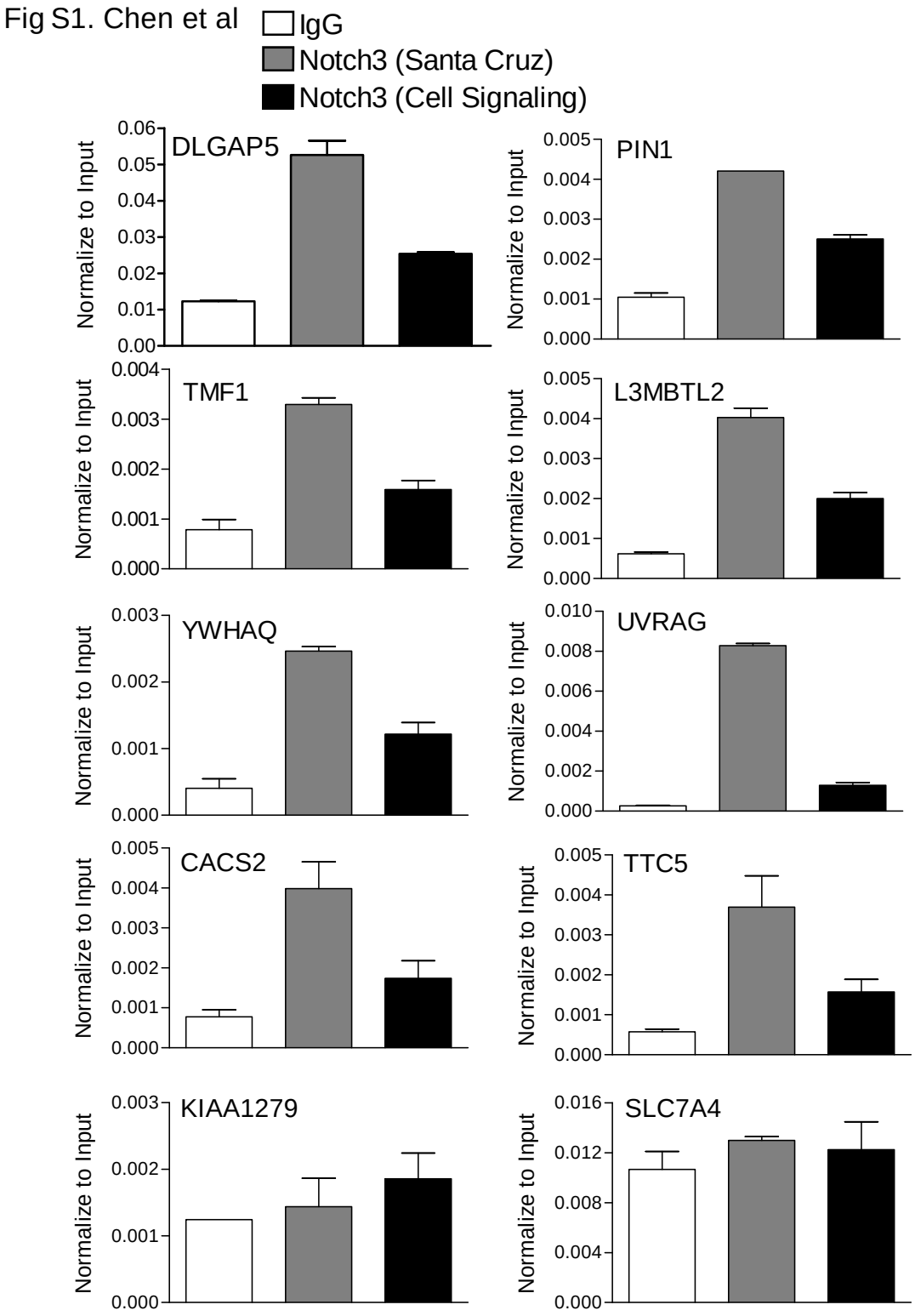

Fig S1. Validation of ChIP-on-chip study by two different anti-NOTCH3 antibodies. ChIP was performed using two different anti-NOTCH3 antibodies. qPCR demonstrates an agreement of sequence enrichment using either of the antibodies.

