A20 undermines alternative NF-κB activity and expression of anti-apoptotic genes in Helicobacter pylori infection

Michelle C.C. Lim¹, Gunter Maubach¹, Anna M. Birkl-Toeglhofer², Johannes Haybäck², Michael Vieth³, Michael Naumann¹#

Supplementary Figure 1: Infection by H. pylori activates the alternative NF-κB pathway. HKC-8 and NCI-N87 cells were infected with H. pylori for the times shown. Total cell lysates (a) or cytosolic (Cyt) and soluble nuclear (N1) fractions (b) were isolated and analyzed for the indicated proteins by IB. A representative blot of at least two experiments was shown.

Supplementary Figure 2: A20 contributes to the down-regulation of alternative NF-κB in H. pylori infection. a WT, A20KO_1 and A20KO_2 AGS cells were infected with H. pylori P12 strain. b AGS cells were transfected with non-target-specific siRNA (scr) or siRNAs targeting A20 (A20si₁,₅ or A20si₁,₉) for 48 h prior to infection with H. pylori P1 strain. c Same procedure as (b), except NCI-N87 cells were used. (a-c) Total cell lysates were analyzed by IB. A representative blot of at least two experiments was shown.

Supplementary Figure 3: Depletion of TRAF3 has no effect on the interaction of A20 with the TIFA/NIK regulatory complex. a AGS cells were transfected with non-target-specific siRNA (scr) or siRNAs targeting TRAF3 for 48 h prior to infection with H. pylori. Total cell lysates were used for IP with an antibody against TIFA or isotype-matched IgG. Eluates were analyzed by IB. A representative blot of at least two experiments was shown.
Supplementary Fig. 1

### a

| Hp [h] | HKC-8 | NCI-N87 |
|---|---|---|
| - | 2.5 | 4 | 9 | 2.5 | 4 | 9 |
| α-NIK | ![Image](image1.png) | ![Image](image2.png) |
| α-P-p100 | ![Image](image3.png) | ![Image](image4.png) |
| α-p100 | ![Image](image5.png) | ![Image](image6.png) |
| α-GAPDH | ![Image](image7.png) | ![Image](image8.png) |

### b

| Hp [h] | HKC-8 | NCI-N87 |
|---|---|---|
| - | 2.5 | 4 | 2.5 | 4 |
| α-p52 | ![Image](image9.png) | ![Image](image10.png) |
| α-GAPDH | ![Image](image11.png) | ![Image](image12.png) |
| α-p52 | ![Image](image13.png) | ![Image](image14.png) |
| α-HDAC1 | ![Image](image15.png) | ![Image](image16.png) |
Supplementary Fig. 2

a

|                | AGS |                |                |
|----------------|-----|----------------|----------------|
|                |     | WT             | A20KO.1        |
|                |     | A20KO.2        |                |
| Hp P12 [min]  | -   | 150 240        | 150 240        |
|                | -   | 150 240        | 150 240        |
| α-A20          |     |                |                |
| α-NIK          |     |                |                |
| α-P-p100       |     |                |                |
| α-p100         |     |                |                |
| α-GAPDH        |     |                |                |

b

|                | AGS |                |                |
|----------------|-----|----------------|----------------|
| A20hi.9        | -   | -              | -              |
| A20hi.5        | -   | +              | +              |
| scr            | +   | -              | -              |
| Hp [min]       | -   | 150 240        | 150 240        |
|                | -   | 150 240        | 150 240        |
| α-A20          |     |                |                |
| α-NIK          |     |                |                |
| α-P-p100       |     |                |                |
| α-p100         |     |                |                |
| α-GAPDH        |     |                |                |

c

|                |                |                |
|----------------|----------------|----------------|
| NCI-N87        |                |                |
| α-A20          | -   | -              | -              |
| α-NIK          | -   | +              | +              |
| α-P-p100       | -   | -              | -              |
| α-p100         | -   | -              | -              |
| α-GAPDH        | -   | -              | -              |
Supplementary Fig. 3

a

| TRAF3si | - | - | - | + | + |
| scr     | + | + | + | - | - |

| IP: TIFA | IgG |
|----------|-----|
| α-clAP1  |     |
| α-TRAF2  |     |
| α-TRAF3  |     |
| α-A20    |     |
| α-TIFA   |     |

