

Table S1 Pathological characteristics of the GC patients based on TCGA

Characteristics	Cases (%)	Low expression of P4HA3 (n=187)	Low expression of P4HA3 (n=188)	P
Gender				0.564
Male	241 (64.3%)	117	124	
Female	134 (35.7%)	70	64	
Age, years				0.438
≤65	164 (44.2%)	86	78	
>65	207 (55.8%)	99	108	
T Stage				0.003*
T1	19 (5.2%)	17	2	
T2	80 (21.8%)	41	39	
T3	168 (45.8%)	85	83	
T4	100 (27.2%)	43	57	
N Stage				0.887
N0	111 (31.1%)	55	56	
N1	97 (27.2%)	51	46	
N2	75 (21.0%)	35	40	
N3	74 (20.7%)	38	36	
M Stage				0.988
M0	330 (93.0%)	166	164	
M1	25 (7.0%)	12	13	
Pathological Stage				0.155
I	53 (15.1%)	34	19	
II	111 (31.5%)	50	61	
III	150 (42.6%)	76	74	
IV	38 (10.8%)	19	19	

Differences between groups were analyzed using the Chi-squared test or Fisher's exact test, as appropriate. *P<0.05 was considered statistically significant for comparisons between two groups.

Table S2 Analysis of clinical characteristics of exosome between GC and normal patients

Characteristics	Cases (%)	Tumor (N=50)	Normal (N=50)	P
Gender				0.109
Male	59 (59%)	37	22	
Female	41 (41%)	13	28	
Age, years				0.124
≤65	30 (30%)	14	16	
>65	70 (70%)	36	34	
BMI/kg·m ²				0.677
≤25	66 (66%)	34	32	
>25	34 (34%)	16	18	
Biomarkers				
AFP	100 (100%)	50.76	11.06	0.244
CEA	100 (100%)	7.97	1.468	0.024*
CA125	100 (100%)	14.93	8.04	0.020*
CA199	100 (100%)	16.37	8.86	0.020*

Differences between groups were analyzed using the Chi-squared test or Fisher's exact test, as appropriate. *P<0.05 was considered statistically significant for comparisons between two groups.

Table S3 Pathologic characteristics of different expression levels of P4HA3 in plasma exosome

Characteristics	Cases (%)	Low expression of P4HA3 (n=24)	Low expression of P4HA3 (n=26)	P
Gender				0.632
Male	37 (74%)	17	20	
Female	13 (26%)	7	6	
Age, years				0.275
≤65	14 (28%)	8	6	
>65	36 (72%)	16	20	
T Stage				0.038*
T1	6 (12%)	5	1	
T2	13 (26%)	8	5	
T3	24 (48%)	8	16	
T4	7 (14%)	3	4	
N Stage				0.091
N0	16 (32%)	10	6	
N1	6 (12%)	4	2	
N2	20 (40%)	7	13	
N3	8 (16%)	3	5	
M Stage				0.904
M0	42 (84%)	20	22	
M1	8 (16%)	4	4	
Pathological Stage				0.477
I	7 (14%)	4	3	
II	10 (20%)	6	4	
III	25 (50%)	10	15	
IV	8 (16%)	4	4	
Biomarkers				
AFP	100 (100%)	103.1	2.44	0.138
CEA	100 (100%)	6.43	9.40	0.553
CA125	100 (100%)	16.07	13.89	0.717
CA199	100 (100%)	15.10	17.54	0.668

Differences between groups were analyzed using the Chi-squared test or Fisher's exact test, as appropriate. *P<0.05 was considered statistically significant for comparisons between two groups.

Table S4 Oligonucleotides used in this study (qPCR primers)

Gene Name	Sequence (5' to 3')	Annealing Temp (°C)	Source or Reference
P4HA3-F	AAG TGG AGT ACC GCA TCA GC	57.7	Sangon
P4HA3-R	TTG GTG ACG TAG CAT GGT CAA	56.5	Sangon
GAPDH-homo-F	TCA TGA CCA CAG TCC ATG CC	57.8	Sangon
GAPDH-homo-R	GGA TGA CCT TGC CCA CAG CC	62.4	Sangon

Table S5 Tumor volume and weight measurements in mice treated with Si-NC or Si-P4HA3

