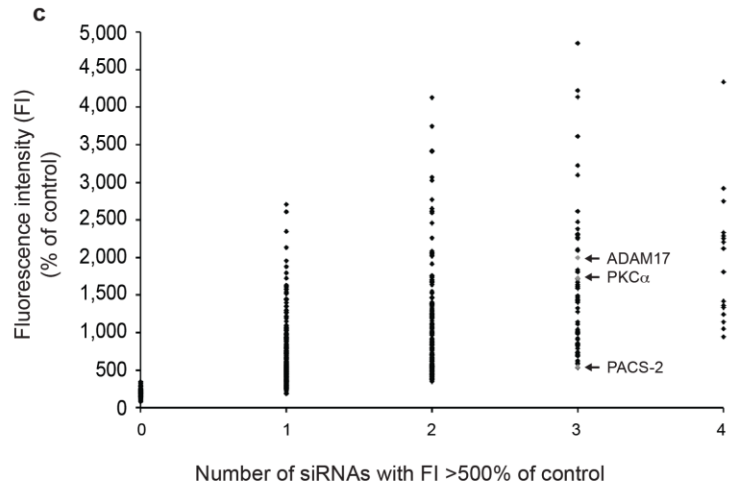
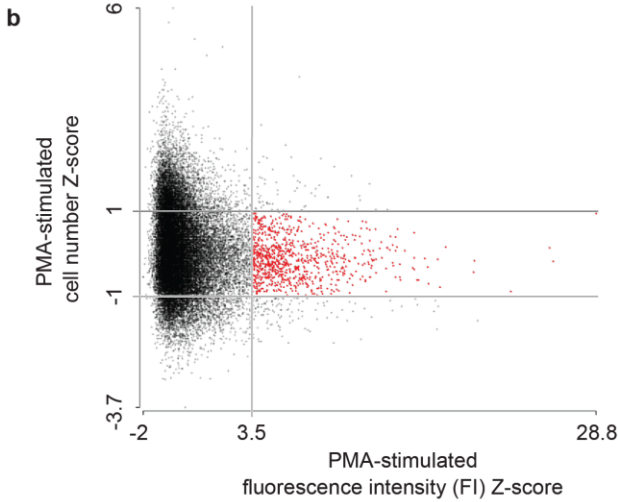


**a** Primary screen hit selection

Number of genes	Selection criteria			
	PMA-stimulated FI Z-score	PMA-stimulated cell number Z-score	Unstimulated FI Z-score	Unstimulated cell number Z-score
637	$\geq 3.5$	$\geq -1.0$ & $\leq 1.0$		$\geq -1.0$ & $\leq 1.0$
8	$\geq 10.0$	$\geq -1.3$		$\geq -1.3$
156		$\geq -1.0$ & $\leq 1.0$	$\geq 3.0$	$\geq -1.0$ & $\leq 1.0$
31		$\geq -1.0$ & $\leq 1.0$	$\leq -3.0$	$\geq -1.0$ & $\leq 1.0$

FI: fluorescence intensity

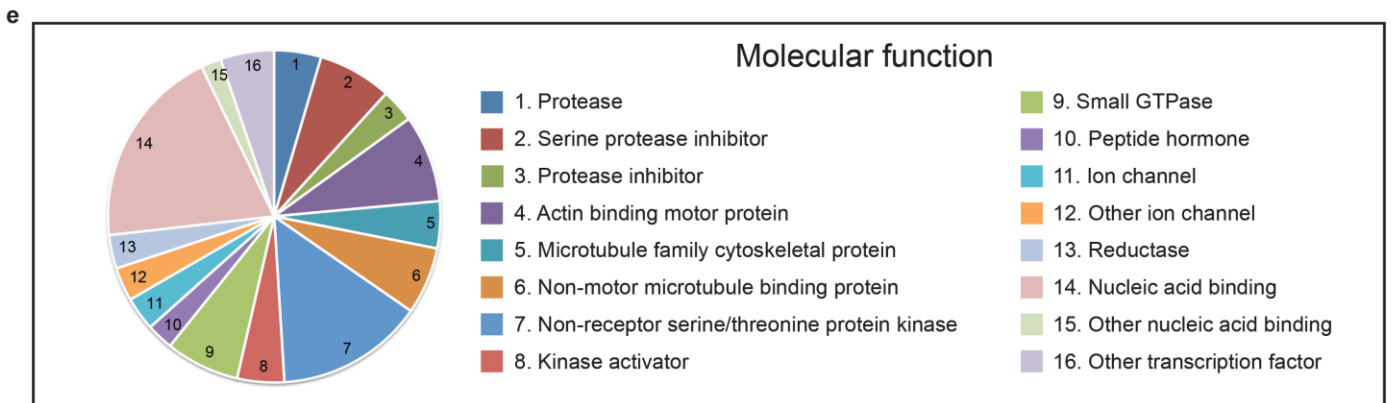


**d** Deconvolution of hits

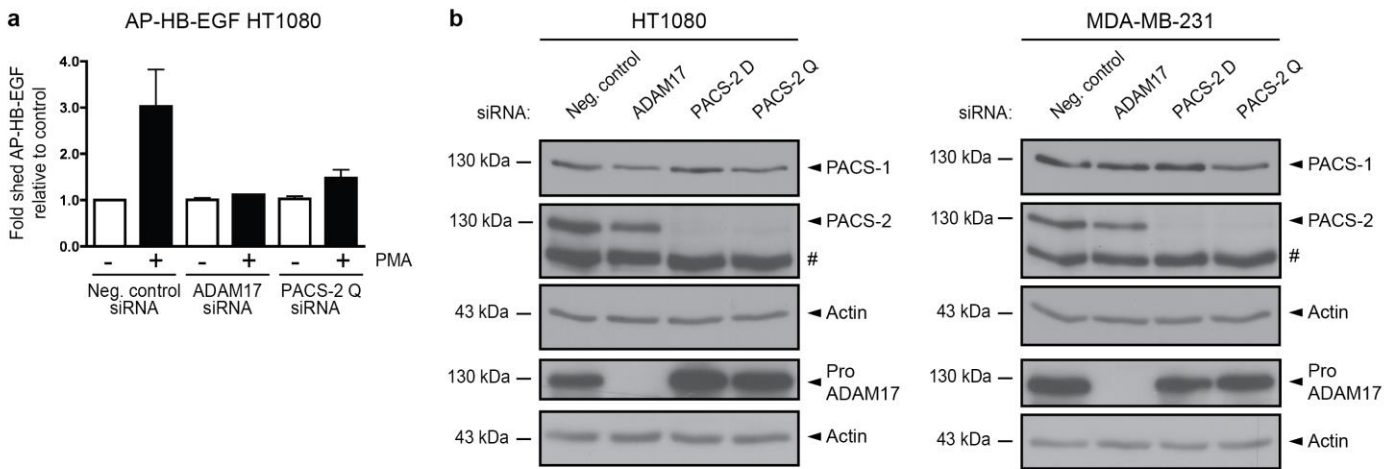
Category	Selected number of genes	*Deconvoluted number of genes	Deconvolution results				
			Number of siRNAs with FI >500% of control				
Increased PMA-stimulated FI	645	548	0	1	2	3	4
			61 (11%)	235 (45%)	171 (31%)	65 (12%)	16 (3%)
Increased unstimulated FI	156	136	Number of siRNAs with FI >200% of control				
			0	1	2	3	4
Reduced unstimulated FI	31	136	Number of siRNAs with FI <50% of control				
			0	1	2	3	4
			56 (41%)	50 (37%)	26 (19%)	4 (3%)	0 (0%)

