Supplemental materials and methods

Tissue microarray analysis

Tissue microarrays (TMAs) specific for gastric cancer containing 31 different paired samples (Gastric cancer and healthy), and tissue microarrays containing 30 samples of 6 types of digestive tract tumors, 5 for each, i.e., gastric cancer, esophagus cancer, colon cancer, rectal cancer, liver cancer and pancreatic cancer were purchased from Shanghai Outdo Biotech Company (Shanghai, China). The tissue samples on the TMAs that we used in this study were all collected from hospitals in Shanghai, China. All the patients had been given informed consent and the collection of tissue samples for research was approved by the ethics committee. The immunohistochemistry (IHC) assay was performed as described [1].Briefly, sections were cut at a thickness of 4 μm and dried for 16 h at 56 ℃ before being dewaxed in xylene and rehydrated through traded ethanol series to water. A heat-induced epitope retrieval step was performed in 0.01 M trisodium citrate solution with heating for 5 min in an induction cooker. After heating, slides were incubated in blocking buffer for 15 min and quickly washed in TBS (pH=7.4). Then TMAs were incubated with mouse rabbit polyclonal to anti-COPS2 (Abcam, Cambridge, USA) and mouse polyclonal to anti-CTSF (R&D, Minneapolis, USA). Specific binding was followed by anti-IgG conjugated with biotin for 1 hr, incubated with horseradish peroxidase (HRP)-conjugated streptavidin and the signal was visualized by using a DAB protein kit (Sigma-Aldrich Inc., Shanghai, China). The specimens were analyzed under a light microscope (Nikon, Tokyo, Japan) by pathologists. The staining intensity was defined from 0 to 4 based on the color shades of

IHC staining performed by two independent pathologists. Statistical analysis was performed by one-tailed Student's t test.

1. Babel I., Barderas R., Díaz-Uriarte R., Martínez-Torrecuadrada JL., Sánchez-Carbayo M., Casal JI. (2012) Identification of tumor-associated autoantigens for the diagnosis of colorectal cancer in serum using high density protein microarrays. *Mol Cell Proteomics*. **8**, 2382-95.

Supplemental Table S1. Eligibility Criteria for Selection of the Subjects.

General inclusion criteria

1. Age ≥ 30 years and ≤ 90 years
2. Not currently residing in an institution, such as a prison, nursing home, or shelter
3. Not severely ill in the intensive care unit
4. With the capability to give informed consent
5. Encountered between August 2008 and June 2013

Healthy donors (healthy group)

1. Had the medical check-up in Ruijin Hospital
2. In healthy condition without malignancy
3. Blood routine examination is normal

Gastric cancer patients (GC group)

1. With surgery treatment
2. Confirmed by pathologic examination result
3. Diagnosed by two experienced pathologists
4. No pre-operative chemotherapy, radiotherapy, transarterial chemoembolization or ablation

GU/GP/CAG group

1. With the signs and symptoms of gastritis
2. With Helicobacter pylori (Hp) detection
3. With gastroscopy Examination
4. Pathological examination result is gastric polyp, intraepithelial neoplasia, gastric epithelial dysplasia, or intestinal metaplasia with diagnosis of chronic superficial gastritis, chronic atrophic gastritis, or gastric ulcer
5. Diagnosed by two experienced pathologists

Supplemental Table S2. Serum samples collected from 7 Hospitals.

Hosptial	**Gastric cancer**	**Healthy**
Ruijin Hospital	220	231
the First Affiliated Hospital of Fujian Medical University	102	110
Shanghai East Hospital	28	27
Tongren Hospital	33	30
Shanghai Putuo Center Hospital	23	25
Shanghai Pudong Gongli Hospital	32	22
the Shanghai Fifth People’s Hospital	99	105
Total	537	550

