# Expanded View Figures

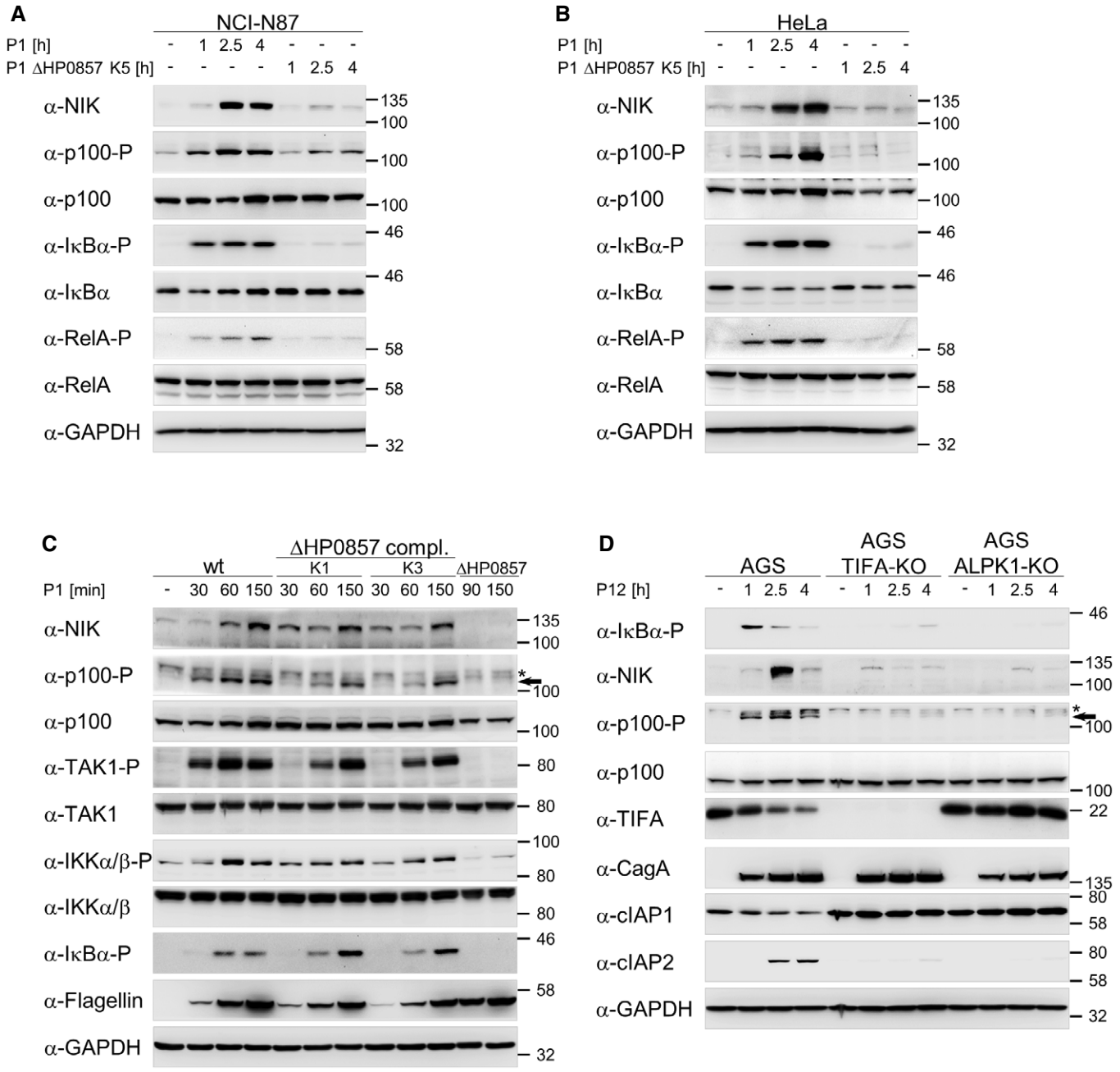


**Figure EV1. Induction of the classical and alternative NF-κB pathways by *H. pylori* depends on ADP-heptose.**

A, B Infection of NCI-N87 and HeLa cells with the *H. pylori* P1 wild-type or isogenic ΔHP0857 (mutated in the *gmhA* gene) strains was performed.

