Supplementary Methods

Hf culture and infection

Hf (strain CS1) were cultured in Brucella broth supplemented with 10% heat-inactivated fetal bovine serum (FBS) using the GasPak EZ Campy Container System (BD Diagnostic Systems, Franklin Lakes, NJ) at 37°C with 120 rpm shaking. The cultures were spun down at an OD600 of 0.6-0.8 and bacteria resuspended in fresh medium. Cultures were checked by phase contrast microscopy for absence of contaminants and bacterial motility. Bacterial concentration was determined by counting aliquots of the suspension fixed in 1% formaldehyde. Mice were gavaged three times at 2-day intervals with either sterile broth (sham treated control) or 10^8 *Hf* in 100 µl medium.

Tissue dissection

Animals were euthanized by isoflurane overdose. The isolated stomachs were opened along the greater curvature and gastric fluid collected in 0.9% NaCl (pH 7.0) for acid titration. Stomachs were rinsed in phosphate buffered saline (PBS), then the corpus dissected and divided along the lesser curvature. One half of the corpus was homogenized in TRIzol solution (Life Technologies, Carlsbad, CA) for RNA extraction. The other half was cut into longitudinal strips for histology from the lesser and greater curvatures. Tissue strips for paraffin-embedding were fixed in 10% formalin. For cryosectioning, samples were fixed in 4% freshly prepared formaldehyde, cryoprotected in 30% sucrose/PBS and snap-frozen in Tissue-Tek O.C.T. compound (Andwin Scientific, Woodland Hills, CA).

Real-time reverse transcription PCR (RT-qPCR)

Total RNA was prepared using TRIzol reagent, treated with deoxyribonuclease and further cleaned up on RNeasy spin columns (Qiagen Sciences, Germantown, MD). RNA was reverse transcribed with Superscript II (Life Technologies) using oligo-dT priming for host gene expression and random hexamers for bacterial gene expression. PCR amplifications were performed in triplicate using a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA) with SYBR Green dye and Platinum Taq DNA polymerase (both from Life Technologies). Amplification specificity was confirmed by melting curve analysis of products. Gene expression of host genes was normalized to *Hprt1* mRNA. The expression of *Hf* genes was normalized to *Hf*-specific 16S rRNA and *flaB*. Primer sequences are listed in Supplemental Table S5.

Immunohistochemical detection of H^+/K^+-ATPase-β

4-µm-thick sections from paraffin-embedded specimens were deparaffinized, rehydrated, and subjected to antigen retrieval in 10 mM citrate buffer, pH 6.0 for 20 min at 95ºC. Slides were washed in 0.01% Triton X-100 in PBS, blocked with 20% donkey serum and incubated with anti-H^+/K^+-ATPase-β (Medical and Biological Laboratories, Woburn, MA). The staining was developed using a rabbit-specific HRP/DAB detection kit (Abcam).

Western blot analysis

Samples of the gastric corpus were homogenized in T-PER extraction reagent containing a cocktail of protease inhibitors (Complete; Roche). Expression of DUOX proteins in the lysates was analyzed by Western blotting as previously described [1].

Figure Legends for Supplementary Data

Fig. S1: ***Hf*-infection induces DUOX protein expression in the gastric surface epithelium**

A) Western blot of DUOX proteins. Note that DUOX proteins in *Duoxa*$^{-/-}$ mice are exclusively in an immature, ER-retained form [1]. B) Immunofluorescent detection of DUOX proteins in the gastric body of *Helicobacter*- and sham-infected mice. CDH1, epithelial E-cadherin. Scale bars: 50 µM.

Fig. S2: **Normal H+/K+-ATPase expression in the stomachs of Duoxa$^{-/-}$ animals**

A) Expression of H^+/K^+-ATPase subunit genes (*Atp4a*, *Atp4b*). B) Immunohistochemical detection of the β-subunit of H^+/K^+-ATPase. Bars represent median and range of expression values. N=5 to 9 mice per group. *NS* $P>.05$.

