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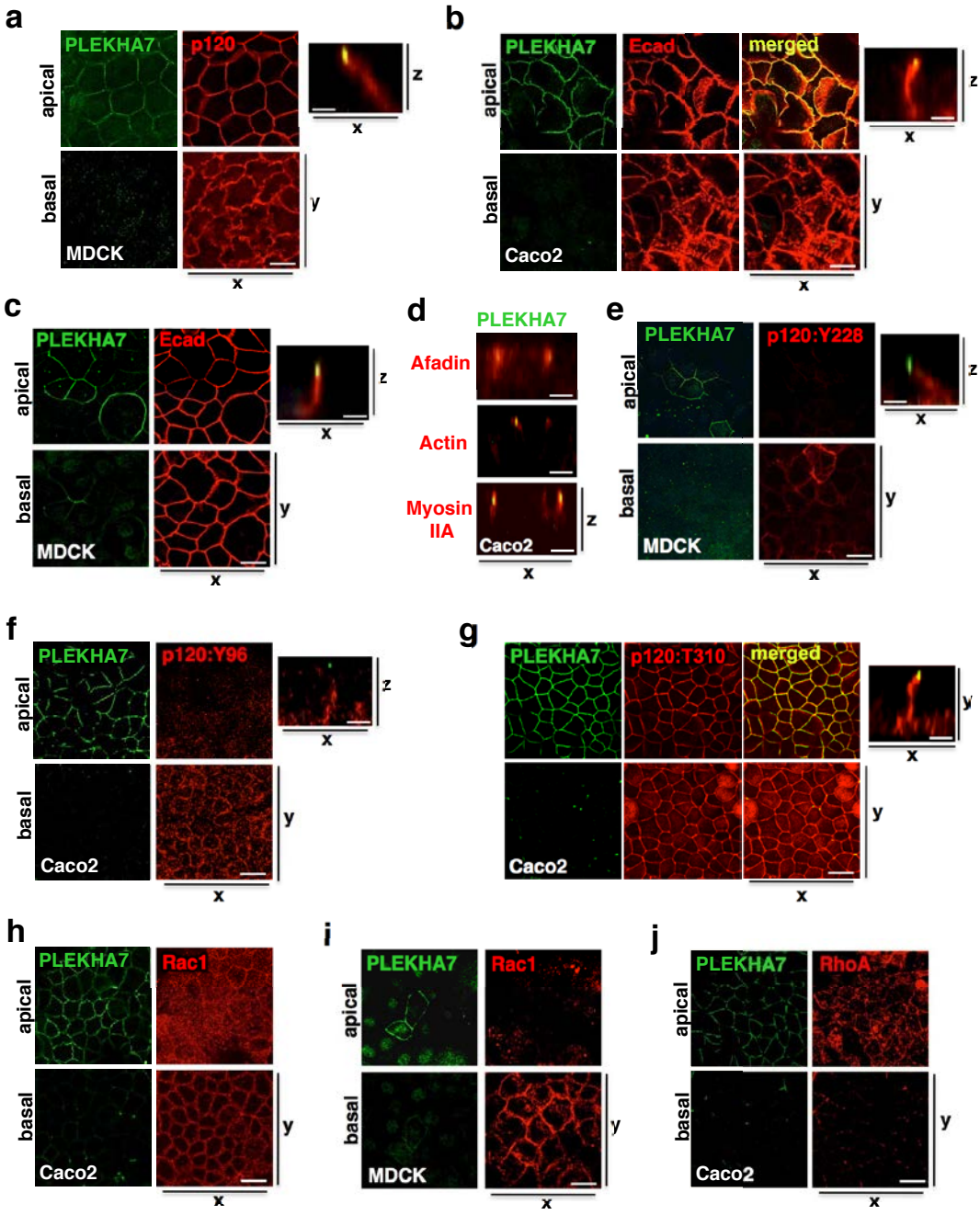
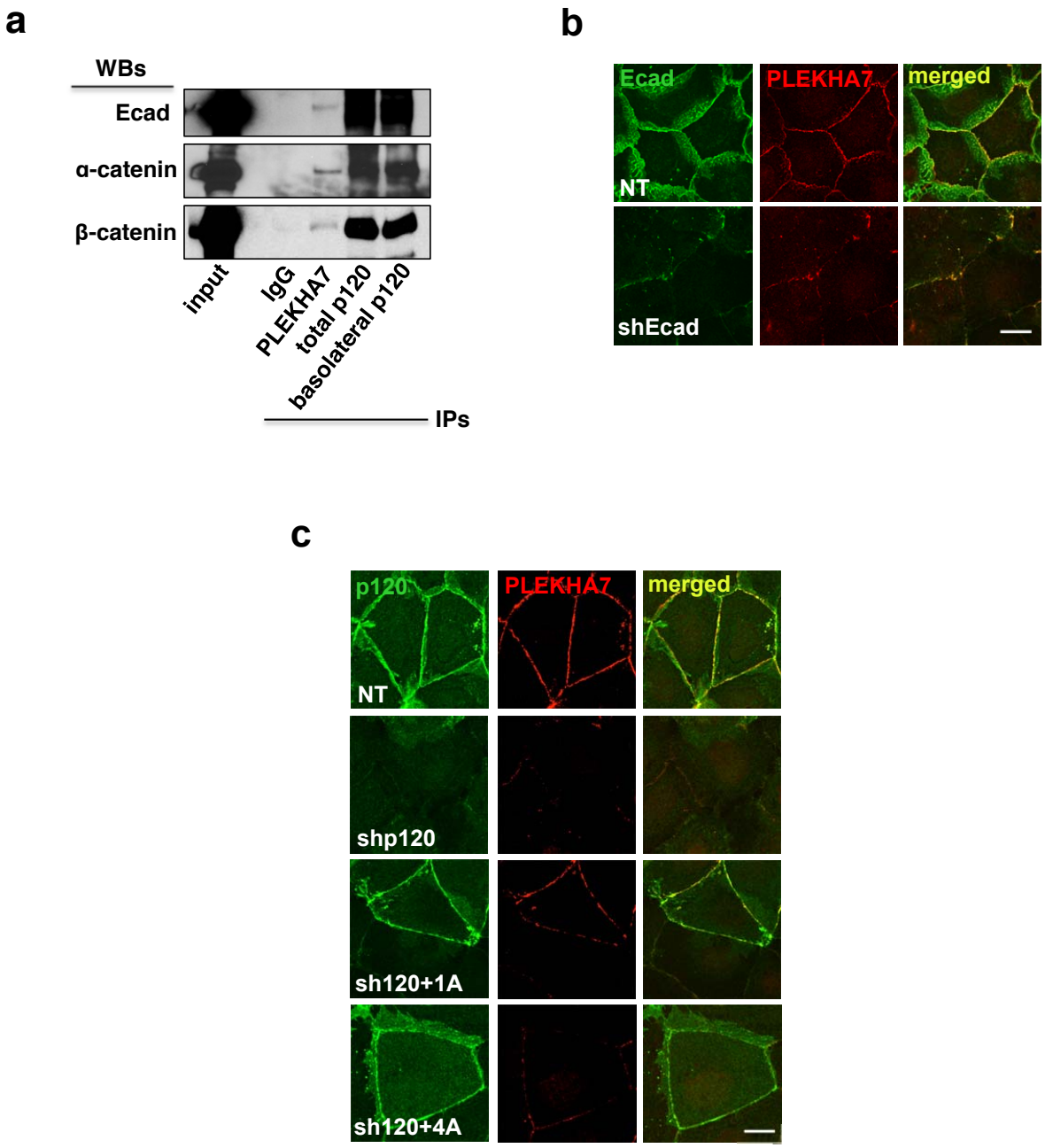
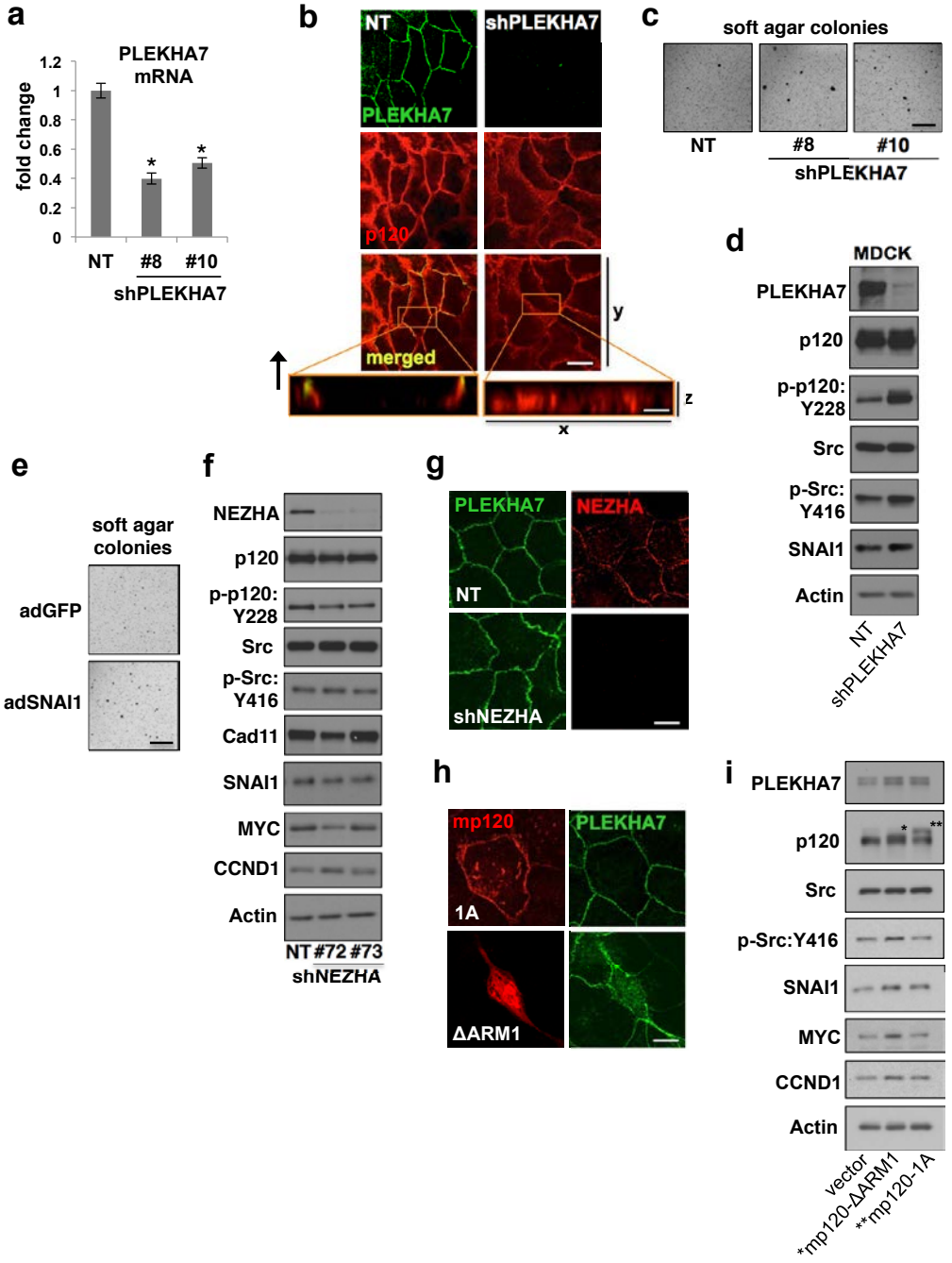


