

Supplementary Materials for

Mitochondrial Membrane Potential Is Required for MAVS-Mediated Antiviral Signaling

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Published 1 February 2011, *Sci. Signal.* **4**, ra7 (2011)

DOI: 10.1126/scisignal.2001147

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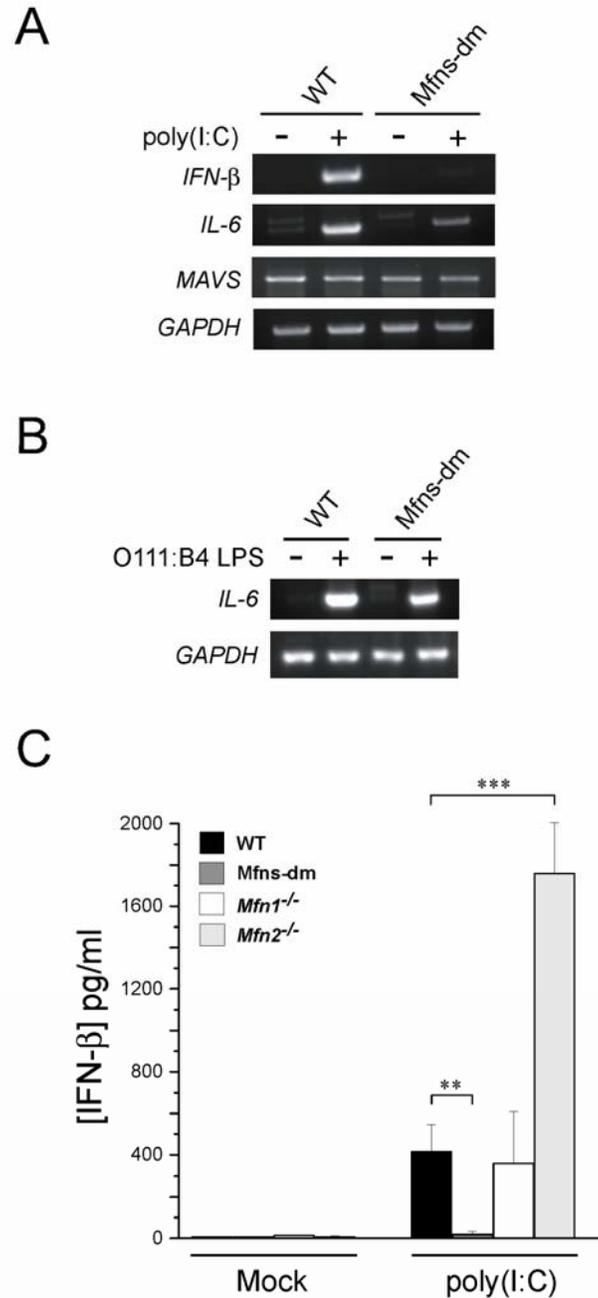


Fig. S1. Suppression of IFN- β production in Mfns-dm MEFs in response to polyI:C. **(A)** Wild-type (WT) or Mfns-dm MEFs were transfected with poly(I:C) (4 μ g) and the isolated total RNAs from each cell type were analyzed by RT-PCR for the expression of *IFN- β* , *IL-6*, *MAVS*, and *GAPDH* (as a control). **(B)** WT or Mfns-dm MEFs had their media exchanged with or without *E. coli* O111:B4 LPS (5 μ g) for 5 hours, and the isolated total RNAs from each cell type were analyzed by RT-PCR for the expression of *IL-6* or *GAPDH* (as a control). Data shown in A and B are representative of 3 experiments. **(C)** WT, Mfns-dm, *Mfn1*^{-/-}, and *Mfn2*^{-/-} MEFs were transfected with polyI:C (4 μ g) and the supernatant was collected to measure IFN- β production by ELISA. Data shown represent mean values \pm SD from three independent experiments. **, $P < 0.01$; ***, $P < 0.001$.

