

Fig. S1. Phenotypic analyses of *Drosophila Formins*. (A-F) Representative images of CIV dendrites in (A) control and (B-F) RNAi knockdowns for *Drosophila Formins* *capu*, *dia*, *Fhos*, *Frl*, and *DAAM*. (G,H) Quantitative neuromorphometric analyses (mean \pm SEM) with N value indicated on the graphs. (I-I'') CIV-specific GAL4477,UAS-mCD8::GFP third instar larval filets triple labeled with (I) Form3, (I') HRP, and (I'') Merge+GFP. Representative image of N=8 dorsal da neuron clusters. Statistical test performed (G,H): one-way ANOVA with Bonferroni correction; ns represents not significant $p > 0.05$.

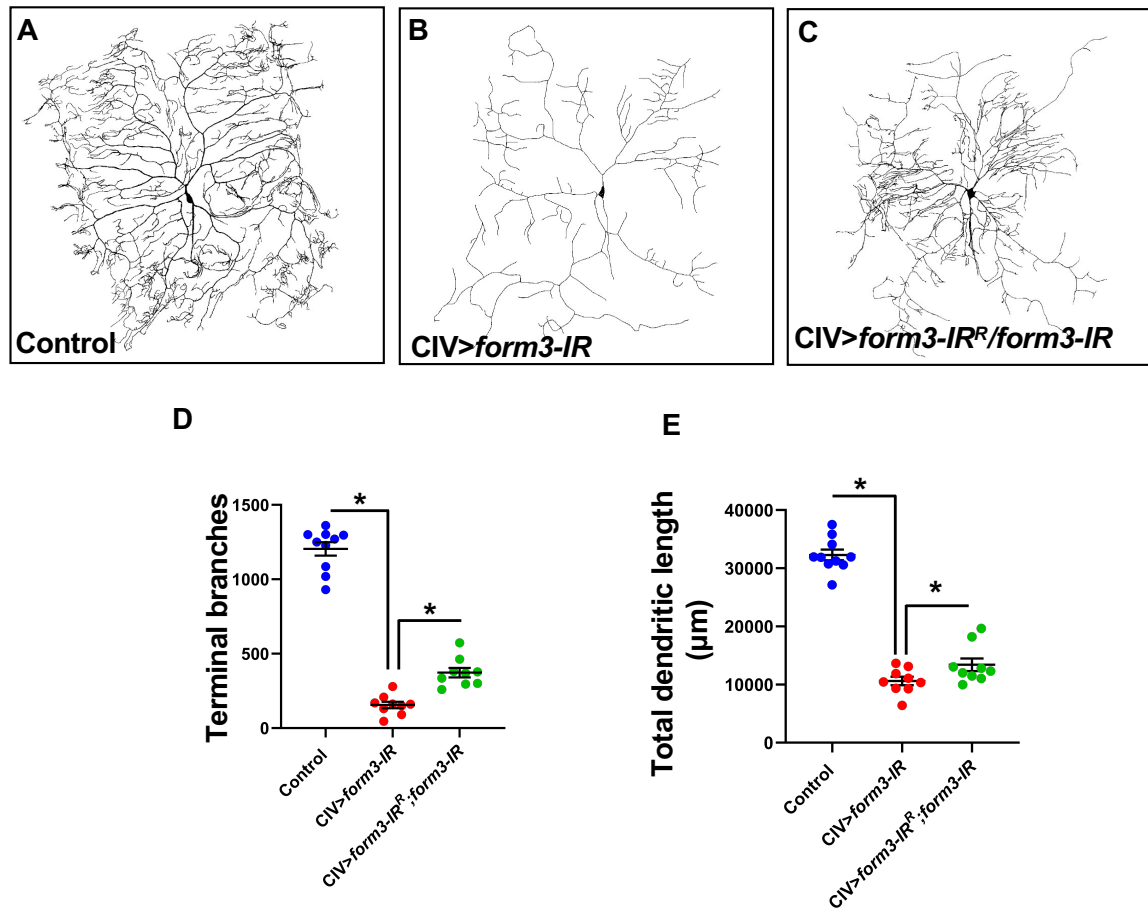


