

Supplementary Data for Gastric Microbiome Alterations Are Associated with Decreased CD8⁺ Tissue-resident Memory T Cells in the Tumor Microenvironment of Gastric Cancer

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Figure S7. Differentially expressed molecules and KEGG pathways between the CD103⁺ (TRM) and CD103⁻ T cell populations.

Figure S8. 5 representative stomach images from sacrificed mice in control and *Methylobacterium* group.

Table S1. Metadata of study subjects.

Table S2. Reagent or Resource.

Table S3. Primer information of RT-PCR.

Table S4. P value and LDA value of gastric cancer and chronic gastritis.

Table S5. Number of tumors in mice stomach.

Supplementary Figure S1

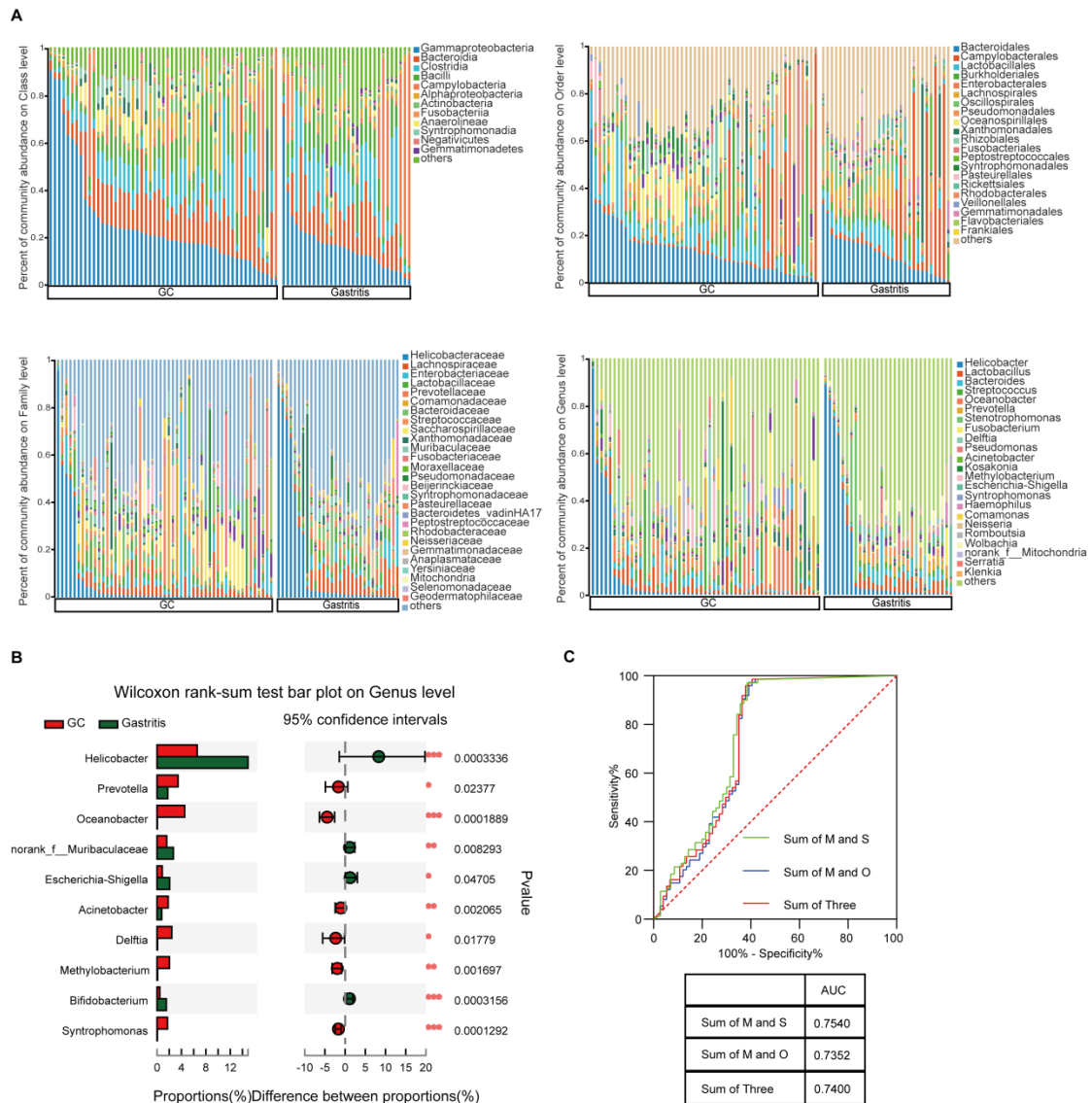


Figure S1. Differences in gastric microbial composition between GC and chronic gastritis. (A) Bar plots of the class, order, family and genus taxonomic levels in gastric cancer and chronic gastritis cohorts of patients. (B) Relative abundance of the top 10 genera differentially enriched in the two clinical settings across gastric cancer and chronic gastritis cohorts. *Significance obtained by wilcoxon rank-sum test and LEfSe analysis at $P < 0.05$. (C) ROC analysis of Taxa relative abundance as predictive of GC status (The genus level of *Methylobacterium*, *Syntrophomonas* and *Oceanobacter*).

