Figure S1

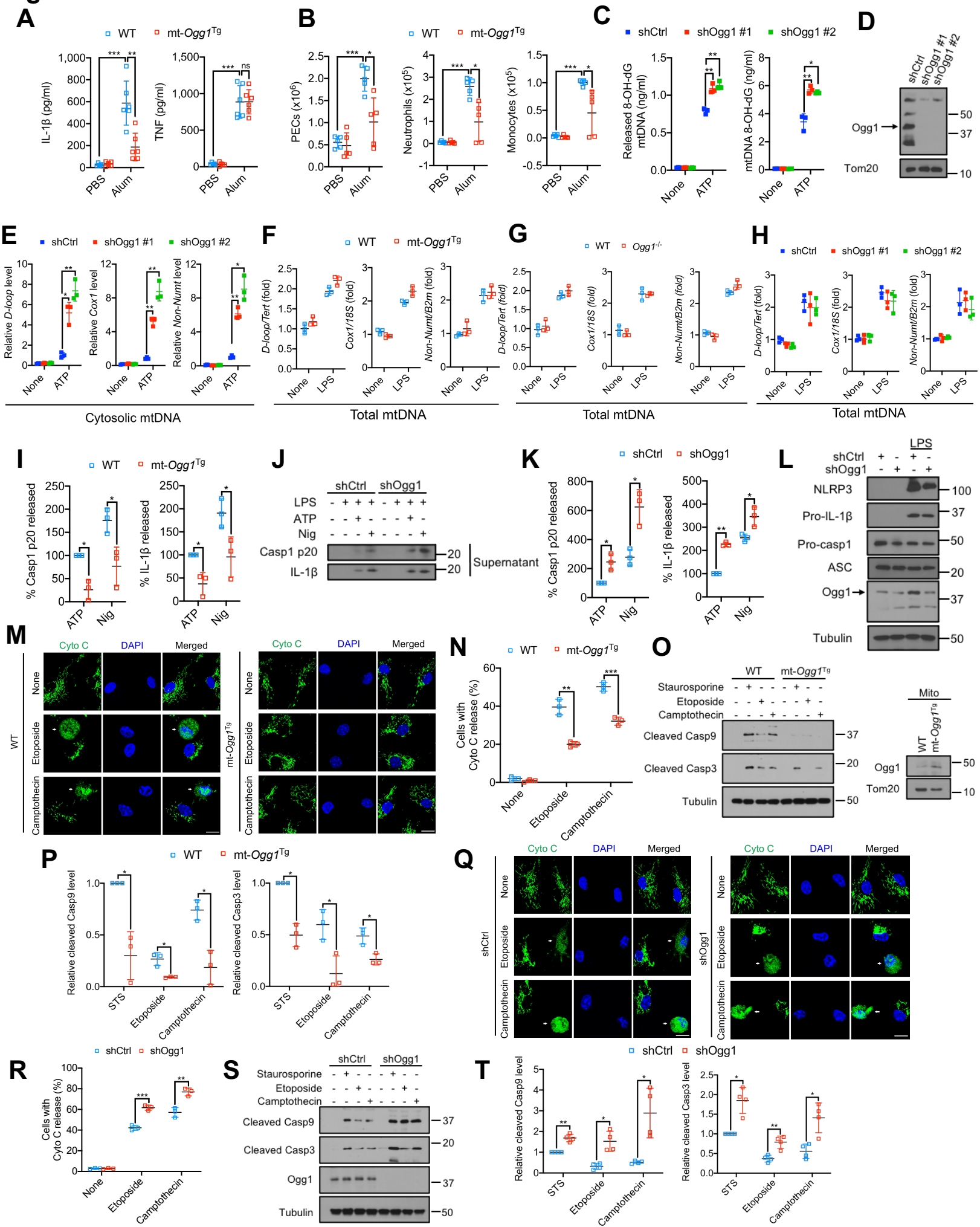
A
WT
mt-Ogg1Tg
IL-1β (pg/ml)
TNF (pg/ml)
PBS
Alum
ns
B
PECs (x10^6)
Neutrophils (x10^5)
Monocytes (x10^5)
C
shCtrl
shOgg1 #1
shOgg1 #2
Released 8-OH-dG mtDNA (ng/ml)
mtDNA 8-OH-dG (ng/ml)
None
ATP
D
Ogg1
Tom20
E
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
Cytosolic mtDNA
F
D-loop/Tert (fold)
Cox1/18S (fold)
Non-Numt/B2m (fold)
LPS
Total mtDNA
G
Ogg1-/-
H
I
% Casp1 p20 released
% IL-1β released
ATP
Nig
J
shCtrl
shOgg1
LPS
ATP
Nig
Casp1 p20
IL-1β
Supernatant
K
L
NLRP3
Pro-IL-1β
Pro-casp1
ASC
Tubulin
M
Cyto C
DAPI
Merged
None
Etoposide
Camptothecin
N
Cells with Cyto C release (%)
O
Staurosporine
Etoposide
Camptothecin
Cleaved Casp9
Cleaved Casp3
Mito
P
Relative cleaved Casp9 level
Relative cleaved Casp3 level
STS
Q
R
S
T

Figure S1. mt-OGG1 reduces Ox-mtDNA content and restrains NLRP3 inflammasome activation, related to Figure 1.

(A) Peritoneal IL-1β and TNF in WT or mt-*Ogg*^{Tg} mice 4 h after i.p. injection of alum (1 mg) or PBS. Data are mean ± s.d. (n=5 in PBS-treated group; n=6 in alum-treated group).

(B) Alum-induced infiltration of peritoneal exudate cells (PEC), neutrophils ($CD11b^+Ly6G^+F4/80^-$) and monocytes ($CD11b^+Ly6C^+Ly6G^-$) in WT and mt-*Ogg*^{Tg} mice 16 h after alum (300 ug) or PBS injection. Data are mean ± s.d. (n=5 for each group).

(C) 8-OH-dG amounts in mtDNA from cytosol (left) and mitochondria (right) of LPS-primed BMDM transduced with Ogg1 (shOgg1 #1 and shOgg1 #2) or control (shCtrl) shRNA, before and after ATP stimulation.

(D) IB analysis of OGG1 and Tom20 in mitochondria isolated from shCtrl- or shOgg1 RNA-transduced BMDM.

(E) Relative cytosolic mtDNA amounts in BMDM treated as (C).

