Supplementary Data

The Role of Prion Protein Expression in Predicting Gastric Cancer Prognosis

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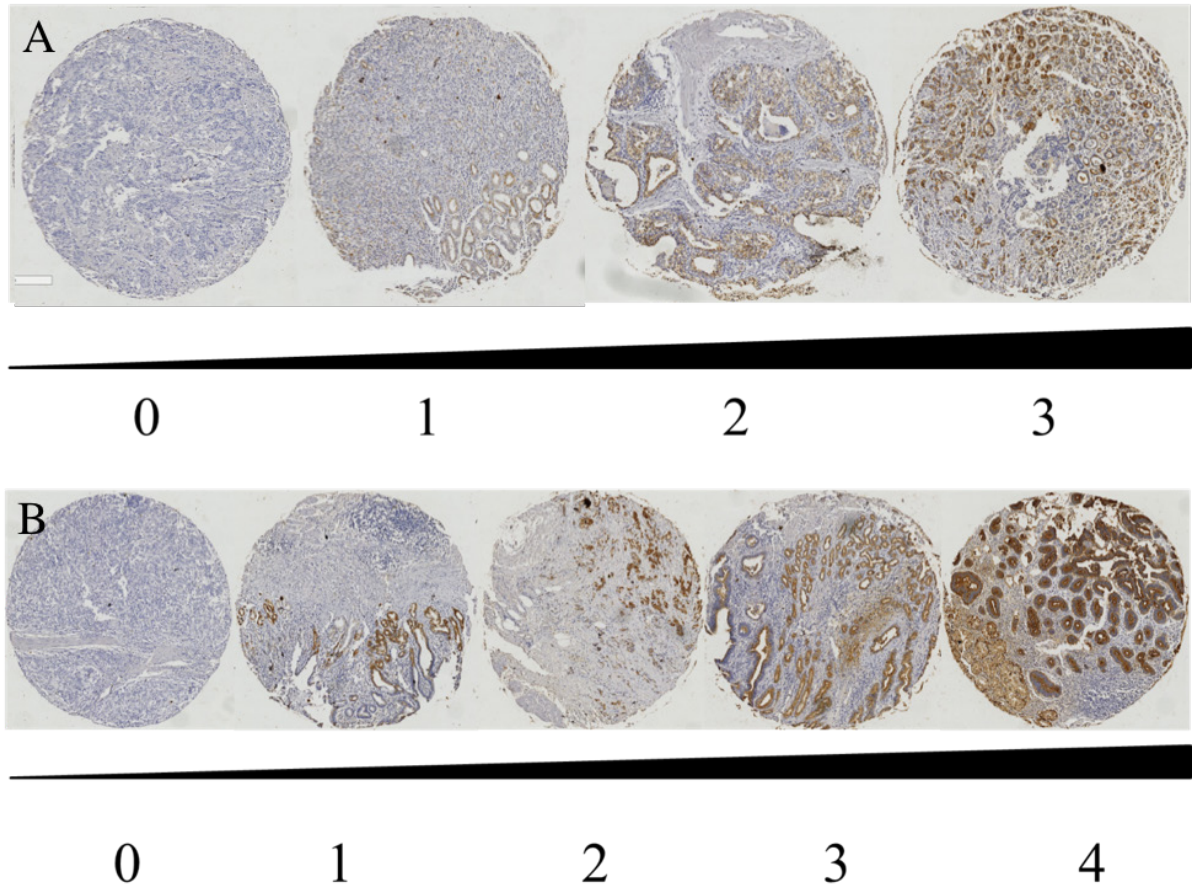
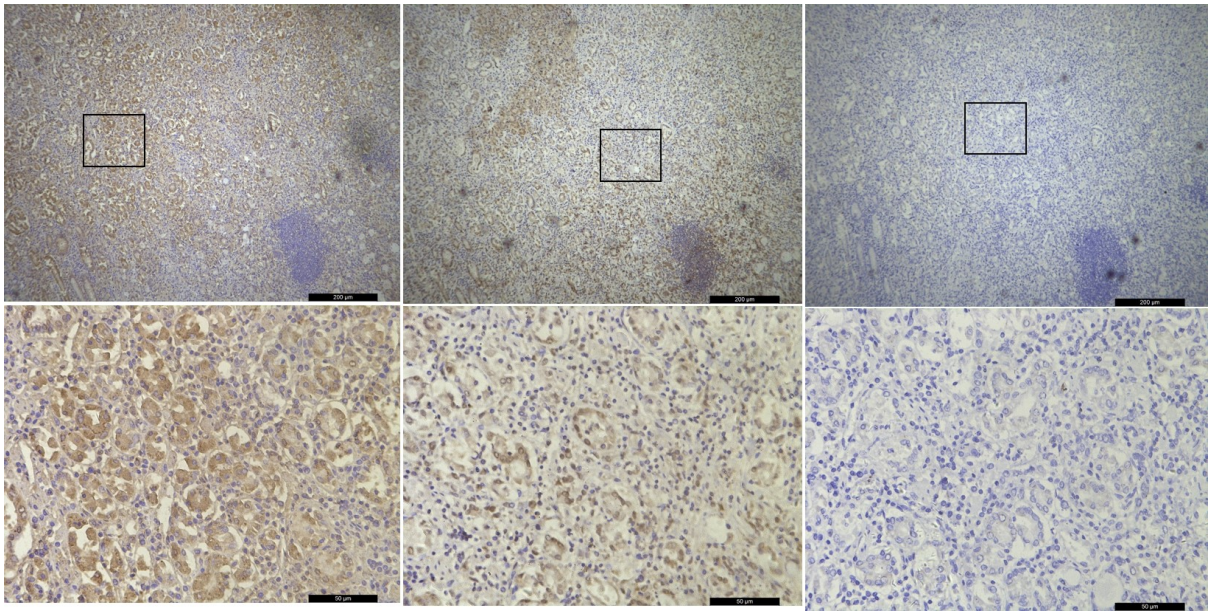


