Supplementary Figure Legends

Materials and Methods

The human colorectal cancer cells: SNU-C5 (KCLB® 0000C5, passage number 04 - 17), SW480 (KCLB® 10228, passage number 04 - 17) and HCT116 (KCLB® 10247, passage number 04 - 17) were obtained from the Korea Cell Line Bank (KCLB; Seoul, Korea). Cell lines purchased from KCLB were analyzed by Short Tandem Repeat (STR) Profiling. HEK293 (ATCC® CRL-1658™, passage number 05 - 15), the human breast carcinoma: MDA-MB 361 (ATCC® HTB-27™, passage number 05 - 15), MDA-MB 231 (ATCC® HTB-26™, passage number 05-15), SK-BR3 (ATCC® HTB-30™, passage number 05-15), osteosarcoma: HOS (ATCC® CRL-1543™, passage number 05 -15), hepatoma: SNU475 (ATCC® CRL-2236™, passage number 05-15) and mouse fibroblast: NIH3T3 (ATCC® CRL-1658™, passage number 05 -15) were obtained from the ATCC (Rockville, MA). Cell lines purchased from ATCC (Rockville, MA) were analyzed by STR Profiling.

Supplementary Figure 1. Ectopic expression of dominant negative TCF-4 and depletion of β-catenin decrease PLD1 expression in HCT116. HCT116 cells were transfected with ΔN30 TCF-4 (*left*) or shRNA for β-catenin (*right*). The expression of the proteins was analyzed by Western blot using the indicated antibodies.

Supplementary Figure 2. Schematic diagram for comparison of TBEs on PLD1 promoter regions from various species. TCF-4 binding elements in 5′ flanking regions of the human PLD1 transcriptional start site were compared with those of the genomes from 5 different species. A core *motif*, CTTTG(A/T)(A/T) [or the complementary sequence (A/T)(A/T)CAAAG] of TCF binding sequences on PLD1 promoter is highly *conserved* across species.

Supplementary Figure 3. PA rescues the TCF transcriptional activity inhibited by overxpression of GSK3β or PLD inhibitor. *A*, SW480 cells were co-transfected with TOP/FOP reporters and GSK3β and then reated with PA (100 μM), and then luciferase activity was measured. $*P < 0.05$ *versus* GSK3β. *B*, The cells were transfected with TOP/FOP reporters, pretreated with PLD inhibitor (VU0155056: 5 μM) and then stimulated with PA (100 μM), and the relative TCF transcriptional activity was determined. The data

represents the mean ± S.D. of four independent experiments. *$P < 0.05$.

Supplementary Figure 4. The effect of PLD1 siRNA on PLD1 expression. Cells were transfected with siRNA for control or PLD1 and PLD1 expression was analyzed by Q-RT-PCR. The data represent the mean ± S.D. of three independent experiments.

Supplementary Figure 5. PLD1 depletion suppresses Wnt3a-induced cell migration. HEK293 cells were transfected with siRNA for control or PLD1 and then seeded in migration chambers and stimulated with Wnt3a for 24 h. The extent of migration was expressed as an average number of cells per microscopic field. Results represent the mean ± S.D. of three independent experiments. *$P < 0.05$ *versus* vehicle; †$P < 0.05$ *versus* Wnt/control-siRNA.

Supplementary Figure 6. β-catenin-induced anchorage-independent growth is suppressed by inhibitor and depletion of PLD1. NIH3T3 cells were transfected with S37A β-catenin and/or PLD1-siRNA, and suspended in agar matrix and treated with or without VU0155056 (10 μM). and then, anchorage-independent growth assay was performed. The data represent the mean ± S.D. of three independent experiments. *$P < 0.05$ *versus* Mock; **$P < 0.05$ *versus* S37A β-catenin; †$P < 0.05$ *versus* S37A β-catenin/control-siRNA.

Supplementary Table 1. Consensus TBE in the PLD1 promoter.

Oligo/plasmid	Position	Seguence (5´ to 3´)
TBE1		
WT	-797 to -791	CTCTGATGACAGTG**TGCAAAG**CTTGAATCTC
MT	-797 to -791	CTCTGATGACAGTG**GCTAAAG**CTTGAATCTC
TBE2		
WT	-522 to -516	GGTAATATACA**CTTTGCT**TGGGATATCACCTACAGAG
MT	-522 to -516	GGTAATATACA**CTTTAGC**TGGGATATCACCTACAGAG
TBE3		
WT	-284 to -278	GAATTACTTATCCAAGTT**AATAAAGT**TGGATGAGAGAAATG
MT	-278 to -284	GAATTACTTATCCAAGTT**GCAAAAGT**TGGATGAGAGAAATG
TBE4		
WT	-25 to -19	CAAAATGCTAAGT**CTTTGTA**ACCGTTTTCTTCTTTCC
MT	-25 to -19	CAAAATGCTAAGT**CTTTAGC**ACCGTTTTCTTCTTTCC

Binding sites : Bold type. Mutations : Underline type

Supplementary Table 2. Primer sets for Chip assay.

