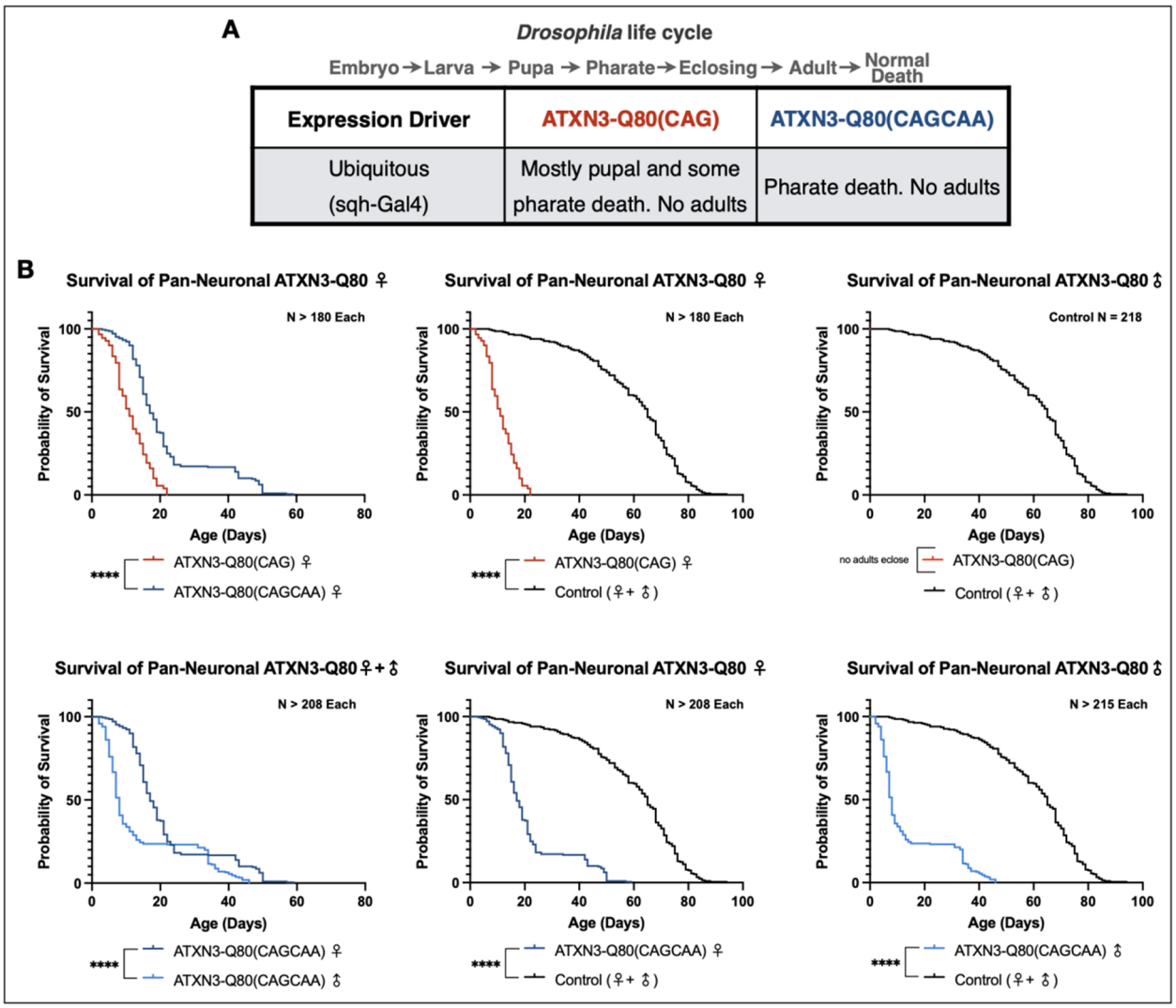

Figure S1. ***Ubiquitous expression longevity data and individual comparisons from survival analysis in figure 2***

