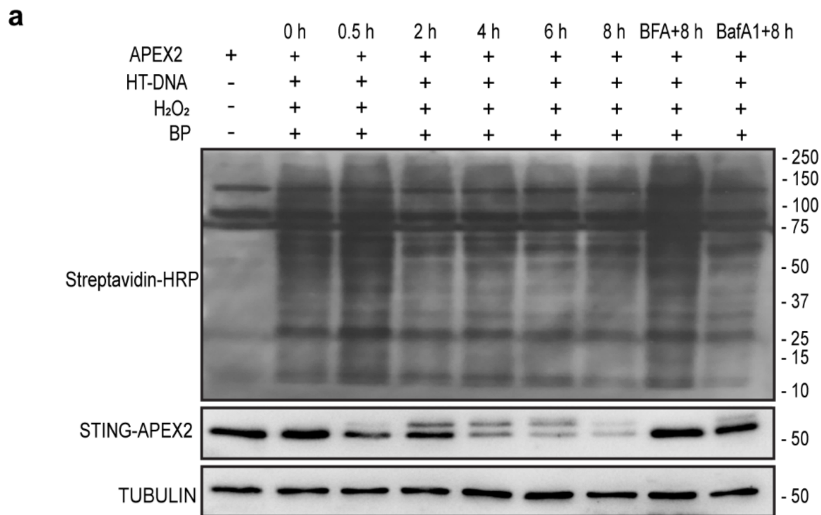
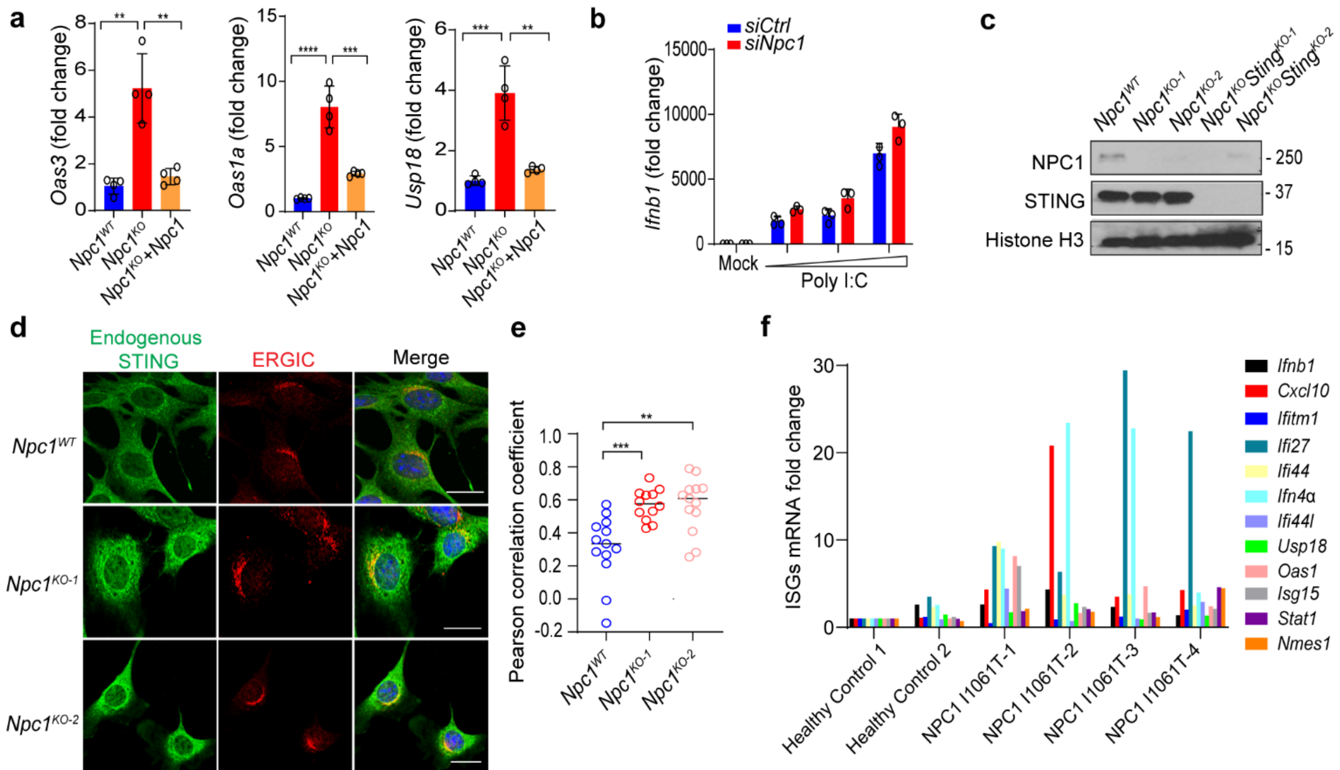
SUPPLEMENTAL MATERIALS

