Supporting Information

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SI Methods

Plasmids, siRNA and Transfection. Plasmids were gifts from the following individuals: rat Dyn2wt-GFP, Dyn2K44A-GFP and Dyn2ΔPRD-GFP from M. A. McNiven (Mayo Clinic, Rochester, MN); myc-Cortactin 9KR and myc-Cortactin 9KQ from E. Seto (H. Lee Moffitt Cancer Center, Tampa, FL); FLAG-cortactin W525K and FLAG-cortactin W22A from D. A. Schafer (University of Virginia, Charlottesville, VA); NTCP-GFP from F. J. Suchy (Mt. Sinai School of Medicine, New York, NY); PLCδ PH-GFP from T. Balla (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD); Eps15WT-GFP and Eps15Δ95/295-GFP from J. S. Bonifacino (National Institute of Child Health and Human Development); and FLAG-Caveolin1WT and FLAG-Caveolin1Y14F from M. A. Schwartz (University of Virginia).

Plasmid and siRNA transfection of monolayers was performed 1 d after confluency using Lipofectamine 2000, according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Monolayers were treated, fixed or imaged live 3d after confluency. Monolayers transfected with temperature sensitive mutants were incubated at 32 °C (permissive temperature) for 1d followed by 40 °C (nonpermissive temperature) for 1 d.

The custom synthesized canine Dyn2-targeted siRNA with the sequence GACATGATCCTGCAGTTCA and ON-Target*plus* Control pool siRNA were from Dharmacon (Lafayette, CO). SiRNA transfection was performed according to Rodriguez-Boulan and colleagues (34) using Nucleofector Solution V and program T23 (Lonza, Switzerland). For suspended cells subjected to two rounds of siRNA transfection, cells were transfected, grown on plates for 3d, and transfected again before culturing on Transwells for an additional 3 d.

RFP and CeFP versions of Dyn2 were constructed by digesting the cDNA with EcoR1 and HindIII and subcloning the fragment in frame with the fluorescent proteins. For single amino acid mutation, 100–200 ng of template DNA (Dyn2wt-CeFP and Dyn2K44A-CeFP) was used to generate mutations using the Quik change site directed mutagenesis protocol (Stratagene). The clones were selected and confirmed by DNA sequencing. Oligo sequences used to generate mutations are as follows:

Dyn2K44A, AGAGCGCCGGC*GCG*AGTTCGGTGCT;
Dyn2S45N, GCGCCGGCAAG*AAT*TCGGTGCTCGA;
Dyn2S61D, TTCCACGAGGA*GAT*GGAATTGTCAC;
Dyn2G146S, AGGTGCCAGTG*TCG*GACCAGCCACC;
Dyn2G273D, CTGACCGCATG*GAT*ACCCCACACTT; and
Dyn2I684K, ATGTGCACCTCATG*AAA*AACAACACAAAGGC.

Primary antibodies and reagents. Rabbit αFLAG and αMyc antibodies were kind gifts from R. Hegde (National Institute of Child Health and Human Development). These antibodies were obtained from the following companies: rabbit αZO-1, rabbit αClaudin-2 and mouse αOccludin from ZYMED Laboratories; rabbit αMyosin IIB-HC from Covance (Berkeley, CA); mouse αDynaminII, αDynamin and αE-cadherin from BD Biosciences; and mouse αTubulin from Sigma-Aldrich. Secondary antibodies were from the following companies: HRP-αmouse, Cy5-goat αmouse and Cy3-goat αmouse from Jackson ImmunoResearch; and TxRed-goat αrabbit from Molecular Probes.

These reagents were obtained from the following companies: FM4–64 (100 μg/ml, 0.5 h), jasplakinolide (1 μM, 1 h), TxRed-phalloidin (4 units/ml), TxRed-dextran (0.5 mg/ml, 1 h), and TxRed-transferrin (40 μg/ml, 1h) from Molecular Probes (Eugene, OR); cytochalasin D (4 μM, 1 h), nocodazole (10 μg/ml, 1 h after 1 h at 4 °C), demecolcine (0.2 μg/ml, 1 h after 1 h at 4 °C), blebbistatin (50 μM, 0.5 h), TSA (5 μM, 5 h, 24 h), TRITC-phalloidin (4 units/ml) and DMSO from Sigma-Aldrich; ML-7 (40 μM, 1 h), Y27632 (10 μM, 1 h), and Digitonin (0.01%, 10 min) from CalBioChem. Dynasore (200 μM, 18 h, serum free) was prepared by H. Pelish in the Kirchhausen laboratory (Harvard University, Cambridge, MA). Secramine (25 μM, 5 h or 24 h, serum free) was jointly prepared by the Kirshhausen laboratory and Hammond laboratory (University of Louisville, Louisville, KY).