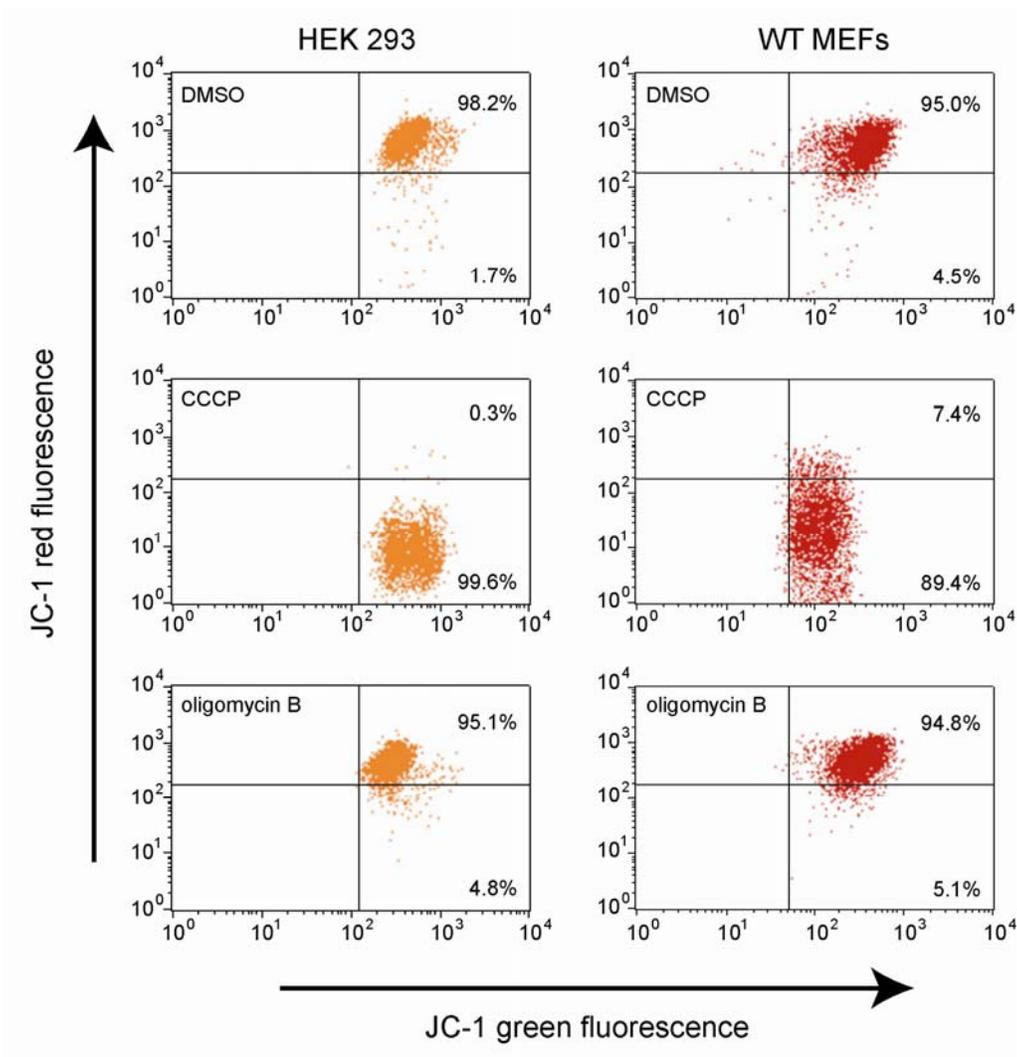


Fig. S2. HEK 293 cells and wild-type MEFs stained with MitoProbe JC-1. HEK293 or WT MEFs were treated with either DMSO (top), CCCP (middle), or oligomycin B (bottom) for about 16 hours at 37°C. After washing, the cells were stained with MitoProbe JC-1 and were analyzed by flow cytometry with FL1 (green) and FL2 (red) channels. Data shown are representative of 3 experiments.