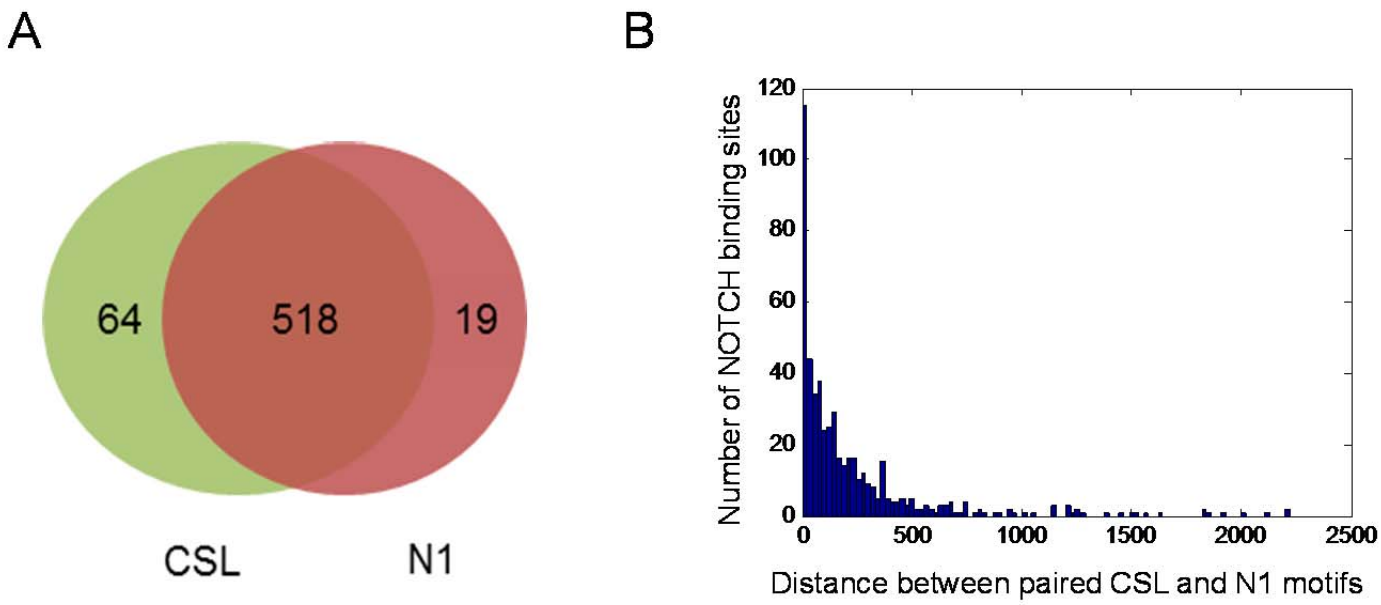

Fig S2. Events and distribution of canonical CSL and N1 motifs at Notch3-bound promoters.

A. NOTCH3-bound promoter sequences were extracted from the BioMart database (601 sequences available), and were screened for the presence of canonical CSL and N1 motifs.

B. Distribution of distance between paired CSL and N1 motifs.

NOTCH1 ChIP Targets

Motif Name	n1B	n2B	n1C	n2C	Enrichment score	p-value (Hypergeometric test)
N1 Motif	79	105000	23550	50279108	1.61	3.26e-005
Canonical CSL Motif	196	105000	18681	50279108	5.02	<1e-013

NOTCH3 ChIP Targets

Motif Name	n1B	n2B	n1C	n2C	Enrichment score	p-value (Hypergeometric test)
N1 Motif	125	131595	23550	50279108	2.03	4.1378e-13
Canonical CSL Motif	64	131595	18681	50279108	1.31	0.0157

n1B: number of occurrence in ChIP-chip bound promoter regions
n2B: total sequence length in ChIP-chip bound promoter regions
n1C: number of occurrence in promoter regions elsewhere
n2C: total sequence length in promoter regions elsewhere
Enrichment score = (n1B/n2B)/(n1C/n2C)
p-value:

$$pvalue = \sum_{i=n1B+1}^{n2B} \binom{n2B}{i} p^i (1-p)^{n-i}.$$

$$where \quad p = n1C / n2C$$

Fig S3. De novo motif discovery in Notch1 and Notch3-ChIP targeted promoter sequences. Motif binding events and sequence length used in determining the enrichment of each motif is shown. Equations for the enrichment score as well as *p* value determined by hypergeometric test are presented.

