Appendix A

Table A1. Detailed IHC protocol for the antibodies used in this study.

Antibody	Clone	Dilution	Antigen retrieval	Incubation time (min)	Incubation tempetaure	Source
CMV	CCH2+DDG9	1:1000	Protease3-8 min + CC1-24 min-97ºC	40 min	37ºC	DAKO
CD4	SP35	RTU	CC1-32 min-95º	24 min	36ºC	Ventana - Roche
CD8	SP57	RTU	CC1-32 min-95º	28 min	37ºC	Ventana - Roche
CD8	L26	RTU	CC1-24 min-95º	24 min	37ºC	Ventana - Roche
CD20	B-A38	RTU	CC1-40 min-95º	32 min	37ºC	Cell Marque
CD138/Syndecan-1	AB54501	1:100	CC1-40 min-95º	36 min	37ºC	Abcam
Foxp3	D13C9	1:200	CC1-64 min-95º	60 min	36ºC	Cell signaling
GATA3	22C3	1:80	CC1-64 min-95º	36 min	37ºC	DAKO
PD-L1	CCH2+DDG9	1:1000	Protease3-8 min + CC1-24 min-97ºC	40 min	37ºC	DAKO

RTU, Ready To Use.

Table A2. Detailed clinico-pathological characteristics of CVID patient cohort.

Patient ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Gender	Female	Male	Female	Female	Male	Male	Female	Female	Male
Age at first CVID symptoms	1	17	13	16	7	2	16	NA	15
Age at CVID diagnosis	22	19	21	18	16	22	30	46	15
Age at IgG replacement therapy	25	25	28	34	29	39	30	46	15
Age at gastric cancer diagnosis	28	43	40	27	45	60	38	62	47
Family history									
Primary immunodeficiency	No	No	No	No	IgA deficiency	No	No	No	No
Autoimmune disorder	Vitiligo	No	SS, ATD	Type 1 DM, ATD	No	No	No	No	No
Malignancy in relatives (years at diagnosis)	Breast (73) Prostate (80)	Colon/rectum (n=2; 80; 75) **Stomach** (n=2; 68; 68)	Breast (51) CNS (57)	Colon/rectum (50) Breast (36)	Breast (69)	RCC (80) Peritoneal carcinomatosis of unknown primary site (60)	Breast (57)	No	**Stomach** (58)
Autoimmune disorders	**Pernicious anaemia** AIP RA-like symptoms	**Pernicious anaemia** Psoriasis	ITP Vasculitis RA-like symptoms	Alopecia RA-like symptoms	**Pernicious anaemia**	No	**Pernicious anaemia**	No	No
Granulomatous disease	Lung/skin granulomas	GLILD	No	GLILD	GLILD	No	No	No	No
Gastrointestinal infection (excluding *H. pylori*)	*C. jejuni* *Salmonella spp.* CMV	*C. jejuni* *Salmonella spp.* *Giardia lamblia* CMV	*C. jejuni* *Salmonella spp.* *Giardia lamblia*	EBV Norovirus CMV	*Giardia lamblia*	*C. jejuni* *Giardia lamblia*	*Giardia lamblia*	*Giardia lamblia*	*C. jejuni* *Giardia lamblia*

***H. pylori* infection**	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative
Prolonged use of PPI	Yes	Yes	Yes	NA	Yes	No	Yes	Yes	NA
Histopathological features before GC diagnosis	Chronic gastritis LGD and HGD	Chronic gastritis IM LGD adenomas	Chronic gastritis Hyperplastic polyp	Chronic gastritis	Chronic gastritis IM	NA	Chronic gastritis IM	Chronic gastritis IM	NA
Surgery	Total gastr.	Total gastr.	Total gastr.	Total gastr.	No (unresectable disease)	No (unresectable disease)	Total gastr.	Distal gastr.	Total gastr.
Chemotherapy	No	No	No	No	No	Palliative	No	No	No
Other malignancy (years at diagnosis)	No	Rectal adenocarcinoma (49)	No	No	HCC (45)	No	No	No	No
Histopathological findings in non-neoplastic mucosa (n=7/9)									
Atrophic chronic gastritis	Present	Present	Present	Present	Present	NA	NA	Present	Present
Lymphocytic gastritis	Exuberant	Present	Present	Exuberant	Present	NA	NA	Present	Present
Lymphoid follicles	Absent	Absent	Absent	Present	Present	NA	NA	Present	Present
Neutrophilic activity	Absent	Absent	Absent	Present	Absent	NA	NA	Absent	Present
Intestinal metaplasia	Absent	Extensive	Extensive	Present	Present	NA	NA	Present	Extensive
EBV infection (EBER-ISH)	Absent	Absent	Absent	Absent	Rare + lymphocytes	NA	NA	Absent	Absent
CMV infection (IHC)	Absent	Absent	Absent	Absent	Absent	NA	NA	Absent	Absent