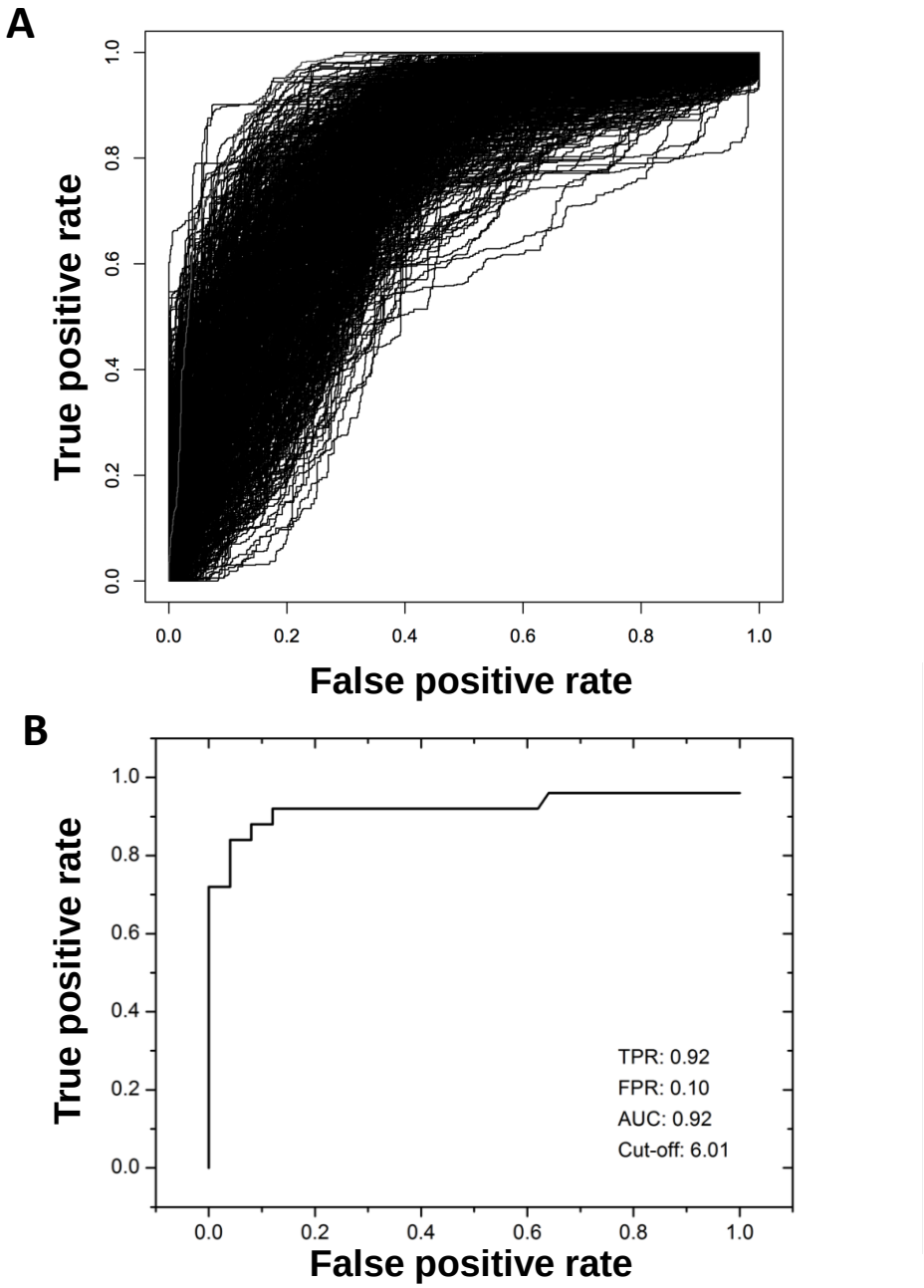
Supplemental Table S3. Candidate proteins identified by human proteome microarray using 87 serum samples.

Name	UniProt ID.	*SNR (Cancer)	*SNR (Healthy)	*P* value (Cancer/Healthy)	**Fold change (Cancer/Healthy)
ZBTB5	O15062	3.01	2.15	<0.001	1.83
ARL6IP4	Q66PJ3	3.24	3.16	<0.001	1.59
SIAH1	Q8IUQ4	3.28	1.73	0.001	1.54
ATG10	Q9H0Y0	3.14	2.94	<0.001	1.48
SPRR1A	P35321	3.71	2.22	<0.001	1.48
OR5BU1	A6ND48	3.59	3.26	<0.001	1.46
COPS2	P61201	3.29	1.92	0.004	1.45
NMI	Q13287	3.01	2.41	<0.001	1.43
PPIL6	Q8IXY8	3.10	2.79	<0.001	1.43
NT5E	P21589	3.35	2.86	0.002	1.43
TERF1	P54274	3.46	3.32	<0.001	1.42
SLC22A24	Q8N4F4	3.18	3.07	0.004	1.42
PIGU	Q9H490	3.32	2.74	<0.001	1.40
CTSF	Q9UBX1	3.53	2.68	<0.001	1.40
ESCO1	Q5FWF5	3.00	2.78	0.002	1.40
SYT3	Q9BQG1	3.26	3.06	<0.001	1.40
C17orf63	Q8WU58	3.73	2.16	<0.001	1.40

