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## Supplemental Data

## Article

# Overexpression of Interleukin-1β Induces Gastric Inflammation and Cancer and Mobilizes Myeloid-Derived Suppressor Cells in Mice

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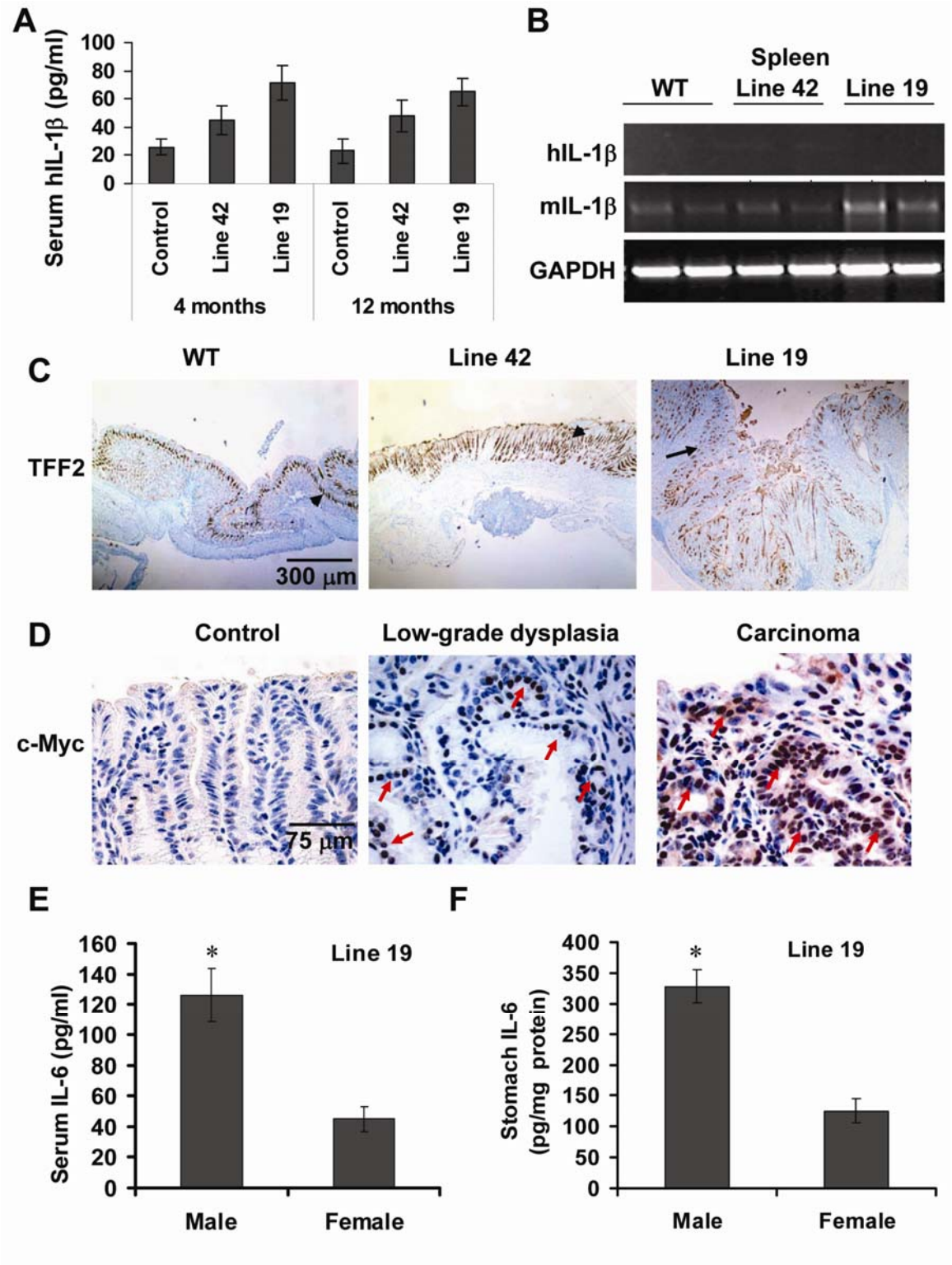
A
Serum hIL-1β (pg/ml)
100
80
60
40
20
0
Control
Line 42
Line 19
Control
Line 42
Line 19
4 months
12 months
B
Spleen
WT
Line 42
Line 19
hIL-1β
mIL-1β
GAPDH
C
WT
Line 42
Line 19
TFF2
300 μm
D
Control
Low-grade dysplasia
Carcinoma
c-Myc
75 μm
E
Serum IL-6 (pg/ml)
160
140
120
100
80
60
40
20
0
*
Line 19
Male
Female
F
Stomach IL-6 (pg/mg protein)
400
350
300
250
200
150
100
50
0
*
Line 19
Male
Female

**Figure S1.**

**Figure S1. Generation of *IL-1*β Transgenic Mice**

(A) Serum concentrations of human IL-1β. Serum was collected from Line 19 and Line 42 transgenic mice and control mice at 4 month and twelve month of age. The concentration of human IL-1β was determined using a human-specific IL-1β ELISA kit. Data represent the mean ± SD of 6 animals.

(B) Expression of hIL-1β mRNA in the spleen. Splenic mRNA was extracted using the TRIZOL kit. Expression of human and mouse IL-1β mRNA in spleen tissues was determined by polymerase chain reaction (PCR).

(C) Increased expression of TFF2 in the stomach of *IL-1*β mice. Stomach sections were examined by immunohistochemical staining with an anti-TFF2 antibody. Arrows show TFF2-positive cells (brown). Note that most mucous metaplastic cells express TFF2 in the stomachs of *IL-1*β mice.

