Supplementary Information

Supplementary Methods

Cell culture and treatments

MEFs were isolated as in (Hadjihannas et al., 2006) from conductin knockout mice described in (Lustig et al., 2002). For treatments, cells were incubated with NaCl (20mM), LiCl (20mM), and Wnt1/Wnt3A-conditioned media (Bilic et al., 2007; Lustig et al., 2002). For depolymerization of actin and microtubules, cells were treated with Latrunculin A (1μg/ml) and Nocodazole (5μg/ml) respectively, for 6 hours prior to fixation and staining.

Transfections of siRNAs and plasmids

For transfection of siRNAs, previously published sequences targeting conductin and β-catenin (Hadjihannas et al., 2006), C-Nap1 and Rootletin (Bahe et al., 2005) as well as Nek2 (Bahe et al., 2005; Mayor et al., 2002) and Luciferase (Eurogentec) were used. Expression plasmids encoding GFP and myc tagged C-Nap1, Rootletin and Nek2 were a gift by Prof. Erich Nigg (Biozentrum, University of Basel). GFP and Flag tagged conductin plasmids were previously described (Behrens et al., 1998; Hadjihannas and Behrens, 2006; Hadjihannas et al., 2006; Lustig et al., 2002). Point mutants of β-catenin were generated using the Quickchange Site-Directed mutagenesis kit (Stratagene).

TOP/FOP assays

TOPFlash carries TCF responsive elements that drive expression of Luciferase, which in turn reflects transcriptional activation by Wnt signalling, whereas FOPFlash contains mutated versions of the TCF responsive elements that do not respond to Wnt signalling activation (van de Wetering et al., 1997). Cells were transfected with TOPFlash and FOPFlash reporters together with the indicated plasmids as shown in Figure 4 for 24 hours thereafter measuring Luciferase activity in the cell lysates.

Supplementary Figure Legends

Fig. S1 Conductin localizes at centrosomes in various cell lines independently of the actin cytoskeleton and microtubules

(A) Immunofluorescence images of various cell lines as indicated, after transfection with either flag tagged conductin (left panels) or GFP tagged conductin (right panels), co-stained with an antibody against γ-tubulin. Nuclei were co-stained with DAPI in merged images. (B) Immunofluorescence images of U2OS cells transfected with GFP tagged conductin (green) and co-stained with γ-tubulin (red) after treatment with Latrunculin A and nocodazole. Control treatment (DMSO) is also shown. On the right low magnification images show similarly transfected and treated U2OS cells stained with antibodies against β-actin and β-tubulin to verify the activities of Latrunculin A and Nocodazole, respectively. N/A, not applicable. Depolymerization of microtubules or the actin cytoskeleton by Nocodazole and Latrunculin A, respectively, as well as preextraction of cells with detergents prior to fixation (Fig. 1B) did not affect the centrosomal localization of conductin, supporting the notion that conductin forms a stable association with centrosomal components. (C) Co-staining of endogenous conductin (red) using rabbit (upper panels) or mouse (lower panels) monoclonal antibodies and γ-tubulin (green) in SW480 cells (left panels) or in SW480 cells transfected with a siRNA against conductin (right panels).

Fig. S2 Conductin and centrosomal cohesion proteins

(A) Western blotting for conductin and myc of anti-myc immunoprecipitation from U2OS cells transfected with plasmids indicated. Top two panels show blots with lysates (Input), and anti-myc immunoprecipitation reactions (IP anti-myc) probed with a conductin antibody. Lower panels show anti-myc immunoprecipitation reactions probed with an antibody against myc. The location of myc tagged proteins is indicated with arrowheads and size markers are shown on the left. (B) Immunofluorescence images of γ-tubulin (red) staining in U2OS cells co-transfected with GFP tagged conductin (green) together with either control siRNA (siControl) (left panels) or siRNA against Rootletin (siRootletin) (right panels). Three representative examples are shown. Cells were treated with pre-extraction buffer containing 0.5% Triton X-100 prior to fixation.

Fig. S3 Small interfering RNA (siRNA) knockdown

(A) Western blotting for myc of lysates from U2OS cells co-transfected with myc tagged C-Nap1/Rootletin together with either siLuc or siC-Nap1/siRoot as indicated. (B) Western blotting for conductin and β-actin of lysates from U2OS cells transfected with siLuc or siCond. (C) Western blottings for GFP of lystates from U2OS cells co-transfected with GFP Nek2 together with either si Nek2 or siLuc.

