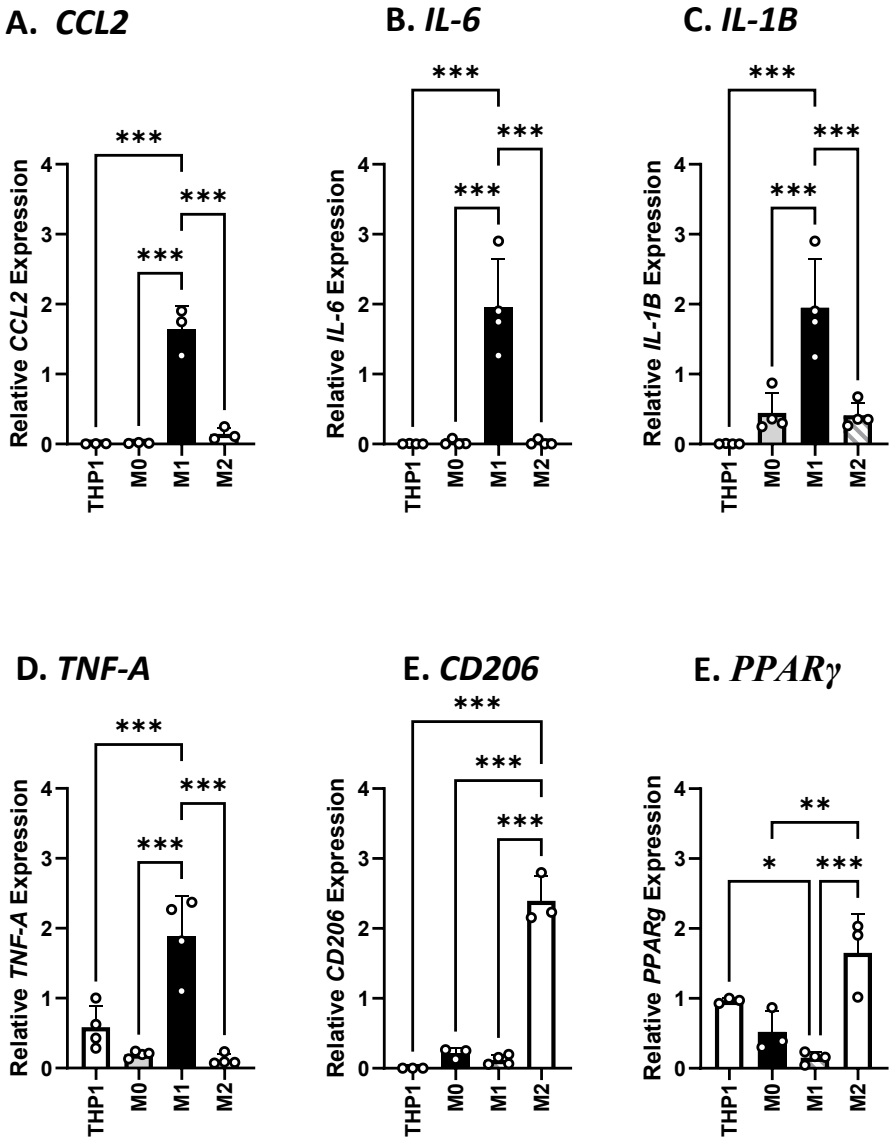
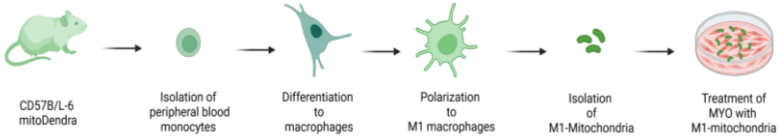
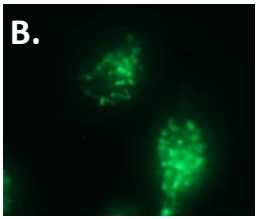
Supplemental Data

