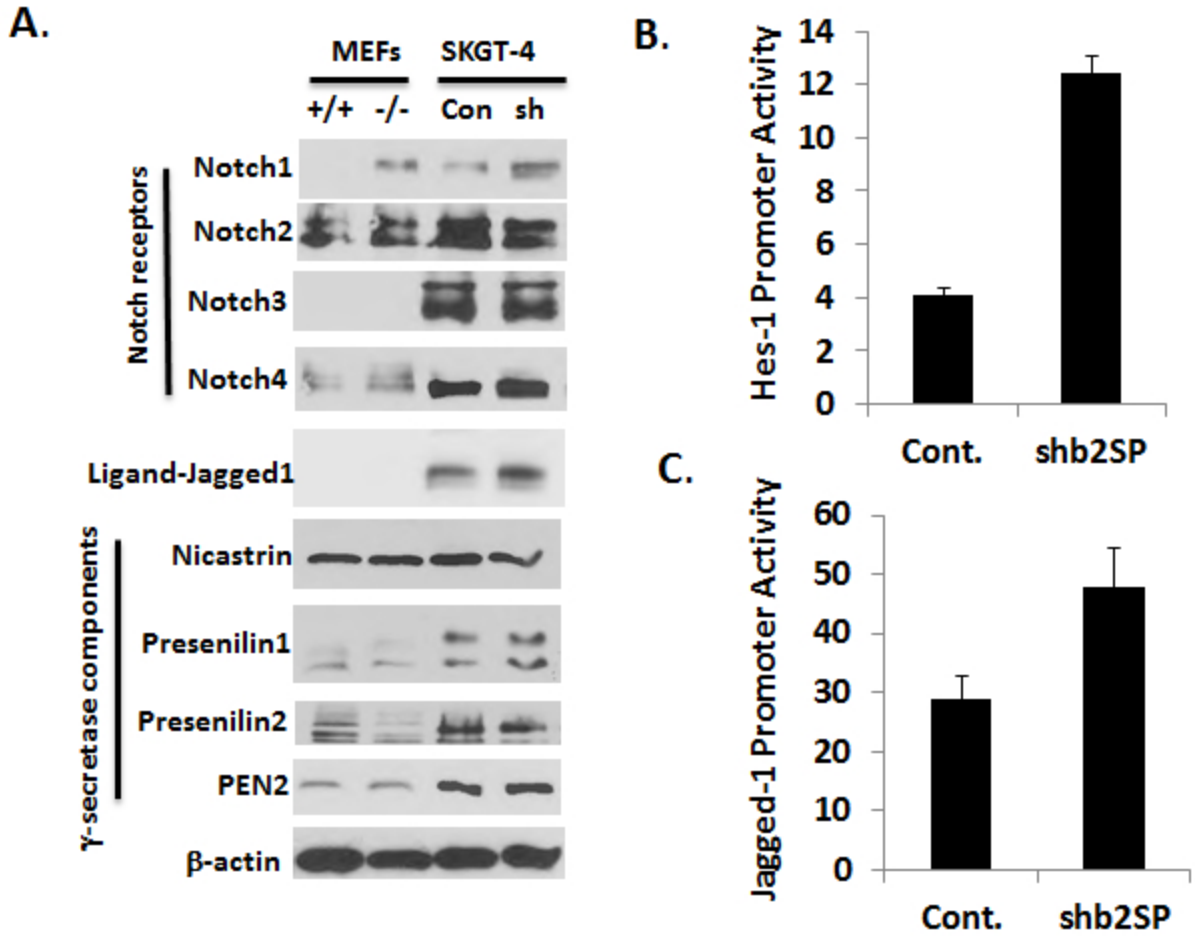
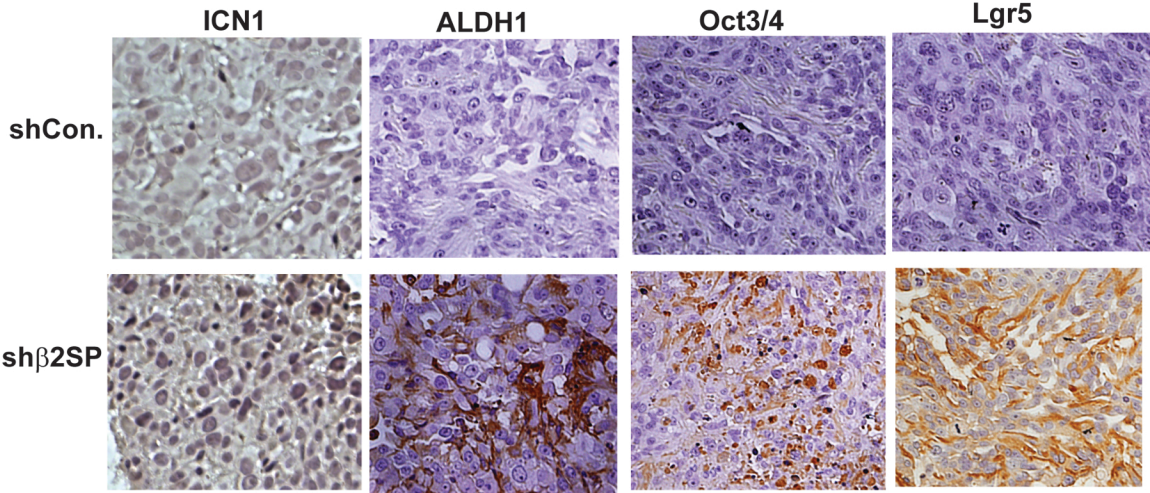
