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Article type : Original Article

Dendritic cell-derived TGF-β mediates the induction of mucosal regulatory T cell response to *Helicobacter* infection essential for maintenance of immune tolerance in mice.

Running title: Dendritic cell-derived TGF-β mediates *H. pylori* induction of immune tolerance.

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/HEL.12763

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ABSTRACT

Background: *Helicobacter pylori* infection leads to regulatory T-cell (Treg) induction in infected mice, which contributes to *H. pylori* immune escape. However, the mechanisms responsible for *H. pylori* induction of Treg and immune tolerance remain unclear. We hypothesized DC-produced TGF-β may be responsible for Treg induction and immune tolerance.

Materials and Methods: To test this hypothesis, we generated TGF-$\beta^{\Delta DC}$ mice ($CD11c^{+}$ DC-specific TGF-β deletion) and assessed the impact of DC-specific TGF-β deletion on DC function during *Helicobacter* infection in vitro and in vivo. To examine the T-cell independent DC

function, we crossed TGF-$\beta^{\Delta DC}$ mice onto Rag1KO background to generate TGF-$\beta^{\Delta DC}$xRag1KO mice.

Results: When stimulated with *H. pylori*, TGF-$\beta^{\Delta DC}$ BMDC/splenocyte cocultures showed increased levels of proinflammatory cytokines and decreased levels of anti-inflammatory cytokines compared to control, indicating a proinflammatory DC phenotype. Following 6 months of *H. felis* infection, TGF-$\beta^{\Delta DC}$ mice developed more severe gastritis and a trend towards more metaplasia compared to TGF-$\beta^{fl/fl}$ with increased levels of inflammatory Th1 cytokine mRNA and lower gastric *H. felis* colonization compared to infected TGF-$\beta^{fl/fl}$ mice. In a T-cell deficient background using TGF-$\beta^{\Delta DC}$xRag1KO mice, *H. felis* colonization was significantly lower when DC-derived TGF-β was absent, revealing a direct, innate function of DC in controlling *H. felis* infection independent of Treg induction.

Conclusions: Our findings indicate that DC-derived TGF-β mediates *Helicobacter*-induced Treg response and attenuates the inflammatory Th1 response. We also demonstrated a previously unrecognized innate role of DC controlling *Helicobacter* colonization via a Treg independent mechanism. DC TGF-β signaling may represent an important target in the management of *H. pylori*.

Introduction

Helicobacter pylori is the most common bacterial infection in humans worldwide and is present in more than half the world's population. Infection is more common in developing countries, affecting up to 80% of individuals, and is thought to be related to poor hygienic conditions[1,2]. Interestingly, the prevalence of *H. pylori* infection is inversely correlated with atopic dermatitis[3], asthma[4–6], IBD[7,8], and rheumatoid arthritis[9], which is hypothesized to be related to the hygiene hypothesis or immunomodulatory effects of the bacterium itself[10–12].

H. pylori is a gram-negative bacterium capable of colonizing the stomach and leading to chronic infection, contributing to the development of peptic ulcer disease, atrophic gastritis, MALT lymphoma, and gastric adenocarcinoma, which is the third leading cause of cancer mortality

worldwide[13]. Though infected individuals generate a robust immune response, failure to eradicate the organism is common[14].

Several mechanisms behind this immune evasion and subsequent persistent infection have been proposed. These include antigenic variation, modulation of adhesion to gastric epithelial cells, evasion of pattern recognition, direct inhibition of T cell proliferation via vacA, and induction of a Treg response that counters T cell immunity[15,16]. The evidence supporting Treg expansion is particularly robust; patients with *H. pylori* infection have demonstrated elevated levels of $CD4^+CD25^+$ Tregs in the gastric and duodenal mucosa compared to non-infected patients[17], and there is a correlation between $Foxp3^+$ Tregs and degree of *H. pylori* colonization[18]. Additionally, depletion of $CD25^+Foxp3^+$ Tregs in *H. pylori*-infected mice leads to increased gastric inflammation and reduced bacterial colonization[19]. Local gastric mucosal infection with *H. pylori* in mice has also been associated with the appearance of peripherally induced Tregs in the lung[20]. We previously showed that *H. pylori* alters the DC-polarized Th17/Treg balance toward a Treg-biased response, which suppresses the effective induction of *H. pylori*-specific Th17 immunity[21]. Treg depletion in a genetic model has resulted in significant inflammatory immune response and spontaneous *H. pylori* clearance[22]. However, the specific mechanisms behind the induction of Treg differentiation in *H. pylori* infection are not well understood.

Emerging evidence demonstrates that dendritic cells (DCs) are involved in the response to *H. pylori* infection[23]. We have shown that DCs are recruited to the gastric mucosa after *H. pylori* infection[21,24]. In another study, DC-depleted neonatally infected mice showed a significant reduction in *H. pylori* CFUs compared to TGF-$\beta^{fl/fl}$ infected mice[25]. DC-depleted mice infected with *H. pylori* also display more severe gastritis and generate stronger Th1 and Th17 responses[26].

DCs are a rich source of TGF-β, which modulates T cell regulation and differentiation[27]. TGF-β is an important immunomodulator for T cell regulation and differentiation, inducing Treg as well as Th17 differentiation[28,29]. *H. pylori* specific immune tolerance requires TGF-β signaling, and mice with a dominant-negative form of the TGF-β receptor II have demonstrated impaired Treg induction and immune tolerance[22]. Hence, we hypothesized that DC-derived TGF-β mediates Treg induction, which conveys host immune tolerance in response to *H. pylori* infection.

