

SUPPLEMENTARY MATERIALS FOR

Inhibition of YTHDF1 by salvianolic acid overcomes gluten induced intestinal inflammation

SUPPLEMENTARY MATERIAL AND METHODS

Human patients and samples

All newly diagnosed adult CD patients had elevated TGA titers and displayed characteristic small intestinal histopathologic abnormalities, including villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis. The samples were obtained after informed consent and using a protocol approved by the Donostia University Hospital. The study was approved by the Basque Country Clinical Research Ethics Board (CEIm-E ref. PI2019133) and analyses were performed after informed consent was obtained from all subjects. All experiments were performed in accordance with relevant guidelines and regulations. Biopsy specimens from the distal duodenum of each patient were obtained during routine diagnosis endoscopy. None of the patients suffered from any other concomitant immunological disease.

***In vitro* experiments**

Intestinal HCT-116 (#91091005) cell line was purchased from Sigma-Aldrich (Poole, UK) and cultured in DMEM media (Lonza, Basel, Switzerland, #12-604F) supplemented with 10 % FBS (Millipore, Burlington, MA, USA #S0115), 100 units/ml penicillin and 100 µg/ml streptomycin (Lonza, #17-602E).

100.000 HCT-116 cells were plated and incubated o/n at 37°C. Next day cells were incubated with a low-dose of 30 µg/mL PT-Gliadin (PTG) or left untreated (NT) and incubated for 48 h. Then treatment with PTG at a final concentration of 350 µg/mL with or without SAC based YTHDF1 inhibitors (50 µM for Y20 and 20 µM for Y22) was performed. After 24 h supernatants and cells were harvested for further RNA and protein analysis.

