

**Figure S1.** Representative transmission electron microscopy images of Hep3B cells. The cells were infected with AdCMV-RB (100 pfu/cell) for 72 h. AdCMV-GFP was used as a control for viral infection and nonspecific protein expression. Note the membrane-bordered vacuoles containing cytoplasmic components (arrows) in the cytoplasm of RB-expressing cells, but not in the mock-treated or GFP-expressing cells. Scale: 500 nm.



**Figure S2.** RB-induced autophagy depends on Beclin 1. The cells were first transfected with the indicated siRNA (10 nM). After 24 h, the cells were infected with AdCMV-RB (100 pfu/cell) for 72 h. Upper panel: Representative images of the cells with the indicated treatment. Scale: 20  $\mu$ m. Lower panel: Quantification of the cells with EGFP-LC3 dots (mean ± SD, \* *P* = 0.004).



**Figure S3.** RB regulated the expression of autophagy-related genes. Saos-2 cells were infected with AdCMV-RB (100 pfu/cell). Seventy-two hours later, total RNA was extracted and examined with the Autophagy PCR Array. AdCMV-GFP was used as a control for viral infection and nonspecific protein overexpression. The data were normalized to mock-treated cells (assigned as 1). Shown are results from two independent experiments.



**Figure S4.** RB increases autophagosome initiation. Shown are representative transmission electron microscopy images of the cells. Saos-2 cells were treated with AdCMV-RB (100 pfu/cell) for 72 h in the presence of Bafilomycin A1 (10 nM). Lower panel is a close-up of parts of the cells in the upper panel. Note that Bafilomycin A1 caused dramatic vacuole accumulation in RB-expressing cells. Scales: Upper panel, 2  $\mu$ m; lower panel, 500 nm.