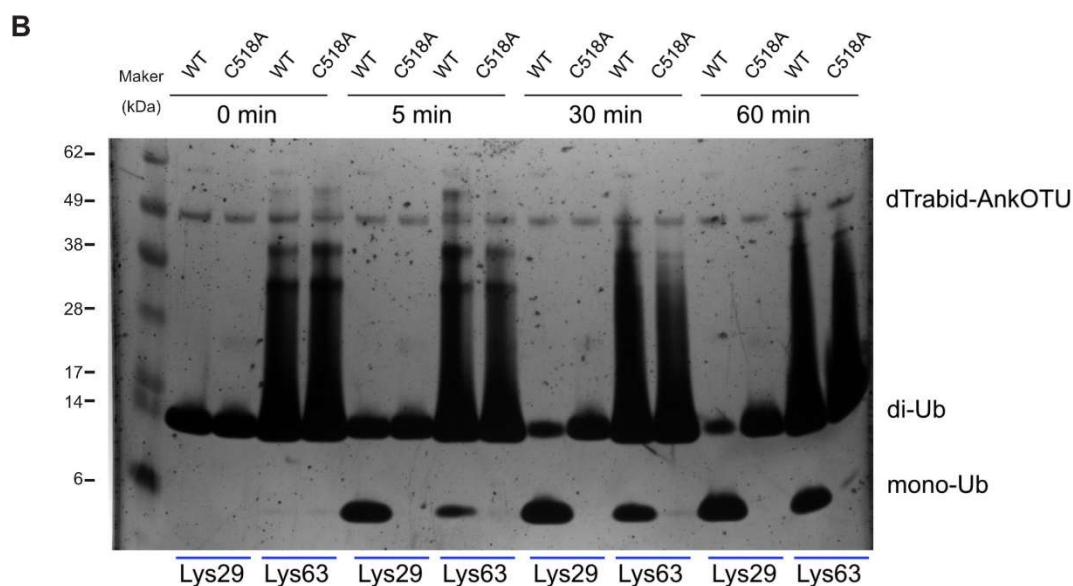
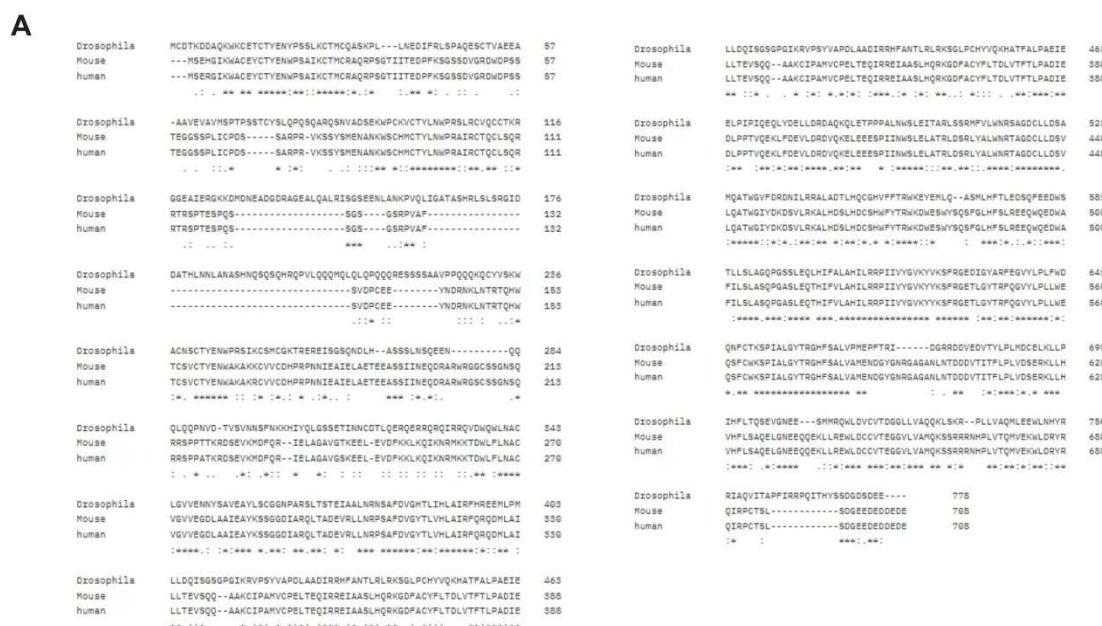