Supplementary Figure S2

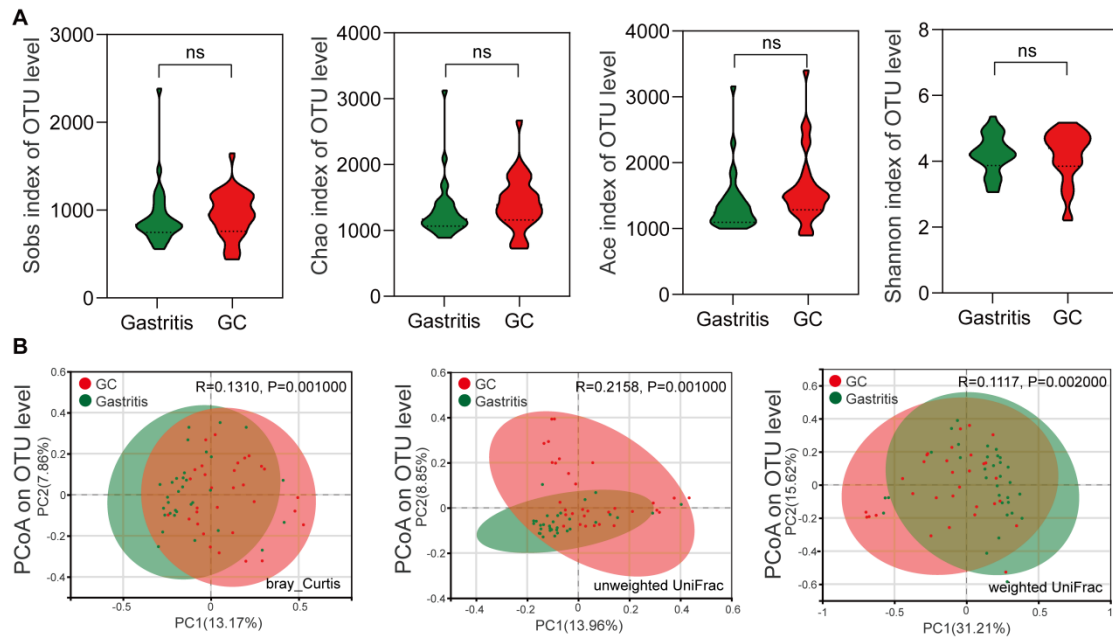


Figure S2. The fecal microbiota diversity of patients with GC and chronic gastritis. 16S rRNA gene sequencing data from the stool samples of 31 patients with GC and 32 patients with chronic gastritis were analyzed. (A) Sobs, Chao, Ace and Shannon index of alpha diversity in stool samples. (B) PCoA using bray_Curtis, unweighted UniFrac and weighted UniFrac distances of beta diversity. The percentage of diversity captured by each coordinate is shown. PCoA, Principal coordinate analysis.

Supplementary Figure S3

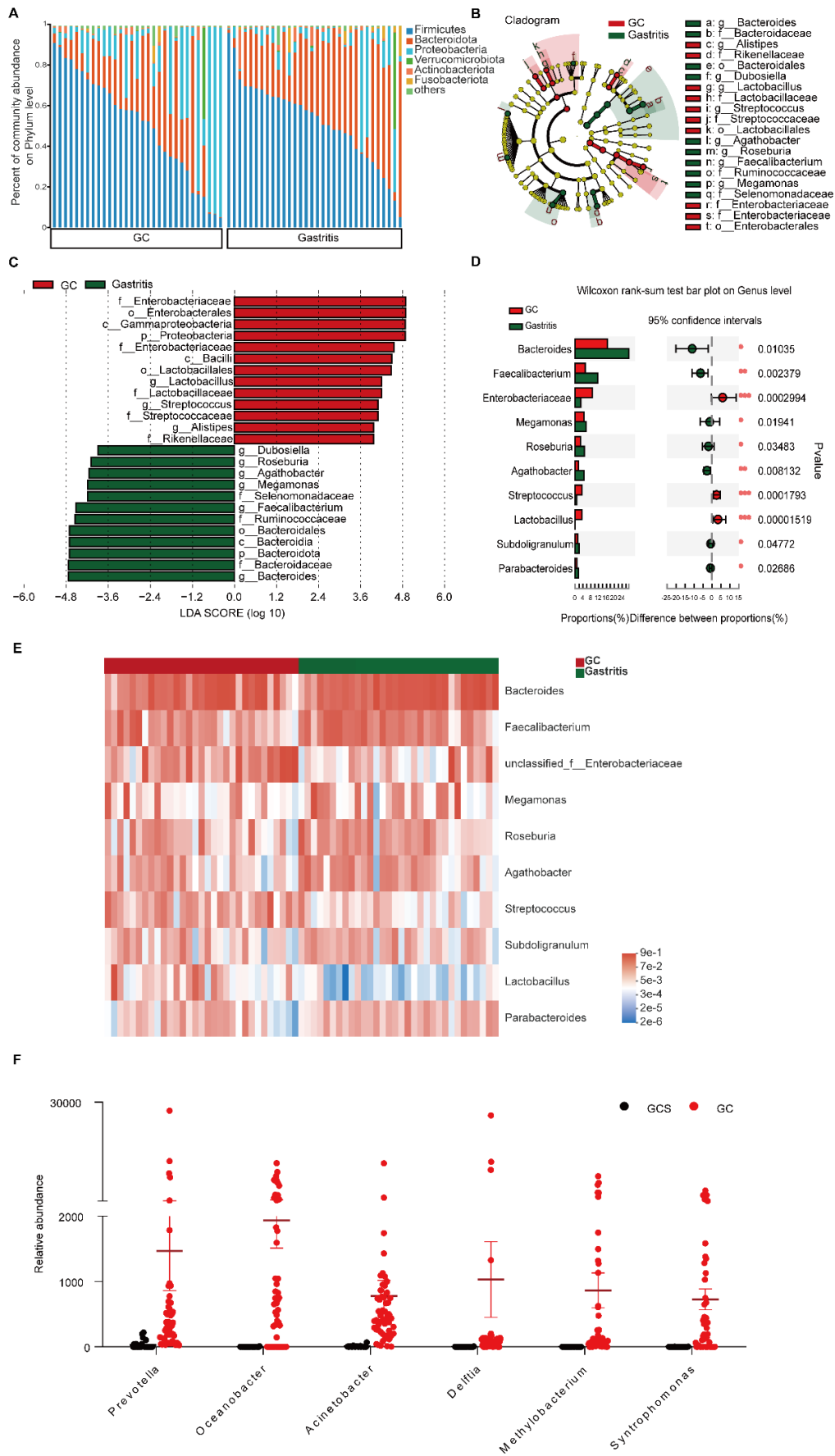


Figure S3. Fecal microbial taxa associated with gastric cancer. (A) Bar plots of the phylum taxonomic levels in gastric cancer and chronic gastritis cohorts of patients. Relative abundance is plotted for each sample. (B) Cladogram representation of the fecal microbiota taxa associated with gastric cancer and chronic gastritis. (C) Association of specific microbiota taxa with the group of gastric cancer and chronic gastritis by linear discriminant analysis (LDA) effect size (LEfSe). (D) Relative abundance of the top 10 genera differentially enriched in the two clinical settings across gastric cancer and chronic gastritis cohorts. *Significance obtained by wilcoxon rank-sum test and LEfSe analysis at $P < 0.05$. (E) Heatmap of selected most differentially abundant features at the genus level. Highlighting three taxa enriched in GC. The blue color represents less abundant, white color represents intermediate abundance and red represents the most abundant. (F) Of the top 10 differential genera between tissues from GC and chronic gastritis patients, we found 6 genera were enriched in GC tissues. The relative abundance of the 6 genera in GC stools and GC tissues were compared. GCS indicates stools from GC patients; GC indicates tumor tissue from GC patients.

