# Figure S1

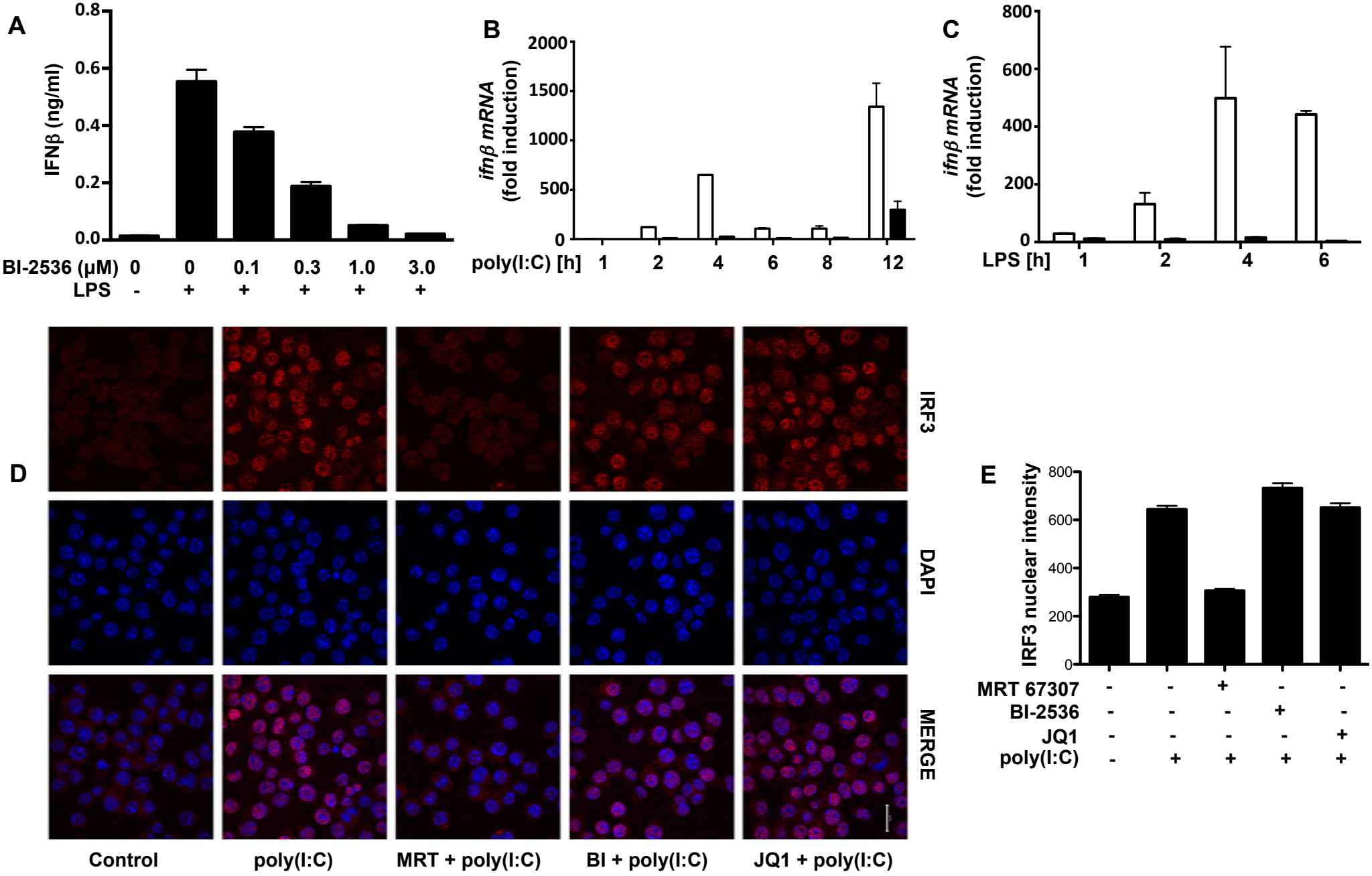
