Fig. S1

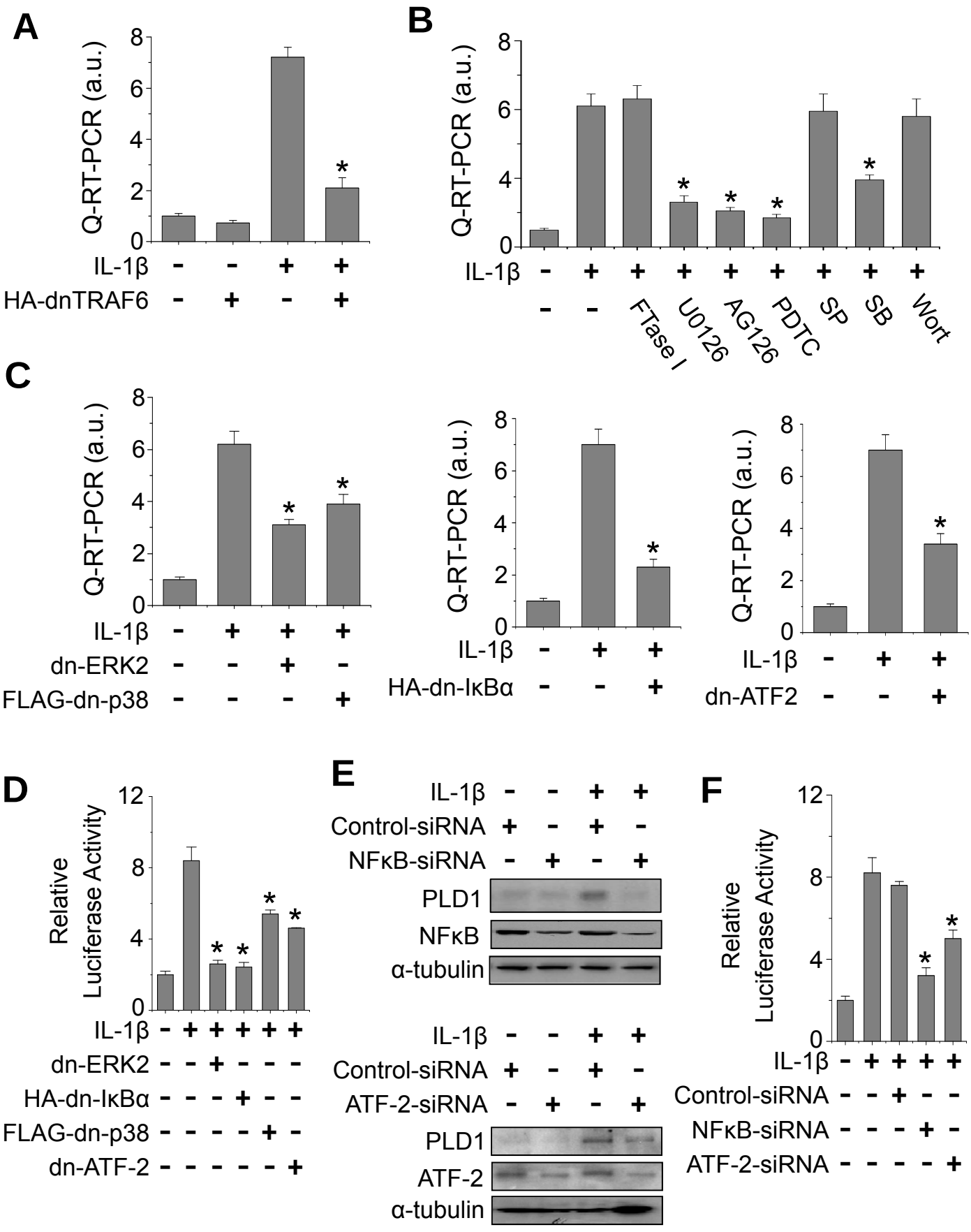
A
Q-RT-PCR (a.u.)
IL-1β
HA-dnTRAF6
B
Q-RT-PCR (a.u.)
IL-1β
FTase I
U0126
AG126
PDTC
SP
SB
Wort
C
Q-RT-PCR (a.u.)
IL-1β
dn-ERK2
FLAG-dn-p38
Q-RT-PCR (a.u.)
IL-1β
HA-dn-IκBα
Q-RT-PCR (a.u.)
IL-1β
dn-ATF2
D
Relative Luciferase Activity
IL-1β
dn-ERK2
HA-dn-IκBα
FLAG-dn-p38
dn-ATF-2
E
IL-1β
Control-siRNA
NFκB-siRNA
PLD1
NFκB
α-tubulin
IL-1β
Control-siRNA
ATF-2-siRNA
PLD1
ATF-2
α-tubulin
F
Relative Luciferase Activity
IL-1β
Control-siRNA
NFκB-siRNA
ATF-2-siRNA

