Supplemental data

Blocking fatty acid-fueled mROS production within macrophages alleviates acute gouty inflammation

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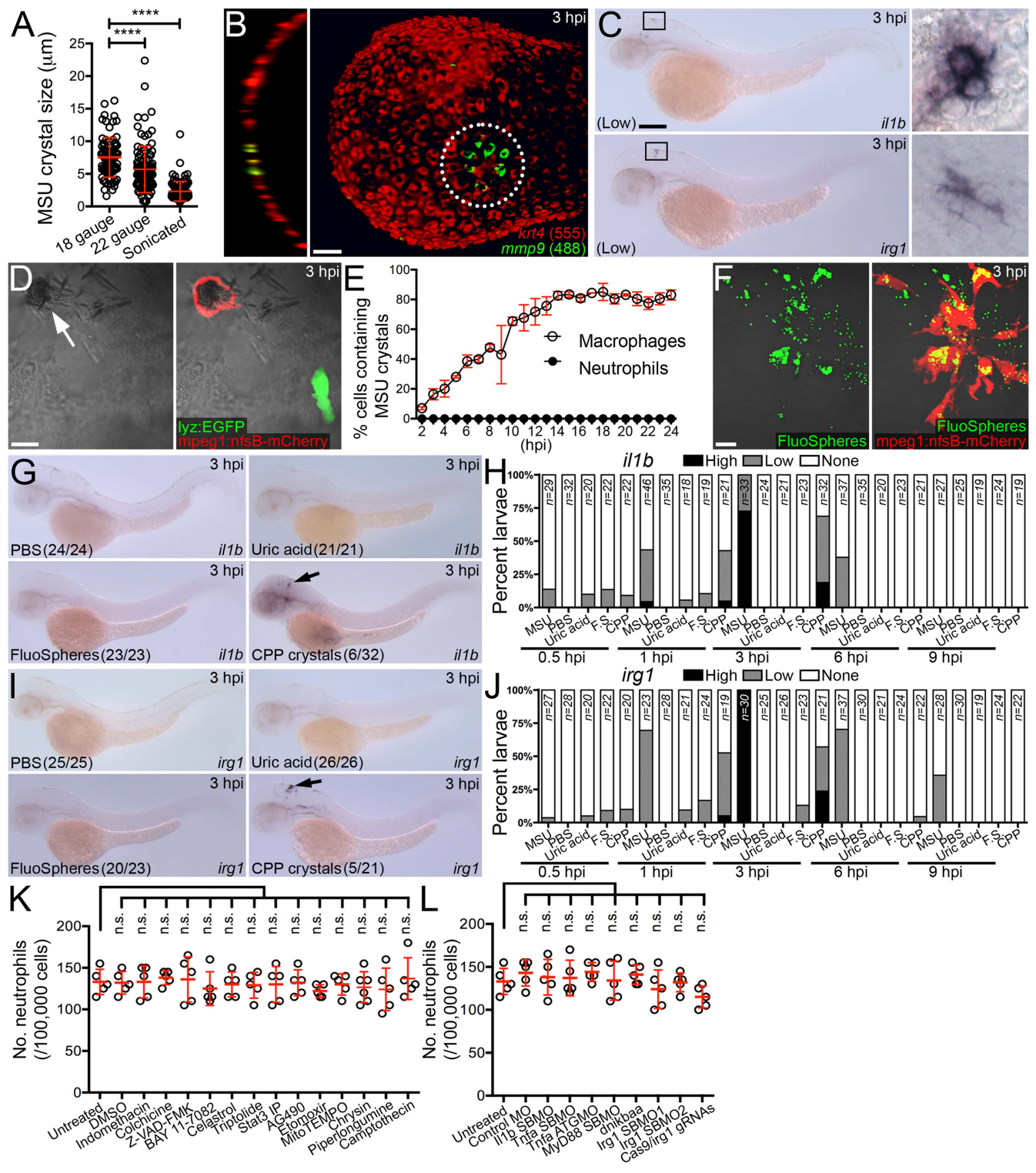
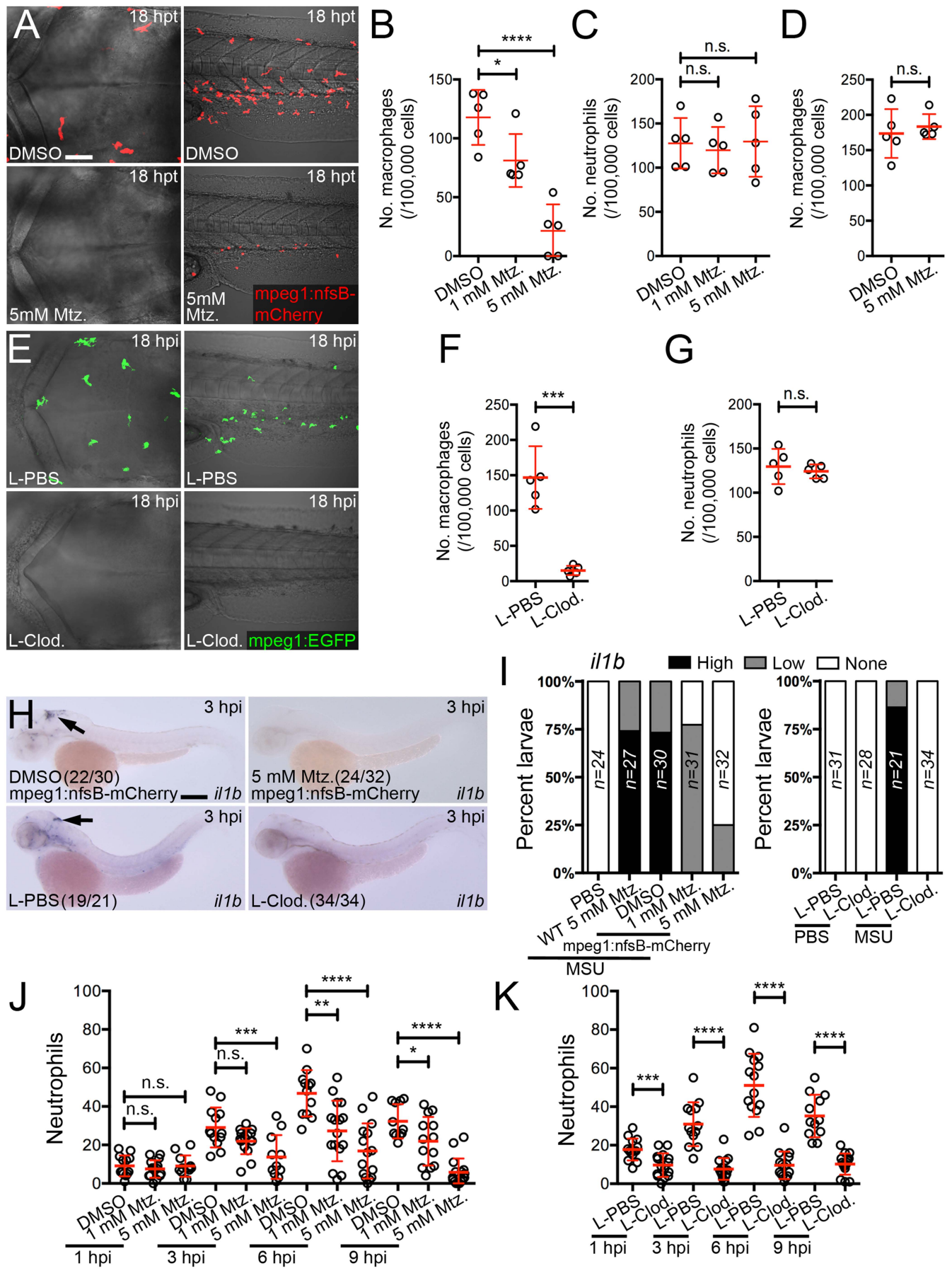
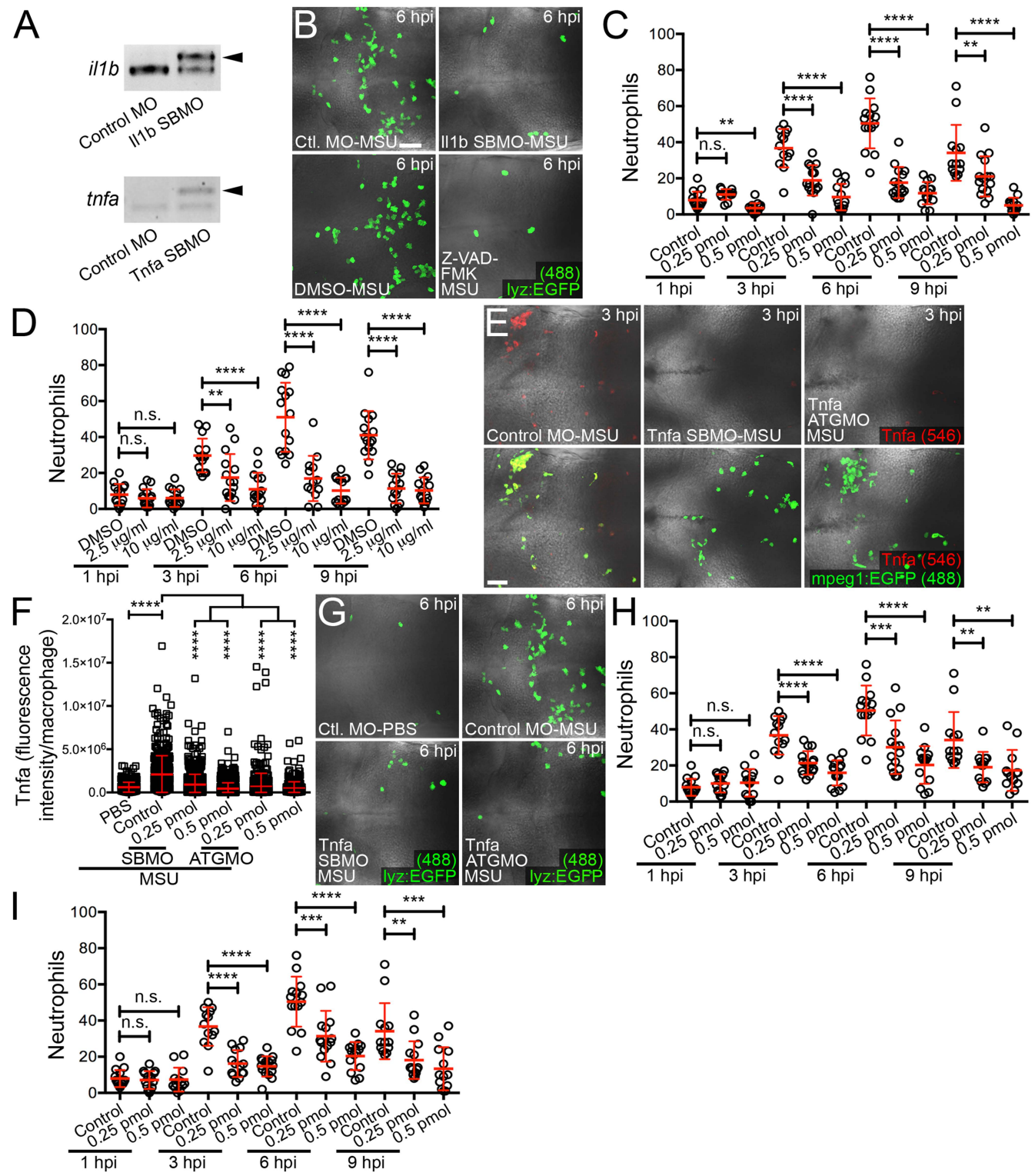