Supplementary Figure S4

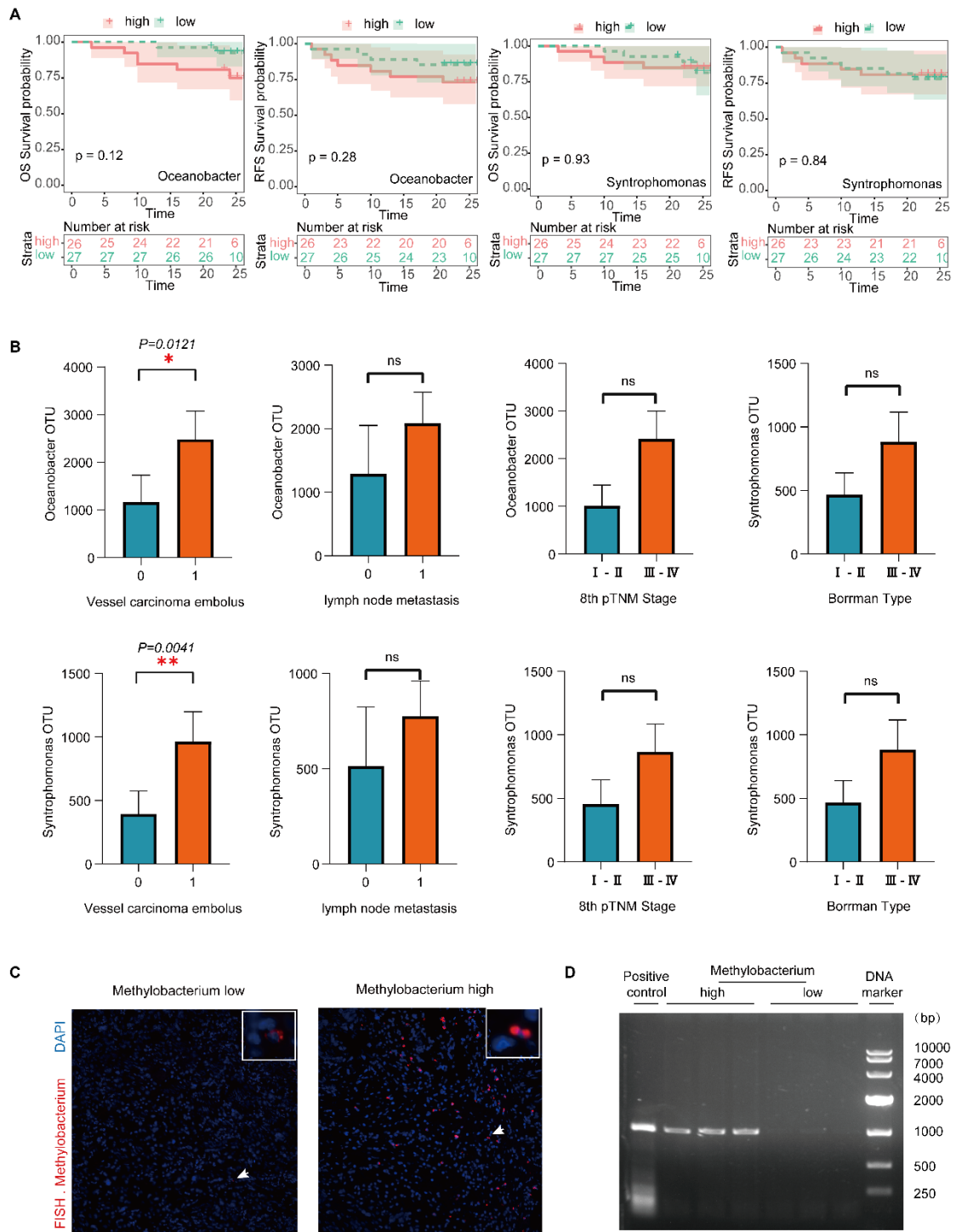


Figure S4. Analysis of correlation between gastric microbiota and clinical features in patients with GC. (A) Clinical gastric cancer patients for survival probability based on the abundance levels of *Syntrophomonas* and *Oceanobacter* enriched at Genus level in GC. Left plot, OS survival probability; and right plot, RFS survival probability. (B) Correlation analysis of

Syntrophomonas and *Oceanobacter* and clinical characteristics (lymph node metastasis, vessel carcinoma embolus, borrmann type and 8th pTNM stage) of patients. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (C) Immunofluorescence with a specific FISH probe for *Methylobacterium* in GC Tissue. Sections were stained with FISH probe (red) and DAPI (blue). (D) PCR detection of *Methylobacterium* in GC tissues with high or low abundance of *Methylobacterium*.