Fig. S1 IL-1β-induced PLD1 expression is mediated by the TRAF6/ERK/NFκB or TRAF6/p38/ATF-2 signaling pathways. (A) RAFLS were transfected with dnTRAF6, treated with IL-1β for 36 h, and PLD1 expression was analyzed by q-PCR. (B) RAFLS were pretreated with the indicated inhibitors for 30 min, treated with IL-1β for 36 h, and PLD1 expression was analyzed by q-PCR (**P*< 0.05 *versus* IL-1β). (C) RAFLS were transfected with expression vectors for dominant negative ERK, p38, IκBα, or ATF-2, stimulated with IL-1β, after which PLD1 expression was analyzed by q-PCR. **P*< 0.05 *versus* IL-1β. (D) After transfection of RAFLS with pGL4-PLD1, cells were pretreated with the indicated inhibitors, stimulated with IL-1β for 24 h, and then luciferase activity was measured (**P*< 0.05 *versus* IL-1β). (E) RAFLS were transfected with siRNAs specific to NFκB or ATF-2, treated with IL-1β, and lysates were immunoblotted with the indicated antibodies. (F) After co-transfection of RAFLS with pGL4-PLD1 and the indicated siRNAs, the cells were treated with IL-1β and luciferase activity was measured (**P*< 0.05 *versus* IL-1β). Data are presented as the mean ± SD of 4 independent experiments.

Fig. S2

Position :	[-1281 ~ -1272] NFκB-1	[-1177 ~ -1168] NFκB-2	[-590 ~ -579] ATF-2	[-515 ~ -506] NFκB-3	[-59 ~ -50] NFκB-4
pGL4 PLD1 (-1930/+1)	TTGAATTTCC	AGCATTTCCC	atACGTCAtt	GGATATCACC	GGAATAGCAT
NFκB-1 *mut*	TT**C**AATTT**AG**	AGCATTTCCC	atACGTCAtt	GGATATCACC	GGAATAGCAT
NFκB-2 *mut*	TTGAATTTCC	A**C**CATTTC**AG**	atACGTCAtt	GGATATCACC	GGAATAGCAT
NFκB-3 *mut*	TTGAATTTCC	AGCATTTCCC	atACGTCAtt	**CT**ATATCA**GG**	GGAATAGCAT
NFκB-4 *mut*	TTGAATTTCC	AGCATTTCCC	atACGTCAtt	GGATATCACC	G**C**AATAG**AG**T
NFκB-1/4 *mut*	TT**C**AATTT**AG**	AGCATTTCCC	atACGTCAtt	GGATATCACC	G**C**AATAG**AG**T
ATF-2 *mut*	TTGAATTTCC	AGCATTTCCC	atA**AA**TCAtt	GGATATCACC	GGAATAGCAT

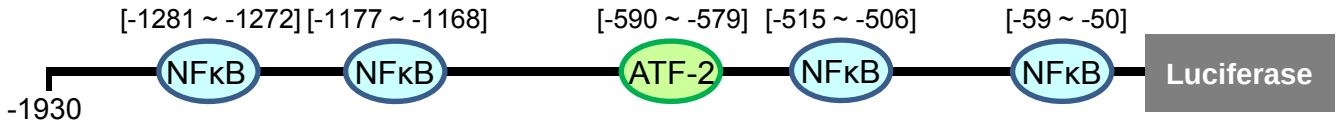

Fig. S2 Schematic representation of putative NFκB- and ATF-2-binding sites in the PLD1 promoter, and its six mutant constructs. A site-specific mutation was introduced into NFκB or ATF-2 binding sites on the PLD1 promoter using a Quick Change Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA) according to the manufacturer's instructions.

Fig. S3

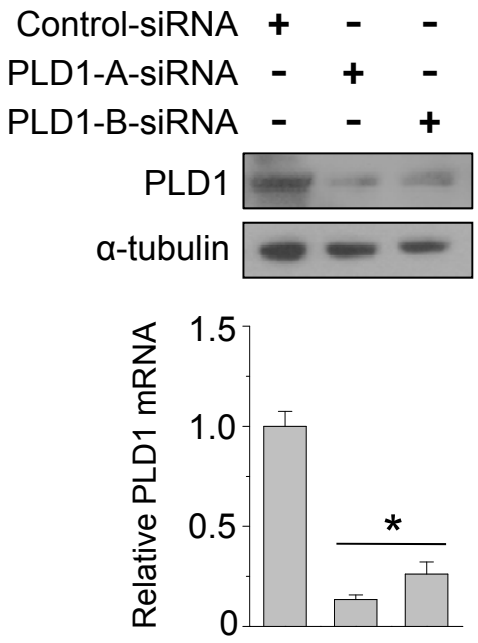
Control-siRNA + - -
PLD1-A-siRNA - + -
PLD1-B-siRNA - - +
PLD1
α-tubulin
Relative PLD1 mRNA
1.5
1.0
0.5
0
*

Fig. S3 The effect of PLD1 siRNAs on PLD1 expression. Cells were transfected with siRNAs for control or two kinds of siRNAs specific to PLD1 and PLD1 expression was analyzed by western blot and Q-RT-PCR. *$P < 0.05$ *versus* control-siRNA. Data are presented as the mean ± SD of 4 independent experiments.

