Single organelle analysis to characterize mitochondrial function and crosstalk during viral infection

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Supplemental Figure 1: Analysis of mitochondria from virus-infected livers.

(**A**) Flow cytometric analysis of purified mitochondria isolated from livers of uninfected and LCMV-infected mice (1 x 10^4 pfu / mouse) mixed with spherotech size beads. Mean size in µm was calculated using linear equation calculated from size beads by linear regression. (**B**) Flow cytometric analysis of purified mitochondria isolated from livers of untreated and poly I:C treated mice (200 µg / mouse) mixed with sperotech size beads. Mean size in µm was calculated using linear equation calculated from size beads by linear regression. (**C**) Flow cytometric analysis of isolated liver mitochondria. Titration of the membrane-potential dependent dye $DiLC_1(5)$ (50nM - 400nM). (**D**) Flow cytometric analysis of the membrane potential by $DiIC_1(5)$ staining of purified mitochondria from uninfected and LCMV infected livers (as in (A)). (**E**) Flow cytometric analysis of the membrane potential by $DiIC_1(5)$ staining of purified mitochondria from livers of untreated and poly I:C treated mice (as in (B)). (**F**) Flow cytometric analysis of purified liver mitochondria for the membrane potential dye $DiIC_1(5)$ (100 nM) against FSC. Population was divided in subpopulation of different FCS ranges to plot MFI-FSC against MFI-$DiIC_1(5)$ for linear regression. (**G**) At d2 after infection with Ad-CMV-GOL (5 x 10^8 PFU/mouse) mitochondria were isolated, challenged with calcium (100 µM) and mitochondrial membrane potential (Rh123 fluorescence intensity) as well as mitochondrial integrity (absorbance at 540 nm) was detected over time.

Representative data from at least three independent experiments.

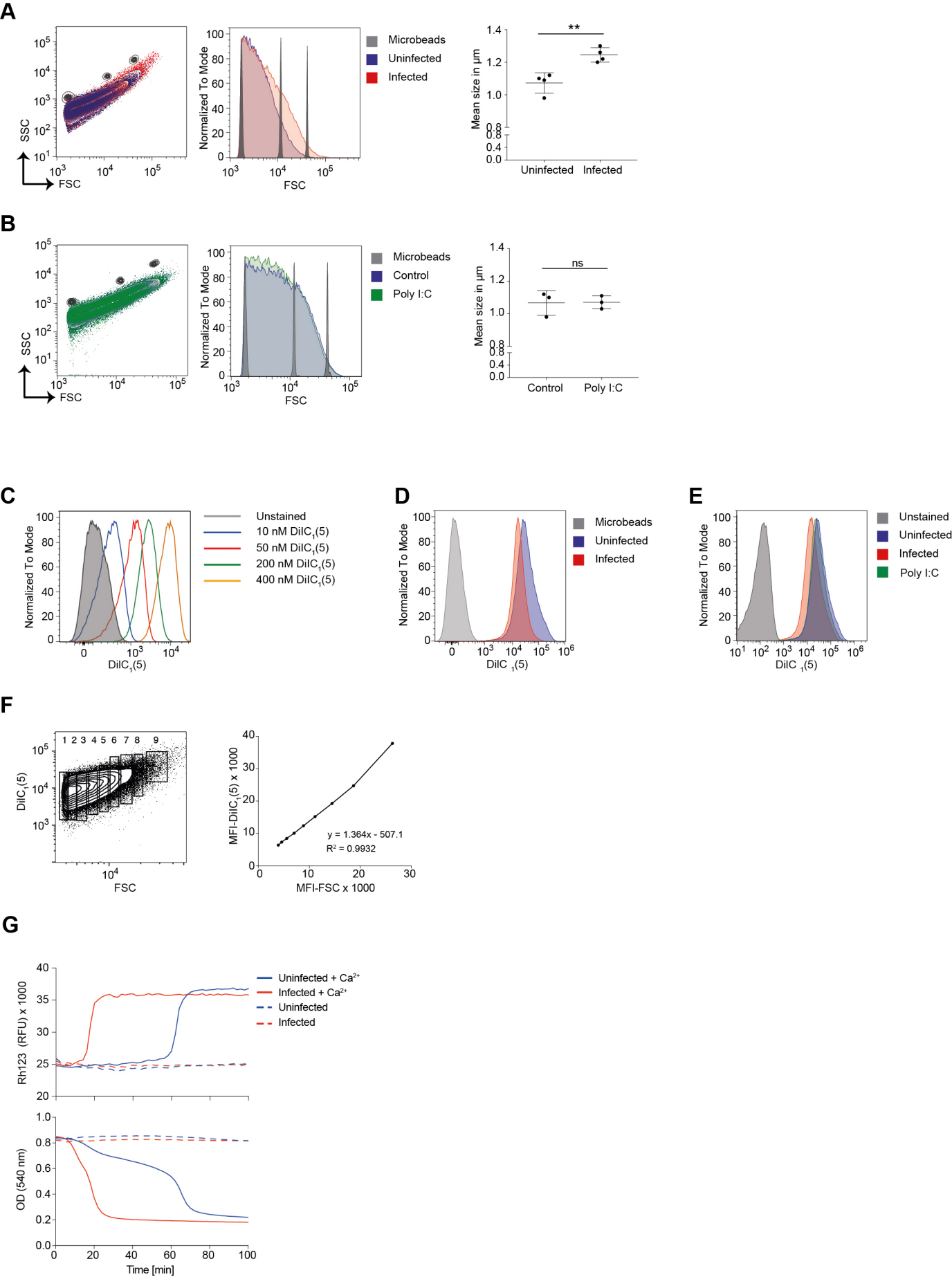
Supplemental Figure 2: Vector map of Ad-CMV-mitoRL.

