Supplementary Figure Legends for:

Gasdermin induces the release of mitochondrial DNA during pyroptosis and apoptosis

de Torre-Minguela et al.

Supplementary Figure S1. GSDMD activation mediates macrophage pyroptosis and mitochondrial damage

A,B Different genotypes of macrophages (as indicated) were primed for 4 h with LPS (10 ng/ml) and then stimulated during 20 min with ATP (3 mM) or nigericin (5 μM), and IL-1β (A) or LDH (B) were detected in the cell-free supernatants.

C Immunoblot for TOMM20, GSDMD and α-tubulin in cell lysates and mitochondria fraction obtained from wild type macrophages primed and activated as in A. Mitochondrial fraction was isolated with ultracentrifugation.

D Immunoblot for TOMM20, GSDMD and α-tubulin in cell lysates and mitochondria fraction obtained from wild type macrophages primed as in A and then stimulated with nigericin (10 μM). Mitochondrial fraction was isolated with an antibody-based kit.

E Different genotypes of macrophages (as indicated) were primed and activated as in A. Maximum intensity projections of z-stack images of macrophages stained with TOMM20 antibodies (green) and DAPI (blue) are shown. Scale bar represents 8 μm.

Data information: Data from panel A and B are means ± SEM from at least ten independent experiments. Statistical analyses were performed using non-parametric Mann–Whitney t-tests. Data were considered significant when ***P < 0.001. Immunoblots of panels C and D are from *n*=1 independent experiment. Microscopy images of panel E are representative of three independent experiments.

Supplementary Figure S2. MitoTEMPO partially blocks MitoSOX fluorescence

A Kinetic of MitoSOX fluorescence of unprimed wild type macrophages stimulated with ATP (3 mM), nigericin (5 μM) or antimycin A (10 μM).

B Kinetic of MitoSOX fluorescence of macrophages primed for 4 h with LPS (10 ng/ml) and then stimulated with nigericin (5 μM) in presence or absence of MitoTEMPO (250 mM). Cells treated with inhibitors were incubated 10 min before and during stimulation of the cells.

Data information: Data are means ± SEM from at least three independent experiments.

Supplementary Figure S3. GSDMD^{NT} and GSDME^{NT} expression

A Immunoblot for Flag (GSDMD and GSDMD^{NT}) and β-Actin in cell lysates of HEK293T transfected for 12 h with a plasmid encoding human GSDMD or human GSDMD^{NT} (1-275).

B YoPro-1 uptake of wild type macrophages primed for 4 h with LPS (10 ng/ml) and stimulated with nigericin (10 μM), or C*asp1/11*$^{-/-}$ macrophages treated with DMSO or staurosporin (1 μM). Measurement of YoPro-1 fluorescence were performed at the indicated times.

C Immunoblot for GSDME and β-Actin in cell lysates of HEK293T transfected for 12 h with a plasmid encoding for human GSDME^{NT} (1-270) or with a control empty vector (pcDNA).

Data information: Immunoblots of panels A and C are representative of three independent experiments. Data of panel B are means ± SEM from three-four independent experiments in triplicates. Statistical analyses were performed using non-parametric Mann–Whitney t-tests. Data were considered significant when ***P < 0.001 or ****P < 0.0001

Supplementary Figure S4. Digitonin induce plasma membrane permeabilization and mitochondrial network fragmentation

A Kinetic of MitoSOX fluorescence and YoPro-1 uptake in wild type macrophages primed for 4 h with LPS (10 ng/ml) and then treated with digitonin (50 μg/ml) when indicate by the arrow

B Maximum intensity projections of z-stack images of wild type macrophages treated as in A and stained with TOMM20 antibodies (green) and DAPI (blue). Scale bar represents 10 μm.

Data information: Data in panel A are means ± SEM from three independent experiments. Microscopy image of panel B is representative of three independent experiments.

Supplementary Figure S5. Uncropped immunoblots of Figure 1A

Supplementary Figure S6. Uncropped immunoblots of Figure 1B

Supplementary Figure S7. Uncropped immunoblots of Figure 1B

Supplementary Figure S8. Uncropped immunoblots of Figure 3A

Supplementary Figure S9. Uncropped immunoblots of Figure 4A

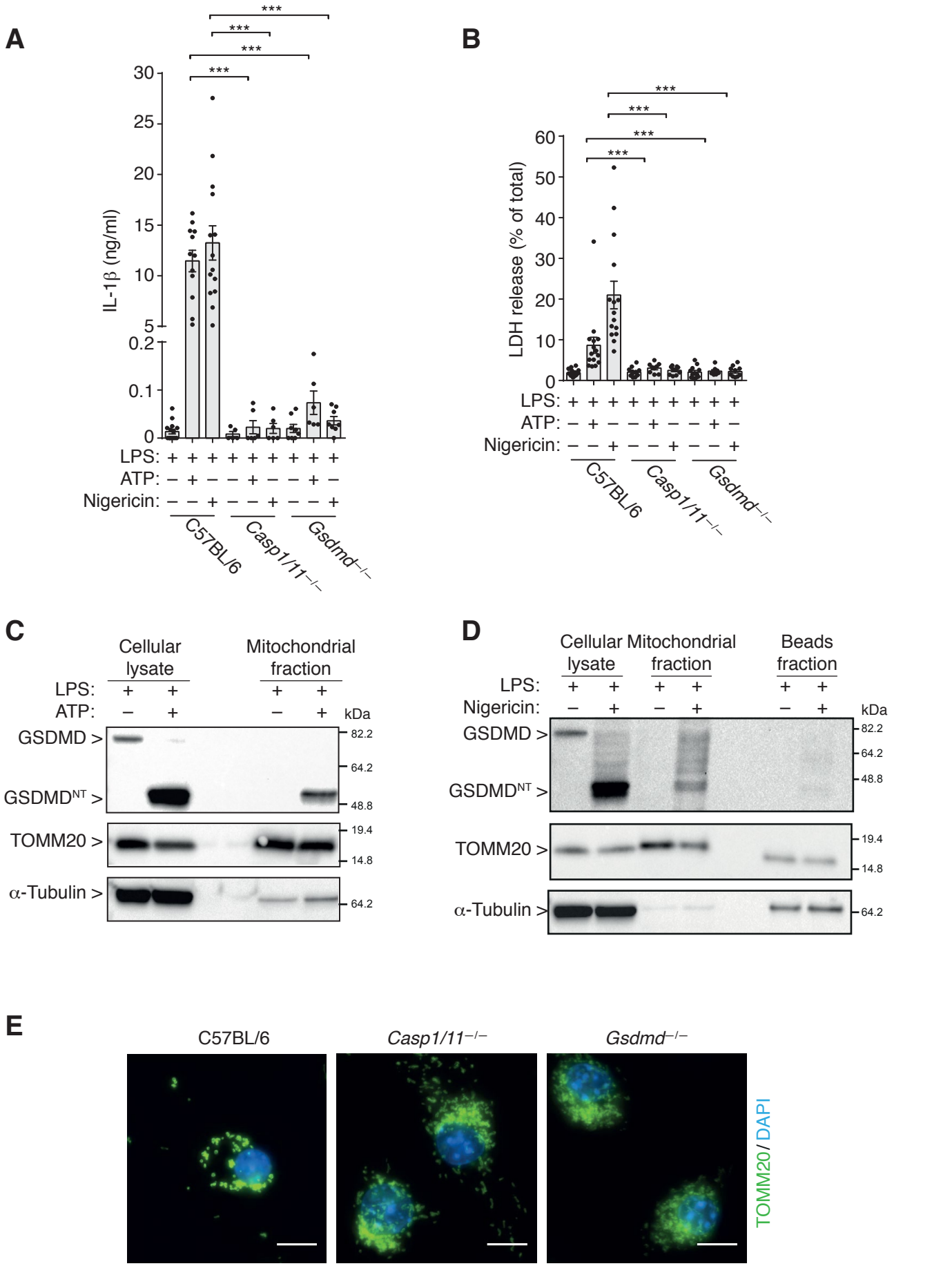
Supplementary Figure S10. Uncropped immunoblots of Figure 5C