Fig. S4

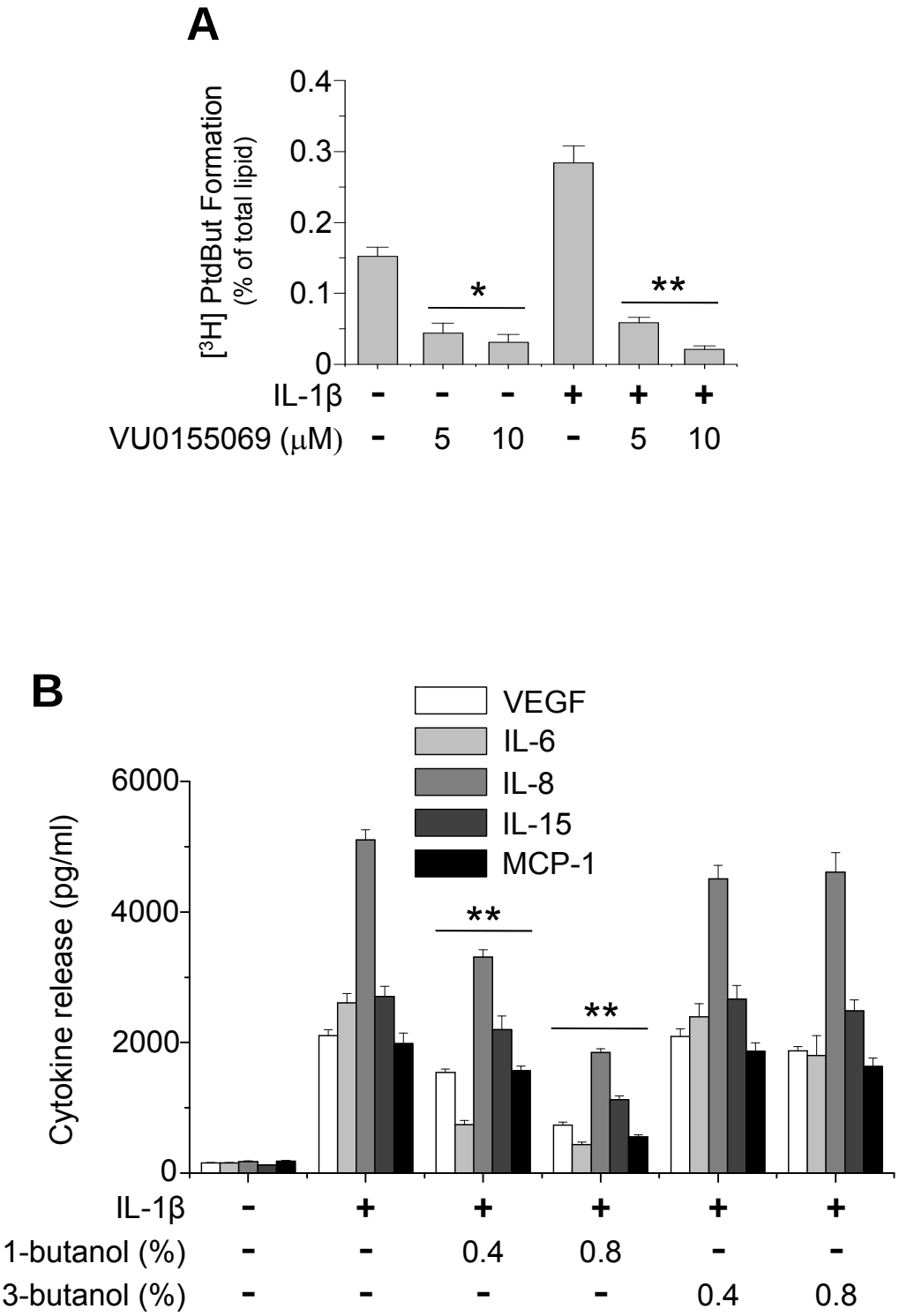
A
[3H] PtdBut Formation
(% of total lipid)
0.4
0.3
0.2
0.1
0
*
**
IL-1β - - - + + +
VU0155069 (μM) - 5 10 - 5 10
B
VEGF
IL-6
IL-8
IL-15
MCP-1
Cytokine release (pg/ml)
6000
4000
2000
0
**
**
IL-1β - + + + + +
1-butanol (%) - - 0.4 0.8 - -
3-butanol (%) - - - - 0.4 0.8