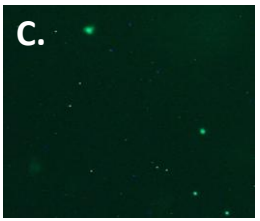

A. CCL2
B. IL-6
C. IL-1B
D. TNF-A
E. CD206
E. PPARγ
Relative CCL2 Expression
Relative IL-6 Expression
Relative IL-1B Expression
Relative TNF-A Expression
Relative CD206 Expression
Relative PPARg Expression
THP1
M0
M1
M2
***
**
*

Supplemental Figure 1: Validation of macrophage polarization to M1 and M2 phenotype. THP-1 monocytes were differentiated into macrophages (M0) using PMA and polarized to M1 using LPS+IFNγ or M2 macrophages with IL-4 stimulation for 48 hours in-vitro. RT-qPCR was conducted to assess polarization markers for M1 (A-D), including *CCL2*, *IL-6*, *IL-1b* and *TNFa*, and for M2 (E,F), including *PPARγ* and *CD206*. Graph shows relative mRNA levels expressed as mean ± SD (N = 3-4 independent experiments). Statistical significance was determined through one-way ANOVA with Tukey's multiple comparisons test to compare different cell types. Statistical significance is denoted as $p < 0.033$ (*), $p < 0.002$ (**), and $p < 0.001$ (***).

A.







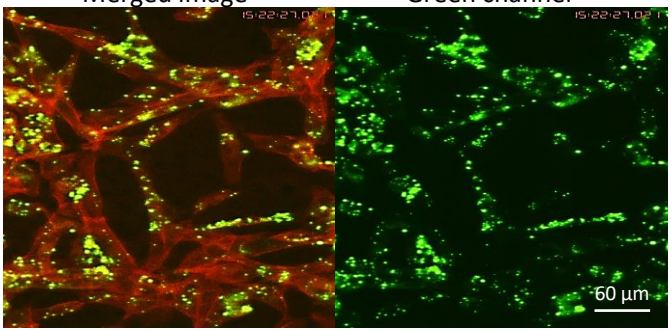
mitoDendra-M1-Macs

Isolated mitoDendra-M1-Mitochondria

D.

