Supplementary material

USP48 and A20 synergistically promote cell survival in *Helicobacter pylori*-infection

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Figure legends

**Figure S1. USP48 stabilises nuclear RelA in *H. pylori* infection**

*a* NCI-N87 cells were transfected with siRNA¹, or *b* siRNA⁵ against USP48 and infected with *H. pylori* for indicated times. Subcellular fractions were subjected to IB for analysis of the indicated proteins. Data information: Data shown are representative for at least two independent experiments. The band intensity was quantified using ImageJ software (NIH). GAPDH, C23 served as load controls and indicate for the purity of the subcellular fractions.

**Figure S2. USP48 deubiquitinylates RelA**

AGS cells were transfected with recombinant human USP48 (rhUSP48) and infected with *H. pylori* for the indicated times. MG132 was added 30 min after infection. IP with an anti-RelA antibody was performed at the indicated times in the presence of NEM and OPT, followed by IB analysis of the indicated proteins. Data information: Data shown are representative of at least two independent experiments.

**Figure S3. USP48 prolongs A20 de novo synthesis**

*a* NCI-N87 cells were transfected with siRNA against USP48 and infected with *H. pylori* for indicated times. *b* AGS cells were transfected with siRNA against USP48 and infected with *H. pylori* strain P12 for indicated times. Whole-cell lysates were subjected to IB for analysis of the indicated proteins. Data information: Data shown are representative for at least two independent experiments.

**Figure S4. Caspase-8 cleavage contributes to *H. pylori* associated apoptotic cell death**

*a* AGS cells were transfected with siRNA against caspase-8 and infected with *H. pylori* for indicated times. Whole-cell lysates were subjected to IB for analysis of the indicated proteins. *b* AGS cells were transfected with siRNA against caspase-8 and infected with *H. pylori* for 24 h. Cleaved caspase-3/7 expression was detected by the IncuCyte® S3 Live-Cell Analysis System. Scale bars = 100 µm. Data shown depict the average of nine pictures from distinct regions. Error bars denote mean ± SD. Data information: Data shown in (a) are representative for at least two independent experiments. Data shown in (b) are from two independent experiments. *P≤0.05 (Student's t-test).

**Figure S5. USP48 suppresses apoptotic cell death**

AGS cells were transfected with siRNA against USP48 (siRNA⁵) and infected with *H. pylori* for 24 h, followed by staining with annexin V/PI. Apoptotic cell death was analysed by flow cytometry. Data shown depict the average of two independent experiments. Error bars denote mean ± SD. Data information: Data shown are representative for at least two independent experiments. *P≤0.05, **P≤0.01 (Student's t-test).
**Figure S1**

### a. NC-N87

| USP48siRNA | P1 h | C | N1 |
|------------|------|---|----|
| -          | -    | 0 | 0  |
| USP48      | -    | 2 | 2  |
| RelA       | +    | 4 | 4  |
| GAPDH      | +    | 6 | 6  |
| C23        | +    | 0 | 0  |

**b. NCI-N87**

| USP48siRNA | P1 [h] | C | N1 |
|------------|--------|---|----|
| -          | 0      | 4 | 4  |
| USP48      | 0      | 6 | 6  |
| RelA       | 4      | 0 | 0  |
| GAPDH      | 6      | 4 | 4  |
| C23        | 0      | 6 | 6  |
Figure S2

| Condition          | 0 h | 2.5 h | 4 h |
|--------------------|-----|-------|-----|
| rhUSP48           | -   | -     | +   |
| MG132             | +   | +     | -   |
| IP: RelA          | +   | +     | +   |
| IB: Ub            |   |       |     |