Extended Data Figures 1-8



Extended Data Fig.1| STING-APEX2 proximity labeling.

a, Immunoblot analysis of cell lysates. STING-APEX2 MEFs were mock-treated or stimulated with HT-DNA (1 μg/ml) for indicated times with or without treatment of Brefeldin A (BFA) or Bafilomycin A1 (BafA1) (top). Then, proximity labeling was performed and biotinylated proteins were detected by streptavidin-HRP.



Extended Data Fig.2| NPC1 deficiency primes STING trafficking and activation of ISGs.

a, qRT-PCR analysis of the baseline ISGs expression (*Oas3*, *Oas1a*, *Usp18*) in *Npc1^{WT}*, *Npc1^{KO}* and *Npc1^{KO}* stably expressing wild-type *Npc1* (n=4).

b, qRT-PCR analysis of *Ifnb1* mRNA expression in WT and *Npc1* knockdown MEFs after stimulation with increasing amount of Poly I:C (0, 1, 2, 4 μg/ml) for 3 h (n=3).

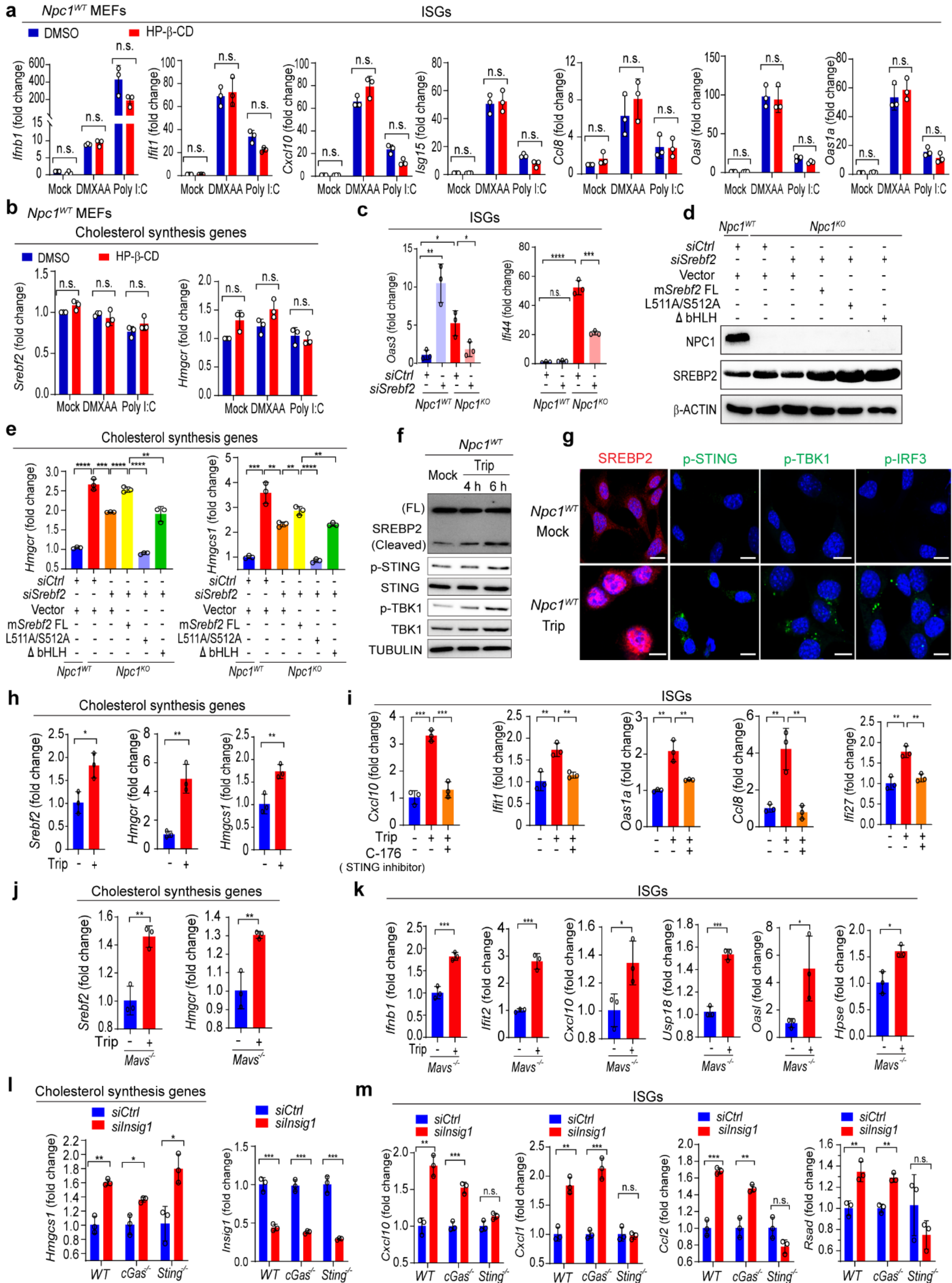
c, Immunoblot analysis of the indicated proteins (left) in *Npc1^{WT}*, *Npc1^{KO}*, *Npc1^{KO}STING^{KO}* MEFs.