To test this hypothesis, we generated DC-specific TGF-β knockout C57BL6 mice (TGF-$\beta^{\Delta DC}$) to demonstrate that this group of DC TGF-β deficient mice exhibit more severe mucosal inflammation and have a lower degree of bacterial colonization. *In vitro* studies using BMDCs from these TGF-$\beta^{\Delta DC}$ mice showed increased levels of pro-inflammatory cytokines following stimulation with *H. pylori* compared to control. To evaluate whether TGF-β can induce immune tolerance independent of Treg response, we crossed TGF-$\beta^{\Delta DC}$ mice onto Rag1 KO background and generated TGF-$\beta^{\Delta DC}$xRag1KO double KO mice. Our studies indicate that DC-derived TGF-β plays an important role in the induction of Treg and attenuation of inflammatory Th1 response following *Helicobacter* infection. Also, TGF-β may modulate immune tolerance independent of Treg, suggesting an innate component to TGF-β signaling.

Methods

Mice

Mice (B6.C-Tg(itgax-cre)1-1Reiz/J, TGF-βtm2.1Doe/J, and Rag1KO) were purchased from Jackson Laboratory for breeding. We used the Cre/lox system to generate DC-specific TGF-β1 knockout C57BL6 mice (TGF-$\beta^{\Delta DC}$) by crossing cCD11c-cre mice with TGF-β1 flox-ex6 mice (Jackson Lab) and generated TGF-$\beta^{\Delta DC}$-Rag1KO mice by crossing TGF-$\beta^{\Delta DC}$ with Rag1KO (Jackson Lab) mice. TGF-β1 flox-ex6 mice served as the TGF-$\beta^{fl/fl}$ control. All animals were housed in the animal maintenance facility at the University of Michigan Health System. This research was undertaken with the approval of the Committee on Use and Care of Animals at the University of Michigan. Mouse genotypes were confirmed by quantitative PCR using mouse tails.

Media and cytokines

For all cell cultures, a complete medium consisted of RPMI-1640 (Sigma, Milwaukee, WI) with 10% heat-inactivated fetal calf serum (ISC Biosciences, Kaysville, UT), 2 mM added Glutamine (4 mM total), and 100 U/mL Penicillin-Streptomycin. The following recombinant cytokines (R&D Systems, Minneapolis, MN) were diluted in complete medium: mGM-CSF (10 ng/mL) and IL-4 (10 ng/mL) for BMDC.

Generation of bone marrow-derived DCs

BMDCs from TGF-$\beta^{fl/fl}$ or TGF-$\beta^{\Delta DC}$ mice were derived using mouse GM-CSF (10 ng/mL) and IL-4 (10 ng/mL) as previously described[19] except BMDCs were cultured with serum free RPMI1640 to exclude exogenous serum TGF-β. and cultured with RPMI1640 containing 10% fetal bovine serum (FBS) BMDCs were harvested and enriched (10^6 cells/mL) by gradient centrifugation using OptiPrep density solution (Sigma, St. Louis, MO) according to the manufacturer's instructions on day 6. For *H. pylori*-stimulated BMDC experiments, 1×10^6 cells/mL of BMDCs were plated in a 12 well plate, treated with 10^7CFU/mL *H. pylori* (DC to *H. pylori* ratio of 1 to 10), 10^7CFU/mL *Escherichia coli* (*E. coli*) (DC to *E. coli* ratio of 1 to 10), PBS, or *E. coli* lipopolysaccharide (LPS). After overnight (18h) culture, the supernatant was harvested and TGF- β was measured using ELISA.

***Helicobacter* culture and infection**

H. pylori SS1 was cultured on Campylobacter-selective agar (BD Diagnostics, Bedford, MA, USA) for 3 days in a humidified microaerophilic chamber at 37°C (BBL Gas System, with CampyPak Plus packs, BD Biosciences San Jose, CA) as previously described[21].

H. felis was cultured in sterile-filtered Brucella broth (BD, Franklin Lakes, NJ) with 10% FBS (Atlanta Biologicals, Lawrenceville, GA) using the GasPak™ EZ Campy Container System (BD) at 37°C at an agitation rate of 150 rpm for 3-5 days. The cultures were spun down at 2700 rpm at room temperature, and the pellets resuspended in Brucella broth plus 10% FBS (Thermo Fisher Scientific, Houston, TX). Bacteria were counted using a hemocytometer by diluting the cells 1:100 in 9:1 HBSS/Formalin solution. TGF-$\beta^{\Delta DC}$, TGF-$\beta^{fl/fl}$, TGF-$\beta^{\Delta DC}$xRag1KO, and control TGF-$\beta^{\Delta DC}$ mice were gavaged 3 times over 5 days with 10^8 CFU *H. felis* in 100 µL of Brucella broth.

Animal studies

After 6 months infection with *H. felis,* the mice were euthanized. The stomach was removed and analyzed. In addition, splenocytes from TGF-$\beta^{fl/fl}$ or TGF-$\beta^{\Delta DC}$ mice were cocultured for 18h with BMDCs from uninfected control mice and 10^7 CFU/mL *H. felis*. The splenocyte-to-BMDC ratio was 10 to 1. After 72 h, the supernatant was collected and IL-12p70, IFN-γ, and TNF-α levels were measured by ELISA (eBioscience/BD Biosciences, San Diego, CA/San Jose, CA).

Splenocytes were collected and the percentages of $CD4^+FoxP3^+$ T cells (Treg) were measured by fluorescence-activated cell sorting (FACS).

Histological scoring

The stomachs of mice were removed and two adjacent full-thickness longitudinal strips were removed from the lesser and greater curvatures of the stomach and fixed in formalin for histologic analysis. The specimens were scored according to previously published protocol[30]. Briefly, 200x microscopic fields were scored individually for the presence or absence of each of the following 4 histological criteria: 1) polymorphonuclear leukocytes neutrophilic (PMN) infiltration, 2) mononuclear infiltration, 3) follicles, and 4) epithelial metaplasia. The gastritis score is defined as the the sum of the percentage of 200x microscopic fields with PMN, mononuclear infiltration, and follicles. The percentage of 200x microscopic fields with epithelial metaplasia was also measured.