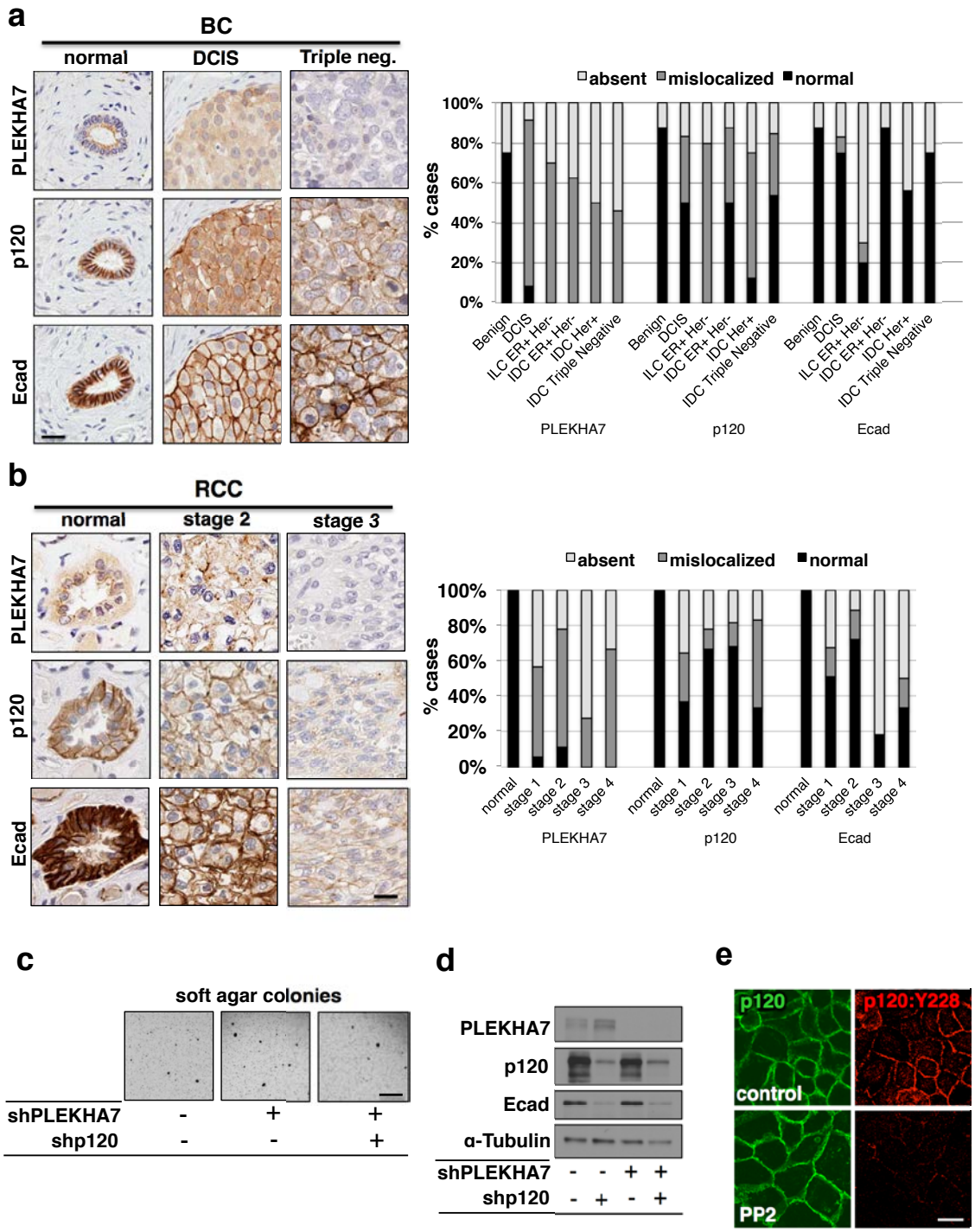
Supplementary Figure 1 Distinct complexes exist at the junctions of epithelial cells. Caco2 or PLEKHA7-GFP transfected MDCK cells were grown to polarize, subjected to IF for PLEKHA7 or GFP respectively and co-stained for (**a**) p120; (**b**,**c**) E-cadherin (Ecad); (**d**) Afadin, Actin (phalloidin), Myosin IIA; (**e**) phosphorylated p120: Y228; (**f**) phosphorylated p120: Y96; (**g**) phosphorylated p120: T310; (**h**,**i**) Rac1; (**j**) RhoA. Stained cells were imaged by confocal microscopy and image stacks were acquired, as in Figure 1. Representative x-y image stacks are shown and/or the merged composite x-z images. Scale bars for x-y images: 20 μM; for x-z images: 5 μM.



