iScience, Volume *24*

Supplemental information

STING regulates peripheral nerve regeneration and colony stimulating factor 1 receptor (CSF1R) processing in microglia

Giulio Morozzi, Julian Rothen, Gauthier Toussaint, Katrina De Lange, Katrin Westritschnig, Arno Doelemeyer, Vanessa Pitiot Ueberschlag, Peter Kahle, Christian Lambert, Michael Obrecht, Nicolau Beckmann, Veronique Ritter, Moh Panesar, Daniela Stauffer, Isabelle Garnier, Matthias Mueller, Danilo Guerini, Caroline Gubser Keller, Judith Knehr, Guglielmo Roma, Michael Bidinosti, Sophie Brachat, Frederic Morvan, and Mara Fornaro

SUPPLEMENTARY FIGURES

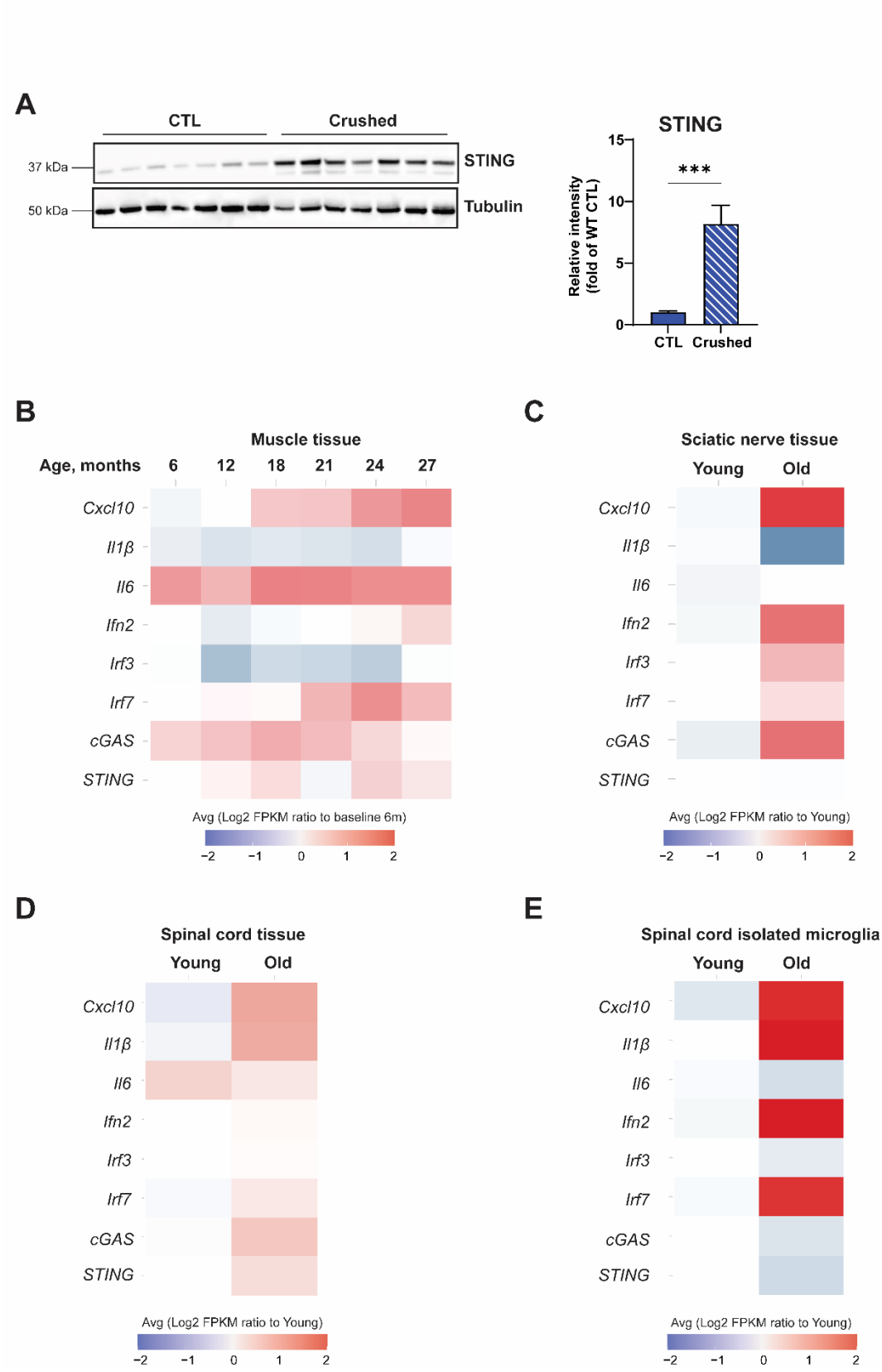

Figure S1. **The cGAS/STING pathway is upregulated in in sciatic nerve crush and aging in rodents, related to Figure 1.**

A) Twelve week old C57BL/6J mice were subjected to SNC. STING protein expression was analyzed by immunoblotting in sciatic nerve 1 day post SNC. Tubulin was used as loading control. The graph shows the quantification of immuno-reactive bands by densitometry. ***$p < 0.001$, t-test. **B**-**E**) The expression pattern of genes regulated by the cGAS/STING pathway was analyzed in neuromuscular tissues during aging. Heatmaps showing expression patterns of genes downstream of cGAS/STING in gastrocnemius rat muscle (Ibebunjo et al. 2013) (**A**) and sciatic nerve of young (2-month-old) and old (19-month-old) rats (**B**), spinal cord (**C**) and FACS isolated brain microglia from young (10-month-old) and old (23-month-old) mice (**D**) (Giorgetti et al. 2019). Data are shown as log2 fold change over control young animal. Each row represents a single gene.

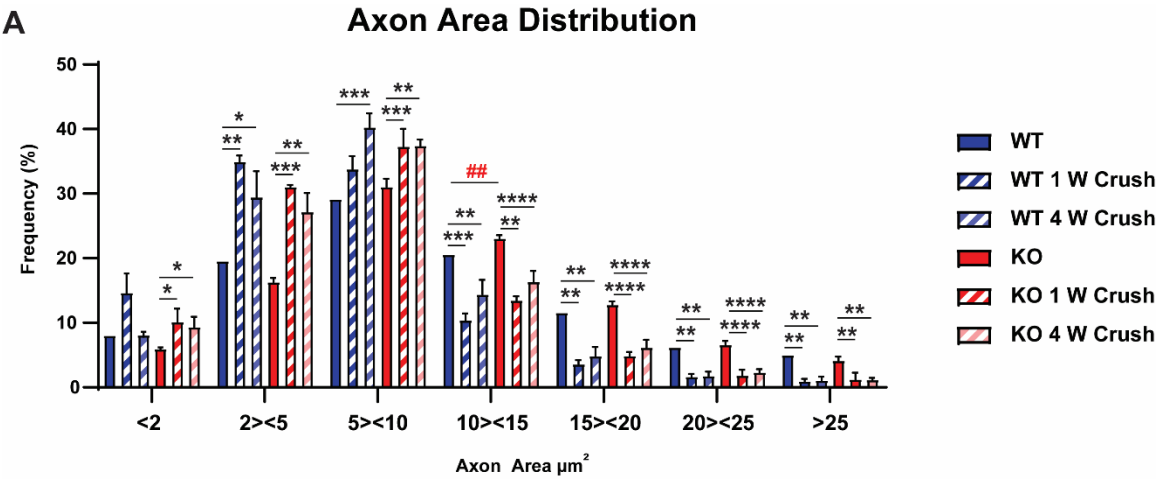

Figure S2. Analysis of axon area distribution in sciatic nerve, related to Figure 3.

Quantification of axon area distribution. Data represent the mean ± SEM. Five mice / group and genotype. Mice were 6 months old at the beginning of the experiment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; one way ANOVA, no crush group versus 1 week and 4 week after crush;# WT versus STING KO; t test.

