Cell Reports Methods, Volume *4*

Supplemental information

Transgenic sensors reveal compartment-specific effects of aggregation-prone proteins on subcellular proteostasis during aging

Michelle Curley, Mamta Rai, Chia-Lung Chuang, Vishwajeeth Pagala, Anna Stephan, Zane Coleman, Maricela Robles-Murguia, Yong-Dong Wang, Junmin Peng, and Fabio Demontis

Supplemental Figures

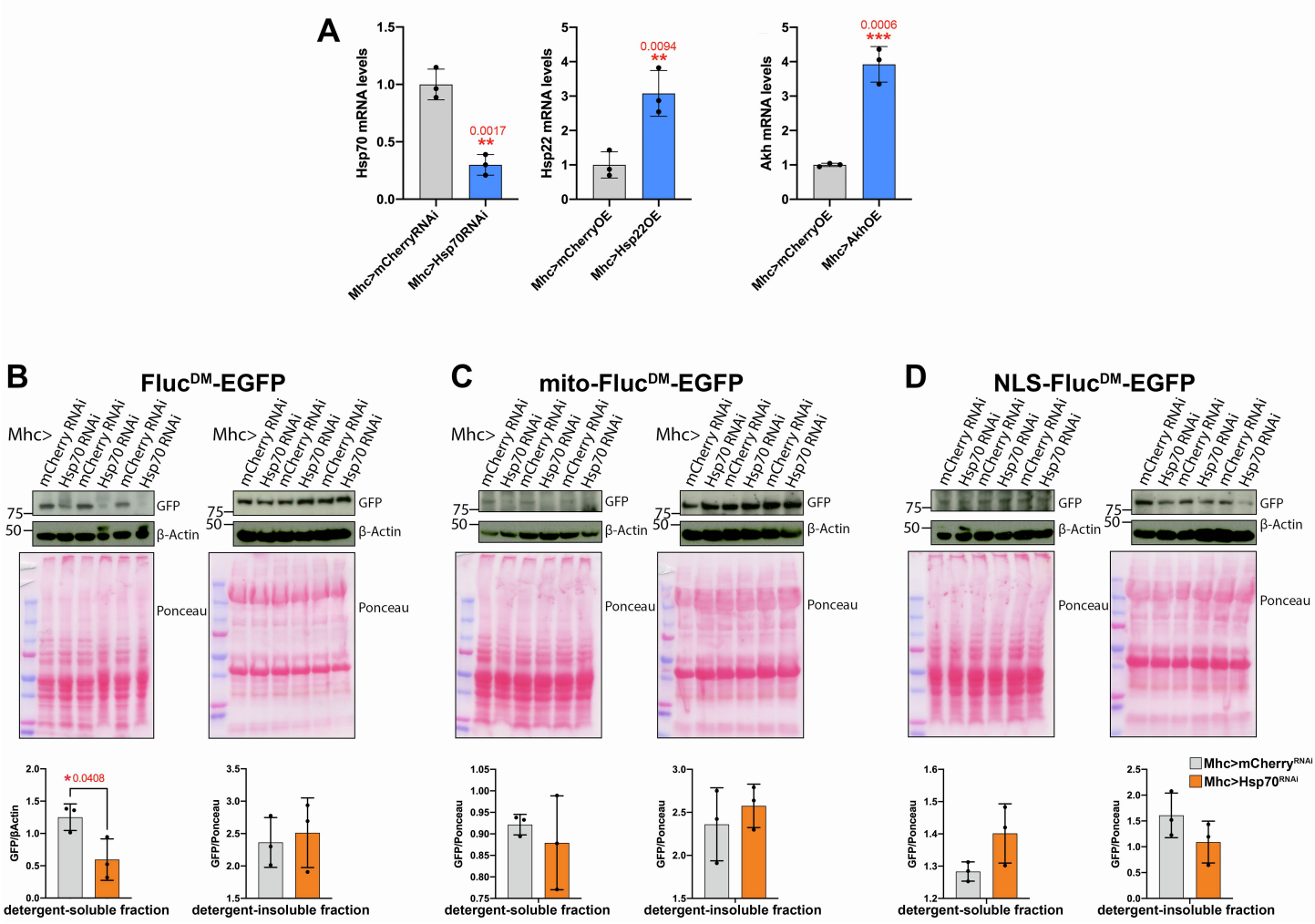

Figure S1. Hsp70 knockdown reduces the detergent-soluble levels of cytoplasmic Fluc^{DM}, related to Fig. 4.