Promoter	Oligo	Direction	Seguence (5´ to 3´)
PLD1	TBE1	Forward	GCACCCATATCAGGTGCTCCTTAATC
		Reverse	GCACATCAGTACCCAGGGCAGAAG
	TBE2	Forward	CACCTCAGATGTCTTTCGGAATAG
		Reverse	CACATATTGAAATATATAGATTTAGAAATAT
	TBE3	Forward	GCTTTCCCAAACCAATCTCCCTTG
		Reverse	TCAGCCTGCTCTGTGTGAGC
	TBE4	Forward	GTCTTTGTAACCGTTTTCTTCTTTCCTAG
		Reverse	GCTCAGATCATCCGTCTTTACC
NOS2	TBE1	Forward	CAGCCTGGCATAGAAACAGATC
		Reverse	CAAGGTCACACAGCAAACAGCC

PLD1 Regions : TBE1 (-1024 to -723), TBE2 (-640 to -437), TBE3 (-330 to -101), TBE4 (-64 to +113)

Supplementary Table 3. Primer sets for Q-RT-PCR.

	Primer	Direction	Seguence (5´ to 3´)
Q-RT-PCR	PLD1	Forward	TGTCGTGATACCACTTCTGCCA
		Reverse	AGCATTTCGAGCTGCTGTTGAA
	c-Myc	Forward	TCC AGCTTGTACCTGCAGGATCTGA
		Reverse	CCTCCAGCAGAAGGTGATCCAGACT
	NOS2	Forward	TGCCAGATGGCAGCATCAGA
		Reverse	TTTCCAGGCCCATTCTCCTGC
	GAPDH	Forward	GTGGTCTCCTCTGACTTCAAC
		Reverse	TCTCTTCCTCTTGTGCTCTTG

