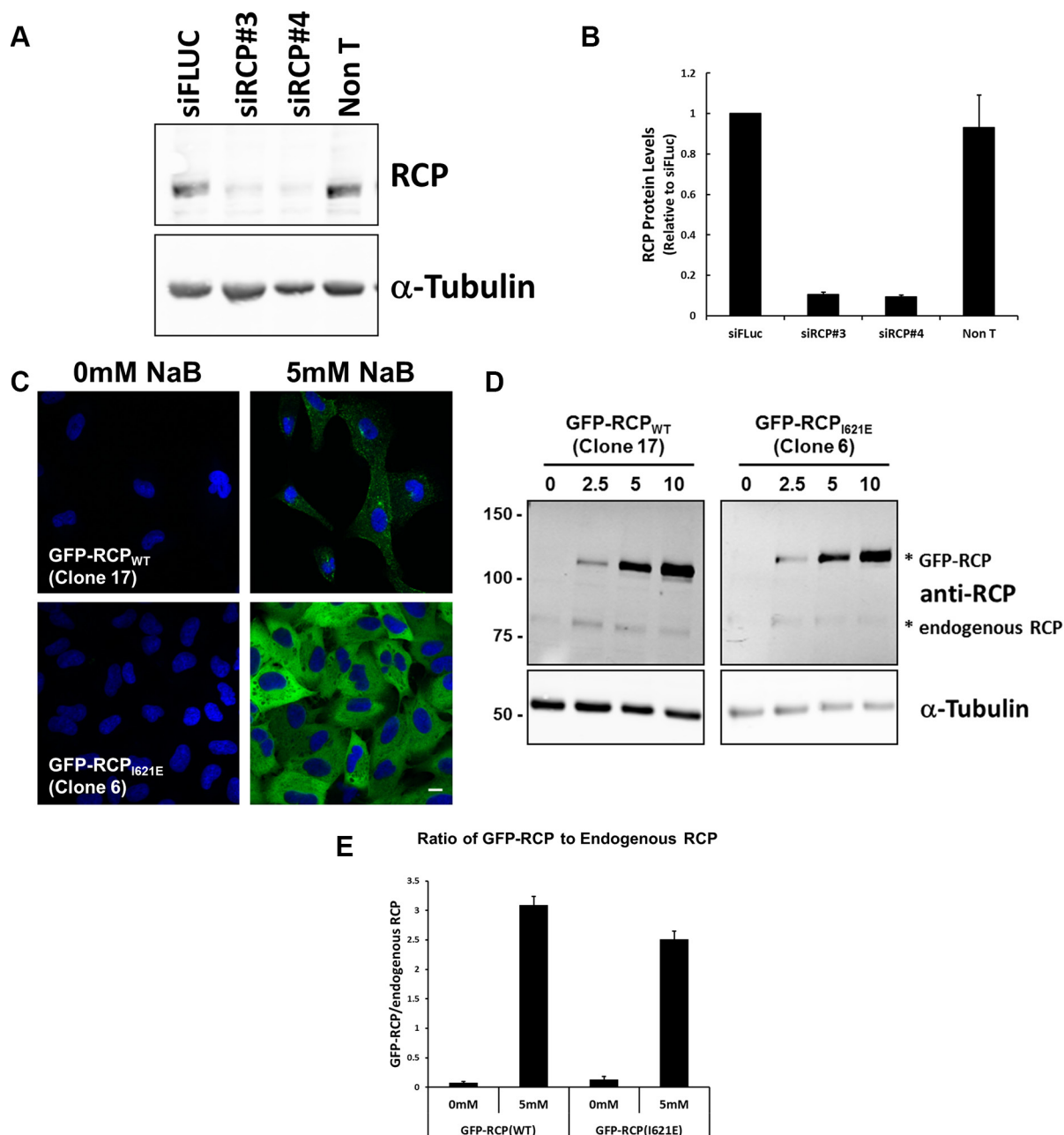


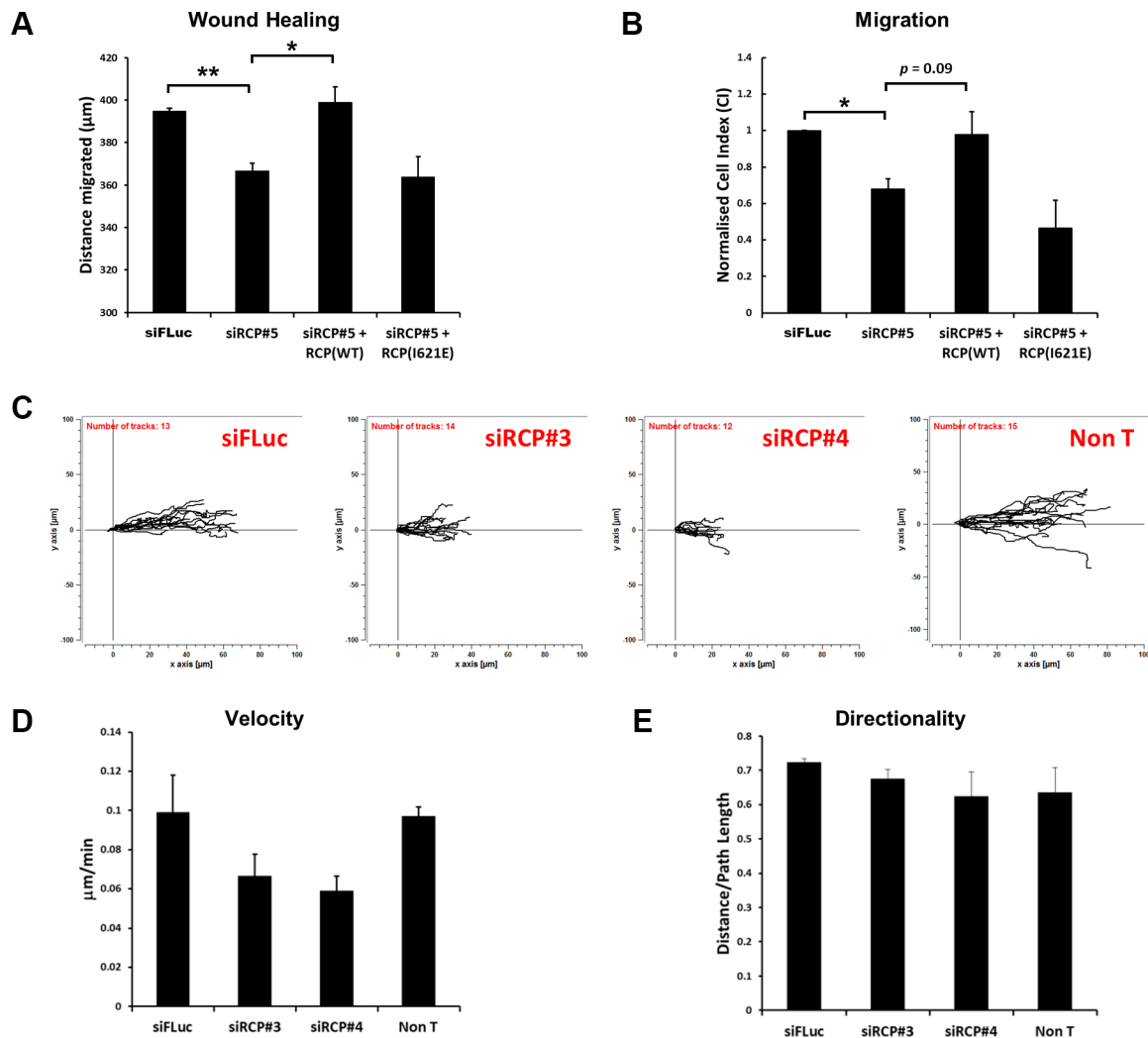
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| Title                       | Rab coupling protein mediated endosomal recycling of N-cadherin influences cell motility                                                                                                                                                                                                                                                                                                     |
| Authors                     | Lindsay, Andrew J.;McCaffrey, Mary W.                                                                                                                                                                                                                                                                                                                                                        |
| Publication date            | 2017                                                                                                                                                                                                                                                                                                                                                                                         |