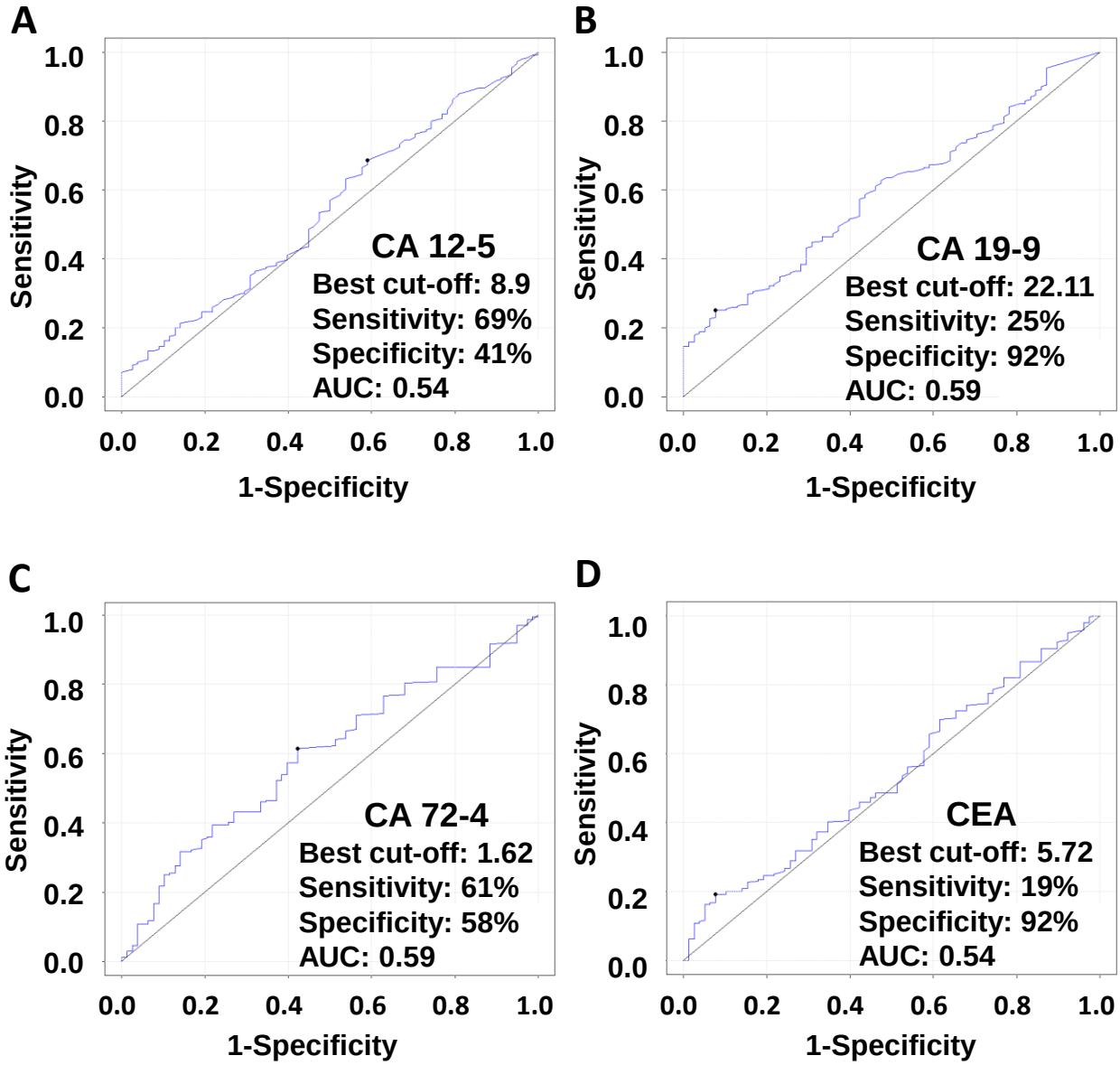
SNR for each group (Healthy and Cancer) was defined as the ratio of the difference between normalized intensities.