(D) Activation of c-Myc in gastric cancer in *IL-1*β mice. The sections from indicated stomach tissues were stained with an anti-c-Myc antibody. Arrows show nuclear c-Myc-positive cells (brown).

(E and F) Serum and gastric levels of murine IL-6 peptide. Serum (E) was collected and stomach protein (F) was extracted from Line 19 male and female **t**ransgenic mice and control mice at 12 month of age. The level of IL-6 was measured using a mouse-specific IL-6 ELISA kit. The data shown represent the mean ± SD of 6 animals (#$p < 0.05$, *$p < 0.01$ versus female mice).

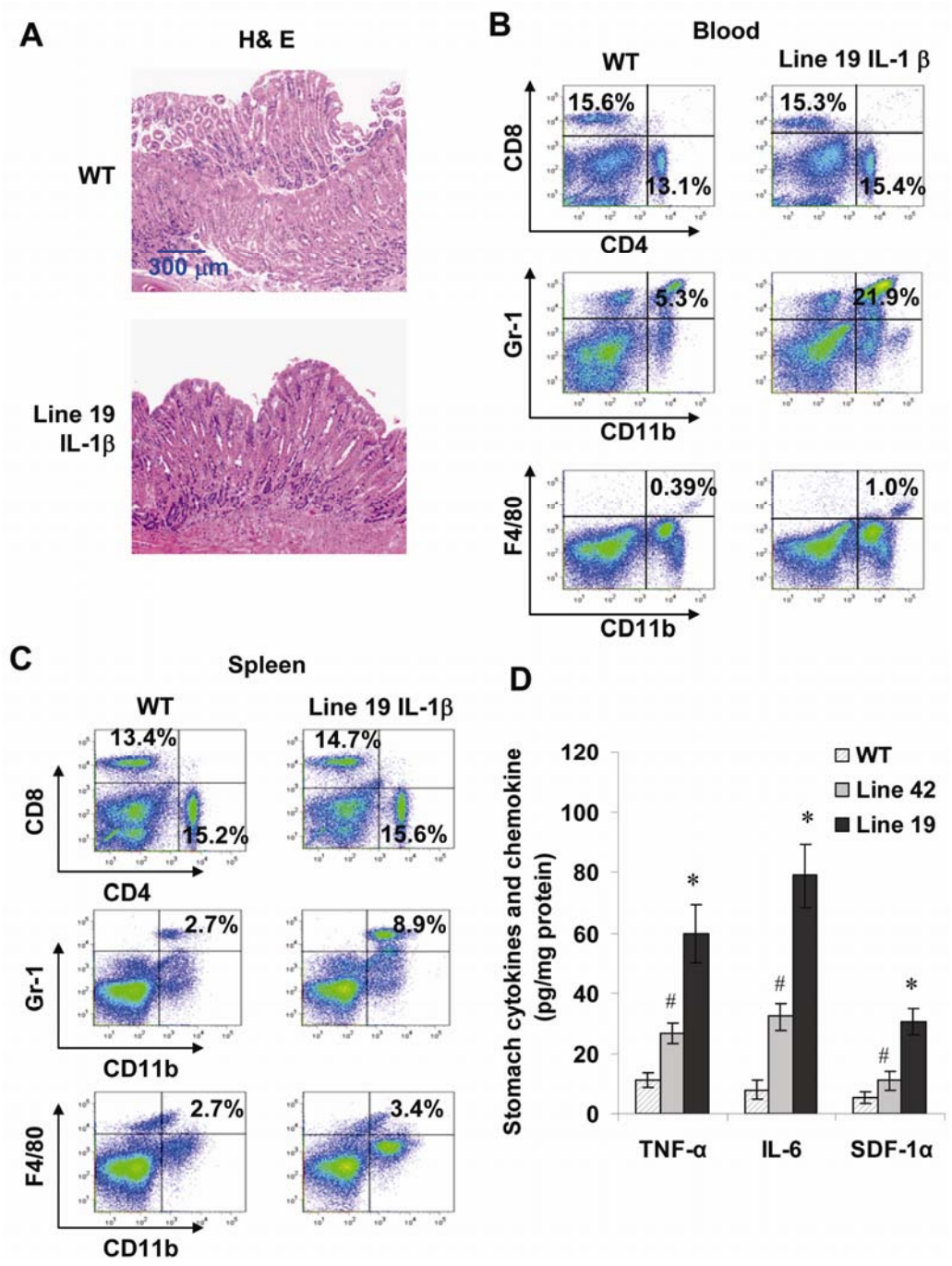


**Figure S2. Overexpression of IL-1β in the Stomach Leads to Mobilization and Recruitment of MDSCs in Young IL-1β Mice**

(A) Two-month-old *IL-1*β transgenic mice developed mild gastritis (H&E staining).

(B and C) Representative FACS blots for detecting lymphoid and myeloid cells in peripheral blood (B) and spleen (C) from two-month-old *IL-1*β mice and age-matched WT mice.

(D) Stomach level of mouse TNF-α, IL-6 and SDF-1α in 2-month-old mice was determined by ELISA. The data shown represent the mean ± SD of 6 animals (*$p < 0.01$ versus WT mice).

