SUPPLEMENTARY FIGURES

Autophagy and leucine promote chronological longevity and respiration proficiency during calorie restriction in yeast

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Abbreviations: CLS, chronological life span; CR, calorie restriction; WT, wild type.

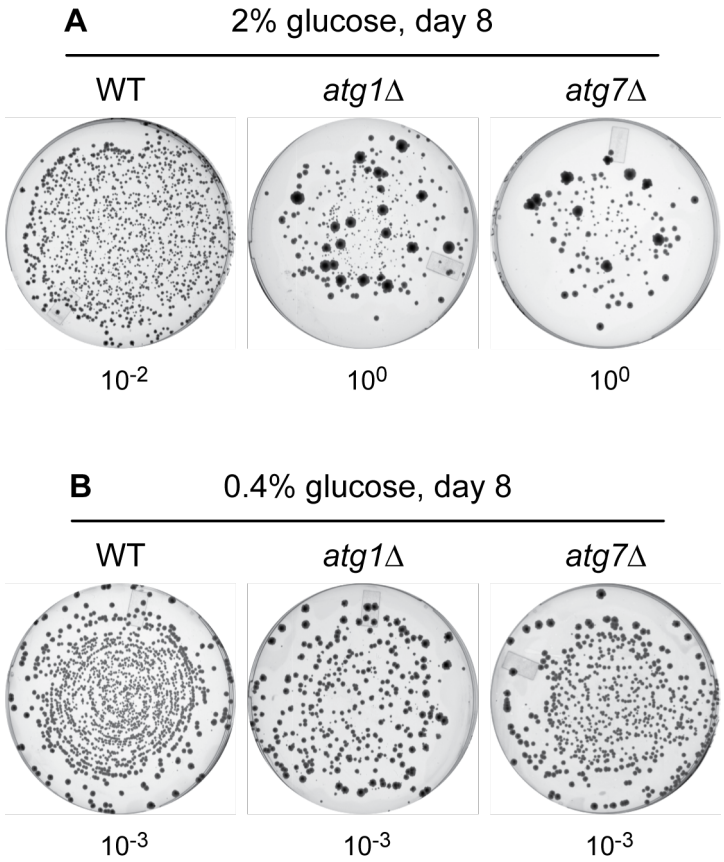

Figure S1. Petite colony formation in autophagy-deficient yeast during chronological aging. A. Plates from day 8 of the CLS experiment shown in Fig. 2 correspond to the gray symbols in panels A and D. B. Plates corresponding to gray symbols in panels C and F in Fig. 2. Cultures were diluted as indicated (10^0 = no dilution) and 100 µl were spread on YPD agar. Plates were incubated for 3 days at 30°C, except plates for *atg1*Δ and *atg7*Δ strains in panel A, which were grown for 5 days to better distinguish grande and petite colonies.

