Supplementary file 1: Protocol details for the immunohistochemistry of CD3, CD4, CD8, c-kit, ITGB7, LPHN2 and MADCAM1.

Immunohistochemistry

CD3, CD4 and CD8

Immunostains on 5 μm-thick sections were done on an automated BOND-MAX system (Leica Microsystems, Mannheim, Germany). Briefly, antigen retrieval was performed by boiling the sections for 20 minutes in citrate-based buffer (BOND Epitope Retrieval Solution 1, pH6; Leica Biosystems, Diegem, Belgium) for CD4 and CD8 detection, or Tris/EDTA (BOND Epitope Retrieval Solution 2, pH9; Leica Biosystems) for CD3 detection. Peroxidase block was then applied for 5 minutes, and the sections were incubated for 30 minutes at room temperature with anti-human CD3 rabbit polyclonal antibody (Dako; ready-to-use), with anti-human CD4 mouse monoclonal antibody (clone 4B12, Dako; ready-to-use), or with anti-human CD8 mouse monoclonal antibody (clone C8/144B). The sections were then incubated for 8 minutes with a post primary rabbit anti-mouse linker followed by incubation for 8 minutes with anti-rabbit horseradish-peroxidase labeled polymer. After incubation for 10 minutes with diaminobenzidine, the slides were counterstained for 5 minutes with hematoxylin (BOND Polymer Refine Detection Kit; Leica Biosystems), dehydrated, cleared and mounted.

C-kit

C-kit staining was performed with the Dako Autostainer Link 48 (Dako). Before staining, sections were dried, deparaffinized and rehydrated followed by epitope retrieval at high pH (Dako PT Link machine, Dako). The automated staining procedure consisted of application of Envision Flex Peroxidase-Blocking Reagent (Dako) for 5 minutes, followed by incubation in first anti-human c-kit rabbit polyclonal antibody (Dako, dilution 1/750) for 20 minutes, and then in a peroxidase-labeled polymer (Envision Flex/HRP; Dako) for 20 minutes, and finally application of the substrate chromogen [Substrate Working Solution (mix), Dako] for 10 minutes. After each step, the sections were rinsed in buffer ((Envision Flex wash buffer, Dako). After the final wash step, the slides were counterstained with haematoxylin, dehydrated, cleared and mounted.

ITGB7, LPHN2 and MADCAM1

Five μm-thick sections were cut from paraffin blocks of fixed endoscopic colonic biopsies. Slides were dried, deparaffinized and rehydrated followed by epitope retrieval at high pH (Dako PT Link machine, Dako Belgium NV, Heverlee, Belgium). Sections were then washed 3 times for 5 minutes (Envision Flex wash buffer) and Envision Flex Peroxidase-Blocking Reagent (Dako) was applied for 10 minutes at room temperature. After a second washing step, slides were incubated with anti-human MADCAM1 mouse monoclonal antibody (clone 17F5; Santa Cruz Biotechnology, Heidelberg, Germany; dilution 1:25) overnight at 4°C, or with anti-human LPHN2 rabbit polyclonal antibody (Atlas Antibodies, Stockholm, Sweden; dilution 1/20) for 1 hour at room temperature, or with anti-human ITGB7 rabbit polyclonal antibody (Atlas Antibodies; dilution 1/1000) for 30 minutes at room temperature. Following a third washing step, slides were incubated with the secondary antibody (Envision Flex/HRP;

Dako) for 30 minutes at room temperature. After a fourth washing step, slides were incubated with the Envision DAB+ Chromogen (Dako) for 10 minutes at room temperature. After rinsing, the slides were counterstained with haematoxylin, dehydrated, cleared and mounted.

For all the stains, negative controls (no application of primary antibodies) were run together with the test samples. Microscopic images were acquired with the Leica Application Suite V4.1.0 software using a Leica DFC290 HD camera (Leica Microsystems Ltd., Heerbrugg, Switzerland) mounted on a Leica DM2000 LED bright field microscope.