Fig. S4

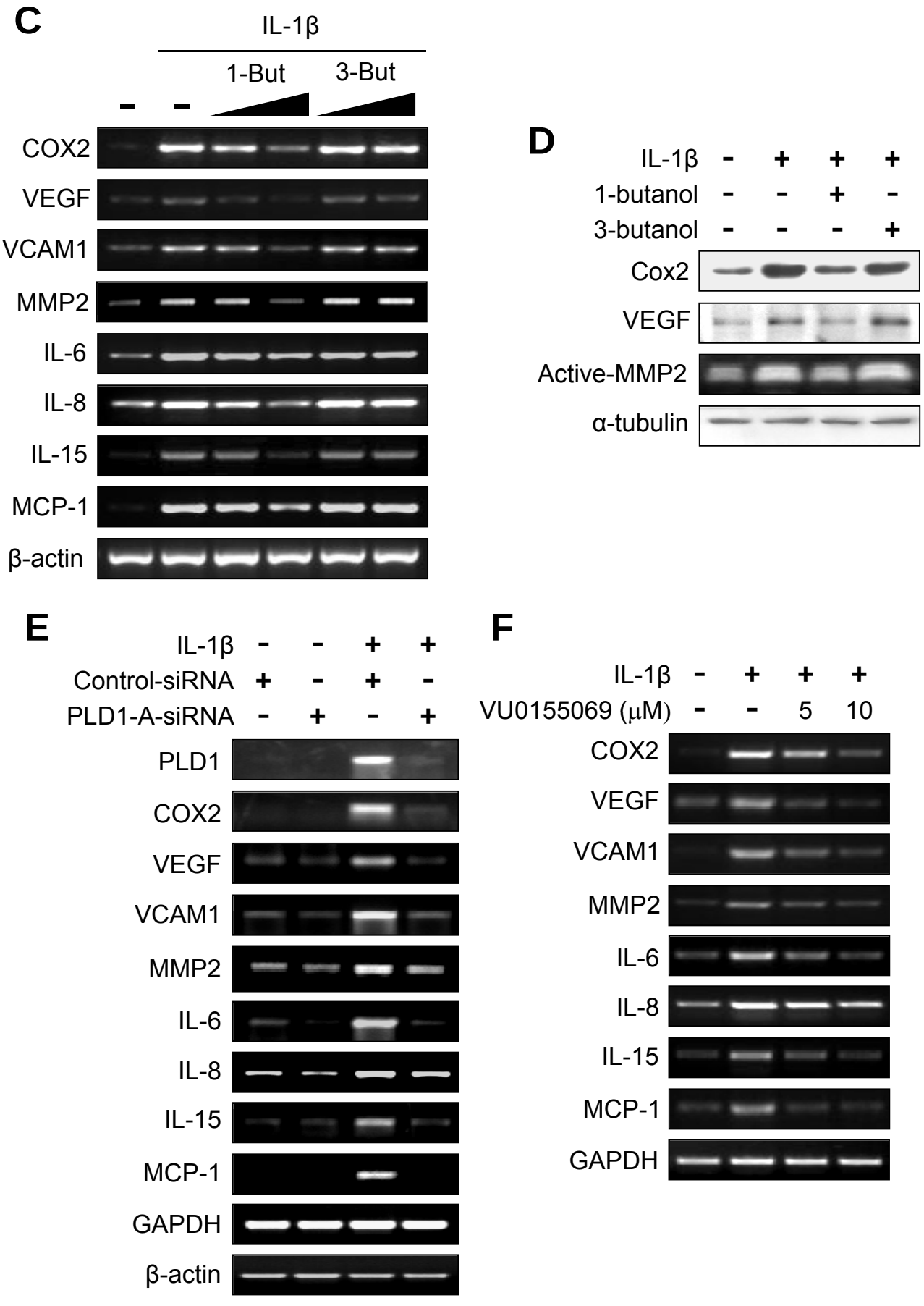
C
IL-1β
1-But
3-But
- -
COX2
VEGF
VCAM1
MMP2
IL-6
IL-8
IL-15
MCP-1
β-actin
D
IL-1β - + + +
1-butanol - - + -
3-butanol - - - +
Cox2
VEGF
Active-MMP2
α-tubulin
E
IL-1β - - + +
Control-siRNA + - + -
PLD1-A-siRNA - + - +
PLD1
COX2
VEGF
VCAM1
MMP2
IL-6
IL-8
IL-15
MCP-1
GAPDH
β-actin
F
IL-1β - + + +
VU0155069 (μM) - - 5 10
COX2
VEGF
VCAM1
MMP2
IL-6
IL-8
IL-15
MCP-1
GAPDH

Fig. S4 PLD activity is required for IL-1β-induced expression of proinflammatory mediators and angiogenic factors in RAFLS. (A) RAFLS were labeled with [^{3}H] myristate for 12 h, pretreated with VU0155069 for 30 min, and then stimulated with IL-1β, after which PLD activity was measured. RAFLS were treated with 1 or 3-butanol and then stimulated with IL-1β for 24 h, after which the secretion and expression of proinflammatory molecules were determined by ELISA (B) and RT-PCR (C). (D) RAFLS were pretreated with 1- or 3-butanol (0.4%) and then treated with IL-1β for 36 h. The expression of COX-2 and VEGF was analyzed by Western blotting, and MMP-2 activity was analyzed by gelatin zymography of the conditioned media. RAFLS were transfected with siRNA specific to PLD1 (E) or pretreated with or without VU0155069 (F), treated with IL-1β for 36 h, and then expression of various genes was analyzed by RT-PCR. *P< 0.01 *versus* vehicle; **P< 0.005 *versus* IL-1β. Data are presented as the mean ± SD of 4 independent experiments.