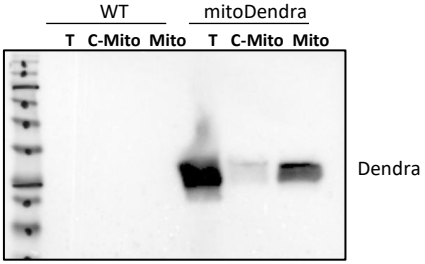
Merged image

Green channel

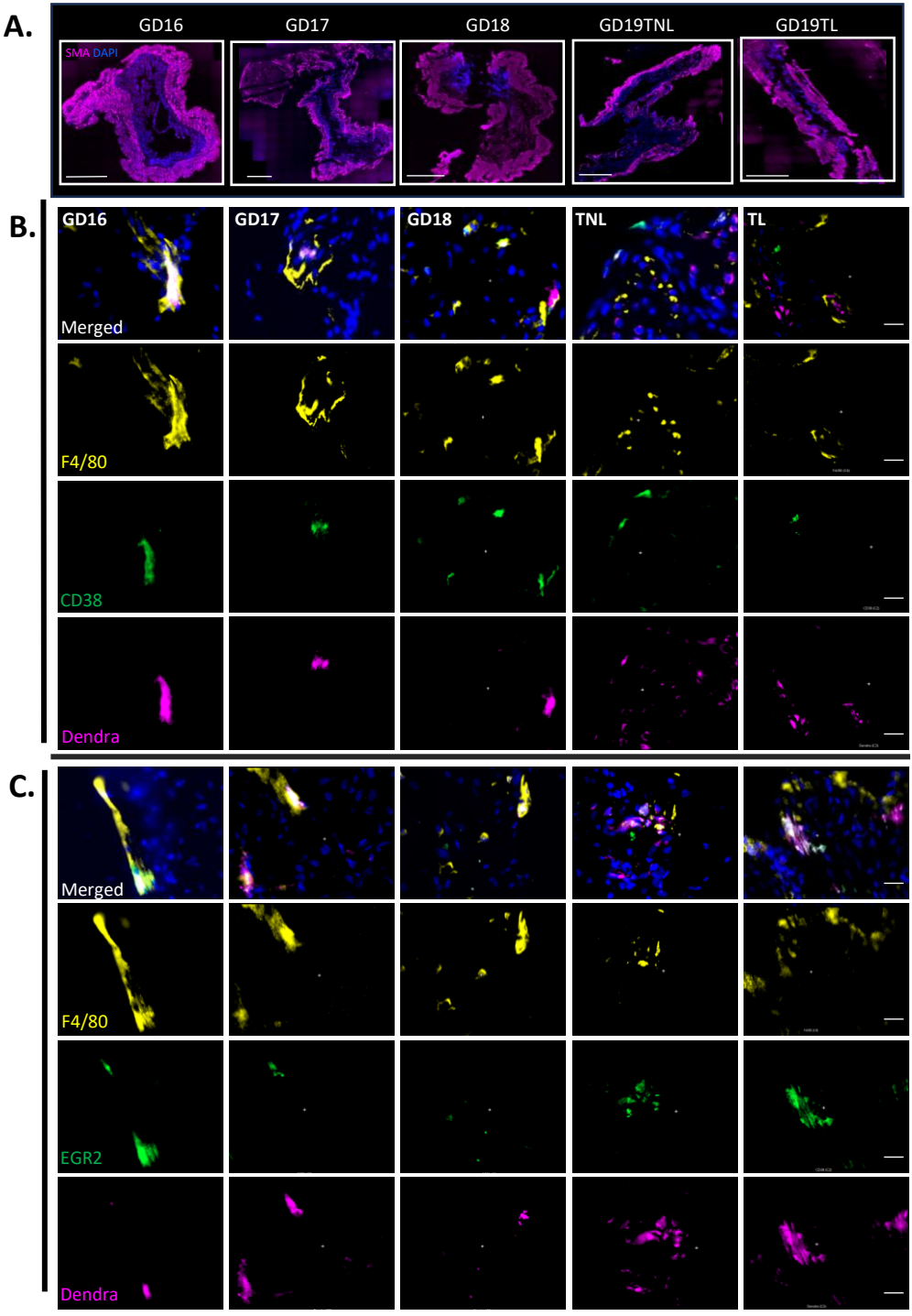


WT-MYO, mitoDendra-M1-Mitochondria

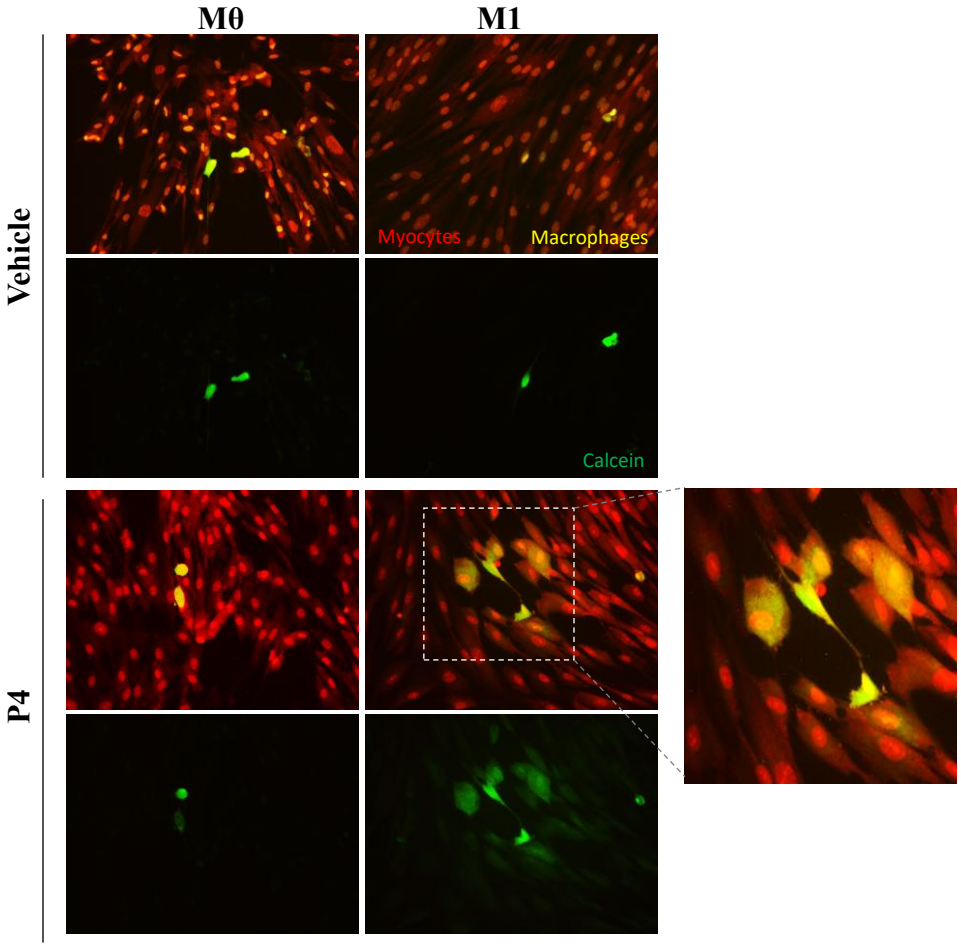
E.