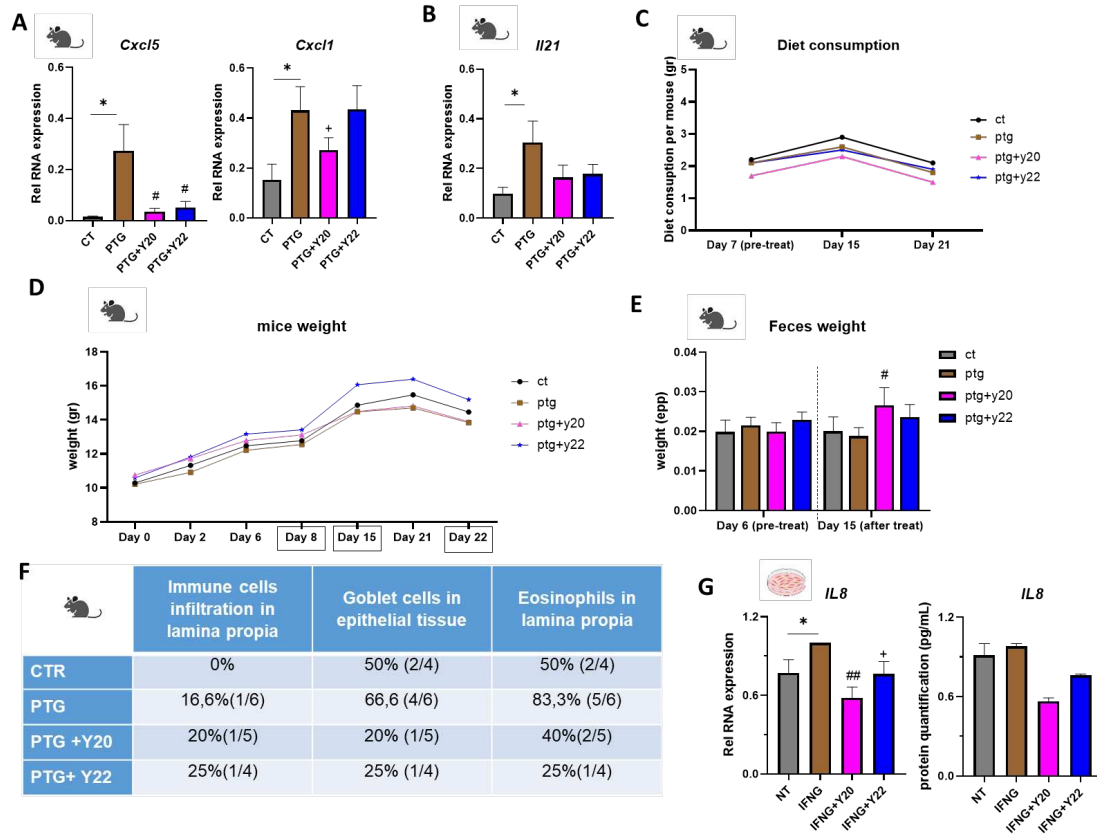
***In vivo* experiments**

Wild Type C57BL/6 JRj (Janvier Labs) mice were used for PTG stimulations. Breeders were maintained on GFD (Altromin #C1074) for at least 4 weeks, and experiments were performed using their progeny. 4 weeks after birth, mice were fasted overnight and then 500 µg PTG only, 500 µg PTG + 72 µM Y20 or 500 µg PTG + 30 µM Y22 was administered by oral gavage together with 25 ng Cholera toxin (CT) (Enzo Life Sciences, #BML-G117-000) to facilitate intestinal permeability. 25 ng CT were used for oral gavage in control mice. Each group of mice was housed in different cages to avoid PTG exposure through feces in control mice. Gavage treatments were done once a week for 3 weeks (F3.E) and mice were sacrificed 3 hours after the last gavage. Duodenum was dissected, perfused with sterile PBS to remove intestinal content and then sectioned and lysed for subsequent RNA and protein extraction.

Organ culture experiments

3 biopsy specimens from the same individual were incubated at 37°C in RPMI media (Gibco, #21875-34) supplemented with 10 % FBS (Millipore, #S0115), 4 mM L-Glutamine (Gibco, #25030-081), 1 mM sodium pyruvate (Lonza, #13-115E), 20 mM HEPES (Lonza, #17-737E), 100 U/mL penicillin/streptomycin (Lonza, #17-602E) and 0.1 U/mL bovine insulin (Sigma-Aldrich, #I0516) alone or with the addition of 50 µM Y20 or 20 µM Y22 inhibitors. After 24h media was collected and biopsies were flash frozen for further RNA and protein expression analysis.

SUPPLEMENTARY FIGURES



Supplementary Figure 1. YTHDF1 inhibitors ameliorate inflammation with no toxicity *in vivo*. **A)** *IL8* murine homologues *Cxcl5* and *Cxcl1* and **B)** *Il21* RNA levels were quantified by RT-qPCR using *Rplp0* as endogenous control. $n \geq 7$ (* $p < 0.05$ compared to control CT mice, according to one-tailed Student's t test; + $p < 0.09$, # $p < 0.05$, compared to PTG mice, according to one-tailed Student's t test). **C-F)** The effect of gluten gavage and YTHDF1 inhibitors in mice was measured. **C)** Diet consumption and **D)** mice weight quantified in different time-points, both before (day 6) and after gavage treatments. $n \geq 7$. Values represent means per group. **E)** Feces weight before first treatment (day 6) and after gavage treatment (day 15). $n \geq 7$ (# $p < 0.05$ compared to PTG after treatment according to two-way ANOVA test). **F)** Summary table of immune cell infiltration in lamina propria, goblet cells in epithelial tissue and eosinophils in lamina propria counts from hematoxylin-eosin staining of small intestinal sections from CT, PTG, PTG+Y20 and PTG+Y22 mice. **G)** HCT-116 intestinal cells were left untreated (NT), treated with interferon gamma (IFNG) or IFNG and the two different YTHDF1 inhibitors (IFNG+Y20 and IFNG+Y22). *IL8* RNA and protein levels were quantified by RT-qPCR using *RPLP0* as endogenous control and ELISA,

respectively. n=2-3 (*p<0.05 compared to control NT, according to one-tailed Student's t test; +p<0.09, ##p<0.01 compared to IFNG according to two-tailed Student's t test).