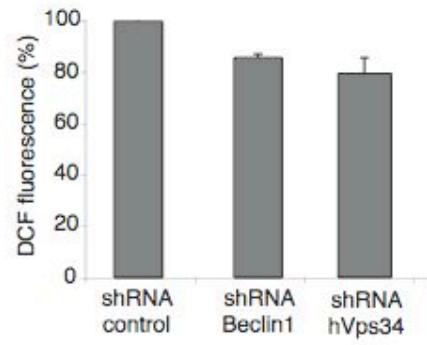
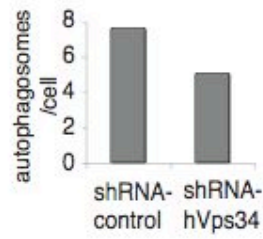
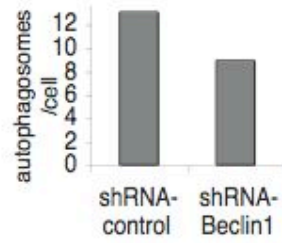
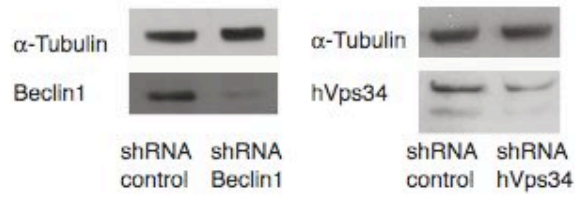


A



B



Supplementary Figure 2. Knockdown of Beclin 1 and hVps34 by shRNA reduces starvation-induced ROS production. (A) HeLa cells were transfected with pSuper-Beclin1 or pSuper-hcRed as control, and HEK 293 cells were transfected with pLKO.1-puro-hVps34 or pLKO.1-puro with a corresponding scrambled sequence as control. At 48-72 h post-transfection, the cells were starved for 2 h in EBSS, treated with DCFDA and analyzed by a fluorimeter. (B) HeLa cells were transfected with each of the 4 constructs mentioned above (experiments with each pair of constructs were conducted separately) before being either lysed in RIPA buffer and analyzed with the indicated antibodies, or starved for 2 h in EBSS, fixed in methanol, permeabilized in acetone and stained with anti-LC3 polyclonal antibodies. Images of the fixed cells were taken using a fluorescent microscope. 150 cells from 2-3 separate experiments were used to quantify the average number of autophagosomes per cell for each transfection. Since we could not identify the transfected cells, the obtained results were relatively variable; however, the differences were significant ($p < 0.001$).