Expanded View Figures

Figure EV1. (corresponding to Fig 1). Ypt1 binds directly to Atg1, Hrr25, Atg11, or Atg17 *in vitro*.

A–D Ni-NTA pulldowns were performed by using purified His_6-tagged TF-Atg1, Atg17, Hrr25, Atg11 CC1(2–321aa), CC2(322–576aa), CC3(577–858aa), or CC4(859–1178aa) with GST or GST-Ypt1 from *E. coli*. Protein samples were separated by SDS–PAGE, and then detected using Coomassie blue staining.

Source data are available online for this figure.

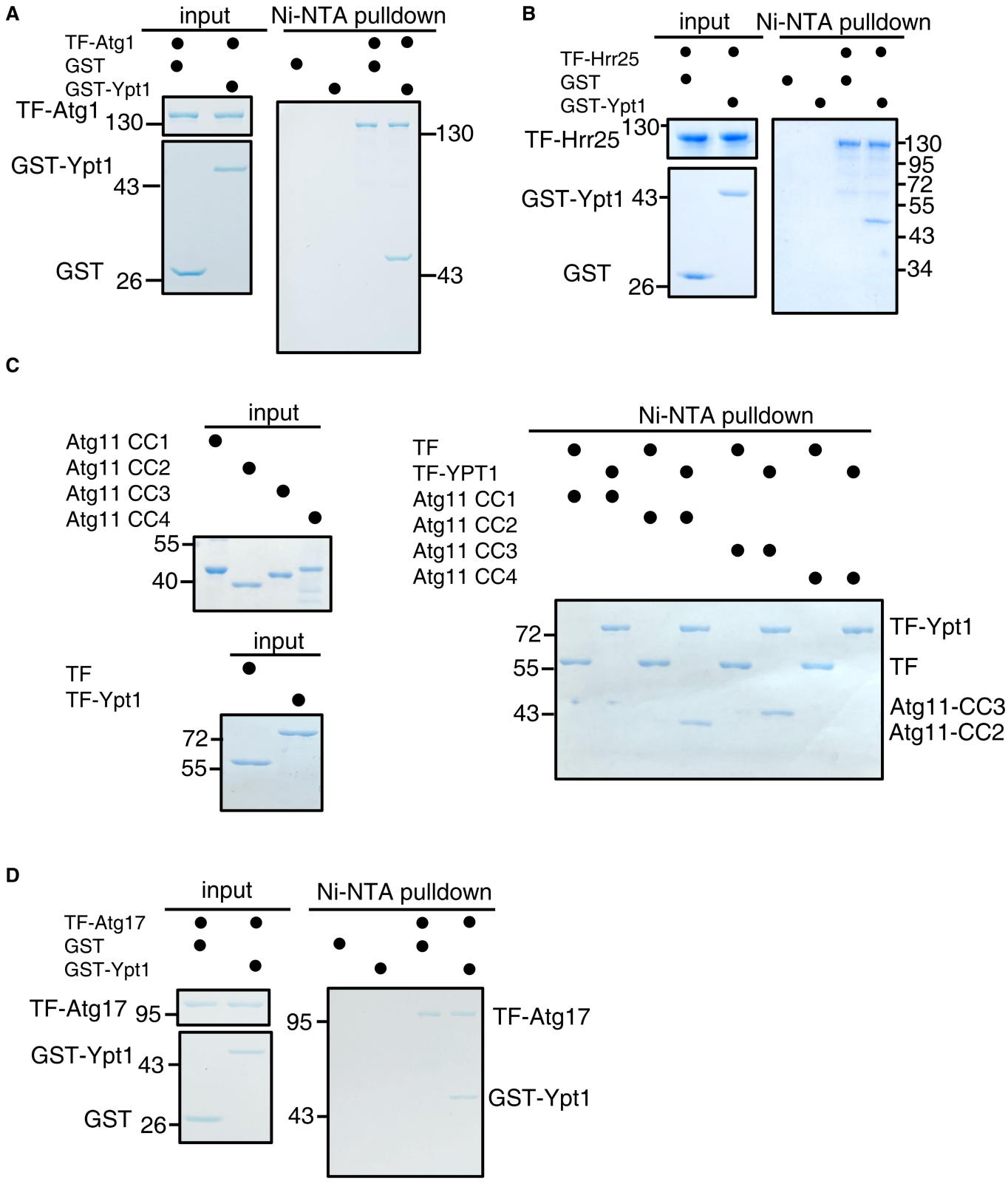

Figure EV1.

