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Supplemental Information

A Rapid Caspase-11 Response Induced by IFNγ Priming Is Independent of Guanylate Binding Proteins

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Figure S1

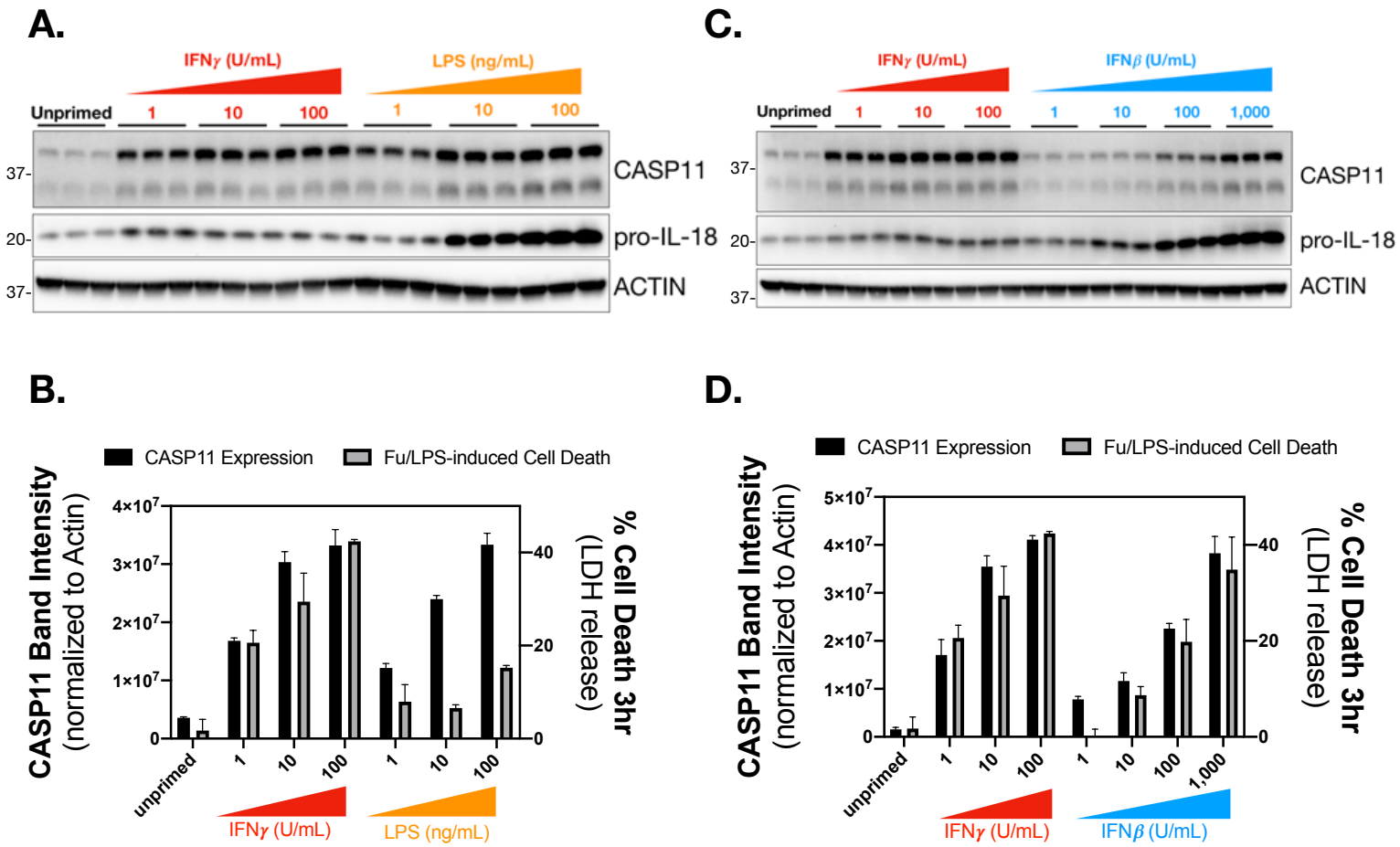

Figure S1. IFNγ is a More Potent Priming Agent for CASP11-dependent inflammasome activation. Related to Figure 1

WT BMDMs were primed as indicated for 16hrs overnight in technical triplicate and cell lysates were collected to determine the expression levels of CASP11 and pro-IL-18 by Western blot (A,C). In parallel, WT BMDMs were primed as indicated for CASP11 activation by LPS (*E. coli* 0111:B4, 25ug/mL) transfection with FugeneHD (Fu/LPS). At 3hrs following inflammasome activation, supernatants were collected to measure release of LDH for percent cell death calculations. Quantitations of CASP11 expression (from A,C) are compared against Fu/LPS-induced cell death (B,D). Bar graphs in black correspond to the left axis, which represents CASP11 expression as band intensity normalized to ACTIN. Percent cell death at 3 hours following LPS transfection is plotted in grey and corresponds to the right axis. Bar graphs depict the mean +/- SD using triplicates for each condition. Molecular weight marker positions are shown to the left of each blot. Data are representative of 4 (A,B) or 3 (C,D) independent experiments.