A
MSU crystal size (µm)
****
****
18 gauge
22 gauge
Sonicated
B
3 hpi
krt4 (555)
mmp9 (488)
C
3 hpi
(Low)
il1b
3 hpi
(Low)
irg1
D
3 hpi
lyz:EGFP
mpeg1:nfsB-mCherry
E
% cells containing MSU crystals
Macrophages
Neutrophils
(hpi)
F
3 hpi
FluoSpheres
FluoSpheres
mpeg1:nfsB-mCherry
G
3 hpi
PBS (24/24)
il1b
Uric acid (21/21)
il1b
FluoSpheres (23/23)
il1b
CPP crystals (6/32)
il1b
H
il1b
High
Low
None
Percent larvae
MSU
PBS
Uric acid
F.S.
CPP
0.5 hpi
1 hpi
3 hpi
6 hpi
9 hpi
I
3 hpi
PBS (25/25)
irg1
Uric acid (26/26)
irg1
FluoSpheres (20/23)
irg1
CPP crystals (5/21)
irg1
J
irg1
High
Low
None
Percent larvae
K
No. neutrophils (/100,000 cells)
n.s.
Untreated
DMSO
Indomethacin
Colchicine
Z-VAD-FMK
BAY 11-7082
Celastrol
Triptolide
Stat3 IP
AG490
Etomoxir
MitoTEMPO
Chrysin
Piperlongumine
Camptothecin
L
No. neutrophils (/100,000 cells)
n.s.
Untreated
Control MO
Il1b SBMO
Tnfa SBMO
Tnfa ATGMO
MyD88 SBMO
dnIkbaa
Irg1 SBMO1
Irg1 SBMO2
Casp1/Irg1 gRNAs

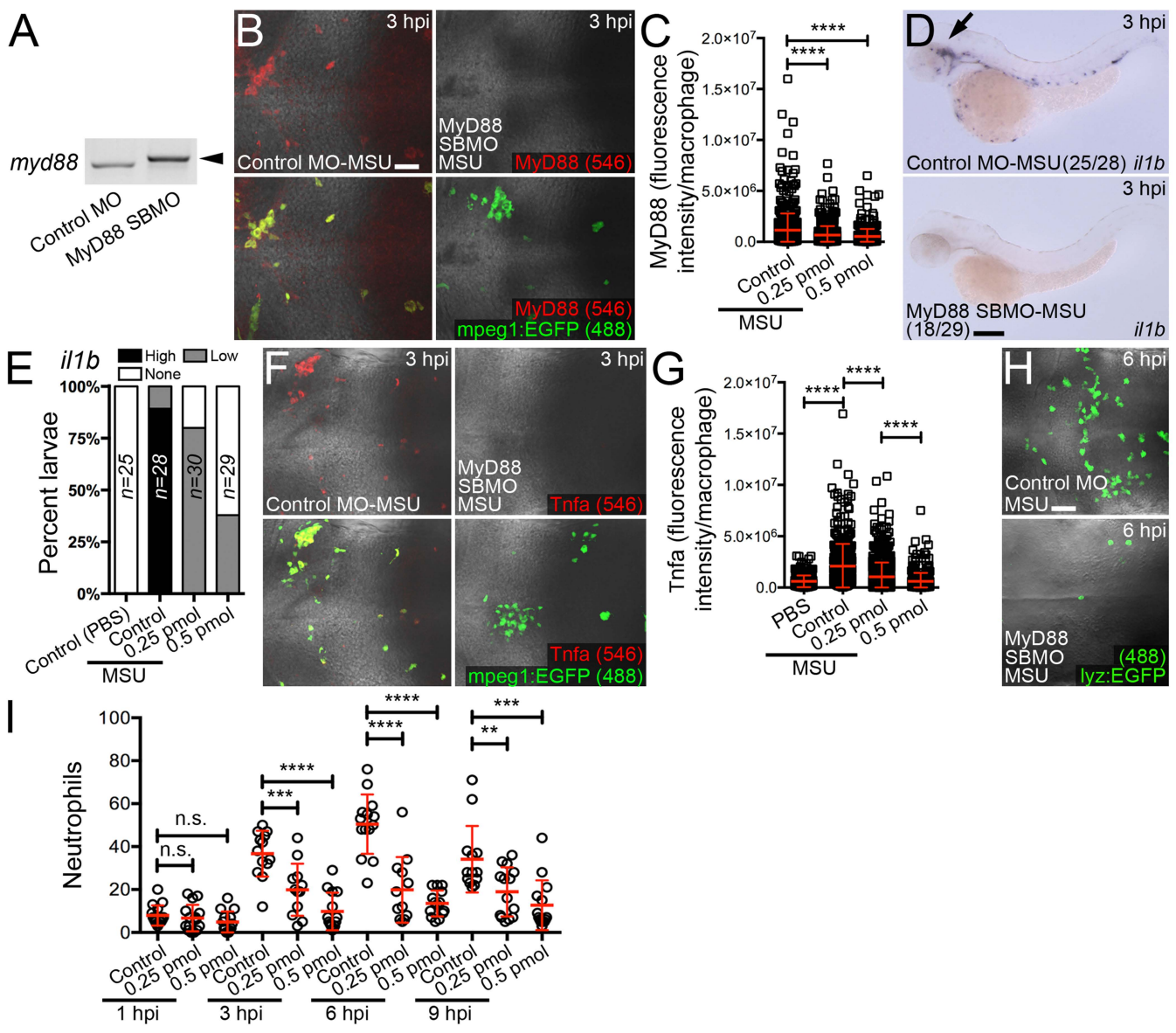
Supplemental Figure 1. MSU crystals activate zebrafish macrophages. (A) Crystal lengths following 18/22 gauge needle dissociation and sonication. (B) Hindbrain expression of *mmp9*/*krt4* (transverse and dorsal views) within MSU crystal-injected larva (circle, microinjection site). (C) MSU crystal-injected larvae demonstrating 'low' *il1b*/*irg1* expression (insets, magnified views of hindbrain). (D) Hindbrain macrophage and neutrophil within MSU crystal-injected *Tg(mpeg1:nfsB-mCherry;lyz:EGFP)* larvae (arrow, intracellular crystals). (E) Temporal quantification of crystal phagocytosis, as in D (n=2 larvae). (F) FluoSpheres within hindbrain macrophages (injected into *Tg(mpeg1:nfsB-mCherry)* larvae). (G and I) Expression of *il1b* (G) and *irg1* (I) within PBS-, uric acid-, FluoSphere (F.S.)- and calcium pyorophosphate (CPP) crystal-injected larvae. PBS images in G and I are the same as in Figures 1B and 1D, respectively. (H and J) Temporal quantification of *il1b* (H) and *irg1* (J) expression, as detected in G and I, respectively. (K and L) Flow cytometry quantification of neutrophils within *Tg(lyz:EGFP)* larvae following indicated treatments (K) and MO/CRISPR-Cas9 injections (L), n=~35 larvae/treatment, 5 biological replicates. Untreated sample in panel L is the same as in panel K. Arrows mark *il1b*/*irg1* expression in hindbrain. Data for D-J pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. ****P<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 50 μm (B), 100 μm (C) and 10 μm (D, F).


A
18 hpt
DMSO
18 hpt
DMSO
18 hpt
5mM Mtz.
18 hpt
5mM Mtz.
mpeg1:nfsB-mCherry
B
No. macrophages (/100,000 cells)
****
*
DMSO
1 mM Mtz.
5 mM Mtz.
C
No. neutrophils (/100,000 cells)
n.s.
n.s.
DMSO
1 mM Mtz.
5 mM Mtz.
D
No. macrophages (/100,000 cells)
n.s.
DMSO
5 mM Mtz.
E
18 hpi
L-PBS
18 hpi
L-PBS
18 hpi
L-Clod.
18 hpi
L-Clod.
mpeg1:EGFP
F
No. macrophages (/100,000 cells)
***
L-PBS
L-Clod.
G
No. neutrophils (/100,000 cells)
n.s.
L-PBS
L-Clod.
H
3 hpi
DMSO (22/30)
mpeg1:nfsB-mCherry
il1b
3 hpi
5 mM Mtz. (24/32)
mpeg1:nfsB-mCherry
il1b
3 hpi
L-PBS (19/21)
il1b
3 hpi
L-Clod. (34/34)
il1b
I
il1b
High
Low
None
Percent larvae
n=24
n=27
n=30
n=31
n=32
PBS
WT 5 mM Mtz.
DMSO
1 mM Mtz.
5 mM Mtz.
mpeg1:nfsB-mCherry
MSU
n=31
n=28
n=21
n=34
L-PBS
L-Clod.
L-PBS
L-Clod.
PBS
MSU
J
Neutrophils
n.s.
n.s.
***
n.s.
**
****
*
****
DMSO
1 mM Mtz.
5 mM Mtz.
1 hpi
3 hpi
6 hpi
9 hpi
K
Neutrophils
***
****
****
****
L-PBS
L-Clod.
1 hpi
3 hpi
6 hpi
9 hpi

