Supplementary Information

Identification of FAM83B as a novel intermediary in EGFR/RAS-mediated transformation.

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Supplementary Figures

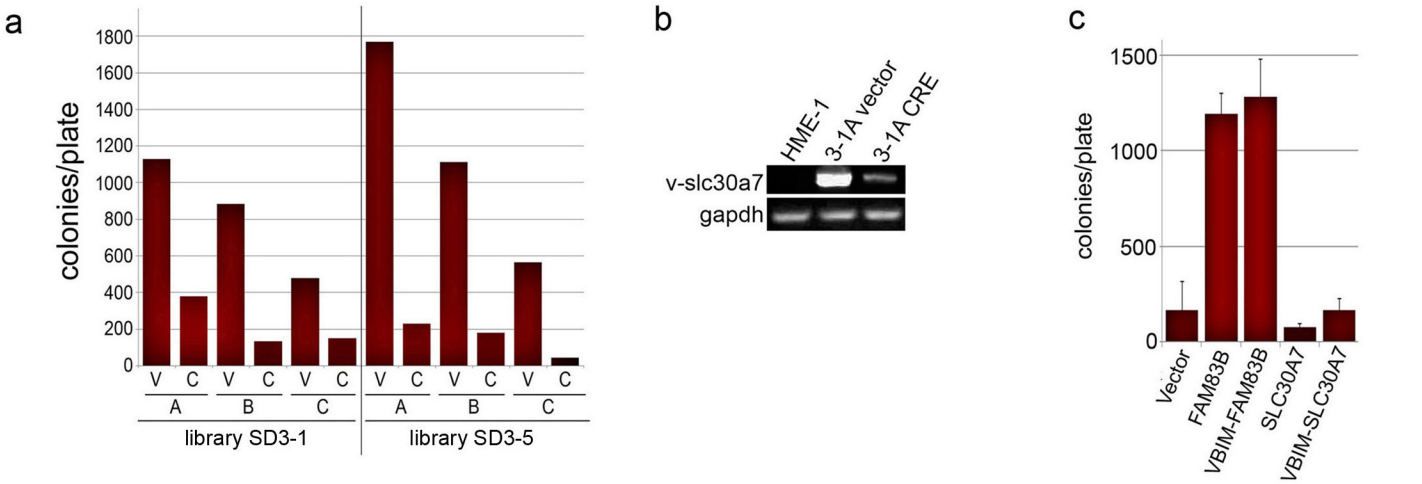

Figure 1. (a) Individual soft agar colonies (A, B, and C) were recovered from pools SD3-1 and SD3-5, expanded and infected with a retrovirus encoding Cre Recombinase (C), or a control (Vector, V). Following puromycin selection, the cells were assessed for AIG. (b) The VBIM viral insertion site in the SD3-1 mutant was identified in the SLC30A7 gene by inverse PCR. RT-PCR was performed on RNA from SD3-1 cells using a primer targeting the 5' region of the VBIM-driven mRNA and a 3' SLC30A7 specific primer, confirming the VBIM-SLC30A7 spliced mRNA (V-SLC30A7) is dependent on the sustained activity of the VBIM promoter. Primers for GAPDH served as a loading control. (c) Naïve HME1 cells expressing full-length FAM83B, VBIM-FAM83B (cloned from SD3-5 mutant), full-length SLC30A7, and VBIM-SLC30A7 (cloned from SD3-1 mutant) were assessed for AIG.

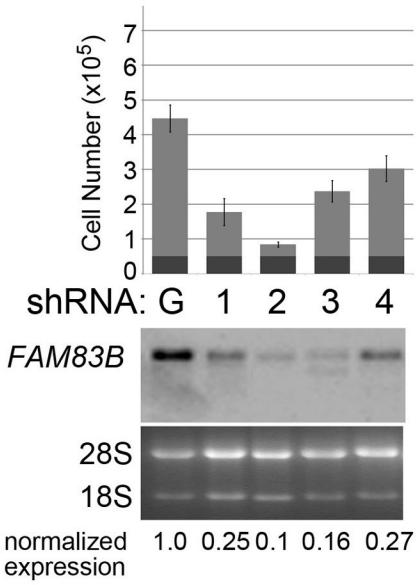

Figure 2. MCF7 cells expressing shRNAs targeting GFP (G) or FAM83B (1, 2, 3, and 4) were plated, grown for 8 days, and the cell number was determined. Northern analysis for FAM83B demonstrated that the inhibitory effect of the FAM83B shRNAs correlated with growth inhibition.

