Characterization of widespread proteome aggregation through aging in mammals

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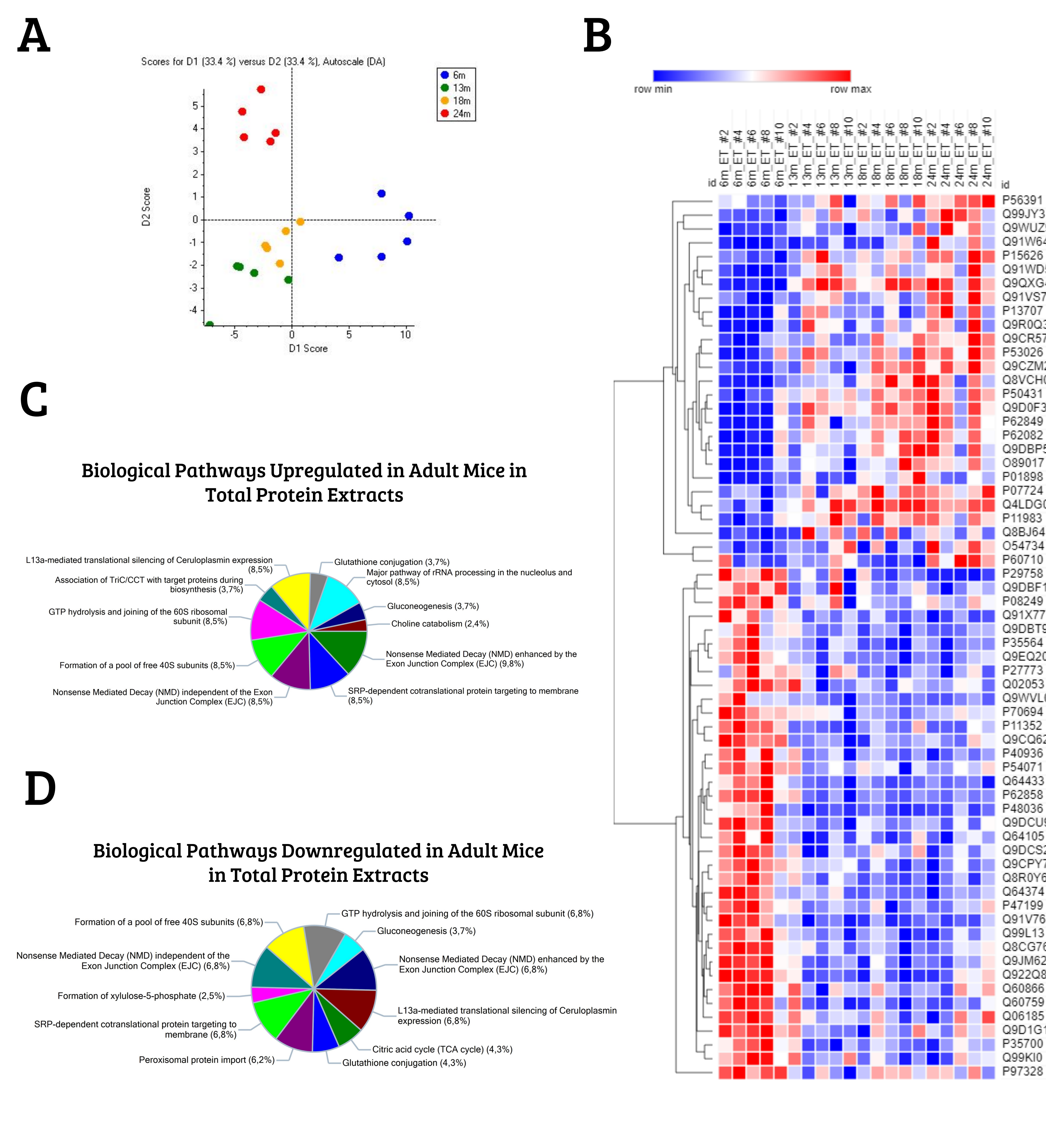2 CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

