

Supplementary Figure S1.

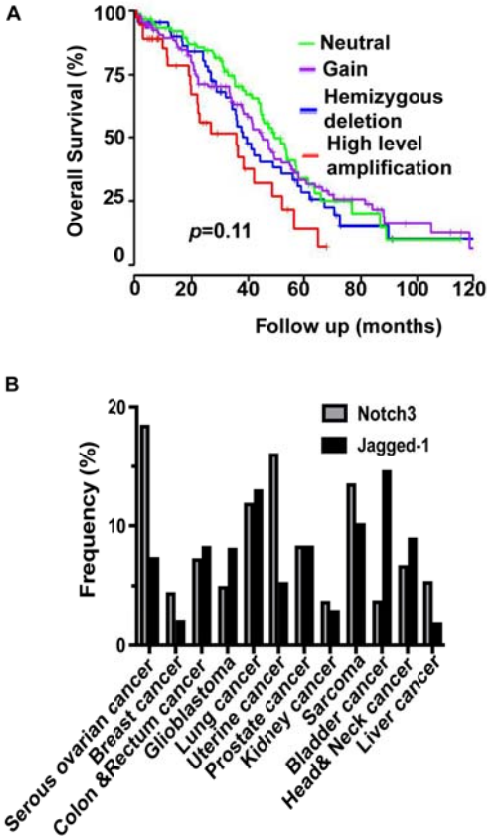


Figure S1. Overall Survival (OS) Analysis in Patients With High-Grade Serous Ovarian Cancer HGS-OvCa (A) OS durations in cases with amplification of Notch 3 versus gain, neutral, or hemizygous deletion of Notch 3. (B) Frequency of Notch 3 and Jag 1 cross human cancers.

Supplementary Figure S2 .

