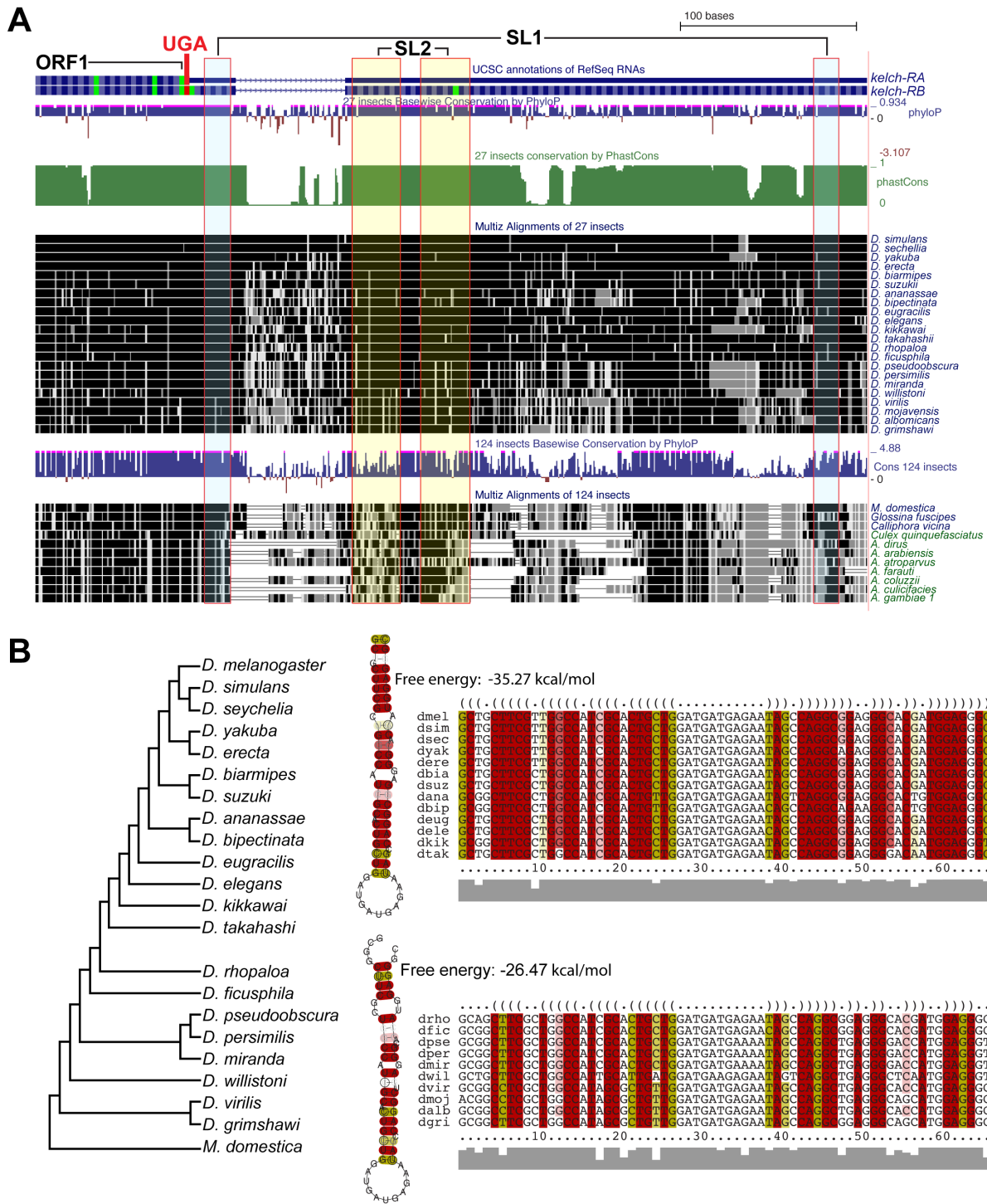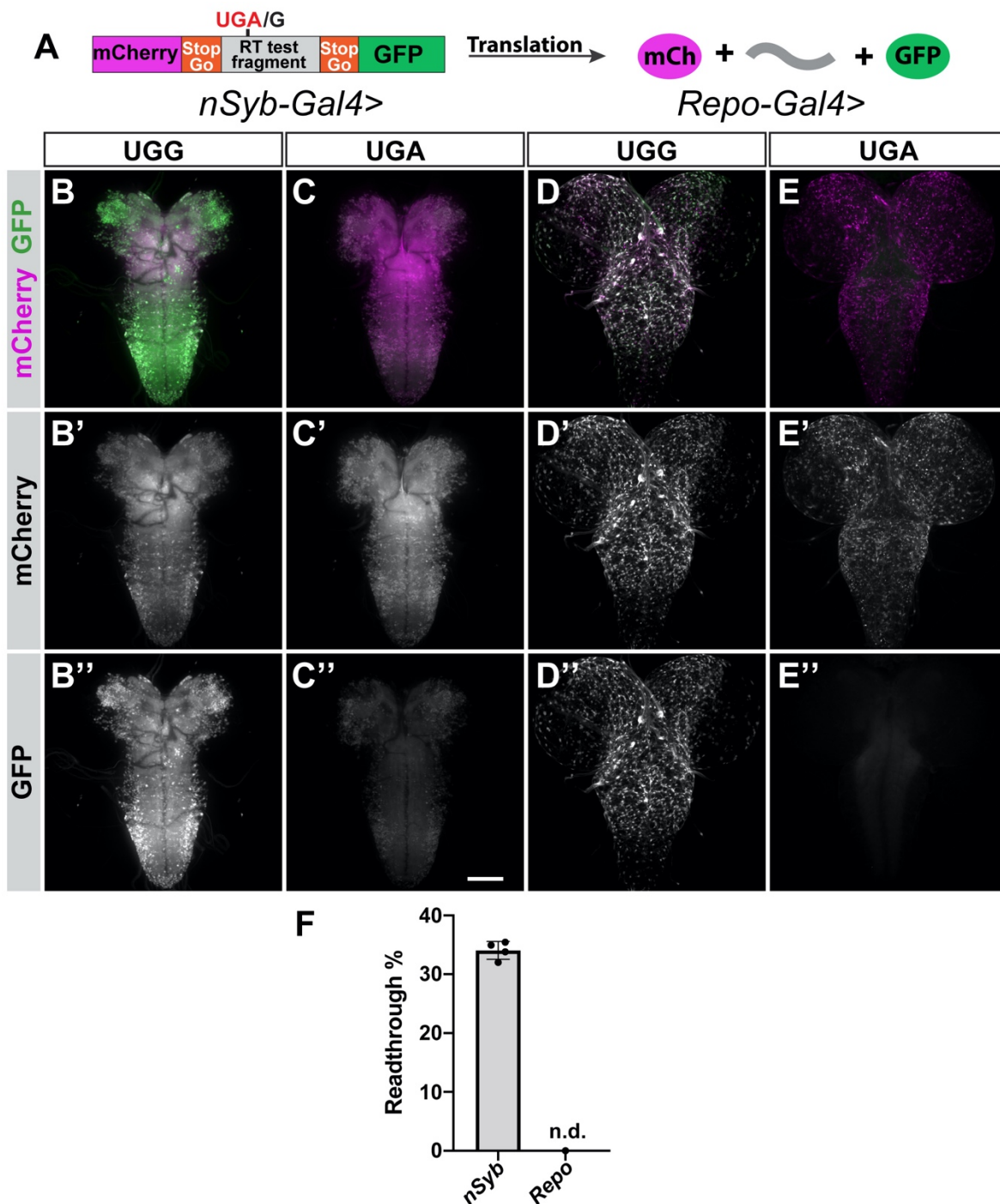## Figure S1