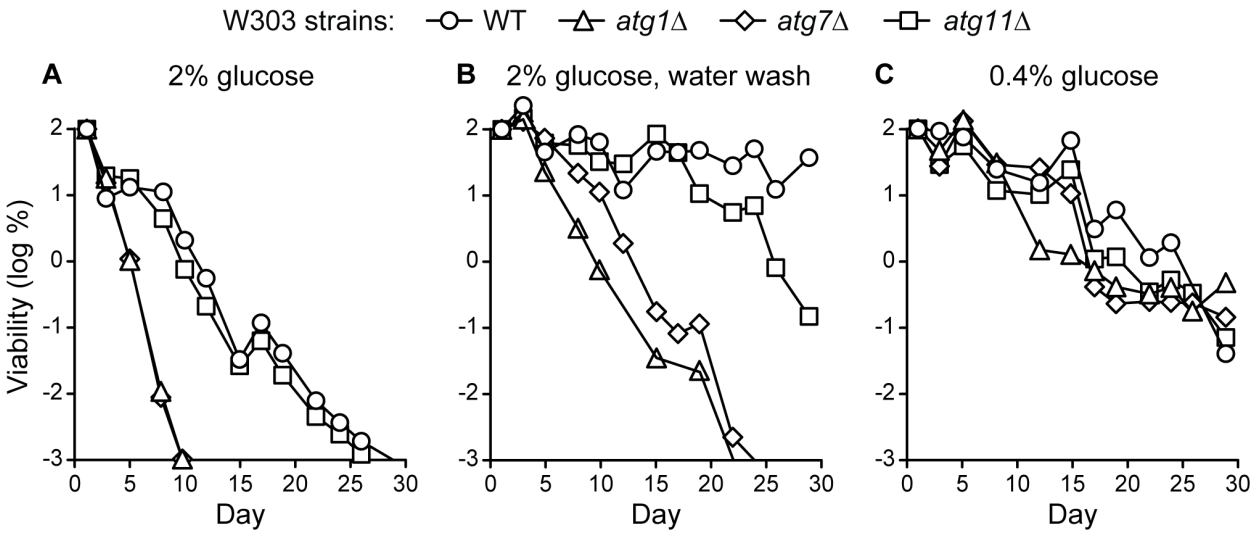

Figure S2. Autophagy is required for full CR-mediated extension of CLS in W303 strains. CLS experiments using WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains in the W303 genetic background were done as described in Fig. 2. Cell viability in CFU/mL is expressed as the log of the percentage of the number of viable cells on day 1 and is plotted as a function of time in days. A. CLS measurements following growth in standard minimal medium containing 2% glucose. B. CLS during water wash CR. Yeast strains were grown in standard 2% glucose minimal medium for 3 days, transferred to sterile water, and washed with water every 2-3 days thereafter. C. CLS during low glucose CR. Yeast strains were grown in standard minimal medium containing 0.4% glucose.

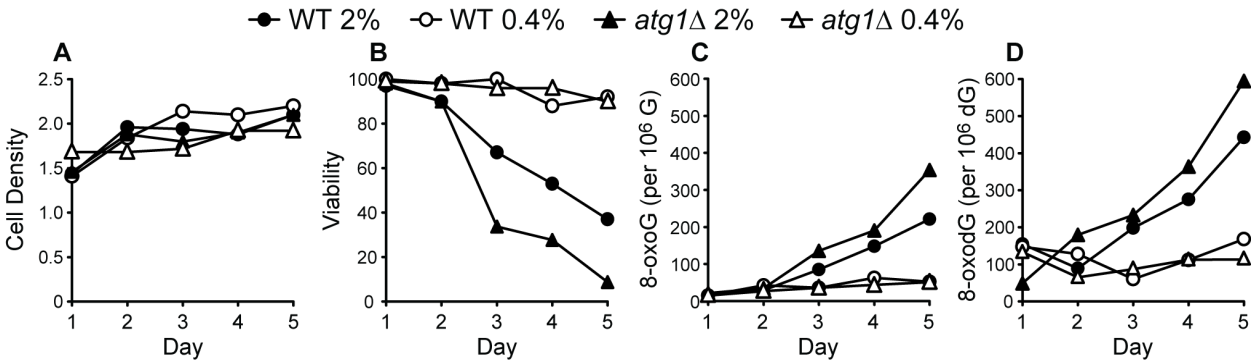

Figure S3. CR reduces oxidative damage to nucleic acids in WT and *atg1*Δ cells. CLS experiments were done using *atg1*Δ and WT strains following growth in minimal medium containing 2% glucose or under conditions of low glucose CR following growth in minimal medium containing 0.4% glucose. A. Cell density (OD_{600}) measurements. B. Cell viability measured using the vital dye Fun1 (see Materials and Methods). C. Oxidative damage to RNA. D. Oxidative damage to DNA. Levels of oxidized guanosine (8-oxo-G) and deoxyguanosine (8-oxo-dG) were measured using an HPLC method following hydrolysis of nucleic acid extracts prepared from yeast spheroplasts (see Materials and Methods). Levels of 8-oxo-guanine nucleosides are expressed per one million unmodified guanine nucleosides.