Refs	human		mouse	
1,2	Y336-p	IHKLDSSyFKNRGIY	Y336-p	IHKLDSSyFKGRGIY
3	S388-p	NENWKRHsYAGEQPE	S388-p	NENWKRHsYAGEQPE
1,3	Y389-p	ENWKRHSyAGEQPET	Y389-p	ENWKRHSyAGEQPET
1	Y399-p	EQPETVPyLLLNRAL	Y399-p	EQPETTPyLLLNRAM
3	S422-p	NWKKPSDsLSVASSS	S422-p	RWRRPSDsLSVASSL
3	S424-p	KKPSDSLsVASSSRE	S424-p	RRPSDSLsVASSLRG
3	S427-p	SDSLSVAsSSREGYV	S427-p	SDSLSVAsSLRGGQG
3	S429-p	SLSVASSsREGYVSH	L429-p	SLSVASSlRGGQGSQ
3	S466-p	RNSNVRRsFNGTDNH	S466-p	RNSNVRRsFNGTDNH
1,4	Y523-p	LGDRFEGyDNPENLK	Y523-p	LGDRFEGyENPEAVK
1	Y639-p	TESNNYIyKTLGVNK	Y639-p	AELNNCIyTNLCVNK
3	S664-p	ENLLKRRsFPLFDNS	S664-p	DNLLKRRsFPSFDHS
1	Y683-p	DPGNSKHyVYSTLTR	Y683-p	EHGNSKNyVYSTLTR
1	Y685-p	GNSKHYVySTLTRNR	Y685-p	GNSKNYVySTLTRNR
1	S686-p	NSKHYVYsTLTRNRV	S686-p	NSKNYVYsTLTRNRI
3	T782-p	LRSLLSLtPDKKENL	T782-p	LRSLLSLtPEKRESL
3	S802-p	PAFYRLCsSSDTLVS	S802-p	PAFYRMCsSSDTLVS
3	S852-p	SKEDVTVsPSQEINA	S852-p	SKEDIAVsASPGISS
3	T867-p	PPDENKRtPSPGPVE	A868-p	AEESRRIaPSPRPVE
3	S869-p	DENKRTPsPGPVESK	S870-p	ESRRIAPsPRPVERR
1,4	Y896-p	FNTEQIQyRDSREIN	-	gap
5	Y972-p	SATMGNSyGRSSPLL	-	gap
3	S976-p	GNSYGRSsPLLNYNT	S977-p	GTAYGRSsPMLNYKT
5	Y981-p	RSSPLLNyNTGVYRS	-	gap
1,4,5	Y986-p	LNYNTGVyRSYQPNE	-	gap

References

1. Guo A, et al. (2008) Signaling networks assembled by oncogenic EGFR and c-Met. Proc Natl Acad Sci U S A 105, 692-7.
2. CST #961 | Cells: HCC1806 | Treatment: serum starvation Ab for IP: P-Tyr-100.
3. Dephoure N, et al. (2008) A quantitative atlas of mitotic phosphorylation Proc Natl Acad Sci U S A 105, 10762-7.
4. CST #1605 | Cells: MKN-45 | Ab for IP: P-Tyr-100
5. Rikova K, et al. (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131, 1190-203

Figure 3. Detection of endogenous FAM83B protein in various cell lines. The FAM83B protein has been detected by mass spectrometry following immunoprecipitation with phospho-Tyr/Ser/Thr antibodies. The human FAM83B peptides recovered are shown alongside the corresponding mouse sequence. The references provided by Phosphosite (http://www.phosphosite.org/) describe the identified peptides and the conditions under which they were identified.

