Supplementary Figure Legends

Supplementary Figure 1. LTβR signaling is activated in *H. pylori*-induced gastritis and gastric tumors.

(A) Whiskers (minimum to maximum) box plots show relative mRNA levels of LTα, LTβ, CD40L, TWEAK, BAFF in healthy human gastric mucosa and *H. pylori*-induced gastritis. Data from 12 (LTα and LTβ) or 6 (CD40L, TWEAK, BAFF) tissue samples of each condition are shown. Significances were calculated using Student's t test. ***p≤0.001. (B) Negative (DapB) and positive controls (+, Pol2A and ++, UBC) used for LTβR *in situ* hybridization. (C) T cells (CD3), B cells (CD20) and macrophages (CD68) detected by immunohistochemistry in normal gastric mucosa, mild, moderate and severe gastritis as well as in tumor-associated gastritis. Representative images are shown. (D) Representative images of LTβ expression in human normal gastric mucosa, mild, moderate and severe gastritis as well as in early gastric adenocarcinoma biopsy samples, detected by immunohistochemistry. Thick arrows indicate expression of LTβ in epithelial cells. Thin arrows show expression of LTβ in immune cells. (Scale bar: 100μm). (E) Quantification of $LT\beta^+$ cells in intestinal-type and diffuse-type tumors. (F) $RelB^+$ and $p52^+$ cells in human normal gastric mucosa, mild, moderate and severe gastritis as well as in early gastric adenocarcinoma biopsy samples (n=10, each). *p≤0.05, **p≤0.01, ***p≤0.001. One-way ANOVA with Bonferroni's multiple comparison test

Supplementary Figure 2. *H. pylori* activates LTβR signaling.

(A) LTβR expression on the surface of MKN45, KATOIII and St3051 GC cells detected by flow cytometry. HepG2 and HEK293 cells were used as positive and negative control, respectively. One representative experiment is shown. (B) p100/p52 basal protein expression levels in GC cell lines analyzed by western blot. β-actin served as loading control. (C) LTβR, TNFR1, TNFα, LTα, LTβ and LIGHT mRNA basal expression levels in gastric cancer cell lines. HepG2 or cells treated with the agonist of LTβR signaling BS-1 (0.5 μg/ml) were used as positive controls. Results (mean ± S.D.) of two independent experiments performed in duplicates are shown. (D) p-p65 and p65 basal protein expression levels in gastric cancer

cell lines analyzed by western blot. β-actin served as loading control. (E) St3051 cells were infected with *H. pylori* G27 at different MOI and p100 processing to p52 was detected by western blot. BS-1 (0.5 µg/ml) was used as a positive control. β-actin served as loading control. One representative blot of three independent experiments is shown. (F) MKN45 and St3051 cell were infected with *H. pylori* G27 strain at the indicated MOI and p-p65 and p65 levels were analyzed by western blot. β-actin was used as loading control. One representative blot from three independent experiments is shown. (G) p100 and p52 levels in MKN45 and St3051 cells detected by western blot after infection with *H. pylori* G27 at MOI 10 for different time points. BS-1 (0.5 µg/ml) was used as a positive control. β-actin served as loading control. (H) Gastric cancer cells MKN45 and St3051 were infected with *H. pylori* G27 at MOI 10 for 12 hours. The mRNA expression levels of LTβR were analyzed by real time PCR. Ct values were normalized to GADPH. Results (mean ± S.D.) of three independent experiments are presented as fold induction. Significances were calculated using One-way ANOVA with Bonferroni's multiple comparison test. (I) St3051 cells were infected with *H. pylori* G27 at MOI 10 for 12 hours. The mRNA expression levels of CXCL13, CXCL10, CCL20 and A20 were analyzed by real time PCR. Ct values were normalized to GADPH. BS-1 (0.5 µg/ml) was used as a positive control. Results (mean ± S.D.) from three independent experiments are presented as fold induction. Control cells were compared to *H. pylori*-infected cells and significances were calculated using Student's t-test. *p≤0.05, **p≤0.01, ***p≤0.001.

Supplementary Figure 3. Activation of LTβR signaling is induced by secreted LT in response to *H. pylori* infection.

(A) MKN45 cells were infected with G27 *H. pylori* strain for 3 hours and lysates were immunoprecipitated with anti-LTβR or anti-CagA antibodies. Irrelevant rabbit IgG were used as control. Immunoprecipitates were subjected to western blot for detection of *H. pylori* proteins. (B) MKN45 cells were incubated LTβR-Ig one hour prior to *H. pylori* infection. LTβ mRNA levels were measured after 12 hours infection. Values were normalized to GADPH.