Fig. S3: **Expression of *Hf*-induced inflammatory markers in the gastric corpus**

Relative qPCR results for A) the murine interleukin-8 equivalent CXCL1 (KC), B) TNF-α, and C) the B-cell marker Cd19. Overlayed bars indicate median expression values. * $P<.05$.

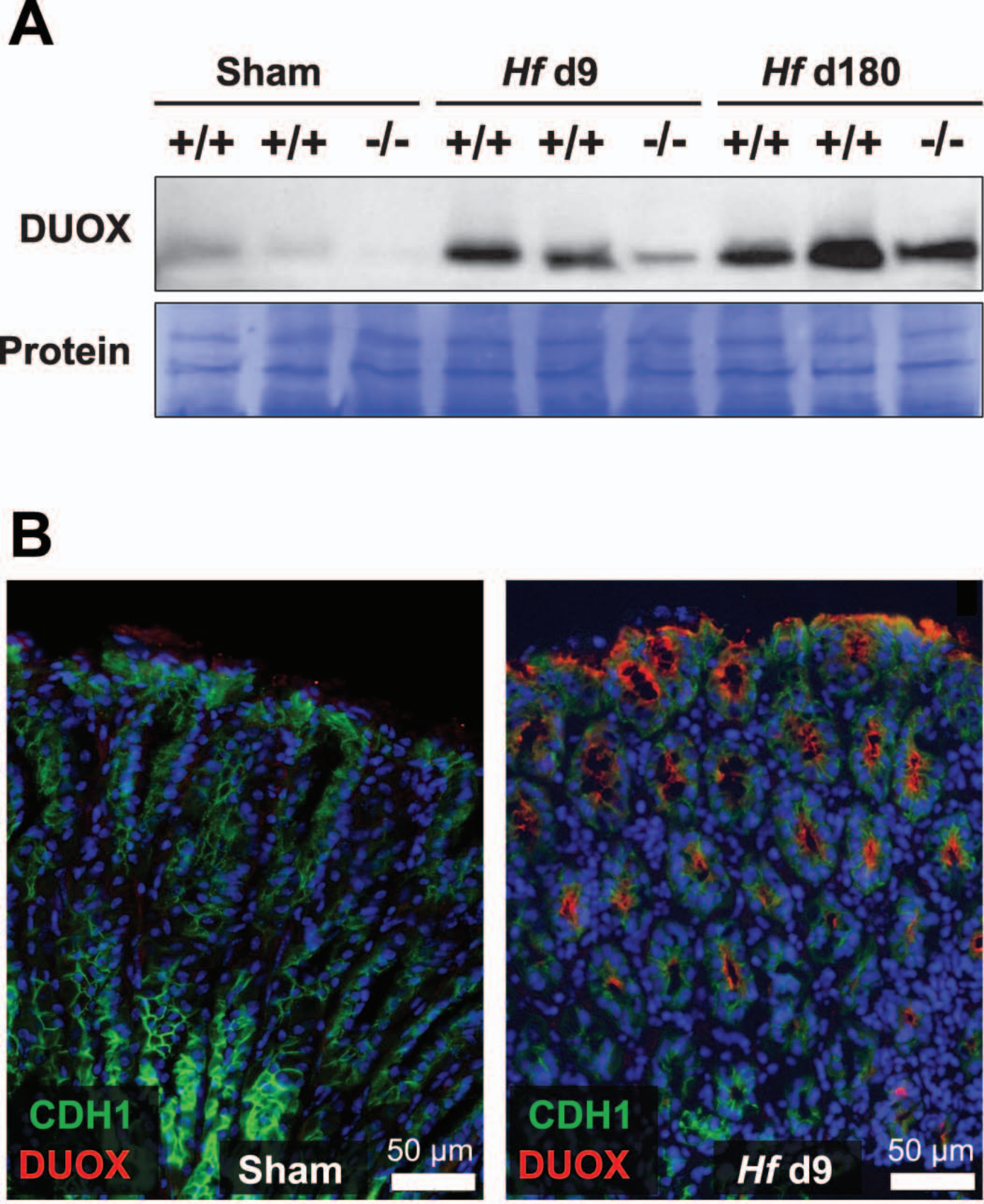
Fig. S4: **Expression of proinflammatory molecules is positively correlated to *Hf* colonization level**