Protein Analysis. Cells were lysed in sample buffer (62.5 mM Tris-HCl pH 6.8, 5% glycerin, 2% SDS, 0.005% bromophenol blue, 2.5% 2-mercaptoethanol) and heating it to 95°C for 5min. Each lysate were analyzed using standard SDS/PAGE followed by transfer to a nitrocellulose membrane. Membranes were probed with the appropriate primary and secondary antibodies. Immunoreactive bands were visualized using an enhanced chemi-luminescent detection system (Thermo Scientific). The same blot was stripped and re-probed for tubulin, which served as protein loading control.

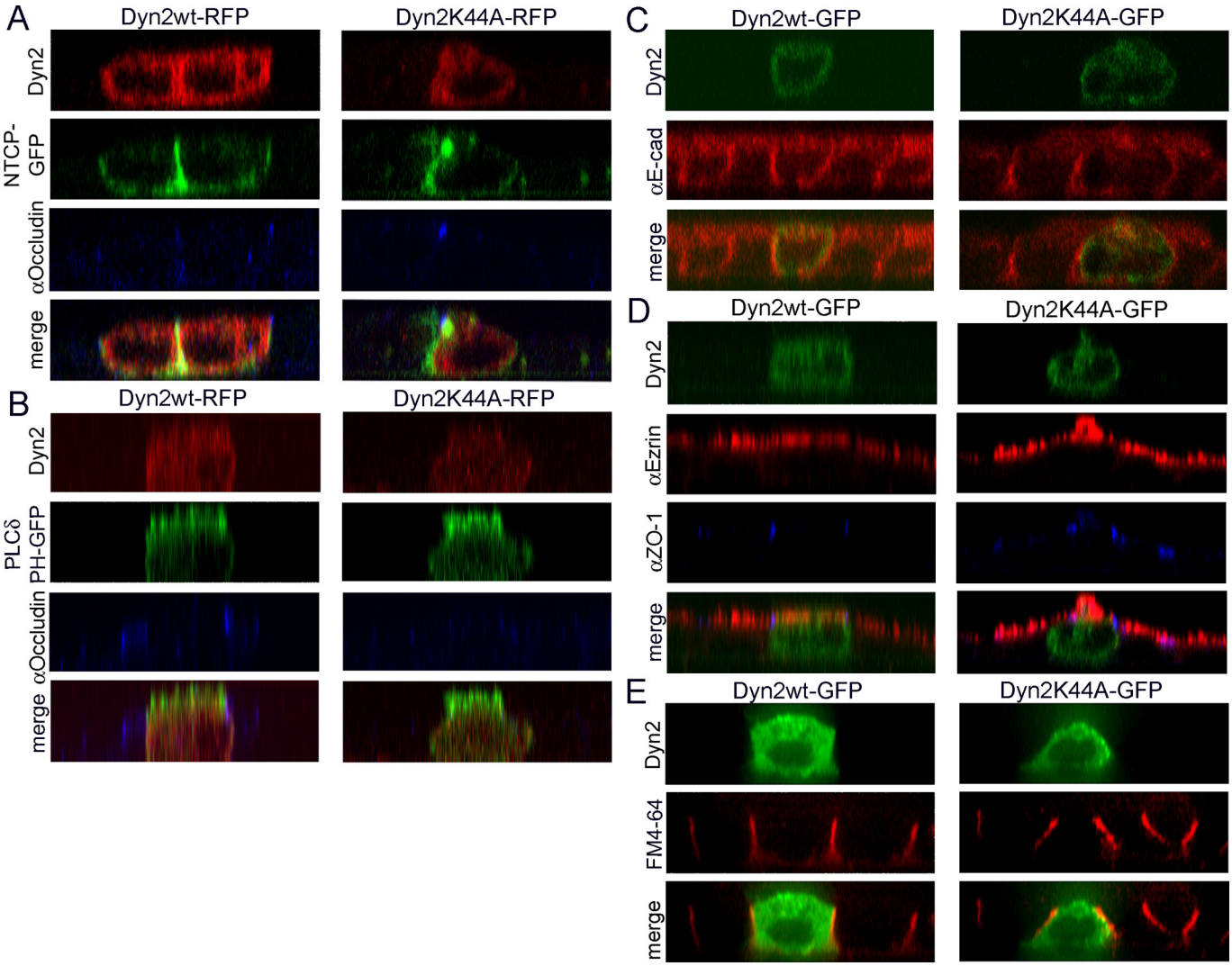


Fig. S1. Tight junctional integrity is maintained in Dyn2K44A-expressing cells. (*A*) XZ section images of Dyn2wt-RFP-expressing (*Left*, red) or Dyn2K44A-RFP-expressing (*Right*, red) cells cotransfected with NTCP-GFP (green) and stained for occludin (blue). (*B*) XZ section images of Dyn2wt-RFP-expressing (*Left*, red) or Dyn2K44A-RFP-expressing (*Right*, red) cells cotransfected with PLCδPH-GFP (green) and stained for occludin (blue). (*C*) XZ section images of Dyn2wt-GFP-expressing (*Left*, green) or Dyn2K44A-GFP-expressing (*Right*, green) cells and stained for E-cadherin (red). (*D*) XZ section images of Dyn2wt-GFP-expressing (*Left*, green) or Dyn2K44A-GFP-expressing (*Right*, green) cells stained for ezrin (red) and ZO-1 (blue). (*E*) XZ section of Dyn2wt-GFP-expressing (*Left*, green) or Dyn2K44A-GFP-expressing (*Right*, green) cells imaged live after FM4–64 (red) was applied basolaterally.