Animal ID	Group	Day	Tumor Length (mm)	Tumor Width (mm)	Tumor Volume (mm ³)	Body Weight (g)	Tumor Weight (g)
Mouse 1	Si-P4HA3	0	-	-	-	17.8	-
		2	-	-	-	17.9	-
		4	-	-	-	17.9	-
		6	3.5	2.5	11	18	-
		8	3.6	2.7	13.1	18.1	-
		10	4.1	2.9	17.2	18.3	-
		12	5.2	3.3	28.3	18.8	-
		14	5.6	3.7	38.3	19.8	-
		16	7	4.8	80.6	21.1	-
		18	7.5	5.1	97.5	21.5	-
		20	7.8	5.4	113.7	22.5	-
		22	8	5.7	130	22.7	0.43
Mouse 2	Si-P4HA3	0	-	-	-	17.8	-
		2	-	-	-	17.8	-
		4	-	-	-	17.8	-
		6	3.6	2.4	10.4	18	-
		8	4	2.6	13.5	18.2	-
		10	4.8	2.8	18.8	18.6	-
		12	5.4	3	24.3	19.4	-
		14	6.3	3.5	38.6	19.9	-
		16	7.2	4.7	79.5	21.2	-
		18	7.2	5.2	97.3	21.6	-
		20	7.9	5.4	115.1	22.5	-
		22	8.2	5.6	128.5	22.7	0.35
Mouse 3	Si-P4HA3	0	-	-	-	17.8	-
		2	-	-	-	17.9	-
		4	2.3	2.1	5.1	18.1	-
		6	3.1	2.6	10.5	18.3	-
		8	3.3	2.7	12	18.7	-
		10	3.9	3.2	20	19.3	-
		12	4.6	3.4	26.6	19.9	-
		14	5.6	3.9	42.6	20.3	-
		16	7.1	4.8	81.8	21.6	-
		18	7.6	5.1	98.8	22	-
		20	8	5.4	116.6	22.4	-
		22	8.4	5.6	131.7	22.9	0.45

Table S5 (continued)

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Animal ID	Group	Day	Tumor Length (mm)	Tumor Width (mm)	Tumor Volume (mm ³)	Body Weight (g)	Tumor Weight (g)
Mouse 1	Si-NC	0	-	-	-	17.8	-
		2	-	-	-	17.8	-
		4	-	-	-	18.1	-
		6	3.9	3	17.6	18.3	-
		8	5.3	3.3	28.9	18.6	-
		10	6.5	3.8	46.9	19.2	-
		12	7.6	4.7	83.9	20.6	-
		14	8.3	5.8	139.6	23	-
		16	9.1	6.3	180.6	24.1	-
		18	9.8	7	240.1	25.5	-
		20	11.1	7.4	303.9	27.3	-
		22	12.1	7.7	358.7	28.9	0.75
Mouse 2	Si-NC	0	-	-	-	17.8	-
		2	-	-	-	17.8	-
		4	-	-	-	18.1	-
		6	4	3.2	20.5	18.5	-
		8	5	3.6	32.4	18.9	-
		10	5.9	3.9	44.8	19.4	-
		12	7.3	4.8	84.1	20.9	-
		14	8.6	5.7	139.7	23	-
		16	9.6	6.1	178.6	24.6	-
		18	10.7	6.7	240.2	26.3	-
		20	11.4	7.3	304.8	27.7	-
		22	12.4	7.6	358.1	30.7	0.77
Mouse 3	Si-NC	0	-	-	-	17.8	-
		2	-	-	-	17.8	-
		4	2	2.2	4.84	17.9	-
		6	4	3.3	21.8	18.5	-
		8	6	3.4	34.6	18.9	-
		10	6.3	3.9	48	19.4	-
		12	8.2	4.6	86.8	20.8	-
		14	9.7	5.4	141.4	22.7	-
		16	10.4	5.9	181	24.1	-
		18	11.2	6.6	243.9	26.2	-
		20	11.8	7.2	305.9	28.4	-
		22	12.2	7.7	361.7	30.4	1.01

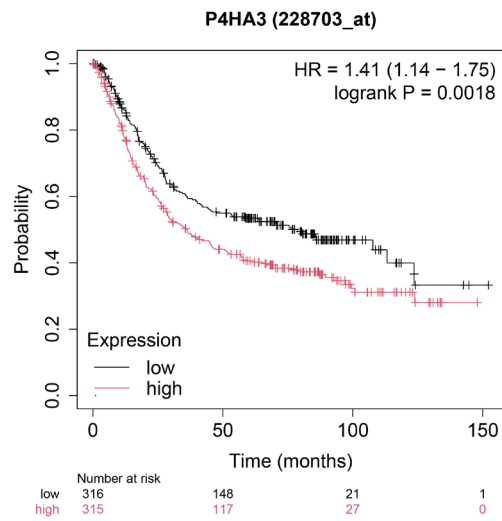


Figure S1 High tissue P4HA3 expression predicts poor overall survival in gastric cancer. Kaplan-Meier survival analysis from the KM-plotter database (<https://kmplot.com/analysis/>). Hazard ratio (HR) with 95% confidence interval and log-rank P value are shown. The number of patients at risk is indicated below.

