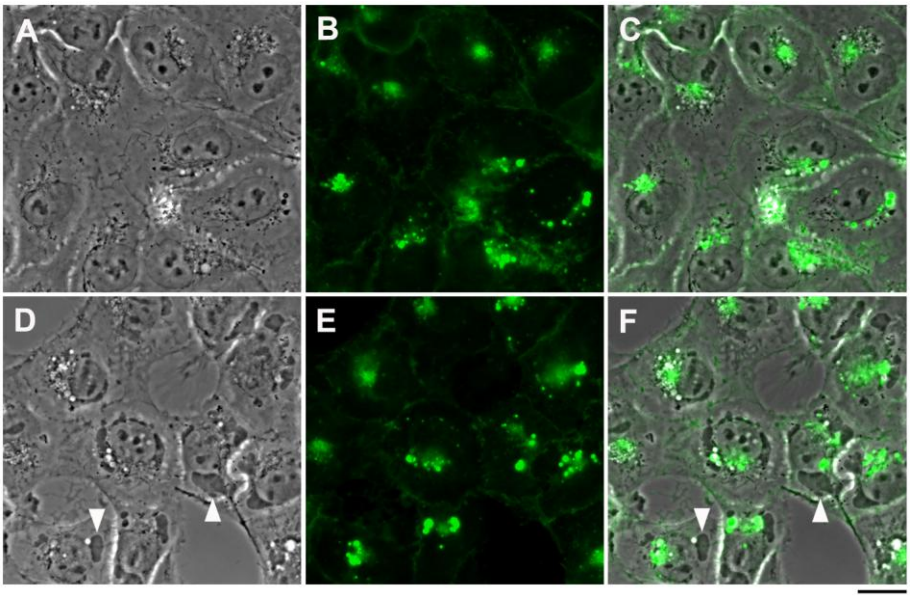

Fig. S1. The effect of bufalin on NT2 cells, analysis with Golgi-specific reagent. NT2 cells were grown in DMEM-F12 medium on glass coverslips for 24 hours. The medium was removed; the cells were washed with PBS and incubated in DMEM-F12 supplemented with 1% FCS, containing 1.25 μM Bodipy FL C5- lactosylceramide (molecular probe). After 30 minutes the cells were washed with PBS and incubated for 4.5 hours with DMEM-F12 drug-free medium (A-C) or medium containing 20 nM bufalin (D-F). After 4.5 hours, the cells were washed with PBS and observed under a fluorescence microscope. The phase contrast images (A and D), the fluorescent images (B and E) and merge images (C and F) were acquired as described in Experimental Procedures. Glycogen clusters are marked by white arrows. Scale bar 20 μm.

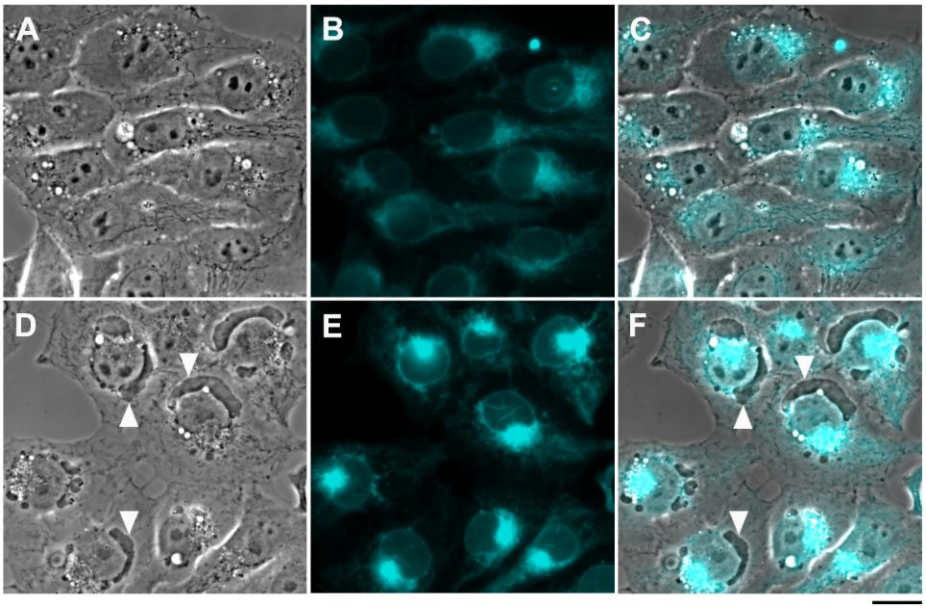

Fig. S2. The effect of bufalin on NT2 cells, analysis with ER-specific reagent. NT2 cells were grown in DMEM-F12 medium on glass coverslips for 24 hours. The DMEM-F12 was then replaced with drug-free medium (A–C) or medium containing 20 nM bufalin (D–F). After 4.5 hours, the medium was removed; the cells were washed with PBS and incubated in PBS containing 1μM ER-tracker (molecular probe) with or without bufalin. After 30 minutes the cells were washed with PBS and observed under a fluorescence microscope. The phase contrast images (A and D), the fluorescent images (B and E) and merged images (C and F) were acquired as described in Experimental Procedures. Glycogen clusters are marked by white arrows. Scale bar 20 μm.