Fig. S5

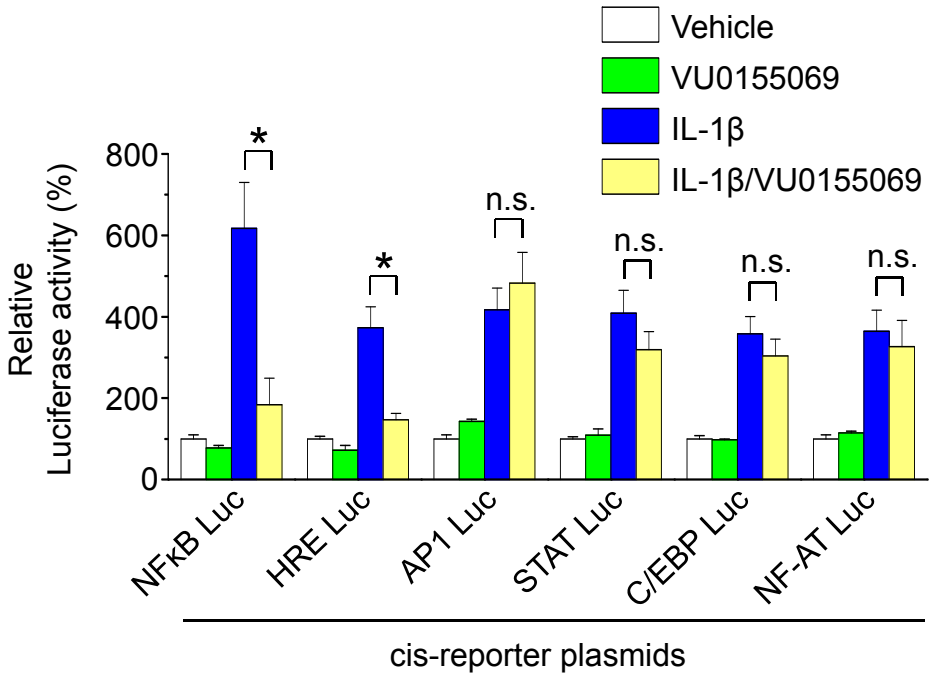
Vehicle
VU0155069
IL-1β
IL-1β/VU0155069
Relative Luciferase activity (%)
800
600
400
200
0
*
*
n.s.
n.s.
n.s.
n.s.
NFκB Luc
HRE Luc
AP1 Luc
STAT Luc
C/EBP Luc
NF-AT Luc
cis-reporter plasmids

Fig. S5 PLD1 inhibitor regulates the transactivation of NFκB, HIF and FoxO but not AP1, STAT, C/EBP, and NF-AT. After RAFLS were transfected with indicated reporter constructs, cells were pretreated with VU0155069, stimulated with IL-1β for 36 h, and then luciferase activity was determined. *P< 0.05 *versus* IL-1β. Data are presented as the mean ± SD of 4 independent experiments.

Fig. S6

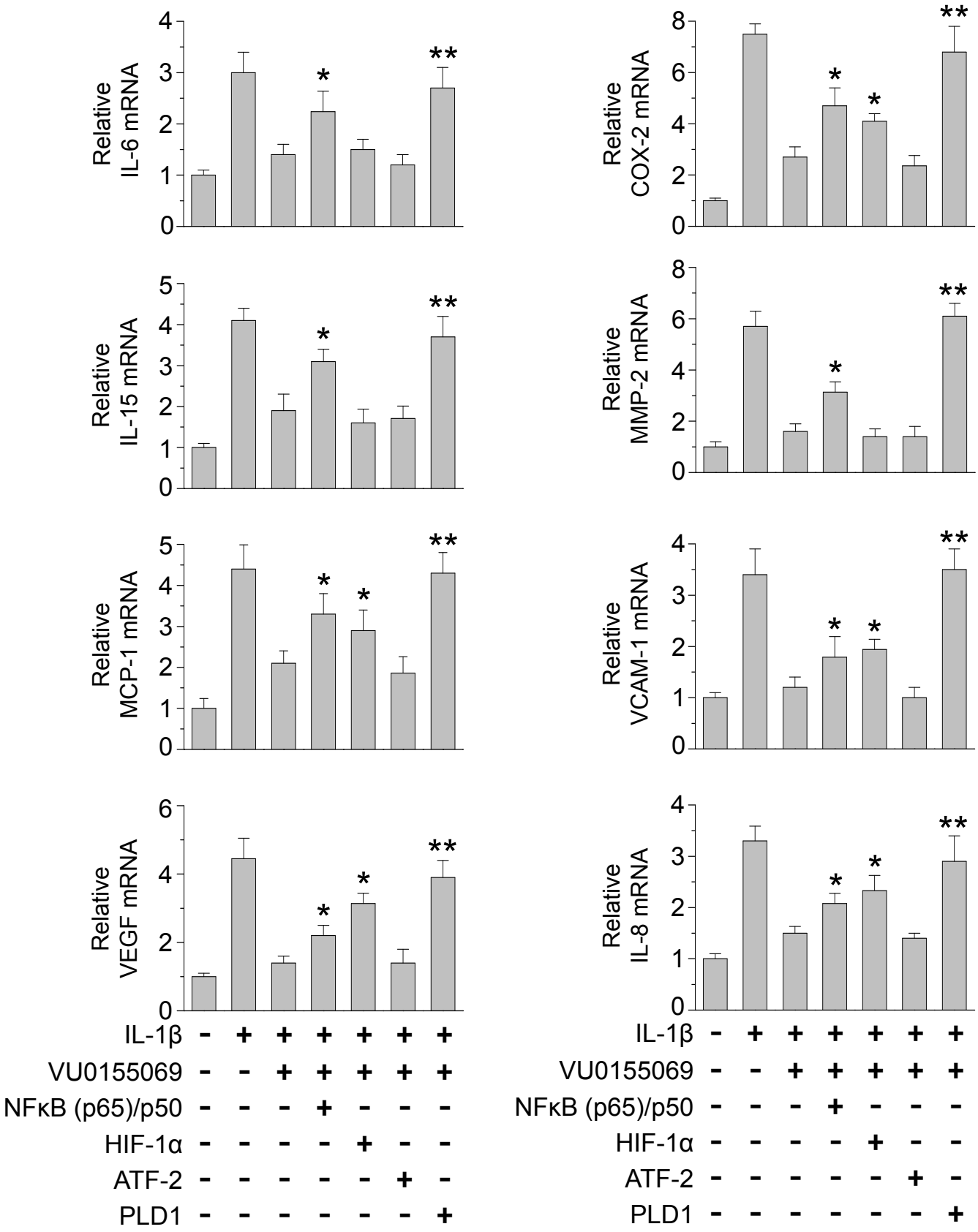
Relative IL-6 mRNA
Relative COX-2 mRNA
Relative IL-15 mRNA
Relative MMP-2 mRNA
Relative MCP-1 mRNA
Relative VCAM-1 mRNA
Relative VEGF mRNA
Relative IL-8 mRNA
IL-1β
VU0155069
NFκB (p65)/p50
HIF-1α
ATF-2
PLD1