Figure S2

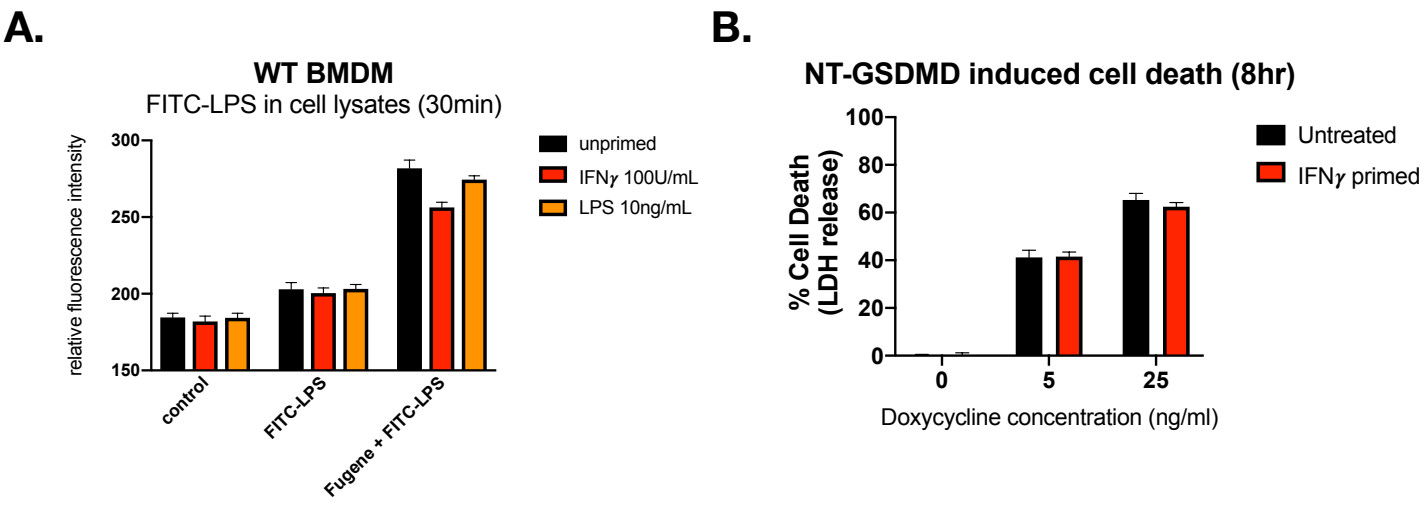

Figure S2. IFNγ Priming Does not Promote Transfection-dependent Delivery of LPS or NT-GSDMD Pore Formation. Related to Figure 2

(A) Transfection-dependent delivery of LPS was determined using a FITC-labelled LPS conjugate. WT BMDMs were primed for 16hrs overnight with the following treatments; unprimed (N/A), IFNγ (100U/mL), or LPS (10ng/mL). The BMDMs were then transfected with a FITC-conjugated LPS (*E. coli* 0111:B4, 25ug/mL) using FugeneHD. Following transfection, cell lysates were collected, and the relative fluorescence intensity of lysates was measured. (B) A Doxycycline-inducible NT-GSDMD cell line was generated in *Gsdmd*-CRISPR/Cas9 KO RAW cells. Prior to Dox-induced expression of NT-GSDMD, cells were treated with or without IFNγ (100U/mL) for 16hrs. Following Dox-induced expression of NT-GSDMD, cell death was determined by measuring release of LDH in the supernatant. Bar graphs show the mean value +/- SD of triplicates (A,B). Data are representative of 2 independent experiments (A,B).