FI: fluorescence intensity

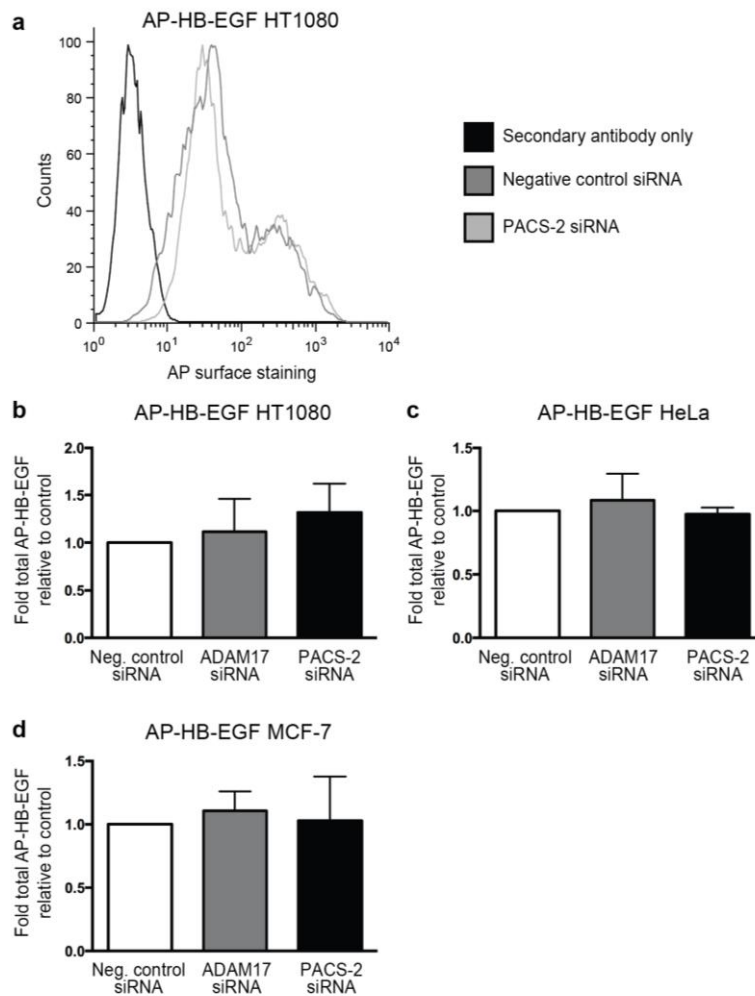
\*Several genes selected in the primary screen were not targeted by the newer deconvoluted library



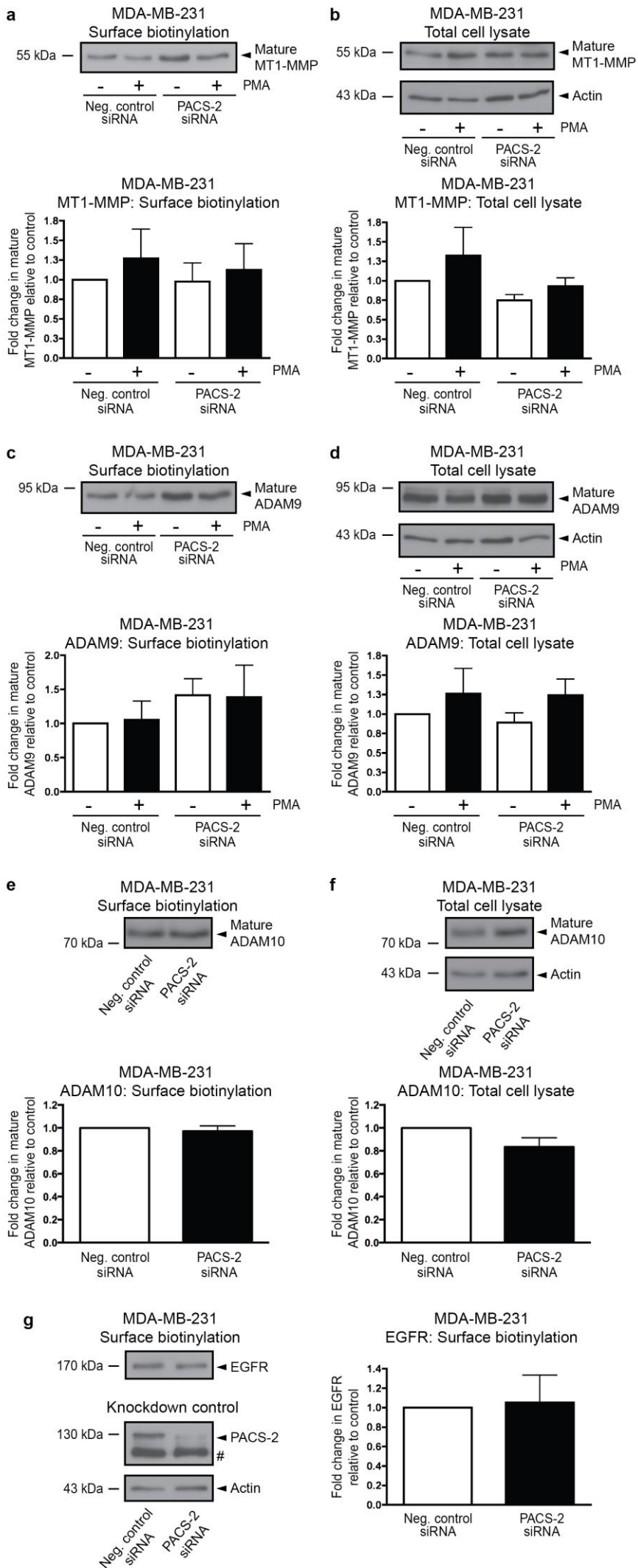
**Supplementary Fig. 1.** *Genome-wide siRNA screen, Related to functional genome-wide screen for ADAM17 regulators and Fig. 1.* (a) Table indicating the number and criteria of primary genome-wide screen hits. (b) Primary genome-wide siRNA screen data depicted as a scatter plot of cell number Z-scores and fluorescence intensity (FI). (c) Deconvolution screen. 548 genes selected in the primary genome-wide screen were re-tested using the 4 individual siRNAs constituting the primary screen SMARTpools. The mean fluorescence intensity after PMA treatment is shown for each of the 4 siRNAs as the percentage increase compared to the negative control siRNA. The data are grouped according to the number of siRNAs (0-4) out of the 4 that caused a  $\geq 500\%$  increased mean FI. ADAM17, PKC $\alpha$  and PACS-2 are indicated with arrows, all with 3 out of 4 siRNAs passing the threshold. (d) Table indicating the number and criteria of hits from the deconvolution screen. (e) Functional clustering of the 81 hits from the deconvolution screen. The annotation was generated using the DAVID Bioinformatics Resources database (<http://david.abcc.ncifcrf.gov/home.jsp>) and is based on Panther Molecular Function GO-terms.