(F-H) Relative total mtDNA amounts in WT and mt-*Ogg*^{Tg} (F), WT and *Ogg1*$^{-/-}$ (G) or shCtrl- and shOgg1 RNA-transduced BMDM (H), before and after LPS priming. The ratios of *D-loop* mtDNA to *Tert* nDNA, *Cox1* mtDNA to *18S* nDNA, or mtDNA that is not inserted into nuclear DNA (*non-NUMT*) to *B2m* nDNA are shown.

(I) Relative Casp1 p20 and IL-1b in culture supernatants of WT and mt-*Ogg*^{Tg} BMDM treated as in Figure 1I.

(J) IB analysis of Casp1 p20 and IL-1β in culture supernatants of shCtrl- or shOgg1 RNA-transduced BMDM treated as indicated.

(K) Relative Casp1 p20 and IL-1b in supernatants of BMDM shown in (J).

(L) IB analysis of NLRP3, pro-IL-1β, pro-caspase-1, ASC and Ogg1 in shCtrl- and shOgg1 RNA-transduced BMDM, before and after LPS priming. Tubulin is loading control.

(M) Representative fluorescent microscopy of WT or mt-*Ogg1*^{Tg} BMDM incubated -/+ etoposide (10 mM) or camptothecin (10 mM) for 16 h and stained for cytochrome c (Cyto C). Arrows indicate Cyto C release. Scale bar, 5 μm.

(N) Percentages of cells in (M) with Cyto C release. n=150 cells per group from 3 independent experiments.

(O) IB analysis of cleaved caspase 9 (Casp9) and cleaved caspase 3 (Casp3) in lysates of WT and mt-*Ogg*^{Tg} BMDM incubated -/+ staurosporine (STS, 100 nM), etoposide (10 μM) or camptothecin (10 μM) for 16 h (left). Mitochondrial OGG1 IB in WT and mt-*Ogg*^{Tg} BMDM (right).

(P) Relative amounts of cleaved Casp9 and Casp3 of BMDM shown in (O).

(Q) Representative fluorescent microscopy of shCtrl- and shOgg1 RNA-transduced BMDM treated as in (M) stained for Cyto C. Arrows indicate Cyto C release. Scale bar, 5 μm.

(R) Percentages of cells from (Q) with Cyto C release. n=150 cells per group from 3 independent experiments.

(S) IB analysis of cleaved Casp9 and Casp3 in lysates of shCtrl- and shOgg1 RNA-transduced BMDM treated as in (O).

(T) Relative amounts of cleaved Casp9 and Casp3 in BMDM shown in (S).

All IBs show one representative out of 3. Results in (C, E-I, K, N, P, R and T) are mean ± s.d. (n=3). *p<0.05; **p<0.01; ***p<0.001. ns, not significant. Two-sided unpaired t-test.

Figure S2

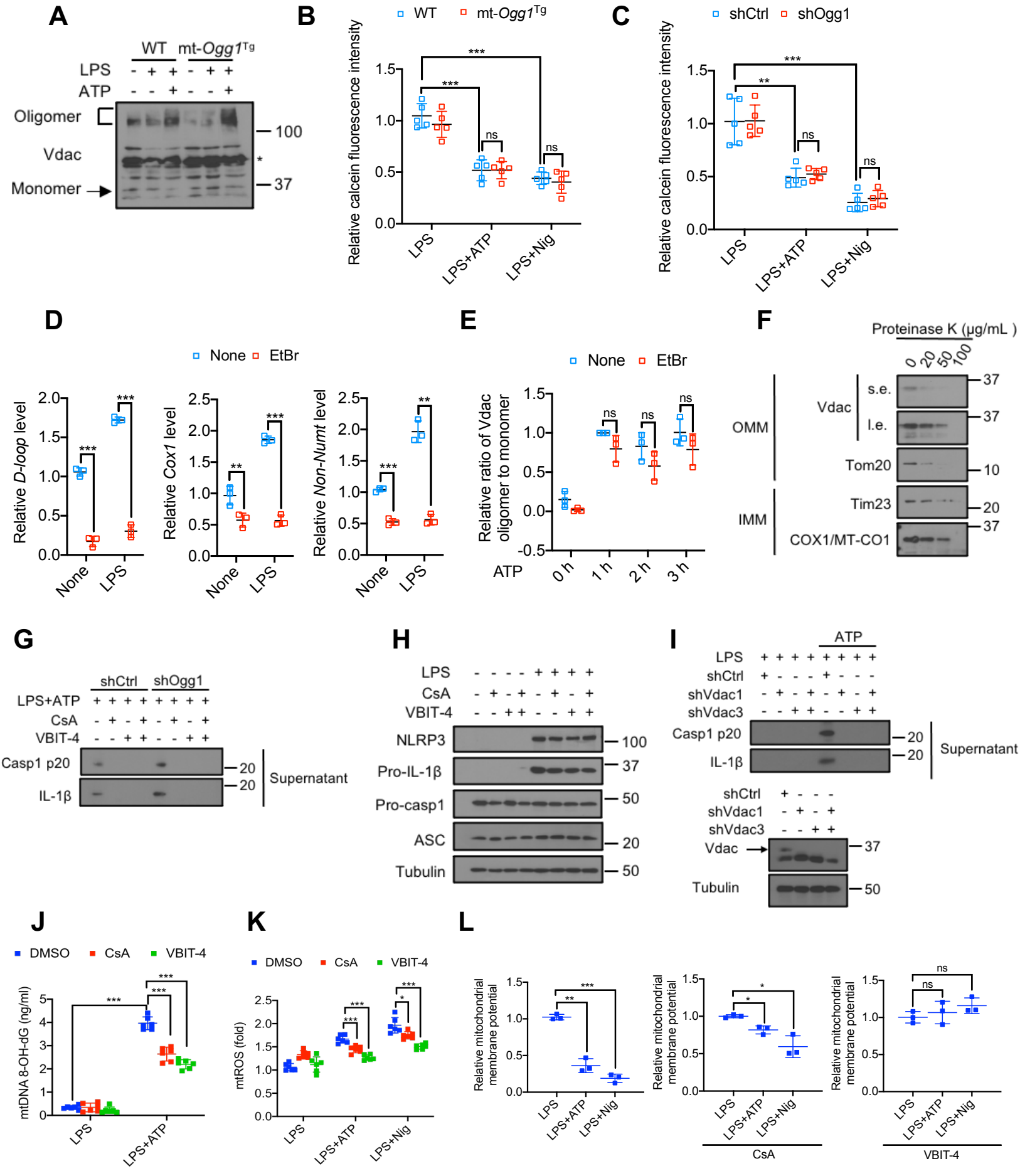
A
WT
mt-Ogg1Tg
LPS
ATP
Oligomer
Vdac
Monomer
100
37
B
WT
mt-Ogg1Tg
Relative calcein fluorescence intensity
LPS
LPS+ATP
LPS+Nig
ns
C
shCtrl
shOgg1
Relative calcein fluorescence intensity
LPS
LPS+ATP
LPS+Nig
ns
D
None
EtBr
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
None
LPS
E
None
EtBr
Relative ratio of Vdac oligomer to monomer
ATP
0 h
1 h
2 h
3 h
F
Proteinase K (μg/mL)
0
20
50
100
OMM
IMM
Vdac
s.e.
l.e.
Tom20
Tim23
COX1/MT-CO1
37
10
20
G
shCtrl
shOgg1
LPS+ATP
CsA
VBIT-4
Casp1 p20
IL-1β
20
Supernatant
H
LPS
CsA
VBIT-4
NLRP3
Pro-IL-1β
Pro-casp1
ASC
Tubulin
100
37
50
20
I
ATP
LPS
shCtrl
shVdac1
shVdac3
Casp1 p20
IL-1β
Supernatant
Vdac
Tubulin
J
DMSO
CsA
VBIT-4
mtDNA 8-OH-dG (ng/ml)
LPS
LPS+ATP
K
DMSO
CsA
VBIT-4
mtROS (fold)
LPS
LPS+ATP
LPS+Nig
L
Relative mitochondrial membrane potential
LPS
LPS+ATP
LPS+Nig
CsA
VBIT-4
ns

