

SUPPLEMENTAL INFORMATION

Table S1, Related to Figure 2: Mitochondrial Morphology and Connectivity.

Morphology	Volume (μm^3)	Surface Area (μm^2)	SA/V (μm^{-1})	Mitochondrial Junction Surface Area (%)^a	Sphericity	n^b
Lipid Droplets	0.15 ± 0.01	1.33 ± 0.08	9.44 ± 0.30	28.67 ± 3.09 (3.96-60.94)	1.01 ± 0.00	26
Paranuclear Mitochondria						
<i>Elongated</i>	1.00 ± 0.16	6.56 ± 0.96	6.78 ± 0.30	11.86 ± 1.71 (1.99-17.52)	*0.75 ± 0.03	10
<i>Compact</i>	0.42 ± 0.10	2.56 ± 0.39	6.58 ± 0.51	12.32 ± 1.67 (5.61-21.25)	1.03 ± 0.03	10
Paravascular Mitochondria						
<i>Elongated</i>	1.76 ± 0.28	8.65 ± 1.11	5.37 ± 0.36	13.5 ± 1.82 (3.55-21.56)	*0.81 ± 0.03	13
<i>Compact</i>	1.07 ± 0.17	5.03 ± 0.60	5.21 ± 0.34	15.81 ± 1.87 (7.83-27.97)	0.98 ± 0.02	13
<i>w/Nanotube</i>	2.23 ± 0.47	*11.64 ± 2.12	5.62 ± 0.29	12.45 ± 1.34 (7.1-21.05)	*0.72 ± 0.03	11
Fiber Parallel Mitochondria						
<i>Elongated</i>	*^2.51 ± 0.66	*^11.20 ± 2.80	^4.67 ± 0.19	*3.68 ± 0.65 (0.38-9.41)	^0.84 ± 0.03	14
<i>Compact</i>	0.93 ± 0.17	4.53 ± 0.57	^5.47 ± 0.34	^13.25 ± 1.79 (4.41-27.79)	0.98 ± 0.01	12
<i>Connector</i>	*^2.40 ± 0.31	*^12.57 ± 1.88	^5.24 ± 0.27	*^5.34 ± 0.64 (1.63-8.56)	*^0.73 ± 0.04	12
<i>Non-connected</i>	*0.32 ± 0.06	*2.28 ± 0.32	*8.69 ± 0.40	*0 ± 0 (0)	0.99 ± 0.02	42

Values are mean ± SE. ^aMax and min values are listed in parentheses. ^bn values include structures from three separate animals. SA/V - surface area to volume ratio. *Significantly different ($p < 0.05$) from Compact. ^Significantly different ($p < 0.05$) from Non-connected. Sphericity is equivalent to 3D form factor.