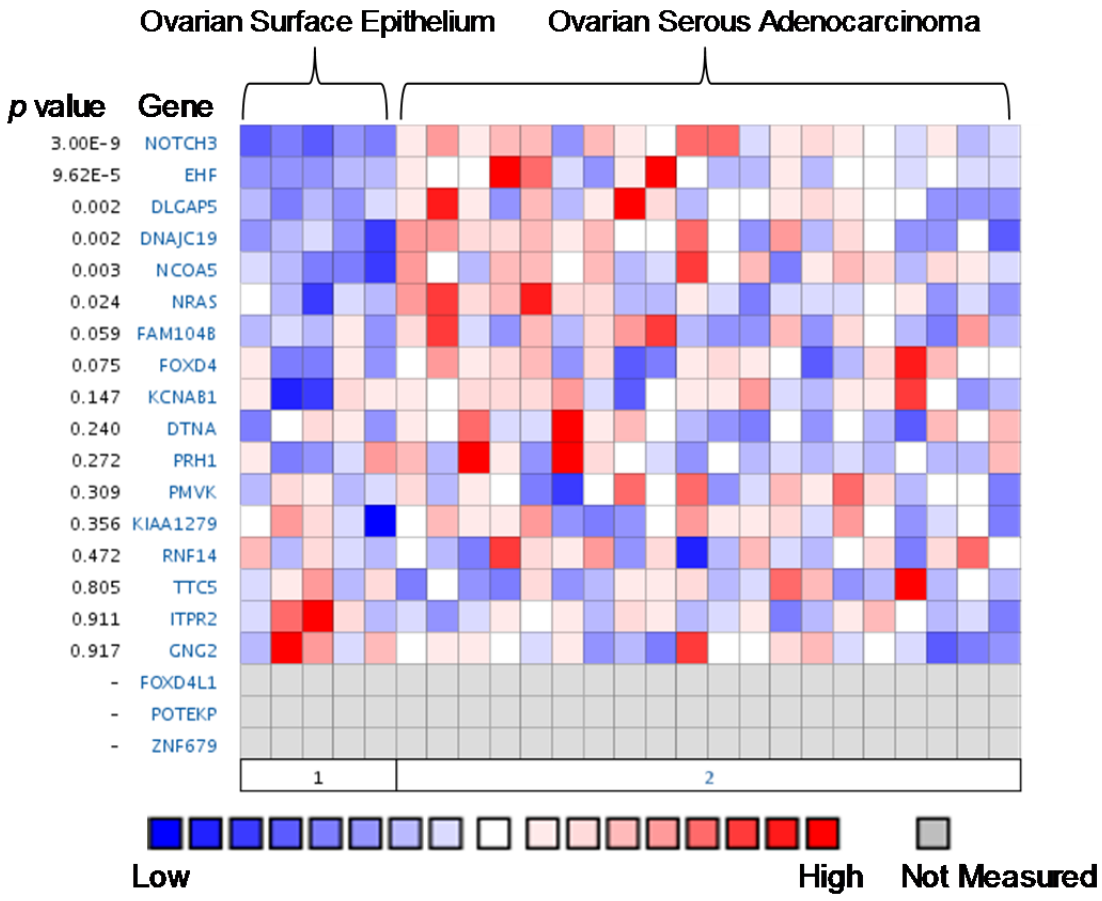

Fig S4. Expression of NOTCH3-upregulated genes in carcinoma tissues. Gene expression analysis to determine the co-upregulation of NOTCH3 and its target genes was performed on a previously published dataset[1] using Oncomine™ web tool (Compendia Bioscience, Ann Arbor, MI). *P* value was determined using Student's *t* test.

[1]Lu KH, Patterson AP, Wang L, Marquez RT, Atkinson EN, et al. (2004) Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res 10:3291-3300.