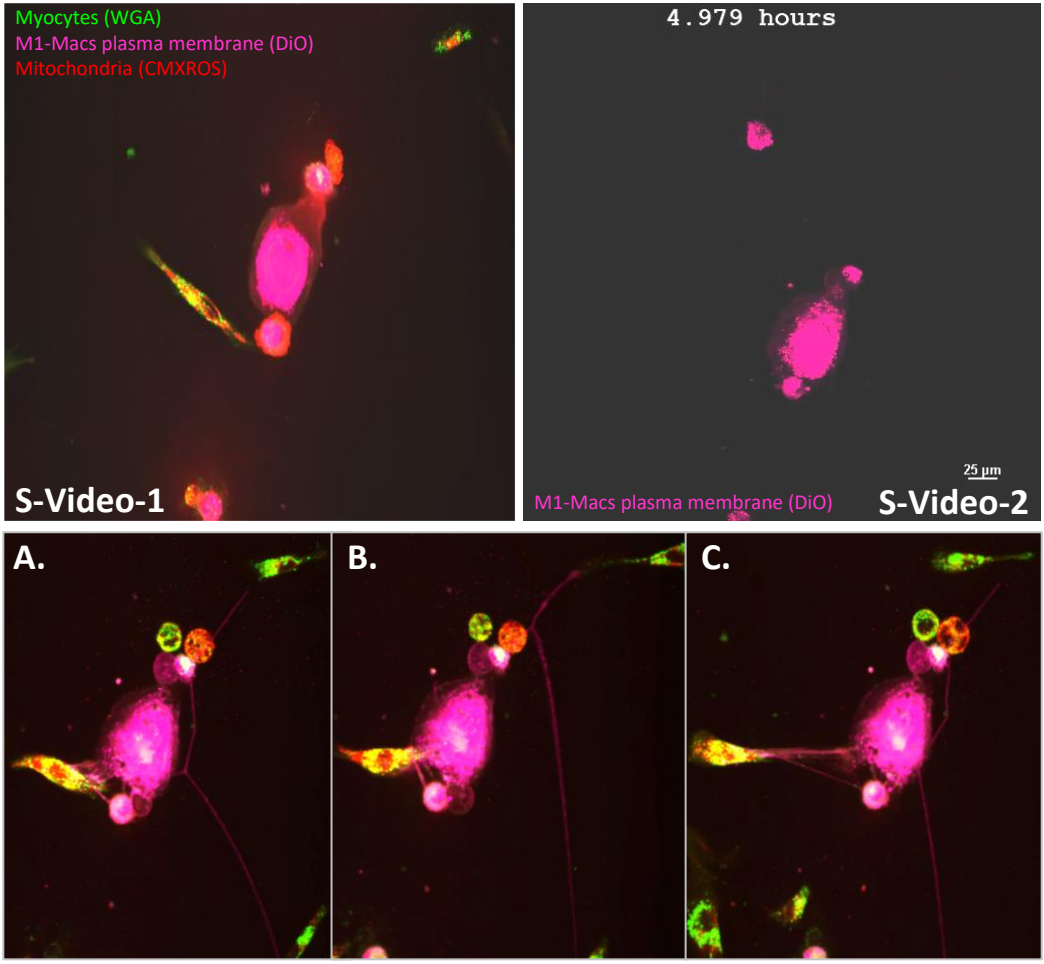
Supplemental Figure 2: Validation of mitochondrial isolation from macrophages and uptake by myocytes following transplant. A) Schematic representation of the experiment workflow. Peripheral blood monocytes were isolated from C57BL/6 mitoDendra mice and differentiated into macrophages in vitro, followed by polarization to M1-Macrophages (mitoDendra-M1-Macs) **(B)**. Mitochondria were isolated from mitoDendra-M1-Macs **(C) and** transplanted on myocytes m(MYO) isolated from wild type C57BL/6 wild-type (WT) mice and pre-stained with the F-actin probe; SPY650 (red). mMYO were washed and imaged after 24 hours post transplant. Representative fluorescence images (merged and green channel) demonstrating the uptake of Dendra-positive mitochondria by mMYO are shown **(D)**. Scale bar =60 μm. **E)** Western blot validation of mitochondria isolation process. Total cell lysates (T) are compared with the cytoplasmic fraction depleted of mitochondria (C-Mito) and isolated mitochondrial fraction (Mito) obtained from WT and mitoDendra-M1-Macs. Anti-dendra was used to verify the presence of Dendra positive Mitochondria in mitochondrial fraction.


