Supplementary Methods

Mice

Mice used in these studies were on the C57BL/6 background. Mice were maintained under specific pathogen-free conditions at the University of Chicago and at the Sainte-Justine University Hospital Research Centre. DQ8-D^d-villin-IL-15tg mice were recently described.[3] DQ8-D^d-villin-IL-15tg-muMT mice were obtained by crossing DQ8-D^d-villin-IL-15tg mice with muMT mice obtained from Jackson Laboratories (Bar Harbor, ME, USA). All strains were maintained from birth on a gluten-free chow (AIN76A; Envigo). For all experiments, mice were used at 10 weeks of age. All experiments were performed in accordance with the Institutional Biosafety Committee, the Institutional Care and Use Committee of the University of Chicago, the Canadian Council on Animal Care guidelines, and the Institutional Committee for Animal Care in Research of the Sainte-Justine University Hospital Research Centre.

Gluten feeding and B-cell depletion

To study the response to dietary gluten, mice were transferred from a gluten-free diet to a standard rodent chow at the beginning of each experiment and allowed to consume the gluten-containing chow ad libitum. In addition, supplemental gluten (20 mg crude gliadin; Sigma-Aldrich) was administered via intragastric gavage every other day for 30 days, with the use of a 22-gauge round-tipped needle (Cadence Science). To study the response to dietary gluten in the absence of mature B cells, DQ8-D^d-villin-IL-15tg mice were injected intraperitoneally every 2 weeks, starting at 7 days before the introduction of gluten, with 250 μg of a CD20-specific monoclonal antibody (anti-CD20 antibody, clone 5D2, Genentech) or its isotype control (IgG2a; BioXCell), as shown in Supplementary Figure 1*A*.

Histology

Hematoxylin and eosin staining was performed on 5μM thick sections of 10% formalin-fixed paraffin-embedded ileum. The segments of the distal ileum analyzed were consistently taken 0.5 cm from the cecum. Slides were analyzed with the use of a Leica DMi8 microscope with HC PL Fluotar L $\times$20/0.40 and HC PL APO $\times$40/0.75 objectives and equipped with the image processing and analysis software LasX (Leica). All assessments were performed blindly by 2 independent investigators. The villous height/crypt depth ratios were obtained from morphometric measurements of 5 well-oriented villi. The villous height-to-crypt ratio was calculated by dividing the villous height by the corresponding crypt depth. Villous height was measured from the tip to the shoulder of the villous or up to the top of the crypt of Lieberkuhn. The crypt depth was measured as the distance from the top of the crypt of Lieberkuhn to the deepest level of the crypt. If the poor orientation of a section prevented a correct morphometric assessment of the sample, additional tissue sections were cut and analyzed. The intraepithelial lymphocyte count was assessed by counting the number of intraepithelial lymphocytes among at least 100 enterocytes.

Epithelial compartment, lamina propria, spleen, mesenteric lymph nodes, Peyer's patches, and blood cell isolation

Epithelial cells including intraepithelial lymphocytes (IELs) and lamina propria cells were isolated as previously described with the use of EDTA containing calcium-free medium and collagenase VIII, respectively. For the analysis of the natural killer cell (NK) receptors by means of flow cytometry, a cell purification step using a 40% Percoll (GE Healthcare) was used to enrich lymphocyte cell populations as previously described.[3] Spleen, mesenteric lymph nodes, and Peyer's patches were dissected, made into a single-cell suspension by means of mechanical disruption, and passed through a 70-μm nylon cell strainer (Corning). Blood was collected from the submandibular vein into heparinized tubes, and red blood cells were lysed with the use of the lysis buffer solution from BD Biosciences.

Flow cytometry

The following conjugated antibodies were purchased from eBioscience: TCRβ APC (H57-597) CD8α APC-eFluor 780 (53-6.7), CD8βPE-Cy5 (eBio H35-17.2), and CD314 (NKG2D) PE (CX5). The following antibodies were purchased from BD Biosciences: CD4 PE-Cy7 (GK1.5), NKG2A/C/E FITC (20d5), IgA FITC (C10–3), CD16/CD32 (Fc Block) (2.4G2), CD3 V500 (UCHT1), and CD19 BUV661 (1D3). CD45 Pacific Blue (30-F11) was purchased from Biolegend. CD8α APC-eFluor 780 (53-6.70), B220 PE-Cyanine7 (RA3-6B2), and Live/Dead Fixable Violet Dead Cell Stain Kit were purchased from Thermo Fisher Scientific. Flow cytometry was performed with a BD LSRFortessa II cell analyzer (BD Biosciences) and data were analyzed with the use of FlowJo software (Treestar).