Fig. S1

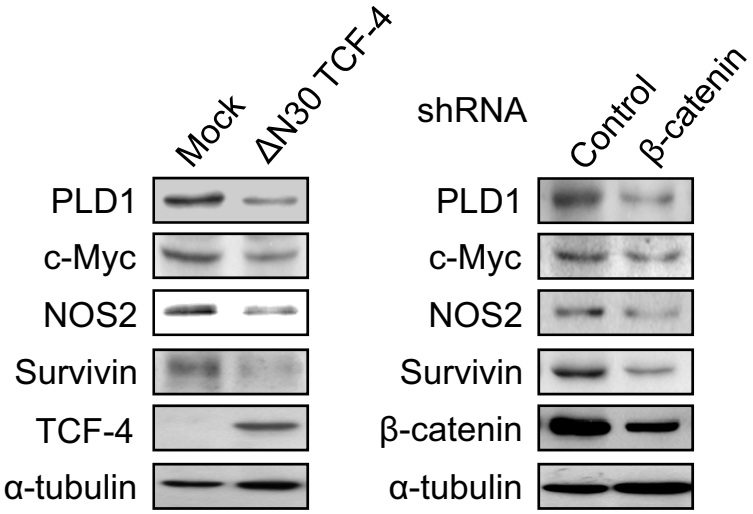
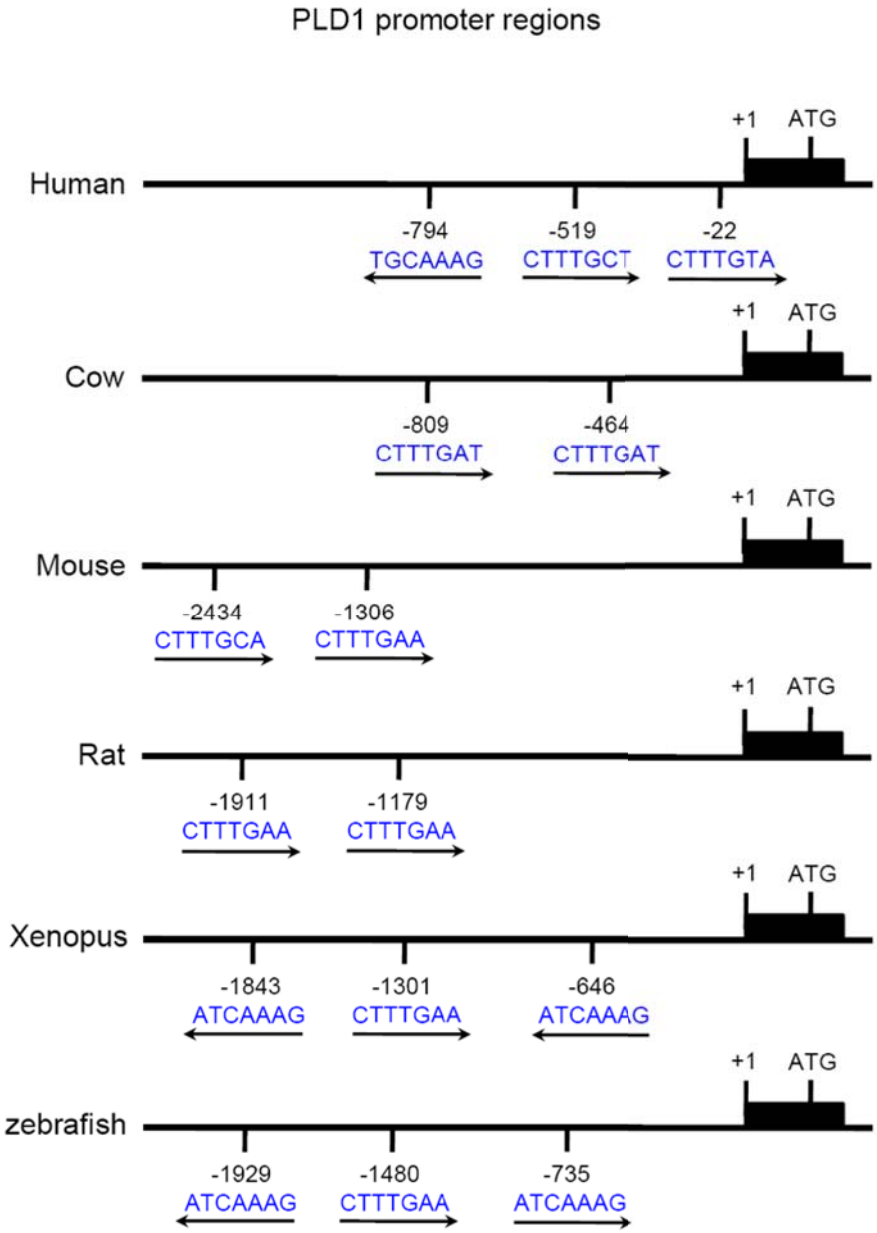

Mock
ΔN30 TCF-4
PLD1
c-Myc
NOS2
Survivin
TCF-4
α-tubulin
shRNA
Control
β-catenin
PLD1
c-Myc
NOS2
Survivin
β-catenin
α-tubulin

Fig. S2


PLD1 promoter regions
Human
+1
ATG
-794
TGCAAAG
-519
CTTTGCT
-22
CTTTGTA
Cow
+1
ATG
-809
CTTTGAT
-464
CTTTGAT
Mouse
+1
ATG
-2434
CTTTGCA
-1306
CTTTGAA
Rat
+1
ATG
-1911
CTTTGAA
-1179
CTTTGAA
Xenopus
+1
ATG
-1843
ATCAAAG
-1301
CTTTGAA
-646
ATCAAAG
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+1
ATG
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-1480
CTTTGAA
-735
ATCAAAG

