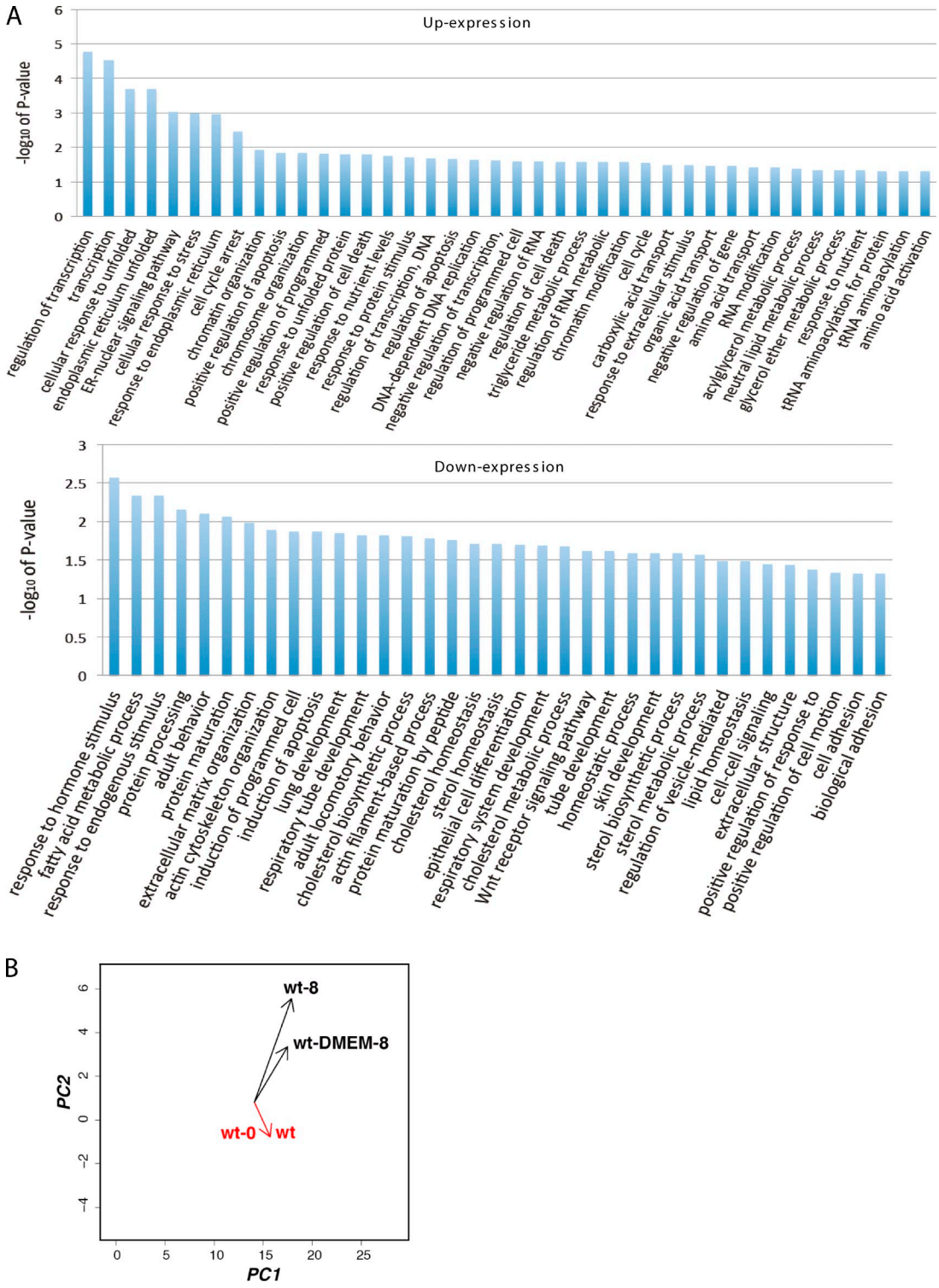

Figure S1. **Gene ontology analysis and principal component analysis of cells undergoing S/Gln starvation.** (A) The RNA of cells treated with DMEM was extracted for transcriptome analysis at 0 and 8 h. Gene ontology analysis was performed on genes that were up-regulated (top) and down-regulated (bottom) during S/Gln starvation. (B) Principal component analysis was performed on the expression data using R (version 3.0.1) prcomp.