Figure EV2. (corresponding to Fig 2). The Atg23 CC1 domain is required for Ypt1-Atg23 binding and Atg23$^{L170N\text{-}L171N\text{-}L173N}$ and Atg23$^{L188N\text{-}L189N}$ mutants impaired the dimerization of Atg23 and its binding with Atg9.

A–C Fluorescence microscopy images of wild-type cells expressing the BiFC constructs VC-Ypt1 and Atg23-VN or the indicated Atg23-VN variants cultured in nitrogen starvation for 1 h. Scale bar: 2 μm.

D The maturation of PrApe1 from Fig 2G were quantified by the ratio of Ape1/Ape1 + PrApe1 and presented as mean ± SD ($n = 3$). ***$P < 0.001$; NS, no significance; two-tailed Student's *t*-tests were used.

E The AH109 strain was transformed with plasmids expressing BD-fused with Atg23 and plasmids expressing AD-fused with Atg23, Atg23$^{L170N\text{-}L171N\text{-}L173N}$, or Atg23$^{L188N\text{-}L189N}$. These strains were grown on SD-Leu-Trp or SD-His-Leu-Trp (3-AT) agar plates at 30°C for 3 days.

F Atg9-TAP (Tandem Affinity Purification tag, CBP-TEV-PA) and Atg23-3×HA, Atg23$^{L170N\text{-}L171N\text{-}L173N}$-3×HA, or Atg23$^{L188N\text{-}L189N}$-3×HA were co-expressed in *atg23*Δ yeast strains. Cells were grown to the log-growth phase and then subjected to nitrogen starvation for 1 h. Cell lysates were immunoprecipitated with anti-Rabbit IgG agarose beads and then analyzed with anti-HA antibody.

G GST pulldowns were performed using purified His_6-tagged TF-Atg9 N (2–318aa), or TF-Atg9 C (747–997aa) with GST, GST-Atg23, GST-Atg23$^{L170N\text{-}L171N\text{-}L173N}$, or GST-Atg23$^{L188N\text{-}L189N}$ from *E. coli*. Protein samples were separated by SDS-PAGE, and then detected using Coomassie blue staining.

H Fluorescence microscopy images of wild-type cells expressing the BiFC constructs Atg23-VC and Atg23-VN or the indicated Atg23-VN variants cultured in nitrogen starvation for 1 h. Scale bar: 2 μm.

Source data are available online for this figure.