| Original Citation           | Lindsay, A. J. and McCaffrey, M. W. (2017) 'Rab coupling protein mediated endosomal recycling of N-cadherin influences cell motility', Oncotarget, 8(62), pp. 104717-104732. doi: 10.18632/oncotarget.10513                                                                                                                                                                                  |
| Type of publication         | Article (peer-reviewed)                                                                                                                                                                                                                                                                                                                                                                      |
| Link to publisher's version | <a href="http://www.oncotarget.com/index.php?journal=oncotarget&amp;page=article&amp;op=view&amp;path[]=10513&amp;path[]=33218">http://www.oncotarget.com/index.php?journal=oncotarget&amp;page=article&amp;op=view&amp;path[]=10513&amp;path[]=33218</a> - 10.18632/oncotarget.10513                                                                                                        |
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| Download date               | 2026-09-18 22:34:46                                                                                                                                                                                                                                                                                                                                                                          |
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# Rab coupling protein mediated endosomal recycling of N-cadherin influences cell motility

## Supplementary Materials

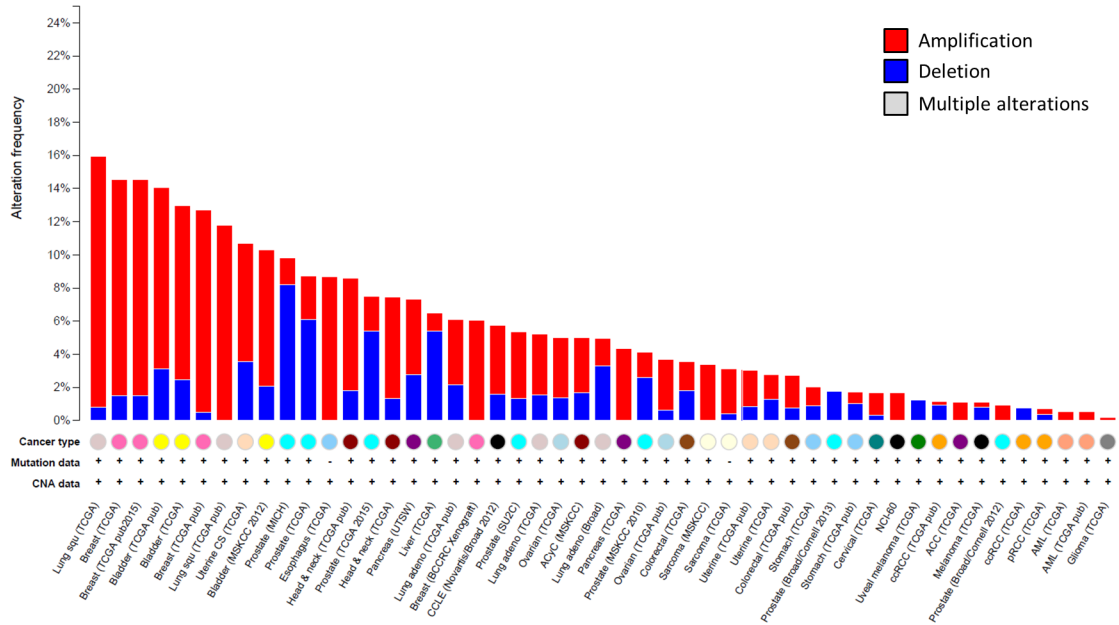


**Supplementary Figure S1: Characterisation of RCP knockdowns and RCP stable cell lines.** (A) Representative Western blot of RCP in lysates generated from cells transfected with the indicated siRNA duplexes for 72 hours,  $\alpha$ -tubulin is used as a loading control. (B) Quantification of RCP protein levels in A549 cells transfected for 72 hours with the indicated siRNA duplexes ( $n = 6$ ). (C) Fluorescence micrographs of the stable cell lines used in this study, treated  $\pm$  5 mM sodium butyrate for 24 hours prior to fixation and labelling with DAPI (blue). (D) Western blots of lysates from the stable cell lines treated with the indicated concentrations of sodium butyrate for 24 hours and probed with anti-RCP.  $\alpha$ -tubulin is used as a loading control. (E) Quantification of the ratio of GFP-RCP to endogenous RCP in the stable cell lines treated  $\pm$  5 mM sodium butyrate. Values are mean  $\pm$  S.E.M. ( $n = 3$ ).

