Immunity, Volume *38*

Supplemental Information

Activation of the Innate Signaling Molecule MAVS by Bunyavirus Infection Upregulates the Adaptor Protein SARM1, Leading to Neuronal Death

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Supplemental Inventory

Supplemental Figures and Tables

Fig. S1.

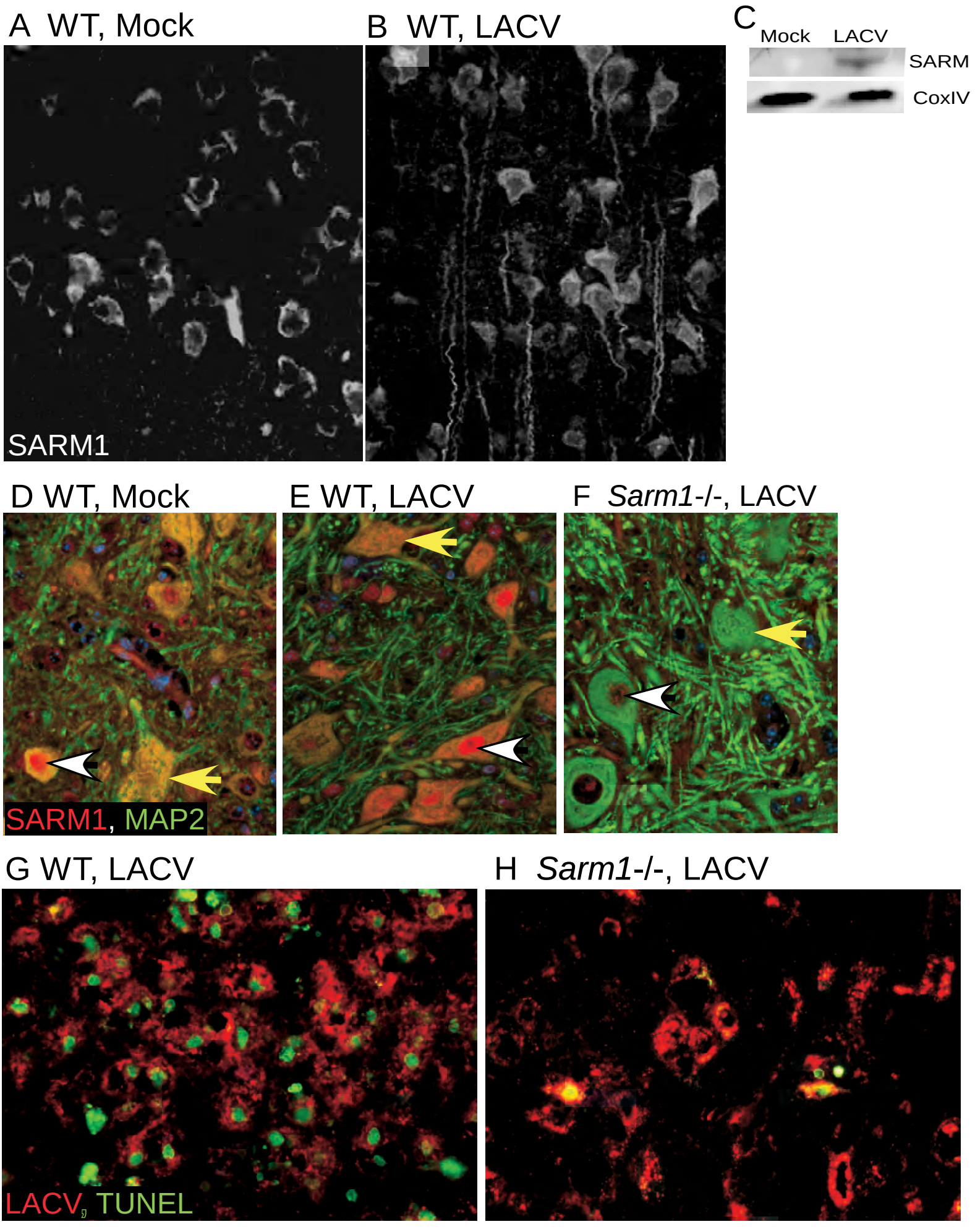

A WT, Mock
B WT, LACV
C
Mock
LACV
SARM
CoxIV
SARM1
D WT, Mock
E WT, LACV
F Sarm1-/-, LACV
SARM1, MAP2
G WT, LACV
H Sarm1-/-, LACV
LACV, TUNEL

