Figure S1 (with Fig. 1)

A Bone marrow-derived macrophages (BMDMs) - genomic DNA

wt Control, Trpm7^{fl} Lck Cre, Trpm7 fl, Trpm7^{fl} (LysM Cre)

Trpm7^{fl}
Trpm7
Trpm7^{fl} (LysM Cre)

B BMDMs - mRNA

Trpm7 (exon 17)

mRNA Fold Change (relative to wt)

$p = 0.001$

Trpm7$^{+/+}$
Trpm7$^{-/-}$

C

273 mOsm/Kg
285 mOsm/Kg

Sampled @ 10kHz
low-pass filter @ 5kHz
HP: 0 mV   +100 mV
-100 mV   400 ms

110 Cs-gluconate
10 HEDTA, 0.5 NaCl
0.75 CaCl$_2$
1.8 Cs$_4$BAPTA
2 Na$_2$ATP
10 HEPES, pH 7.3

135 Na-MeSO4
5 Cs-gluconate
2 CaCl$_2$
10 HEPES, pH 7.3

D Isolated bone marrow

Cells per mouse mass (× 10^6/g)

n.s.

Trpm7$^{+/+}$ Trpm7$^{-/-}$

E BMDM - Day 7 Post-isolation

F4/80 / CD11b

3.27 — 92.4
1.22 — 3.06

3.09 — 93.2
0.90 — 2.85

F4/80 FMO
7.82E-3 — 1.13
3.16 — 95.7

CD11b FMO
87.7 — 0.51
11.7 — 8.81E-3

F

% CD11b+ F4/80+

n.s.   n.s.

d4   d7
Days post-isolation

G

Cells per plate (×10^6)

n.s.

Trpm7$^{+/+}$
Trpm7$^{-/-}$

Days post-isolation

H *Trpm7 (exon 17)*

mRNA Fold Change (relative to untreated)

Trpm7$^{+/+}$
Trpm7$^{-/-}$

100 ng/mL LPS   - +   - +

I

Il1b   *Il6*   *Tnfa*

mRNA Fold Change (relative to untreated)

n.s.   n.s.   n.s.

100 ng/mL LPS   - + - +

Trpm7$^{fl/fl}$
Trpm7$^{+/+}$ (LysM Cre)

J

Nlrp3   *Tnfa*

mRNA Fold Change (relative to untreated)

$p=0.0003$ $p=0.008$

Trpm7$^{+/+}$
Trpm7$^{-/-}$

100 ng/mL LPS
3 hours   - + - +

K *Rsad2*

mRNA Fold Change (relative to untreated)

Trpm7$^{+/+}$
Trpm7$^{-/-}$

n.s.   n.s.

Pam3CSK4   ODN 1826
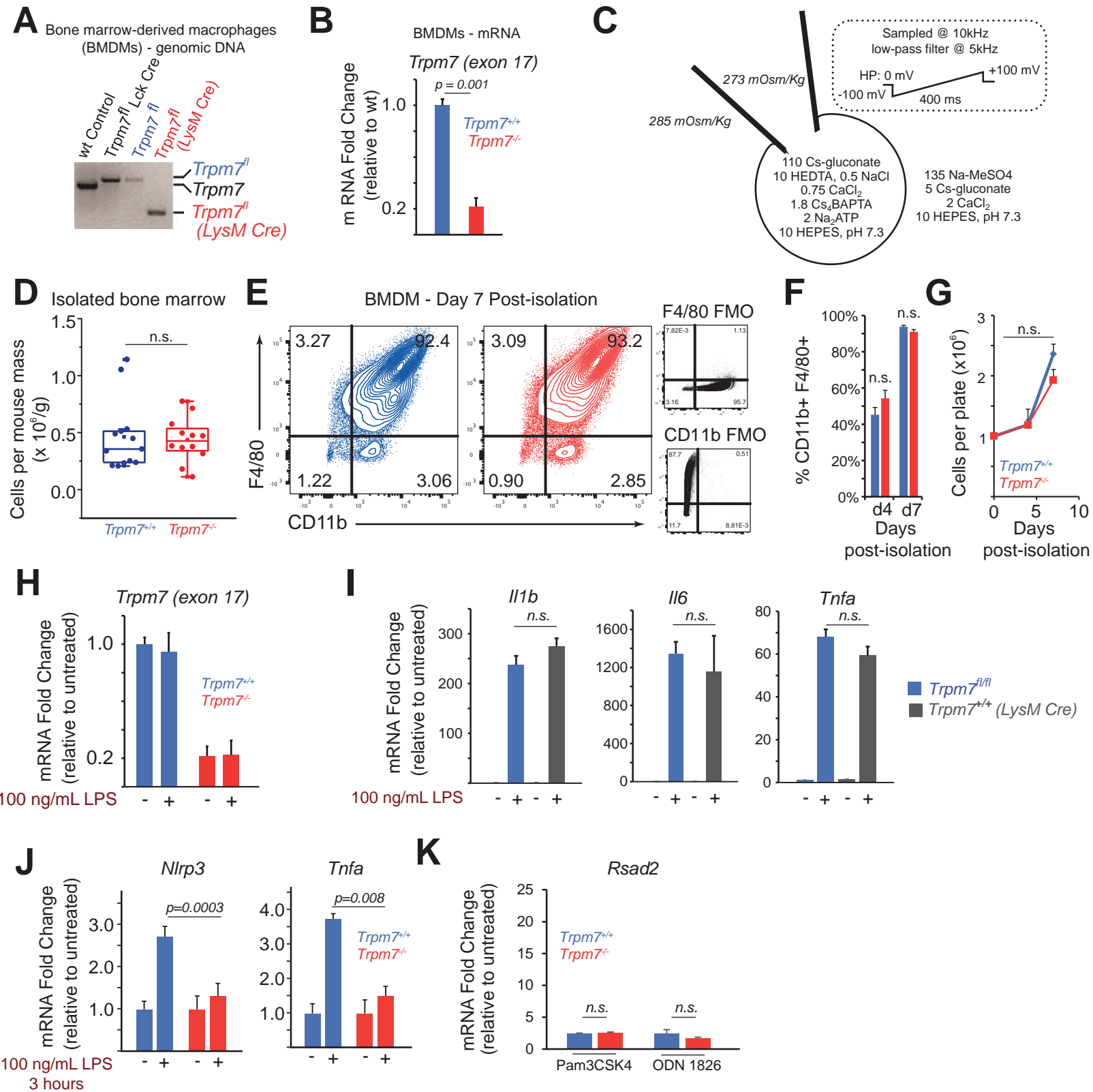
Figure S1 (with Fig. 1)

(A) Genotyping of the targeted *Trpm7* genomic locus amplified via PCR from indicated BMDMs. Increased amplicon size reflects the insertion of *loxP* sites in macrophages obtained from *Trpm7*$^{fl/fl}$ mice. Decreased size reflects Cre-mediated excision of loxP-flanked *exon 17* of *Trpm7* in macrophages obtained from *Trpm7*$^{fl/fl}$ *(LysM Cre)* mice but not in macrophages isolated from *Trpm7*$^{fl/fl}$*(Lck Cre)* mice, used as controls.