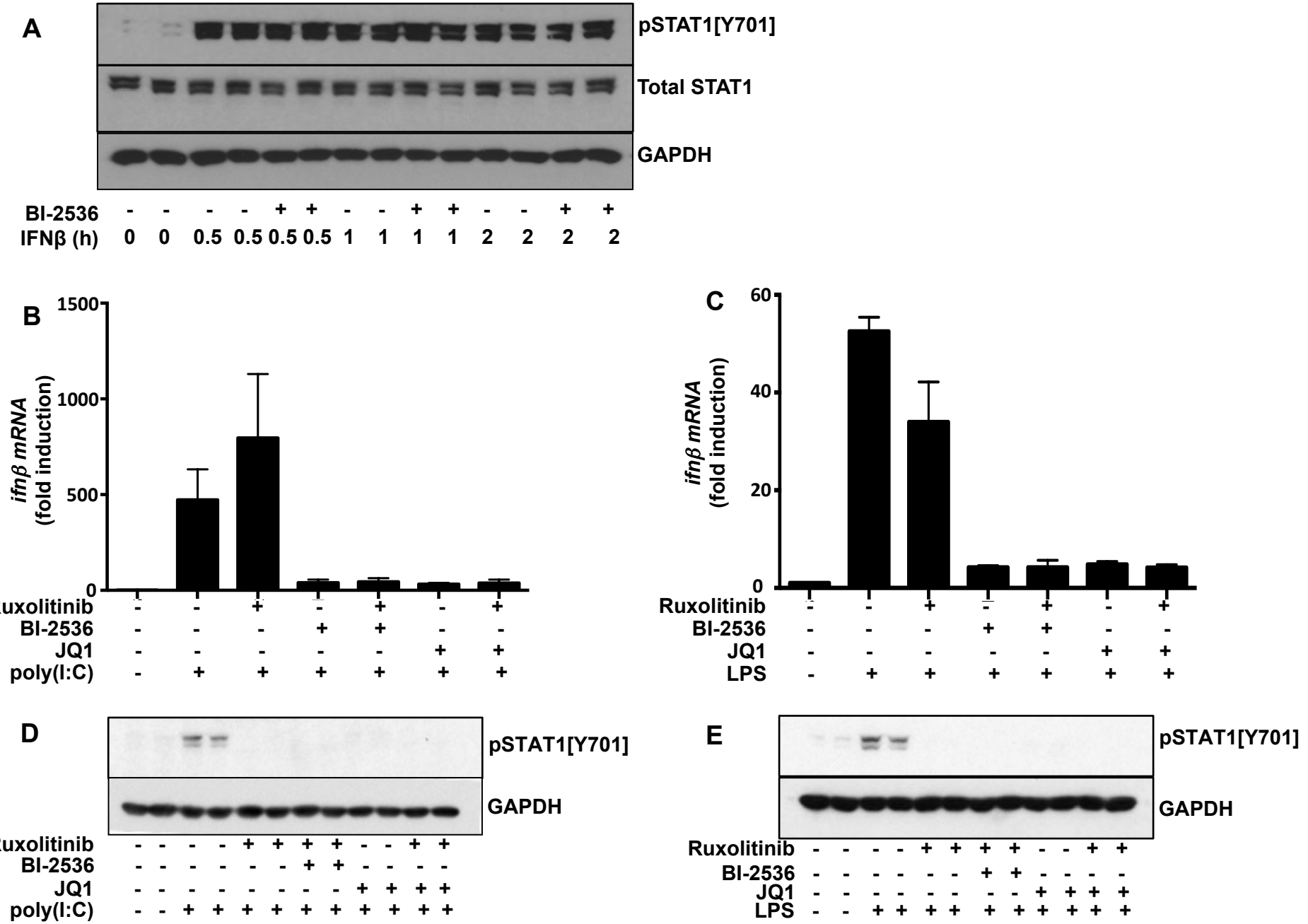
