Expanded View Figures

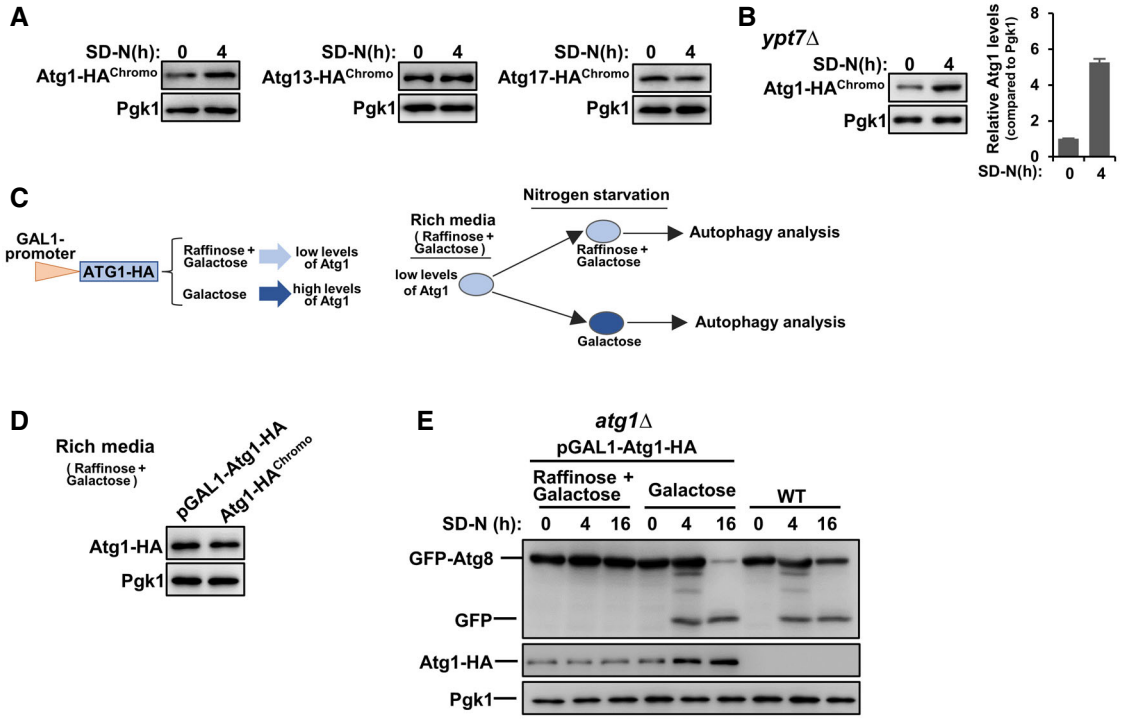


Figure EV1. Induced transcription of ATG1 upon starvation is important for autophagy activity.

A Atg1, Atg13, and Atg17 were HA-tagged, respectively, at the C-terminal by chromosome recombination and their expression levels were analyzed before and after nitrogen starvation in SD-N medium.

B Atg1 was HA-tagged at the C-terminal by chromosome recombination in *ypt7*Δ cells and its protein levels were analyzed before and after nitrogen starvation in an SD-N medium.

C–E Expression of exogenous Atg1 is controlled by the galactose promoter, and thus the expression levels of exogenous Atg1 in *atg1*Δ cells are controlled by different types of sugar added in the culture medium. Raffinose combined with galactose (2 and 0.01%, respectively) will induce low levels of Atg1 proteins while complete galactose (2%) will induce high levels of Atg1 proteins. Autophagic degradation of GFP-Atg8 was measured.

Figure EV2. Rpb9 is essential for autophagy.

A N-terminally GFP-tagged Atg8 was checked for its autophagic degradation in indicated yeast cells by GFP processing assays after 0, 4, and 16 h starvation. Yeast cells with *ATG1* deletion were used as positive controls.

B C-terminally GFP-tagged Pgk1 was checked for its autophagic degradation in indicated yeast cells by GFP processing assays after 0, 4, and 16 h starvation. The PVDF membrane was subject to Ponceau staining and used as a loading control.

C–E Expression determination of HA-Rpb9 in experiments Fig 2D, F, and G.

F Autophagic transfer of endogenous Ape1 in indicated yeast cells was analyzed at rich medium conditions.