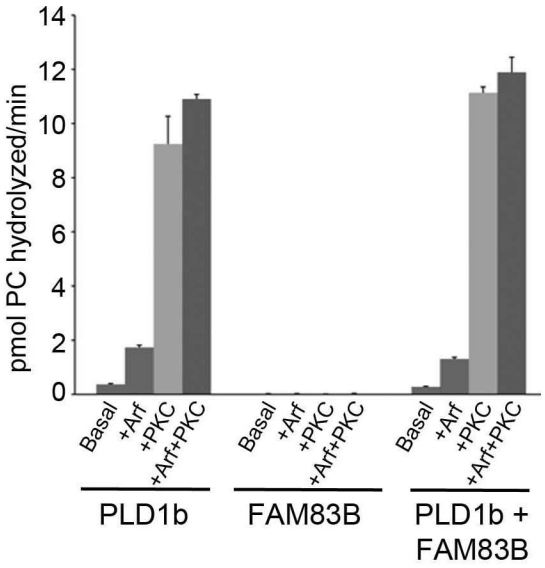

Figure 4. Recombinant FAM83B does not catalyze in vitro choline release or increase hPLD1b activity. Recombinant FAM83B-482 (comprising amino acids 1-482) was purified from E. Coli and assayed for [3H]-choline release under basal conditions or with a combination of known PLD stimulators, ARF GTPase and protein kinase C (PKC). Insect-cell purified human PLD1b was used as a positive control.

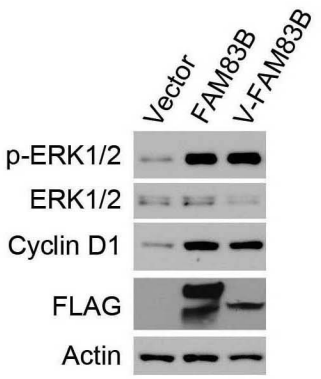

Figure 5 . Confirmation of MAPK activation by VBIM-FAM83B. HME1 cells expressing FAM83B and VBIM-FAM83B have elevated ERK1 and ERK2 phosphorylation. In addition, the level of Cyclin D1 protein, which is transcriptionally elevated by MAPK signaling, is also elevated following ectopic FAM83B expression.

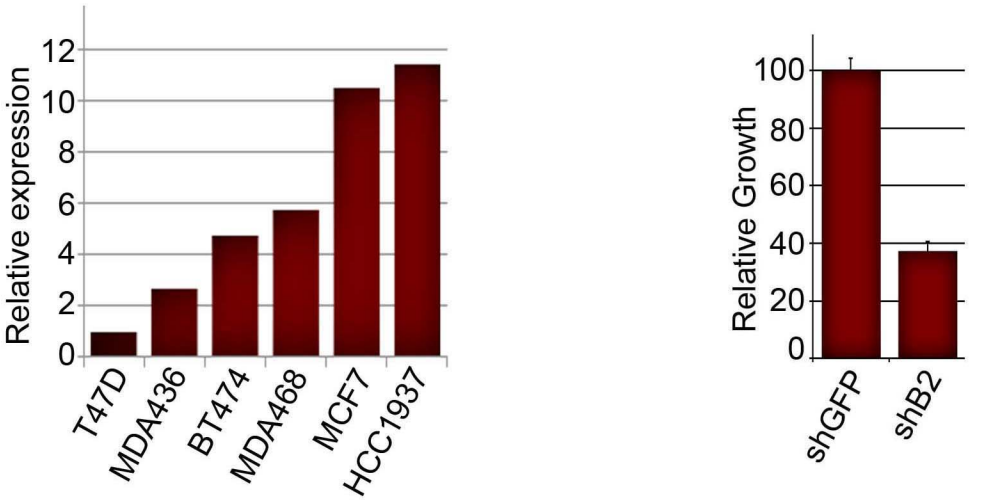

Figure 6 . Real Time PCR analysis was performed on a panel of breast cancer cell lines (T47D, MDA436, BT474, MDA468, MCF7, and HCC1937) for *FAM83B* expression. HCC1937 cells were infected with lentiviruses encoding a shRNA targeting *GFP* or *FAM83B*. The cells were plated for 5 days, and growth was assessed.