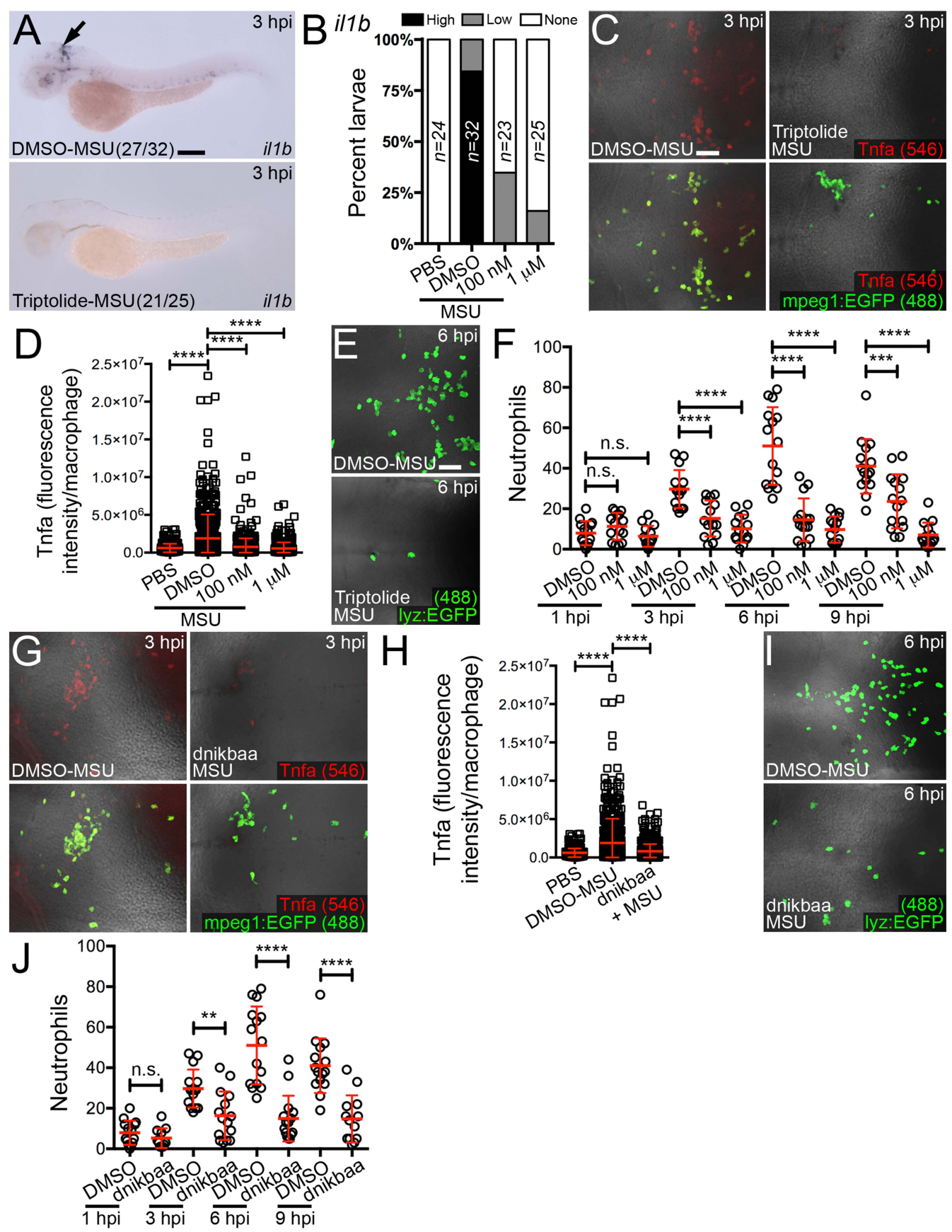
Supplemental Figure 2. MSU crystal-driven neutrophil recruitment is suppressed in macrophage-depleted larvae. (A) Hindbrain and tail of DMSO- and metronidazole (Mtz.)-treated *Tg(mpeg1:nfsB-mCherry)* larvae, 18 hours post treatment (hpt). (B and C) Flow cytometry quantification of macrophages (B) and neutrophils (C) from *Tg(mpeg1:nfsB-mCherry)* (B) and *Tg(lyz:EGFP)* (C) larvae as treated in A. (D) Flow cytometry quantification of macrophages from Mtz.-treated *Tg(mpeg1:EGFP)* larvae. (E) Hindbrain and tail of liposomomal PBS (L-PBS)- and liposomal clodronate (L-Clod.)-injected *Tg(mpeg1:EGFP)* larvae, 18 hpi. (F and G) Flow cytometry quantification of macrophages (F) and neutrophils (G) from *Tg(mpeg1:EGFP)* and *Tg(lyz:EGFP)* larvae, respectively, as treated in E. (H) Expression of *il1b* within MSU crystal-injected DMSO- and metronidazole(Mtz.)-treated *Tg(mpeg1:nfsB-mCherry)* larvae and following L-PBS and L-clodronate (L-Clod.) injection. (I) Quantification of *il1b* expression, as detected in H. (J and K) Temporal quantification of neutrophils within the hindbrain of MSU crystal-injected DMSO- and Mtz.-treated *Tg(lyz:EGFP;mpeg1:nfsB-mCherry)* larvae (J) and in L-PBS- and L-clodronate-injected *Tg(lyz:EGFP)* larvae (K), n=13-15 larvae/treatment. For flow cytometry, n=5 groups ~30 larvae/treatment. Arrows mark *il1b* expression in hindbrain. Data for H-K pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test (B, C, J) and Student's *t* test (F, G, K). Scale bars, 50 μm (A) and 100 μm (H).


A
il1b
Control MO
Il1b SBMO
tnfa
Control MO
Tnfa SBMO
B
6 hpi
Ctl. MO-MSU
Il1b SBMO-MSU
DMSO-MSU
Z-VAD-FMK MSU
(488) lyz:EGFP
C
Neutrophils
n.s.
Control
0.25 pmol
0.5 pmol
1 hpi
3 hpi
6 hpi
9 hpi
D
Neutrophils
DMSO
2.5 µg/ml
10 µg/ml
E
3 hpi
Control MO-MSU
Tnfa SBMO-MSU
Tnfa ATGMO MSU
Tnfa (546)
mpeg1:EGFP (488)
F
Tnfa (fluorescence intensity/macrophage)
PBS
SBMO
ATGMO
MSU
G
Ctl. MO-PBS
Control MO-MSU
Tnfa SBMO MSU
Tnfa ATGMO MSU
H
Neutrophils
I
Neutrophils

Supplemental Figure 3. MSU crystal-driven neutrophil recruitment is dependent on macrophage *il1b* expression and Tnfa production. (A) RT-PCR for *il1b* and *tnfa* from MSU-injected controlMO-, Il1bSBMO- and TnfaSBMO-injected larvae (arrowheads, alternatively spliced transcripts). (B) Immunofluorescence detection of neutrophils within the hindbrain of control MO- and Il1b SBMO-injected *Tg(lyz:EGFP)* larvae following MSU crystal injection and MSU crystal-injected DMSO- and Z-VAD-FMK-treated *Tg(lyz:EGFP)* larvae. (C and D) Quantification of neutrophils, as detected in B, for Il1b SBMO-injected (C) and Z-VAD-FMK-treated (D) larvae (n=13-15 larvae/treatment). DMSO-MSU samples in panel D are the same as in Figure, 2B/2E/2F. (E) Immunofluorescence of Tnfa within controlMO-, TnfaSBMO- and TnfaATGMO-injected *Tg(mpeg1:EGFP)* larvae following MSU crystal injection. (F) Quantification of Tnfa, as detected in E (n=15 larvae/treatment). (G) Immunofluorescence detection of neutrophils within the hindbrain of control MO/PBS-injected and control MO-, Tnfa SBMO- and Tnfa ATGMO-injected *Tg(lyz:EGFP)* larvae following MSU crystal injection. Control MO-MSU image is the same as in panel B. (H and I) Temporal quantification of neutrophils, as detected in G, for Tnfa SBMO-(H) and TnfaATGMO-(I)injected larvae (n=13-15 larvae/treatment). Control MO-MSU samples are the same as in panel C. Data for B-I pooled from 2 independent experiments. All error bars, means ± SD. ***P*<0.01; ****P*<0.001; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 50 μm (B, E).