$\mathrm{SNR} = \frac{I - \sigma}{\sigma}$ *I* is the normalized intensity and σ is its variation.

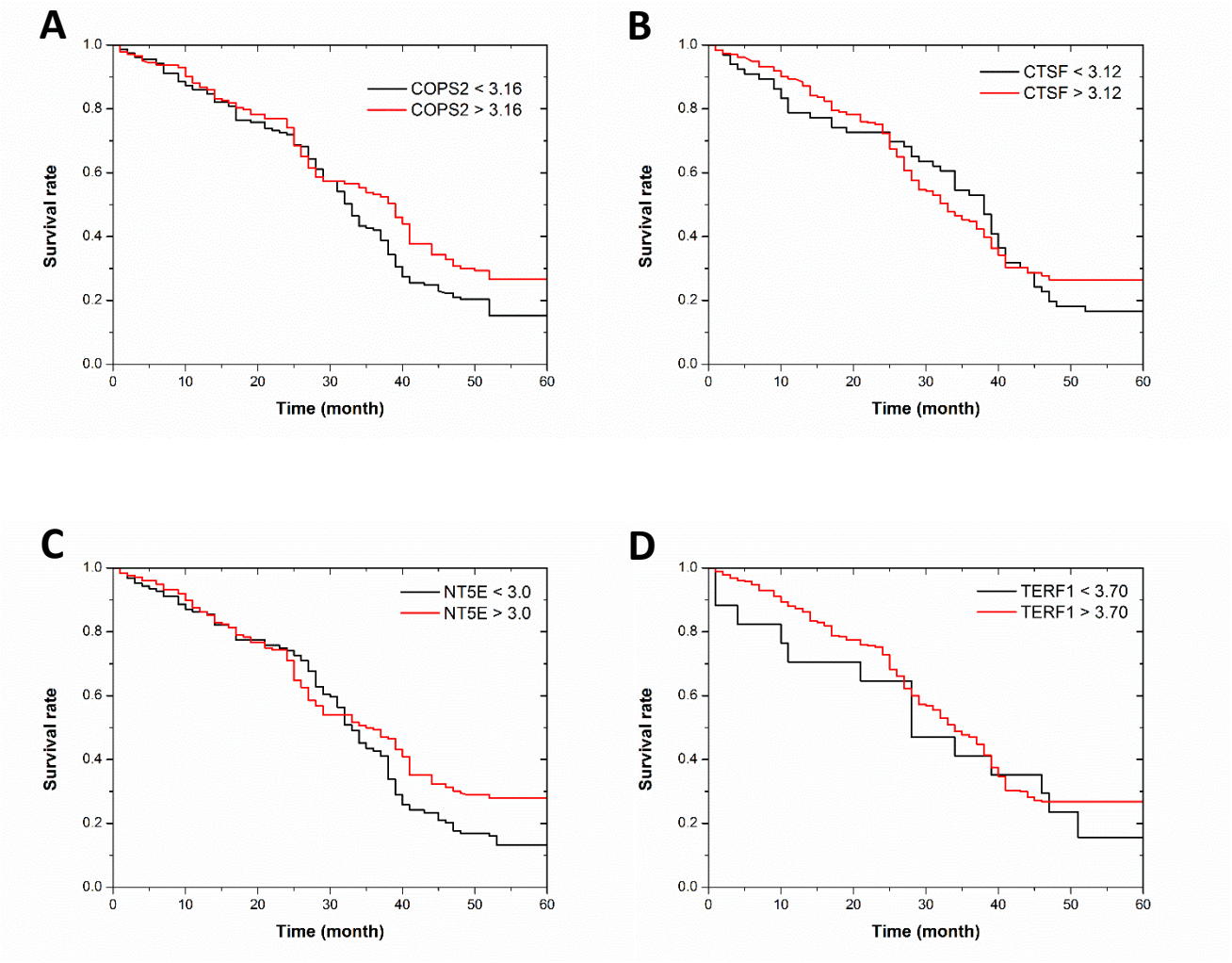
*SNR=Log10(SNR).
**Fold change= SNR(Cancer)/ SNR(Healthy)



