Supplemental material

Feng et al., https://doi.org/10.1085/jgp.201812176

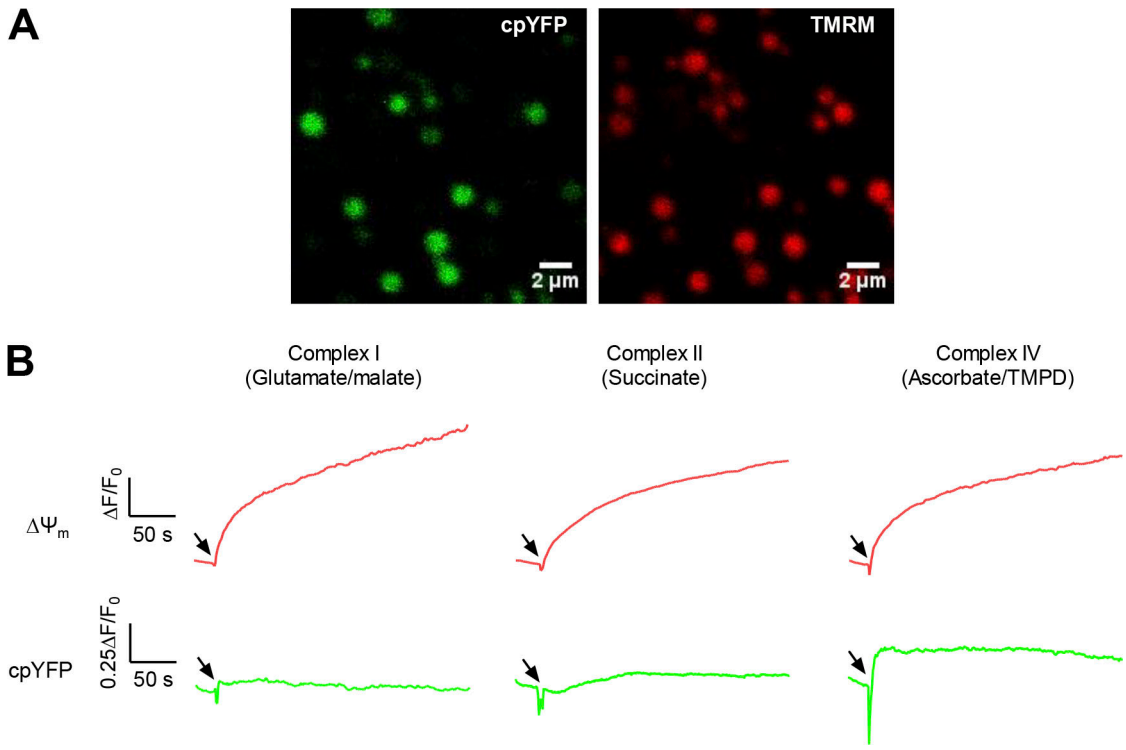


Figure S1. **Establishment of $\Delta\Psi_m$ and mild matrix alkalization in isolated cardiac mitochondria upon adding substrates in state II/IV respiration. (A)** Representative images of single cardiac mitochondria isolated from a cpYFP transgenic mouse loaded with TMRM. Mitochondria were energized with 5 mM glutamate and 5 mM malate in state II/IV respiration. **(B)** Averaged time courses of cpYFP fluorescence and $\Delta\Psi_m$ indexed by TMRM fluorescence in response to addition of different substrates without ADP. Arrows indicate the time of substrate administration; 5 mM glutamate/5 mM malate were added for Complex I, 2.5 mM succinate for Complex II, or 2.5 mM ascorbate/0.5 mM TMPD for Complex IV. Each trace used three image series from three mice. Error bars are omitted for clarity.