Extraction of RNA, reverse transcription, and quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA from stomach samples was prepared using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). Samples were reverse-transcribed using iScript™ cDNA Synthesis Kit (BIO-RAD, Hercules, California). Expression of *H. felis*, TGF-β, TNF-α, IFN-γ, IL-12, IL-6, IL-1β, IL-10, and HPRT RNA was measured using iQ™SYBR Green Supermix Kit obtained from BIO-RAD. Primers are shown in **Table 1**. Finally, quantitation of relative differences in expression was calculated using the comparative $2^{-\Delta\Delta CT}$ method[31].

Statistical analysis

The results were evaluated using unpaired Student's t-tests (Mean±SEM). Statistics were performed in the GraphPad Prism program suite (GraphPad Software, Inc., La Jolla, CA). Significant values were indicated as follows: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

RESULTS

TGF-$\beta^{\Delta DC}$ DCs produce diminished TGF-β and exhibit an inflammatory phenotype

We previously showed that BMDCs produced TGF-β at homeostasis as well as when exposed to *H. pylori* [21], suggesting DC production of TGF-β may contribute to immune tolerance in *H. pylori* infection. To test this hypothesis, we generated a DC-specific TGF-β knockout murine model (**Figure 1A**). We verified DC-specific TGF-β depletion by comparing BMDC TGF-β production in TGF-$\beta^{fl/fl}$ vs TGF-$\beta^{\Delta DC}$ mice (**Figure 1B**).

When stimulated with PBS, *H. pylori*, *E. coli,* or LPS *in vitro*, TGF-$\beta^{\Delta DC}$ BMDC and splenocyte coculture supernatant contained markedly lower levels of TGF-β than control TGF-$\beta^{fl/fl}$ BMDC coculture supernatant (**Figure 2A**). Proinflammatory cytokine levels were significantly higher in the TGF-$\beta^{\Delta DC}$ group when stimulated with *H. pylori*, *E. coli*, and LPS (**Figure 2B**). Anti-inflammatory IL-10 levels were decreased in the TGF-$\beta^{\Delta DC}$ group compared to control when stimulated with *H. pylori*, *E. coli,* and LPS (**Figure 2C**). Overall, this decrease in anti-inflammatory cytokine levels and an increase in proinflammatory cytokine levels indicates a proinflammatory DC phenotype.

TGF-$\beta^{\Delta DC}$ mice infected with *H. felis* develop more severe gastritis compared to infected TGF-$\beta^{fl/fl}$ control mice

Next, we infected the TGF-$\beta^{\Delta DC}$ mice and TGF-$\beta^{fl/fl}$ mice with *H. felis* (10^8 CFU/mL *H. felis* via gavage 3 times over 5 days). *H. felis* was used as it produces more severe gastritis in mice and achieves higher levels of colonization compared to *H. pylori*[32–34]. Our data show that after 6 months of *H. felis* infection, TGF-$\beta^{\Delta DC}$ mice developed more severe gastritis compared to control TGF-$\beta^{fl/fl}$ mice, as evidenced by increased neutrophils, gland distortion, and metaplasia. Gastric TGF-β mRNA expression was confirmed to be significantly decreased in the TGF-$\beta^{\Delta DC}$ mice compared to wildtype (**Figure 3A**). Representative micrographs of gastric histology are shown in **Figure 3B**. The gastritis score for the TGF-$\beta^{\Delta DC}$ group was 2.7 fold higher than in the control group ($p<0.01$) (**Figure 3C**), showing that in the absence of DC-TGF-β, infected mice developed more severe gastritis compared to control. Additionally, there was a trend towards increased metaplasia in the TGF-$\beta^{\Delta DC}$ mice although values that did not reach statistical significance ($p=0.11$) (**Figure 3D**). These findings indicate DC-derived TGF-β plays a role in modulating gastric inflammation and likely subsequent metaplasia in *Helicobacter* infection.

TGF-β$^{\Delta DC}$ mice infected with *H. felis* display elevated Th1 cytokine production and decreased *H. felis*-specific Treg response and gastric colonization.

We next examined the *in vivo* TGF-β$^{\Delta DC}$ mouse cytokine response to *H. felis* infection vs control TGF-β$^{fl/fl}$ mouse cytokine response. Stomach samples taken after euthanasia at 6 months showed higher levels of IFN-γ, TNF-α, and IL-12 compared to the levels in the control TGF-β$^{fl/fl}$ group (**Figure 4A**), indicating a stronger Th1 response. In the TGF-β$^{\Delta DC}$ mice, levels of IFN-γ, TNF-α, and IL-12 were significantly higher than levels observed in the control group ($p<0.05$) (**Figure 4A**). Moreover, the spleens of *H. felis*-infected TGF-β$^{\Delta DC}$ mice showed a decreased *H. felis*-specific Treg response compared to control TGF-β$^{fl/fl}$ spleens (**Figure 4B**, $p<0.05$). Also, we determined that increased gastritis severity and inflammatory cytokine production were associated with decreased *H. felis* colonization. We quantified gastric *H. felis* mRNA using RT-PCR on the stomach samples from infected mice to measure colonization. Infected TGF-β$^{\Delta DC}$ mice had a lower degree of gastric *H. felis* mRNA compared to infected TGF-β$^{fl/fl}$ mice (**Figure 4C**) indicating lower colonization in the knockout mice. These data support the hypothesis that TGF-β plays an important role in immune tolerance leading to persistent *Helicobacter* infection.

