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Supplemental Information

hGBP1 Coordinates *Chlamydia* Restriction and Inflammasome Activation through Sequential GTP Hydrolysis

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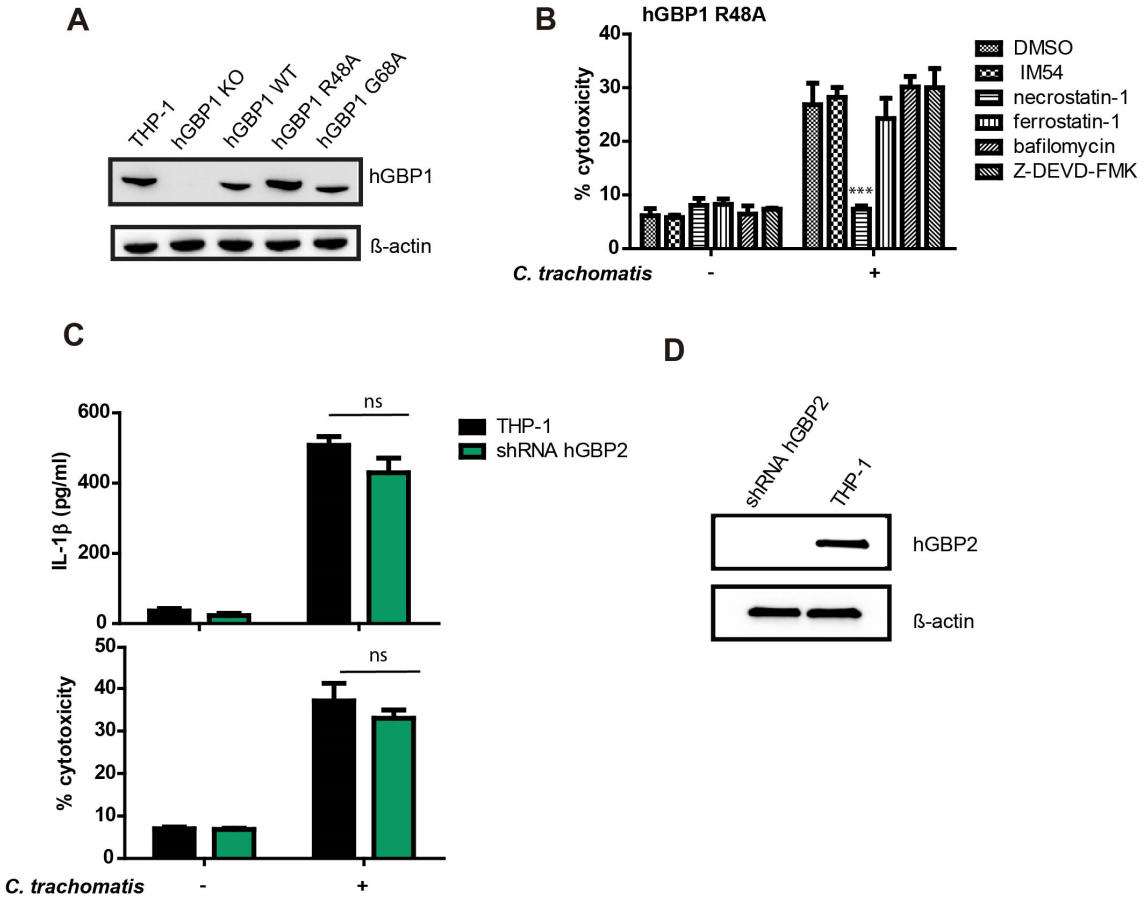

Figure S1. hGBP1 restricts *C. trachomatis*, related to Figure 2.

(A) CRISPR-Cas9-mediated knock-out of hGBP1 and stable overexpression of hydrolysis mutants as shown by immunoblotting. Cell lines were stimulated with IFN-γ for 16 h.

(B) LDH release of THP-1 hGBP1 R48A cell line stimulated with IFN-γ + LPS for 16 h, followed by infection with *C. trachomatis* at MOI 30 for 24 h. Cells were co-treated with indicated cell death inhibitors for 24 h, n=3; two-way ANOVA. Cell death inhibitors - IM54, necrostatin-1, ferrostatin-1, bafilomycin and Z-DEVD-FMK that inhibit necrosis, necroptosis, ferroptosis, autophagy and apoptosis, respectively.

(C) hGBP2 knock-down by shRNA in THP-1 cells. IL-1β ELISA and LDH release following infection with *C. trachomatis* at MOI 30 for 24 h, n=3; two-way ANOVA.