Fig. S2. Co-expression of RNAi-resistant *form3* and *form3-IR* partially rescues *form3-IR*-induced dendritic defects. (A-C) Representative images of CIV neurons in (A) control, (B) *form3-IR*, and (C) *form3-IR^R;form3-IR*. (D,E) Quantitative analyses of the terminal branches and total dendritic length of control (N=11), *form3-IR* (N=10), and *form3-IR^R;form3-IR* (N=10) CIV neurons. Statistical tests performed: (D) unpaired t-test $p < 0.0001$ (control vs. *form3-IR* and *form3-IR* vs. *form3-IR^R;form3-IR*); (E) unpaired t-test $p < 0.0001$ (control vs. *form3-IR*); Mann-Whitney *U* test $p < 0.05$ (*form3-IR* vs. *form3-IR^R;form3-IR*).

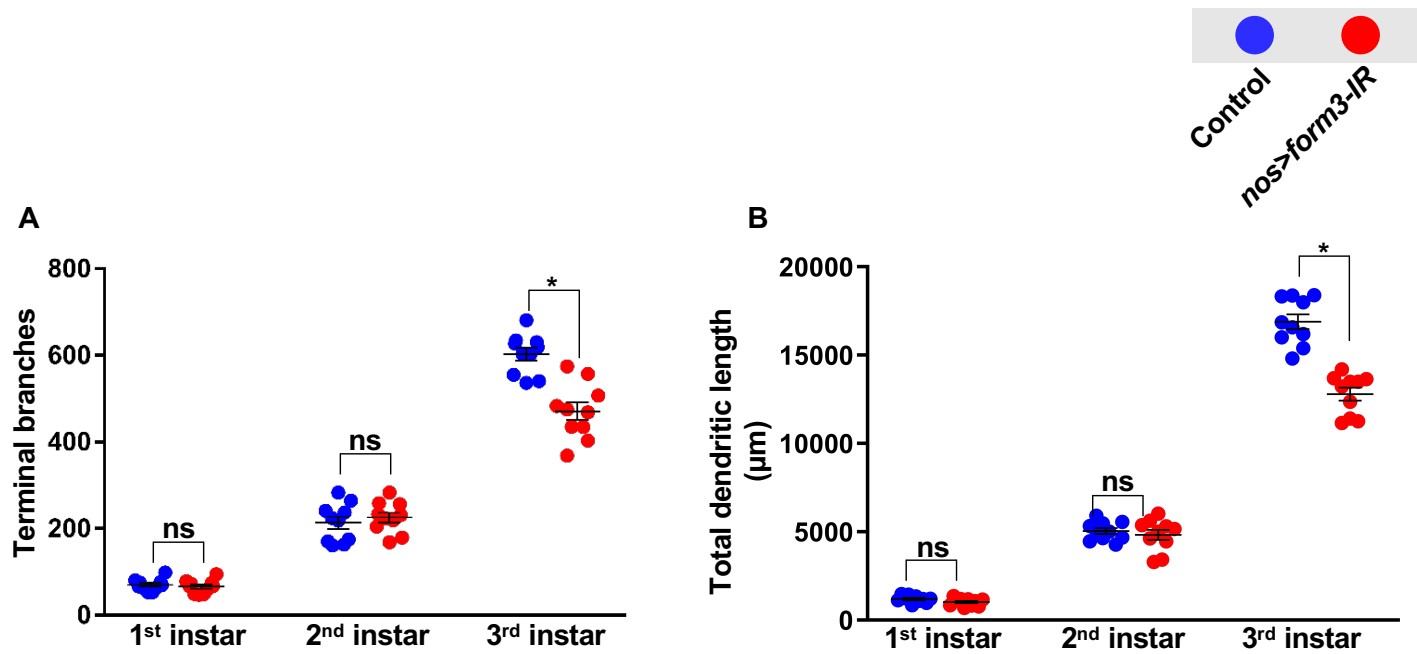


Fig. S3. *Form3* is required to maintain higher order dendritic branching over developmental time.

