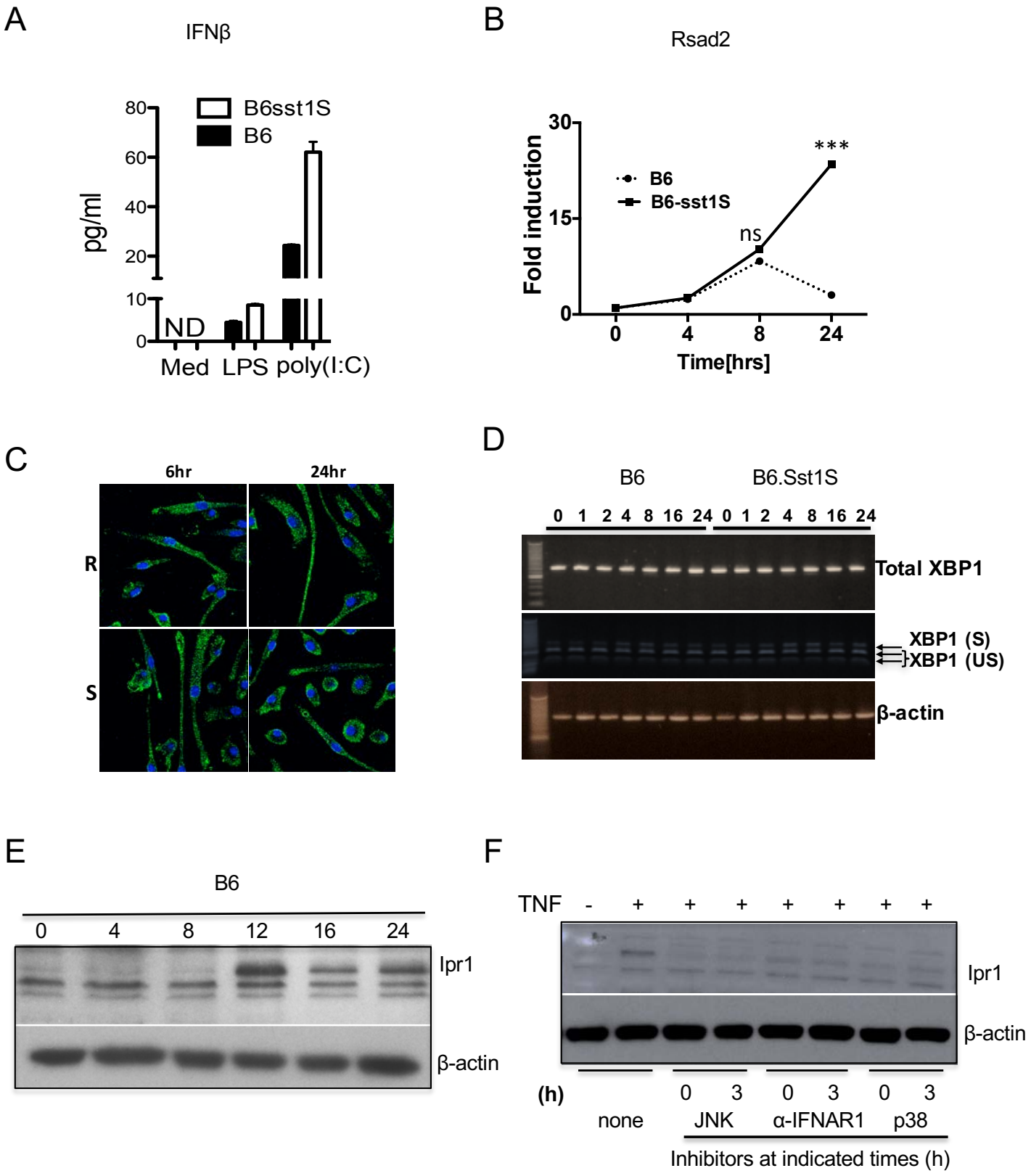
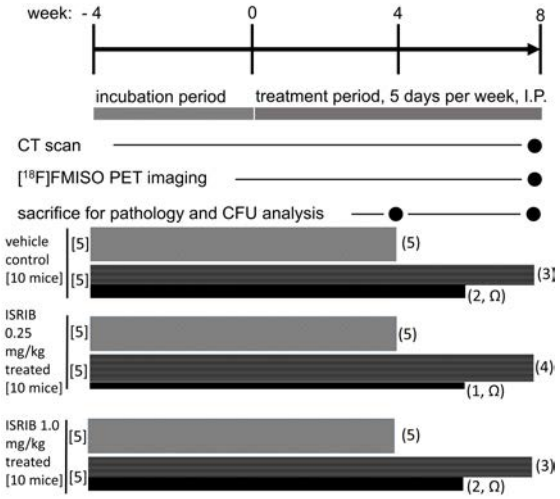
