Supporting Information

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SI Materials and Methods

Cell Culture. Hepatocellular cell lines HepG2 and HLE were obtained from the Japanese Collection of Research Bioresources Cell Bank (National Institute of Health Sciences). The hepatocellular cell line SK-HEP-1 and breast cancer cell line MDA-MB-231 were obtained from the American Type Culture Collection. Breast cancer cell line MCF7 was obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer at Tohoku University. Cells were maintained at 37 °C in a 5% (vol/vol) CO_2 humidified atmosphere in RPMI Medium 1640 (Invitrogen) supplemented with 10% (vol/vol) FBS.

Plasmids, siRNA, and Transfection. The ORF of human or mouse phospholipase Cδ1 (PLCδ1) cDNA was amplified by PCR and subcloned into the pcDNA3.1 vector (Invitrogen). PLC activity-dead construct of mouse PLCδ1, which is introduced in the mutation in catalytic residue (H356A), was also subcloned into pcDNA3.1 vector. The ORF of human PLCδ3 cDNA was amplified by PCR and subcloned into the pcDNA3.1 vector (Invitrogen). Negative control siRNA (Qiagen), siRNA for E-cadherin (Qiagen), siRNA for PLCδ1 (Qiagen), and siRNA for Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (Sigma) as well as siRNA for PLCδ1 (Hokkaido System Science) were used at final concentrations of 20–40 nM. Transient transfections were performed with the Lipofectamine 2000 and Lipofectamine RNAi Max (Invitrogen) according to the manufacturer's protocol. In every experiment, the total amounts of transfected DNA or siRNA were adjusted with relevant empty vectors or negative control siRNA, respectively. To obtain stable cell lines, transfected cells were expanded sparsely, and 1,000 μg/mL G418 (Invitrogen) was added to the culture medium for 8 d.

Immunohistochemistry. Human colon carcinoma tissue array with matched adjacent normal colon tissue was purchased from US Biomax. Immunohistochemical assays for human PLCδ3 were performed using anti-PLCδ3 antibody (GeneTex).

Immunofluorescence Microscopy. Cells were fixed with 4% (wt/vol) paraformaldehyde at room temperature for 15 min. These samples were permeabilized with 0.1% Triton X-100, blocked with 5% (wt/vol) normal BSA (Wako), and then incubated with primary antibodies at 4 °C overnight and subsequently, Alexa Fluor-conjugated secondary antibodies (Invitrogen). Nuclei were counterstained with Hoechst33342 (Dojindo). Images were obtained on a fluorescent microscope (BioZero; Keyence) or confocal laser microscope (FV1000; Olympus). Primary antibodies for Vimentin (Santa Cruz) and E-cadherin (BD Biosciences) were used.

RNA Isolation, cDNA Synthesis, and Quantitative Real-Time PCR. Total RNA was isolated from cells using the ReliaPrep RNA Cell Miniprep System (Promega) and reverse-transcribed to generate cDNA (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) according to the manufacturer's protocol. Real-time PCR was performed using the THUNDERBIRD SYBR qPCR Mix (Toyobo) with a CFX96 thermocycler (Bio-Rad). The primers sequence used are listed: *CDH1* (E-cadherin), F: GGACTTTGGCGTGGGCCAGG, R: CCCTGTCCAGCTCAGCCCGA; *ACTB* (β-actin), F: GCCCTGGCACCCAGCACAAT, R: GGAGGGGCCGGACTCGTCAT; *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), F: AGCCTCCCGCTTCGCTCTCT, R: CCAGGCGCCCAATACGACCA; *PLCD1* (PLCδ1), F: CACGGAGTTTGCGTTTGAGG, R: ACAAAGAGGGTGGCTGATGG; *PLCD3* (PLCδ3), F: CCAGAACCACTCTCAGCATCCA, R: GCCATTGTTGAGCACGTAGTCAG; *VIM* (Vimentin), F: AACAATGACGCCCTGCGCCA, R: TCTCATCCTGCAGGCGGCCA; *TGFB1*, F: CAAGGACCTCGGCTGGAA, R: CCGGGTTATGCTGGTTGTACA; *SNAI1* (Snail1), F: CTGCGGGAAGGCCTTCTCT, R: CGCCTGGCACTGGTACTTCTT; *SNAI2* (Slug), F: CGGACCCACACATTACCTTGTGTTT, R: CACAGCAGCCAGATTCCTCATGTTT; and *ZEB1*, F: GCCAATAAGCAAACGATTCTG, R: TTTGGCTGGATCACTTTCAAG.