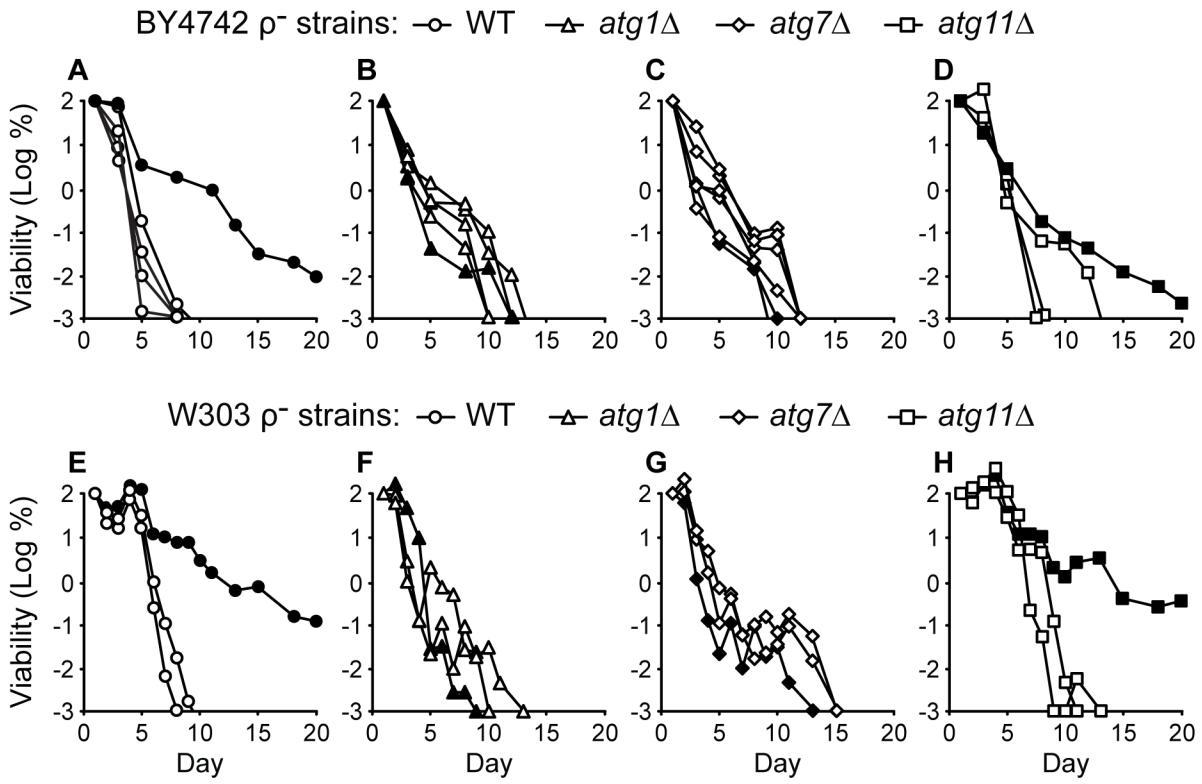

Figure S4. Respiration-deficient strains isolated from chronologically old cultures are short-lived. Respiration-deficient strains were isolated from petite colonies that arose during chronological aging of WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains in the BY4742 (A-D) and W303 (E-H) genetic background (see Materials and Methods). CLS determinations were done following growth in standard 2% glucose minimal medium as described in Fig. 2. Respiration-deficient (ρ^{-}) strains are shown in open symbols; respiration-proficient (ρ^{+}) parental strains are shown in filled symbols.

