**Supplemental material:**

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**Figure S1.** RELA regulates the transcription of *BECN1*. (**A**) H1299 cells were transfected with pcDNA3.0-*RELA* plasmid and the mRNA levels of *BECN1* and *RELA* were detected by Q-PCR. Data represent the average of 3 independent experiments (mean±SD). (**B**) The pcDNA3.0-*RELA* plasmid was transfected into H1299 cells. The expression of the indicated proteins was determined by western blot. (**C**) H1299 cells were transfected with control siRNA (CTL) or *RELA* siRNAs, and the mRNA levels of *BECN1* and *RELA* were detected by Q-PCR. Data represent the average of 3 independent experiments (mean±SD). (**D**) The expression of the indicated proteins was detected in H1299 cells with or without *RELA* knockdown.

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**Figure S2.** TRIM59 affects the ubiquitination level of BECN1. (**A**) H1299 cells were co-transfected with a plasmid encoding His-*UB* and control siRNA or *TRIM59* siRNAs. After 42 h, the cells were treated with MG132 for 6 h and the lysates were immunoprecipitated with BECN1 antibody. Western blot was performed using the indicated antibodies. (**B**) H1299 cells were co-transfected with a plasmid encoding His-*UB* and HA-*TRIM59* or empty vector. After 42 h, the cells were treated with MG132 for 6 h and the proteins were immunoprecipitated with BECN1 antibody. The protein expression was detected with the indicated antibodies.

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**Figure S3.** TRIM59 affects the stability of TRAF6. (**A**) H1299 cells were treated with 25μg/ml CHX alone or 25μg/ml CHX plus 20μM MG132 or 25μg/ml CHX plus 20μM chloroquine (CQ) for 6 h. The TRAF6 expression was detected by western blot (top panel). TRAF6 expression relative to ACTB was quantified. Data represent the average of 3 independent experiments (mean±SD) (bottom panel). (**B**) H1299 cells were transfected with or without a plasmid encoding HA-*TRAF6*. After 48 h, the cell lysate was immunoprecipitated with HA antibody. The expression of the indicated proteins was detected by western blot.

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**Figure S4.** TRIM59 induces the K48-linked ubiquitination of TRAF6. (**A**) H1299 cells were co-transfected with a plasmid encoding His-*UB-K48R*, HA-*TRAF6* and either tGFP-*TRIM59* or empty vector. After 42 h, the cells were treated with or without MG132 for 6 h. Proteins were immunoprecipitated with HA antibody. The ubiquitination was detected using an antibody specific for ubiquitin. TRAF6 and TRIM59 were detected using anti-HA and anti-tGFP antibodies. (**B**) H1299 cells were co-transfected with a plasmid encoding His-*UB-K63R*, HA-*TRAF6* and either tGFP-*TRIM59* or empty vector. After 42 h, the cells were treated with or without MG132 for 6 h. Proteins were immunoprecipitated with HA antibody. The ubiquitination was detected using an antibody specifically targeting ubiquitin. TRAF6 and TRIM59 were detected using anti-HA and anti-tGFP antibodies.

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**Figure S5.** TRIM59 affects the expression of BECN1 in a TRAF6-independent manner. (**A**) Plasmids encoding HA-*TRIM59* and HA-*TRAF6* were transfected separately or co-transfected into H1299 cells. After 48 h, the mRNA levels of the indicated genes were detected by Q-PCR. Data represent the average of 3 independent experiments (mean±SD). (**B**) Plasmids encoding HA-*TRIM59* and HA-*TRAF6* were transfected separately or co-transfected into H1299 cells. After 48 h, the cells were lysed and the expression of indicated proteins was detected by western blot.

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**Figure S6.** The RING domain of TRIM59 is essential for regulating TRAF6-induced autophagy. (**A**) H1299 cells stably expressing GFP-LC3B were co-transfected with a plasmid encoding HA-*TRAF6* and either HA-*ΔR* or empty vector. After 48 h, the cells were analyzed by fluorescence microscopy (Olympus IX83). Scale bar: 10μm (top panel). The cell numbers with GFP-LC3B puncta were counted under 200×magnification. \*\*\**P*≤0.001; ns, *P*> 0.05 (bottom figure). (**B**) Plasmids encoding His-*UB* and HA-*BECN1* with or without GFP-*ΔR* were co-transfected into H1299 cells. After 42 h, the cells were treated with MG132 for 6 h. The proteins were immunoprecipitated with HA antibody and blotted with the indicated antibodies. (**C**) H1299 cells stably expressing GFP-LC3B were transfected with a plasmid encoding HA-*ΔR* or empty vector. After 48 h, cells were lysed and western blots were performed using the indicated antibodies.

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**Figure S7.** The binding of TRAF6 to BECN1 is essential for autophagy induction. (**A**) Plasmids encoding HA-*BECN1-WT*, HA-*BECN1-K117R* and HA-*BECN1-E299A* were separately transfected into H1299 cells stably expressing GFP-LC3B. After 48 h, the cells were analyzed by fluorescence microscopy (Olympus IX83). Scale bar: 10μm. (**B**) The cell numbers with GFP-LC3B puncta in (**A**) were counted under 200×magnification. \*\**P*≤0.01, \*\*\**P*≤0.001. (**C**) The indicated plasmids were transfected into H1299 cells stably expressing GFP-LC3B and the expression of related proteins was examined by western blot.