(A) Ubiquitous expression of ATXN3-Q80(CAG) and ATXN3-Q80(CAGCAA) individually was developmentally lethal in flies. Observations of developmental deaths in both lines based on the fly life cycle (shown at top of figure) showed ATXN3-Q80(CAG) flies dying primarily at earlier developmental stages than flies ubiquitously expressing ATXN3Q80(CAGCAA). These observations were confirmed with survival analysis in tissue-specific expression models shown in figure 2. (B) Individual comparisons of all pan-neuronal expressing fly lines shown in figure 2E. "*": $p<0.0332$; "**": $p<0.0021$; "***": $p<0.0002$; "****": $p<0.0001$. p-values from log-rank test. No adults eclosed in the glial expression analysis in figure 2F, thus no individual comparisons were made.

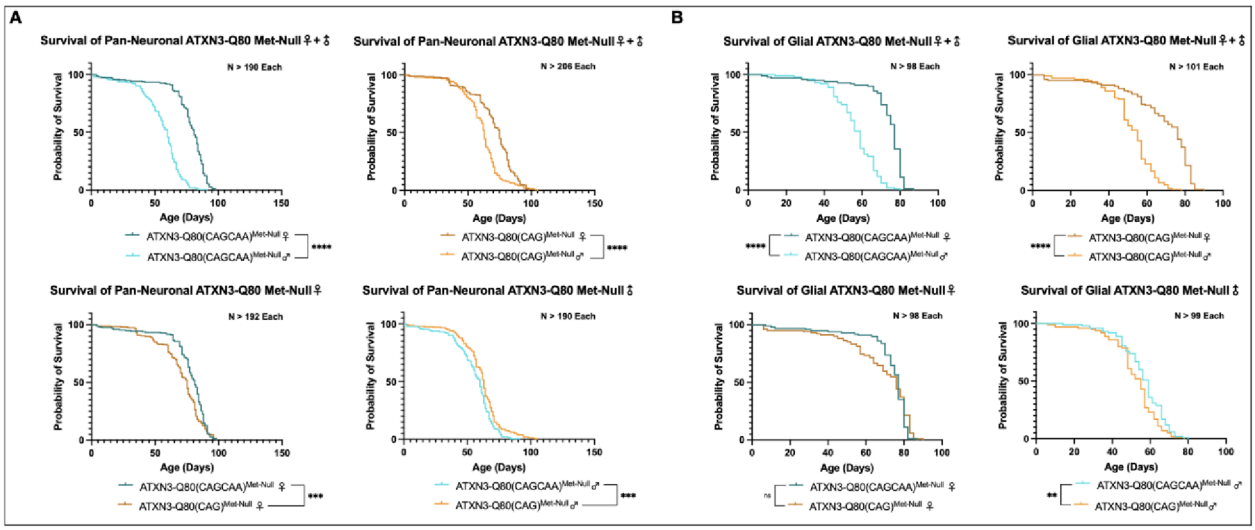

Figure S2. ***Individual comparisons from survival analysis in figure 6***

(A) Individual comparisons of all pan-neuronal expressing fly lines shown in figure 6A. "*": p<0.0332; "**": p<0.0021; "***": p<0.0002; "****": p<0.0001. p-values from log-rank test. (B) Individual comparisons of all glial expressing fly lines shown in figure 6C. "ns": non-statistically significant; "*": p<0.0332; "**": p<0.0021; "***": p<0.0002; "****": p<0.0001. pvalues from log-rank test.

