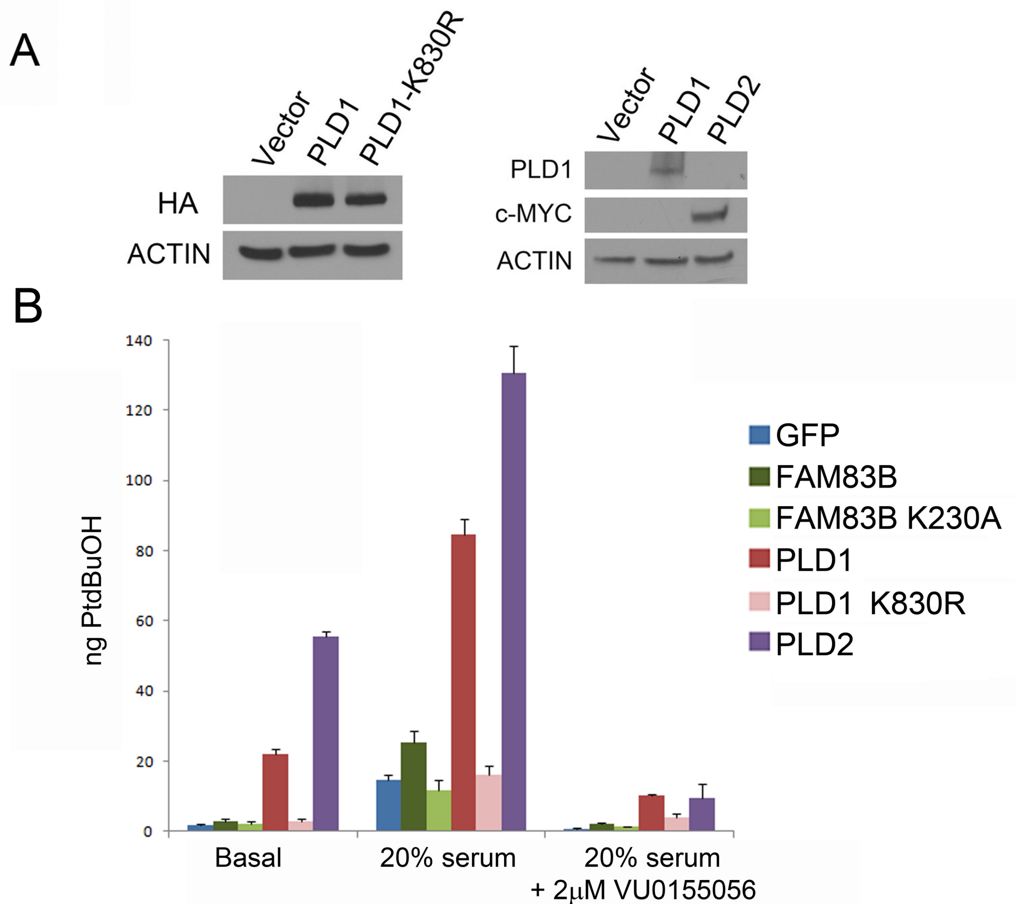
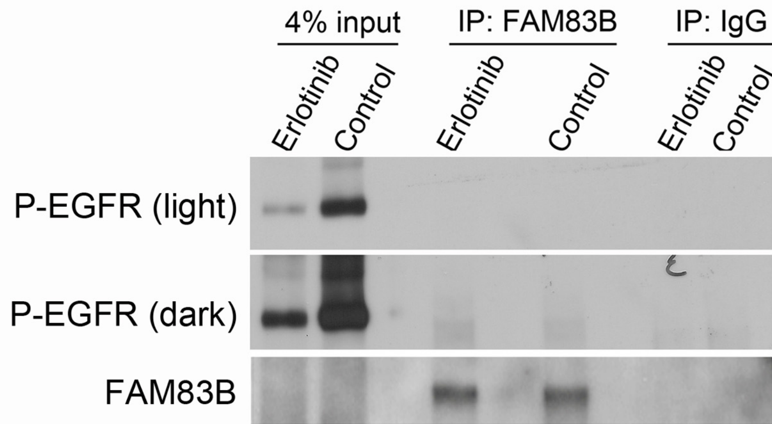
Supplementary Information

Hyperactivation of EGFR and downstream effector phospholipase D1 by oncogenic FAM83B.