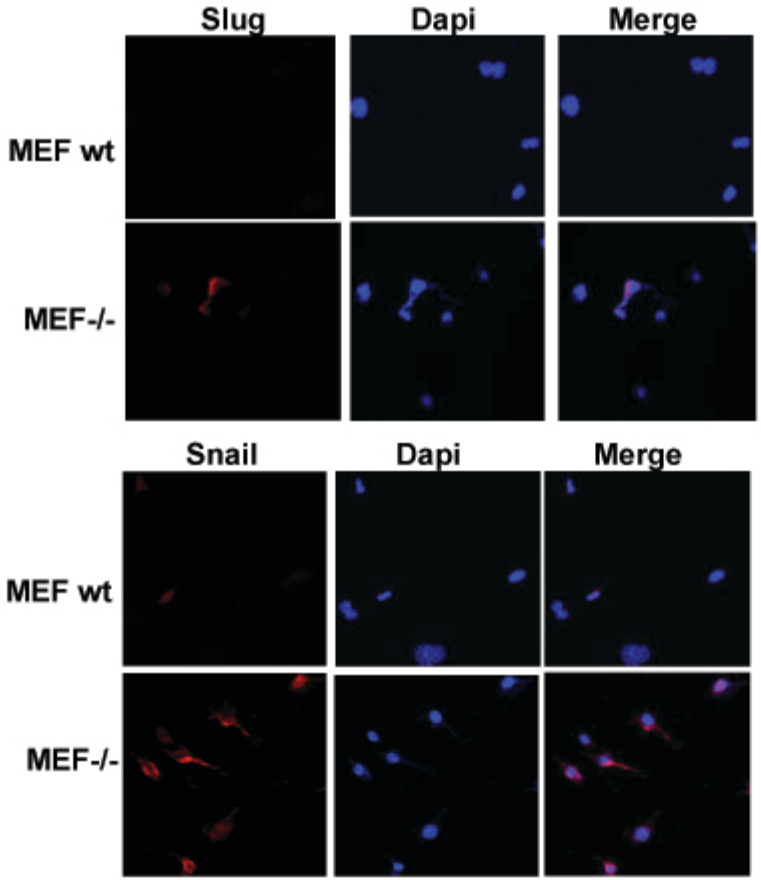