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Introduction

Proteome and proteostasis network disruptions lead to proteostasis alterations, leading to accumulation of protein aggregates, characteristic of several age-related diseases. Previous work in Caenorhabditis elegans and zebrafish has unveiled asymptomatic protein aggregation (ARPA), characterized by generalized increased accumulation of insoluble proteins through aging (1,2,3). However, the consequences of alterations in the PN in the context of healthy aging remains mostly unexplored in mammals. To elucidate if accumulation of insoluble proteins also occurs throughout aging in mammals, C57BL/6 mice of different ages corresponding to young (6 months old), adult (13 months old), and old stages (18 and 24 months old) were used. Detergent-insoluble fractions were isolated from total protein extracts of tissues, followed by characterization of both total and detergent-insoluble protein profiles. Our results show tissue-specific proteome alterations during aging. For example, protein-insoluble fractions increase through aging in the liver. We performed SWATH mass spectrometry analysis to identify differential proteome signatures of aging and determine which proteins are more prone to aggregate in order to further elucidate the functions and biological processes affected by ARPA.

Methodology

1. Sample Preparation
Female C57BL/6 Mice
- Tissues conserved in liquid nitrogen

6 months | 13 months | 18 months | 24 months

2. Total Protein Extract

3. Isolation of Insoluble Fraction

4. SWATH Mass Spectrometry

Results

1) Proteomic Characterization of Total Protein Extract in Liver

A — Scores for D1 (33.4 %) versus D2 (33.4 %), Autoscale (DA)

C — Biological Pathways Upregulated in Adult Mice in Total Protein Extracts

D — Biological Pathways Downregulated in Adult Mice in Total Protein Extracts
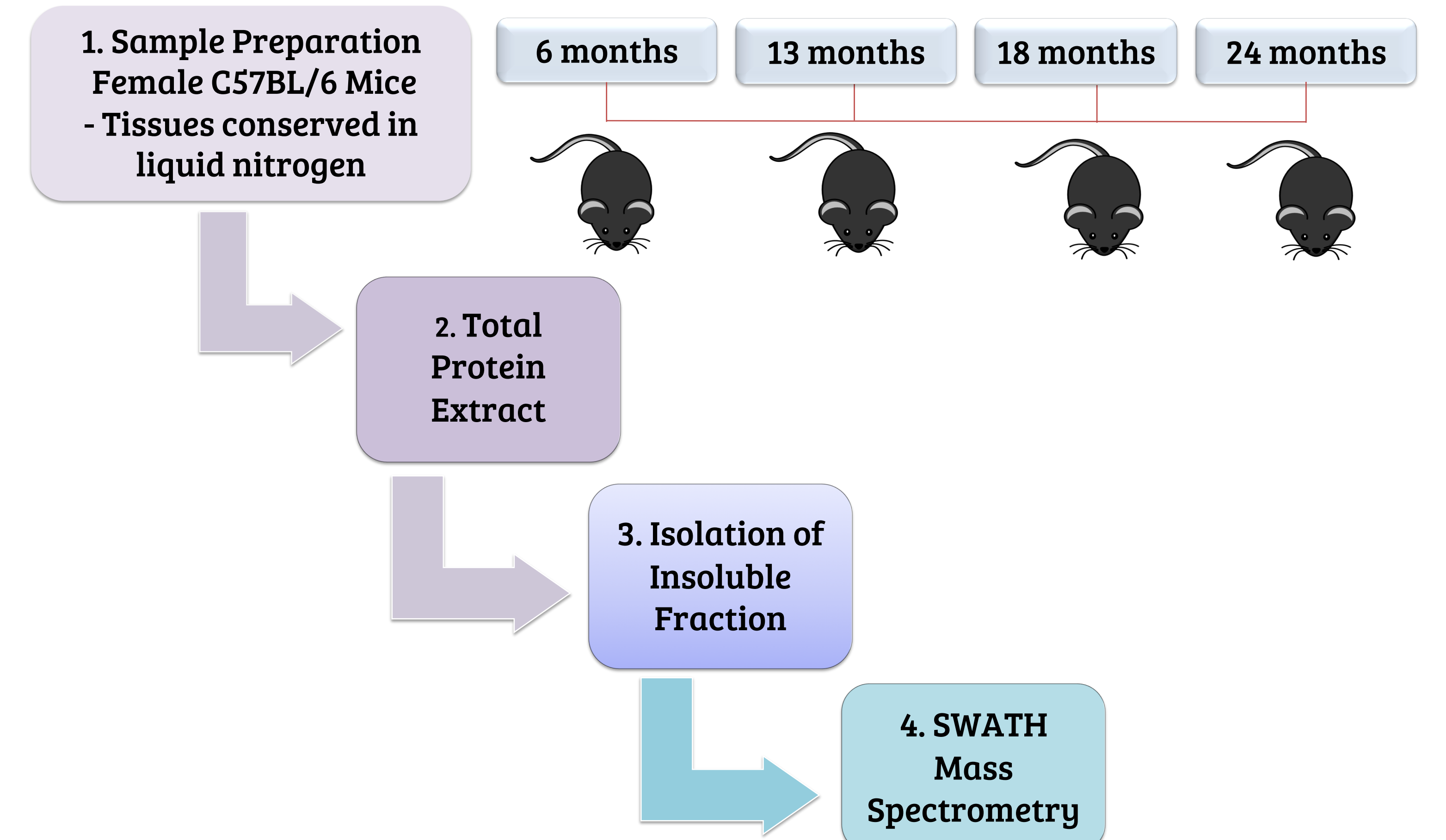
B — row min / row max