Supplementary Figure S11. Uncropped immunoblots of Figure 6A

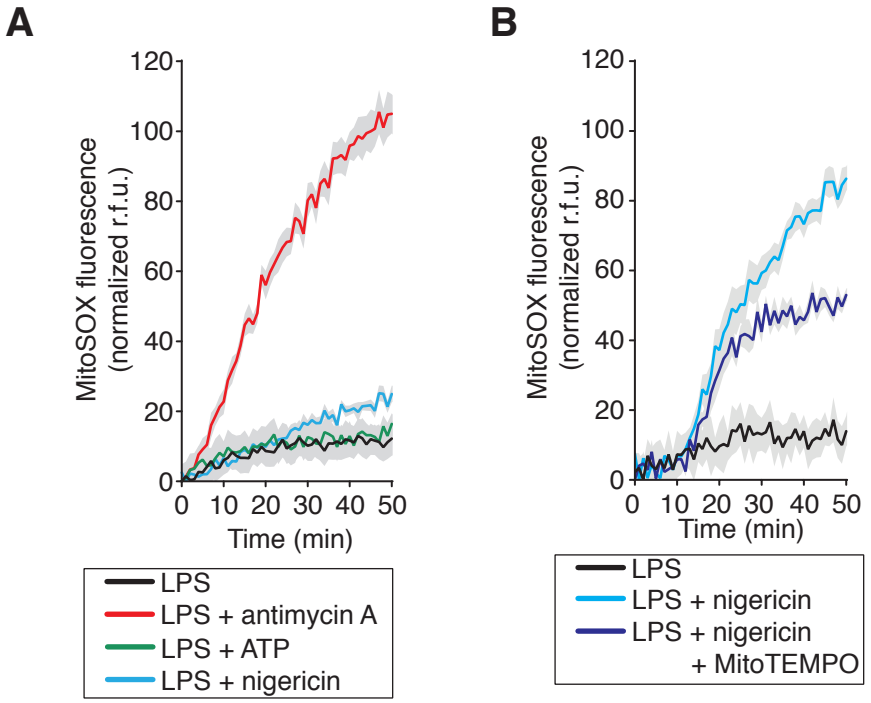
Supplementary Figure S12. Uncropped immunoblots of Supplementary Figure S1C and S1D

Supplementary Figure S13. Uncropped immunoblots of Supplementary Figure S3A and S3B