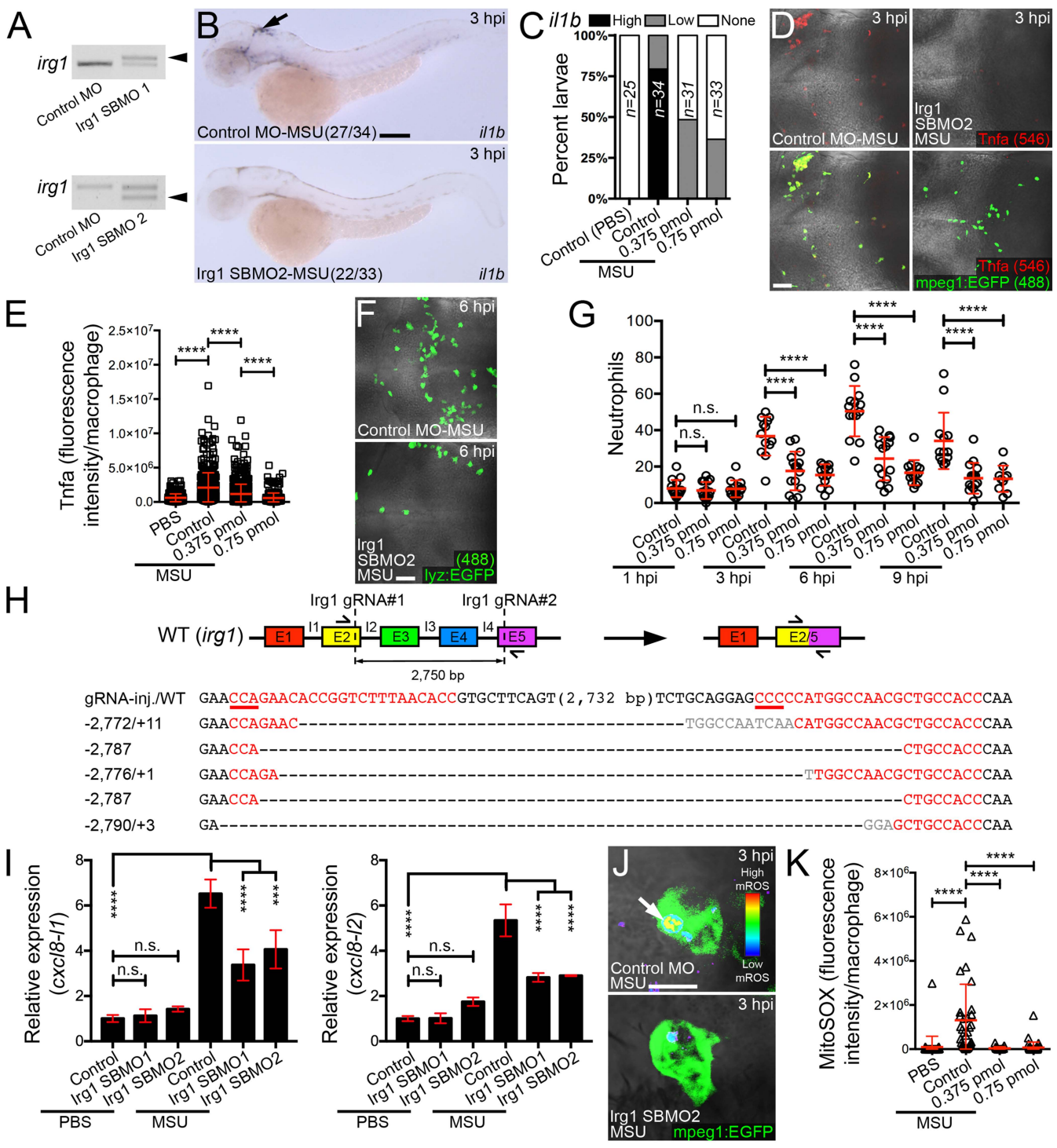

A
myd88
Control MO
MyD88 SBMO
B
3 hpi
Control MO-MSU
MyD88
SBMO
MSU
MyD88 (546)
mpeg1:EGFP (488)
C
MyD88 (fluorescence intensity/macrophage)
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
****
Control
0.25 pmol
0.5 pmol
MSU
D
3 hpi
Control MO-MSU(25/28) il1b
MyD88 SBMO-MSU (18/29)
il1b
E
il1b
High
Low
None
Percent larvae
100%
75%
50%
25%
0%
n=25
n=28
n=30
n=29
Control (PBS)
Control
0.25 pmol
0.5 pmol
MSU
F
3 hpi
Control MO-MSU
MyD88
SBMO
MSU
Tnfa (546)
mpeg1:EGFP (488)
G
Tnfa (fluorescence intensity/macrophage)
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
****
****
PBS
Control
0.25 pmol
0.5 pmol
MSU
H
6 hpi
Control MO
MSU
MyD88
SBMO
MSU
(488)
lyz:EGFP
I
Neutrophils
100
80
60
40
20
0
n.s.
n.s.
****
***
****
****
***
**
Control
0.25 pmol
0.5 pmol
1 hpi
3 hpi
6 hpi
9 hpi

Supplemental Figure 4. MSU crystal-driven macrophage activation and neutrophil recruitment is dependent on MyD88. (A) RT-PCR for *myd88* from MSU crystal-injected controlMO- and MyD88SBMO-injected larvae (arrowhead, alternatively spliced transcript). (B) Immunofluorescence of MyD88 within controlMO- and MyD88SBMO-injected *Tg(mpeg1:EGFP)* larvae following MSU crystal injection. (C) Quantification of MyD88, as detected in B (n=15 larvae/treatment). (D) Expression of *il1b* within controlMO-, and MyD88SBMO-injected larvae following MSU crystal injection. (E) Quantification of *il1b* expression, as detected in D. (F) Immunofluorescence of Tnfa within controlMO- and MyD88SBMO-injected *Tg(mpeg1:EGFP)* larvae following MSU crystal injection. Control MO-MSU image is the same as in Supplemental Figure 3E. (G) Quantification of Tnfa, as detected in F (n=15 larvae/treatment). Control MO-MSU sample is the same as in Supplemental Figure 3F. (H) Immunofluorescence detection of neutrophils within controlMO- and MyD88SBMO-injected *Tg(lyz:EGFP)* larvae following MSU crystal injection. Control MO-MSU image is the same as in Supplemental Figure 3B/3G. (I) Quantification of neutrophils, as detected in H (n=13-15 larvae/treatment). Control MO-MSU samples are the same as in Supplemental Figure 3C/3H/3I. Arrow marks *il1b* expression in hindbrain. Data for B-I pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. ***P*<0.01; ****P*<0.001; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 50 μm (B, H) and 100 μm in (D).


A
3 hpi
DMSO-MSU(27/32)
il1b
3 hpi
Triptolide-MSU(21/25)
il1b
B
il1b
High
Low
None
Percent larvae
100%
75%
50%
0%
n=24
n=32
n=23
n=25
PBS
DMSO
100 nM
1 μM
MSU
C
3 hpi
DMSO-MSU
3 hpi
Triptolide
MSU
Tnfa (546)
Tnfa (546)
mpeg1:EGFP (488)
D
Tnfa (fluorescence intensity/macrophage)
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
****
****
PBS
DMSO
100 nM
1 μM
MSU
E
6 hpi
DMSO-MSU
6 hpi
Triptolide
MSU
(488)
lyz:EGFP
F
Neutrophils
100
80
60
40
20
0
n.s.
n.s.
****
****
****
****
***
DMSO
100 nM
1 μM
1 hpi
3 hpi
6 hpi
9 hpi
G
3 hpi
DMSO-MSU
3 hpi
dnikbaa
MSU
Tnfa (546)
Tnfa (546)
mpeg1:EGFP (488)
H
Tnfa (fluorescence intensity/macrophage)
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
****
PBS
DMSO-MSU
dnikbaa
+ MSU
I
6 hpi
DMSO-MSU
6 hpi
dnikbaa
MSU
(488)
lyz:EGFP
J
Neutrophils
100
80
60
40
20
0
n.s.
**
****
****
DMSO
dnikbaa
1 hpi
3 hpi
6 hpi
9 hpi

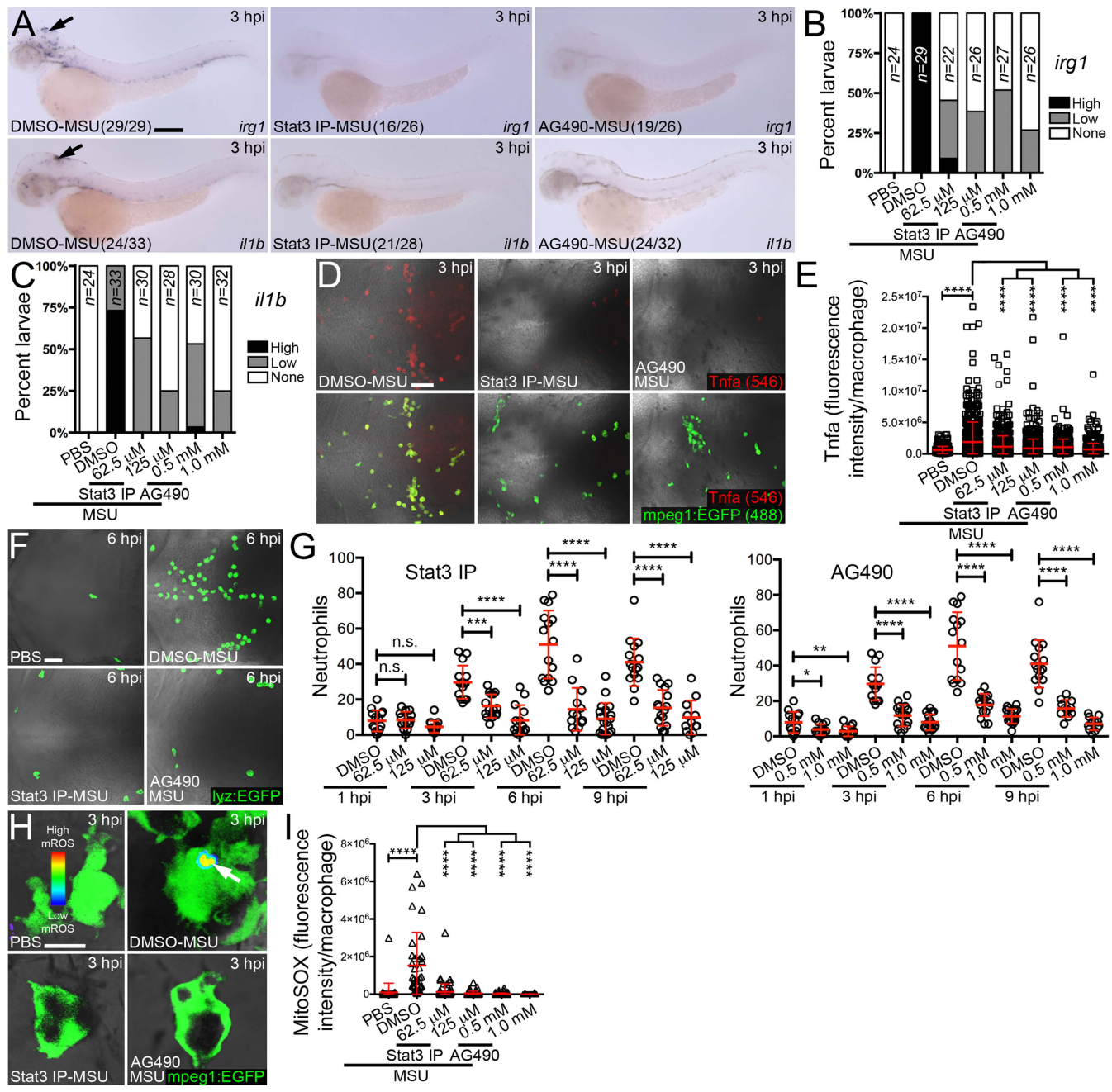
Supplemental Figure 5. Blocking NF-κB signaling suppresses MSU crystal-driven macrophage activation and neutrophil recruitment. (A) Expression of *il1b* within MSU-injected DMSO-, and triptolide-treated larvae. DMSO-MSU image is the same as in Figure 3A. (B) Quantification of *il1b* expression, as detected in A. DMSO-MSU sample is the same as in Figure 3B. (C) Immunofluorescence of Tnfa within MSU crystal-injected DMSO- and triptolide-treated *Tg(mpeg1:EGFP)* larvae. DMSO-MSU image is the same as in Figure 3C. (D) Quantification of Tnfa, as detected in C (n=15 larvae/treatment). DMSO-MSU sample is the same as presented in Figures 1G (3 hpi)/3D. (E) Immunofluorescence detection of neutrophils within MSU crystal-injected DMSO- and triptolide-treated *Tg(lyz:EGFP)* larvae. DMSO-MSU image is the same as in Figure 4A and Supplemental Figure 3B. (F) Quantification of neutrophils, as detected in E (n=13-15 larvae/treatment). DMSO-MSU samples are the same as in Figures, 2B/2E/2F/4B/4C and Supplemental Figure 3D. (G) Immunofluorescence of Tnfa within the hindbrain of MSU crystal-injected DMSO-treated and *mpeg1:dnikbaa*-injected *Tg(mpeg1:EGFP)* larvae. (H) Quantification of Tnfa, as detected in G (n=15 larvae/treatment). DMSO-MSU sample is the same as in panel D and Figures 1G (3 hpi)/3D. (I) Immunofluorescence detection of neutrophils within the hindbrain of DMSO-treated and *mpeg1:dnikbaa*-injected *Tg(lyz:EGFP)* larvae following MSU crystal injection. DMSO-MSU image is the same as in Figure 2D. (J) Temporal quantification of neutrophils, as detected in I (n=13-15 larvae/treatment). DMSO-MSU samples are the same as in panel F, Figure 2B/2E/2F and Supplemental Figure 3D. Arrow marks *il1b* expression in

