Supplemental Figures

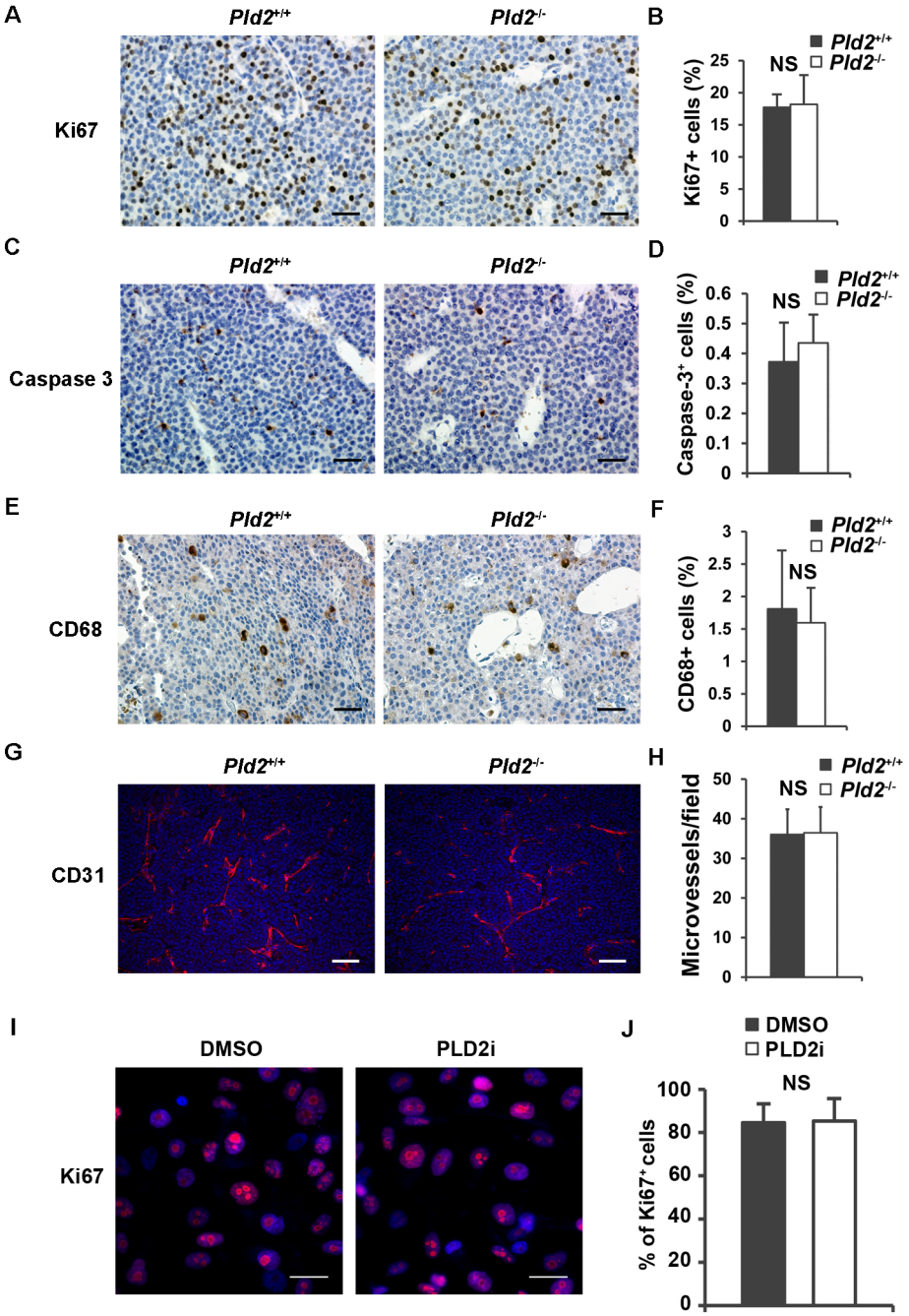

Figure S1. PLD2 deficiency does not affect proliferation, apoptosis, macrophage infiltration and angiogenesis in primary tumors and MDA-MB-231 cells. **Related to Figure 1.**
Representative immunohistochemical staining pictures of Ki67 (A), active caspase-3 (C), and CD68 (E), in mammary tumors from *MMTV-Neu*;*Pld2*$^{+/+}$ and *MMTV-Neu*;*Pld2*$^{-/-}$ mice, and their respective quantitation (B, D & F). (G) Immunofluorescent staining of microvessels and nuclei using an anti-CD31 antibody and DAPI, respectively, in mammary tumors from *MMTV-Neu*;*Pld2*$^{+/+}$ and *MMTV-Neu*;*Pld2*$^{-/-}$ mice. (H) Quantitation of staining results