Supplemental Figure 1

Motility assays validate non-toxic presence of N-VCP in Drosophila.

Negative geotaxis assays conducted in flies pan-neuronally expressing N-VCP. Driver: elav-Gal4. Tests were performed weekly starting on day 7 and continuing through day 42. N-VCP-expressing flies were compared to flies with the same genetic background and pan-neuronal driver but lacking N-VCP. Means +/- SD. No statistical significance was found under any conditions; two-tailed student's t-tests. N>70 flies per genotype.
Supplemental Figure 2

Daily alive adult numbers from Figure 2C.

Another graphical representation of the survival analyses shown in figure 2C. Whereas figure 2C shows the ‘probability of survival’, these curves show the ‘daily alive percentages’ of flies in each group. As discussed in ‘Results’, flies denoted by green color likely represent the effect of N-VCP more accurately as improvements in longevity begin during development. As the zoomed-in portion of the longevity curve shows, the strongest flies expressing N-VCP (green) outlive those expressing pathogenic ataxin-3 alone (red), approaching ‘Control’ flies, denoted by black color in the upper portion of the graph.

A point to address when comparing the results from the ubiquitous driver (this graph and figure 2C) to those with the pan-neuronal drivers (figure 2D,E): a handful of flies that co-express pathogenic ataxin-3 alongside N-VCP in all tissues live markedly longer than those that express the same proteins only in neurons. This outcome may indicate non-neural aspects of lethality from ataxin-3, which deserve future attention. Indeed, we recently reported that pathogenic ataxin-3 causes different extents of lethality in neuronal versus glial cells in Drosophila (Johnson et al., 2020).
Supplemental Figure 3

Motility assays with the ‘stronger’ pathogenic ataxin-3.

Negative geotaxis performed with flies expressing the ‘stronger’ pathogenic ataxin-3, without or with N-VCP. Means +/- SD. Statistics: one-tailed student’s t-tests comparing “red” and “green” histograms to each other. P value not shown if not <0.05. N>70 flies per genotype.
Supplemental Figure 4

VCP increases pathogenic ataxin-3 toxicity in a dose-dependent manner.

(A, B) Longitudinal observation of developmental stage death among flies ubiquitously expressing the noted version of pathogenic ataxin-3 with zero, one, or two copies of exogenous full-length VCP. Driver: sqh-Gal4. Means +/- standard deviations. P-values: one-tailed student’s t-tests, shown below graphs.