

Table S1. primer sequences for quantitative PCR analysis

Gene	Forward primer	Reverse primer
<i>Notch-3</i>	GTGTGTGTCAATGGCTGGAC	GTGACACAGGAGGCCAGTCT
<i>Jagged-1</i>	ATCGTGCTGCCTTTCAGTTT	GGTCACGCGGATCTGATACT
<i>Jagged-2</i>	AGCTGGAACGAGACGAGTGT	TCTTGCCACCAAAGTCATCA
<i>DLL1</i>	ACTCCTACCGCTTCGTGTGT	ATGCTGCTCATCACATCCAG
<i>DLL3</i>	TGAGCATGGCTTCTGTGAAC	CATTCAAAGGACCTGGGTGT
<i>DLL4</i>	GCACTCCCTGGCAATGTACT	CTCCAGCTCACAGTCCACAC
<i>App</i>	GTGAAGATGGATGCAGAATTCCG	AAAGAACTTGTAGGTTGGATTTTCG

Fig. S1

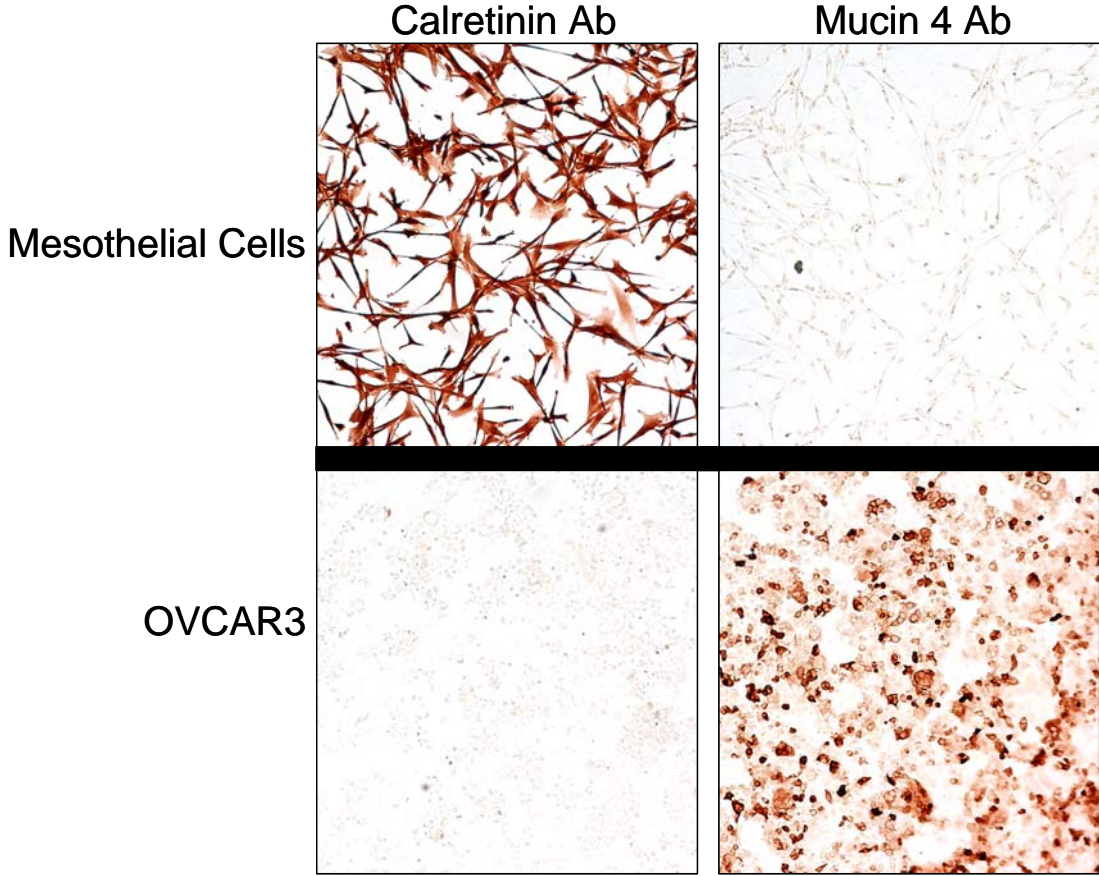


Figure S1. Expression of calretinin and mucin 4 in mesothelial cell culture using immunocytochemistry. A representative primary mesothelial cell culture and an ovarian cancer cell line OVCAR3 were incubated with anti-calretinin and anti-mucin 4 antibodies. Strong immunoreactivity for calretinin can be detected in mesothelial cells, but not in OVCAR3. Conversely, mucin 4 staining is detected in OVCAR3 but not in mesothelial cells.