Figure S2. CsA and VBIT-4 restrict NLRP3 inflammasome activation, related to Figure 2

(A) VDAC oligomerization in WT or mt-*Ogg1*^{Tg} BMDM that were primed -/+ LPS and stimulated -/+ ATP was analyzed as in Figure 2A. Asterisk indicates nonspecific band.

(B and C) Relative calcein fluorescent intensity in LPS-primed WT and mt-*Ogg*^{Tg} (B) or shCtrl- or shOgg1 RNA-transduced (C) BMDM stimulated -/+ ATP or nigericin, using the calcein-quenching assay for mPTP opening (n=5).

(D) Relative mtDNA amounts in BMDM from Figure 2E, before or after LPS priming, demonstrating the efficacy of mtDNA depletion by EtBr.

(E) Relative VDAC oligomerization in BMDM from Figure 2E (n=3).

(F) Mitochondria isolated from BMDM were digested with the indicated concentrations of proteinase K for 30 min on ice and IB analyzed with antibodies against the OMM proteins VDAC and Tom20 and the IMM proteins Tim23 and COX1/MT-CO1.

(G) IB analysis of Casp1 p20 and IL-1β in supernatants of shCtrl- or shOgg1 RNA-transduced BMDM that were LPS primed and challenged with ATP, -/+ CsA (1 μM, 16 h) or VBIT-4 (10 μM, 16 h) pretreatment.

(H) IB analysis of NLRP3, pro-IL-1β, pro-caspase-1, ASC, and tubulin in lysates of BMDM before and after LPS priming, -/+ CsA or VBIT-4 pretreatment as above.

(I) IB analysis of Casp1 p20 and IL-1β in supernatants of LPS-primed BMDM transduced with shCtrl, shVdac1, shVdac3, or shVdac1 plus shVdac3 RNA, -/+ ATP stimulation (top) and validation of VDAC silencing (bottom).

(J) 8-OH-dG amounts in mtDNA from mitochondria of LPS-primed BMDM pretreated -/+ CsA (1 μM, 16 h) or VBIT-4 (1 μM, 16 h), before and after ATP stimulation (n=6).

(K) Relative mtROS amounts in LPS-primed BMDM pretreated -/+ CsA or VBIT-4 as above and challenged with ATP or nigericin (n=6).

(L) Mitochondrial membrane potential of BMDM treated as in (K) (n=3).

All IBs show one representative out of 3. Results in (B-E and J-L) are mean ± s.d.. *p<0.05; **p<0.01; ***p<0.001. ns, not significant. Two-sided unpaired t-test.