Figure S3

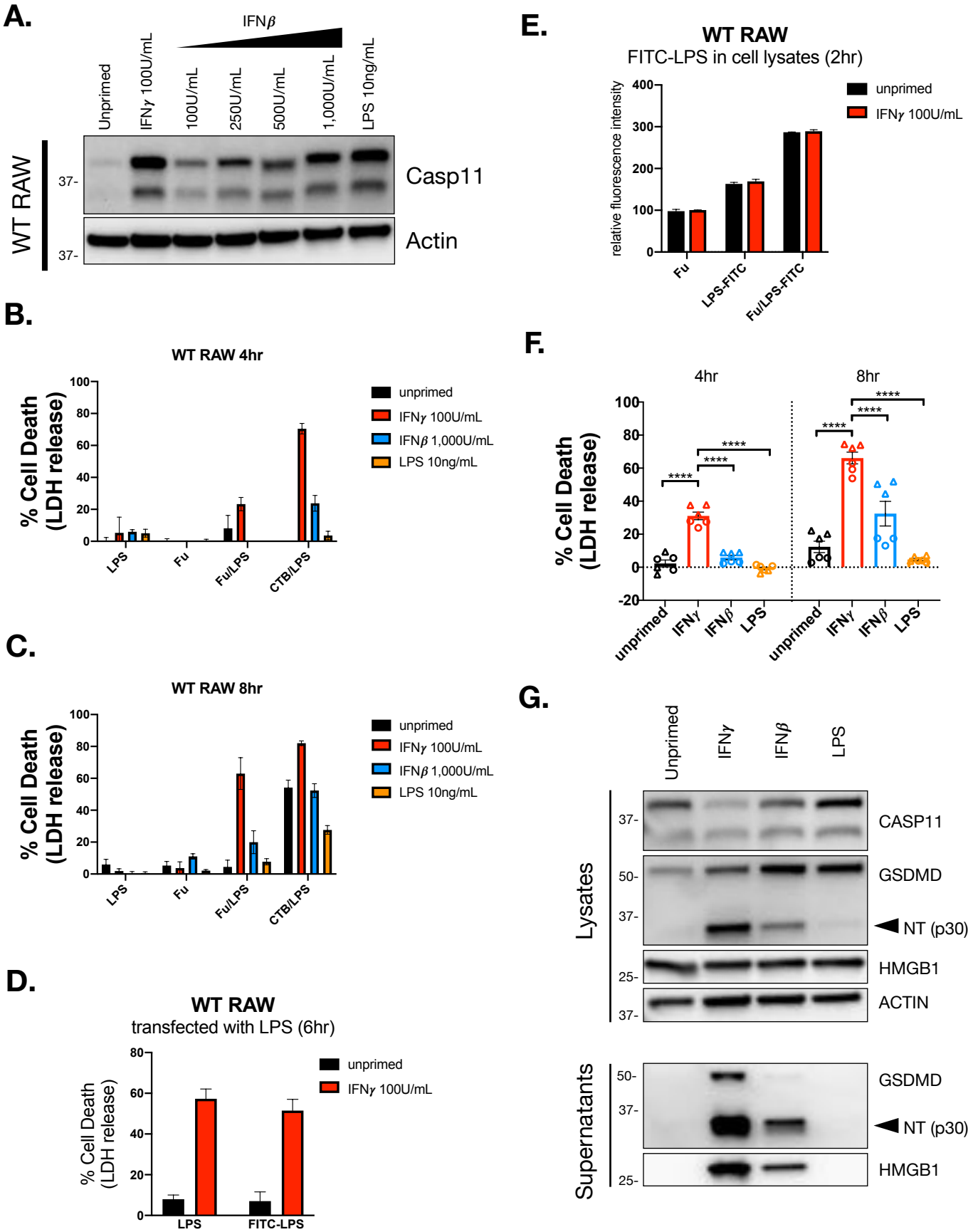
A.
IFNβ
Unprimed
IFNγ 100U/mL
100U/mL
250U/mL
500U/mL
1,000U/mL
LPS 10ng/mL
WT RAW
37-
37-
Casp11
Actin
B.
WT RAW 4hr
% Cell Death (LDH release)
unprimed
IFNγ 100U/mL
IFNβ 1,000U/mL
LPS 10ng/mL
LPS
Fu
Fu/LPS
CTB/LPS
C.
WT RAW 8hr
% Cell Death (LDH release)
unprimed
IFNγ 100U/mL
IFNβ 1,000U/mL
LPS 10ng/mL
LPS
Fu
Fu/LPS
CTB/LPS
D.
WT RAW
transfected with LPS (6hr)
% Cell Death (LDH release)
unprimed
IFNγ 100U/mL
LPS
FITC-LPS
E.
WT RAW
FITC-LPS in cell lysates (2hr)
relative fluorescence intensity
unprimed
IFNγ 100U/mL
Fu
LPS-FITC
Fu/LPS-FITC
F.
4hr
8hr
% Cell Death (LDH release)
****
unprimed
IFNγ
IFNβ
LPS
G.
Unprimed
IFNγ
IFNβ
LPS
Lysates
37-
50-
37-
25-
37-
CASP11
GSDMD
NT (p30)
HMGB1
ACTIN
Supernatants
50-
37-
25-
GSDMD
NT (p30)
HMGB1

Figure S3. IFNγ Priming Enhances CASP11-dependent Cell Death in RAW 264.7 Independently of CASP11 Expression. Related to Figure 3

(A) WT RAW 264.7 cells were left unprimed or treated with IFNγ, IFNβ, or LPS at the indicated concentrations for 16hrs and cell lysates were collected to determine the expression of CASP11 by Western blot. (B-C) WT RAW 264.7 cells were primed for 16hrs overnight with the following treatments: unprimed (N/A), IFNγ (100U/mL), IFNβ (1,000U/mL), or LPS (10ng/mL). Cells were treated with LPS (*E. coli* 0111:B4, 25ug/mL), FugeneHD (0.5%), LPS and FugeneHD, or LPS with CTB (Cholera Toxin B Subunit, 20ug/mL). At 4hrs (B) and 8hrs (C) following inflammasome activation, supernatants were collected to measure release of LDH for percent cell death calculations. (D) WT RAW 264.7 cells were left unprimed or treated with IFNγ (100U/mL) for 16hrs and CASP11 inflammasome activation was triggered by FITC-conjugated or standard LPS (*E. coli* 0111:B4, 25ug/mL) transfection using FugeneHD. Supernatants were collected to measure release of LDH for percent cell death calculations. (E) Transfection-dependent delivery of LPS was determined using the FITC-labeled LPS conjugate. WT RAW 264.7 cells were primed with or without IFNγ (100U/mL) for 16hrs and FITC-LPS was transfected with FugeneHD. Following transfection, cell lysates were collected, and the relative fluorescence intensity of lysates was measured. (F-G) The constitutive CASP11-expressing cell line was primed for 16hrs overnight with the following treatments: unprimed (N/A), IFNγ (100U/mL), IFNβ (1,000U/mL), or LPS (10ng/mL). CASP11 inflammasome activation was triggered with a mixture of LPS (*E. coli* 0111:B4, 50ug/mL) and CTB (20ug/mL). (F) At 4 and 8hrs following inflammasome activation, supernatants were collected to measure release of LDH for percent cell death calculations. (G) Alternatively, supernatants and lysates were collected 6hrs post-transfection to monitor for the cleavage and release of inflammasome-related proteins by SDS-PAGE and Western blot. Molecular weight marker positions are shown to the left of each blot, and arrows indicate a cleavage product. Bar graphs (B-E) show the mean value +/- SD of technical triplicates and are representative of at least 2 independent experiments. Bar graphs (F) show the mean value +/- SEM along with individual data points pooled from 2 independent experiments depicted with different shapes. Western blots (A,G) are representative of 2 independent experiments. Statistical analysis performed using a Two-way ANOVA and Tukey's multiple comparisons test; **** < 0.0001