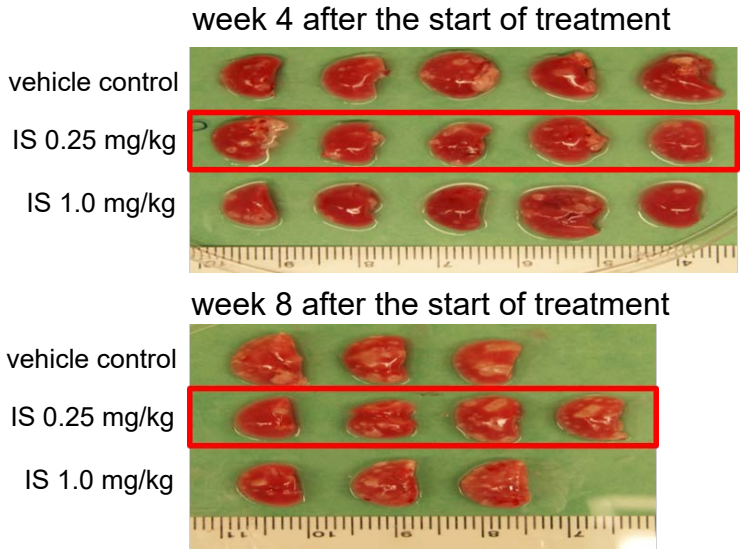