(D) Stable-knock down of hGBP2 in THP-1 cells shown by immunoblotting against hGBP2. Cells were treated with IFN-γ for 16 h.

All error bars indicate SEM. *P<0.05, **P<0.01, ***P<0.001.

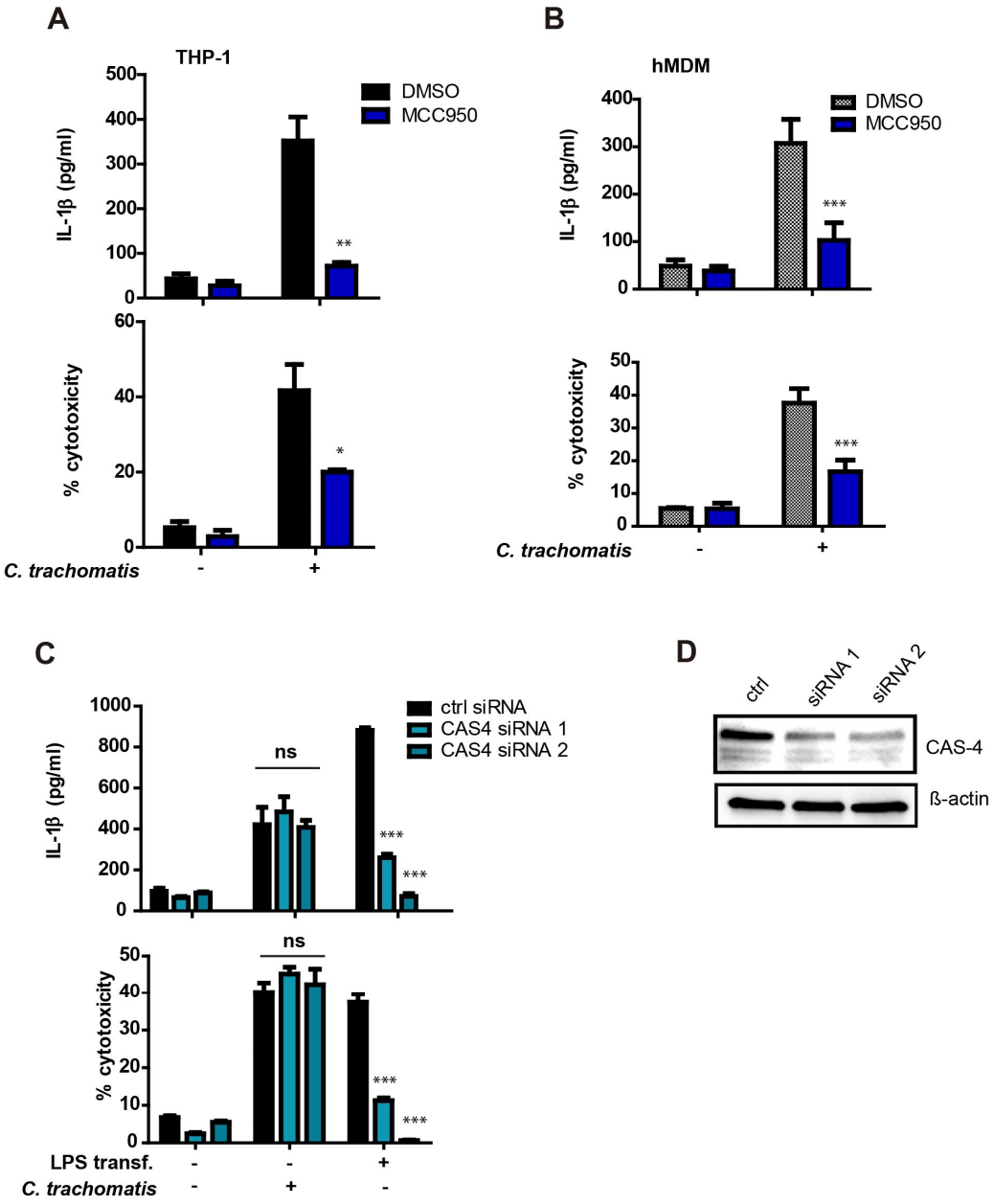

Figure S2. hGBP1 activates the caspase-4-independent NLRP3 inflammasome pathway upon *C. trachomatis* infection, related to Figure 3

(A) IL-1β ELISA and LDH release for THP-1 cells treated with MCC950 and infected with *C. trachomatis* at MOI 30 for 24 h, n=3; two-way ANOVA.

(B) IL-1β ELISA and LDH release for hMDMs treated with MCC950 and infected with *C. trachomatis* at MOI 30 for 24 h, n=3; two-way ANOVA.