C AGS cells were infected with the *H. pylori* P1 wild-type strain, isogenic strain mutated in the *gmhA* gene (ΔHP0857) alone or complemented with P1 *gmhA* gene. Asterisk denotes an unspecific band and the arrow indicates the band corresponding to phosphorylated p100.

D Infection of AGS, TIFA-KO, and ALPK1-KO cells with the *H. pylori* P12 wild-type strain was performed. Asterisk denotes an unspecific band and the arrow indicates the band corresponding to phosphorylated p100.

Data information: Total cell lysates were analyzed by immunoblotting with the indicated antibodies. Data shown are representative for at least two independent experiments. GAPDH served as loading control.
Source data are available online for this figure.

**Figure EV2. TIFA is required for the activation of NF-κB upon *H. pylori* infection.**

A The nuclear translocation of transcription factors RelA and RelB was analyzed in nuclear fractions of AGS and TIFA-KO cells. C23 served as loading control.

B Analysis of the activation of ERK1/2, p38, and JNK in AGS and TIFA-KO cells by immunoblot. CagA demonstrated a similar infection rate of different cells.

C–E Analysis of IL-1β (C), TNF (D), and $LT\alpha_1\beta_2$ (E) treatment of AGS and TIFA-KO cells by immunoblotting revealed the specificity of TIFA for *H. pylori* infection. Cell lysates were analyzed by immunoblotting with the indicated antibodies. Asterisk denotes an unspecific band and the arrow indicates the band corresponding to phosphorylated p100 (E).

F Transfection of His-tagged recombinant TIFA into TIFA-KO cells rescued the activation of classical and alternative NF-κB pathways upon *H. pylori* infection. Cell lysates were analyzed by immunoblotting with the indicated antibodies.

G AGS cells were transfected with siRNA against TRAF2 and infected with the *H. pylori* P1 wild-type strain. Cell lysates were analyzed by immunoblotting using the indicated antibodies.

H AGS cells were transfected with siRNA against TRAF6 and infected with the *H. pylori* P1 wild-type strain. Cell lysates were analyzed by immunoblotting using the indicated antibodies.

Data information: Data shown are representative for at least two independent experiments. GAPDH served as loading control.
Source data are available online for this figure.