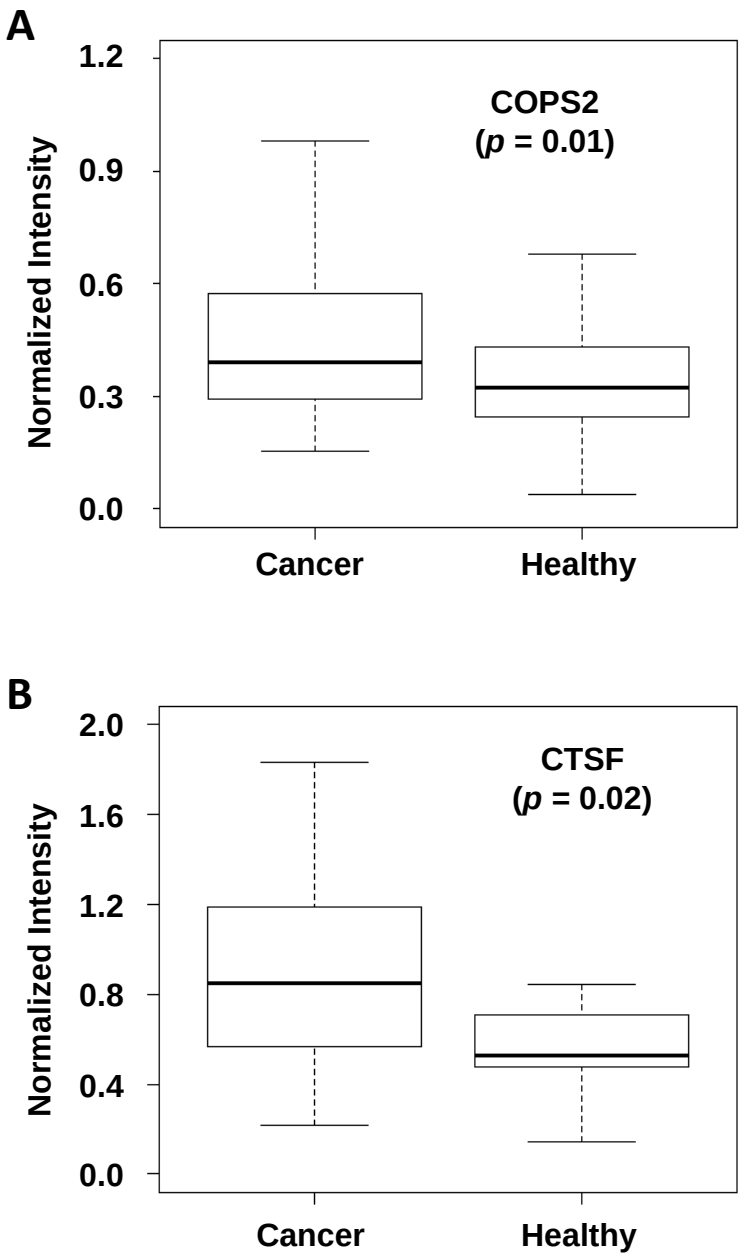
Supplemental Figure S1. Receiver operating characteristic curve analysis of the computational cross validation using the 4-marker panel. (A) The 100 splits, (B) The best model and the classifier. Based on the cross validation, a best model ((0.835*expression level of COPS2) + (0.792*expression level of CTSF) + (0.817*expression level of NT5E) + (0.671*expression level of TERF1)) was generated according to the linear combination of the final 4 candidates using multiple linear regression in “lm” package implemented in R.