(C) IL-1β ELISA and LDH release for THP-1 and THP-1 caspase-4 knock down cells, transfected with LPS or infected with *C. trachomatis* at MOI 30 for 24 h, n=3; two-way ANOVA.

(D) Transient knock down of caspase-4 in THP-1 cells was validated by immunoblotting against caspase-4. Cells were primed with IFN-γ and LPS.

*P<0.05, **P<0.01, ***P<0.001.

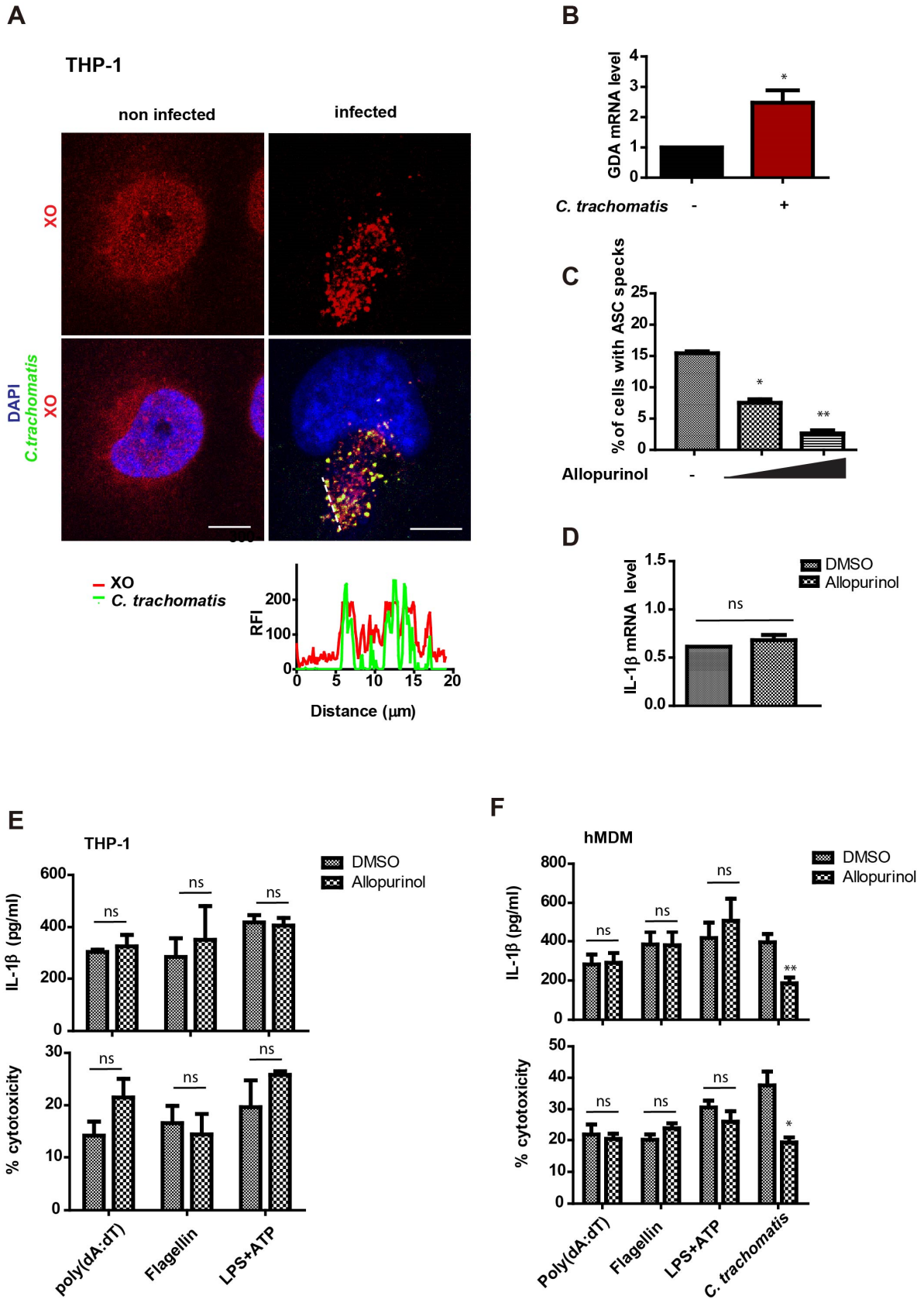
A
THP-1
non infected
infected
XO
DAPI
C.trachomatis
XO
XO
C. trachomatis
RFI
200
100
0
0 5 10 15 20
Distance (μm)
B
GDA mRNA level
4
3
2
1
0
*
C. trachomatis - +
C
% of cells with ASC specks
25
20
15
10
5
0
*
**
Allopurinol -
D
IL-1β mRNA level
1.5
1.0
0.5
0.0
ns
DMSO
Allopurinol
E
THP-1
DMSO
Allopurinol
IL-1β (pg/ml)
600
400
200
0
ns
ns
ns
% cytotoxicity
30
20
10
0
ns
ns
ns
poly(dA:dT)
Flagellin
LPS+ATP
F
hMDM
DMSO
Allopurinol
IL-1β (pg/ml)
800
600
400
200
0
ns
ns
ns
**
% cytotoxicity
50
40
30
20
10
0
ns
ns
ns
*
Poly(dA:dT)
Flagellin
LPS+ATP
C. trachomatis

