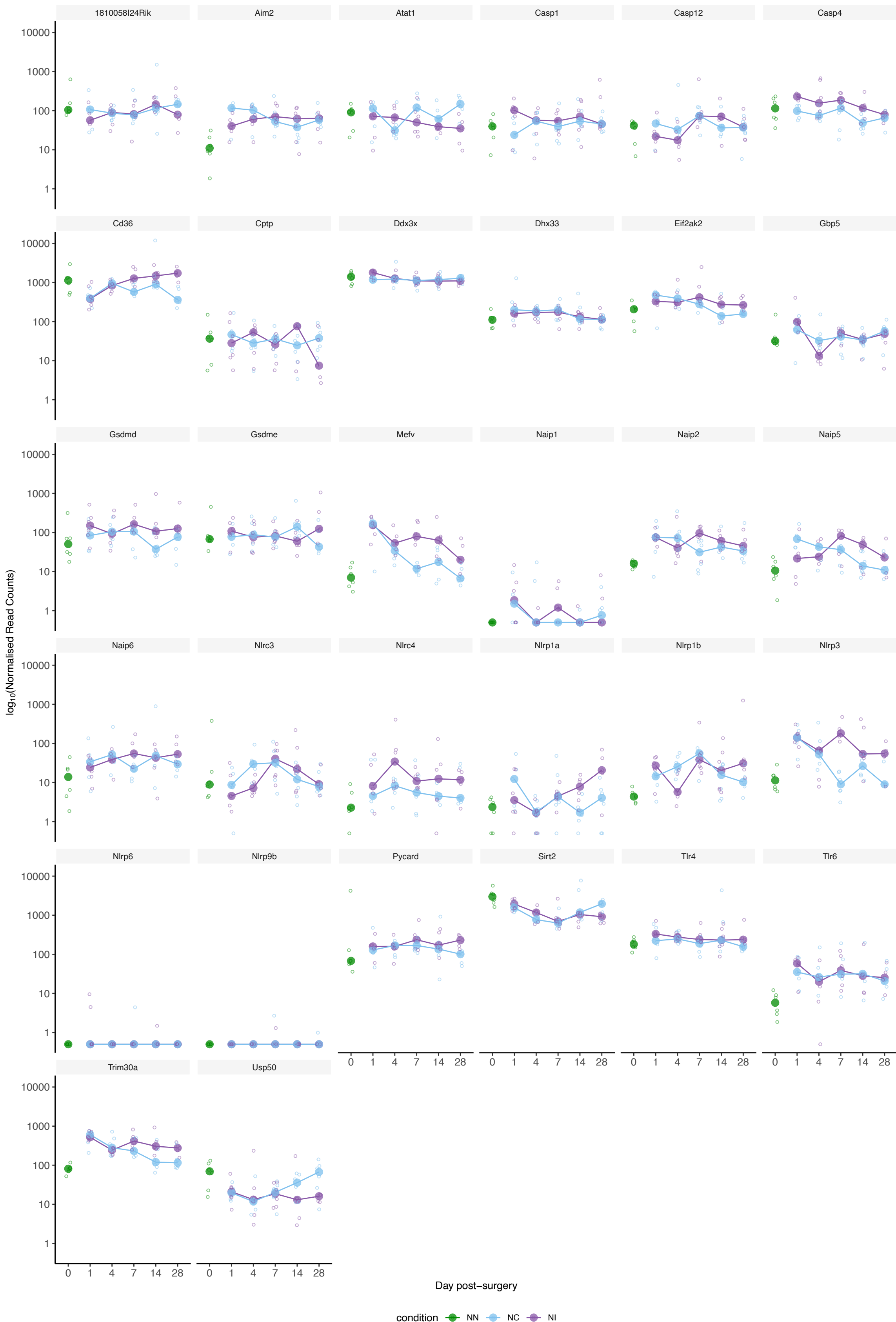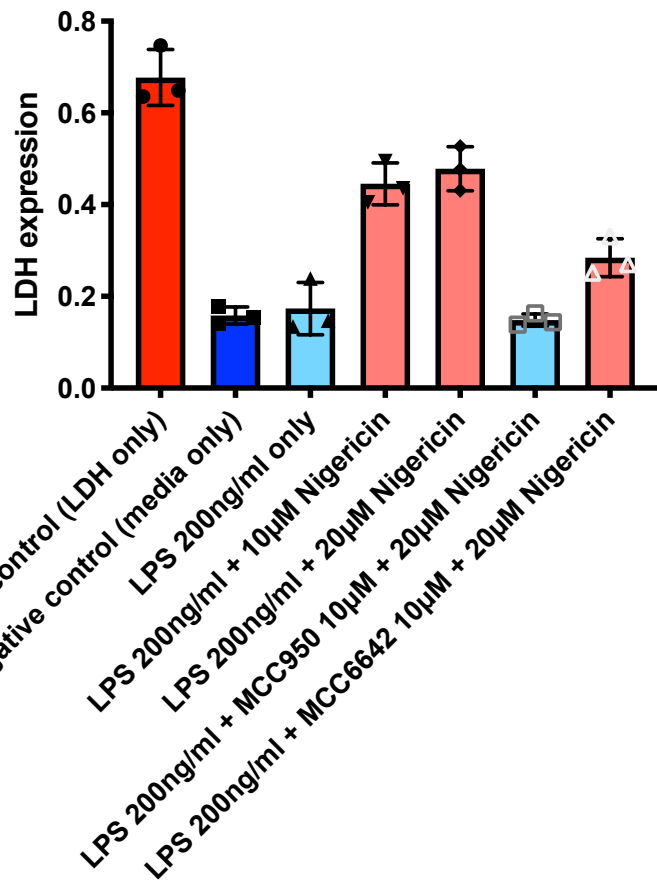