A
IFNβ
B6sst1S
B6
pg/ml
80
60
40
20
10
0
ND
Med
LPS
poly(I:C)
B
Rsad2
Fold induction
30
15
0
B6
B6-sst1S
ns
***
0
4
8
24
Time[hrs]
C
6hr
24hr
R
S
D
B6
B6.Sst1S
0 1 2 4 8 16 24 0 1 2 4 8 16 24
Total XBP1
XBP1 (S)
XBP1 (US)
β-actin
E
B6
0
4
8
12
16
24
Ipr1
β-actin
F
TNF - + + + + + + +
Ipr1
β-actin
(h)
0 3 0 3 0 3
none
JNK
α-IFNAR1
p38
Inhibitors at indicated times (h)

Suppl.Fig. 1

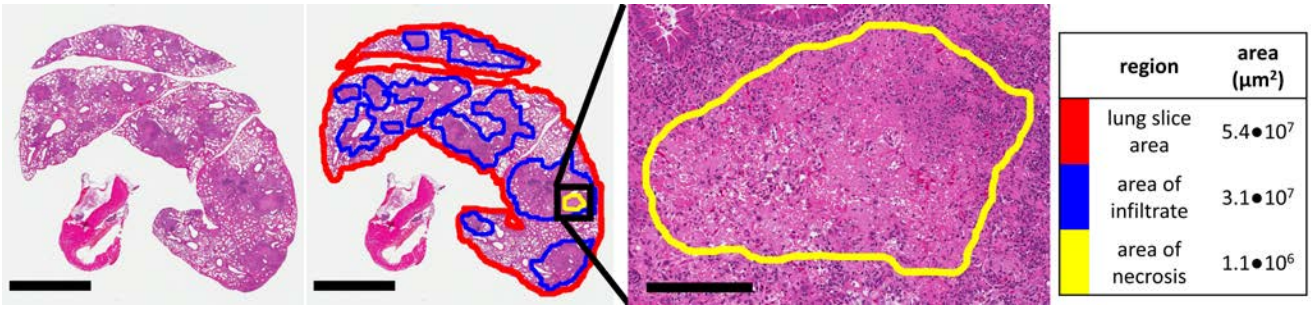
a



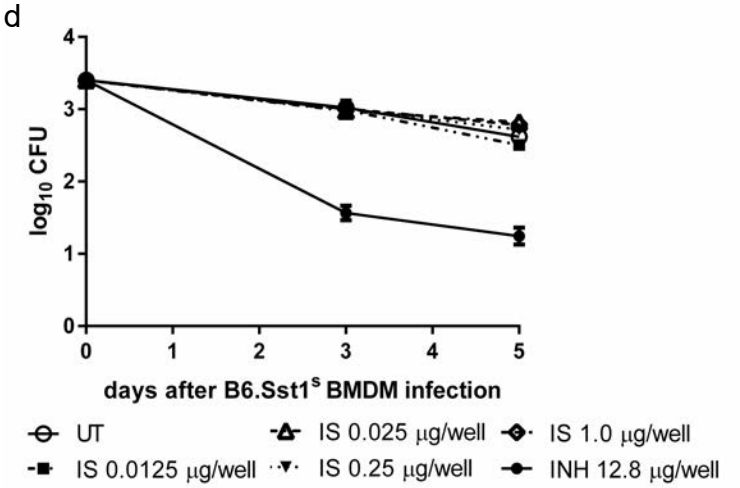
b



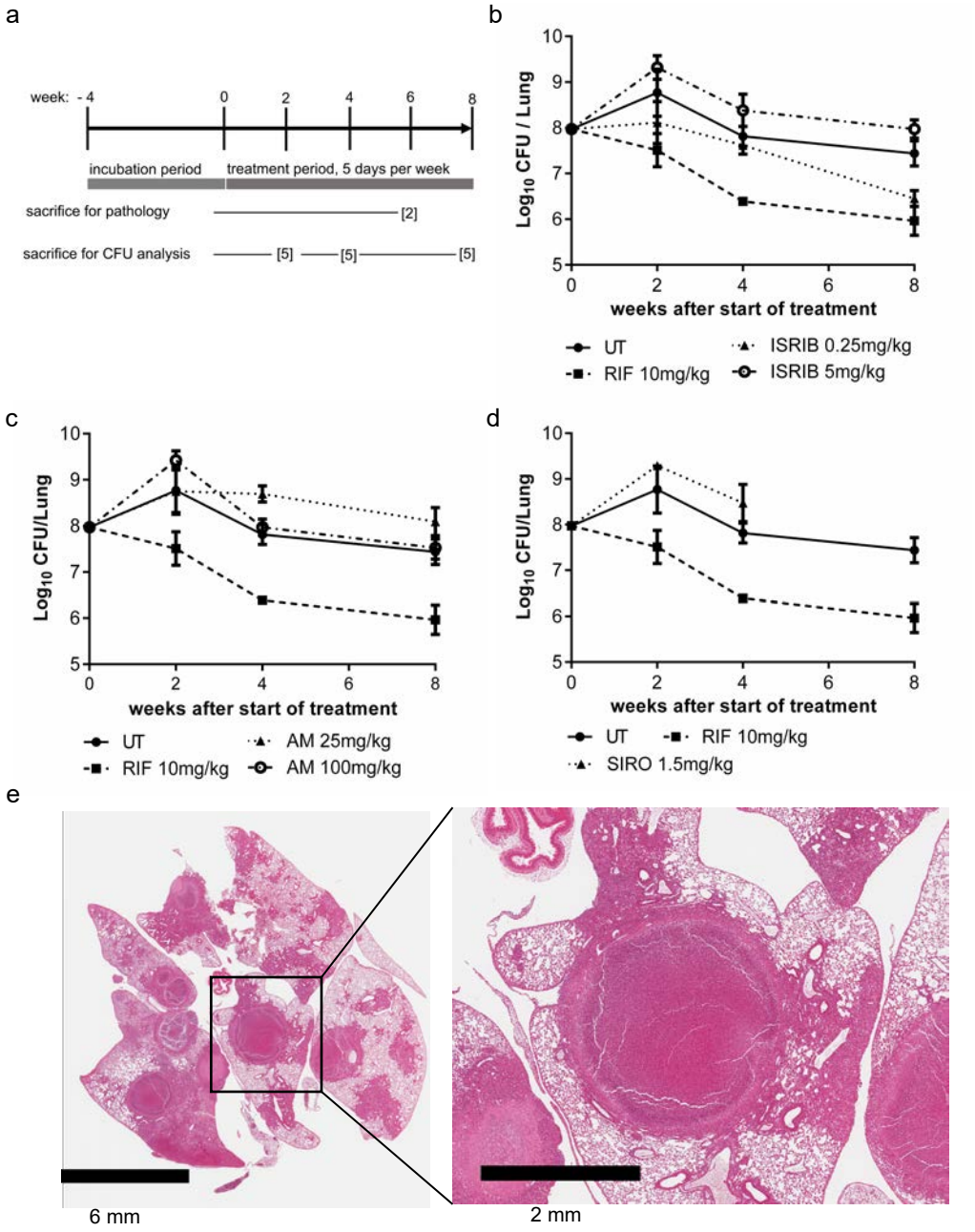
c



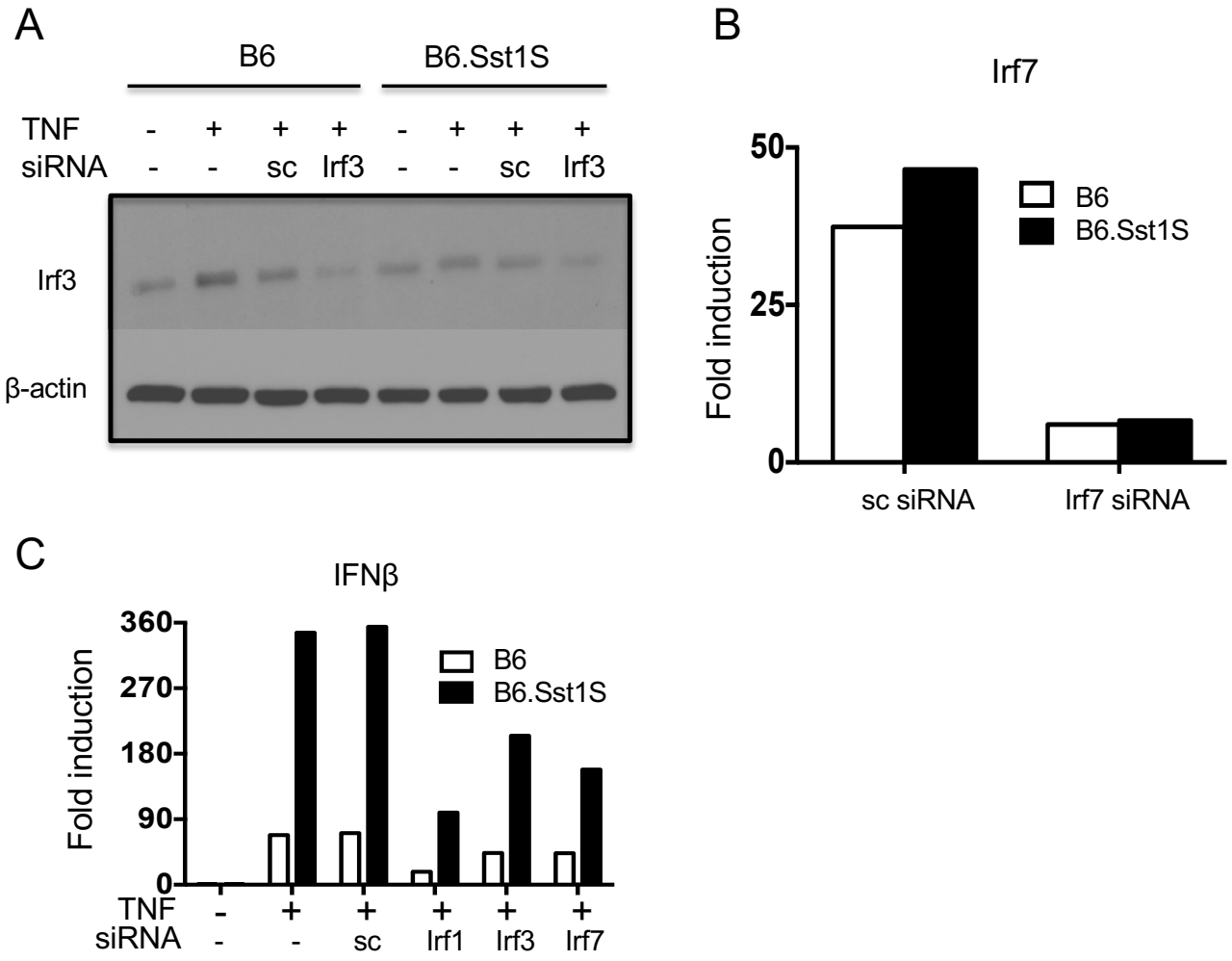
d

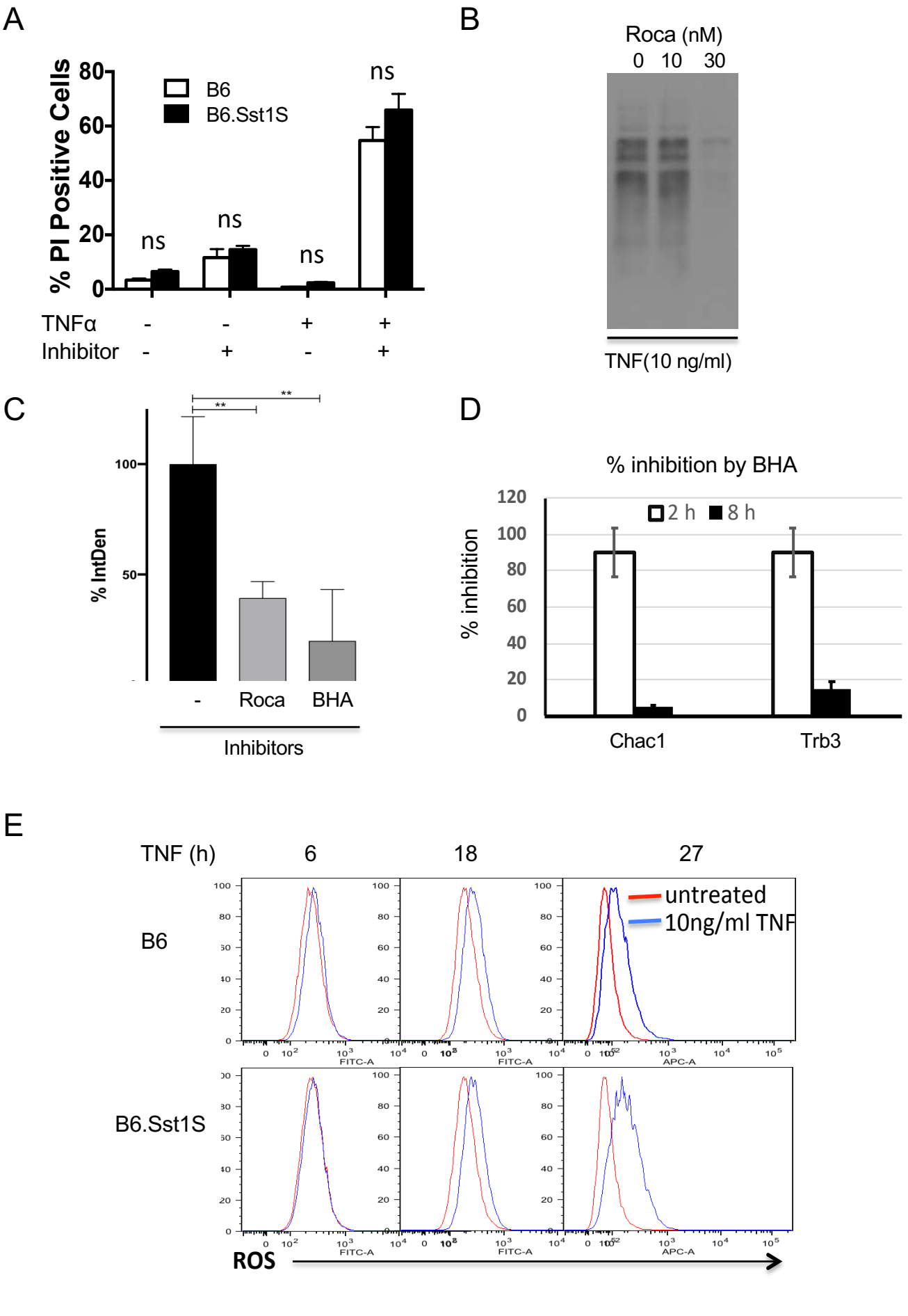


Suppl.Fig.2


a
week: -4
0
2
4
6
8
incubation period
treatment period, 5 days per week
sacrifice for pathology
[2]
sacrifice for CFU analysis
[5]
[5]
[5]
b
Log10 CFU / Lung