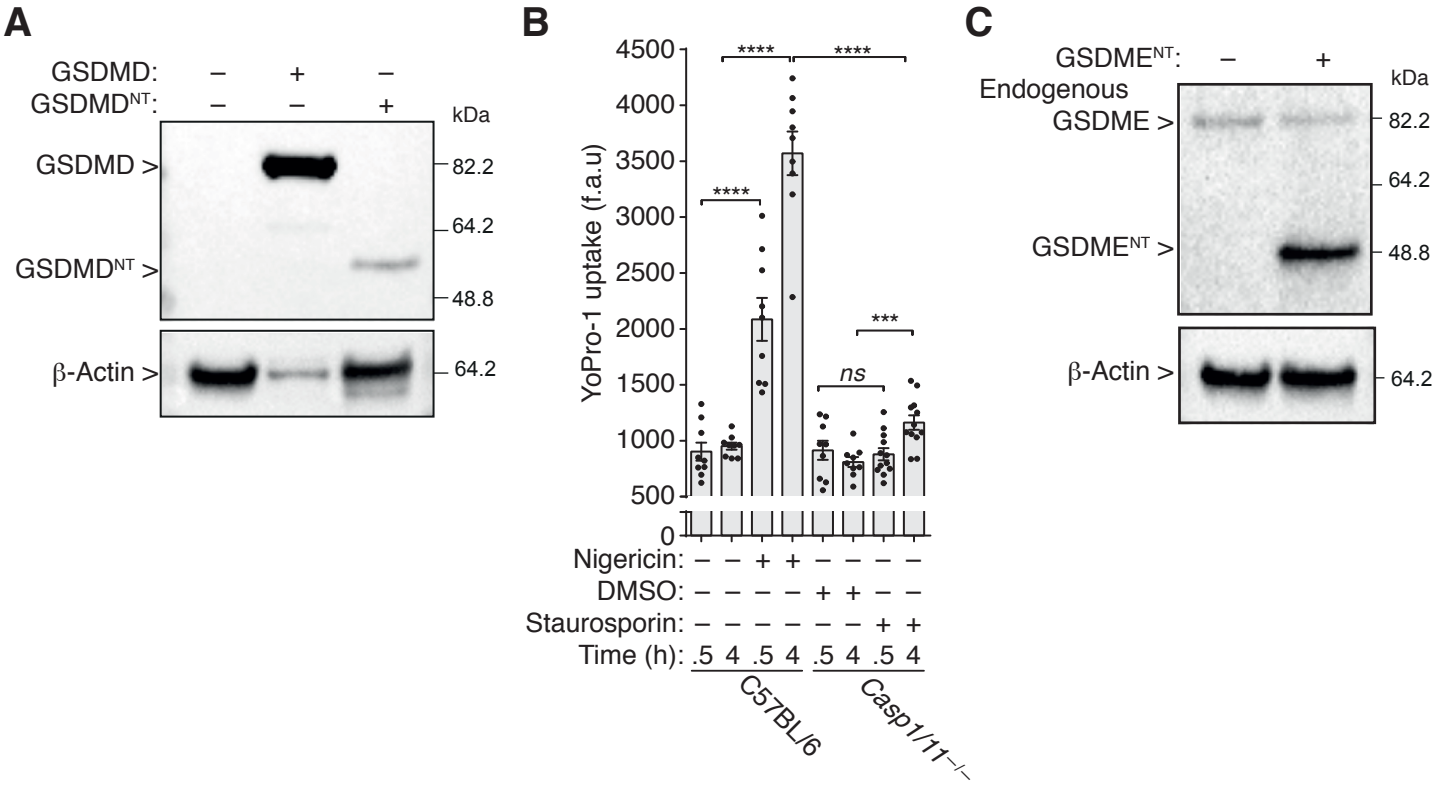
Supplementary Figure S1



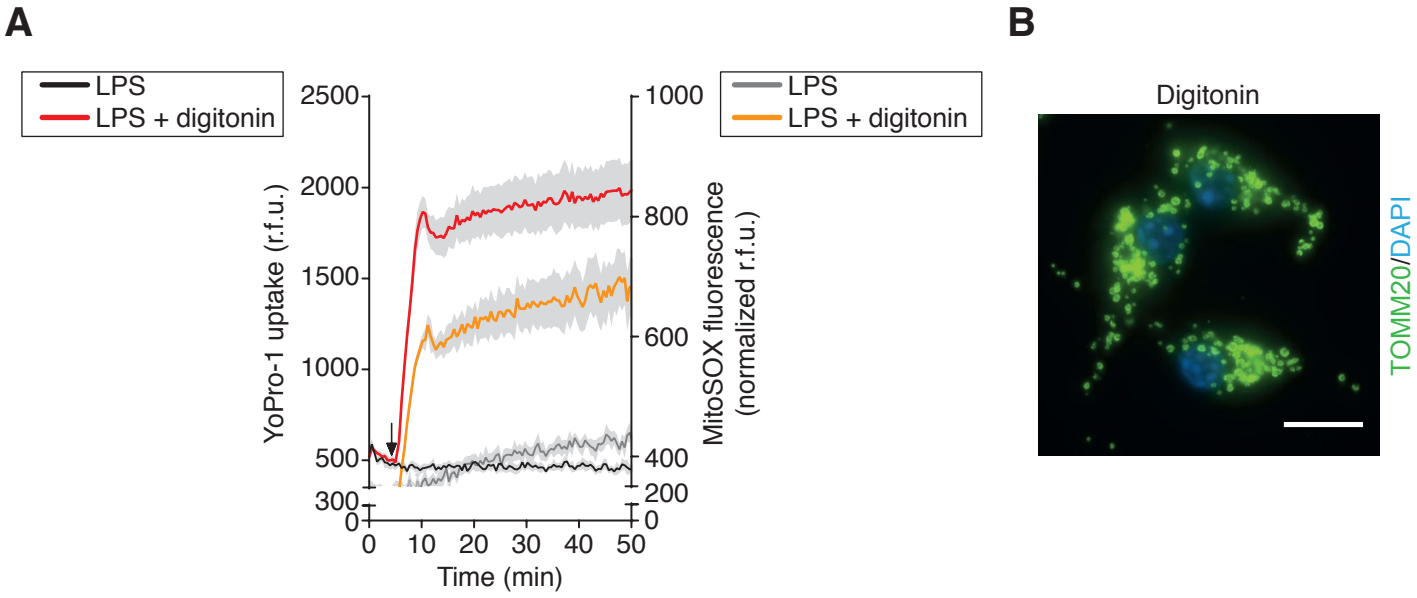
Supplementary Figure S2



Supplementary Figure S3



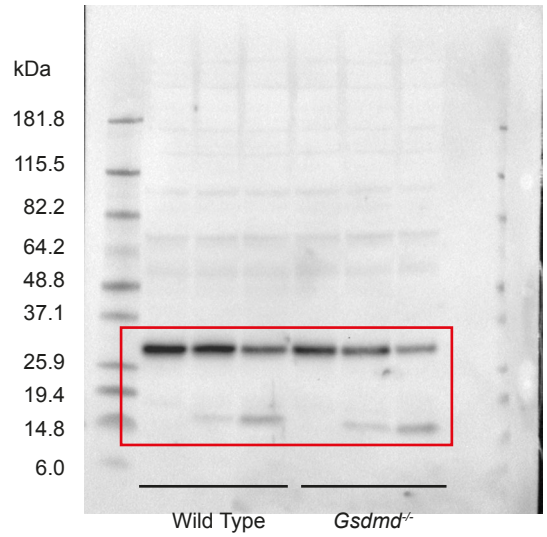
Supplementary Figure S4



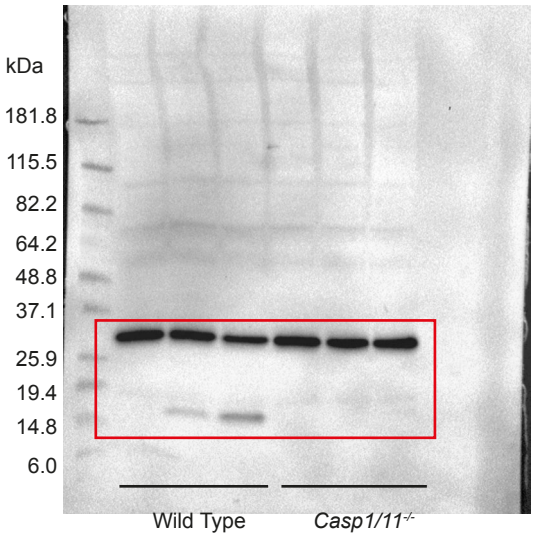
Supplementary Figure S5

Uncropped membranes Fig 1A
Membranes were probed consecutively with stripping between Bid and Gsdmd or Casp1.

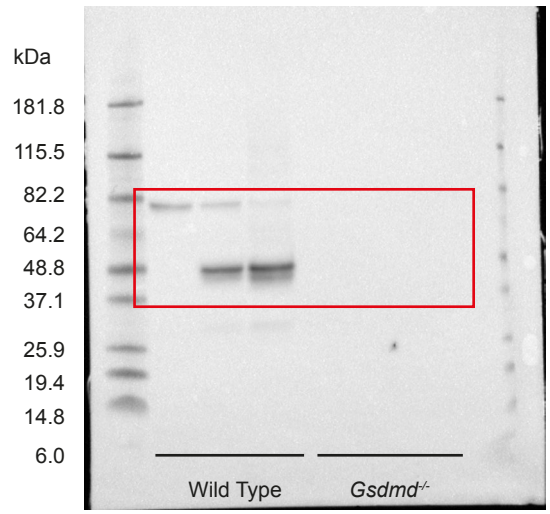
1st



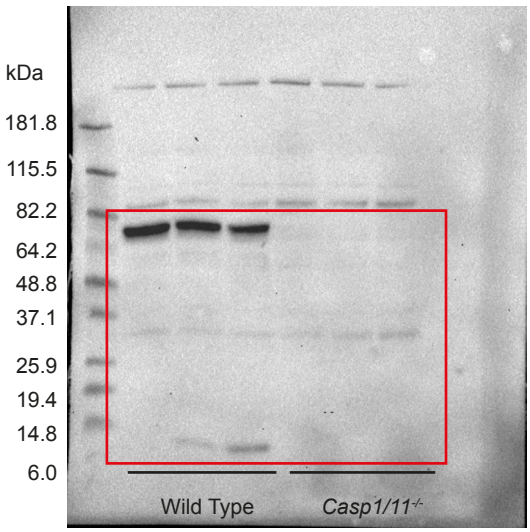
Anti-BID antibody (#91508)
MAB860
monoclonal rat