A.
GD16
GD17
GD18
GD19TNL
GD19TL
SMA DAPI
B.
GD16
GD17
GD18
TNL
TL
Merged
F4/80
CD38
Dendra
C.
Merged
F4/80
EGR2
Dendra

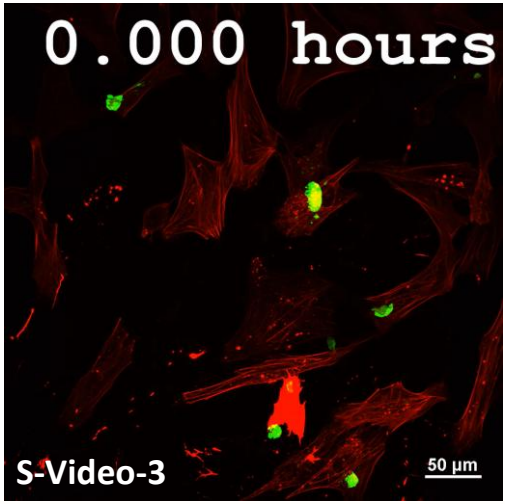
Supplemental Figure 3: Adoptive transfer and *in-vivo* tracking of monocyte infiltration, differentiation, polarization and mitochondrial transfer in myometrium. Peripheral blood monocytes were isolated from pregnant mitoDendra-CD57-BL/6 (mitoDendra) mice on GD15 and infused to wild type C57BL/6 (WT) on GD15 through tail vein. Uterine tissues were collected every 24 hour from gestational day (GD16) to post partum (PP) from N = 3 mice per GD. Cryosections were stained with smooth muscle actin (pink) representing myometrium. Scale bar = 1 mm. **B-C)** Multiplexed immunostaining with markers to identify murine Macrophages (mMacs) (F4/80, yellow), mM1- Macs (CD38, green, B), mM2-Macs (EGR2, green, **C**) Mitochondria from infused monocytes (Dendra, pink), nuclei were stained with Dapi. Scale bar = 20 μm