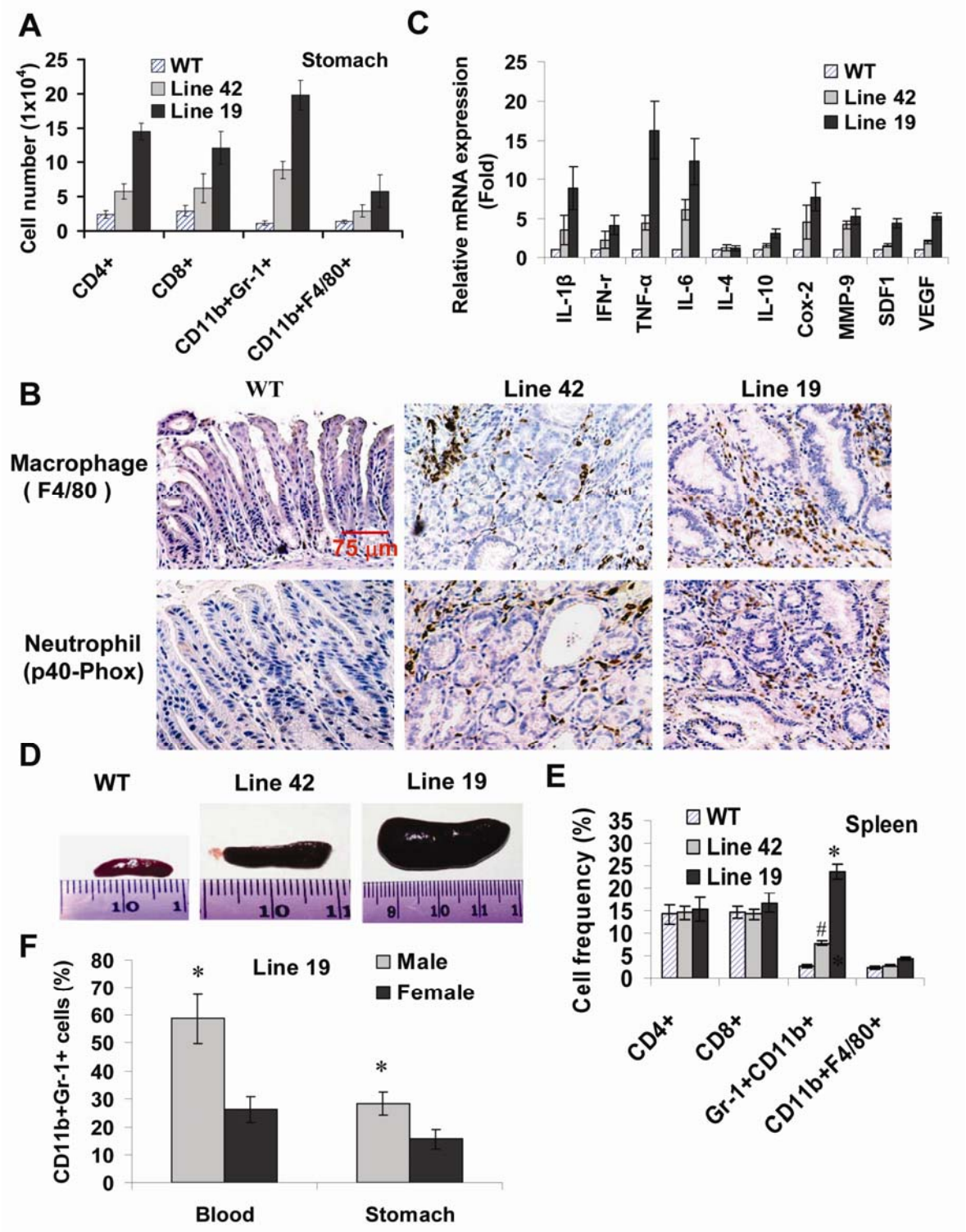
A
Stomach
WT
Line 42
Line 19
Cell number (1x10⁴)
CD4+
CD8+
CD11b+Gr-1+
CD11b+F4/80+
C
Relative mRNA expression (Fold)
IL-1β
IFN-r
TNF-α
IL-6
IL-4
IL-10
Cox-2
MMP-9
SDF1
VEGF
B
WT
Line 42
Line 19
Macrophage ( F4/80 )
75 μm
Neutrophil (p40-Phox)
D
WT
Line 42
Line 19
E
Spleen
Cell frequency (%)
F
Line 19
Male
Female
CD11b+Gr-1+ cells (%)
Blood
Stomach

**Figure S3.**

**Figure S3. Overexpression of IL-1β Increases Infiltration of Inflammatory Cells in the Stomach of Aged *IL-1*β Transgenic Mice**

(A) Quantitative analysis of gastric mucosal lymphoid and myeloid cells in 12-month-old indicated mice using FACS. The data shown represent the mean ± SD of cell number per stomach derived from 6 animals (*$p < 0.01$ versus control mice).

(B) Increased gastric mucosal macrophages and neutrophil in *IL-1*β mice. Gastric sections from 12-month-old transgenic and control mice were stained with anti-F4/80 antibody (macrophages) and anti-P40 antibody (neutrophils).

(C) Gastric mRNA expression of cytokines, chemokines and growth factors from 12-month-old *IL-1*β transgenic and control mice as assessed by real time PCR. The data shown are normalized to WT mice and represent the mean ± SD of 6 mice.

(D) Splenomegaly in *IL-1*β transgenic mice. Photograph of representative spleen from 12-month-old mice.

(E) Increased frequency of MDSCs in spleen of *IL-1*β transgenic mice. Isolated single splenocytes were stained with indicated fluorescent-conjugated antibodies and analyzed by FACS. The data shown represent the mean ± SD of 6 animals (#$p < 0.05$, *$p < 0.01$ versus control mice).

(F) Increased frequency of MDSCs in the blood and stomach of male *IL-1*β transgenic mice. Single nucleated cells isolated from 12-month-old male and female Line 19 male *IL-1*β transgenic were stained with indicated fluorescent-conjugated CD11b and Gr-1 antibodies and analyzed by FACS. The data shown represent the mean ± SD of 6 animals (#$p < 0.05$, *$p < 0.01$ versus female mice).