2nd

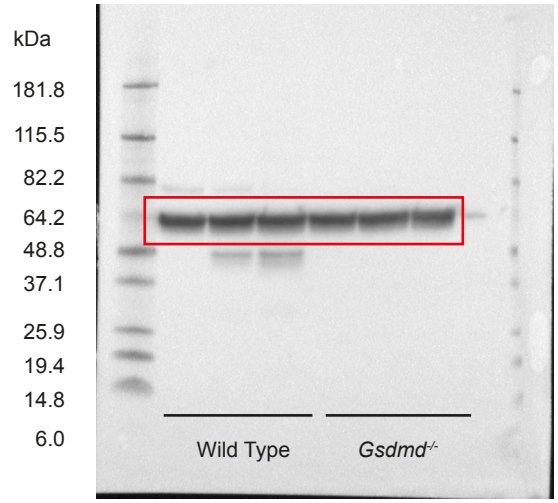


Anti-Gsdmd antibody
[EPR19828] ab209845
monoclonal rabbit

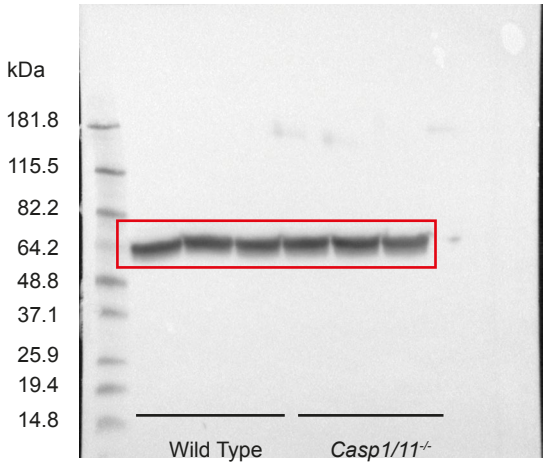


Anti-caspase-1 p10 (M-20):
sc-514
polyclonal rabbit

3rd



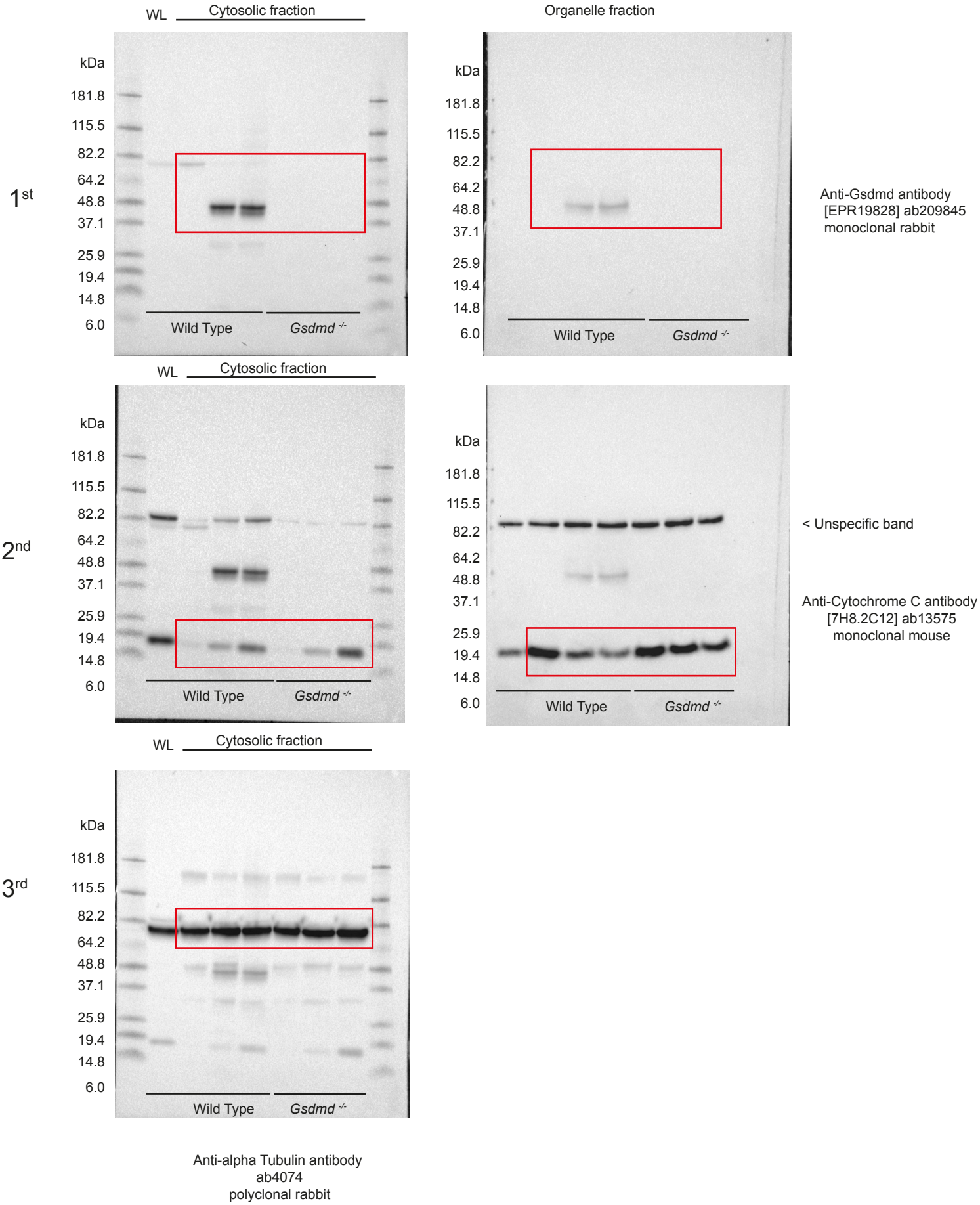
Anti-β-Actin antibody
sc-47778



Supplementary Figure S6

Uncropped membranes Fig 1B *Gsdmd*$^{-/-}$

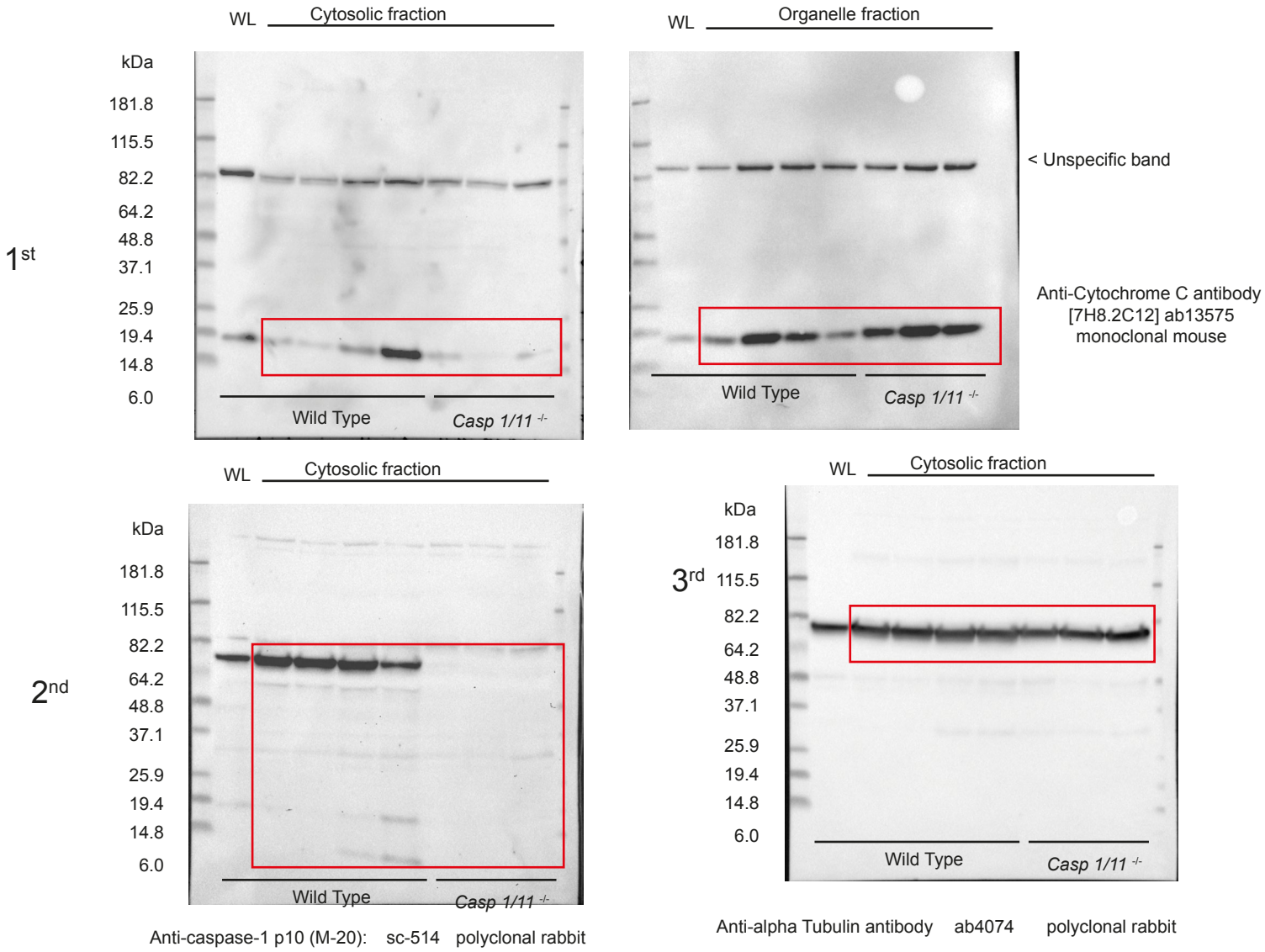
Membranes were probed consecutively without stripping



Supplementary Figure S7