Results (mean ± S.D.) of three independent experiments are shown. p values were calculated using One-way ANOVA with Bonferroni's multiple comparison test. (C) LTα, LTβ, A20 and LIGHT mRNA expression levels in MKN45 cells after stimulation with 10ng/ml of TNFα for 12h. Values were normalized to GADPH. Results (mean ± S.D.) of three independent experiments are shown. p values were calculated using Student's t-test. *p≤0.05. (D) MKN45 and St3051 cells were incubated with the inhibitor of human IκB kinase 2 (IKK2) TPCA-1 to block canonical NF-κB signaling. Cells were stimulated with 50ng/ml of TNFα or the LTβR agonist BS-1 (0.5µg/ml) where indicated. Cell lysates were obtained after 12h infection with the *H. pylori* strain G27 and levels of p-p65 and p65 were assessed by western blot. β-actin was used as a loading control. One representative blot and quantification from three independent experiments is shown. (E) St3051 cells were incubated with TPCA-1 1h prior to infection with the *H. pylori* strain G27 (MOI 10). Cell lysates were obtained after 12h infection and levels of p100/p52 were assessed by western blot. β-actin served as loading control. One representative blot of three independent experiments is shown. (F) TWEAK mRNA expression in MKN45 cells after inhibition of canonical NF-κB using TPCA-1. Values were normalized to GADPH. Results from three independent experiments are shown (mean ± S.D.).

Supplementary Figure 4. Activation of non-canonical NF-κB is independent of CagA.

(A) LIGHT mRNA expression in MKN45 and St3051 cells infected for 12 hours with the *H. pylori* strain G27 and the isogenic CagA and CagE mutants. Values were normalized to GADPH. Results from three independent experiments are shown (mean ± S.D.) (B) p100 processing to p52 detected by western blot in St3051 cells infected for 12 hours with *H. pylori* G27, the isogenic mutant strains G27*ΔCagA*, G27*ΔCagE*, G27*ΔBabA,* G27*ΔSabA,* G27*ΔVacA,* G27*ΔgGT,* G27*ΔUreA/B* or the SS1 and PMSS1 wild type strains at MOI of 10. β-actin was used as a loading control. (C) p100 processing to p52 detected by western blot in MKN45 cells infected with *H. pylori* G27 or the isogenic mutant strains G27*ΔCagA*, G27*ΔCagE*, G27*ΔCagF*, G27*ΔCagI* at MOI of 10. β-actin was used as a loading control. (D)

Expression of CagA and isocitrate dehydrogenase (ICD), used as a housekeeping gene, detected by PCR in different *H. pylori* clinical isolates. (E) p100 processing to p52 detected by western blot in MKN45 cells infected with *H. pylori* P12 or the peptidoglycan mutant P12*slt*. Quantification of three independent experiments is shown.

Supplementary Figure 5. Blocking LTβR during *H. pylori* infection reduces gastric inflammation.

(A) Time schedule of LTβR-Ig treatment. C57BL/6 mice were injected with 100µg of LTβR-Ig or the isotype control MOPC-21 at the indicated time points ( ). At day 0, 2 and 5, mice were infected with a 10^9 orogastric dose of the *H. pylori* strain PMSS1. Mice were sacrificed at day 36 for analyses. (B) Immunohistochemical staining of FDC-M1 or CD21/35 in cryosections of spleens to detect follicular dendritic cells from MOPC-21 or LTβR-Ig-treated mice, infected with *H. pylori* PMSS1. (C) Representative pictures of CD4 T cells and macrophages (F4/80) detected in gastric tissue samples of control, MOPC-21 and LTβR-Ig-treated mice infected with *H. pylori* PMSS1. (Scale bar 100µm).

Supplementary Figure 6. Agonistic activation of LTβR-signaling during *H. pylori* infection increases gastric inflammation.

(A) Representative pictures of CD4 T cells and macrophages (F4/80) detected in gastric tissue samples of control, *H. pylori* PMSS1-infected and ACH6-treated and PMSS1-infected mice. (Scale bar 100µm). (B) B220 positive cells calculated per area of tissue (mm^2) in untreated and ACH6-treated control mice. Significances were calculated using Student's t-test. *p≤0.05. (C) Relative mRNA expression levels of KC and CCL20 in the stomach of control, *H. pylori*-infected mice and *H. pylori*-infected mice and treated with ACH6. Ct values were normalized to GADPH. Statistical significance was calculated by using One-way ANOVA Kruskal-Wallis with Dunn's multiple comparison test. *p≤0.05, **p≤0.01.

Supplementary Figure 7. Representative model of the alternative NF-κB activation via LTβR-activation upon *H. pylori* infection.