d, Fluorescent microscopy analysis of endogenous STING localization at the resting-state in *Npc1^{WT}* and two independent clones of *Npc1^{KO}* MEFs. Endogenous STING in green, an ERGIC marker (ERGIC53) in red and DAPI in blue. Scale bar,10 μm.

e, Quantification of STING colocalization with the ERGIC in **d**.

f, qRT-PCR analysis of the baseline ISGs expression in fibroblasts from healthy controls (n=2) and unrelated *NPC1^{I1061T}* patients (n=4).

a, **b**, Data are shown as means ± s.d. **a**, **e**, Unpaired two-tailed Student's *t*-test. **p < 0.01, ***p < 0.001, ****p < 0.0001. Data are representative of at least two independent experiments.


a
Npc1^WT MEFs
ISGs
DMSO
HP-β-CD
Ifnb1 (fold change)
Ifit1 (fold change)
Cxcl10 (fold change)
Isg15 (fold change)
Ccl8 (fold change)
Oasl (fold change)
Oas1a (fold change)
n.s.
Mock DMXAA Poly I:C
b
Npc1^WT MEFs
Cholesterol synthesis genes
Srebf2 (fold change)
Hmgcr (fold change)
c
ISGs
Oas3 (fold change)
Ifi44 (fold change)
siCtrl
siSrebf2
Npc1^WT Npc1^KO
d
Npc1^WT
Npc1^KO
siCtrl
siSrebf2
Vector
mSrebf2 FL
L511A/S512A
Δ bHLH
NPC1
SREBP2
β-ACTIN
e
Cholesterol synthesis genes
Hmgcr (fold change)
Hmgcs1 (fold change)
f
Npc1^WT
Mock
Trip
4 h
6 h
(FL)
SREBP2
(Cleaved)
p-STING
STING
p-TBK1
TBK1
TUBULIN
g
SREBP2
p-STING
p-TBK1
p-IRF3
Npc1^WT Mock
Npc1^WT Trip
h
Cholesterol synthesis genes
Srebf2 (fold change)
Hmgcr (fold change)
Hmgcs1 (fold change)
Trip
i
ISGs
Cxcl10 (fold change)
Ifit1 (fold change)
Oas1a (fold change)
Ccl8 (fold change)
Ifi27 (fold change)
Trip
C-176
(STING inhibitor)
j
Cholesterol synthesis genes
Srebf2 (fold change)
Hmgcr (fold change)
Trip
Mavs^-/-
k
ISGs
Ifnb1 (fold change)
Ifit2 (fold change)
Cxcl10 (fold change)
Usp18 (fold change)
Oasl (fold change)
Hpse (fold change)
Trip
Mavs^-/-
l
Cholesterol synthesis genes
siCtrl
siInsig1
Hmgcs1 (fold change)
Insig1 (fold change)
WT cGas^-/- Sting^-/-
m
ISGs
siCtrl
siInsig1
Cxcl10 (fold change)
Cxcl1 (fold change)
Ccl2 (fold change)
Rsad (fold change)
WT cGas^-/- Sting^-/-

Extended Data Fig.3| SREBP2 primes STING signaling independently of cGAS or MAVS in *Npc1*-deficient cells.