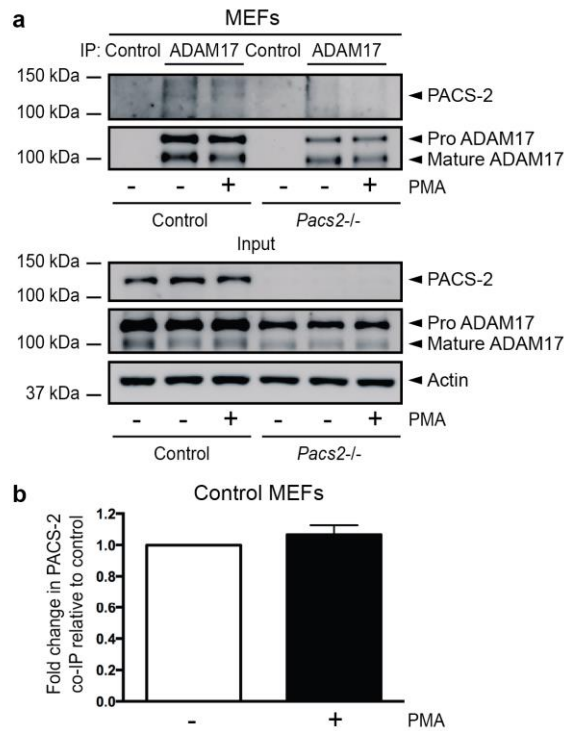
**Supplementary Fig. 2. Validation of PACS-2 siRNAs. Related to Fig. 1. (a)** AP-HB-EGF HT1080 cells were PMA-stimulated, and the cell medium analysed for AP activity as in Fig. 1b, but here using a PACS-2 siRNA pool from Qiagen (PACS-2 Q). The graph shows mean values  $\pm$  standard error of the mean and represents 2 individual experiments each done in triplicate. **(b)** HT1080 and MDA-MB-231 cells were transfected with negative control, ADAM17, or PACS-2 siRNA pools from either Dharmacon (PACS-2 D) or Qiagen (PACS-2 Q), and cell lysates were western blotted for PACS-1 to demonstrate that PACS-2 knockdown does not knock down PACS-1. The experiment was performed more than 3 times. Knockdown was confirmed by western blot and # denotes a non-specific band.



**Supplementary Fig. 3.** *PACS-2 knockdown does not affect expression of AP-HB-EGF. Related to Fig. 1. (a)* Cell-surface levels of AP-HB-EGF were analysed by flowcytometry on AP-HB-EGF HT1080 cells transfected with negative control or PACS-2 siRNA. The graph shows cell counts versus AP cell surface staining. **(b-d)** AP-HB-EGF HT1080 (b), AP-HB-EGF HeLa (c), and AP-HB-EGF MCF-7 (d) cells were transfected with negative control, ADAM17 or PACS-2 siRNA, lysed, and total AP-HB-EGF expression assessed by addition of colorimetric alkaline phosphatase substrate to the lysates. Data in (b) were compiled from 4 individual experiments, from 3 individual experiments in (c), and from 2 individual experiments in (d). Graphs show mean values  $\pm$  standard error of the mean. Data were analysed by ANOVA.

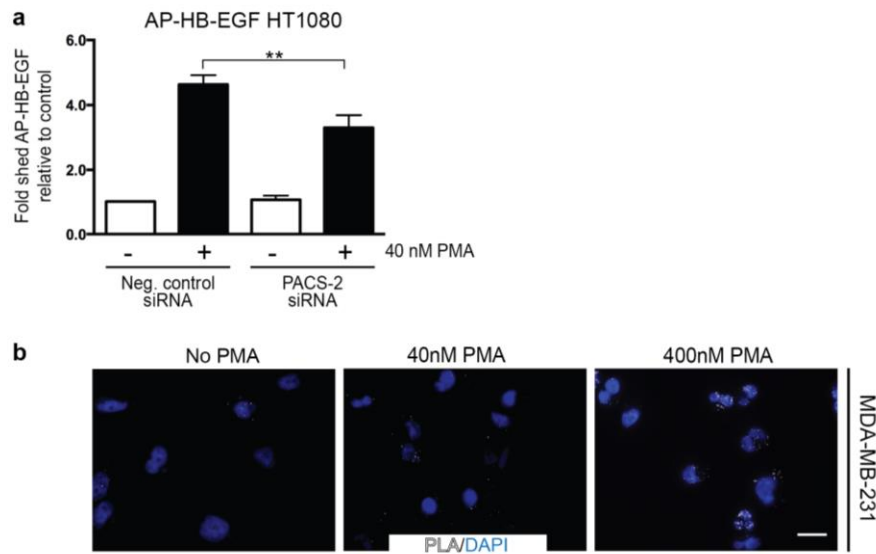


**Supplementary Fig. 4.** *PACS-2 does not regulate MT1-MMP, ADAM9, ADAM10 or EGFR levels. Related to Fig. 3.* MDA-MB-231 cells were PMA-stimulated and surface biotinylated as for Fig. 3a, and cell surface and total levels of mature MT1-MMP (**a+b**), ADAM9 (**c+d**), and ADAM10 (**e+f**) as well as cell-surface levels of EGFR (**g**) were analysed in the same manner as described for Fig. 3a. Data in (a-d) were compiled from 4 individual experiments, from 3 individual experiments in (e-f) and from 5 individual experiments in (g). PACS-2 knockdown was verified by western blot, as exemplified in (g). Graphs show mean values  $\pm$  standard error of the mean. Data were analysed by ANOVA or unpaired two-tailed Student's t-test, as appropriate.