(A) *H. pylori* type IV secretion system-mediated activation of canonical NF-κB induces the expression of LTα and LTβ and low levels of LIGHT in gastric epithelial cells. Secreted $LT\alpha_1\beta_2$ and LIGHT bind to LTβR expressed on the gastric epithelium leading to activation of alternative NF-κB and up-regulation of chemokines such as CXCL13, CCL17, CCL20 or CXCL10 in cis and in trans. These chemokines contribute to the recruitment of B and T cells and macrophages to the stomach, which once activated in turn secrete LTs (Chiang et al., 2009) and possibly also LIGHT, enhancing LTβR-activation and chemokine secretion resulting in a feed-forward loop driving gastric inflammation. Treatment with LTβR-Ig increases bacterial load, while decreasing gastric inflammation and inflammatory chemokine expression. Conversely, agonistic activation of LTβR by ACH6 reduces *H. pylori* colonization, but increases gastric inflammation as well as chemokine expression. (B) Inhibition of canoncical NF-κB during *H. pylori* infection enhances the expression of LIGHT and strongly reduces LTβ expression. Under these conditions, LIGHT can signal through LTβR activating the expression of target chemokines such as CXCL13, leading to a positive feed-forward loop sustaining gastric inflammation.

Figure S1

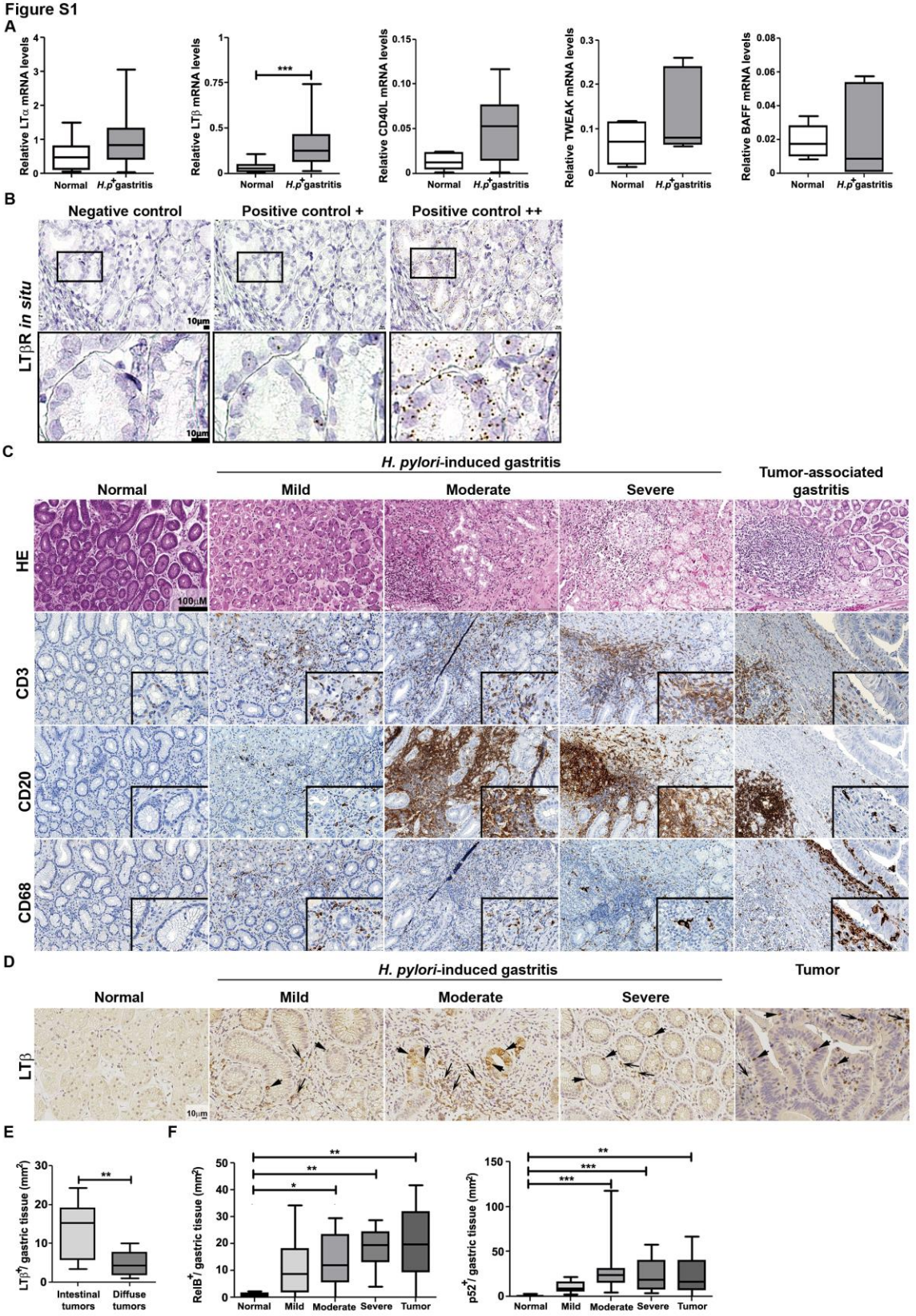

A
Relative LTα mRNA levels
Relative LTβ mRNA levels
Relative CD40L mRNA levels
Relative TWEAK mRNA levels
Relative BAFF mRNA levels
Normal
H.p+gastritis
***
B
Negative control
Positive control +
Positive control ++
LTβR in situ
10μm
C
H. pylori-induced gastritis
Normal
Mild
Moderate
Severe
Tumor-associated gastritis
HE
CD3
CD20
CD68
100μM
D
H. pylori-induced gastritis
Tumor
Normal
Mild
Moderate
Severe
LTβ
10μm
E
LTβ+/ gastric tissue (mm2)
Intestinal tumors
Diffuse tumors
**
F
RelB+/ gastric tissue (mm2)
p52+/ gastric tissue (mm2)
Normal
Mild
Moderate
Severe
Tumor
*
**
***