Supplementary Figure S5

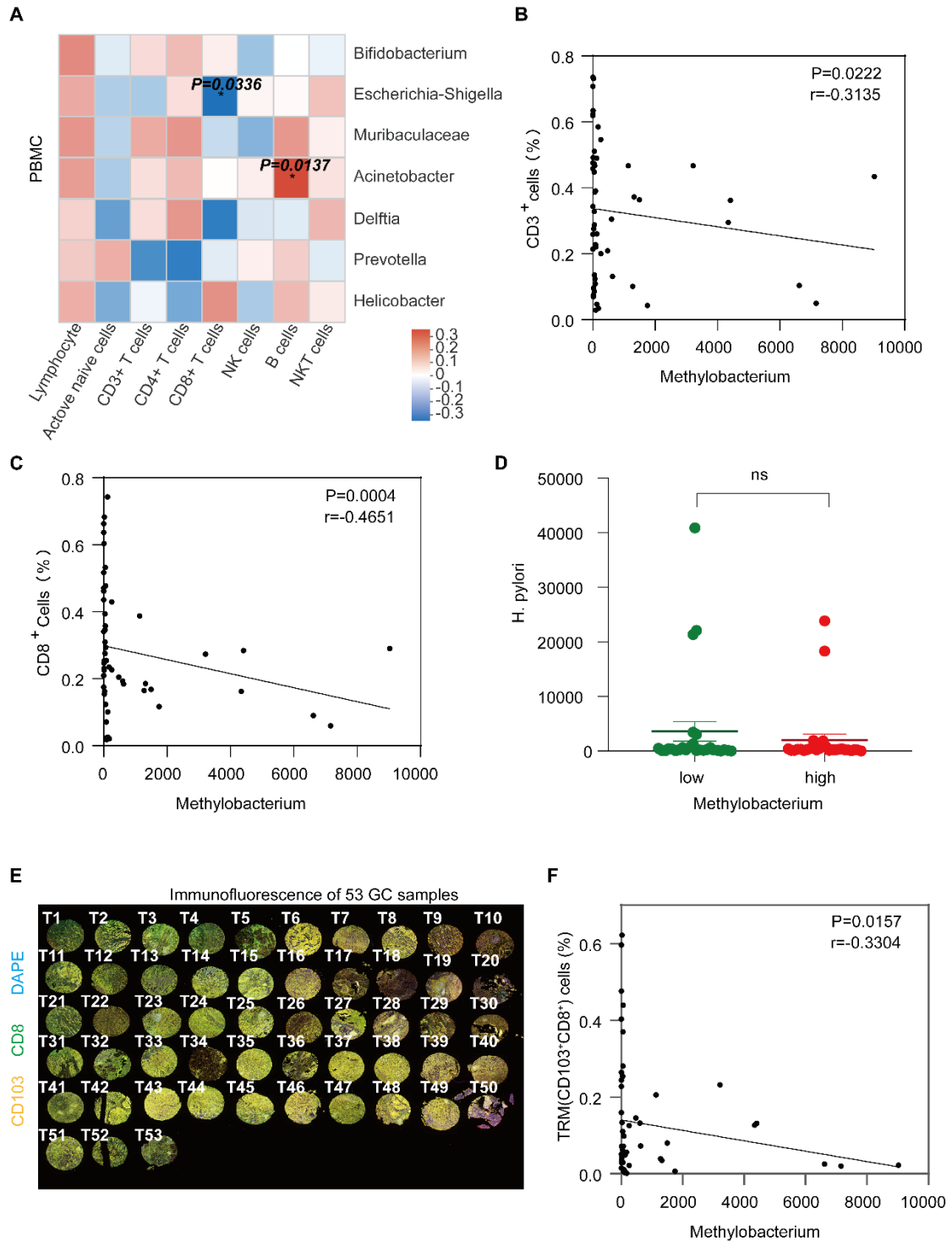


Figure S5. *Methylobacterium* correlates with T cell subsets in tissues from GC patients. (A) Correlation heatmap between other seven GC enrichment genus and immunocytes of PBMCs. (B-C) Linear relationship between CD3 and CD8 cells and *Methylobacterium* in tumor tissues. (D) *H. pylori* abundance in the *Methylobacterium*^{high} group and *Methylobacterium*^{low} group. (E) Immunofluorescence imaging of 53 cases of tumor tissue. (F) Linear relationship between TRM cells and *Methylobacterium* in tumor tissues.

Supplementary Figure S6

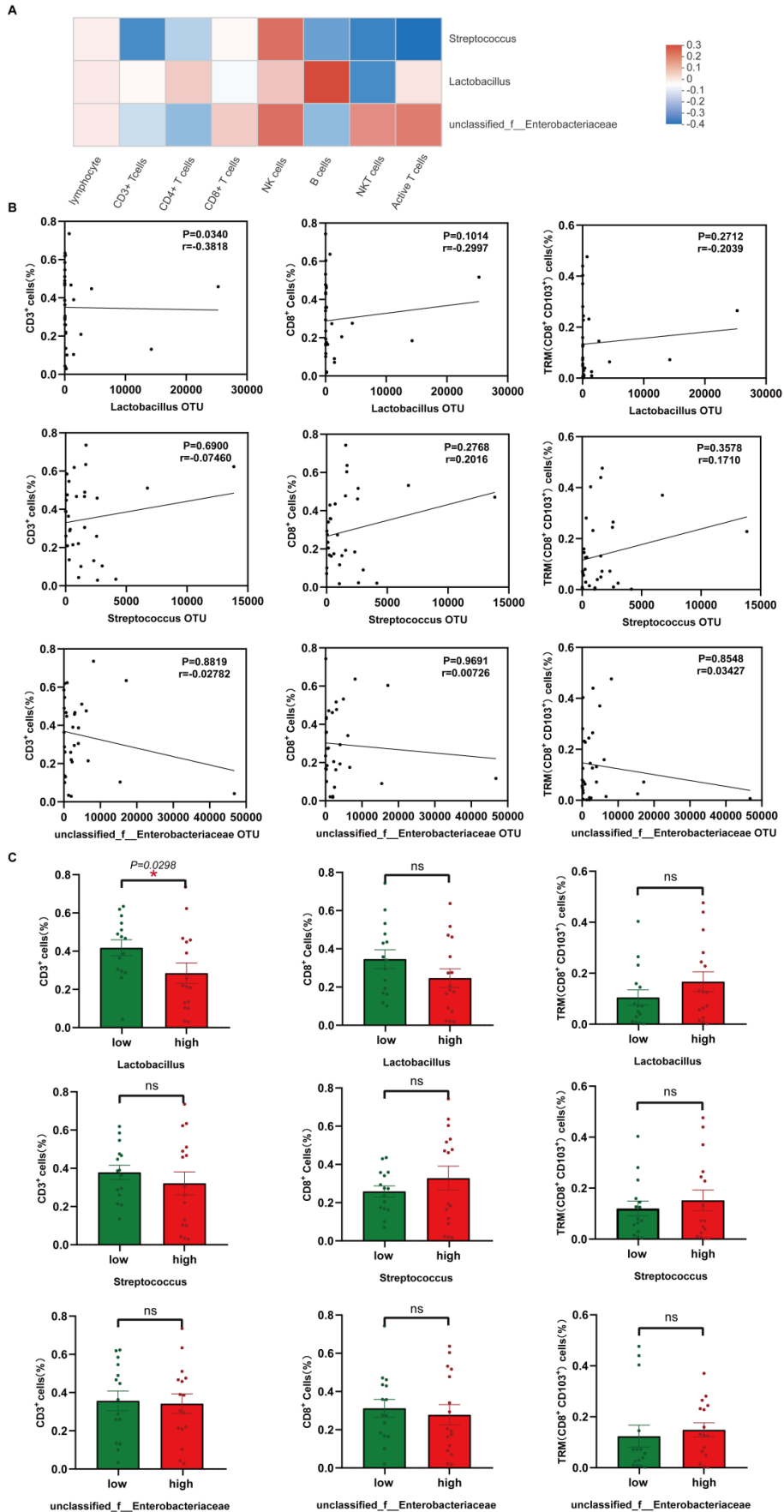
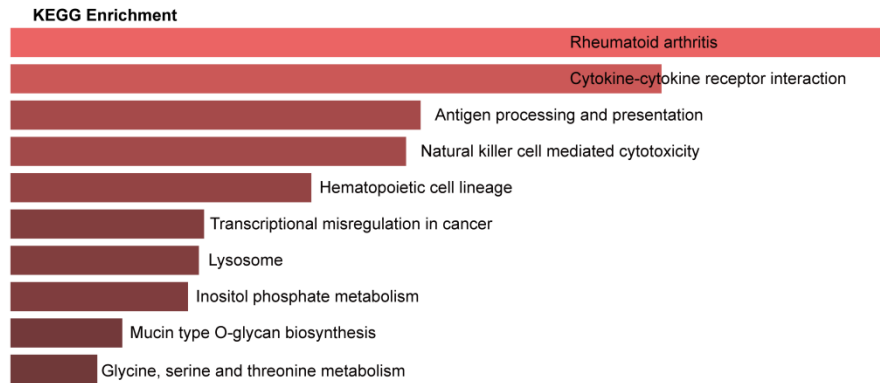