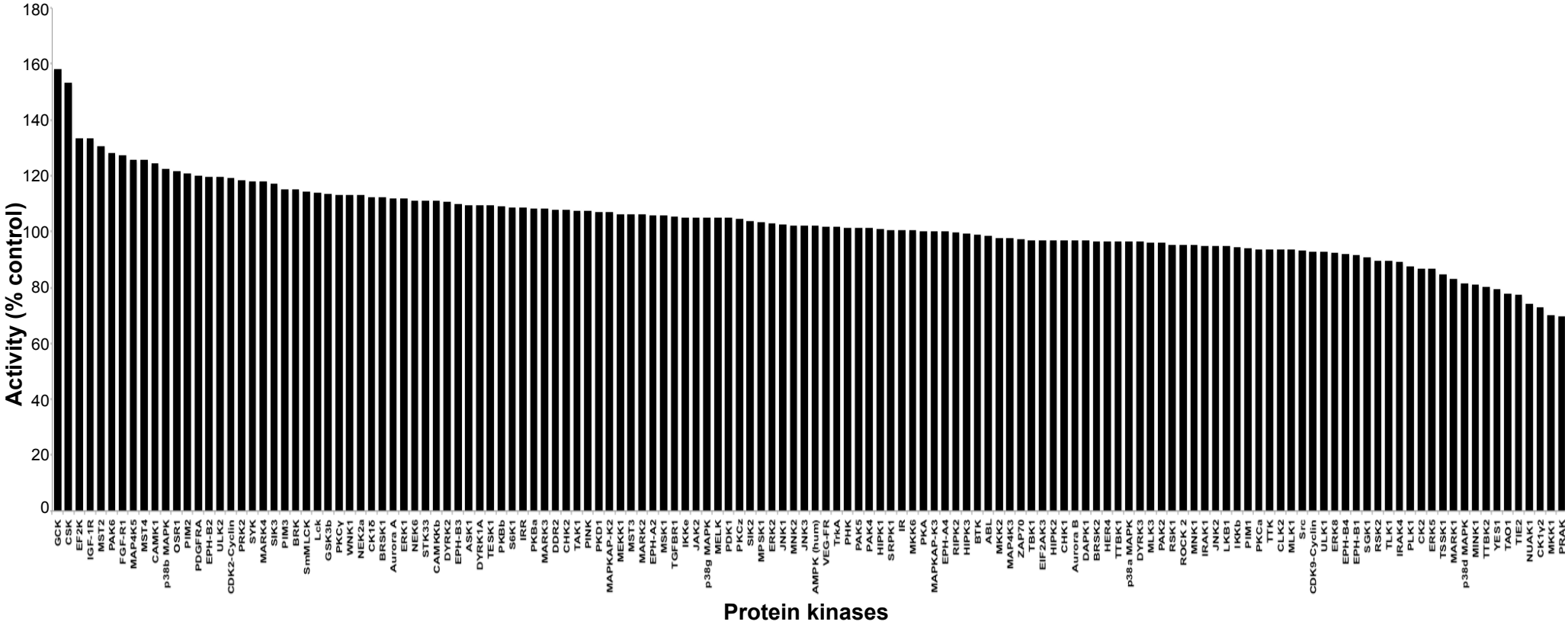