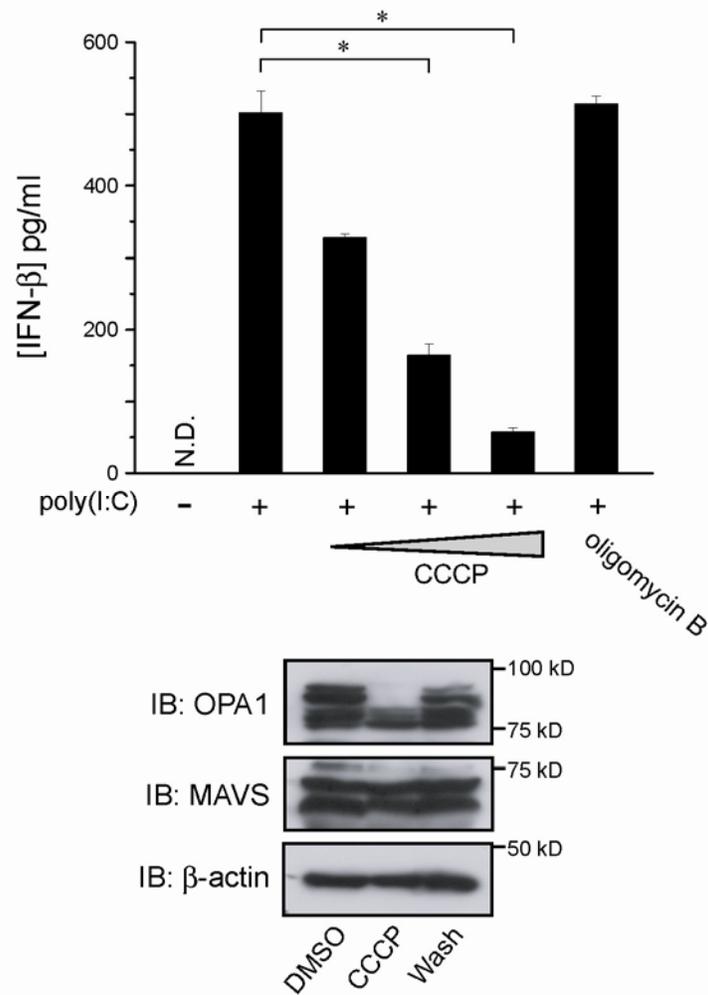


Fig. S3. CCCP inhibits the MDA-5-dependent production of IFN- β in a dose-dependent manner. WT MEFs that were treated with increasing amounts (10, 20, and 40 μ M) of CCCP were transfected with poly(I:C) (4 μ g) and the amount of IFN- β produced was analyzed by ELISA as described in the legend for Fig. 1B. Data shown in the far right lane are from cells treated with oligomycin B (10 μ M) instead of CCCP. The blots at the bottom represent the processing of OPA1 to confirm the dissipation of $\Delta\Psi_m$. Data shown represent mean values \pm SD from three independent experiments. *, $P < 0.05$; N.D., not detected.

