

Supplementary figure 1

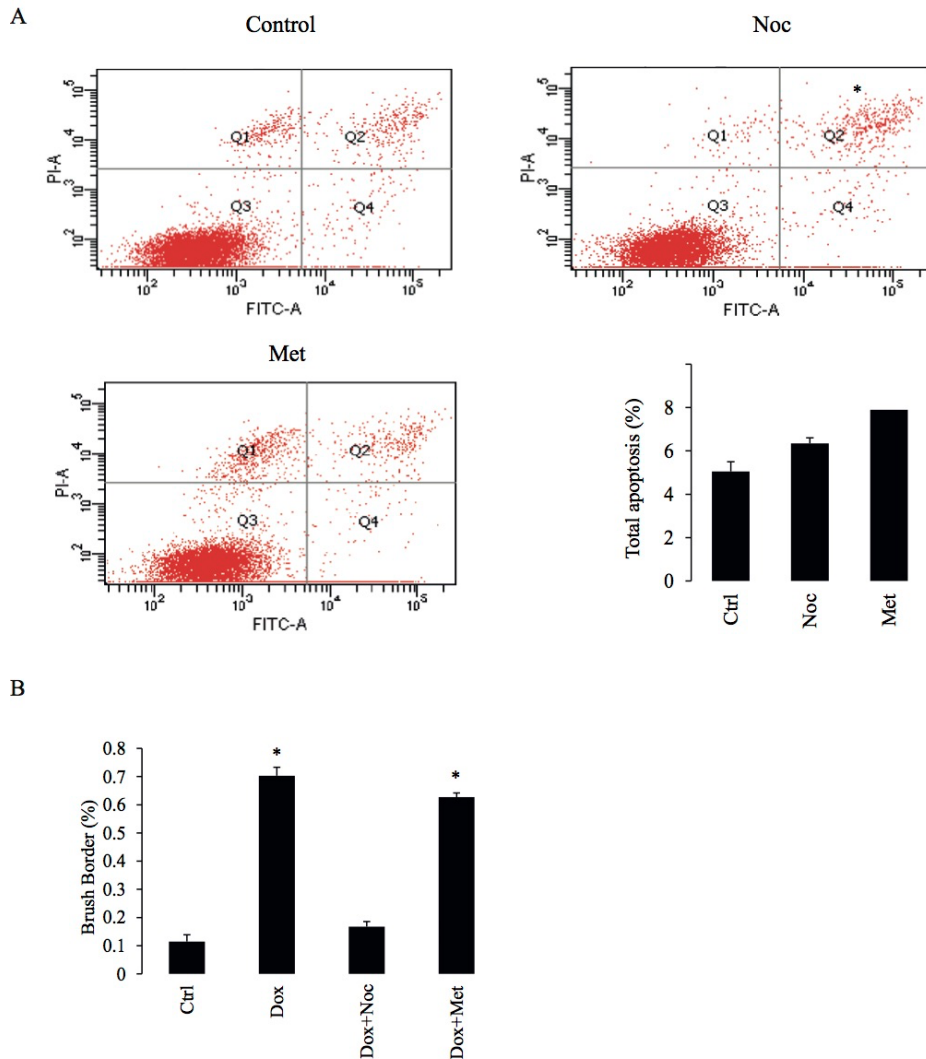


Figure S1. Apoptotic effects of nocodazol do not account for its inhibitory effects on brush border formation. Ls174T-W4 cells were incubated with nocodazol (17 μ M) or metformin (5 μ M) 30 min previous and during activation with doxycyclin. After 6 h of doxycyclin activation cells were stained with Annexin V-FITC and propidium iodide (PI) and analyzed by flow cytometry (A) or fixed and stained for brush border analysis by immunofluorescence microscopy (B). A, Outputs of the cytometric analysis showing the populations of cells undergoing early apoptosis (bottom right, Q4), late apoptosis (upper right, Q2) and primary necrosis (upper left, Q1) of an experiment representative of 2 independent experiments. Bars represent the total apoptosis in each condition. (B) Bars represent the percentage of cells which developed brush border in each condition.

Supplementary figure 2

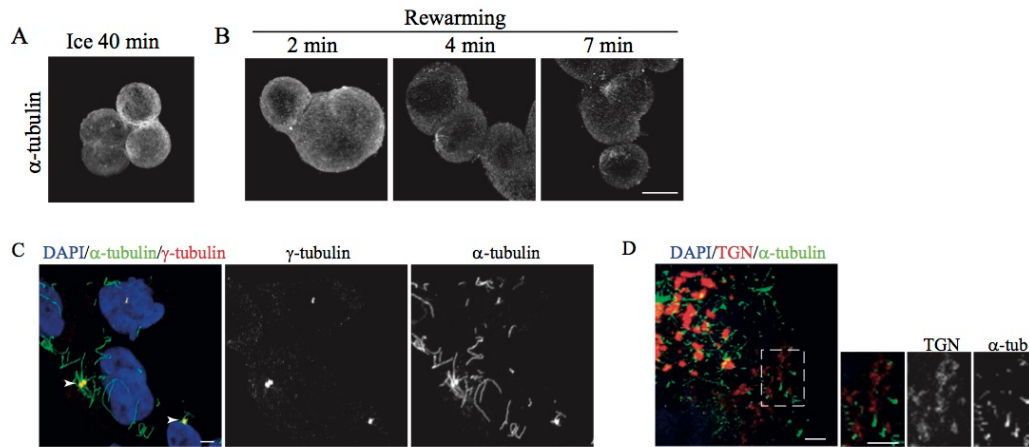


Figure S2. Centrosomes and Golgi apparatus behave as MTOCs in Ls174T-W4 cells. Ls174T-W4 cells grown on coverslips for 24 h were incubated on ice 40 min. (A) Cells were fixed with glutaraldehyde immediately after ice incubation and stained with anti α -tubulin antibody. (B) After ice incubation, cells were incubated at room temperature for the indicated periods and fixed and stained as in (A). Arrowheads indicate newly nucleated microtubules. After ice incubation, Ls174T-W4 cells (C) or Ls174T-W4 cells expressing the Golgi marker TGN-RFP (D) were incubated at room temperature for 7 min. Cells were fixed and stained for α -tubulin (green) and γ -tubulin (C, red). (C) Arrowheads point centrosome-nucleated microtubules. (D) Microtubules nucleated at the Golgi apparatus were identified as those associated with this organelle (inbox). Scale bars, 10 μ m (A,B) 2.5 μ m (C,D).