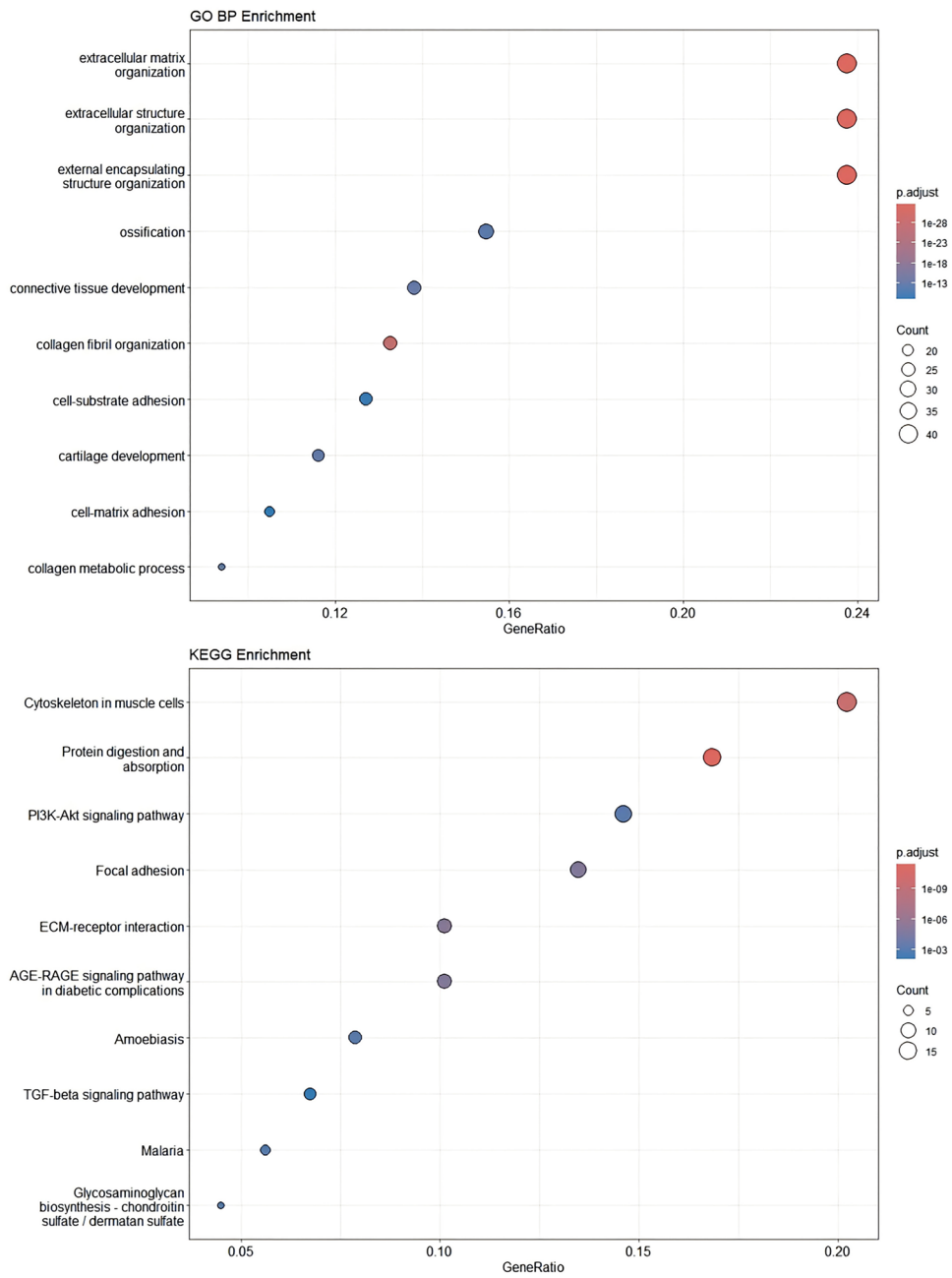


Figure S2 Functional enrichment analysis of tissue P4HA3 ($r>0.5$).