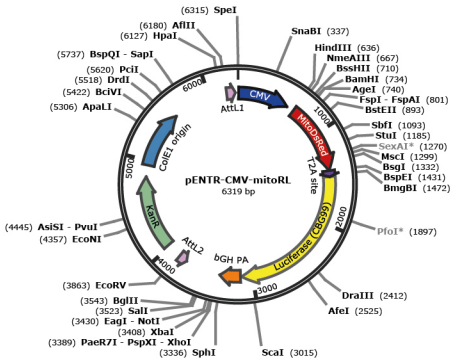
pENTR vector with expression cassette consisting of CMV promoter, dsRed linked to mitochondrial targeting site and luciferase gene linked by P2A sequences and followed by an bGH polyadenylation signal.

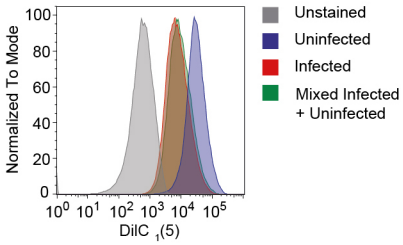
Supplemental Figure 3: Change of membrane potential after mixing of LCMV-infected and uninfected mitochondria

Flow cytometric analysis of membrane potential by $DiIC_1(5)$ staining of mitochondria from uninfected livers with mitochondria from LCMV infected livers at a ratio 1:1. Samples were mixed before staining procedure (measurement 1h post mixing) .

Representative data from three independent experiments.


A
SSC
FSC
Normalized To Mode
Microbeads
Uninfected
Infected
Mean size in µm
**
Uninfected
Infected
B
Control
Poly I:C
ns
C
Unstained
10 nM DiIC1(5)
50 nM DiIC1(5)
200 nM DiIC1(5)
400 nM DiIC1(5)
DiIC1(5)
D
DiIC 1(5)
E
F
1 2 3 4 5 6 7 8 9
MFI-DiIC1(5) x 1000
y = 1.364x - 507.1
R² = 0.9932
MFI-FSC x 1000
G
Uninfected + Ca2+
Infected + Ca2+
Rh123 (RFU) x 1000
OD (540 nm)
Time [min]


(6315) SpeI
(6180) AflII
(6127) HpaI
(5737) BspQI - SapI
(5620) PciI
(5518) DrdI
(5422) BciVI
(5306) ApaLI
(4445) AsiSI - PvuI
(4357) EcoNI
(3863) EcoRV
(3543) BglII
(3523) SalI
(3430) EagI - NotI
(3408) XbaI
(3389) PaeR7I - PspXI - XhoI
(3336) SphI
ScaI (3015)
AflII (2412)
AfeI (2525)
PfoI* (1897)
SnaBI (337)
HindIII (636)
NmeAIII (667)
BssHII (710)
BamHI (734)
AgeI (740)
FspI - FspAI (801)
BstEII (893)
SbfI (1093)
StuI (1185)
SexAI* (1270)
MscI (1299)
BsgI (1332)
BspEI (1431)
BmgBI (1472)
1000
2000
3000
4000
5000
6000
AttL1
CMV
mitoRFP
T2A site
Luciferase (cdGP9)
bGH PA
AttL2
KanR
ColE1 ori
pENTR-CMV-mitoRL
6319 bp


Normalized To Mode
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4 10^5
DilC $_1$(5)
Unstained
Uninfected
Infected
Mixed Infected
+ Uninfected