in g. Angiogenesis was quantified as the mean microvessel density per field. Quantitation results are shown as mean + SD. n=6 per group. The scale bars correspond to 50 μm. (I) PLD2 inhibitor does not affect the proliferation rate of MDA-MB-231 cells. Representative immunofluorescent staining pictures of Ki67 in MDA-MB-231 breast cancer cells in the absence and presence of PLD2 inhibitor. (J) Quantitation of staining results in a. The scale bars correspond to 20 μm. All quantifications are presented as mean ± SD; t-test, NS (not significant, p> 0.05).

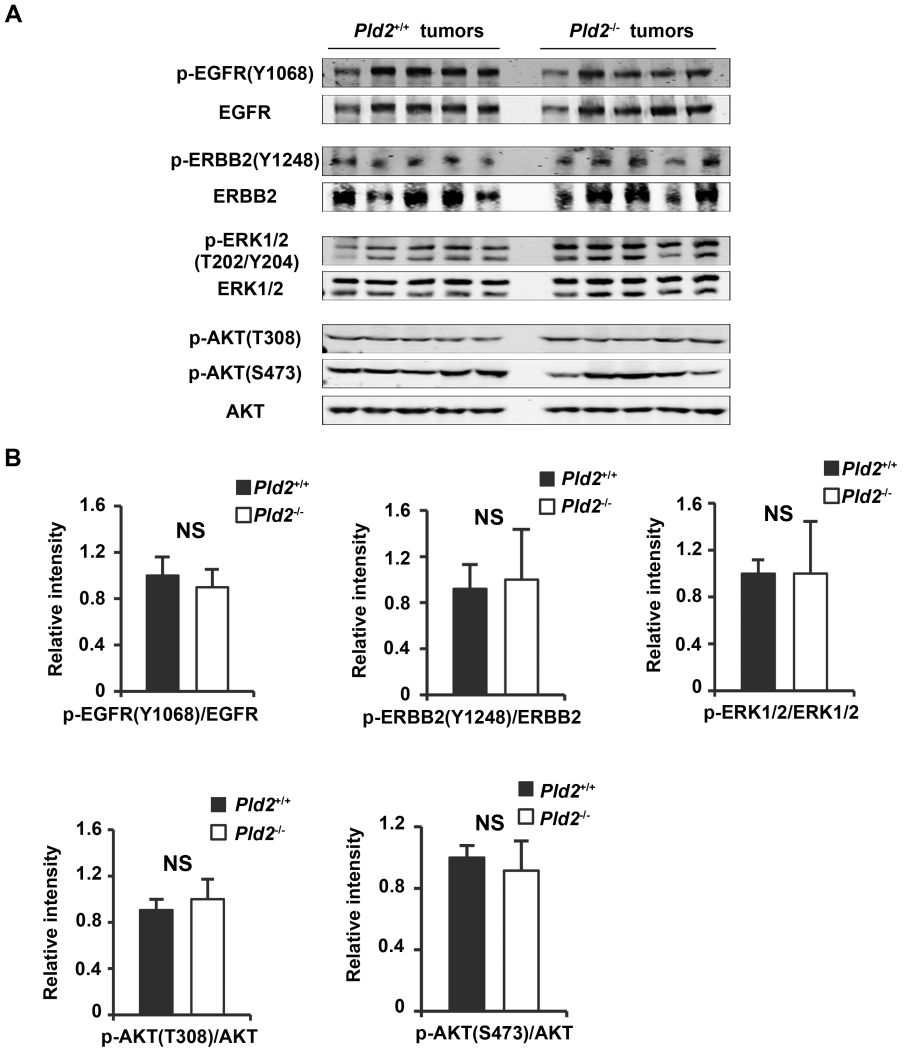

Figure S2. PLD2 knockout has no effect on the activity of the common oncogenic signaling pathways.