hindbrain. All data pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. ** P<0.01; *** P<0.001; **** P<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test (D, F, H) and Student's t test (J). Scale bars, 100 μm in (A) and 50 μm (C, E).


A
irg1
Control MO
Irg1 SBMO 1
irg1
Control MO
Irg1 SBMO 2
B
3 hpi
Control MO-MSU(27/34)
il1b
3 hpi
Irg1 SBMO2-MSU(22/33)
il1b
C
il1b
High
Low
None
Percent larvae
100%
75%
50%
25%
0%
n=25
n=34
n=31
n=33
Control (PBS)
Control
0.375 pmol
0.75 pmol
MSU
D
3 hpi
3 hpi
Control MO-MSU
Irg1
SBMO2
MSU
Tnfa (546)
Tnfa (546)
mpeg1:EGFP (488)
E
Tnfa (fluorescence intensity/macrophage)
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
****
****
PBS
Control
0.375 pmol
0.75 pmol
MSU
F
6 hpi
Control MO-MSU
6 hpi
Irg1
SBMO2
MSU
(488)
lyz:EGFP
G
Neutrophils
100
80
60
40
20
0
n.s.
n.s.
****
****
****
****
****
****
Control
0.375 pmol
0.75 pmol
1 hpi
3 hpi
6 hpi
9 hpi
H
WT (irg1)
Irg1 gRNA#1
Irg1 gRNA#2
E1
I1
E2
I2
E3
I3
E4
I4
E5
2,750 bp
E1
E2/5
gRNA-inj./WT
GAACCAGAACACCGGTCTTTAACACCGTGCTTCAGT(2,732 bp)TCTGCAGGAGCCCCCATGGCCAACGCTGCCACCCAA
-2,772/+11
GAACCAGAAC----------TGGCCAATCAACATGGCCAACGCTGCCACCCAA
-2,787
GAACCA----------CTGCCACCCAA
-2,776/+1
GAACCAGA----------TTGGCCAACGCTGCCACCCAA
-2,787
GAACCA----------CTGCCACCCAA
-2,790/+3
GA----------GGAGCTGCCACCCAA
I
Relative expression (cxcl8-l1)
8
6
4
2
0
****
n.s.
n.s.
****
****
***
Control
Irg1 SBMO1
Irg1 SBMO2
Control
Irg1 SBMO1
Irg1 SBMO2
PBS
MSU
Relative expression (cxcl8-l2)
8
6
4
2
0
****
n.s.
n.s.
****
****
Control
Irg1 SBMO1
Irg1 SBMO2
Control
Irg1 SBMO1
Irg1 SBMO2
PBS
MSU
J
3 hpi
High
mROS
Low
mROS
Control MO
MSU
3 hpi
Irg1 SBMO2
MSU
mpeg1:EGFP
K
MitoSOX (fluorescence intensity/macrophage)
8×10^6
6×10^6
4×10^6
2×10^6
0
****
****
****
PBS
Control
0.375 pmol
0.75 pmol
MSU

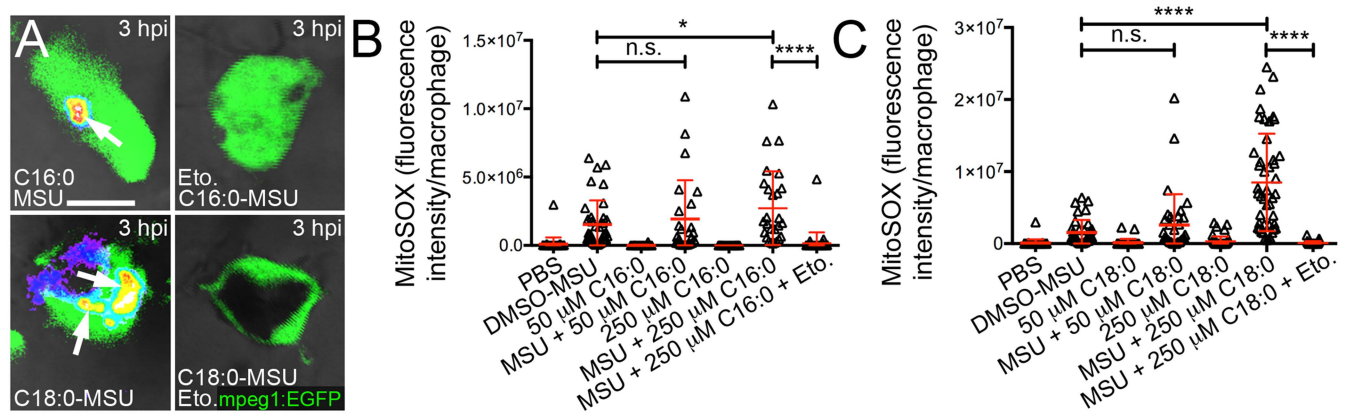
Supplemental Figure 6. Irg1 contributes to MSU crystal-driven macrophage activation and neutrophil recruitment. (A) RT-PCR for *irg1* from control MO-, Irg1 SBMO1- and Irg1 SBMO2-injected larvae following MSU crystal injection (arrowheads, alternatively spliced transcripts). (B) Expression of *il1b* within control MO- and Irg1 SBMO2-injected larvae following MSU crystal injection. Control MO-MSU image is the same as in Figure 5A. (C) Quantification of *il1b* expression, as detected in B. Control MO-MSU sample is the same as in Figure 5B. (D) Immunofluorescence of Tnfa within the hindbrain of control MO- and Irg1 SBMO2-injected *Tg(mpeg1:EGFP)* larvae following MSU crystal injection. Control MO-MSU image is the same as in Figure 5D and Supplemental Figures 3E/4F. (E) Quantification of Tnfa, as detected in D (n=15 larvae/treatment). Control MO-MSU sample is the same as in Figure 5E and Supplemental Figures 3F/4G. (F) Immunofluorescence detection of neutrophils within the hindbrain of control MO- and Irg1 SBMO2-injected *Tg(lyz:EGFP)* larvae following MSU crystal injection. Control MO-MSU image is the same as in Figure 6A and Supplemental Figures 3B/3G/4H. (G) Temporal quantification of neutrophils, as detected in F (n=13-15 larvae/treatment). Control MO-MSU samples are the same as in Figure 6B and Supplemental Figures 3C/3H/3I/4I. (H) Target sites for Irg1 gRNAs#1/2 and examples of deletions detected by sequencing amplicons generated from individual gRNA/cas9-injected larvae using highlighted primers, compared to gRNA-only-injected larvae (-/+, deletions and additions [in grey], respectively). I, intron; E, exon; red sequence, gRNA targets (PAM sequence underlined). (I) Expression of *cxcl8-l1 and cxcl8-l2* within control MO-, Irg1 SBMO1 and Irg1

SBMO2-injected larvae following PBS and MSU crystal injection (as detected by qPCR at 3 hpi, n=25-30 larvae per sample, 3 biological replicates). (J) Macrophage mROS production (white arrow) within the hindbrain of control MO- and Irg1 SBMO2-injected *Tg(mpeg1:EGFP)* larvae following MSU crystal injection (MitoSOX signal displayed as a heatmap, warmer colors represents higher levels of mROS). Control MO-MSU image is the same as in Figure 6D. (K) Quantification of macrophage-specific mROS production, as detected in J (n=10 larvae/treatment). Control MO-MSU sample is the same as in Figure 6E. Black arrow marks *il1b* expression in hindbrain. Data for B-G and J,K pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. Error bars, means ± SD. ****P*<0.001; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 100 μm (B), 50 μm (D, F) and 10 μm (J).