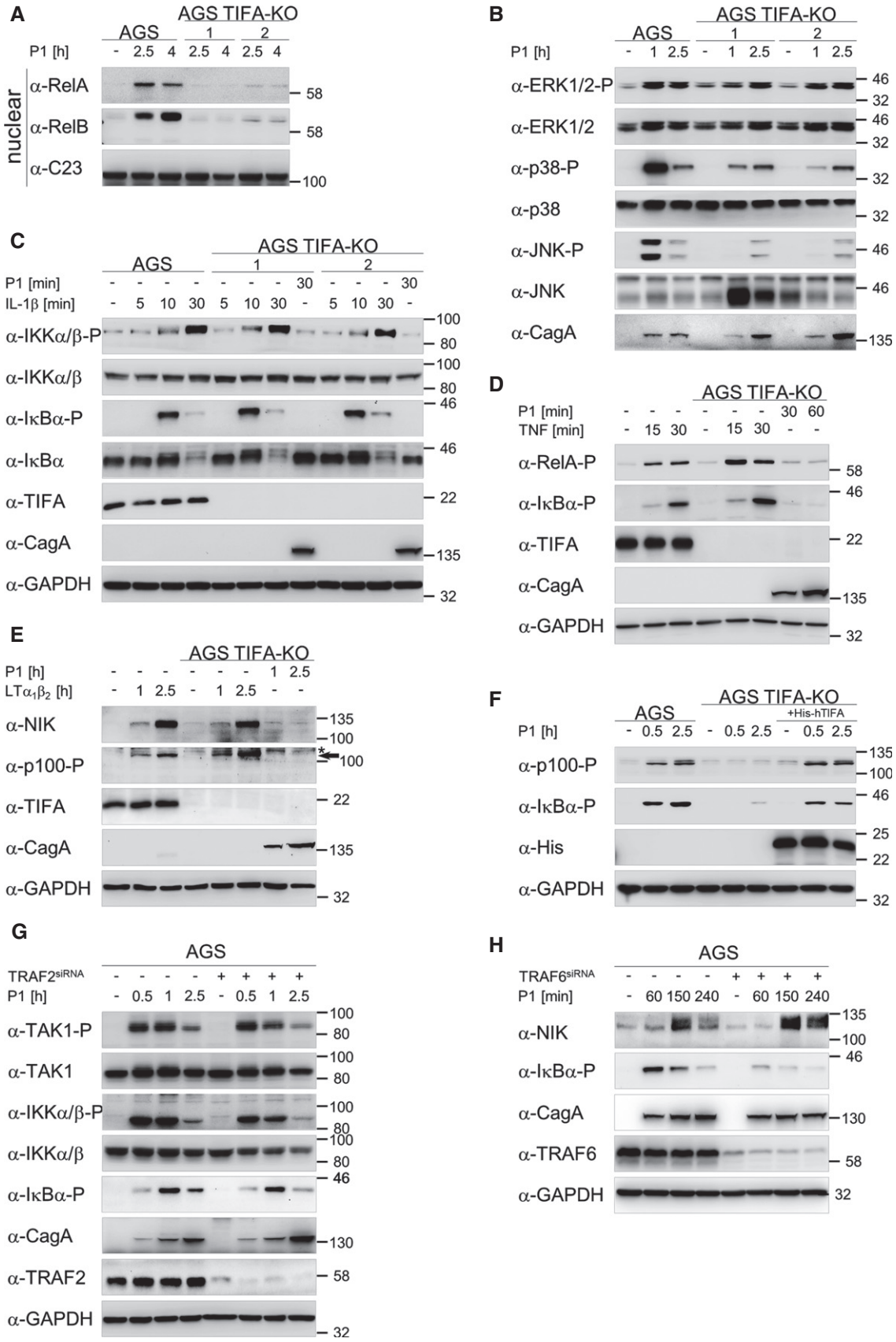
A
AGS TIFA-KO
AGS 1 2
P1 [h] - 2.5 4 2.5 4 2.5 4
nuclear
α-RelA
α-RelB
α-C23
58
58
100
B
AGS TIFA-KO
AGS 1 2
P1 [h] - 1 2.5 - 1 2.5 - 1 2.5
α-ERK1/2-P
α-ERK1/2
α-p38-P
α-p38
α-JNK-P
α-JNK
α-CagA
46
32
46
32
32
32
46
46
135
C
AGS TIFA-KO
AGS 1 2
P1 [min] - - - - - - - 30 - - - - 30
IL-1β [min] - 5 10 30 5 10 30 - 5 10 30 -
α-IKKα/β-P
α-IKKα/β
α-IκBα-P
α-IκBα
α-TIFA
α-CagA
α-GAPDH
100
80
100
80
46
46
22
135
32
D
AGS TIFA-KO
P1 [min] - - - - - - 30 60
TNF [min] - 15 30 - 15 30 - -
α-RelA-P
α-IκBα-P
α-TIFA
α-CagA
α-GAPDH
58
46
22
135
32
E
AGS TIFA-KO
P1 [h] - - - - - - 1 2.5
LTα1β2 [h] - 1 2.5 - 1 2.5 - -
α-NIK
α-p100-P
α-TIFA
α-CagA
α-GAPDH
135
100
100
22
135
32
F
AGS TIFA-KO
+His-hTIFA
AGS
P1 [h] - 0.5 2.5 - 0.5 2.5 - 0.5 2.5
α-p100-P
α-IκBα-P
α-His
α-GAPDH
135
100
46
25
22
32
G
AGS
TRAF2siRNA - - - - + + + +
P1 [h] - 0.5 1 2.5 - 0.5 1 2.5
α-TAK1-P
α-TAK1
α-IKKα/β-P
α-IKKα/β
α-IκBα-P
α-CagA
α-TRAF2
α-GAPDH
100
80
100
80
100
80
100
80
46
130
58
32
H
AGS
TRAF6siRNA - - - - + + + +
P1 [min] - 60 150 240 - 60 150 240
α-NIK
α-IκBα-P
α-CagA
α-TRAF6
α-GAPDH
135
100
46
130
58
32