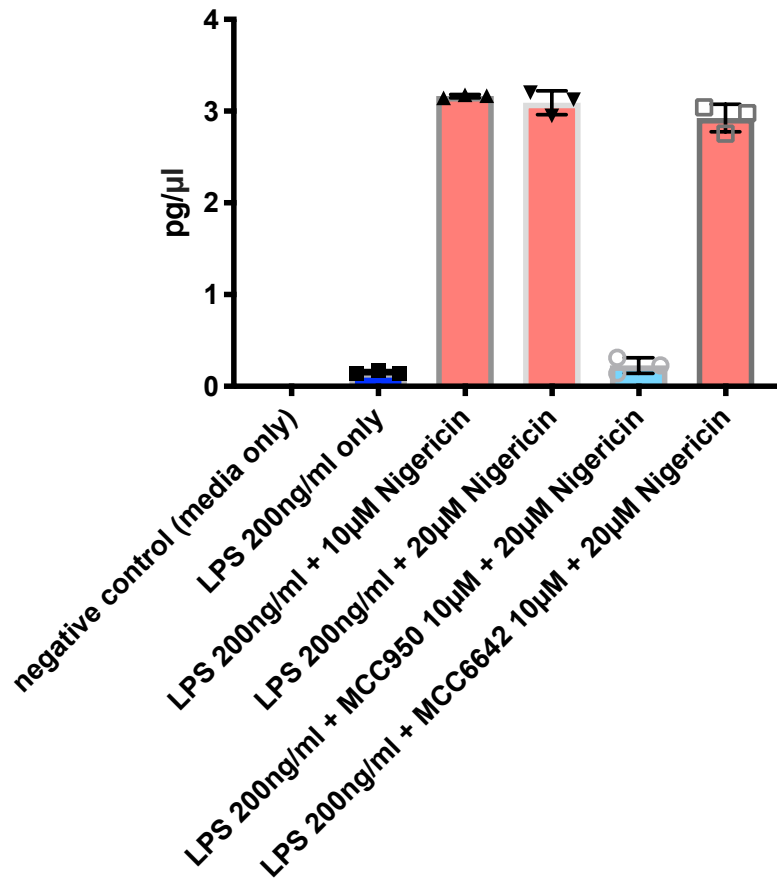# Inflamasome GO Terms