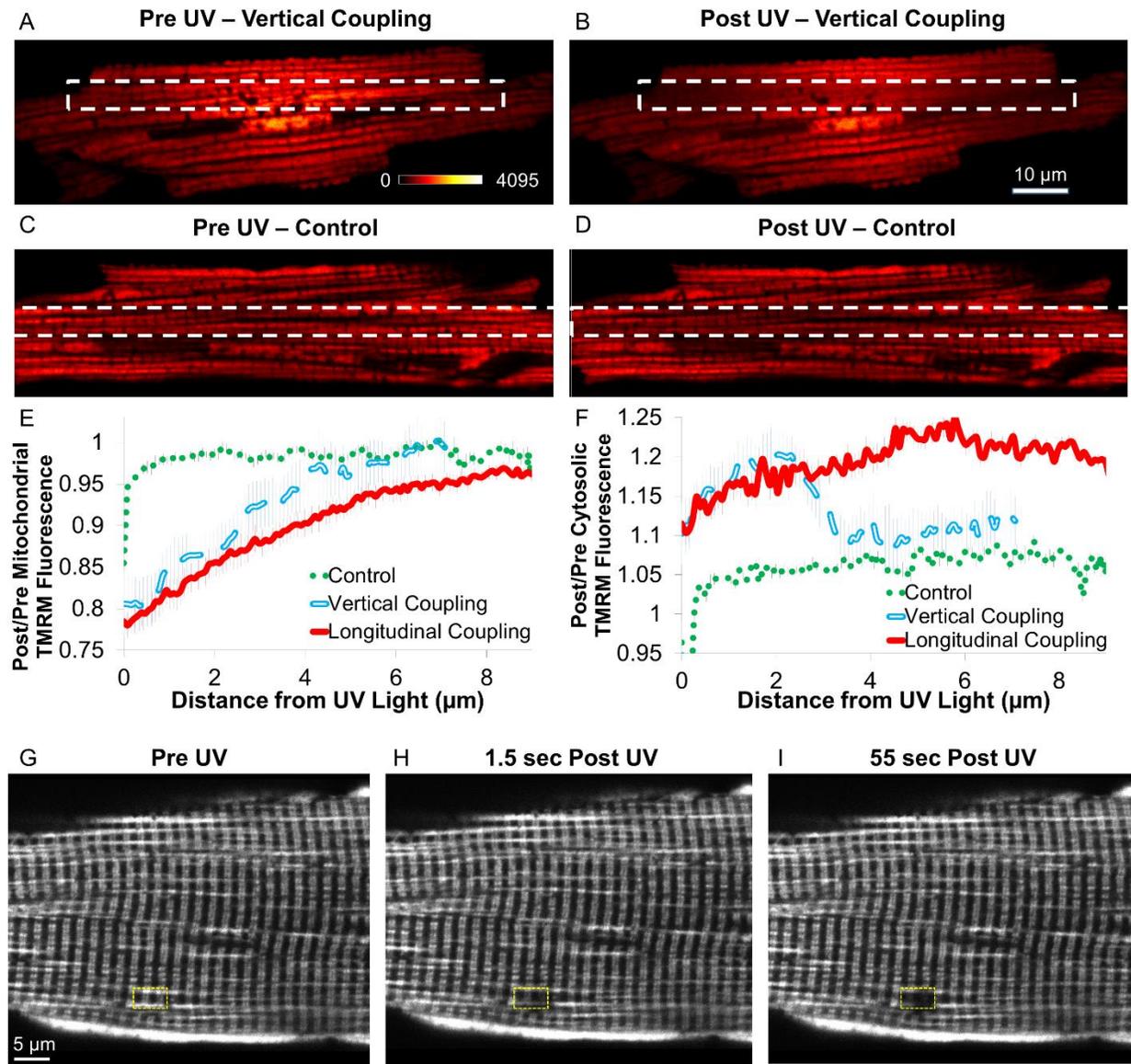


Figure S1, Related to Figure 3: MitoPhotoDNP depolarization. A) Confocal image of a TMRM and MitoPhotoDNP loaded cardiomyocyte prior to UV irradiation to assess Vertical coupling. Dashed lines represent the UV irradiation zone. B) Confocal image of a TMRM and MitoPhotoDNP loaded cardiomyocyte immediately after UV irradiation. C) Confocal image of a TMRM loaded cardiomyocyte without MitoPhotoDNP prior to UV irradiation. D) Confocal image of a TMRM loaded cardiomyocyte without MitoPhotoDNP immediately after UV irradiation. E) Post/pre mitochondrial TMRM signal as a function of distance outside UV irradiation zone. F) Post/pre cytosolic TMRM signal as a function of distance outside UV irradiation zone. G) Confocal image of TMRM fluorescence in a MitoPhotoDNP-loaded isolated mouse soleus fiber before UV irradiation. H) TMRM fluorescence immediately after UV activation of MitoPhotoDNP in a small “single mitochondria-sized” region (yellow dotted lines). I) TMRM fluorescence 55 seconds after MitoPhotoDNP activation.