Fig. S6 PLD1 inhibitor regulates the expression of proinflammatory molecules via NFκB, HIF-1α, ATF-2 or PLD1. RAFLS were transfected with the indicated expression vectors, after which the cells were pretreated with VU0155069 and treated with IL-1β for 36 h. Expression of the indicated molecules was analyzed by q-PCR. **P*< 0.05 *versus* IL-1β and VU0155069. Data are presented as the mean ± SD of 4 independent experiments.

Fig. S7

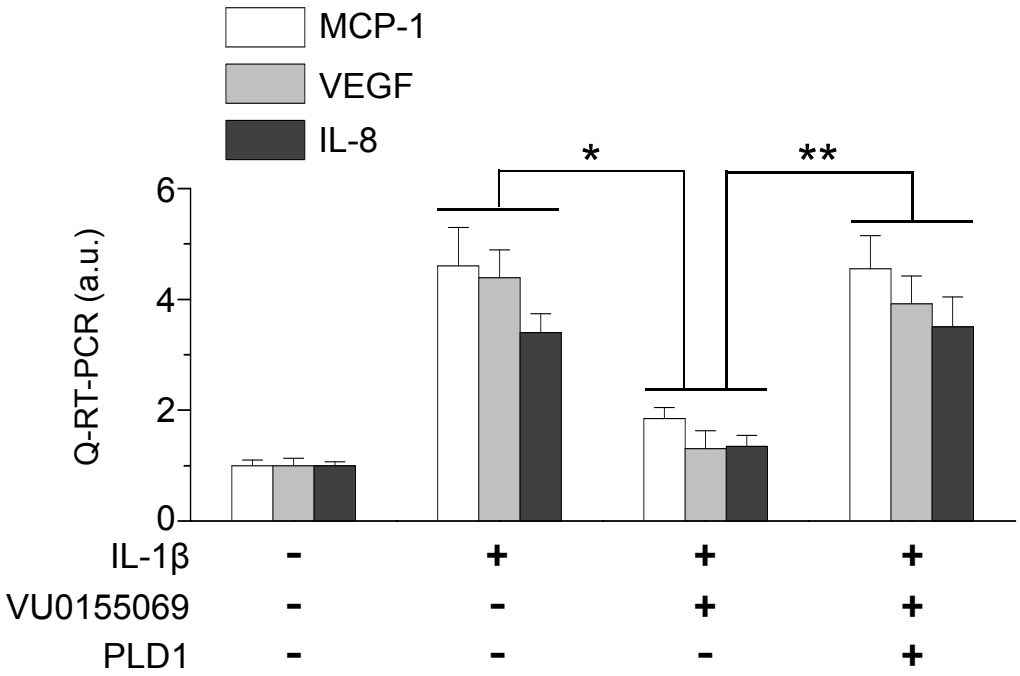

Fig. S7 Ectopic expression of PLD1 recovers the suppressive effect of PLD1 inhibitor on IL-1β mediated gene expression via PLD1. RAFLS were transfected with or without PLD1, and then pretreated with or without VU0155069 (10 μM) and treated with IL-1β for 36 h. Expression levels of MCP-1, IL-8, and VEGF were analyzed by q-PCR. **P*< 0.05 *versus versus* IL-1β; ***P*< 0.05 *versus* IL-1β and VU0155069. Data are presented as the mean ± SD of 4 independent experiments.