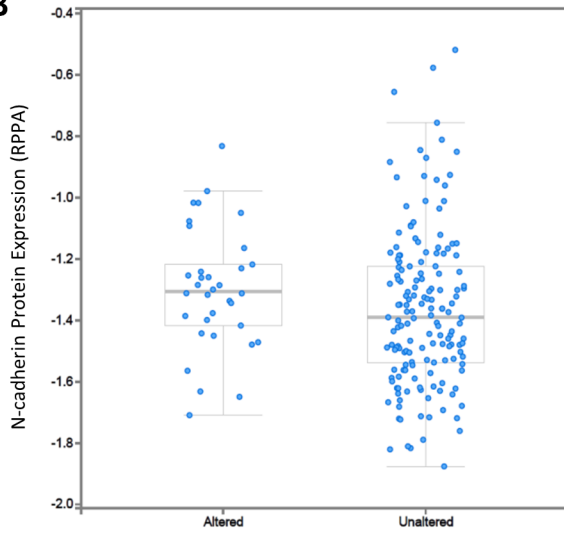


**Supplementary Figure S2: Rescue of siRNA-mediated inhibition of cell motility.** (A) The A549 RCP<sub>WT</sub> and A549 RCP<sub>I621E</sub> stable cell lines were transfected with siFLuc or siRCP#5 for 72 hours. The cell monolayers were wounded and brightfield images were recorded. The cells were returned to 37°C for 18 hours and imaged again. The distance migrated by the wound front is plotted in the bar graph. GFP-RCP<sub>WT</sub> or GFP-RCP<sub>I621E</sub> expression was induced by treatment with 5 mM sodium butyrate for the final 24 hours of the experiment. Error bars indicate the standard error of the means (\* $p < 0.05$ , \*\* $p < 0.01$ ;  $n = 3$ ). (B) The A549 RCP<sub>WT</sub> and A549 RCP<sub>I621E</sub> stable cell lines were transfected with siFLuc or siRCP#5 duplexes for 72 hours, detached and seeded, in duplicate, on CIM-16 Transwell plates and subjected to real-time migration assays (xCelligence). The histogram depicts the Normalised Cell Index (CI) after 48 hours of migration. GFP-RCP<sub>WT</sub> or GFP-RCP<sub>I621E</sub> expression was induced by treatment with 5 mM sodium butyrate for 24 hours prior to seeding on the CIM-16 plate, and 5 mM sodium butyrate was maintained in the medium in the upper chamber of the plate for the duration of the migration assay. Error bars indicate the standard error of the means (\* $p < 0.05$ ;  $n = 3$ ). (C) Representative trajectories of live A549 cells, transfected with the indicated siRNA duplexes, migrating into a wound for 10 hours (1 frame every 10 minutes). (D) Quantification of average velocity of the A549 cells extracted from > 50 track plots per condition, from 3 independent experiments. Values shown are mean  $\pm$  SEM. (E) Quantification of directionality (distance travelled divided by path length) of the A549 cells extracted from > 50 track plots per condition, from 3 independent experiments. Values are mean  $\pm$  SEM.