Figure S1. SARM1 Expression in the Brain and Spinal Cord of LACV-Infected Mice, Related to Figure 3

(A-B) Brain tissue from (A) mock and (B) LACV-infected wildtype mice at 5-7 dpi were analyzed for SARM1 expression as described in methods. SARM1 staining was observed around cell bodies in (A) mock-infected mice, but was also found in axons of localized regions in the cortex in (B) LACV-infected mice. Images are 200X.

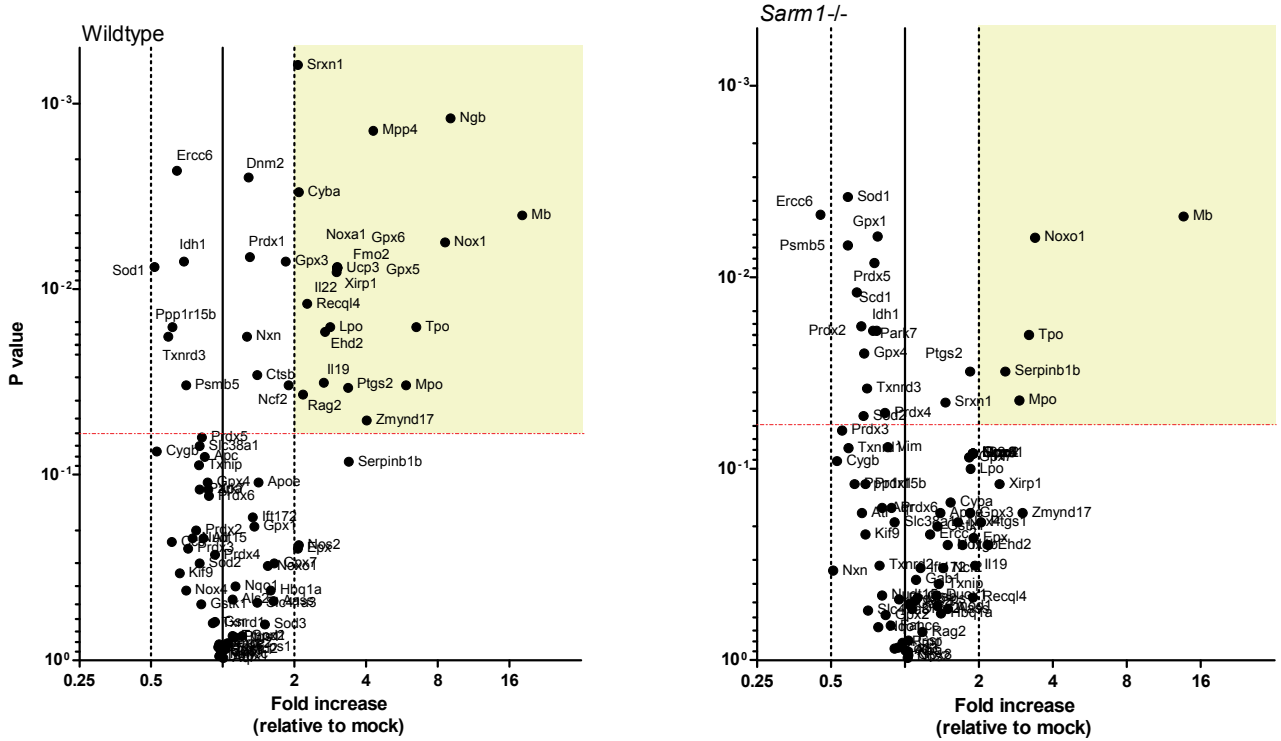
(C) Western blot analysis of mitochondrial fractions from lysates of brain tissue from mock or LACV-infected wildtype mice at 5 dpi stained for SARM1 or mitochondrial protein CoxIV.