(A) qRT-PCR validation of transgene expression. As expected, *Hsp70* mRNA levels decrease in response to Hsp70 RNAi in skeletal muscle whereas *Hsp22* mRNA levels increase in response to *Hsp22* overexpression. *Akh* mRNA levels increase upon *Akh* overexpression. In (A-B), n=3 (biological replicates) with the mean ±SD indicated; ***P*<0.01, ****P*<0.001 (unpaired two-tailed t-test).

(B-D) Western blots of detergent-soluble and insoluble fractions from skeletal muscle with Hsp70 knockdown (*Mhc>Hsp70^{RNAi}*; orange) versus controls (*Mhc>mCherry^{RNAi}*; gray). Anti-GFP antibodies were used to detect the EGFP-tagged Fluc^{DM} variants targeted to the cytoplasm (Fluc^{DM}; (B)), the mitochondria (mito-Fluc^{DM}; (C)), and the nucleus (NLS-Fluc^{DM}; (D)). Ponceau staining and β-actin are shown as normalization control. Hsp70 RNAi significantly decreases the detergent-soluble levels of the untargeted (~cytoplasmic) Fluc^{DM} whereas NLS-Fluc^{DM} and mito-Fluc^{DM} are not affected. N=3 (biological replicates) with the mean ±SD indicated; **P*<0.05 (unpaired two-tailed t-test).

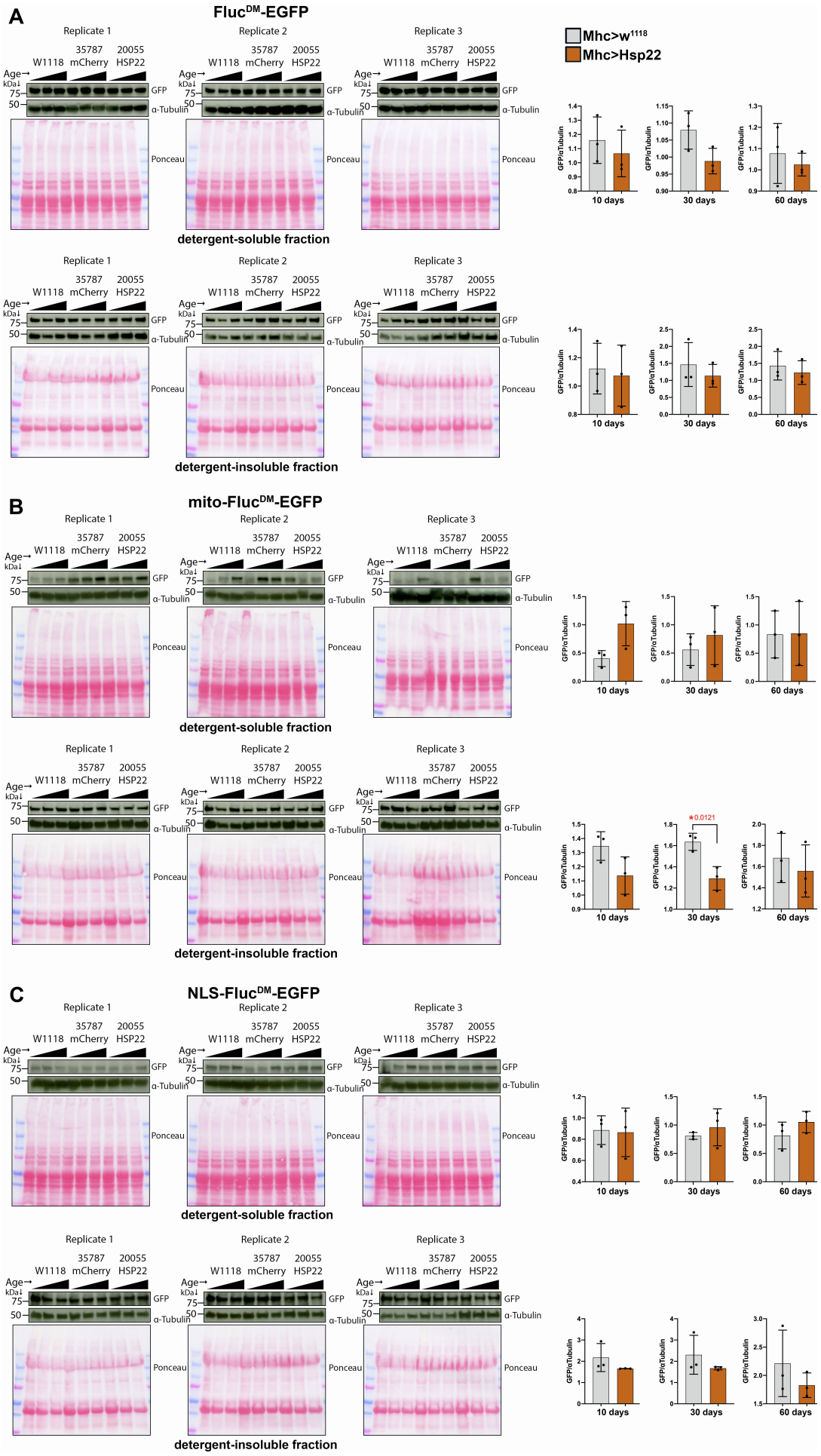
A
Fluc^DM-EGFP
Replicate 1
Replicate 2
Replicate 3
W1118
35787 mCherry
20055 HSP22
Age→
kDa↓
75
50
GFP
α-Tubulin
Ponceau
detergent-soluble fraction
detergent-insoluble fraction
Mhc>w1118
Mhc>Hsp22
GFP/αTubulin
10 days
30 days
60 days
B
mito-Fluc^DM-EGFP
C
NLS-Fluc^DM-EGFP
*0.0121