Fig. S2

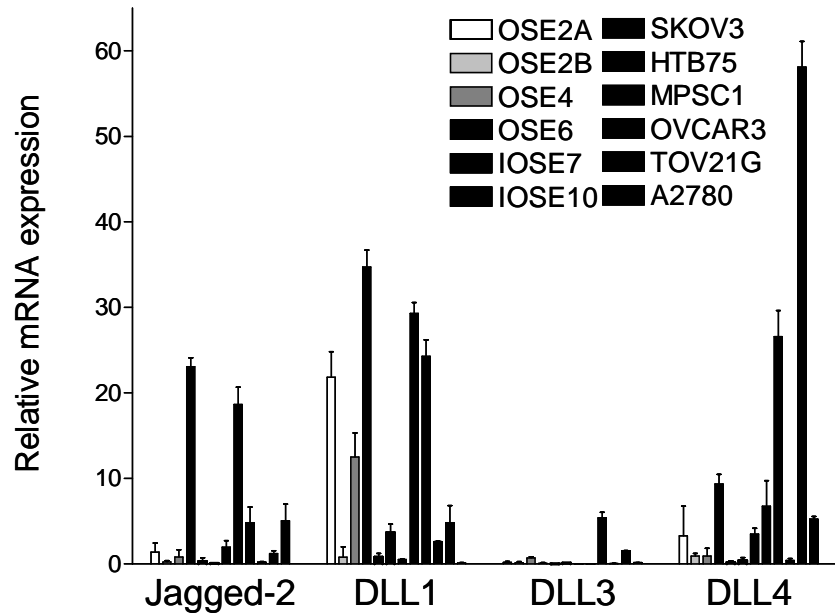


Figure S2. Expression of Notch ligands other than Jagged-1 in ovarian cancer cells. mRNA expression levels of Notch ligands including Jagged-2, DLL1, DLL3, and DLL4 were measured by quantitative RT-PCR in six immortalized ovarian surface epithelial (OSE) cell lines and six ovarian cancer cell lines. Note: The scale used to plot is different than Fig. 1.

Fig. S3

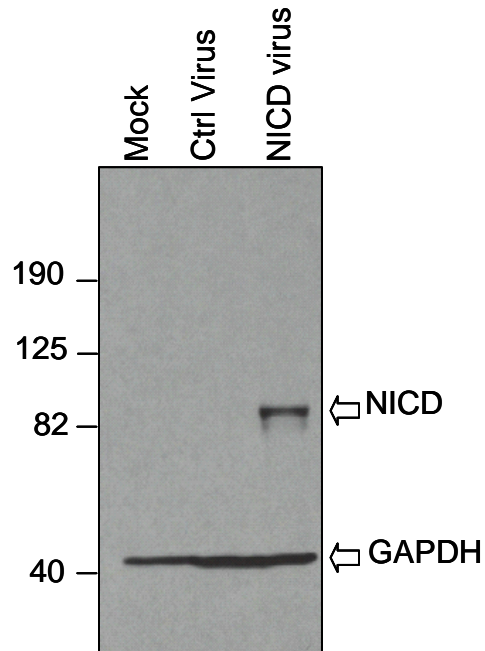


Figure S3. Determination of Notch3 antibody specificity. To validate the antibody specificity of the Notch3 antibody, Western blot was performed in HeLa cells transduced with a retrovirus expressing the intracellular domain of Notch3 (NICD). A single band corresponding to the predicted molecular weight of NICD construct (~84 kD) was detected. This band is absent in non-transduced HeLa (mock) or empty vector-transduced HeLa cells.