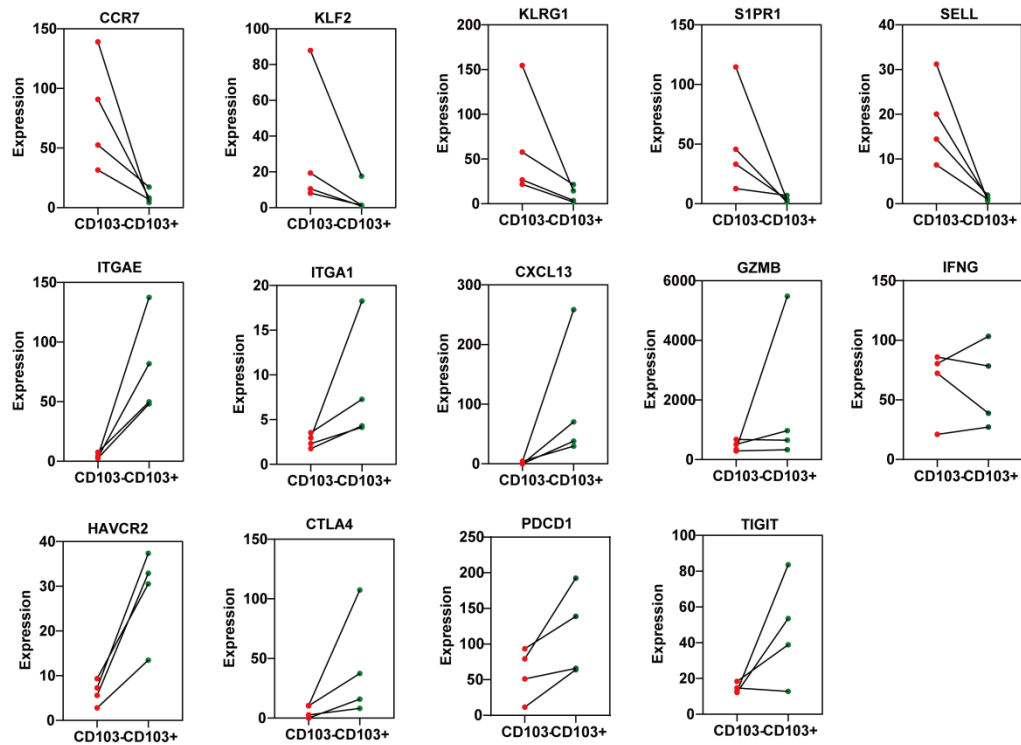
Figure S6. Relationship between fecal microorganisms and tumor infiltrating TRM cells. (A) Correlation heatmap between three Fecal enrichment genus and immunocytes of PBMCs. (B) Linear relationship between CD3⁺ T, CD8⁺ T and TRM cells and 3 different bacteria in GC fecal. (C) Expression of CD3⁺T, CD8⁺ T and TRM cells in high or low cohorts of three fecal enrichment genus. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Figure S7

A



B



C

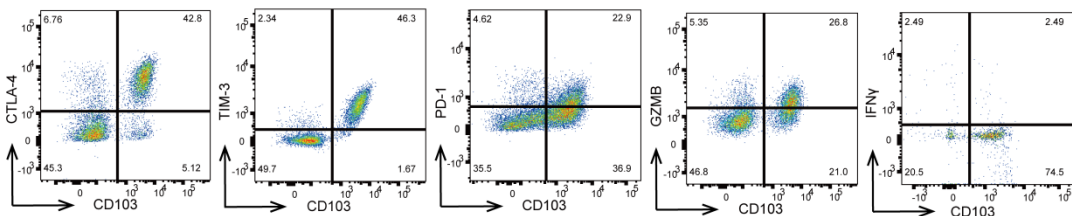


Figure S7. Differentially expressed molecules and KEGG pathways between the CD103⁺ (TRM) and CD103⁻ T cell populations. (A) KEGG enrichment analysis of up- and downregulated genes uniquely altered in tumor TRM cells. (B) Statistical chart of characteristic gene expression related to TRM cells (RNA seq). (C) Flow cytometry scatter diagram of cytokines in Tim-3, CTLA4, PD-1, GZMB and IFN- γ .