Figure S4

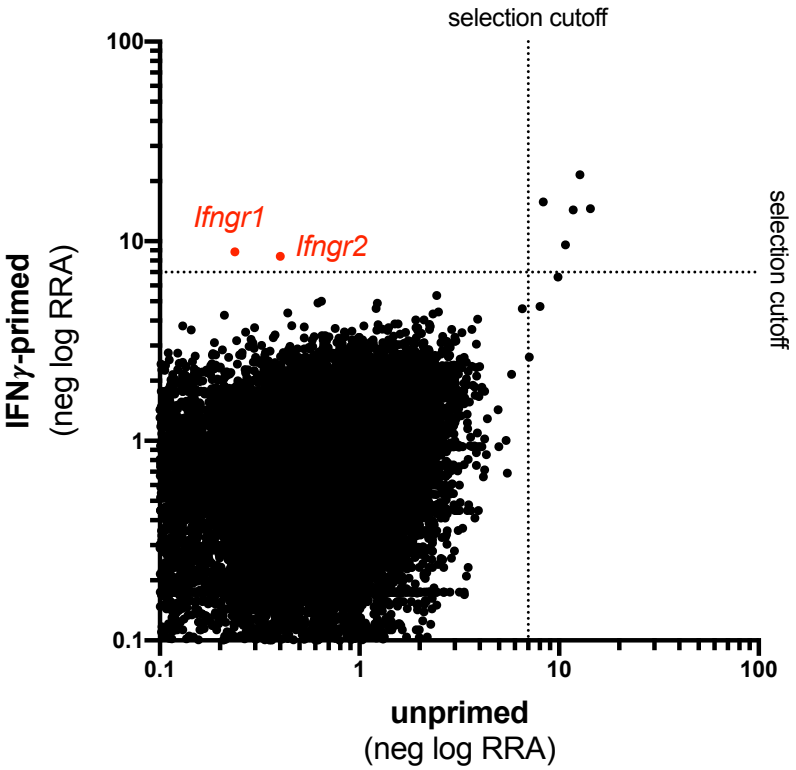

Figure S4. Genome-wide CRISPR KO Survival Screen Identifies Genes Encoding IFNγ Receptor. Related to Figure 4

We generated a genome-wide CRISPR knockout library in the constitutive CASP11 expressing cells (described in Figure 3) and selected for surviving cell populations following treatment with or without IFNγ priming and transfected with LPS. Each axis depicts the enrichment score for gRNAs present in the surviving population calculated using MAGeCK and an arbitrary selection cutoff was made at a negative log RRA enrichment score of 7. Cell populations with gRNAs targeting *Ifngr1* and *Ifngr2* (highlighted in red) were only selected following Casp11 activation with IFNγ priming (y-axis) compared with an unprimed selection (x-axis).

Figure S5

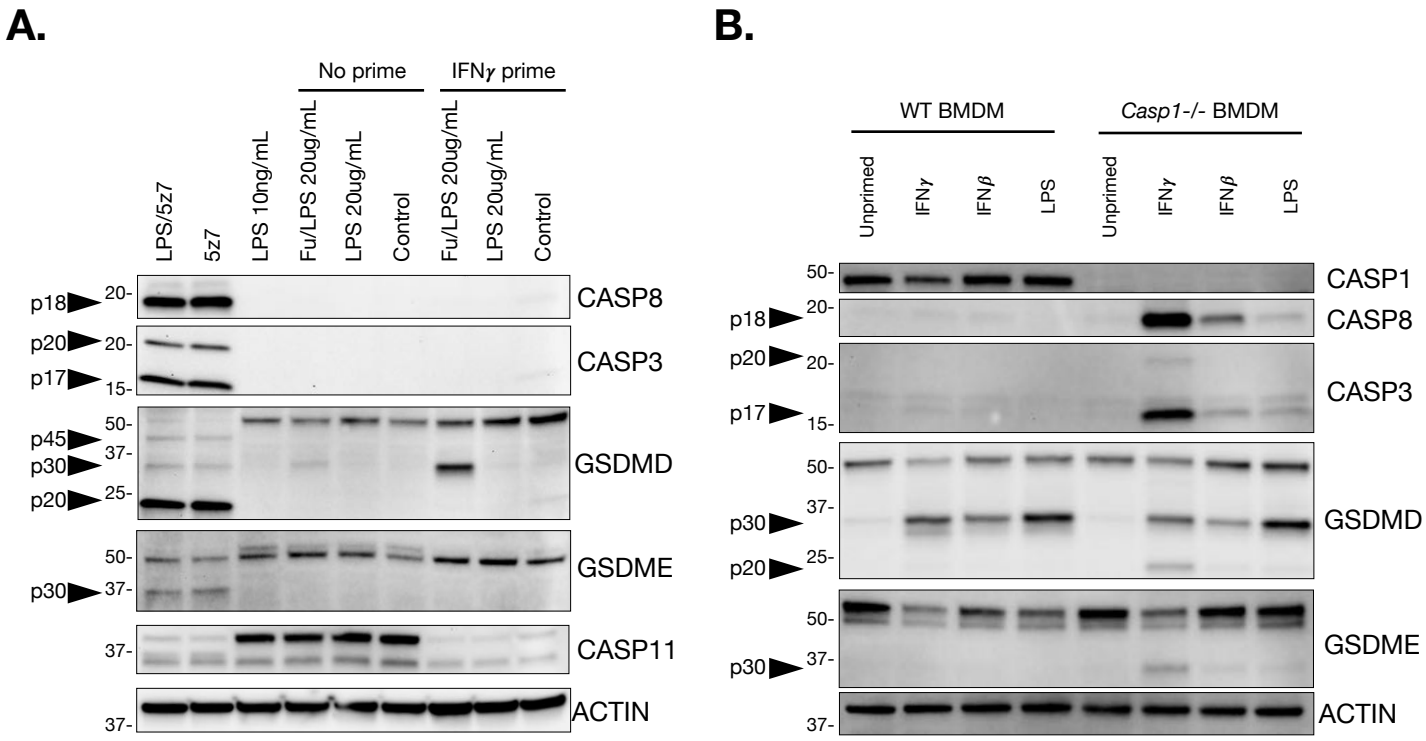

Figure S5. IFNγ Enhancement of CASP11-dependent Cell Death is not Associated with GSDME Cleavage. Related to Figure 5