Figure S3

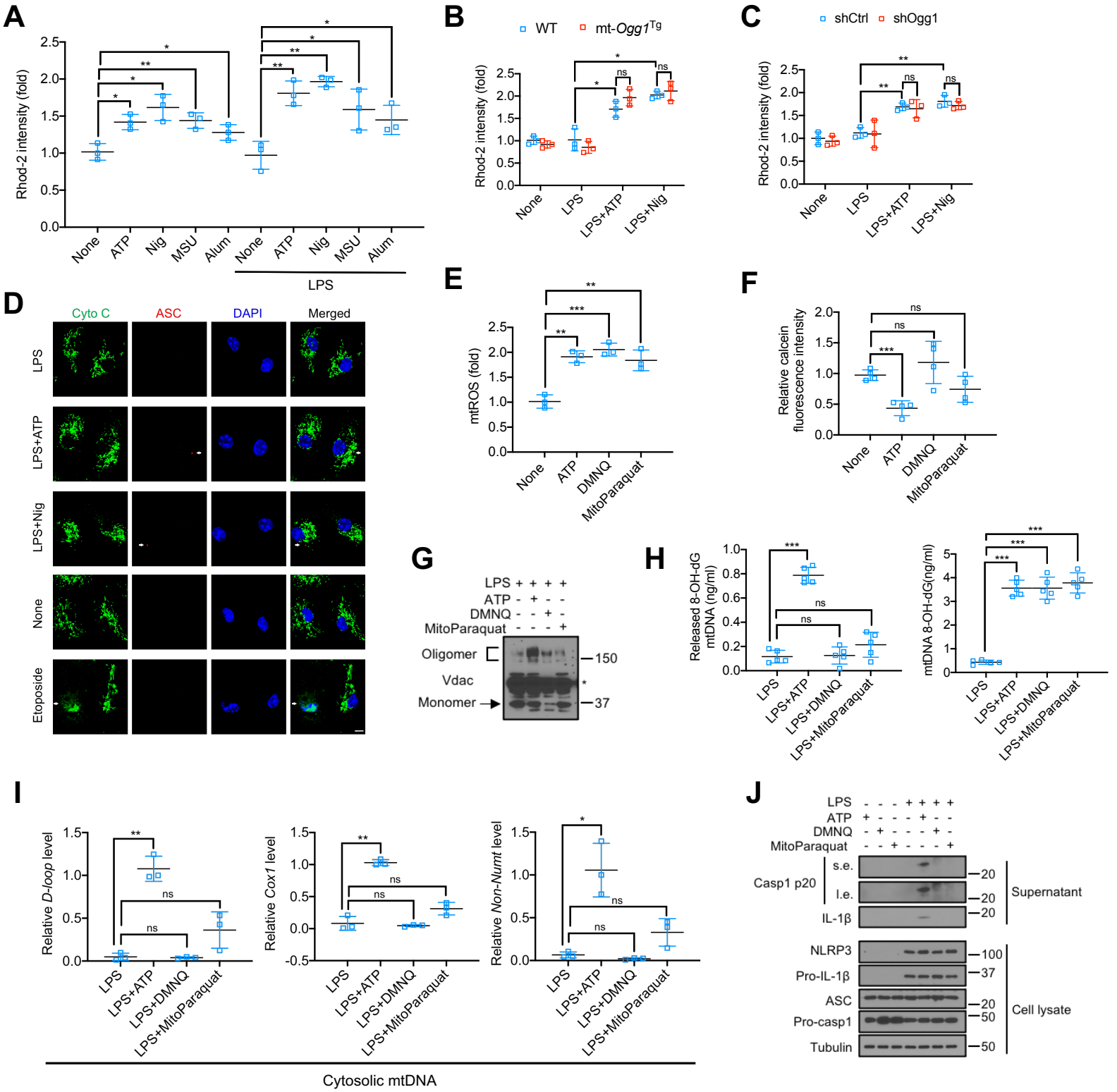
A
Rhod-2 intensity (fold)
None
ATP
Nig
MSU
Alum
LPS
B
WT
mt-Ogg1Tg
Rhod-2 intensity (fold)
None
LPS
LPS+ATP
LPS+Nig
C
shCtrl
shOgg1
Rhod-2 intensity (fold)
D
Cyto C
ASC
DAPI
Merged
LPS
LPS+ATP
LPS+Nig
None
Etoposide
E
mtROS (fold)
None
ATP
DMNQ
MitoParaquat
F
Relative calcein fluorescence intensity
G
LPS
ATP
DMNQ
MitoParaquat
Oligomer
Vdac
Monomer
150
37
H
Released 8-OH-dG mtDNA (ng/ml)
mtDNA 8-OH-dG(ng/ml)
LPS
LPS+ATP
LPS+DMNQ
LPS+MitoParaquat
I
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
Cytosolic mtDNA
J
Casp1 p20
s.e.
l.e.
IL-1β
Supernatant
NLRP3
Pro-IL-1β
ASC
Pro-casp1
Tubulin
Cell lysate

Figure S3. mtROS alone do not induce channel opening and consequent mtDNA release and apoptosis is not involved either, related to Figure 3

(A) Relative Rhod-2 fluorescent intensity in BMDM that were challenged with different NLRP3 agonists, -/+ LPS priming (n=3).

(B and C) Relative Rhod-2 fluorescent intensity in WT and mt-*Ogg*^{Tg} (B) or shCtrl and shOgg1 transduced (C) BMDM before and after LPS priming, -/+ ATP or nigericin stimulation (n=3).

(D) Representative fluorescent microscopy images of LPS-primed BMDM -/+ ATP or nigericin stimulation that were co-stained for cytochrome c (Cyto C) and ASC. DAPI stains nuclei. BMDM treated with etoposide were used as a positive control for Cyto C release. Arrows indicate ASC specks or Cyto C release. Scale bar, 5 μm.

(E and F) Relative mtROS amounts (n=3) (E) and calcein fluorescent intensity (n=4) (F) in LPS-primed BMDM treated with ATP or the ROS generators DMNQ (20 μM, 16 h) and MitoParaquat (5 μM, 1 h).

(G) VDAC oligomerization in BMDM treated as in (E) was IB analyzed as in Figure S2A. Asterisk indicates nonspecific band.

(H) 8-OH-dG amounts in cytosolic (left) or mitochondrial (right) mtDNA in LPS-primed BMDM treated as in (E) (n=5).

(I) Relative cytosolic mtDNA in BMDM treated as in (H) (n=3).

(J) IB analysis of Casp1 p20 and IL-1β in supernatants and NLRP3, pro-IL-1β, ASC, pro-caspase-1, and tubulin in lysates of BMDM -/+ LPS priming, followed by ATP, DMNQ or MitoParaquat treatment as above.

All IBs show one representative out of 3. Results in (A-C, E, F, H and I) are mean ± s.d.. *$p<0.05$; **$p<0.01$; ***$p<0.001$. ns, not significant. Two-sided unpaired t-test.