Figure S2

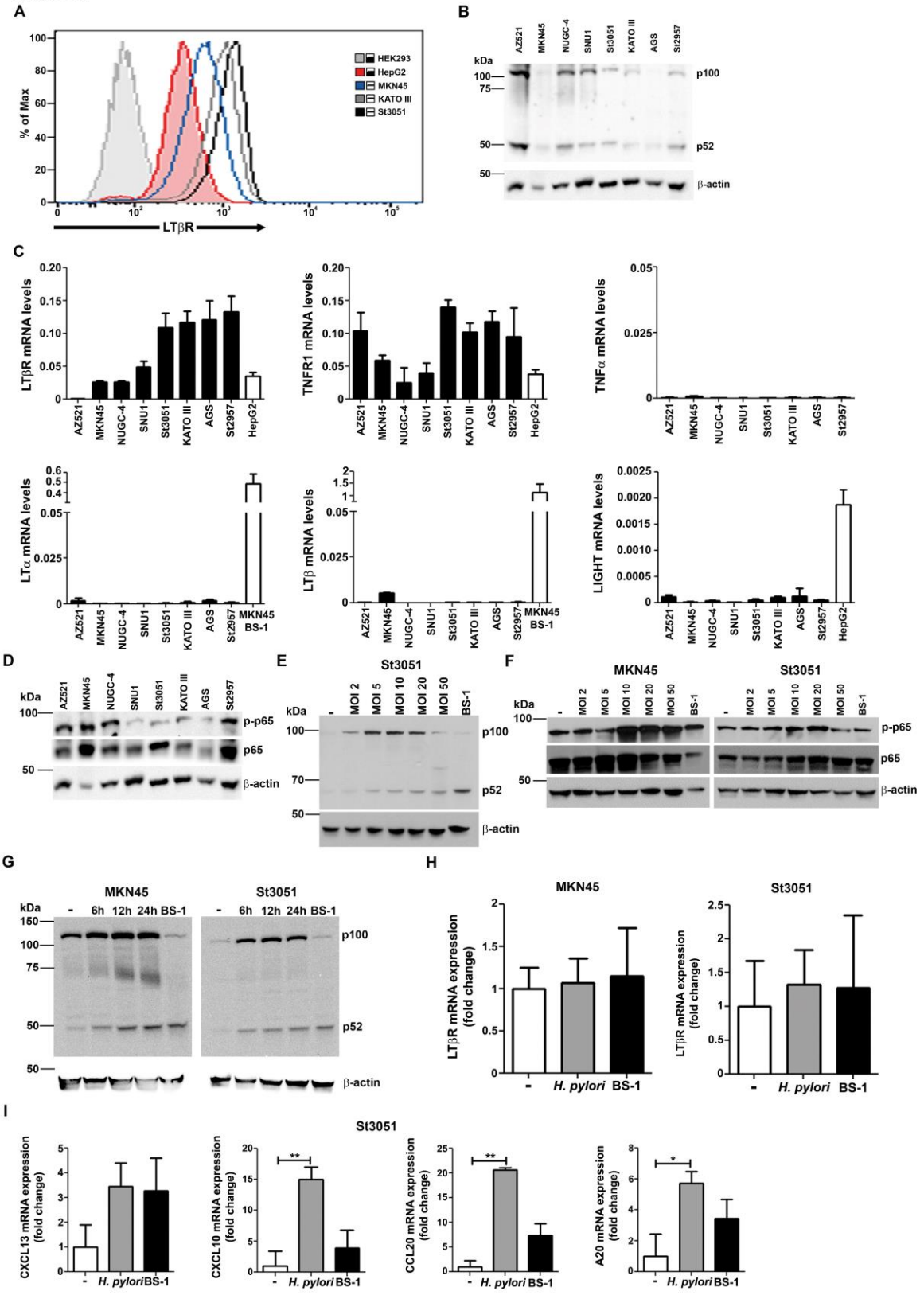

A
% of Max
HEK293
HepG2
MKN45
KATO III
St3051
LTβR
B
kDa
AZ521
MKN45
NUGC-4
SNU1
St3051
KATO III
AGS
St2957
p100
p52
β-actin
C
LTβR mRNA levels
TNFR1 mRNA levels
TNFα mRNA levels
LTα mRNA levels
LTβ mRNA levels
LIGHT mRNA levels
HepG2
MKN45 BS-1
D
p-p65
p65
β-actin
E
St3051
MOI 2
MOI 5
MOI 10
MOI 20
MOI 50
BS-1
F
MKN45
St3051
G
6h
12h
24h
H
LTβR mRNA expression (fold change)
H. pylori
I
CXCL13 mRNA expression (fold change)
CXCL10 mRNA expression (fold change)
CCL20 mRNA expression (fold change)
A20 mRNA expression (fold change)