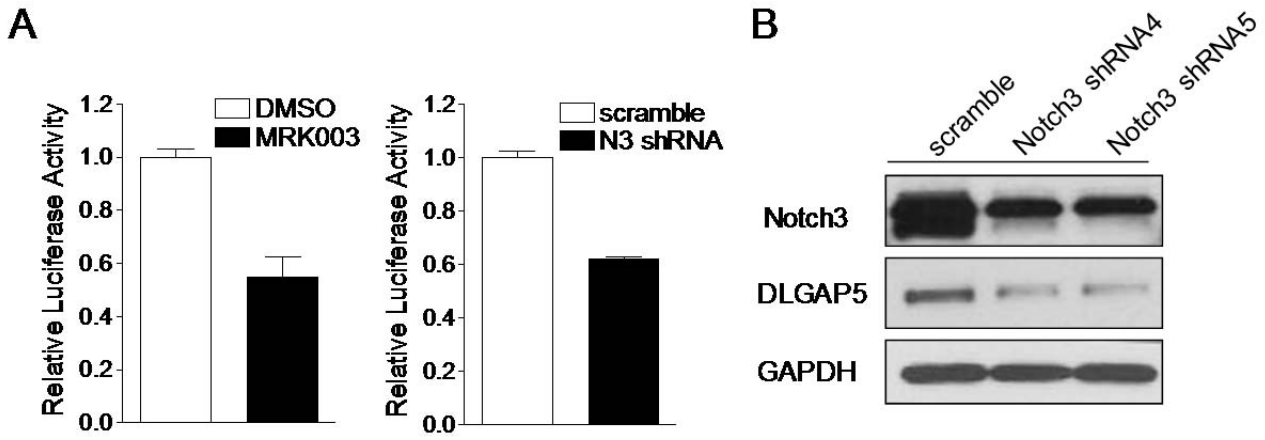

Fig S5. Regulation of DLGAP5 transcription and protein expression by NOTCH3. DLGAP5 luciferase reporter activity is suppressed by either MRK-003 or NOTCH3 shRNA in OVCAR3 cells. Luciferase activity was normalized to either DMSO or scrambled shRNA. **B.** NOTCH3 shRNA reduces NOTCH3 protein levels and concomitantly decreases DLGAP5 protein levels in OVCAR3.

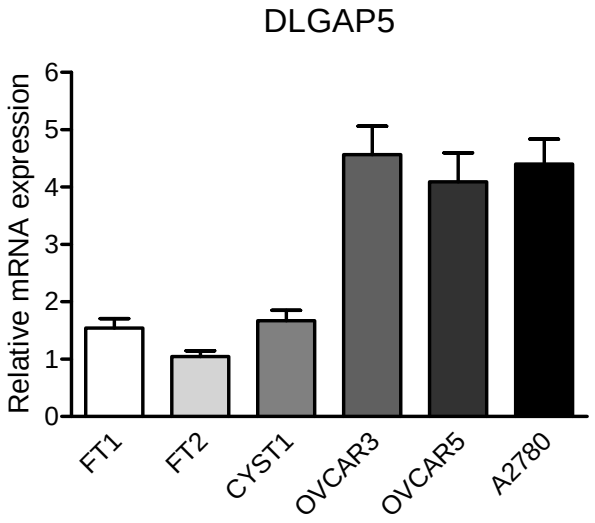

Fig S6. DLGAP5 expression in three ovarian cancer cell lines. mRNA expression was determined by quantitative RT-PCR in three ovarian cancer cell lines (OVCAR3, OVCAR5, and A2780). Primary fallopian tube epithelial cell cultures (FT1 and FT2) and a primary benign serous cystadenoma culture (Cyst1) were also analyzed for comparison. In this experiment, expression values were normalized to that of FT2.

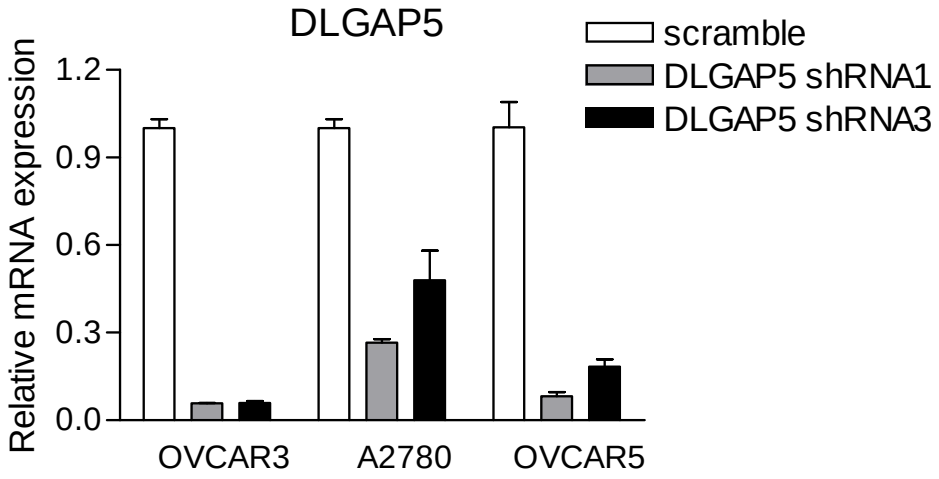

Fig S7. Knockdown efficiency of DLGAP5 shRNAs. Both DLGAP5 shRNAs (1 and 3) significantly reduce the level of DLGAP5 transcript expression as compared to scramble shRNA in a panel of ovarian cancer cell lines.

Fig S8.