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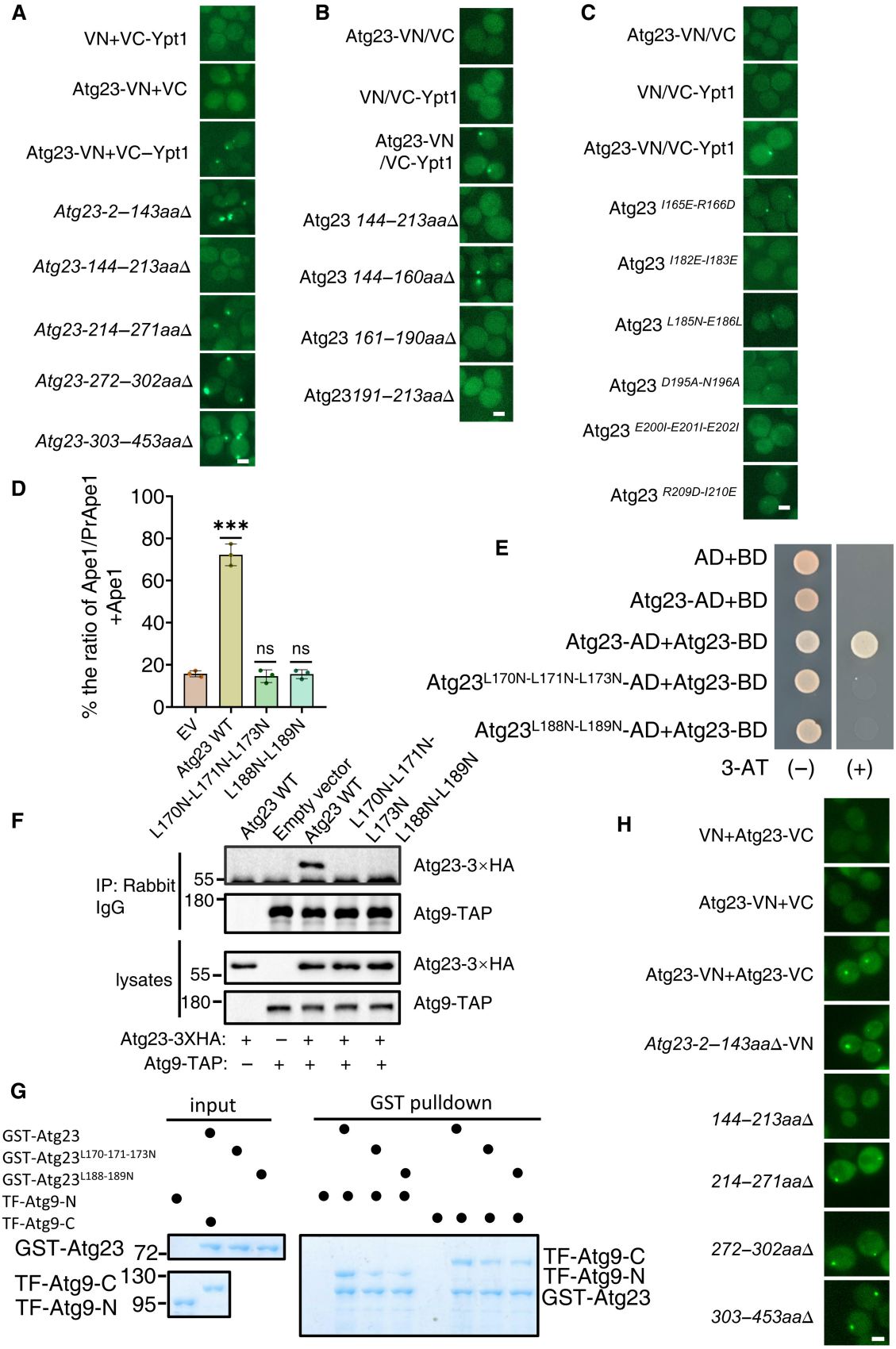

Figure EV2.

Figure EV3. (corresponding to Fig 5). Ypt1 binds directly with Atg17.

A Fluorescence microscopy images of wild-type cells expressing the BiFC VC-Ypt1 and Atg17-VN, cultured in nutrient-rich medium. Scale bar: 2 μm.

B Atg17-3×FLAG and HA-Ypt1 were co-expressed in wild-type yeast strains. Cells were grown to the log-growth phase. Cell lysates were immunoprecipitated with anti-FLAG agarose beads and then analyzed with anti-HA antibody.

C GST pulldowns were performed using purified His_6-tagged TF-Atg17 with GST, GST-Ypt1^{S22N}, or Ypt1^{Q67L} from *E. coli*. Protein samples were separated by SDS–PAGE, and then detected using Coomassie blue staining.

D, E Fluorescence microscopy images of wild-type cells expressing the BiFC VC-Ypt1 and the indicated Atg17-VN variants, cultured in nutrient-rich medium. Scale bar: 2 μm.

