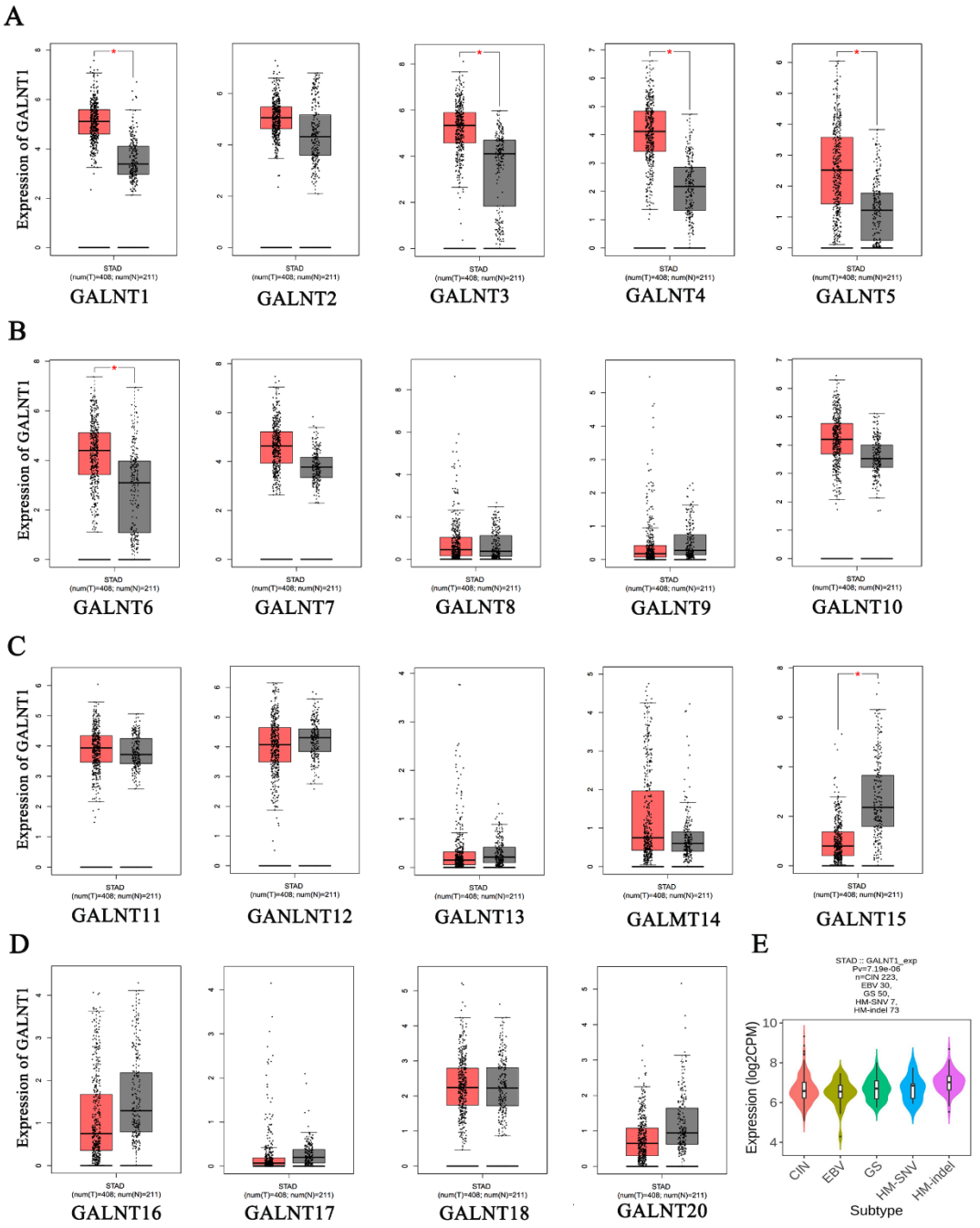
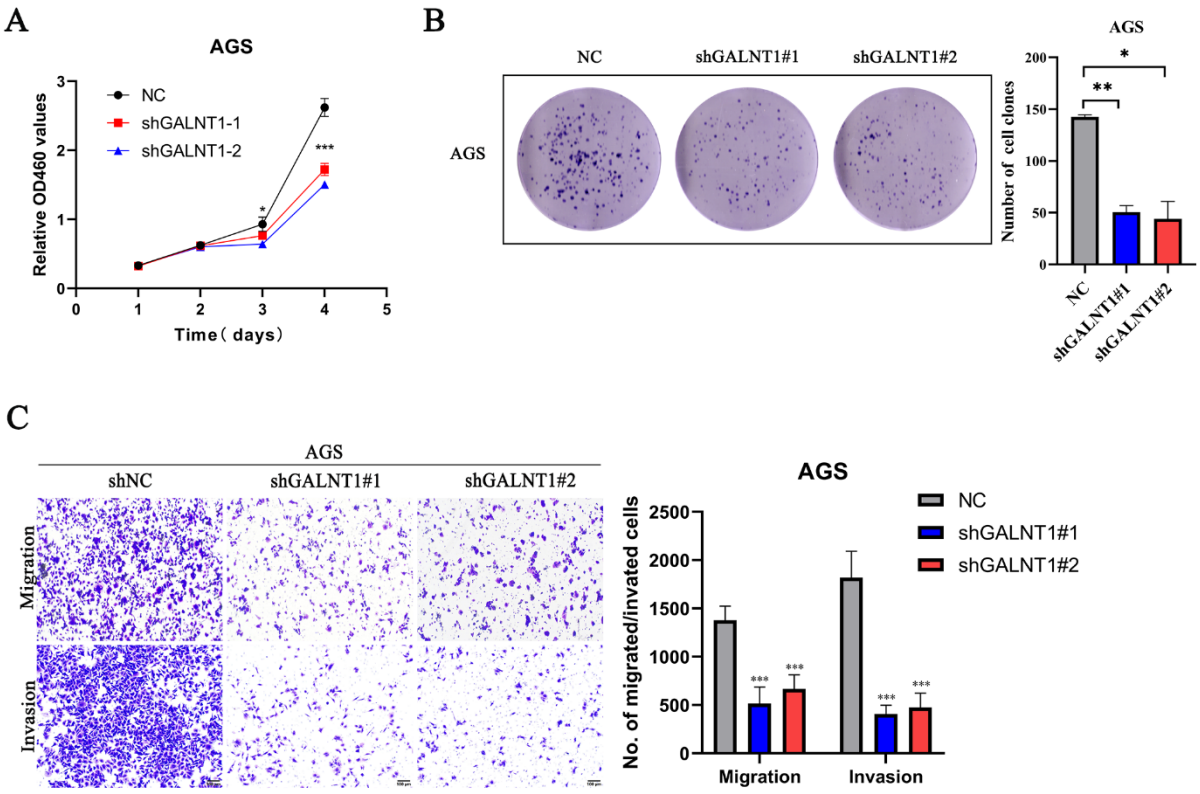