weeks after start of treatment
UT
RIF 10mg/kg
ISRIB 0.25mg/kg
ISRIB 5mg/kg
c
Log10 CFU/Lung
weeks after start of treatment
UT
RIF 10mg/kg
AM 25mg/kg
AM 100mg/kg
d
Log10 CFU/Lung
weeks after start of treatment
UT
RIF 10mg/kg
SIRO 1.5mg/kg
e
6 mm
2 mm

Suppl.Fig.3


A
B6
B6.Sst1S
TNF - + + + - + + +
siRNA - - sc Irf3 - - sc Irf3
Irf3
β-actin
B
Irf7
Fold induction
50
25
0
B6
B6.Sst1S
sc siRNA
Irf7 siRNA
C
IFNβ
Fold induction
360
270
180
90
0
B6
B6.Sst1S
TNF - + + + + +
siRNA - - sc Irf1 Irf3 Irf7


A
% PI Positive Cells
B6
B6.Sst1S
ns
TNFα
Inhibitor
B
Roca (nM)
0 10 30
TNF(10 ng/ml)
C
% IntDen
Roca
BHA
Inhibitors
D
% inhibition by BHA
2 h
8 h
% inhibition
Chac1
Trb3
E
TNF (h)
6
18
27
B6
B6.Sst1S
untreated
10ng/ml TNF
FITC-A
APC-A
ROS

Suppl. Fig.5

Supplemental Figure legends