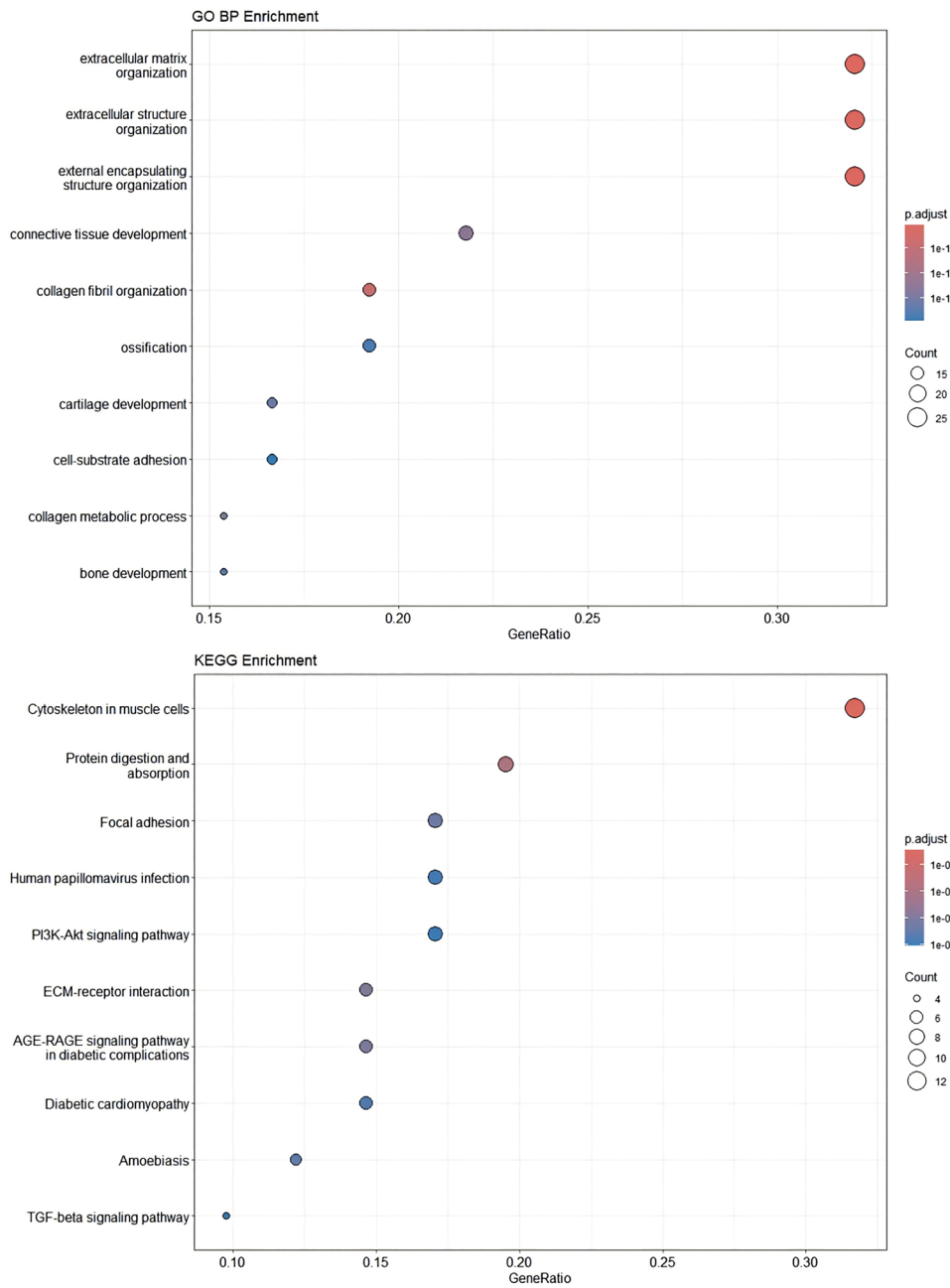


Figure S3 Functional enrichment analysis of tissue P4HA3 ($r > 0.6$).

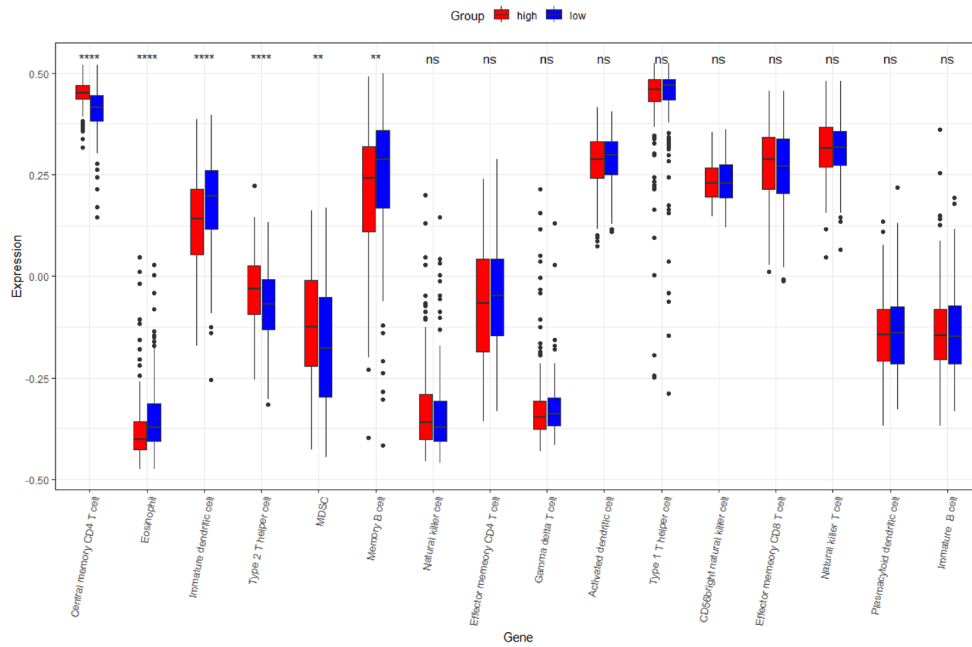


Figure S4 Statistical comparison of immune cell infiltration between P4HA3-high and P4HA3-low gastric cancer samples.

