Appendix A. Supplementary data

Fig. S1. GR protein absorption test showing the disappearance of immunohistochemical positivity of GR after the absorption by the immunizing peptide. Measure bar = 20 μ m in both.

Supplementary of the methods.

The method of the immunofluorescence (IF) was briefly as follows. After deparaffinization and blocking procedures, heat-mediated antigen retrieval was performed in sodium citrate buffer pH 6. The primary antibody was applied and incubated overnight at 4°C, followed by the incubation with the secondary antibody (anti-goat Texas Red, 1:200; Abcam, ab7123) for 1 hour at RTs. The fluorescence was digitally observed and photographed using a fluorescence microscope system (BZ-X810, KEYENCE, Osaka, Japan).

The in situ hybridization (ISH) assay was carried out briefly as follows. The target probe against human CCKBR (Probe-Hs-CCKBR; 311101, ACD, CA, USA), positive control probe (positive control probe–Hs-PPIB; 313901, ACD, CA, USA), and negative control probe (negative control probe-DapB; 310043, ACD, CA, USA) were applied respectively and incubated at 40°C for 2 hours. Positive and negative control probes were applied on the FFPE sections from a randomly selected colonic carcinoma case, which was resected endoscopically and previously diagnosed at our department. mRNA signals were evaluated by the presence of dot-like cytoplasmic and/or nuclear staining in colon carcinoma cells on the positive control slides or in parietal cells of the oxyntic glands, with confirmation of no signals on the slides of negative control probe.

Supplementary of the results.

Upon the categorization of GR expression patterns in oxyntic gland mucosa, we checked the intensity of immunohistochemical membranous linear positivity of the parietal cells, scoring according to the criteria shown in Figure S2 (GR intensity score 0 to 3). The intensity scores were evaluated as follows (shown in the order of Score 0/1/2/3): 2/0/4/28 in the upper half (mainly the isthmus-neck region) of the oxyntic gland area, and 20/4/4/6 in the lower half (mainly the base region) of the oxyntic gland area. Considering that most of the intensity scores were 2 and 3, and the positivity was unambiguously recognized even in the score 1 regions, every intensity score except 0 was included in the positive expression.

Fig. S2. GR intensity scores. A, Score 0: no membranous linear expression. B, Score1: weak but discernable membranous linear expression (arrows). C, Score2: weak but defined membranous linear expression. D, Score 3: intense and distinctive membranous linear expression. Measure bar = 50 μm in A to D.