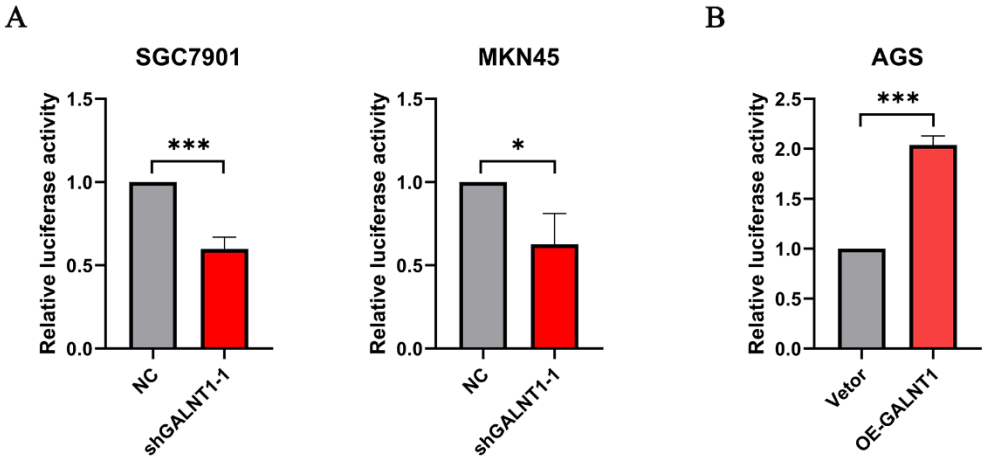