A
IFNβ (ng/ml)
0.8
0.6
0.4
0.2
0.0
BI-2536 (μM) 0 0 0.1 0.3 1.0 3.0
LPS - + + + + +
B
ifnβ mRNA (fold induction)
2000
1500
1000
500
0
poly(I:C) [h] 1 2 4 6 8 12
C
ifnβ mRNA (fold induction)
800
600
400
200
0
LPS [h] 1 2 4 6
D
IRF3
DAPI
MERGE
Control
poly(I:C)
MRT + poly(I:C)
BI + poly(I:C)
JQ1 + poly(I:C)
E
IRF3 nuclear intensity
800
600
400
200
0
MRT 67307 - - + - -
BI-2536 - - - + -
JQ1 - - - - +
poly(I:C) - + + + +

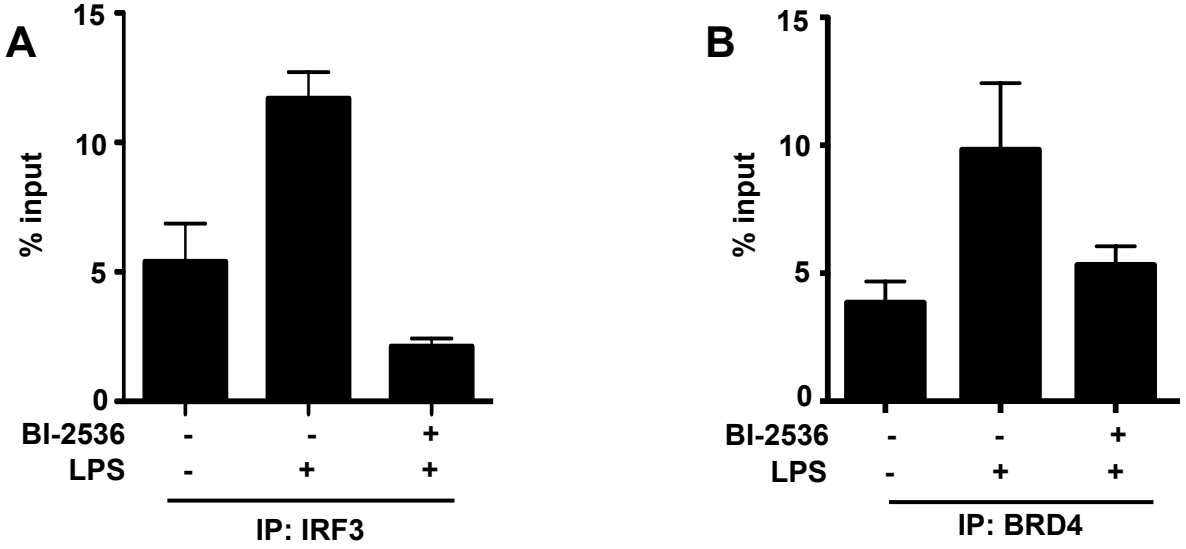
Figure S2


A
pSTAT1[Y701]
Total STAT1
GAPDH
BI-2536 - - - - + + - - + + - - + +
IFNβ (h) 0 0 0.5 0.5 0.5 0.5 1 1 1 1 2 2 2 2
B
ifnβ mRNA (fold induction)
1500
1000
500
0
Ruxolitinib - - + - + - +
BI-2536 - - - + + - -
JQ1 - - - - - + +
poly(I:C) - + + + + + +
C
ifnβ mRNA (fold induction)
60
40
20
0
Ruxolitinib - - + - + - +
BI-2536 - - - + + - -
JQ1 - - - - - + +
LPS - + + + + + +
D
pSTAT1[Y701]
GAPDH
Ruxolitinib - - - - + + + + - - + +
BI-2536 - - - - - - + + - - - -
JQ1 - - - - - - - - + + + +
poly(I:C) - - + + + + + + + + + +
E
pSTAT1[Y701]
GAPDH
Ruxolitinib - - - - + + + + - - + +
BI-2536 - - - - - - + + - - - -
JQ1 - - - - - - - - + + + +
LPS - - + + + + + + + + + +