Figure S3

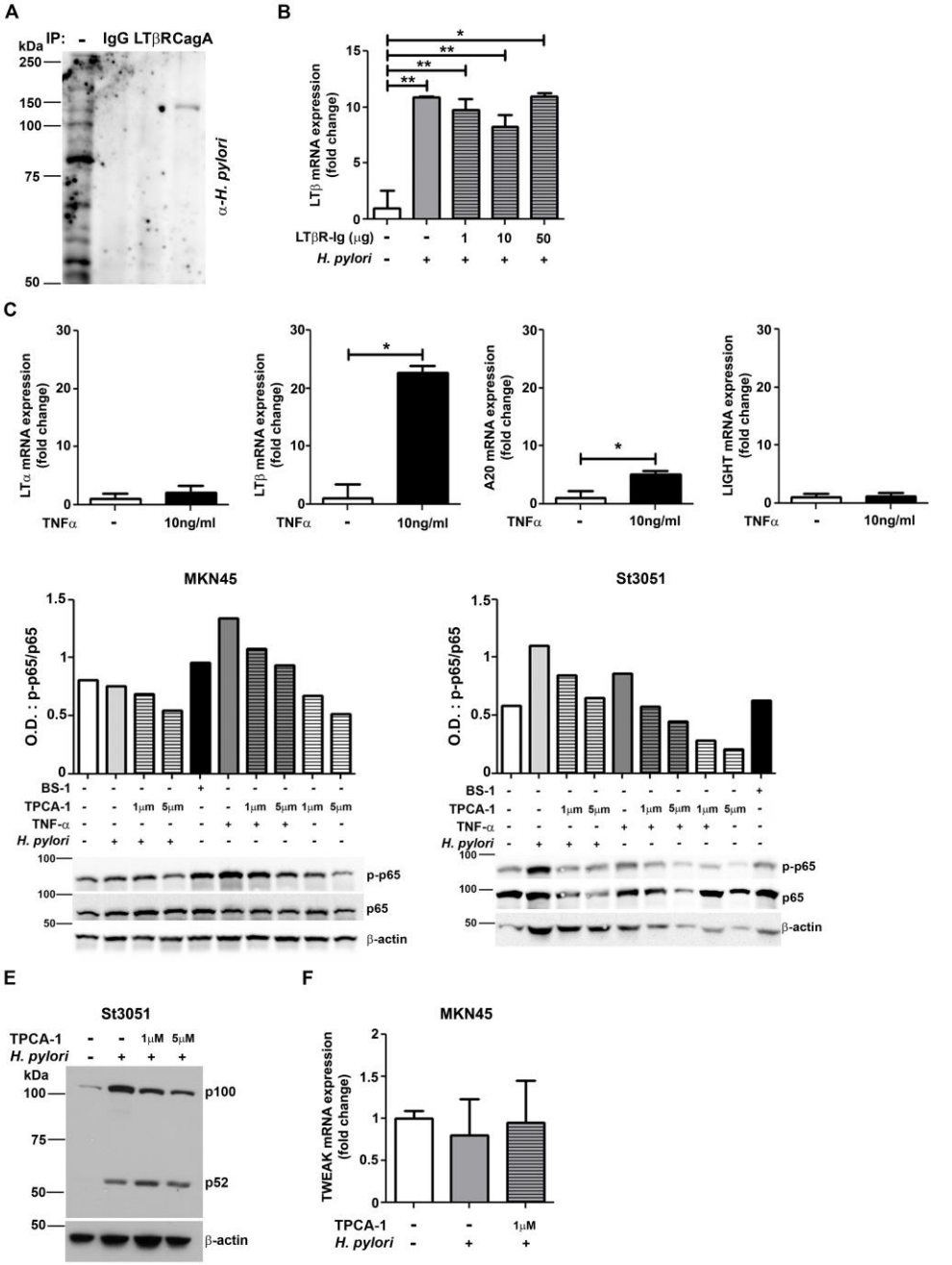

A
IP: - IgG LTβRCagA
kDa
250
150
100
75
50
α-H. pylori
B
LTβ mRNA expression (fold change)
LTβR-Ig (μg) - - 1 10 50
H. pylori - + + + +
C
LTα mRNA expression (fold change)
LTβ mRNA expression (fold change)
A20 mRNA expression (fold change)
LIGHT mRNA expression (fold change)
TNFα - 10ng/ml
MKN45
St3051
O.D. : p-p65/p65
BS-1
TPCA-1
TNF-α
H. pylori
p-p65
p65
β-actin
E
