Supplemental Material

Quantitative super-resolution microscopy reveals promoting mitochondrial interconnectivity protects against AKI

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Supplemental Material Table of Contents:

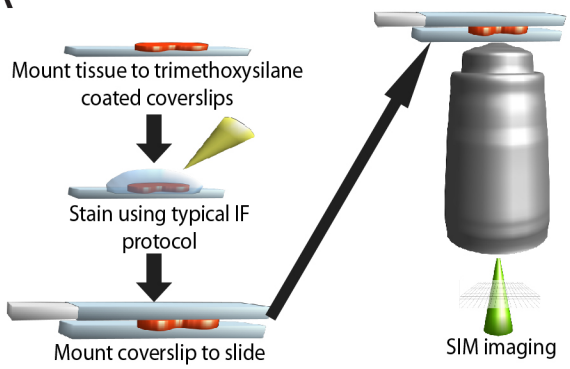
Supplemental Figure 1. SRRF and SoRa imaging can resolve tubule cell mitochondria and be used for quantitative analysis of mitochondrial volume. A. Schematic representation of the process of mounting tissue to coverslips. **B**. SRRF imaging of uninjured mouse kidney tissue. Nikon TiE, 100x objective, Andor IXon with SRRF, 1024x1024, processed to 4096x4096. **C.** Comparison of the original widefield image to the SRRF processed image using line scans. Note the increased resolution of the peaks following SRRF processing. **D.** Uninjured tubule cells imaged with SoRa, top panels, and quantitative data, bottom panel. Scale bar=10μm. **E**. Low magnification EM micrograph of the area chose for serial sectioning in Figure 2.

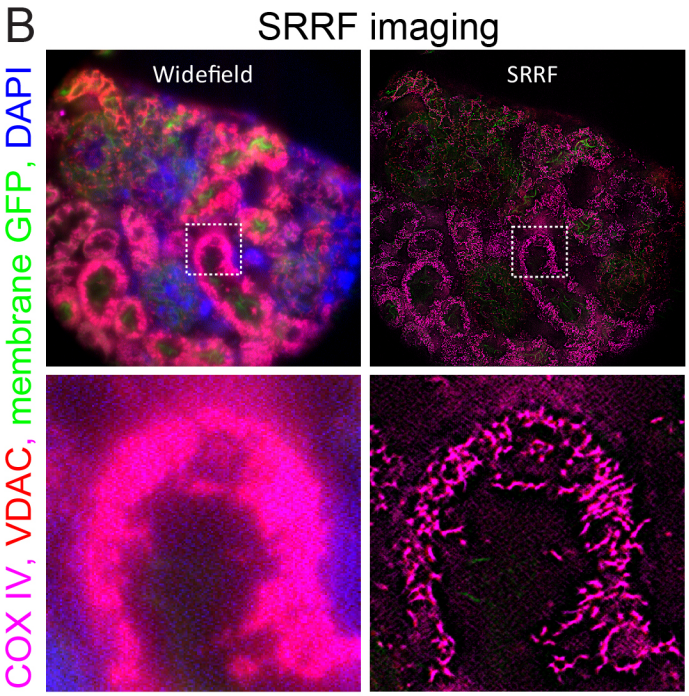
Supplemental Figure 2. Tissue SIM can resolve cardiomyocyte mitochondria and quantify mitochondrial surface area alterations with injury. A. SIM can image cardiac muscle cell mitochondria. Hearts were collected from db/m or db/db mice at 6 months of age and fixed, stained and imaged using the same protocol used for kidney. Scale bar = 5μm. **B**. Quantification of maximum mitochondrial network area and total mitochondrial surface area from A. n = 5.