Uncropped membranes Fig 1B *Casp1/11*$^{-/-}$

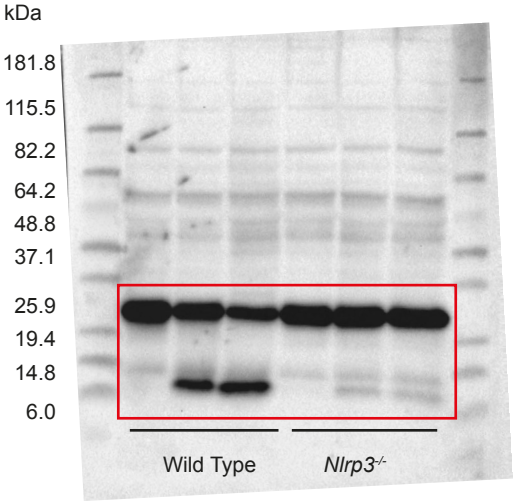
Membranes were probed consecutively without stripping



Supplementary Figure S8

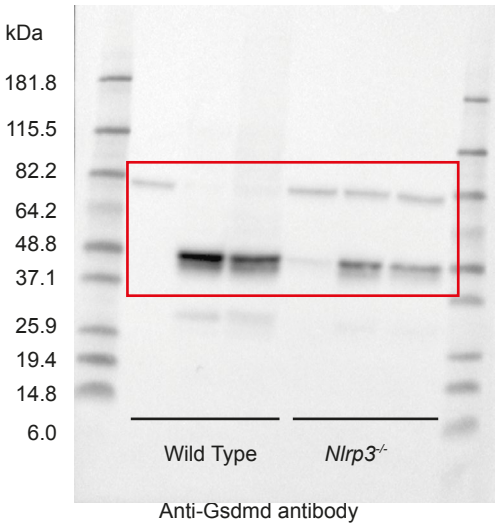
Uncropped membranes Fig 3A
Membranes were probed consecutively with stripping between Bid and Gsdmd

1st



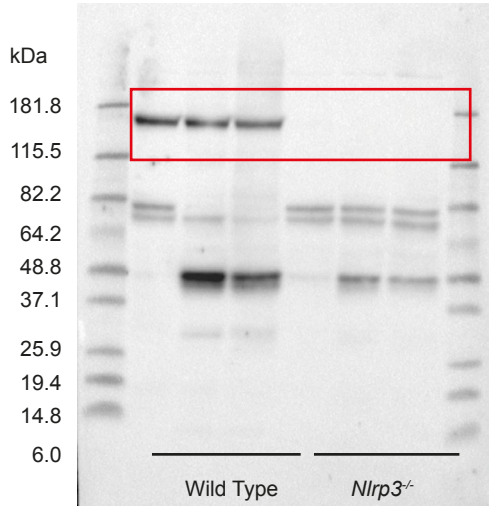
Anti-BID antibody (#91508)
MAB860
monoclonal rat

2nd



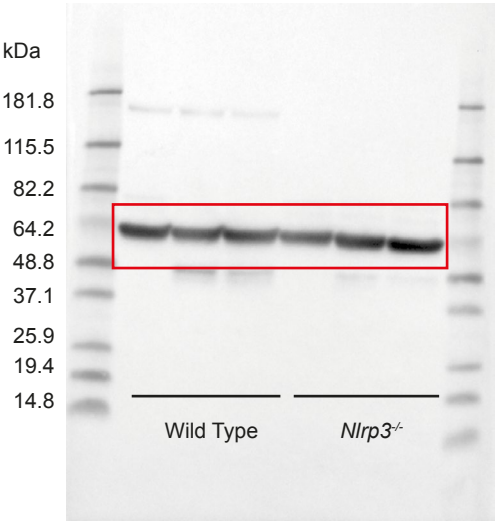
Anti-Gsdmd antibody
[EPR19828] ab209845
monoclonal rabbit