(A) Constitutive CASP11-expressing RAW cells (see Figure 3) were primed +/- IFNγ for 16hrs and CASP11 activation was triggered by LPS (*E. coli* 0111:B4, 25ug/mL) transfection with FugeneHD. Following transfection (7hrs) cell lysates and supernatants were collected and pooled to determine Caspase and Gasdermin cleavage by Western blot. As a positive control for CASP8, CASP3, and GSDME cleavage, cells were treated with the TAK1 inhibitor 5z7. (B) WT and *Casp1-/-* BMDMs were treated with the indicated priming agents for 16hrs and transfected with LPS (*E. coli* 0111:B4, 25ug/mL). Following transfection, cell lysates were collected at 3hrs and analyzed by Western blot to determine Caspase and Gasdermin cleavage. Molecular weight marker positions are shown to the left of each blot, and arrows indicate a cleavage product. Data represent results from single experiments (A-B).

Figure S6

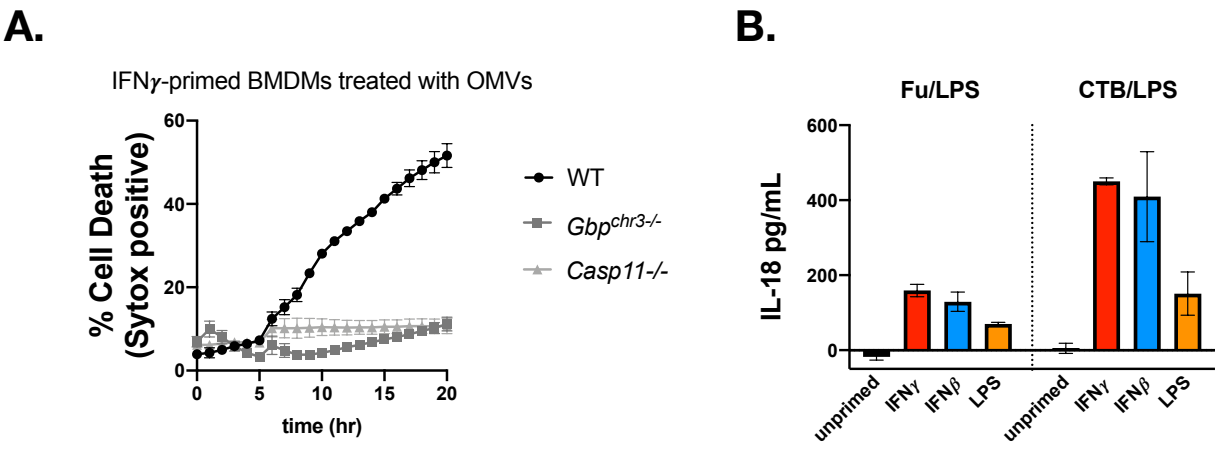

Figure S6. IFN-Inducible GBPs do not Fully Account for Enhanced CASP11-dependent Cell Death Triggered by IFNγ Priming. Related to Figure 6

(A) WT, *Gbp*$^{chr3-/-}$, and *Casp11-/-* BMDMs were primed for 16hrs overnight with IFNγ (100U/mL) and CASP11 inflammasome activation was triggered by treating cells with OMVs (*E. coli* DH5α). Cell death kinetics were monitored over time by measuring the incorporation of SYTOX Green. (B) *Gbp*$^{chr3-/-}$ BMDMs were primed for 16hrs overnight with the following treatments: unprimed (N/A), IFNγ (100U/mL), IFNβ (100U/mL), LPS (10ng/mL). CASP11 inflammasome activation was triggered by LPS (*E. coli* 0111:B4, 25ug/mL) transfection with FugeneHD, or LPS (*E. coli* 0111:B4, 25ug/mL) mixed with CTB (Cholera Toxin B Subunit, 20ug/mL). At 3hrs following inflammasome activation, supernatants were collected to measure release of IL-18. Line graph shows the mean value +/- SEM pooled from 2 independent experiments with technical replicates (A). Bar graph shows the mean value +/- SD of samples in triplicate from one experiment (B).

Transparent Methods:

Experimental Models

Cell lines and primary cell cultures:

Primary BMDMs were differentiated from flushed femurs as described previously (Broz and Monack 2013). All animal care and the bone marrow isolation procedures used in this study for differentiating BMDMs were approved and are in accordance with the guidelines set out by Stanford University's Administrative Panel on Laboratory Animal Care (APLAC). Briefly 6-14 week old mice were euthanized and following bone marrow, isolation cells were cultured in differentiation media: Dulbecco's Modified Eagle Media (DMEM) containing L-Glutamine 4mM, D-Glucose 25mM, Sodium Pyruvate 1mM (ThermoFisher catalogue #11995073) supplemented with 10% Fetal Bovine Serum (FBS) and 20% conditioned media from MCSF-producing 3T3 cells (provided by Anita Sil UCSF). Differentiation was conducted over 6 days in non-"TC treated" culture plates, BMDMs were lifted by gentle scraping in ice-cold PBS, and frozen down in cryovials at $1x10^7$ cells/mL in FBS containing 10% DMSO. MCSF conditioned media from 3T3 cells was generated by splitting a confluent flask of MCSF-producing 3T3 cells 1:10 in DMEM containing L-Glutamine 4mM, D-Glucose 25mM, Sodium Pyruvate 1mM (ThermoFisher catalogue #11995073) supplemented with 10% FBS. Supernatants were collected on day four and the media was replenished for a second collection on day seven. MCSF conditioned media from day four and seven were pooled, filter sterilized (0.2 μm filter flask), and stored at -80°C. Both male and female mice were used for BMDM differentiation. We have not observed inflammasome activation differences *in vitro*