**Figure EV2.**

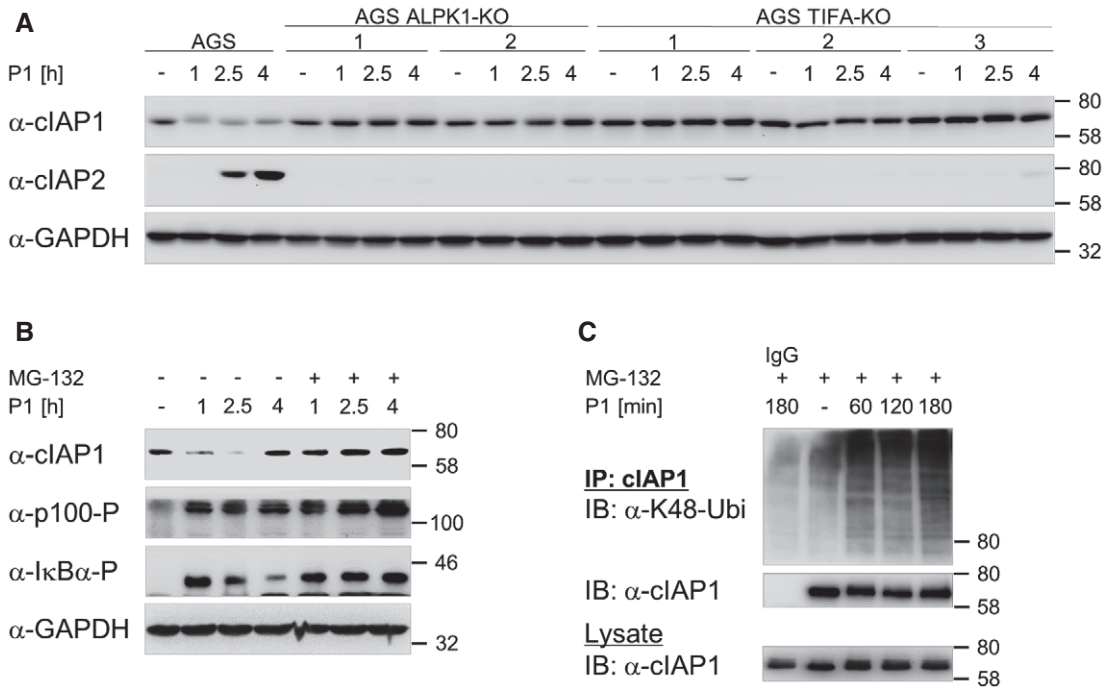


**Figure EV3. Degradation of cIAP1 and synthesis of cIAP2 upon *H. pylori* infection in AGS, ALPK1-KO, and TIFA-KO cells.**

A AGS, ALPK1-KO, and TIFA-KO cells were infected with *H. pylori*. Total cell lysates were analyzed by immunoblotting for the expression of cIAP1 and cIAP2.