Blots:
- Lysates
- USP48: 130kDa
- RelA: 55kDa
- GAPDH: 34kDa

Note: MG132 is a proteasome inhibitor.
Figure S3

a

| USP48siRNA1 | NC-N87 |
|-------------|--------|
| P1 [h]      |        |
| 0 0.5 2 4 6 | 0 0.5 2 4 6 |
| USP48       | + + + + |
| A20         | + + + + |
| IkBα        | + + + + |
| RelA-P      | + + + + |
| RelA        | + + + + |
| GAPDH       | + + + + |

b

| USP48siRNA1 | P12 [h] |
|-------------|---------|
| 0 0 1 2 4 6 | 0 0 1 2 4 6 |
| USP48       | + + + + |
| A20         | + + + + |
| IkBα        | + + + + |
| RelA-P      | + + + + |
| RelA        | + + + + |
| GAPDH       | + + + + |
Figure S4

(a)

|                | scRNA | Casp8RNA | P1 [24h] |
|----------------|-------|----------|----------|
| Casp8          | +     | -        | +        |
| C-Casp8        | -     | +        | -        |
| Casp3          | -     | +        | -        |
| C-Casp3        | -     | -        | +        |
| GAPDH          | -     | -        | -        |

Apoptotic cell death (Fluorescence intensity, A.U.)

(b) No infection vs P1 [24h]

|                | scRNA | Caspase-8RNA |
|----------------|-------|--------------|
| No infection   |       |              |
| P1 [24h]       |       |              |

Apoptotic cell death (Fluorescence intensity, A.U.)
Figure S5

Apoptotic cell death (%)

USP48siRNA5

P1 [24h]

Annexin V

P1 [24h]

**

*
| P1 [h] | C   | N1  | N2  |
|-------|-----|-----|-----|
| 0     |     |     |     |
| 1     |     |     |     |
| 2     |     |     |     |
| 4     |     |     |     |
| 6     |     |     |     |

**RelA-P**

**RelA**

**IκBα**

**GAPDH**

**C23**

**LaminB2**
Uncropped blot for Fig 1d

|        | C  |     |     |     |     | N2  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|--------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| P1 [h] | 0  | 1   | 2   | 4   | 6   | 0   | 1   | 2   | 4   | 6   | 0   | 1   | 2   | 4   | 6   | 0   | 1   | 2   | 4   | 6   | 0   |
| MG132  | -  | +   | +   | +   | +   | -   | +   | +   | +   | +   | -   | +   | +   | +   | +   | -   | +   | +   | +   | +   |
| LMB    | -  | +   | +   | +   | +   | -   | +   | +   | +   | +   | -   | +   | +   | +   | +   | -   | +   | +   | +   | +   |

**IP: RelA**

**IB: Ub**

**IB: RelA**

**Lysates**

RelA

IkBα-P

IkBα

GAPDH

C23

LaminB2
Uncropped blot for Fig 1e

| P1 [h] | 0 | 0 | 2 | 2 | 4 | 4 | 6 | 6 |
|--------|---|---|---|---|---|---|---|---|
| Elongin B or RNA | - | + | - | + | - | + | - | + |
| MG132 | - | - | + | + | + | + | + | + |

**IP: RelA**

**IB: Ub**

**IB: RelA**

**Lysates**

**Elongin B**

**RelA**

**GAPDH**
Uncropped blot for Fig 2a

| P1 [h] | 0 | 0 | 2 | 2 | 4 | 4 | 6 | 6 | 8 | 8 |
|--------|---|---|---|---|---|---|---|---|---|---|
| USP48RNA1 | - | + | - | + | - | + | - | + | - | + |

USP48 (C)

USP48 (N1)

USP48 (N2)

RelA (C)

RelA (N1)

RelA (N2)

GAPDH (C)

GAPDH (N1)

GAPDH (N2)
### Uncropped blot for Fig 2a

| USP48siRNA1 | 0 | 0 | 2 | 2 | 4 | 4 | 6 | 6 | 8 | 8 |
|-------------|---|---|---|---|---|---|---|---|---|---|
| P1 [h]      | - | + | - | + | - | + | - | + | - | + |

**C23 (C)**

![BLot Image](image1)

**C23 (N1)**

![BLot Image](image2)