Intraepithelial lymphocytes expressing NKG2D were identified as TCR$\alpha\beta^+$ CD8$^+$ NKG2D$^+$ by means of flow cytometry. The quantification of NKG2D-expressing CD8$^+$ IELs per 100 intestinal epithelial cells was determined as previously described.[3]

Anti-gliadin enzyme-linked immunosorbent assay

Serum was harvested 30 days after mice received the first gliadin feeding. High-binding 96-well plates enzyme-linked immunosorbent assay (Nunc; Thermo-Scientific) were coated with 50 μL of 100 μg/ml of pepsin-trypsin–digested gliadin in 100 mmol/L Na_2HPO_4 overnight at 4°C. Plates were washed 3 times with phosphate-buffered saline solution containing 0.05% Tween-20 (PBS-T) and blocked with 200 μL of 2% bovine serum albumin in PBS-T for 2 hours at room temperature. Serum was assessed in duplicate and at 2 dilutions, typically 1:50 and 1:200. Sera were incubated overnight at 4°C, and plates were washed 3 times with PBS-T. Anti–mouse Ig–horseradish peroxidase (HRP; Southern Biotech) in blocking buffer was added to the plates and incubated for 1 hour at room temperature. The

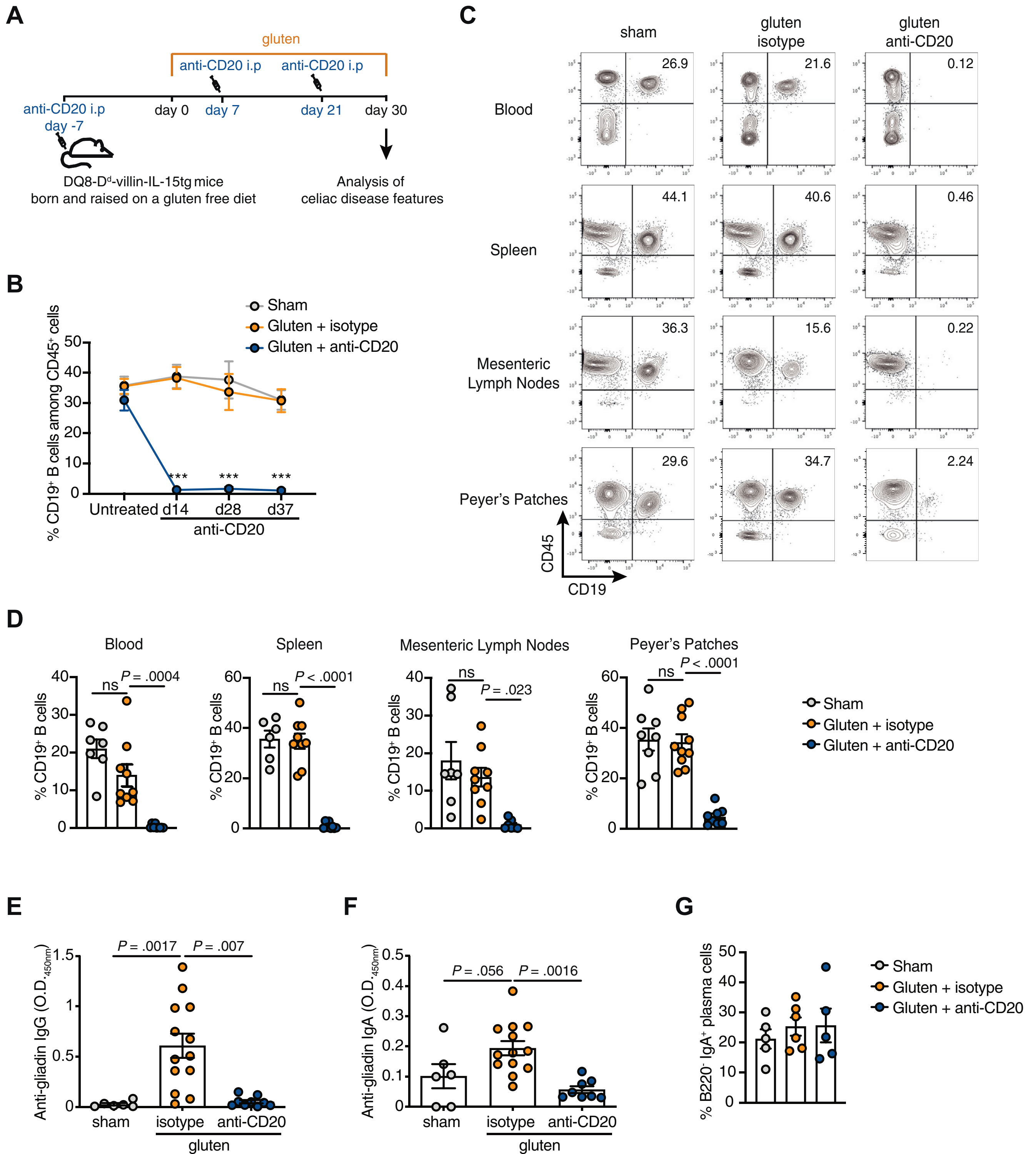
plates were washed 5 times with PBS-T. Fifty μL HRP substrate TMB (Thermo-Scientific) was added and the reaction stopped by the addition of 50 μL 2N H_2SO_4 (Fluka Analytical). Absorbance was read at 450 nm on a Thermo Scientific Multiskan Go microplate reader. Levels of anti-gliadin IgG and IgA were expressed in optical density values.