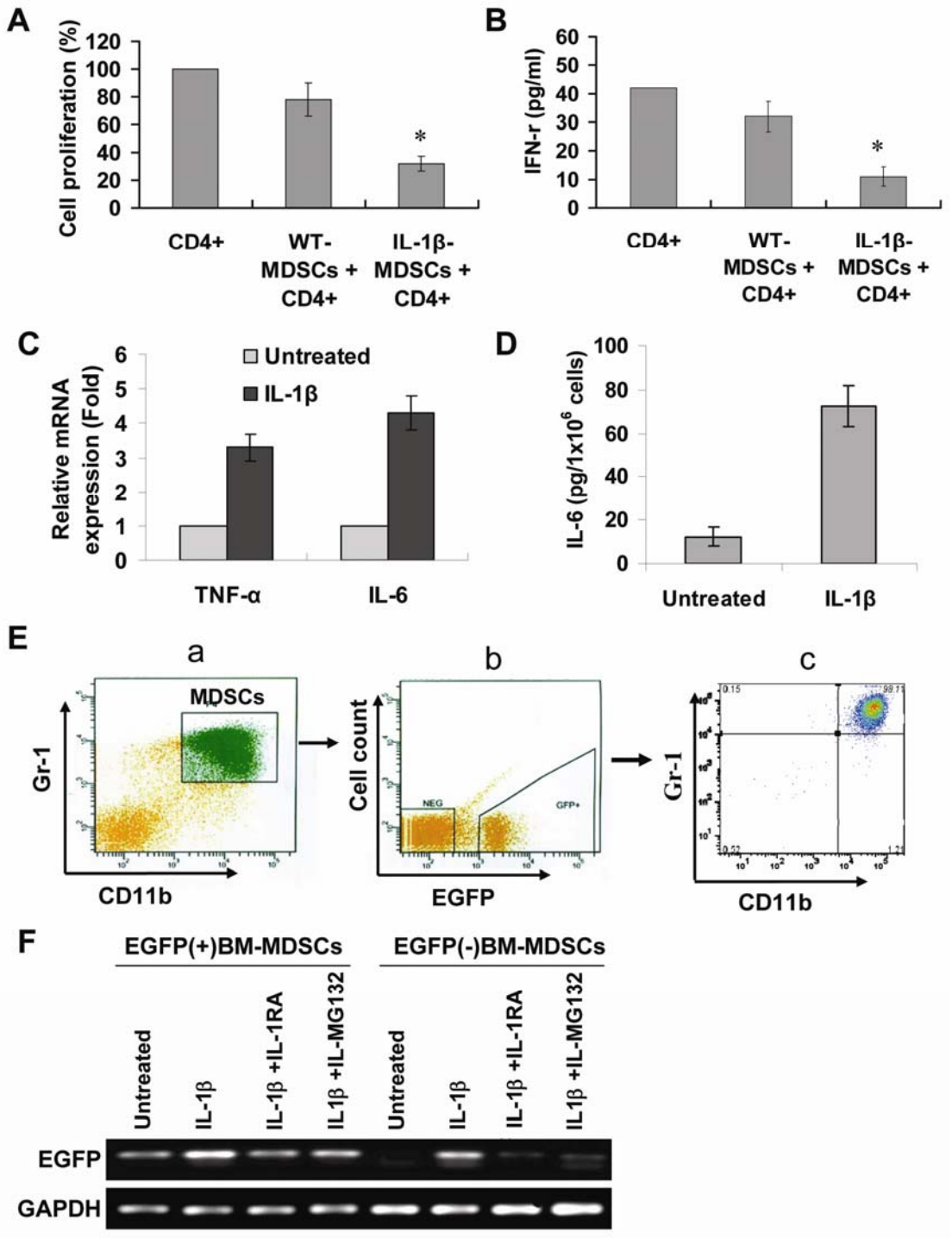
A
Cell proliferation (%)
120
100
80
60
40
20
0
*
CD4+
WT-MDSCs + CD4+
IL-1β-MDSCs + CD4+
B
IFN-r (pg/ml)
60
50
40
30
20
10
0
*
CD4+
WT-MDSCs + CD4+
IL-1β-MDSCs + CD4+
C
Relative mRNA expression (Fold)
6
5
4
3
2
1
0
Untreated
IL-1β
TNF-α
IL-6
D
IL-6 (pg/1x10^6 cells)
100
80
60
40
20
0
Untreated
IL-1β
E
a
b
c
MDSCs
Gr-1
CD11b
Cell count
NEG
GFP+
EGFP
Gr-1
CD11b
F
EGFP(+)BM-MDSCs
EGFP(-)BM-MDSCs
Untreated
IL-1β
IL-1β +IL-1RA
IL1β +IL-MG132
Untreated
IL-1β
IL-1β +IL-1RA
IL1β +IL-MG132
EGFP
GAPDH

**Figure S4.**

**Figure S4. MDSCs Inhibit Cell Proliferation and Function of CD4 T Cells, and IL-1β Activates MDSCs In Vitro**

(A) Splenic MDSCs inhibit CD4 T cell proliferation. Splenic MDSCs were sorted spleen from *IL-1*β mice and WT mice, and naive CD4 cells were sorted from WT mice. $1 \times 10^6$ MDSCs and CD4 cells (1:1) were cocultured in six-well plates in the presence of Con A (2.5 μg/ml) for 48 hours. Cell proliferation was measured by MTT method. The data shown are normalized to single culture CD4 T cells and represent the mean ± SD of 3 independent experiments.

(B) Splenic MDSCs inhibits Con A-induced IFN-γ secretion. $1 \times 10^6$ MDSCs and CD4 cells (1:1) were cocultured in six-well plates in the presence of Con A (2.5 μg/ml) for 48 hours. Supernatant were collected and IFN-γ secretion was detected by ELISA. The data shown represent the mean ± SD of 3 independent experiments (*$p < 0.01$ versus WT MDSCs).