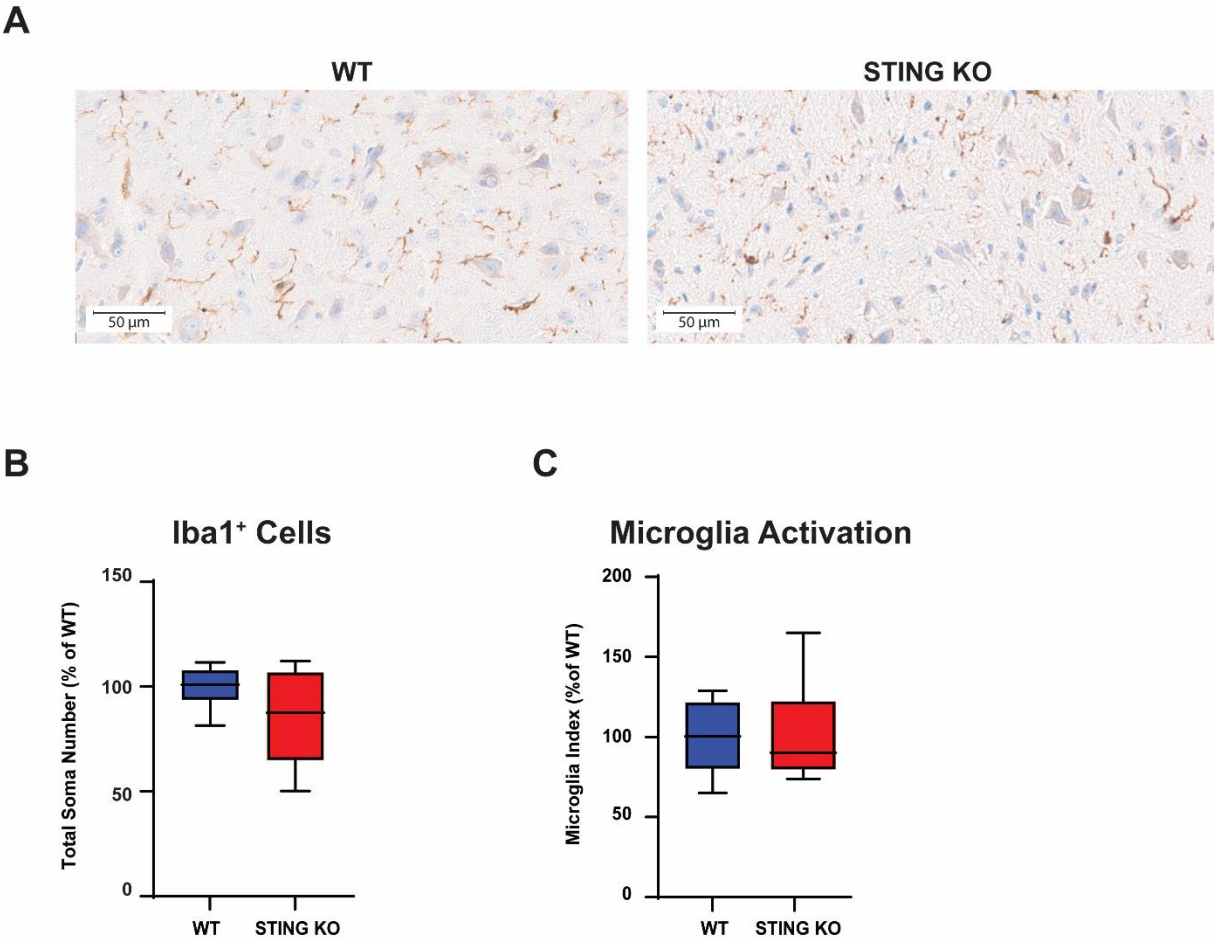

Figure S3. Microglia number and activation index in naive WT and STING KO mice, related to Figure 4.

Representative images of Iba1 immunostaining of two month old WT and STING KO spinal cord (**A**). Scale bar in A is 50 µm; n = six-eight mice / group and genotype. Quantitative analysis of Iba1^{+} cells in spinal cord (lumbar region L5-L6) (**B-C**). The microglia activation index is the ratio of the distal area and the soma plus the proximal area. Data represent the mean ± SEM.