TGF-β$^{\Delta DC}$xRag1KO mice (DC-TGF-β deficient and T cell-deficient) display lower degree of gastric *H. felis* colonization compared to Rag1 KO mice

To investigate whether TGF-β acts solely via modulation of the adaptive immune response to induce immune tolerance to *H. felis*, generated TGF-β$^{\Delta DC}$xRag1KO mice by crossing T and B cell-deficient Rag1KO mice with TGF-β$^{\Delta DC}$ mice (**Figure 5A**). TGF-β$^{fl/fl}$ mice served as the control. We compared *H. felis* colonization after 6 months in these two groups. As T cells are required for gastritis, neither group of mice displayed evidence of significant histological gastritis (data not shown and previously reported[35]) and inflammatory cytokines mRNA (IFN-γ, IL-6, IL-1β, IL-10, and TNF-α) measured were not significantly different between the two groups (**Figure 5B**). However, independent of gastric inflammation, the infected DKO (TGF-β$^{\Delta DC}$xRag1KO) mice had lower levels of *H. felis* mRNA in the gastric tissue compared to infected Rag1KO mice ($p<0.05$, **Figure 5C**). This suggests that while Treg response contributes to *Helicobacter* immune tolerance, DC-derived TGF-β has an additional innate role independent of Treg expansion or modification of T cell response which supports Helicobacter colonization.

DISCUSSION

H. pylori colonizes half of the world's population and most of those infected are asymptomatic. However, *H. pylori* infection can cause decades-long gastritis. This long term infection and chronic inflammation result in the development of peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma[36–38]. Despite persistent gastric inflammation with vigorous humoral and cellular immune responses, humans frequently fail to clear the bacterium and colonization persists for life unless treated. This failure to eradicate *H. pylori* has been attributed to ineffective host immune response and the induction of immune tolerance.

DCs are recruited to the gastric epithelium during *H. pylori* infection[21,24,39]. These antigen-presenting cells can migrate from the peripheral tissue to the draining lymph node or spleen with the captured antigen, where they present the antigen to naïve T-cells and initiate host immunity[40]. As such, they function as a link between the innate and adaptive immune responses. Depending on the local environment and costimulatory signals, DCs may activate cytotoxic/helper T cells and B cells[41]. They also help maintain immunologic tolerance to self and commensal bacteria by presenting these antigens in the absence of inflammatory cytokines[42]. We have previously shown that dendritic cells are recruited to the gastric mucosa following *H. pylori* infection and that *H. pylori* can induce tolerogenic programming of DCs to inhibit the host immune response[21,24]. Using a mouse model of *H. pylori* infection, we showed that *H. pylori* DNA downregulates DC production of pro-inflammatory cytokines IL-12 and type 1 interferon[43]. This may be mediated by increased frequency of an immunoregulatory sequence, TTTAGGG, which likely activates the DNA-sensing TLR-9 signaling pathway[44]. In addition to its DNA, *H. pylori* cell wall LPS activates DC TLR-2 to inhibit Th1 immunity and induce immune tolerance[45]. However, the mediators behind this immunoregulatory function have not been fully elucidated.

Since TGF-β induces naïve T cell differentiation into $Foxp3^+$ regulatory T cells, we hypothesized that TGF-β produced by BMDCs is the key mediator in Treg activation and inhibition of the immune response, leading to the immune tolerance commonly observed in *H. pylori* infection. To test this hypothesis, we generated DC-specific TGF-β knockout mice and verified the successful knockdown of TGF-β from BMDCs in vitro. When infected with *H. felis*, these mice developed more severe gastritis accompanied by enhanced Th1 response with marked elevation

in IFN-γ and TNF-α production. They also displayed 77% lower colonization compared to wild type mice. The spleen from these TGF-$\beta^{\Delta DC}$ mice had a 29% decrease in FoxP3^{+}Tregs compared to wildtype. Taken together, these in vivo and in vitro studies showed that BMDC-derived TGF-β plays an important role in *H. pylori* infection by modulating gastric inflammation and inducing Treg differentiation, leading to immune tolerance and *Helicobacter* persistence. This observation is consistent with the known immunomodulatory roles of TGF-β in suppressing effector T cell proliferation and inducing Treg differentiation[46,47].

Following *H. pylori* infection, TGF-β production is upregulated in many cells, including gastric fibroblasts, FoxP3^{+}Tregs, macrophages, and DCs[20,48,49]. In this study, we demonstrated a clear role for DC-derived TGF-β in Treg expansion. Based on our observations and other findings reported in the literature[50–52], we propose that following *H. pylori* infection, DCs migrate to peripheral lymphoid tissue, release TGF-β, stimulate Treg induction, and thus influence systemic immunity, which may lead to a reduction of inflammatory Th1 cytokines and enhanced colonization.

To examine whether DC-derived TGF-β acts solely by affecting T cell differentiation to induce immune tolerance following *H. pylori* infection, we generated double knock out mice by crossing TGF-$\beta^{\Delta DC}$ with Rag1KO and infected these DKO mice with *H. felis*. We reasoned that if DC-derived TGF-β acts to induce immune tolerance via Treg induction, the degree of *H. felis* colonization would be similar between the TGF-$\beta^{\Delta DC}$xRag1KO and Rag1KO mice. As expected, we did not detect gastritis in either group of mice because T cells are required for the development of mucosal inflammation. IFN-γ, IL-6, IL-1β, IL-10, and TNF-α mRNA levels were not significantly different between the Rag1KO and TGF-$\beta^{\Delta DC}$xRag1KO mice, supporting the absence of gastritis. However, we observed a lower *H. felis* colonization in the TGF-$\beta^{\Delta DC}$xRag1KO mice compared to the Rag1KO mice. This suggests that in addition to acting on adaptive immunity, DC-derived TGF-β may also exert its effects via a T cell-independent pathway. This reveals a direct innate immune function of DCs in the response to *Helicobacter* infection. It is conceivable that DC-derived TGF-β may act via autocrine signaling pathways that further upregulate DC TGF-β expression, and may have wide-ranging effects on the innate immune populations similar to the effect of Tregs in suppressing innate lymphoid cells[53,54].