Figure S2. Hsp22 overexpression reduces the detergent-insoluble levels of mitochondrial Fluc^{DM}, related to Fig. 4.

(A-C) Western blots of detergent-soluble and insoluble fractions from skeletal muscle with Hsp22 overexpression (*Mhc>Hsp22*; brown) versus controls (*Mhc>w^{1118}*; gray). Anti-GFP antibodies were used to detect the EGFP-tagged Fluc^{DM} variants targeted to the cytoplasm (Fluc^{DM}; (A)), the mitochondria (mito-Fluc^{DM}; (B)), and the nucleus (NLS-Fluc^{DM}; (C)). Ponceau staining and α-tubulin are shown as normalization control. Hsp22 overexpression decreases the detergent-insoluble levels of mito-Fluc^{DM} (B). N=3 (biological replicates) with the mean ±SD; *P<0.05 (unpaired two-tailed t-test).

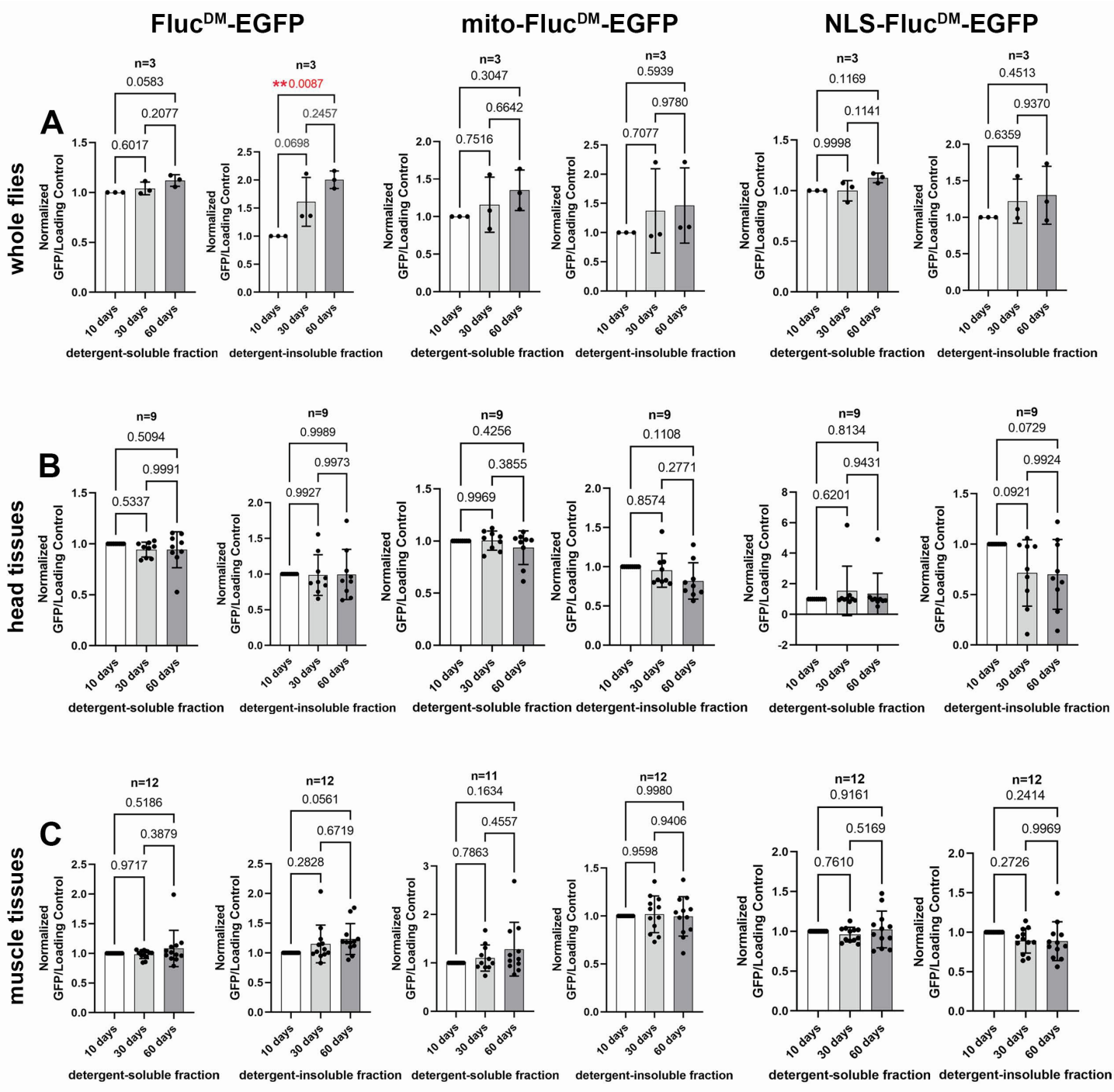

Figure S3. The solubility of Fluc^{DM} sensors is overall maintained during normal aging in *Drosophila*, related to Fig. 4.

(A-C) Western blots of detergent-soluble and insoluble fractions from whole flies (A), heads (B), and thoraces (C) at different ages (10, 30, and 60 days of age). There is a significant increase in the detergent-insoluble levels of Fluc^{DM} (but not of mito-Fluc^{DM} and NLS-Fluc^{DM}) with aging in extracts from whole flies. However, there is no modulation of the detergent-soluble and insoluble-levels of Fluc^{DM}, mito-Fluc^{DM}, and NLS-Fluc^{DM} in heads and thoraces, which are enriched respectively for tissues of the central nervous system and skeletal muscle. The n(biological replicates) is indicated in each figure, with the mean ±SD; $^{**}P<0.01$ (one-way ANOVA).