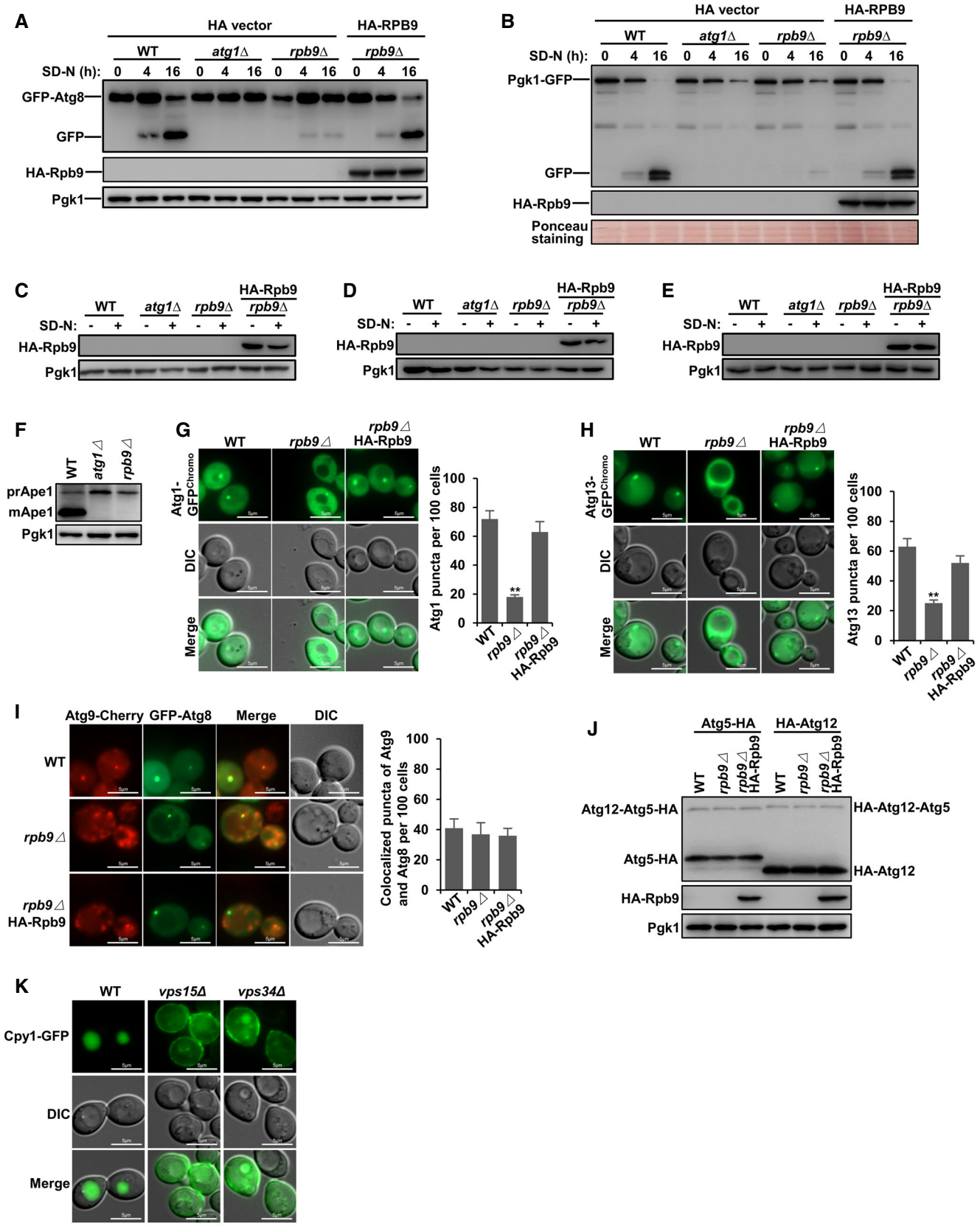
G Atg1 puncta was regulated by Rpb9. Atg1 was C-terminally GFP tagged in chromosome and its puncta in indicated yeast cells after 4-h SD-N starvation was observed and quantified. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test, **indicates $P < 0.01$ ($n = 5$ biological replicates). Scale bars: 5 μm.

H Atg13 puncta was regulated by Rpb9. Atg13 was C-terminally GFP tagged in chromosome and its puncta in indicated yeast cells after 4-h SD-N starvation was observed and quantified. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test, **indicates $P < 0.01$ ($n = 5$ biological replicates). Scale bars: 5 μm.