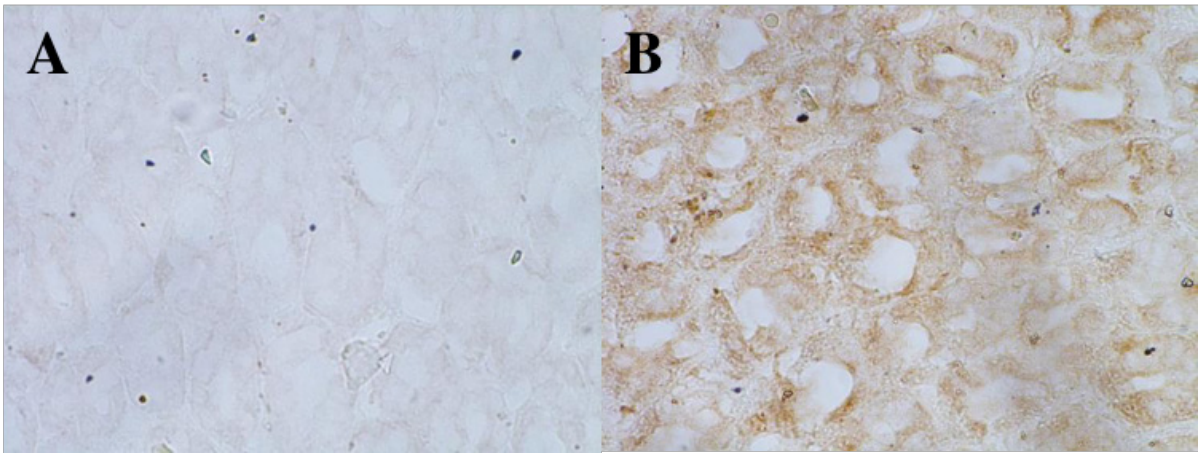
Supplementary Figure S1. Evaluation of immunostaining scores

The protein expression was visualized and classified based on the percentage of positive cells and the intensity of staining. A: The intensity of staining was divided into four grades (intensity scores): negative (0), weak (1), moderate (2) and strong (3). B: The percentage of positive cells was divided into five grades (percentage scores): <1% (0), 1–25% (1), 26–50% (2), 51–75% (3) and >75% (4). The histological score (H-score) was determined using the following formula: overall scores = percentage score × intensity score. An overall score of 0–12 was calculated and graded as negative (score:0) or positive (score:1-12).



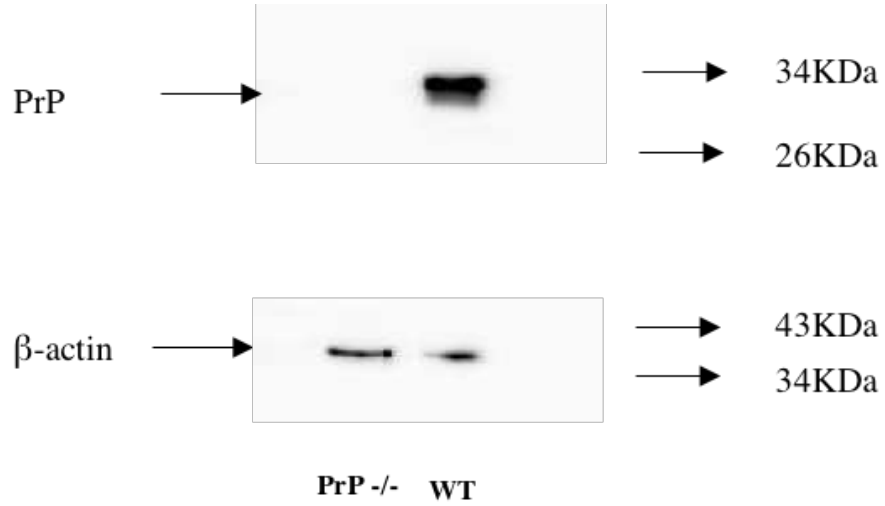
Supplementary Figure S2. Comparison of different anti-PrP antibodies