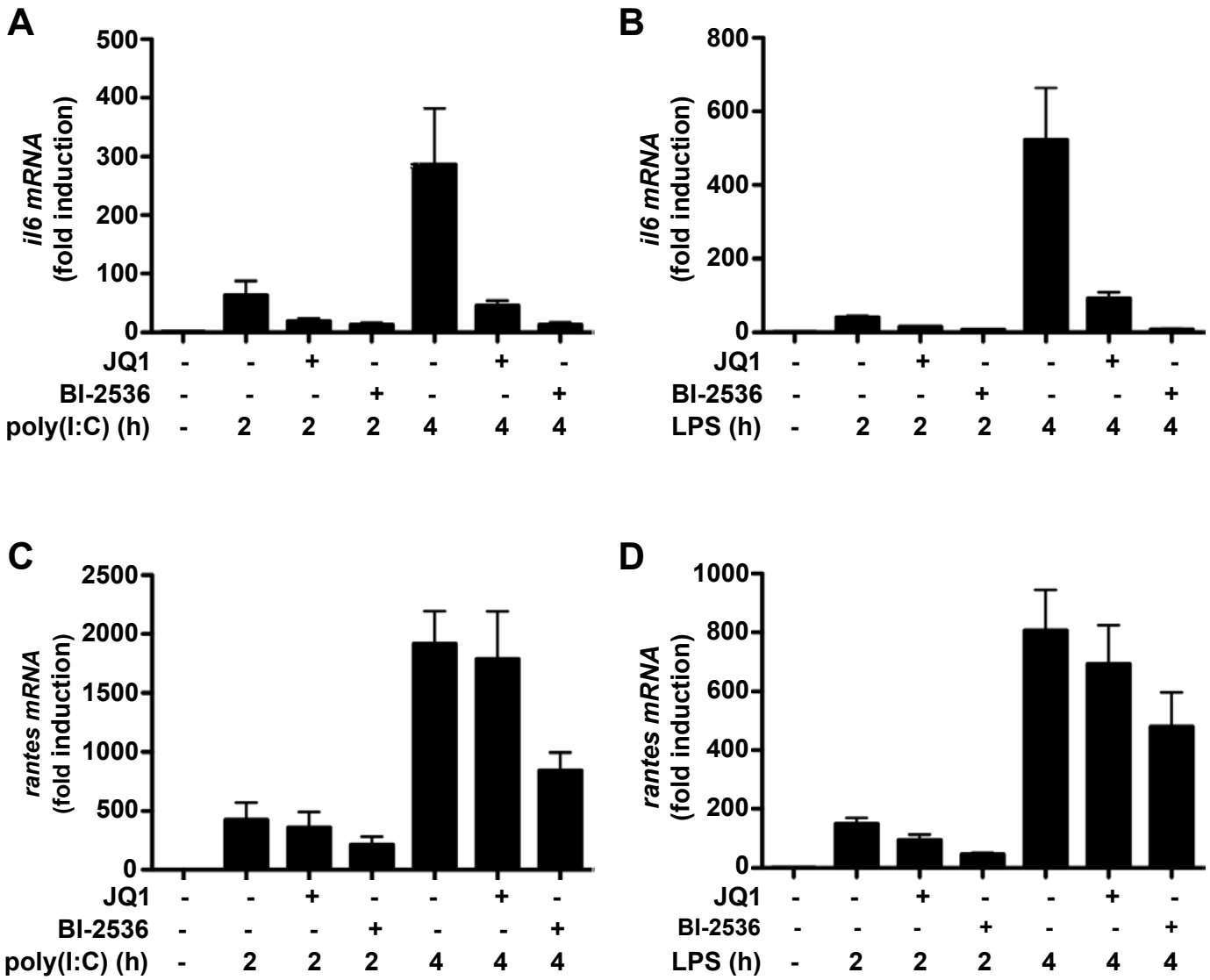
# Figure S3


Activity (% control)
180
160
140
120
100
80
60
40
20
0
GCK
CSK
EF2K
IGF-1R
MST2
PAK6
FGF-R1
MAP4K5
MST4
CAMK1
p38b MAPK
OSR1
PIM2
PDGFRA
EPH-B2
ULK2
CDK2-Cyclin
PRK2
SYK
MARK4
SIK3
PIM3
BRK
SmMLCK
Lck
GSK3b
PKCγ
WNK1
NEK2a
CK1δ
BRSK1
Aurora A
ERK1
NEK6
STK33
CAMKKb
DYRK2
EPH-B3
ASK1
DYRK1A
TESK1
PKBb
S6K1
IRR
PKBa
MARK3
DDR2
CHK2
TAK1
PINK
PKD1
MAPKAP-K2
MEKK1
MST3
MARK2
EPH-A2
MSK1
TGFBR1
IKKε
JAK2
p38g MAPK
MELK
PDK1
PKC2
SIK2
MPSK1
ERK2
JNK1
MNK2
JNK3
AMPK (hum)
VEG-FR
TrkA
PHK
PAK5
PAK4
HIPK1
SRPK1
IR
MKK6
PKA
MAPKAP-K3
EPH-A4
RIPK2
HIPK3
BTK
ABL
MKK2
MAP4K3
ZAP70
TBK1
EIF2AK3
HIPK2
CHK1
Aurora B
DAPK1
BRSK2
HER4
TTBK1
p38a MAPK
DYRK3
MLK3
PAK2
RSK1
ROCK 2
MNK1
IRAK1
JNK2
LKB1
IKKb
PIM1
PKCa
TTK
CLK2
MLK1
Src
CDK9-Cyclin
ULK1
ERK8
EPH-B4
EPH-B1
SGK1
RSK2
TLK1
IRAK4
PLK1
CK2
ERK5
TSSK1
MARK1
p38d MAPK
MINK1
TTBK2
YES1
TAO1
TIE2
NUAK1
CK1γ2
MKK1
PRAK
Protein kinases