(C) IL-1β upregulates expression of TNF-α and IL-6 in MDSCs. MDSCs sorted from bone marrow of WT mice were treated with 10 ng/ml IL-1β for 3 hours. The mRNA expression was determined by real time-PCR. The data are normalized to untreated MDSCs and represent the mean ± SD of four independent experiments.

(D) IL-1β stimulates secretion of IL-6 in MDSCs. MDSCs were treated with IL-1β for 36 hours. The level of IL-6 in culture medium was measured by ELISA.

(E) Sorting EGFP+ and EGFP- bone marrow MDSCs from NF-κB$^{EGFP}$ mice. Single nucleated cells isolated from bone morrow of NF-κB$^{EGFP}$ were stained with PerCP-Gr-1+ and APC-CD11b+ antibodies (a). EGFP+ and EGFP- MDSCs were further sorted by FACS (b). The purification of MDSCs sorted was more than 98% examined by FACS (c).

(F) IL-1β upregulates EGFP expression in MDSCs from NF-κB$^{EGFP}$ mice. EGFP+ MDSCs and EGFP- MDSCs were treated with 10 ng/ml IL-1β in absence or presence of 50 ng/ml IL-1RA or 1 μM MG132 for 3 hours, respectively. mRNA expression was determined by semi-quantity PCR. The image shown was representative of three independent experiments.

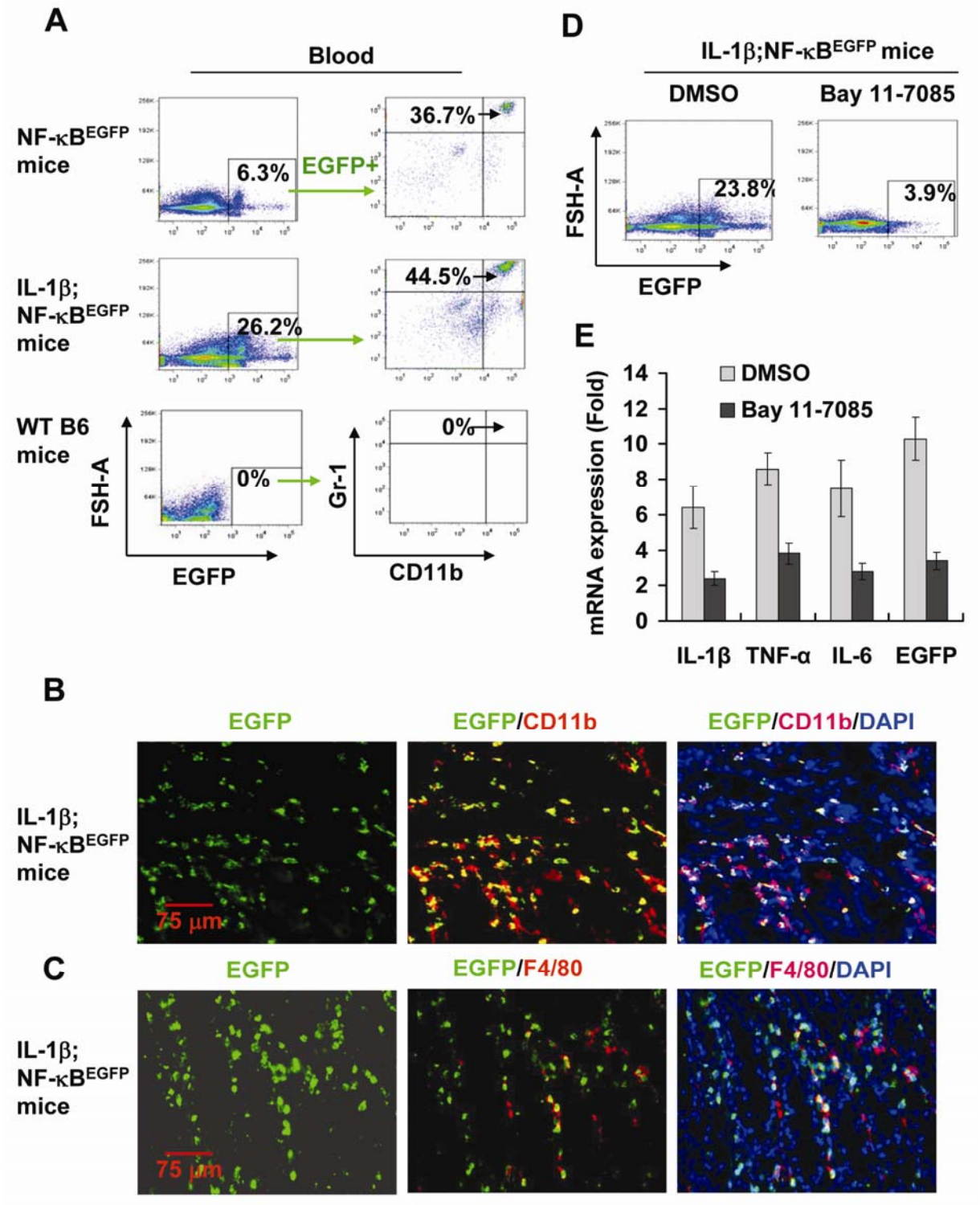
A
Blood
NF-κB^EGFP mice
6.3%
EGFP+
36.7%
IL-1β; NF-κB^EGFP mice
26.2%
44.5%
WT B6 mice
0%
0%
FSH-A
EGFP
Gr-1
CD11b
D
IL-1β;NF-κB^EGFP mice
DMSO
Bay 11-7085
23.8%
3.9%
FSH-A
EGFP
E
DMSO
Bay 11-7085
mRNA expression (Fold)
14
12
10
8
6
4
2
0
IL-1β
TNF-α
IL-6
EGFP
B
EGFP
EGFP/CD11b
EGFP/CD11b/DAPI
IL-1β; NF-κB^EGFP mice
75 μm
C
EGFP
EGFP/F4/80
EGFP/F4/80/DAPI
IL-1β; NF-κB^EGFP mice
75 μm