Figure S4

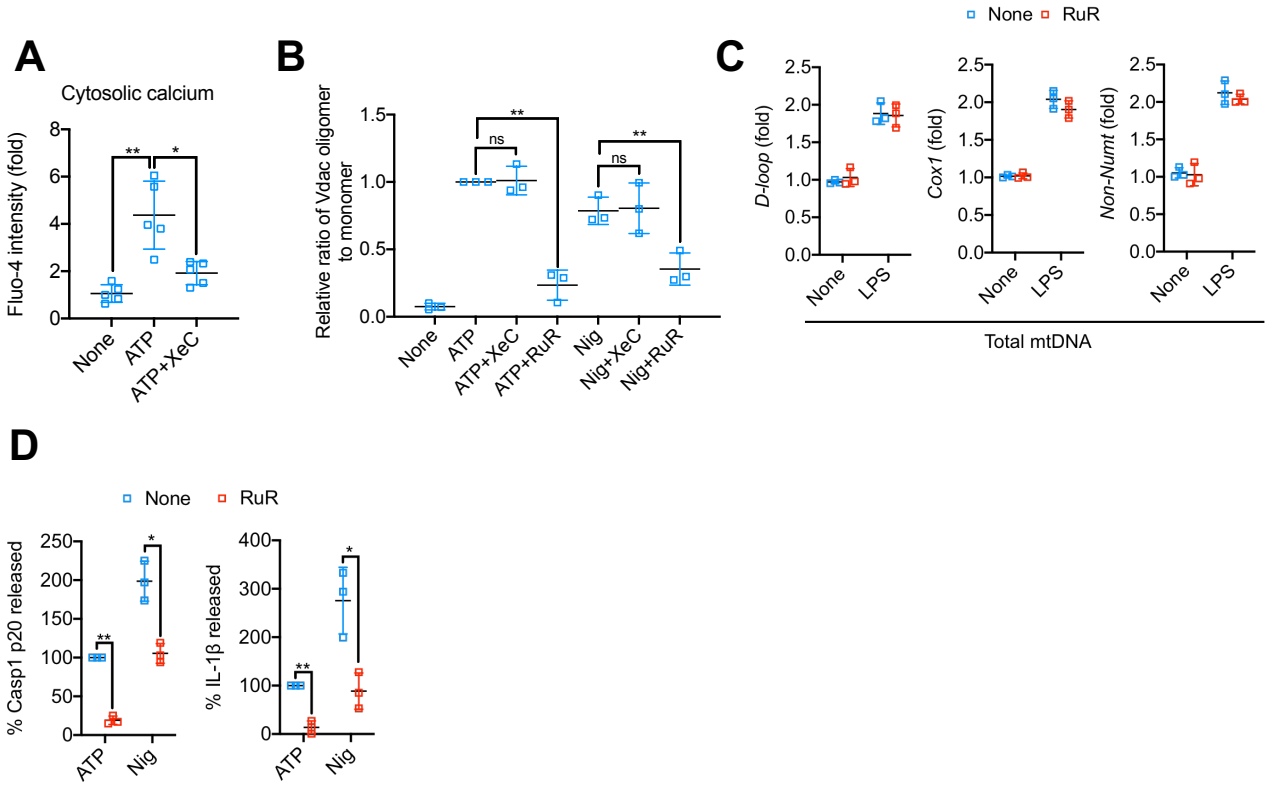

Figure S4. MCU inhibitor blunts mPTP-VDAC channel opening and NLRP3 inflammasome activation, related to Figure 4

(A) Relative Fluo-4 fluorescent intensity of BMDM pretreated -/+ XeC (5 μM, 16 h) and challenged with ATP (n=5).

(B) Relative VDAC oligomerization in the BMDM from Figure 4C (n=3).

(C) Relative mtDNA amounts in BMDM pretreated -/+ RuR (10 μM, 16 h) before and after LPS priming (n=3).

(D) Relative Casp1 p20 and IL-1b in supernatants of BMDM from Figure 4F (n=3).

Results are mean ± s.d.. *p<0.05; **p<0.01. ns, not significant. Two-sided unpaired t-test.

Figure S5

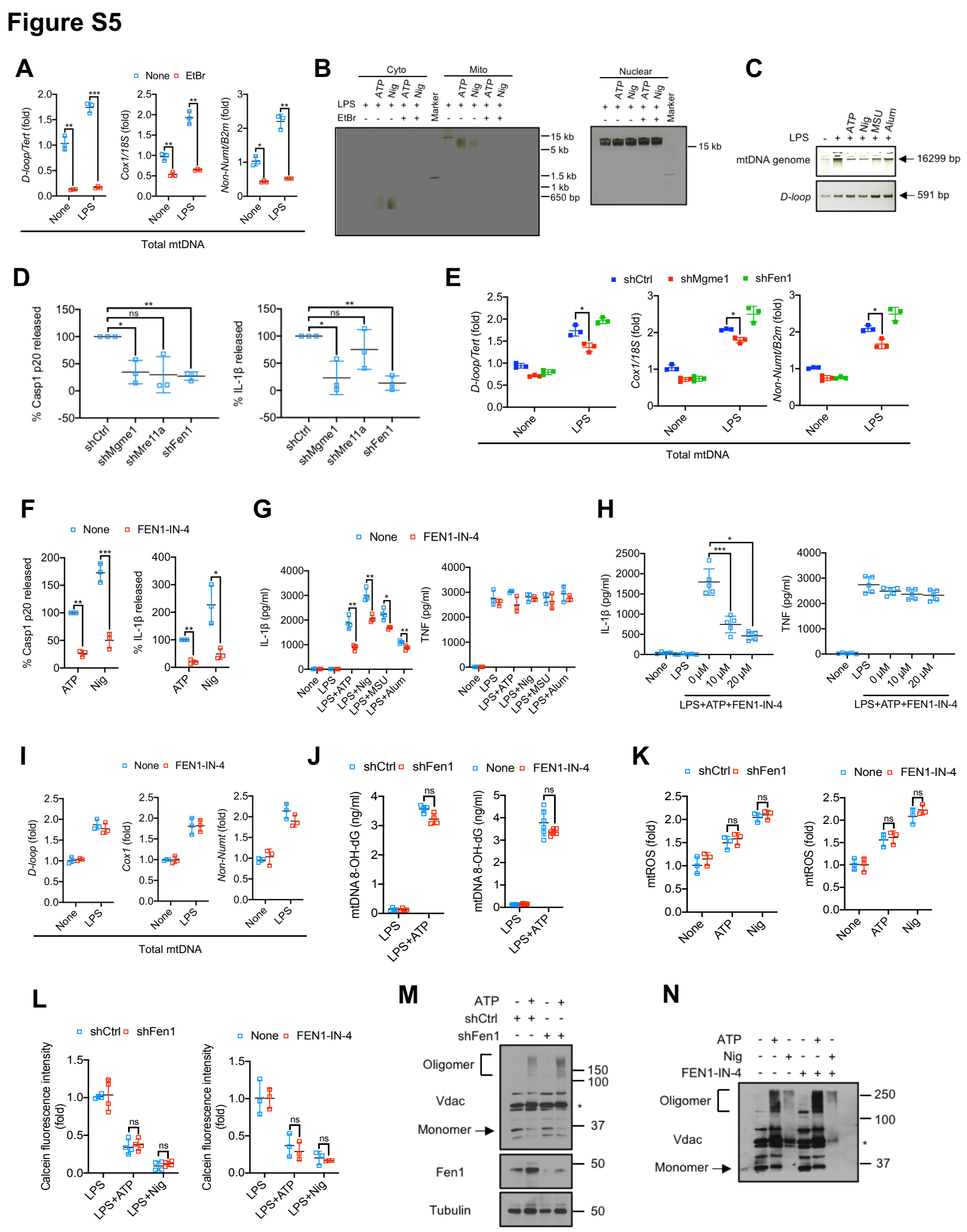
A
None
EtBr
D-loop/Tert (fold)
Cox1/18S (fold)
Non-Numt/B2m (fold)
None
LPS
Total mtDNA
B
Cyto
Mito
Nuclear
LPS
EtBr
ATP
Nig
Marker
15 kb
5 kb
1.5 kb
1 kb
650 bp
C
LPS
ATP
Nig
MSU
Alum
mtDNA genome
16299 bp
D-loop
591 bp
D
% Casp1 p20 released
% IL-1β released
shCtrl
shMgme1
shMre11a
shFen1
E
shCtrl
shMgme1
shFen1
D-loop/Tert (fold)
Cox1/18S (fold)
Non-Numt/B2m (fold)
None
LPS
Total mtDNA
F
None
FEN1-IN-4
% Casp1 p20 released
% IL-1β released
ATP
Nig
G
None
FEN1-IN-4
IL-1β (pg/ml)
TNF (pg/ml)
None
LPS
LPS+ATP
LPS+Nig
LPS+MSU
LPS+Alum
H
IL-1β (pg/ml)
TNF (pg/ml)
None
LPS
0 μM
10 μM
20 μM
LPS+ATP+FEN1-IN-4
I
None
FEN1-IN-4
D-loop (fold)
Cox1 (fold)
Non-Numt (fold)
None
LPS
Total mtDNA
J
shCtrl
shFen1
None
FEN1-IN-4
mtDNA 8-OH-dG (ng/ml)
LPS
LPS+ATP
K
shCtrl
shFen1
None
FEN1-IN-4
mtROS (fold)
None
ATP
Nig
L
shCtrl
shFen1
None
FEN1-IN-4
Calcein fluorescence intensity (fold)
LPS
LPS+ATP
LPS+Nig
M
ATP
shCtrl
shFen1
Oligomer
Vdac
Monomer
Fen1
Tubulin
150
100
37
50
50
N
ATP
Nig
FEN1-IN-4
Oligomer
Vdac
Monomer
250
100
37