To select the anti-PrP antibody for this study, we compared 8H4 (Sigma, Left), 3F4 (Sigma, middle), and 8B4(Sigma, Right) antibody for IHC staining in consecutive sections. The 8H4 antibody was found to be suitable for staining PrP in GC samples because of its low background and clear positive staining.



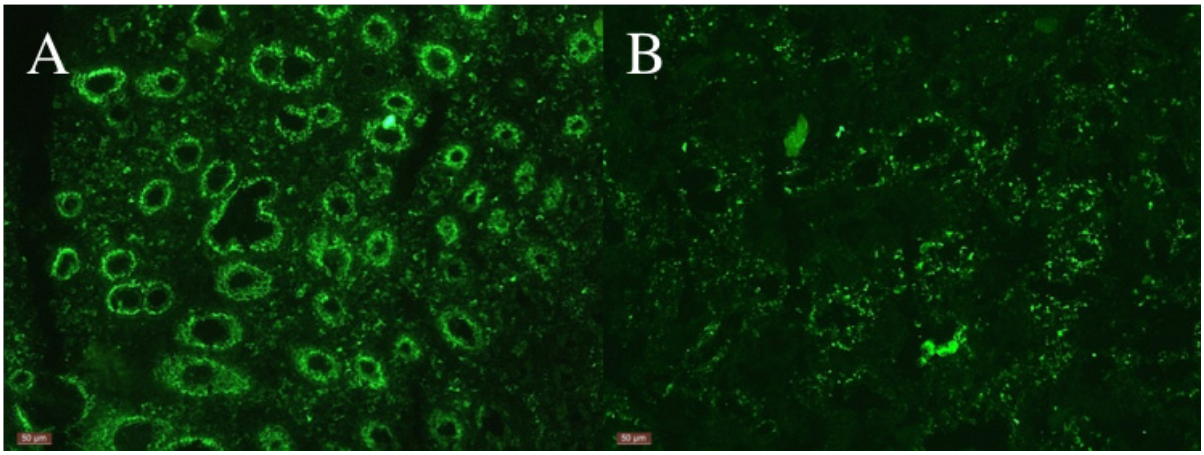
Supplementary Figure S3.The expression of PrP in the epithelial cells of murine stomach detected by IHC

Paraffin sections of stomach tissue from wide-type (WT) or PrP knock-out (PrP-/-) mice (5 μm) were stained with the 8H4 anti-PrP antibody (8H4, Sigma, 1:200) followed by a biotinylated anti-Ig secondary antibody and streptavidin-HRP/DAB. **A.** Gastric sections from PrP-/- mice. **B.** Gastric sections from WT mice. The positive staining (brown) was detected in the epithelial cells of WT mice, but not in PrP-/- mice.



Supplementary Figure S4.The expression of PrP in the murine brain detected by western blot analysis

Brain tissues were dissected from WT or PrP-/- mice. Total protein were isolated and detected by the 8H4 anti-PrP antibody (8H4, Sigma, 1:1000) or anti- β -actin antibody (Sigma, 1:5000) respectively. A total of 40μg protein from brain was loaded in each lane for western blot analysis. This analysis confirmed PrP expression in WT mice, but not in PrP-/- mice.



Supplementary Figure S5. The expression of PrP in noncancerous(A) and cancerous(B) tissues of stomach detected by immunofluorescence analysis.

Non-cancerous and cancerous tissues of a GC patient were frozen sectioned at 5 μm. The sections were stained with the 8H4 anti-PrP antibody (8H4, Sigma, 1:200) followed by a Cy2-conjugatedgoatanti-mouse secondary antibody (Jackson ImmunoResearch, 1:200). The expression of PrP is mostly located in the crypt epithelial cells. Scale bar = 50 μm.