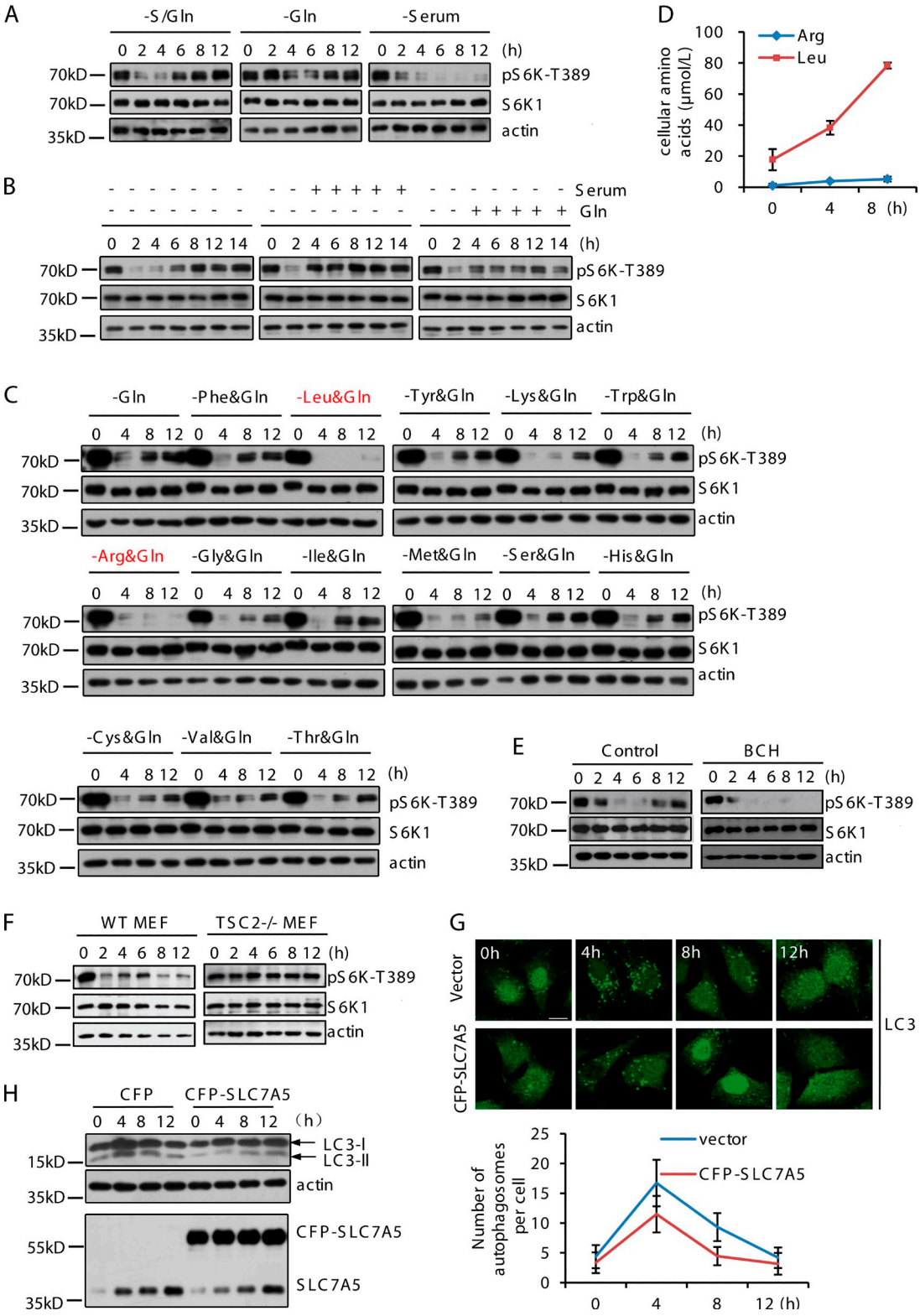

Figure S2. **SLC7A5 is required for S/Gln starvation–induced mTOR reactivation and regulating autophagy.** (A) CFP-LC3 NRK cells were treated with DMEM in the absence or presence of glutamine or serum. Proteins from cell lysates were analyzed by Western blotting for S6K1-T389 (phosphorylated S6K1), S6K1, and β-actin. (B) CFP-LC3 NRK cells were pretreated with DMEM for 4 h, and then glutamine or serum was added. Proteins from cell lysates were analyzed for S6K1-T389, S6K1, and β-actin. (C) CFP-LC3 NRK cells were treated with DMEM deficient in different amino acids, as indicated. Proteins from cell lysates were analyzed for S6K1-T389, S6K1, and β-actin. (D) CFP-LC3 NRK cells were treated with DMEM for the indicated times. The absolute levels of intracellular free Leu and Arg were quantified using LC/MS/MS. Error bars indicate the SD. (E) CFP-LC3 NRK cells were treated with DMEM in the absence or presence of the SLC7A5 inhibitor 2-aminobicyclo-(2, 2, 1)-heptane-2-carboxylic acid (BCH). Proteins from cell lysates were analyzed by Western blotting for SLC7A5 and β-actin. (F) Wild-type MEF and TSC2$^{-/-}$ MEF cells were starved with DMEM for the indicated times. Proteins from cell lysates were analyzed by Western blotting for S6K1-T389, S6K1, and β-actin. (G) NRK cells and stable CFP-SLC7A5 expressing cells were starved with DMEM for the indicated times, and then stained with antibodies against LC3 to reveal autophagosomes. The mean number of LC3 puncta per cell was assessed in a blind fashion after starvation and quantified. 100 cells from three independent experiments were analyzed. Error bars indicate the SD. Bar, 10 μm. (H) NRK cells and stable CFP-SLC7A5–expressing cells were starved with DMEM for the indicated times, and proteins from cell lysates were analyzed by Western blotting for LC3 and β-actin.