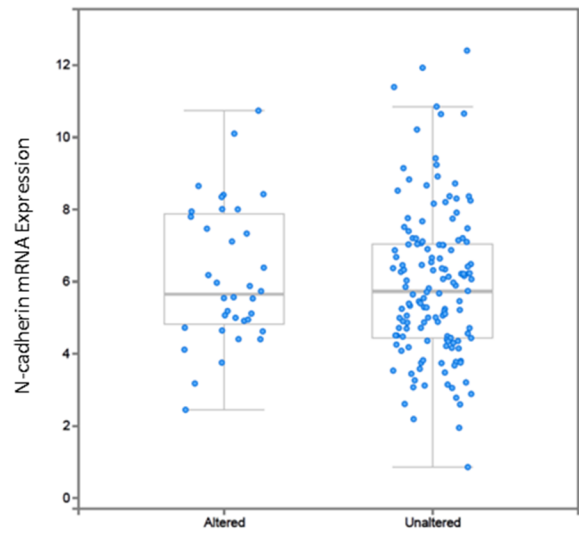
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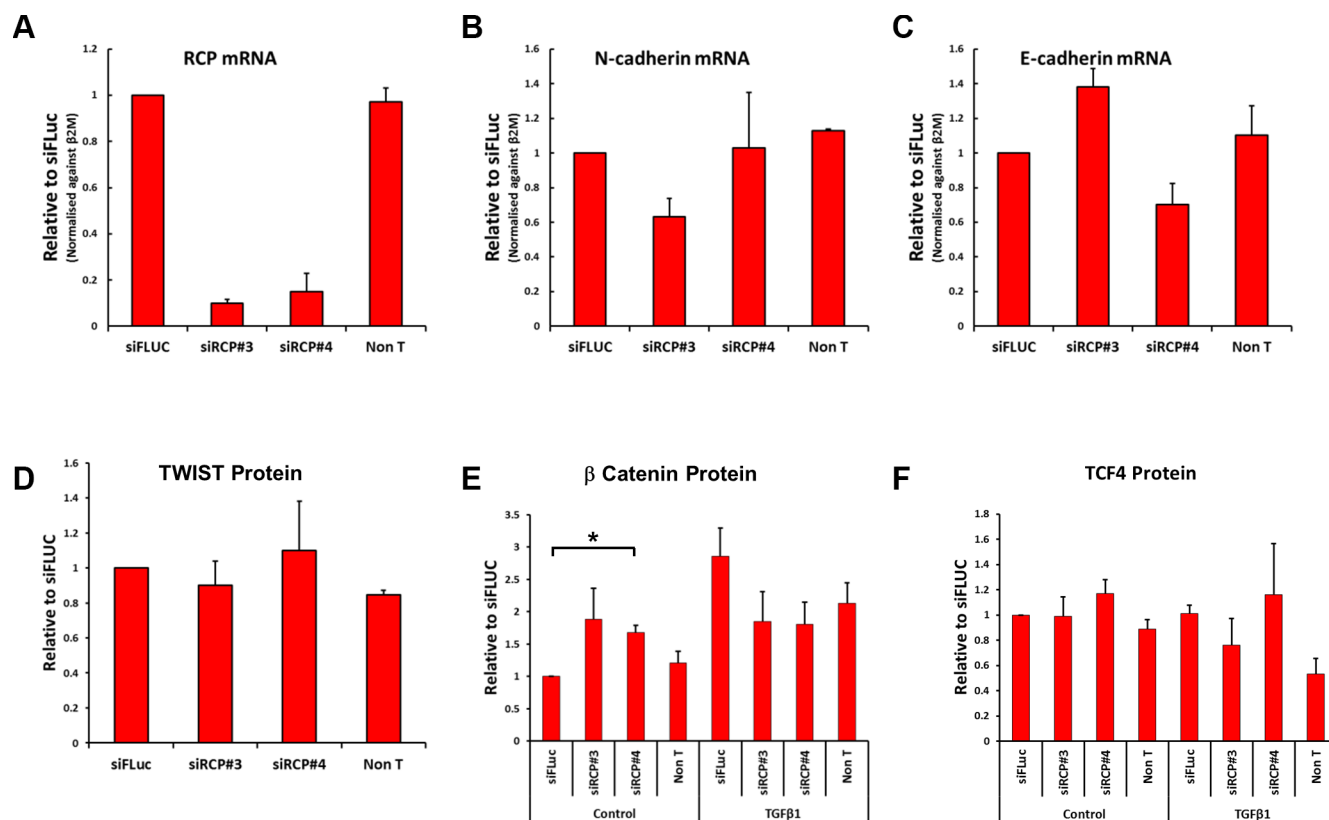
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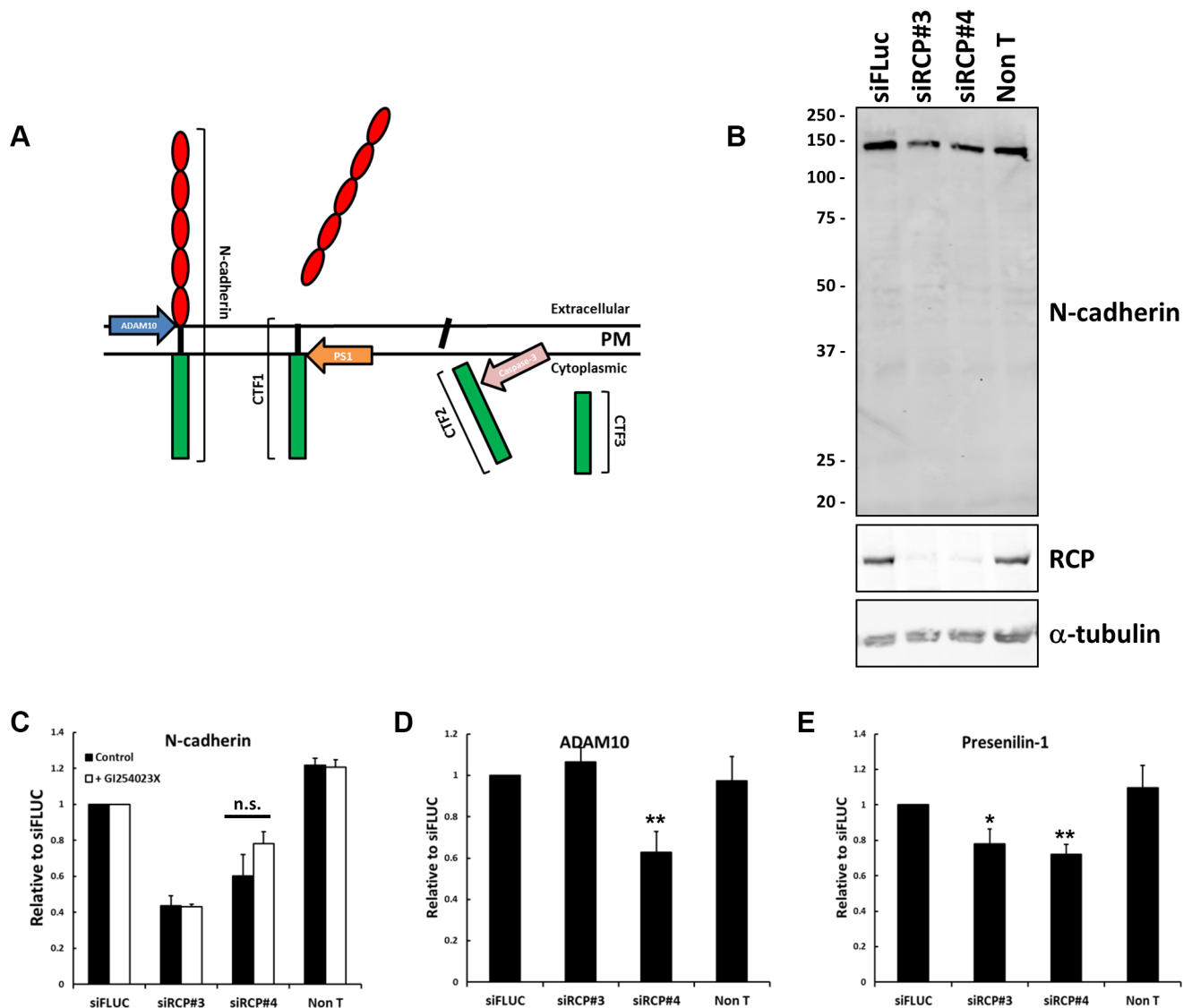