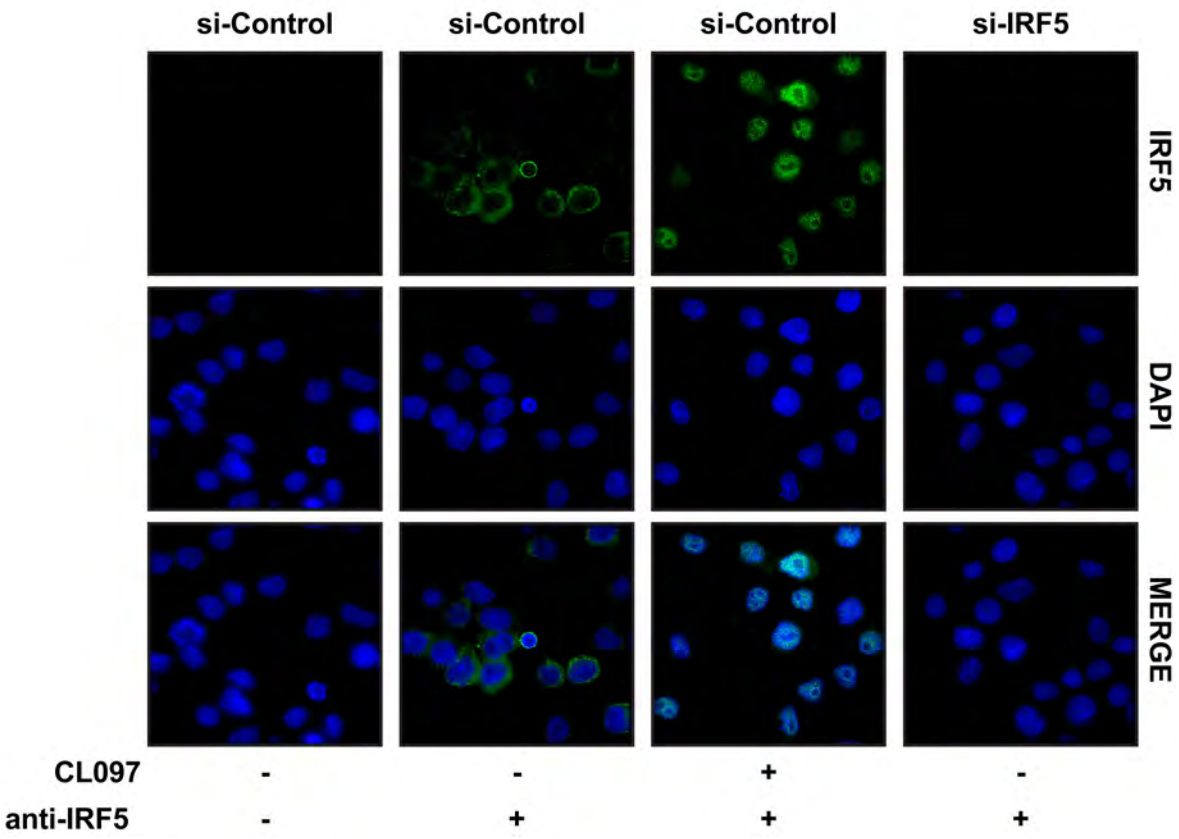
# Figure S4


A
15
10
5
0
% input
BI-2536 - - +
LPS - + +
IP: IRF3
B
15
10
5
0
% input
BI-2536 - - +
LPS - + +
IP: BRD4

# Figure S5


A
il6 mRNA
(fold induction)
500
400
300
200
100
0
JQ1 - - + - - + -
BI-2536 - - - + - - +
poly(I:C) (h) - 2 2 2 4 4 4
B
il6 mRNA
(fold induction)
800
600
400
200
0
JQ1 - - + - - + -
BI-2536 - - - + - - +
LPS (h) - 2 2 2 4 4 4
C
rantes mRNA
(fold induction)
2500
2000
1500
1000
500
0
JQ1 - - + - - + -
BI-2536 - - - + - - +
poly(I:C) (h) - 2 2 2 4 4 4
D
rantes mRNA
(fold induction)
1000
800
600
400
200
0
JQ1 - - + - - + -
BI-2536 - - - + - - +
LPS (h) - 2 2 2 4 4 4

# Figure S6


si-Control
si-Control
si-Control
si-IRF5
IRF5
DAPI
MERGE
CL097 - - + -
anti-IRF5 - + + +

**Supplementary Figures**

**Figure S1. BI-2536 prevents LPS or poly(I:C)-stimulated IFNβ secretion and mRNA production but not the nuclear translocation of IRF3.** **(A)** RAW cells were incubated for 1 h with the indicated concentrations of BI-2536, and then stimulated for 4 h without (-) or with (+) 100 ng/ml LPS. At each time point, the amounts of IFNβ secreted into the cell culture medium, were measured by ELISA. The results are shown + S.E.M for triplicate determinations. **(B, C)** RAW cells were incubated for 1 h with (black bars) or without (white bars) 1.0 μM BI2536 and then stimulated for the times indicated with 10 μg/ml poly(I:C) **(B)** or 100 ng/ml LPS **(C)**. At each time point, the total RNA was extracted from the cells and mRNA encoding *ifnβ* was quantitated by qRT-PCR. The figures show the fold increase in mRNA levels relative to the values measured in RAW cells not stimulated with poly(I:C) or LPS (+ S.E.M for triplicate determinations). (**D**) RAW cells were plated on cover slips and were treated for 1 h with either 2.0 μM MRT67307 (MRT), 1.0 μM BI-2536 (BI) or 1.0 μM JQ1, or without any inhibitor. The cells were then stimulated for 90 min with 10 μg/ml poly(I:C) or left unstimulated (control). The cells were fixed, stained and examined by confocal microscopy (see Methods). Typical fields are shown. Similar results were obtained in two independent experiments. (**E**) Mean quantities of nuclear IRF3 obtained from analysis with Volocity Software. The results are shown + SD for n=10 determinations. Similar results for **A-E** were obtained in two independent experiments.

