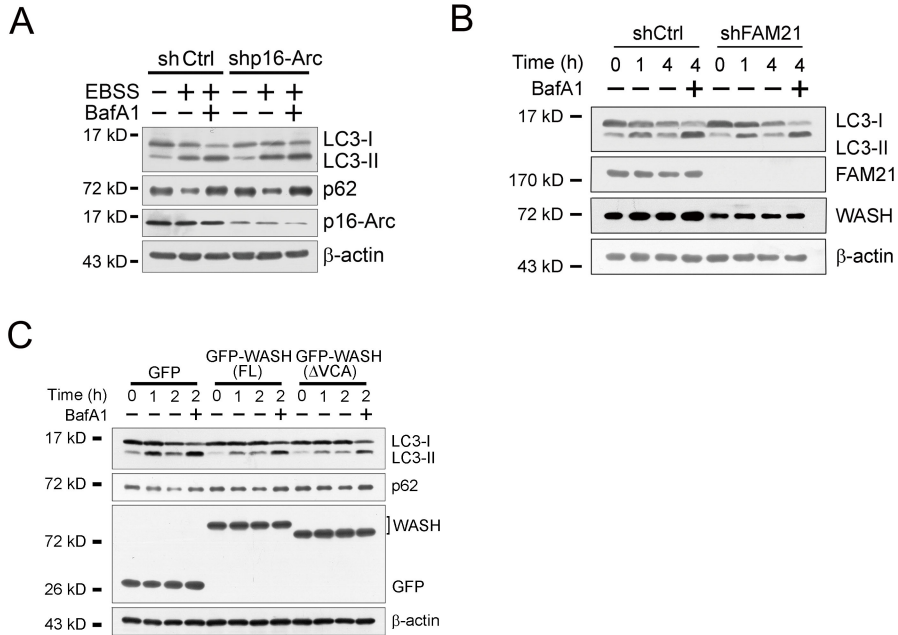


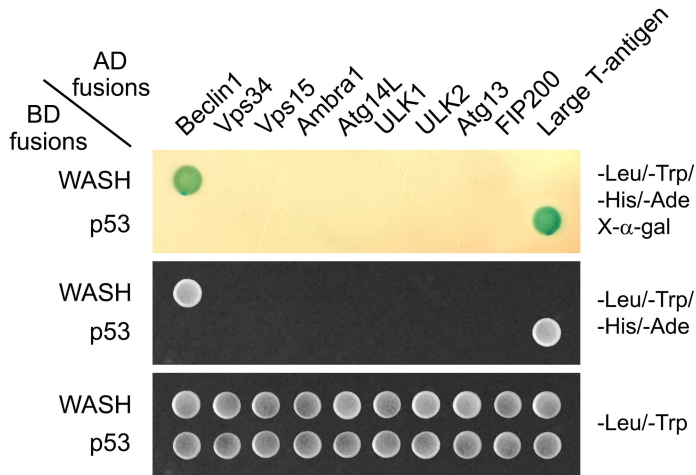
**Figure S1. WASH expression profiles were detected by immunoblotting.** Expression levels of WASH in mouse tissues (A) and developmental stages of embryos (B). Experiments were repeated for three independent times with similar results.



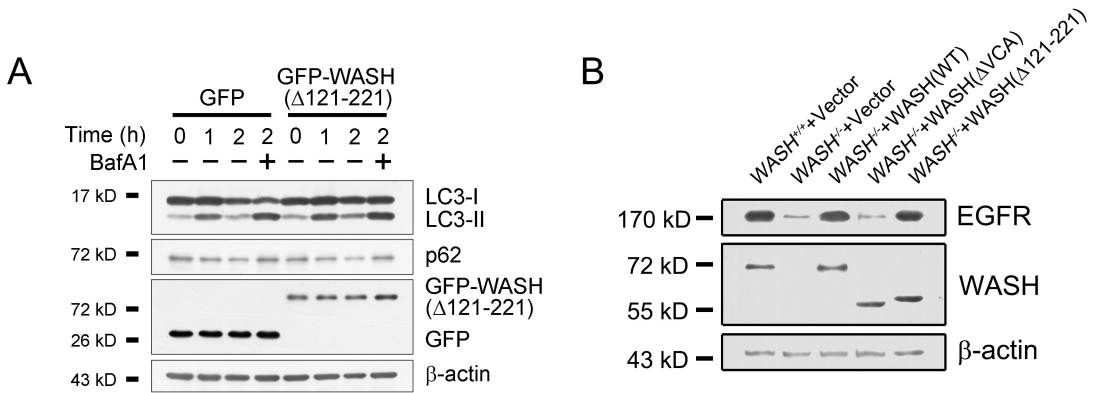
**Figure S2. Starvation-induced autophagy is independent of its endosomal sorting function.** (A) p16-Arc knockdown does not affect autophagy induction. p16-Arc knockdown or shCtrl HeLa cells were treated with EBSS for the indicated times with or without BafA1 for 1 h, followed by immunoblotting with the indicated antibodies. (B) FAM21 knockdown does not affect autophagy induction. FAM21 knockdown or shCtrl HeLa cells were treated with EBSS for the indicated times and detected as above. WASH expression level was diminished after FAM21 knockdown as previously reported. (C) The VCA domain-truncated WASH ( $\Delta$ VCA) mutant could still rescue the accelerated autophagy induction in WASH KO MEFs. *WASH*<sup>-/-</sup> MEFs were rescued with GFP control, GFP-WASH (full length, FL), and WASH ( $\Delta$ VCA) and treated with EBSS for the indicated times with or without BafA1, followed by immunoblotting with the indicated antibodies. Data were repeated for three times with similar results.