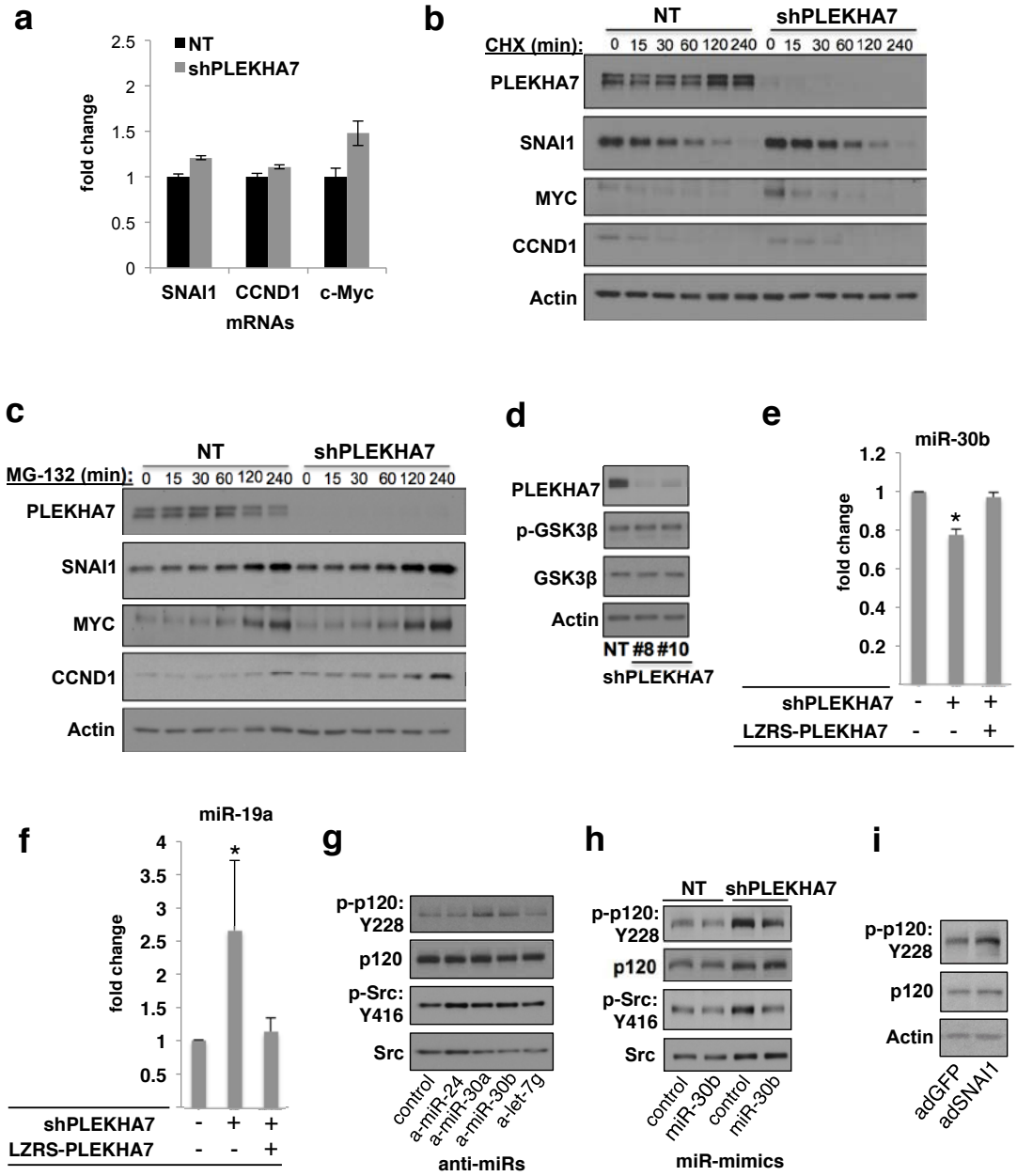
Supplementary Figure 2 PLEKHA7 localization to the junctions is E-cadherin- and p120-dependent. (**a**) Western blot of the lysates from the separated apical and basolateral fractions shown in Figure 2 for E-cadherin (Ecad), α-catenin, and β-catenin. (**b**) Caco2 control (NT) or E-cadherin knockdown (shEcad) cells, stained by IF for E-cadherin and PLEKHA7. (**c**) p120 and PLEKHA7 IF stainings of Caco2 control (NT) cells, p120 knockdown (shp120) cells, and of p120 knockdown cells transfected either with the full length murine mp120-1A isoform (shp120+1A) or the murine mp120-4A isoform that lacks the N-terminal PLEKHA7-binding domain (shp120+4A). All scale bars: 20 μM.