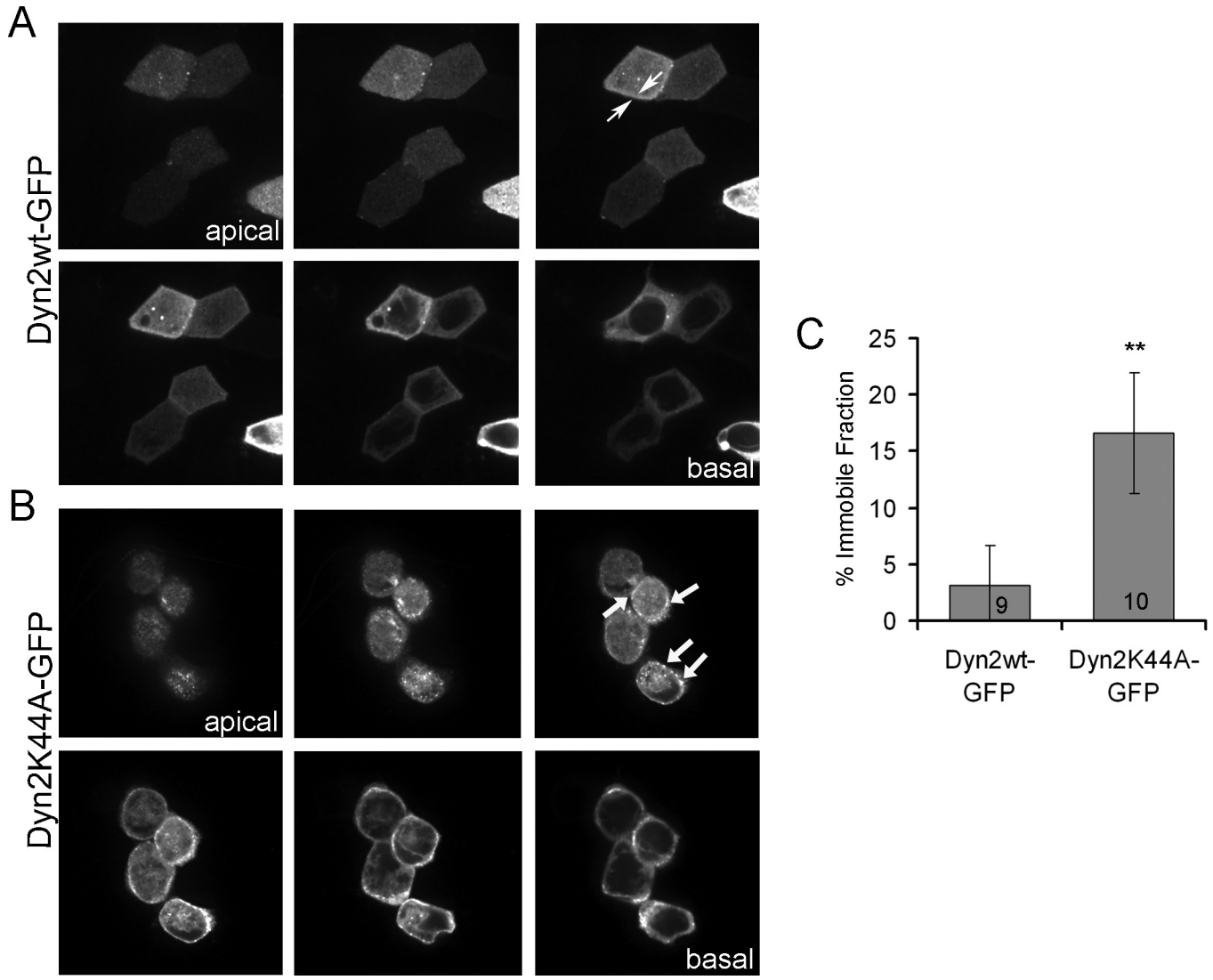


Fig. S2. Dynamin is enriched at the apical junctional region and in intracellular structures. (*A* and *B*) Z sections of cells expressing Dyn2wt-GFP (*A*) or Dyn2K44A-GFP (*B*). Note puncta and enrichment at the AJC (arrows). (*C*) Mean percent immobile fraction of Dyn2wt-GFP and Dyn2K44A-GFP calculated from the experiment in Fig. 3H. **, $P < 0.001$.