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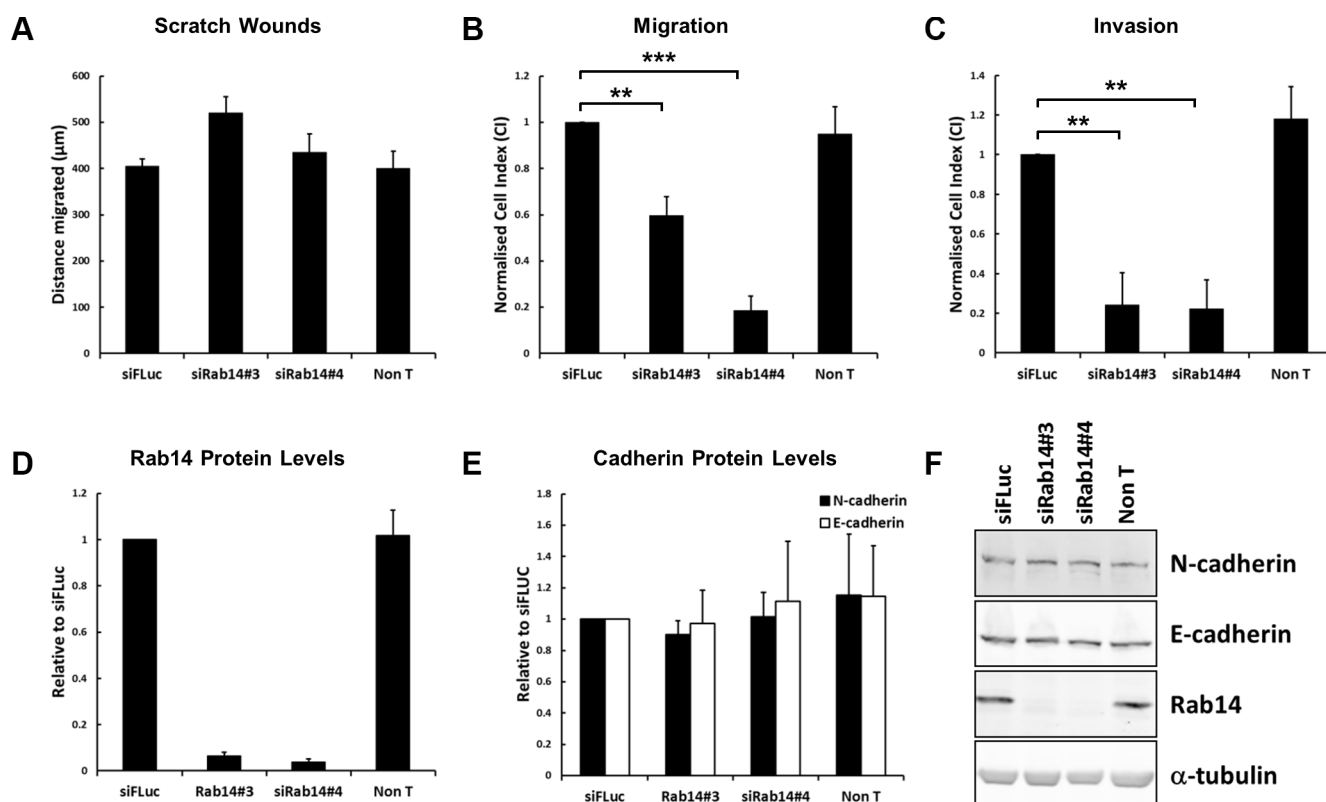
**Supplementary Figure S3: The RCP gene is frequently altered in cancer.** (A) Cross cancer histogram of the RCP gene in human cancer (cBioPortal). (B) N-cadherin protein expression in lung squamous cell tumours in which the RCP gene is altered or unaltered. (C) N-cadherin mRNA levels in lung squamous cell tumours in which the RCP gene is altered or unaltered.