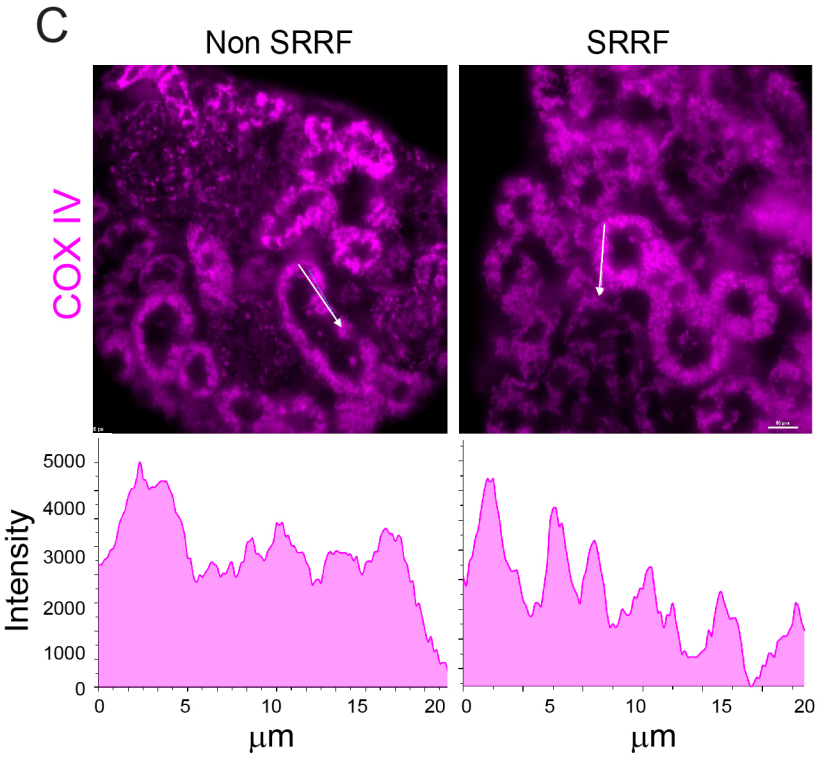
Supplemental Video 1: Video showing a full 3D rotation of the single mitochondrion imaged by SIM in Figure 2C.

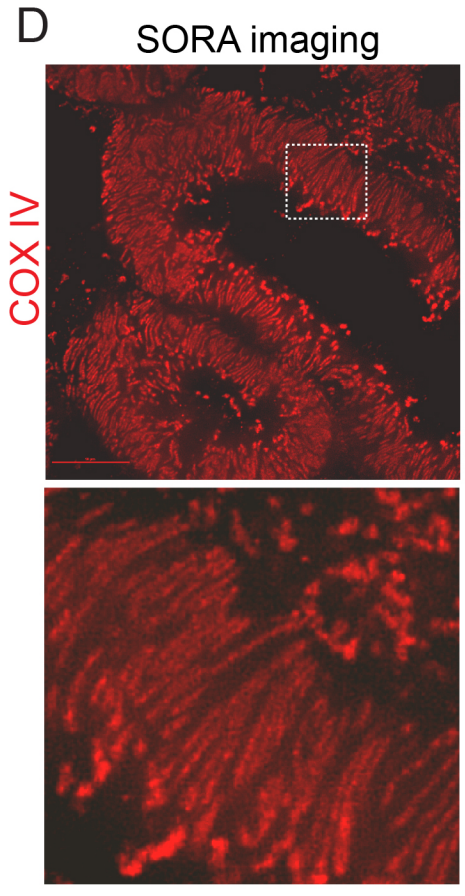
Supplemental Video 2: Video showing a full 3D rotation of the single mitochondrion imaged by in Figure 2G.