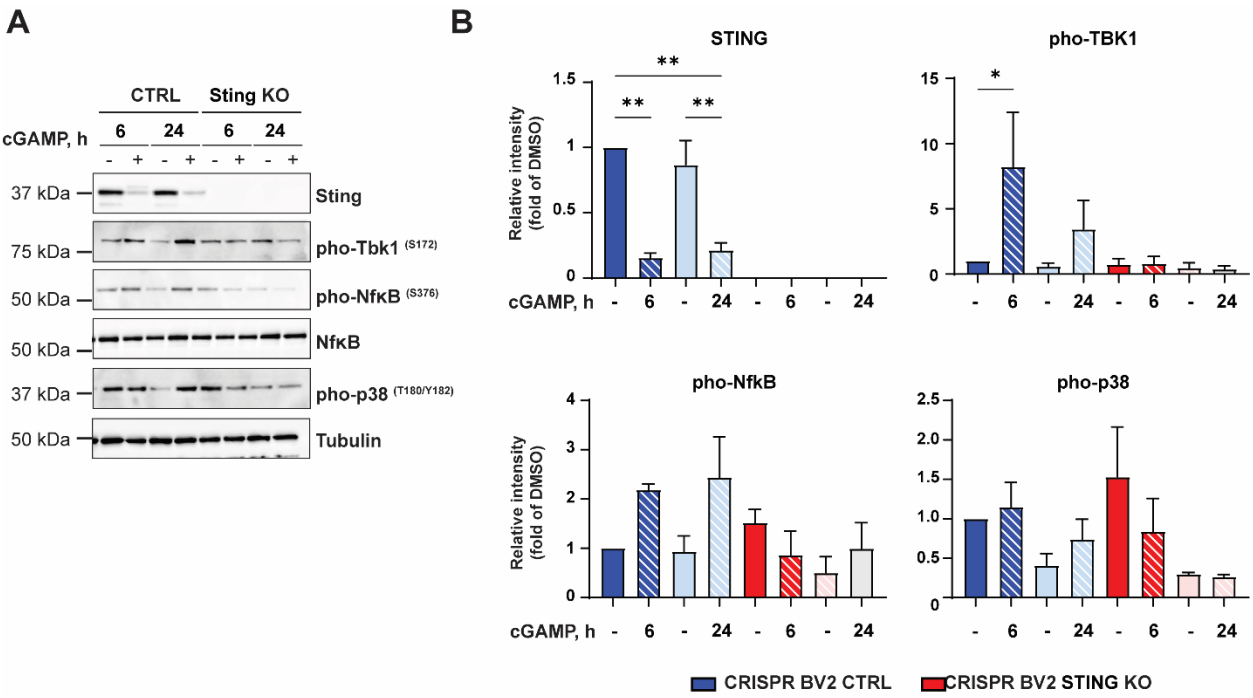

Figure S4. Analysis of signaling downstream STING, related to Figure 5.

A) BV2 STING CRISPR KO and control (CTRL) cells were treated with 50 µM of cGAMP or DMSO for the indicated time. Protein expression was analyzed by immunoblotting. The experiment was repeated three times and representative blots are shown. Tubulin was used as a loading control. **B**) The graph shows the quantification of immune-reactive bands by densitometry as fold of increase over DMSO CRISPR CTRL cells 6h after cGAMP treatment. Data are mean band intensity ± SEM. *$p < 0.05$, **$p < 0.01$, two way ANOVA.