(D-F) SARM1 expression in spinal cord of (D) uninfected and (E-F) LACV-infected (D-E) wildtype and (F) *Sarm1*-/- mice. SARM1 (red fluorescence) localized to the (D-E) cell body and (E) axons of MAP-2-positive (green fluorescence) neurons from wildtype mice (yellow arrow), but not *Sarm1*-/- mice. Some staining of nuclei was also observed (white arrow), but this was also observed in *Sarm1*-/- mice suggesting non-specificity. Images are 200X.

(G-H) Immunohistochemistry of brain tissue from LACV-infected (G) wildtype and (H) *Sarm1*-/- mice stained for TUNEL (green fluorescence) and the G2 protein of LACV (red fluorescence). Images are 400X.

Fig. S2

A Oxidative stress response genes



B type I IFN response genes

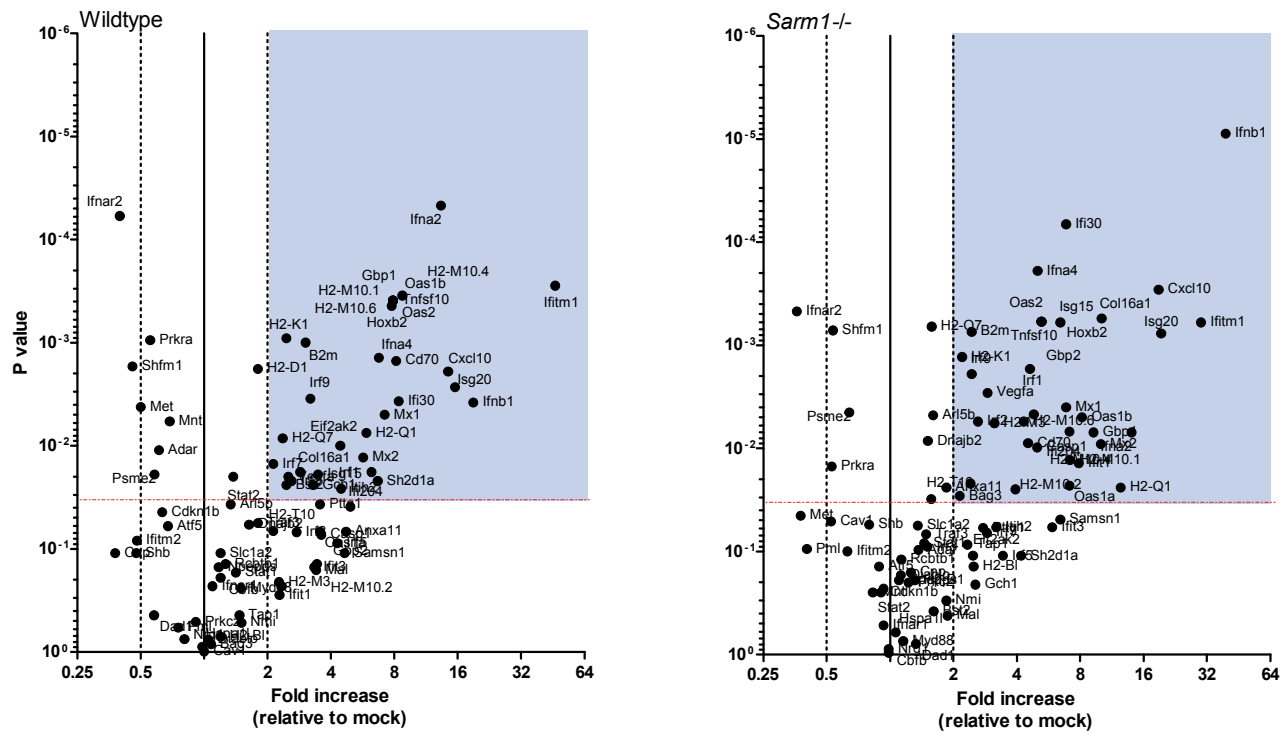


Figure S2. SARM1 Expression Is Necessary for LACV-Induced Upregulation of Oxidative Stress Response Genes, but Not Type I IFN Response Genes, Related to Figure 4

(A-B) Primary cortical neurons from wildtype and *Sarm1*-/- mice were infected with LACV at a MOI of 0.01. At 30 hpi, RNA was extracted and used to generate cDNA. cDNA from 3 mock and 3 LACV-infected neuron cultures generated from wildtype and *Sarm1*-/- mice were analyzed for the expression of (A) oxidative stress response genes or (B) type I IFN response genes. Results are shown as a volcano plot of mock vs LACV-infected cells for each mouse strain. Data are shown as the fold increases in gene expression of LACV-infected samples relative to mock-infected samples. Yellow or blue areas indicate regions of a 2-fold difference relative to mock-infected controls (X axis), with significance between samples indicated by *P* values of <0.05 (Y axis). The only significant difference between wildtype and *Sarm1*-/- mice in the IFN response gene analysis was annexin A11, which was increased by two-fold in wildtype cells compared to *Sarm1*-/- cells.

