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# TREM2<sup>hi</sup> resident macrophages protect the septic heart by maintaining cardiomyocyte homeostasis

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**Supplementary Table 1: List of primers used for RT-qPCR.**

All primers are purchased from Sangon Biotech.

<b>Gene</b>	<b>Forward Primer (5'→3')</b>	<b>Reversed primer (5'→3')</b>
<i>β-actin</i>	CAGCTTCTTTGCAGCTCCTT	GCAGCGATATCGTCATCCA
<i>Trem2</i>	TGGGTCCCCGAGGAGTCATC	AAACTTGCTCAGGAGAACGC
<i>Anp</i>	CACAGATCTGATGGATTTCAAGA	CCTCATCTTCTACCGGCATC
<i>Bnp</i>	GTCAGTCGTTTGGGCTGTAAC	AGACCCAGGCAGAGTCAGAA
<i>αMhc</i>	TGCACTACGGAAACATGAAGTT	CGATGGAATAGTACACTTGCTGT
<i>βMhc</i>	GATGTTTTTGTGCCCGATGA	CAGTCACCGTCTTGCCATTCT
<i>Il1b</i>	CTTCCCCAGGGCATGTTAAG	ACCCTGAGCGACCTGTCTTG
<i>Il6</i>	TTCCATCCAGTTGCCTTCTTG	TTGGGAGTGGTATCCTCTGTGA
<i>Ccl2</i>	TAAAAACCTGGATCGGAACCAAA	GCATTAGCTTCAGATTTACGGGT
<i>Tnfa</i>	ATGGCCTCCCTCTCATCAGT	CTTGGTGGTTTGCTACGACG

**Supplementary Table 2: List of Antibodies.**

<b>Antibodies</b>	<b>Source</b>	<b>Catalog</b>	<b>dilution</b>
AF700 Rat anti-Mouse CD45 (30-F11)	BD Biosciences	560510	1 : 200
PE-Cy5 Rat anti-Mouse CD45R/B220 (RA3-6B2)	BD Biosciences	553091	1 : 200
BV510 Hamster anti-Mouse CD3e (145-2C11)	BD Biosciences	563024	1 : 200
BV786 Mouse anti-Mouse NK-1.1 (PK136)	BD Biosciences	740853	1 : 200
BV711 Rat anti-Mouse CD11b (M1/70)	BD Biosciences	563168	1 : 200
FITC Rat anti-Mouse Ly-6G (1A8-Ly6g)	Thermo Fisher Scientific	11-9668-82	1 : 200
PE Rat anti-Mouse F4/80 (BM8)	BioLegend	123110	1 : 200
BV650 Rat anti-Mouse I-A/I-E (M5/114.15.2)	BD Biosciences	563415	1 : 200
BV605 Hamster anti-Mouse CD11c (N418)	BD Biosciences	744179	1 : 200
BV421 Rat anti-Mouse Ly-6C (HK1.4)	BioLegend	128031	1 : 200
PerCP-Cyanine5.5 Rat anti- Mouse/Human CD11b (M1/70)	BioLegend	101228	1 : 200
PE-Cyanine7 Rat anti-Mouse F4/80 (BM8)	Thermo Fisher Scientific	25-4801-82	1 : 200

Super Bright 600 Rat anti-Mouse CD163 (TNKUPJ)	Thermo Fisher Scientific	63-1631-82	1 : 200
PerCP-Cy5.5 Mouse anti-Mouse CD45.1 (A20)	BD Biosciences	560580	1 : 200
BV605 Mouse anti-mouse CD45.2 (104)	BD Biosciences	563051	1 : 200
Biotinylated Rabbit anti-Murine RELM $\alpha$	PeproTech	500-P214BT	1 : 200
BV421 Mouse anti-Ki-67 (B56)	BD Biosciences	565929	1 : 200
BV421 Mouse IgG1, k Isotype Control (X40)	BD Biosciences	562438	1 : 200
Rat anti-Mouse CD68 (FA-11)	Abcam	ab53444	1:250
Mouse anti-Mouse CD163 (ED2)	Santa Cruz Biotechnology	sc-58965	1:100
Goat anti mouse TREM2 Antibody(polyclonal)	Abcam	ab95470	1:100
Rat anti Human/Mouse TREM2 (237920)	R and D Systems	MAB17291	1:100
Rabbit anti-Mouse TOMM20 (ERP15581-54)	Abcam	ab186735	1:200/1:250
Rabbit anti-Cardiac Troponin I (polyclonal)	Abcam	ab47003	1:250
Rat anti-Mouse LAMP1	DSHB	P11438	1:50

Alexa Fluor 594 Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	A21209	1:500
Alexa Fluor 488 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	A21202	1:500
Alexa Fluor 488 Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	A21206	1:500
Alexa Fluor Plus 647 Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	A48272	1:500
Alexa Fluor 594 Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	A11005	1:500