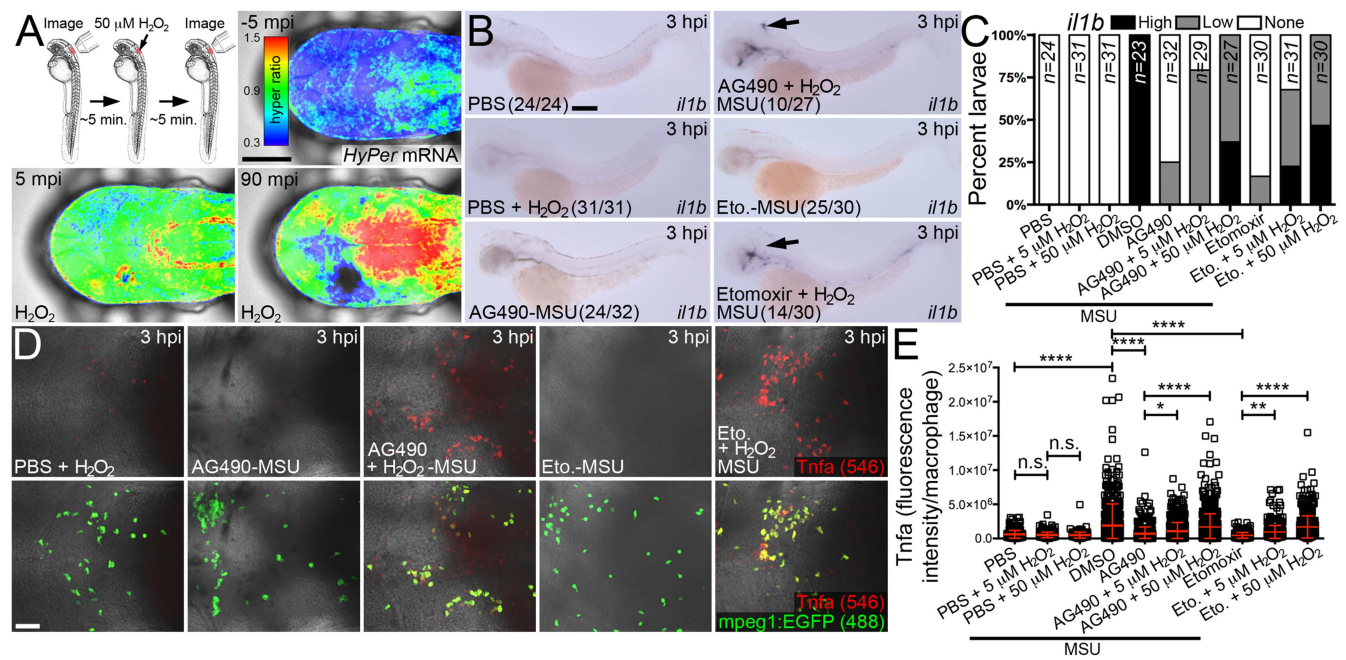

A
3 hpi
DMSO-MSU(29/29)
irg1
Stat3 IP-MSU(16/26)
AG490-MSU(19/26)
DMSO-MSU(24/33)
il1b
Stat3 IP-MSU(21/28)
AG490-MSU(24/32)
B
Percent larvae
irg1
High
Low
None
n=24
n=29
n=22
n=26
n=27
n=26
PBS
DMSO
62.5 μM
125 μM
0.5 mM
1.0 mM
Stat3 IP
AG490
MSU
C
il1b
n=24
n=33
n=30
n=28
n=30
n=32
D
DMSO-MSU
Stat3 IP-MSU
AG490 MSU
Tnfa (546)
mpeg1:EGFP (488)
E
Tnfa (fluorescence intensity/macrophage)
F
6 hpi
PBS
DMSO-MSU
Stat3 IP-MSU
AG490 MSU
lyz:EGFP
G
Stat3 IP
AG490
Neutrophils
n.s.
1 hpi
3 hpi
6 hpi
9 hpi
H
High mROS
Low mROS
mpeg1:EGFP
I
MitoSOX (fluorescence intensity/macrophage)

Supplemental Figure 7. JAK/STAT signaling contributes to macrophage activation and neutrophil recruitment in response to MSU crystals. (A) Expression of *irg1* and *il1b* within MSU crystal-injected DMSO-, Stat3 inhibitor peptide(Stat3 IP)- and AG490-treated larvae. (B and C) Quantification of *irg1* (B) and *il1b* (C) expression, as detected in A. (D) Immunofluorescence of Tnfa within the hindbrain of MSU crystal-injected DMSO-, Stat3 IP- and AG490-treated *Tg(mpeg1:EGFP)* larvae. DMSO-MSU image is the same as in Figure 3C and Supplemental Figure 5C. (E) Quantification of Tnfa, as detected in D (n=15 larvae/treatment). DMSO-MSU sample is the same as in Figures 1G (3 hpi)/3D/5F and Supplemental Figures 5D/5H. (F) Immunofluorescence detection of neutrophils within the hindbrain of PBS-injected and MSU crystal-injected DMSO-, Stat3 IP- and AG490-treated *Tg(lyz:EGFP)* larvae. PBS image is the same as in Figure 4A. (G) Temporal quantification of neutrophils, as detected in F (n=13-15 larvae/treatment). DMSO-MSU samples are the same as in Figures, 2B/2E/2F/4B/4C/6C and Supplemental Figures 3D/5F/5J. (H) Macrophage mROS production (white arrow) within the hindbrain of MSU crystal-injected DMSO-, Stat3 IP- and AG490-treated *Tg(mpeg1:EGFP)* larvae (MitoSOX signal displayed as a heatmap, warmer colors represents higher levels of mROS). (I) Quantification of macrophage-specific mROS production, as detected in H (n=10 larvae/treatment). DMSO-MSU sample is the same as in Figure 6F. Black arrows mark *irg1*/*il1b* expression in hindbrain. All data pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. **P*<0.05; ***P*<0.01; ****P*<0.001;

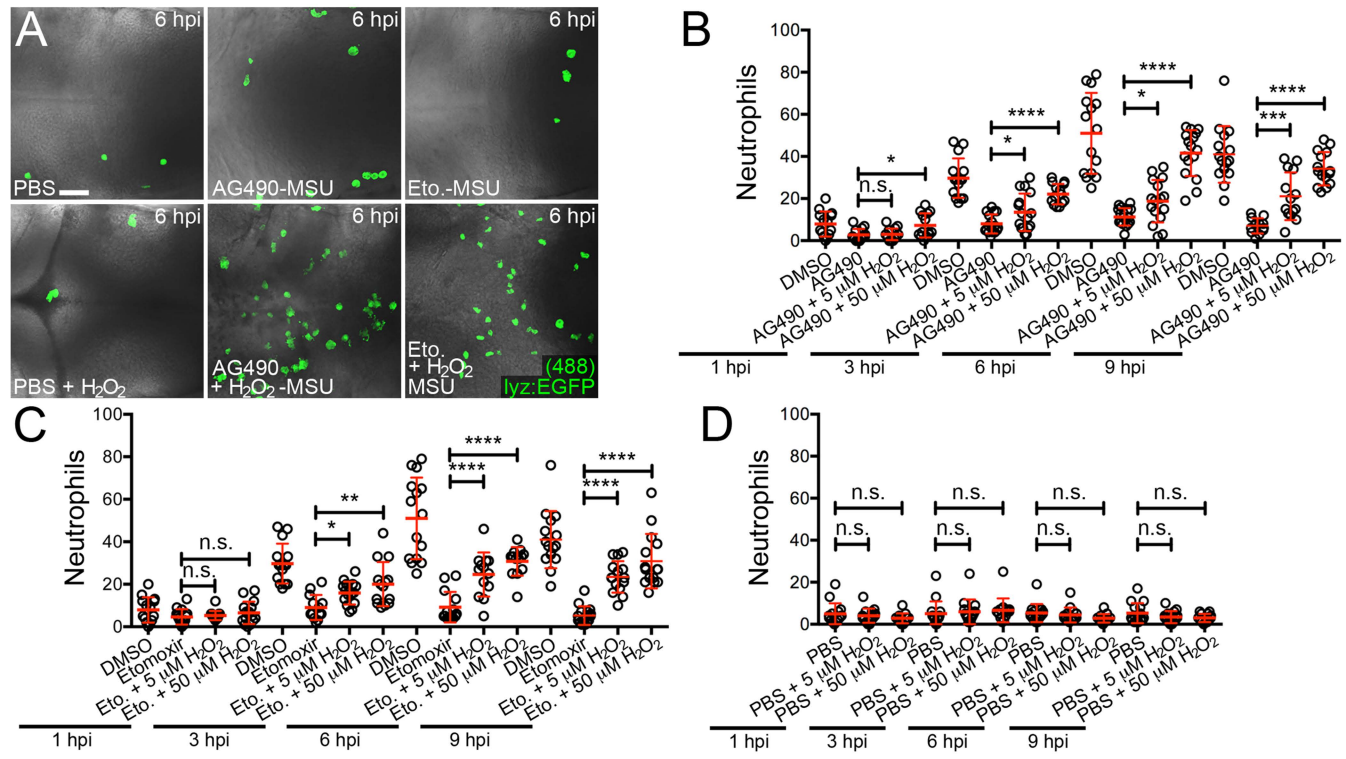
****P<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 100 μm (A), 50 μm (D, F) and 10 μm (H).


A
3 hpi
C16:0
MSU
3 hpi
Eto.
C16:0-MSU
3 hpi
C18:0-MSU
3 hpi
C18:0-MSU
Eto.mpeg1:EGFP
B
MitoSOX (fluorescence intensity/macrophage)
1.5×10^7
1.0×10^7
5.0×10^6
0.0
n.s.
*
****
PBS
DMSO-MSU
50 μM C16:0
MSU + 50 μM C16:0
250 μM C16:0
MSU + 250 μM C16:0
MSU + 250 μM C16:0 + Eto.
C
MitoSOX (fluorescence intensity/macrophage)
3×10^7
2×10^7
1×10^7
0
n.s.
****
****
PBS
DMSO-MSU
50 μM C18:0
MSU + 50 μM C18:0
250 μM C18:0
MSU + 250 μM C18:0
MSU + 250 μM C18:0 + Eto.