RNA extraction, cDNA synthesis, and quantitative real-time PCR

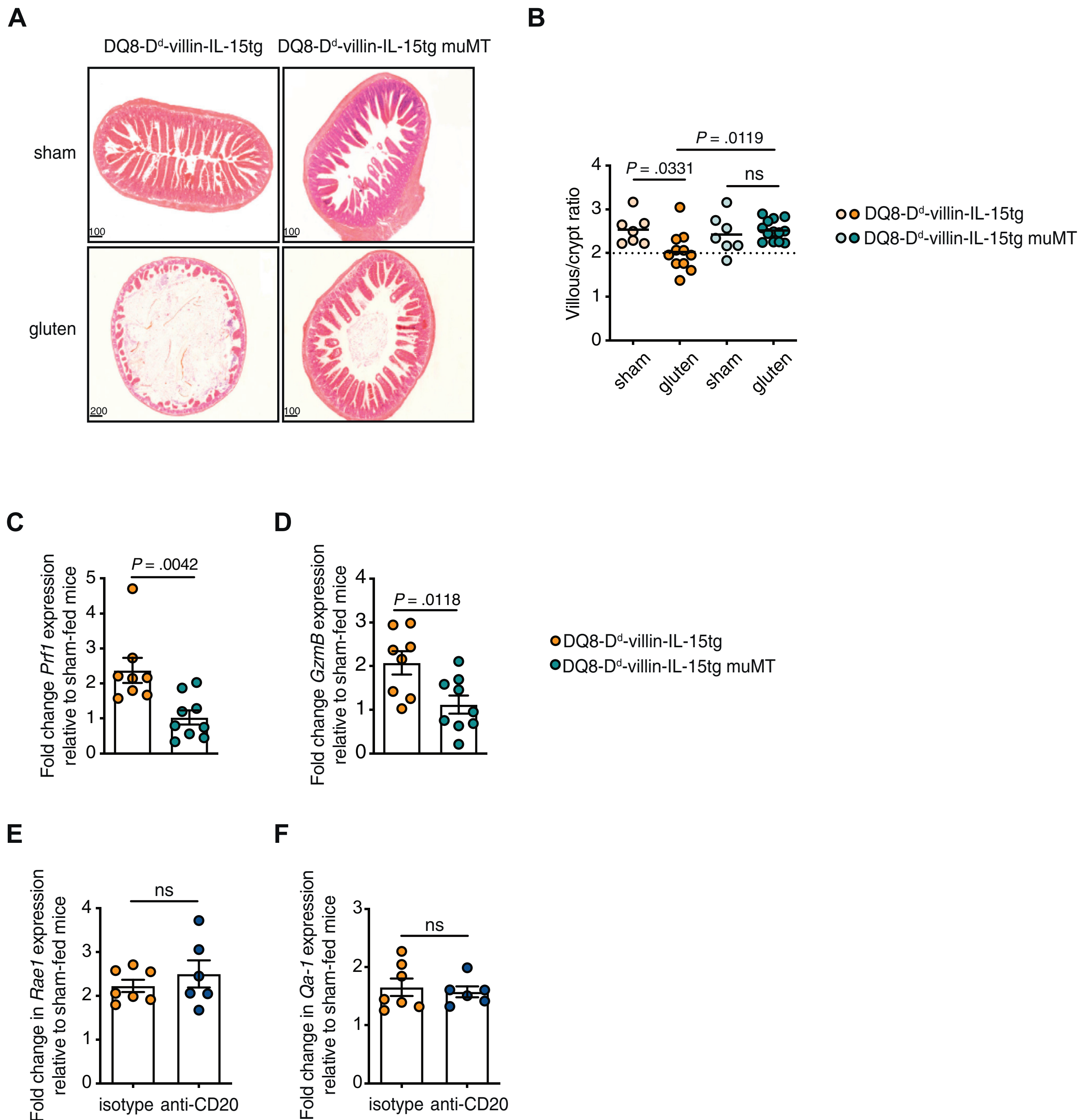
Total RNA isolation was performed on epithelial cells with the use of the RNeasy Mini Kit (Qiagen). RNA concentration and quality were determined by means of ultraviolet spectrophotometry (Epoch Microplate Spectrophotometer; BioTek). cDNA synthesis was performed with the use of qScript cDNA SuperMix (QuantaBio) according to the manufacturer's instructions. Expression analysis for murine *gzm*, *prf1*, *rae1*, and *qa1* was performed with the use of TaqMan gene expression assays and normalized to *gapdh* (Thermo Fisher Scientific). Relative gene expression levels were determined using the deltadelta Ct method to calculate the relative changes in gene expression relative to sham-fed mice.