(B) qRT-PCR analysis using primers directed against loxP-flanked *exon 17* shows the relative difference in the TRPM7 mRNA in BMDMs from *Trpm7*$^{fl/fl}$ *(LysM Cre)* (*Trpm7*$^{-/-}$) mice and *Trpm7*$^{fl/fl}$ *(Trpm7*$^{+/+}$) with intact exon 17. Means representative of n=5 measurements. Error bars represent SD.

(C) Schematic of electrophysiology conditions used for whole-cell patch clamp recordings shown in *Figure 1B*.

(D) Live cell counts from freshly isolated bone marrow (day 0) shown as statistical box charts. Trypan blue-excluded cell counting was carried out using an automated cell counter.

(E) Flow cytometry-based phenotypic characterization of BMDMs on day 7 after *ex vivo* differentiation. Representative bivariant cytographs are shown (n=4).

(F) Quantification of measurements depicted in *panel E*, error bars represent SEM (n=4).

(G) Live cell counts of BMDMs in culture during differentiation. After isolation, BMDMs were plated at a density of 1 x 10^6 cells per plate and collected for counting at indicated time points. Cell counts were determined via trypan blue exclusion. Error bars represent SEM (n=4).

(H) qRT-PCR analysis of LPS-induced changes in TRPM7 mRNA expression. BMDMs were treated with LPS (100 ng/mL, 3h) prior to RNA isolation. Error bars represent SD (n=3).
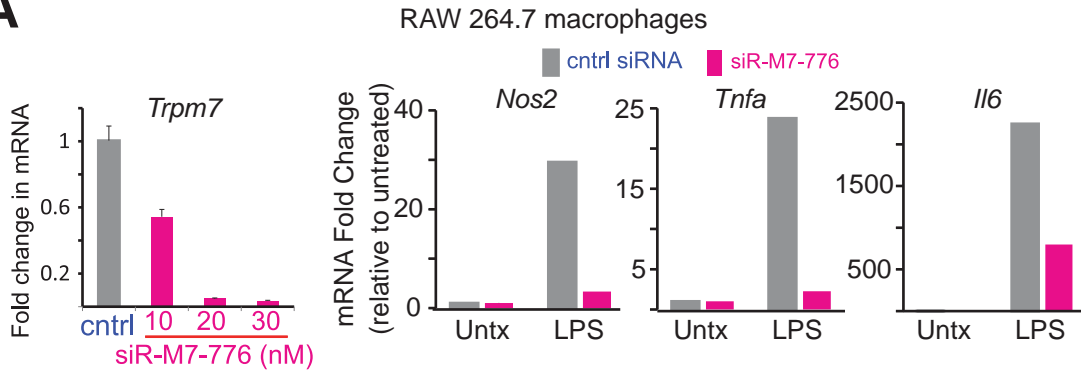
(I) qRT-PCR analysis of LPS-induced inflammatory gene expression in *Trpm7*$^{fl/fl}$ and *Trpm7*$^{+/+}$ *(LysM Cre)* BMDMs. Error bars represent SD. Means represent n=2 independent experiments.

(J) Additional mRNA expression analysis from genes as shown in *Fig. 1E*.
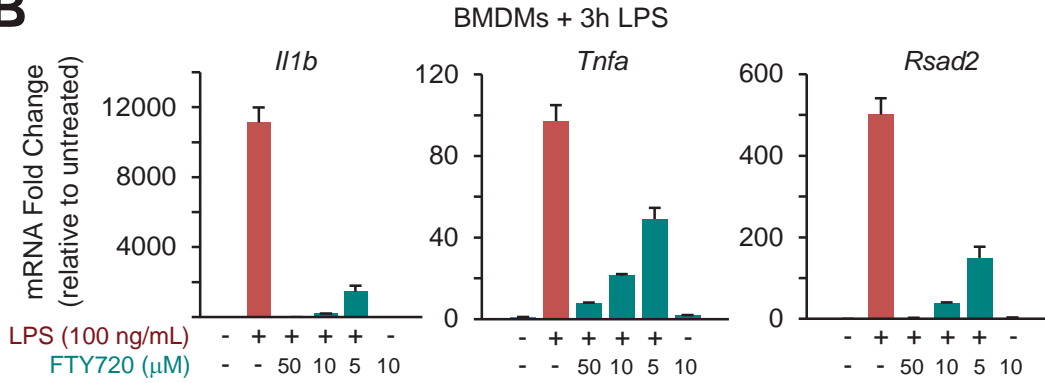
(K) Additional mRNA expression analysis of *Rsad2* as shown in *Fig. 1F*.
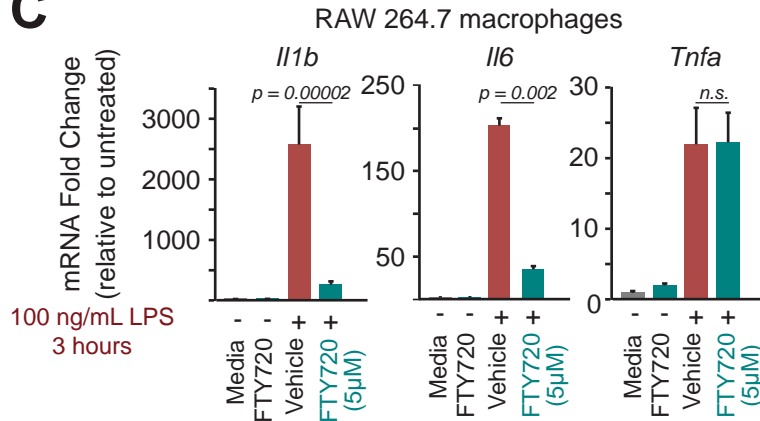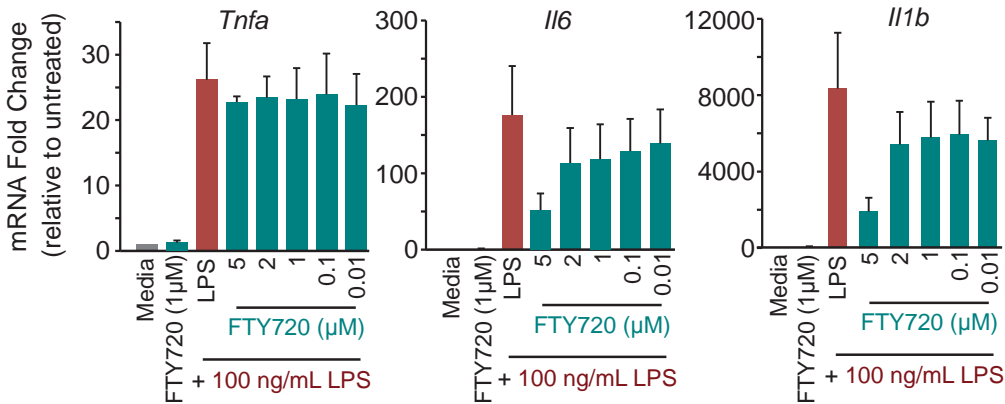
Figure S2 (with Fig. 1)