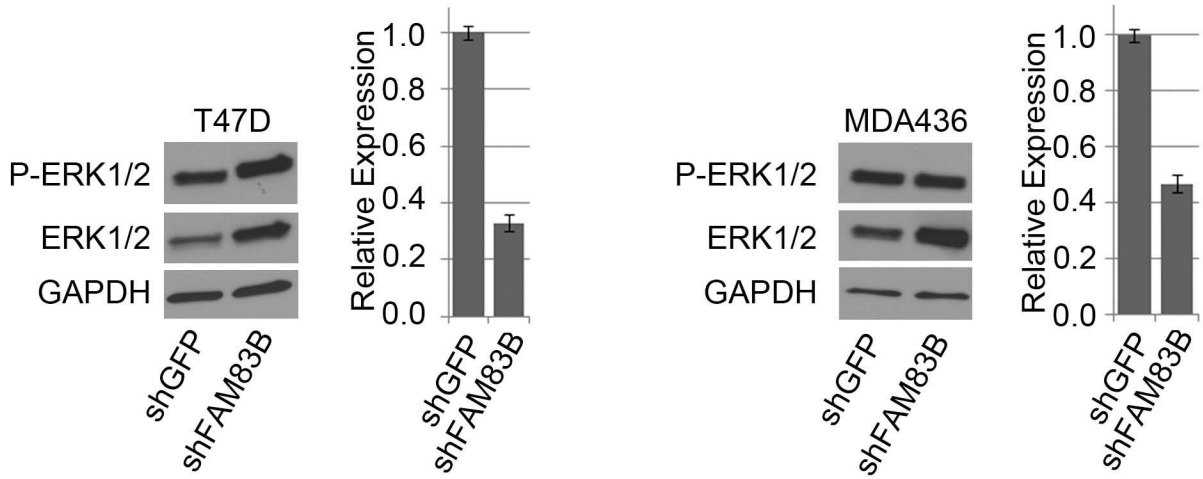

Figure 7. Western anaylsis of T47D and MDA436 breast cancer cells expressing shRNA targeting GFP or FAM83B. Real-time PCR was performed to confirm the level of *FAM83B* knockdown in T47D and MDA436 expressing shRNA targeting FAM83B or GFP.

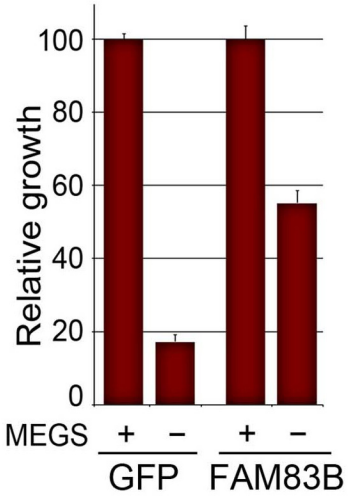

Figure 8 . FAM83B expression promotes growth factor-independent proliferation. HME1 cells expressing GFP or FAM83B were plated in the presence and absence of mammary epithelial growth supplement (MEGS) and cell number quantified 5 days later.