**C23 (N2)**

![BLot Image](image3)

**LaminB2 (C)**

![BLot Image](image4)

**LaminB2 (N1)**

![BLot Image](image5)

**LaminB2 (N2)**

![BLot Image](image6)
Uncropped blot for Fig 2b

| Time (h) | 0 | 0 | 2 | 2 | 4 | 4 | 6 | 6 |
|----------|---|---|---|---|---|---|---|---|
| USP48RNA1 | - | + | - | + | - | + | - | + |
| MG132     | - | + | + | + | + | + | + | + |

**IP: RelA**

**IB: Ub**

**IB: RelA**

**Lysates**

**USP48**

**RelA**

**GAPDH**
Uncropped blot for Fig 2c

|        | P1 [4h] | C  | Nt |
|--------|---------|----|----|
| IP: RelA | - + + + | - | + |
| NEM     | - + - - | - | - |
| rhUSP48 | - + + - | - | - |

**Lysate**

|        | P1 [4h] | C  | Nt |
|--------|---------|----|----|
| RelA   | - + + + | - | + |
| rhUSP48| - - + + | - | + |

**Western Blot Images**

- IB: Ub
- IB: RelA
- IB: USP48
- LaminB2
- GAPDH
Uncropped blot for Fig 2e

**Input Flag**

**IB: USP48**

**IP: USP48**

**IB: Flag**

| CSN1 | CSN2 | CSN3 | CSN4 | CSN5 | CSN6 | CSN7A | CSN7B | CSN8 |
|------|------|------|------|------|------|-------|-------|------|
|      |      |      |      |      |      |       |       |      |
Uncropped blot for Fig 2f

| P1 [h] | 0 | 0 | 2 | 2 | 4 | 4 | 6 | 6 |
|--------|---|---|---|---|---|---|---|---|
| CSN2siRNA | - | + | - | - | + | - | - | + |
| MG132 | - | - | + | + | + | + | + | + |

**IP: RelA**

**IB: Ub**

**IB: RelA**

**Lysates**

**CSN2**

**CSN1**

**CSN5**

**CSN6**

**RelA**

**GAPDH**
Uncropped blot for Fig 2g

| P1 [h] | CSN2siRNA |
|--------|-----------|
| 0      | -         |
| 0      | +         |
| 2      | -         |
| 2      | +         |
| 4      | -         |
| 4      | +         |
| 6      | -         |
| 6      | +         |

|          | C          |
|----------|------------|
| 0        | 0 0 0      |
| 0        | 0 0 0      |
| 2        | 0 0 0      |
| 2        | 0 0 0      |
| 4        | 0 0 0      |
| 4        | 0 0 0      |
| 6        | 0 0 0      |
| 6        | 0 0 0      |

- **CSN2**
- **RelA**
- **GAPDH**
- **C23**
- **Lamin B2**
Uncropped blot for Fig 2g

| P1 [h] | CSN2siRNA | N1 |
|-------|-----------|----|
|       | 0 0 2 2 4 4 6 6 |
| 0     | - + - + - + - + |
| 1     | - + - + - + - + |
| 2     | - + - + - + - + |
| 3     | - + - + - + - + |
| 4     | - + - + - + - + |
| 5     | - + - + - + - + |
| 6     | - + - + - + - + |

- **CSN2**: Molecular weight standard
- **RelA**: Molecular weight standard
- **GAPDH**: Molecular weight standard
- **C23**: Molecular weight standard
- **Lamin B2**: Molecular weight standard
Uncropped blot for Fig 3c

| RNAi | USP48 | USP48 | USP48 | P1 [24h] |
|------|-------|-------|-------|----------|
|       | -     | +     | -     | +        |
|       | -     | -     | +     | -        |
|       | -     | -     | -     | +        |
|       | -     | -     | -     | +        |