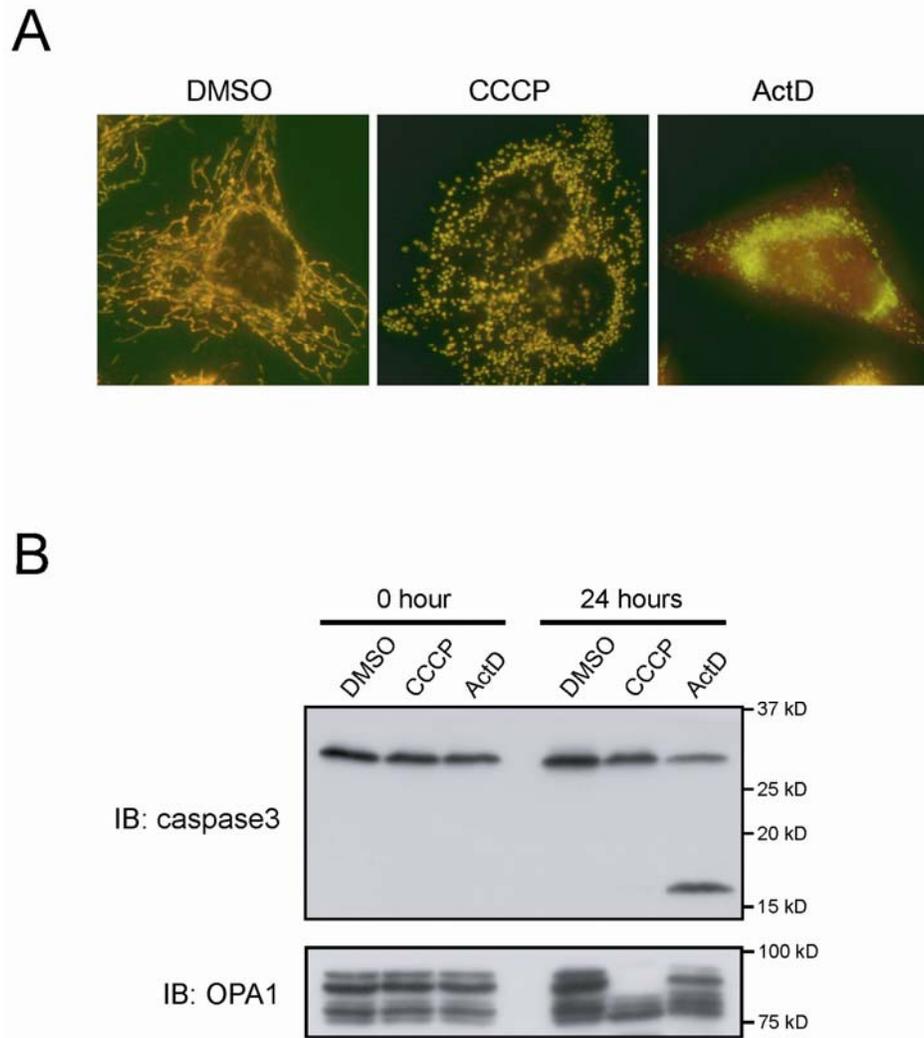


Fig. S4. Analysis of cytochrome *c* release and caspase-3 activation in wild-type MEFs. **(A)** WT MEFs were incubated for 24 hours with DMSO, CCCP (40 μ M), or actinomycin D (ActD, 20 μ M) and the cells were examined by immunofluorescence microscopy for the presence of endogenous cytochrome *c* (red) and mtHsp70 (green). Scale bar: 10 μ m. **(B)** Lysates from the cells shown in (A) were analyzed by Western blotting with antibodies against caspase-3. The blots at the bottom represent the processing of OPA1 to confirm the dissipation of $\Delta\Psi_m$. Data shown are representative of 3 experiments.