a, **b**, qRT-PCR analysis of mock-, DMXAA- (50 μg/ml, 2 h) or poly(I:C)- (1 μg/ml, 2 h) induced expression of ISGs (**a**) and cholesterol synthesis genes (**b**) with mock- or HP-β-CD treatment (8 h) in $Npc1^{WT}$ MEFs (n=3).

c, qRT-PCR analysis of baseline ISGs expression in $Npc1^{WT}$ and $Npc1^{KO}$ MEFs transfected with *si-control* or *si-Srebf2* for 48 h (n=3).

d, Immunoblot analysis of the indicated proteins (left) in $Npc1^{WT}$, $Npc1^{KO}$, $Npc1^{KO}Srebf2^{KD}$ and $Npc1^{KO}Srebf2^{KD}$ MEFs reconstituted with SREBP2 wild-type or transcription-inactive mutants (L511A/S512A, ΔbHLH).

e, qRT-PCR analysis of cholesterol synthesis genes expression in $Npc1^{WT}$, $Npc1^{KO}$, $Npc1^{KO}Srebf2^{KD}$ and $Npc1^{KO}Srebf2^{KD}$ MEFs reconstituted with SREBP2 wild-type or transcription-inactive mutants (L511A/S512A, ΔbHLH) (n=3).

f, Immunoblot analysis of the SREBP2 activation and STING signaling cascade in $Npc1^{WT}$ MEFs treated with mock or triparanol (14 μM) for indicated time (shown on the top). Cleaved SREBP2 and phospho- and total- proteins of the STING pathway blotted are showing on the left.

g, Fluorescent microscopy analysis of cleaved SREBP2 nuclear translocation (red) as well as p-STING (green), p-TBK1(green) and p-IRF3 (green) staining in $Npc1^{WT}$ MEFs treated with mock or triparanol (14 μM) for 6 h. DAPI (blue), Scale bar, 10 μm.

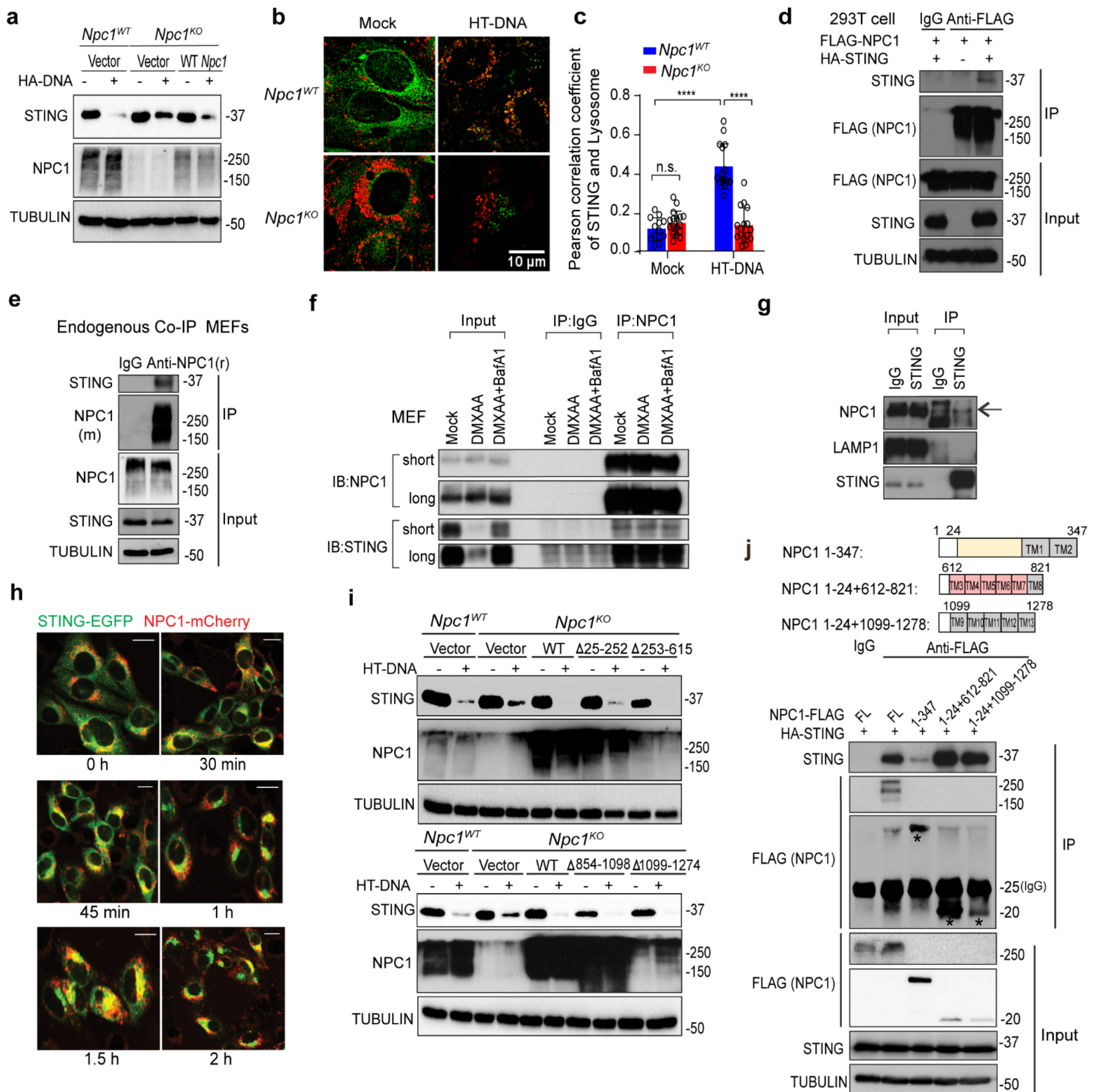
h, qRT-PCR analysis of cholesterol synthesis genes in BMDMs mock-treated or triparanol-treated (14 μM) for 12 h (n=3).