Fig. S4 Phosphorylated and total β-catenin at centrosomes

(A) Immunofluorescence co-stainings of phospho β-catenin (red) and γ-tubulin (green) in 293T, HCT116 and SW480 cells as indicated. Merged pictures are shown on the third column. DNA was stained with DAPI. (B) Co-staining of endogenous total β-catenin (red) and γ-tubulin (green) in wildtype (+/+) and conductin knockout (-/-) MEFs. A representative example is shown. (C) Fluoresence images of U2OS cells transfected with β-catenin plasmids (green) as indicated and stained for γ-tubulin (red). Merged images are shown on the right and white arrowheads point to centrosomes. (D) Nek2 can induce phosphorylation in the β-catenin armadillo repeat domain, an event that correlates with centrosome splitting. We asked whether conductin is involved in this phosphorylation. Knockdown of conductin did not alter Nek2-induced β-catenin phosphorylation. This panel shows western blots of lysates from 293T cells transfected with indicated plasmids and siRNAs, and incubated with antibodies shown. Total β-catenin (arrow) and phosphorylated β-catenin (bracket) are indicated. CIP; Calf intestinal phosphatase treatment. (E) Quantification of centrosomal splitting in U2OS cells transfected with siLuc, siRoot, siC-Nap1 and co-transfected with either GFP centrin2 or GFP conductin as indicated. Error bars show SEM from at least 3 independent experiments (n>300).

Movie S1,S2. Centrosomal localization of GFP tagged Conductin and γ-tubulin

For 3D reconstruction of γ-tubulin stained centrosomes in U2OS cells transfected with GFP tagged conductin images were acquired on an Axioplan2 from Zeiss, fitted with a motorized stage at 100x magnification at 0.2μm z-axis intervals. Following deconvolution, z-stacks were assembled from these images and a 3D reconstruction module from metamorph was used to generate movies. γ-Tubulin is shown in red.

Supplementary References

Bahe, S., Stierhof, Y.D., Wilkinson, C.J., Leiss, F. and Nigg, E.A. (2005) Rootletin forms centriole-associated filaments and functions in centrosome cohesion. *J Cell Biol*, **171**, 27-33.

Behrens, J., Jerchow, B.A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D. and Birchmeier, W. (1998) Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. *Science*, **280**, 596-599.

Bilic, J., Huang, Y.L., Davidson, G., Zimmermann, T., Cruciat, C.M., Bienz, M. and Niehrs, C. (2007) Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science*, **316**, 1619-1622.

