

# The effects of TNF- $\alpha$ /TNFR2 in regulatory T cells on the microenvironment and progression of gastric cancer

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### Supplementary Table S1 Purification and RNA-Sequencing of Tregs in GC patients

Tissue	Subset	Sorting phenotype	Number of collected cells
Tumor	CD4 <sup>+</sup> Treg	CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>-</sup>	7252
Blood	CD4 <sup>+</sup> Treg	CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>-</sup>	2340

Tumor tissue and peripheral blood were collected from three GC patients (2 men and 1 woman) for scRNA sequencing. Tissue of origin, cell subsets, surface marker combinations used for sorting, as well as number of collected cells are indicated.

### Supplementary Table S2. The sequencing coverage and quality statistics

Sample ID	Total number of sequenced reads per sample	Total number of uniquely mapped reads per sample*	Total number of called cells	Median number (and range) of uniquely mapped reads per called cell	Median rRNA rate (and range) per called cell	Median number (and range) of detected genes per called cell
N712	547680763	214392981.4	2824	53012 (17601 - 250858)	0.401204 (0.001744 - 0.823044)	1162 (70 - 3690)
N711	500941463	99316630.49	545	80623 (28059 - 1250357)	0.0149792 (0.0000551 - 0.6505409)	655 (42 - 4171)
pbmc	509299528	297941995.1	11192	19888 (7052 - 145472)	0.1319728 (0.0005069 - 0.4250594)	1025 (36 - 3585)
tumour	672216281	412062890	7816	37703 (13080 - 892712)	0.0921786 (0.0001285 - 0.3023369)	1093 (44 - 5819)
blood_566709	563624569	271459202.9	2735	85141 (18680 - 342302)	0.2137984 (0.0001346 - 0.4082517)	1217 (67 - 3801)
tumor_566709	1686069446	563854524.6	697	271137 (103066 - 29392094)	0.0003975 (0 - 0.5643967)	249 (43 - 4347)

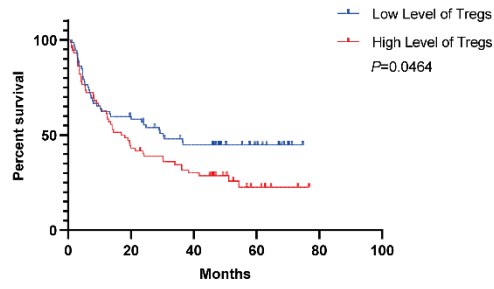
\*GRCh38 was the reference genome.

### Supplementary Table S3 Multivariate Cox regression analysis of the level of TNFR2+ Tregs infiltration on the influence of OS and DFS in gastric cancer patients

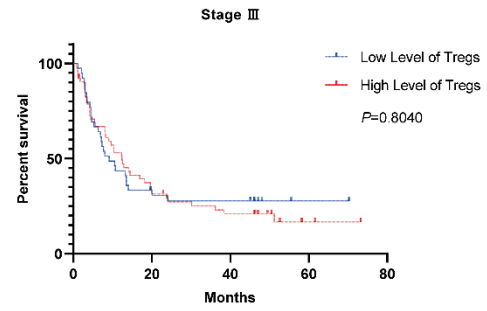
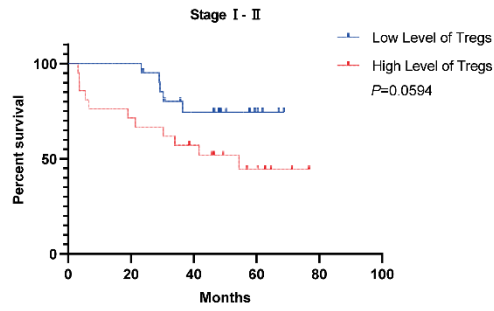
Variables	OS			DFS		
	P value	Hazard Ratio*	95.0% CI	P value	Hazard Ratio*	95.0% CI
Age	0.482	1.163	0.763-1.775	0.652	1.099	0.729-1.659
Gender	0.25	0.769	0.492-1.203	0.878	1.035	0.667-1.607
N stage	0.484	1.13	0.802-1.593	0.608	1.081	0.803-1.455
TNM stage	0.025	1.512	1.054-2.169	0.024	1.445	1.0491-1.990
TNFR2 <sup>+</sup> Treg	0.001	2.259	1.408-3.624	<0.001	2.88	1.787-4.640

OS: overall survival; HR: Hazard ratio; DFS: Disease-free survival. \*HR adjusted for age, gender, N stage, and TNM stage.