i, qRT-PCR analysis of baseline ISGs expression in BMDMs mock-treated or triparanol-treated (14 μM) alone or in combination with STING inhibitor C-176 (0.5 μM) for 12 h (n=3).

j, **k**, qRT-PCR analysis of cholesterol synthesis genes (**j**) and ISGs (**k**) expression in $Mavs^{-/-}$ BMDMs mock-treated or triparanol-treated (14 μM) for 12 h (n=3).

l, **m**, qRT-PCR analysis of cholesterol synthesis gene *Hmgcs1*, knockdown efficiency of *Insig1* (**l**), and ISGs expression (**m**) in WT, $cGas^{-/-}$ and $Sting^{-/-}$ MEFs (n=3).

a-c, **e**, **h-m**, Data are shown as means ± s.d. Unpaired two-tailed Student's *t*-test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, n.s. not significant. Data are representative of at least three independent experiments.



Extended Data Fig.4| NPC1 interacts with STING and mediates STING lysosomal degradation.

a, Immunoblot analysis of STING degradation in *Npc1*^{WT}, *Npc1*^{KO} or *Npc1*^{KO} MEFs stably expressing wild-type NPC1. Cells were mock-treated or stimulation with HT-DNA (1 μg/ml) for 8 h.

b, Live-cell microscopy images of STING-EGFP and lysosomes. *Npc1^{WT}* and *Npc1^{KO}* MEFs stably expressing STING-EGFP were mock-treated (left) or stimulated with HT-DNA (right, 4 μg/ml). STING-EGFP in green and LysoTracker-Red in red. Scale bar, 10 μm.

c, Quantification of STING and lysosome colocalization in **b**.

d, Immunoblot analysis of NPC1 and STING co-IP in HEK293T cells. HEK293T cells were transfected with indicated plasmids (top), and 24 h later, anti-IgG (mouse) or anti-FLAG was used to pull down. HA-STING co-IP was analyzed by anti-STING immunoblot. Whole-cell lysates were blotted by anti-FLAG (NPC1), anti-STING and anti-TUBULIN as input.

e, Immunoblot analysis of endogenous STING and NPC1 co-IP in wild-type MEFs. Anti-IgG (rabbit) or Anti-NPC1 (rabbit) was used to pull down. Both IP and lysate were blotted for endogenous STING (rabbit), NPC1 (mouse) and TUBULIN.

