## Supplementary information, Figure S4

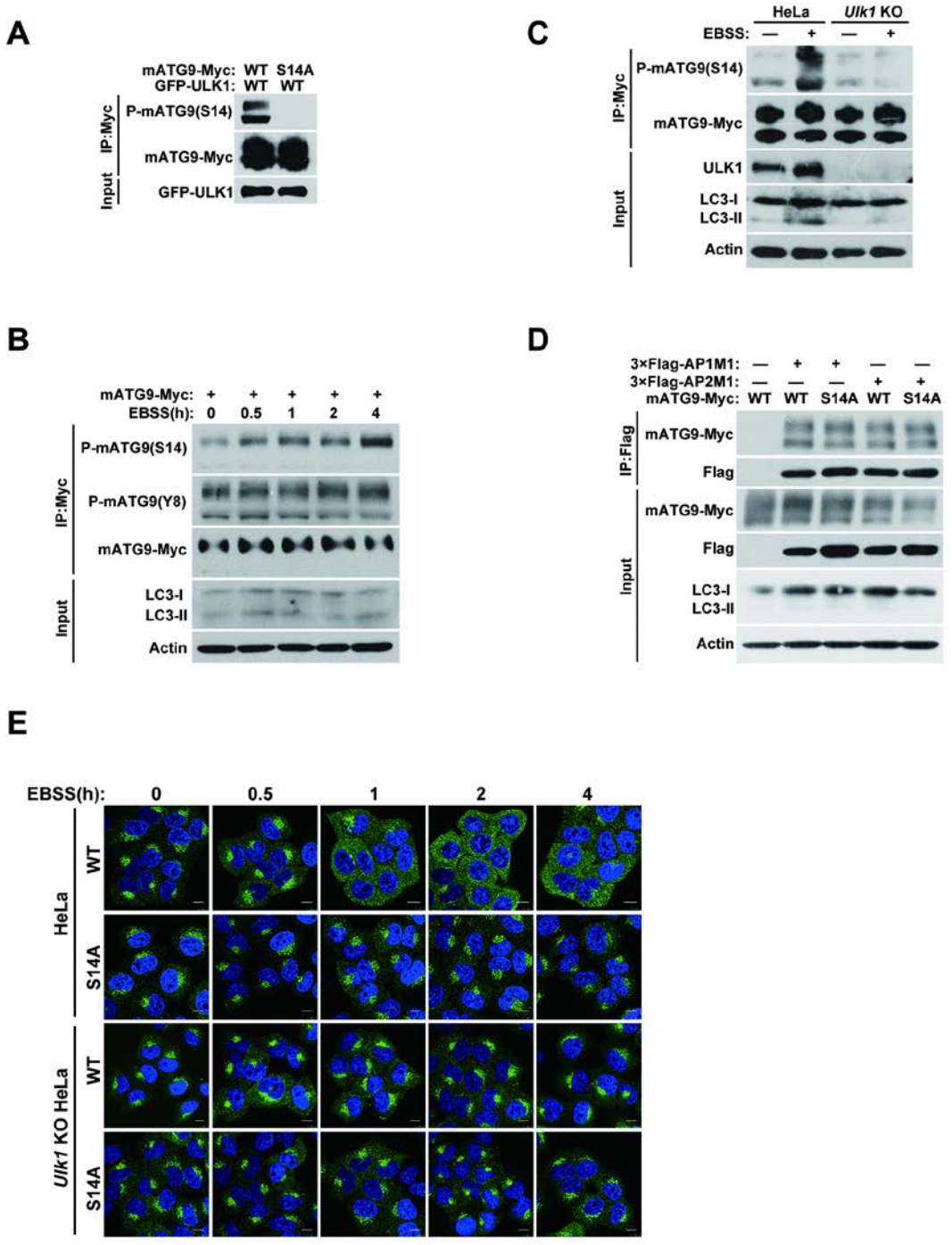


**Figure S4** S14 phosphorylation of mATG9 is essential for its redistribution under starvation stress. **(A)** HEK293T cells were co-transfected with WT mATG9-Myc or S14A and GFP-ULK1 for 24 h, and then collected for immunoprecipitation with anti-Myc antibody. Phospho-mATG9 was assessed by immunoblotting with a specific

antibody against S14 of mATG9. **(B)** HeLa cells stably expressing mATG9-Myc were starved in EBSS for the indicated time, and then collected for immunoprecipitation with anti-Myc antibody. The proportion of phospho-mATG9 was assessed by immunoblotting with specific antibodies against Y8 and S14 of mATG9. **(C)** HeLa cells or *Ulk1* KO HeLa cells were transfected with mATG9-Myc for 24 h, and then starved in EBSS for 2 h. Cells were collected for immunoprecipitation with anti-Myc antibody, and phospho-mATG9 was assessed by immunoblotting with a specific antibody against S14. **(D)** HeLa cells were co-transfected with WT mATG9-Myc or the S14A mutant and 3×Flag-AP1/2M1 for 24 h, and then collected for immunoprecipitation with anti-Flag antibody. **(E)** HeLa or *Ulk1* KO cells stably expressing mATG9-Myc or S14A mutant were starved in EBSS for the indicated time. Cells were fixed and immunostained with anti-Myc (green) antibody. Cells were counterstained with DAPI (blue). Scale bar, 10 μm.