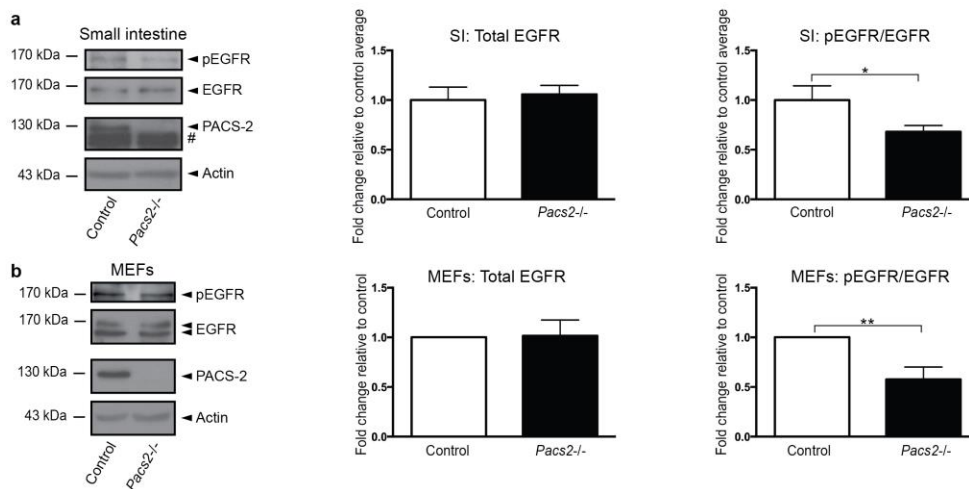
**Supplementary Fig. 5. Validation of PACS-2/ADAM17 co-immunoprecipitation.**

Related to Fig. 4. **(a)** ADAM17 was immunoprecipitated from unstimulated or PMA-stimulated control and *Pacs2*<sup>-/-</sup> MEFs, and co-immunoprecipitation of PACS-2 detected by western blot. The blot shown is representative of three individual experiments and # denotes a non-specific band. **(b)** The effect of PMA stimulation on PACS-2/ADAM17 co-immunoprecipitation in control MEFs in (a) was quantified. The amount of co-immunoprecipitated PACS-2 was normalized to the amount of immunoprecipitated mature ADAM17. The unstimulated negative control for each experiment was then set to 1, the other raw data were normalized to this value, and finally the average of all individual experiments was calculated. Data were analysed by unpaired two-tailed Student's t-test.



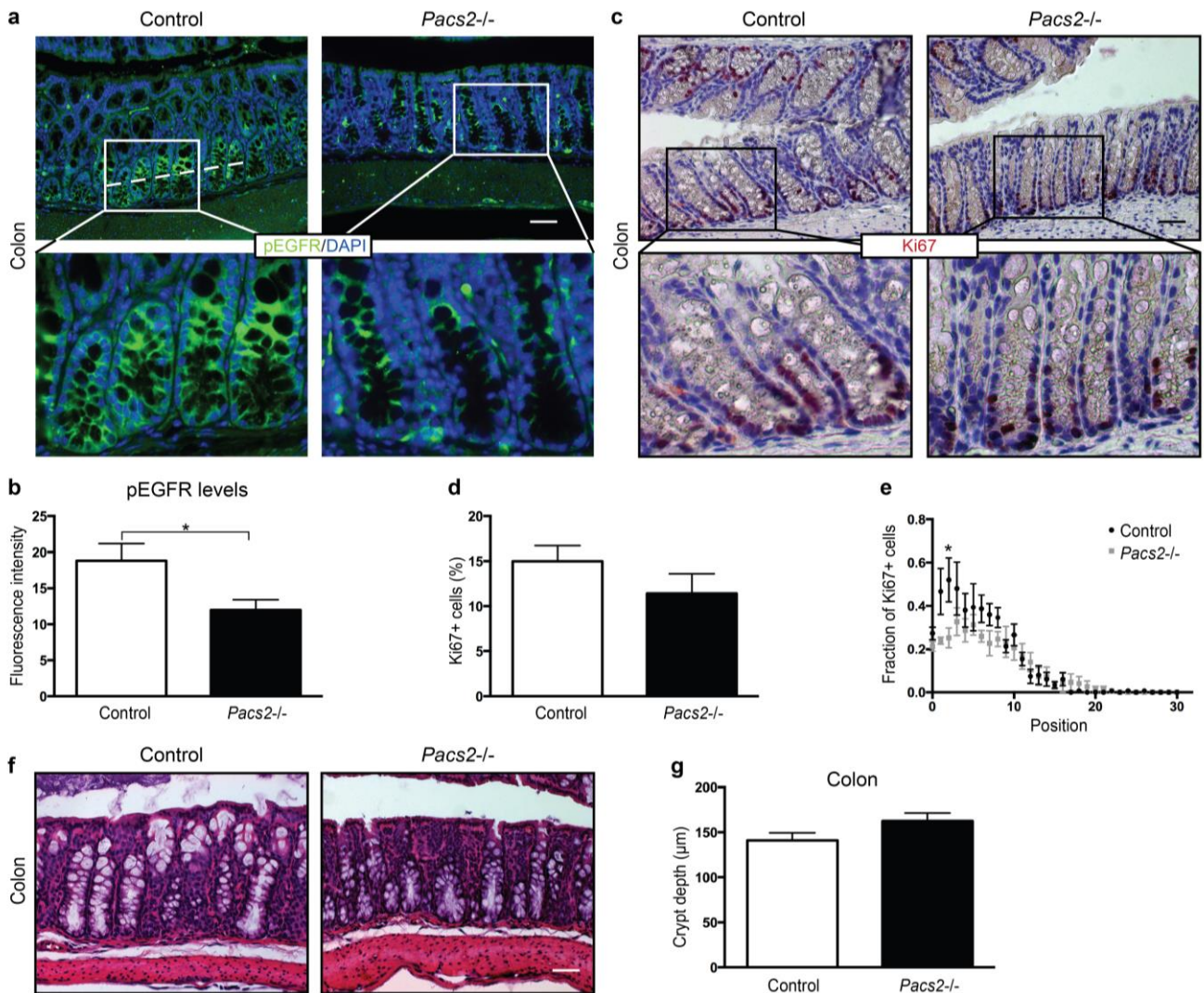
**Supplementary Fig. 6.** *PACS-2 regulates shedding and co-localizes with ADAM17 also in response to low-dose PMA. Related to Fig. 4. (a)* siRNA-transfected AP-HB-EGF HT1080 cells were stimulated with 40 nM PMA, and the cell medium analysed for AP activity as in Fig. 1b. Data were compiled from 3 individual experiments, each performed in triplicate. The graph shows mean values  $\pm$  standard error of the mean. Data were analysed by ANOVA. \*\* $p < 0.01$ . (b) MDA-MB-231 cells were subjected to Proximity Ligation Assay (PLA) without PMA stimulation or using 40 nM or 400 nM PMA. The experiment was performed 4 times in duplicate. Scale bar represents 17  $\mu\text{m}$ .