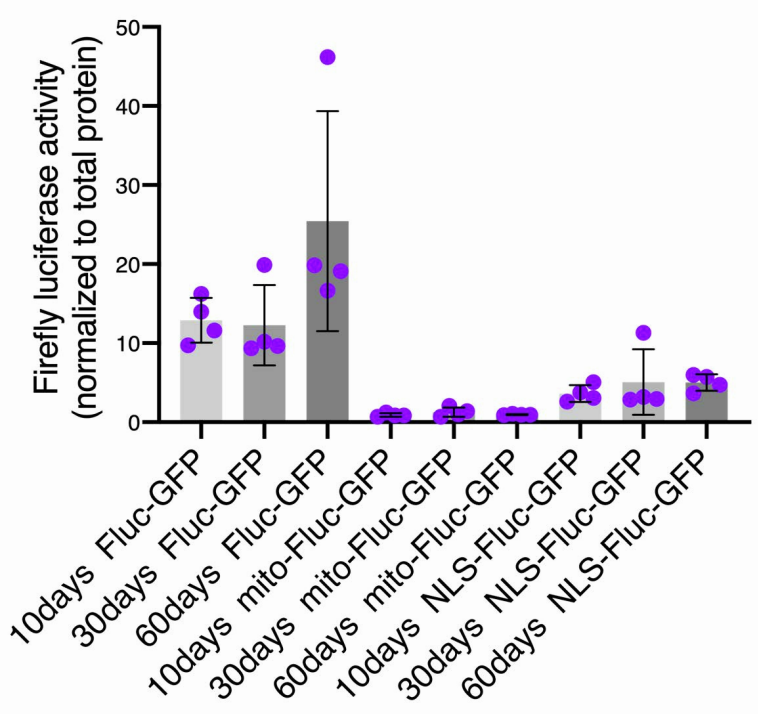

Figure S4. Monitoring the folding and activity of compartment-targeted Fluc^{DM} variants with luciferase assays, related to Fig. 4. Firefly luciferase assays from thoraces of flies with Fluc^{DM}-GFP, mito-Fluc^{DM}-GFP, and NLS-Fluc^{DM}-GFP at different ages (10, 30, and 60 days). The luminescence is normalized by the total protein content. There is no significant age-associated change in the luciferase activity of each Fluc^{DM} sensor in skeletal muscle (thoraces), consistent with the western blot results in Fig. S3C. The mean ±SD is shown with n=4.

The activity of the mitochondrial and nuclear luciferases (mito-Fluc^{DM}-GFP and NLS-Fluc^{DM}-GFP) is lower than that of the general, ~cytoplasmic Fluc^{DM}-GFP luciferase, presumably because the nuclear and mitochondrial luciferases are less active or less stable than the general Fluc^{DM} due to compartment-specific challenges to their activity and/or stability.

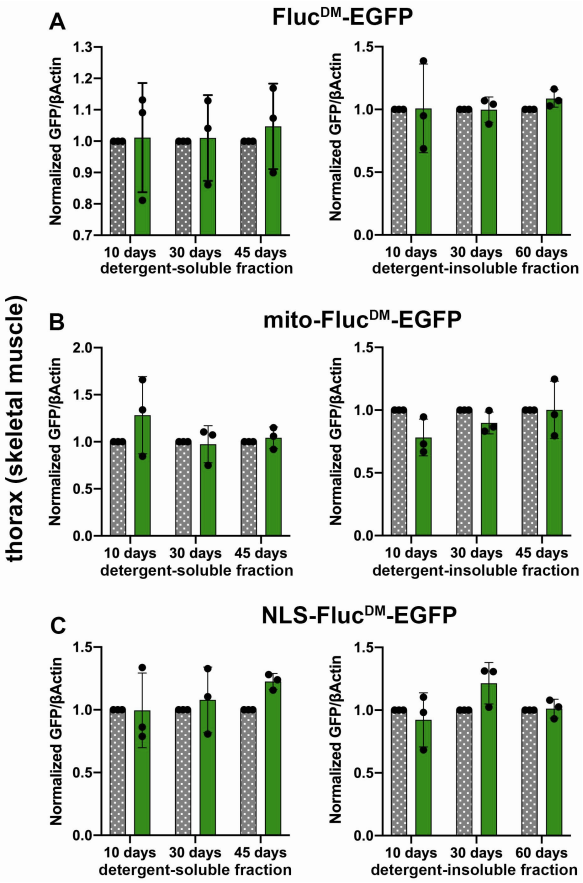

Figure S5. Amyrel does not regulate Fluc^{DM} solubility in skeletal muscle during aging, related to Fig. 5. (A-C) Quantification of normalized GFP/β-actin levels from western blots of thoracic extracts indicates that Amyrel (green) does not regulate proteostasis in skeletal muscles when compared to controls (gray).