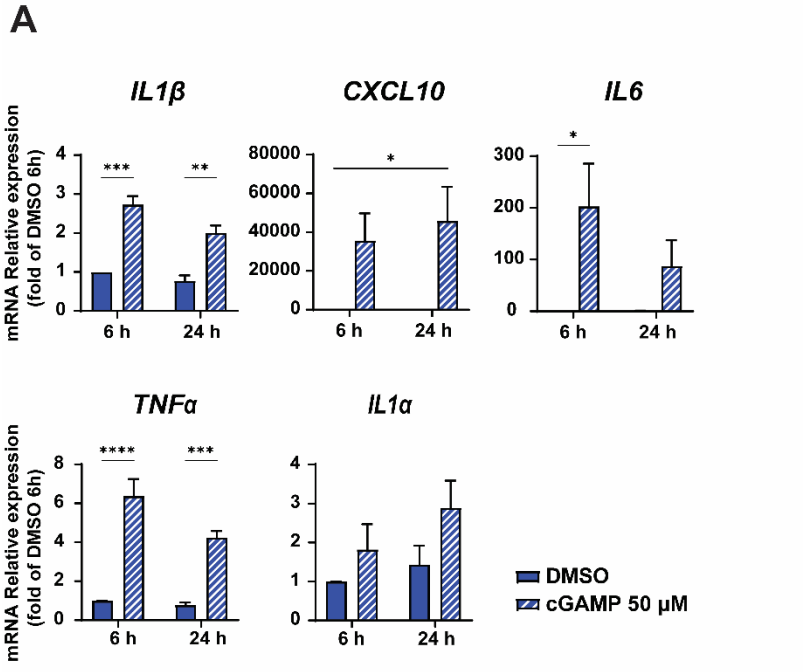

Figure S5. cGAMP upregulates pro-inflammatory cytokines in iPSC-derived microglia, related to Figure 5.

Human iPSC-derived microglia were treated with 50 µM cGAMP or DMSO for 6 and 24 h. Analysis of inflammatory cytokines and markers of microglia activation was performed by qRT-PCR. The experiment was repeated 3 times and the data represent means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001; two way ANOVA.

Table S3. Oligonucleotides used for CRISPR.

ID	Sequence 5'-3'	
Mm.Cas9.TMEM173.1.AD	ATATTTGGAGCGGTGACCTC	Guide RNA (crRNA)
Mm.Cas9.TMEM173.1.AG	GTGGATCCTTTGCCACCCAA	
mTMEM173_Fwd	CTCCAACCTGCATCCAGCC	Genotyping
mTMEM173_Rev	GTTCGTGCGAGGCTAGGTG	
mTMEM173_Fwd_long	GAAGGACAGGAGAACCCCAG	
mTMEM173_Rev_long	GGAGACCACAGAGGGTTACCTG	

Table S4. Taqman probes (in bold are reported housekeeping genes).

TaqMan ID	Gene symbol
Mm00475988_m1	*Arg1*
Mm00445235_m1	*Cxcl10*
Hs99999049_m1	*CXCL10*

Mm00432688_m1	*Csf1*
Mm99999915_g1	***Gapdh***
Hs99999905_m1	***GAPDH***
Mm03024075_m1	***Hprt***
Mm00439620_m1	*Il1α*
Hs00174092_m1	*IL1α*
Mm00434228_m1	*Il1β*
Hs01555410_m1	*IL1β*
Mm00446190_m1	*Il6*
Hs00174131_m1	*IL6*
Mm00440502_m1	*Nos2*
Mm00725448_s1	***Rplp0***
Hs99999902_m1	***RPLP0***
Mm00443258_m1	*Tnfα*
Hs00174128_m1	*TNFα*

Table S5. Antibodies for western blot analysis.

Antibody	**Reference**
GAPDH (6C5), Mouse, mAbs	Ambion Cat# AM4300, RRID:AB_437392
M-CSF Receptor, Rabbit, pAbs	Cell Signaling Technology Cat# 3152, RRID:AB_2085233
NF-κB p65 (D14E12) XP®, Rabbit mAbs	Cell Signaling Technology Cat# 8242, RRID:AB_10859369
NF-κB p65 (Phospho Ser536) (93H1), Rabbit, mAbs	Cell Signaling Technology Cat# 3033, RRID:AB_331284
TBK1/NAK (Phospho Ser172) (D52C2) XP® Rabbit, mAbs	Cell Signaling Technology Cat# 5483, RRID:AB_10693472
STING (D1V5L), Rabbit, mAbs	Cell Signaling Technology Cat# 50494, RRID:AB_2799375
Tubulin-α (DM1A), Mouse, mAb	Sigma-Aldrich Cat# T6199, RRID:AB_477583