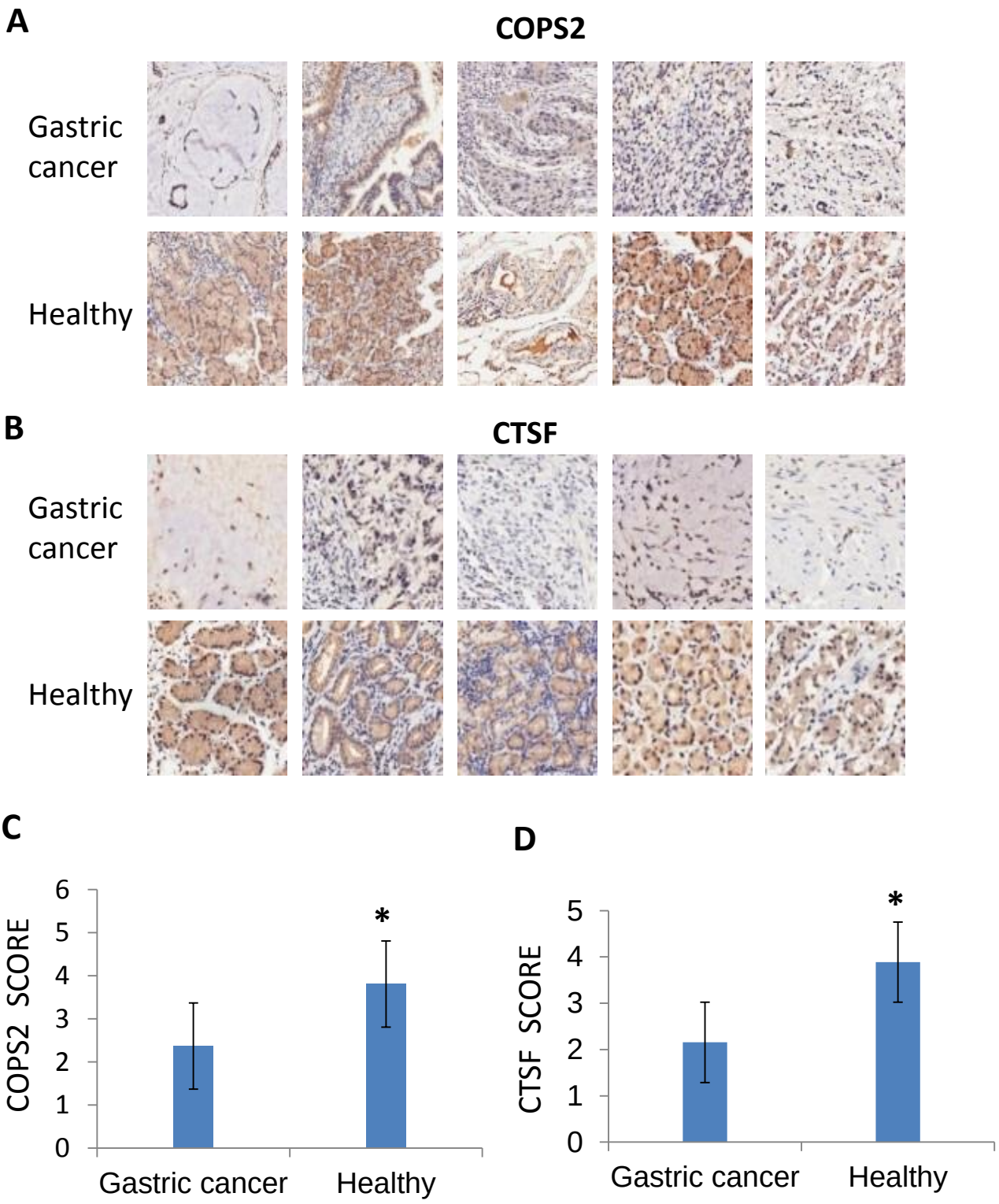
Supplemental Figure S2. Receiver operating characteristic curve analysis of the four routinely used serum biomarkers for gastric cancer diagnosis: (A) CA 12-5, (B) CA 19-9, (C) CA 72-4, and (D) CEA. The data for this analysis were obtained by ELISA.