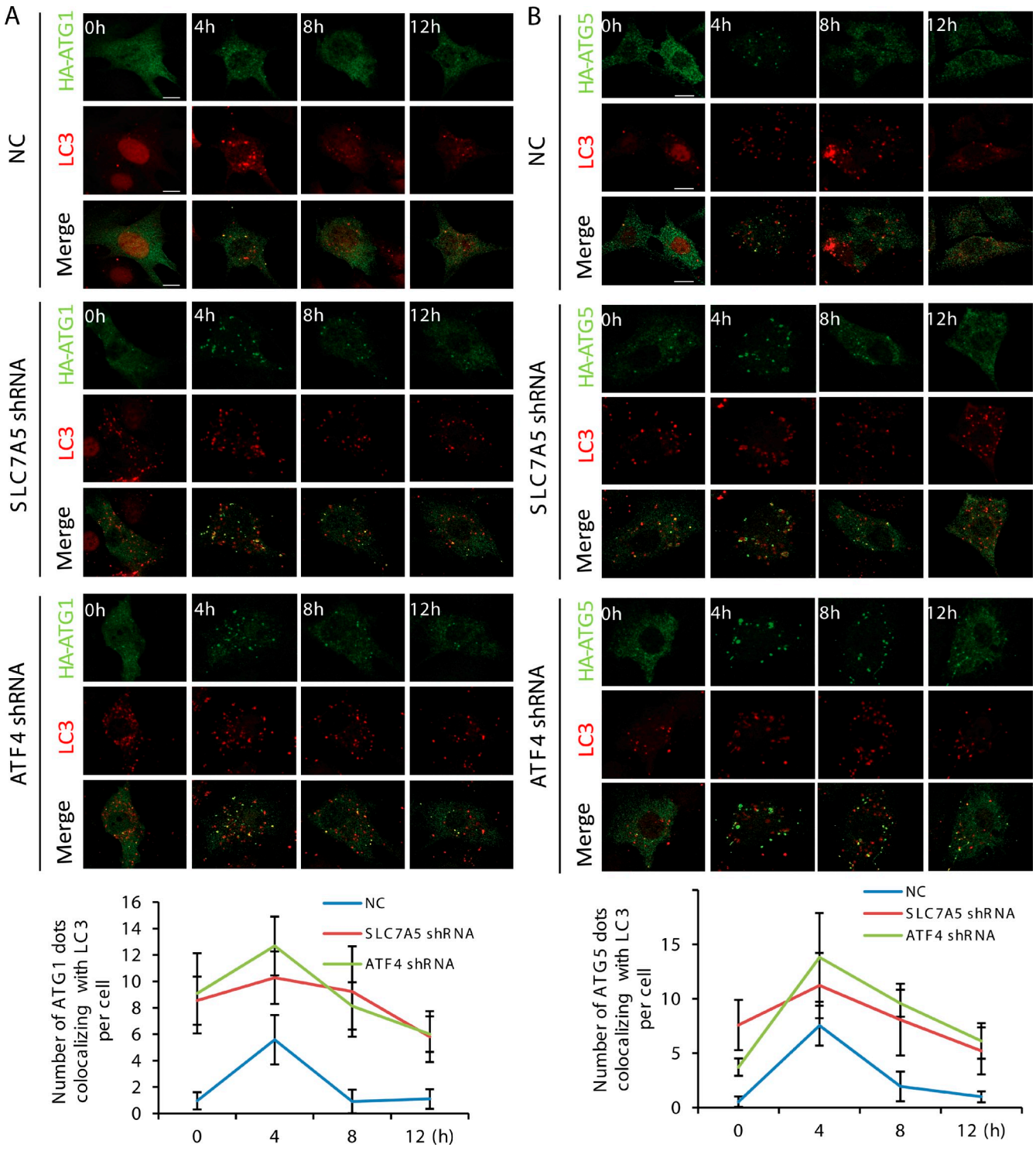

Figure S3. **ATF4 and SLC7A5 regulate the strength and duration of autophagy.** (A and B) CFP-LC3 NRK cells, stable SLC7A5 knockdown cells, and stable ATF4 knockdown cells were transfected with HA-ATG1 or HA-ATG5 plasmids, starved with DMEM for the indicated times, and then stained with antibodies against HA and GFP. The mean number of ATG1 dots colocalizing with LC3 per cell was assessed in a blind fashion after starvation and quantified. 50 cells were analyzed. Error bars indicate the SD. Bars, 10 μm.