Fig. S8

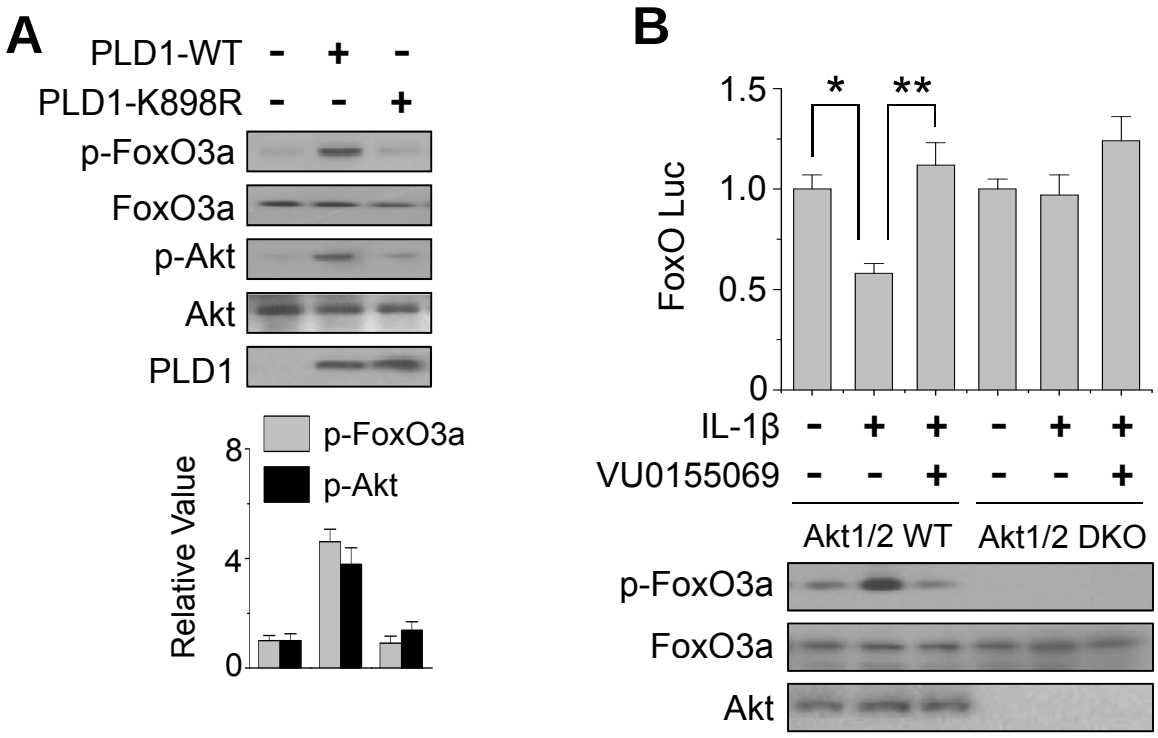
A
PLD1-WT - + -
PLD1-K898R - - +
p-FoxO3a
FoxO3a
p-Akt
Akt
PLD1
p-FoxO3a
p-Akt
Relative Value
8
4
0
B
1.5
1.0
0.5
0
FoxO Luc
*
**
IL-1β - + + - + +
VU0155069 - - + - - +
Akt1/2 WT
Akt1/2 DKO
p-FoxO3a
FoxO3a
Akt

FIG S8 Inhibition of PLD1 suppresses IL-1β-induced phosphorylation FoxO3a and enhances cell cycle arrest via transactivation of FoxO3a. (A) RAFLS were transfected with WT or K898R mutant of PLD1. Lysates were immunoblotted with the indicated antibodies. The relative levels of indicated proteins were quantitated by densitometer analysis. Data shown are representative of 4 independent experiments. (B) Akt1/2$^{+/+}$ and Akt1/2$^{-/-}$MEFs were transfected with FoxO-Luc and treated with VU0155069 and/or IL-1β. Luciferase activity assay and immunoblotting were performed. *P< 0.01; **P< 0.05. Data are presented as the mean ± SD of 4 independent experiments.

Supplementary Table

Table S1 Primer sets for ChIP assay on PLD1 promoter region

Promoter	Oligo	Direction	Seguence (5´ to 3´)
PLD1	NFκB-1	Forward	CCAGCGAGGTGCATTCTAAA
		Reverse	AGCCCTTATTATAACTCAATGAGTCT
	NFκB-2	Forward	CGAGACTCATTGAGTTATAATAAGGGCT
		Reverse	CAAGGTCTTAGGCTTCTTGAGAATG
	NFκB-3	Forward	GATGTCTTTCGGAATAGGTATATTAATCAA
		Reverse	GGAGATTGGTTTGGGAAAGCTTA
	NFκB-4	Forward	CACACAGAGCAGGCTGAATTG
		Reverse	GCTCAGATCATCCGTCTTTACC
	CRE	Forward	CTGCCCTGGGTACTGATGTG
		Reverse	GAGATTTTGGCCATGACTTTACGTG

