

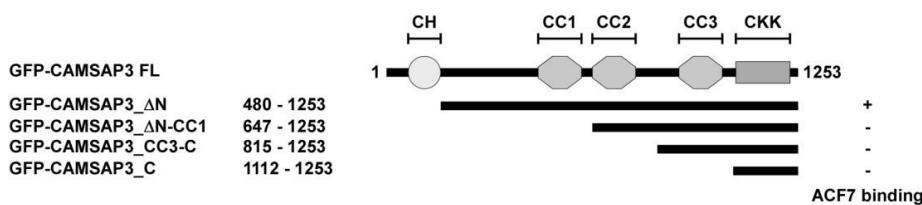
Supplemental Information

Control of apico-basal epithelial polarity by the microtubule minus-end binding protein CAMSAP3 and spectraplakin ACF7

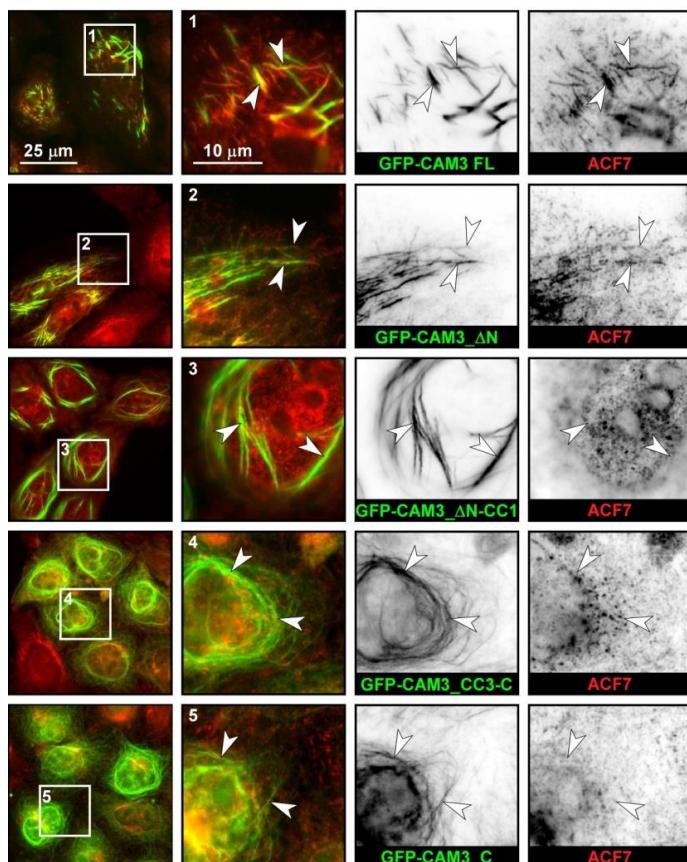
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Figure s1