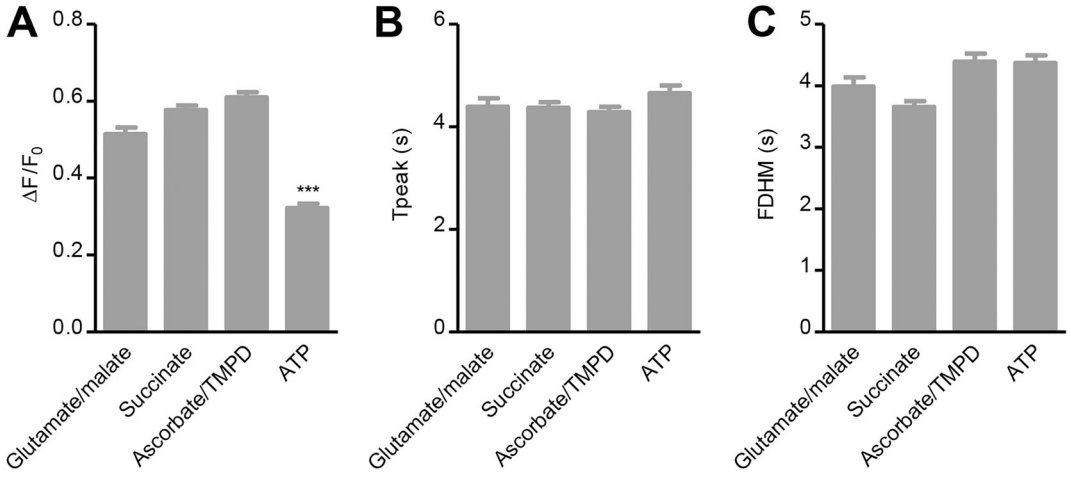


Figure S2. **Unitary properties of mitoflashes under different conditions. (A)** Mitoflash amplitude. Data are reported as $\Delta F/F_0$ of cpYFP fluorescence intensity, where F_0 refers to the baseline level. One-way ANOVA with post hoc Tukey was used to compare the differences among four groups. ***, $P < 0.001$ versus the other three groups. **(B)** Rise time. T_{peak}: time from the onset to the peak of a mitoflash. **(C)** Duration. FDHM: the full duration at half maximum of a mitoflash. Data are mean ± SEM, *n* = 213–845 mitoflash events for each group.

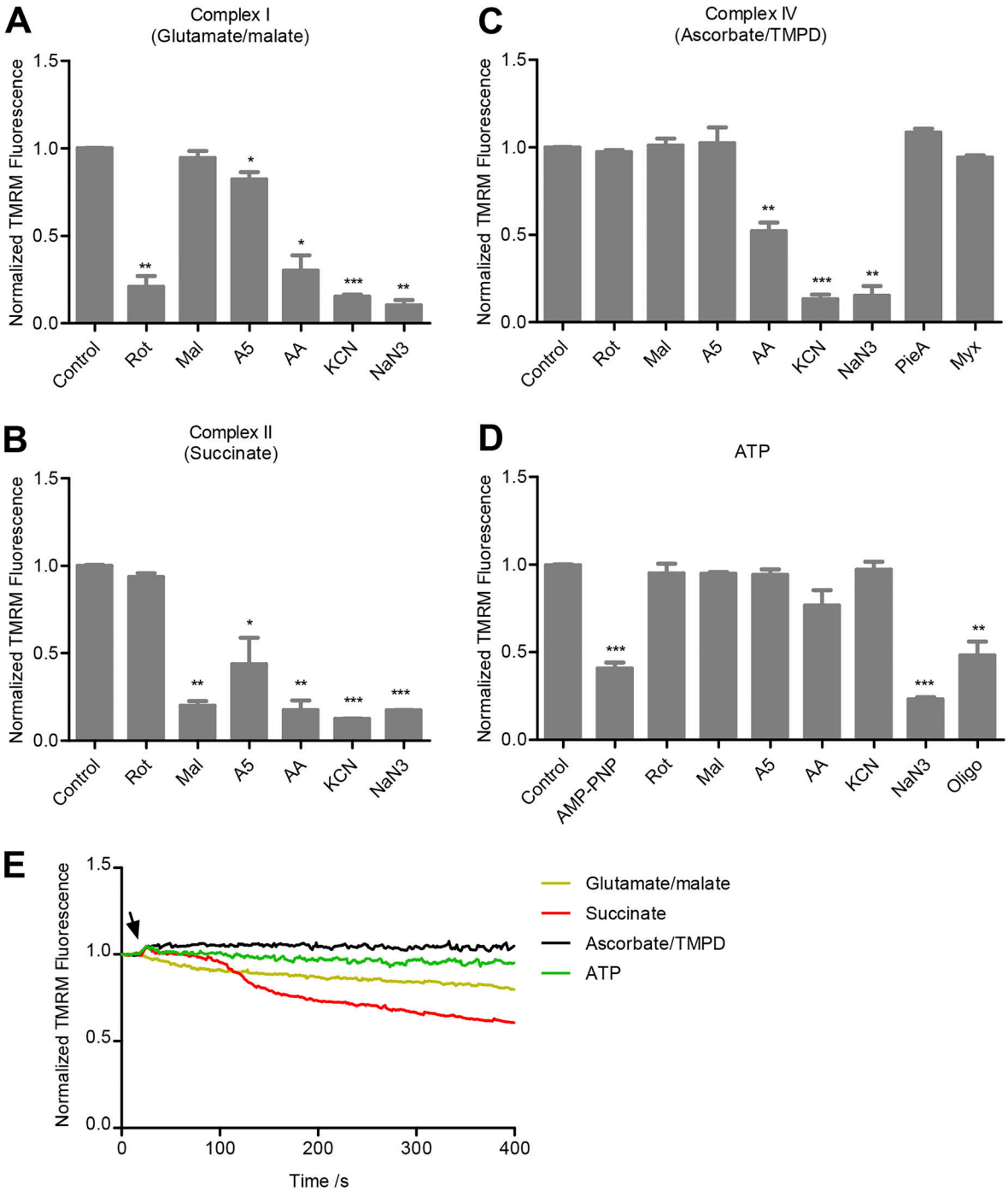
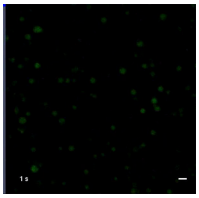
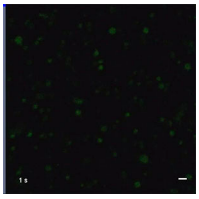