F Atg29-TAP, Atg31-GFP and Atg17-2×GFP, Atg17$^{L286N\text{-}V290N}$-2×GFP, or Atg17$^{I293E\text{-}I294E}$-2×GFP were co-expressed in *atg17*Δ yeast strains. Cells were grown to the log-growth phase. Cell lysates were immunoprecipitated with anti-Rabbit IgG agarose beads and then analyzed with anti-GFP antibody.

G Fluorescence microscopy images of *atg17*Δ cells co-expressing the RFP-Ape1 and Atg17-2×GFP, Atg17$^{L286N\text{-}V290N}$-2×GFP, or Atg17$^{I293E\text{-}I294E}$-2×GFP, cultured in nutrient-rich medium. Scale bar: 2 μm.

Source data are available online for this figure.

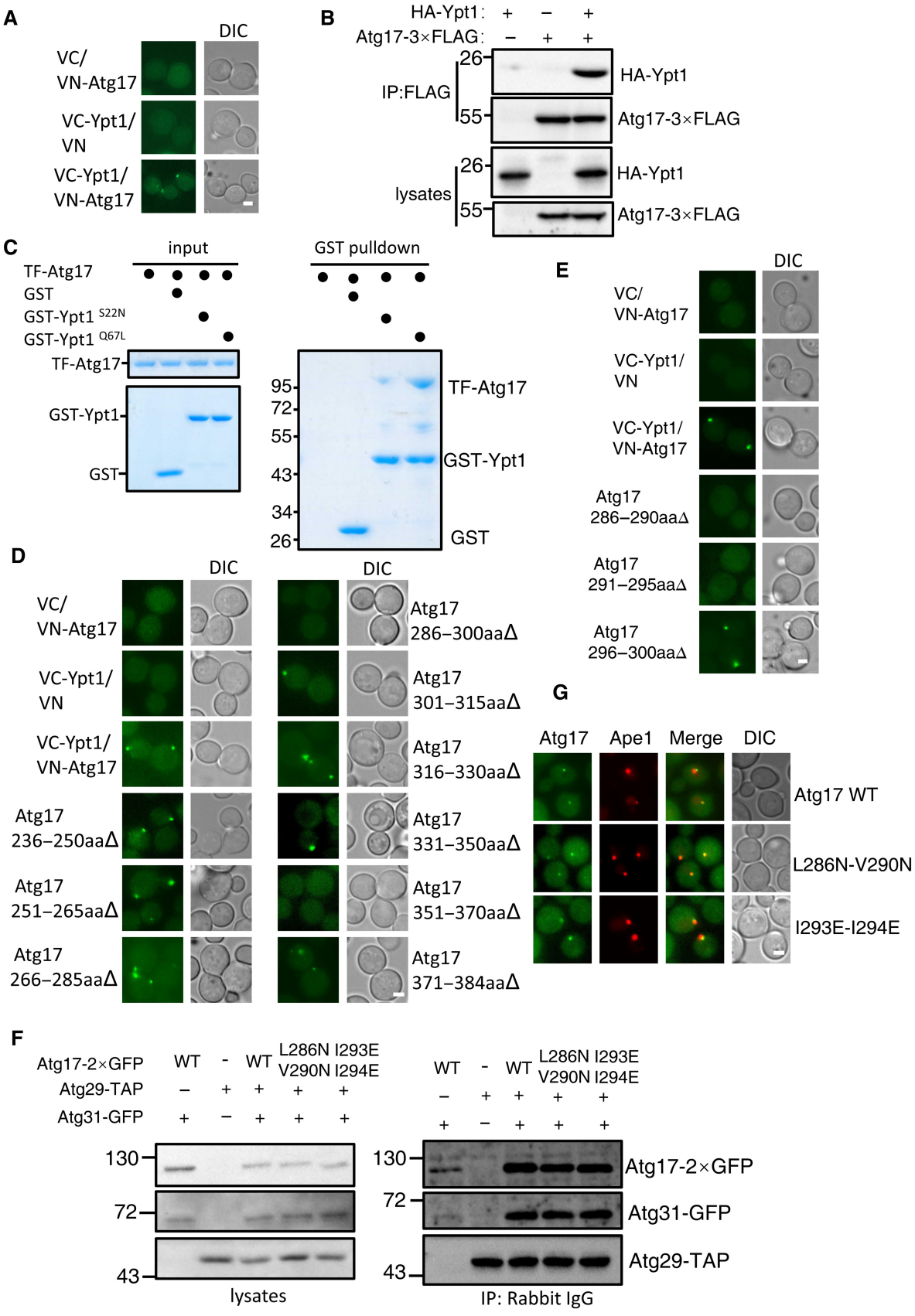

Figure EV3.