Dotplots represent mRNA expression of inflammation markers versus expression of the *Hf* housekeeping gene *flaB*. Linear regressions are shown with 95% confidence intervals.

Tbl. S5: **Primers used in qPCR assays**

References:

1. Grasberger H, De Deken X, Mayo OB, et al. Mice deficient in dual oxidase maturation factors are severely hypothyroid. Molecular endocrinology 2012;26:481-492.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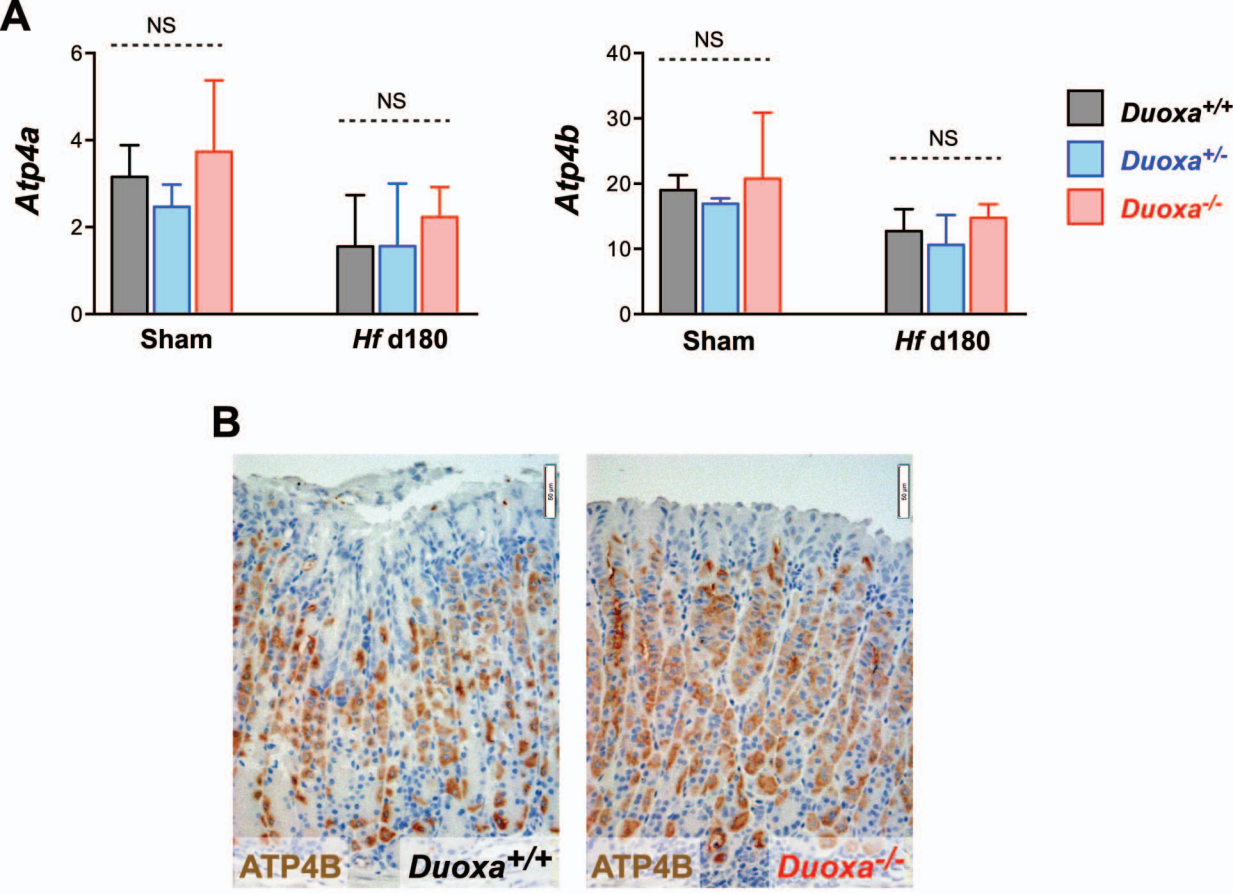
Hf d180
+/+ +/+ -/- +/+ +/+ -/- +/+ +/+ -/-
DUOX
Protein
B
CDH1
DUOX
Sham
50 µm
CDH1
DUOX
Hf d9
50 µm