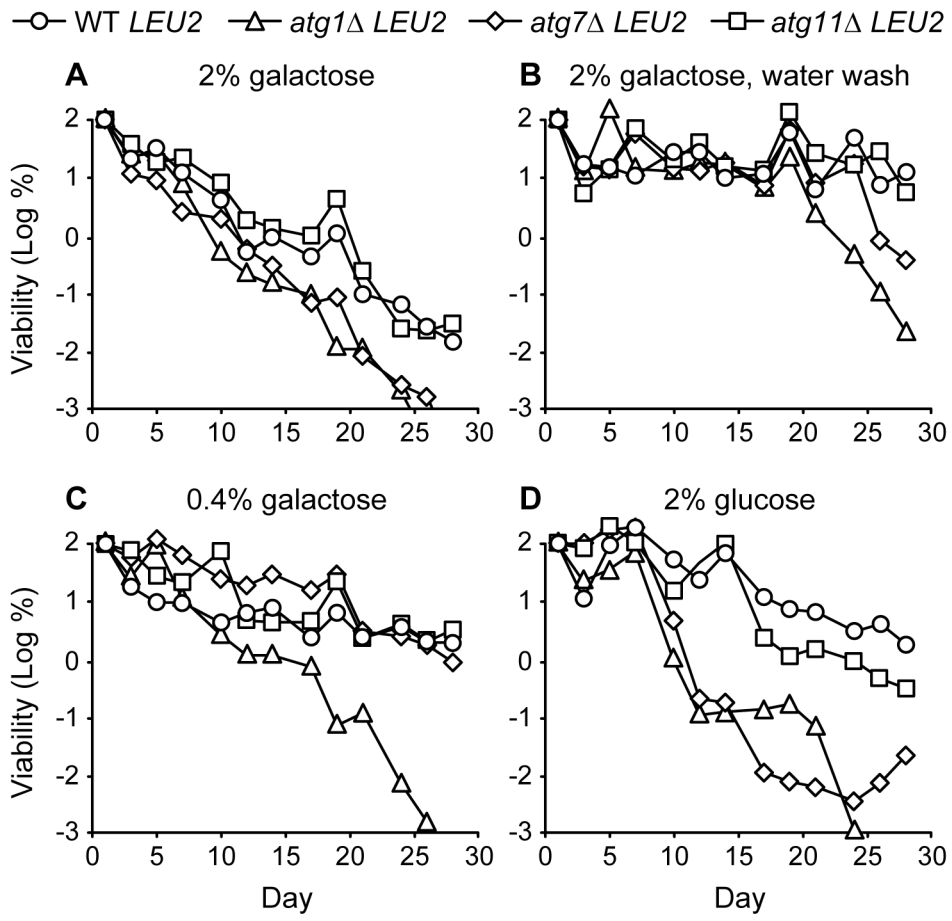

Figure S5. Requirements for autophagy during CR-mediated CLS extension in leucine prototrophs grown in galactose medium. WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains with a restored *LEU2* locus were previously described (Alvers et al 2009a). A. CLS was measured following growth in standard synthetic medium containing 2% galactose but lacking leucine. B. CLS during water wash CR following growth in 2% galactose medium and transferred to sterile water on day 3, followed by washing with sterile water every 2-3 days. C. CLS during low galactose CR following growth in minimal medium containing 0.4% galactose. D. CLS of yeast strains grown in 2% glucose minimal medium. Cell viability in CFU/mL is expressed as the log of the percentage of the number of viable cells on day 1 and is plotted as a function of time in days.