St3051
TPCA-1 - - 1μM 5μM
H. pylori - + + +
p100
p52
β-actin
F
MKN45
TWEAK mRNA expression (fold change)
TPCA-1 - - 1μM
H. pylori - + +

Figure S4

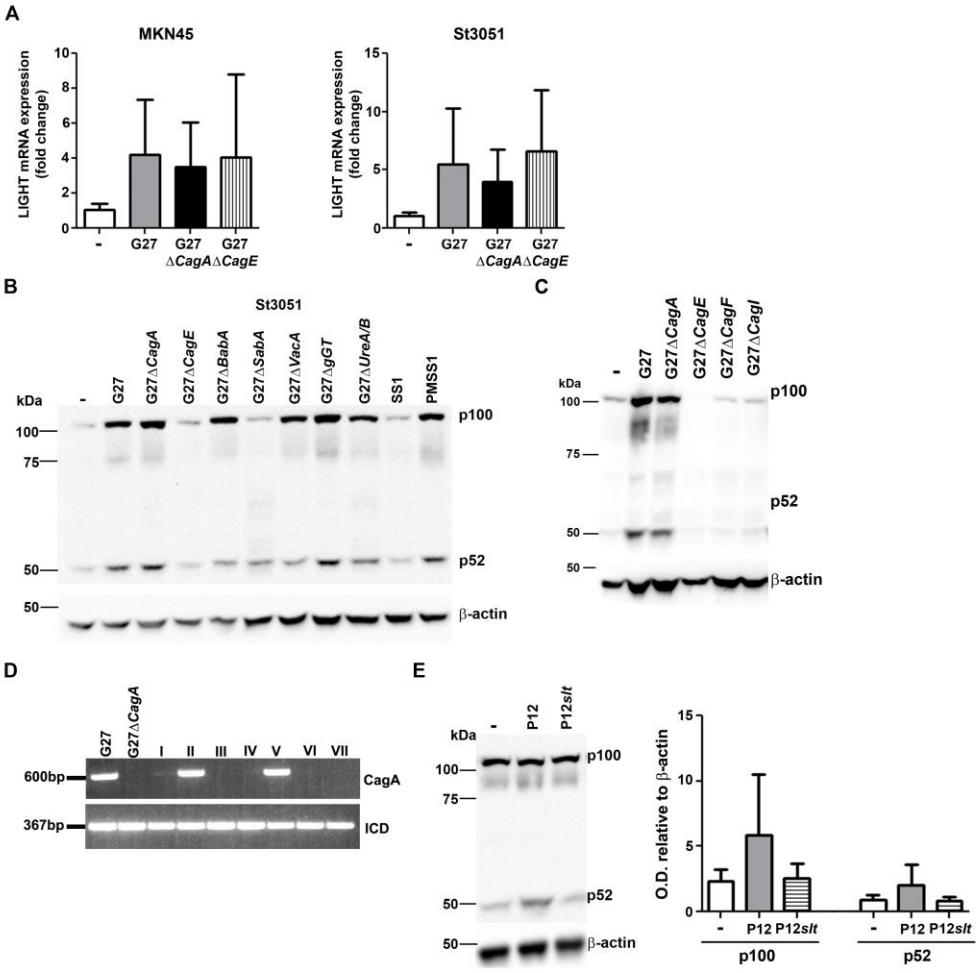

A
MKN45
St3051
LIGHT mRNA expression (fold change)
-
G27
G27 ΔCagAΔCagE
B
St3051
kDa
p100
p52
β-actin
C
p100
p52
β-actin
D
600bp
CagA
367bp
ICD
I
II
III
IV
V
VI
VII
E
kDa
p100
p52
β-actin
O.D. relative to β-actin
P12
P12slt
p100
p52