Figure 1 – A - PCA analysis of the 135 proteins considered as altered in the total protein extract of liver samples, produced in MarkerView software from Sciex, discriminant analysis using autoscaling and no weighing of the data.
B – Heat map analysis of SWATH MS data of the total protein extract. Heatmap produced in Morpheus software from Broad institute using one minus Pearson correlation for the hierarchical clustering of the quantitative information of the 64 proteins considered as altered between the 24 months and 6 months in the total extract. 6m, 6 months; 13m, 13 months; 18m, 18 months; 24m, 24 months.
C - Common biological pathways upregulated (Funrich Software) in the total protein extracts of older mice (24m, 18m, 13m) compared to young mice (6m). D - Common biological pathways downregulated (Funrich Software) in older mice (24m, 18m, 13m) compared to young mice (6m).
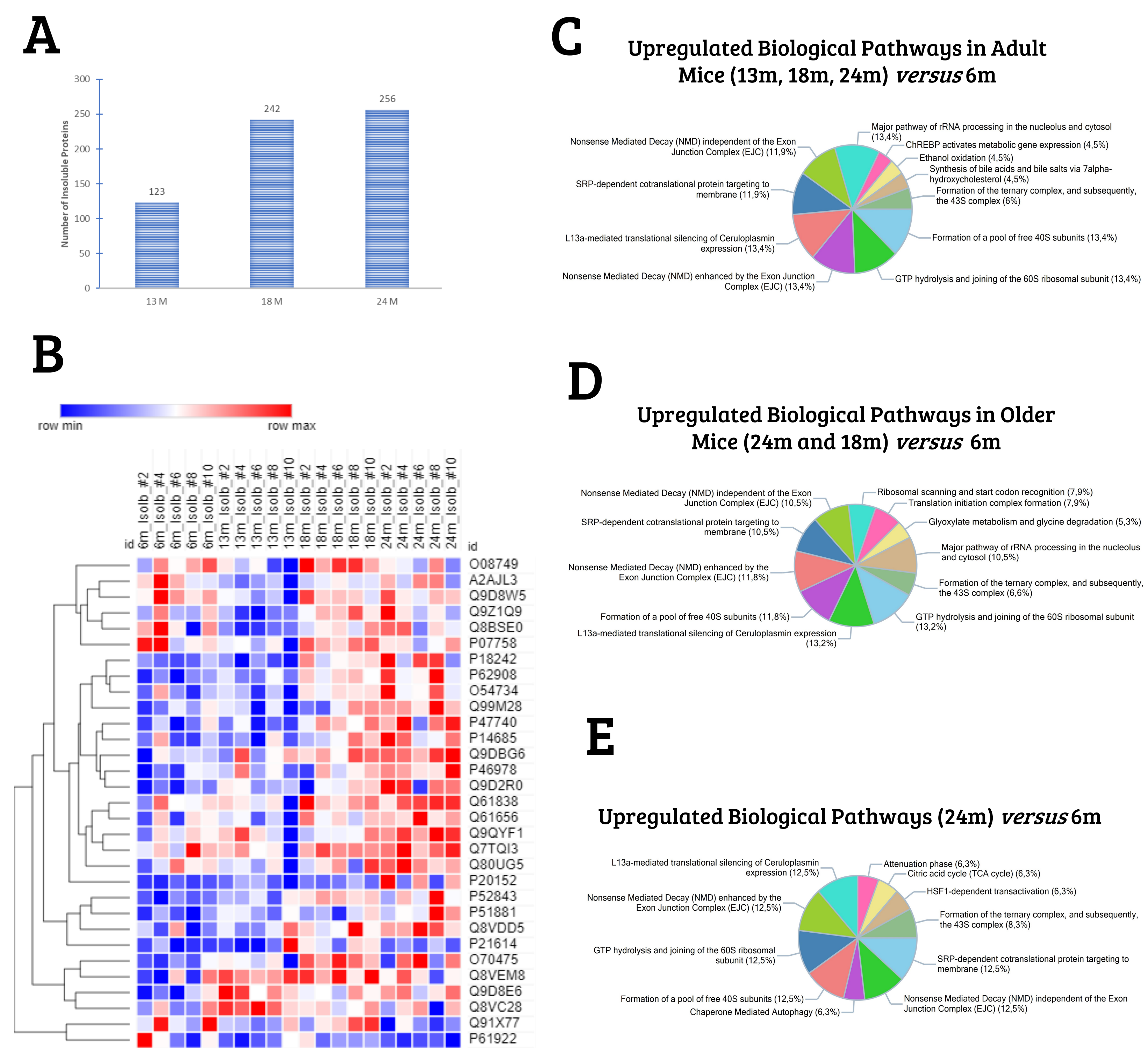
2) Proteomic Characterization of Insoluble Proteins In Liver

