FIGURE LEGEND

Supplementary figure 1: p120ctn overexpression in rat normal intestinal epithelial cells (IEC) induces cell proliferation defects associated with enhanced Cyclin E expression and polyploidy.

(A) Wild-type IEC cells (IEC-WT) were stably transfected with GFP-p120ctn3A plasmids as described in material and methods (IEC GFP-p120ctn). **Left panel:** expression levels of p120ctn and GFP were analyzed by Western-blotting whole cell lysates. Actin blotting was used as a loading control. **Right panel:** IEC GFP-p120ctn cells cultured for 48h on coverslips were fixed in 3% paraformaldehyde, mounted in mowiol-DAPI and GFP-p120ctn fluorescence was assessed using epifluorescent microscopy, bar is 10 μm.

(B) Wild-type IEC and IEC GFP-p120ctn cells were seeded at 2.10^4 cells per well, grown for the indicated times and living cells were then counted using trypan blue solution. Each point is the mean +/- s.e. of three independent experiments.

(C) Upper panel: centrosomes fractions were enriched from mitotic IEC-WT and IEC GFP-p120ctn cells using a discontinuous sucrose gradient (see material and methods). Centrosomes and cell membranes enriched fractions were analyzed by western-blotting using antibodies against GFP, cyclin E and cdk2. The cyclin E levels are more important in both fractions of the IEC GFP-p120ctn than in wild types IEC. **Lower panel:** Mitotic IEC GFP-p120ctn cells were observed under epifluorescent microscopy, bar is 10 μm. The GFP-p120ctn accumulates in centrosome-like organites at each side of the division axis.

(D) Asynchronized IEC-WT and IEC GFP-p120ctn cells (asy) or cells synchronized at G1/S transition with Aphidicolin blockade and released in fresh medium for indicated times were lyzed. Total lysates were analyzed for cyclin E, cdk2 and actin expression by western-blotting. The cyclin E expression levels are more important in IEC GFP-p120ctn cells than in IEC-WT all along the cell cycle.

(E) Left panel: IEC-WT and IEC GFP-p120ctn cells were cultured for 48 hours and observed under phase contrast microscopy, bar is 100 μm. Whereas IEC-WT have only one nucleus, many IEC GFP-p120ctn cells are much larger and show two or more nucleus (red arrows).
Right panel: IEC GFP-p120ctn cells cultured for 48h on coverslips were fixed in 3% paraformaldehyde, mounted in mowiol-DAPI and GFP-p120ctn fluorescence was assessed using epifluorescent microscopy, bar is 10 μm. Positive GFP-p120ctn cells are largely spread and show two nucleus. The GFP-p120ctn accumulates in perinuclear structures.