Supplementary Figure 1. Bubbleplot of expression values of all genes in the the inflammasome complex gene ontology term (GO:0061702), as well as Gasdermin E (Gsdme). Obtained through RNA sequencing of mice which had undergone microchannel cuff implantation (NI), crush nerve injury (NC), or naïve nerves (NN), at various days post-surgery. A subset of these plots are shown in Fig. 2e. Their combined expression is shown under inflammasome complex gene ontology (Fig. 1e, Fig. 2b).

Cytotoxic Assay (MCC950) — bar chart. Y-axis: LDH expression (0.0 to 0.8). X-axis categories: positive control (LDH only), negative control (media only), LPS 200ng/ml only, LPS 200ng/ml + 10µM Nigericin, LPS 200ng/ml + 20µM Nigericin, LPS 200ng/ml + MCC950 10µM + 20µM Nigericin, LPS 200ng/ml + MCC6642 10µM + 20µM Nigericin.

In vitro IL1b production (MCC950) — bar chart. Y-axis: pg/µl (0 to 4). X-axis categories: negative control (media only), LPS 200ng/ml only, LPS 200ng/ml + 10µM Nigericin, LPS 200ng/ml + 20µM Nigericin, LPS 200ng/ml + MCC950 10µM + 20µM Nigericin, LPS 200ng/ml + MCC6642 10µM + 20µM Nigericin.
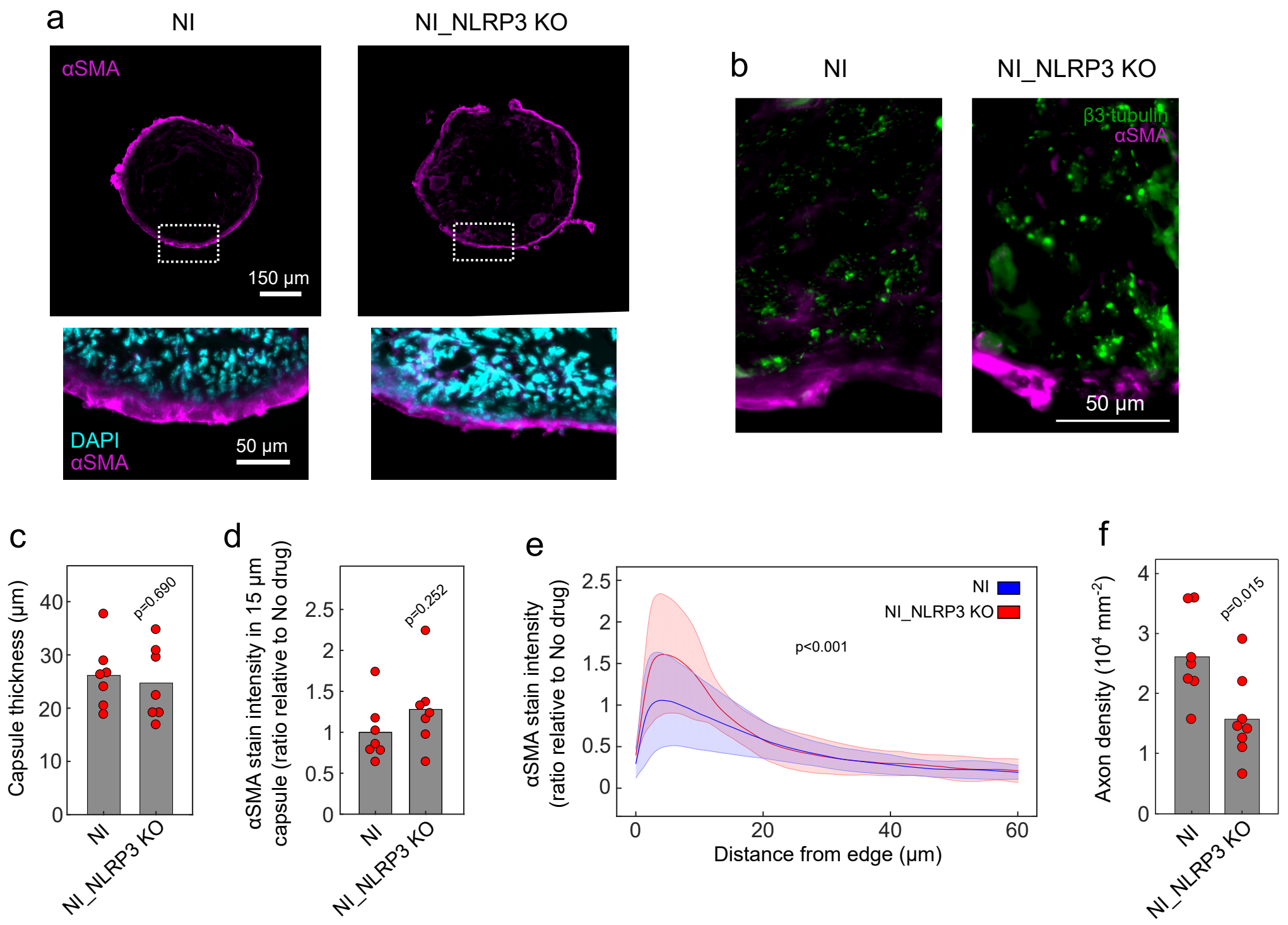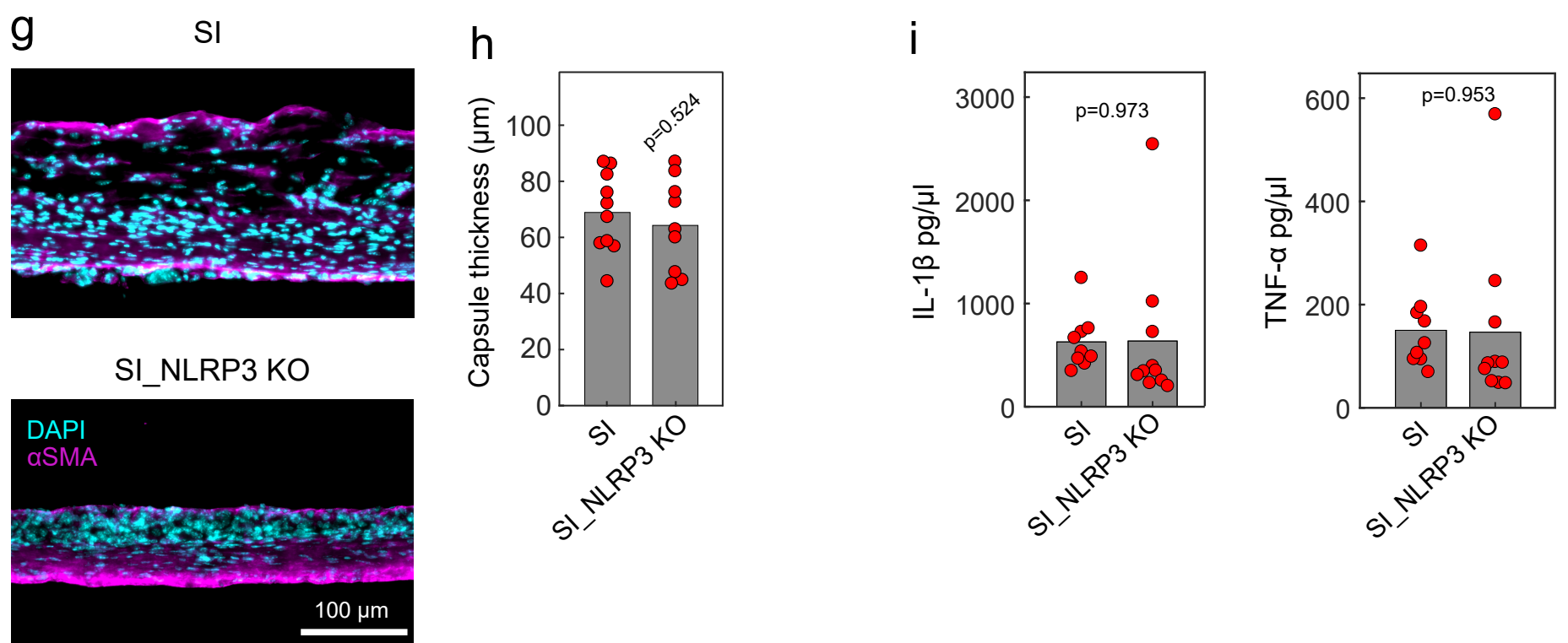
Supplementary Figure 2. Validation of MCC950 inhibiting NLRP3. MCC950 inhibits the production of the inflammasome product IL-1b *in vitro* (right), analyzed by ELISA. The inactive form MCC6642 used as a control does not affect its production. MCC950 does not produce any cytotoxicity *in vitro* (left), as characterized by LDH expression in a cytotoxic assay. Bar indicates sample mean, with bars indicating standard deviation. *In vitro* replicates represented as data points.