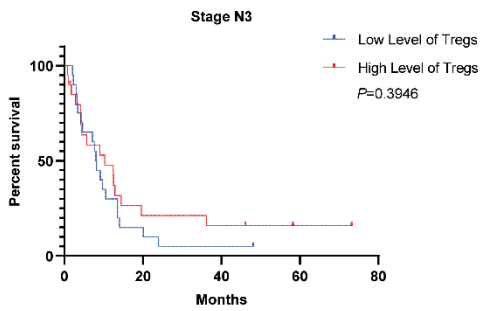
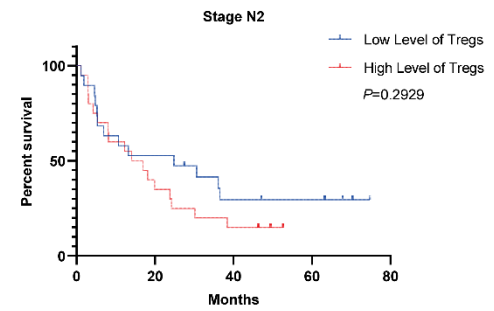
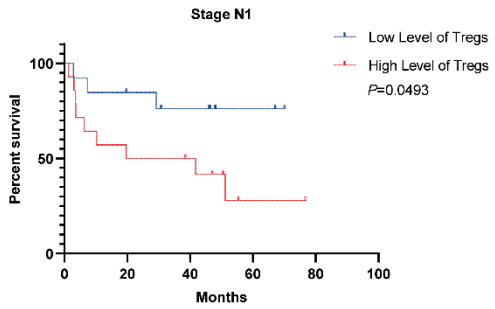
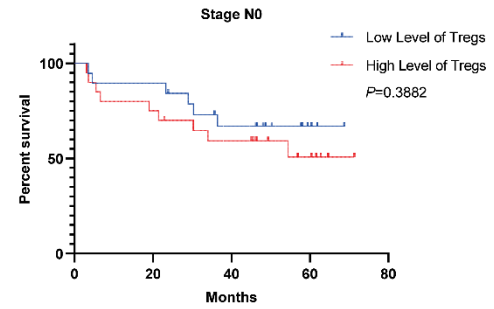
(A)