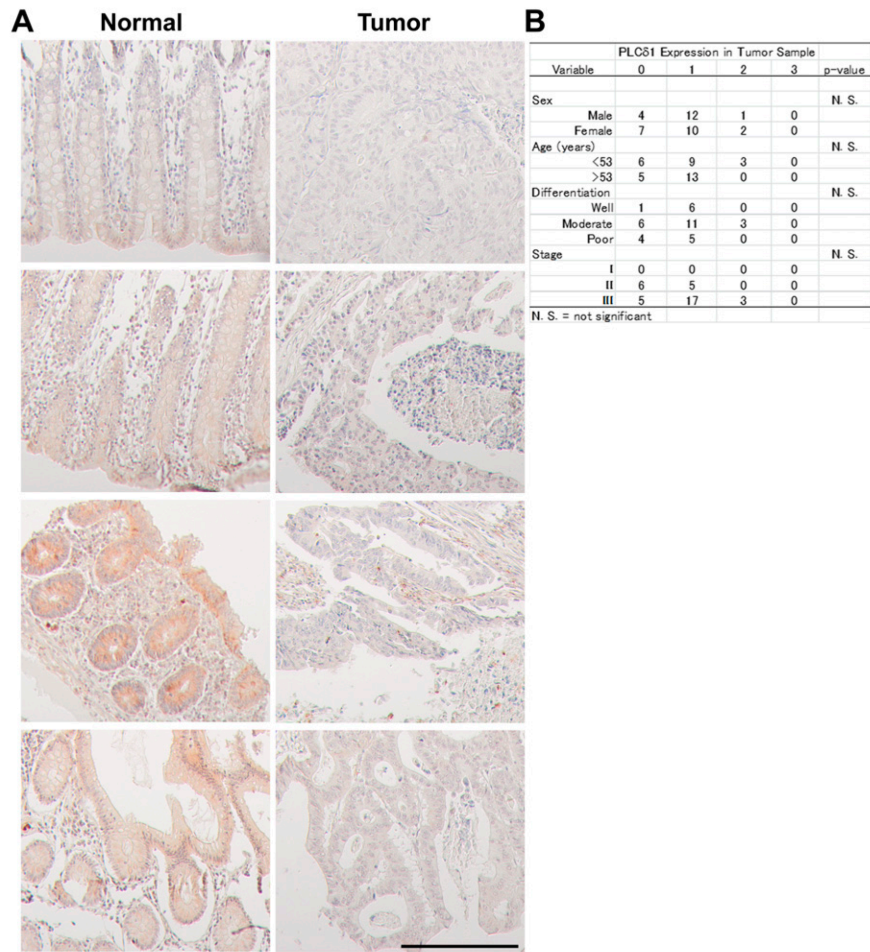
Luciferase Reporter Assay. Luciferase reporter constructs, TOP-FLASH and FOP-FLASH (Upstate), were used to evaluate T-cell factor/lymphocyte enhancer factor transcriptional activity. Cells were transiently transfected in triplicate with the luciferase reporter plasmid and the phRL-TK plasmid (Promega) as an internal control using the Lipofectamine 2000 reagent (Invitrogen). Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega).

Cell Proliferation Assay. Cells were plated in 24-well plates at 10,000 cells/well in triplicate. Cells were trypsinized, and the total cell number was determined every 24 h by cell counting.

Soft Agar Colony Formation Assay. The cells were layered (1,000 cells/well) in base agar (0.5%) -coated six-well plates with room temperature agar (0.4%) in RPMI Medium 1640. The plate was incubated at 37 °C for 14 d, the number of colonies was counted, and the images were obtained.

Animal Experiments. Ten million SW620 cells that stably express PLCδ1 or empty vector were suspended in 0.1 mL PBS and s.c.-inoculated into the flanks of 5-wk-old female BALB/c nu/nu nude mice (CLEA). Tumor volume was determined every 1 wk using the formula $V = 1/2(A \times B^2)$, where A and B represent the largest and smallest dimensions of the tumor, respectively. Animal experiments were reviewed by the institutional ethics committee and performed in compliance with the Guidelines for Laboratory Animal Research of the Tokyo University of Pharmacy and Life Sciences.