3rd



Anti-NLRP3/NALP3 antibody
[Cryo-2] AG-20B-0014
monoclonal mouse

4th



Anti-β-Actin antibody
sc-47778
monoclonal mouse

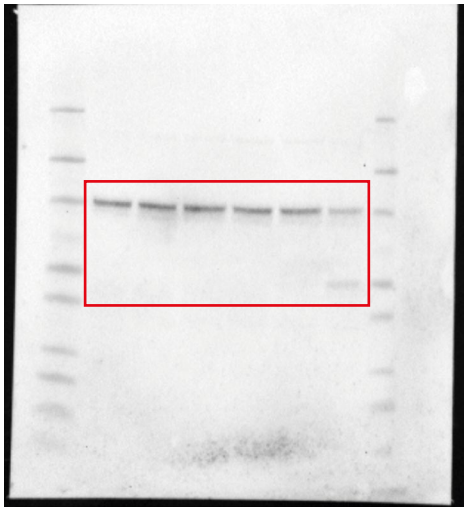
Supplementary Figure S9

Uncropped membranes Fig 4A
Membranes were probed consecutively .

1st

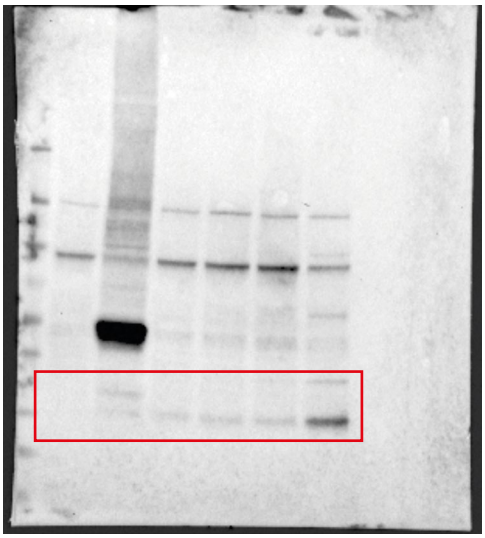
Anti-Gsdmd antibody
[EPR19828] ab209845
monoclonal rabbit

1st



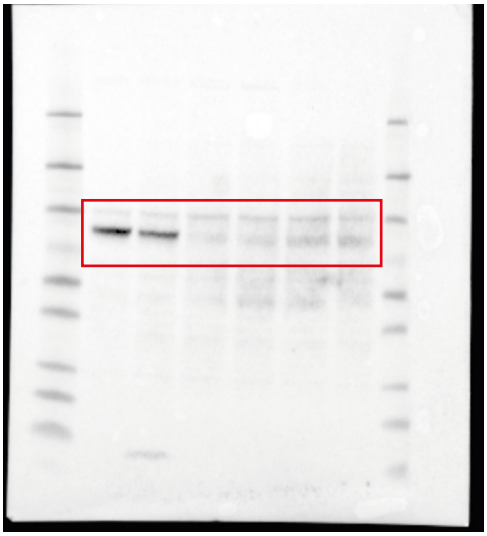
Anti-GsdmE antibody
[EPR19859] ab215191
monoclonal rabbit

2nd



Anti-cleaved Caspase-3
rabbit polyclonal
(#9661,Cell signalling)