B AGS cells were treated with 10 μM MG-132 thirty minutes after infection with *H. pylori*. Total cell lysates were analyzed by immunoblotting for the expression of cIAP1 and phosphorylation of p100 and IκBα.

C AGS cells were treated with 10 μM MG-132 thirty minutes after infection with *H. pylori*. Cells were harvested in lysis buffer containing 1% SDS. Lysates were diluted 1:10 in lysis buffer without SDS and subjected to IP with antibody against cIAP1 or an isotype-matched antibody (IgG). Eluates and total cell lysates were analyzed by immunoblotting using the indicated antibodies.

Data information: Data shown are representative for at least two independent experiments. GAPDH served as loading control.
Source data are available online for this figure.

**Figure EV4. Co-localization of TRAF6 with TIFAsomes formed upon *H. pylori* infection.**

A TIFA-KO cells transiently expressing TIFA-tdTomato were left uninfected or infected with *H. pylori* for 60 min. TIFAsomes were detected by epifluorescence microscopy. Secondary antibody control with no primary antibody added was also shown. The nuclei were counterstained with DAPI. Scale bar = 10 μm.

B TIFA-KO cells were transiently transfected with TIFA-tdTomato and infected with *H. pylori* for 60 min, followed by immunofluorescence detection of TRAF6. Specific regions of interest (ROIs) in the micrographs were analyzed for co-localization between TRAF6 and TIFAsomes. The Fiji software with the plug-in JACoP using Costes' automatic threshold was used. Presented are the values for Pearson's coefficient (r) and Manders's coefficient (M1&M2). Scale bar = 20 μm (left) and 10 μm (right).

 