A

RAW 264.7 macrophages

cntrl siRNA   siR-M7-776

Trpm7 — Fold change in mRNA — cntrl / siR-M7-776 (nM) 10 20 30

Nos2, Tnfa, Il6 — mRNA Fold Change (relative to untreated) — Untx / LPS

B

BMDMs + 3h LPS

Il1b, Tnfa, Rsad2 — mRNA Fold Change (relative to untreated)

LPS (100 ng/mL)   - + + + + -
FTY720 (μM)   - - 50 10 5 10

C

RAW 264.7 macrophages

Il1b (p = 0.00002), Il6 (p = 0.002), Tnfa (n.s.) — mRNA Fold Change (relative to untreated)

100 ng/mL LPS 3 hours

Media / FTY720 / Vehicle + / FTY720 (5μM) +

D

Tnfa, Il6, Il1b — mRNA Fold Change (relative to untreated)

Media / FTY720 (1μM) / LPS / FTY720 (μM) 5 2 1 0.1 0.01
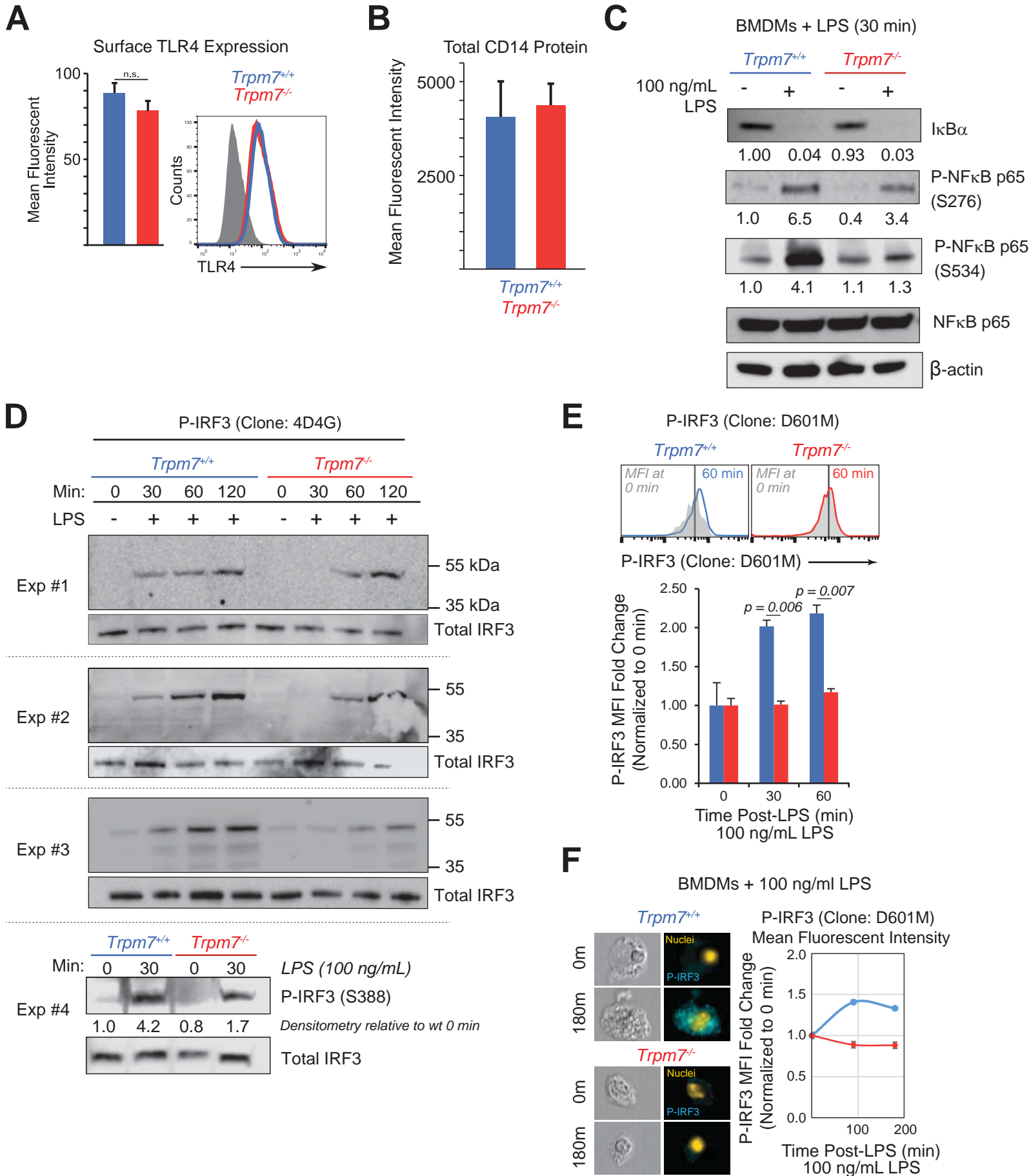
+ 100 ng/mL LPS

Figure S2 (with Fig. 1)

(A) Gene expression analysis (qRT-PCR) of indicated inflammatory genes in RAW 264.7 cells. Left panel is dose-dependent effect of TRPM7-directed siRNA (siR-M7-776) on TRPM7 mRNA levels. Right panels are RAW 264.7 cells treated with LPS (500 ng/mL, 12h). The cells were treated with 20 nM siR-M7-776 (TRPM7-directed) or control (scrambled) siRNA prior to LPS treatment.