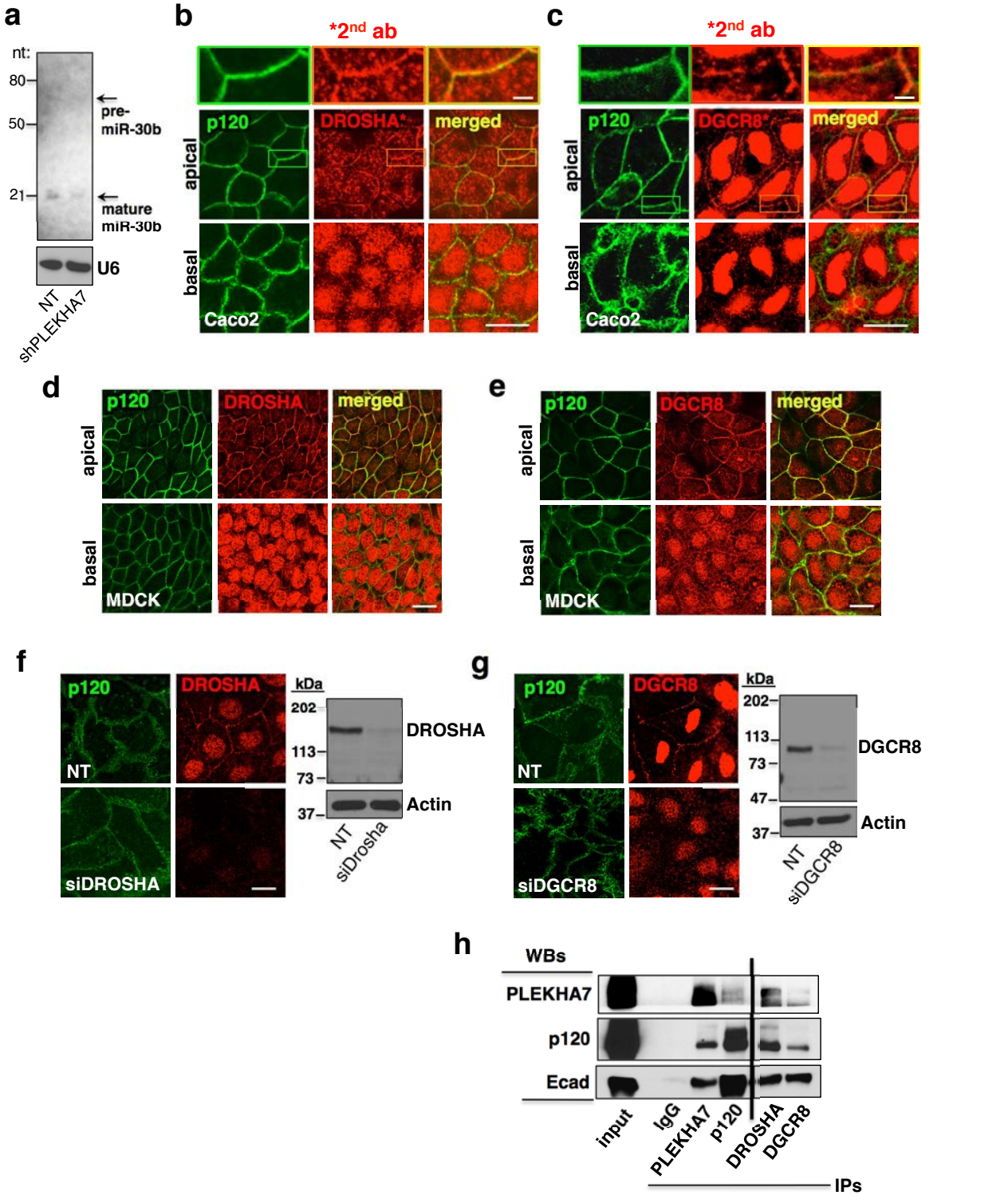
Supplementary Figure 3 PLEKHA7 loss from the junctions results in increased anchorage-independent growth and related signalling. (**a**) Demonstration of the PLEKHA7 mRNA knockdown by qRT-PCR after infection of Caco2 cells with two PLEKHA7 shRNAs (shPLEKHA7 #8 and #10) or non-target control shRNA (NT) (mean ± s.d. from n=3 independent experiments; *P<0.0001, Student's two-tailed t-test). Source data are provided in Supplementary Table 2. (**b**) Caco2 control (NT) and PLEKHA7 shRNA knockdown (shPLEKHA7) cells were stained and imaged for PLEKHA7 and p120. (**c**) Caco2 control (NT) or PLEKHA7 knockdown cells (shPLEKHA7 #8, #10) grown on soft agar and imaged for colony formation (images are in 2x magnification; see Fig. 3c for quantitation). (**d**) MDCK cells were infected with either control (NT) or PLEKHA7 shRNA (shPLEKHA7#8) and subjected to western blot for the markers shown. Phosphorylation sites are denoted by p-. Actin is the loading control. (**e**) Soft agar assay of Caco2 cells infected with either vector control (adGFP) or a SNAI1-expressing construct (adSNAI1) (images are in 2x magnification; see Fig. 3h for quantitation). (**f**) Western blot of control (NT) or NEZHA knockdown (shNEZHA#72 and #73 shRNAs) Caco2 cells for the markers shown; Actin is the loading control. (**g**) IF of control (NT) or NEZHA knockdown (shNEZHA) Caco2 cells for PLEKHA7 and NEZHA. (**h**) IF of Caco2 cells transfected with either wild type murine mp120-1A (1A) or the murine mp120-ΔARM1 (ΔARM1) construct that cannot bind E-cadherin, stained with the murine-specific p120 antibody 8D11 (mp120) and co-stained with PLEKHA7. (**i**) Western blot of pcDNA (vector control), mp120-ΔARM1, or mp120-1A transfected cells, for the markers shown; Actin is the loading control. Single and double stars on the p120 blot indicate the 1A and ΔARM1 bands, respectively, right above the endogenous p120 bands. Scale bars for x-y images: 20 μM; for x-z images: 2.5 μM; for panels **c** and **e**: 2 mm.