Suppl. Fig. S1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Sham
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NS
NS
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30
20
10
0
Sham
Hf d180
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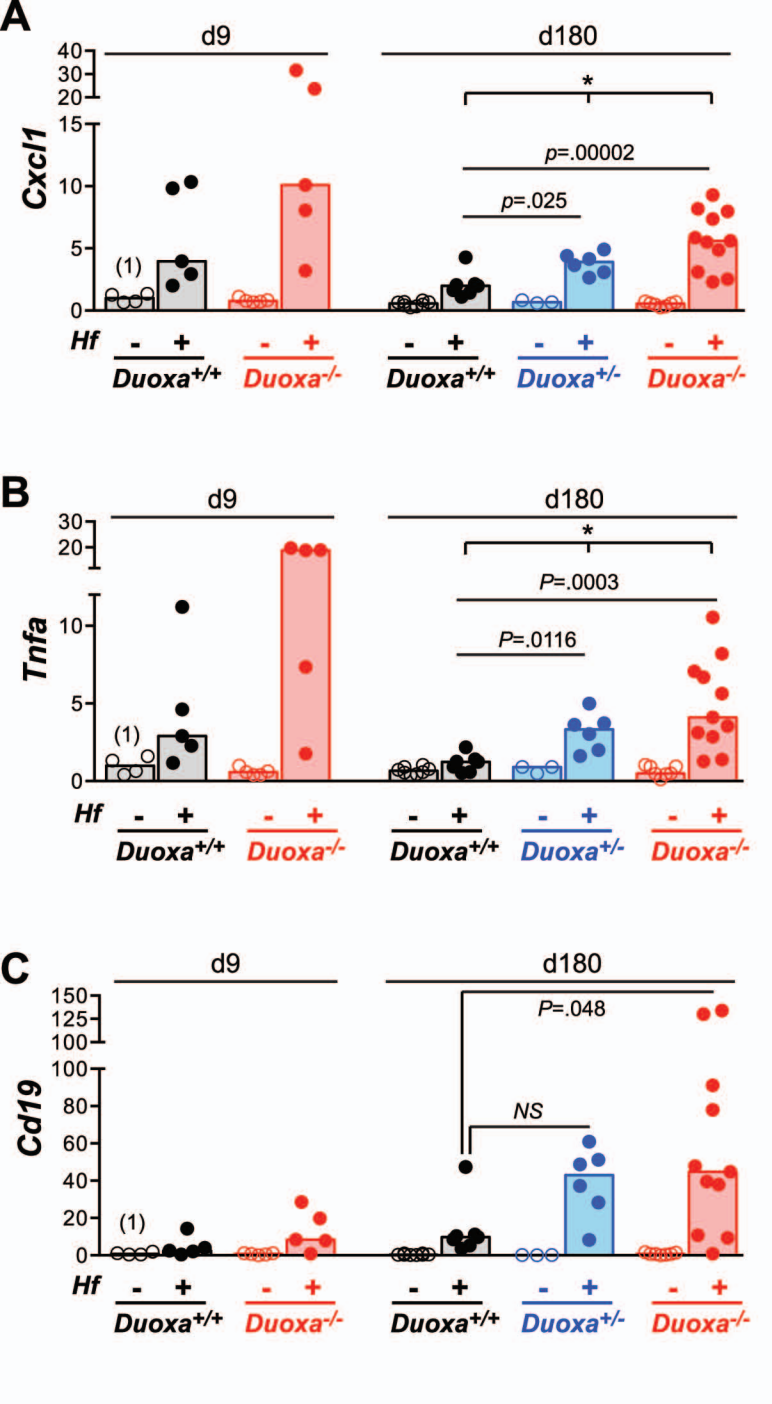
Duoxa+/-
Duoxa-/-
B
ATP4B
Duoxa+/+
ATP4B
Duoxa-/-