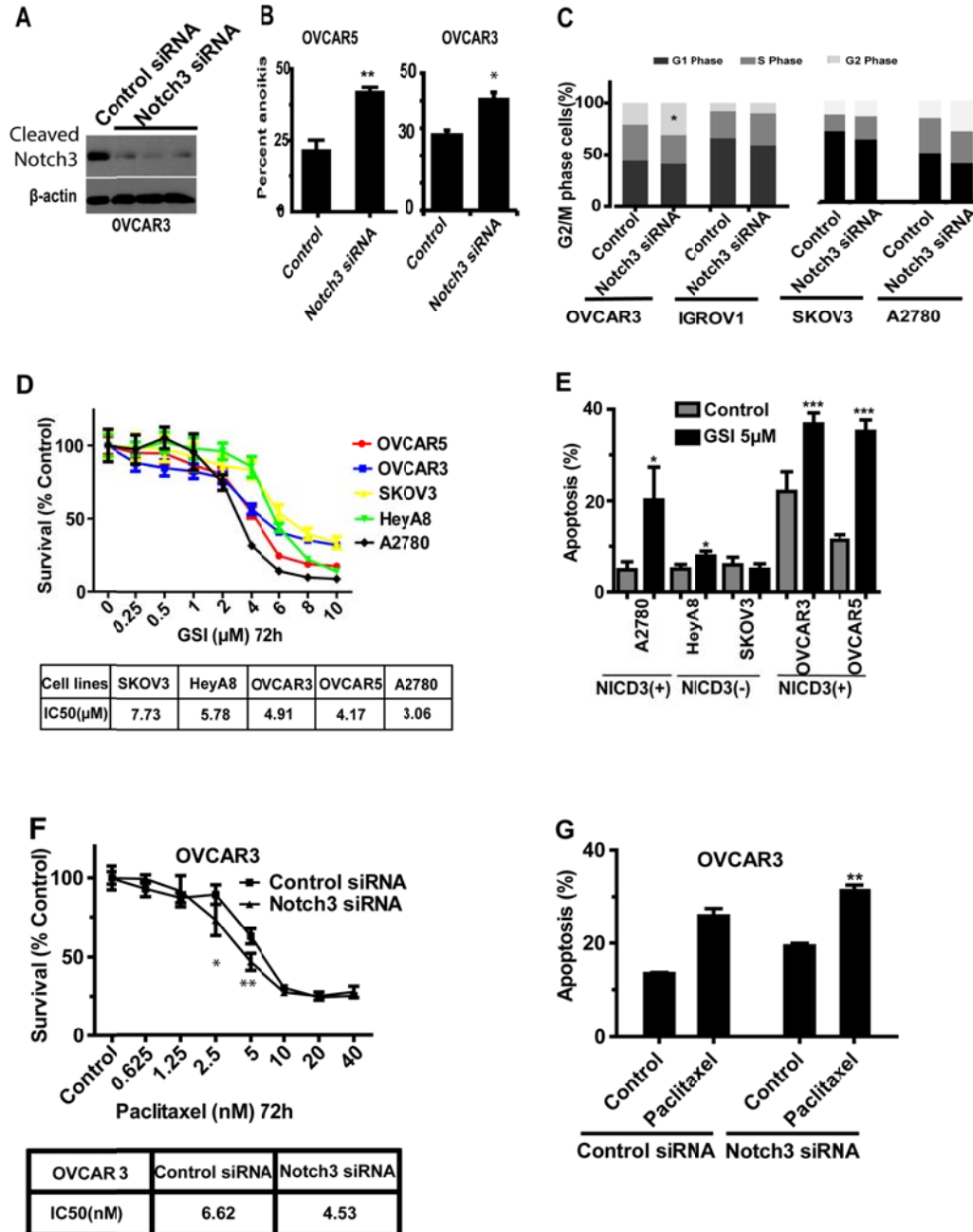


Figure S2. In Vitro Functional Studies of Notch3 siRNA or GSI and combined with Paclitaxel in OvCa Cells

(A) Western blot analysis of intracellular domain (NICD3) expression in OVCAR3 cells transfected with different Notch3 siRNAs. **(B)** Anoikis in NICD3+ cells (OVCAR5 and OVCAR3) cells with and without transfection of Notch siRNA as detected using Flow cytometry. **p < 0.01; * p<0.05 **(C)** Cell-cycle analysis of NICD3+ cells (OVCAR3, A2780) and NICD3- cells (IGROV1 and SKOV3) after exposure to Notch3 siRNA as detected using flow cytometry. *p < 0.05. **(D)** MTT-based survival analysis of GSI-treated OvCa cells. **(E)** Apoptosis in A2780 cells after exposure to GSI at 5µM as detected using flow cytometry. *p < 0.05; ***p < 0.001. **(F)** MTT analysis of Notch3 silencing in combination with paclitaxel-based treatment in OVCAR3 cells. **p < 0.01; * p<0.05 **(G)** Apoptosis in OVCAR3 cells after exposure to Notch3 siRNA and paclitaxel as detected using flow cytometry. **p<0.01.

Supplementary Figure S3.