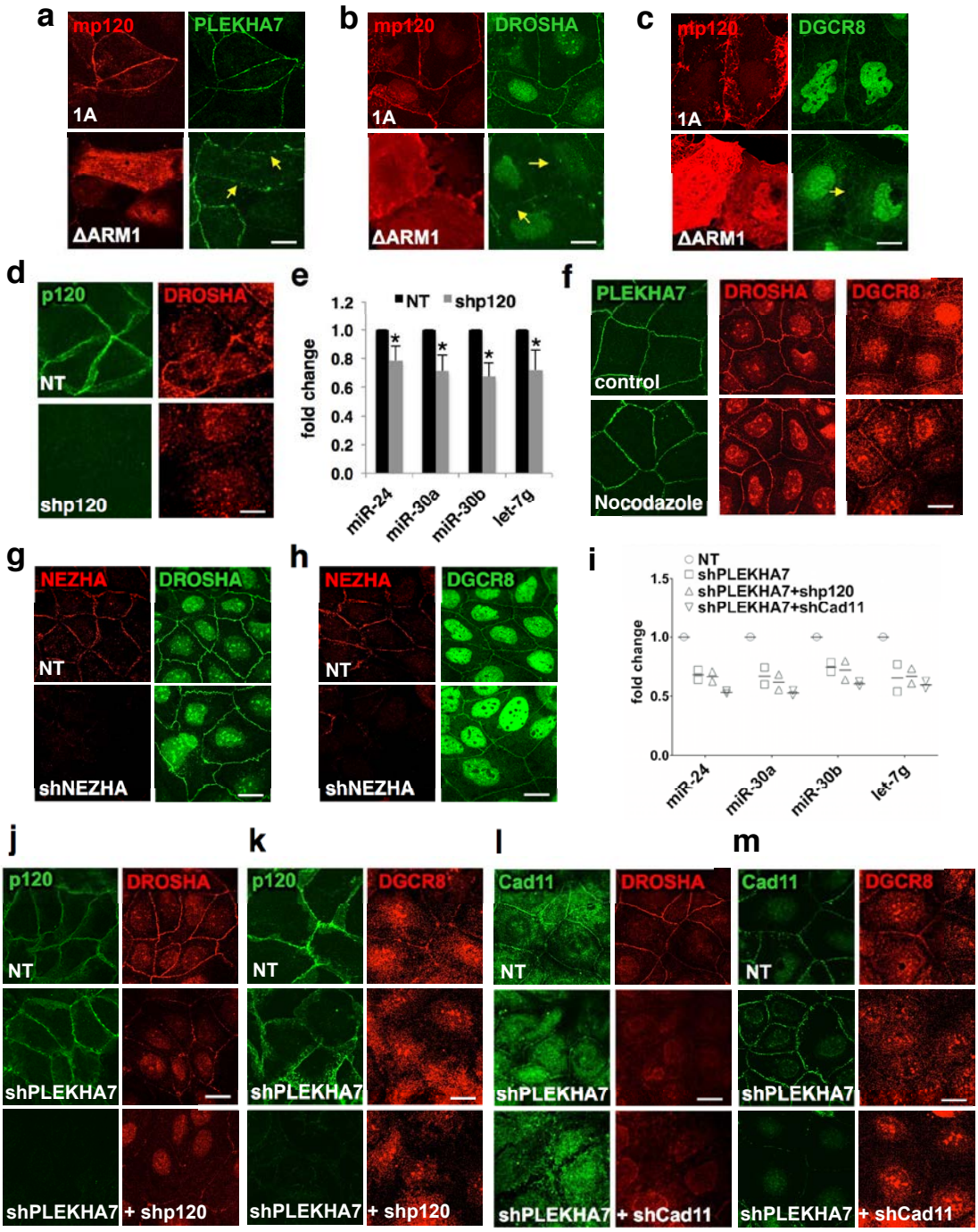
Supplementary Figure 4 PLEKHA7 is mis-localized or lost in breast and renal tumour tissues. Representative immunohistochemistry images of (**a**) breast and (**b**) kidney (renal), normal and cancer tissues stained for PLEKHA7, p120 and E-cadherin (Ecad) (left panels) and the percentage of tissues that exhibit presence, absence, or mis-localization of the three markers examined (right panels). BC: breast cancer; RCC: renal cell carcinoma. Scale bars: 20 μM. Number of tissues examined per cancer type/stage; BC TMA: Benign, *n*=8; DCIS, *n*=12; ILC ER+ Her-, *n*=10; IDC ER+, Her-, *n*=16; IDC Her+, *n*=16; IDC Triple Negative, n=13; RCC TMA: Matched normal, n=119; stage 1, n=71; stage 2, *n*=20; stage 3, *n*=22; stage 4, *n*=6. (**c**) Caco2 control (NT), PLEKHA7 knockdown (shPLEKHA7), and PLEKHA7, p120 (shPLEKHA7, shp120) double knockdown cells were grown on soft agar for colony formation assay (images are shown in 2x magnification; see Fig. 4a for quantitation) and (**d**) examined by western blot for E-cadherin (Ecad) levels; α-Tubulin is the loading control. (**e**) Caco2 cells treated with either vehicle (DMSO) or the Src inhibitor PP2 (10 μM) were stained for p120 and phosphorylated p120: Y228. Scale bars 20 μM; for panel **c**: 2 mm.