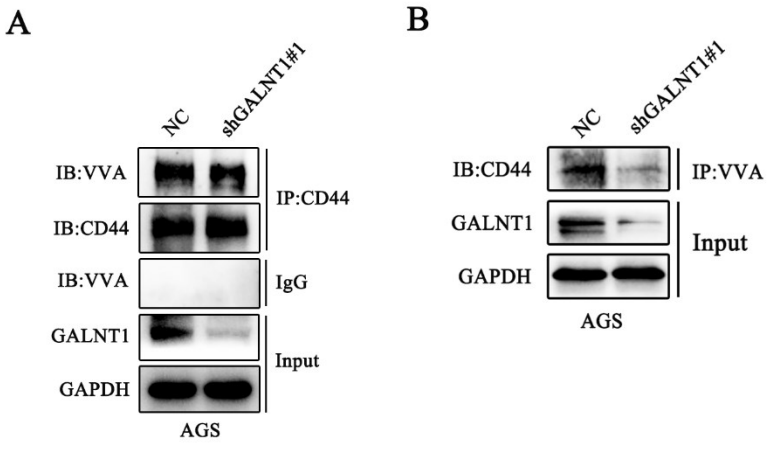
Supplementary figure S1: (**A–D**) Analysis of *GALNT*s expression in gastric cancer using GEPIA database. **E**, Analysis of GALNTs expression in gastric cancer each molecular subtype.