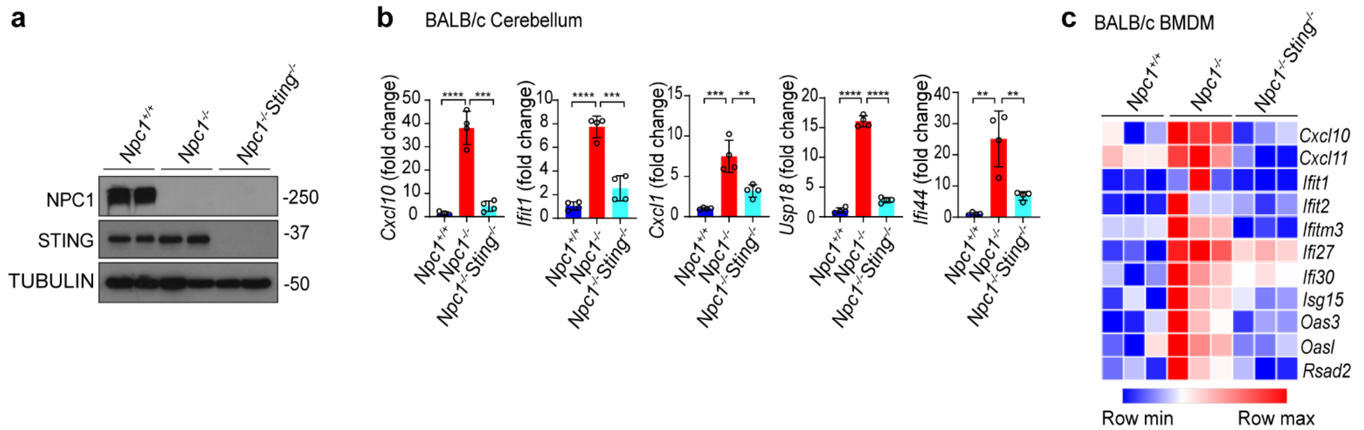
f, Immunoblot analysis of endogenous STING and NPC1 co-IP in wild-type MEFs with mock-treated, DMXAA-(30 μg/ml, 8 h) treated or DMXAA combined with Bafilomycin A1 treated (to prevent STING degradation). Anti-IgG (rabbit) or Anti-NPC1 (rabbit) was used to pull down. Both IP and lysate input were blotted for endogenous STING (rabbit) and NPC1 (mouse).

g, Immunoblot analysis of endogenous STING interaction with NPC1 or LAMP1 in wild-type MEFs. Anti-IgG (rabbit) or Anti-STING (rabbit) was used for pull-down. Both IP and lysate input were blotted for endogenous STING, NPC1 or LAMP1.

h, Live-cell fluorescent microscopy analysis of STING-EGFP and NPC1-mCherry localization. *Npc1^{KO}Sting^{KO}* MEFs stably expressing STING-EGFP (green) and NPC1-mCherry (red) were stimulated with HT-DNA (4 μg/ml) and imaged at indicated time. Scale bar,10 μm.

i, Immunoblot analysis of STING degradation in *Npc1^{WT}*, *Npc1^{KO}* or *Npc1^{KO}* MEFs stably expressing indicated NPC1 truncations. Cells were mock-treated or stimulated with HT-DNA (1 μg/ml) for 8 h. Cell lysates were analyzed for proteins indicated on the left.

j, Immunoblot analysis of STING interaction with NPC1 transmembrane bundles in HEK293T cells. HEK293T cells were transfected with indicated plasmids (top), and 24 h later, anti-IgG (mouse) or anti-FLAG (mouse) was used for pull down. Both IP and whole-cell lysates were analyzed by anti-FLAG, anti-STING and anti-TUBULIN. Data are representative of at least two independent experiments.



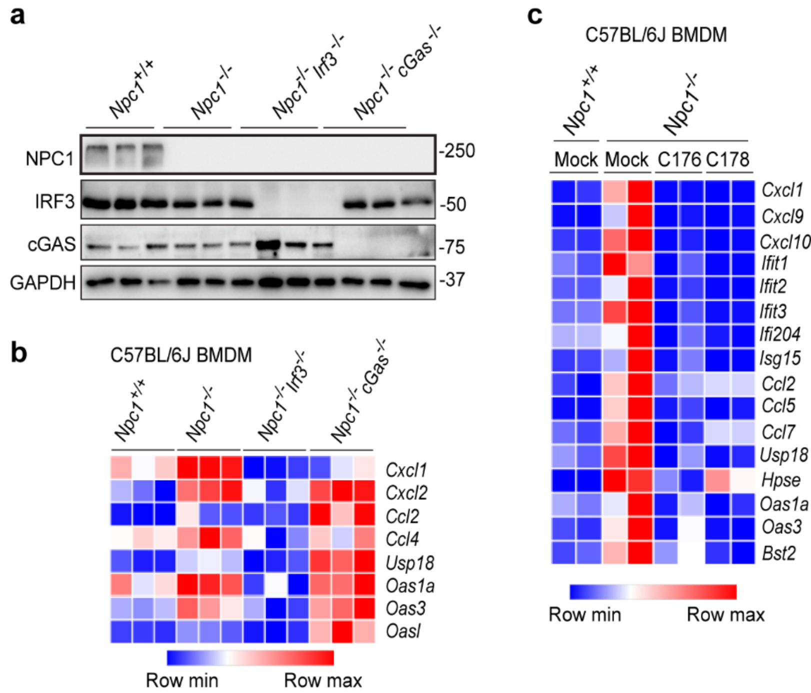
Extended Data Fig.5| STING drives neuroinflammation in *Npc1*$^{-/-}$ mice.