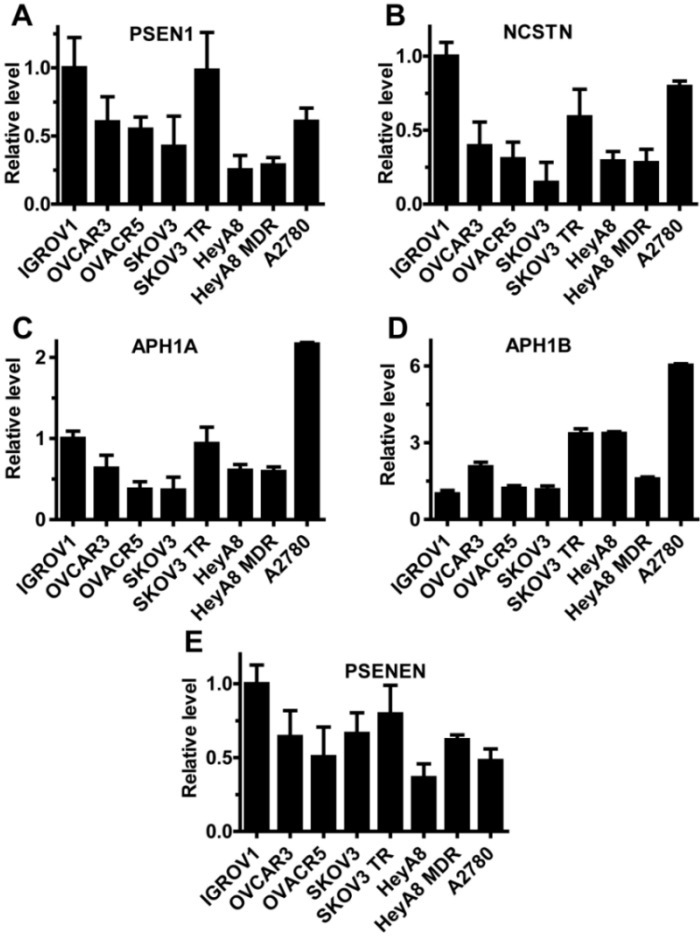
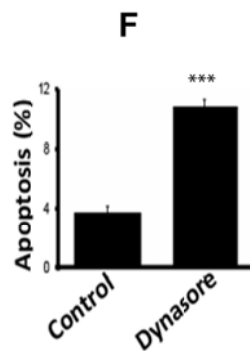
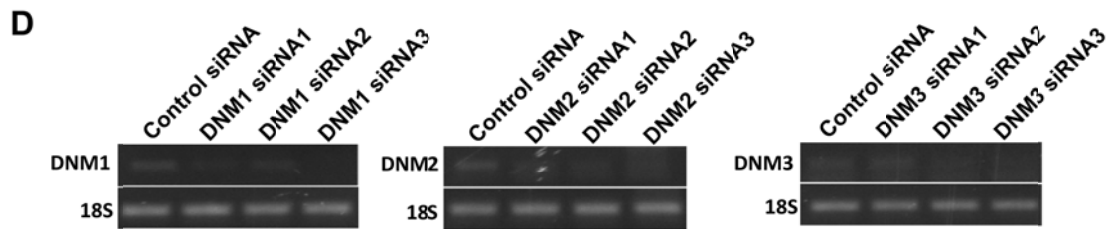
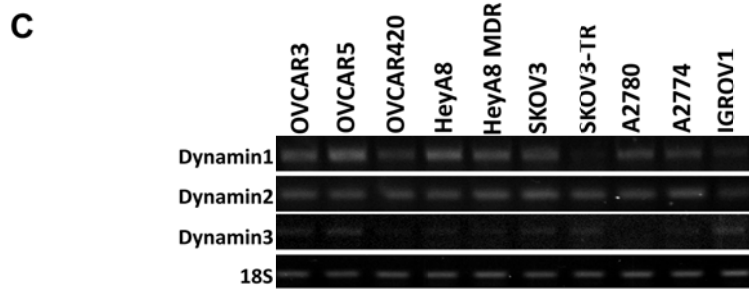
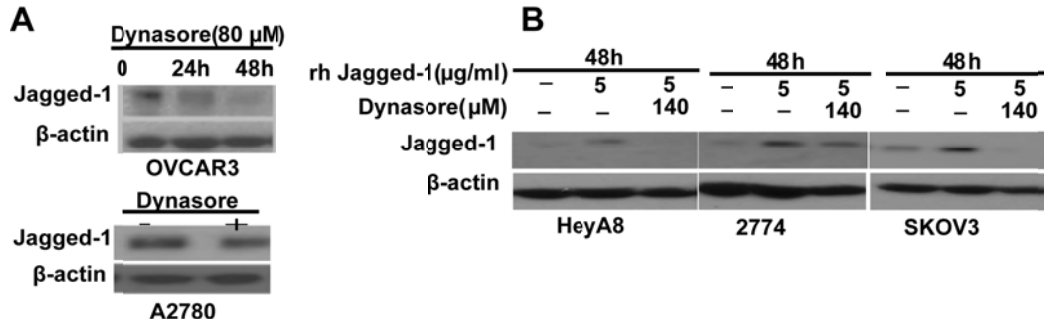


Figure S3. qRT-PCR Analysis of Expression of Gamma-Secretase Complex in Ovarian Cancer Cells

(A) PSEN1 expression. (B) NCSTN expression. (C) APH1A expression. (D) APH1B expression. (E) PSENEN expression.

Supplementary Figure S4.



Supplementary Figure S4. In vitro effects of blocking endocytosis on Jagged-1/Notch3 pathway in cancer cells.

(A,B) Western blot analysis of Jagged-1 expression in ovarian cancer cells after exposure to Dynasore, or rh Jagged-1 or rhJagged-1 plus dynasore.

(C) Expression of DNM1, DNM2, and DNM3 in a panel of Ovarian cancer cells.

(D) Expression of DNM1, DNM2, and DNM3 in OVCAR3 cells treated with DNM siRNAs .

(E) Western blot analysis of expression of cleaved Notch3 and Jag1 in uterine cancer cells (Ishkawa) .

(F) Flow cytometry analysis of apoptosis in uterine cancer cells treated with or without dynasore.

Supplemental Figure 5.