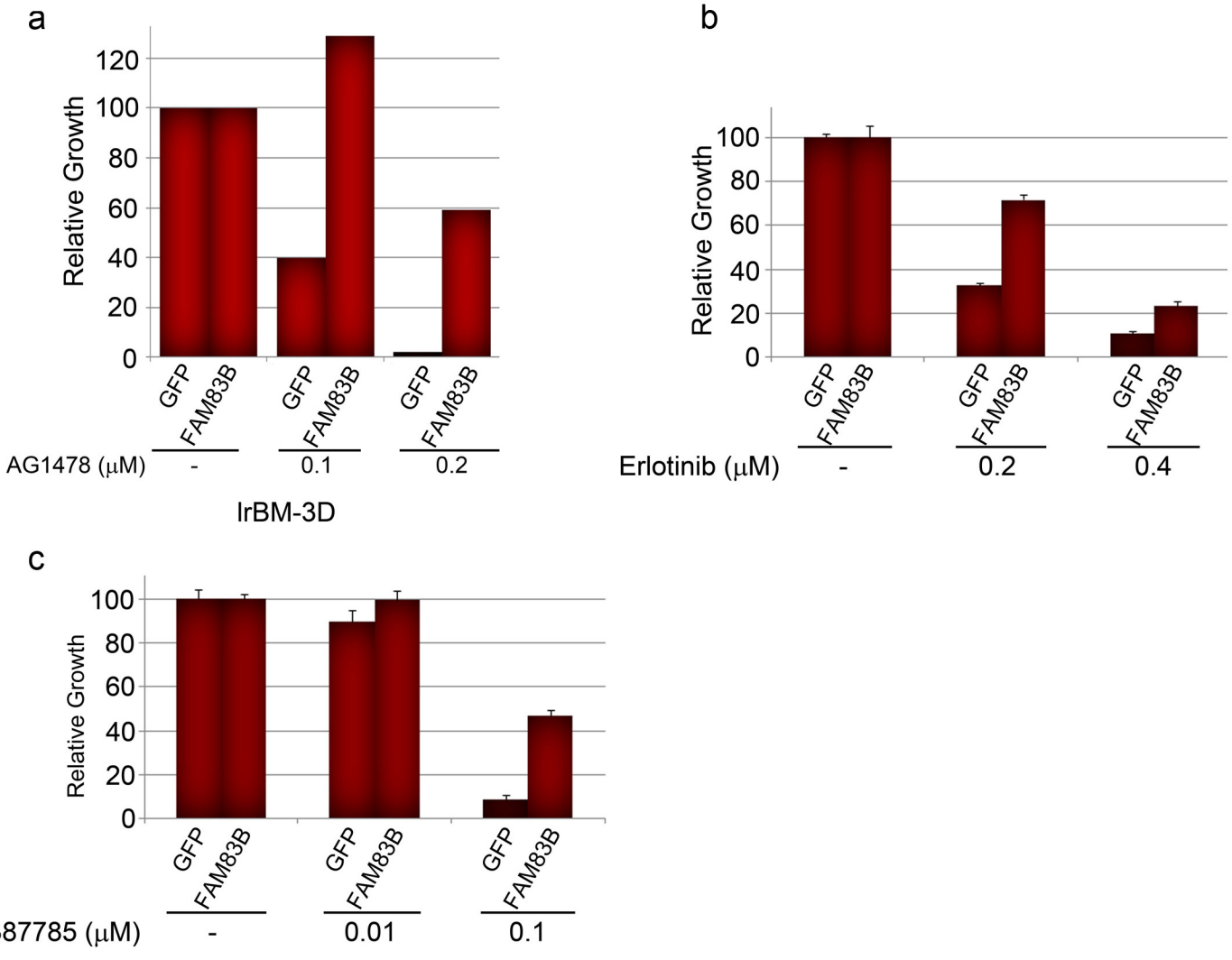

Figure 9. FAM83B expression confers resistance to various EGFR-TKIs. (a) HME1 cells expressing either GFP or FAM83B were grown in lrBM in the presence and absence of AG1478 and cell number was quantified 3 weeks later. (b and c) HME1 cells expressing either GFP or FAM83B were grown in the presence and absence of erlotinib (b) or CL387784 (c) and cell number quantifiedf 7 days later.