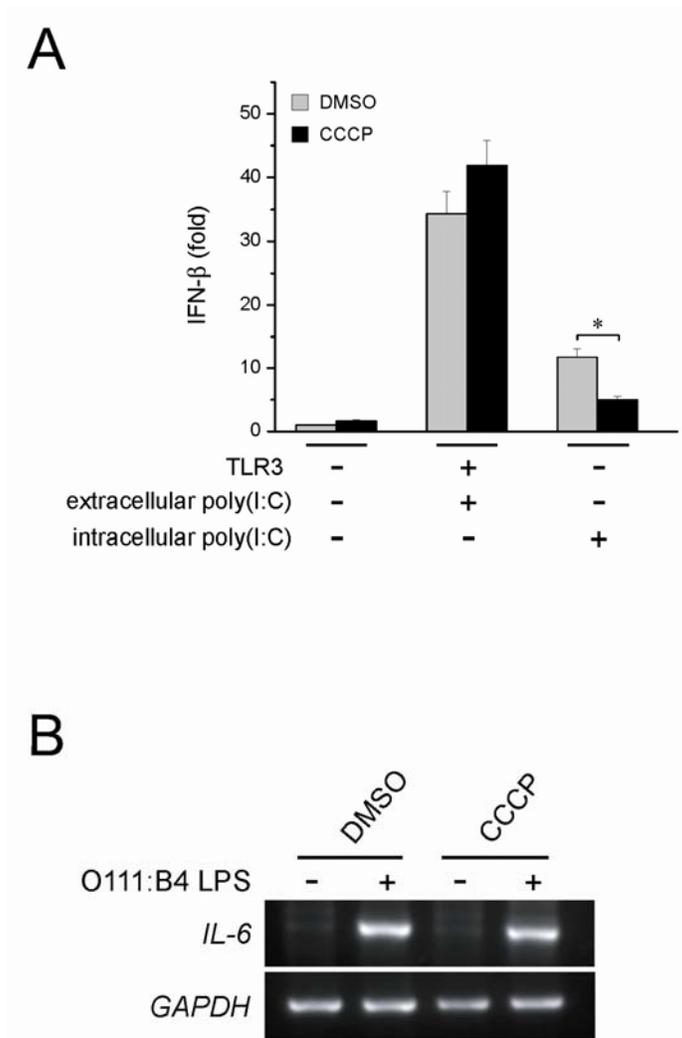


Fig. S5. CCCP-treated cells are unaffected by extracellular polyI:C or LPS. **(A).** HEK 293 cells were transfected with either 100 ng of plasmid encoding human TLR3 or pcDNA3.1, together with the luciferase reporter plasmid p125luc. Twenty-four hours after transfection, the cells were untreated or they had their media exchanged with polyI:C (20 μ g) and CCCP (40 μ M) for 6 hours. In the control (right panel), cells were transfected with 2 μ g of polyI:C instead of being treated with extracellular reagent. The data shown represent the mean values \pm SD of IFN- β reporter activity from 3 independent experiments. *, $P < 0.05$. **(B).** WT MEFs treated with DMSO (control) or CCCP (40 μ M) were left alone or had their media exchanged with *E. coli* O111:B4 LPS (5 μ g) for 6 hours, and the isolated total RNAs from each cell were analyzed by RT-PCR for the expression of *IL-6* or *GAPDH* (as a control).

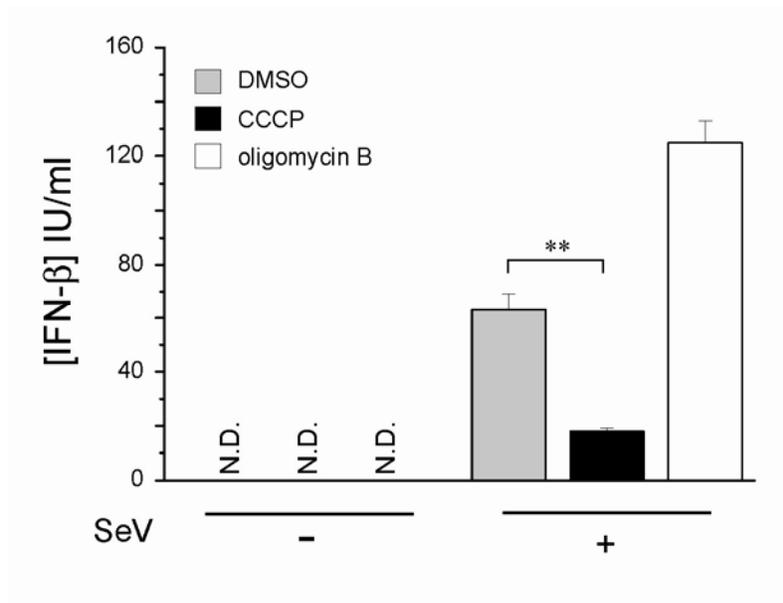


Fig. S6. CCCP inhibits antiviral signaling in HEK 293 cells. HEK 293 cells treated with DMSO, CCCP (40 μ M), or oligomycin B (10 μ M) were infected with SeV (16 HA units/ml) and the culture supernatants were harvested 20 hours after infection and analyzed by ELISA to measure the amounts of IFN- β produced. Data shown represent mean values \pm SD from 3 independent experiments. **, $P < 0.01$; N.D., not detected.