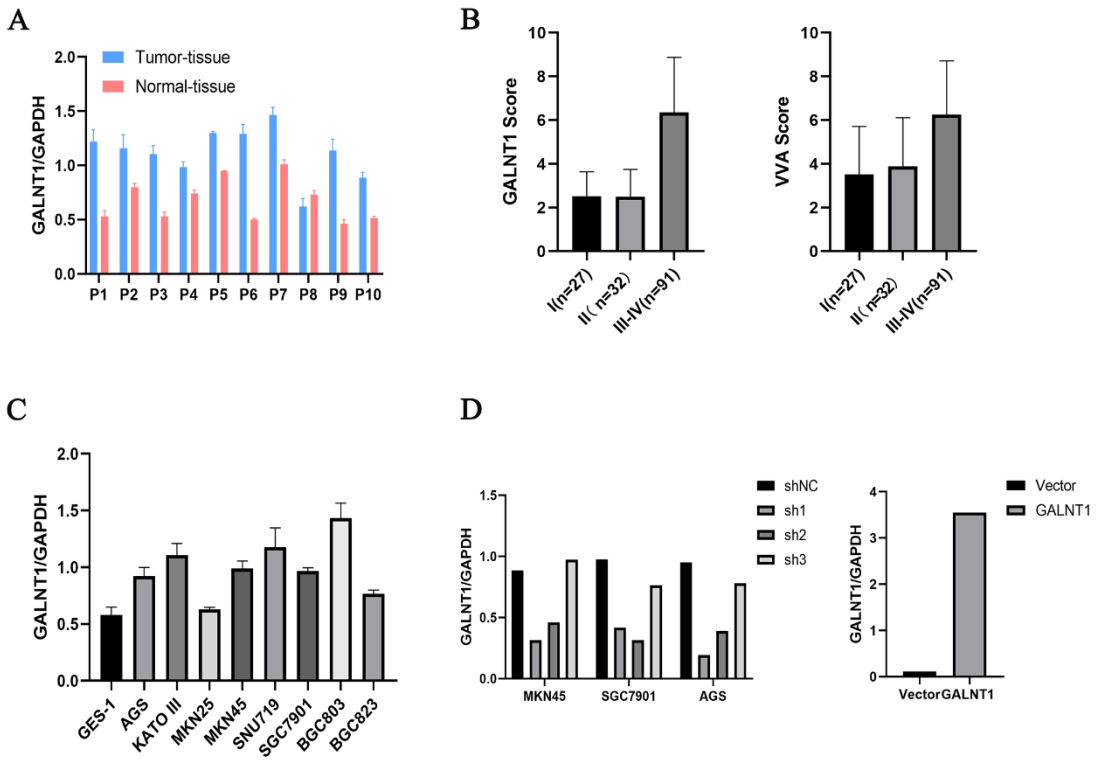
Supplementary figure S2: GALNT1 promotes the growth and metastatic ability of AGS cells and modifies O-glycosylation of CD44. **A**–**B**, AGS cells' growth abilities after *GALNT1* knockdown were determined by CCK8 assay (**A**) and colony formation assay (**B**). **C**, *GALNT1* knockdown decreased the abilities of migration and invasion of AGS cells.