Related to Figures 4.

(A) Representative Western blot measuring the phosphorylation status of the indicated signaling proteins. Five tumors were analyzed for each genotype. Results are representative of at least three independent experiments. (B) Quantitation of Western blot results (mean ± SD) in a. T-test, NS (not significant, $p > 0.05$).

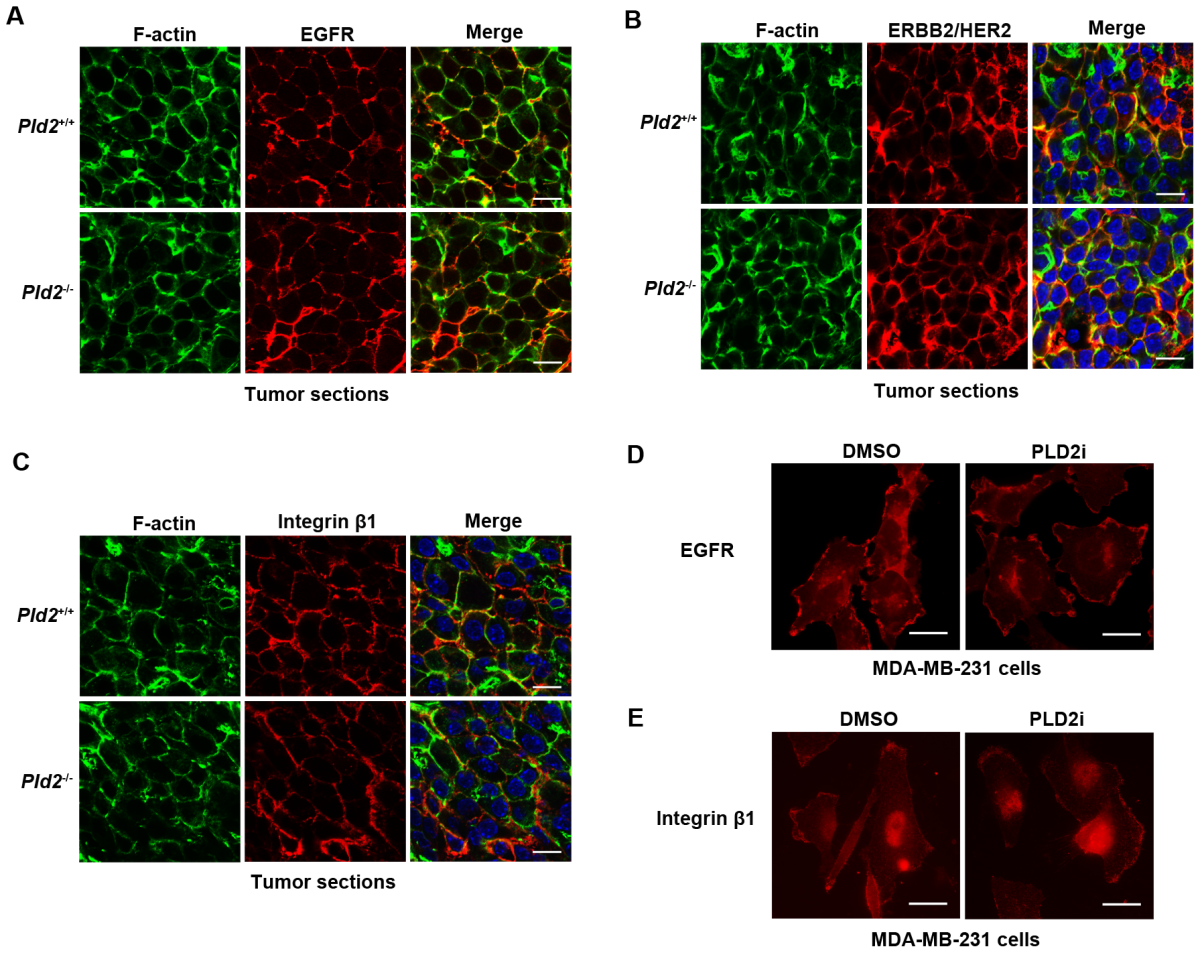

Figure S3. PLD2 deficiency does not change the localizations of EGFR, ERBB2, and integrin β1.

Related to Figure 4.

(A-C) Cryosections of mammary tumors from *MMTV-Neu*;*Pld2*$^{+/+}$ and *MMTV-Neu*;*Pld2*$^{-/-}$ mice were stained with Alexa 488-phalloidin and EGFR (A), ERBB2 (B), or integrin β1 (C). The representative images were selected from at least six different fields from four mice for each genotype. The scale bars correspond to 10 μm. (D and E) MDA-MB-231 cells treated with DMSO or PLD2 inhibitor (5μM) were stained for EGFR (D) or integrin β1 (E). The scale bars correspond to 10 μm.