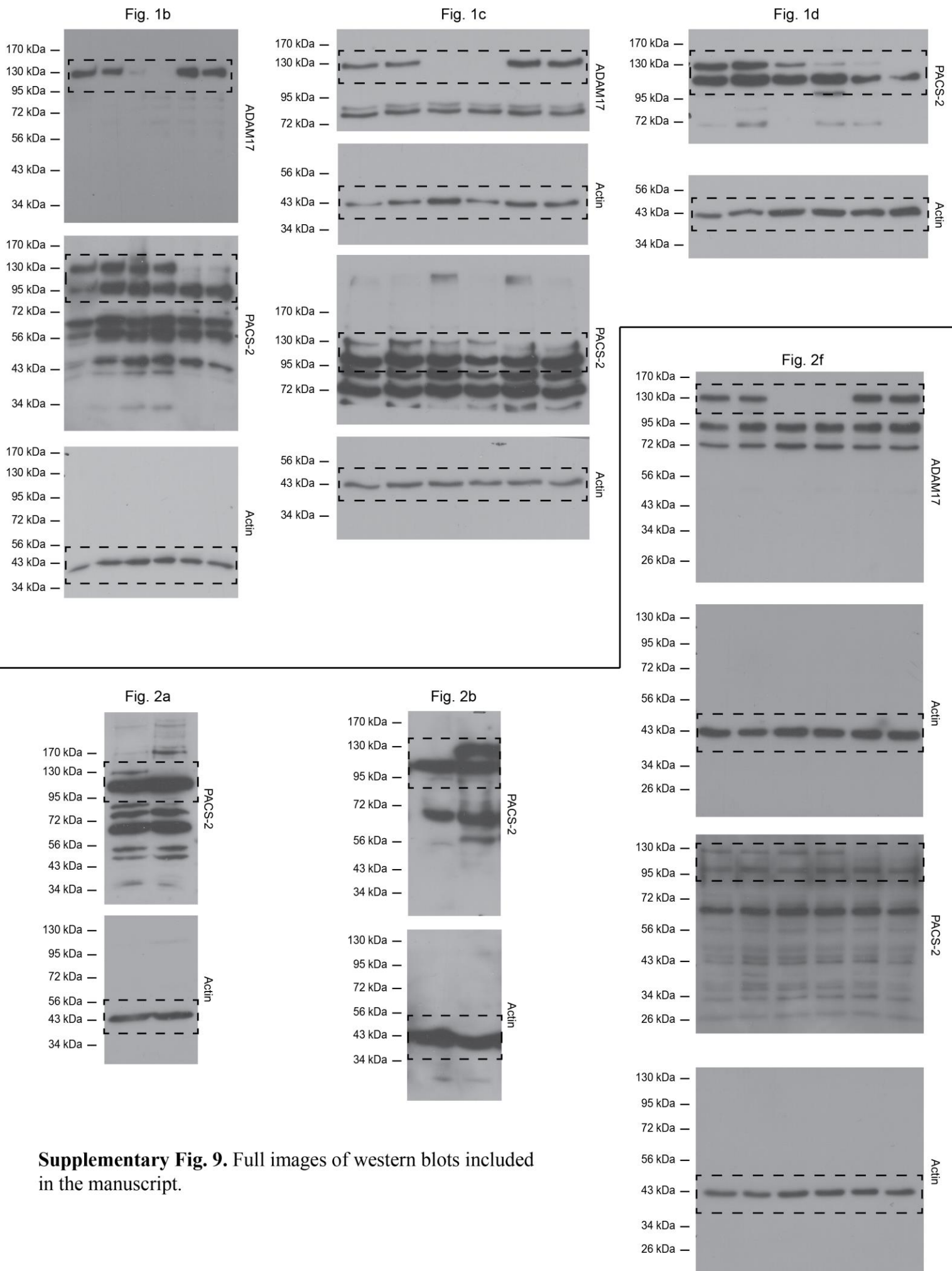


**Supplementary Fig. 7.** *Small intestinal lysates and MEFs from Pacs2-deficient mice show reduced levels of pEGFR. Related to Fig. 6.* Levels of total EGFR were assessed in small intestinal (SI) lysates from 6 control and 6 *Pacs2*<sup>-/-</sup> mice. The fold change was obtained by normalizing EGFR levels to actin, calculating the average value in controls and setting this to 1, then normalizing all the other raw data to this value, and finally calculating the average for each genotype. EGFR pY1068 levels were assessed in intestinal lysates from 5 control and 7 *Pacs2*<sup>-/-</sup> mice. pEGFR levels were quantified and shown as a ratio relative to total EGFR levels. The fold change was obtained by first calculating the average value in controls and setting this to 1, then normalizing all the other raw data to this value, and finally calculating the average for each genotype. **(b)** Total cellular levels of EGFR were assessed in control and *Pacs2*<sup>-/-</sup> MEFs serum-starved overnight. Samples were normalized to input actin, and the fold change calculated by setting the control for each experiment to 1, normalizing the *Pacs2*<sup>-/-</sup> data to this value, and calculating the average of all individual experiments. As in (b), pEGFR levels are shown as a ratio relative to total EGFR levels. Data were compiled from 5 individual experiments for total EGFR and from 3 individual experiments for pEGFR. On blots, # denotes a non-specific band. Graphs show mean