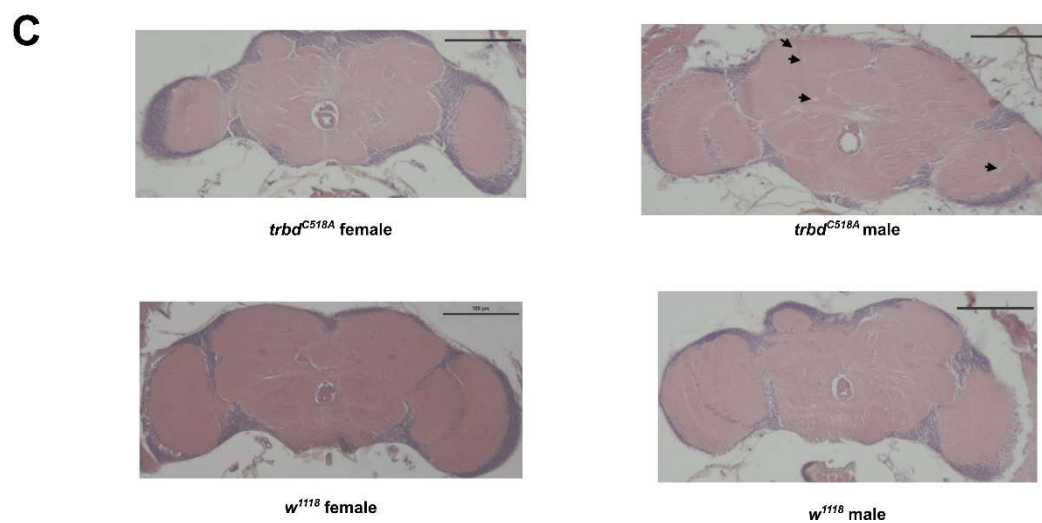
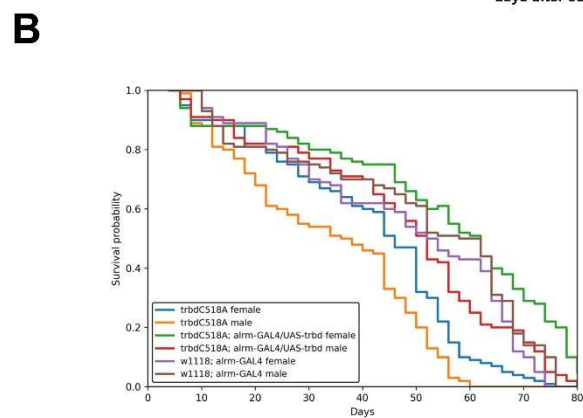
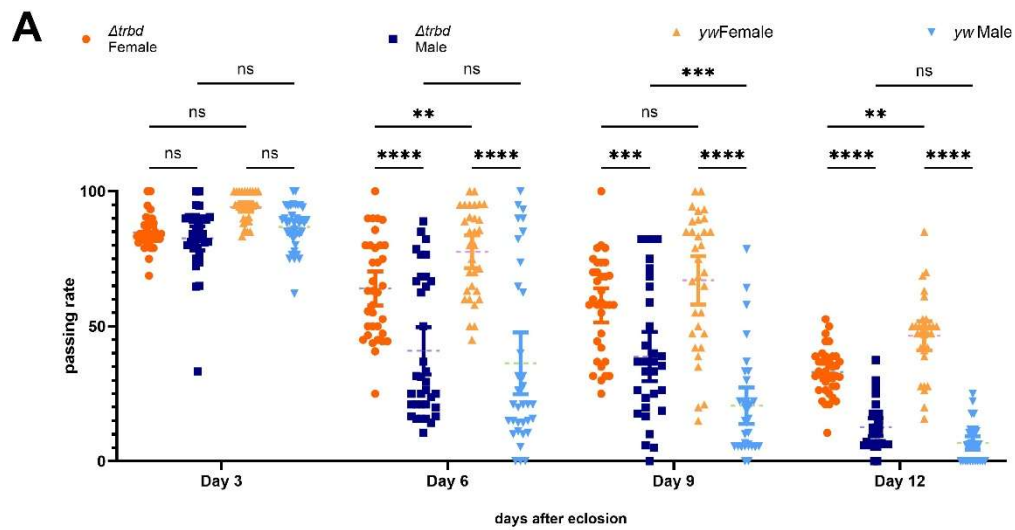
2 **Supplementary Figure 1.**