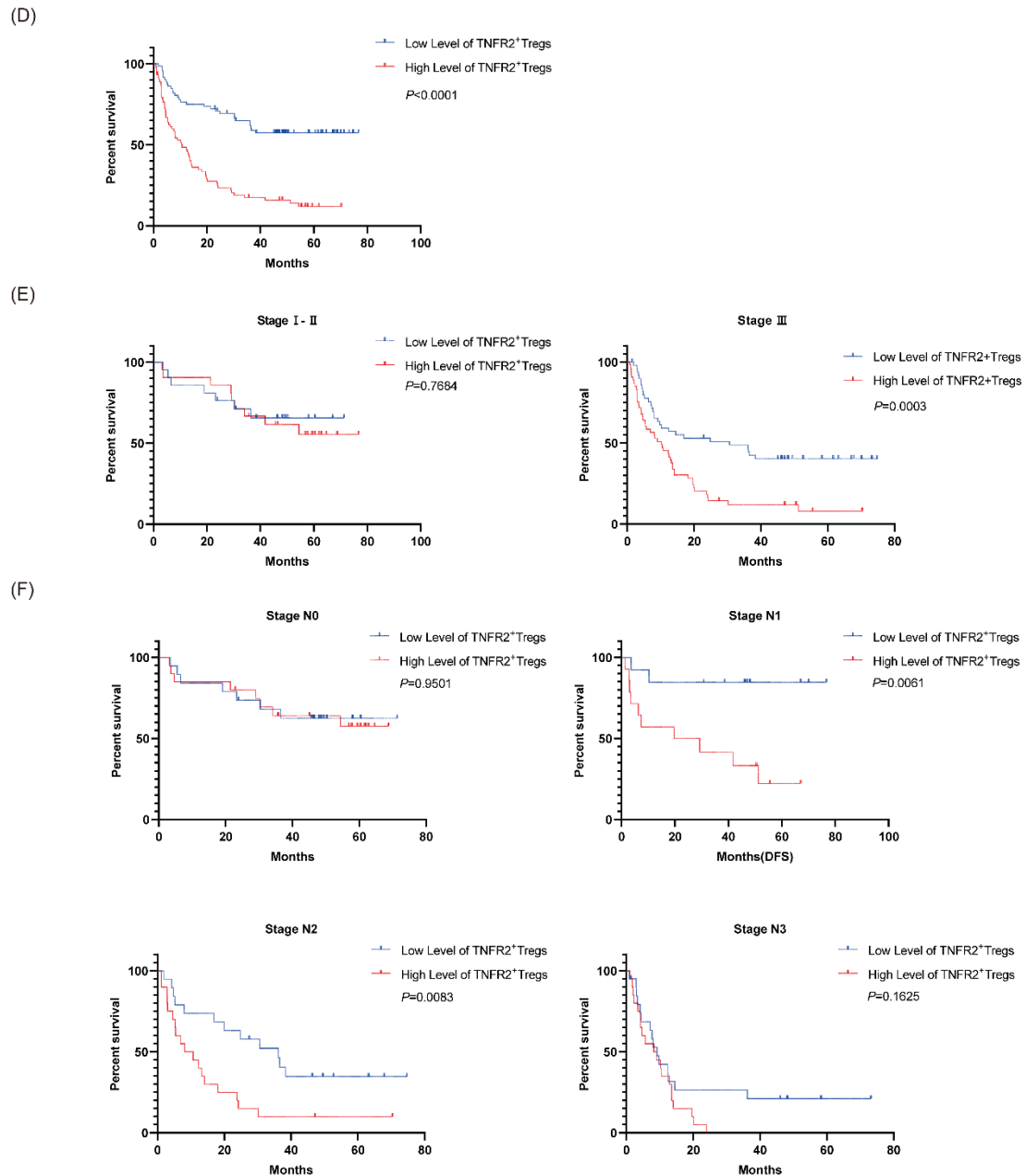


(B)



(C)





**Fig. S1**

**Correlations of the Treg and TNFR2<sup>+</sup> Treg infiltration levels with DFS**

(A) Patients were divided into high- and low-infiltration groups according to the median Treg infiltration ratio. The Kaplan-Meier survival curves showed a difference in the DFS between patients with high and low levels of tumor-infiltrating Tregs (log-rank test,  $P < 0.05$ ).

(B) Subgroup analysis. The DFS curves for patients stratified by TNM stage were compared between patients with high and low levels of tumor-infiltrating Tregs (log-rank