**Supplementary Figure S4: Protein and mRNA levels in RCP knockdown cells.** RCP (A) N-cadherin (B) and E-cadherin (C) mRNA levels (normalised to  $\beta_2$  microglobulin) in A549 cells treated with the indicated siRNA duplexes for 72 hours and assessed by RT-qPCR ( $n = 3$ ). (D) Levels of TWIST protein in A549 cells transfected with the indicated siRNA duplexes for 72 hours. Values are mean  $\pm$  S.E.M. ( $n = 3$ ). Histogram indicating the levels of  $\beta$ -catenin (E) and TCF4 (F) proteins in A549 cells transfected with the indicated siRNA duplexes for 24 hours and treated  $\pm$  5 ng/ml TGF $\beta$ i for a further 48 hours. Values are normalised to siFLUC Control ( $*p < 0.05$ ;  $n = 5$ ).



**Supplementary Figure S5: RCP Knockdown Does Not Affect Processing of N-cadherin at the Cell Surface.** (A) Schematic diagram depicting the proteolytic processing of N-cadherin that takes place at the plasma membrane. (B) Full length Western blot of lysates generated from cells transfected with the indicated siRNA duplexes for 72 hours and probed with an N-cadherin antibody that detects the C-terminus. (C) Quantification of N-cadherin protein levels in cells transfected with the indicated siRNA duplexes for 72 hours and treated with or without the ADAM10 inhibitor, GI254023X, for the final 24 hours ( $n = 3$ ). Quantification of ADAM10 (D) and Presenilin-1 (E) protein levels in A549 cells transfected with the indicated siRNA duplexes for 72 hours ( $n = 6$ ).



**Supplementary Figure S6: Effect of Rab14 knockdown on A549 cell motility and cadherin protein levels.** (A) A549 cells in 24-well plates were transfected with a control siRNA or two siRNA duplexes targeting Rab14. 72 hours post-transfection the cell monolayers were wounded and brightfield images recorded. The cells were returned to 37°C for 18 hours and imaged again. The distance migrated by the wound front is plotted in the bar graph. Error bars indicate the standard error of the means ( $n = 3$ ). (B) A549 cells were transfected with the indicated siRNA duplexes for 72 hours, detached and seeded in duplicate on CIM-16 Transwell plates and subjected to real-time migration assays (xCelligence). The histogram depicts the Normalised Cell Index (CI) after 24 hours of migration. Error bars indicate the standard error of the means ( $**p < 0.01$ ,  $***p < 0.001$ ;  $n = 4$ ). (C) A549 cells transfected with the indicated siRNA duplexes for 72 hours, were detached and seeded, in duplicate, on CIM-16 Transwell plates that had been coated with Matrigel. The histogram depicts the Normalised Cell Index (CI) after 48 hours of migration. Error bars indicate the standard error of the means ( $**p < 0.01$ ;  $n = 3$ ). (D) Histogram represents the levels of Rab14 protein in lysates from cells transfected with the indicated Rab14 siRNA duplexes for 72 hours ( $n = 3$ ). (E) Histogram represents the level of N-cadherin (black) and E-cadherin (white) protein in lysates from cells transfected for 72 hours with the indicated siRNA duplexes. Values are normalised to siFLuc ( $n = 3$ ). (F) Representative Western blots of lysates from A549 cells transfected with the indicated siRNA duplexes for 72 hours,  $\alpha$ -tubulin was used as a loading control.