Table S2 Primer sets for Q-RT-PCR

	Primer	Direction	Seguence (5´ to 3´)
Q-RT-PCR	PLD1	Forward	TGTCGTGATACCACTTCTGCCA
		Reverse	AGCATTTCGAGCTGCTGTTGAA
	PLD2	Forward	CATCCAGGCCATTCTGCAC
		Reverse	GTGCTTCCGCAGACTCAAGG
	COX-2	Forward	TTCAAATGAGATTGTGGAAAAAT
		Reverse	AGATCATCTCTGCCTGAGTATCTT
	VEGF	Forward	AATCCAAATGCGGCATCT
		Reverse	GAGTATGCCTGCCGTGTG
	VCAM-1	Forward	GGCTGTGAATCCCCATCTTT
		Reverse	TCCACCTGGATTCCCTTTTC
	MMP-2	Forward	TGATCTTGACCAGAATACCATCGA
		Reverse	GGCTTGCGAGGGAAGAAGTT
	IL-6	Forward	GGTACATCCTCGACGGCATCT
		Reverse	GTGCCTCTTTGCTGCTTTCAC
	IL-8	Forward	TCTCTTGGCAGCCTTCCTGATTTC
		Reverse	TCCAGACAGAGCTCTCTTCCATCA
	IL-15	Forward	GTTTCAGTGCAGGGCTTCCTAAA
		Reverse	TACTTTGCAACTGGGGTGAACAT
	MCP-1	Forward	GATCTCAGTGCAGAGGCTCG
		Reverse	TGCTTGTCCAGGTGGTCCAT
	P27Kip1	Forward	GCAGGAGAGCCAGGATGTCAG
		Reverse	ATGCGTGTCCTCAGAGTTAGCC
	P21Cip1	Forward	GCAGGGGACAGCAGAGGAAG
		Reverse	CGGCGTTTGGAGTGGTAGAAATC
	β-actin	Forward	GACTACCTCATGAAGATC
		Reverse	GATCCACATCTGCTGGAA

Table S3 Primer sets for RT-PCR

	Primer	Direction	Seguence (5´ to 3´)
RT-PCR	PLD1	Forward	AATCGTTGGAGGTTGGACTG
		Reverse	AGACGGTGGAT GACACATGA
	PLD2	Forward	CTCTTCCCCACTGGGGACGAA
		Reverse	GACAGAATACAGAGTGCAGGTTCCCAC
	COX-2	Forward	GTTCCACCCGCAGTACAG
		Reverse	GGAGCGGGAAGAACTTGC
	VEGF	Forward	ATCGAGACCCTGGTGGACA
		Reverse	TGTGCTGGCCTTGGTGAG
	VCAM-1	Forward	ATGACATGCTTGAGCCAGG
		Reverse	GTGTCTCCTTCTTTGACACT
	MMP-2	Forward	GGCCCTGTCACTCCTGAGAT
		Reverse	GGCATCCAGGTTATCGGGGA
	IL-6	Forward	CAGATGAGTACAAAAGTCCTG
		Reverse	CTACATTTGCCGAAGAGCGC
	IL-8	Forward	CAGTTTTGCCAAGGAGTGCTAA
		Reverse	AACTTCTCCACAACCCTCTGC
	IL-15	Forward	GAGTTACAAGTTATTTCACTTGAG
		Reverse	CAAGAAGTGTTGATGAACATTTGG
	MCP-1	Forward	ATGGAGGTCCCTGTCATG
		Reverse	GCTTGAGGTGGTTGTGGA
	GAPDH	Forward	GTGGTCTCCTCTGACTTCAAC
		Reverse	TCTCTTCCTCTTGTGCTCTTG
	β-actin	Forward	GACTACCTCATGAAGATC
		Reverse	GATCCACATCTGCTGGAA

Table S4 Primer sets for Chip assay on NFκB or HIF1α target genes

Promoter	Oligo	Direction	Seguence (5´ to 3´)
IL-6	NFκB	Forward	ACCCTCACCCTCCAACAAAGATTTATC
		Reverse	GGGCTAAGGATTTCCTGCACTTACTT
IL-15	NFκB	Forward	CGGCAGGTAGAGGAGGAGACCGGT
		Reverse	TGAAAGAGAAAGAGCCGGGAGCATAGG
MMP-2	NFκB	Forward	GCATCTCGCACTATACGAGGCCAAGT
		Reverse	CAGAGACAGTGGAAGGTCCCAGGTTG
VCAM-1	NFκB	Forward	CTTTTGCCAGGACAGAGAGAGGAGC
		Reverse	GTATTCAGCTCCTGAAGCCAGTGAGG
VEGF	NFκB	Forward	GGGAGGAAGAGTAGCTCGCC
		Reverse	AAGTTCATGGTTTCGGAGGC
	HRE	Forward	CCTTTGGGTTTTGCCAGACTCCACAG
		Reverse	CACCAAGTTTGTGGAGCTGAGAACG
IL-8	NFκB	Forward	GGCCATCAGTTGCAAATCGTG
		Reverse	TTCCTTCCGGTGGTTTCTTCCTG
	HRE	Forward	GTGTGATGACTCAGGTTTGCCCT
		Reverse	CAGTGAGATGGTTCCTTCCGGTGGT
MCP-1	NFκB	Forward	GTAAACACAGGGAAGGTGAAGGGTATG
		Reverse	GGAACTTCCAAAGCTGCCTCCTCAGAG
	HRE	Forward	CAGACGTGGTACCCACAGTCTTGC
		Reverse	GAAGCAGCTGGGGGAGTAACTGCGC
COX-2	NFκB	Forward	GGGAGGGATCAGACAGGAGAGTGG
		Reverse	AAGCCCATGTGACGAAATGACTGTTTC
	HRE	Forward	CGACGTGACTTCCTCGACCCTCTAAAG
		Reverse	CAGAAGGACACTTGGCTTCCTCTCC