(B) qRT-PCR analysis of indicated inflammatory genes in BMDMs after 15 min pre-treatment with FTY720, at indicated concentrations, for 3h. Error bars reflect SD of independent experiments (n=3). Changes in mRNA levels, relative to untreated macrophages are shown. Error bars represent SD (Means representative of n=3 independent experiments).

(C) Inflammatory gene expression analysis (qRT-PCR) from RAW 264.7 cells after treatment with 100 ng/mL LPS (3h). Cells were treated with FTY720 or vehicle (EtOH) in media prior to LPS treatment.

(D) qRT-PCR analysis of indicated inflammatory genes in RAW 264.7 cells after treatment with FTY720, at indicated concentrations, for 3h. Error bars reflect SD of independent experiments (n=3).

Figure S3 (with Fig. 2)

A

Surface TLR4 Expression

Mean Fluorescent Intensity

Trpm7+/+
Trpm7-/-

n.s.

Counts

TLR4 →

B

Total CD14 Protein

Mean Fluorescent Intensity

Trpm7+/+
Trpm7-/-

C

BMDMs + LPS (30 min)

	Trpm7+/+		*Trpm7*-/-	
100 ng/mL LPS	-	+	-	+

IκBα
1.00   0.04   0.93   0.03

P-NFκB p65 (S276)
1.0   6.5   0.4   3.4

P-NFκB p65 (S534)
1.0   4.1   1.1   1.3

NFκB p65

β-actin

D

P-IRF3 (Clone: 4D4G)

	Trpm7+/+				*Trpm7*-/-			
Min:	0	30	60	120	0	30	60	120
LPS	-	+	+	+	-	+	+	+

Exp #1
55 kDa
35 kDa
Total IRF3

Exp #2
55
35
Total IRF3

Exp #3
55
35
Total IRF3

	Trpm7+/+		*Trpm7*-/-	
Min:	0	30	0	30

LPS (100 ng/mL)

Exp #4
P-IRF3 (S388)
1.0   4.2   0.8   1.7
Densitometry relative to wt 0 min

Total IRF3

E

P-IRF3 (Clone: D601M)

Trpm7+/+   *Trpm7*-/-

MFI at 0 min   60 min   MFI at 0 min   60 min

P-IRF3 (Clone: D601M) →

P-IRF3 MFI Fold Change (Normalized to 0 min)

$p = 0.006$ $p = 0.007$

Time Post-LPS (min)
100 ng/mL LPS

F

BMDMs + 100 ng/ml LPS

Trpm7+/+
0m   Nuclei
     P-IRF3
180m Nuclei
      P-IRF3

Trpm7-/-
0m   Nuclei
     P-IRF3
180m Nuclei
      P-IRF3

P-IRF3 (Clone: D601M) Mean Fluorescent Intensity

P-IRF3 MFI Fold Change (Normalized to 0 min)

Time Post-LPS (min)
100 ng/mL LPS

Figure S3 (with Fig. 2)

(A) Flow cytometry-derived mean fluorescence intensities (MFI) of TLR4 staining in *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs, reflecting cell surface TLR4 levels (left panel). Error bars reflect SEM (n=4). Representative overlays of TLR4 histograms are shown in the right panel. The control histogram derived from unstained cells is depicted in gray.

(B) Flow cytometry-derived MFI of CD14 staining in *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs, reflecting cell CD14 levels. Error bars represent SEM (n=3).

(C) Immunoblot analysis of NFκB p65 phosphorylation at S276 and S534 (in humans S536) and IkBa protein levels from whole cells lysates of *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs stimulated with LPS (100 ng/ml; 30 min). Values for signal intensity are normalized to β-actin and total protein relative to untreated sample. Representative of n=3 independent experiments.

(D) Immunoblot analysis of P-IRF3 (S388) and total IRF3 protein levels from whole cells lysates of *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs stimulated with LPS (100 ng/ml), as indicated. Blots for P-IRF3 and total IRF3 are shown from individual experiments. The densitometric values for P-IRF3 were calculated by taking the ratios of phospho-protein levels relative to total protein and then normalizing the ratios to *Trpm7*$^{+/+}$ at 0 min. The statistics are shown in *Figure 2E*.