Fig. S3

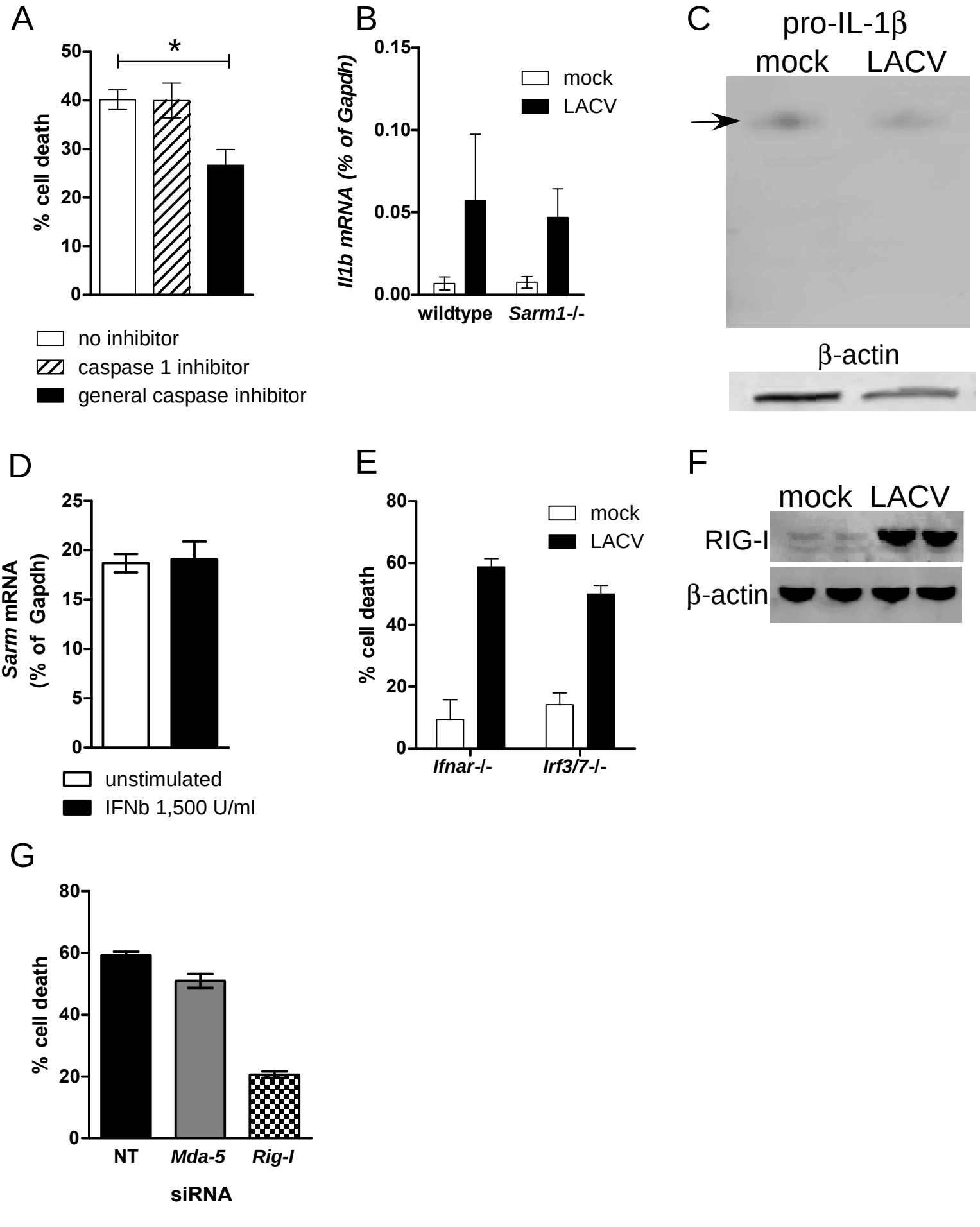

A
*
% cell death
50
40
30
20
10
0
no inhibitor
caspase 1 inhibitor
general caspase inhibitor
B
Il1b mRNA (% of Gapdh)
0.15
0.10
0.05
0.00
mock
LACV
wildtype
Sarm1-/-
C
pro-IL-1β
mock
LACV
β-actin
D
Sarm mRNA (% of Gapdh)
25
20
15
10
5
0
unstimulated
IFNb 1,500 U/ml
E
% cell death
80
60
40
20
0
mock
LACV
Ifnar-/-
Irf3/7-/-
F
mock
LACV
RIG-I
β-actin
G
% cell death
80
60
40
20
0
NT
Mda-5
Rig-I
siRNA

Figure S3. IL-1 and Type I IFN Are Not Responsible for SARM1-Mediated Cell Death, Related to Figure 2

(A) Wildtype neurons were treated with 200 uM of general caspase-inhibitor (ZVAD-FMK) and 10 uM of caspase-1 inhibitor (Z-WEHD-FMK) at the time of LACV infection and after 36 hpi cell viability was measured by MTT assay. Data are the mean +/- SEM of 3-6 samples per group.

(B) RNA isolated at 30 hpi from wildtype and *Sarm1*$^{-/-}$ neurons infected with LACV or with mock supernatants were analyzed for expression of *Il-1b* mRNA. Data are presented as gene expression relative to *Gapdh* mRNA expression and are the mean +/- SEM of 3-4 replicates per group.

(C) Analysis of IL-1β from whole cell lysates of mock and LACV-infected neurons at 36 hpi showing pro-IL1β, but not cleavage products, during LACV infection. β-actin was used as loading control (lower panel).