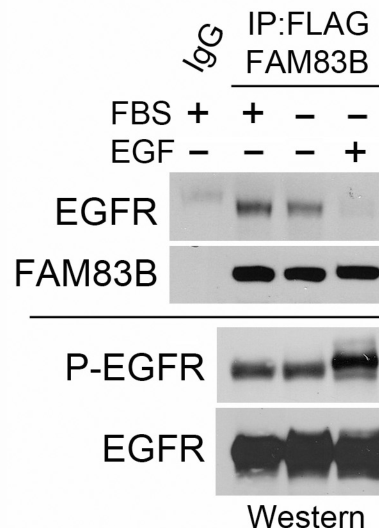
Rocky Cipriano, Benjamin L. Bryson, Kristy L.S. Miskimen, Courtney A. Bartel, Wilnelly Hernandez-Sanchez, Ronald C. Bruntz, Sarah A. Scott, Craig W. Lindsley, H. Alex Brown, and Mark W. Jackson



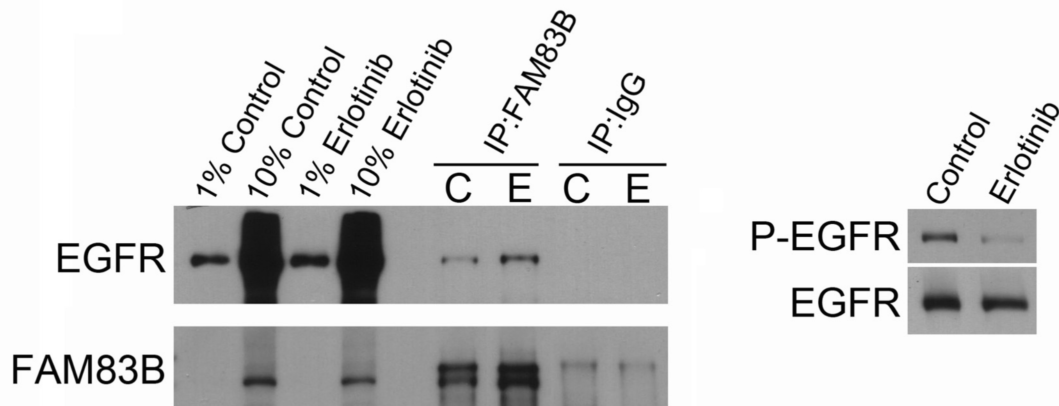
Supplementary Figure S1. To compare PLD activities, cells expressing GFP (as a control), FAM83B, FAM83B K230A, PLD1, PLD1 K830R, or PLD were created. (A) Western analysis confirmed the expression of each protein (also see Fig. 1A). (B) Cells were left untreated (basal) or stimulated with serum (20% serum) in the presence of 1-butanol-d10 to trap PLD product as phosphatidylbutanol-d9 (PtdBuOH), which was detected by mass spectrometric analysis. Addition of a dual PLD1/PLD2 inhibitor VU0155056 (2 μM) significantly diminished PLD activity in all HME derivatives. Data is plotted as ng PtdBuOH produced.