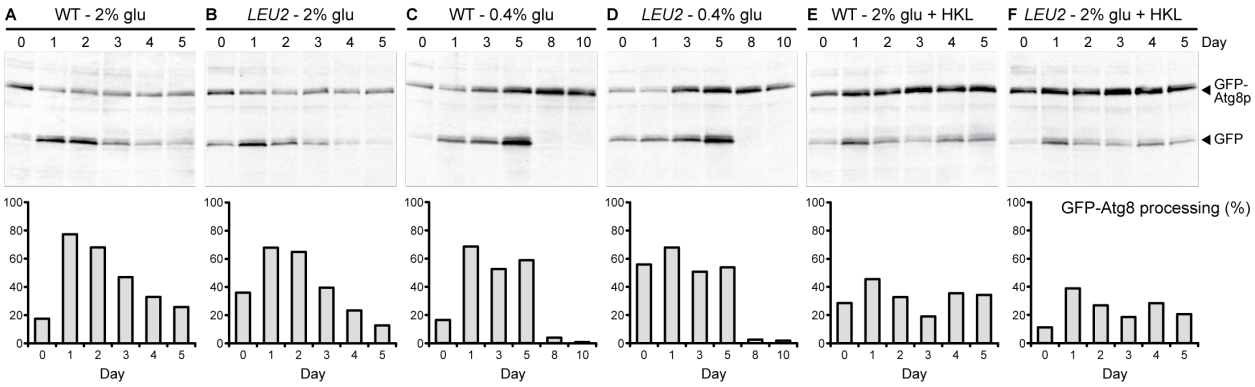

Figure S6. Induction of macroautophagy during chronological aging of leucine prototrophs. Macroautophagy was measured in WT (*leu2*Δ) and *LEU2* yeast transformed with plasmid pCuGFPAUT7(416) and grown under the following conditions: A-B. Minimal medium containing 2% glucose. C-D. Low glucose CR in 0.4% glucose minimal medium. E-F. Minimal medium containing 2% glucose and three-fold elevated levels of histidine, lysine, and leucine (+ HKL). Uracil is not present in media because the plasmid carries the *URA3* selectable marker. Western blotting with anti-GFP antibody is described in Fig. 1. The percent conversion of GFP-Atg8p to GFP for each day is graphed below western blots and reflects the extent of autophagic activation. See Materials and Methods for quantitation methodology. Day zero samples were collected during mid-log to late-log growth phase.