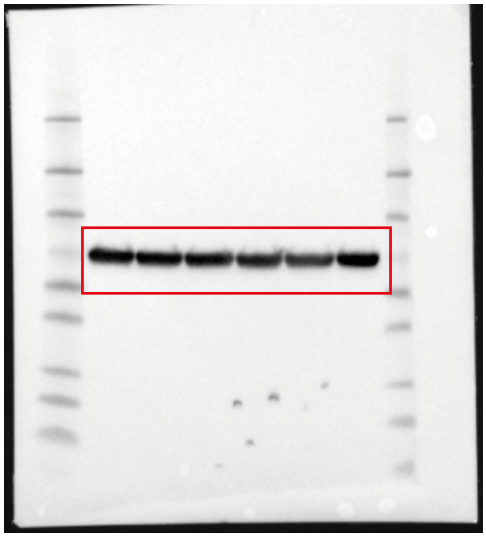
2nd



Anti-caspase-1 p10 (M-20):
sc-514
polyclonal rabbit

3rd

Anti-β-Actin antibody
sc-47778

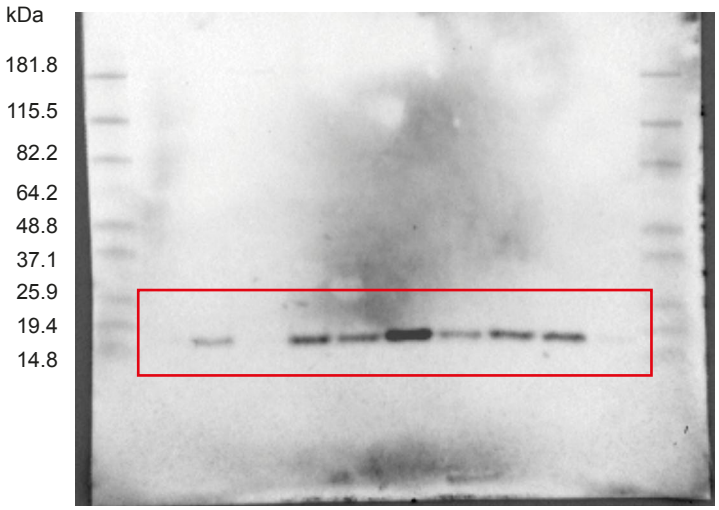


Anti-β-Actin antibody
sc-47778

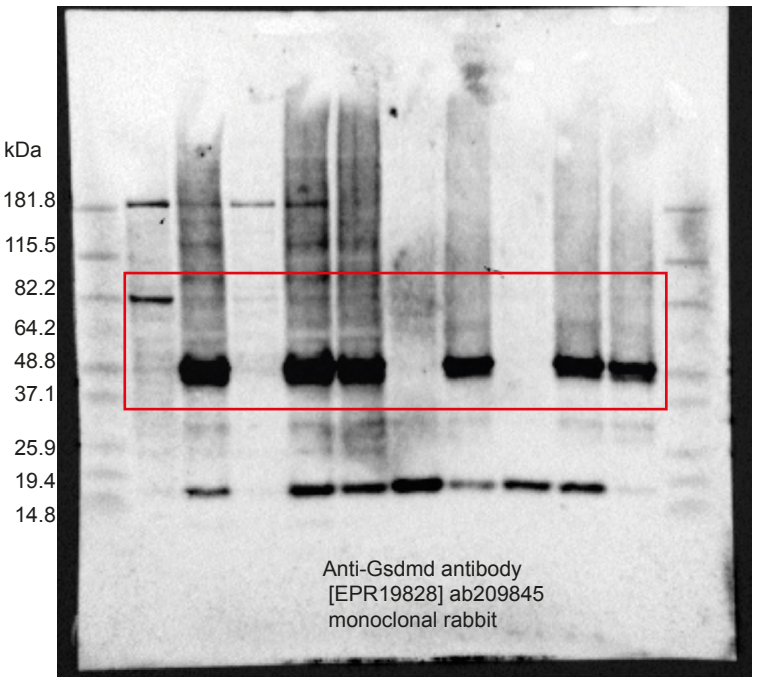
Supplementary Figure S10

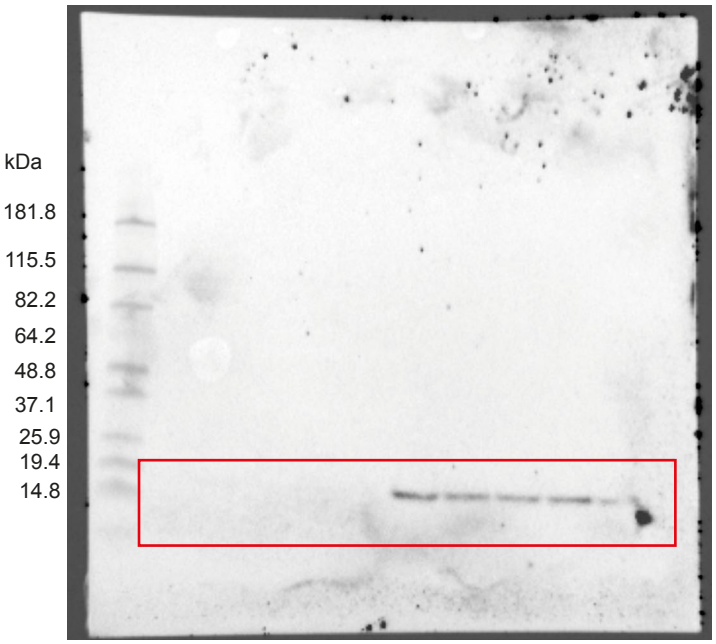
Uncropped membranes Fig 5C

Membranes were probed consecutively .

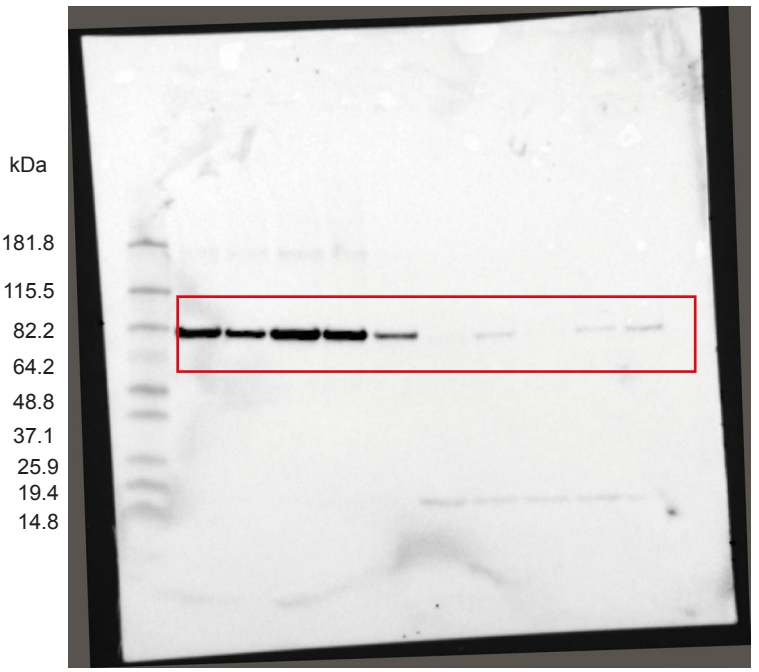


Anti-Cytochrome C antibody
[7H8.2C12] ab13575
monoclonal mouse





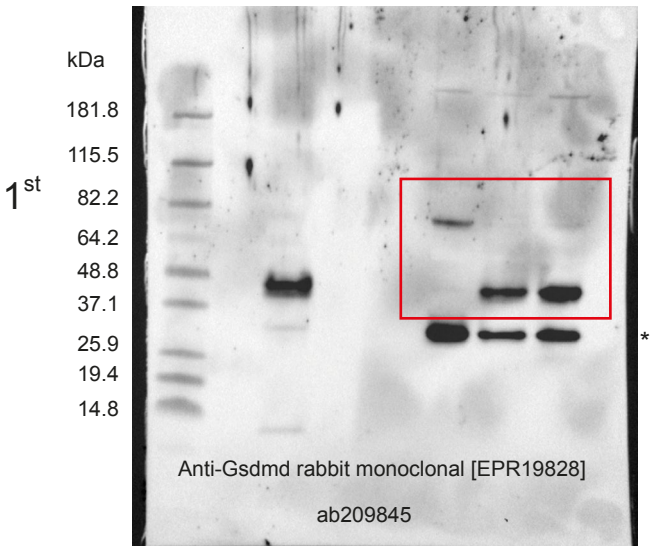
Anti-TOMM20 antibody
EPR15581-54 ab4074
monoclonal rabbit



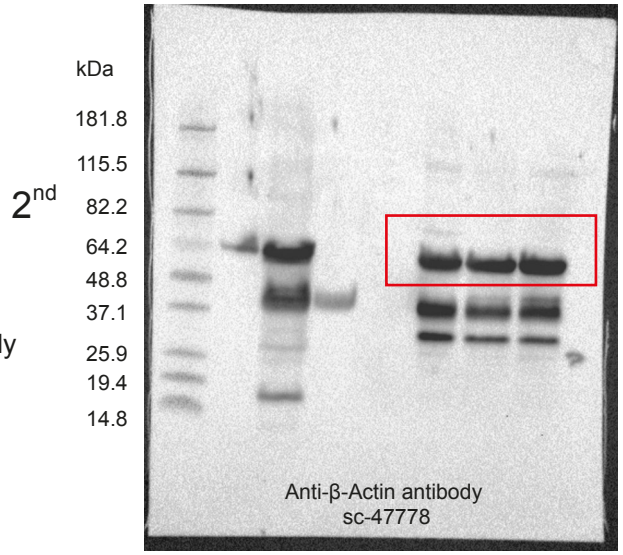
Anti-alpha Tubulin antibody
ab4074
polyclonal rabbit

Supplementary Figure S11

Uncropped membranes Fig 6A
Membranes were probed consecutively without stripping .