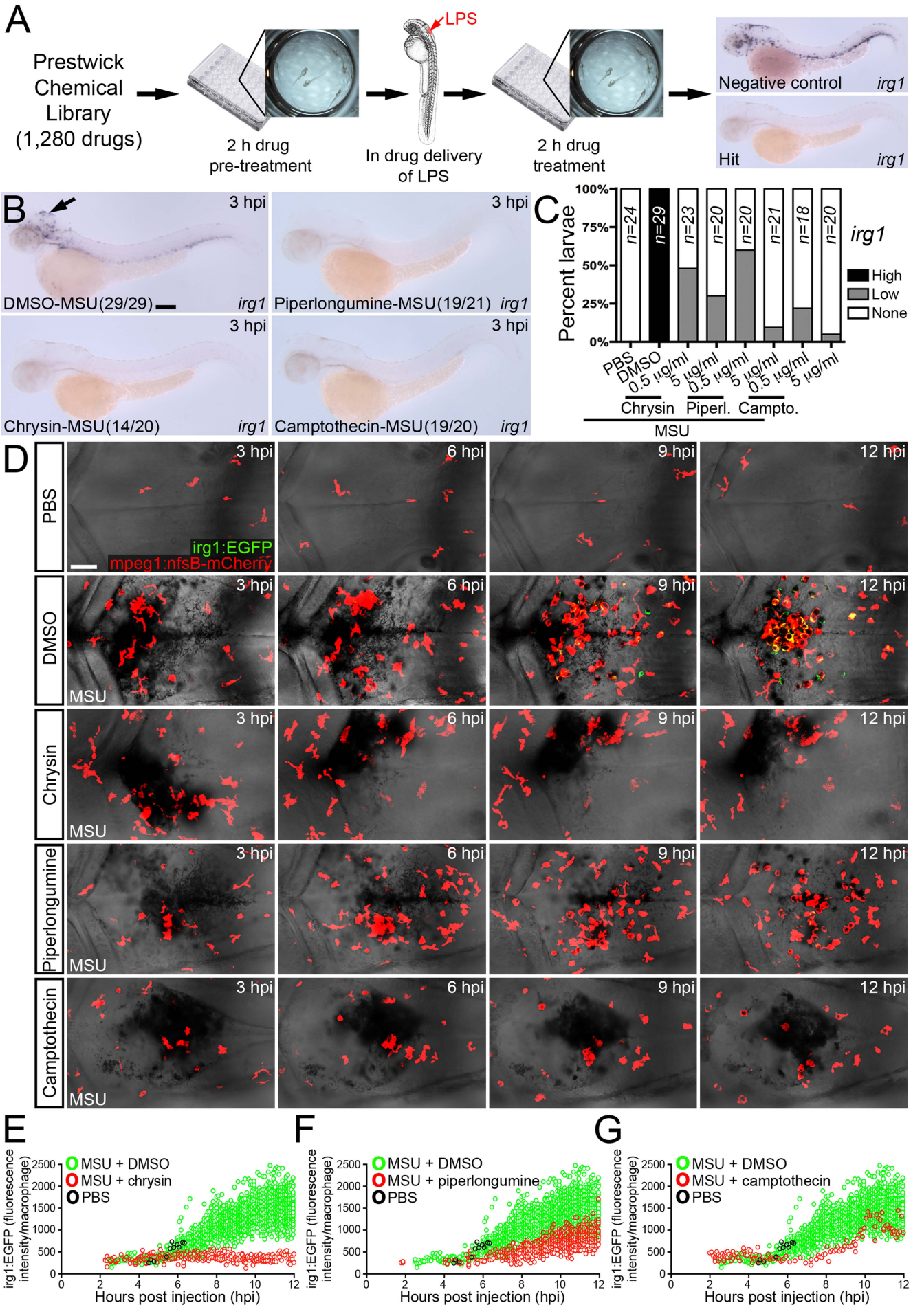
Supplemental Figure 8. Hindbrain injection of C16:0 or C18:0 elevates MSU crystal-driven macrophage-specific mROS production through a FAO-dependent mechanism. (A) Live imaging of macrophage mROS production within the hindbrain of *Tg(mpeg1:EGFP)* larvae co-injected with MSU crystals and C16:0 or C18:0 with and without etomoxir (Eto.) treatment. (B and C) Quantification of macrophage-specific mROS production, as detected in A, for C16:0 (B) and C18:0 (C) treatments (n=10 larvae/treatment). DMSO-MSU samples are the same as in Figures 6F/8E and Supplemental Figure 7I. All data pooled from 2 independent experiments. All error bars, means ± SD. **P*<0.05; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bar, 10 μm (A).


A
Image
50 μM H2O2
Image
~5 min.
~5 min.
-5 mpi
1.5
hyper ratio
0.9
0.3
HyPer mRNA
5 mpi
90 mpi
H2O2
H2O2
B
3 hpi
PBS (24/24)
il1b
AG490 + H2O2
MSU (10/27)
PBS + H2O2 (31/31)
Eto.-MSU (25/30)
AG490-MSU (24/32)
Etomoxir + H2O2
MSU (14/30)
C
il1b
High
Low
None
Percent larvae
100%
75%
50%
25%
0%
n=24
n=31
n=31
n=23
n=32
n=29
n=27
n=30
n=31
n=30
PBS
PBS + 5 μM H2O2
PBS + 50 μM H2O2
DMSO
AG490
AG490 + 5 μM H2O2
AG490 + 50 μM H2O2
Etomoxir
Eto. + 5 μM H2O2
Eto. + 50 μM H2O2
MSU
D
3 hpi
PBS + H2O2
AG490-MSU
AG490 + H2O2 -MSU
Eto.-MSU
Eto. + H2O2 MSU
Tnfa (546)
mpeg1:EGFP (488)
E
Tnfa (fluorescence intensity/macrophage)
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
n.s.
*
**
PBS
PBS + 5 μM H2O2
PBS + 50 μM H2O2
DMSO
AG490
AG490 + 5 μM H2O2
AG490 + 50 μM H2O2
Etomoxir
Eto. + 5 μM H2O2
Eto. + 50 μM H2O2
MSU

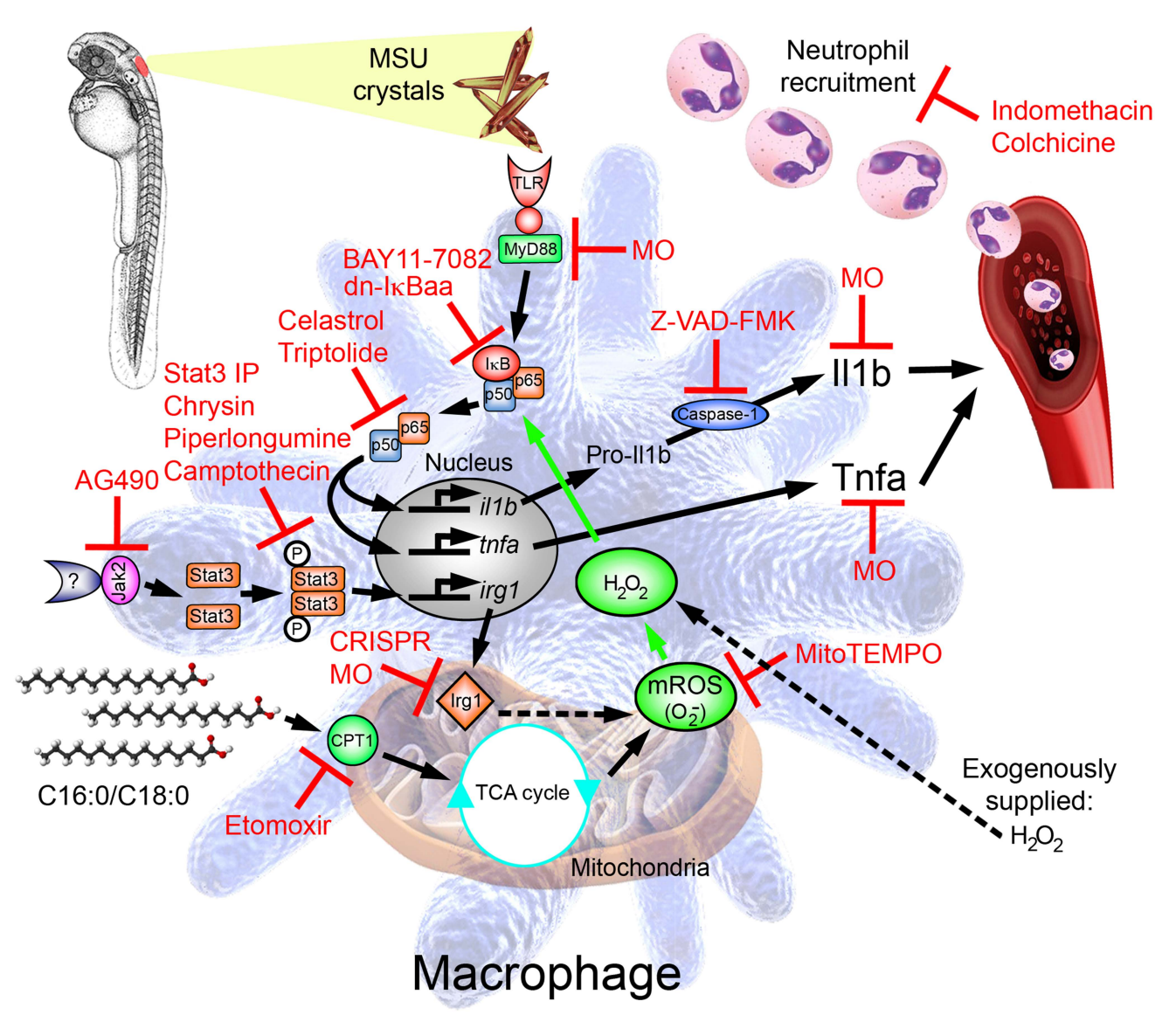
Supplemental Figure 9. Exogenous H_2O_2 can rescue MSU crystal-driven macrophage activation following endogenous mROS depletion. (A) Schematic showing delivery of exogenously supplied H_2O_2 to the hindbrain region and ratiometric HyPer imaging of H_2O_2 levels (488/405 nm ratio is displayed as a heat map, with warmer colors representing higher levels of H_2O_2) within the hindbrain region of *HyPer* mRNA-injected larvae 5 min before (-5 min post injection, mpi) and after (5 and 90 mpi) injection of 50 μM H_2O_2. (B) Expression of *il1b* within PBS-injected (with and without co-injected 50 μM H_2O_2) and MSU crystal-injected AG490(1 mM)- and etomoxir(Eto., 250 μM)-treated (with and without co-injected 50 μM H_2O_2) larvae. PBS image is the same as in Figures 1B/7A and Supplemental Figure 1G. (C) Quantification of *il1b* expression, as detected in B. DMSO-MSU sample is the same as in Figure 10E. (D) Immunofluorescence of Tnfa within the hindbrain of PBS/H_2O_2(50 μM)-injected *Tg(mpeg1:EGFP)* larvae and MSU crystal-injected AG490(1.0 mM)- and etomoxir(250 μM)-treated larvae, with and without co-injected 50 μM H_2O_2. (E) Quantification of Tnfa, as detected in D (n=15 larvae/treatment). DMSO-MSU sample is the same as in Figures 1G (3 hpi)/3D/5F/7D/11B and Supplemental Figures 5D/5H/7E. Arrows mark *il1b* expression in hindbrain. Data for B-E pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. All error bars, means ± SD. **P*<0.05; ***P*<0.01; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bars, 100 μm (A, B) and 50 μm (D).