Statistical Analysis. Data were analyzed statistically using the R statistical software package (version 3.0.1). To examine the difference in PLCδ1 expression scores between paired samples, the Wilcoxon signed rank test was performed. Associations between PLCδ1 expressions and clinicopathological features were examined using the Fisher exact test. In other experiments, differences between the means of two groups were tested by using a Student t test. Either the Dunnett or Tukey test was used for posthoc multiple comparisons. $P < 0.05$ was considered statistically significant. Averages + SDs are shown in bar graphs.



	PLCδ1 Expression in Tumor Sample				
Variable	0	1	2	3	p-value
Sex					N. S.
Male	4	12	1	0	
Female	7	10	2	0	
Age (years)					N. S.
<53	6	9	3	0	
>53	5	13	0	0	
Differentiation					N. S.
Well	1	6	0	0	
Moderate	6	11	3	0	
Poor	4	5	0	0	
Stage					N. S.
I	0	0	0	0	
II	6	5	0	0	
III	5	17	3	0	

N. S. = not significant

Fig. S1. PLCδ1 was down-regulated in colon adenocarcinoma. (*A*) Immunohistochemical staining of human colon carcinoma tissue arrays, which contain 36 matched normal and adenocarcinoma tissues, was performed using anti-PLCδ1 antibody. Four pairs of specimens are shown. (Scale bar: 200 μm.) (*B*) Associations between PLCδ1 expression and clinicopathological features were examined using the Fisher exact test. No clinicopathological features were associated with PLCδ1 expression levels.

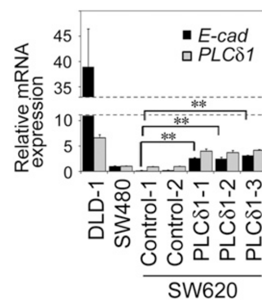


Fig. S2. PLCδ1 overexpression induced E-cadherin expression. The relative expression levels of E-cadherin (E-cad) and PLCδ1 genes, normalized to *GAPDH* (as an internal control), in DLD-1, SW480, and the established SW620 cells were determined using quantitative real-time PCR. The value for SW480 cells was set as one. Statistical analysis was performed using the Dunnett multiple comparison of means test. $^{**}P < 0.005$ (vs. Control-1).

Fig. S3. Knockdown of PLCδ1 repressed E-cadherin expression. SW480 cells were transfected with negative control siRNA (siNeg) or siRNA for PLCδ1 (siδ1-1 or -2). After 7 d, the expression levels of E-cadherin (E-cad) and PLCδ1 genes were determined using quantitative real-time PCR (n = 3). The relative mRNA expression, normalized to β-actin gene, is shown. Western blots for E-cadherin, PLCδ1, and β-actin (loading control) are shown. $^{**}P < 0.005$.

Fig. S4. PLCδ3 was down-regulated in colon adenocarcinoma. (*A*) Immunohistochemical staining of human colon carcinoma tissue arrays, which contain 33 matched normal and adenocarcinoma tissues, was performed using anti-PLCδ3 antibody. Two pairs of specimens are shown. (Scale bar: 200 μm.) (*B*) The expression level of PLCδ3 in each sample was scored as described in Fig. 1. The scored PLCδ3 levels were compared between normal and tumor samples (33 pairs) using the Wilcoxon signed rank test.