a, Immunoblot analysis of the indicated proteins (left) in BALB/c *Npc1*$^{+/+}$, *Npc1*$^{-/-}$ and *Npc1*$^{-/-}$*Sting*$^{-/-}$ mouse whole brain lysates (n=2).

b, qRT–PCR analysis of ISGs expression in BALB/c *Npc1*$^{+/+}$, *Npc1*$^{-/-}$ and *Npc1*$^{-/-}$*Sting*$^{-/-}$ mouse cerebellum (n=4).

c, A heatmap showing ISGs expression in BALB/c *Npc1*$^{+/+}$, *Npc1*$^{-/-}$ and *Npc1*$^{-/-}$*Sting*$^{-/-}$ mouse BMDMs (n=3). Each ISG mRNA expression level was measured by qRT–PCR.

b, Data shown as means ± s.d. Unpaired two-tailed Student's *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Data are representative of at least two independent experiments.

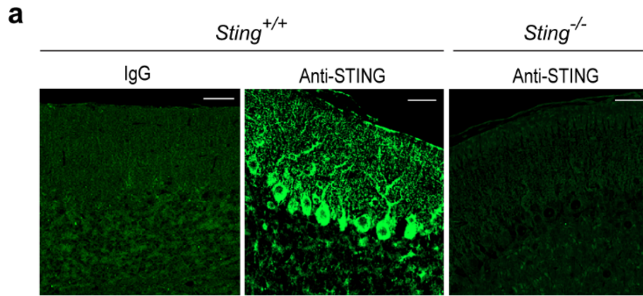


Extended Data Fig.6| STING and IRF3, but not cGAS, is required for immune activation in *Npc1*$^{-/-}$ BMDMs.

a, Immunoblot analysis of proteins (left) in whole brain lysates of indicated genotypes of mice (C57BL/6J, n=3).