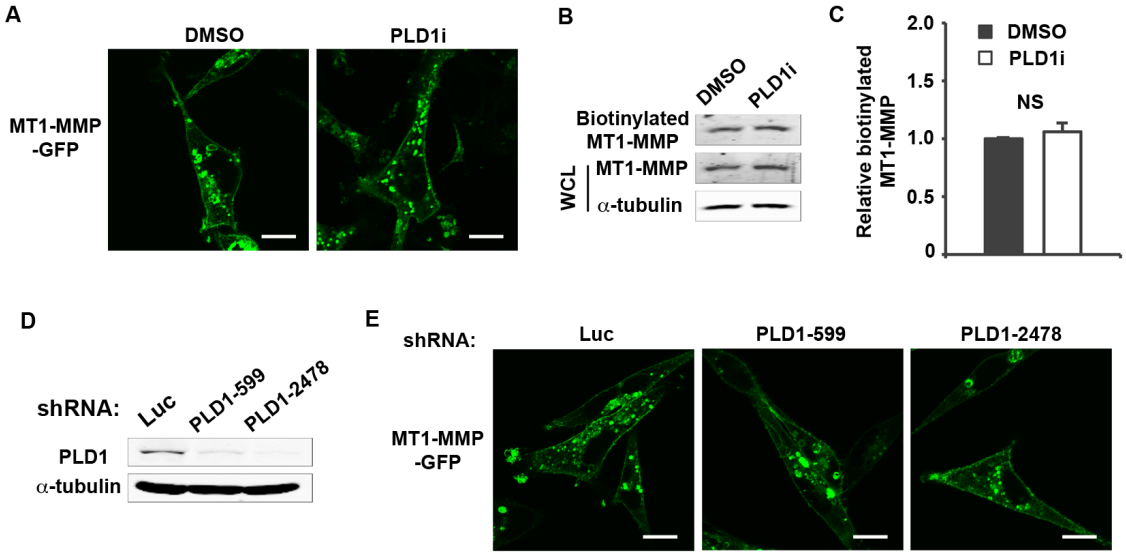

Figure S4. The surface level of MT1-MMP is not regulated by PLD1.

Related to Figure 4.

(A) Localization of MT1-MMP-GFP in MDA-MB-231 cells treated with DMSO or a PLD1 inhibitor, VU0359595 (5μM). The scale bars correspond to 10 μm. (B) Measurement of surface MT1-MMP levels. Cell surface proteins were labeled with biotin on ice for 15 min, and then recovered by streptavidin beads. Biotinylated and total MT1-MMP were analyzed by Western blotting using an MT1-MMP antibody. (C) Quantification of results in (B). The intracellular labeled MT1-MMP was normalized to total labeled MT1-MMP. Quantitation results are shown as mean + SD. n=3. (D) PLD1 knockdown by two independent shRNAs. (E) No change in the localization of MT1-MMP-GFP in MDA-MB-231 cells expressing the control (Luciferase, Luc) and PLD1 shRNAs. The scale bars correspond to 10 μm. Quantifications are presented as mean ± SD; t-test, NS ($p > 0.05$).