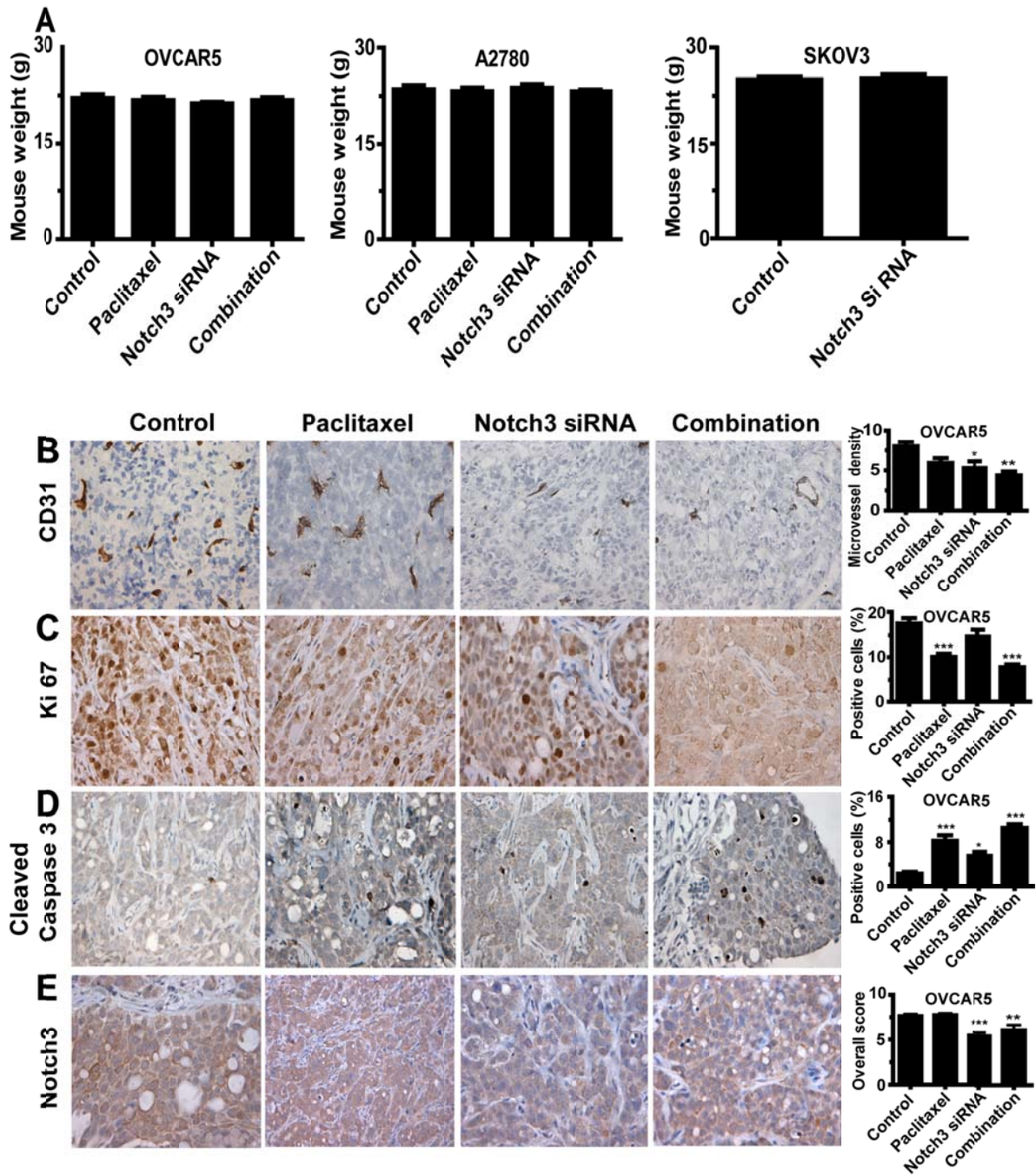


Figure S5. In vivo study of Notch3 siRNA in orthotopic mouse models of ovarian cancer.

(A) *In vivo* effect of Notch3 siRNA and/or paclitaxel on tumor weight in models of OVCAR5,

A2780, and SKOV3 ovarian cancer. Ovarian cancer cells were injected intraperitoneally into the mice. Seven days later, the mice were randomly allocated to four treatment groups (n = 10/group): 1) control siRNA, 2) paclitaxel (given intraperitoneally once a week), 3) human Notch3 siRNA, and 4) human Notch3 siRNA plus paclitaxel. Mouse weight was recorded. The error bars represent standard error of the mean (SEM).

(B-E). Biological effect of treatment with Notch3 siRNA on angiogenesis (CD31), cell proliferation (Ki67), apoptosis (Cleaved Caspase-3) and Notch3 expression in mouse models of HGS ovarian cancer. Original magnification, 200x. The graphs correspond sequentially to the images at left. The error bars represent SEM. *p <0.05; **p <0.01; ***p <0.001.