4 **(A) The amino acid alignment of *Drosophila* Trabad, human Trabad and mouse**
5 **Trabad using Clustal Omega.**

6 **(B) The *Drosophila* deubiquitylase (DUB) Trabad hydrolyzes Lys29 and Lys63 di-**
7 **ubiquitin chains.** An *in vitro* DUB assay (uncropped gel) showing the catalytic activity
8 of 600 nM dTrabad-AnkOTU and its catalytically inactive mutant, dTrabad-AnkOTU-
9 C518A. Samples were incubated with Lys29- or Lys63-linked di-ubiquitin (di-Ub)
10 chains and analyzed at 0, 5, 30, and 60 minutes. dTrabad-AnkOTU exhibited a higher
11 cleavage rate toward Lys29 di-ubiquitin chains compared to Lys63 chains, as
12 evidenced by the faster accumulation of mono-ubiquitin (mono-Ub). The C518A
13 mutation completely abolished the hydrolytic activity of dTrabad-AnkOTU across all
14 time points.

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(A) Climbing assay of *yw*; $\Delta trbd$ and *yw* adult flies at days 3, 6, 9, and 12. From day 6 onwards, males exhibited a significantly lower climbing passing rate than females across both genotypes ($p < 0.0001$). *yw* females showed higher passing rates than $\Delta trbd$ females at days 6 and 12 ($p < 0.01$) (mean $\pm$ 95% CI; two-way ANOVA with Tukey's HSD).

(B) Kaplan–Meier survival curves showing the lifespan of *trbd*^{C518A} mutants, *trbd*^{C518A} flies with astrocyte-specific *trbd* rescue (*alrm-GAL4/UAS-trbd*), and wild-type background controls (*w*¹¹¹⁸; *alrm-GAL4*). In females, the median lifespan of *trbd*^{C518A} (46 days) was significantly extended by astrocyte-specific expression of *UAS-trbd* (62 days; $\chi^2 = 33.85$, $p = 5.97 \times 10^{-9}$), exceeding the control lifespan (52 days). In males, the median lifespan of *trbd*^{C518A} (36 days) was partially rescued by astrocyte-specific expression of *UAS-trbd* (52 days), compared to the control (58 days). Pairwise log-rank tests indicate that *trbd*^{C518A} mutants of both sexes are significantly shorter-lived than controls ($p < 0.001$), and that restoring Trbd function specifically in astrocytes significantly mitigates these longevity defects. ($n = 100$ flies per cohort).

(C) Representative eosin staining images of brain lesions for *trbd*^{C518A} females and males as well as control *w*¹¹¹⁸ females and males (scale bar 100 μ m).