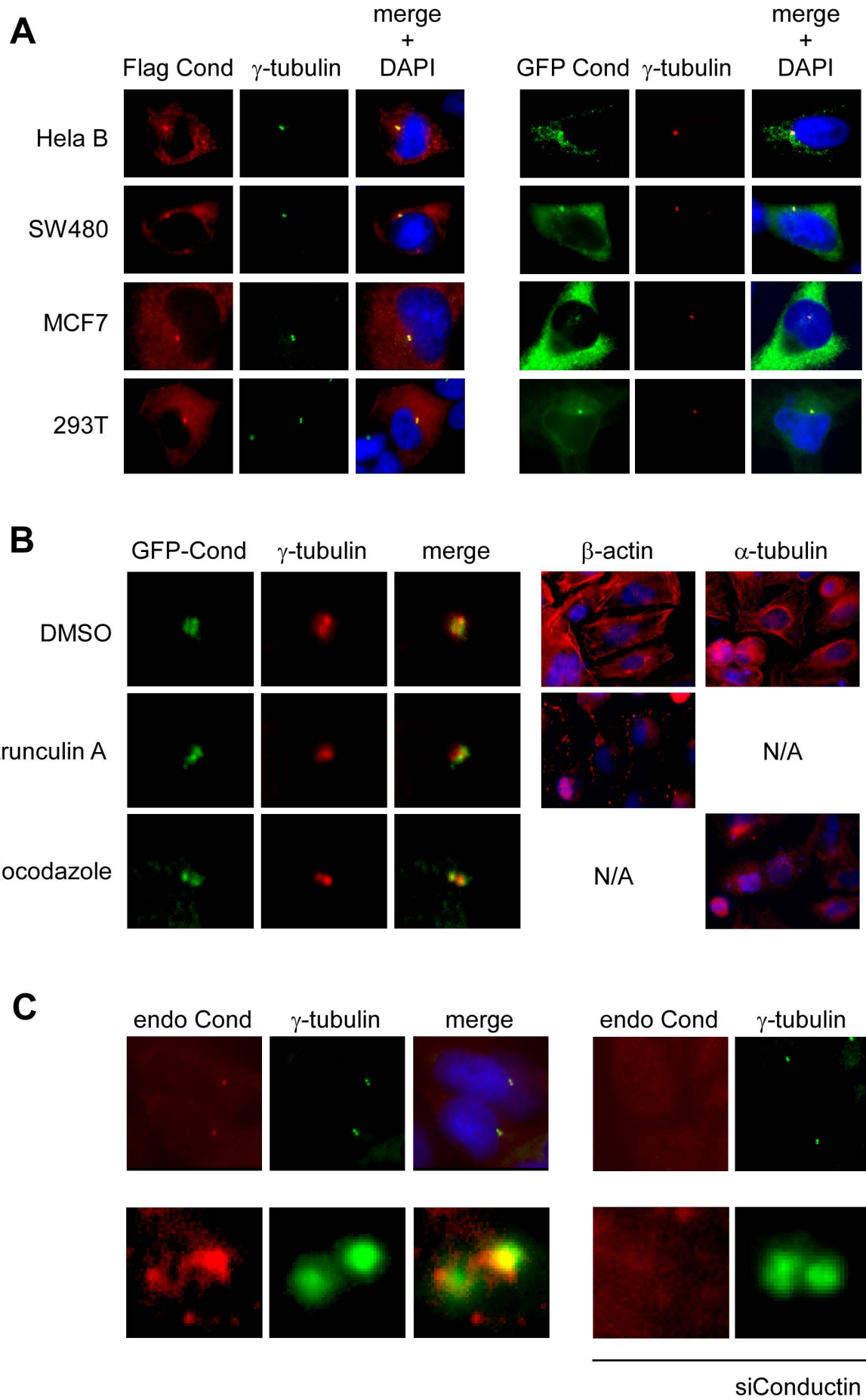
Hadjihannas, M.V. and Behrens, J. (2006) CIN By WNT: growth pathways, mitotic control and chromosomal instability in cancer. *Cell Cycle*, **5**, 2077-2081.

Hadjihannas, M.V., Bruckner, M., Jerchow, B., Birchmeier, W., Dietmaier, W. and Behrens, J. (2006) Aberrant Wnt/beta-catenin signaling can induce chromosomal instability in colon cancer. *Proc Natl Acad Sci U S A*, **103**, 10747-10752.

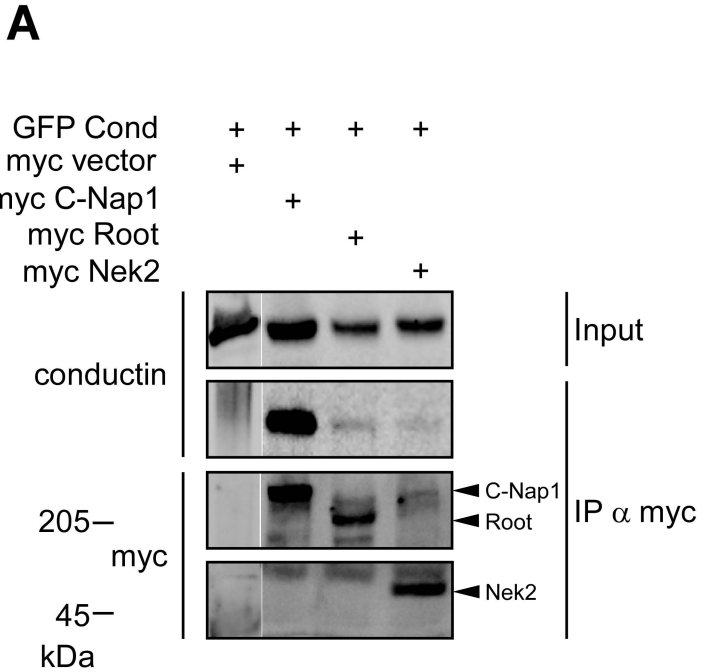
Lustig, B., Jerchow, B., Sachs, M., Weiler, S., Pietsch, T., Karsten, U., van de Wetering, M., Clevers, H., Schlag, P.M., Birchmeier, W. and Behrens, J. (2002) Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol Cell Biol*, **22**, 1184-1193.