(E) Analysis of IRF3 phosphorylation at S388 in BMDMs by flow cytometry. *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs were stimulated with LPS (100 ng/ml) for indicated times, stained for viability (Live/Dead stain), and fixed prior to analysis. Later, the cells were stained with anti-P-IRF3 (Clone D601M). *(Top)* Representative histograms of P-IRF3 MFI; grey-filled histograms indicate P-IRF3 protein levels at 0 min (untreated). *(Bottom)* Quantification of P-IRF3 protein levels. Fold change is normalized to P-IRF3 MFI at 0 min. Error bars are SEM (n=3).
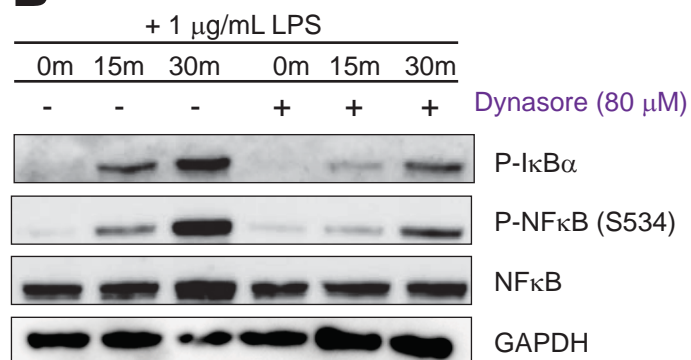
(F) Representative images (n> 5000 cells) taken from ImageStream flow cytometry analysis of indicated BMDMs, stimulated with LPS (100 ng/mL for indicated time) and stained with anti-P-IRF3 antibody (Clone: D601M) and DRAQ-5 (nuclei) *(left panels)*. Levels of P-IRF3 at indicated time points after LPS treatment were quantified based on MFI at 0 min *(right panel)*. Error bars represent SEM (n>5000 cells).
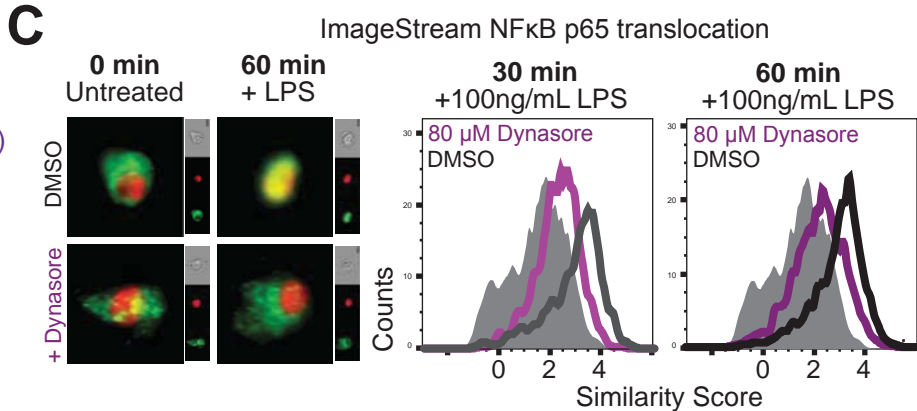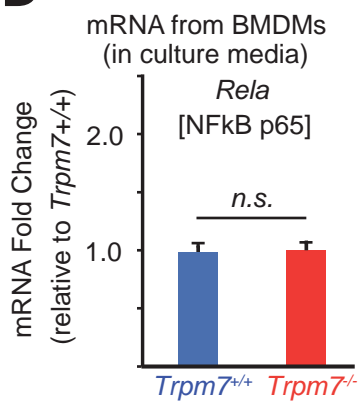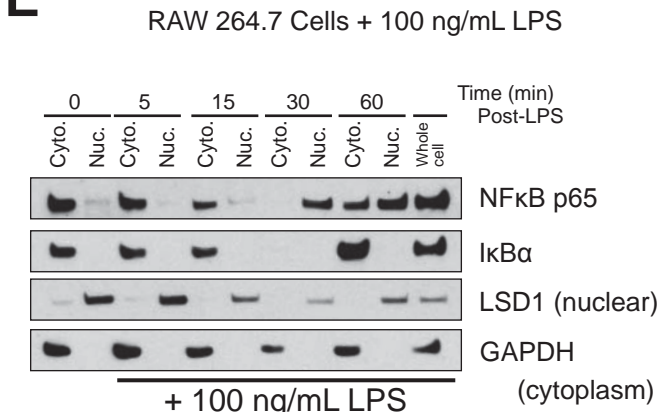
Figure S4 (with Fig. 3)

A

BMDMs + LPS [3h]

Il1b

p = 0.0003

Trpm7+/+
Trpm7-/-

n.s.

Il6

p = 0.001

n.s.

Rsad2

p = 0.0005

n.s.

LPS (100 ng/mL)	-	+	+	-	-	+	+	-
Dynasore(80 µM)	-	-	+	+	-	-	+	+

B

+ 1 µg/mL LPS

0m 15m 30m   0m 15m 30m

Dynasore (80 µM)

− − −   + + +

P-IκBα
P-NFκB (S534)
NFκB
GAPDH

C

ImageStream NFκB p65 translocation

0 min Untreated   60 min + LPS

DMSO

+ Dynasore

30 min +100ng/mL LPS

60 min +100ng/mL LPS

80 µM Dynasore
DMSO

Counts

Similarity Score

D

mRNA from BMDMs (in culture media)

Rela [NFkB p65]

n.s.

mRNA Fold Change (relative to Trpm7+/+)

Trpm7+/+   Trpm7-/-

E

RAW 264.7 Cells + 100 ng/mL LPS

Time (min) Post-LPS

0   5   15   30   60   Whole cell

Cyto. Nuc.

NFκB p65
IκBα
LSD1 (nuclear)
GAPDH (cytoplasm)

+ 100 ng/mL LPS

F

0 min   15 min   Time (min) Post-LPS

Cyto. Nuc.   Cyto. Nuc.

Trpm7+/+

IRF3

0.09   0.31   Nuclear:Cytoplasm

LSD1

Trpm7-/-

IRF3

0.19   0.19   Nuclear:Cytoplasm

LSD1

G

Trpm7+/+

0 min LPS   60 min LPS

Nuclei IRF3

Trpm7-/-

0 min LPS   60 min LPS

H

NFkB Translocation + ODN 1826 (1 µM)

Trpm7+/+
Trpm7-/-

Unstimulated
+ 30 min ODN 1826

Events

Similarity Score (Degree of translocation)

Trpm7+/+
Trpm7-/-

n.s.   n.s.

Similarity Score (Degree of translocation)

0 min   30 min
+ODN 1826
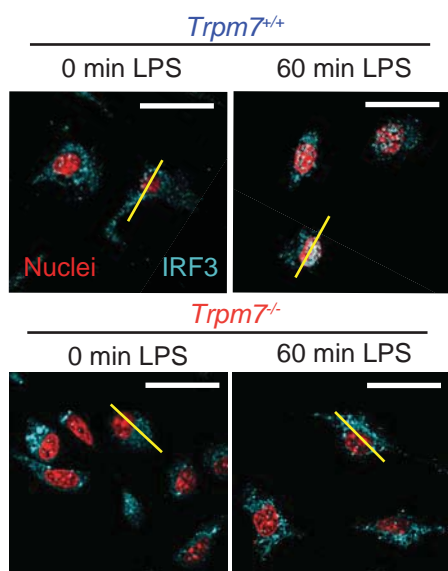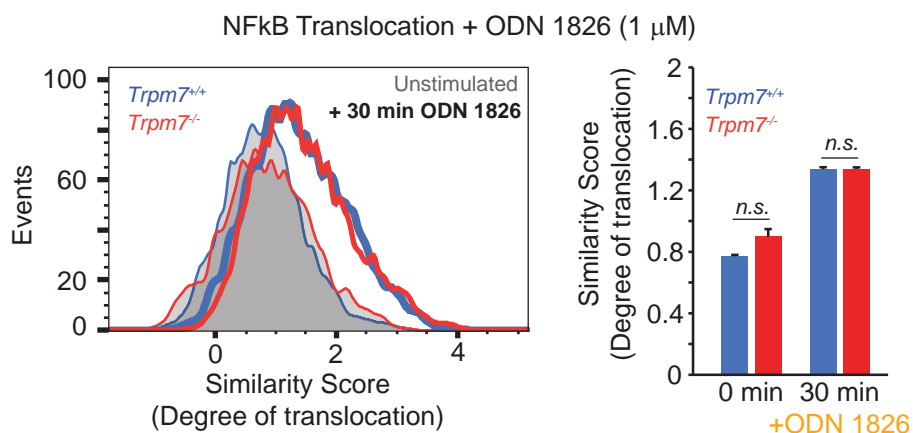
Figure S4 (with Fig. 3)