A
6 hpi
PBS
AG490-MSU
Eto.-MSU
PBS + H2O2
AG490 + H2O2 -MSU
Eto. + H2O2 MSU
(488) lyz:EGFP
B
Neutrophils
DMSO
AG490
AG490 + 5 μM H2O2
AG490 + 50 μM H2O2
n.s.
1 hpi
3 hpi
6 hpi
9 hpi
C
Neutrophils
DMSO
Etomoxir
Eto. + 5 μM H2O2
Eto. + 50 μM H2O2
D
Neutrophils
PBS
PBS + 5 μM H2O2
PBS + 50 μM H2O2

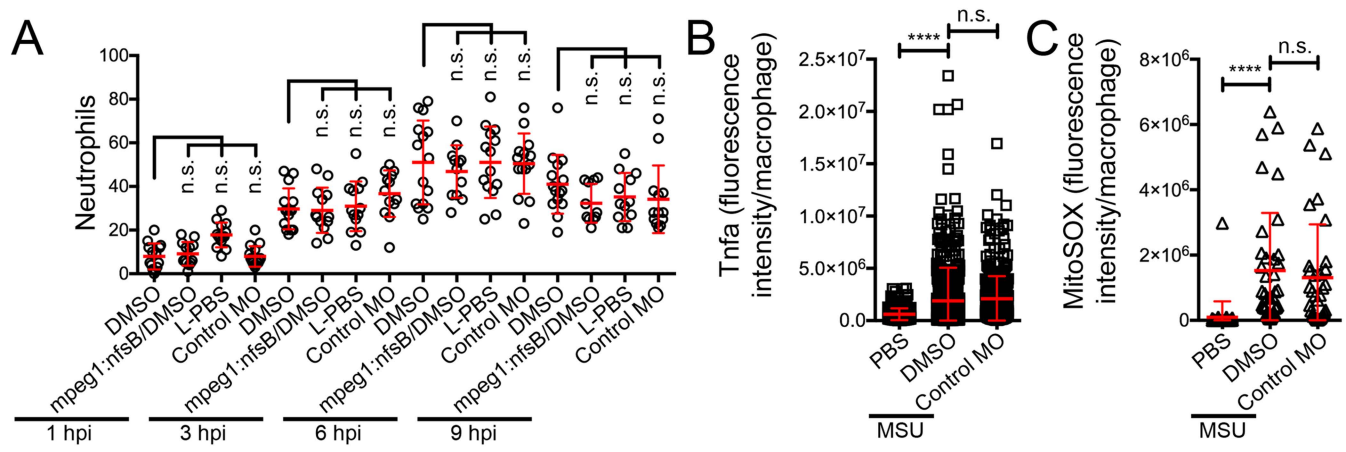
Supplemental Figure 10. Exogenous H_2O_2 can rescue MSU crystal-driven neutrophil recruitment following endogenous mROS depletion. (A) Immunofluorescence detection of neutrophils within the hindbrain of PBS-injected *Tg(lyz:EGFP)* larvae (with and without co-injected 50 μM H_2O_2) and MSU crystal-injected AG490(1.0 mM)- and etomoxir(250 μM)-treated larvae (with and without co-injected 50 μM H_2O_2). PBS image is the same as in Figure 8A. (B and C) Quantification of neutrophils, as detected in A, for AG490/H_2O_2- (B) and etomoxir/H_2O_2- (C) treatments (n=13-15 larvae/treatment). DMSO-MSU samples are the same as in Figures, 2B/2E/2F/4B/4C/6C/8B/8C/11D/11E and Supplemental Figures 3D/5F/5J/7G. (D) Quantification of neutrophils, as detected in A, for PBS/H_2O_2-treatments (n=13-15 larvae/treatment). All data pooled from 2 independent experiments. All error bars, means ± SD. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test. Scale bar, 50 μm (A).


A
Prestwick Chemical Library (1,280 drugs)
2 h drug pre-treatment
LPS
In drug delivery of LPS
2 h drug treatment
Negative control irg1
Hit irg1
B
3 hpi
DMSO-MSU(29/29) irg1
Piperlongumine-MSU(19/21) irg1
Chrysin-MSU(14/20) irg1
Camptothecin-MSU(19/20) irg1
C
Percent larvae
100%
75%
50%
25%
0%
n=24
n=29
n=23
n=20
n=20
n=21
n=18
n=20
irg1
High
Low
None
PBS
DMSO
0.5 µg/ml
5 µg/ml
0.5 µg/ml
5 µg/ml
0.5 µg/ml
5 µg/ml
Chrysin
Piperl.
Campto.
MSU
D
PBS
DMSO
Chrysin
Piperlongumine
Camptothecin
3 hpi
6 hpi
9 hpi
12 hpi
irg1:EGFP
mpeg1:nfsB-mCherry
MSU
E
F
G
irg1:EGFP (fluorescence intensity/macrophage)
MSU + DMSO
MSU + chrysin
MSU + piperlongumine
MSU + camptothecin
PBS
Hours post injection (hpi)

Supplemental Figure 11. Phenotypic drug screen uncovers chrysin, piperlongumine and camptothecin as inhibitors of *irg1* expression in activated macrophages. (A) Schematic of drug screen to uncover repurposed drugs that suppress *irg1* expression in activated macrophages. (B) Expression of *irg1* within MSU crystal-injected DMSO-, chrysin-, piperlongumine- and camptothecin-treated larvae. DMSO-MSU image is the same as in Supplemental Figure 7A. (C) Quantification of *irg1* expression, as detected in B. DMSO-MSU sample is the same as presented in Supplemental Figure 7B. (D) Time-lapse confocal imaging of macrophage activation (EGFP expression within $mCherry^{+}$ macrophages) within the hindbrain of MSU crystal-injected DMSO-, chrysin-, piperlongumine- and camptothecin-treated *Tg(irg1:EGFP;mpeg1:nfsB-mCherry)* larvae. (E-G) Quantification of macrophage activation (measured as fluorescence intensity of EGFP within individual $mCherry^{+}$ macrophages), as detected in D, within PBS-, MSU crystal + DMSO-, MSU crystal + chrysin- (E), MSU crystal + piperlongumine- (F) and MSU crystal + camptothecin-treated (G) *Tg(irg1:EGFP;mpeg1:nfsB-mCherry)* larvae. MSU + DMSO and PBS samples are the same in panels E-G. Arrow marks *irg1* expression in hindbrain. Data for B and C pooled from 2 independent experiments. Numbers in parentheses, frequency of larvae with displayed phenotype. Scale bars, 100 μm (B) and 50 μm (D).


MSU
crystals
Neutrophil
recruitment
Indomethacin
Colchicine
TLR
MyD88
MO
BAY11-7082
dn-IκBaa
Celastrol
Triptolide
Stat3 IP
Chrysin
Piperlongumine
Camptothecin
AG490
IκB
p50
p65
Nucleus
il1b
tnfa
irg1
Pro-Il1b
Z-VAD-FMK
Caspase-1
Il1b
MO
Tnfa
MO
?
Jak2
Stat3
Stat3
P
H_2O_2
CRISPR
MO
Irg1
mROS
(O_2^-)
MitoTEMPO
CPT1
C16:0/C18:0
Etomoxir
TCA cycle
Mitochondria
Exogenously
supplied:
H_2O_2
Macrophage

Supplemental Figure 12. Model of Irg1-dependent, FAO/mROS-driven activation of macrophages in response to MSU crystals. Pharmacologic and genetic interventions used in this study to support this model are shown in red.


A
Neutrophils
100
80
60
40
20
0
n.s.
DMSO
mpeg1:nfsB/DMSO
L-PBS
Control MO
1 hpi
3 hpi
6 hpi
9 hpi
B
Tnfa (fluorescence intensity/macrophage)
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
****
n.s.
PBS
DMSO
Control MO
MSU
C
MitoSOX (fluorescence intensity/macrophage)
8×10^6
6×10^6
4×10^6
2×10^6
0
****
n.s.
PBS
DMSO
Control MO
MSU

Supplemental Figure 13. Comparisons between control groups used in this study. (A) Control groups used when assessing the effects of pharmacologic treatments (DMSO), genetic depletion of macrophages (mpeg1:nfsB/DMSO), liposomal clodronate-mediated depletion of macrophages (L-PBS) and MO-mediated depletion studies (Control MO) on the temporal recruitment of neutrophils following MSU crystal injection (n=13-15 larvae/treatment). DMSO-MSU samples are the same as in Figures, 2B/2E/2F/4B/4C/6C/8B/8C/11D/11E/13B-D and Supplemental Figures 3D/5F/5J/7G/10B/10C. mpeg1:nfsB/DMSO and L-PBS samples are the same as in Supplemental Figure 2J and 2K, respectively. Control MO samples are the same as in Figure 6B and Supplemental Figures 3C/3H/3I/4I/6G. (B) Control groups used when assessing the effects of pharmacologic treatments (DMSO) and MO-mediated depletion studies (Control MO) on macrophage-specific Tnfa production following MSU crystal injection (n=15 larvae/treatment). DMSO-MSU sample is the same as in Figures 1G (3 hpi)/3D/5F/7D/11B/12D and Supplemental Figures 5D/5H/7E/9E. Control MO-MSU sample is the same as in Figure 5E and Supplemental Figures 3F/4G/6E. (C) Control groups used when assessing the effects of pharmacologic treatments (DMSO) and MO-mediated depletion studies (Control MO) on macrophage-specific mROS production (MitoSOX signal) following MSU crystal injection (n=10 larvae/treatment). DMSO-MSU sample is the same as in Figures 6F/8E/13F and Supplemental Figures 7I/8B/8C. Control MO-MSU sample is the same as in Figure 6E and Supplemental Figure 6K. All data pooled from 2 independent experiments. All

error bars, means ± SD. ****P<0.0001; n.s., not significant, one-way ANOVA, Dunnett's post hoc test.