Supplemental Figure 1.

a) IFNβ concentrations in supernatants of B6wt and B6.Sst1S BMDM treated with 100ng/ml LPS and 1ug/ml poly IC for 24h, as determined by ELISA.

b) Time course of Rsad2 mRNA expression in B6wt and B6.Sst1S BMDM treated with 10ng/ml TNF (representative of two independent experiments).

c) Staining with dsRNA-specific J2 antibodies of B6wt and B6.Sst1S BMDMs treated with 10ng/ml TNF for 6 and 24 h. Cells were stained with J2 antibody (green); nuclei are counterstained with DAPI (blue). All microscopic images represent data from two independent experiments.

d) Time course analysis of XBP-1 mRNA expression and splicing in TNF-stimulated B6wt and B6.Sst1S BMDMs. Total RNA samples isolated from TNF stimulated B6wt and B6.Sst1S BMDM were amplified using RT-PCR and digested with Pst1, as described in Methods. The resultant PCR products were separated by electrophoresis on 2% agarose gel. The PCR products of spliced XBP1(S) remained intact, whereas the PCR products of unspliced XBP1(U) mRNA were cut into two fragments as indicated by arrows.

e) The kinetics of the IPR1 protein expression in B6wt macrophages after TNF stimulation. No IPR1 protein was detected in corresponding samples of B6.Sst1S BMDMs.

f) Effects of JNK and p38 inhibitors and IFNAR blockade on IPR1 protein induction in B6wt BMDM treated with 10ng/mL of TNFα for 24 h. The inhibitors were added at the beginning (0hr) or after 3hrs of TNF stimulation. Immunoblotting was carried out using rabbit anti-IPR1 polyclonal antibody and represents two independent experiments.