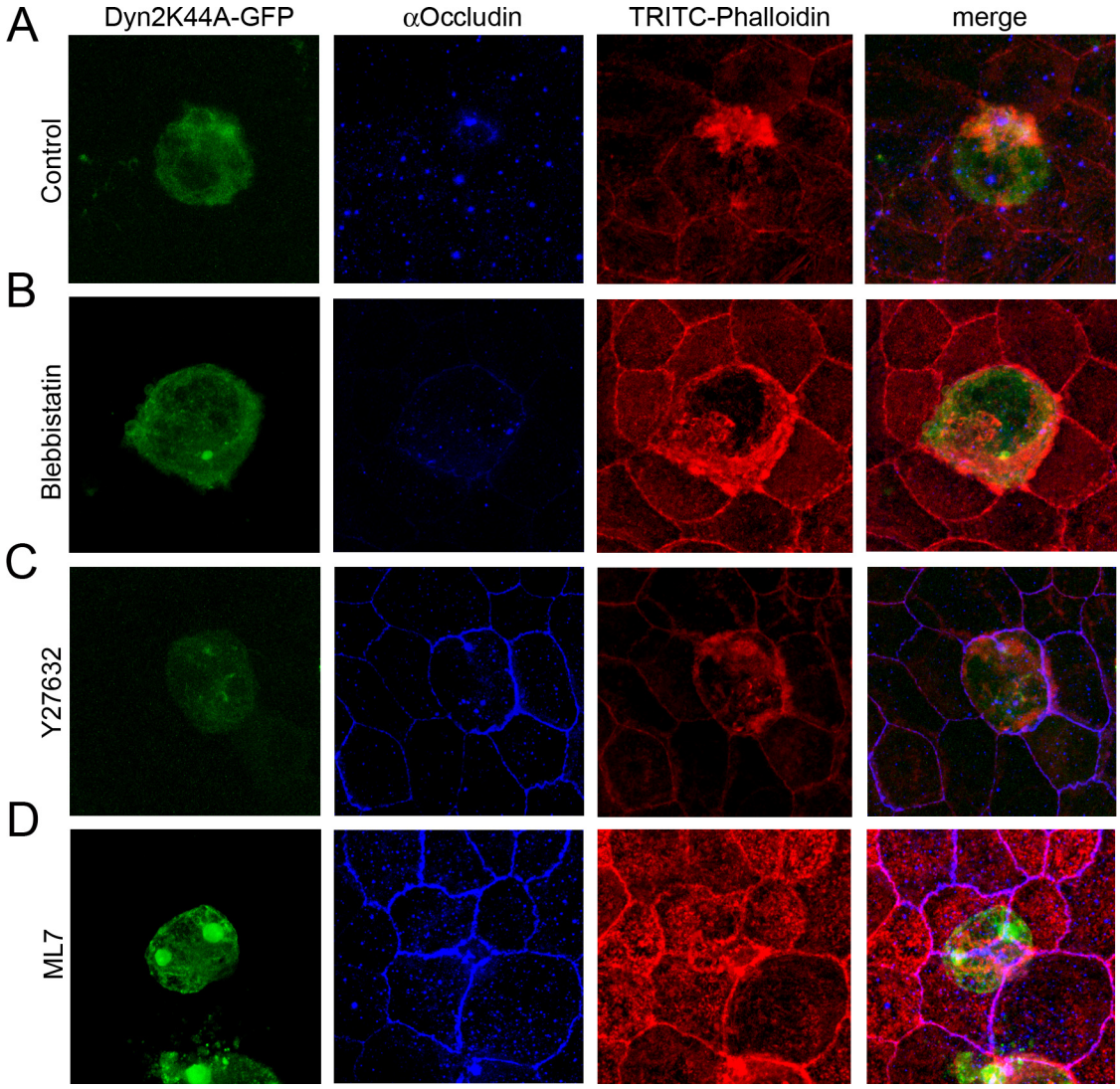


Fig. S3. Examining actin localization when MyosinII and ROCK are inhibited. Maximum intensity projection images of Dyn2K44A-GFP-expressing cells treated with blebbistatin (*B*), Y27632 (*C*), or ML7 (*D*), or with no treatment (*A*), and stained with TRITC-phalloidin (red) and occludin (blue).