Histopathological features of gastric cancer									
Location	Antrum	Antrum	Corpus	Antrum	Antrum	Corpus	Corpus	Antrum	Corpus
pT stage	pT1a	pT1a	pT2	pT1a	NA	NA	pT1a	pT1b	pT1b
Greatest dimension	4.5 cm	0.5 cm	1.5 cm	1.8 cm	NA	NA	1.0 cm	2.5 cm	0.6 cm
pN stage	pN0	pN0	pN0	pN0	NA	NA	pN0	pN0	pN0
Laurén classification	Intestinal	Diffuse	Intestinal	Indeterminate	Intestinal	Intestinal	Intestinal	Indeterminate	Intestinal
WHO classification	Tub/Pap	Poorly cohesive	Tub/Pap	Mucinous	Tub/Pap	Tub/Pap	Tub/Pap	Mucinous	Tub/Pap
Grading	Low grade	NA	High grade	NA	High grade	High grade	Low grade	NA	Low grade
Precursor lesion	Intestinal adenoma LGD	Absent	Absent	Absent	NA	NA	Absent	Intestinal adenoma LGD	Absent
EBV infection (EBER-ISH)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
CMV infection (IHC)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

AIP, Autoimmune pancreatitis; **ATD**, Autoimmune Thyroid Disease; ***C. jejuni***, *Campylobacter jejuni*; **CMV**, Cytomegalovirus; **CNS**, Central Nervous System; **DM**, Diabetes Mellitus; **EBER-ISH**, EBV Enconded RNA *In Situ* Hybridization; **EBV**, Epstein-Barr Virus; **Gastr**, Gastrectomy; **GC**, Gastric Cancer; **GLILD**, Granulomatous Lymphocytic Interstitial Lung Disease; ***H. pilory***, Helicobacer pylori; **HCC**, Hepatocelular Carcinoma; **HGD**, High Grade Dysplasia; **IHC**, Immunohistochemistry; **IM**, Intestinal Metaplasia; **ITP**, Immune Thrombocytopenia; **LGD**, Low Grade Dysplasia; **NA**, Not Available; **PPI**, Proton-pump Inhibitor; **RA**, Rheumatoid Arthritis; **RCC**, Renal Cell Carcinoma; **RLH**, Reactive Lymphoid Hyperplasia; **SS**, Sjogren's syndrome; **Tub/pap**, Tubular/Papillary.

Table A3. Detailed results of the immune cell counting (Foxp3, GATA3, CD4, CD8, CD20) in non-neoplastic mucosa (distant from and adjacent to tumour) and adenocarcinomas of CVID patients in comparison with non-CVID patients infected by *Helicobacter pylori*.

Immune cell biomarker	**CVID patients** *H. Pylori* **positive** **n=8**	**Non-CVID patients** *H. Pylori* **positive** **n=6**	*p value*
1. Non-neoplastic mucosa distant from gastric cancer (intraepithelial)			
	n=6/8 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	n=6/6 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	
Foxp3	3.0 (3.0-16.0)	0.0 (0.0-1.0)	**0.015***
GATA3	32.0 (2.0-38.0)	1.5 (0.0-5.0)	**0.015***
CD4	5.5 (2.0-9.0)	11.0 (2.0-34.0)	0.818
CD8	77.0 (40.0-374.0)	36.5 (11.0-52.0)	0.132
CD20	0.0 (0.0-0.0)	6.0 (0.0-21.0)	**0.015***
2. Non-neoplastic mucosa distant from gastric cancer (lamina propria)			
	n=6/8 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	n=6/6 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	
Foxp3	68.05 (24.0-181.0)	7.0 (3.0-11.5)	**0.004***
GATA3	181.5 (18.4-570.0)	11.5 (3.0-25.0)	**0.004***
CD4	159.5 (116.0-509.0)	67.0 (2.2-101.0)	**0.004***
CD8	161.5 (84.0-186.0)	36.5 (24.0-43.0)	**0.002***
CD20	10.5 (7.5-89.0)	23.0 (14.3-5.1)	0.589
CD138	2.0 (0.0-40.0)	110.0 (61.0-141.0)	**0.002***
3. Non-neoplastic mucosa adjacent to gastric cancer (intraepithelial)			
	n=6/8 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	n=6/6 *(95% CI)* *Median value* *(No. positive cells per 20x PF)*	
Foxp3	1.0 (0.0-1.0)	0.0 (0.0-1.0)	0.310