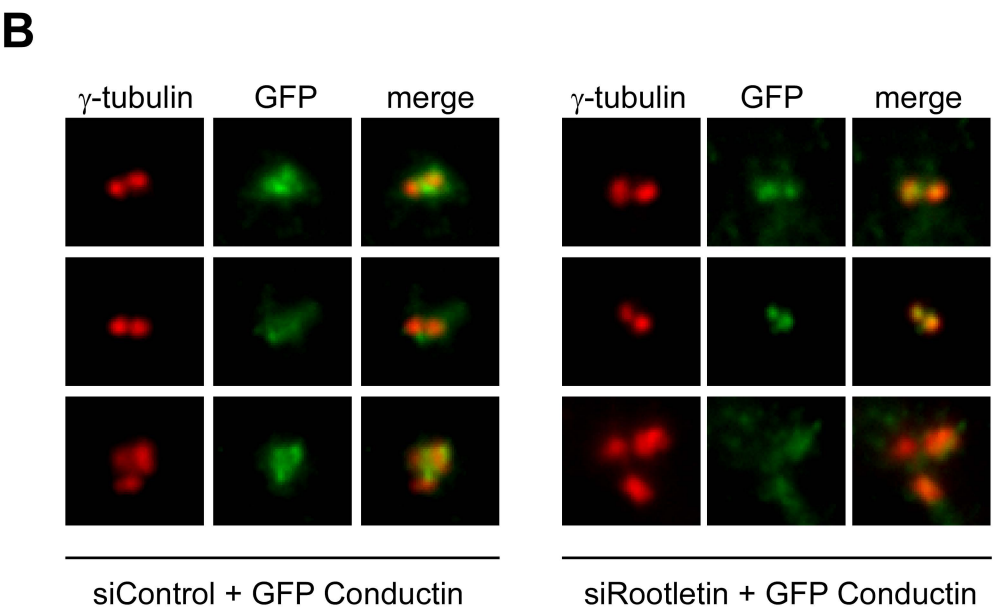
Mayor, T., Hacker, U., Stierhof, Y.D. and Nigg, E.A. (2002) The mechanism regulating the dissociation of the centrosomal protein C-Nap1 from mitotic spindle poles. *J Cell Sci*, **115**, 3275-3284.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M. and Clevers, H. (1997) Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. *Cell*, **88**, 789-799.

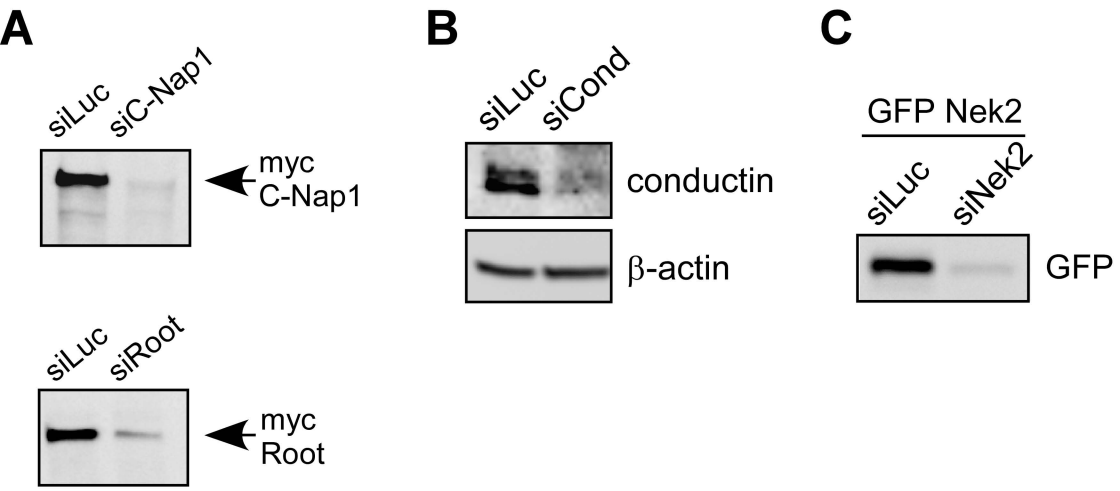


Supplemental Figure 1 - Hadjihannas et al.