Suppl. Fig. S2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d180
Cxcl1
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15
10
5
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(1)
*
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p=.025
Hf
-
+
Duoxa+/+
Duoxa-/-
Duoxa+/+
Duoxa+/-
Duoxa-/-
B
d9
d180
Tnfa
30
20
10
5
0
(1)
*
P=.0003
P=.0116
C
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Cd19
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100
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20
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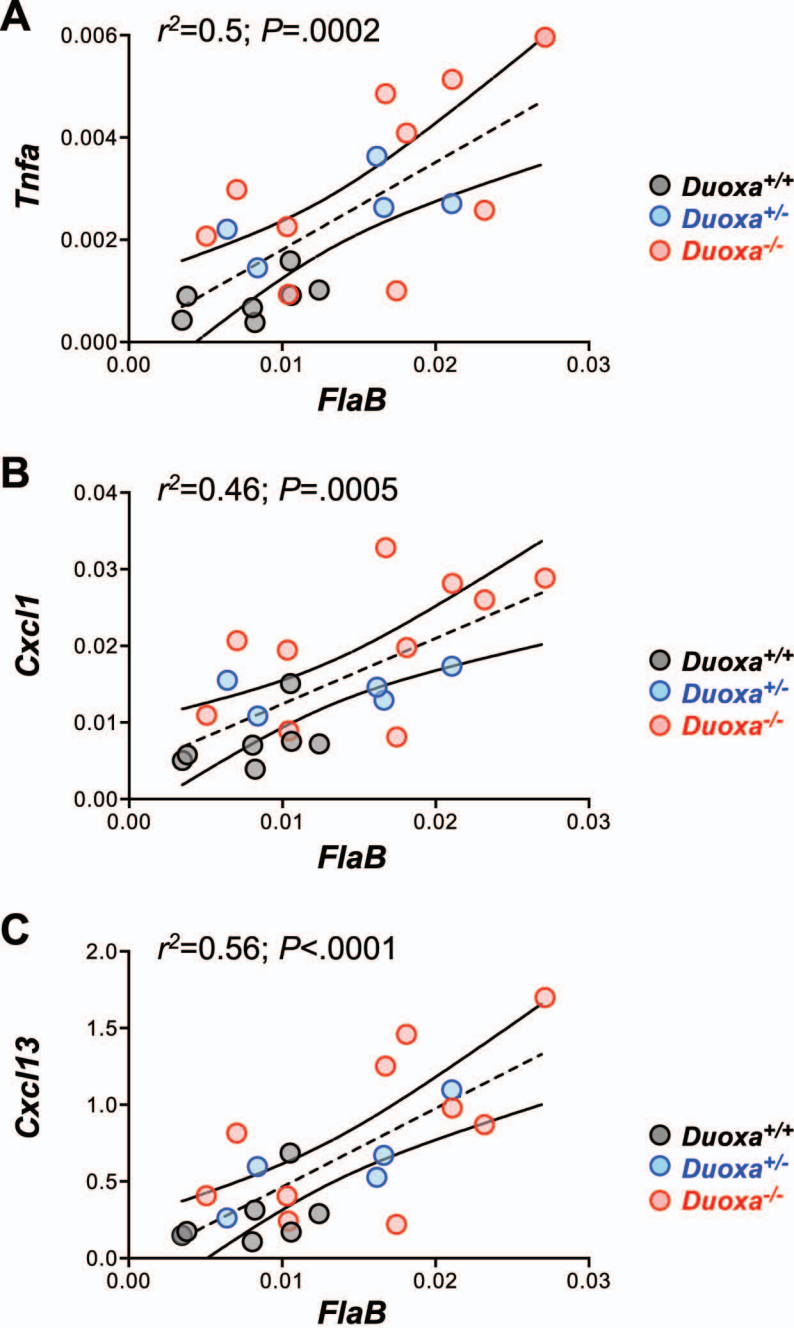
P=.048
NS