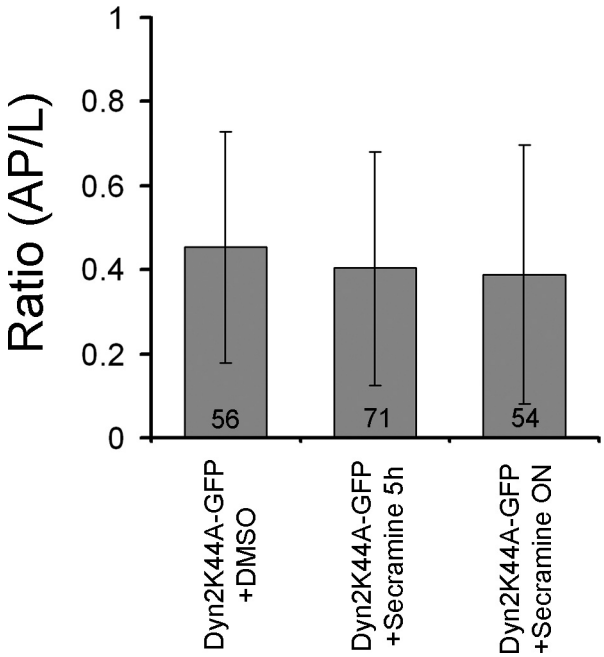


Fig. S4. Examining the role of Cdc42 in Dyn2K44A-induced apical constriction. Mean ratio (AP/L) of Dyn2K44A-expressing cells treated with DMSO or secramine for 5 h or overnight.

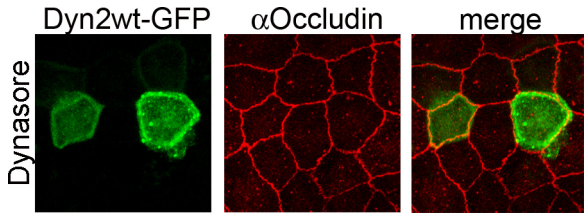
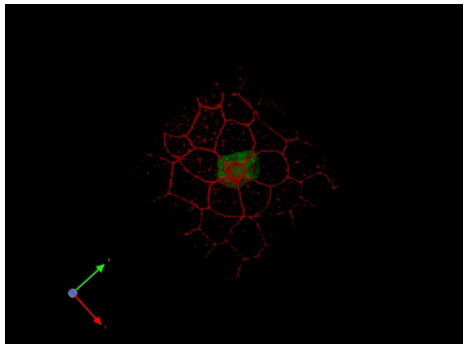
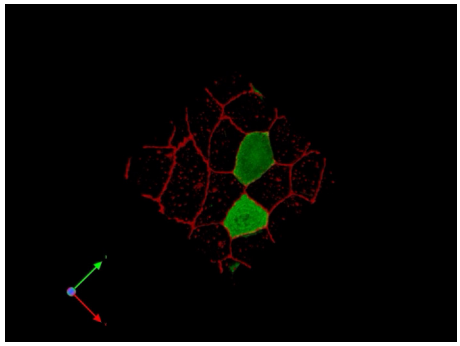


Fig. S5. Maximum intensity projection images of cells expressing Dyn2wt-GFP (green) treated with dynasore and stained for occludin (red).



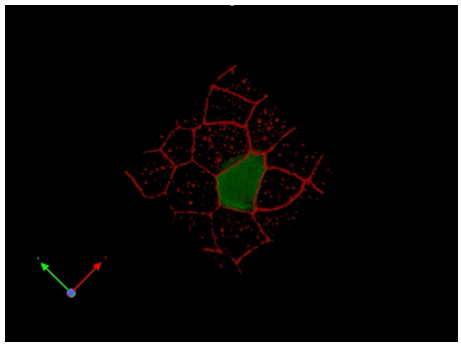
Movie S1. Dyn2K44A-GFP-expressing cell (green) in a monolayer and stained for occludin (red).

Movie S1 (AVI)



Movie S2. Dyn2wt-GFP-expressing cells (green) in a monolayer and stained for occludin (red).

Movie S2 (AVI)



Movie S3. GFP-expressing cell (green) in a monolayer and stained for occludin (red).

Movie S3 (AVI)