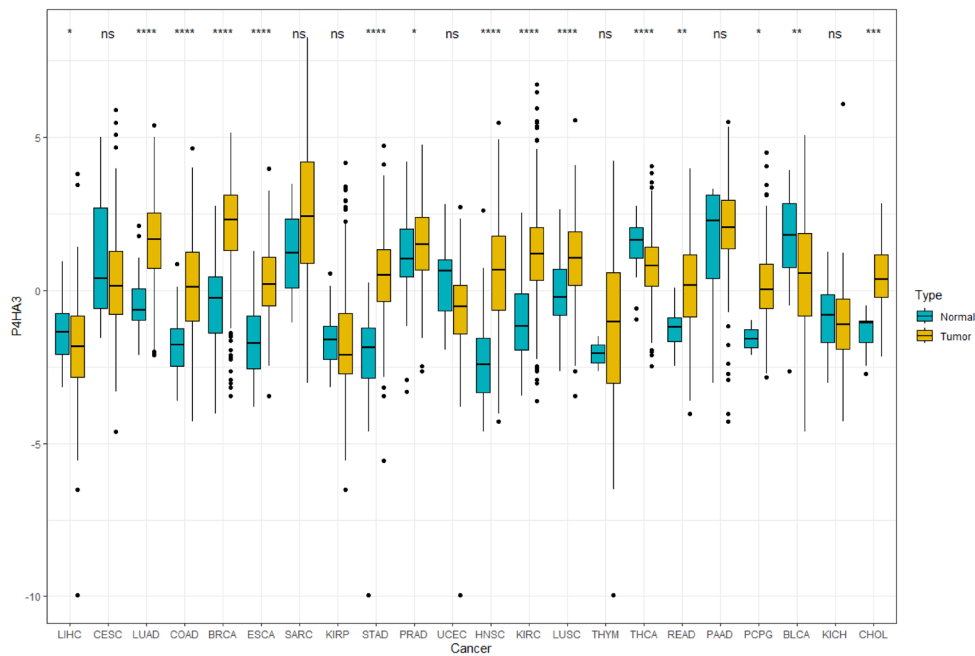


Figure S5 Boxplot of tissue P4HA3 expression across cancer types from The Cancer Genome Atlas (TCGA).

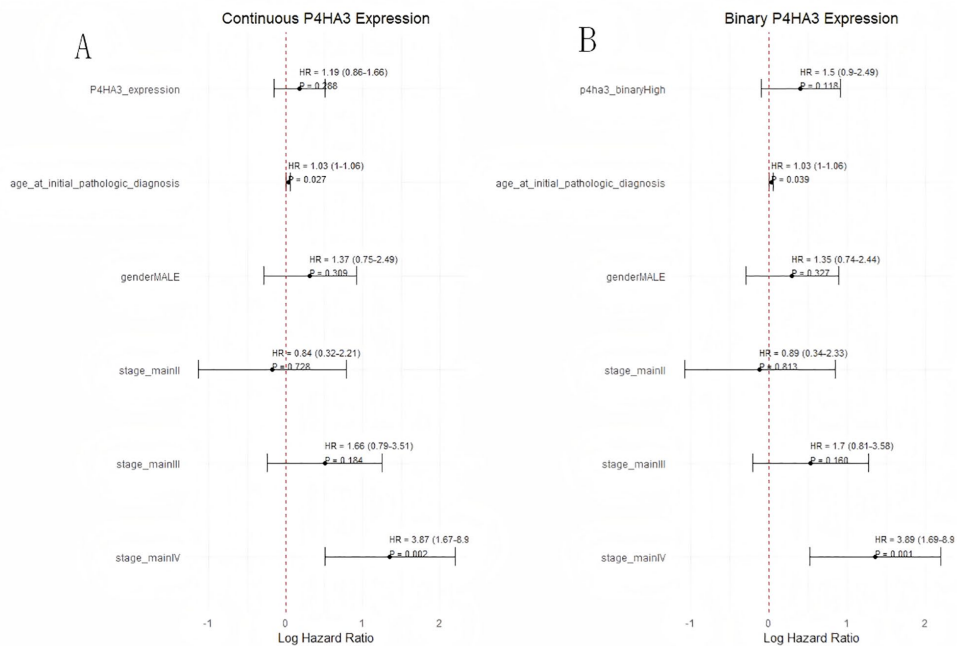


Figure S6 Forest plots of multivariate analyses assessing the association between tissue P4HA3 expression and clinical stage in the TCGA-STAD cohort. (A) Multivariable linear regression model with tissue P4HA3 expression as a continuous variable. (B) Multivariable model with tissue P4HA3 expression dichotomized as high *vs.* low based on the median. Both models are adjusted for age and gender. Data points represent coefficient estimates, horizontal lines indicate 95% confidence intervals. Black data points indicate statistically significant associations ($P < 0.05$). The analysis includes $n = 375$ gastric adenocarcinoma samples from the TCGA-STAD dataset.

