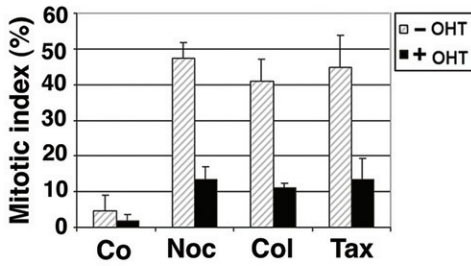
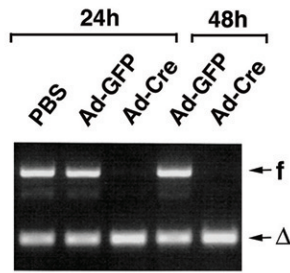
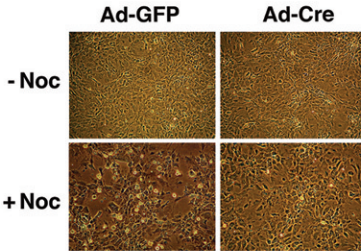
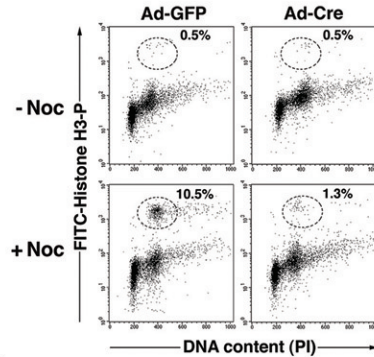
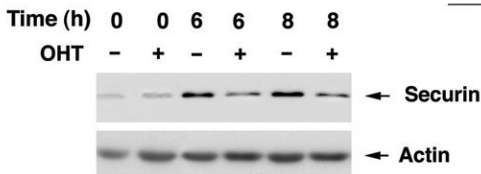
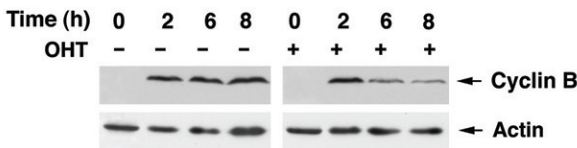


A**B****C****D****E****F**

Supplementary Figure S1 Loss of Trrap in both primary and immortalized cells compromises mitotic checkpoint. (A) Trrap deficiency abolishes mitotic checkpoint in immortalized cells in the presence of different spindle poisons. CER9 cells were grown in the presence or absence of OHT for 48 h and nocodazole (Noc), colcemid (Col) or taxol (Tax) were added and cultured for further 10 h. After DAPI staining, the mitotic index was determined by scoring at least 100 cells. Data are means of triplicate samples \pm standard deviations and are representative of two independent experiments. (B) Generation and analysis of primary Trrap "conditional" knockout MEFs. Primary Trrapf/D MEFs were kept uninfected (PBS) or infected with adenovirus expressing GFP (Ad-GFP) or Cre (Ad-Cre) for 48 h, and Trrap deletion was analyzed by PCR as described previously (Herceg et al., 2001). (C) Primary Trrap-deficient MEFs exhibit mitotic checkpoint defect. Primary Trrap "conditional" knockout MEFs were infected with Ad-GFP and Ad-Cre for 48 h, incubated in the presence of nocodazole for further 10 h, and photographs were taken at phase-contrast microscope. (D) The cells were treated as in (C) and analyzed by flow cytometry for DNA content (PI) and the phospho-histone H3 fluorescence (FITC-Histone H3-P) associated specifically with mitotic cells. Mitotic cells expressed as a percentage of total cells were indicated. (D,E) Trrap-deficient cells degrade securin and cyclin B in the presence of spindle poison. CER9 cells, pre-incubated in the presence or absence of OHT for 48 h, were incubated in the presence of nocodazole for indicated time and protein levels of securin (D) and cyclin B (E) were analyzed by western blotting. Equal loading was verified by anti-actin antibody.