Figure S3. Allopurinol specifically abrogates inflammasome activation upon *C. trachomatis* infection, related to Fig. 4.

(A) Representative images of xanthine oxidase (red) immunostaining with or without *C. trachomatis* (green) infection. The line tracing quantification of the white dashed line is shown at the bottom. Scale bar = 10 µm.

(B) mRNA levels of GDA in THP-1 cells upon infection or not with *C. trachomatis* at MOI 30 for 24 h, n=3; two-tailed Student´s t-test.

(C) Quantification of inflammasome ASC specks in THP-1 cells upon infection with *C. trachomatis* at MOI 30. The cells were co-treated with increasing concentrations of allopurinol (100 µM and 500 µM) for 24 h, n=3,; one-way ANOVA.

(D) mRNA levels of IL-1β in THP-1 stimulated and treated or untreated with allopurinol (500 µM), n=3; two-tailed Student´s t-test.

(E) IL-1β ELISA and LDH release of THP-1 cells, which were transfected with Flagellin, poly (dA:dT) or stimulated with LPS + ATP and co-treated with allopurinol (500 µM) as indicated, n=3; two-tailed Student´s t-test.

(F) IL-1β ELISA and LDH release of THP-1 cells transfected with Flagellin, poly (dA:dT) or stimulated with LPS + ATP or infected with *C. trachomatis* and co-treated with allopurinol (500 µM) as indicated, n=3; two-tailed Student´s t-test.