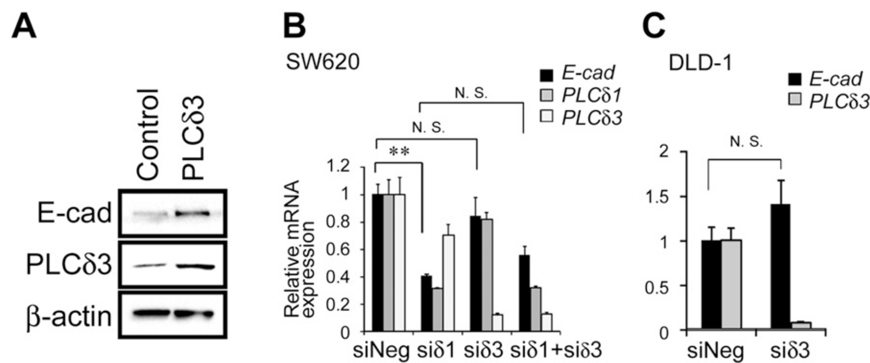


Fig. S5. The effect of PLCδ3 on E-cadherin expression. (*A*) SW620 cells were transfected with either PLCδ3-expression vector or the relevant empty vector and then selected using G418 treatment for 8 d. The expressions of E-cadherin (E-cad), PLCδ3, and β-actin (loading control) were determined using Western blotting. (*B*) The expression levels of E-cadherin, PLCδ1, and PLCδ3 genes in SW620 cells transfected with negative control siRNA (siNeg), siRNA for PLCδ1 (siδ1), or siRNA for PLCδ3 (siδ3) were determined by quantitative real-time PCR ($n = 3$). The relative mRNA expression normalized to β-actin gene is shown. E-cadherin expression was decreased by knockdown of PLCδ1 but not PLCδ3. A synergistic effect of siδ1 and siδ3 was not observed. (*C*) DLD-1 cells were transfected with siNeg or siδ3, and quantitative real-time PCR was performed as described in *B*. E-cadherin expression was not decreased by PLCδ3 knockdown. Statistical analysis was performed using (*B*) Tukey multiple comparison of means test or (*C*) Student *t* test. N.S., not significant. $^{**}P < 0.005$.

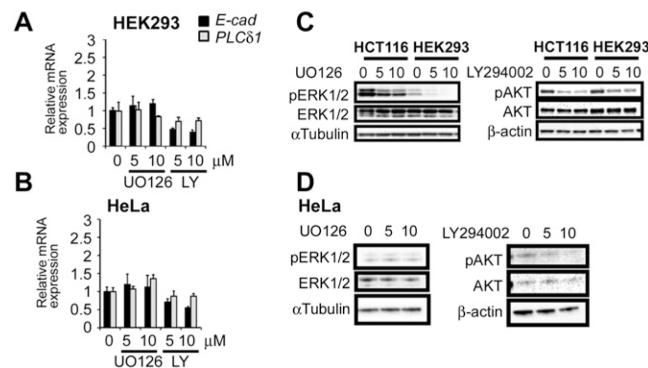


Fig. S6. PLCδ1 expression was not induced by mitogen-activated protein kinase kinase or phosphatidylinositol-3 kinase inhibitors in HEK293 (KRAS WT) or HeLa (KRAS WT) cells. (*A* and *B*) Cells were treated with UO126 (5 or 10 μM) or LY294002 (5 or 10 μM) for 24 h, and then, mRNA expression levels were determined. The relative expression of PLCδ1 and E-cadherin (E-cad) genes normalized to β-actin gene is shown. (*C* and *D*) Western blots for the indicated proteins are shown.

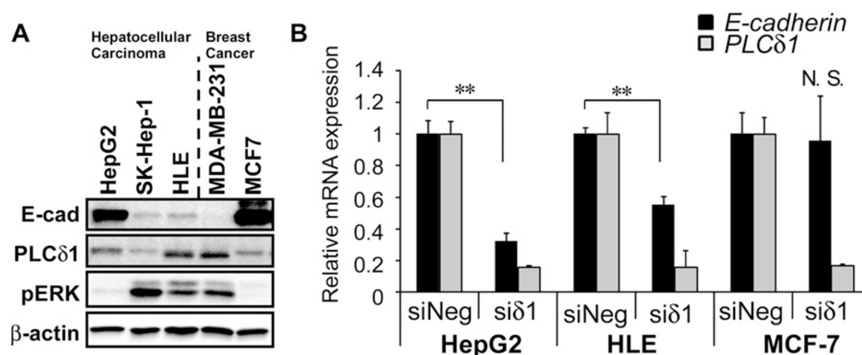


Fig. S7. The link between E-cadherin and PLCδ1 in other cell lines. (*A*) To investigate the link between the expression of PLCδ1 and E-cadherin (E-cad) in other cancer types, Western blotting was performed to assess their levels in hepatocellular carcinoma cell lines HepG2 (with *KRAS* mutation), SK-Hep-1, and HLE and breast cancer cell lines MDA-MB-231 (with *KRAS* mutation) and MCF-7. (*B*) The expression levels of E-cadherin and PLCδ1 genes in HepG2, HLE, and MCF-7 cells transfected with negative control siRNA (siNeg) or siRNA for PLCδ1 (siδ1) were determined using quantitative real-time PCR ($n = 3$). The relative mRNA expression, normalized to β-actin gene, is shown. E-cadherin expression was decreased by PLCδ1 knockdown in HepG2 and HLE cells but not MCF-7 cells. Statistical analysis was performed using Student *t* tests. N.S., not significant. $^{**}P < 0.005$.