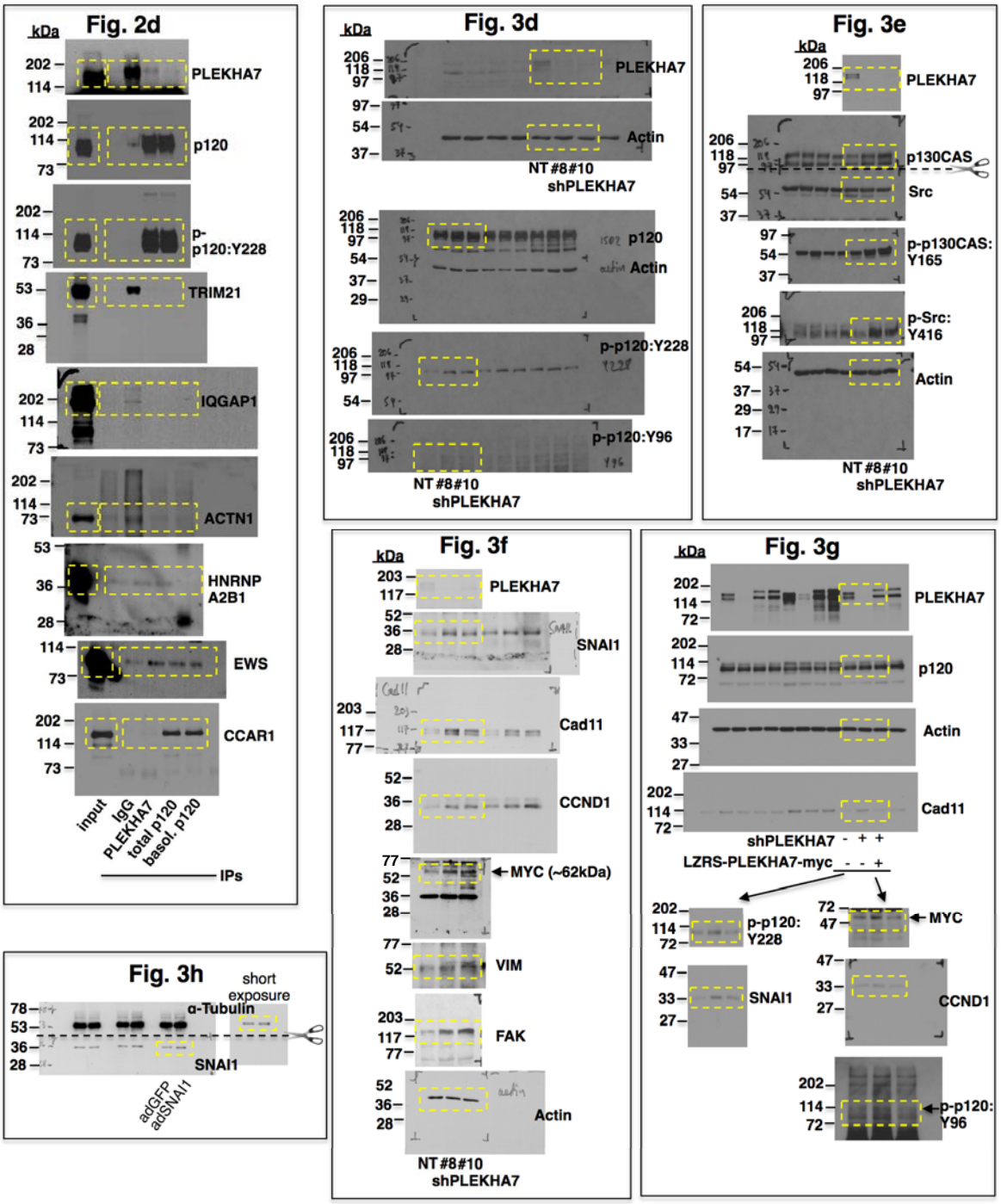
Supplementary Figure 5 PLEKHA7 acts via miRNAs but not post-translational modification mechanisms. (**a**) qRT-PCR analysis for the indicated mRNAs of Caco2 control (NT) or PLEKHA7 knockdown cells (shPLEKHA7) (mean ± s.d. from *n*=3 independent experiments). (**b**) Western blot for the markers shown (Actin: loading control) of control (NT) or PLEKHA7 knockdown (shPLEKHA7) Caco2 cells treated with 20 μg/ml cycloheximide (CHX) or (**c**) 10 μM MG-132, for the indicated time points. (**d**) Western blot of control (NT) or PLEKHA7 knockdown (shPLEKHA7) Caco2 cells for the markers shown; Actin is the loading control. (**e**,**f**) miR-30b and miR-19a qRT-PCR analysis of PLEKHA7-knockdown (shPLEKHA7) Caco2 cells after ectopic re-expression of PLEKHA7 (LZRS-PLEKHA7) (mean ± s.d. from *n*=3 independent experiments; **P*<0.05, Student's two-tailed *t*-test) (**g**) Caco2 cells transfected with the indicated anti-miRs (a-miR) were subjected to western blot for the markers shown. (**h**) Caco2 control (NT) and PLEKHA7 knockdown (shPLEKHA7) cells were transfected with either control or miR-30b mimic and blotted for the markers shown. (**i**) Caco2 cells infected with either vector control (adGFP) or a SNAI1-expressing construct (adSNAI1) were subjected to western blot for the markers shown; Actin is the loading control. Source data for panels **a**, **e**, **f** are provided in Supplementary Table 2.