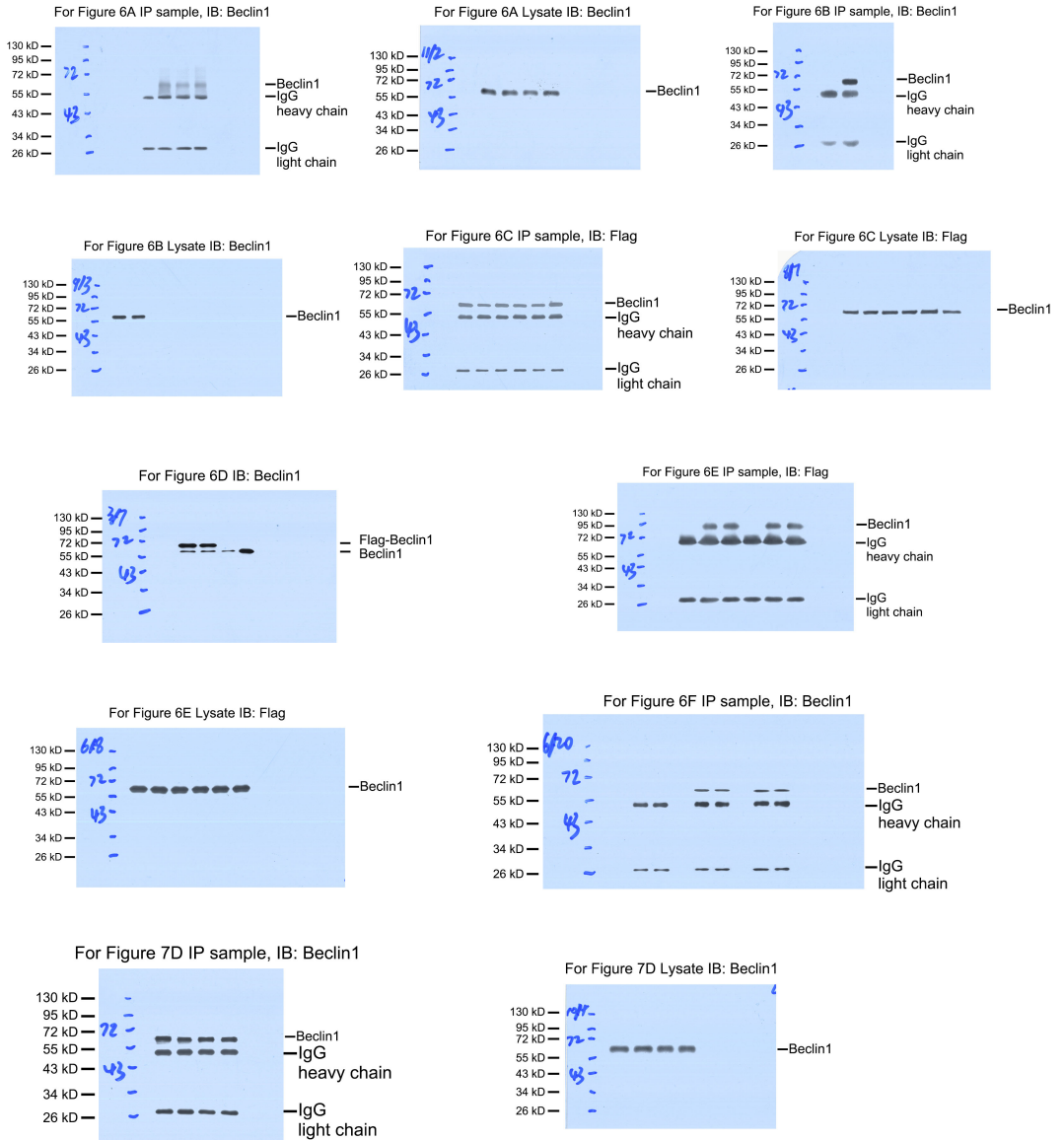
**Figure S3. WASH does not affect the stability of Beclin 1.** *WASH<sup>+/+</sup>* and *WASH<sup>-/-</sup>* MEFs were treated with EBSS for the indicated times, followed by immunoblotting with the indicated antibodies. Data are representative of three independent experiments.



