Supplementary Figure 1

A) Strep-FLAG tagged N-terminal (aa 1-1260), middle (aa 1261-2544) and C-terminal (aa 2545-3526) regions of ALFY were expressed in HEK293T cells. 24h after transfection, total cell lysates and StrepTactin precipitates were subjected to immunoblot analysis with anti-FLAG and anti-GABARAP antibodies.

B) A series of Strep-FLAG tagged C-terminal deletion mutants were expressed in HEK293T cells. 24h after transfection, total cell lysates and StrepTactin precipitates were subjected to immunoblotting as described in (b).

C) STREP-FLAG-tagged ALFY deletion mutants with or without LIR mutations were expressed in HEK293T cells. 24h after transfection, total cell lysates and StrepTactin precipitates were subjected to immunoblot analysis with anti-FLAG and -GABARAP antibodies. Data are representative of three independent experiments.

D) A representative isothermal calorimetry (ITC) profile of the titration of Atg8 homologs with ALFY-LIR peptide. The upper panel shows the raw ITC thermograms, and the lower panel shows the fitted binding isotherms.