(A) Gene expression analysis (qRT-PCR) of $Trpm7^{+/+}$ and $Trpm7^{-/-}$ BMDMs stimulated as indicated. BMDMs were pre-treated in serum-free media with or without dynasore (80 μM; 30 min) prior to LPS stimulation (100 ng/ml; 3h). Error bars represent SD (n=3)

(B) Immunoblot analysis of whole cell lysates from BMDMs treated as indicated. BMDMs were pre-treated in serum-free media with or without dynasore (80 μM; 30 min) prior to LPS stimulation.

(C) Representative images (n> 5000 cells) taken from ImageStream flow cytometry analysis of untreated and Dynasore treated WT BMDMs, stimulated with LPS (100 ng/mL, 60m) and stained with anti-NFκB p65 antibody and DRAQ5 (a nuclear stain) are shown *(left panels)*. An overlaid image of NFκB p65 staining (green) and DRAQ5 (red) is shown and the single channel images of that cell are shown on the right of that image in smaller sizes. The right panel shows an overlay of histograms depicting the similarity scores derived for each condition, 30m and 60m after LPS treatment. The filled histogram represents untreated cells.

(D) qRT-PCR analysis of NFκB p65 gene expression in indicated BMDMs. Error bars reflect SD (n=3).

(E) Immunoblot analysis of NFκB p65 and IκBα present in cytosolic and nuclear fractions obtained from RAW 264.7 cells at various time points after LPS (100 ng/ml) treatment. Immunoblots of LSD1 (a nuclear protein) and GAPDH (a cytosolic protein) are also shown.

(F) Immunoblot analysis of IRF3 present in cytosolic and nuclear fractions from indicated BMDMs, untreated and treated with LPS (100 ng/ml, 15m). Immunoblots of LSD1, a nuclear marker are also shown. These are representative results of independent experiments (n=3).
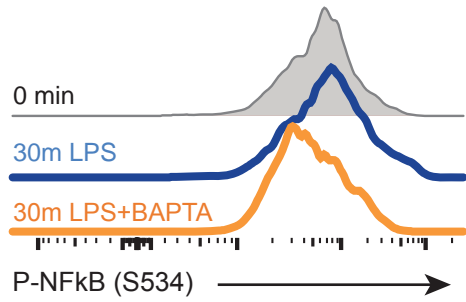
(G) Representation of linear intensity analysis in *Fig 3F*. Images are relabeled from *Fig 3D* with region of interest annotated (yellow).

(H) Representative histograms taken from ImageStream flow cytometry (n> 7000 cells) analysis of $Trpm7^{+/+}$ and $Trpm7^{-/-}$ BMDMs, stimulated with ODN 1826 (1 μM, 30m). Histograms depict similarity scores after staining with anti-NFκB p65 antibody and DRAQ5 (a nuclear stain) are shown *(left panels)*; the filled histogram represents untreated cells. The right panel depicts quantification of similarity scores. Error bars are SEM (n=4).
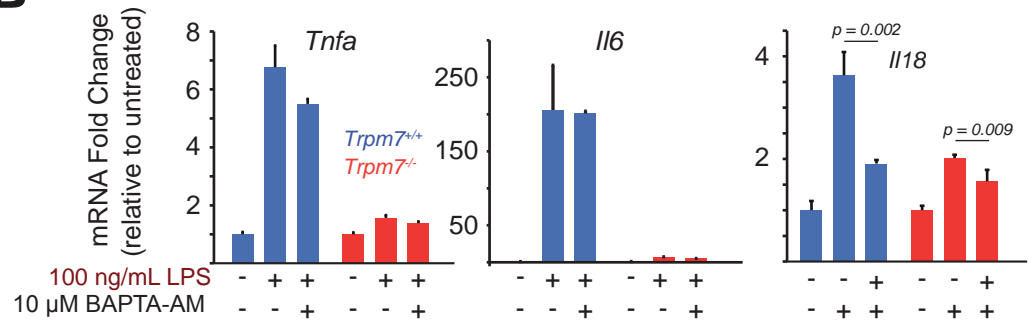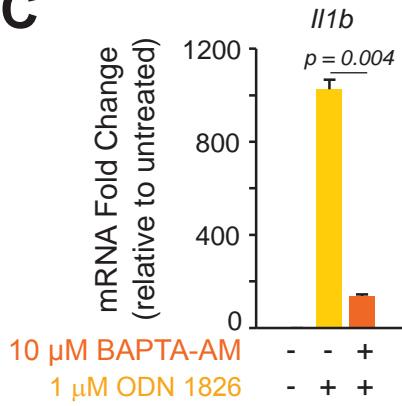
Figure S5 (with Fig. 4)

A

0 min
30m LPS
30m LPS+BAPTA

P-NFkB (S534) →

B

Tnfa

mRNA Fold Change (relative to untreated)

8
6
4
2

$Trpm7^{+/+}$
$Trpm7^{-/-}$

100 ng/mL LPS   - + +   - + +
10 µM BAPTA-AM - - +   - - +

Il6

250
150
50

- + +   - + +
- - +   - - +

Il18

$p = 0.002$
$p = 0.009$

4

2

- - +   - - +
- + +   - + +

C

Il1b

mRNA Fold Change (relative to untreated)