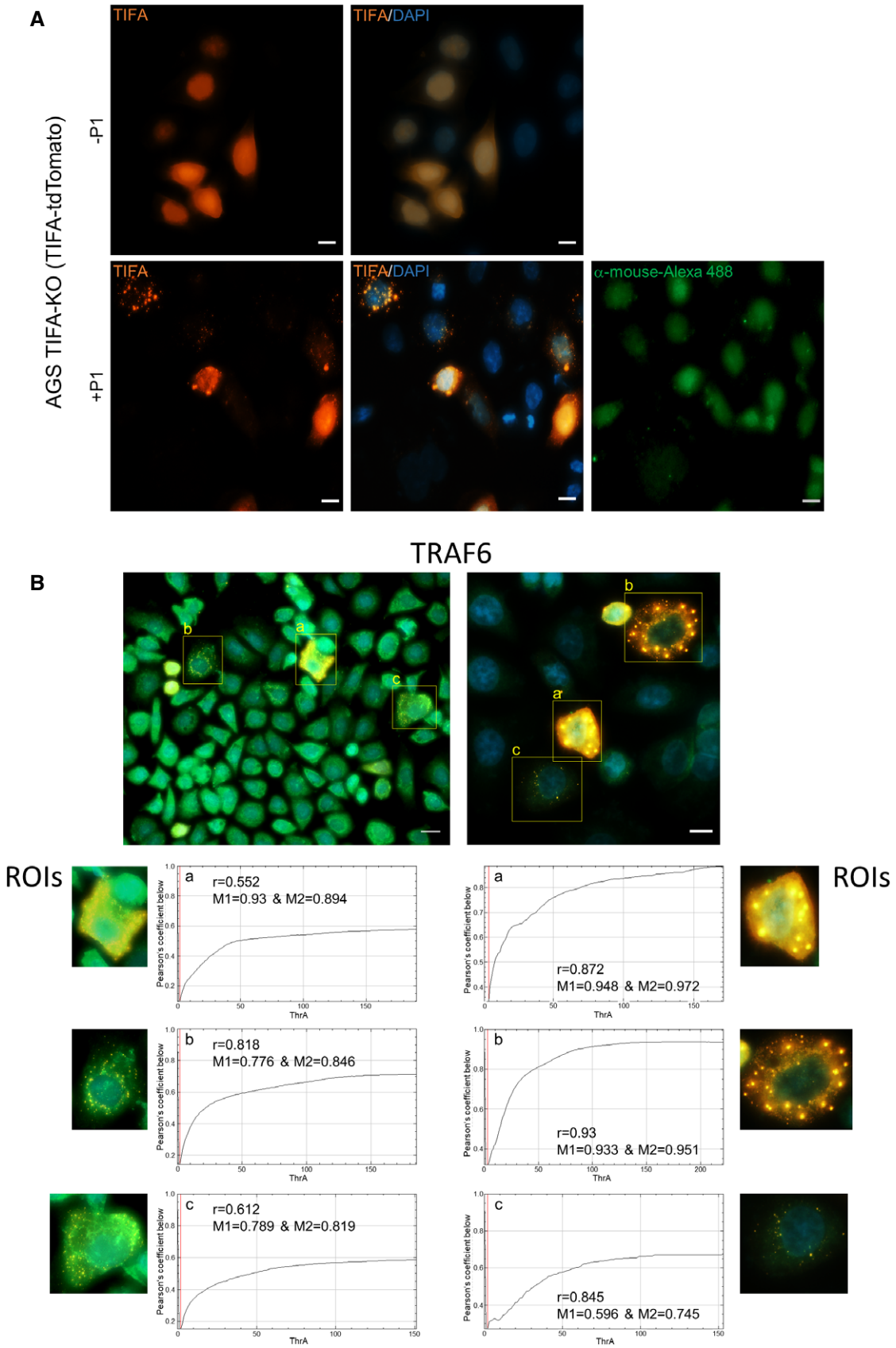
A
AGS TIFA-KO (TIFA-tdTomato)
-P1
+P1
TIFA
TIFA/DAPI
α-mouse-Alexa 488
B
TRAF6
ROIs
ROIs
a
b
c
r=0.552
M1=0.93 & M2=0.894
r=0.872
M1=0.948 & M2=0.972
r=0.818
M1=0.776 & M2=0.846
r=0.93
M1=0.933 & M2=0.951
r=0.612
M1=0.789 & M2=0.819
r=0.845
M1=0.596 & M2=0.745
Pearson's coefficient below
ThrA

**Figure EV4.**

**Figure EV5. Co-localization of TRAF3, TRAF2, or cIAP1 with TIFAsomes formed upon *H. pylori* infection.**

TIFA-KO cells were transiently transfected with TIFA-tdTomato and infected with *H. pylori* for 60 min, followed by immunofluorescence detection of TRAF2, cIAP1, and TRAF3. Specific regions of interest (ROIs) in the micrographs were analyzed for co-localization of TRAF2, cIAP1, or TRAF3 with TIFAsomes. The Fiji software with the plug-in JACoP using Costes' automatic threshold was used. Presented are the values for Pearson's coefficient (r) and Manders's coefficient (M1&M2). Scale bar = 20 μm (TRAF2, cIAP1) and 10 μm (TRAF3).