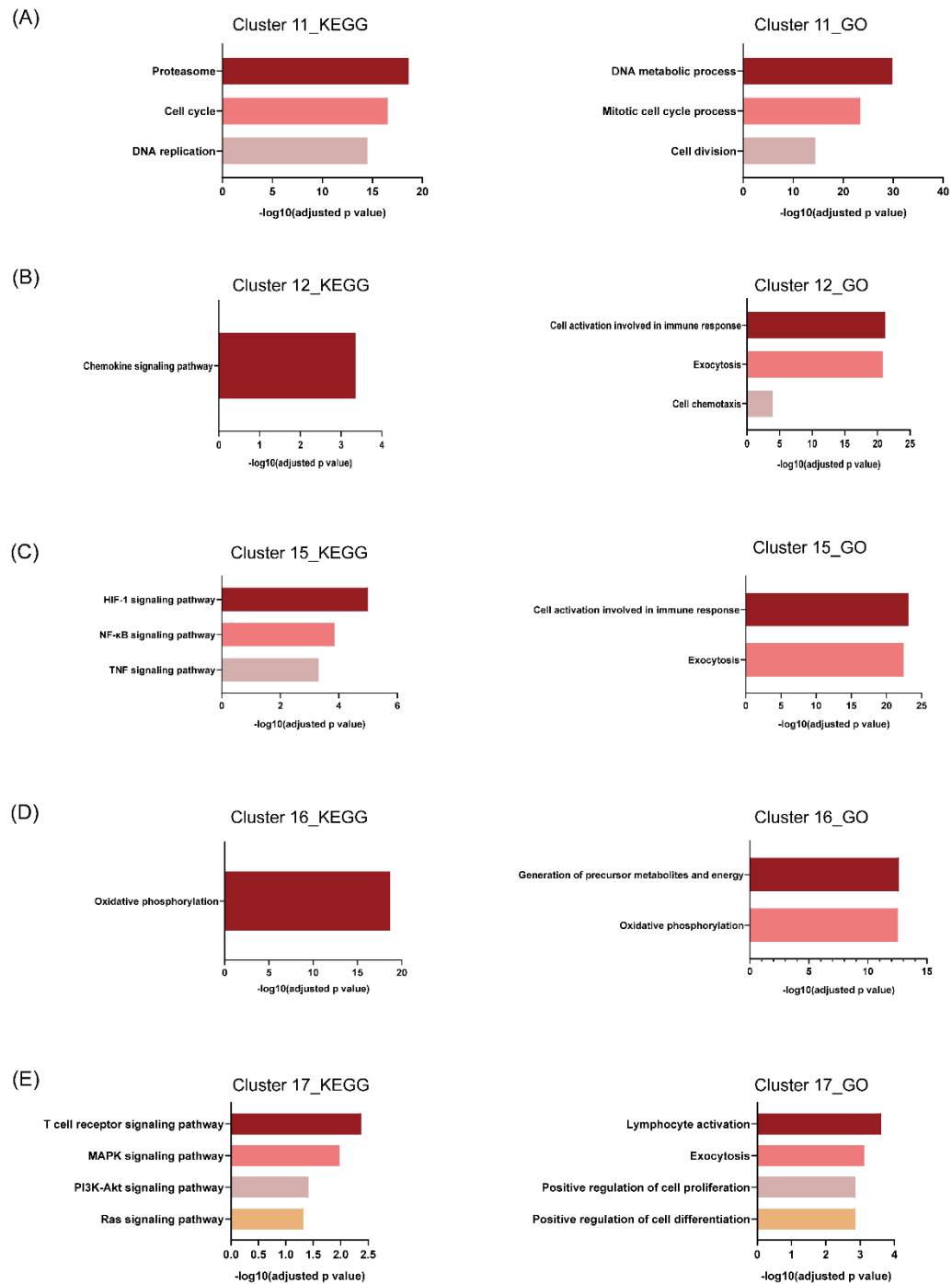
test,  $P < 0.05$ ).

(C) Subgroup analysis. The DFS curves for patients stratified by N stage were compared between patients with high and low levels of tumor-infiltrating Tregs (log-rank test,  $P < 0.05$ ).

(D) Patients were divided into high- and low-infiltration groups according to the median TNFR2<sup>+</sup> Treg infiltration ratio. The Kaplan-Meier survival curves showed a difference in the DFS between patients with high and low levels of tumor-infiltrating TNFR2<sup>+</sup> Tregs (log-rank test,  $P < 0.05$ ).

(E) Subgroup analysis. The DFS curves for patients stratified by TNM stage were compared between patients with high and low levels of tumor-infiltrating TNFR2<sup>+</sup> Tregs (log-rank test,  $P < 0.05$ ).

(F) Subgroup analysis. The DFS curves for patients stratified by N stage were compared between patients with high and low levels of tumor-infiltrating TNFR2<sup>+</sup> Tregs (log-rank test,  $P < 0.05$ ).



**Fig. S2**

**Enrichment analysis**

(A-E) KEGG and GO enrichment analyses of the upregulated genes in clusters 11, 12, 15, 16 and 17 after merging and reclustered all Tregs. In these clusters, tumor-infiltrating Tregs accounted for more than 95% of the total Treg population.