between BMDMs isolated from male or female mice. WT C57BL/6NJ (Stock No. 005304) and *Ifngr1-/-* (Stock No. 003288) mice were acquired from Jackson Labs, femurs and/or bone marrow from knockout mice were provided by Vishva Dixit (*Casp11-/-, Casp1-/-, Gsdmd-/-, and Gsdme-/-*), Petr Broz and Igor Brodsky ($Gbp^{chr3-/-}$).

RAW 264.7 macrophages are an Abelson murine leukemia virus transformed cell line derived from male mice and were obtained from ATCC (TIB-71). RAW cells were cultured in DMEM containing L-Glutamine 4mM, D-Glucose 25mM, Sodium Pyruvate 1mM (ThermoFisher catalogue #11995073) supplemented with 10% FBS.

Method Details:

Macrophage treatments:

Macrophages were seeded into 96-well (40,000 cells/well) or 6-well ($1x10^6$ cells/well) plates with macrophage media: DMEM, containing L-Glutamine 4mM, D-Glucose 25mM, Sodium Pyruvate 1mM (ThermoFisher catalogue #11995073) supplemented with 10% Fetal Bovine Serum (FBS) and 10% conditioned media from MCSF-producing 3T3 cells (see above). Once macrophages adhered to the plate, cells were primed (16hr) with the indicated priming agents by adding a 1:1 volume of a 2x working stock diluted in bone marrow macrophage media. In our hands, transfection efficiency is improved with the use of Opti-MEM media and LDH assays are more robust with media that does not contain Phenol red. Therefore, all inflammasome activation assays were carried out in Phenol red-free Opti-MEM (Gibco #11058021 and referred to as Opti-MEM throughout) to match the optimized conditions for LPS

transfection. Prior to transfection (LPS) or treatment (Nigericin, ATP, CTB/LPS, OMV), macrophage media in the plate was removed and replaced with Opti-MEM. For LPS transfection in BMDMs and WT RAW 264.7 macrophages, LPS (*E. coli* 0111:B4; Invivogen LPS-EB VacciGrade Cat #vac-3pelps; 20-25ug/mL final concentration) and FuGENE HD (0.5-0.6% final concentration) were complexed in Opti-MEM by briefly vortexing and allowing the solution to incubate for 15-30min at room temperature. The LPS/FuGENE HD complex was then overlayed in each well containing Opti-MEM. For transfection in *Casp1*,*Casp11*-DKO *+Casp11* RAW cells, the same amounts of LPS and FuGENE HD were used, however the final volume of Opti-MEM was reduced by half to increase transfection efficiency. For NLRP3 inflammasome activation, ATP (5mM final concentration) or Nigericin (10uM final concentration) were prepared in Opti-MEM and added to macrophages in Opti-MEM. For CTB/LPS treatments, LPS (20-25ug/mL final concentration) and CTB (List Biological Laboratories #104; 20ug/mL final concentration) were mixed in Opti-MEM by pipetting up and down and allowed to incubate for 15-30min at room temperature. The CTB/LPS complex was then overlayed on macrophages in wells containing Opti-MEM. For OMV treatments, *E. coli* derived OMVs (10uL/well 96well) resuspended in PBS were added to BMDMs in Opti-MEM (see more on OMV isolation below). As a positive control to activate CASP8/CASP3/Gsdme, cells were treated with the TAK1 inhibitor (5z7) as described previously (Sarhan et al). Briefly, 5z7 (125nM) with or without LPS (10ng/mL) in serum-free media was added to cells for 5hr. Proteins in the supernatant were precipitated with TCA and combined with cell lysates to determine CASP8/CASP3/Gsdme activation by Western blot(Broz and

Monack, 2013). To quantify the amount of LPS delivered to macrophages, $1x10^6$ cells were transfected with a FITC-labelled LPS conjugate following the procedure described above. At the timepoint indicated, the media in each well was removed, cells were washed twice with 4mL of PBS, lysates were collected in 300uL of RIPA buffer by gently scraping, and the relative fluorescence intensity for each sample was measured with a fluorometric plate reader.

Cell Death assays:

Cell death assays were conducted in 96-well format with BMDM or RAW cells plated at 40,000 cells per well. Media was collected at the indicated timepoints and the relative amount of LDH was determined using the CytoTox (Promega Cat# G1780) assay following manufacturer instructions. Alternatively, SYTOX Green (ThermoFisher Cat# S7020; 20nM) was included in Opti-MEM media added prior to treatment for inflammasome activation. Following treatment for inflammasome activation, SYTOX positive cells were enumerated using the IncuCyte S3 Live Cell Analysis Imaging System. Triton X-100 (0.05% final concentration) was added to a set of control wells with corresponding priming conditions to generate a total cell count and to calculate percent cell death (% cell death = ((experimental count)/(count from total lysis)) x 100).

