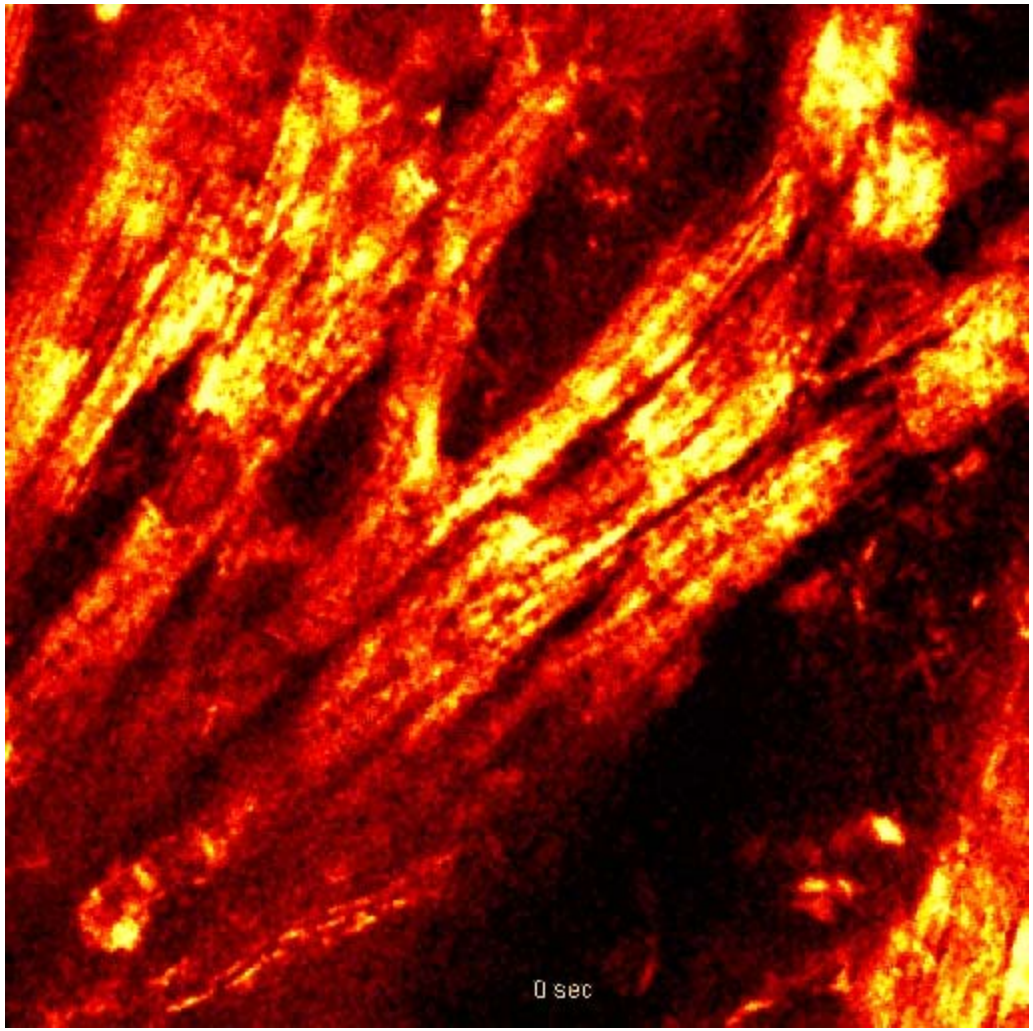
Flashed region



Plot of $\Delta\Psi$ and NADH
(left hand flashed cell)