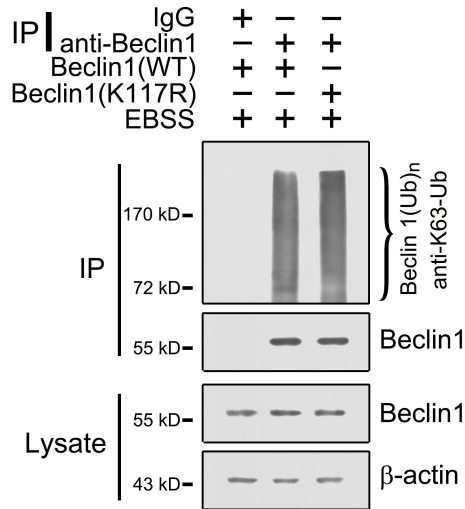
**Figure S4. WASH does not interact with other components of the Vps34 or the Ulk1/Ulk2 complex besides Beclin 1.** Yeast strain AH109 was co-transfected with Gal4 DNA-binding domain (BD) fused WASH and Gal4 activating domain (AD) fused Beclin 1 and other indicated autophagic proteins. p53 and large T antigen was introduced as a positive control. Experiments were repeated for three independent times with similar results.



**Figure S5. WASH exerts its autophagy regulation independently of its endosomal sorting role.** (A) The aa121-221 deleted WASH ( $\Delta$ 121-221) fails to rescue the enhanced autophagy process in WASH KO cells. *WASH*<sup>-/-</sup> MEFs rescued with GFP control or WASH ( $\Delta$ 121-221) mutant were starved with EBSS for the indicated times with or without BafA1. Cells were lysed for immunoblotting with the indicated antibodies. (B) The aa121-221 of WASH ( $\Delta$ 121-221) is able to rescue the enhanced EGFR degradation in WASH KO MEFs. *WASH*<sup>+/+</sup> and *WASH*<sup>-/-</sup> MEFs were transfected with the indicated plasmids, followed by examination of the protein level of EGFR. Experiments were repeated for three independent times with similar results.

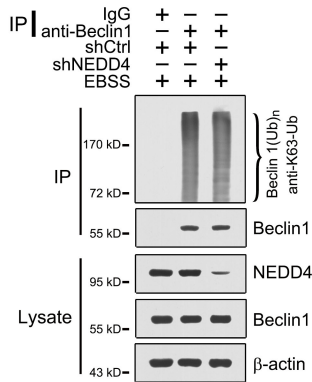


**Figure S6.** Uncropped anti-Beclin 1 western blots as shown in Fig. 6 and Fig. 7D.

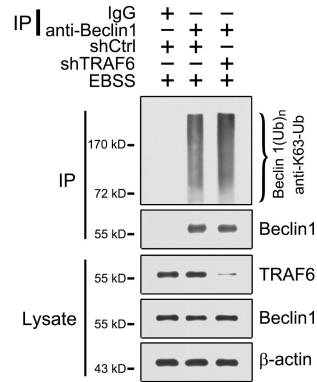


**Figure S7. K117R-Beclin 1 mutant still undergoes ubiquitination upon autophagy induction in HeLa cells.** Beclin 1 silenced HeLa cells stably expressing WT- or K117R-Beclin 1 were stimulated with EBSS for 1 h and immunoprecipitated with anti-Beclin 1 antibody. Immunoprecipitates were dissociated with 1% SDS and reimmunoprecipitated with anti-Beclin 1 antibody, followed by immunoblotting with the indicated antibodies. Data represent three separate experiments.

A



B



**Figure S8. NEDD4 or TRAF6 knockdown does not affect Beclin 1 ubiquitination in starvation-induced autophagy.** (A) HeLa cells with NEDD4 knockdown were stimulated with EBSS for 1 h and immunoprecipitated with anti-Beclin 1 antibody. Immunoprecipitates were dissociated with 1% SDS and reimmunoprecipitated with anti-Beclin 1 antibody, followed by immunoblotting with the indicated antibodies. (B) HeLa cells with TRAF6 knockdown were stimulated with EBSS for 1 h and detected as above. Experiments were repeated for three independent times with similar results.