Supplementary Figure S8

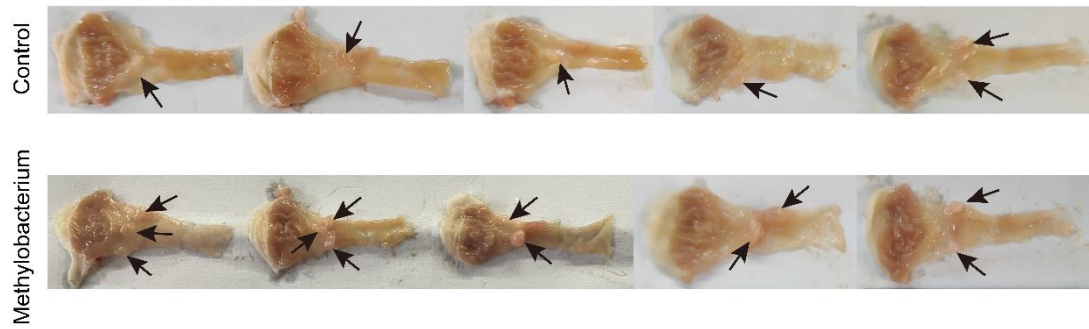


Figure S8. 5 representative stomach images from sacrificed mice in control and *Methylobacterium* group. Black arrows indicated the tumors. Mice in *Methylobacterium* group showed more stomach tumors and larger tumor size compared with mice in control group.

Supplementary Table S1. Metadata of study subjects for 16S rRNA sequencing

53 cases of gastric cancer for tissue samples

Number	Gender	Age	Tumor size	T	N	M	TNM	Lauren type	Vessel carcinoma embolus	Nerve invasion	Stool samples
GC_1	F	56	6	4a	1	1	IV	1	+	-	N
GC_2	F	64	5	3	0	0	II	1	-	-	N
GC_3	M	65	10	3	0	0	II	3	-	-	N
GC_4	M	65	4	4a	0	0	II	2	-	-	N
GC_5	M	66	4	2	2	0	II	2	-	-	N
GC_6	F	69	6	2	2	0	II	2	+	-	Y
GC_7	F	68	10	4a	3b	1	IV	3	+	+	Y
GC_8	M	55	6	3	3a	0	III	3	-	+	Y
GC_9	M	51	4	3	1	0	II	1	+	+	N
GC_10	M	65	6	1b	0	0	I	3	-	-	Y
GC_11	M	62	4	4a	0	0	II	1	+	+	Y
GC_12	M	74	5	4a	0	0	II	2	-	-	Y
GC_13	M	69	5	4a	2	0	III	3	+	+	Y
GC_14	F	80	10	3	3b	0	III	3	+	+	N
GC_15	M	74	5	4a	2	0	III	3	-	-	N
GC_16	M	65	5	2	1	0	II	2	+	-	Y
GC_17	M	55	4	1b	0	0	I	2	-	-	Y
GC_18	F	56	6	4a	2	0	III	2	+	+	N
GC_19	M	71	5	4a	3a	0	III	2	+	+	Y
GC_20	M	62	4	4a	0	0	II	1	+	+	Y
GC_21	M	73	6	4a	1	0	III	1	+	+	Y
GC_22	F	79	10	4a	3b	0	III	2	+	+	Y
GC_23	M	49	2	4a	1	0	III	2	-	+	Y
GC_24	M	30	6	4a	1	0	III	2	-	+	Y
GC_25	M	55	2.5	4a	3a	0	III	3	+	-	Y
GC_26	M	62	8	4a	2	0	III	2	+	-	N
GC_27	M	67	3	4a	2	0	III	3	-	-	Y
GC_28	F	59	6	3	1	0	II	2	-	+	Y
GC_29	M	64	8	4a	2	0	III	3	+	+	Y
GC_30	F	49	6	4a	3a	0	III	2	+	-	Y
GC_31	M	53	6	3	3a	0	III	1	+	+	Y
GC_32	M	69	2	1b	0	0	I	1	+	-	Y
GC_33	M	60	5	4a	3a	0	III	1	+	+	N
GC_34	M	66	10	4a	3a	0	III	3	+	-	Y
GC_35	M	74	5	4a	3a	0	III	3	-	+	Y
GC_36	M	62	6	4a	1	0	III	3	-	-	N

GC_37	F	44	6	4a	2	0	III	2	-	-	N
GC_38	M	57	6	3	3a	0	III	1	+	+	N
GC_39	F	69	10	3	3b	0	III	3	+	+	N
GC_40	M	62	6	3	1	0	II	2	+	-	N
GC_41	F	50	6	4a	3a	0	III	2	+	+	Y
GC_42	M	63	6	3	2	0	III	3	+	+	N
GC_43	M	62	4	4a	0	0	II	3	-	-	N
GC_44	M	70	6	4a	3a	0	III	3	-	-	Y
GC_45	M	74	8	4a	3a	0	III	3	-	-	Y
GC_46	F	53	4	3	2	0	III	1	+	-	Y
GC_47	M	53	4	3	1	0	II	1	-	-	Y
GC_48	F	63	5	3	1	0	II	2	-	-	N
GC_49	M	63	5	4a	1	0	III	1	-	+	Y
GC_50	M	53	5	4a	2	0	III	1	+	+	Y
GC_51	M	55	6	4a	2	0	III	3	+	+	N
GC_52	M	64	3.5	3	3a	0	III	3	+	+	N
GC_53	M	62	6	4a	3b	0	III	2	+	+	N

* We also performed 16S rRNA sequencing for 31 stool samples of patients with GC, which were all included in the 53 GC patients in this table.