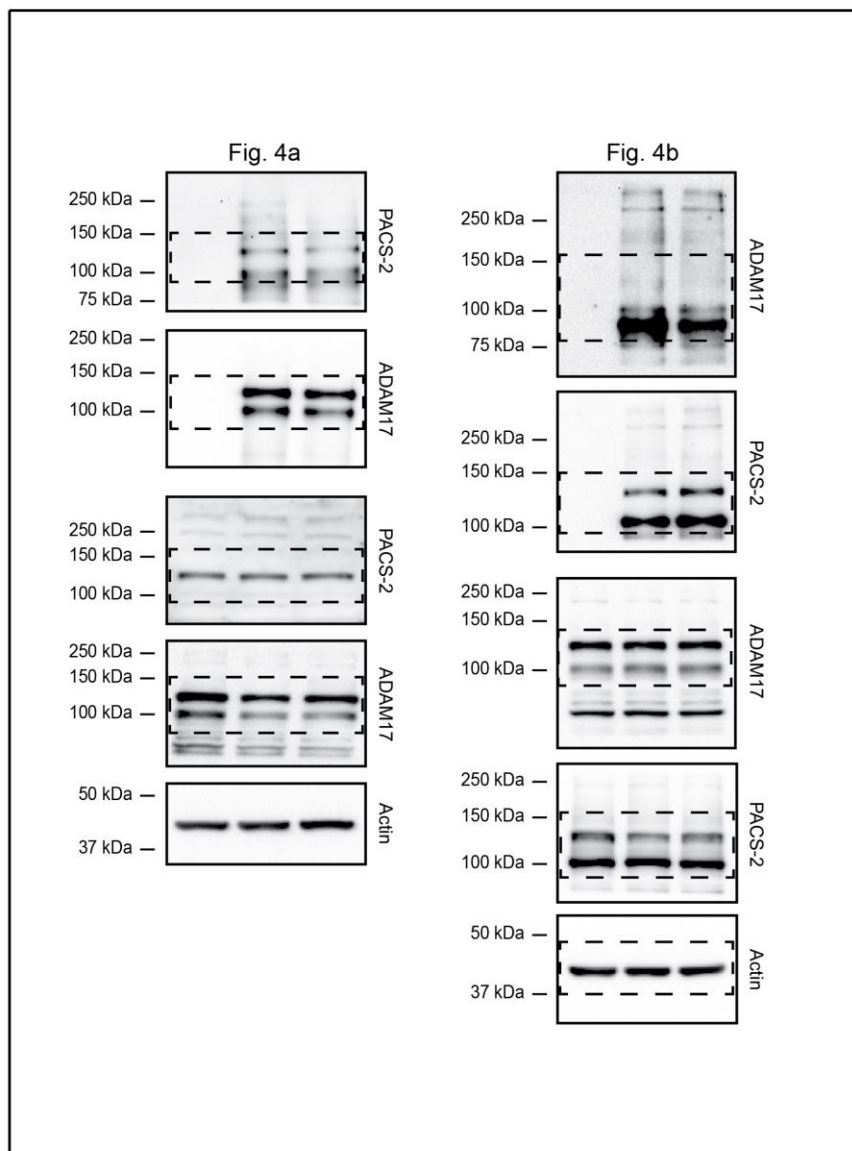
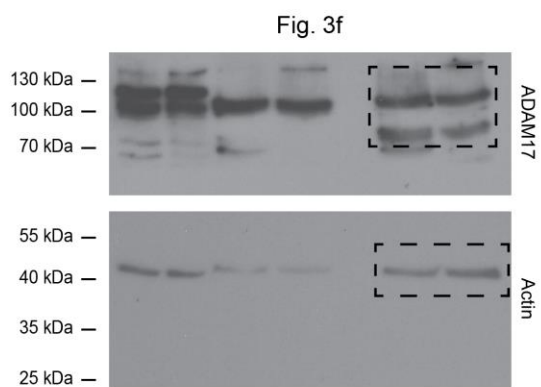
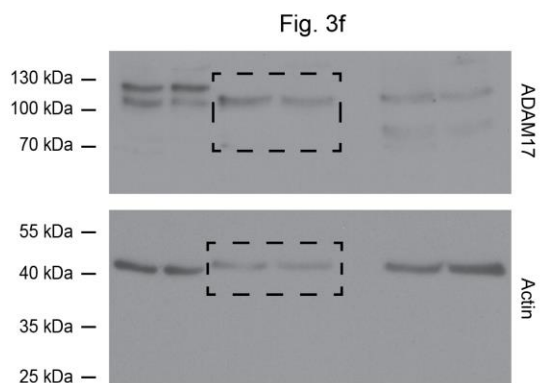
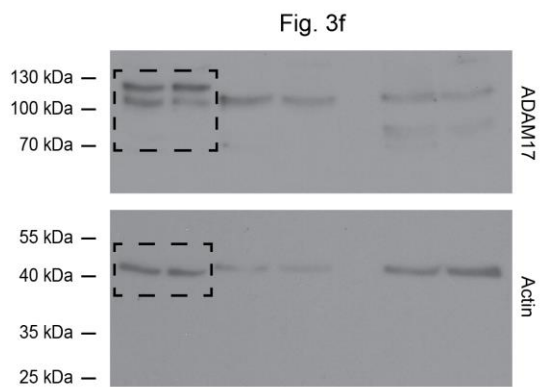
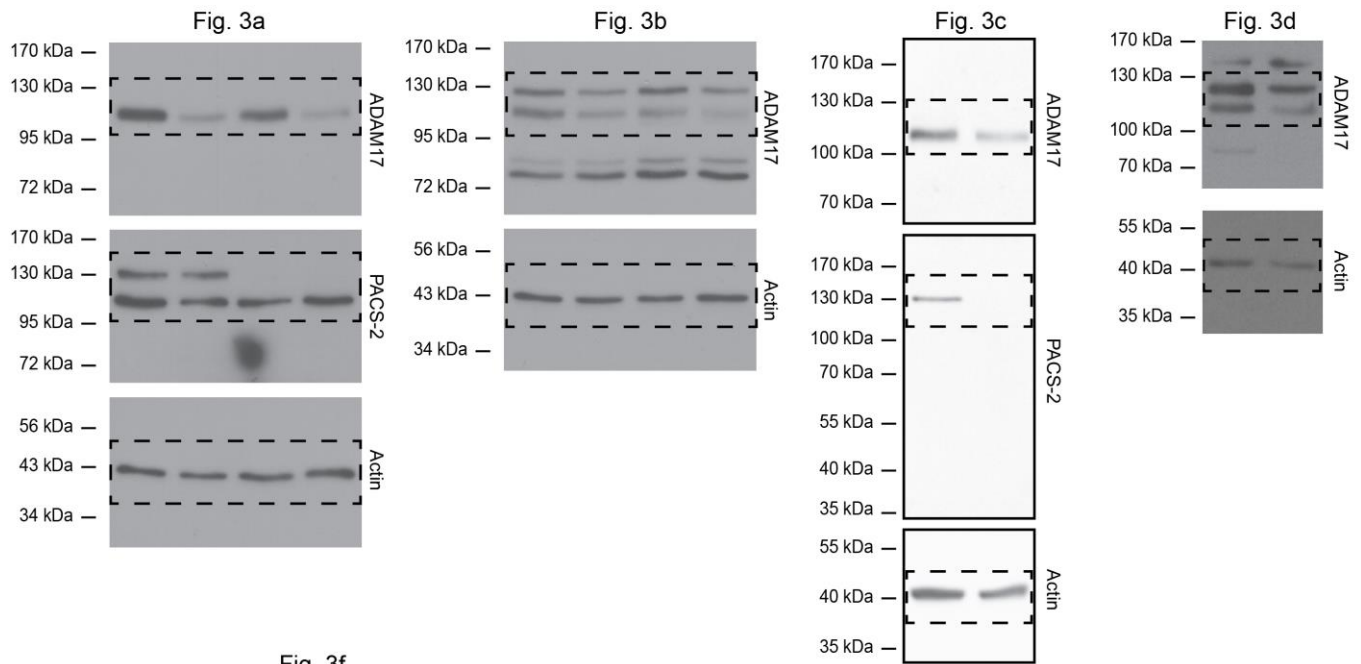
values  $\pm$  standard error of the mean. Data were analysed by unpaired two-tailed Student's t-test. \* $p < 0.05$ , \*\* $p < 0.01$ .