A — Number of Insoluble Proteins: 13M 123, 18M 242, 24M 256

B — row min / row max

C — Upregulated Biological Pathways in Adult Mice (13m, 18m, 24m) versus 6m

D — Upregulated Biological Pathways in Older Mice (24m and 18m) versus 6m

E — Upregulated Biological Pathways (24m) versus 6m

Figure 2 . A – Graph of the number of insoluble proteins upregulated throughout aging. The number of insoluble proteins steadily increases with age. B- Heatmap analysis of SWATH MS data of insoluble fraction. Rows display proteins and columns represent the analyzed liver samples. Heatmap was produced in Morpheus software from Broad institute using one minus Pearson correlation for the hierarchical clustering of the quantitative information of proteins considered as altered in the insoluble fraction. Proteins significantly increased are displayed in red, while the proteins that significantly decreased are displayed in blue. 6m, 6 months; 13m, 13 months; 18m, 18 months; 24m, 24 months. C - Common biological pathways upregulated (Funrich Software) in the insoluble protein fractions of adult mice (24m, 18m, 13m) compared to young mice (6m). D - Common biological pathways upregulated (Funrich Software) in the insoluble protein fractions of older mice (only 18m and 24m) compared to 6m. E - Biological pathways upregulated (Funrich Software) in in the insoluble protein fraction of 24m compared to 6m.

Conclusion

Our results suggest that:
- As mice age, metabolic processes are deregulated.
- The accumulation of detergent-insoluble proteins steadily increases with aging in the mouse liver.
- Most insoluble proteins are involved in translation.

References

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3. Reverendo M, Soares AR, Pereira PM, Carreto L, Ferreira V, Gatti E, Pierre P, Moura GR, and Santos MA (2014). TRNA mutations that affect decoding fidelity deregulate development and the proteostasis network in zebrafish. RNA biology 11(9): 1199–1213.

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