A



B

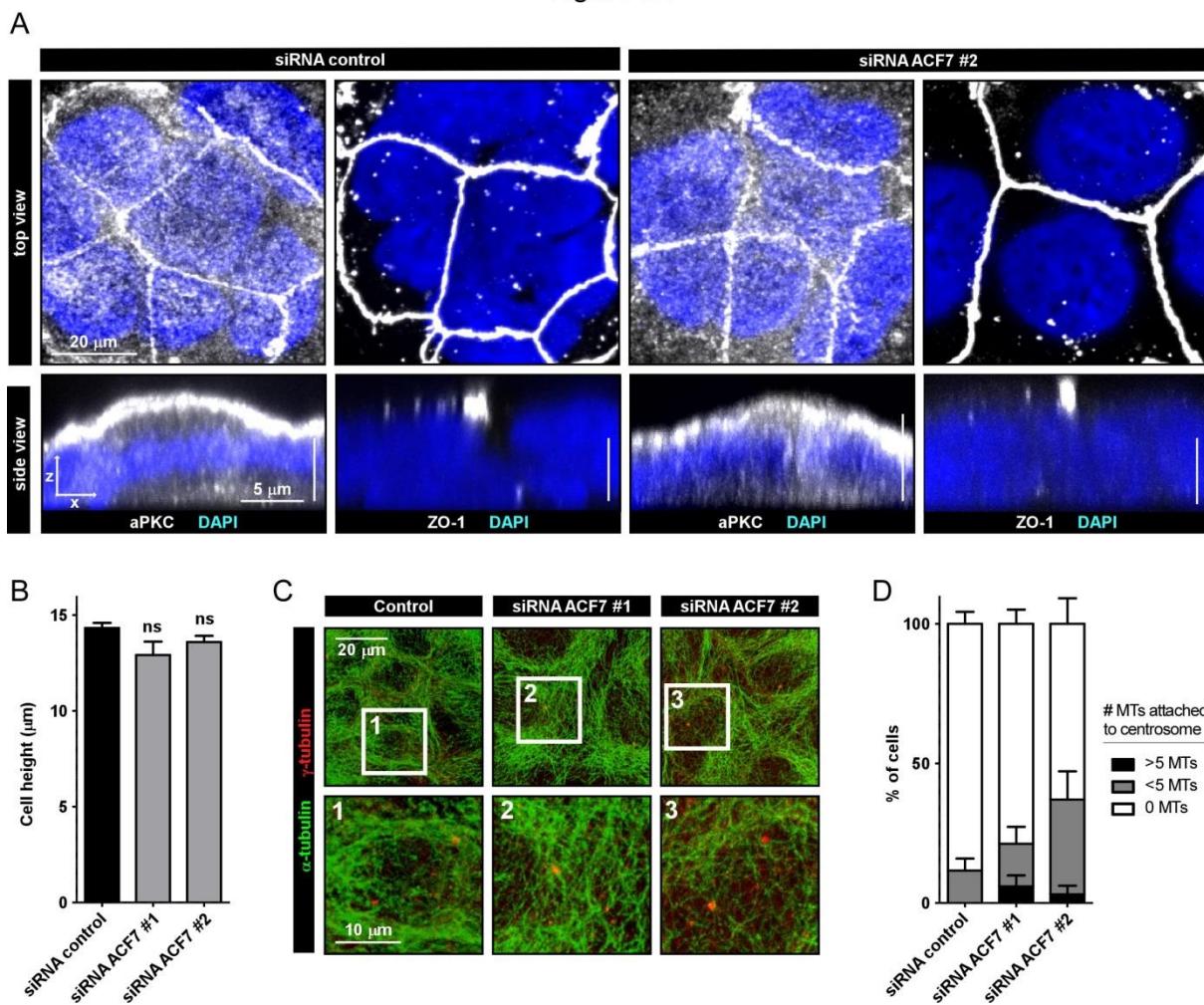


Supplemental Figure S1. Endogenous ACF7 is recruited to CAMSAP3-induced microtubule bundles in manner dependent on the CC1 region of CAMSAP3.

A. Domain organization of GFP-CAMSAP3 deletion mutants used to study binding to ACF7 and a summary of detected interactions. FL, full length, CH, calponin homology domain.

B. HeLa cells transiently expressing GFP-CAMSAP3 fragments (as in A, green) and stained for ACF7 (red). White arrowheads indicate the same positions in different panels.

Figure s2



Supplemental Figure S2. ACF7 depletion does not affect general polarity in 2D monolayer culture.