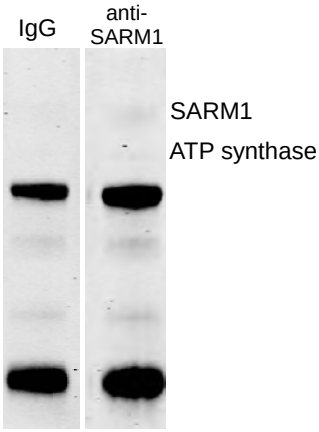
(D) Neuron cultures were stimulated with 1,500 U/ml of IFN-beta and RNA isolated at 6, 24 (not shown) and 48 hpi (shown). Expression of *Sarm1* mRNA was measured by real-time PCR analysis with no difference of expression observed at any time point.

(E) Neurons from *Ifnar* or *Irf3/Irf7* deficient mice were infected with LACV and analyzed for cell death at 36 hpi as described in Fig. 2. The level of LACV-induced cell death was comparable to that observed in wildtype controls at 36 hpi. Data are the average of 4-6 wells per group and are representative of at least two experiments per strain.

(F) RIG-I levels in brain homogenate from mock and LACV-infected mice at 5 dpi. Brain tissue from mock and LACV-infected mice was homogenized and analyzed for RIG-I protein levels by western blot analysis. Equal volume of protein was loaded in each lane

(G) siRNA specific to *Rig-I*, but not *Mda-5*, inhibits LACV-induced cell death. Cells were treated as described as in Fig. 2, except that siRNA against *Rig-I* or *Mda-5* were utilized. Data are shown as the mean +/-SEM of 4-6 replicates per group.

Fig. S4.