Protein analysis by Western blot:

Macrophages were plated in 6-well format at $1x10^6$ cells per well. Cell lysates were collected by removing the media in each well, washing with PBS, and lysing in a

low volume (50uL/well) of RIPA buffer containing protease inhibitors (Roche cOmplete inhibitor cocktail). Protein concentrations were determined by Bradford assay (Pierce Cat# 23236) according to manufacturer instructions and equal concentrations were loaded for separation by standard SDS-PAGE. For experiments characterizing released protein into the supernatant, a TCA precipitation protocol was used as previously described(Broz and Monack, 2013) and equal fractions were loaded for SDS-PAGE. For a complete list of the antibodies used for Western Blot in this publication, please refer to the Key Resource Table.

Measurement of cytokine release by ELISA:

BMDM inflammasome activation for determining cytokine release were conducted in triplicate within a 96-well format and were plated at 40,000 cells per well as described above in the Cell Death assays section. Following inflammasome activation supernatants were collected at the indicated timepoints and stored at -20ºC for future processing. The amount of IL-18 or IL-1β present in the supernatants was determined with commercially available ELISA kits by following manufacturer instructions (Invitrogen: IL-18 - Cat# BMS618-3TEN, IL-1β - Cat# 88-7013-88).

Cell Lines Generated for this Manuscript:

RAW 264.7 KO cell lines (*Casp1/11* DKO, *Gsdmd* KO) were generated as previously described(Napier et al., 2016) and gRNAs are listed in the Key Resource Table. The cell line reconstituted with constitutive CASP11 expression was generated

on the *Casp1/11* DKO background with VSV pseudotyped retroviral constructs generated in 293T cells. The cDNA encoding CASP11 was isolated from BMDMs stimulated for 4hrs with LPS (100ng/mL) and cloned into pMSCV-IRES-GFP with XhoI/NotI. Transduced cells were isolated by FACS based on GFP expression and clonal populations were isolated. Untagged mouse NT-GSDMD PCR-amplified from Flag-GSDMD was Gibson cloned (NEB) into the pLenti CMVTRE3G Puro DEST inducible expression system using the EcoRV site. The cloned construct was introduced into *Gsdmd*-CRISPR/Cas9 KO RAW cells stably expressing the reverse tetracycline repressor-VP16 transactivator fusion protein pLenti CMV rtTA3G Blast using lentiviral delivery. Stable transductants were selected with puromycin and dilution plating was used to isolate the *Gsdmd*-CRISPR/Cas9 KO RAW Tet-On-NT-GSDMD clonal cell line.

Genome-wide CRISPR screen:

Our genome-wide CRISPR screen was conducted in the cell line that was created to constitutively express CASP11 (See above). Cells were stably transduced with lentiCas9-Blast (Addgene plasmid #52962) and selected with blasticidin to generate cells constitutively expressing Cas9. Lentiviral production of the Mouse CRISPR Knockout Pooled gRNA Library(Sanjana et al., 2014) (GeCKO V2; Addgene Cat# 1000000053) was generated by co-transfecting 150 million 293FT cells with a mixture of the plasmid library, ΔVPR, VSV-G, pAdVAntage packaging plasmids and FugeneHD. Lentiviral-containing supernatants were pooled from the total 293FT cell populations on day two and three following transfection, passed through a 0.45uM filter,

and stored at 4ºC. This pooled lentiviral gRNA library was used to transduce a total of 400 million constitutive CASP11-,Cas9-expressing cells at a MOI of 0.3 along with protamine sulfate (1ug/mL). Three days following transduction, cells containing the lentiviral gRNA constructs were selected with a five-day course of puromycin (5ug/mL). Each screen was conducted with a starting population of >100 million cells of the pooled gRNA library population to ensure sufficient knockout diversity by having an estimated >500-fold coverage of each gRNA in the library. For each screen two rounds of selection were conducted consisting of: 1) +/- IFNγ priming 2) LPS transfection to activate CASP11 inflammasome 3) a recovery period. Briefly, cells were treated +/- IFNγ (100 U/mL) for 16hrs in DMEM supplemented with 10%FBS and then the media was replaced with Opti-MEM just prior to LPS transfection. To trigger CASP11 inflammasome activation, LPS (*E. coli* 0111:B4, InvivoGen) was complexed with FuGENE HD and transfected into the knockout library. Following transfection, Opti-MEM media containing LPS and FuGENE HD were removed and replaced with fresh DMEM 10% FBS once a substantial level of cell death was observed by eye. Cells surviving the CASP11 activation were allowed to expand prior to a second round of selection or prior to collection for isolating genomic DNA. An untreated starting population of the mutagenized library was used as the unselected reference for each screen. Total genomic DNA was isolated from the surviving cell populations as well as from the untreated starting population using QIAamp DNA MiniKits (Qiagen). Two rounds of PCR were used to amplify the gRNA sequences from each population and append barcodes for next-generation sequencing by MiSeq (Illumina). Finally, the

previously described MAGeCK algorithm was used to analyze our sequencing results and generate enrichment scores for each gRNA(Li et al., 2014).

OMV isolation:

E. coli DH5α was grown for 8 hours in LB broth at 200 rpm at 37°C. This starter culture was then back-diluted 1:1000 into 200 ml LB and grown shaking at 200 rpm overnight at 37°C. Bacteria were removed by pelleting at 5000 xg for 15 minutes, and the supernatant was passed through a 0.2 μm filter. OMVs were isolated by centrifuging the filtered supernatant in thick-wall polycarbonate tubes (Beckman Coulter Catalog No. 355631) in a Type 70 Ti rotor in an Optima L-90K ultracentrifuge (Beckman Coulter) at 149,000 xg at 4°C for 3 hours. Following centrifugation, the supernatant was removed and the resulting OMV pellet was resuspended in cell culture-grade 1x PBS pH 7.4 (final concentration = 440x concentrated from culture broth). The resuspended OMVs were then passed through a 0.2 μm filter and stored at -80°C. This process was repeated to generate three batches of OMVs. Prior to addition to mammalian cells, the three batches were combined in a ratio of 1:1:1.