Figure EV4. (corresponding to Fig 7). The phosphorylation of Ypt1 S174 by TOR negatively regulates autophagy.

A–D (A, C) AID-3×HA-Ypt1 yeast strains co-expressing GFP-Atg8 and Vph1-mCherry were transformed into empty vector, FLAG-Ypt1, FLAG-Ypt1^{S174A}, or FLAG-Ypt1^{S174D} plasmids. These yeast strains were grown to early log phase, IAA was then added to induce the degradation of AID-3×HA-Ypt1 for 2 h. These yeast cells were then subjected to rapamycin treatment for 1 or 4 h. The autophagic activity of cells were analyzed by western blot for the cleavage of GFP-Atg8. Pgk1 served as a loading control. (B, D) The cleavage of GFP-Atg8 from (A, C) were quantified and presented as mean ± SD (n = 3). ***P < 0.001; **P < 0.01; NS, no significance; two-tailed Student's t-tests were used.

E AID-3×HA-Ypt1 ALP yeast strain transformed with FLAG-Ypt1, FLAG-Ypt1^{S174D}, Ypt1^{S174A}, or empty vector were grown to OD_{600} = 0.6 ~ 0.8. After treatment with 0.5 mM IAA for 2 h, the cells were subjected to nitrogen starvation for the indicated timepoints. Autophagy activity was tested by ALP assay.

Source data are available online for this figure.