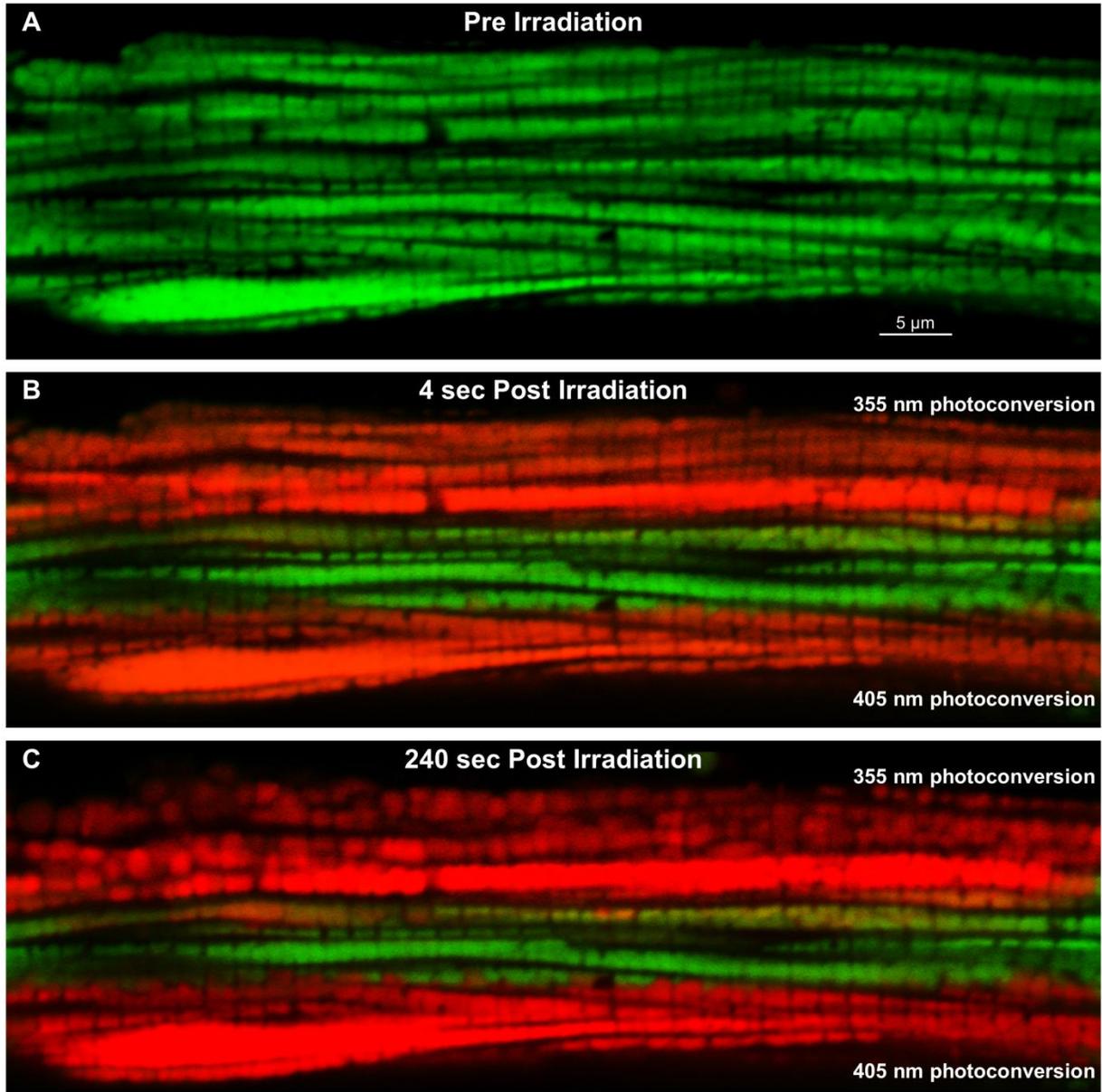


Figure S2, Related to Figure 5: Cardiomyocyte Mitochondrial Network Disconnection. A) Murine left ventricular cardiomyocyte MitoDendra2 fluorescence prior to irradiation of isolated cardiomyocyte loaded with MitoPhotoDNP. B) Cardiomyocyte MitoDendra2 fluorescence immediately after photoconversion of MitoDendra2 (green to red) with 355 nm (upper) and 405 nm (lower) light. Note that 355 nm light photoactivates MitoPhotoDNP and photoconverts MitoDendra2 while 405 nm light only photoconverts MitoDendra2. C) Cardiomyocyte MitoDendra2 fluorescence 240 seconds after photoconversion. Representative of 9 experiments from 3 mice.