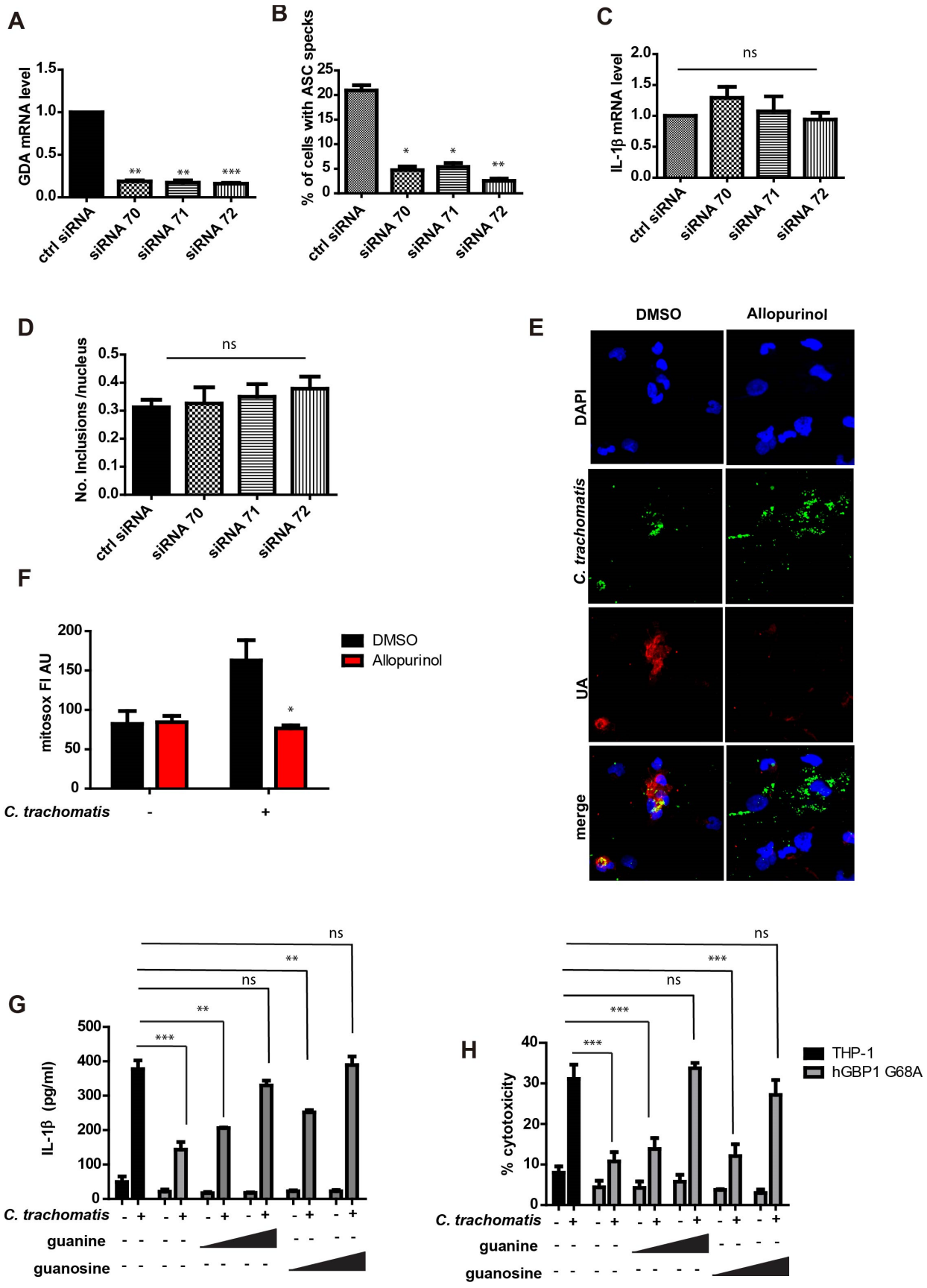
A
GDA mRNA level
1.5
1.0
0.5
0.0
**
**
***
ctrl siRNA
siRNA 70
siRNA 71
siRNA 72
B
% of cells with ASC specks
25
20
15
10
5
0
*
*
**
ctrl siRNA
siRNA 70
siRNA 71
siRNA 72
C
IL-1β mRNA level
2.0
1.5
1.0
0.5
0.0
ns
ctrl siRNA
siRNA 70
siRNA 71
siRNA 72
D
No. Inclusions /nucleus
0.5
0.4
0.3
0.2
0.1
0.0
ns
ctrl siRNA
siRNA 70
siRNA 71
siRNA 72
E
DMSO
Allopurinol
DAPI
C. trachomatis
UA
merge
F
mitosox FI AU
200
150
100
50
0
*
DMSO
Allopurinol
C. trachomatis - +
G
IL-1β (pg/ml)
500
400
300
200
100
0
ns
**
ns
**
***
C. trachomatis - + - + - + - + - + - +
guanine - - - - - - - -
guanosine - - - - - - - -
H
% cytotoxicity
40
30
20
10
0
ns
***
ns
***
***
THP-1
hGBP1 G68A
C. trachomatis - + - + - + - + - + - +
guanine - - - - - - - -
guanosine - - - - - - - -

Figure S4. Uric acid production upon *C. trachomatis* infection, related to Fig. 4.
(A) GDA knock down verification. GDA mRNA levels in THP-1 cells transfected with control or 3 different GDA siRNAs, n=3; one-way ANOVA.
(B) Quantification of inflammasome ASC specks in THP-1 transfected with 3 different GDA siRNAs, and infected with *C. trachomatis* at MOI 30 for 24 h, n=3; one-way ANOVA.
(C) mRNA levels of IL-1β in THP-1 cells transfected with three different GDA siRNAs (n=3; one-way ANOVA.
(D) Inclusion count of THP-1 cells transfected with three different GDA siRNAs and infected with *C. trachomatis* at MOI 5 for 24 h, n=3, one-way ANOVA.
Representative images of uric acid fluorescence intensity quantification, as in Fig. 4B.
(E) THP-1 cells were stimulated with IFN-γ + LPS, infected with *C. trachomatis* at MOI 30, and treated with allopurinol or DMSO. Cells were stained for *C. trachomatis* (green), uric acid (red) and DAPI (blue).
(F) Fluorescence intensity measurements of mitochondrial ROS in THP-1 cells infected or not with *C. trachomatis* and treated or not with 500 μM allopurinol, n=3; two-way ANOVA.
(G, H) IL-1β ELISA (G) and LDH release (H) of hGBP1 WT or G68A mutant cells infected with *C. trachomatis* at MOI 30 for 24 h and treated with increasing concentrations of guanine or guanosine (0.25 mM and 0.5 mM), n=3; significance was determined in comparison to the parental cells by two-way ANOVA.
All error bars indicate SEM. *P<0.05, **P<0.01, ***P<0.001.