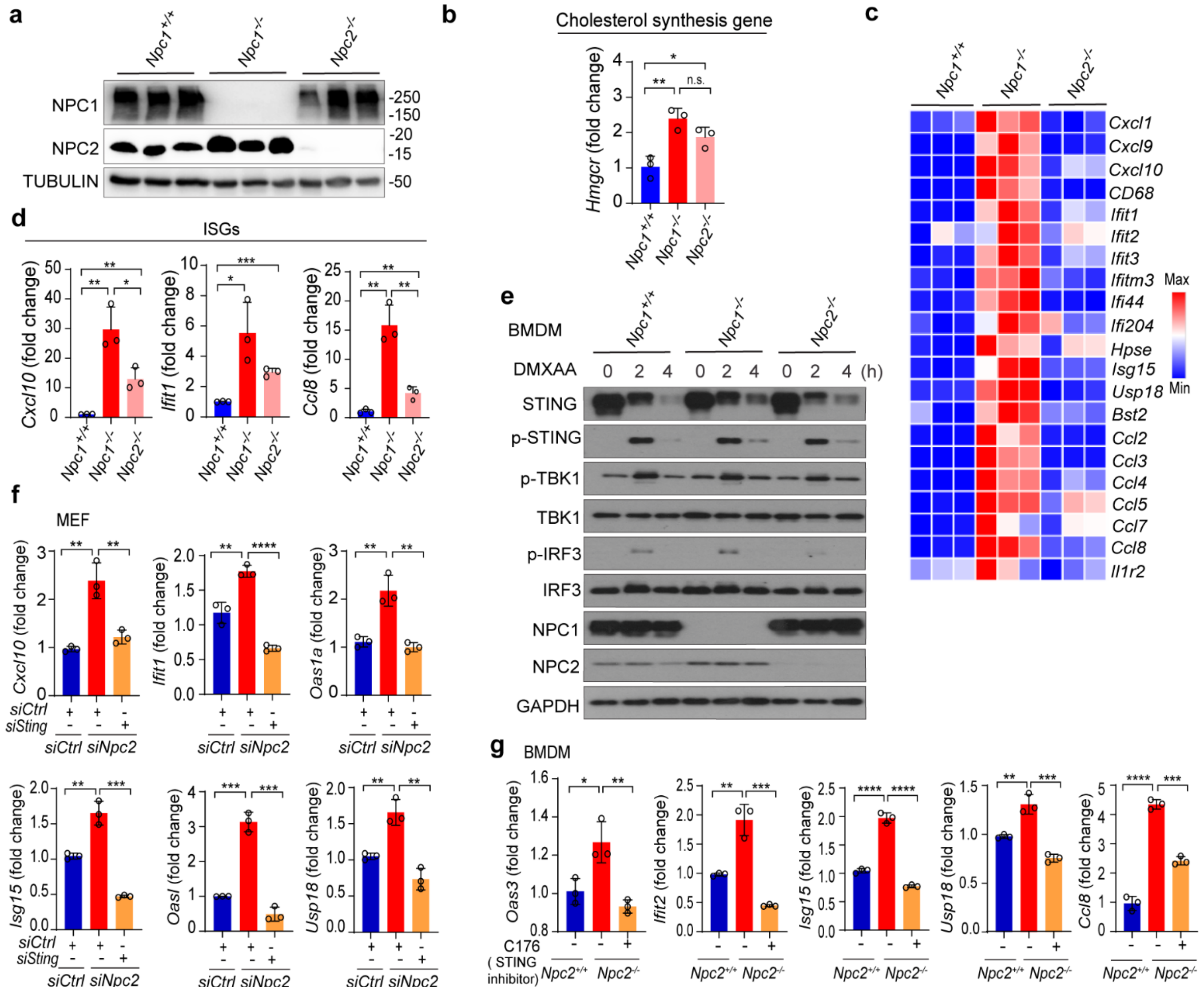
b, A heatmap showing ISGs expression in indicated genotypes of mice BMDMs (C57BL/6J, n=3). Each ISG mRNA expression level was measured by qRT–PCR.

c, A heatmap showing ISG expression in *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ BMDMs mock-treated or treated with STING inhibitor C-176 (0.5 μM) or C-178 (0.5 μM) overnight (n=2). Each ISG mRNA expression was measured by qRT–PCR.



Extended Data Fig.7| Validation of STING antibody for fluorescent IHC staining of mouse cerebellum.

a, Fluorescent IHC staining with IgG (rabbit), anti-STING (rabbit) in WT C57BL/6J cerebellum or staining with anti-STING (rabbit) in *Sting*$^{-/-}$ C57BL/6J cerebellum. Scale bars, 30 μm.



Extended Data Fig.8| STING signaling activation in *Npc1*$^{-/-}$ and *Npc2*$^{-/-}$ cells and mice.

a, Immunoblot analysis of proteins (left) in indicated genotype of BALB/c mouse whole brain lysates (n=3).

b, qRT–PCR analysis of cholesterol synthesis gene (*Hmgcr*) expression in the cerebellums of indicated genotype mice (BALB/c) (n=3)

c, A heatmap showing ISGs expression in BALB/c *Npc1*$^{+/+}$, *Npc1*$^{-/-}$ and *Npc2*$^{-/-}$ mouse cerebellum (n=3). Each ISG mRNA expression was measured by qRT–PCR.

d, qRT–PCR analysis of some ISGs expression in the cerebellum of indicated genotype mice (BALB/c) (n=3).

e, Immunoblot analysis of the STING signaling cascade. BALB/c *Npc1*$^{+/+}$, *Npc1*$^{-/-}$ and *Npc2*$^{-/-}$ mouse BMDMs were stimulated with DMXAA (50 μg/ml) for 0, 2, 4 h. Phospho- and total- proteins blotted are showing on the left.

f, qRT–PCR analysis of baseline ISGs expression in *Npc2*^{WT}, *Npc2*^{KD}, *Npc2*^{KD}*Sting*^{KD} MEFs (n=3).

g, qRT–PCR analysis of baseline ISGs expression in BALB/c *Npc1*$^{+/+}$ and *Npc2*$^{-/-}$ mouse BMDMs with mock-treated or treated with STING inhibitor C-176 (0.5 μM) overnight (n=3).

b, **d**, **f**, **g**, Data shown as means ± s.d. Unpaired two-tailed Student's t-test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Data are representative of at least two independent experiments.