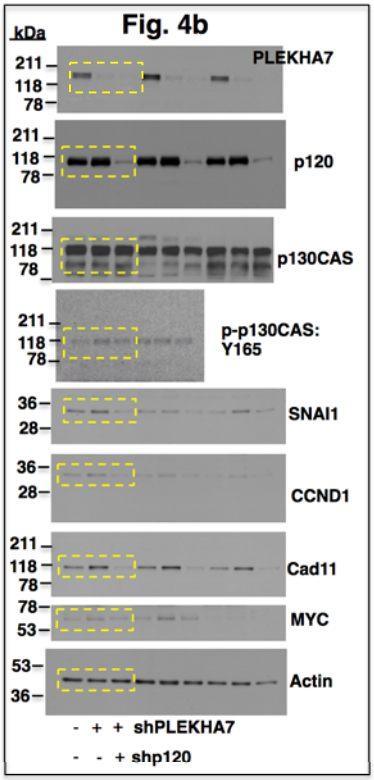
Supplementary Figure 6 The microprocessor complex localizes at the ZA. (**a**) Northern blot analysis of control (NT) or PLEKHA7 knockdown (shPLEKHA7) Caco2 cells using a miR-30b probe, indicating the pre-miR-30b and the mature miR-30b. U6 is the loading control. (**b**,**c**) IF of polarized Caco2 cells for p120 and DROSHA or DGCR8. Antibodies used here: DROSHA: Sigma and DGCR8: Sigma. The indication *2nd ab refers to the use here of a different antibody for DROSHA and DGCR8, compared to the antibodies used in Fig. 6d,e (for antibody details, see Supplementary Table 3). Enlarged details in boxes are shown on top of apical fields. The x-z composite image of panel **b** is shown in Fig. 6f and of **c** in Fig. 6g. (**d**,**e**) IF of polarized MDCK cells for p120 and DROSHA (antibody: Abcam) or DGCR8 (antibody: Abcam). (**f**,**g**) siRNA-mediated knockdown of DROSHA (siDROSHA) and DGCR8 (siDGCR8) in Caco2 cells, shown both by IF (left side of each panel), and western blot (right side of each panel). Non-target (NT) siRNA is the control. p120 was used as a co-stain for the IF; Actin is the loading control for the blots; molecular weights (kDa) are indicated on the left side of each blot. (**h**) Western blots of PLEKHA7, p120, DROSHA, and DGCR8 IPs for PLEKHA7, p120 and E-cadherin (Ecad). IgG is the negative IP control. Scale bars: 20 μM; for enlarged parts of panels **b** and **c**: 3 μM.

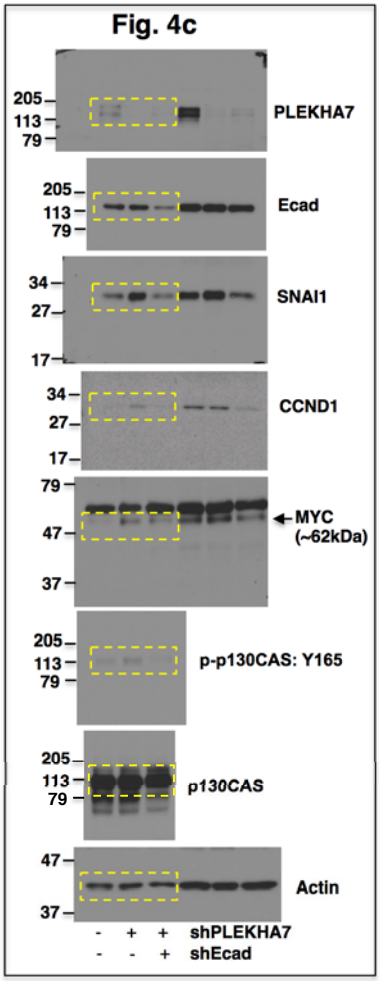


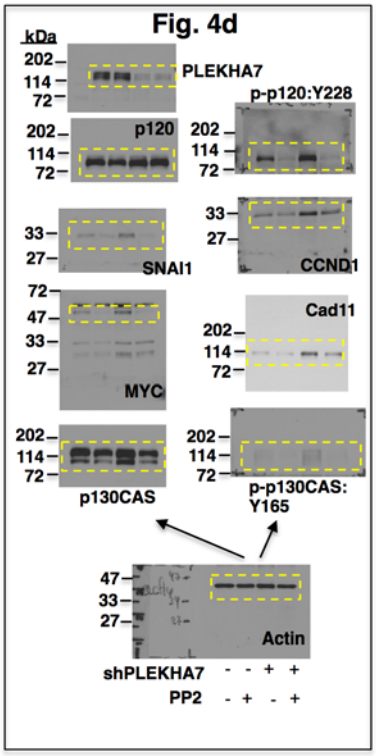
Supplementary Figure 7 Regulation of the microprocessor at the ZA is PLEKHA7-depended. (**a-c**) IF of Caco2 cells transfected with either the wild type murine mp120-1A (1A) construct or the murine mp120-ΔARM1 (ΔARM1) construct that cannot bind E-cadherin, both stained with the murine-specific p120 antibody 8D11 (mp120) and co-stained with PLEKHA7, DROSHA, or DGCR8. Arrows indicate affected junctional staining of the indicated markers in transfected cells. (**d**) IF of control (NT) or p120 knockdown (shp120) Caco2 cells for DROSHA and p120. (**e**) qRT-PCR of control (NT) and p120 knockdown (shp120) Caco2 cells for the miRNAs shown (mean ± s.d. from n=3 independent experiments; *P<0.05, Student's two-tailed t-test). (**f**) IF of control (DMSO) or Nocodazole (10 μM for 8h) treated Caco2 cells for PLEKHA7, DROSHA, or DGCR8. (**g**,**h**) IF of control (NT) or NEZHA knockdown (shNEZHA) Caco2 cells for DROSHA and DGCR8, co-stained with NEZHA. (**i**) qRT-PCR of control (NT), PLEKHA7 knockdown (shPLEKHA7), PLEKHA7+p120 (shPLEKHA7+shp120), and PLEKHA7+Cadherin-11 (shPLEKHA7+shCad11) double knockdown Caco2 cells for the indicated miRNAs (individual data points and mean from n=2 independent experiments are shown). (**j**,**k**) IF of control (NT), PLEKHA7 knockdown (shPLEKHA7), and PLEKHA7+p120 double knockdown (shPLEKHA7+shp120) Caco2 cells for DROSHA and DGCR8, co-stained with p120. (**l**,**m**) IF of control (NT), PLEKHA7 knockdown (shPLEKHA7), and PLEKHA7+Cadherin-11 double knockdown (shPLEKHA7+Cad11) Caco2 cells for DROSHA and DGCR8, co-stained with Cadherin 11 (Cad11). Non-specific cytoplasmic or nuclear background appears respectively for the two Cadherin-11 antibodies used in the IF (see Methods for antibody details). All scale bars: 20 μM. Source data for panels **e** and **i** are provided in Supplementary Table 2.