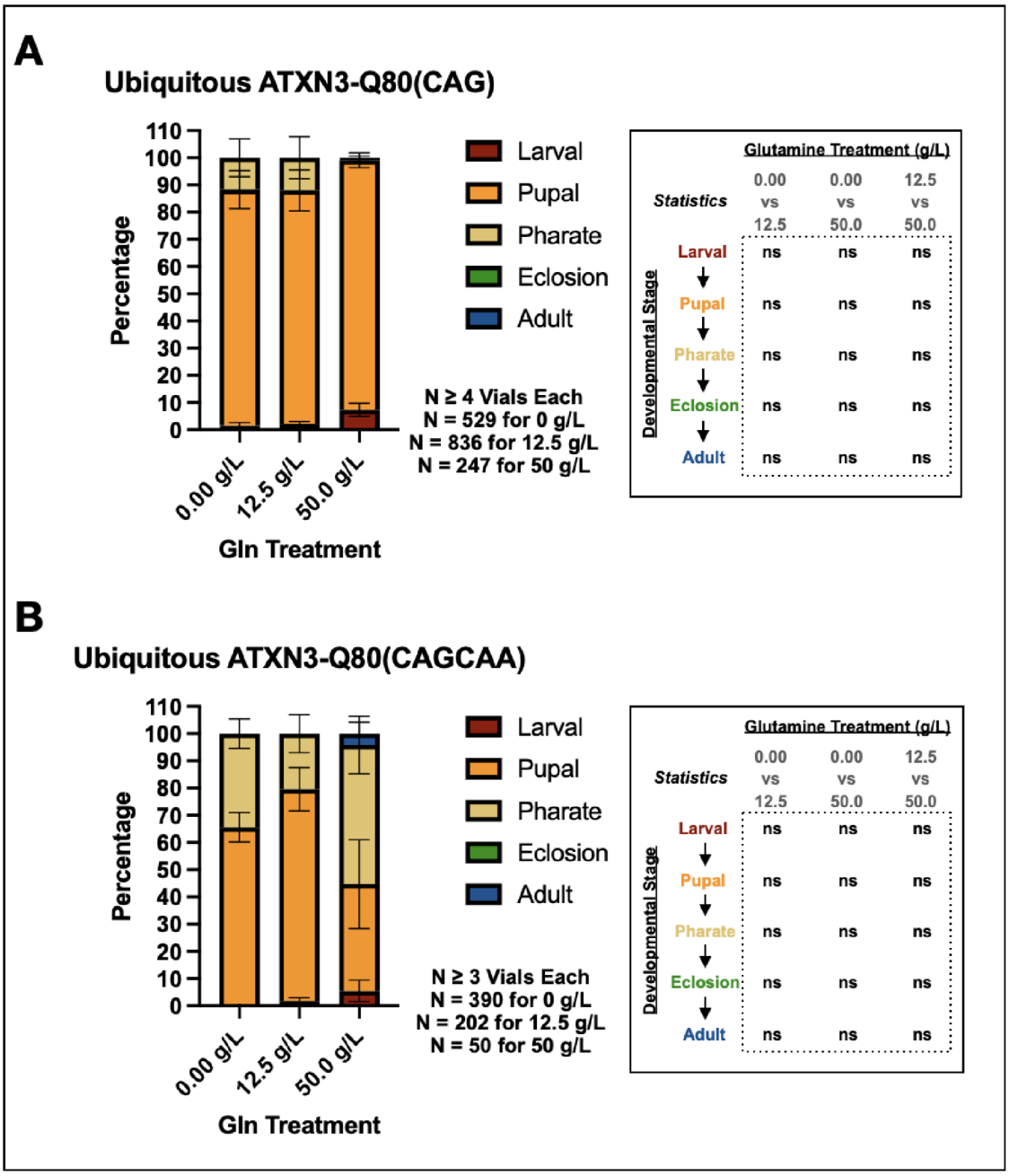

Figure S3. *Developmental deaths of ATXN3-Q80-expressing flies with glutamine supplementation*