**Figure S5.**

**Figure S5. Overexpression of IL-1β Activates NF-κB in MDSCs In Vivo**

(A) Increased frequencies of EGFP+ MDSCs in the blood of *IL-1β;NF-κB*$^{EGFP}$ mice. Single nucleated cells were isolated from peripheral blood in WT, NF-κB$^{EGFP}$ and *IL-1β;NF-κB*$^{EGFP}$ mice. Cells were stained for APC-CD11b and PerCP-Gr-1 antibodies. Endogenous EGFP fluorescence and CD11b+Gr-1+ cells were analyzed by FACS. Present FACS blots are representative of 6 animals (*$p < 0.01$ versus *NF-κB*$^{EGFP}$ mice.)

(B and C) Enhanced EGFP expression in CD11b+ MDSCs from *IL-1β;NF-κB*$^{EGFP}$ mice. Frozen gastric sections from 6-month-old *IL-1β;NF-κB*$^{EGFP}$ mice were subjected to double staining with anti-EGFP (*green*) and CD11b (*red*) antibodies (B) or F4/80 (*red*) antibody (C), and counterstained with DAPI (*blue*). The overlay of green and red (yellow staining) indicates NF-κB activation in CD11b+MDSCs or F4/80+ macrophage.

(D and E) Blocking NF-κB activity prevents expression of EGFP and cytokines in *IL-1β;NF-κB*$^{EGFP}$ mice. Two-month-old *IL-1β;NF-κB*$^{EGFP}$ mice were injected i.p. three times weekly with the NF-κB inhibitor Bay 11-7085 (5 mg/kg) or vehicle control (DMSO) for 5 weeks. Single nucleated cells isolated from peripheral blood (Endogenous EGFP) were analyzed by FACS (D). Stomach mRNA were extracted and detected by real time PCR (E). The data shown are normalized to GAPDH and represent the mean ± SD of 6 mice (*$p < 0.01$ versus MDSO treated group).

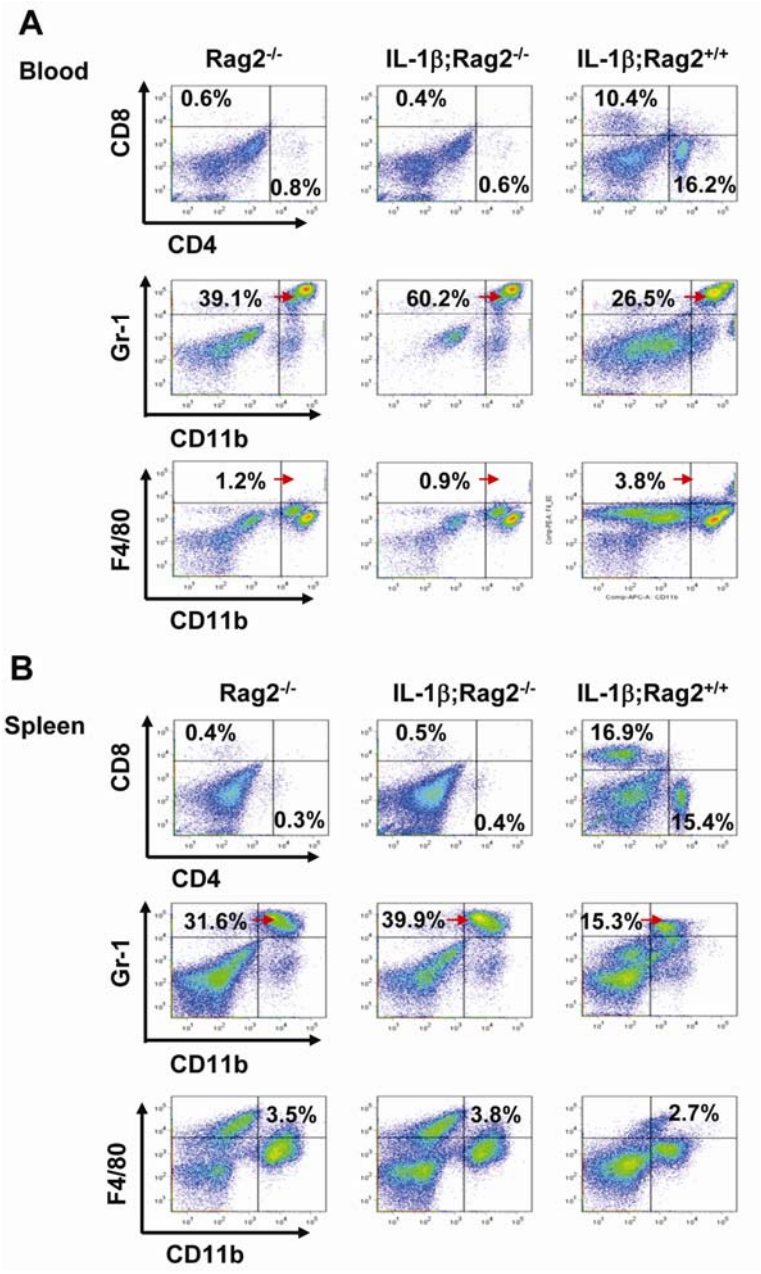


**Figure S6. Overexpression of IL-1β Increases the Mobilization and Recruitment of MDSCs in *IL-1β;Rag2*$^{-/-}$ Mice**

Representative FACS blots for detecting lymphoid and myeloid cells in the blood (A) and spleen (B) from 6-month-old *Rag2*$^{-/-}$ mice, *IL-1β;Rag2*$^{-/-}$ mice and *IL-1β;Rag2*$^{+/+}$ mice. Cells were stained with fluorescence-labeled antibodies, as indicated, and analyzed by FACS. The data shown are representative of 6 animals.