**Supplementary Table 3: List of chemicals and assays.**

<b>REAGENT</b>	<b>Source</b>	<b>Catalog</b>
FITC TUNEL Assay Kit	Abcam	ab66108
MitoTracker™ Green FM	Thermo Fisher Scientific	M7514
CytoTracker Orange (CMTMR)	Thermo Fisher Scientific	C2927
NIR Mitochondrial Membrane Potential Assay Kit	Abcam	ab112149
Liberase™ TL Research Grade	Sigma-Aldrich	5401020001
Percoll	Yeadon	40501ES60
Dnase I	Sinopharm Chemical Reagent Co.	64002860
HEPES	Sigma-Aldrich	V900477
Tamoxifen Citrate	Meilunbio	MB1233
DAPI-Fluoromount-G	Southern Biotech	0100-20
Corning® Matrigel® Basement Membrane Matrix, LDEV-free, 5 mL	Corning	356234
Mouse Interleukin Array Q1	Ray Biotech	QAM-INT-1
Lactic Acid assay kit	Nanjing Jiancheng Bioengineering Institute	A019-2-1
Lactate dehydrogenase assay kit	Nanjing Jiancheng Bioengineering Institute	A020-2-2
Mouse IL-1 beta Valukine ELISA	Novus	VAL601
Mouse IL-6 Valukine ELISA Kit	Novus	VAL604

Mouse TNF-a ELISA Kit	MULTI SCIENCES	EK282
Mouse CCL2/MCP-1 ELISA Kit	MULTI SCIENCES	EK287
ELISA Kit for Atrial Natriuretic Peptide (ANP)	USCN Life Science	SEA225Mu
ELISA Kit for N-Terminal Pro-Brain Natriuretic Peptide (NT-ProBNP)	USCN Life Science	SEA485Mu
ELISA Kit for Cardiac Troponin I (cTnI)	USCN Life Science	SEA478Mu
ATP Assay Kit	Beyotime	S0026
UltraComp eBeads™ Compensation Beads	Thermo Fisher Scientific	01-2222-42
eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set	Thermo Fisher Scientific	00-5523-00
Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1, 16 rxns	10x Genomics	PN-1000121
Chromium Next GEM Chip G Single Cell Kit, 48rxns	10x Genomics	PN-1000120
TRizol™ Reagent	Ambion	15596018
PrimeScript™ RT reagent Kit (Perfect Real Time)	Takara	RR037A
TB Green® Premix Ex Taq™ (Tli RNaseH Plus)	Takara	RR420A
eBioscience™ Calcein Violet 450 AM Viability Dye	Thermo Fisher Scientific	65-0854-39



Vybrant™ DyeCycle™ Ruby Stain	Thermo Fisher Scientific	V10309
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3 **Supplementary Video 1. Sepsis induces the release of massively cardiomyocyte-derived exophers.**

3D reconstruction of the heart slices from Card<sup>RED</sup> mice showed the presence of  
6 mitochondria (Tom20, cyan) in cardiomyocyte-derived exophers (red) and the accumulation of Tomato<sup>+</sup> exophers co-localized with Tom20 in septic hearts.

9 **Supplementary Video 2. Mac1 cells take up cardiac exophers containing mitochondria in SICM.**

3D reconstruction of the heart slices from Card<sup>RED</sup> mice. Mac1 cells (TREM2, green) phagocytosed cardiomyocyte-derived exophers (red). Exophers in Mac1 cells included  
12 mitochondria (Tom20, white).

**Supplementary Video 3. Cardiomyocyte-derived mitochondria transfer to Mac1 cells.**

15 3D reconstruction of the heart slices from MitoCard mice. Mac1 cells (TREM2, red) took up cardiomyocyte-derived mitochondria (mtDendra2, green).

18 **Supplementary Video 4. Cardiomyocyte-derived mitochondria processed with LAMP1<sup>+</sup> phagolysosomes in Mac1 cells.**

3D reconstruction of the heart slices from MitoCard mice. Cardiomyocyte-derived mitochondria (mtDendra2, green) phagocytosed by Mac1 cells (TREM2, red) partially localized in lysosomes (LAMP1, white).

**Supplementary Video 5. *Trem2* deficiency impairs the uptake of cardiomyocyte-derived mitochondria by Mac1 subset in septic heart.**

Part 1: Schematic illustration of the cardiomyocyte mitochondria labeled with AAV9-Tnnt2-mt-Keima virus. Keima-tagged mitochondria were indicated by different fluorescence in neutral (Keima 458nm, green) and acidic (Keima 561nm, red) environments. Part 2: 3D reconstruction showed that TREM2<sup>+</sup> macrophages (green) took up cardiomyocyte-derived mitochondria (mtKeima-458, cyan) and some mitochondria in an acidic environment (mtKeima-561, red) in the hearts of AAV9-Tnnt2-mt-Keima infected mice. Part 3: CD163<sup>+</sup> macrophages (green) took up cardiomyocyte-derived mitochondria (mtKeima-458, cyan; mtKeima-561, red) in hearts of WT and *Trem2*<sup>-/-</sup> mice infected with AAV9-Tnnt2-mt-Keima.

**Supplementary Video 6. Transplanted Mac1 cells engulf cardiomyocyte-derived mitochondria in SICM.**

3D reconstruction of the heart slices from MitoCard mice. Injected Mac1 cells (CMTMR, red) phagocytosed cardiomyocyte-derived mitochondria (mtDendra2, green).