Supplementary figure S3: TOP luciferase reporter assays were measured in GALNT1-silenced (**A**) and -overexpressing cells (**B**). Normalization was based on internal Renilla luciferase activity.



Supplementary figure S4: *GALNT1* knockdown decreased the O-glycosylation levels of CD44 by co-immunoprecipitation in AGS cells (**A-B**).



Supplementary figure S5: (A) The quantification of *GALNT1* levels in paired primary tumors and adjacent normal tissues. **(B)** Statistical analysis of *GALNT1* staining at different clinical stages. **(C)** The quantification of *GALNT1* levels in gastric cancer cell lines. **(D)** The quantification of the silencing efficiency of GALNT1 in MKN45, AGS, and SGC7901 cells as well as the overexpression of GALNT1 in AGS cells.

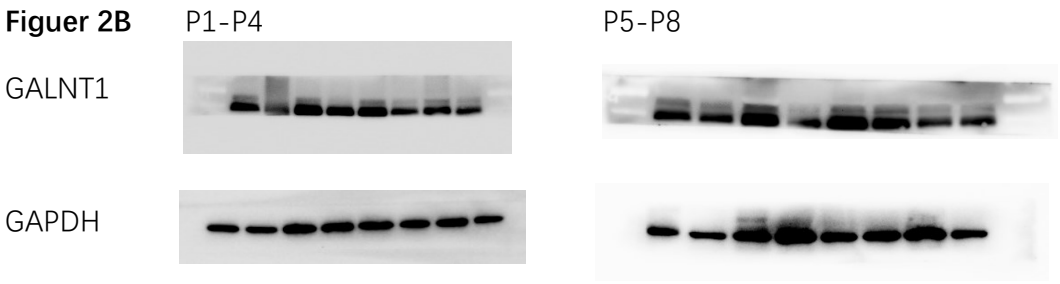
Table S1. The target sequence of sh*GALNT1* and shCD44

Gene	Target sequence
sh*GALNT1*-1	5′− GCTTGGATGTTTCCAAACTTA −3’
sh*GALNT1*-2	5′− ATTGATCAGAGCTAGATTAAA −3’
sh*GALNT1*-3	5′− GACGTGAAACTGCATAGTAAT −3’
ShCD44-1	5′− GGCGCAGATCGATTTGAATAT−3'
shCD44-2	5′− CCAGTATGACACATATTGCTT−3'
shCD44-3	5′−CCATTCAAATCCGGAAGTGCT−3'

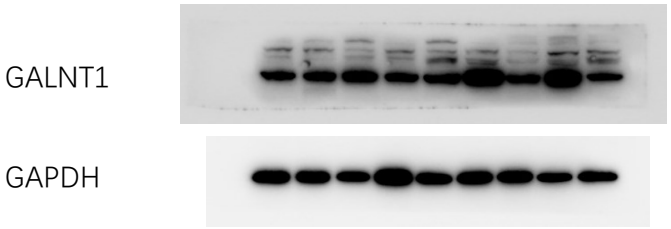
Table S2. Sequences of primers used in this study for qRT-PCR.