GATA3	8.0 (0.0-21.0)	1.5 (0.0-7.0)	0.180
CD4	11.5 (3.5-37.0)	8.0 (2.1-33.3)	0.699
CD8	32.0 (21.0-189.0)	13.5 (11.0-38.5)	0.310
CD20	0.0 (0.0-0.0)	4.5 (2.0-9.4)	**0.002***
4. Non-neoplastic mucosa adjacent to gastric cancer (lamina propria)			
	n=6/8 *Median value (95% CI)* *(No. positive cells per 20x PF)*	n=6/6 *Median value (95% CI)* *(No. positive cells per 20x PF)*	
Foxp3	48.0 (18.0-109.0)	5.5 (2.1-10.0)	**0.002***
GATA3	36.0 (27.0-145.0)	12.0 (2.0-29.6	**0.065**
CD4	75.5 (60.0-409.0)	51.0 (3.0-97.1)	**0.004***
CD8	132.05 (128.0-494.0)	21.5 (13.2-40.0)	**0.002***
CD20	3.0 (3.0-17.0)	30.0 (8.3-37.8)	**0.026***
CD138	0.0 (0.0-0.0)	57.0 31.8-130.3)	**0.002***
5. Gastric cancer (intraepithelial and stromal compartments)			
	n=8/8 *Median value (95% CI)* *(No. positive cells per 20x PF)*	n=6/6 *Median value (95% CI)* *(No. positive cells per 20x PF)*	
Foxp3	66.5 (54.0-75.0)	8.0 (6.0-16.0)	**0.001***
GATA3	74.5 (53.0-65.0)	26.0 (3.0-28.0)	**0.008***
CD4	291.5 (222.0-372.0)	41.5 (32.1-101.0)	**0.003***
CD8	115.0 (45.0-303.0)	34.5 (24.2-48.7)	**0.005***
CD20	8.5 (1.0-11.0)	22.5 (15.1-51.2)	**0.029**
CD138	2.0 (2.0-26.0)	16.5 8.5-52.0)	**0.108**

Statistically significant results are highlighted by an asterisk (*). Differences in comparison with the analyses performed in the totality of CVID and non-CVID patients (Table 2) are highlighted in red.

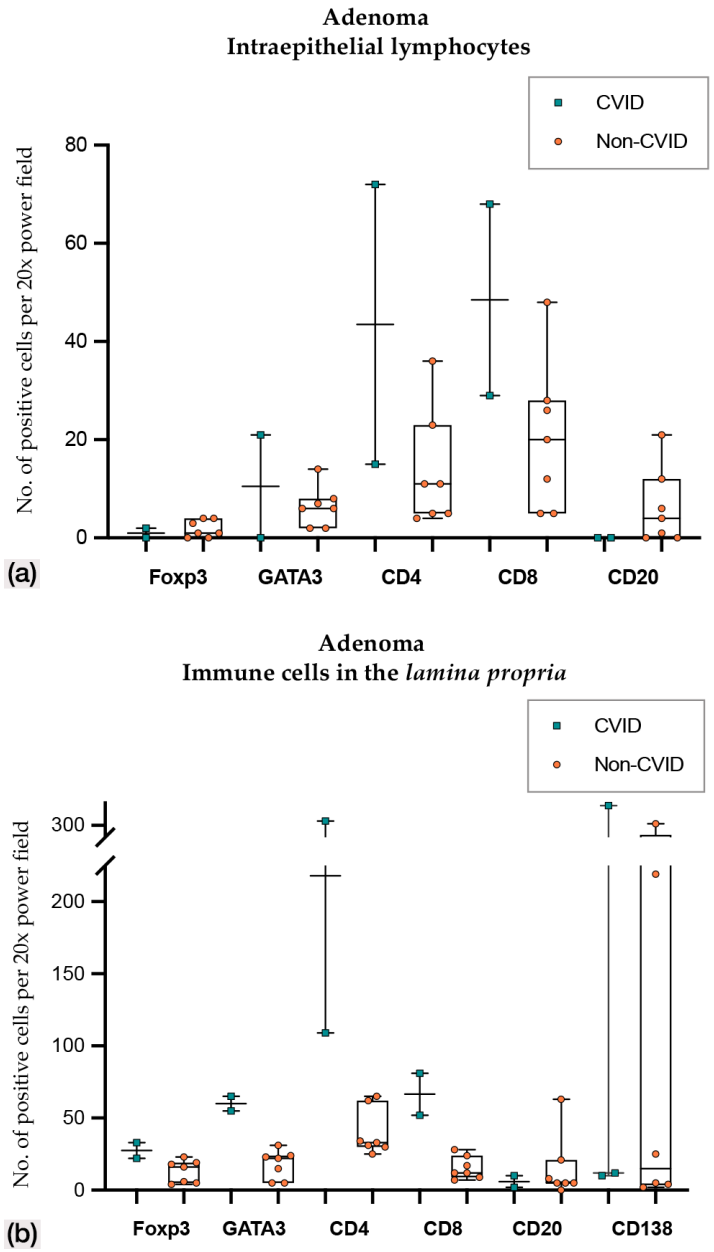

Figure A1. Immune cell counting in epithelial and lamina propria compartments in adenomatous lesions from CVID and non-CVID patients. No statistically significant differences were found when CVID and non-CVID patients were compared.