Fig. S4

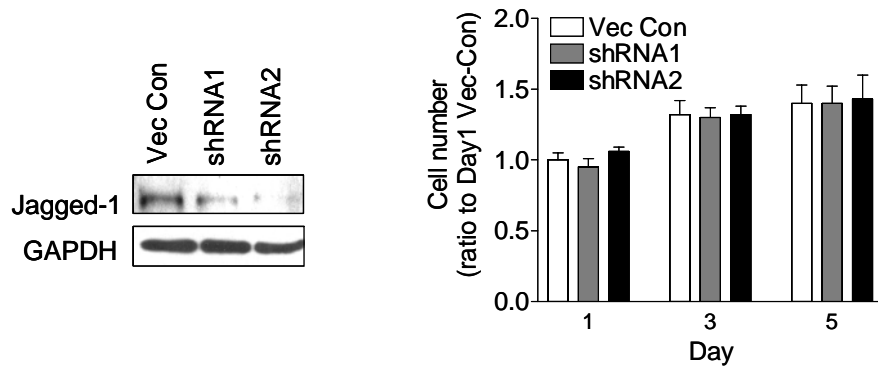


Figure S4. Jagged-1 shRNA does not affect growth of mesothelial cells.

Jagged-1 shRNAs were found to reduce Jagged-1 protein expression in mesothelial cells (left panel). Treatment of Jagged-1 shRNAs in mesothelial cells, however, does not affect growth of mesothelial cells (right panel).

Fig. S5

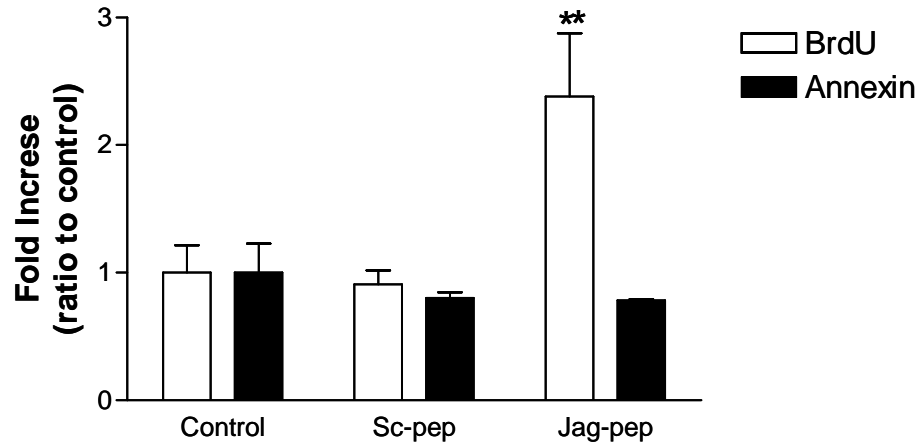


Figure S5. Jagged-1 peptide stimulates BrdU incorporation in OVCAR3 cells.

OVCAR3 cells were incubated with Jagged-1 or scramble peptide, and 48 hours after, the cells were analyzed for BrdU incorporation and membrane annexin V staining. Increased BrdU incorporation was observed in Jagged-1 peptide- treated group as compared to the scramble peptide-treated group or mock control ($p < 0.01$, Student's *t* test). However, Jagged-1 peptides did not affect the percentage of cells stained with annexin V in the OVCAR3 cells.