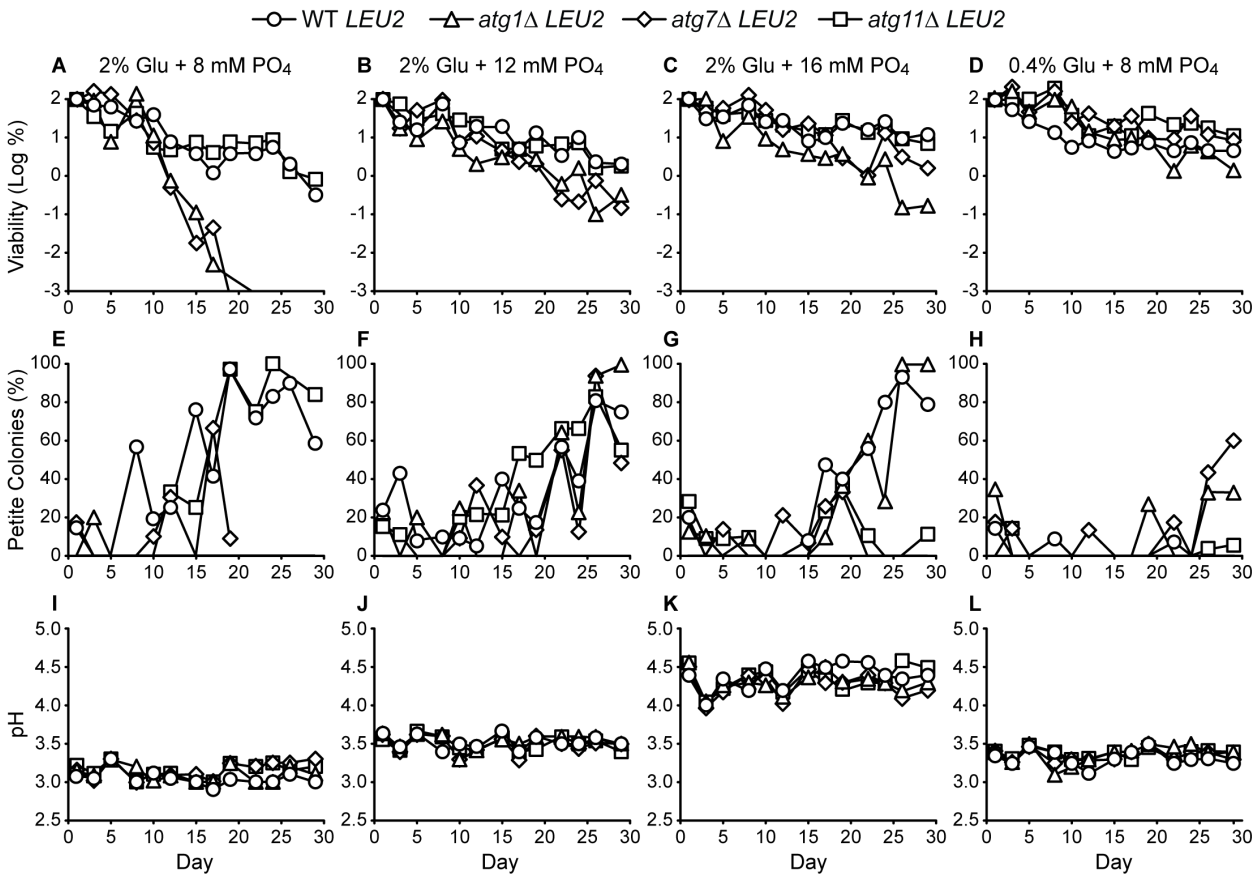

Figure S7. Autophagy is required for longevity of leucine prototrophs in less acidic medium. WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains with a restored *LEU2* locus were previously described (Alvers et al 2009a). A-C. CLS was measured in WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains following growth in 2% glucose minimal medium containing 8, 12, or 16 mM dibasic potassium phosphate. D. CLS during low glucose CR. Yeast strains were grown in minimal medium containing 0.4% glucose containing 8 mM dibasic potassium phosphate. Cell viability in CFU/mL is expressed as the log of the percent of the number of viable cells on day 1 and is plotted as a function of time in days. E-H. Percentages of petite colonies for panels A-D, respectively, are plotted as described in Fig. 2. I-L. Media pH values for panels A-D, respectively. This experiment was done in parallel with experiments shown in Figs. 7, S8, and S9.

