Fig. S1. Three large fragments encompassing the full-length Rubicon (Myc tagged) were coexpressed with Flag-tagged hVps34 in HEK 293T cells. The cell extracts fro these cells were immunoprecipitated with anti-Flag M2 beads, followed by Western blotting with Flag (hVps34) and Myc (Rubicon fragments) antibodies.

Fig. S2. The expression of N terminus of Rubicon suppresses LC3 puncta formation. **(A).** Vector alone, the N-terminal region (1-300aa), and the C-terminal region (181-972) of Rubicon were co-expressed with GFP-LC3 in U_2OS cells. GFP-LC3 puncta were observed under a fluorescence microscope. **(B).** Quantitative analysis (summarized from 100 cells) of GFP-LC3 puncta in cells described in (A).

Fig. S3. Rubicon depletion promotes the fusion between autophagosome and endolysosomes. Myc-LC3 construct was co-expressed with either GFP-Rab5-WT or GFP-Rab7-WT into Rubicon knockdown (KD) or control cells (WT). 24 hours post-transfection, cells were fixed and stained for Myc-LC3 using a monoclonal antibody against Myc (9E10) and a Rhodamine-red conjugated secondary antibody. Endogenous LAMP-1 was stained by a rabbit antibody and followed by FITC-conjugated secondary antibody. Scale bar (10μm).

Fig. S4. Rubicon protein level was detected in Rubicon wild type, Doxycycline (DOX) inducible RNAi knockdown (KD), or Rubicon (RNAi resistant) complemented (OE) cells by anti-Rubicon antibody.

Figure S1. Sun et al.

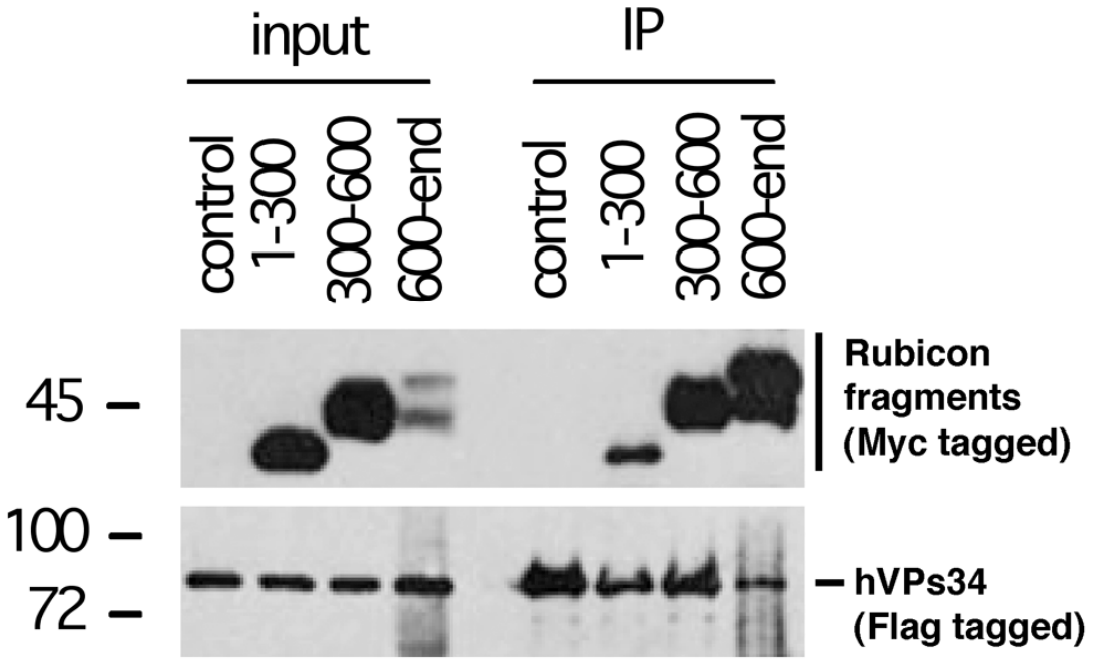

Figure S2. Sun et al.

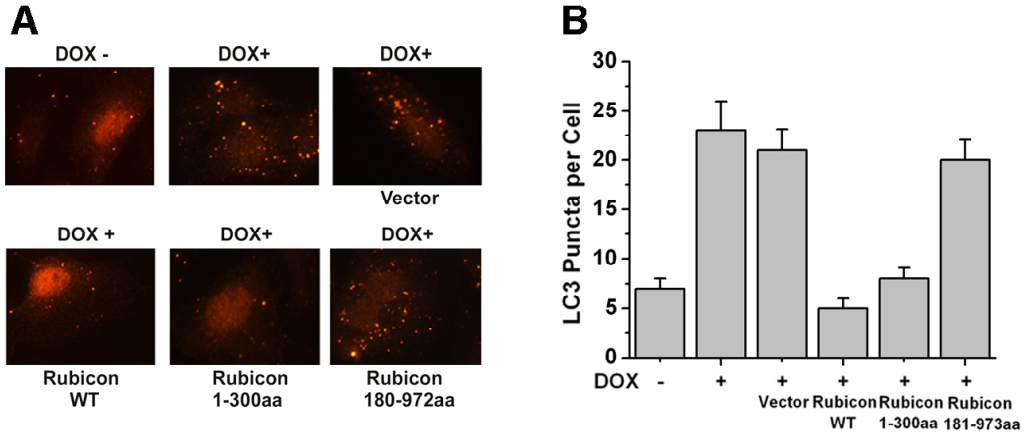

Figure S3. Sun et al.

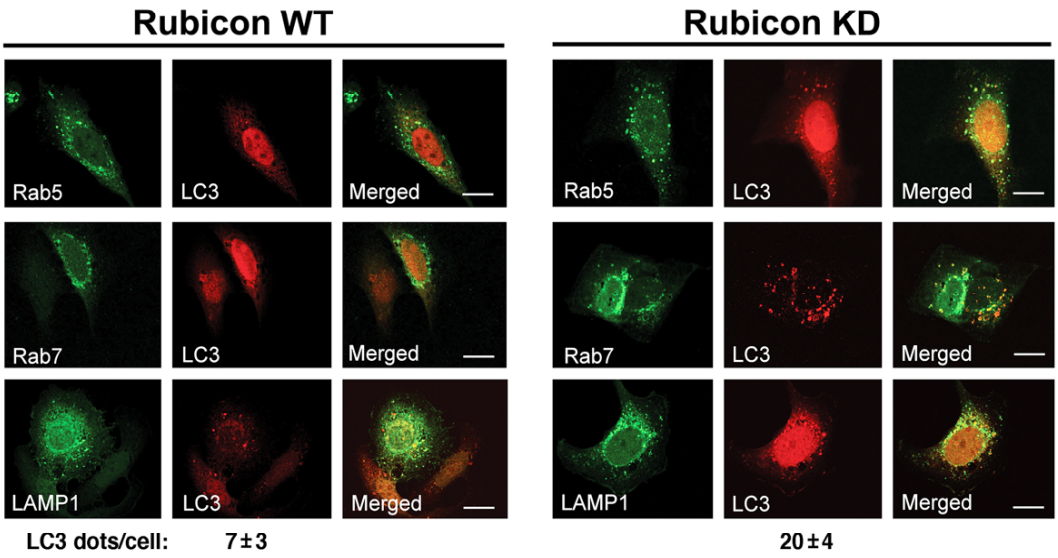

Figure S4. Sun et al.