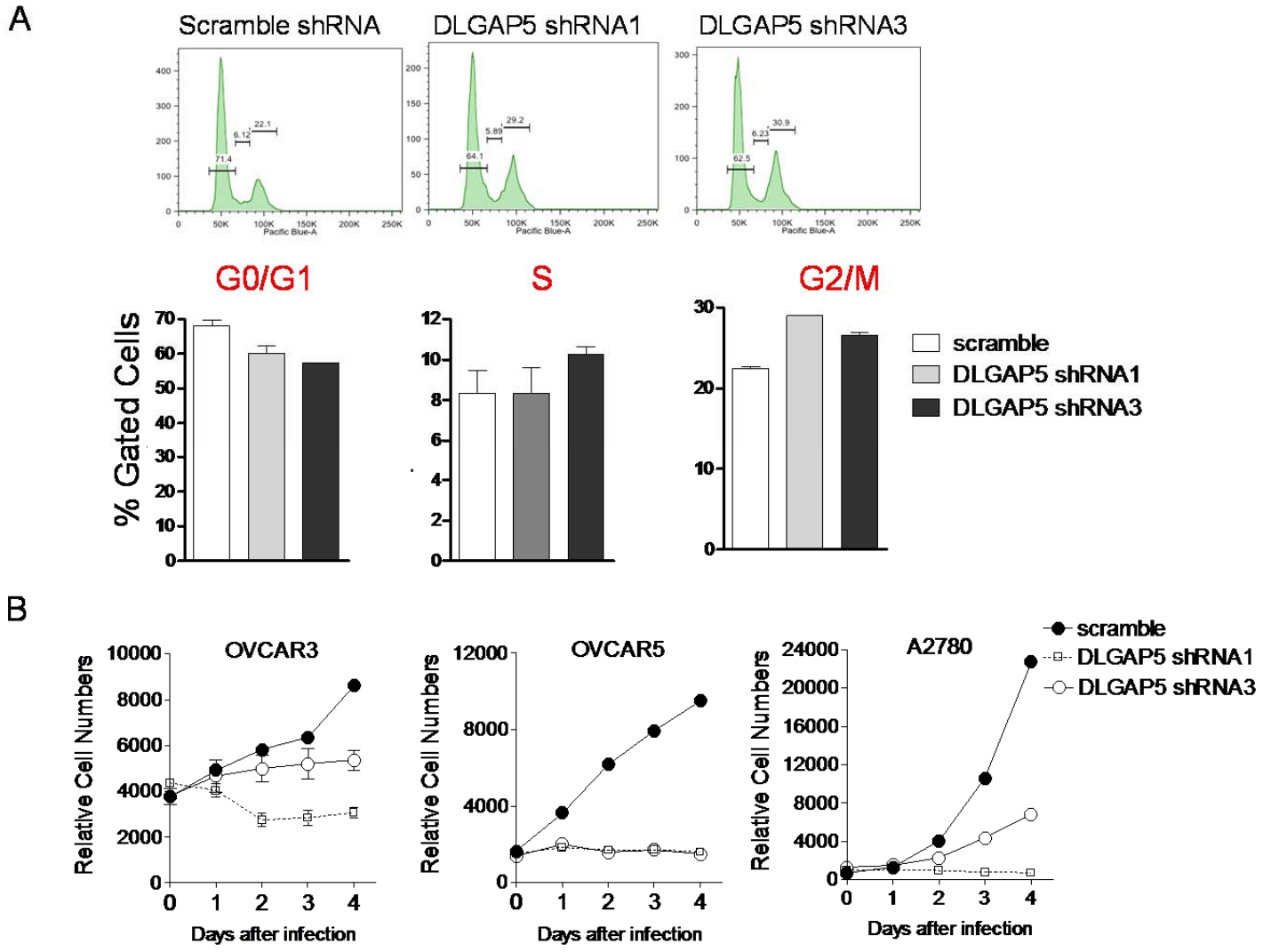

Fig S8. Knockdown of DLGAP5 leads to cell cycle arrest at the G2/M phase and reduces cellular proliferation. A. The effects of DLGAP5 shRNAs (shRNA1 and shRNA3) on cell cycle progression in A2780 ovarian cancer cells. Top: representative plots of cell cycle analysis. Bottom: percentage of cells in G0/G1, S, and G2/M phases. Individual viable cells were gated to exclude debris, dead cells, and cell aggregates. Data shown represent mean plus one standard deviation from three replicates. **B.** Growth curve analysis shows a decrease in cell numbers of shRNA-treated OVCAR3, OVCAR5 and A2780 cells as compared to scramble shRNA-treated cells.