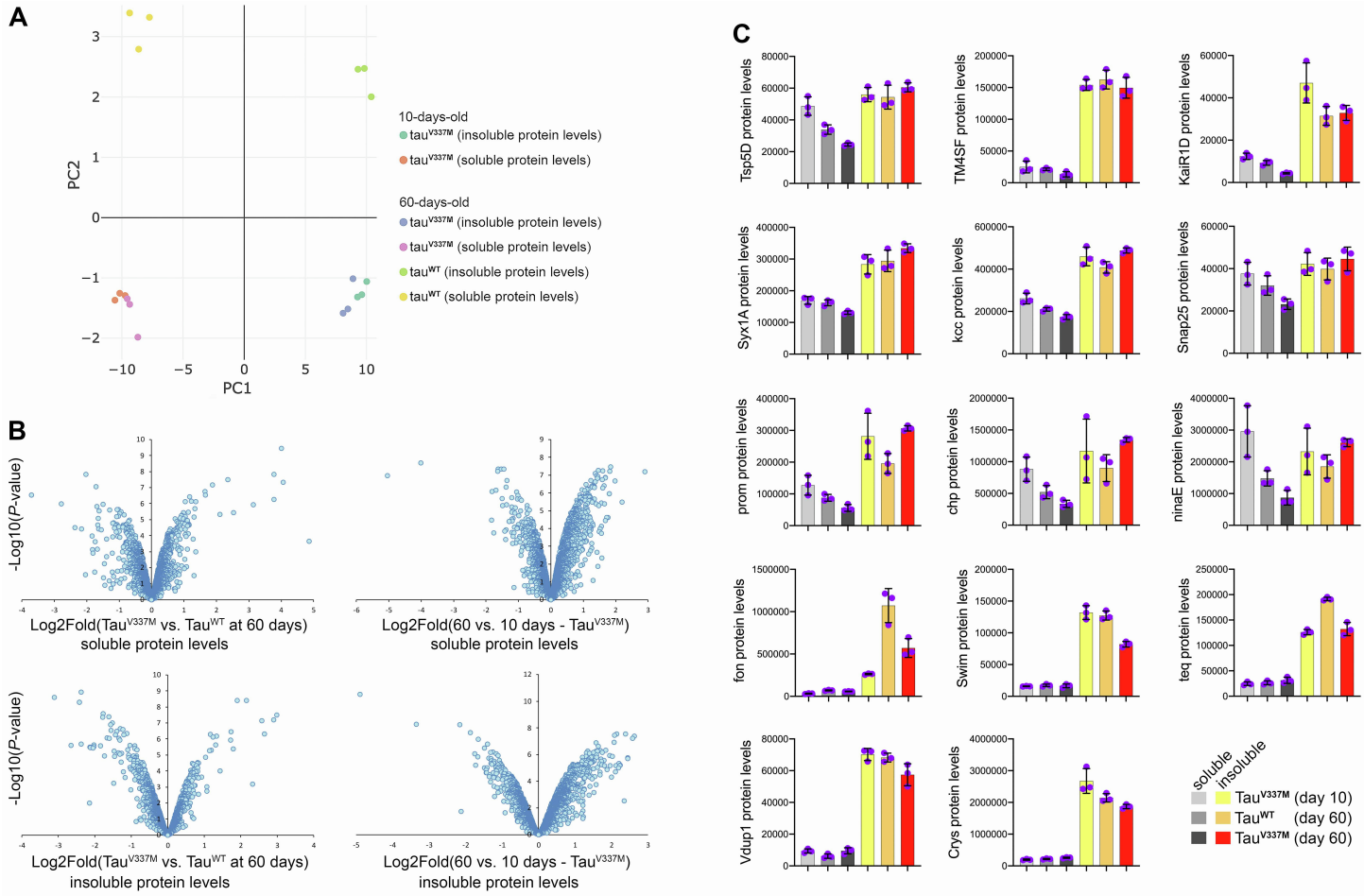

Figure S6. Changes in protein solubility induced by pathogenic tau^{V337M}, tau^{WT}, and aging in *Drosophila*, related to Fig. 7. (A) PCA of proteomics of detergent-soluble and insoluble fractions. (B) Overall representation of the proteomic changes induced by tau in detergent-soluble and insoluble fractions. The *y*-axis reports the -log$_{10}$(*P*-value) whereas the *x*-axis displays the log$_2$Fold changes induced by tau^{V337M} versus tau^{WT} in old age (left panels), and by tau^{V337M} at ~60 days versus 10 days (right panels) for the soluble (top panels) and insoluble (bottom panels) protein levels. (C) Graphs of detergent-soluble and insoluble levels of selected proteins with tau^{V337M}-induced changes in solubility. These graphs refer to the examples shown in Fig. 7H-I and report the detergent-soluble and insoluble levels of significantly regulated proteins (all *P*<0.05). N=3 (biological replicates) with the mean ±SD indicated.