Additionally, TGF-β has been shown to suppress TLR signaling and inhibit myeloid cell activation[55,56]. These possibilities would be worthwhile targets for investigation, though are beyond the scope of our current study.

In conclusion, our findings demonstrate that DC-derived TGF-β mediates Treg response in *H. pylori* infection, resulting in an attenuated Th1 inflammatory response. Using a double knockout mouse model, we also demonstrated a previously unrecognized innate role of DCs orchestrating response to Helicobacter colonization via a Treg-independent mechanism.

AUTHORSHIP

SYO and MZ performed the experiments, analyzed the data, and wrote the manuscript. KAE provided the histological interpretation of the mouse stomach. MEZ, GH, HG, and SB provided critiques on the study design and data interpretation. JYK conceived and designed the study and critically revised the manuscript.

ACKNOWLEDGEMENTS

This study was funded in full by National Institutes of Health, grant number R01 DK087708-01 (JYK), P30-DK034933-35 (University of Michigan Center for GI Research), and funding from the Department of Internal Medicine, Michigan Medicine, University of Michigan.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant competing interests.

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FIGURE LEGENDS

Figure 1. Generation of TGF-$\beta^{\Delta DC}$ mice, which are TGF-β-deficient. (A) TGF-$\beta^{\Delta DC}$ mice were generated by crossing cD11c-cre mice with TGF-β1 flox-ex6 mice, which were used as the TGF-$\beta^{fl/fl}$ control. (B) BMDCs derived from wt (TGF-$\beta^{fl/fl}$) vs TGF-$\beta^{\Delta DC}$ mice were cultured for 18h and supernatant TGF-β was quantified using enzyme-linked immune absorbent assay (ELISA), confirming deficient TGF-β production in the TGF-$\beta^{\Delta DC}$ DCs. *DC = dendritic cells, PBS = phosphate-buffered saline. Results are shown as mean ± S.E.M. *P < 0.05.*

Figure 2. TGF-$\beta^{\Delta DC}$ bone marrow-derived DCs produce diminished TGF-β and exhibit an inflammatory phenotype. BMDCs derived from control (TGF-$\beta^{fl/fl}$) vs TGF-$\beta^{\Delta DC}$ mice were cultured for 18h with *H. pylori* (10^7 CFU *H. pylori* to DC ratio 1:10), *E. coli* (10^7 CFU, *E. coli* to DC ratio 1:10), PBS, or LPS. Supernatant levels of (A) TGF-β were measured. When these BMDCs were cocultured with splenocytes, (B) IL-23p19 and IL-12 as well as (C) IL-10 were measured via ELISA. Data are representative of the results of three independent experiments. *DC = dendritic cells, PBS = phosphate-buffered saline, EC = E. coli, HP = H. pylori. Results are shown as mean ± S.E.M. *P < 0.05, ** P<0.01, ***P<0.001.*

Figure 3. TGF-$\beta^{\Delta DC}$ mice infected with *H. felis* show reduced gastric TGF-β expression and develop more severe gastritis compared to infected control (TGF-$\beta^{fl/fl}$) mice. TGF-$\beta^{\Delta DC}$ or TGF-$\beta^{fl/fl}$ C57BL/6 mice (n = 10 per group) were orally challenged with *H. felis* (10^8 CFU/mL) 3 times over 5 days starting on day 0. Stomach samples were analyzed after sacrifice at 6 months. Gastritis scores were determined in a blinded fashion. (A) TGF-β expression as measured via quantitative PCR. (B) Micrographs of gastric histology. (C) Gastritis score and (D) metaplasia in

stomach samples from wt (TGF-$\beta^{fl/fl}$) vs TGF-$\beta^{\Delta DC}$ mice infected with *H. felis*. Results are shown as mean ± S.E.M. $^{**}P < 0.01$.

Figure 4. *H. felis*-infected TGF-$\beta^{\Delta DC}$ mice show increased Th1 responses, decreased Treg responses, and increased *H. felis* colonization compared to control. After chronic *H. felis* infection of 6-month duration, stomachs from TGF-$\beta^{fl/fl}$ and TGF-$\beta^{\Delta DC}$ mice were removed. Splenocytes from these mice were cocultured with BMDCs from uninfected control mice and 10^7 CFU/mL *H. felis*. After 18h, expression of (A) IFNγ, TNFα, and IL-12 mRNA was measured via qPCR. (B) Splenocytes were stimulated with *H. felis* lysate and *H. felis*-specific $CD4^+FoxP3^+$ T cells via flow cytometry, and (C) *H. felis* mRNA via quantitative PCR (n=10 mice per group). Results are shown as mean ± S.E.M.$^{*}P<0.05, ^{***}P<0.0001$.

Figure 5. TGF-$\beta^{\Delta DC}$xRag1KO double knockout mice (TGF-β deficient and T cell-deficient) show a lower degree of gastric *H. felis* colonization independent of inflammatory cytokine levels. After chronic 6-month *H. felis* infection, the stomachs from Rag1KO and Rag1KO/TGF-$\beta^{\Delta DC}$ mice were removed (n=10 mice per group). (A) Schematic representation TGF-$\beta^{\Delta DC}$xRag1KO generation. (B) Gastric cytokine levels including IFN-γ, IL-6, IL-1β, IL-10, and TNF-α were measured via quantitative PCR and were not significantly different between the Rag1KO and TGF-$\beta^{\Delta DC}$xRag1KO mice. (C) *H. felis* mRNA was measured via quantitative PCR to assess colonization (n=10 mice per group). Results are shown as mean ± S.E.M. * P<0.05.