**Supplementary Fig. 8. Reduced EGFR activity and decreased cell proliferation in the colon of *Pacs2*<sup>-/-</sup> mice. Related to Fig. 6. (a+b)** Immunohistochemistry for pEGFR as in Fig. 6, on colon sections from 5 control and 5 *Pacs2*<sup>-/-</sup> mice. Fluorescence intensity of pEGFR was quantified along a line averaging 226 μm across colonic crypts, represented by the dotted line in (a). **(c-e)** Immunohistochemistry for Ki67 on colon sections from 5 control and 5 *Pacs2*<sup>-/-</sup> mice, performed as in Fig. 6. **(f)** Histology of the colon of *Pacs2*<sup>-/-</sup> mice. Scale bar represents 52 μm. **(g)** Crypt depth was measured on colonic sections from 5 control and 5 *Pacs2*<sup>-/-</sup> mice. Approximately 75 colon crypts were measured for each genotype. Graphs show mean values ± standard error of the mean. Data were analysed by unpaired two-tailed Student's t-test. \*p<0.05.



**Supplementary Fig. 9.** Full images of western blots included in the manuscript.



**Supplementary Fig. 9 (continued).** Full images of western blots included in the manuscript.

Fig. 5a

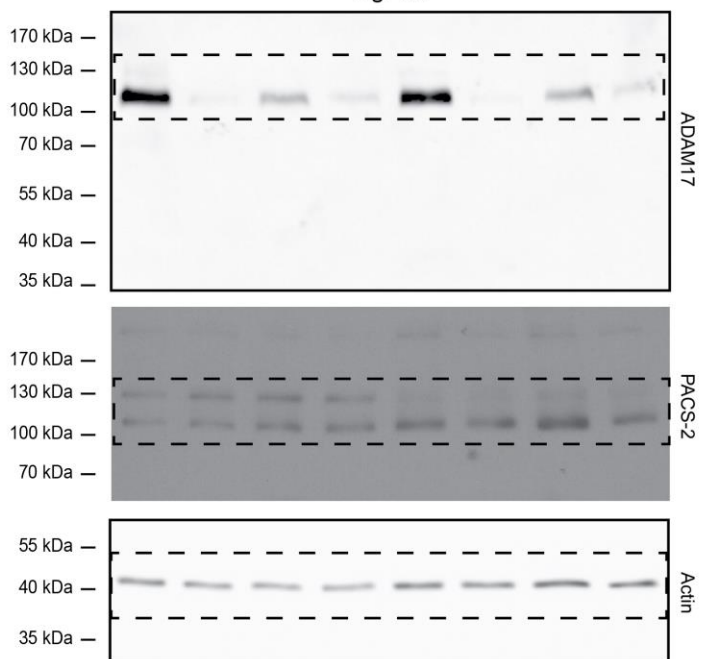
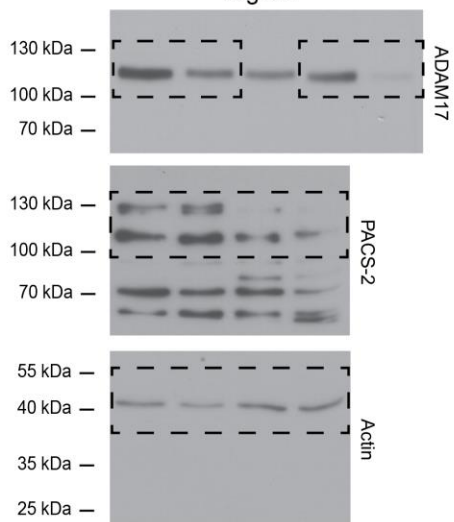