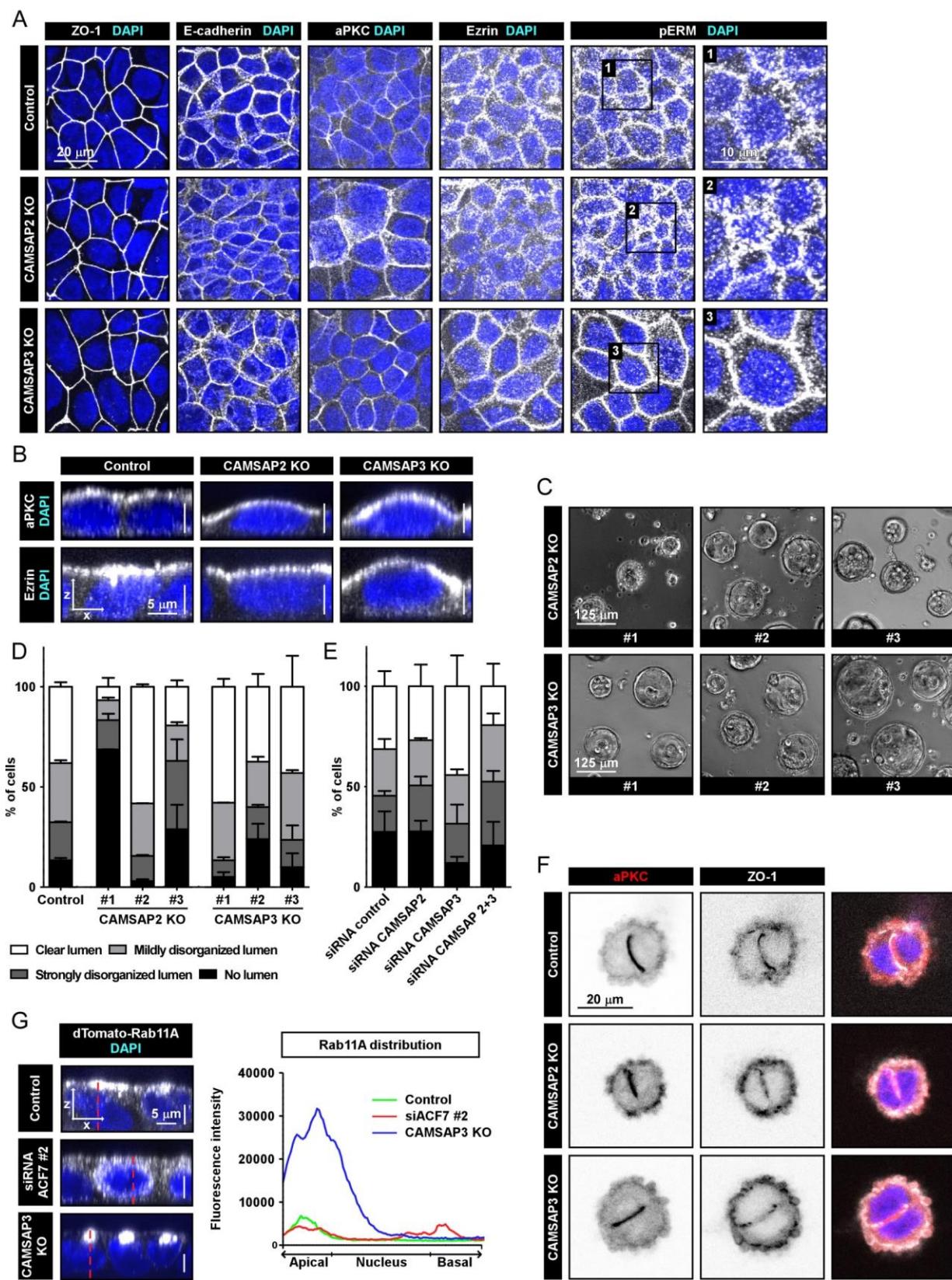
A. Staining of aPKC, ZO-1 (white) and DNA (blue) in Caco-2 cells transfected with control or ACF7 siRNAs. Both top and side views are shown.

B. Quantification of cell height of Caco-2 cells transfected with control or ACF7 siRNAs. Control, n=36; siRNA ACF7 #1, n=36; siRNA ACF7 #2, n=36; ns, not significant; Mann-Whitney U test; Error bars, SEM.

C. Staining of microtubules (green, α -tubulin) and centrosomes (red, γ -tubulin) in Caco-2 cells transfected with control or ACF7 siRNAs.