Fig. S6

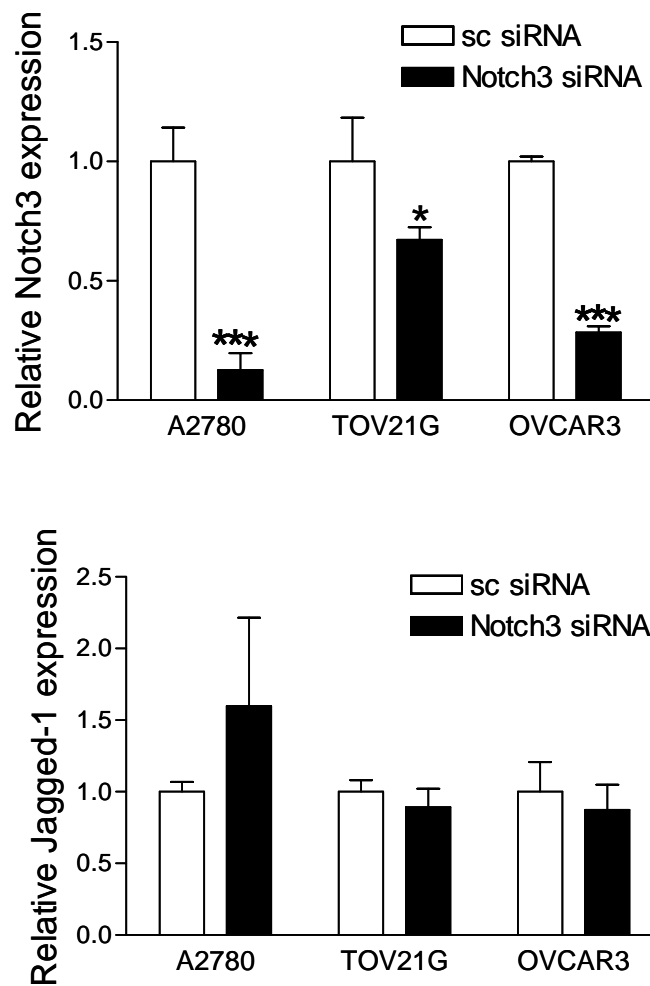


Figure S6. Notch3 does not affect Jagged-1 mRNA expression. Notch3-specific siRNA was applied to ovarian cancer cell line, A2780, TOV21G, and OVCAR3. The effect of Notch3 siRNA on Notch3 mRNA expression (upper panel) as well as its ability to affect Jagged-1 mRNA expression (lower panel) was determined using quantitative real-time PCR.

Fig. S7

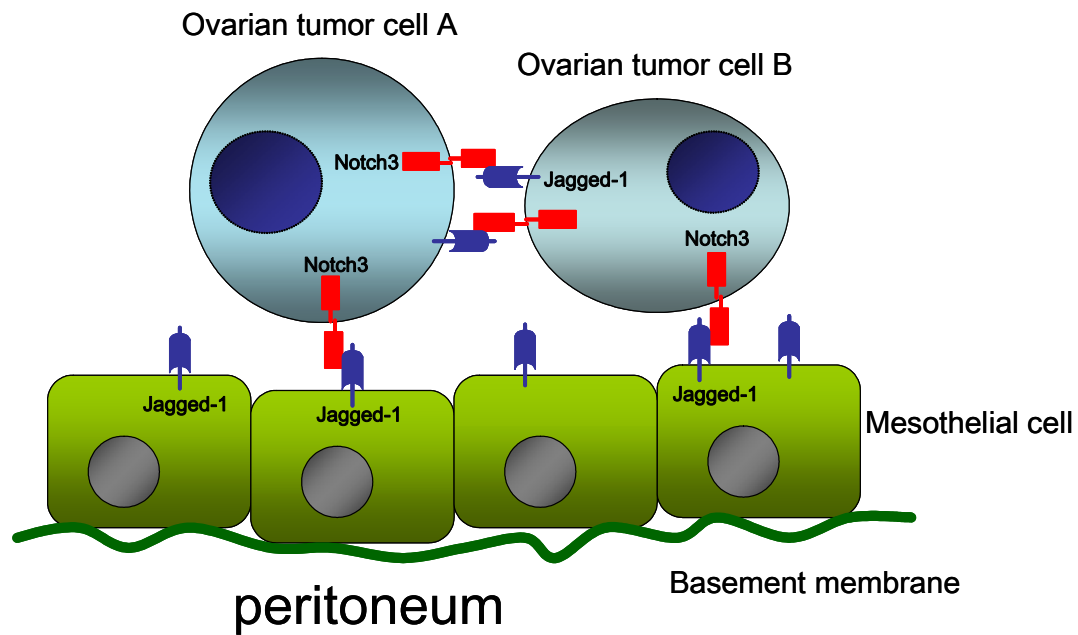


Figure S7. Notch3/Jagged-1 interaction among ovarian tumor cells and between neighboring mesothelial cells and tumor cells. Jagged-1 and Notch3 forms a juxtacrine loop among ovarian tumor cells. During tumor cell dissemination in the peritoneal cavity, Notch3 expressed in ovarian cancer cells binds to Jagged-1 expressed in neighboring mesothelial cells. The receptor-ligand interaction enhances tumor cell binding to and growth on the peritoneal surface, thus facilitating intra-peritoneal tumor dissemination.