1200
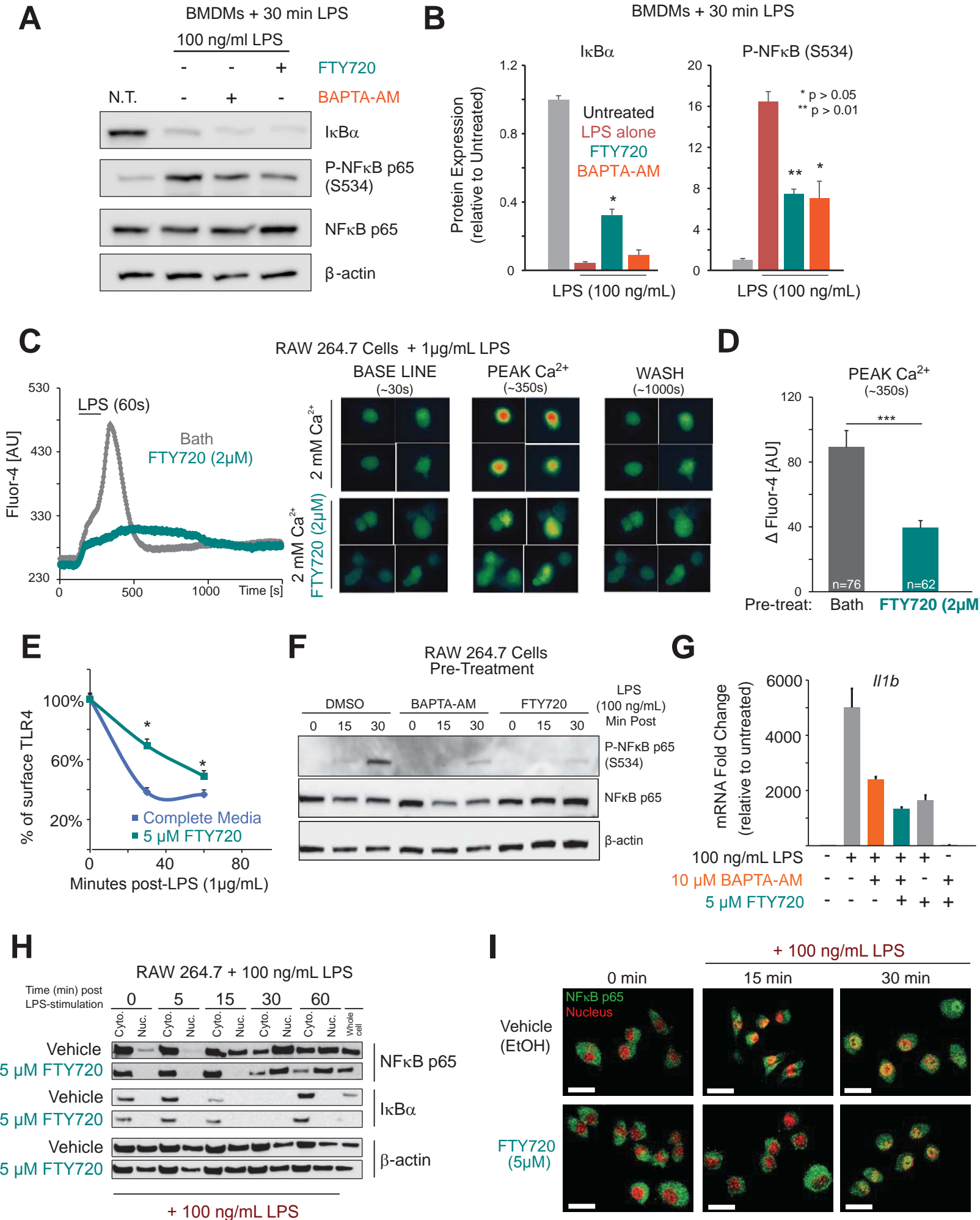800
400
0

$p = 0.004$

10 µM BAPTA-AM - - +
1 µM ODN 1826   - + +

Figure S5 (with Fig. 4)

(A) An extension of *Fig. 4A*, representative histograms of phospho-NFκB p65 (S534) staining in indicated BMDMs.

(B) Expression of indicated inflammatory genes (qRT-PCR) in *Trpm7*$^{+/+}$ and *Trpm7*$^{-/-}$ BMDMs, treated as indicated in *Fig. 4D*. Prior to LPS treatment (100 ng/mL, 3h), BMDMs were loaded with vehicle or BAPTA-AM for 30m in serum-free media. Error bars represent SD (n=3).

(C) qRT-PCR analysis of *Il1b* mRNA expression in WT BMDMs. Prior to ODN 1826 treatment (1 μM, 3h), BMDMs were loaded with vehicle (DMSO) or BAPTA-AM for 30m in serum-free media. Error bars represent SD (n=3).

Figure S6 (with Fig. 6)

A

BMDMs + 30 min LPS

100 ng/ml LPS

| | | − | − | + | FTY720 |
| N.T. | − | + | − | BAPTA-AM |

IκBα

P-NFκB p65 (S534)

NFκB p65

β-actin

B

BMDMs + 30 min LPS

IκBα

P-NFκB (S534)

Untreated
LPS alone
FTY720
BAPTA-AM

* p > 0.05
** p > 0.01

LPS (100 ng/mL)

Protein Expression (relative to Untreated)

C

RAW 264.7 Cells + 1µg/mL LPS

BASE LINE (~30s)   PEAK Ca^{2+} (~350s)   WASH (~1000s)

2 mM Ca^{2+}

2 mM Ca^{2+} FTY720 (2µM)

LPS (60s)

Bath
FTY720 (2µM)

Fluor-4 [AU]
Time [s]

D

PEAK Ca^{2+} (~350s)

ΔFluor-4 [AU]

***

n=76   n=62

Pre-treat: Bath   **FTY720 (2µM)**

E

% of surface TLR4

Complete Media
5 µM FTY720

*   *

Minutes post-LPS (1µg/mL)

F

RAW 264.7 Cells
Pre-Treatment

| DMSO | BAPTA-AM | FTY720 | LPS (100 ng/mL) |
| 0 | 15 | 30 | 0 | 15 | 30 | 0 | 15 | 30 | Min Post |

P-NFκB p65 (S534)

NFκB p65

β-actin

G

Il1b

mRNA Fold Change (relative to untreated)

100 ng/mL LPS	−	+	+	+	+	−
10 µM BAPTA-AM	−	−	+	+	−	+
5 µM FTY720	−	−	−	+	+	+

H

RAW 264.7 + 100 ng/mL LPS

Time (min) post LPS-stimulation

| 0 | 5 | 15 | 30 | 60 |
| Cyto. | Nuc. | Cyto. | Nuc. | Cyto. | Nuc. | Cyto. | Nuc. | Cyto. | Nuc. | Whole cell |

Vehicle
5 µM FTY720
NFκB p65

Vehicle
5 µM FTY720
IκBα

Vehicle
5 µM FTY720
β-actin

+ 100 ng/mL LPS

I

+ 100 ng/mL LPS

0 min   15 min   30 min

NFκB p65
Nucleus

Vehicle (EtOH)

FTY720 (5µM)

Figure S6 (with Fig. 6)

(A) Immunoblot analysis of LPS-induced NFκB phosphorylation at S534 and IκBα protein in the whole cell lysates of BMDMs pre-treated as follows: BAPTA-AM (10 µM, 30m), FTY720 (5 µM, 15m), DMSO as vehicle control. These data are representative of n=3 independent experiments.