Supplemental Figure 2. Effect of ISRIB on TB progression in B6.Sst1S mice and on *M.tb* replication in B6-sst1S BMDMs.

a) Experimental overview of mouse chronic infection model used to test effects of ISRIB monotherapy on *M. tb* bacterial growth. At left, [#] indicates number of mice in each experimental group and subgroup in the experimental plan. At right, (#) indicates number of mice in each analysis group. (#, Ω) indicates number of mice excluded from analysis because of morbidity and mortality based on IRB and study criteria.

b) Lung gross pathology of B6-sst1S mice infected with *M. tb* following 4 and 8 weeks of ISRIB treatment (8 and 12 weeks post infection).

c) Overview of quantitative lung pathology analysis. Areas were drawn by eye and validated by a veterinary pathologist. Analysis was conducted using Aperio ImageScope (Leica Biosystems)

d) BMDM infection ISRIB dose ranging experiment. B6.Sst1S mice bone marrow derived macrophages were infected with *M. tb* and treated with ISRIB (0.0125, 0.025, 0.25 or 1 μg/ml) 1 hour after infection. Bacillary loads were enumerated in day 0, 3 and 5 after infection. INH=isoniazid, UT=untreated infected macrophage. IS = ISRIB. Data plotted as means±SD.

Supplemental Figure 3. Effect of small molecule inhibitors on TB progression in C3HeB/FeJ mice.

a) Overview of experiments and data presented in b – e. [#] indicates number of mice/analysis group at each endpoint.

(**b - d**) *M. tb*-infected C3HeB/FeJ mice were treated with ISRIB (IS) at doses 0.25 or 5 mg/kg, Amlexanox (AM) at doses 25 or 100 mg/kg, sirolimus (SIRO) at dose 1.5 mg/kg, and untreated (UT). All doses were given 5 days per week during the treatment period. Data plotted as means ± SEM.

e) Histopathology exemplifying necrotic lung granulomas in a mouse treated with Amlexanox 25mg/kg at week 6 of treatment (H&E staining).

Supplemental Figure 4.

a) IRF3 expression and validation of IRF3 knockdown using immunoblotting with IRF3-specific antibodies (sc – scrambled control; Irf3 – specific siRNA).

b) Validation of Irf7 knockdown in B6wt and B6-sst1S BMDM. Irf7 mRNA expression was quantified using qRT-PCR, normalized to expression of 18S rRNA and presented relative to expression in untreated cells (set as 1). The data are representative of two independent experiments.

c) Effects of the Irf1, Irf3 and Irf7 knockdowns on TNF-induced expression of IFNβ mRNA in B6 and B6-sst1S BMDMs 18 h after stimulation with TNF (10ng/ml).

Supplemental Figure 5.

a) Synergistic effect of TNF and HSF1 inhibitor KRIB11 on macrophage death. B6 and B6-sst1S BMDM were treated with 10ng/ml TNFα for 24 hrs in presence or absence of 10uM KRIB11. Cell death was assessed using automated microscopy as % of PI positive cells. Data represents results from two independent experiments.

b) Inhibition of protein translation in TNF-stimulated BMDM by the rocaglate treatment. TNF and rocaglate at indicated concentrations were added simulataneouly for 24 h. The protein biosynthesis rate was determined using puromycylation assay.

c) Quantification of the effects of rocaglate and BHA treatment on aggressome formation induced by TNF in B6.Sst1S BMDMs using particle analysis module in ImageJ software.

d) Effects of an oxidative stress inhibitor BHA on TNF-induced Trb3 and Chac1 mRNA levels in B6.Sst1S BMDMs. BHA was added 2 or 8 hours after TNF stimulation. The mRNA levels were determined using qRT-PCR at 18 h of TNF stimulation. % inhibition was calculated as compared to TNF stimulated untreated cells.

f) ROS production in B6wt and B6.Sst1S BMDM stimulated with 10ng/ml TNFα for the indicated times. Flow cytometry analysis was performed using DCFDA (dichlorofluorescindiacete) probe (Abcam).