Nerve implantation

a

NI   NI_NLRP3 KO

αSMA

150 μm

DAPI
αSMA
50 μm

b

NI   NI_NLRP3 KO

β3-tubulin
αSMA

50 μm

c

Capsule thickness (μm)

p=0.690

NI   NI_NLRP3 KO

d

αSMA stain intensity in 15 μm capsule (ratio relative to No drug)

p=0.252

NI   NI_NLRP3 KO

e

αSMA stain intensity (ratio relative to No drug)

NI
NI_NLRP3 KO

$p < 0.001$

Distance from edge (μm)

f

Axon density (10^4 mm^{-2})

p=0.015

NI   NI_NLRP3 KO

Subcutaneous implantation

g

SI

SI_NLRP3 KO

DAPI
αSMA

100 μm

h

Capsule thickness (μm)

p=0.524

SI   SI_NLRP3 KO

i

IL-1β pg/μl

p=0.973

SI   SI_NLRP3 KO

TNF-α pg/μl

p=0.953

SI   SI_NLRP3 KO

Supplementary Figure 3. NLRP3 knockout mice develop normal levels of FBR. **a-b**, Z-stack confocal images (cross-sections) of FBR in nerve 3 months post-implantation of PDMS conduit. Conduits were implanted into wild type (NI) or into NLRP3$^{-/-}$ (NI_NLRP3 KO) mice. The tissue was fluorescently labelled for the myofibroblast marker αSMA (magenta), as well as cell nuclei (DAPI, cyan) in the insets (a); or for axon marker β3 tubulin (green) and αSMA (b). Inset highlighted by white dashed box. FBR to the conduit is characterized by a ring of myofibroblasts around the edge of the nerve **c**, Quantification of FBR capsule thickness around nerves, based on αSMA stain. **d-e**, Quantification of FBR marker αSMA stain intensity. Plot in c consists of average intensity over the 15 μm closest to the implant edge. **f**, Quantification of axon density (β3 tubulin stain pattern) in implanted nerves. **g**, Images of FBR capsules formed around subcutaneously implanted PDMS disks (SI), 3 months post-implantation, fluorescently stained for myofibroblasts (αSMA, magenta) and cell nuclei (DAPI, cyan). **i**, ELISA of protein content in subcutaneous disk implants. For plots c-d, f, h, and i: circles indicate average value per mouse, and grey bar the average of all animals. Statistical comparisons carried out via Student's t-test. For plot e: solid lines correspond to average intensity for N = 7 mice at an increasing distance from implant edge, and the shaded envelope corresponds to the standard deviation. Statistical comparison done through two-way ANOVA.

Resource	URL
GRCm38	http://mar2016.archive.ensembl.org/index.html
FastQC	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Trim_galore	http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
STAR	http://dx.doi.org/10.1093/bioinformatics/bts635
HTSeq-counts	http://dx.doi.org/10.1093/bioinformatics/btu638
Feature_counts	http://dx.doi.org/10.1093/bioinformatics/btt656
Qualimap	http://dx.doi.org/10.1093/bioinformatics/bts503
ClusterFlow	http://dx.doi.org/10.12688/f1000research.10335.2
MultiQC	http://dx.doi.org/10.1093/bioinformatics/btw354
DESeq2	http://dx.doi.org/10.1186/s13059-014-0550-8
UpSetR	http://dx.doi.org/10.1093/bioinformatics/btx364
DeconRNASeq	http://dx.doi.org/10.1093/bioinformatics/btt090
Immune cell Reference	http://dx.doi.org/10.1038/srep40508

Supplementary Table 1. Software packages used in RNA sequencing data analysis.

Antibody	Dilution	Source	Code
Primary antibodies			
Rabbit anti-beta Tubulin	1:200	Abcam	ab6046
Mouse anti-αSMA	1:100	Abcam	ab7817
Secondary antibodies			
Alexa Fluor donkey anti-mouse 555	1:1,000	Invitrogen	ab150106
Alexa Fluor donkey anti-rabbit 488	1:1,000	Invitrogen	ab150073
Hoechst-33342 nuclear stain	1:10,000	Sigma	14533

Supplementary Table 2. Antibodies used in immunohistochemistry experiments.