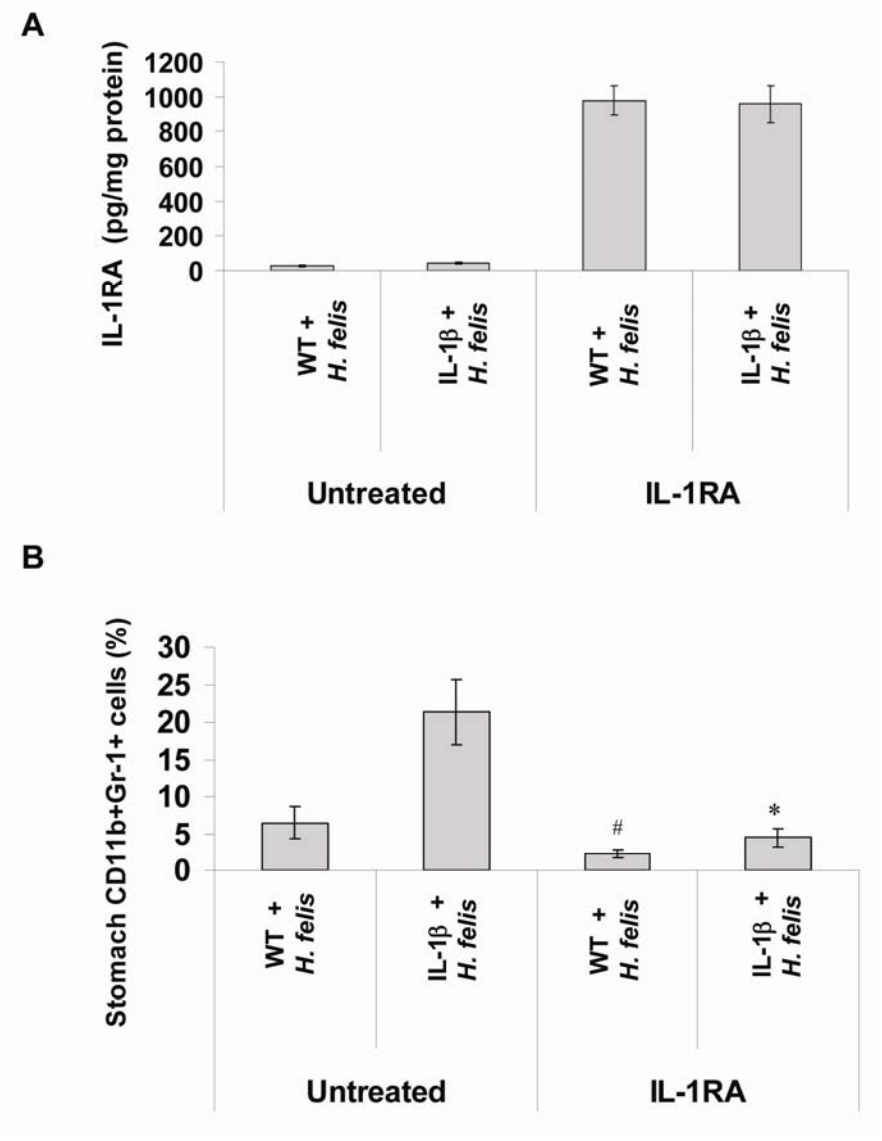


**Figure S7. IL-1RA Inhibits the Development of Gastric Carcinoma and Represses the MDSCs Mobilization in *H. felis*-Infected *IL-1*β Mice**

(A) IL-1RA treatment increases the level of human IL-1RA in the stomach. Stomach proteins were extracted from 7-month-old Line 19 *IL-1*β transgenic and control mice infected with *H. felis* for 5 months with or without preventive administration of IL-1RA. The level of IL-1RA was measured using the human IL-1RA ELISA kit. The data shown represent the mean ± SD of 6 animals.

(B) IL-1RA treatment inhibits the recruitment of MDSCs in the blood in *H. felis*-infected mice. Single nucleated cells in peripheral blood were isolated from 7-month-old Line 19 *IL-1*β transgenic and control mice infected with *H. felis* for 5 months with or without preventive administration of IL-1RA. Cells were stained with fluorescence-labeled CD11b and Gr-1 antibodies and analyzed by FACS. The data shown represent the mean ± SD of 6 animals (#$p < 0.01$ versus untreated *H. felis*-infected WT mice; *$p < 0.01$ versus untreated *H. felis*-infected *IL-1*β mice).

**Table S1.** Gastric pathology in *IL-1β* transgenic mice

| | Sex | Age (Months) | No. | Hyalinosis | Epithelial defects | Foveolar Hyperplasia | Acute Inflam. | Chronic Inflam. | Oxnt Atrophy | Metaplasia | Dysplasia | HGG | Cancer | Lymphoid follicles |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | F | 15.6±4.2 | 8 | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | M | 15.2±3.8 | 12 | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| **Line 42** | F | 15.2±3.7 | 19 | 0% | 11% | 22% | 11% | 22% | 16% | 16% | 0% | 0% | 0% | 0% |
| | M | 14.3±3.4 | 16 | 25% | 31% | 50% | 31% | 38% | 38% | 38% | 0% | 0% | 0% | 25% |
| **Line 19** | F | 13.9±4.6 | 16 | 33% | 68% | 68% | 81% | 88% | 88% | 88% | 44% | 0% | 0% | 30% |
| | M | 14.2±3.5 | 20 | 50% | 80% | 90% | 90% | 90% | 90% | 90% | 70% | 15% | 15% | 70% |

**Note:** The section of stomach were stained with H&E. The pathological changes in the gastric corpus were analyzed according to the diagnosis criterion described in Methods. HGG, high-grade GIN; GIN, gastrointestinal epithelial neoplasia (carcinoma in situ).