SARM Q6PDS3

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  1 MVLTLLFSAYKLCRFFTMSGPRPGADRLTVPGPDRSGGASPWWAAGGRGSREVSPGVGTE  60
 61 VQGALERSLPELQQALSELKQASAARAVGAGLAEVFQLVEEAWLLPAVGREVAQGLCDAI 120
121 RLDGGLDLLLRLLQAPELETRVQAARLLEQILVAENRDRVARIGLGVILNLAKEREPVEL 180
181 ARSVAGILEHMFKHSEETCQRLVAAGGLDAVLYWCRRTDPALLRHCALALANCALHGGQT 240
241 VQRCMVEKRAAEWLFPLAFSKEDELLRLHACLAVAVLATNKEVEREVEHSGTLALVEPLV 300
301 ASLDPGRFARCLVDASDTSQGRGPDDLQSLVLLLDSSRLEAQCIGAFYLCAEAAIKSLQG 360
361 KTKVFSDIGAIQSLKRLVSYSTNGTTSALAKRALRLLGEEVPRRILPCVASWKEAEVQTW 420
421 LQQIGFSQYCENFREQQVDGDLLLRLTDEELQTDLGMKSSITRKRFFRELTELKTFASYA 480
481 TCDRSNLADWLGSLDPRFRQYTYGLVSCGLDRSLLHRVSEQQLLEDCGIRLGVHRTRILS 540
541 AAREMLHSPLPCTGGKLSGDTPDVFISYRRNSGSQLASLLKVHLQLHGFSVFIDVEKLEA 600
601 GKFEDKLIQSVIAARNFVLVLSAGALDKCMQDHDCKDWVHKEIVTALSCGKNIVPIIDGF 660
661 EWPEPQALPEDMQAVLTFNGIKWSHEYQEATIEKIIRFLQGRPSQDSSAGSDTSLEGATP 720
721 MGLP
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ATP synthase Q03265

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  1 MLSVRVAAAVARALPRRAGLVSKNALGSSFVGARNLHASNTRLQKTGTAEMSSILEERIL  60
 61 GADTSVDLEETGRVLSIGDGIARVHGLRNVQAEEMVEFSSGLKGMSLNLEPDNVGVVVFG 120
121 NDKLIKEGDVVKRTGAIVDVPVGEELLGRVVDALGNAIDGKGPIGSKTRRRVGLKAPGII 180
181 PRISVREPMQTGIKAVDSLVPIGRGQRELIIGDRQTGKTSIAIDTIINQKRFNDGTDEKK 240
241 KLYCIYVAIGQKRSTVAQLVKRLTDADAMKYTIVVSATASDAAPLQYLAPYSGCSMGEYF 300
301 RDNGKHALIIYDDLSKQAVAYRQMSLLLRRPPGREAYPGDVFYLHSRLLERAAKMNDSFG 360
361 GGSLTALPVIETQAGDVSAYIPTNVISITDGQIFLETELFYKGIRPAINVGLSVSRVGSA 420
421 AQTRAMKQVAGTMKLELAQYREVAAFAQFGSDLDAATQQLLSRGVRLTELLKQGQYSPMA 480
481 IEEQVAVIYAGVRGYLDKLEPSKITKFENAFLSHVISQHQSLLGNIRSDGKISEQSDAKL 540
541 KEIVTNFLAGFEP                                                  600
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Figure S4. Immunoprecipitation of SARM1 Results in Pull Down of ATP Synthase, Related to Figure 5

Immunoprecipation was performed as described in Fig. 6 and run on an SDS-PAGE gel. Two bands were identified in the anti-SARM1 immunoprecipitation that were not observed in the IgG control. The top band was identified as SARM1 based on 3 peptides (blue sequence) detected in that sample by tandem mass spectroscopy. The second band was identified as ATP synthase based on detection of 3 peptides (shown in blue).

Fig. S5.