Statistical analysis

Tests were performed as indicated in the figure legends with the use of GraphPad Prism. Data are presented as mean $\pm$ SEM. The statistical tests used and *P* values are indicated in each figure or figure legend. *P* values $<.05$ were considered to be statistically significant.



Supplementary Figure 1. Anti-CD20 treatment eliminates B cells and anti-gluten antibodies while preserving mucosal plasma cells. (*A*) Experimental scheme. (*B*) Frequency of blood $CD19^+$ $B220^+$ B lymphocytes, demonstrating the efficacy of B-cell depletion. ****P* < .001. (*C*) Representative dot-plots showing depletion efficacy in the blood, spleen, mesenteric lymph nodes, and Peyer's patches. (*D*) Frequency of $CD19^+$ B lymphocytes in the blood, spleen, mesenteric lymph nodes, and Peyer's patches. (*E*, *F*) Serum anti-gliadin IgG (*E*) and IgA (*F*) levels were measured by means of enzyme-linked immunosorbent assay 30 days after gluten feeding. (*G*) Percentage of $B220^-$ IgA^+ plasma cells among $CD45^+$ $CD3^-$ cells found in the lamina propria of the different groups of mice. Results are representative of 3 (*C–F*) or 2 (*G*) independent experiments pooled together and shown as mean ± SEM. Analysis of variance/Tukey multiple comparison.



Supplementary Figure 2. Gluten-fed DQ8-D^{d}-villin-IL-15tg-muMT mice have decreased levels of cytotoxic molecules and intestinal tissue destruction. (*A–D*) DQ8-D^{d}-villin-IL-15tg mice and DQ8-D^{d}-villin-IL-15tg-muMT were maintained on a gluten-free diet (sham), or fed with gluten every other day for 30 days (gluten). (*A*) Hematoxylin-stained ileum sections. (*B*) Villous height-to-crypt depth ratio measured from ileum sections of sham and gluten-fed DQ8-D^{d}-villin-IL-15tg and DQ8-D^{d}-villin-IL-15tg-muMT mice. Four independent experiments are presented as mean $\pm$ SEM. Analysis of variance/Tukey multiple comparison. (*C*) Expression of perforin (*prf1*) in the epithelial compartment was measured by means of quantitative polymerase chain reaction (qPCR). Relative expression levels in gluten-fed mice were normalized to the expression levels observed in sham-fed mice. (*D*) The expression of granzyme B (*gzmb*) was measured as in (*C*). (*E*, *F*) DQ8-D^{d}-villin-IL-15tg mice were maintained on a gluten-free diet (sham), or fed with gluten every other day for 30 days and treated with 250 μg anti-CD20 antibody (gluten + anti-CD20) or its isotype control (gluten + isotype) every 2 weeks: (*E*) expression of retinoic acid early inducible gene 1 (*rae1*) was measured as in (*C*); (*F*) expression of major histocompatibility class Ib molecule *qa-1* was measured as in (*C*). qPCR results are representative of 3 independent experiments, shown as mean $\pm$ SEM. Unpaired Student *t* test.