Figure S5. Mgme1 is needed for mtDNA synthesis but FEN1 has no impact on total mtDNA content, Ox-mtDNA generation and pore opening, related to Figure 5

(A) Relative mtDNA amounts in BMDM pretreated -/+ EtBr (450 ng/mL, 4 days) before or after LPS priming.

(B) Analysis of DNA in the cytosolic (Cyto), mitochondrial (Mito), or nuclear fractions of LPS-primed BMDM pretreated -/+ EtBr (450 ng/mL, 4 days), followed by ATP or nigericin stimulation. The agarose gel is representative of 3 independent experiments.

(C) PCR products generated by amplification of mtDNA (entire genome, 16299 bp or *D-loop* region, 591 bp) from BMDM before or after LPS priming, followed by stimulation with different NLRP3 activators.

(D) Relative Casp1 p20 and IL-1b in supernatants of BMDM from Figure 5C.

(E) Relative mtDNA amounts in shCtrl, shMgme1, or shFen1 transduced BMDM -/+ LPS priming.

(F) Relative Casp1 p20 and IL-1b in supernatants of BMDM from Figure 5M.

(G) IL-1β and TNF secretion by BMDM pretreated -/+ FEN1-IN-4 (10 μM, 16 h), primed -/+ LPS, and stimulated with different NLRP3 activators (n=3).

(H) IL-1β and TNF secretion by BMDM pretreated -/+ FEN1-IN-4 (10 μM or 20 μM, 16 h), primed -/+ LPS, followed by ATP challenge (n=3).

(I) Relative mtDNA amounts in BMDM pretreated -/+ FEN1-IN-4 (10 μM, 16 h), before or after LPS priming (n=3).

(J and K) 8-OH-dG content of mtDNA from mitochondria (J) and relative mtROS amounts (K) in LPS-primed BMDM transduced with Fen1 (shFen1) or control (shCtrl) shRNA (left) or pretreated -/+ FEN1-IN-4 (10 μM, 16 h) (right), and stimulated -/+ ATP or nigericin (n=3-6).

(L) Calcein fluorescent intensity of LPS-primed BMDM treated as in (K) (n=3).

(M and N) VDAC oligomerization in LPS-primed BMDM transduced with shFen1 or shCtrl RNA (M) or pretreated -/+ FEN1-IN-4 (10 μM, 16 h) (N), and stimulated -/+ ATP or nigericin, was IB analyzed as in Figure S2A. Asterisk indicates nonspecific band.

The agarose gel in (B and C) is representative of 3 independent experiments. Results in (A and D-L) are mean ± s.d. *p<0.05; **p<0.01; ***p<0.001. ns, not significant. Two-sided unpaired t-test.