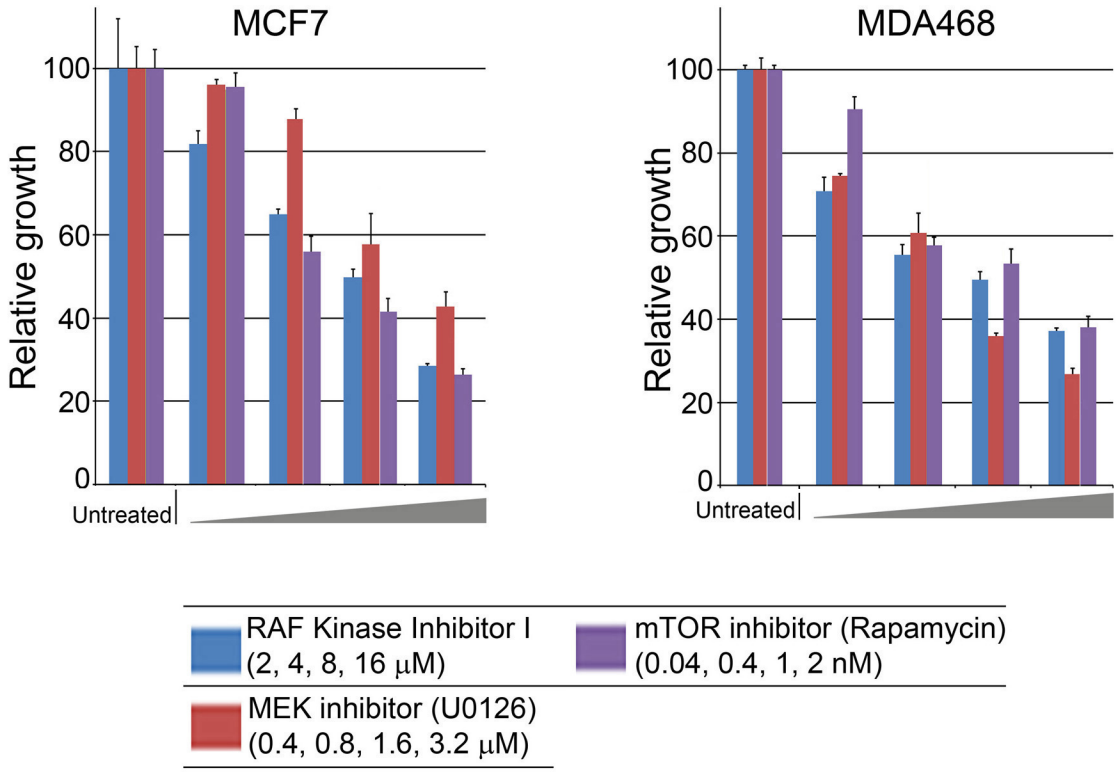

Figure 10. MCF7 and MDA468 cells were plated for 5 days and treated with RAF inhibitor (RAF Kinase Inhibitor I), MEK inhibitor (U0126), or mTOR inhibitor (Rapamycin) at the indicated concentrations and cell number was assessed 5 days later.

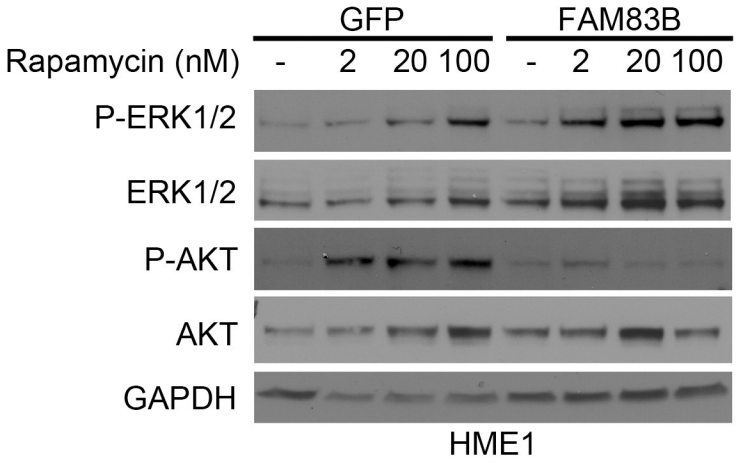

Figure 11. Western anaylsis of HME1 cells expressing GFP or FAM83B treated with various doses (2, 20, and 100nM) of Rapamycin for 24 hours.