I Atg9 recruitment to Atg8 puncta was not regulated by Rpb9. C-terminal Cherry tagged Atg9 and N-terminal GFP tagged Atg8 were expressed in indicated yeast cells and their puncta co-localization was observed and quantified. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test ($n = 5$ biological replicates). Scale bars: 5 μm.

J Atg12-Atg5 conjugation was not regulated by Rpb9. C-terminal HA-tagged Atg5 and N-terminal HA-tagged Atg12 were expressed in indicated yeast cells and the Atg12-Atg5 conjugation levels were analyzed.

K Vacuole transfer of Carboxypeptidases Y (Cpy1) by the secretory pathway was analyzed in WT, *vps15*Δ, and *vps34*Δ cells. Scale bars: 5 μm.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rpb9Δ
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HA-Atg12
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HA-Atg12-Atg5
Atg5-HA
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Pgk1
K
WT
vps15Δ
vps34Δ
Cpy1-GFP
DIC
Merge

Figure EV2.

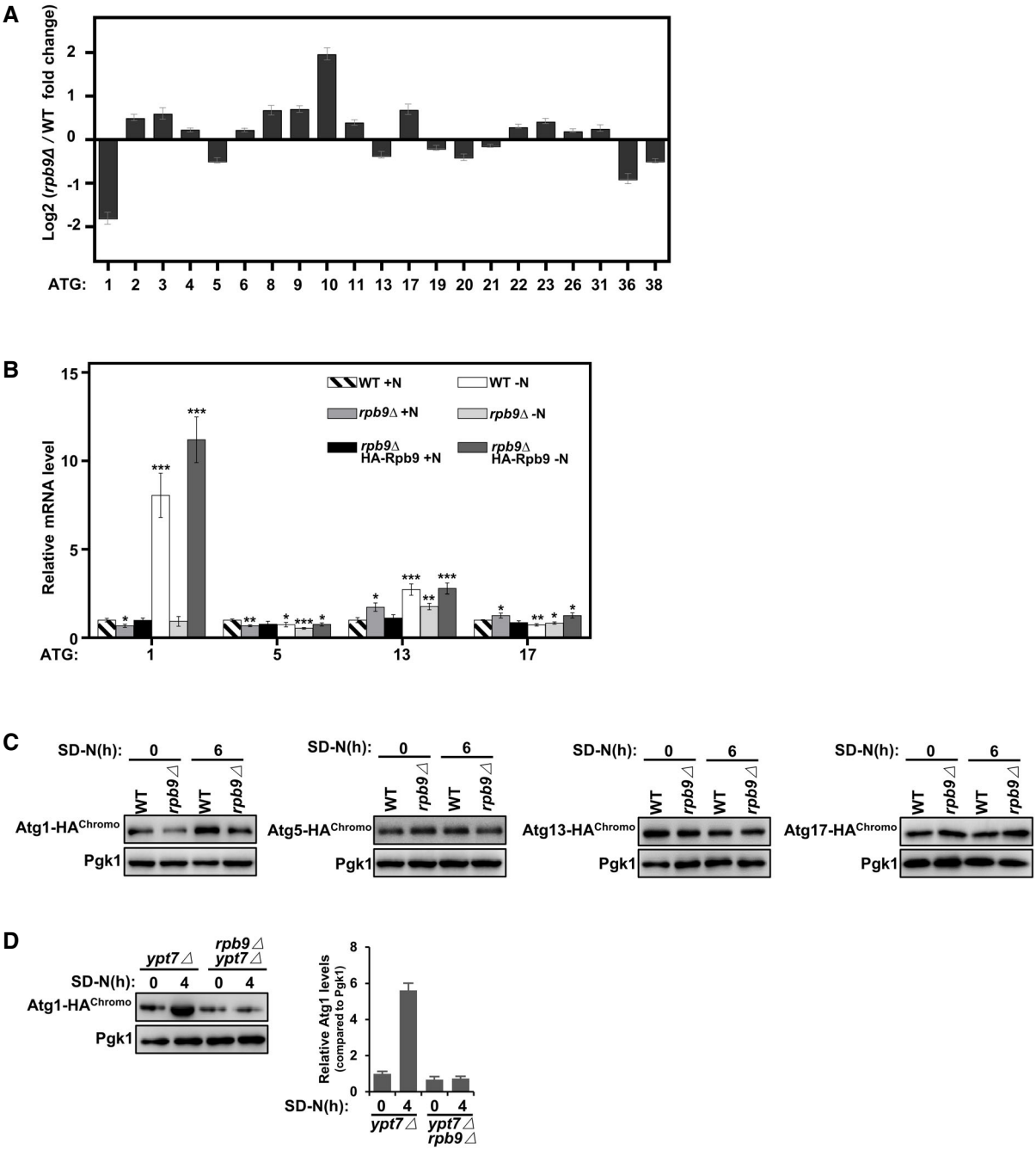


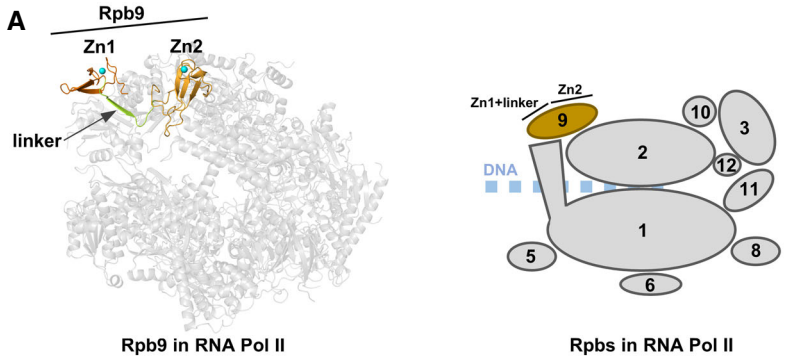
Figure EV3. Rpb9 is important for *ATG1* transcription.

A Data from genome-wide analysis of differential gene expression in *rpb9*Δ yeast cells compared to WT cells showed Rpb9 deletion caused specific downregulation of the *ATG1* gene. Bars represent mean, error bars represent standard deviation (n = 5 biological replicates).