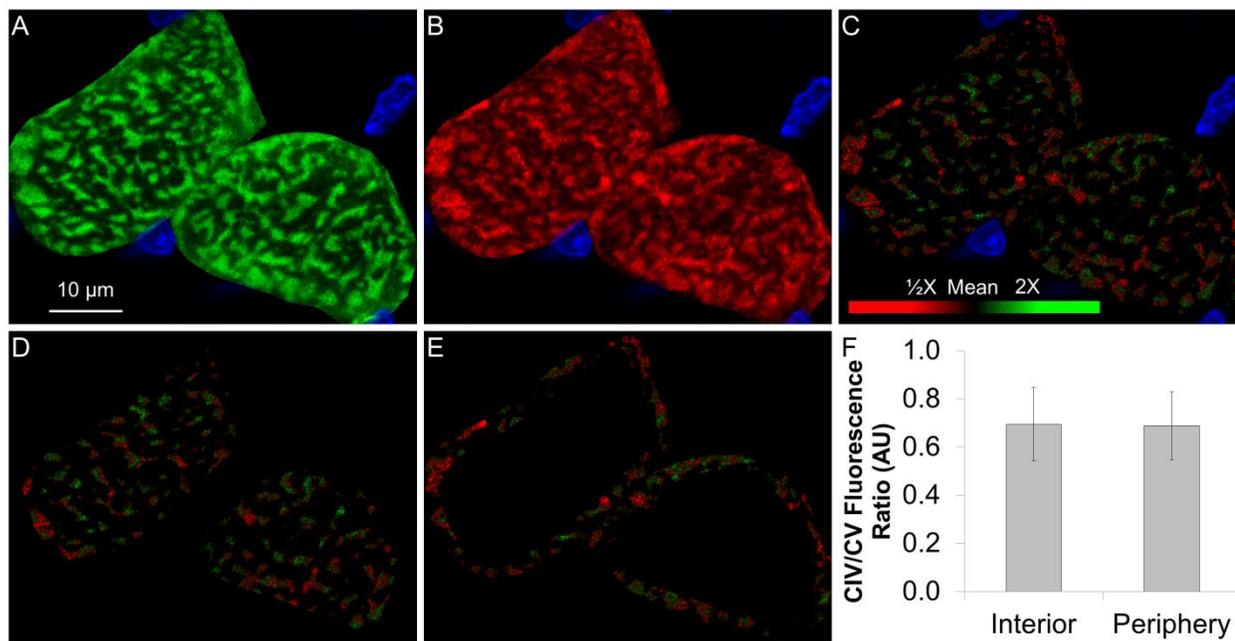


Figure S3, Related to Figure 3: Regional capacity for membrane potential generation and utilization. A) Confocal image of two fixed mouse heart cells immunostained for Complex IV. Nuclei: blue. B) Confocal image of the same mouse heart section immunostained for Complex V. C) Ratiometric image of the Complex IV/V immunofluorescence signals. Red signifies relatively higher Complex V and green signifies relatively higher Complex IV. D) Complex IV/V immunofluorescence ratio in the heart cell interior. E) Complex IV/V immunofluorescence ratio in the heart cell periphery. F) There are no regional differences in the Complex IV/V immunofluorescence ratio in the heart. Images are representative of and values are mean \pm SE from 11 cells from 3 mice.

Supplemental Movie and Dataset Legends

Movie S1, Related to Figures 1 and 2: Upper: A 3D FIB-SEM image stack of a mouse left ventricle. Longitudinal images are shown and time represents sequential images moving deeper into the fiber. Data shown has been binned by 3 in x and y and by 10 in z to reduce file size. A 2.5 Gb raw image stack is available as Supplementary Dataset 1. Lower: 360° rotation of mitochondrial subnetworks within part of a single heart cell. Each color represents a different subnetwork of tens to hundreds of mitochondria. White mitochondria are not network connected.

Movie S2, Related to Figure 3: Top) Timecourse loop of confocal microscopy images of the TMRM signal in a live, isolated cardiomyocyte before and after UV light induced release of MitoPhotoDNP in the cell interior to assess Longitudinal coupling. Bottom) Timecourse loop of confocal microscopy images of the TMRM signal in a live, isolated cardiomyocyte before and after UV light induced release of MitoPhotoDNP in the cell interior to assess Vertical coupling.

Movie S3, Related to Figure 4: Upper) TMRM fluorescence in an isolated mouse left ventricular cardiomyocyte before and after UV activation of MitoPhotoDNP. Middle) TMRM fluorescence in an isolated mouse soleus fiber before and after UV activation of MitoPhotoDNP. Lower: TMRM fluorescence in an isolated mouse soleus fiber before and two minutes after UV photodamage (~8-fold higher 355 nm laser power per μm^2 than used to activate MitoPhotoDNP).

Movie S4, Related to Figure 5: Upper three panels: MitoDendra2 fluorescence in an isolated mouse soleus fiber before and two minutes after green to red photoconversion of MitoDendra2. Lower panel: MitoDendra2 fluorescence in an isolated mouse soleus fiber before and two minutes after simultaneous green to red photoconversion of MitoDendra2 and photoactivation of MitoPhotoDNP.

Movie S5, Related to Figure 5: Upper: MitoDendra2 fluorescence in the paravascular region of an isolated mouse soleus fiber before and two minutes after simultaneous green to red photoconversion of MitoDendra2 and photoactivation of MitoPhotoDNP. Lower: MitoDendra2 fluorescence in the intrafibrillar region of an isolated mouse soleus fiber before and two minutes after simultaneous green to red photoconversion of MitoDendra2 and photoactivation of MitoPhotoDNP.