- USP48
- A20
- Casp8
- Cl. Casp8
- Casp3
- Cl. Casp3
- GAPDH
Uncropped blot for Fig 3d

rhUSP48  
P1 [24h]  

|       | - | - | + | + | + |
|-------|---|---|---|---|---|

|       | USP48 | Casp8 | Cl. Casp8 | Casp3 | Cl. Casp3 | GAPDH |
|-------|-------|-------|-----------|-------|-----------|-------|
| rhUSP48 | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| P1 [24h] | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) | ![Image](image11) | ![Image](image12) |
Uncropped blot for Fig 3e

| USP48siRNA1 | - | - | - | + | + | + | IgG |
|-------------|---|---|---|---|---|---|-----|
| P1 [h]      | 0 | 4 | 8 | 24| 0 | 4 | 8 | 24 |

**IP: Casp8**

**IB: K63 Ub**

**IB: Casp8**

**IB: Cl. Casp8**

**Lysate**

**USP48**

**A20**

**Casp8**

**GAPDH**
Uncropped blot for Fig 4a

| Gene      | A20 | rhUSP48 | P1 [h] |
|-----------|-----|---------|--------|
| A20 siRNA | -   | +       | -      |
| rhUSP48   | -   | -       | -      |
| P1 [h]    | -   | +       | 8      |

**Gene Expression Blot Images:**

- **A20**
- **USP48**
- **Casp8**
- **Cl. Casp8**
- **Casp3**
- **Cl. Casp3**
- **GAPDH**
Uncropped blot for Fig 4c

| USP48| rhA20 | P1 [h] |
|------|-------|--------|
| -    | -     | -      |
| +    | +     | 8      |
| -    | +     | 8      |
| +    | +     | 16     |
| -    | -     | 16     |

A20

USP48

Casp 8

Cl. Casp 8

Casp 3

Cl. Casp 3

GAPDH
Uncropped blot for Fig S1a

| USP48siRNA1 | C | N1 |
|-------------|---|----|
| P1 [h]      | 0 | 0  |
|             | 2 | 2  |
|             | 2 | 4  |
|             | 4 | 6  |
|             | 6 | 6  |
|             | 8 | 8  |
|             | 8 | 0  |

- **USP48**
- **RelA**
- **GAPDH**
- **C23**
Uncropped blot for Fig S1a

| USP48siRNA1 | N1 | C |
|-------------|----|---|
| P1 [h]      |    |   |
| 0           | +  | - |
| 0           | -  | + |
| 2           | +  | - |
| 4           | +  | - |
| 4           | +  | - |
| 6           | +  | - |
| 6           | +  | - |
| 8           | +  | - |
| 8           | +  | - |

- **USP48**
- **RelA**
- **GAPDH**
- **C23**
Uncropped blot for Fig S1b

| USP48 [RNAi] | C | N1 |
|--------------|---|----|
| USP48        | - | -  | +  | +  |
| P1 [h]       | 0 | 4  | 6  | 0  | 4  | 6  |

**Protein Expression**

**USP48**

**RelA**

**GAPDH**

**C23**
Uncropped blot for Fig S3a

| USP48siRNA1 | NCI-N87 |
|-------------|---------|
| P1 [h]      | 0 0.5  2 4 6 0 0.5 2 4 6 |

- **USP48**
- **A20**
- **IκBα**
- **RelA-P**
- **RelA**
- **GAPDH**
Uncropped blot for Fig S3b

| USP48 | A20 | IκBα | RelA-P | RelA | GAPDH |
|-------|-----|------|--------|------|-------|
| -     | +   | -    | -      | -    | +     |
| P12 [h] | 0  | 0    | 1      | 1    | 2     | 4     | 6     | 6     |

- USP48
- A20
- IκBα
- RelA-P
- RelA
- GAPDH
Uncropped blot for Fig S4a

| SCPiRNA | + | - | + | - | + | - | + | - |
|---------|---|---|---|---|---|---|---|---|
| Casp8iRNA | - | + | - | - | + | - | + | - |
| P1 [24h] | - | - | + | - | - | + | - | + |