Gene	Sequence
GALNT1	F:5′- GTGAGATGATTGCACTCAACAGA -3′
	R:5′- ACTATGGACAGTTCGCAGAAGT -3′
GAPDH	F:5′- CGGAGTCAACGGATTTGGTCGT -3′
	R:5′- TCTCAGCCTTGACGGTGCCA -3′
CD44	F:5′- TCCTCACATCCAACACCTCC-3′
	R:5′- GCTGCTCACGTCATCATCAG -3′

Figuer 2B



Figuer 3A



Figuer 3B

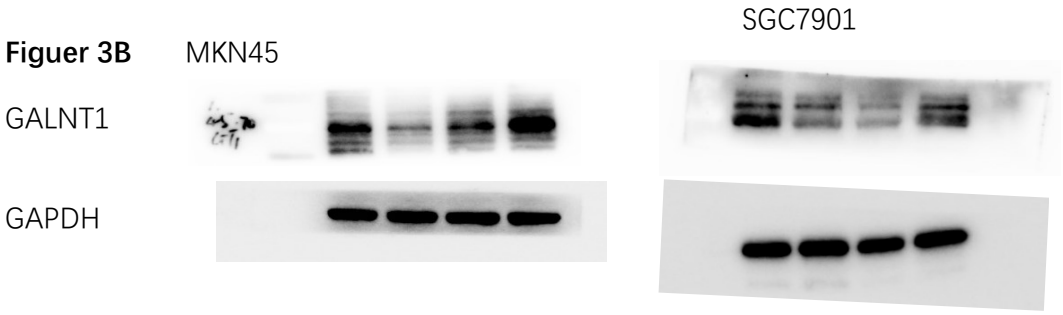
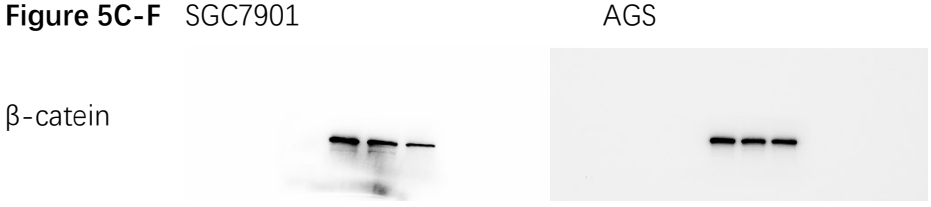
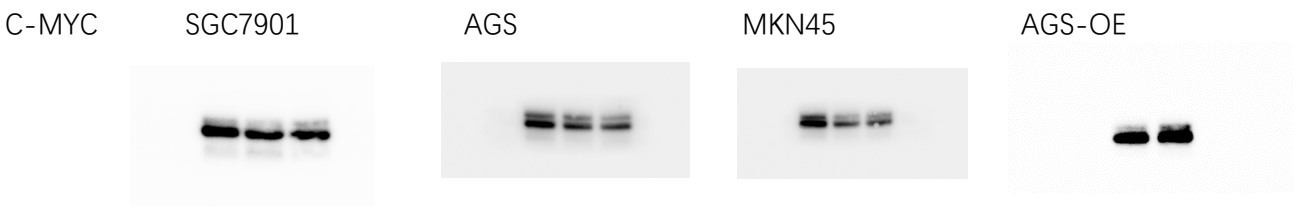
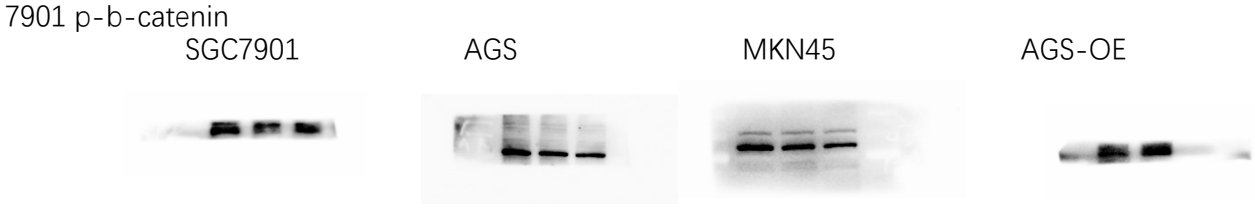
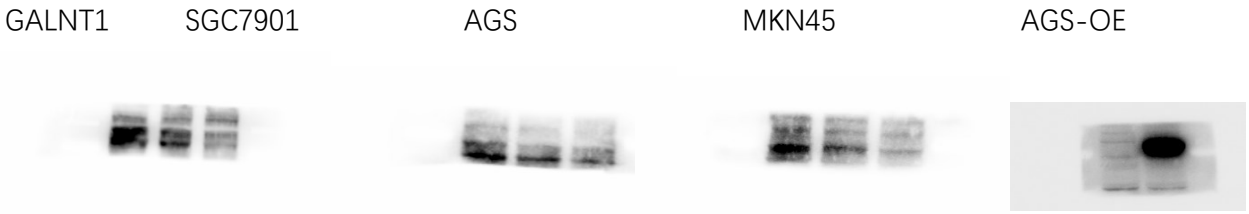