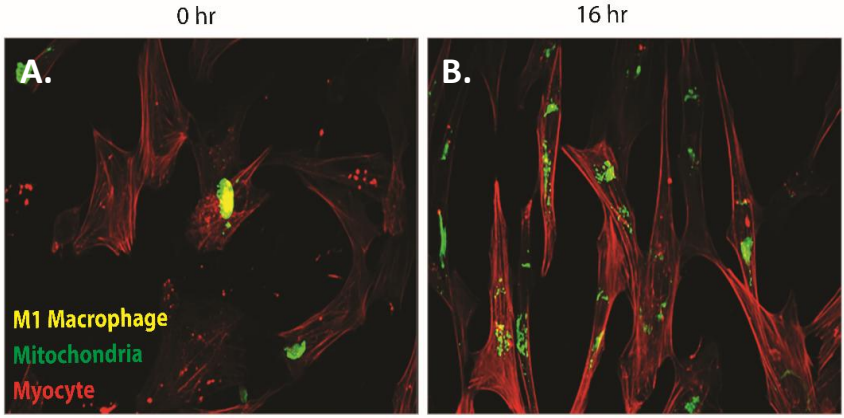


Supplemental Figure 4: M1 macrophages enhance cell connectivity among myocytes. hTERT-HM$^{A/B}$ cells (HM, red) were co-cultured in the presence of vehicle or progesterone (P4, 100 nM) with THP-1 derived M0 or M1 Macrophages which were preloaded with calcein dye (green). Images were taken at two hours post co-culture. Magnification = 200 X.

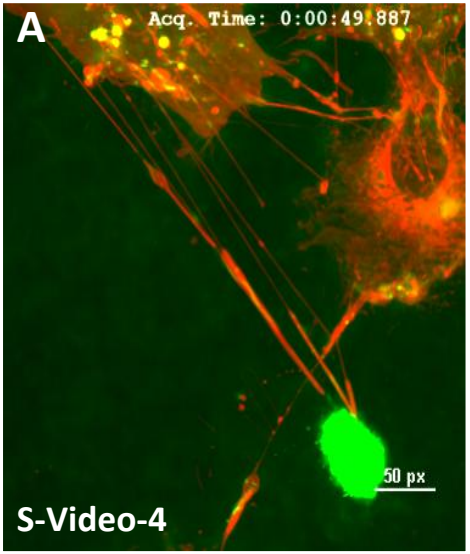


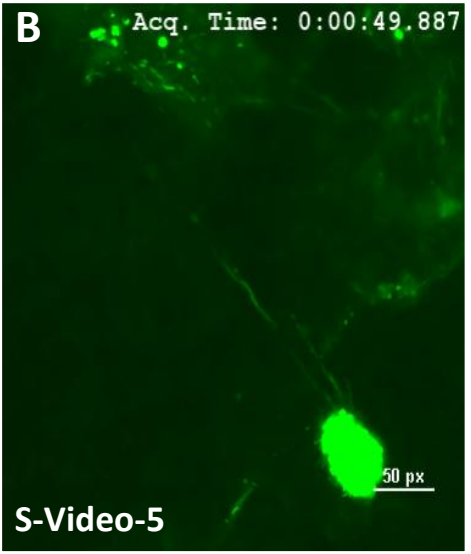
Supplemental Video 1,2: Time-lapse video demonstrating real-time formation of tunneling nanotube (TNT) protrusions connecting multiple cells simultaneously. Human PBMC-derived M1 macrophages (M1-Macs) were labeled with MitoTracker (red), and a plasma membrane marker (pink) and co-cultured with wheat germ agglutinin–labeled human myocytes (green) from hTERT-HM$^{A/B}$ cell line (MYO) in the presence of progesterone (100 nM). Time-lapse imaging was performed over approximately 15 hours. **S-Video-1)** Representative time-lapse video capturing dynamic TNT formation; **S-Video-2)** Corresponding far red channel staining showing plasma membrane of M1-Macs (only) and demonstrating multiple TNTs originating from M1-Macs **A–C)** Snapshots from the video illustrating various stages of TNT formation and mitochondrial transfer between M1-Macs and MYO. Scale bar = 25 µm.