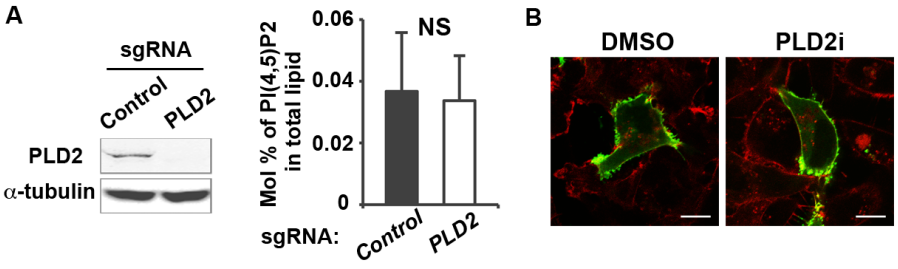

Figure S5. PLD2 inhibition does not change the levels and distribution of PI4,5P2.

Related to Figure 5.

(A) PLD2 knockout did not change PI4,5P2 levels. Left, PLD2 knockout by CRISPR/Cas9 in MDA-MB-231 cells. Right, measurement of PI4,5P2 levels by high-performance liquid chromatography. Quantitation results are shown as mean + SD. n=3. (B) PLD2 inhibitor did not affect the plasma membrane association and distribution of the PI4,5P2 biosensor, GFP-PLCδ-PH. The scale bars correspond to 10 μm. Quantifications are presented as mean ± SD. T-test, NS (not significant, $p > 0.05$).

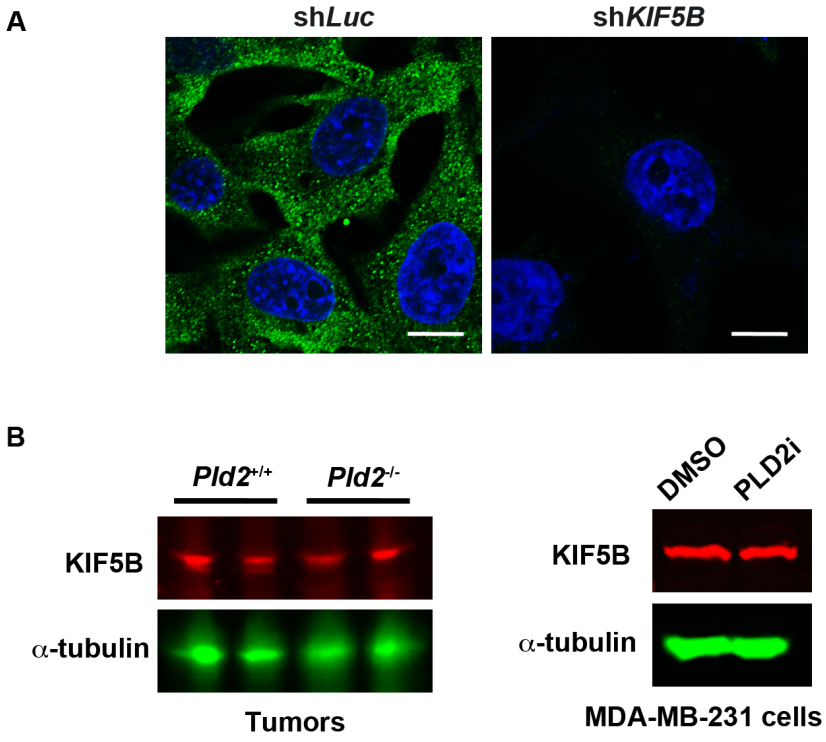

Figure S6. PLD2 does not regulate the expression level of KIF5B.

Related to Figure 6.