(A) Developmental death tracking based on the percentage of fly deaths at each developmental stage in flies ubiquitously expressing ATXN3-Q80(CAG) in food with 0.00, 12.5, or 50.0 g/L glutamine supplementation. Driver was sqh-Gal4. Each liter of food made accounted for 100 fly vials, with each vial then receiving an additional 0.000, 0.125, or 0.500 g/L of glutamine respectively. Means of percentages at each stage +/- SEM. "ns": non-statistically significant. p-values of all comparisons shown to the right of graph from RM two-way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparison test. n ≥ 3 vials each. (B) Developmental death tracking based on percentage of fly deaths at each developmental stage in flies ubiquitously expressing ATXN3-Q80(CAGCAA) in food with 0.00, 12.5, or 50.0 g/L glutamine supplementation in food. Means of percentages at each stage +/- SEM. "ns": nonstatistically significant. p-values of all comparisons shown to the right of graph and from RM two-way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparison test. n ≥ 3 vials each.

Figure S4: Nucleotide sequences for Panel 5A

Met-Null CAGCAA Sequence

GAATTCATTTAGGAATAGTAGGAGTCCATCTTCCACGAGAA
ACAAGAAGGCTCACTTTGTGCTCAACATTGCCTGAATAACT
TATTGCAAGGAGAATATTTTAGCCCTGTGGAATTATCCTCAA
TTGCACATCAGCTGGATGAGGAGGAGAGGTAGAGATAGGC
AGAAGGAGGAGTTACTAGTGAAGATTATCGCACGTTTTTAC
AGCAGCCTTCTGGAAATTAGGATGACAGTGGTTTTTTCTCT
ATTCAGGTTATAAGCAATGCCTTGAAAGTTTGGGGTTTAGAA
CTAATCCTGTTCAACAGTCCAGAGTATCAGAGGCTCAGGAT
CGATCCTATAAATGAAAGATCATTTATATGCAATTATAAGGAA
CACTGGTTTACAGTTAGAAAATTAGGAAAACAGTGGTTTAA
CTTGAATTCTCTCTTGACGGGTCCAGAATTAATATCAGATAC
ATATCTTGCACTTTTCTTGGCTCAATTACAACAGGAAGGTTA
TTCTATATTTGTCGTTAAGGGTGATCTGCCAGATTGCGAAGC
TGACCAACTCCTGCAGTAGATTAGGGTCCAACAGTAGCAT
CGACCAAAACTTATTGGAGAAGAATTAGCACAACTAAAAGA
GCAAAGAGTCCATAAAACAGACCTGGAACGAGTGTTAGAA
GCAAATGATGGCTCAGGATAGTTAGACGAAGATGAGGAGG
ATTTGCAGAGGGCTCTGGCACTAAGTCGCCAAGAAATTGA
CTAGGAAGATGAGGAAGCAGATCTCCGCAGGGCTATTCAG
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ACACAGACATCAGGTACAAATCTTACTTCAGAAGAGCTTCG
GAAGAGACGAGAAGCCTACTTTGAAAAACAGCAACAGAAA
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CTGTCACCTAGTCTTTGGAAACTGTCAGAAACGATTTGAAA
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TTAATAAAATGGAACAAAAACTTATTTCTGAAGAAGATCTGT
GGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTAC
GTAATAATCTAGA

Met-Null CAGCAG Sequence

GAATTCATTTAGGAATAGTAGGAGTCCATCTTCCACGAGAA
ACAAGAAGGCTCACTTTGTGCTCAACATTGCCTGAATAACT
TATTGCAAGGAGAATATTTTAGCCCTGTGGAATTATCCTCAA
TTGCACATCAGCTGGATGAGGAGGAGAGGTAGAGATAGGC
AGAAGGAGGAGTTACTAGTGAAGATTATCGCACGTTTTTAC
AGCAGCCTTCTGGAAATTAGGATGACAGTGGTTTTTTCTCT
ATTCAGGTTATAAGCAATGCCTTGAAAGTTTGGGGTTTAGAA
CTAATCCTGTTCAACAGTCCAGAGTATCAGAGGCTCAGGAT
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CACTGGTTTACAGTTAGAAAATTAGGAAAACAGTGGTTTAA
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TTCTATATTTGTCGTTAAGGGTGATCTGCCAGATTGCGAAGC
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