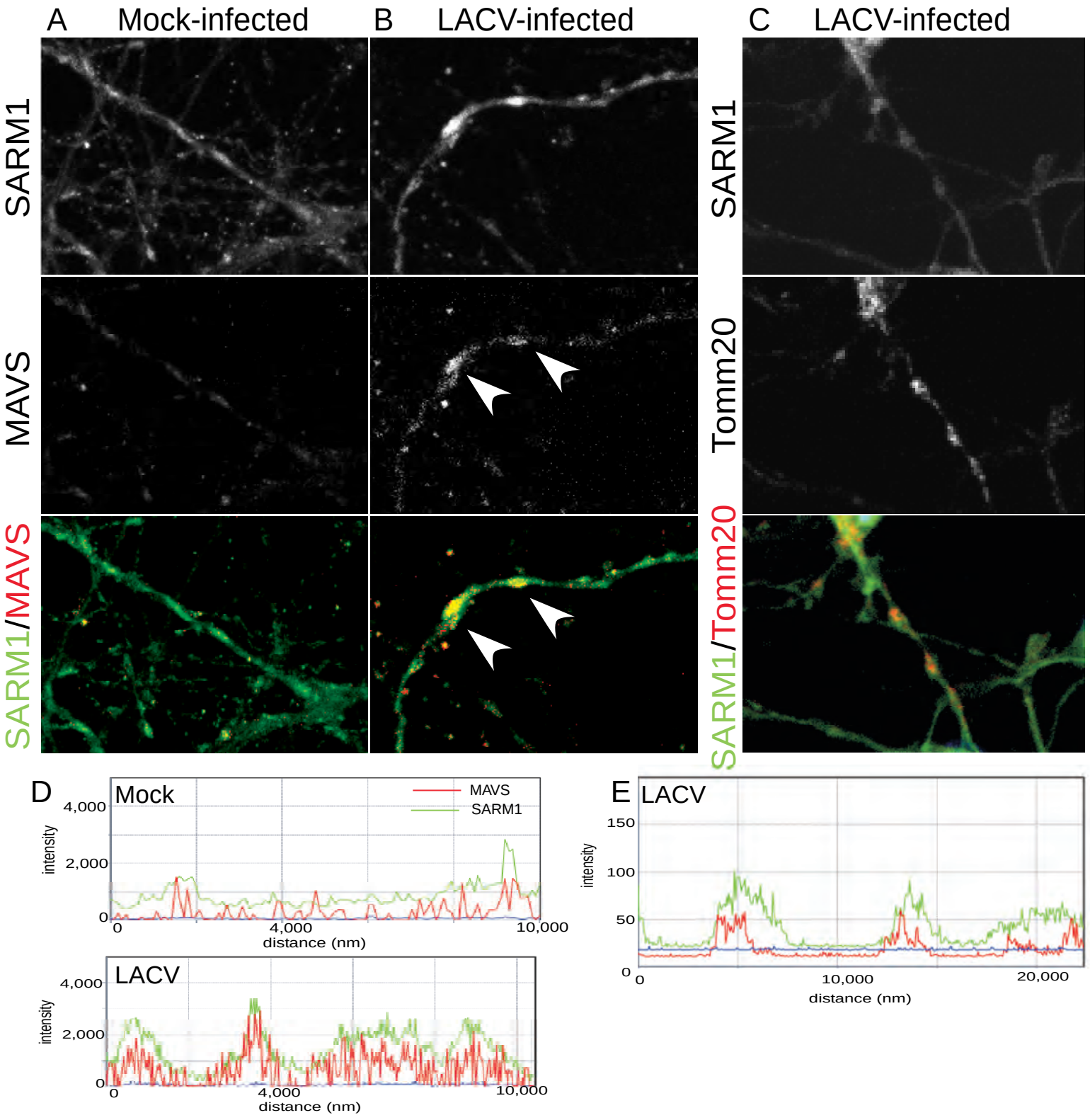
A Mock-infected
B LACV-infected
C LACV-infected
SARM1
MAVS
SARM1/MAVS
SARM1
Tomm20
SARM1/Tomm20
D
Mock
MAVS
SARM1
intensity
4,000
2,000
0
0
4,000
10,000
distance (nm)
LACV
4,000
2,000
0
0
4,000
10,000
distance (nm)
E
LACV
150
100
50
0
intensity
0
10,000
20,000
distance (nm)

Figure S5. MAVS Colocalizes with SARM1 in Neurons during LACV Infection, Related to Figure 6

(A-C) Mock and LACV-infected neurons from wildtype mice at 36 hpi were stained anti-SARM1 (green fluorescence) and (A-B) anti-MAVS (red fluorescence) or (C) anti-Tomm20 (red fluorescence) and analyzed by confocal microscopy. Shown are axons from (A) mock or (B-C) LACV-infected neurons.

(D-E) Histograms from a 10,000 nm stretch of axon are shown below for both (D) MAVS and SARM1 colocalization and (E) Tomm20 and SARM1 colocalization. Images are representative of 3-4 images per culture from two to four separate experiments.

Fig. S6.