Supplemental Figure 1. Effects of loss of β2SP on Notch receptors, ligand and γ-secretase complex. **A.** Notch receptors (Notch 1-4), Ligand-Jagged1 and γ-secretase complex components-Nicastrin, Presenilin1/2 and PEN2 were detected by immune-blotting as described in Material&Methods. **B&C.** Hes-1 and Jagged1 promoter activities (luciferase) were detected after transient transfection of their luciferase promoters into SKGT-4 cells with Cont. or shβ2SP knock down as described in Material&Methods.

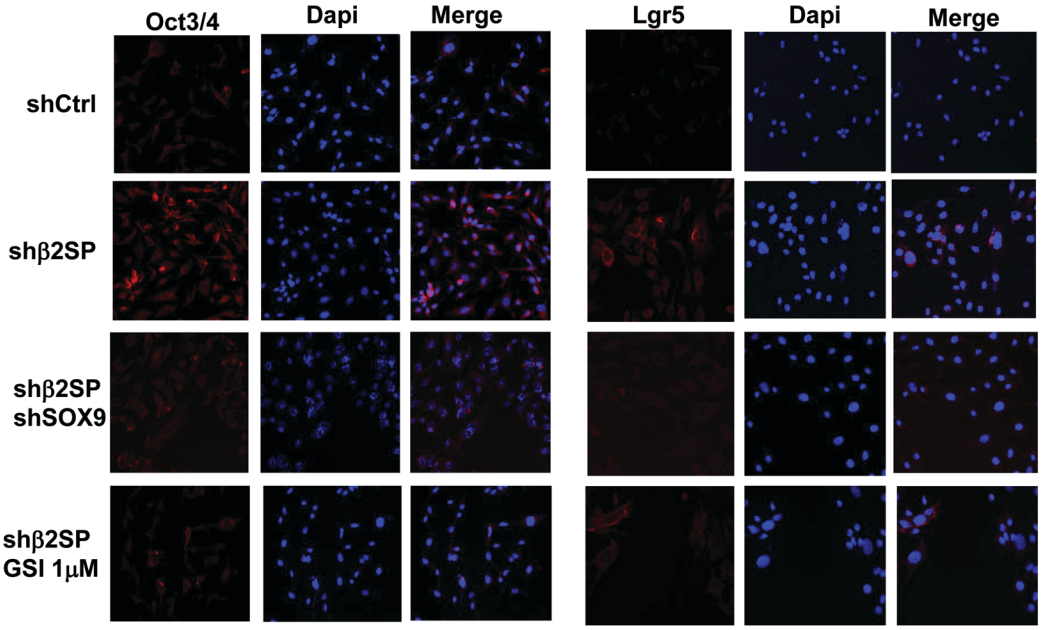
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



P=0.030		SOX9 Total by 5		
		<5	>=5	Total
Pathological N	N0	10	18	28
	N1	12	63	75
Total		22	81	103

N0: Lymph node negative
N1: Lymph node positive

Supplemental Table 1. Pathological lymph node positivity categorized by SOX9 nuclear staining intensity; The tumors were categorized into SOX9-low (combined scores <5) and SOX9-high (combined score ≥ 5) based on the scoring of nuclear staining in tumor cells and staining intensity. High SOX9 nuclear expression correlates with lymph node metastasis (p=0.03).

Figure legends for Supplemental Figures

Supplemental Figure 1. Deletion of β2SP in MEFs leads to upregulation of EMT transcription factors. Indirect immunofluorescence was performed on MEFs cell with or without b2SP deletion cells using anti-Slug or anti-Snail antibodies and followed by DAPI counterstaining (blue). The merge of Slug or Snail (red) with DAPI (blue) is also shown.

Supplemental Figure 2. Increased putative stem cell markers was in mice tumors with knockdown β2SP in the nude mouse of xenograft. A. Tumor weight of four groups in xenograft mouse model was recorded by end of experiments. Significant indicated in the bar graph. B. Expression of ALDH-1, Oct3/4 and Lgr5 were determined by immunohistochemistry as described in materials&Methods.

Supplemental Figure 3. Increased expression of stem cell markers in shβ2SP EA cells, while shSOX9 and Notch inhibitor GSI rescue the phenotype of shβ2SP. . Indirect immunofluorescence was performed on SKGT-4 cells with shControl, shb2SP, double shβ2SP and shSOX9 and shβ2SP treated with GSI 5μM using anti-Oct3/4 or anti-Lgr5 antibodies and followed by DAPI counterstaining (blue). The merge of Slug or Snail (red) with DAPI (blue) is also shown.

Supplemental Figure 4. loss of β2SP increases EA cell tumor sphere formation and invasion. A&B. SKGT-4 and BE3 cells ($1\text{-}3x10^3$) were seeded in triplicate onto a 6-well ultra-low attachment plate. After 10-14 days of culture, the number of tumor spheres formed (diameter >100 μm) was counted under microscopy. D&E. SKGT-4 and BE3 cells ($1x10^5$) in which β2SP expression was knocked down by shRNA (shβ2SP) or vector control (Con.) were seeded onto Matrigel-coated invasion chambers; 24 hours later, invading cells that adhered on the lower surface of the membrane were fixed, stained with Diff-Quick stain, and counted. Representative fields are shown left panel (D) and the Bar graph in right panel (E). Experiments performed in triplicate; bars, standard errors, *$p<0.001$.