SUPPLEMENTAL DATA

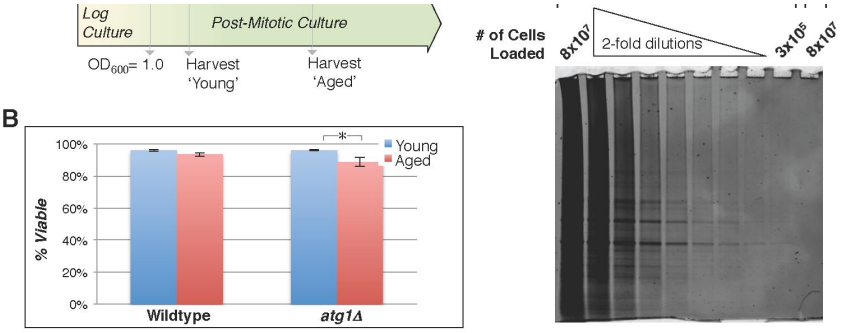

Figure S1- ***(A) Chronological aging model*** - Cells were grown into stationary phase (determined empirically to be approximately 12 hours after culture density reached OD_{600} of 1.0) and either harvested (Young) or incubated for an additional 48 hours at 30°C before being harvested (Aged). ***(B) Young and aged cultures have similar viability*** - 'Young and 'Aged' cells of both wildtype and *atg1Δ* strains were incubated with propidium iodide before being measured for the uptake and binding of the dye. The percent unstained cells are reported here as a measure of viability. WT cells show on average a decrease in viability of 2.5% during this time course, while *atg1Δ* cells decrease by 7.4% *(n=3)*. *p-value <0.01. ***(C) Aging causes over 200x increase in insoluble protein levels*** - Two-fold serial dilutions of the aged sample (replicate 1) were separated by SDS-PAGE and compared to SDS-insoluble protein that accumulates in the young sample (replicate 1). The resulting gel was stained for total protein and over-exposed when imaged so as to determine the dynamic range of protein detection using this method while examining if any protein could be detected in the young sample. The amount of insoluble protein loaded from both young and aged cultures was equivalent to $8x10^7$ cells. The final dilution of the aged sample represented the equivalent to $3x10^5$ cells loaded, a 256-fold dilution.

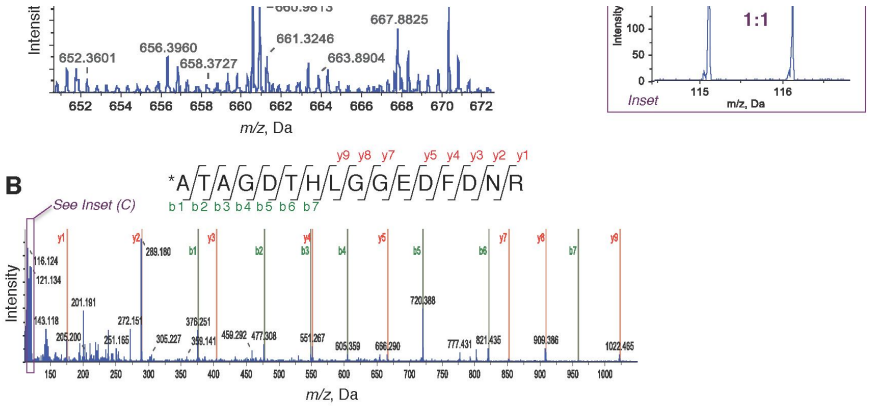

Figure S2 - *iTRAQ analysis of insoluble fraction (A)* Representative fragmentation, identification, and quantitation of the peptide ATAGDTHLGGEDFDNR from the protein Ssa1 is shown. This peptide was identified in the formic acid-soluble (FAS) fractions of the SDS-insoluble cellular protein and compared between post-mitotically aged biological replicates. FAS fractions resuspended in an aqueous buffer were reduced, alkylated, and trypsinized before chemically labeling primary amines in each sample with differential, but isobaric iTRAQ tags (Replicate 1 and 2 labeled with iTRAQ reagents 115 and 116, respectively). Labeled peptides were then mixed together, passed through a SCX cartridge to exclude unbound iTRAQ label, and subjected to HPLC/MS analysis using an Applied Biosystems QSTAR Elite mass spectrometer. The differentially isotope mass-tagged but isobaric peptides are chemically indistinguishable, resulting in co-elution under SCX fractionation and final reverse phase chromatography, yielding a single isotopic distribution at the MS level of analysis ***(A)***. Collision Induced Dissociation of the parent ions creates b- and y-ions used for identification of the peptide **(*B*)** as well as generating labile iTRAQ reporter ions for each peptide/protein pool at low mass, *m/z* 113-121 (purple box). The relative concentration of peptides from the differentially labeled samples is determined by comparing the intensity of the signal between these distinct reporter ions ***(C)***. In this example, the peptide ATAGDTHLGGEDFDNR was labeled on the amino terminus resulting in an initial triply charged molecular ion at *m/z* 660.6485 **(*A*)** and the corresponding reporter ions at *m/z* 115 and 116 **(*B*)** were distributed in a 1:1 ratio between two aged biological replicate samples ***(C)***.

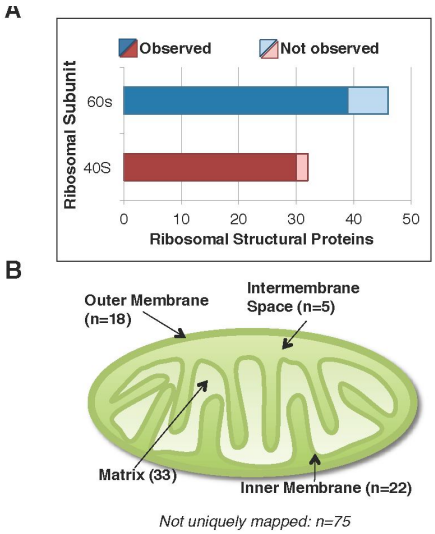

Figure S3 – ***Ribosomal and mitochondrial constituents of the age-dependent insoluble fraction suggest complex sequestration.*** ***(A)*** The number of structural ribosomal proteins identified in the age-dependent insoluble fraction was compared to the total number of structural ribosomal proteins in the large (60S) or small (40S) ribosomal subunits. ***(B)*** Insoluble proteins that are annotated with the 'Mitochondrion' GO term (n=153) were queried for sub-organelle localization. While the majority of those proteins associated with the 'Mitochondrion' GO term have not been mapped to a specific compartment or membrane within the organelle, several insoluble proteins were found to map distinctly to either luminal spaces in the organelle (intermembrane space and matrix) or to one of the membranes of the mitochondrion. The number of proteins found uniquely in each part of the mitochondria is shown.