D. Quantification of microtubule organization presented as a number of microtubules attached to the centrosome. Quantification based on staining shown in (C). Number of analyzed cells: control, n=41; siRNA ACF7 #1, n=38; siRNA ACF7 #2, n=60; Error bars, SEM; #, number; MTs, microtubules.

Figure s3



Supplemental Figure S3. Formation of 3D cysts and the distribution of polarity markers in control, CAMSAP2 and CAMSAP3 knockout cells.

- A. Staining for ZO-1, E-cadherin, aPKC, ezrin en pERM (white) and DNA (blue) in control, CAMSAP2 and CAMSAP2 knockout (KO) cells.
- B. Side view of aPKC, ezrin (white) and DNA (blue) staining in cells as in (A).
- C. 3D cyst formation 1 day after dense seeding of different CAMSAP2 and CAMSAP3 knockout clones.
- D. Quantification of 3D cyst formation of cells in (C). Control, n=200; CAMSAP2 KO #1, n=172; CAMSAP2 KO #2, n=245; CAMSAP2 KO #3, n=143; CAMSAP3 KO #1, n=173; CAMSAP3 KO #2, n=204; CAMSAP3 KO #3, n=213.
- E. Quantification of 3D cyst formation of Caco-2 cells transfected with siRNA targeting CAMSAP2, CAMSAP3 or CAMSAP2 and 3 simultaneously. siRNA control, n=320; siRNA CAMSAP2, n=226; siRNA CAMSAP3, n=316; siRNA CAMSAP2+3, n=252.
- F. Staining of aPKC (red), ZO-1 (white) and DNA (blue) in cells as in (A) seeded in a 3D matrix.
- G. Side view of dTomato-Rab11A (white) and DNA (blue) in control cells, cells transfected with siRNA targeting ACF7 and CAMSAP3 knockout cells. dTomato-Rab11A intensity profiles along the line indicated in red are plotted in the graph.

Supplemental table 1

Primary antibodies

| Target | Source | Dilution | Company | Catalog number |
|-------------------------------|--------|--------------------|------------------|----------------|
| Tyrosinated α -tubulin | Rat | IF 1:300 | Abcam | #ab6160 |
| mCherry | Mouse | WB 1:1000 | Clontech | #632543 |
| ACF7 | Mouse | IF 1:400 | Abnova | #H00023499-A01 |
| CAMSAP3 | Mouse | IF 1:200 WB 1:1000 | Sigma-Aldrich | #SAB4200415 |
| γ -tubulin | Mouse | IF 1:100 | Sigma-Aldrich | #T5326 |
| Ku80 | Mouse | WB 1:2000 | BD Biosciences | #611360 |
| ZO-1 | Mouse | IF 1:500 | BD Biosciences | #610966 |
| E-cadherin | Mouse | IF 1:500 | BD Biosciences | #610181 |
| Ezrin | Mouse | IF 1:500 | BD Biosciences | #610602 |
| GFP | Rabbit | WB 1:1000 | Abcam | #ab290 |
| CAMSAP2 | Rabbit | IF 1:300 | Novus | #NBP1-21402 |
| CAMSAP2 | Rabbit | WB 1:1000 | Proteintech | #17880-1-AP |
| aPKC | Rabbit | IF 1:300 | Santa Cruz | #H00023499-A01 |
| Actin | Rabbit | IF 1:200 | Sigma-Aldrich | #20-33 |
| CEP135 | Rabbit | IF 1:300 | Sigma-Aldrich | #SAB4503685 |
| Phosphorylated ERM | Rabbit | IF 1:500 | Cell Signaling | #3141 |
| E-cadherin | Rabbit | IF 1:1000 | Gift from A. Yap | - |

IF: Immunofluorescence staining

WB: Western blot

Secondary antibodies

All secondary antibodies used for immunofluorescence staining were diluted 1:300

All secondary antibodies used for western blot were diluted 1:15000