B WT, *rpb9*Δ, and *rpb9type="InMathematical_Operators">*Δ+HA-Rpb9 yeast cells were grown to log phase in YPD (+N) and then shifted to nitrogen starvation (−N) for 3 h. mRNA levels of *ATG1*, *ATG5*, *ATG13*, and *ATG17* were quantified by qRT-PCR. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test, *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$ (n = 5 biological replicates).

C Protein expression levels of chromosome tagged Atg1, Atg5, Atg13, and Atg17 in WT and *rpb9*Δ yeast cells were analyzed before and after SD-N starvation.

D Atg1 was HA-tagged at the C-terminal by chromosome recombination in *ypt7*Δ cells and in *ypt7*Δ *rpb9*Δ cells and its protein levels were analyzed before and after nitrogen starvation in an SD-N medium.



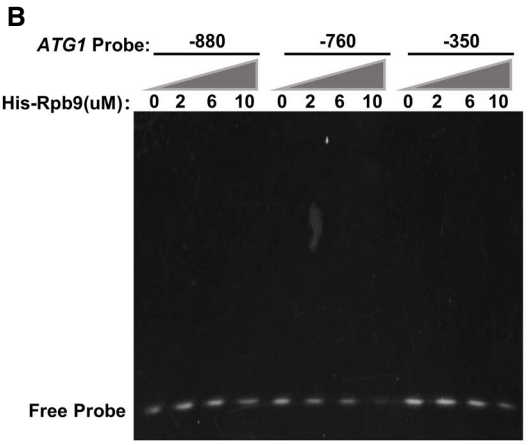


Figure EV4. Rpb9 alone cannot bind the *ATG1* promoter.

A Schematic diagram of Rpb9 in RNA polymerase II. The 10 subunits of yeast RNA polymerase II were shown as ribbon diagrams (this figure was prepared with PyMOL). Rpb9 was shown with orange color while the other subunits were shown in gray.

B His-tagged Rpb9 was purified from *Escherichia coli* cells and subject to an electrophoretic mobility shift assay (EMSA) together with DNA probes spanning the indicated promoter regions of *ATG1*.