**Table S2.** Gastric inflammation and histology scores in aged *IL-1β* mice

| | Foveolar hyperplasia | Acute inflammation | Chronic inflammation | Oxyntic Atrophy | Metaplasia | Dysplasia |
|---|---|---|---|---|---|---|
| **Control** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Line 42** | 0.7 ± 0.5* | 0.6 ± 0.6 * | 0.4 ± 0.3* | 0.3 ± 0.3 * | 0.3 ± 0.4* | 0 |
| **Line 19** | 1.6 ± 0.6 # * | 1.5 ± 0.5 #* | 1.7 ± 0.6* | 1.1 ± 0.7 # * | 1.4 ± 0.6 #* | 1.4 ± 0.5#* |

**Note**: Sections of stomach from aged transgenic and control mice were stained with H&E. Gastric inflammation and pathology scores were graded according the diagnosis criterion described in Material and Method. The results represent the mean ± SD of 20-36 mice. *p < 0.01, *vs* Control; #p < 0.05, *vs* Line 42. Control mice, n = 20; Line 42 mice, n =35; Line 19 mice, n = 36.

**Table S3.** Gastric inflammation and pathology scores in *H. felis*-infected *IL-1β* mice

| | Foveolar hyperplasia | Acute inflammation | Chronic inflammation | Oxyntic Atrophy | Metaplasia | Dysplasia |
|---|---|---|---|---|---|---|
| **WT Control** | 0 | 0 | 0 | 0 | 0 | 0 |
| **WT+*H. felis*** | 0.7 ± 0.5 ** | 0.8±0.6 ** | 1.2±0.7** | 0.8 ± 0.5 ** | 0.9 ± 0.5** | 0 |
| **Line 42** | 0.5±0.4* | 0.6±0.4 * | 0.3±0.3* | 0.3±0.2 * | 0.3±0.2* | 0 |
| **Line 42 +*H. felis*** | 1.6±0.6 # ** | 1.2±0.4 # ** | 3.0±0.6# ** | 2.1±0.7# ** | 2.2±0.6#** | 1.7 ± 0.5#** |

**Note:** Gastric sections from 12 month old *H. felis*-infected transgenic and control mice were stained with H&E. Gastric inflammation and pathology scores were graded by two pathologists according the diagnosis criterion described in Methods. The results represent the mean ± SD of 6-10 mice. *p < 0.05, *vs* WT control; **p < 0.01, *vs* uninfected mice; #p < 0.01, *vs H. felis*- infected WT mice. WT mice, n = 6; Line 42 mice, n = 10.

**Table S4.** The primers for PCR and Real-time PCR

| Genes | Sequences | Size of Productions |
|---|---|---|
| **hIL-1β** | Forward: 5'-TGCGAATCTCCGACCACCACTACA-3' | 295bp |
| | Reverse: 5'-TGGAGGTGGAGAGCTTTCAGTTCATAT-3' | |
| **mIFN-γ** | Forward: 5'-CATGGCTGTTTCTGGCTGTTACTG-3' | 226bp |
| | Reverse: 5'-GTTGCTGATGGCCTGATTGTCTTT-3' | |
| **mTNF-α** | Forward: 5'-TGGCCCAGACCCTCACACTCAG-3' | 180bp |
| | Reverse: 5'-ACCCATCGGCTGGCACCACT-3' | |
| **mIL-1β** | Forward: 5'-GGAGAACCAAGCAACGACAAAATA-3' | 211bp |
| | Reverse: 5'-TGGGGAACTCTGCAGACTCAAAC-3' | |
| **mIL-6** | Forward: 5'-GTTTTCTGCAAGTGCATCATCG-3' | 236bp |
| | Reverse: 5'-GGTTTCTGCAAGTGCATCATCG-3' | |
| **mIL-4** | Forward: 5'-ATCGGCATTTTGAACGAGGTCA-3' | 221bp |
| | Reverse: 5'-CATCGAAAAGCCCGAAAGAGTCT-3' | |
| **mCox-2** | Forward: 5'-GCTGCCCGACACCTTCAACATT-3' | 150bp |
| | Reverse: 5'-CACATTTCTTCCCCCAGCAACC-3' | |
| **mMMP-9** | Forward: 5'-GCCGGCGTTCAGGGAGATG-3' | 160bp |
| | Reverse: 5-TGTGGTGCAGGCCGAATAGGA-3' | |
| **mSDF-1α** | Forward: 5'-TGAGTCAACACAAGATCCGGCAGA-3' | 154bp |
| | Reverse: 5'-GATGAAGCATGCGTTTGGAGGCAA-3' | |
| **mVEGF** | Forward: 5'-TCCAGGAGTACCCCGACGAGATAG-3' | 142bp |
| | Reverse: 5'-TGCTGGCTTTGGTGAGGTTTGAT-3' | |
| **mGAPDH** | Forward: 5'-GGAGGAACCTGCCAAGTATG-3' | 256bp |
| | Reverse: 5'-TGGGAGTTGCTGTTGAAGTC-3' | |
| **mIL-1RI** | Forward: 5'-AAGTAATGCTGTCCTGGGCTGCACT-3' | 649bp |
| | Reverse: 5'-TTTCCTGACACTGGCTCTGTTCCA-3' | |

(Note: h:human, m:mouse)