Supplementary Figure 1. Conservation of predicted stem-loop sequences 3' of the *kelch* UGA stop codon. (A) Conservation of the 407 bp *kelch* ORF1 stop codon readthrough fragment displayed as phastCons and Multiz alignments to the dm6 *Drosophila* genome on the UCSC genome browser (1-3). SL1 is highlighted in cyan, SL2 in yellow. (B) Sequences contributing to the predicted *kelch* SL2 stem loop are conserved among *Drosophila* species. A multiple sequence alignment of the displayed sequences was analyzed using RNAalifold from the ViennaRNA package (4). Species closely related to *D. melanogaster* show conserved stem loop structure (top alignment). More distantly related species retain similar potential to form a similar stem loop.
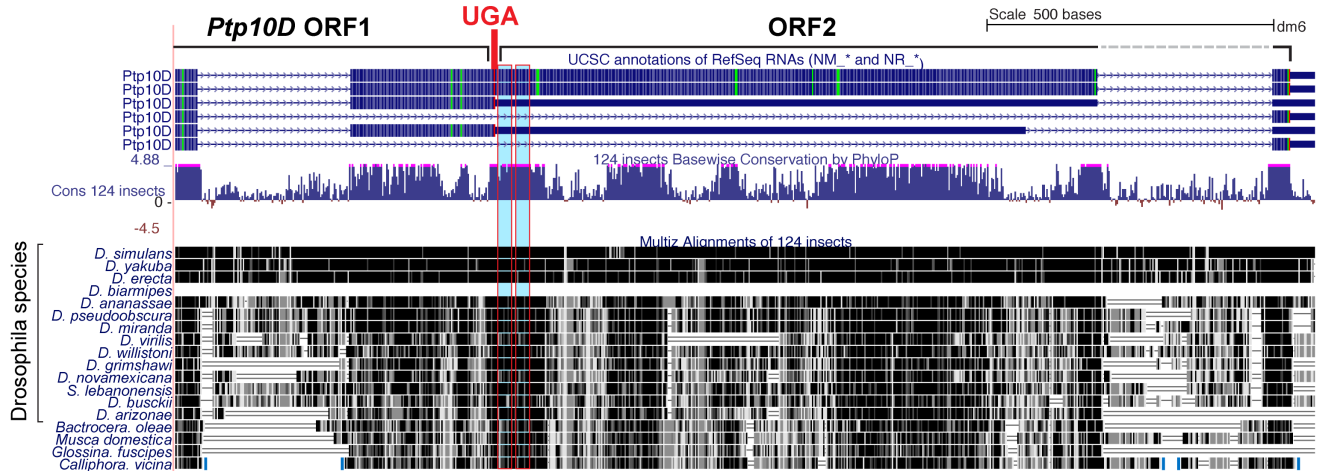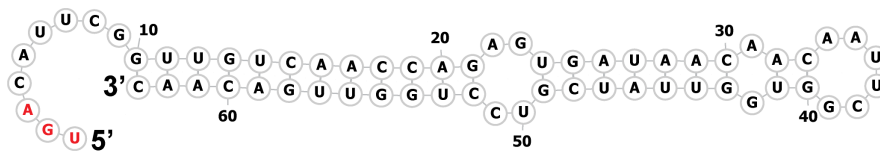
Figure S2