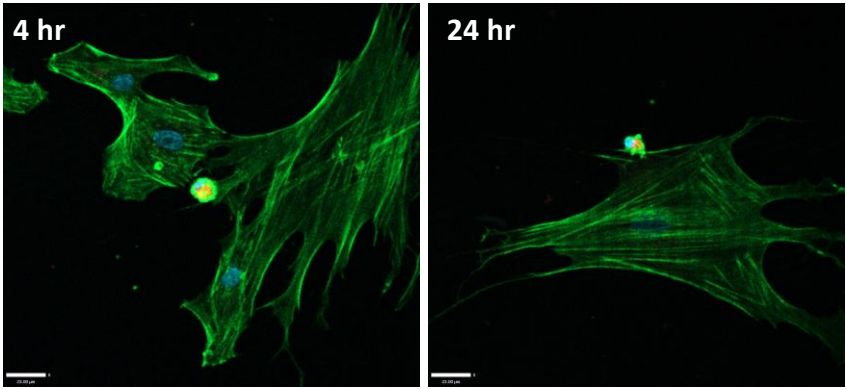
Supplemental Figure 5 : Cargo transferred from M1-Macrophages to Myocytes is distributed among Myocytes over time. Mitodendra expressing peripheral mouse mitoDendra-Monocyte derived M1 macrophages (mitoDendra-M1-Macs, green) were co-cultured with SPY-650 labelled murine Myocytes. **Video-3** and representative images (**A,B**) show the green cargo contained within the mitoDendra-M1-Macs at the time of co-culture (0 hour) and distributed between the mMYOs after 16 hours post co-culture.



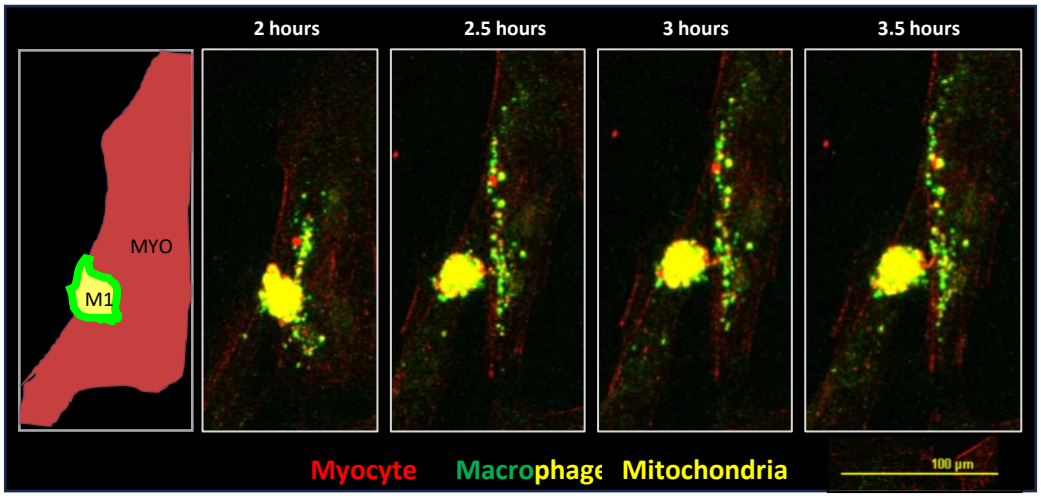


Supplemental Video 4,5: Time lapse imaging of primary murine Macrophages (mitoDendra-M1-Macs) with Dendra-labeled mitochondria (green) co-cultured with wild-type myocytes (WT-mMYO) labelled with F-actin probe SPY650 (red).