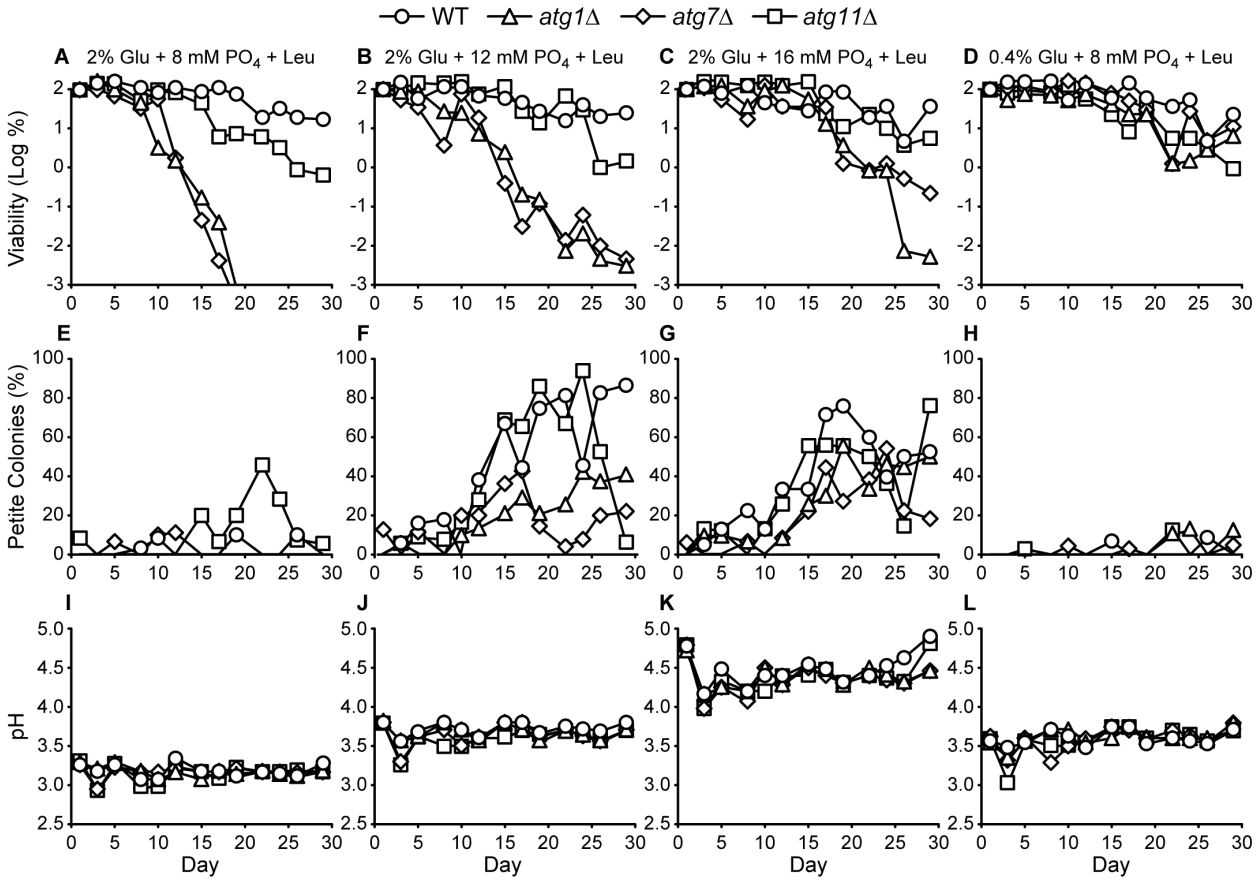

Figure S8. Autophagy is required for longevity in less acidic medium containing extra leucine. A-C. CLS was measured in WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains following growth in 2% glucose minimal medium containing 8, 12, or 16 mM dibasic potassium phosphate. D. CLS during low glucose CR. Yeast strains were grown in minimal medium containing 0.4% glucose containing 8 mM dibasic potassium phosphate. All media contained three-fold elevated levels of leucine (final concentration = 90 µg/mL). Cell viability in CFU/mL is expressed as the log of the percent of the number of viable cells on day 1 and is plotted as a function of time in days. E-H. Percentages of petite colonies for panels A-D, respectively, are plotted as described in Fig. 2. I-L. Media pH values for panels A-D, respectively. This experiment was done in parallel with experiments shown in Figs. 7, S7, and S9.