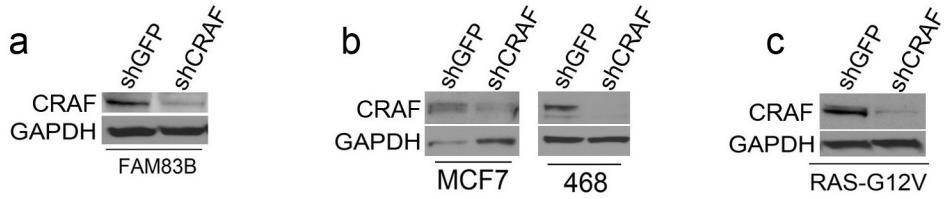

Figure 12. (a) Western analysis of FAM83B-expressing HME1 cells expressing shGFP and shCRAF. (b) Western analysis of MDA468 and MCF7 cells expressing shGFP or shCRAF. (c) Western analysis of RAS-expressing HME1 cells expressing shGFP and shCRAF.

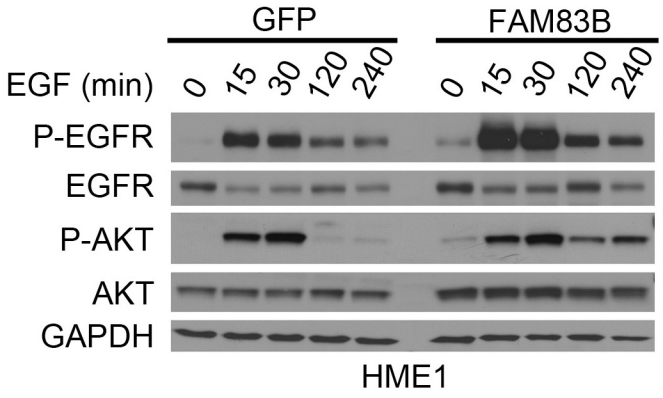

Figure 13. HME1 cells expressing GFP or FAM83B were grown in the absence of mammary epithelial growth supplement (MEGS) for 24 hours and then treated with 10ng/mL epidermal growth factor (EGF) for 15, 30, 120, and 240 minutes. Immunoblot analysis of phospho-EGFR, EGFR, phospho-AKT, AKT, and GAPDH was performed.

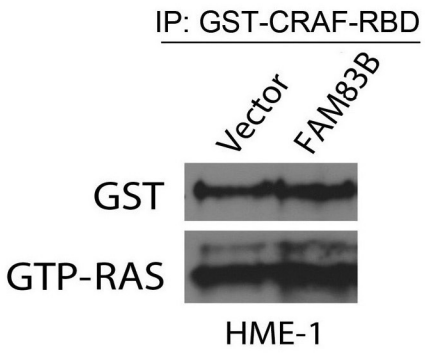

Figure 14. RAS activation assay was performed according to the procedures recommended by Upstate. Lysates from cells expressing Vector or FAM83B were precipitated using a glutathione S-transferase (GST) fusion protein containing the RAS-binding domain (RBD) of CRAF (GST-CRAF-RBD) bound to agarose beads, and the bead-associated proteins were analyzed by SDS page electrophoresis followed by immunoblotting using anti-RAS and anti-GST antibodies.

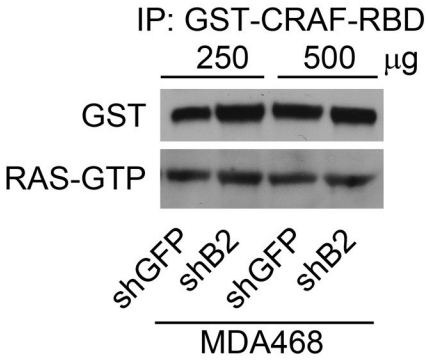

Figure 15. RAS activation assay was performed according to the procedures recommended by Upstate. Lysates from MDA468 cells expressing shRNA targeting GFP or FAM83B were precipitated using glutathione S-transferase (GST) fusion protein containing the RAS-binding domain (RBD) of CRAF (GST-CRAF-RBD) bound to agarose beads, and the bead associated proteins were analyzed by SDS page electrophoresis followed by immunoblotting using anti-RAS and anti-GST antibodies.