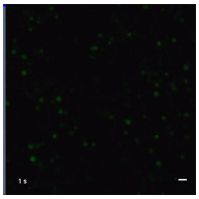
Figure S3. **Effects of ETC inhibitors on $\Delta\Psi_m$. (A–D)** Baseline levels of TMRM fluorescence in isolated mitochondria in the presence of different ETC inhibitors. Note that $\Delta\Psi_m$ was established by substrates of Complex I (A), Complex II (B), or Complex IV (C), or by ATP (D). *n* = 3–5 mice for each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus control group. **(E)** Effects of atpenin A5 on $\Delta\Psi_m$ indexed by TMRM fluorescence in mitochondria supported by different substrates. Arrow indicates the time of adding 50 nM atpenin A5. Each averaged trace used image data from 3–5 mice. Error bars are omitted for clarity.



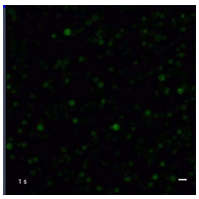
Video 1. **Mitoflashes supported by Complex I substrates in state II/IV respiration.** The video shows cpYFP-reported mitoflashes in isolated heart mitochondria supported by Complex I substrates (5 mM glutamate and 5 mM malate) in state II/IV respiration. Bar, 2 µm.



Video 2. **Mitoflashes supported by Complex II substrates in state II/IV respiration.** The video shows cpYFP-reported mitoflashes in isolated heart mitochondria supported by Complex II substrate (2.5 mM succinate) in state II/IV respiration. Bar, 2 µm.



Video 3. **Mitoflashes supported by Complex IV substrates in state II/IV respiration.** The video shows cpYFP-reported mitoflashes in isolated heart mitochondria supported by Complex IV substrates (2.5 mM ascorbate and 0.5 mM TMPD) in state II/IV respiration. Bar, 2 µm.



Video 4. **Mitoflashes supported by Complex V operating as an ATPase.** The video shows cpYFP-reported mitoflashes in isolated heart mitochondria supported by 3 mM ATP. Bar, 2 µm.