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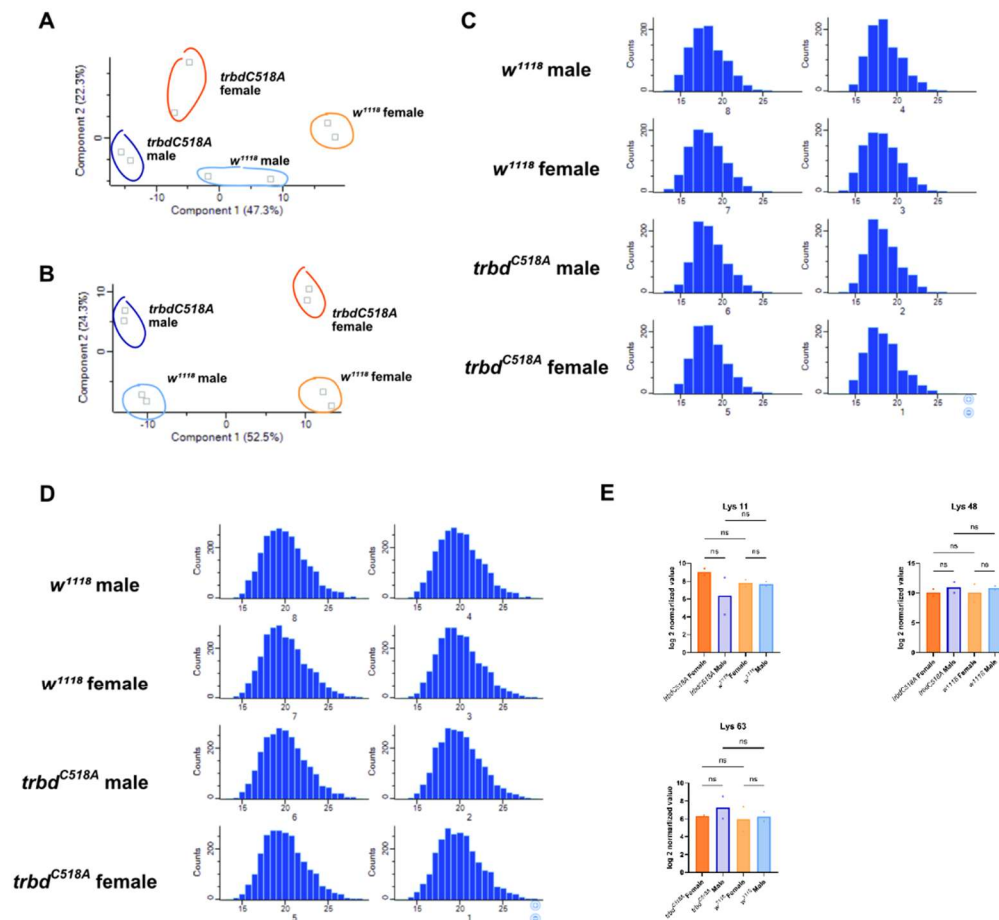


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46 **(C)** Venn diagram displaying the overlap of significantly up-regulated and down-
47 regulated proteins between *trbd*^{C518A} males and females. Numbers in parentheses
48 indicate the total count of significant hits for each group.

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Supplementary Figure 4. Proteome quality control and ubiquitin linkage analysis.