Figure S6

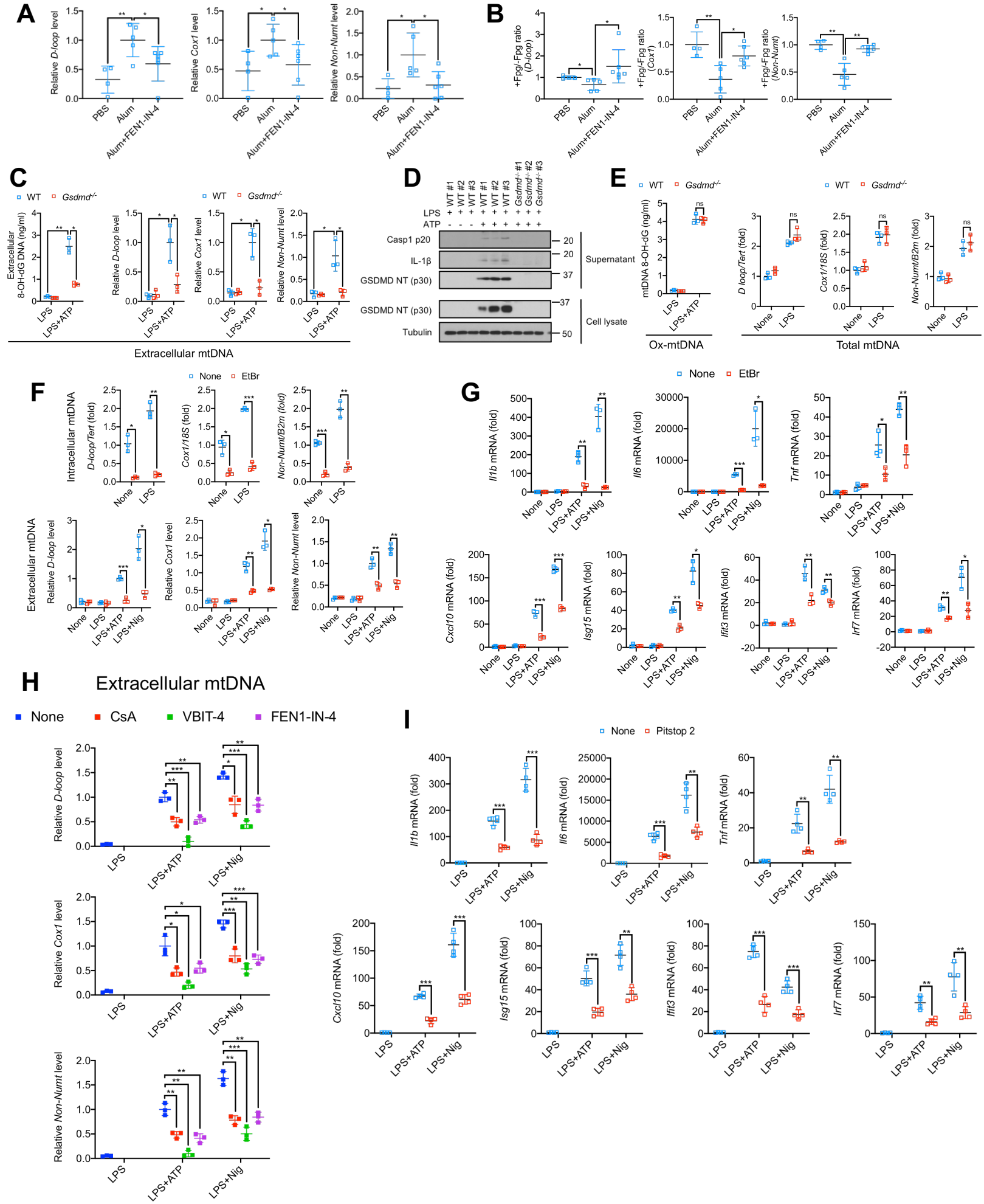
A
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
PBS
Alum
Alum+FEN1-IN-4
B
+Fpg/-Fpg ratio (D-loop)
+Fpg/-Fpg ratio (Cox1)
+Fpg/-Fpg ratio (Non-Numt)
C
WT
Gsdmd-/-
Extracellular 8-OH-dG DNA (ng/ml)
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
LPS
LPS+ATP
Extracellular mtDNA
D
WT #1
WT #2
WT #3
Gsdmd-/- #1
Gsdmd-/- #2
Gsdmd-/- #3
LPS
ATP
Casp1 p20
IL-1β
GSDMD NT (p30)
Supernatant
GSDMD NT (p30)
Tubulin
Cell lysate
E
mtDNA 8-OH-dG (ng/ml)
Ox-mtDNA
D loop/Tert (fold)
Cox1/18S (fold)
Non-Numt/B2m (fold)
Total mtDNA
ns
None
LPS
F
None
EtBr
Intracellular mtDNA
D-loop/Tert (fold)
Cox1/18S (fold)
Non-Numt/B2m (fold)
Extracellular mtDNA
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
LPS+Nig
G
Il1b mRNA (fold)
Il6 mRNA (fold)
Tnf mRNA (fold)
Cxcl10 mRNA (fold)
Isg15 mRNA (fold)
Ifit3 mRNA (fold)
Irf7 mRNA (fold)
H
Extracellular mtDNA
None
CsA
VBIT-4
FEN1-IN-4
Relative D-loop level
Relative Cox1 level
Relative Non-Numt level
I
None
Pitstop 2
Il1b mRNA (fold)
Il6 mRNA (fold)
Tnf mRNA (fold)
Cxcl10 mRNA (fold)
Isg15 mRNA (fold)
Ifit3 mRNA (fold)
Irf7 mRNA (fold)

Figure S6. Immunostimulatory cell-free Ox-mtDNA secretes via pyroptosis and is up-taken by stander cells through endocytosis, related to Figure 6

(A and B) Relative ccf-mtDNA amounts (A) and the ratio of Fpg-treated (+) to nontreated (-) mtDNA, indicating the fraction of cell-free non-Ox-mtDNA (Fpg-resistant) relative to total secreted mtDNA (B) in peritoneal exudates from Figure 5O (n=4-5 for PBS- and alum-treated groups; n=6 for alum+FEN1-IN-4-treated group).

(C) Relative amounts of extracellular Ox-mtDNA (left) or mtDNA in LPS-primed WT and *Gsdmd*$^{-/-}$ BMDM stimulated -/+ ATP (n=3).

(D) IB analysis of Casp1 p20, IL-1β and cleaved GSDMD [GSDMD NT (p30)] in culture supernatants of LPS-primed WT and *Gsdmd*$^{-/-}$ BMDM -/+ ATP challenge (n=3)

(E) 8-OH-dG amounts in mtDNA from mitochondria of LPS-primed WT or *Gsdmd*$^{-/-}$ BMDM before and after ATP stimulation (left). Relative total mtDNA amounts in WT and *Gsdmd*$^{-/-}$ BMDM, before and after LPS priming (n=3).

(F) Relative amounts of intracellular (top panel) or secreted (bottom panel) mtDNA in BMDM pretreated -/+ EtBr (450 ng/mL, 4 days), primed -/+ LPS and stimulated -/+ ATP or nigericin. Intracellular mtDNA content validates effective mtDNA depletion by EtBr (n=3).

(G) qPCR quantitation of *Il1b*, *Il6*, *Tnf*, *Cxcl10*, *Isg15*, *Ifit3* and *Irf7* mRNAs in BMDM incubated for 24 h with cell free DNA collected from (F) (n=3).

(H) Relative mtDNA amounts released by LPS-primed BMDM pretreated -/+ CsA, VBIT-4 or FEN1-IN-4 as above and challenged with ATP or nigericin (n=3).

(I) qPCR quantitation of *Il1b*, *Il6*, *Tnf*, *Cxcl10*, *Isg15*, *Ifit3* and *Irf7* mRNAs in BMDM -/+ Pitstop 2 (2 uM) incubated for 24 h with extracellular DNA collected from LPS-primed donor BMDM stimulated -/+ ATP or nigericin (n=4).

Results are mean ± s.d.. *p<0.05; **p<0.01; ***p<0.001. ns, not significant. Two-sided unpaired t-test.