A) mM1-Macs were derived from peripheral blood monocytes of pregnant mitoDendra2 C57BL/6 (mitoDendra) mice, which ubiquitously express Dendra fluorescence in mitochondria, on gestational day (GD) 15. Primary murine myocytes were isolated from wild-type C57BL/6 (WT) mice on GD15 and stained with the F-actin probe SPY650. Co-culture and time-lapse imaging revealed the formation of tunneling nanotubes (TNTs) both between mMYOs and between mM1-Macs and mMYOs. Real-time movement and accumulation of Dendra-labelled mitochondria within mMYOs were observed over time. The green fluorescence represents mitochondria originating from mitoDendra-M1-Macs, as visualized in **Video 5**. Scale bar = 50 px = 50 μm.



Supplemental Figure 6: M2-Macrophages do not form TNT connection with Myocytes. Human PBMC-derived M2 macrophages (hM2-Macs, labeled with MitoTracker, red) were co-cultured with primary human Myocytes (hMYO) in the presence of WGA-488. Representative images show no TNT formation and no mitochondrial transfer from hM2-Macs to hMYO 4 or 24 hours post co-culture. Scale bar = 23 μm.



Supplemental Figure 7: Mitochondria is not the only cargo transferred from M1-Macs to MYOs. Human PBMC-derived M1 macrophages (hM1-Macs, labelled with MitoTracker red (yellow) and plasma-membrane label wheat germ agglutinin (green) were co-cultured with SPY-650 labelled primary human Myocytes (hMYO, red). Representative images demonstrate the transfer of both membrane-associated (green) and mitochondrial (yellow) cargo from hM1-Macs to hMYO over a period of 3.5 hours. Scale bar =100 µm.

Supplementary Table S1: Details of the primer sets used in this study

Gene Symbol	Forward primer	Reverse primer
CCL2	AAACTGAAGCTCGCACTCTCGC	AGGTGACTGGGGCATTGATTG
TNFα	CAGGCGCCACCACGCTCTTC	CCTGGGGAACTCTTCCCTCTGGGG
IL1β	GTGGCAATGAGGATGACTTGTTCT	TGTAGTGGTGGTCGGAGATTCG
IL-6	CAAGCCAGAGCTGTGCAGATGAGT	GCTTCGTCAGCAGGCTGGCAT
CD206/MRC1	GGGTTGCTATCACTCTCTATGC	TTTCTTGTCTGTTGCCGTAGTT
PPARγ	ACCAAAGTGCAATCAAAGTGGA	ATGAGGGAGTTGGAAGGCTCT
HPRT	TGACACTGGCAAAACAATGCAG	GGTCCTTTTCACCAGCAAGCT
SDHA	TGGGAACAAGAGGGCATCTGC	CCACCACTGCATCAAATTCATGATCC
TBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA

Supplementary Table S2: Details of the antibodies used in this study

Antibody	Company	Catalogue #	Used in	Dilution
Rat anti-mouse monoclonal F4/80 antibody	Serotec/Cedarlane	MCA497GA	IF	1:500
CD38 (E9F5A) XP® Rabbit mAb	NEB/Cell Signaling	68336T	IF	1:200
Recombinant Anti-EGR2 antibody [EPR23228-40]	Abcam	ab245228	IF	1:200
Anti-alpha smooth muscle Actin antibody [1A4]	Abcam	ab7817	IF	1:500
Anti-Dendra2 antibody	Evrogen	AB821	IF, WB	1:200
M-Sec/Tumor Necrosis Factor alpha-Induced Protein 2 (TNFα-IP2)	Santa Cruz	sc-28318	WB	1:1000
Anti-AKR1C1/20α hydroxysteroid Dehydrogenase (20α-HSD)	GeneTex	GTX105620	WB	1:1000
Phospho-Progesterone Receptor (Ser345) Antibody (p-PR$^{S344/345}$)	NEB/Cell Signaling	12783S	WB	1:1000
Progesterone Receptor (A/B) Antibody	Santa Cruz	sc-7208	WB	1:500
Anti-Connexin 43	Abcam	AB1728	WB	1:1000
Anti Tubulin	NEB/Cell Signaling	2144S	WB	1:1000
Anti-ERK2	Santa Cruz	sc-1647	WB	1:500