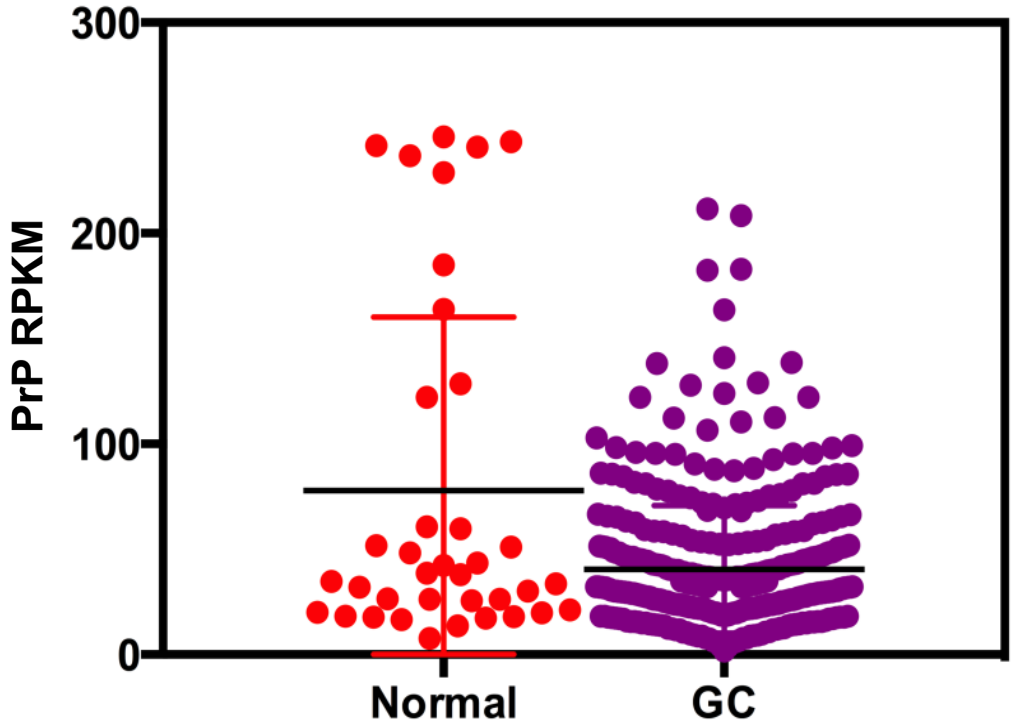
Supplementary Table S1. **PrP expression in cancerous and noncancerous tissues.**

	Positive: n(percent)	**P（χ^2 test）**
Cancerous (n=480)	213 (44.4%)	< 0.001
Noncancerous (n=458)	304 (66.4%)	

Supplementary Table S2. Cox regression analysis in 480 GC patients.

		Gender	Age	Differentiation	Histological type	TNM Stage	Tumor location	PrP expression	Status	Survival time
Gender	**Correlation Sig.**	1								
Age	**Correlation Sig.**	0.078 0.089	1							
Differentiation	**Correlation Sig.**	0.032 0.484	0.170 <0.001**	1						
Histological type	**Correlation Sig.**	-0.104 0.123	-0.200 <0.001**	-0.386 <0.001**	1					
TNM Stage	**Correlation Sig.**	0.100 0.028*	0.047 0.300	-0.190 <0.001**	-0.121 0.008**	1				
Tumor location	**Correlation Sig.**	-0.017 0.713	-0.002 0.969	0.005 0.922	0.033 0.477	-0.042 0.353	1			
PrP expression	**Correlation Sig.**	-0.067 0.142	-0.006 0.894	0.002 0.968	-0.028 0.548	-0.123 0.007**	0.055 0.227	1		
Status	**Correlation Sig.**	0.033 0.470	0.098 0.032*	-0.049 0.279	-0.031 0.502	0.416 <0.001**	-0.074 0.103	-0.140 0.002**	1	
Survival time	**Correlation Sig.**	-0.093 0.042*	-0.077 0.094	0.138 0.002**	0.038 0.410	-0.499 <0.001**	0.002 0.968	0.180 <0.001**	-0.812 <0.001**	1

*, P < 0.05; **, P < 0.01.



Supplementary Figure S6. The mRNA levels of PrP in noncancerous (Normal) and cancerous (GC) tissues of stomach analysis using data from The Cancer Genome Atlas (TCGA) project

The level 3 RNA-seq data of GC (n = 409) and noncancerous gastric tissues (n = 37) was download from TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). The methods of RNA sequencing and data processing were described in Ref 19 (there were only 295 samples published). We extracted the value of reads per kilobases per million mapped reads (RPKM, which represents the mRNA abundance) from the downloading level 3 data, and compared RPKM of the two groups using t-test. The data show the mRNA level of PrP in GC is significant lower than that in noncancerous tissue (40.55±30.24 vs 77.75±82.49, $p<0.001$).