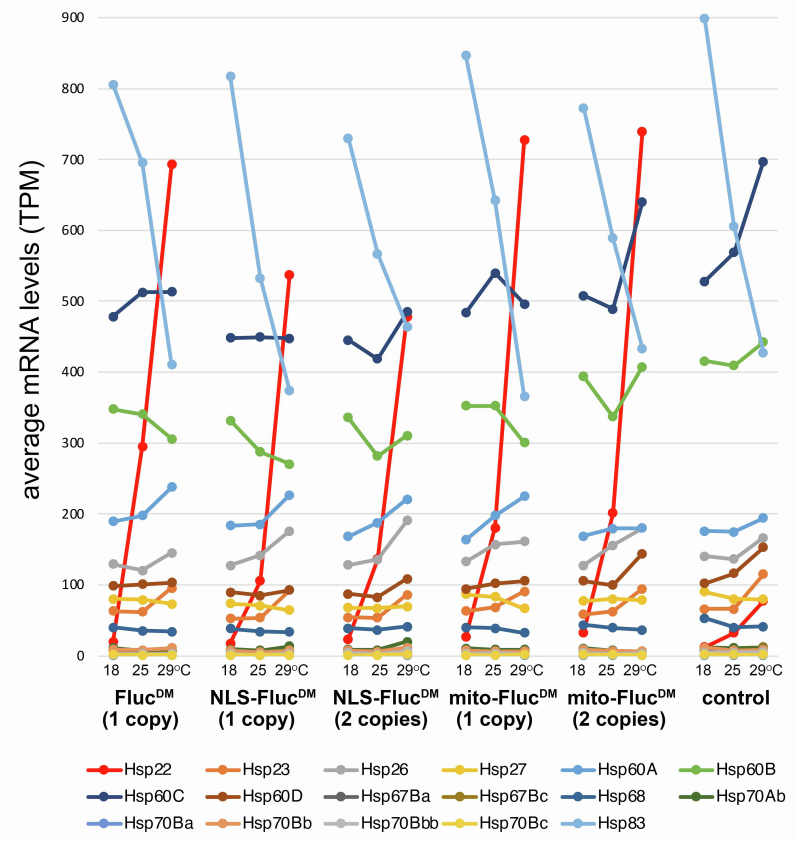

Figure S7. Fluc^{DM} proteins do not induce a heat shock response, related to Fig. 3. The average mRNA levels (TPM) of heat shock proteins were obtained from 3 biological replicates (Table S1). Compartment-targeted Fluc^{DM} variants do not have a major impact on the expression of heat shock proteins, compared to controls with no Fluc^{DM} expression. However, all Fluc^{DM} variants strongly induce the mitochondrial chaperone Hsp22 (red): this occurs at 18ºC and even more noticeably at 25ºC and 29ºC. Similar changes are induced by 1 versus 2 copies of the Fluc^{DM} transgenes. Apart from Hsp22, Fluc^{DM} variants do not appear to induce a heat shock-like response as they do not upregulate the expression of multiple chaperones but only of Hsp22.