(A) Validation of KIF5B antibody for immunofluorescent staining. MDA-MB-231 cells were infected with either a control luciferase (sh*Luc*) or KIF5B shRNA (sh*KIF5B*), and stained with a KIF5B mouse IgM monoclonal antibody and followed by an Alexa488 goat anti-mouse IgM antibody (green). Nuclei were stained with DAPI (blue). The scale bars correspond to 10 μm. (B) *Pld2* knockout or inhibition had no effect on KIF5B protein level in tumors (left) or in MDA-MB-231 cells (right).

Supplemental table S4. DNA oligos used for plasmid constructs and genotyping.

Related to Key Resources Table.

Application	Primer name	Primer sequence
pGEX-4T-1-KIF5B-C-A1	KIF5B-C-A1F	ccgctgtgaacttcctgctttggaagctgcacttgcagctacagctgagagagtg
	KIF5B-C-A1R	cactctctcagctgtagctgcaagtgcagcttccaaagcaggaagttcacagcgg
pGEX-4T-1-KIF5B-C-A2	KIF5B-C-A2F	cttcgagctacagctgaggctgtggcagctttggaatcagcac
	KIF5B-C-A2R	gtgctgattccaaagctgccacagcctcagctgtagctcgaag
pGEX-4T-1-KIF5B-C-A3	KIF5B-C-A3F	ctttggaatcagcactggcagaagctgctgaaaatgcatctcgtg
	KIF5B-C-A3R	cacgagatgcattttcagcagcttctgccagtgctgattccaaag
pGEX-4T-1-KIF5B-C-A4	KIF5B-C-A4F	gctaaagaaaatgcatctgctgatgcagctgcttatcagcaagaagtag
	KIF5B-C-A4R	ctacttcttgctgataagcagctgcatcagcagatgcattttctttagc
pGEX-4T-1-KIF5B-C-A5 & pCDH-Myc-KIF5B-PA$^-$	KIF5B-C-A5F	cagcaagaagtagatgcaatagcagaagcagtcgcttcagcaaatatggccagaagagg
	KIF5B-C-A5R	cctcttctggccatatttgctgaagcgactgcttctgctattgcatctacttcttgctg
pCDH-KIF5B-GFP and pCDH-KIF5B wobble mutants	KIF5B wobble-F	gacgtgttggagggatataatggaacaatatttgc
	KIF5B wobble-R	ttatatccctccaacacgtctttaacaatcttctttgc
pCDH-KIF5B-GFP	KIF5B-5'-NheI	ctagagctagcaccatggcggacctggccgagtgc
	KIF5B-3'-AgeI	gactgaccggtcccacttgtttgcctcctccacc
pCDH-KIF5B & pCDH-KIF5B-PA$^-$ (no tag)	KIF5B-Pst	gtcaatgtggagttaactgcagaacag
	KIF5B-3'stop	cgcagatccttcgcggccgcttacacttgtttgcctcctccac
pcDNA-ARF6-N48R & pHAGE-ARF6-N48R	ARF6-N48R-F	cccacggtgggcttcagagtggagacggtgac
	ARF6-N48R-R	gtcaccgtctccactctgaagcccaccgtggg
pHAGE-ARF6 wt and N48R	ARF6-N-F	caggtgttgtgagctagcccgaccatatggggaaggtgc
	ARF6-C-R	ggggggggcggaattctctagaactagtggatctctgcg
LentiGuide-*PLD2*-puro	PLD2-KO-F-F	caccgtattctgtccgcttgactca
	PLD2-KO-F-R	aaactgagtcaagcggacagaatac
Pld2$^{+/+}$ and *Pld2*$^{-/-}$ genotyping	*Pld2*-F	aaggctcctcctctgcatctt
	Pld2-R	caagcatcctcaaaacaccagc
	Neomycin-R	gaggagtggttggctctggag
*Ne*u transgene genotyping	oIMR0386 (Neu-F)	tttcctgcagcagcctacgc
	oIMR0387 (Neu-R)	cggaacccacatcaggcc
	oIMR8744 (internal control forward)	caaatgttgcttgtctggtg
	oIMR8745 (internal control reverse)	gtcagtcgagtgcacagttt