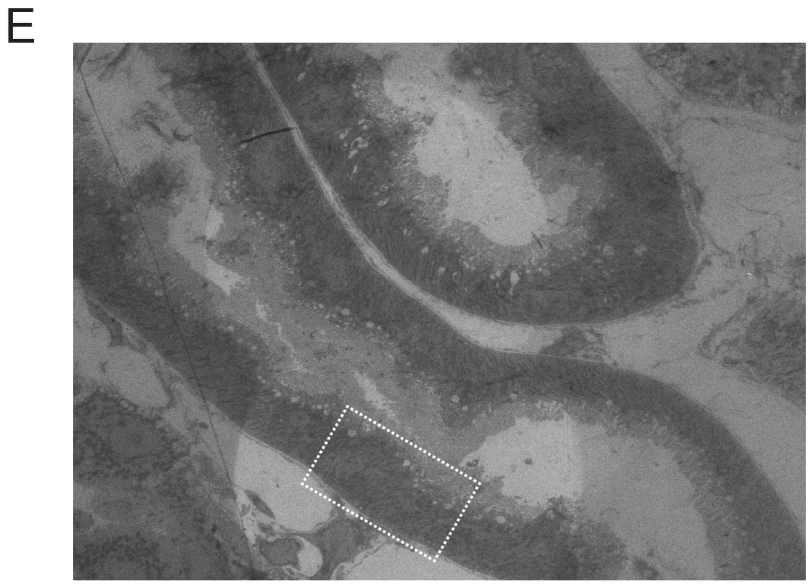
Supplemental Figure 1





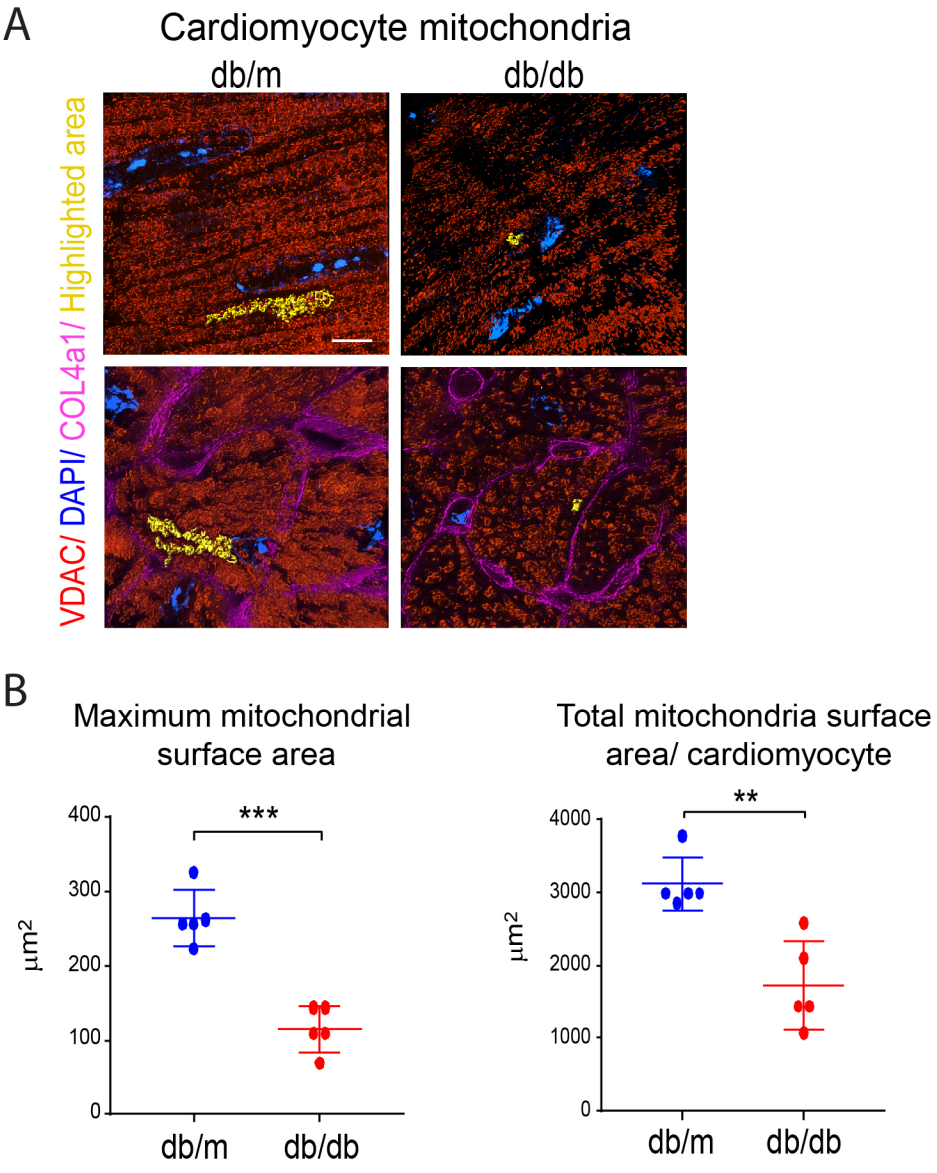






	Average ± SE
Maximum mitochondria network (µm^3)	70.2 ± 2.86
Total mitochondria vol. / tubule (µm^3)	67.1 ± 2.43
Mitochondrial number / tubule (n)	92.8 ± 7.53

Supplemental Figure 2



Supplemental Table1

	Reason for Biopsy	BUN (mg/dL)	Cr (mg/dL)	Proteinuria (g/ g/gCr)	Pathological diagnosis
Tubular injury patient #1	Acute pyelonephritis	8	0.46	5.4	Diffuse acute tubular injury
Tubular injury patient #2	Kidney transplant	26	1.9	0.22	Multifocal acute tubular injury
Tubular injury patient #3	Kidney transplant	61	13.6	1.5	Diffuse acute tubular injury
Uninjured tubule patient #1	Kidney transplant	25	1.06	0.14	Minimal histologic abnormalities
Uninjured tubule patient #2	Kidney transplant	21	2.5	0.96	Minimal histologic abnormalities
Uninjured tubule patient #3	IgA-N	N/A	N/A	0.3	Minimal histologic abnormalities

Supplemental Table1

Characteristics and diagnosis of patients with or without tubular injury. AKI, acute kidney injury; IgA-N, IgA nephropathy; BUN, blood urea nitrogen; Cr, creatinine; N/A, not available.