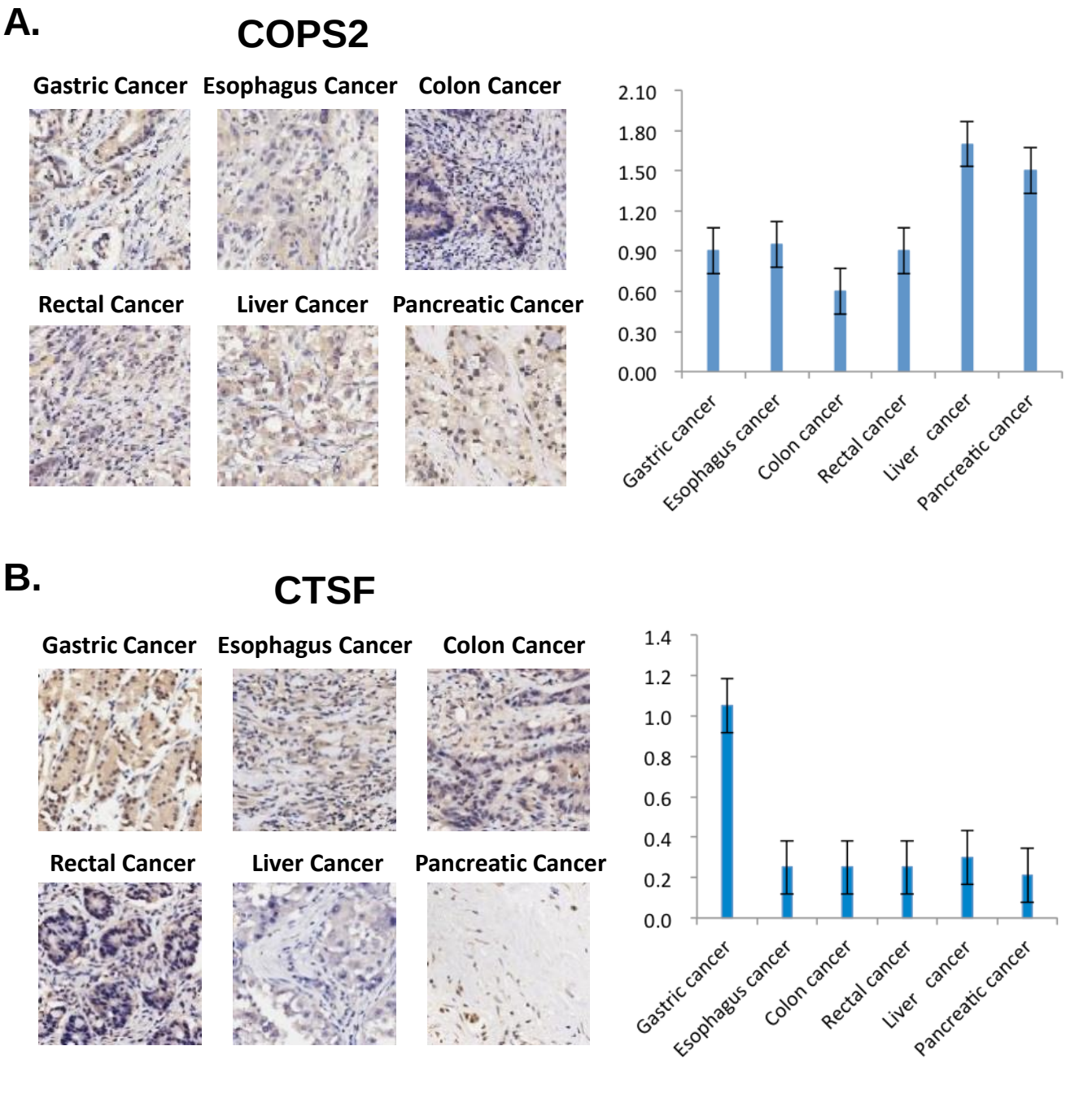
Supplemental Figure S3. Kaplan-Meier Survival curve. (A) COPS2, (B) CTSF, (C) NT5E and (D) TERF1.



Supplemental Figure S4. Reactivity of the two serum biomarkers with sera of ELISA validation phase I (100 healthy people and 100 GC patients). (A) COPS2, and (B) CTSF.



Supplemental Figure S5. Tissue microarray analysis with anti-COPS2 and anti-CTSF. Immuno-staining with anti-COPS2 (A,C), and anti-CTSF (B,D).



Supplemental Figure S6. Tissue microarray analysis with anti-COPS2 and anti-CTSF for testing the gastric cancer specificity on 6 types of digestive tract tumors.