30 cases of gastritis for tissue samples

Number	Gender	Age	Diagnose
G_1	M	45	Chronic superficial gastritis
G_2	F	50	Chronic superficial gastritis
G_3	M	74	Chronic superficial gastritis
G_4	M	62	Chronic superficial gastritis
G_5	M	62	Chronic superficial gastritis
G_6	M	38	Chronic superficial gastritis
G_7	M	43	Chronic superficial gastritis
G_8	F	67	Chronic superficial gastritis
G_9	F	50	Chronic superficial gastritis
G_10	M	62	Chronic superficial gastritis
G_11	F	37	Chronic superficial gastritis
G_12	F	51	Chronic superficial gastritis
G_13	M	63	Chronic superficial gastritis
G_14	F	50	Chronic superficial gastritis
G_15	F	54	Chronic superficial gastritis
G_16	M	44	Chronic superficial gastritis
G_17	F	54	Chronic superficial gastritis
G_18	F	61	Chronic superficial gastritis

G_19	M	64	Chronic atrophic gastritis with intestinal metaplasia
G_20	M	61	Chronic atrophic gastritis with intestinal metaplasia
G_21	M	49	Chronic atrophic gastritis with intestinal metaplasia
G_22	F	46	Chronic atrophic gastritis with intestinal metaplasia
G_23	F	44	Chronic atrophic gastritis with intestinal metaplasia
G_24	F	48	Chronic atrophic gastritis with intestinal metaplasia
G_25	M	57	Chronic atrophic gastritis with intestinal metaplasia
G_26	M	56	Chronic atrophic gastritis with intestinal metaplasia
G_27	M	66	Chronic atrophic gastritis with intestinal metaplasia
G_28	M	59	Chronic atrophic gastritis with intestinal metaplasia
G_29	M	62	Chronic atrophic gastritis with intestinal metaplasia
G_30	F	50	Chronic atrophic gastritis with intestinal metaplasia
* We also performed 16S rRNA sequencing for 32 stool samples of patients with chronic gastritis, in which all the 30 cases in the table were included.			

Flt3L-derived cDC1 are demonstrated to be superior to GM-CSF-generated DCs as a cell-based vaccine. Flt3L-cDC1 acquire tumor antigen, migrate to tumor-draining lymph nodes, and directly prime antitumor T-cell responses, highlighting their potential as a cancer therapeutic.

Supplementary Table S2. Reagent or Resource

Antibodies	SOURCE	IDENTIFIER
Recombinant anti-human CD103 Antibody	abcam	Cat#ab129202
Recombinant Anti-human CD8 alpha Antibody	abcam	Cat#ab237710
Recombinant anti- human CD3 Antibody	abcam	Cat#ab135372
HU anti-human IFN-Gama BV711 4S.B3 50Tst	BD Pharmingen	Cat#564793
PE/Cyanine5 anti human/mouse Granzyme B Recombinant Antibody	BioLegend	Cat#372226
Alexa Fluor® 700 anti-human CD45 Antibody	BioLegend	Cat#368513
FITC anti-human CD3 Antibody	BioLegend	Cat#300306
PerCP/Cyanine5.5 anti-human CD8	BioLegend	Cat#980918
APC anti-human CD103 (Integrin α E) Antibody	BioLegend	Cat#350215
PE anti-human CD49a Antibody	BioLegend	Cat#328303
Hu anti-human CD279 (PD-1) BV786 EH12.1 50Tst	BD Pharmingen	Cat#563789
Hu anti-human CD152(CTLA-4) PE-CF594 BNI3 50Tst	BD Pharmingen	Cat#562742
PE/Cyanine5 anti-human CD366 (Tim-3) Antibody	BioLegend	Cat#345051
FITC anti-mouse CD45 Antibody	BioLegend	Cat#157214
APC anti-mouse CD3 Antibody	BioLegend	Cat#100236
PE anti-mouse CD8 Antibody	BioLegend	Cat#140408
Alexa Fluor® 700 anti-mouse CD69 Antibody	BioLegend	Cat#104539
Brilliant Violet 421™ anti-mouse CD103 Antibody	BioLegend	Cat#156915
PE/Cyanine7 anti-mouse PD-1 Antibody	BioLegend	Cat#135216
Recombinant anti-mouse CD69 Antibody	abcam	Cat# ab224202
Recombinant anti-mouse CD103 Antibody	abcam	Cat# ab202909
Recombinant anti-mouse CD8 Antibody	abcam	Cat# ab217344
Bacterial		
<i>Methylobacterium</i>	BNCC	BNCC195932
Staining kit		
PanoPANEL Kits	PANOVUE	0002100100

Supplementary Table S3. Primer information of RT-PCR.

RT-PCR	Primer base sequence
GAPDH	Forward: 5'-GTCTCCTCTGACTTCAACAGCG-3' Reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3'.
IFNG	Forward: 5'-CAGGTCATTCAGATGTAGCGGAT-3' Reverse: 5'-ACTCTCCTCTTTCCAATTCTTCAAAA-3'.
TNF	Forward: 5'-CCTCTCTCTAATCAGCCCTCTG-3' Reverse: 5'-GAGGACCTGGGAGTAGATGAG-3'.
GZMB	Forward: 5'-TACCATTGAGTTGTGCGTGGG-3' Reverse: 5'-GCCATTGTTTCGTCCATAGGAGA-3'.
TGFB	Forward: 5'-TTGTGCGGCAGTGGTTGAG -3' Reverse: 5'-TTGCAGTGTGTTATCCCTGCT-3'.
IL2	Forward: 5'-AACTCCTGTCTTGCATTGCAC-3' Reverse: 5'-GCTCCAGTTGTAGCTGTGTTT-3'.
IL12A	Forward: 5'-AGCAACATGCTCCAGAAGG-3' Reverse: 5'-CAATCTCTTCAGAAGTGCAAGG-3'.
IL17A	Forward: 5'-TGGGAAGACCTCATTGGTGT-3' Reverse: 5'-GGATTCGTGGGATTGTGAT-3'.
IL15	Forward: 5'-TGCAGGGCTTCCTAAAACAGA-3' Reverse: 5'-CAACTGGGGTGAACATCACTTT-3'.
CXCL13	Forward: 5'-GCTTGAGGTGTAGATGTGTCC-3' Reverse: 5'-CCCACGGGGCAAGATTTGAA-3'.

Supplementary Table S4. P value and LDA value of gastric cancer and chronic gastritis.