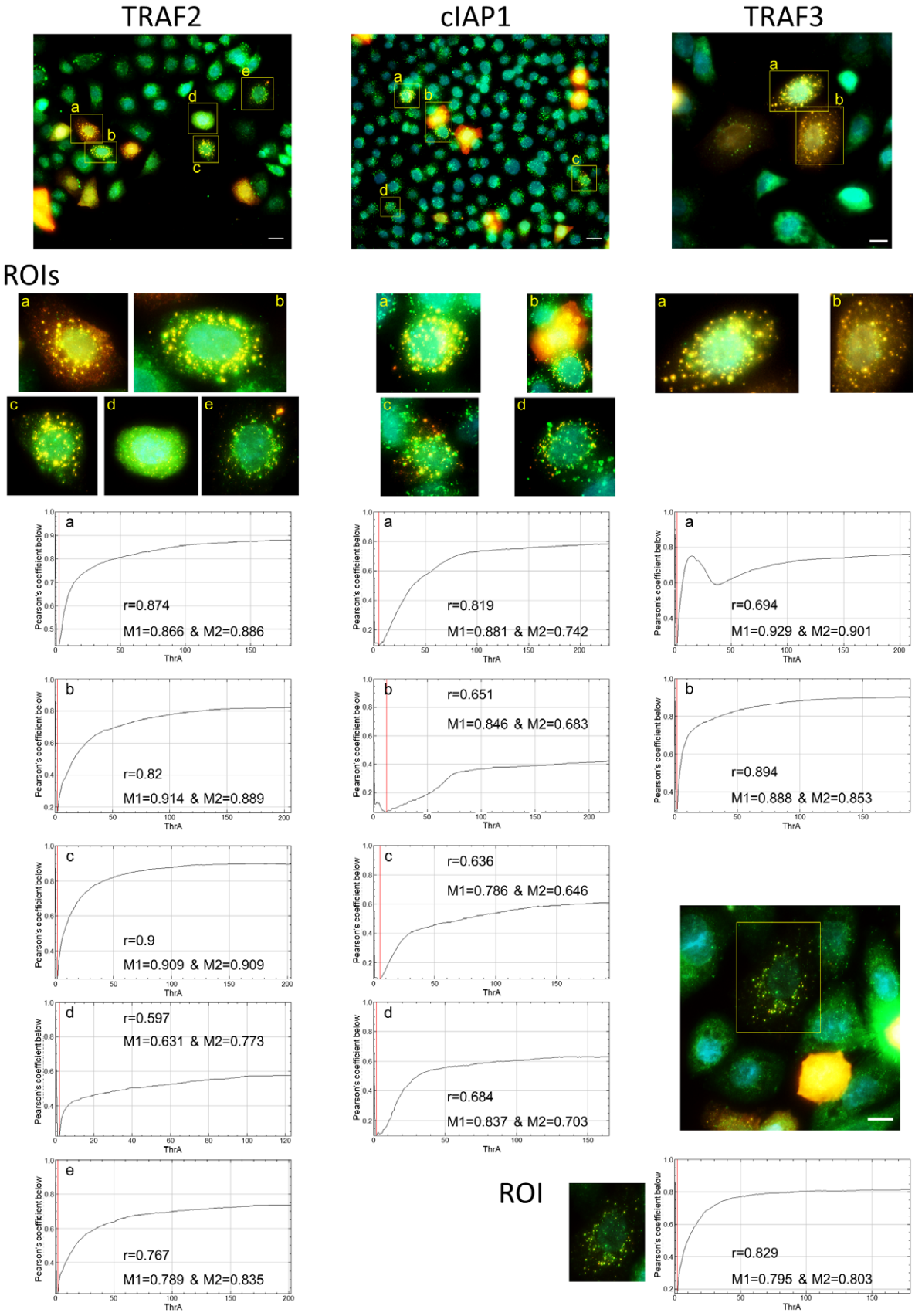
TRAF2
cIAP1
TRAF3
ROIs
Pearson's coefficient below
ThrA
r=0.874
M1=0.866 & M2=0.886
r=0.819
M1=0.881 & M2=0.742
r=0.694
M1=0.929 & M2=0.901
r=0.82
M1=0.914 & M2=0.889
r=0.651
M1=0.846 & M2=0.683
r=0.894
M1=0.888 & M2=0.853
r=0.9
M1=0.909 & M2=0.909
r=0.636
M1=0.786 & M2=0.646
r=0.597
M1=0.631 & M2=0.773
r=0.684
M1=0.837 & M2=0.703
r=0.767
M1=0.789 & M2=0.835
ROI
r=0.829
M1=0.795 & M2=0.803

**Figure EV5.**