Figure S7

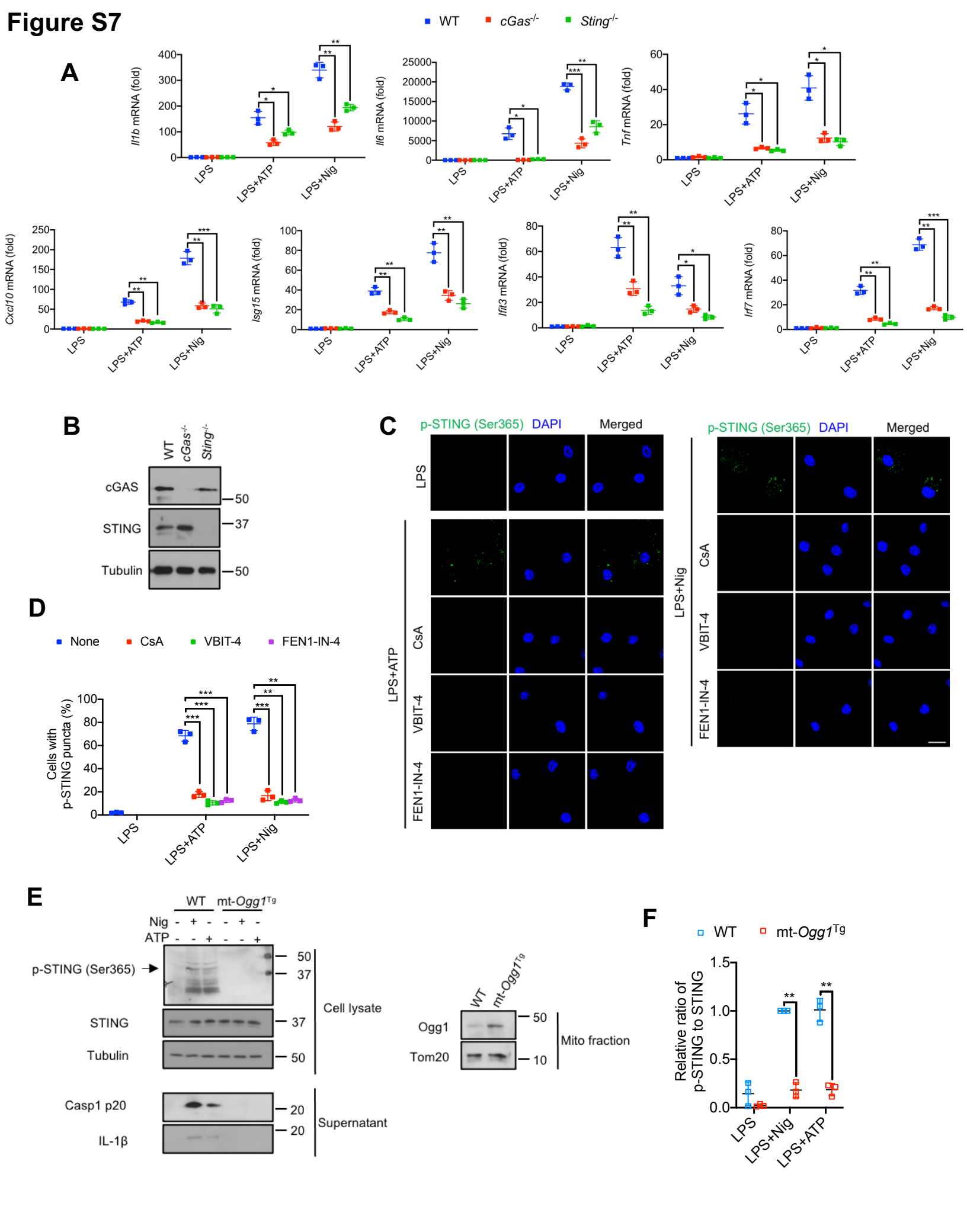
A
WT
cGas-/-
Sting-/-
Il1b mRNA (fold)
Il6 mRNA (fold)
Tnf mRNA (fold)
Cxcl10 mRNA (fold)
Isg15 mRNA (fold)
Ifit3 mRNA (fold)
Irf7 mRNA (fold)
LPS
LPS+ATP
LPS+Nig
B
WT
cGas-/-
Sting-/-
cGAS
STING
Tubulin
50
37
50
C
p-STING (Ser365)
DAPI
Merged
LPS
LPS+ATP
LPS+Nig
CsA
VBIT-4
FEN1-IN-4
D
None
CsA
VBIT-4
FEN1-IN-4
Cells with p-STING puncta (%)
E
WT
mt-Ogg1Tg
Nig
ATP
p-STING (Ser365)
STING
Tubulin
Cell lysate
Casp1 p20
IL-1β
Supernatant
Ogg1
Tom20
Mito fraction
F
WT
mt-Ogg1Tg
Relative ratio of p-STING to STING

Figure S7. Induction of mtDNA release by NLRP3 inflammasome activators leads to STING Ser^{365} phosphorylation, related to Figure 7

(A) qPCR quantitation of *Il1b*, *Il6*, *Tnf*, *Cxcl10*, *Isg15*, *Ifit3* and *Irf7* mRNAs in WT, *cGas*$^{-/-}$ or *Sting*$^{-/-}$ BMDM incubated for 24 h with extracellular DNA collected from LPS-primed donor BMDM stimulated -/+ ATP or nigericin (n=3), showing cGAS-STING dependence.

(B) IB analysis of cGAS and STING in lysates of BMDM as in (A).

(C) Representative fluorescent microscopy images of LPS-primed BMDM pretreated-/+ CsA (1 μM, 16 h), VBIT-4 (10 μM, 16 h) or FEN1-IN-4 (10 μM, 16 h) and stained for p-STING (Ser^{365}) before or after ATP or nigericin stimulation. DAPI stains nuclei. Scale bar, 5 μm.

(D) Percentages of cells with p-STING (Ser^{365}) puncta from (C). n=150 cells per group were analyzed from 3 independent experiments.

(E) IB analysis of STING Ser^{365} phosphorylation in lysates and Casp1 p20 and IL-1β in supernatants of LPS-primed WT or mt-*Ogg1*^{Tg} BMDM stimulated -/+ ATP or nigericin (left) (n=3). Mitochondrial OGG1 IB in WT and mt-*Ogg1*^{Tg} BMDM (right).

(F) Relative p-STING (Ser^{365}) to total STING amounts as indicated in (E).

Results in (A, D and F) are mean ± s.d.. *p<0.05; **p<0.01; ***p<0.001. Two-sided unpaired t-test.