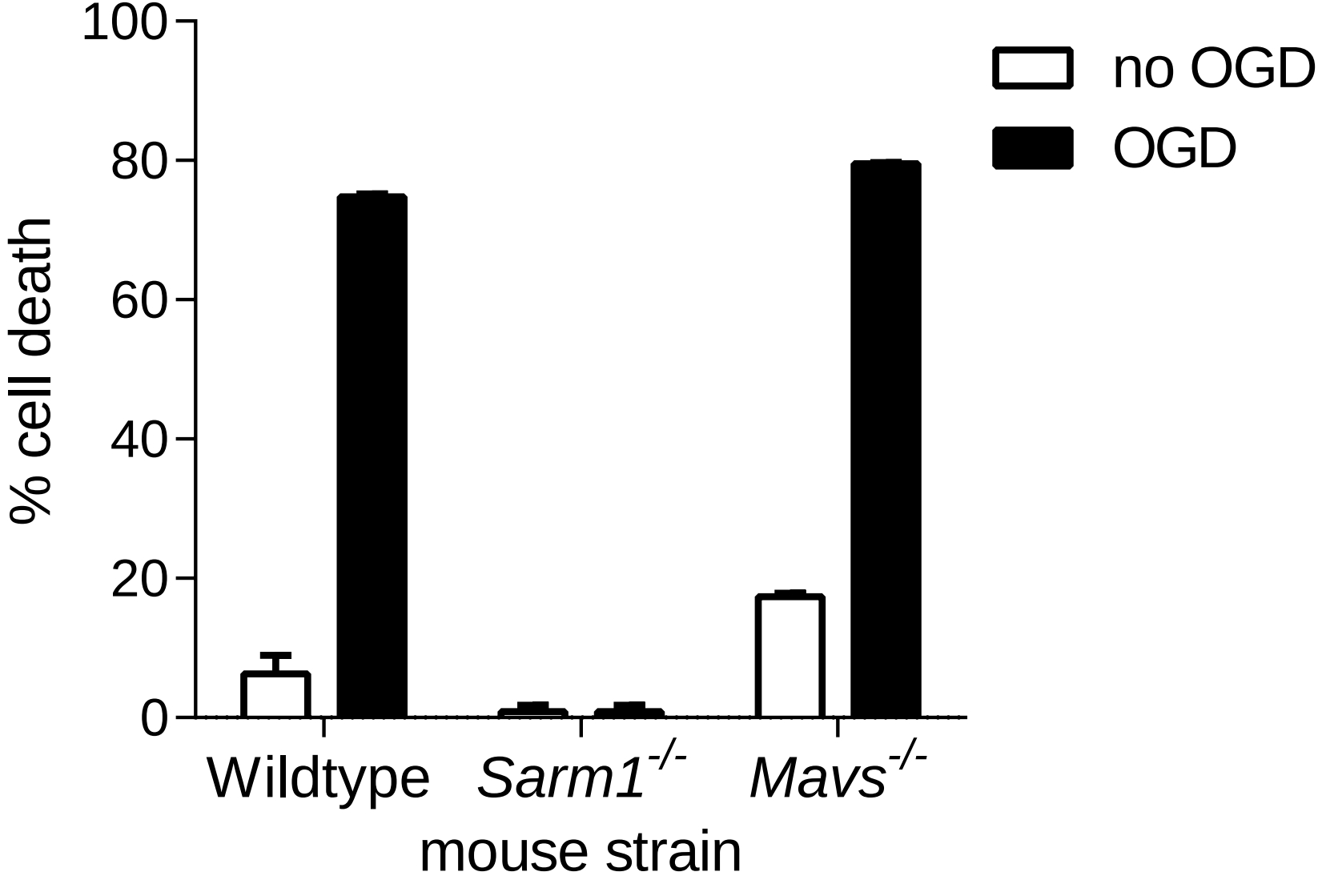
100
80
60
40
20
0
% cell death
no OGD
OGD
Wildtype
Sarm1-/-
Mavs-/-
mouse strain

Figure S6. MAVS Does Not Influence OGD-Induced Neuronal Death, Related to Figure 7

Neurons from wildtype, *Sarm1*-/-or *Mavs*-/-mice were cultured under normal or OGD conditions as described in the methods for 1-3 hrs and then analyzed for cell death using an MTT assay. Data are from the 3 hour time point and are the mean+/-SEM of 3-8 samples per treatment group per strain.