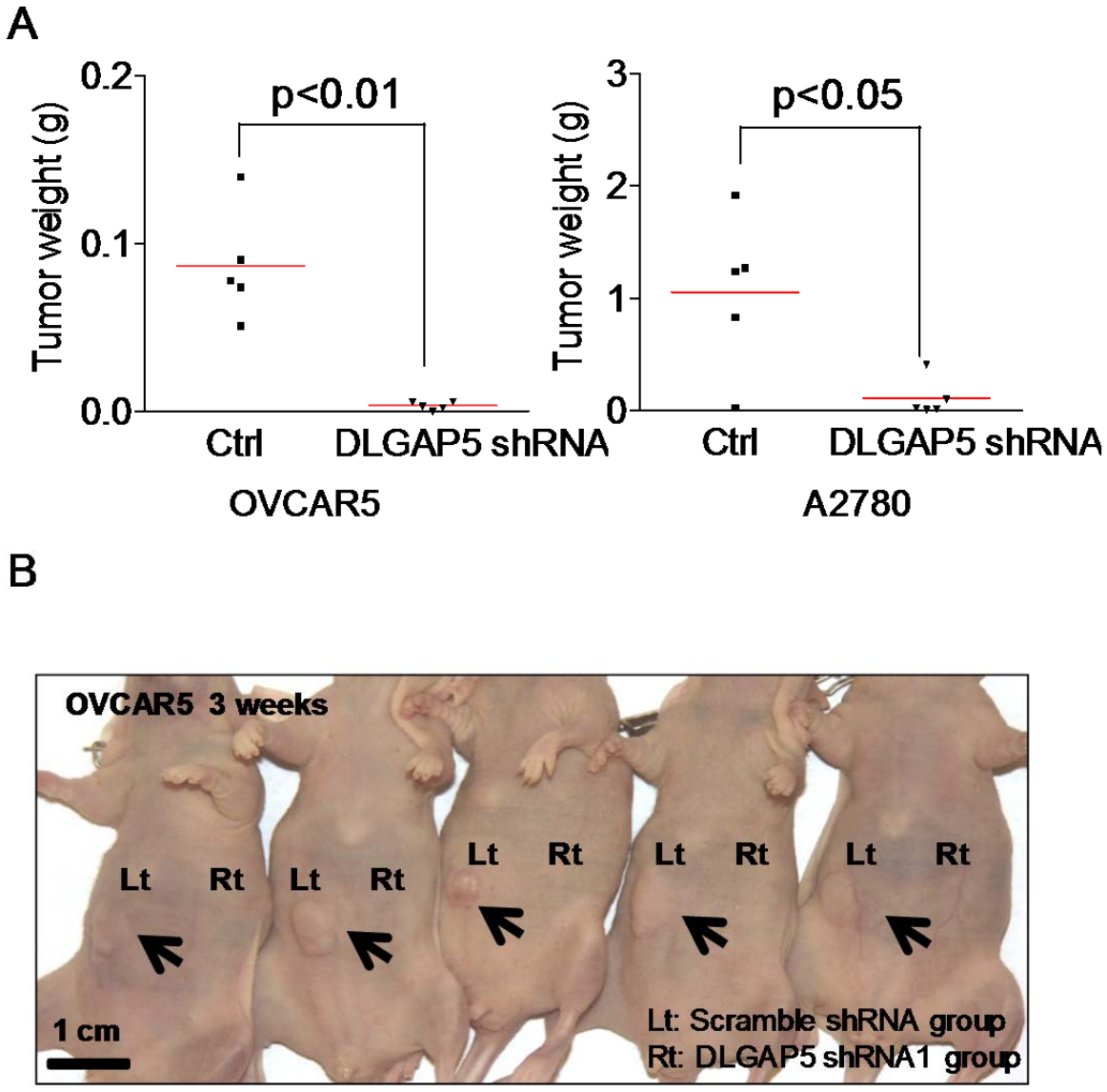

Fig. S9. Knockdown of DLGAP5 reduces tumorigenicity. A. Tumor weight was measured in excised OVCAR5 and A2780 subcutaneous tumor xenografts from nude mice. The difference between DLGAP5 and control shRNA-treated groups was determined using Mann-Whitney test. B. Representative photos of mice bearing OVCAR5 subcutaneous tumors in control shRNA group (left flank, Lt); most mice receiving DLGAP5 shRNA treated cells do not develop tumors, or their tumors are very small (right flank, Rt).