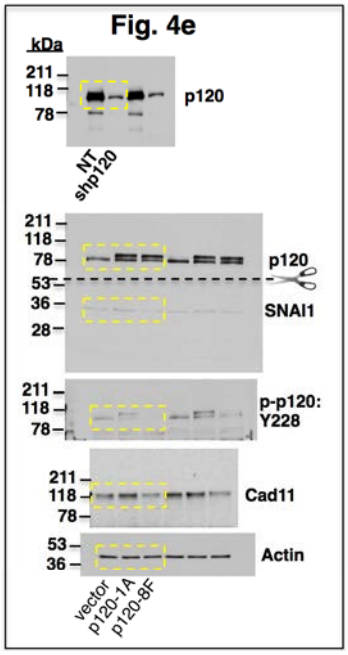


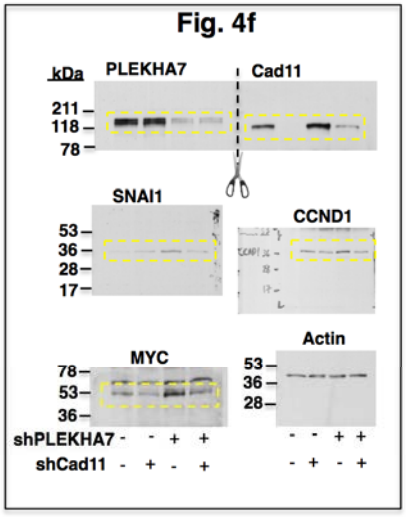
Supplementary Figure 8 Full western blot scans. Multiple experiments were routinely run on every gel and the membranes were cut and blotted for multiple antibodies for each experiment. The cut blots for each antibody are shown here per case. Molecular weights are indicated on the left; the cropped images shown in the respected Figure panels are indicated in yellow boxes.

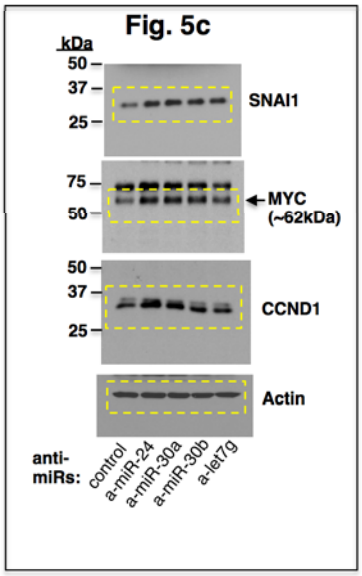




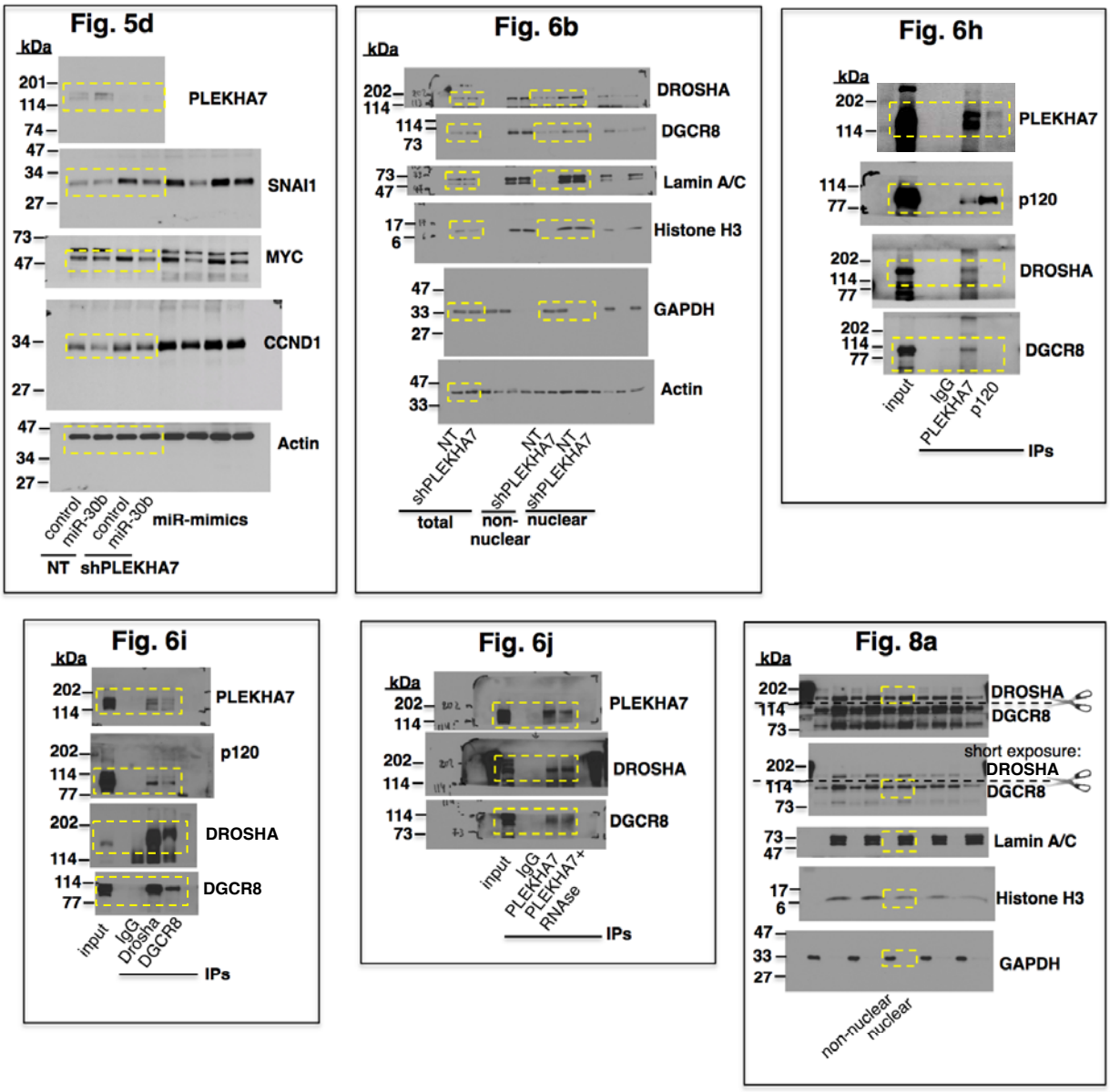








Supplementary Figure 8 continued



Supplementary Figure 8 continued

Supplementary Table Legends

Supplementary Table 1 The proteins identified by proteomics of junctional fractions.
Supplementary Table 2 The source data file.
Supplementary Table 3 The antibodies used in the study.