Figure 5C-F









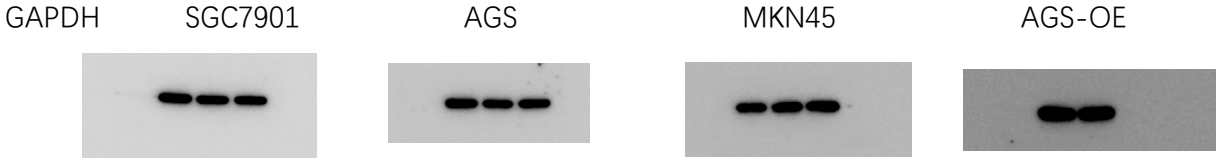


Figure 5G

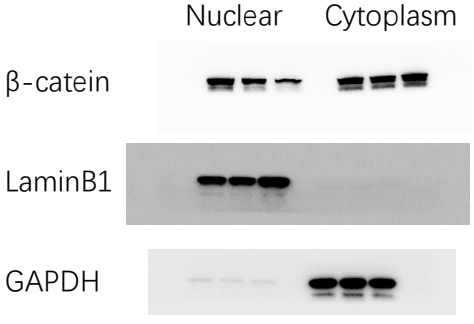


Figure 5H

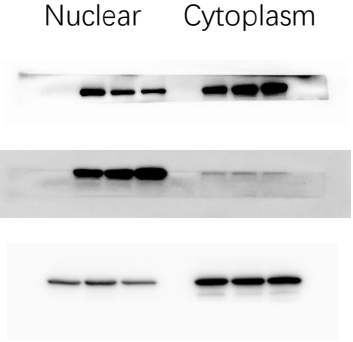


Figure 5I AGS

Nuclear Cytoplasm

β-catein

LaminB1

GAPDH

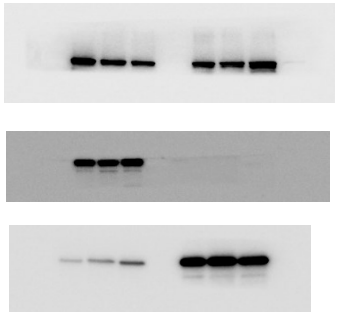


Figure 5J AGS-OE

Nuclear Cytoplasm

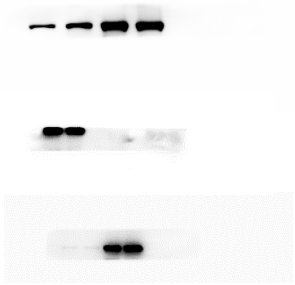


Figure 6A MKN45

INPUT IP:CD44 IP:CD44

CD44

IB:VVA

GALNT1

GAPDH

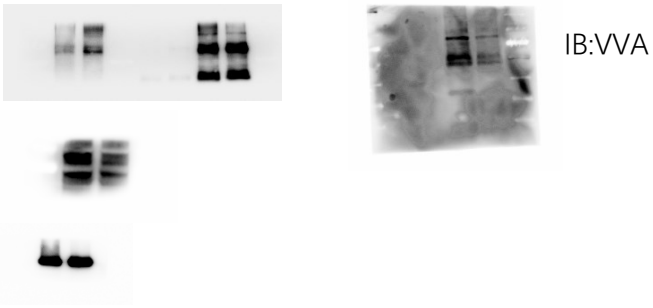


Figure 6B SGC7901

INPUT IP:CD44 IP:CD44

CD44

IB:VVA

GALNT1

GAPDH

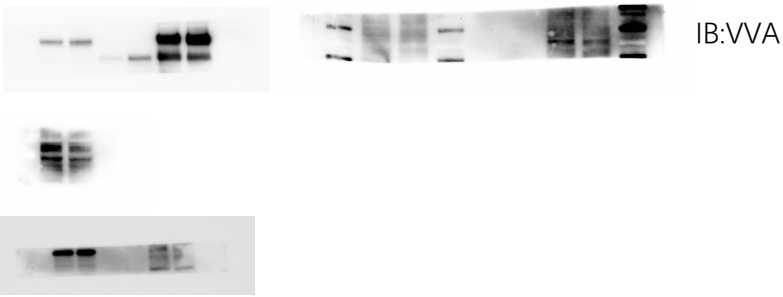


Figure 6C AGS-OE

INPUT IP:CD44 IP:CD44

CD44

IB:VVA

GALNT1

GAPDH

Figure 6D MKN45

IP:VVA

IB:CD44

GALNT1

GAPDH

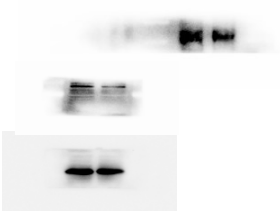


Figure 6E SGC7901

IP:VVA

IB:CD44

GALNT1

GAPDH

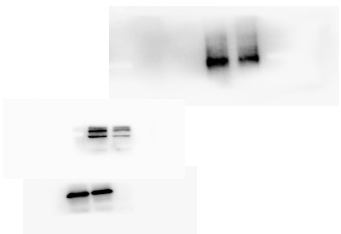


Figure 6F

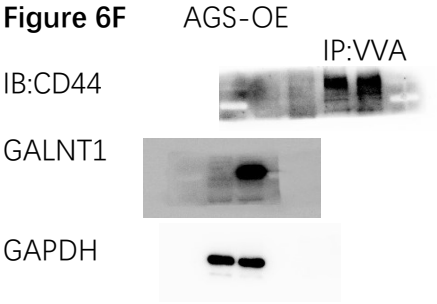


Figure 6G

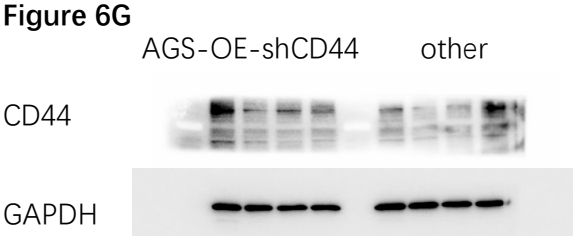


Figure 6K

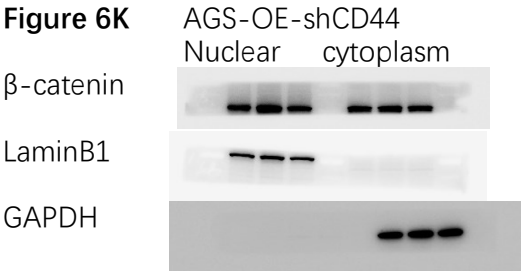


Figure S4A

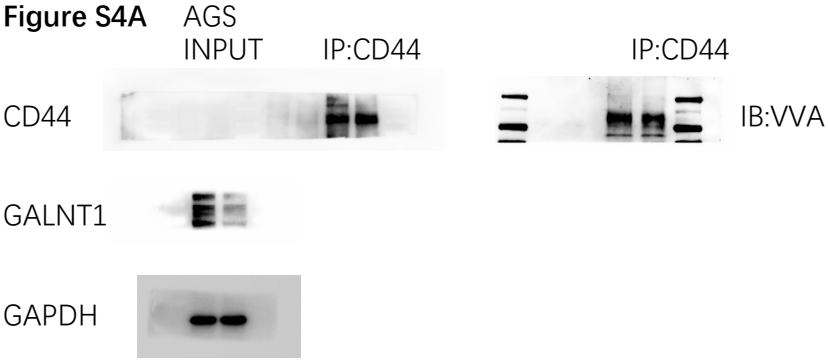


Figure S4B