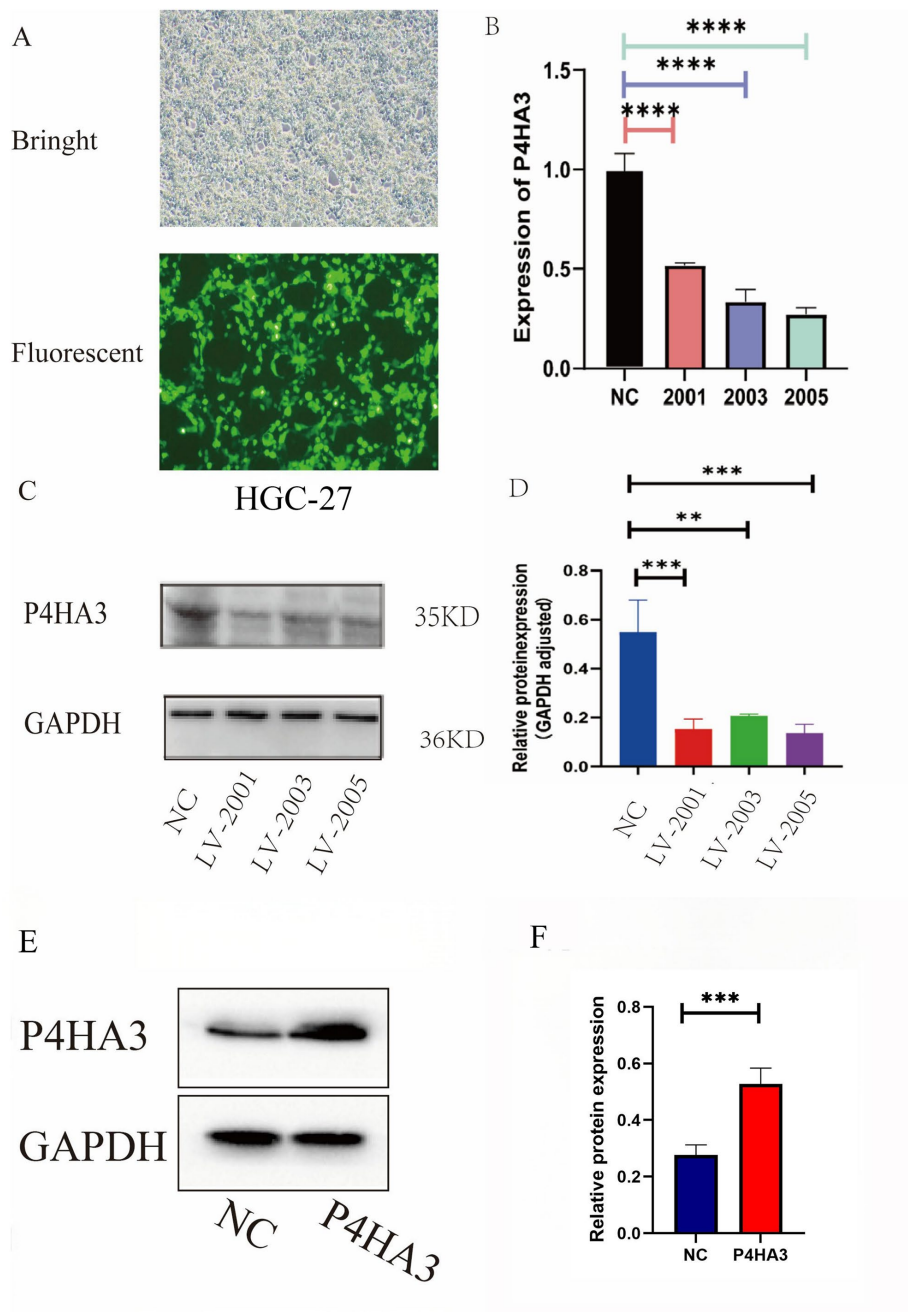


Figure S7 Efficient modulation of P4HA3 expression in HGC-27 cell lines. (A) Fluorescence microscopy images confirming successful lentiviral transduction in HGC-27 cells (scale bar, 100 μ m). (B) Quantitative RT-PCR (qRT-PCR) analysis of P4HA3 mRNA expression after transduction with three independent shRNAs (LV-2001, LV-2003, LV-2005) or negative control (sh-NC). (C) Representative Western blot images showing P4HA3 protein levels after transfection with negative control or three independent P4HA3-targeting lentiviral shRNAs. (D) Densitometric quantification of P4HA3 protein levels normalized to GAPDH. (E) Representative Western blot images showing P4HA3 protein levels, cells were transfected with an empty vector control (Vector) or a P4HA3-overexpressing plasmid (P4HA3-OE), GAPDH served as a loading control. (F) Quantitative analysis of P4HA3 protein expression. (* P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001).