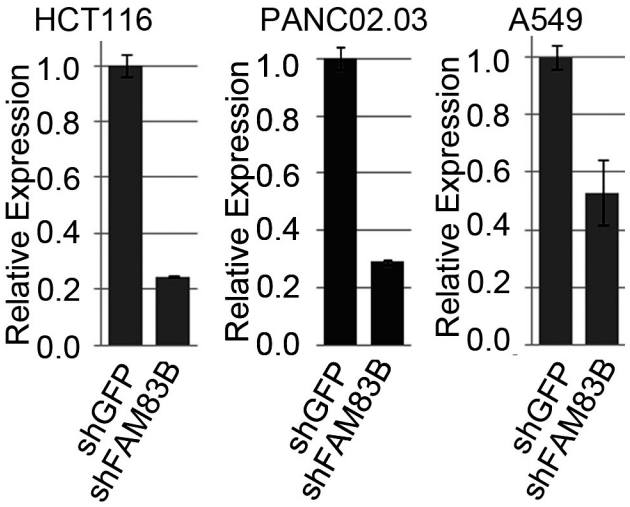

Figure 16. Real-time PCR anaylsis for *FAM83B* knockdown in HCT116, PANC02.03, and A549 cancer cell lines expressing either shRNA targeting GFP or FAM83B.

FAM83B			
Study	**Difference**	**p-value**	**Experiment Type**
Bittner_Breast	Elevated in ER-negative Breast Carcinoma vs. ER-positive.	3.0E-06	mRNA
Bittner_Breast	Elevated in PR-negative Breast Carcinoma vs. PR-positive.	6.3E-05	mRNA
Pyeon_Multi-cancer	Elevated in Cervical Cancer vs. nomal cervix.	3.5E-04	mRNA
Bittner_Breast	Elevated in Breast Carcinoma with increasing grade.	5.3E-04	mRNA
Bild_Lung	Elevated in Squamous Cell Lung Carcinoma relative to Lung Adenocarcinoma.	8.4E-04	mRNA
Watanabe_Colon	Elevated in Colorectal Carcinoma with Instable Microsattelite Status vs. Stable Microsattelite Status.	8.8E-04	mRNA
Oudes_Prostate	Elevated in Benign Basal Epithelial Prostate Cancer relative to Benign Endothelial, Luminal secretory, and stromal fibromuscular.	9.7E-04	mRNA
Bittner_Ovarian	Elevated in Ovarian Mucinous Adenocarcinoma relative to Clear Cell Adenocarcinoma, Endometrioid Adenocarcinoma, Surface Papillary Adenocarcinoma, and Serous Adenocarcinoma.	3.0E-03	mRNA
Bittner_Endometrium	Elevated in Endometrial Adenosquamous Carcinoma relative to Clear Cell Adenocarcinoma, Mucinous Adenocarcinoma, Serous Adenocarcinoma, Serous Surface Papillary.	6.0E-03	mRNA
Bittner_Lung	Elevated in Lung Carcinoma with increasing T-stage.	1.6E-02	mRNA
Chin_Breast2	Elevated with increasing grade.	2.1E-02	DNA
Chin_Breast2	Elevated with increasing Nottingham Prognostic Index.	2.6E-02	DNA
Richardson_Breast_2	Elevated in breast Carcinoma vs. Normal Breast.	3.2E-02	mRNA
Richardson_Breast_2	Elevated in ER-negative Breast Carcinoma vs ER-positive .	3.4E-02	mRNA
Chin_Breast2	Decreased in patients with decreased 5-year survival.	3.6E-02	DNA
Chin_Breast2	Increased with Estrogen-receptor negativity.	3.7E-02	DNA
Finak_Breast	Elevated in Breast Carcinoma Stroma from individuals with recurrance at 5 years vs. no recurrance.	3.8E-02	mRNA
Finak_Breast	Elevated in Breast Carcinoma Stroma from individuals with ER-negative vs. ER-positive.	4.5E-02	mRNA

Table 1. Summary of tumor microarray datasets obtained from Oncomine (www.oncomine.org) demonstrating significant association of FAM83B expression with specific clinical or pathologic parameters.