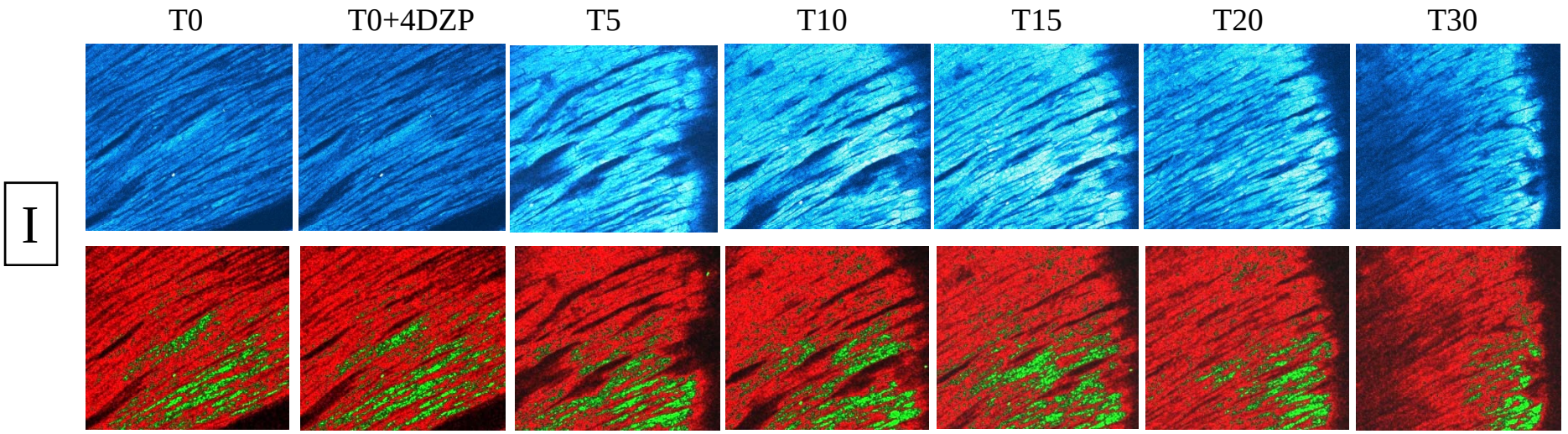
Slodzinski et al Figure S2

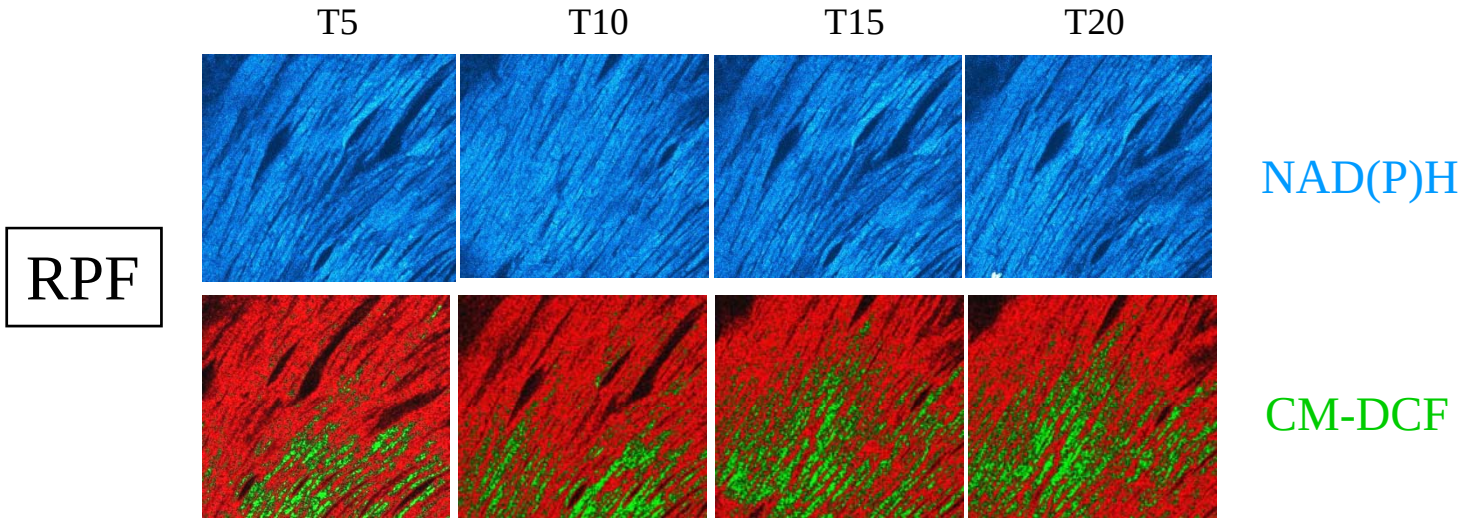
Wave of sustained $\Delta\Psi$ depolarization in reperfused heart



Slodzinski et al Figure S3

Two-photon imaging whole heart: I/R in the presence of 4'-Chl DZP





Supplemental Figure S4