**Figure S2. BI-2536 and JQ1 suppress IFNβ-stimulated mRNA production by a mechanism that is independent of the positive feedback loop.** **(A)** RAW cells were incubated without (-) or with (+) 1.0 μM BI-2536 and then stimulated with IFNβ (500 units/ml) for the times indicated. Cell lysates (20 μg protein) were subjected to SDS/PAGE, transferred to PVDF membranes and immunoblotted with antibodies recognizing STAT1 phosphorylated at Tyr701, all forms of STAT1 and GAPDH. (**B, C**) RAW cells were incubated for 1 h without (-) or with (+) 1.0 μM Ruxolitinib, 1.0 μM BI2536 and/or JQ1 and then stimulated for 2 h with 10 μg/ml poly(I:C) (**B**) or 100 ng/ml LPS (**C**) or left unstimulated. Total RNA was isolated from the cells and qRT-PCR carried out to quantitate *ifnβ* mRNA levels. The figures show the fold increase in mRNA levels relative to the values measured in RAW cells not stimulated with poly(I:C) or LPS (+ S.E.M for triplicate determinations). (**D, E**) The experiment was performed as in **B,C**, but after stimulation for 2 h with poly(I:C) or LPS, the cells were lysed and 20 μg of cell extract protein was subjected to SDS/PAGE and immunoblotted as in **A**. Similar results were obtained in two independent experiments for **A-E**.

**Figure S3**. **The compound JQ1 is not a protein kinase inhibitor**. The effect of JQ1 on the activities of 140 protein kinases was examined by the International Centre for Protein Kinase Profiling, MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee (http//www.kinase-screen.mrc.ac.uk). The result obtained for each protein kinase tested is presented as the % activity remaining in the presence of JQ1 compared to control incubations lacking JQ1. The assays were performed in duplicate and the average value is shown.

**Figure S4**. **The LPS-induced association of IRF3 and BRD4 with the IFNβ gene promoter is prevented by the compound BI-2536.** RAW cells were incubated for 1 h without (-) or with (+) 1.0 μM BI-2536, then

stimulated for 2 h without (-) or with (+) LPS (100 ng/ml). The cells were cross-linked and lysed, and the chromatin sheared by sonication. Chromatin immunoprecipitation (ChIP) was performed using antibodies that recognizes IRF3 (**A**) or BRD4 (**B**). Enrichment of the IFNβ promoter was measured by qRT-PCR, normalizing to input (see experimental section).

**Figure S5. Effect of BI-2536 and JQ1 on poly(I:C) and LPS-stimulated IL-6 and RANTES mRNA production in RAW cells.**
**(A, B)** Cells were incubated for 1 h with the indicated concentrations of BI-2536 or JQ1 and then stimulated for the times indicated without (-) or with (+) poly(I:C) (10 μg/ml) (**A**) or LPS (100 ng/ml) (**B**). Total RNA was isolated and *il6* mRNA levels were quantitated by qRT-PCR. **(C, D)** As for **A**, **B**, except that *rantes* mRNA (**C, D**) was quantitated. The figures show the fold-increase in mRNA levels relative to the values measured in cells not stimulated with poly(I:C) or LPS. The results show the ± S.E.M for two independent experiments, each performed in triplicate.

**Figure S6**. **The IRF5 antibody recognizes the endogenous IRF5 specifically**. Gen2.2 cells were transfected with siRNAs for IRF5 and Cy3-labeled negative control, as described [25]. After 72 h, the cells were treated for 1 h with or without the TLR7 agonist CL097 (1.0 μg/ml). The cells were fixed, centrifuged on to pre-coated slides, permeabilised and incubated with or without the anti-IRF5 antibody. Then, the cells were stained with the secondary antibody Alexa448, counterstained with DAPI to reveal nuclei and analyzed by deconvolution microscopy.