Quantification and Statistical Analysis:

Statistical calculations were conducted using GraphPad Prism software program. Two-way ANOVA statistical tests were conducted with multiple comparisons as indicated in each figure legend.

References:

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SANJANA, N. E., SHALEM, O. & ZHANG, F. 2014. Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods,* 11**,** 783-4.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-beta-Actin	Sigma-Aldrich	Cat# A1978, RRID:AB_476692
Rabbit polyclonal anti-Caspase1 p10	Santa Cruz Biotechnology	Cat# sc-514, RRID:AB_2068895
Rabbit polyclonal anti-Cleaved Caspase3	Cell Signaling Technology	Cat# 9661, RRID:AB_2341188
Rabbit monoclonal anti-Cleaved Caspase8	Cell Signaling Technology	Cat# 8592, RRID:AB_10891784
Rat monoclonal anti-Caspase11 (clone 17D9)	Sigma-Aldrich	Cat# C1354, RRID:AB_258736
Rabbit polyclonal anti-GBP2	Proteintech	Cat# 11854-1-AP, RRID:AB_2109336
Rabbit monoclonal anti-GSDMD	Abcam	Cat# ab209845, RRID:AB_2783550
Rabbit monoclonal anti-GSDME	Abcam	Cat# ab215191, RRID:AB_2737000
Rabbit monoclonal anti-HMGB1	Abcam	Cat# ab79823, RRID:AB_1603373
Goat polyclonal anti-IL-1β	R&D Systems	Cat# AF-401-NA RRID:AB_416684
Rabbit monoclonal anti-IL-18	Abcam	Cat# ab207323
Mouse monoclonal anti-NLRP3	AdipoGen	Cat# AG-20B-0014, RRID:AB_2490202
Biological Samples		
LPS VacciGrade Lipopolysaccharide from E. coli 0111:B4	InvivoGen	Cat# vac-3pelps
Cholera Toxin B Subunit in Low Salt	List Biological Labs	Cat# 104
Chemicals, Peptides, and Recombinant Proteins		
Recombinant IFNγ Mouse Protein	ThermoFisher Scientific	Cat# PMC4031
Recombinant IFNβ1 Mouse Protein	BioLegend	Cat# 581302
FuGENE HD transfection reagent	Promega	Cat# E2311
SYTOX Green Nucleic Acid Stain	ThermoFisher Scientific	Cat# S7020
Opti-MEM, no phenol red	ThermoFisher Scientific	Cat# 11058021
Nigericin	InvivoGen	Cat# tlrl-nig
ATP	InvivoGen	Cat# tlrl-atpl
(5Z)-7-Oxozeaenol (5z7)	Sigma-Aldrich	Cat# 499610
Critical Commercial Assays		
CytoTox 96 Non-Radioactive Cytotoxicity Assay	Promega	Cat# G1780
IL-1β Mouse Uncoated ELISA Kit	Invitrogen	Cat# 88-7013-88
Il-18 Mouse ELISA Kit	Invitrogen	Cat# BMS618-3TEN
Experimental Models: Cell Lines		
RAW 264.7	ATCC	Cat# TIB-71

Constitutive CASP11 expressing cell line: *Casp1,Casp11* CRISPR DKO +CASP11	This manuscript	
Experimental Models: Mouse Strains for BMDM isolation		
WT C57BL/6NJ	Jackson Labs	Stock No. 005304
Ifngr1-/-	Jackson Labs	Stock No. 003288
Casp1-/-	Gift of Vishva Dixit	
Casp11-/-	Gift of Vishva Dixit	
Gsdmd-/-	Gift of Vishva Dixit	
Gsdme-/-	Gift of Vishva Dixit	
$Gbp^{chr3-/-}$	Gift of Petr Broz and Igor Brodsky	
Oligonucleotides		
gRNA Casp1: TGTCTCTAAAAAAGGGCCCC	Napier et al 2016	
gRNA Casp11: CTGAACGCAGTGACAAGCGT	Napier et al 2016	
gRNA Gsdmd: TCGTGGGGATGACCTGTTTG	GeCKO V2 library Sanjana et al. 2014	
Primer pair to clone constitutive Casp11: fwd- AAAACTCGAGACTCTGTCAAGCTGTCTTCACGGT rev- AAAAGCGGCCGCTCAGTTGCCAGGAAAGAGGTAGA AATAT	This manuscript	
Primer pair to clone NT-Gsdmd: fwd- CAGTGTGGTGGAATTCTGCAGATGCCACCATGCCA TCGGCCTTTGAGAAAG rev- GCGGCCGCCACTGTGCTGGATCTAATCTGACAGGA GACTGAGCTGCTTTC	This manuscript	
Recombinant DNA		
Plasmid: MSCV-IRES-GFP	Addgene	Plasmid # 20672; RRID:Addgene_20672
Plasmid: pLenti CMVTRE3G Puro DEST	Addgene	Plasmid # 27565; RRID:Addgene_26429
Plasmid: pLenti CMV rtTA3G Blast	Addgene	Plasmid # 26429; RRID:Addgene_26429
Plasmid: Flag-Gsdmd	Addgene	Plasmid # 80950; RRID:Addgene_80950
Plasmid: lentiCas9-Blast	Addgene	Plasmid #52962; RRID:Addgene_52962
Plasmid: Mouse CRISPR Knockout Pooled Library (GeCKO v2)	Addgene; Sanjana et al. 2014	Cat #1000000053
Software and Algorithms		
Prism8	GraphPad	
MAGeCK	Li et al. 2014	