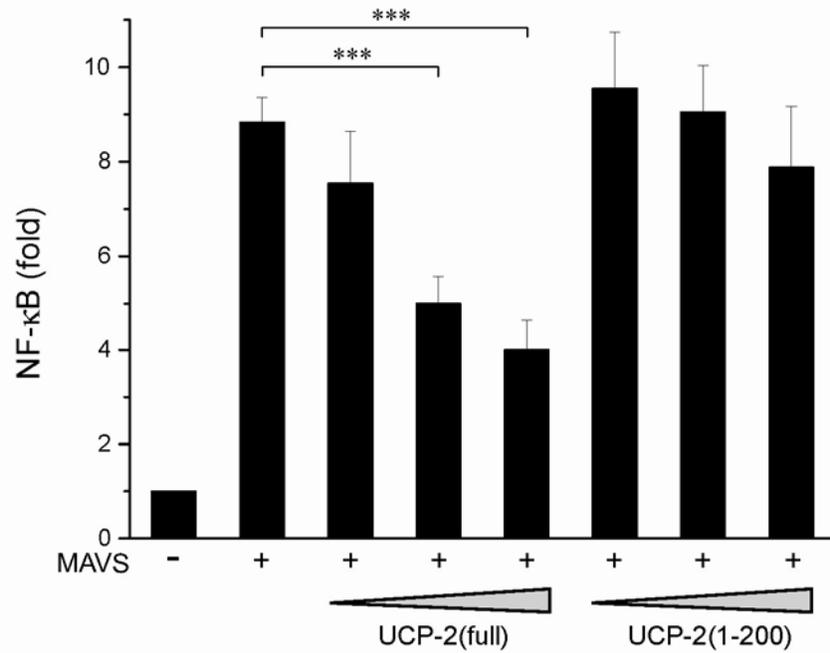


Fig. S7. UCP-2 inhibits MAVS-mediated activation of the NF- κ B reporter. HEK 293 cells were cotransfected with 50 ng of plasmid encoding MAVS together with increasing amounts (20, 50, and 100 ng) of a plasmid encoding UCP-2(full) or UCP-2(1-200) and the NF- κ B luciferase reporter plasmid. Data shown represent mean values \pm SD from 3 independent experiments. ***, $P < 0.001$.

Table S1. Primers were used in the study.

Primer	Sequence	Description
TK300	5'-aaaaGCGGCCGCcatgacatttgctgaggacaagacc	5' Oligo for <i>mMAVS</i> (A.A.1) with Not I site
TK305	5'-ttttGATATCtcaaccagacatcctcgegcaggg	3' Oligo for <i>mMAVS</i> (A.A.173) with Eco RV and stop
TK350	5'-aaaGCGGCCGCccccccagaccactggaccc	5' Oligo for <i>hMAVS</i> (A.A.103) with Not I site
TK354	5'-tttGATATCtcatgtgtccttctctgatgccc	3' Oligo for <i>hMAVS</i> (A.A.200) with Eco RV and stop
TK535	5'-ccacagccctctccatcaactataagc	5' Oligo for <i>mIFN-β</i> (RT-PCR)
TK536	5'-agctcttcaactggagagcagttgagg	3' Oligo for <i>mIFN-β</i> (RT-PCR)
TK537	5'-accacagtccatgccatcac	5' Oligo for <i>mGAPDH</i> (RT-PCR)
TK538	5'-tccaccacctgttgetgta	3' Oligo for <i>mGAPDH</i> (RT-PCR)
TK550	5'-gtcagtggtggacctgacc	5' Oligo for <i>hGAPDH</i> (RT-PCR)
TK551	5'-aggggtctacatggcaactg	3' Oligo for <i>hGAPDH</i> (RT-PCR)
TK552	5'-gattcatctagcactggctgg	5' Oligo for <i>hIFN-β</i> (RT-PCR)
TK553	5'-cttcaggtaatgcagaatcc	3' Oligo for <i>hIFN-β</i> (RT-PCR)
TK554	5'-cctctctgcaagagacttcc	5' Oligo for <i>mIL-6</i> (RT-PCR)
TK555	5'-actcctctgtgactccagc	3' Oligo for <i>mIL-6</i> (RT-PCR)
TK556	5'-atggcctcgeccctttgctttactg	5' Oligo for <i>hIFN-α</i> (RT-PCR)
TK557	5'-tttctgctctgacaacctcccagg	3' Oligo for <i>hIFN-α</i> (RT-PCR)
TK571	5'-aaGCGGCCGCcatggttggttcaaggccacagatgtgc	5' Oligo for <i>hUCP2</i> (A.A.1) with Not I site
TK572	5'-ttGATATCtcaagaaggagcctctcgggaagtgc	3' Oligo for <i>hUCP2</i> (A.A.308) with Eco RV and stop
TK575	5'-ttGATATCtcatgatgaggtcataggtcaccag	3' Oligo for <i>hUCP2</i> (A.A.200) with Eco RV and stop