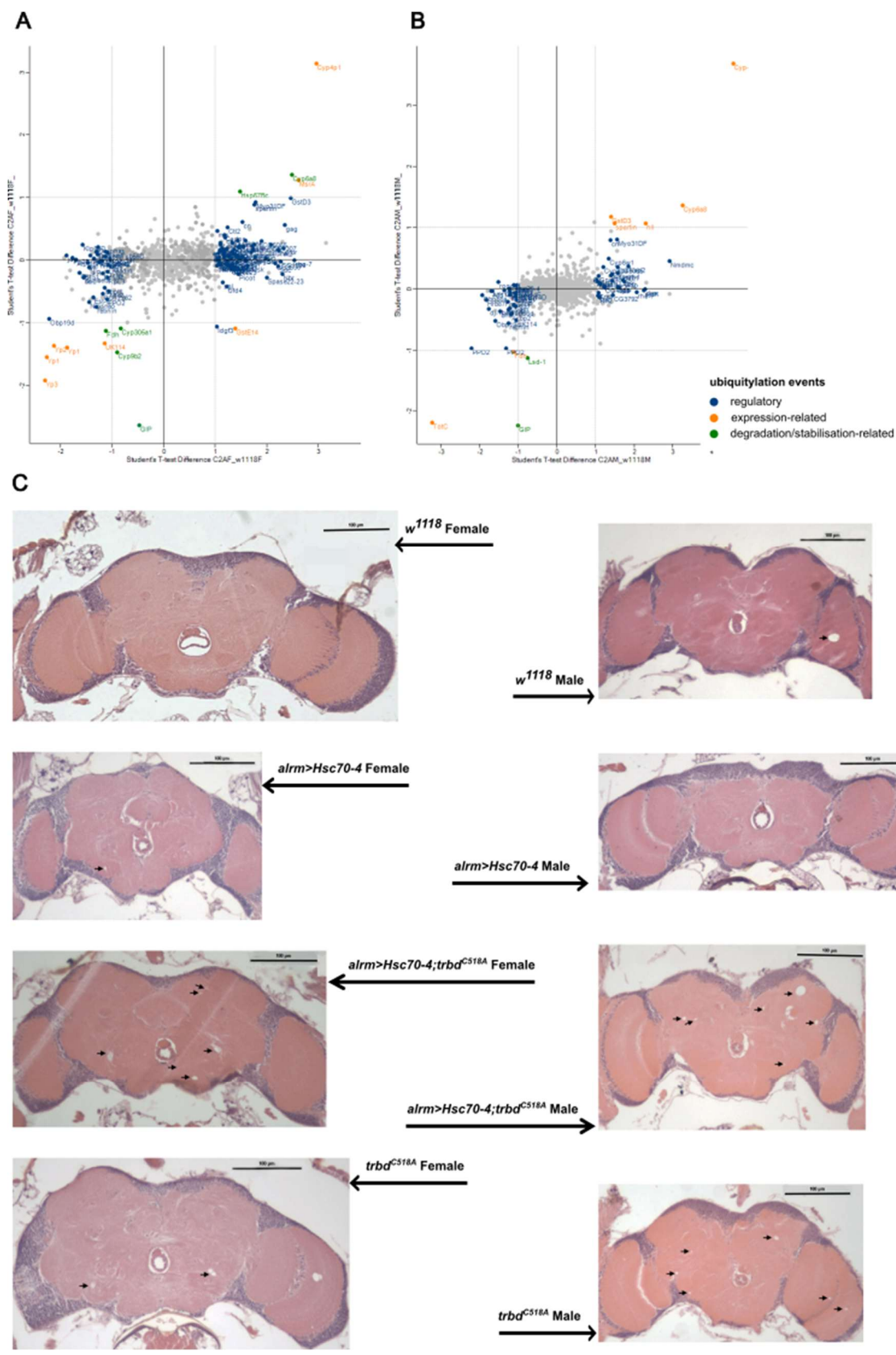
(A–B) Principal Component Analysis (PCA) of the (A) ubiquitinome and (B) proteome. Data points represent individual biological replicates, showing high reproducibility within groups and clear separation between genotypes.

(C–D) Frequency distributions of quantified proteins in the (C) ubiquitinome and (D) proteome. All samples exhibit comparable distribution patterns following normalization.

58 **(E)** Quantitative analysis of ubiquitin chain linkages, which is normalized against
59 proteome. No significant differences were observed in Lys11, Lys48, or Lys63 chain
60 accumulation between *trbd*^{C518A} and *w*¹¹¹⁸ flies of either sex (non-parametric Kruskal-
61 Wallis test with uncorrected Dunn's test).

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Supplementary Figure 5. Sex matched comparison of ubiquitinome and proteome.



66 Hits from ubiquitinome (Fig 5. A and B) were matched to hits from proteome
67 (Supplementary Fig 3A and B). Proteins showing significant changes only in the
68 ubiquitinome (navy). Proteins showing significant changes in both the ubiquitinome
69 and proteome (orange). Proteins showing significant changes only in the proteome
70 (green).

71 **(A)** The result of female *trbd*^{C518A} and *w*¹¹¹⁸.

72 **(B)** The result of male *trbd*^{C518A} and *w*¹¹¹⁸.

73 **(C)** Representative images of eosin-stained brains for *w*¹¹¹⁸, *alrm>Hsc70-4*,
74 *alrm>Hsc70; trbd*^{C518A} and *trbd*^{C518A} (top to bottom). The females are on the left and
75 males are on the right.

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83 **Supplementary data 1. A list and GO data analysis for proteins with significant**
84 **representation in the proteome and/or ubiquitinome. Supplementary Data 1**
85 **contains all the data for Fig 5 and Supplementary Fig 3, 5A and B.**

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87 **Supplementary data 2. Raw data for all quantitative analysis in the study.**

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89 **Supplementary data 3. Material list for the study.**

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91 **Supplementary data 4. Uncropped gels and blots.**