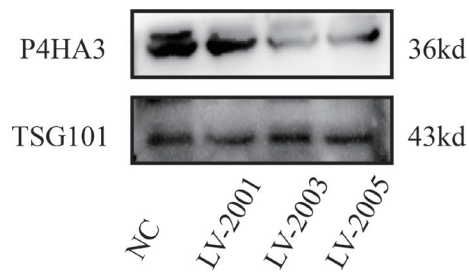


Figure S8 P4HA3 knockdown reduces its level in cell-derived exosomes. Western blot analysis of P4HA3 in exosomes isolated from HGC-27 cells transduced with a control (NC) or three independent P4HA3-targeting shRNAs (LV-2001, LV-2003, LV-2005). TSG101 serves as a loading control. The data demonstrate that P4HA3 is secreted in exosomes and that its packaging is diminished upon P4HA3 knockdown.

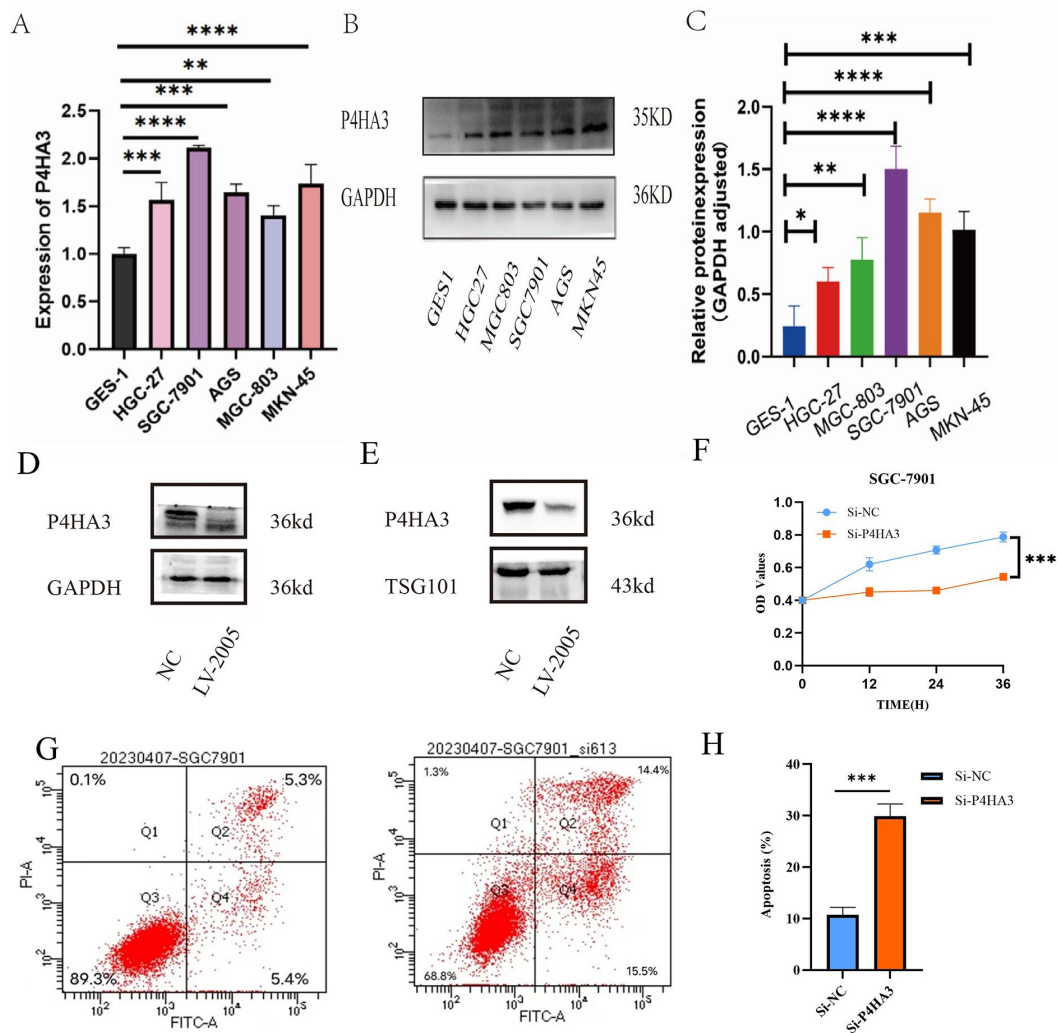


Figure S9 Validation of P4HA3 function in SGC-7901 gastric cancer cells. (A) Relative mRNA levels of P4HA3 in a normal gastric epithelial cell line (GES-1) and various gastric cancer cell lines (HGC-27, SGC-7901, AGS, MGC-803, MKN-45) as determined by qRT-PCR. (B-C) Western blot (B) and quantification (C) of P4HA3 protein expression in the indicated cell lines. (D) Efficient knockdown of P4HA3 in SGC-7901 cells using specific shRNAs. (E) Reduced level of P4HA3 in exosomes derived from P4HA3-knockdown SGC-7901 cells. TSG101 serves as an exosomal loading control. (F) P4HA3 knockdown inhibits proliferation of SGC-7901 cells (CCK-8 assay). (G) P4HA3 knockdown induces apoptosis in SGC-7901 cells (flow cytometry). Data are mean \pm SD; **, $P < 0.01$; ***, $P < 0.001$ vs. sh-NC group.