Fig. 5b



**Supplementary Fig. 9 (continued).** Full images of western blots included in the manuscript.

## Supplementary Methods

### Functional Genome-wide Screen for ADAM17 Regulators

#### *Primary genome-wide siRNA screen*

For the siRNA screen, we used the Dharmacon siGenome SMARTpool siRNA Library (Thermo Scientific), which contains 21,121 pools of four distinct siRNAs against non-overlapping regions of a target mRNA. SMARTpools against ADAM17 and PKC $\alpha$  serving as positive controls and RNA-induced silencing complex (RISC)-free negative controls (all from Dharmacon) were included on each plate. HT1080 cells stably expressing alkaline phosphatase-tagged proHB-EGF (AP-HB-EGF) were transiently reverse transfected with siRNA SMARTpools. In brief, 10  $\mu$ l siRNAs (375 nM diluted in HBSS (Invitrogen)) were transferred in quadruplicate from Dharmacon library plates into black 96-well plates (Greiner cat#655090). Ten  $\mu$ l Interferin transfection reagent (PolyPlus) diluted in Opti-MEM $^{\circledR}$  (final concentration 0.3  $\mu$ l/well) was added into each well by a Wellmate liquid dispenser (Thermo Scientific). Plates were briefly centrifuged at 1000 rotations per minute, and incubated at room temperature (RT) for 20 minutes. HT1080 cells were trypsinized, washed, and 80  $\mu$ l of cells in culture medium without Geneticin was added (final concentration of  $5 \times 10^3$  cells/well) and plates were incubated at 37 $^{\circ}$ C and 10% CO $_2$ . Seventy-two hours later, plates were washed twice with phosphate buffered saline (PBS) using a ELx405 plate washer (BioTek) and treated with 400 nM phorbol 12-myristate 13-acetate (PMA) or DMSO control in serum-free medium (SFM) each in duplicate plates for 30 minutes at 37 $^{\circ}$ C, fixed in 4% formaldehyde for 15 minutes at RT, and stored at 4 $^{\circ}$ C overnight.

Cell-surface expression of AP-HB-EGF was determined by immunofluorescent staining of membrane bound AP-HB-EGF. In brief, cells were

blocked with 5% bovine serum albumin (BSA) in PBS, incubated for 2 hours at RT with a primary anti-alkaline phosphatase monoclonal antibody (clone 8B6 from Sigma) diluted 1:1000 in 1% BSA in PBS, washed in PBS, and incubated with the secondary antibody, Alexa Fluor 488-donkey anti-mouse IgG (Invitrogen) 1:2000 in 1% BSA in PBS for 1 hour at RT. For identification of intact cells, 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen, 1 ng/ $\mu$ l) was also added with the secondary antibody. Fluorescence intensities were determined using Acumen <sup>c</sup>X3 laser scanning microplate cytometer (TTP LabTech Ltd.), with DAPI and Alexa 488 fluorescence detected using 405–470 nm and 500–530 nm bandpass filters, respectively. A sampling resolution of 0.5  $\mu$ m in the X-direction and 7  $\mu$ m in the Y-direction was used and the whole well was selected for scanning. To distinguish nuclei from cellular debris and larger clumps of cells, cell counts were restricted to objects measuring 5–100  $\mu$ m in both width and depth. The total cell number per well was estimated by dividing the total area of the cells by the average area of a single cell. To quantify cell-surface expression of Alexa 488-labelled AP-HB-EGF, the object size was restricted to 7–222  $\mu$ m in both width and depth and additional fluorescent parameters including peak, mean and total intensity measurements were used to gate the population.

#### *Criteria used to define positive hits*

All data were processed using the cellHTS2 software in R Bioconductor (<http://www.bioconductor.org/packages/devel/bioc/html/cellHTS2.html>). Raw fluorescence intensity values were normalized to the plate median value and Z-scores were calculated by subtracting the population mean from the individual raw values and dividing the difference by the population standard deviation. Z-scores from plate



replicates were averaged and genes preventing loss of AP-HB-EGF cell-surface levels after PMA treatment with no effect on cell number were then selected according to the criteria: mean Z-score  $\geq 3.5$  after PMA treatment and no effect on cell number (mean Z-score  $\geq -1$  and  $\leq 1$ ) (Supplementary Fig. 1a+b). In addition, a few genes with mean Z-score  $\geq 10$  after PMA treatment were allowed a minor loss in cell number (mean Z-score  $\geq -1.3$ ) (Supplementary Fig. 1a). Finally, genes affecting unstimulated AP-HB-EGF levels were identified using the following criteria: mean Z-score  $\geq 3.0$  or  $\leq -3.0$  without PMA treatment and no effect on cell number (Supplementary Fig. 1a). Based on this selection, the primary screen resulted in the identification of 645 candidate genes affecting AP-HB-EGF levels after PMA treatment and 187 genes affecting unstimulated AP-HB-EGF levels.

#### *Deconvolution screen*

To validate the selected screen hits, the deconvoluted siGENOME library from Dharmacon was used. Several genes selected in the primary screen were not targeted by the newer deconvoluted library. In brief, the 4 individual siRNAs constituting the SMARTpool were each tested in triplicate using the same assay conditions as for the primary screen, except that depending on whether selected genes affected unstimulated or PMA-stimulated AP-HB-EGF cell-surface levels, they were only tested under those conditions. Moreover, rather than normalizing raw values to the plate median, normalization to mean RISC-free negative controls on each plate was performed. In order to qualify as a hit, at least 3 out of the 4 individual siRNAs from the pool had to give rise to more than 500% increased mean fluorescence intensity as compared to the negative control (Supplementary Fig. 1c).

A total of 81 genes (15% of the 548 deconvoluted hits) were confirmed with at

least 3 independent siRNA oligonucleotides resulting in more than 500% increased mean fluorescence intensity after PMA treatment, as compared to non-targeting siRNA controls (Supplementary Fig. 1c+d and Supplementary Table 1). Both ADAM17 and protein kinase C (PKC)  $\alpha$  were among these, ratifying the screen design. Moreover, 7 genes affected the surface level of AP-HB-EGF in unstimulated cells (3 independent siRNAs causing more than 200% increased mean fluorescence intensity or exhibiting less than 50% mean fluorescence intensity, as compared to non-targeting siRNA controls) (Supplementary Fig. 1d). Yet, these 7 genes showed opposite effects in the deconvolution screen versus the primary screen – i.e. increased levels in the primary screen and reduced levels in the secondary screen, and were therefore excluded. Hits were further evaluated using an independent colorimetric AP shedding assay (see experimental procedure below), which revealed that knockdown of 24 genes phenocopied ADAM17 knockdown (Supplementary Table 1).

We functionally categorized the genes using the DAVID bioinformatics database (<http://david.abcc.ncifcrf.gov/>). Multiple categories of genes were enriched based on Panther Molecular Function Gene Ontology terms<sup>1</sup> (Supplementary Fig. 1e and Supplementary Table 1). No major signalling pathways appeared using Ingenuity pathway analysis ([www.ingenuity.com](http://www.ingenuity.com)). All screen data are available at <http://hts.cancerresearchuk.org/db/public/index.php>.

### **Supplementary Reference**

- 1 Huang da, W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44-57, (2009).