Supplementary information, Figure S3

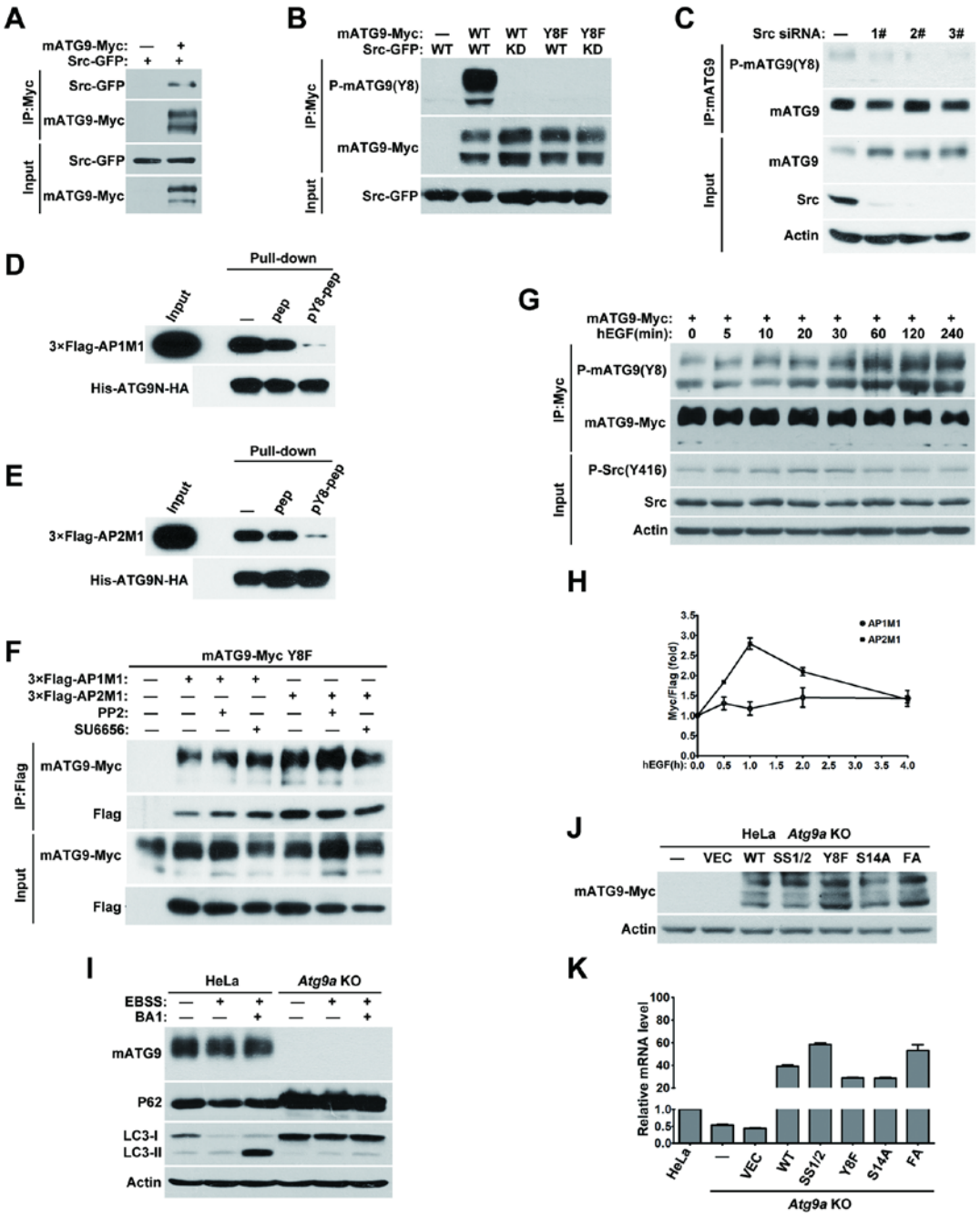

Figure S3 Active Src promotes mATG9 trafficking. **(A)** HEK293T cells were transfected with mATG9-Myc and Src-GFP for 24 h, and then collected for immunoprecipitation with anti-Myc antibody. **(B)** HEK293T cells were co-transfected with WT mATG9-Myc or Y8F and Src-GFP or KD Src for 24 h, and then collected

for immunoprecipitation with anti-Myc antibody. Phosphorylation at Y8 was assessed by immunoblotting with a specific antibody against phospho-Y8 of mATG9. **(C)** HeLa cells were transfected with scrambled or Src targeted siRNA for 72 h, and then collected for immunoprecipitation with anti-mATG9 antibody. **(D-E)** Cell lysates of HEK293T cells transfected with 3×Flag-AP1/2M1 were incubated with immobilized His-ATG9N-HA protein at 4 °C in the presence or absence of the indicated synthesized mATG9 N-terminal peptides (10 μM, aa1-18). **(F)** HeLa cells were co-transfected with mATG9 Y8F mutant and 3×Flag-AP1/2M1 for 24 h, and then treated with vehicle or the Src kinase inhibitors PP2 (10 μM) or SU6656 (10 μM) for 12 h. Cells were collected for immunoprecipitation with anti-Flag antibody. **(G)** HeLa cells stably expressing mATG9-Myc were serum-starved for 24 h, and then stimulated with 50 ng/mL hEGF for the indicated time. Cells were collected for immunoprecipitation with anti-Myc antibody and phospho-mATG9 was assessed by immunoblotting with a specific antibody against phosphorylated Y8. **(H)** Quantitative analysis of the co-immunoprecipitated mATG9 protein levels in **Figure 4B** by Image J (normalized to immunoprecipitated 3×Flag-AP1M1 or 3×Flag-AP2M1 levels). **(I)** An *Atg9a* KO HeLa cell line was produced using the CRISPR-Cas9 system. HeLa cells and *Atg9a* KO HeLa cells were treated with or without EBSS starvation for 2 h in the presence or absence of the lysosome inhibitor BA1 (20 nM). **(J)** *Atg9a* KO HeLa cells were reconstituted with empty vector, WT mATG9 or the indicated mutants, and then collected for western blotting with anti-Myc antibody. **(K)** The mRNA levels of mATG9 in the indicated cells were analyzed by RT-qPCR.