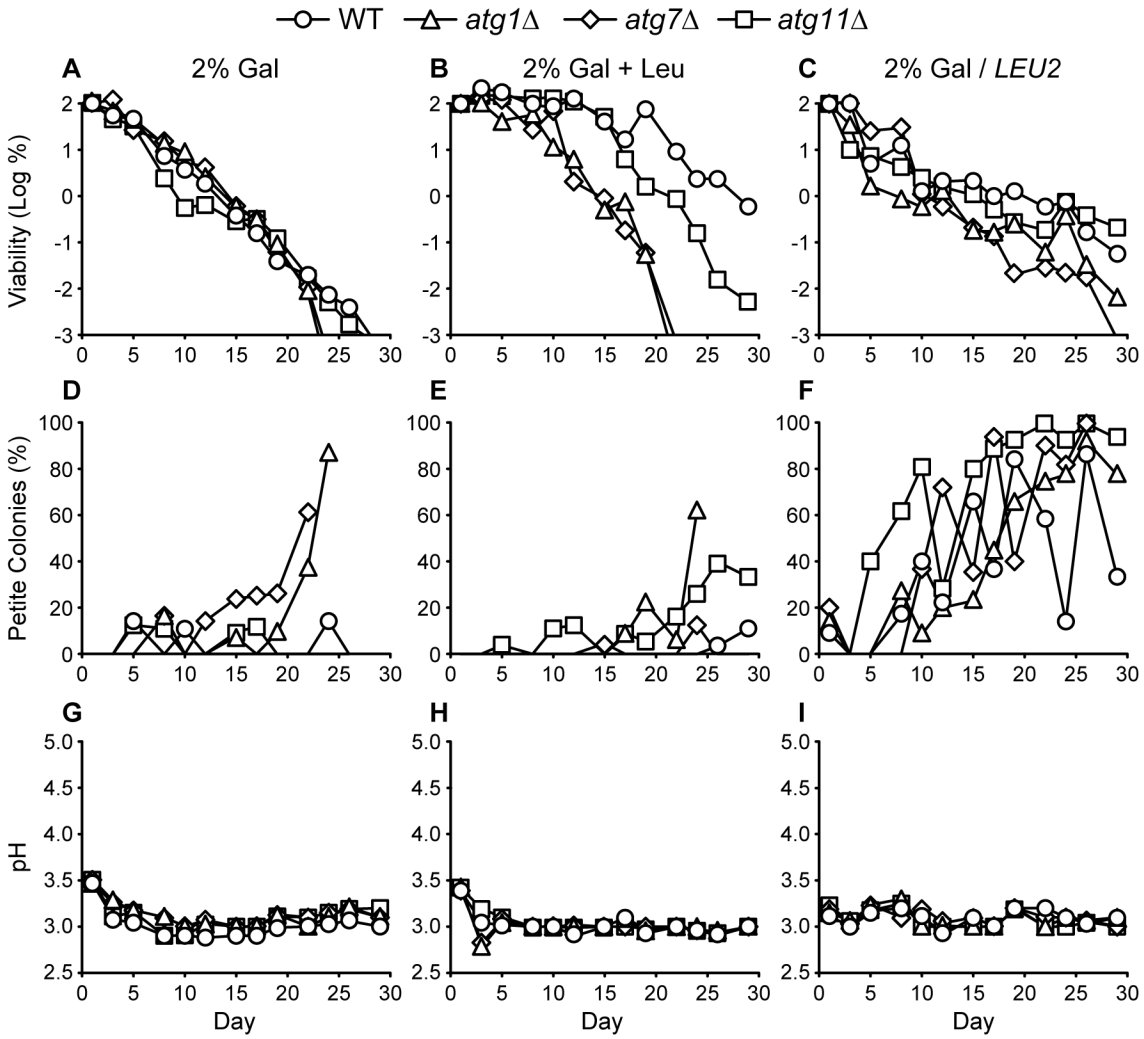

Figure S9. Galactose and increased leucine availability extend CLS in acidic medium. A. CLS was measured in WT, *atg1*Δ, *atg7*Δ, and *atg11*Δ strains following growth in synthetic minimal medium containing 2% galactose. Cell viability in CFU/mL is expressed as the log of the percent of the number of viable cells on day 1 and is plotted as a function of time in days. B. CLS was measured following growth in medium containing 2% galactose with three-fold elevated levels of leucine (final concentration = 90 µg/mL). C. CLS was measured in *LEU2* strains following growth in minimal medium containing 2% galactose. D-F. Percentages of petite colonies for panels A-C, respectively, are plotted as described in Fig. 2. G-I. Medium pH values in aging cultures. This experiment was done in parallel with experiments shown in Figs. 7, S7, and S8.