Fig. S3

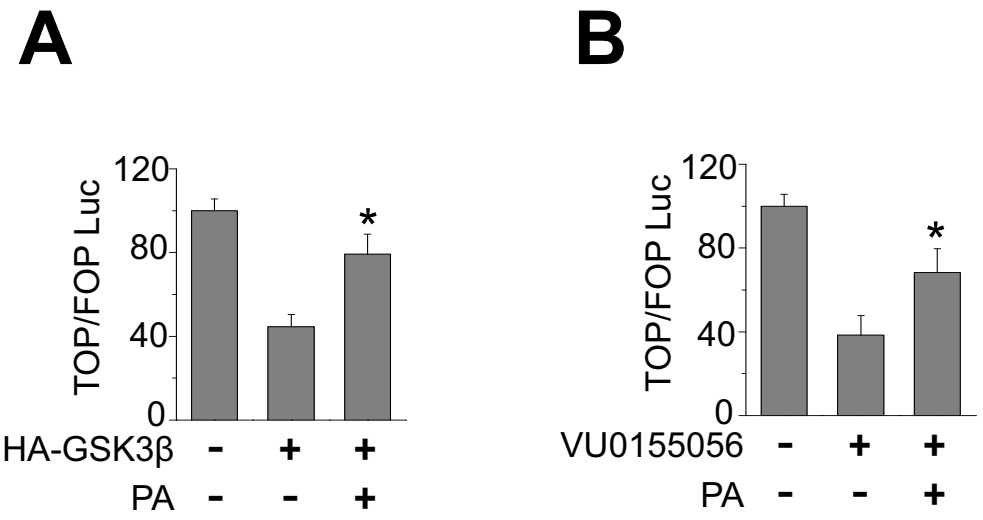


Fig. S4

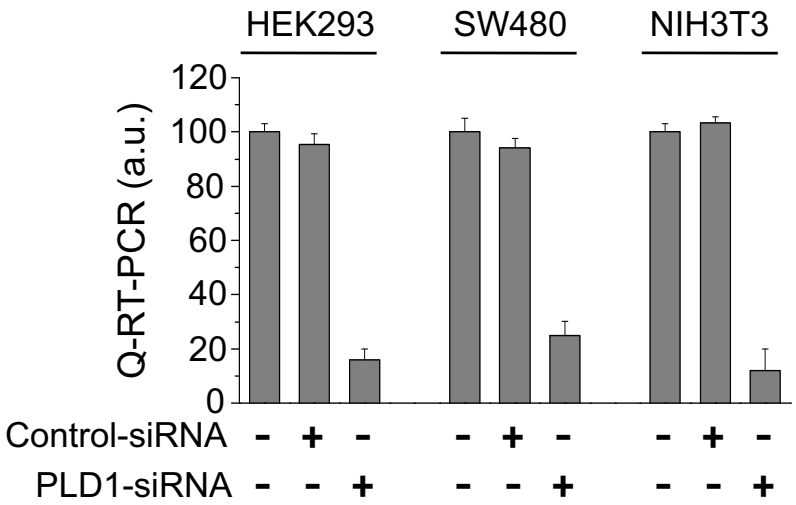

HEK293
SW480
NIH3T3
Q-RT-PCR (a.u.)
120
100
80
60
40
20
0
Control-siRNA - + - - + - - + -
PLD1-siRNA - - + - - + - - +

Fig. S5

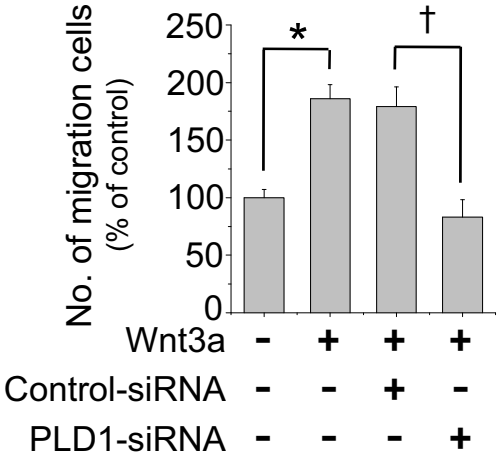
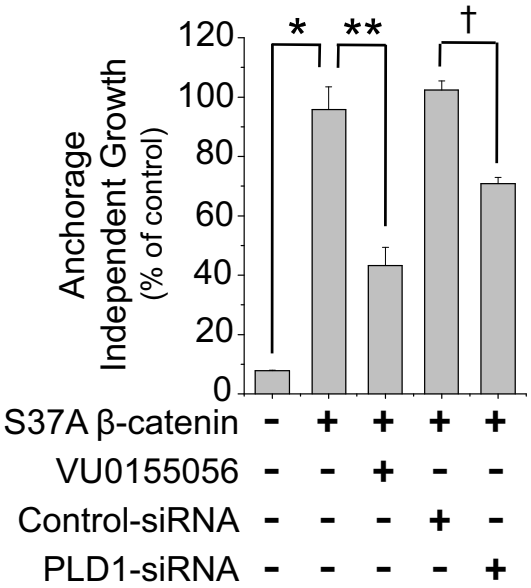

No. of migration cells
(% of control)
250
200
150
100
50
0
*
†
Wnt3a - + + +
Control-siRNA - - + -
PLD1-siRNA - - - +

Fig. S6


Anchorage Independent Growth (% of control)
120
100
80
60
40
20
0
*
**
†
S37A β-catenin - + + + +
VU0155056 - - + - -
Control-siRNA - - - + -
PLD1-siRNA - - - - +