Lysate

| α-clAP1  |     |
| α-TRAF2  |     |
| α-TRAF3  |     |
| α-A20    |     |
| α-TIFA   |     |
| α-GAPDH  |     |
Fig. 1 Uncropped blots

a

\( \alpha \)-NIK
\( \alpha \)-P-p100
\( \alpha \)-p100
\( \alpha \)-P-I\( \kappa \)B\( \alpha \)
\( \alpha \)-I\( \kappa \)B\( \alpha \)
\( \alpha \)-P-RelA
\( \alpha \)-RelA
\( \alpha \)-A20
\( \alpha \)-GAPDH

b

\( \alpha \)-p52
\( \alpha \)-RelB
\( \alpha \)-GAPDH
\( \alpha \)-p52
\( \alpha \)-RelB
\( \alpha \)-HDAC1

\( \alpha \)-NIK
\( \alpha \)-p100
\( \alpha \)-GAPDH
\( \alpha \)-p100
\( \alpha \)-GAPDH

f

\( \alpha \)-p52
\( \alpha \)-RelB
\( \alpha \)-GAPDH
\( \alpha \)-p52
\( \alpha \)-RelB
\( \alpha \)-HDAC1

\( \alpha \)-NIK
\( \alpha \)-p100
\( \alpha \)-p100
\( \alpha \)-GAPDH

\( \alpha \)-A20
\( \alpha \)-NIK
\( \alpha \)-p100
\( \alpha \)-p100
\( \alpha \)-GAPDH

\( \alpha \)-A20
\( \alpha \)-NIK
\( \alpha \)-p100
\( \alpha \)-p100
\( \alpha \)-GAPDH
**Fig. 2 Uncropped blots**

|        | a                                               | b                                           |
|--------|-------------------------------------------------|---------------------------------------------|
| **IP: TRAF2** | **α-CLAP1**                                        | **α-CLAP1**                                    |
|        | **α-TRAF3**                                     | **α-TRAF3**                                   |
|        | **α-TIFA**                                      | **α-TIFA**                                    |
|        | **α-A20**                                       | **α-A20**                                     |
|        | **α-TRAF2**                                     | **α-TRAF2**                                    |
|        | **Lysates**                                     | **α-CLAP1**                                   |
|        | **α-CLAP1**                                     | **α-CLAP1**                                   |
|        | **α-TRAF3**                                     | **α-TRAF3**                                   |
|        | **α-TIFA**                                      | **α-TIFA**                                    |
|        | **α-A20**                                       | **α-A20**                                     |
|        | **α-TRAF2**                                     | **α-TRAF2**                                   |
|        | **α-GAPDH**                                     | **α-GAPDH**                                    |

*This is the same IB strip where CLAP1 was probed first, followed by TRAF3*
Fig. 2 Uncropped blots

c

| IP: TIFA | α-clAP1 | α-TRAF2 | α-A20 | α-TIFA | Lysates | α-clAP1 | α-TRAF2 | α-A20 | α-TIFA | α-GAPDH |
|---------|---------|---------|-------|--------|---------|---------|---------|-------|--------|---------|

d

| IP: TIFA | α-A20 | α-TIFA | α-A20 |
|---------|-------|--------|-------|

e

| IP: A20 | α-TIFA | α-A20 |
|---------|--------|-------|

f

| IP: TIFA | α-clAP1 | α-TRAF2 | α-TRAF3 | α-A20 | α-TIFA | Lysates | α-clAP1 | α-TRAF2 | α-TRAF3 | α-A20 | α-TIFA | α-GAPDH |
|---------|---------|---------|---------|-------|--------|---------|---------|---------|---------|-------|--------|---------|


Supplementary Fig. 1

a

- α-NIK
- α-P-p100
- α-p100
- α-GAPDH

b

- α-p52
- α-GAPDH
- α-p52
- α-HDAC1

Cyto

N1
Supplementary Fig. 2

a

*α-A20
α-NIK
α-P-p100
*α-p100
α-GAPDH

* This is the same IB strip where A20 was probed first, followed by p100

b

c

α-A20
α-NIK
α-P-p100
α-p100
α-GAPDH
Supplementary Fig. 3

a

**IP: TIFA**
- α-clAP1
- α-TRAF2
- α-TRAF3
- α-A20
- α-A20
- α-TIFA

**Lysates**
- α-clAP1
- α-TRAF2
- α-TRAF3
- α-A20
- α-TIFA
- α-GAPDH