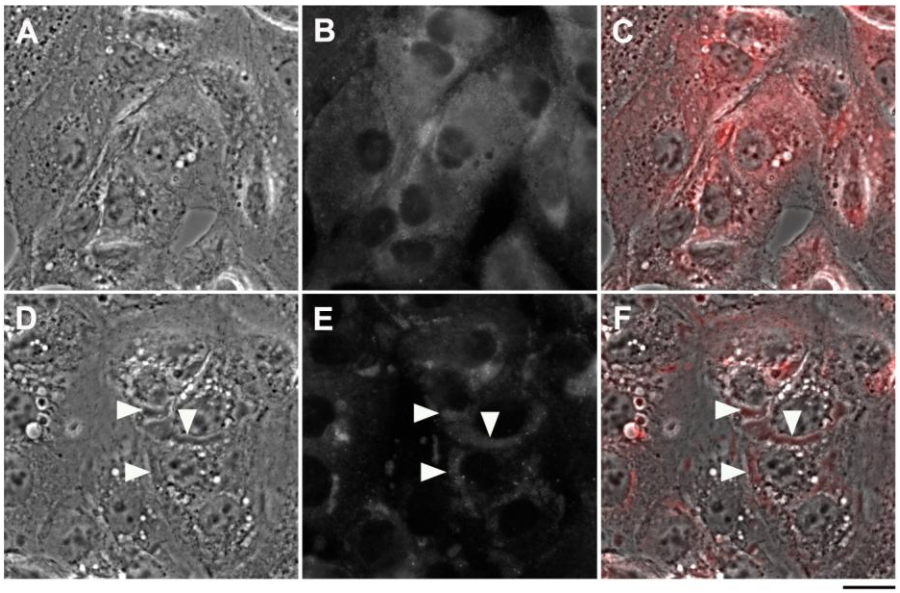

Fig. S3. Effect of bufalin on glycogen and glycogen synthase localization in ACHN cells. The experiments were conducted as described in the legend to Fig. 1. PAS histological staining of control ACHN cells (A) and bufalin-treated cells (B) are shown. Immunostaining of glycogen synthase was performed on ACHN cells grown in DMEM-F12 on glass coverslips for 24 hours (C-H). The DMEM-F12 was then replaced with medium with (F, G, H) or without (C, D, E) 20 nM bufalin for 4.5 hours. The cells were fixed with 1.5% glutaraldehyde, stained with anti-glycogen synthase rabbit monoclonal antibodies and images were acquired. Phase microscopy- C, F, fluorescence microscopy- D, G, the merged phase contrast and fluorescence images -E, H. White arrowheads point to the glycogen clusters. Scale bar, 20 μm.

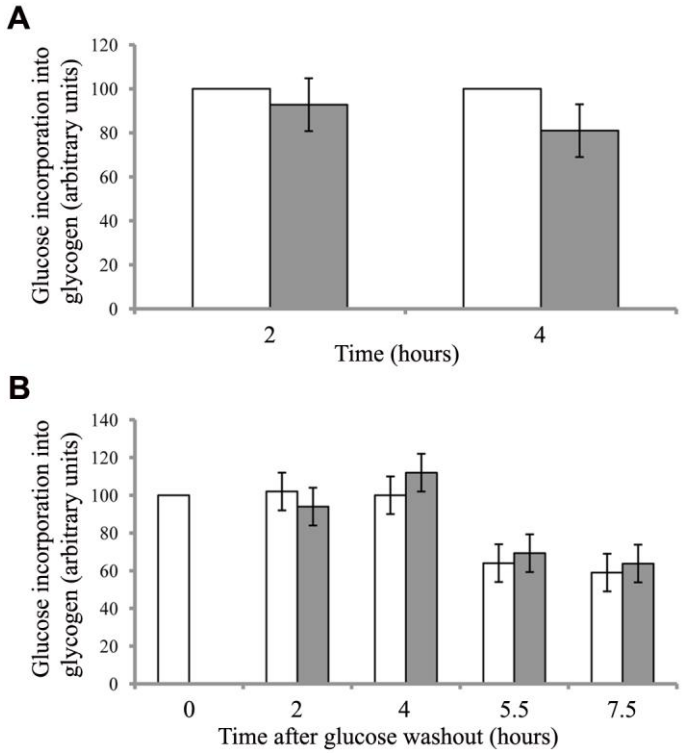

Fig. S4. Effect of bufalin on glycogen metabolism in NT2 cells. NT2 cells were grown in DMEM-F12 medium in 35 mm tissue culture plates to a density of $0.5x10^6$ cells/plate. The medium was then replaced with serum-free medium. After 5 hours, the medium was replaced with medium containing 0.5 μCi/ml glucose D-[U-^{14}C] (1.43mCi/mg GE Healthcare) with (dark bars) or without (white bars) 20 nM bufalin. After the indicated periods, glycogen was extracted as previously described (Kotova et al., 2006) and ^{14}C-glucose incorporation was determined (A). The results are expressed as percent incorporation at 2 hours in the absence of bufalin. In the washout experiments (B) the cells were incubated with ^{14}C-glucose for 24 hours, washed three times, incubated with medium without radioactive glucose, and glycogen extraction was performed at the indicated times. The results are expressed as percent incorporation at the end of the washout (time 0). Each bar represents the average ± S.D of 3 experiments.