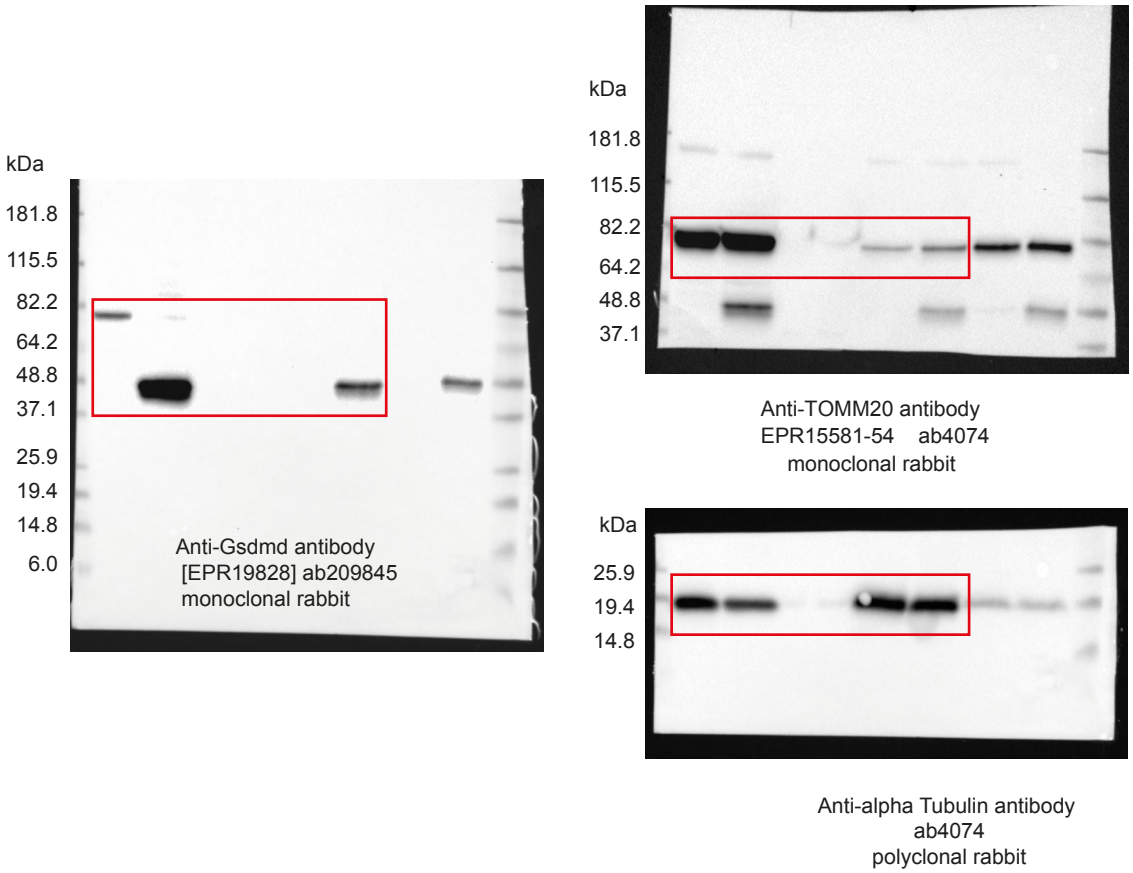


* other antibody used before



Supplementary Figure S12

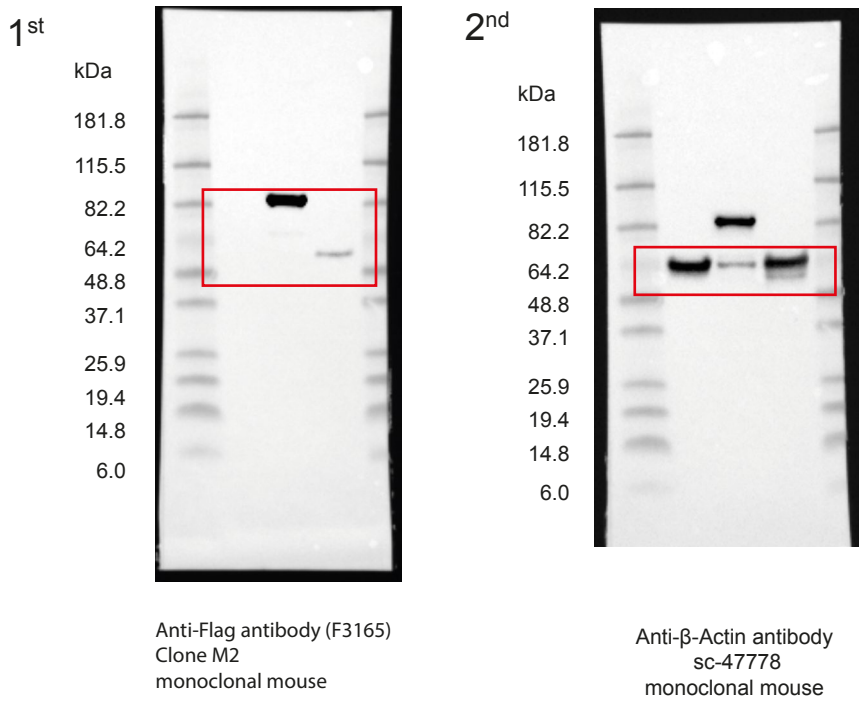
Uncropped membranes Supplementary Fig S1C



Uncropped membranes Supplementary Fig S1D

Supplementary Figure S13

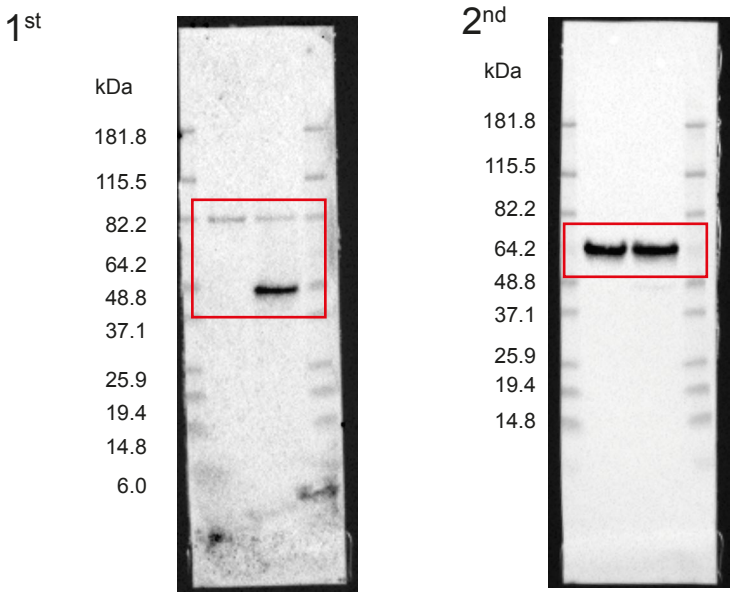
Uncropped membranes Supplementary Fig S3A
Membranes were probed consecutively without stripping



Anti-Flag antibody (F3165)
Clone M2
monoclonal mouse

Anti-β-Actin antibody
sc-47778
monoclonal mouse

Uncropped membranes Supplemnentary Fig S3B



Anti-GsdmE antibody
[EPR19859] ab215191
monoclonal rabbit

Anti-β-Actin antibody
sc-47778
monoclonal mouse