Supplementary Figure 2. Dual-fluorescent readthrough reporter. (A) Diagram of dual-fluorescent readthrough reporter. T2A StopGo sequences flank readthrough test fragment, resulting in production of mCherry and GFP without associated fusion sequence. When expressed in neurons (B, C), GFP expression produced from UGA construct (C'') reveals ~30% readthrough efficiency relative to UGG in-frame control (B''). Expression in glial cells (D-E) produced no detectable GFP (E''). (F) Quantification based on summed fluorescence intensities of neuronal expression. n.d. indicates not determined.
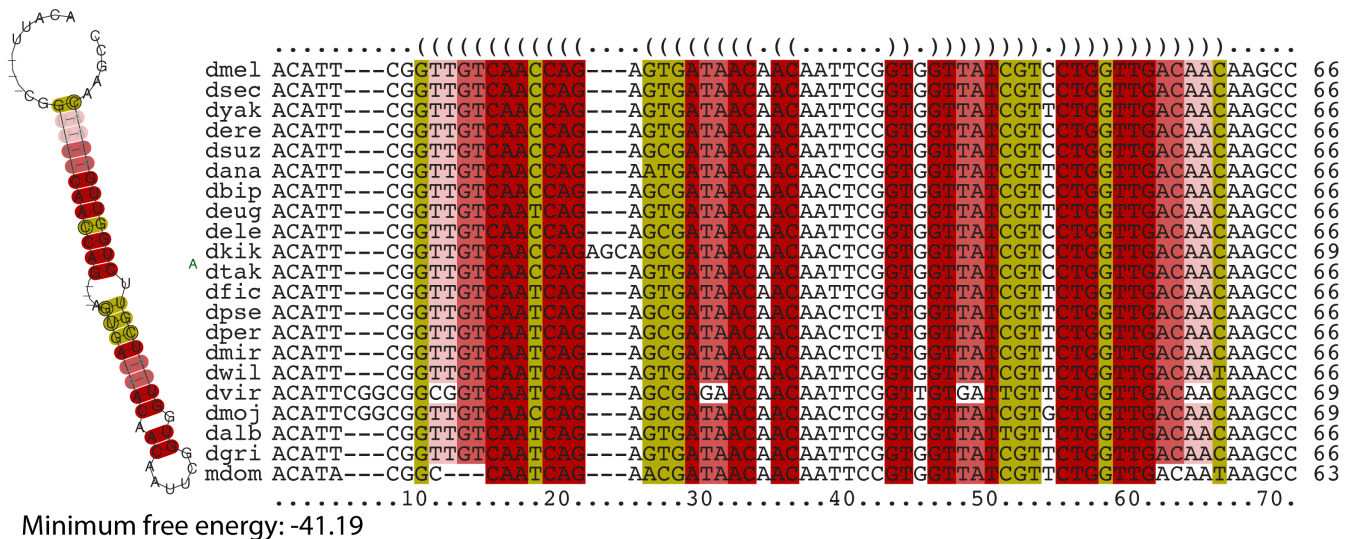
Figure S3

A

B

C

Minimum free energy: -41.19

Supplementary Figure 3. Conservation of predicted stem-loop sequences 3' of the *Ptp10D* UGA stop codon. (A) Conservation of the sequence 3' of the *Ptp10D* ORF1 stop codon displayed as phastCons and Multiz alignments to the dm6 *Drosophila* genome on the UCSC genome browser (1-3). (B) Predicted *Ptp10D* RNA stem loop structure generated using the RNAfold prediction tool (4). Sequence begins immediately following termination codon. (C) Conservation of Ptp10D stem loop structural feature. A multiple sequence alignment of insect sequences was analyzed using RNAalifold from the ViennaRNA package (4). Conservation of predicted pairing sequences is present throughout *Drosophila*, and conservation is also evident in *Musca domestica* (mdom).

Supplementary Information References

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4.	A. R. Gruber, S. H. Bernhart, R. Lorenz, The ViennaRNA web services. *Methods Mol Biol* **1269**, 307-326 (2015).