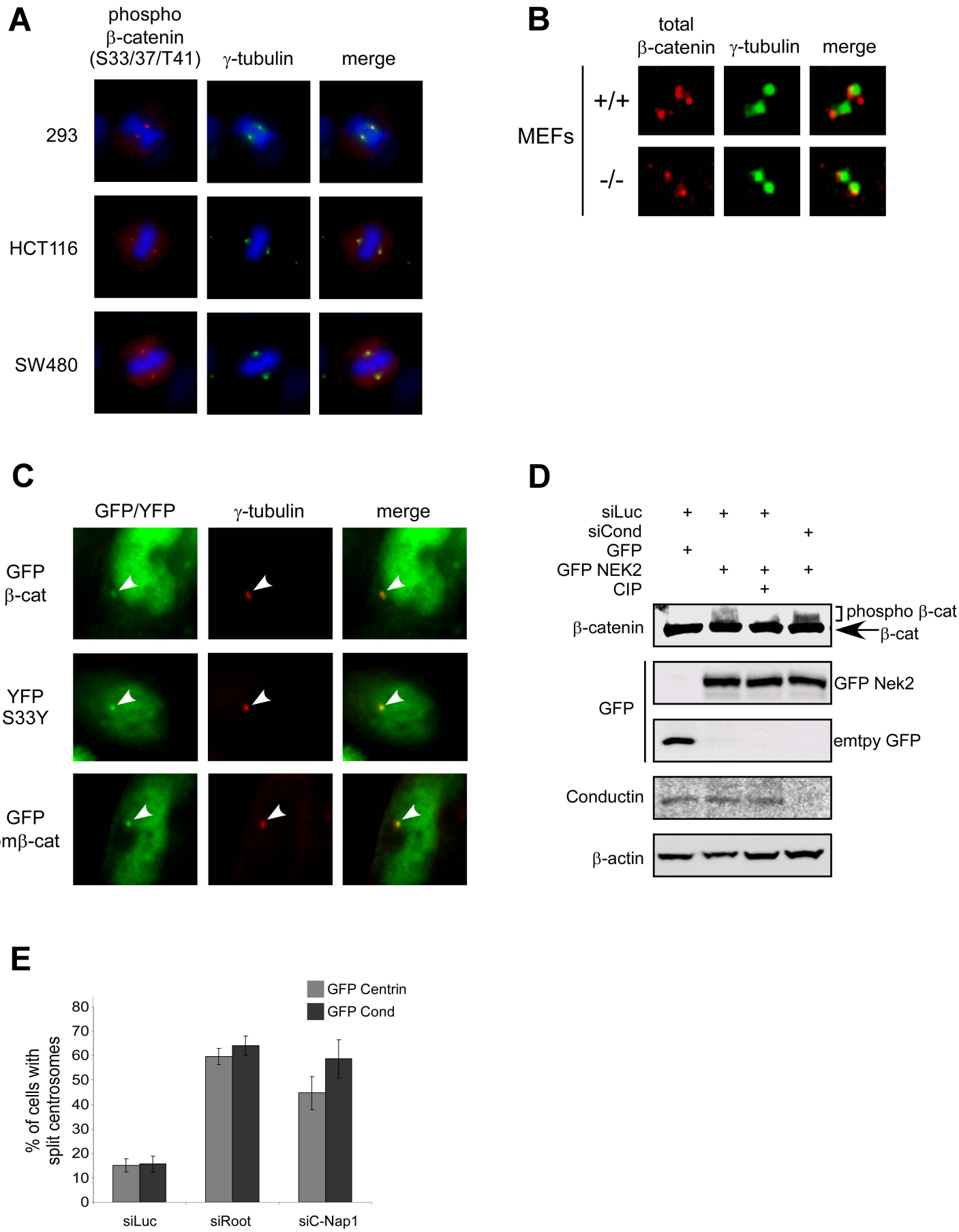




Supplemental Figure 2 - Hadjihannas et al.



Supplemental Figure 3 - Hadjihannas et al.



Supplemental Figure 4 - Hadjihannas et al.