Figure S5

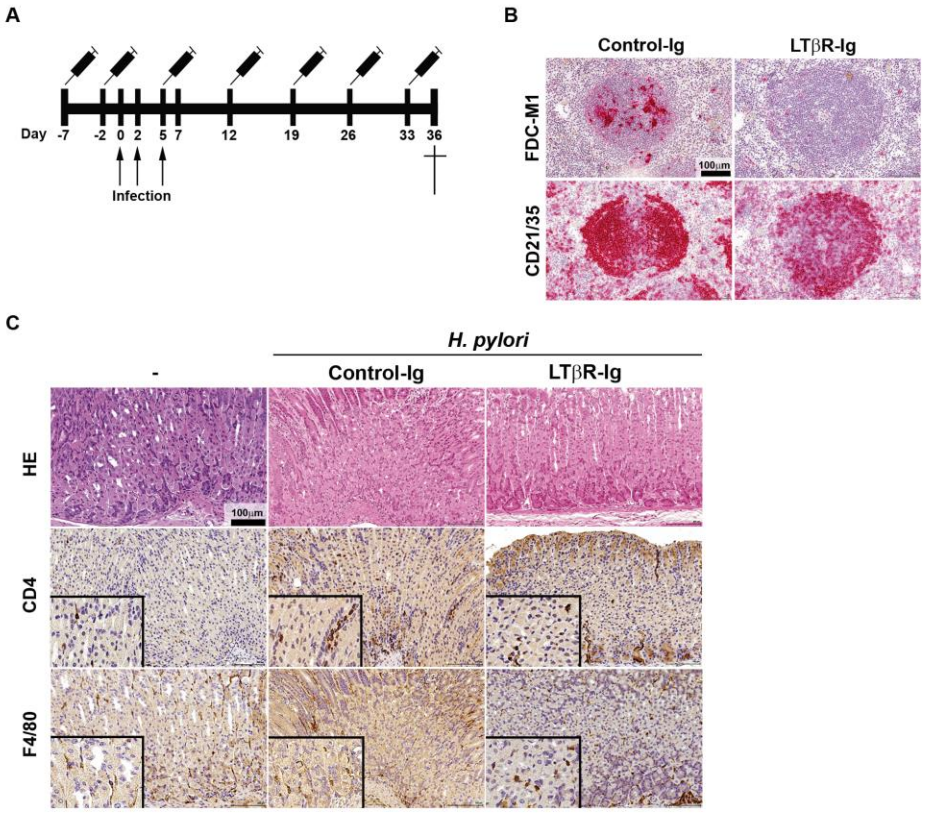

A
Day -7 -2 0 2 5 7 12 19 26 33 36
Infection
B
Control-Ig
LTβR-Ig
FDC-M1
CD21/35
100μm
C
H. pylori
-
Control-Ig
LTβR-Ig
HE
CD4
F4/80
100μm