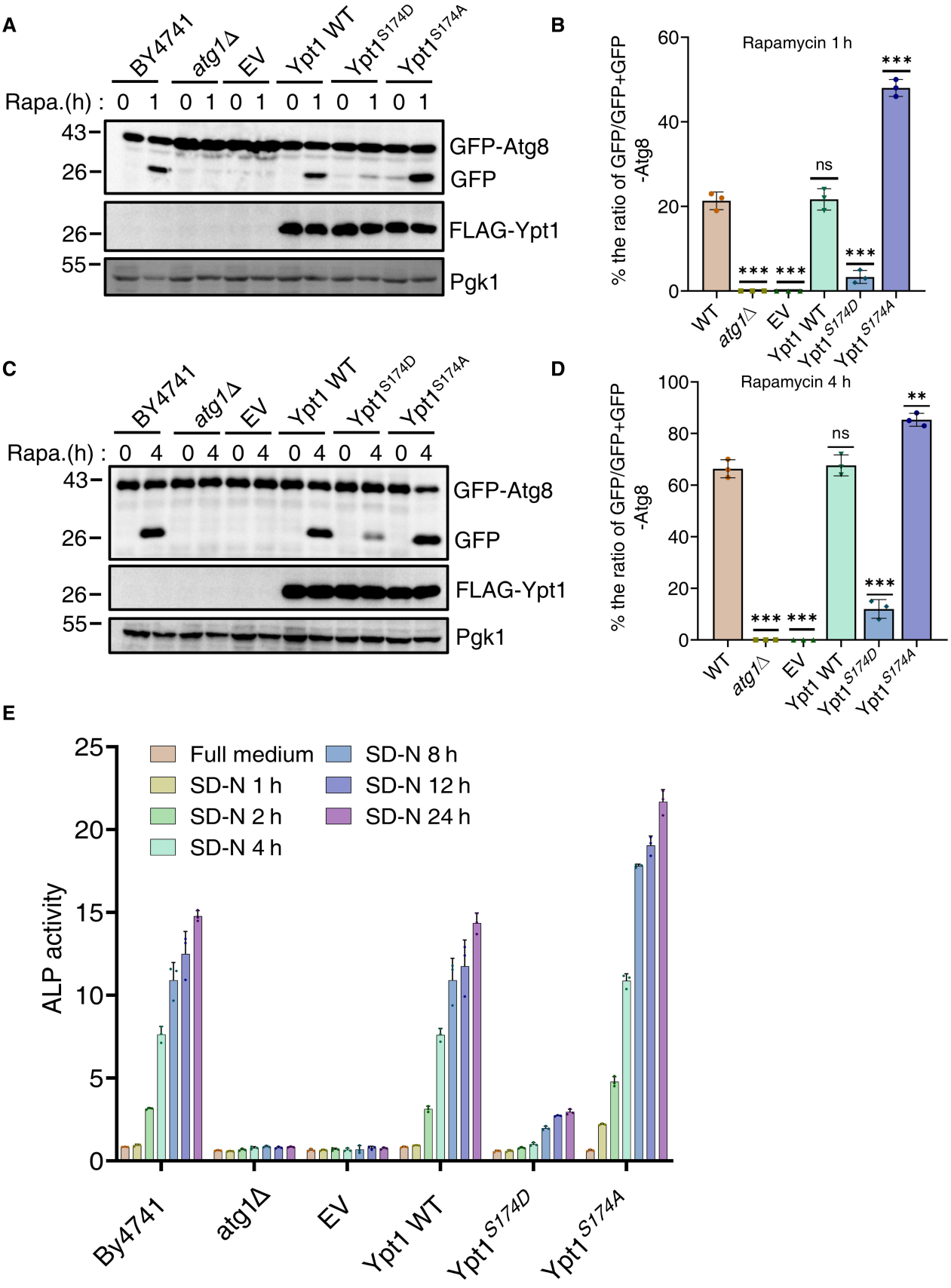

Figure EV4.

Figure EV5. (corresponding to Fig 8). The phosphorylation of Ypt1 by TOR negatively regulates the PAS recruitment of Hrr25.

A, B The protein samples from Fig 8D (A) and G (B) were analysis by western-blot using the corresponding antibody. Pgk1 served as a loading control.

C *atg1*Δ yeast cells expressing empty vector, wild-type FLAG-Atg1 (WT), or FLAG-Atg1^{T226} plasmids were grown to the early log phase, and then subjected to SD-N for 0 or 1 h. Cell lysates were immunoprecipitated with anti-FLAG agarose beads and then the phosphorylation level of Atg1 T226 was detected using an anti-phos-T226 Atg1 antibody.

D The phosphorylation level of Atg1 T226 from Fig 8J were quantified by calculating the ratio of p-Atg1-T226/FLAG-Atg1 ($n = 3$). Data are presented as mean ± SD. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; two-tailed Student's *t*-tests were used.

E AID-3×HA-Ypt1 yeast cells co-expressing Atg17-2×mCherry and Hrr25-GFP were transformed into empty vector, HA-Ypt1 WT, S174A, or S174D plasmids, these yeast strains were grown to log phase and then subjected to nitrogen starvation for 1 h. Images were obtained by fluorescence microscopy. Scale bar: 2 μm.

F Cells from (E) were quantified for the number of cells with puncta that were double-positive for Hrr25 and Atg17. $n = 300$ cells were pooled from three independent experiments. Data are presented as mean ± SD. ***$P < 0.001$; *$P < 0.05$; two-tailed Student's *t*-tests were used.

G The protein samples from (E) were analysis by western-blot using the corresponding antibody. Pgk1 served as a loading control.

H AID-3×HA-Ypt1 yeast cells co-expressing HA-Ypt1, HA-Ypt1^{S174A} or HA-Ypt1^{S174D} and Hrr25-3×FLAG were grown to log phase and then subjected to nitrogen starvation for 0 or 1 h. Cell lysates were immunoprecipitated with anti-FLAG agarose beads and then analyzed by western blot using an anti-HA antibody.