Suppl. Fig. S3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0.002
0.000
0.00
0.01
0.02
0.03
FlaB
Duoxa+/+
Duoxa+/-
Duoxa-/-
B
r^2=0.46; P=.0005
Cxcl1
0.04
0.03
0.02
0.01
0.00
0.00
0.01
0.02
0.03
FlaB
Duoxa+/+
Duoxa+/-
Duoxa-/-
C
r^2=0.56; P<.0001
Cxcl13
2.0
1.5
1.0
0.5
0.0
0.00
0.01
0.02
0.03
FlaB
Duoxa+/+
Duoxa+/-
Duoxa-/-

Suppl. Fig. S4

Suppl. Tbl. S5

mouse-specific primers		
name	**5'-3' sequence**	**gene**
DUOXA2-F	GCCTGGCTTTGCTCACCA	*Duoxa2*
DUOXA2-R	GAGGAGGAGGCTCAGGAT	
DUOXA1-F	CATCACCCTCACAGGCACC	*Duoxa1*
DUOXA1-R	GGAATGCCACCCACAGCA	
DUOX1-F	CCCACGTTACCATTTCCATCA	*Duox1*
DUOX1-R	CATCTGCATAGCTGGCTGGA	
DUOX2-F	GGACAGCATGCTTCCAACAAGT	*Duox2*
DUOX2-R	GCCTGATAAACACCGTCAGCA	
CD19-F	GAGAGGCACGTGAAGGTCATTG	*Cd19*
CD19-R	CATGGCTCTGAGCTCCAGTATC	
IFNG-F	GCGTCATTGAATCACACCTG	*Ifng*
IFNG-R	TGAGCTCATTGAATGCTTGG	
CXCL13-F	TGGCTGCCCCAAAACTGA	*Cxcl13*
CXCL13-R	TGGCACGAGGATTCACACAT	
CXCL1-F	CTGCACCCAAACCGAAGTCAT	*Cxcl1*
CXCL1-R	TTGTCAGAAGCCAGCGTTCAC	
TNF-F	GTCCCCAAAGGGATGAGAAGT	*Tnf*
TNF-R	CTCCACTTGGTGGTTTGCTAC	
IL1B-F	GGACAGAATATCAACCAACAAGTG	*Il1b*
IL1B-R	GAACTCTGCAGACTCAAACTCCA	
IL17A-F	GGACTCTCCACCGCAATGA	*Il17a*
IL17A-R	GGCACTGAGCTTCCCAGATC	
***Hf*-specific primers**		
name	**5'-3' sequence**	**gene**
FlaB-F	TTCGATTGGTCCTACAGGCTCAGA	*flaB*
FlaB-R	TTCTTGTTGATGACATTGACCAACGCA	
HF16S-F	CTAGCTTGCTAGGCGGATTAGT	16S rRNA
HF16S-R	CCTCACCAACAAGCTGATAGGA	
KATA-F	CAAGATGTCCTCACAGCAGGA	*katA*
KATA-R	CCACGGTGGAAAAGCGCA	