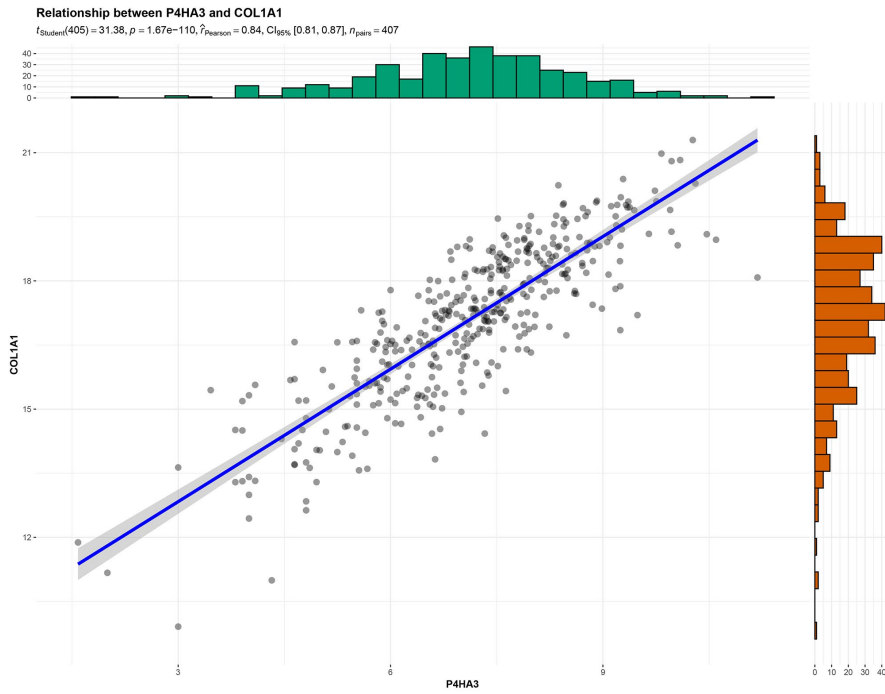


Figure S10 Association between tissue P4HA3 expression and COL1A1.

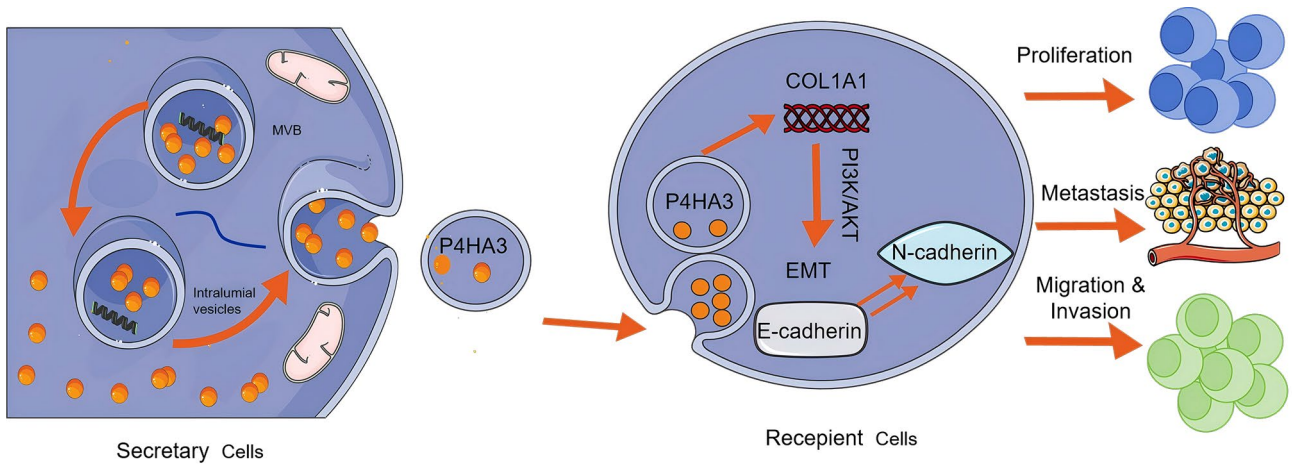


Figure S11 P4HA3-induced COL1A1 expression and epithelial-mesenchymal transformation.