Source data are available online for this figure.

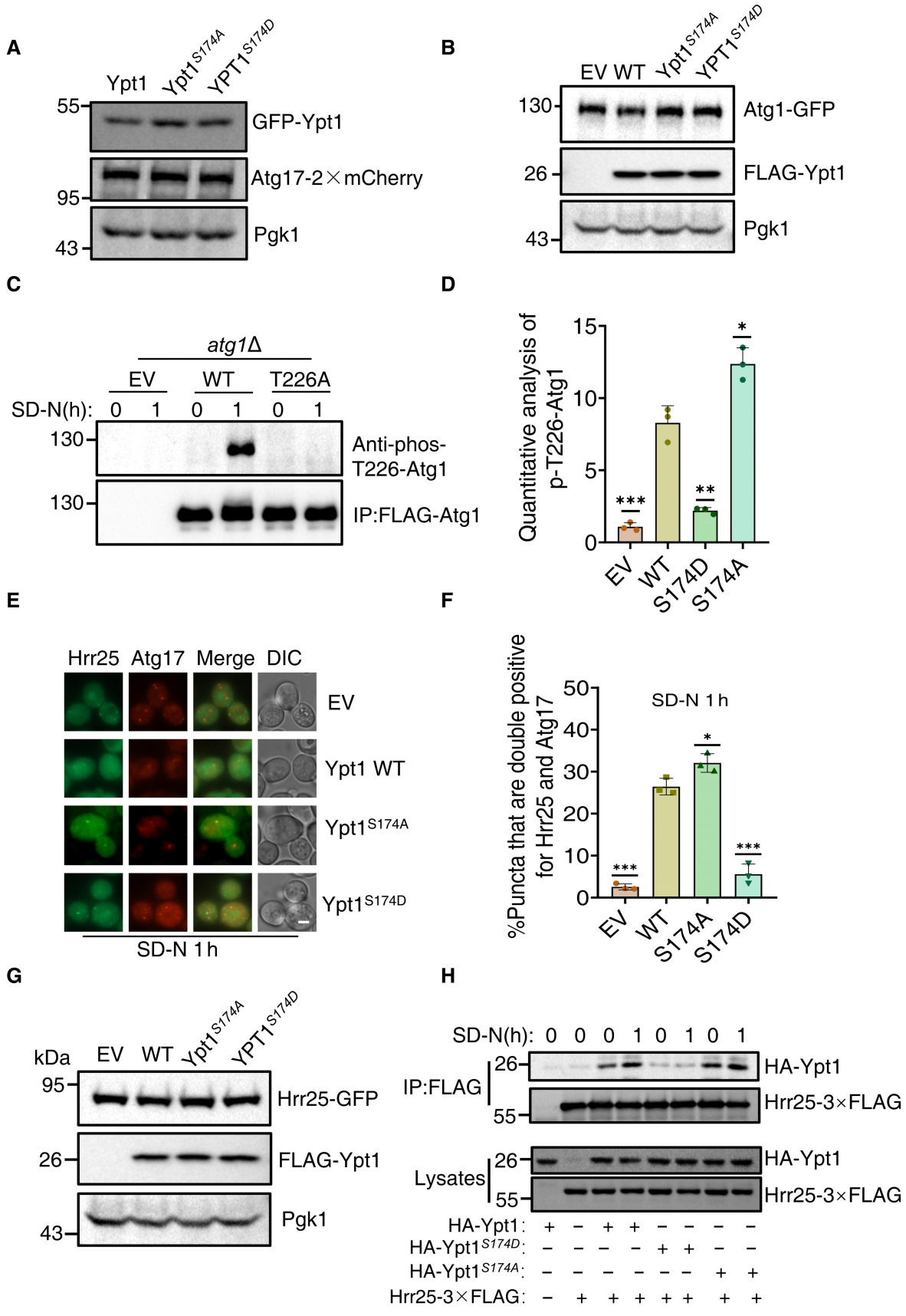

Figure EV5.