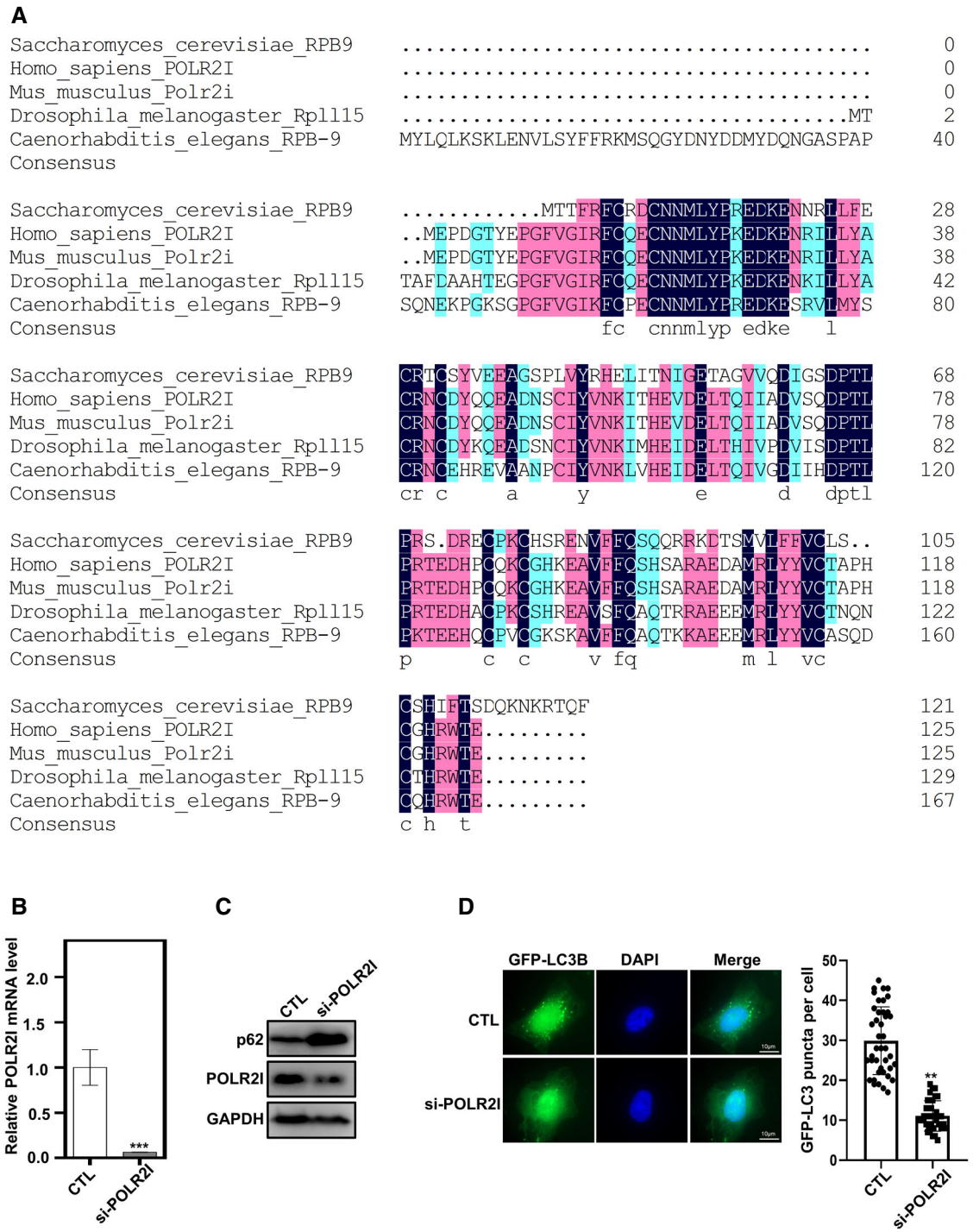


Figure EV5. Rpb9 is conserved in eukaryotes.

A Protein alignment of Rpb9 orthologs from *Saccharomyces cerevisiae*, *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, and *Caenorhabditis elegans*.

B HEK293T cells were transfected with siRNA targeting POLR2I for 72 h and then the mRNA levels of POLR2I were analyzed. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test, ***indicates $P < 0.001$ ($n = 5$ biological replicates).

C POLR2I knockdown increased the protein levels of autophagy receptor p62 which was subject to autophagic degradation. HEK293T cells were transfected with siRNA targeting POLR2I for 72 h and then the protein levels of p62 were analyzed.

D POLR2I knockdown reduced the number of autophagosomes shown by LC3 puncta. HEK293T cells with expression of GFP-LC3B were transfected with siRNA targeting POLR2I for 72 h and then the LC3 puncta was observed and quantified. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test, **indicates $P < 0.01$ ($n = 5$ biological replicates). Scale bars: 10 μm.