(B) Quantification of immunoblots as shown in *Fig S5A*. Relative protein expression is normalized to corresponding total protein and then 0 min treatment. Bar charts represent means from n = 3 independent experiments. Error bars are SEM (n=3). T-test for significance compared to LPS alone.

(C) Relative changes in LPS-induced intracellular Ca^{2+} concentration [Ca^{2+}]$_i$ in untreated or FTY720-treated RAW 264.7 cells (*left panel*). Representative images of Fluo-4 fluorescence are shown on the right.

(D) Quantification of LPS-induced peak fluorescent intensities in indicated conditions, relative to untreated cells (*** indicates $p < 0.001$).

(E) Flow cytometry based measurement of cell surface TLR4 levels in BMDMs, pre-treated with either vehicle or FTY720 for 15 min, and then stimulated with LPS as indicated. The relative change in the percentage of cell surface TLR4 levels was inferred based on MFI values. Error bars represent SEM (n=3; * indicates $p < 0.05$).
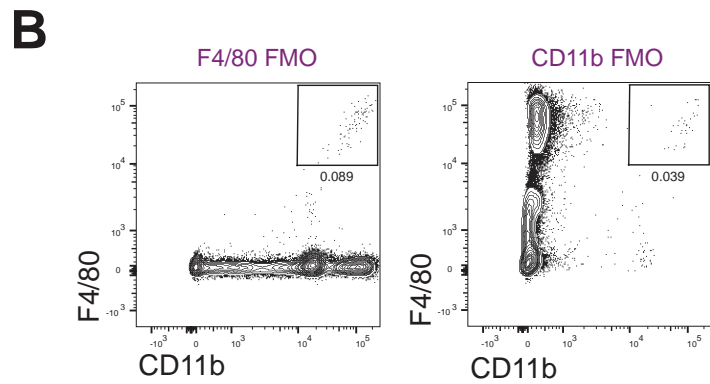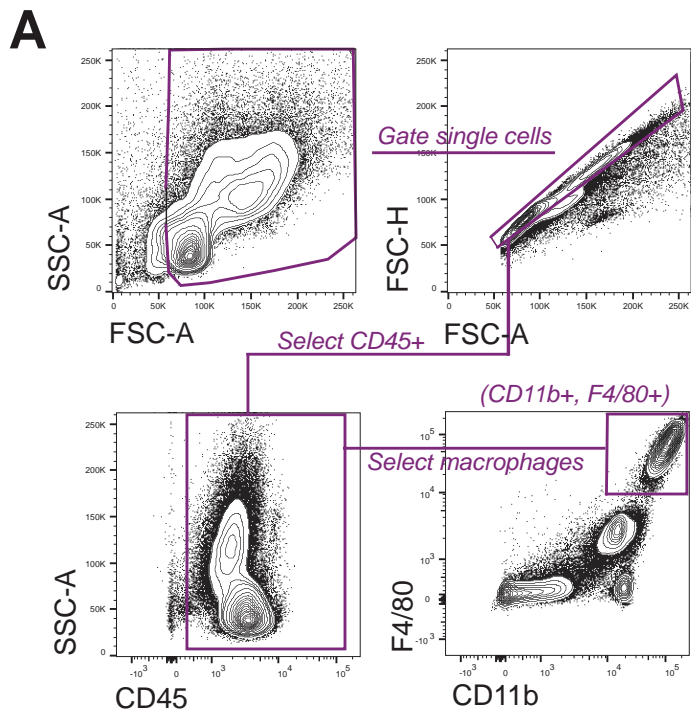
(F) Immunoblot analysis of LPS-induced NFκB phosphorylation at S534 in the whole cell lysates of RAW 264.7 cells pre-treated as follows: BAPTA-AM (10 µM, 30m), FTY720 (5 µM, 15m), DMSO as vehicle control. These data are representative of n=3 independent experiments.

(G) Expression analysis (qRT-PCR) of *Il1b* in RAW 264.7 cells pre-treated as indicated prior to LPS (100 ng/ml, 3h). Error bars represent SD (n=3).

(H) Immunoblot analysis of NFκB p65 and IκBα in cytosolic and nuclear fractions of RAW 264.7 cells pre-treated with FTY720 or Vehicle (EtOH), and then stimulated with LPS (100 ng/ml) for indicated times. The results represent typical results seen in n=3 independent experiments.

(I) Immunofluorescence confocal microscopy images showing NFκB p65 nuclear translocation in RAW 264.7 cells, pre-treated with FTY720 or Vehicle (EtOH) prior to LPS stimulation for indicated times. Scale bar = 10 μm. These are representative images from n=2 independent experiments.

Figure S7 (with Fig. 7)

A

SSC-A / FSC-A

Gate single cells

FSC-H / FSC-A

Select CD45+

SSC-A / CD45

Select macrophages

(CD11b+, F4/80+)

F4/80 / CD11b

B

F4/80 FMO

F4/80 / CD11b

0.089

CD11b FMO

F4/80 / CD11b

0.039

Figure S7 (with Fig. 7)

(A) Flow cytometry gating strategy used in Fig. 7F-H

(B) Fluorescence minus one (FMO) gating controls for flow cytometry analysis.

Table S1 (Relates to Fig. 7)

Clinical scoring guidelines. All clinical scores were determined by double-blinded observation.

Scoring Parameter	0	1	2
Conjunctivitis	Normal	Single eye open with visible discharge	Eyes closed with discharge and swelling
Lethargy	Normal locomotion and reaction, >3 steps	Inactive, <3 steps after moderate stimulation	Only lifting of head after moderate stimulation, <1 step
Hair coat	Well groomed with smooth coat	Rough coat, minor ruffling	Unkempt fur, dull coat
Grimace pain scale	Normal	Moderate orbital tightening or nose bulge	Severe orbital tightening, nose bulge, and collapsed ear position