Table 1. Primers and annealing temperatures used for the amplification of each gene.

Gene	Primer(5'-3')	Annealing temperature
HPRT	F:5'-AGGACCTCTCGAAGTGTTGGATAC-3' R:5'-AACTTGCGCTCATCTTAGGCTTTG-3'	65
IL-6	F:5'-GAGGATACCACTCCCAACAGACC-3' R:5'-AAGTGCATCATCGTTGTTCATACA-3'	65
IL-10	F:5'-AGTGGAGCAGGTGAAGAGTG-3' R:5'-TTCGGAGAGAGGTACAAACG-3'	58
IFN-γ	F:5'TCAAGTGGCATAGATGTGGAAGAA--3' R:5'-TGGCTCTGCAGGATTTTCATG-3'	65
FoxP3	F:5'-TCTCCAGGTTGCTCAAAGTC-3' R:5'-GCAGAAGTTGCTGCTTTAGG-3'	58
TNF-α	F:5'-CATCTTCTCAAAATTCGAGTGACAA-3' R:5'-TGGGAGTAGACAAGGTACAACCC-3'	65
TGF-β	F: 5'-GCTACCATGCCAACTTCTGT-3' R: 5'-CGTAGTAGACGATGGGCAGT-3'	58

Figure 1

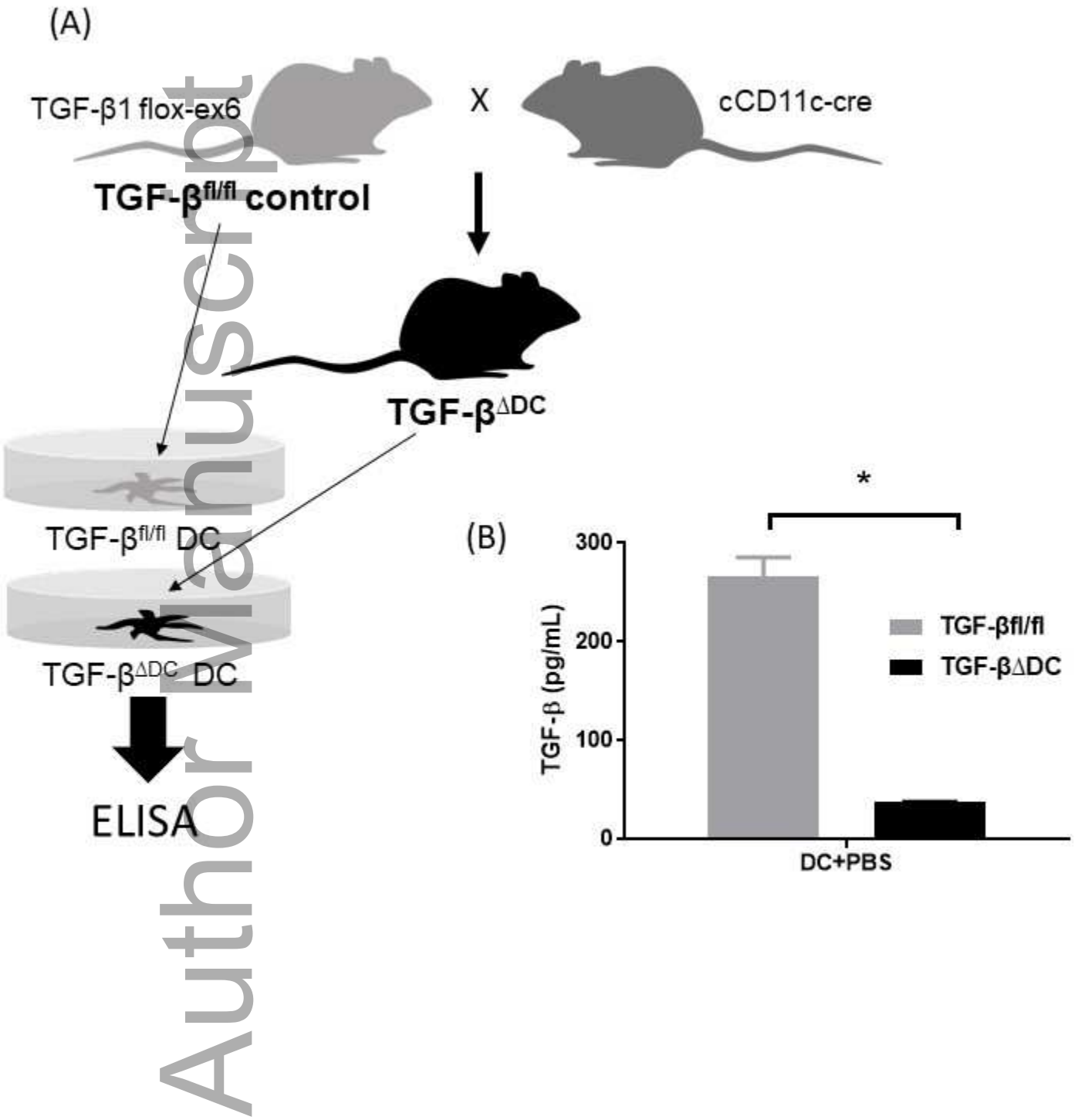

Figure 2

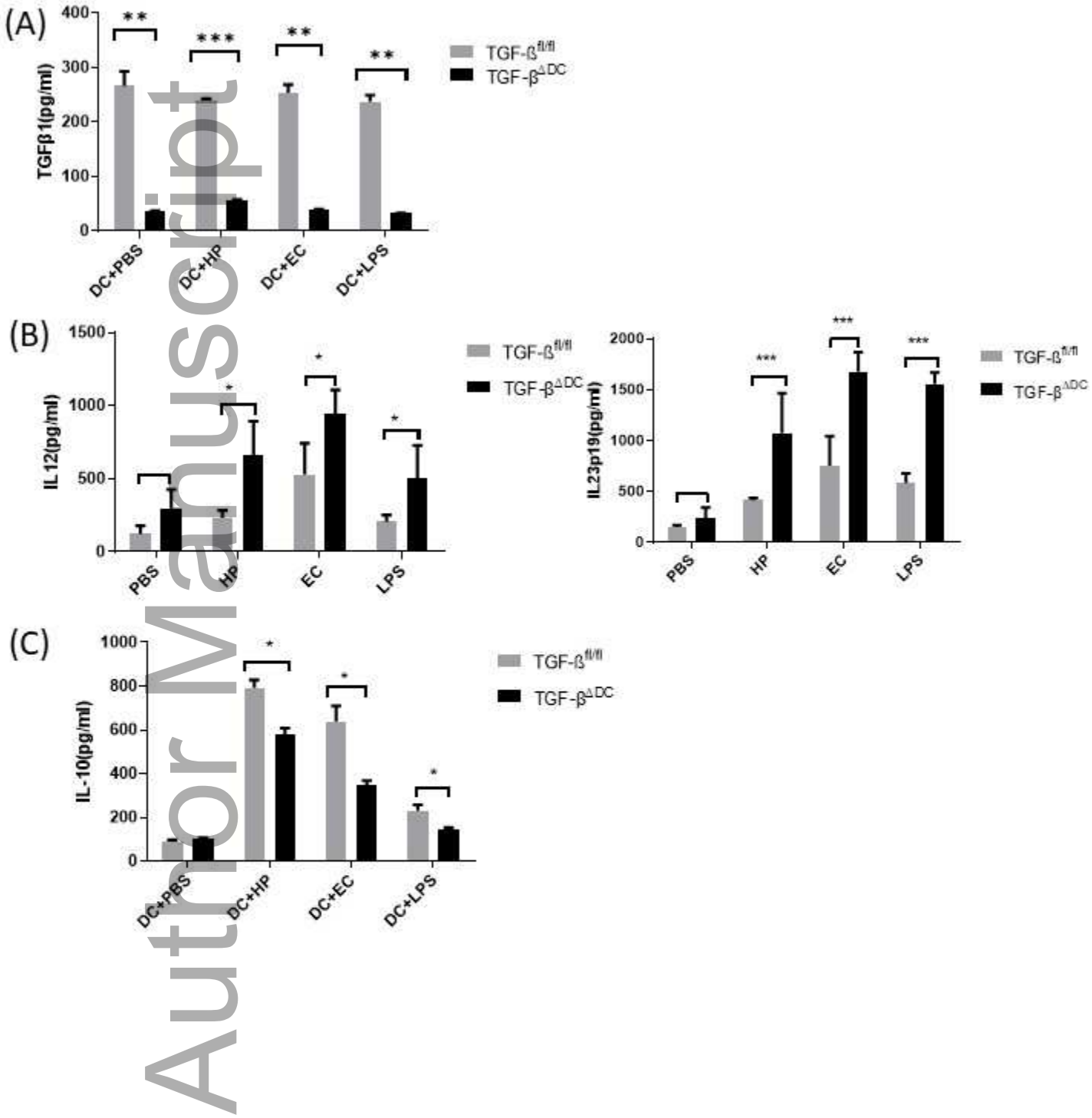

Figure 3

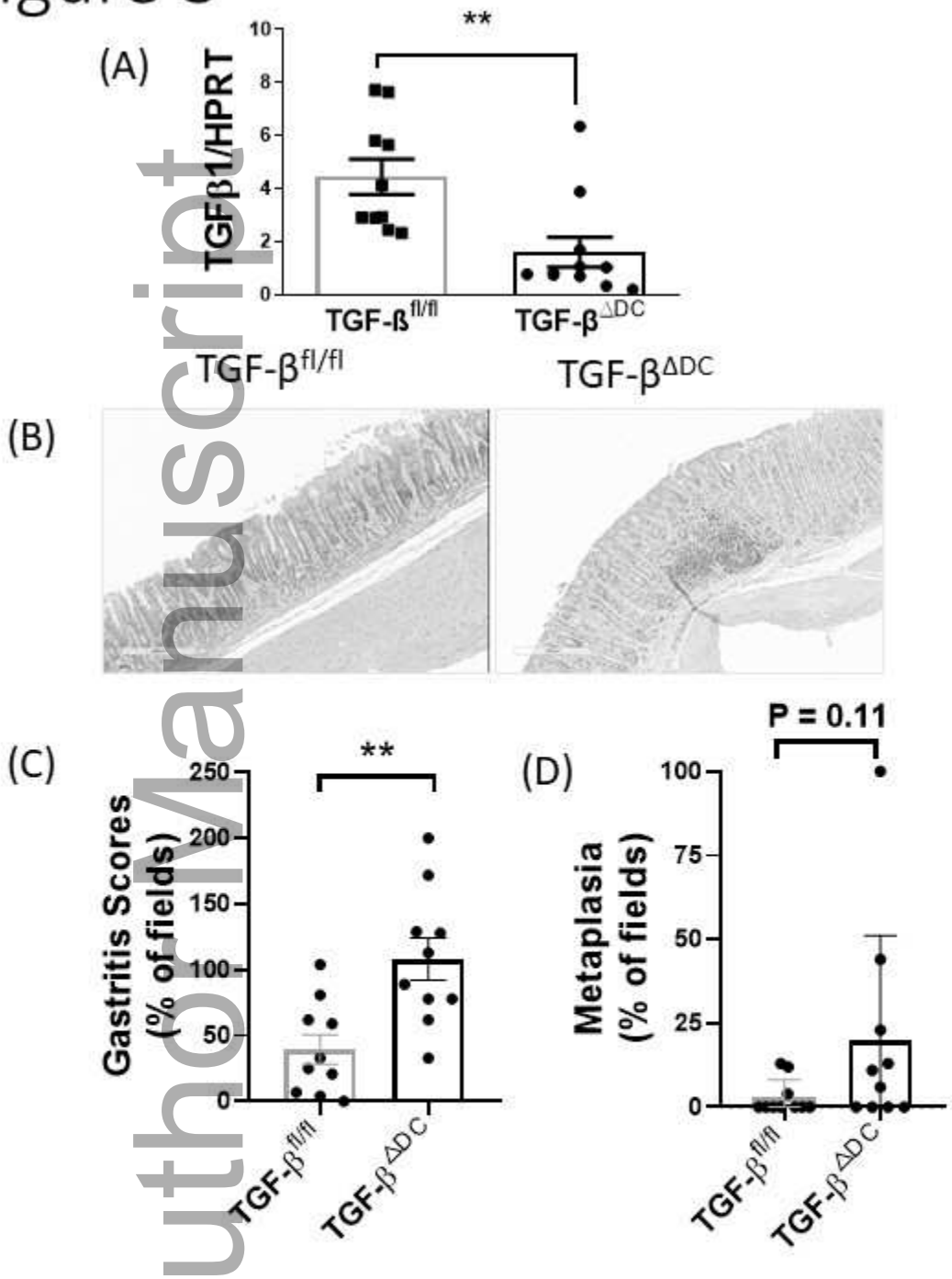

Figure 4

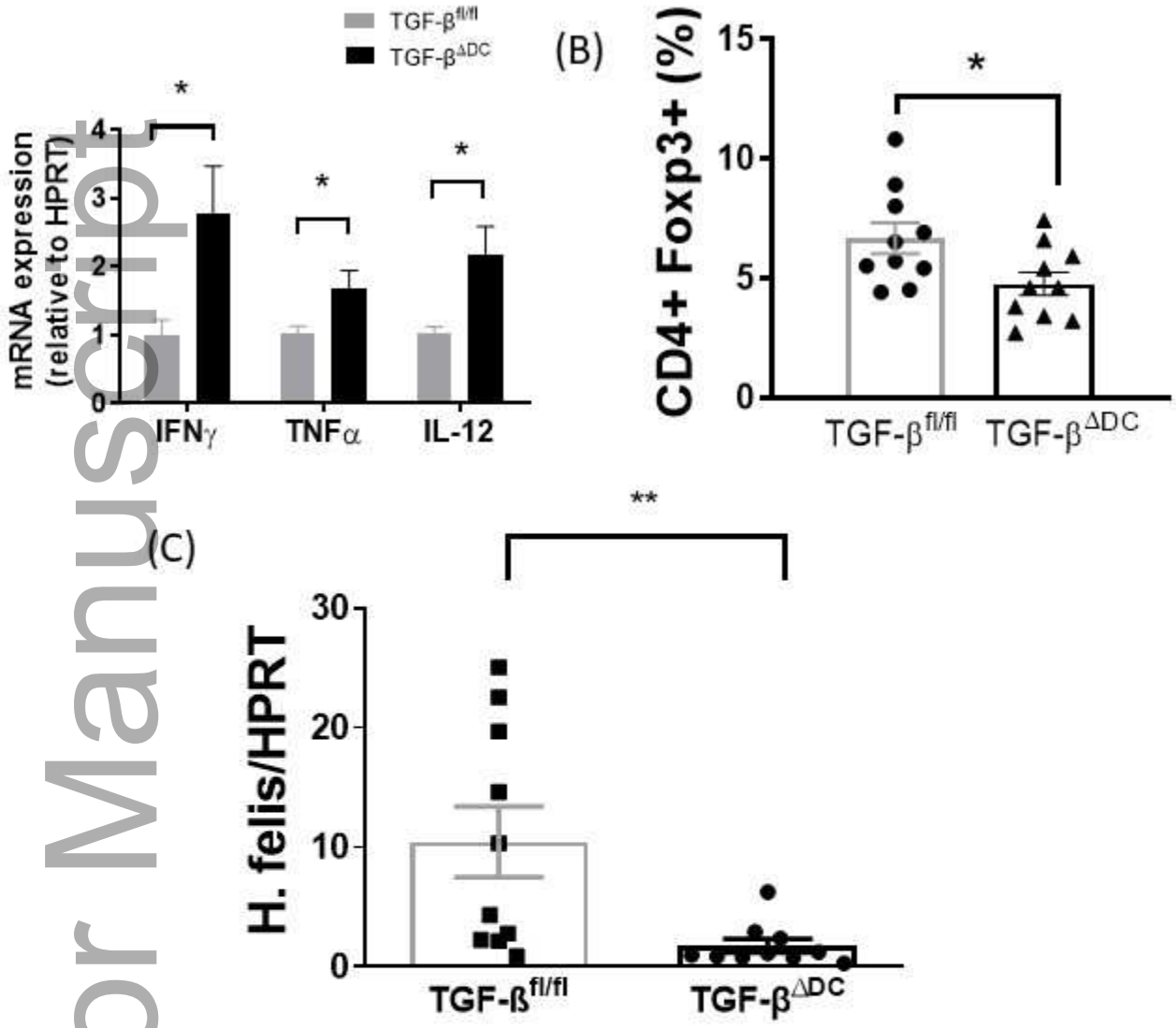

Figure 5