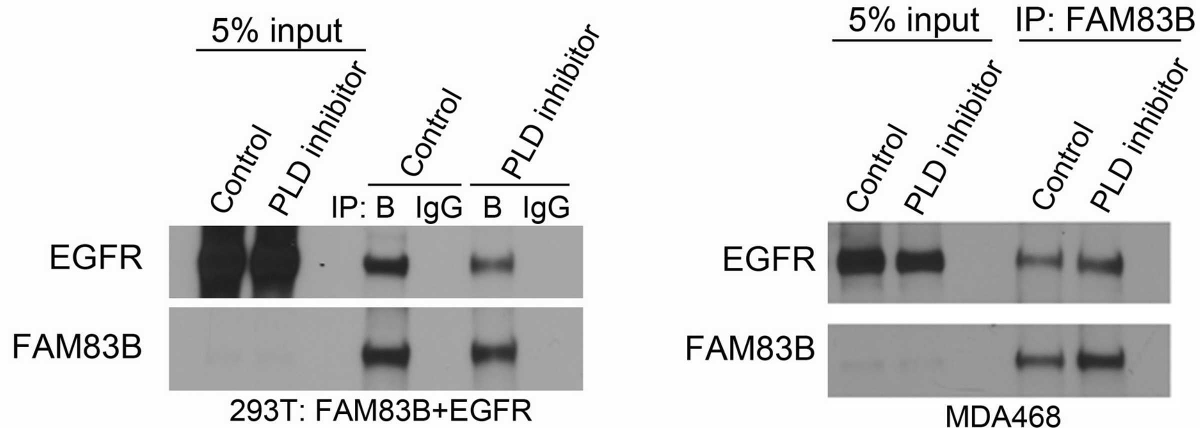
Supplementary Fig. S2. To assess whether FAM83B complexes with phopshorylated EGFR, MDA468 cells were treated with 10 μM Erlotinib for 16 hours or left untreated. Immunoprecipitation was performed using a FAM83B-specific or IgG (control) antibody. Precipitated proteins were analyzed by Western analysis to determine the amount of phosphorylated EGFR (Y1068) complexed with FAM83B.



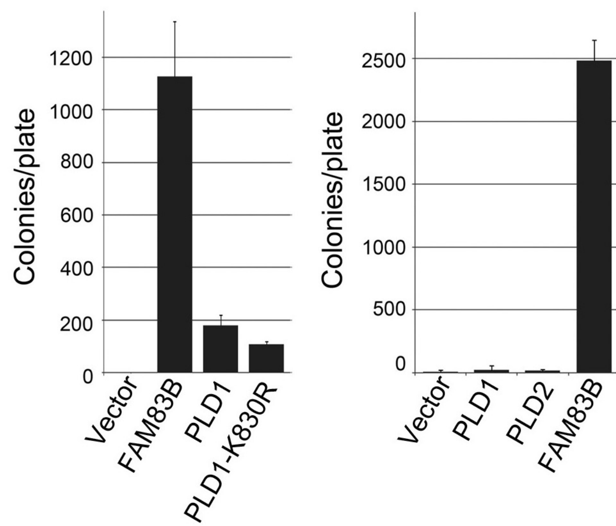
Supplementary Fig. S3. To assess whether EGF stimulation alters FAM83B-EGFR complex formation, 293T cells were transfected with expression constructs encoding EGFR and FLAG-FAM83B. FBS was removed and recombinant EGF (10 ng/ml) was added for 1 hour. Immunoprecipitation was performed using a FLAG or IgG (control) antibody, and precipitated proteins analyzed by Western analysis to determine the amount of EGFR bound to FAM83B. Western analysis of each sample demonstrates the increase in phospho-EGFR following EGF stimulation.



Supplementary Fig. S4. To assess whether FAM83B complexes with phopshorylated EGFR, MDA468 cells were treated with 10 μM Erlotinib for 16 hours or left untreated (Control). Immunoprecipitation was performed using a FAM83B-specific or IgG (control) antibody and the precipitated proteins were analyzed by Western analysis to determine the amount of EGFR complexed with FAM83B. Western analysis of each sample demonstrates the decrease in phospho-EGFR following Erlotinib treatment (right panel).



Supplementary Fig. S5. To assess whether PLD inhibition alters FAM83B-EGFR complex formation, 293T cells were transfected with expression constructs encoding EGFR and FLAG-FAM83B (left panel). Endogenous proteins from MDA468 cells were examined in the right panel. PLD inhibitor (10 μM VU0155069) was added to the cells for 16 hours, and FAM83B was immunoprecipitated using a FAM83B-specific or IgG (control) antibody. Precipitated proteins were analyzed by Western analysis to determine the amount of EGFR bound to FAM83B.



Supplementary Fig. S6. To assess whether PLD activity is sufficient to drive HME1 cell transformation, HME1 cells expressing FAM83B, PLD1, a catalytically inactive PLD1 mutant (PLD1-K830R), and PLD2 were plated in soft agar to assess AIG. Western analysis and PLD activity are shown in Supplementary Fig. 1.