Figure S6

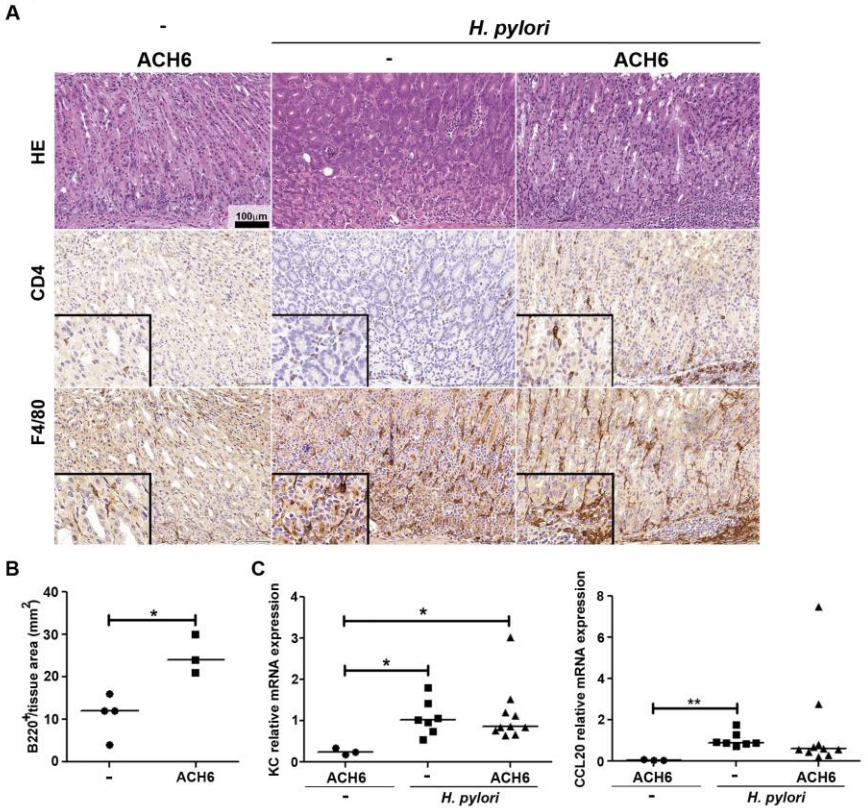
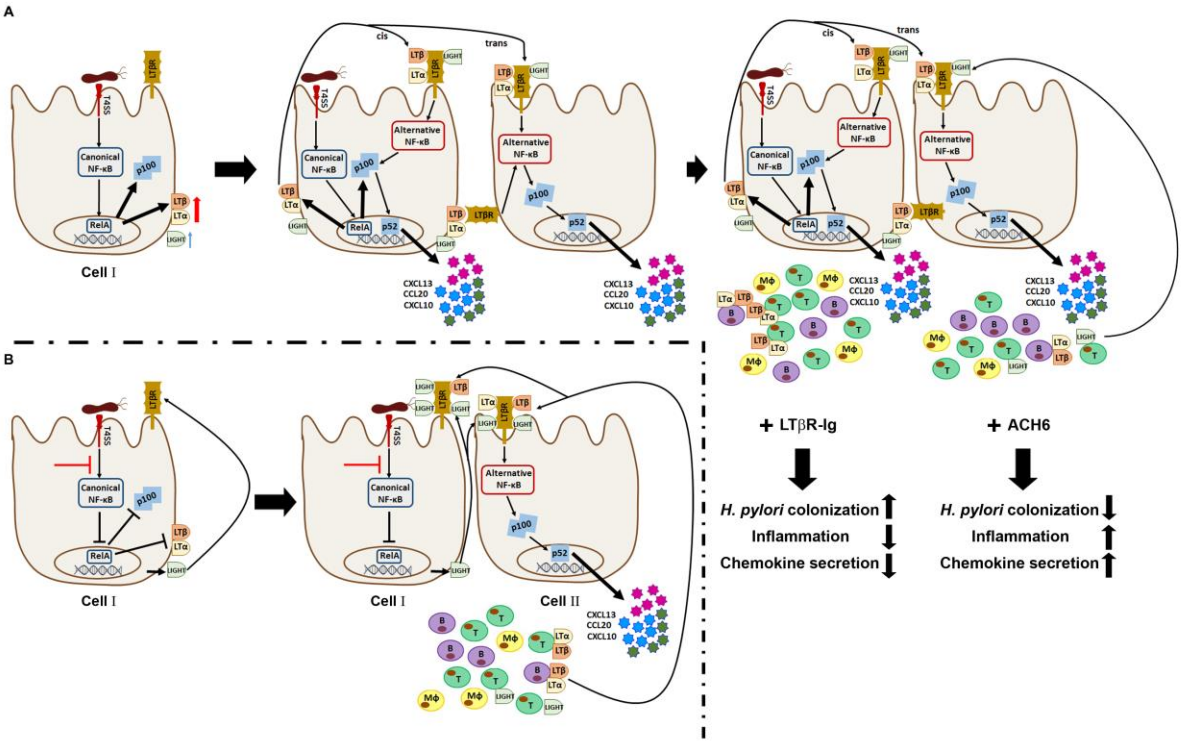

A
-
H. pylori
ACH6
-
ACH6
HE
CD4
F4/80
100μm
B
B220+tissue area (mm2)
40
30
20
10
0
*
-
ACH6
C
KC relative mRNA expression
4
3
2
1
0
*
*
ACH6
-
ACH6
-
H. pylori
CCL20 relative mRNA expression
8
6
4
2
0
**
ACH6
-
ACH6
-
H. pylori

Figure S7


A
cis
trans
T4SS
LTβR
LTβ
LTα
LIGHT
Canonical NF-κB
Alternative NF-κB
p100
RelA
p52
CXCL13
CCL20
CXCL10
Cell I
B
Cell I
Cell II
+ LTβR-Ig
+ ACH6
H. pylori colonization
Inflammation
Chemokine secretion
Mφ
T
B