Species name	Group	Mean	LDA value	P value
f__Rhodobacteraceae	GC	4.046182823	3.680264783	0.046531077
c__Syntrophomonadia	GC	4.32238901	4.02358021	0.000216508
o__Anaerolineales	GC	4.366852777	3.960454988	0.017790799
p__Proteobacteria	GC	5.53462558	4.648034501	0.013012017
o__Syntrophomonadales	GC	4.32238901	4.02358021	0.000216508
c__Gammaproteobacteri a	GC	5.439205681	4.56790411	0.032177057
g__Catenisphaera	GC	3.914074643	3.605223257	0.000371713
f__Saccharospirillaceae	GC	4.695314526	4.380112306	0.000109685
f__Dysgonomonadaceae	GC	4.133889224	3.724672411	0.017302162
g__Acinetobacter	GC	4.28196645	3.715985524	0.003298881
g__Flexilinea	GC	3.827309408	3.494465305	0.000864768
g__Prevotella	GC	4.545879398	3.950588256	0.018264293
f__Syntrophomonadacea e	GC	4.32238901	4.02358021	0.000216508
o__Christensenellales	GC	3.964836662	3.57264815	0.009142894
f__Christensenellaceae	GC	3.964836662	3.57264815	0.009142894
g__Delftia	GC	4.40089257	3.987213246	0.024670855
g__Syntrophomonas	GC	4.270629607	3.97268877	0.000122236
g__Oceanobacter	GC	4.695049475	4.379837273	0.000216508
f__Comamonadaceae	GC	4.722974398	4.301013656	0.001312163
o__Rhodobacterales	GC	4.046182823	3.680264783	0.046531077
g__Methylobacterium	GC	4.329062208	4.013171908	0.00187334
g__Proteiniphilum	GC	3.993270251	3.550638203	0.041321018
f__Anaerolineaceae	GC	4.093568402	3.639177543	0.049682882
f__Prevotellaceae	GC	4.626506092	3.880232834	0.021798276
g__Vibrio	GC	3.792374359	3.456798249	0.017802771
f__Anaerolineaceae	GC	4.366852777	3.960454988	0.017790799
g__Comamonas	GC	4.096697675	3.791369781	7.37934E-09

o__Pseudomonadales	GC	4.608794217	4.01646334	0.026552488
f__Moraxellaceae	GC	4.339516144	3.721503446	0.006335575
o__Oceanospirillales	GC	4.704389033	4.380728878	2.04558E-06
f__Beijerinckiaceae	GC	4.344073832	4.0104615	0.042512359
g__Christensenellaceae	GC	3.945298494	3.555958289	0.009930845
o__Oscillospirales	Gastritis	4.689587873	4.020001545	0.009142894
g__Haemophilus	Gastritis	3.976098995	3.472931812	0.003614458
f__Bifidobacteriaceae	Gastritis	4.212199682	3.714455974	0.00062199
g__Bifidobacterium	Gastritis	4.206746643	3.724579649	0.000226695
o__Campylobacterales	Gastritis	5.19804917	4.563219823	0.000540668
f__Gemmatimonadaceae	Gastritis	4.195091567	3.910048766	4.33518E-06
o__Gemmatimonadales	Gastritis	4.231392011	3.9328832	5.26958E-06
c__Campylobacteria	Gastritis	5.19804917	4.563219823	0.000540668
f__Neisseriaceae	Gastritis	4.17469541	3.784946826	0.000283012
f__Mycoplasmataceae	Gastritis	3.998678884	3.472569156	3.03686E-05
o__Mycoplasmatales	Gastritis	3.998678884	3.472569156	3.03686E-05
c__Gemmatimonadetes	Gastritis	4.231392011	3.9328832	5.26958E-06
o__Pasteurellales	Gastritis	4.138429274	3.571511095	0.009142894
c__Vicinamibacteria	Gastritis	4.039322784	3.563684264	0.000155429
g__Romboutsia	Gastritis	4.153303815	3.706496715	0.004077924
f__Helicobacteraceae	Gastritis	5.186429792	4.554997041	0.000272796
p__Actinobacteriota	Gastritis	4.717358912	3.821846525	0.037907577
o__Bifidobacteriales	Gastritis	4.212199682	3.714455974	0.00062199
f__Nitrosomonadaceae	Gastritis	3.863932508	3.528076004	1.75394E-05
f__Oscillospiraceae	Gastritis	4.239544294	3.612644537	0.008892927
g__Helicobacter	Gastritis	5.186385744	4.554985725	0.000272796
o__Vicinamibacterales	Gastritis	3.992671073	3.504085954	0.000226523
g__Blautia	Gastritis	4.049729122	3.527380566	0.000283012
c__Dehalococcoidia	Gastritis	3.997710187	3.584837663	0.001035785
g__Escherichia-Shigella	Gastritis	4.336304561	3.752513321	0.035353242

o__Lachnospirales	Gastritis	4.811525297	4.06829762	0.026552488
p__Gemmatimonadota	Gastritis	4.269675934	3.950177935	6.98115E-06
c__Blastocatellia	Gastritis	3.852263857	3.453783241	1.82856E-05
f__Muribaculaceae	Gastritis	4.469362818	3.782765979	0.005809844
f__Ruminococcaceae	Gastritis	4.394070529	3.716589161	0.004201921
o__Rickettsiales	Gastritis	4.167269603	3.5913515	0.002496655
f__Lachnospiraceae	Gastritis	4.811074651	4.068052701	0.025912997
f__Muribaculaceae	Gastritis	4.465214912	3.777905509	0.006335575
g__Alloprevotella	Gastritis	3.992352655	3.448550676	0.012009109
c__Acidobacteriae	Gastritis	3.992522674	3.539992719	0.000378611
o__Xanthomonadales	Gastritis	4.670183763	4.268803844	0.032177057
g__Wolbachia	Gastritis	4.139751899	3.833822909	5.61223E-11
f__Gemmatimonadaceae	Gastritis	4.231392011	3.9328832	5.26958E-06
g__Faecalibacterium	Gastritis	4.022492329	3.460323954	0.000144119
f__Anaplasmataceae	Gastritis	4.140084563	3.834090205	5.61223E-11
p__Acidobacteriota	Gastritis	4.542725669	4.055187107	0.00097163
f__Pasteurellaceae	Gastritis	4.138429274	3.571511095	0.009142894
g__Mycoplasma	Gastritis	3.975909222	3.474980148	1.82856E-05
g__Neisseria	Gastritis	4.155000489	3.781658457	5.13261E-06
p__Campilobacterota	Gastritis	5.198075078	4.563244092	0.000540668

Supplementary Table S5. Number of tumors in mice stomach.

Mice in control group		Mice in <i>Methylobacterium</i> group	
Mouse 1	1	Mouse 1	3
Mouse 2	1	Mouse 2	3
Mouse 3	1	Mouse 3	3
Mouse 4	1	Mouse 4	2
Mouse 5	2	Mouse 5	2
Mouse 6	2		
Mouse 7	1		