(A,B) Quantitative analyses of the number of branches (A) and total dendritic length (B) of control (N=10) and *nos-GAL4>form3-IR* (N=10) at different stages of development as indicated on the figure. Statistical test performed: (A,B) unpaired t-test, (A_{3rd-instar}) and (B_{3rd-instar}) $p < 0.0001$. ns represents not significant $p > 0.05$. Error bars indicate SEM.

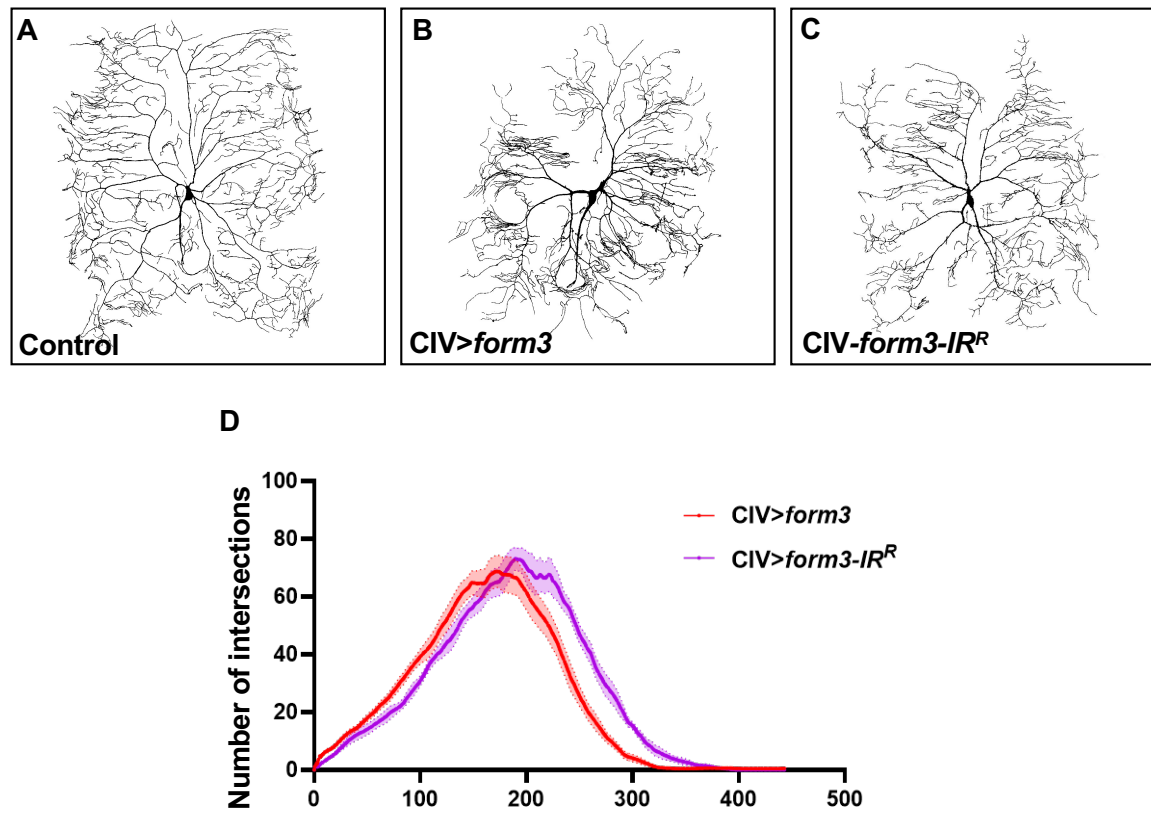


Fig. S4. Overexpression of *form3* RNAi resistant transgene. (A-C) Representative images of CIV neurons in (A) control, (B) *form3* overexpression, and (C) *form3-IR^R* overexpression. (D) Sholl profile of control (N=11), *form3* (N=11), and *form3-IR^R* (N=5). Statistical tests performed: (D) unpaired t-test $p > 0.05$, not significant for critical value or radius.

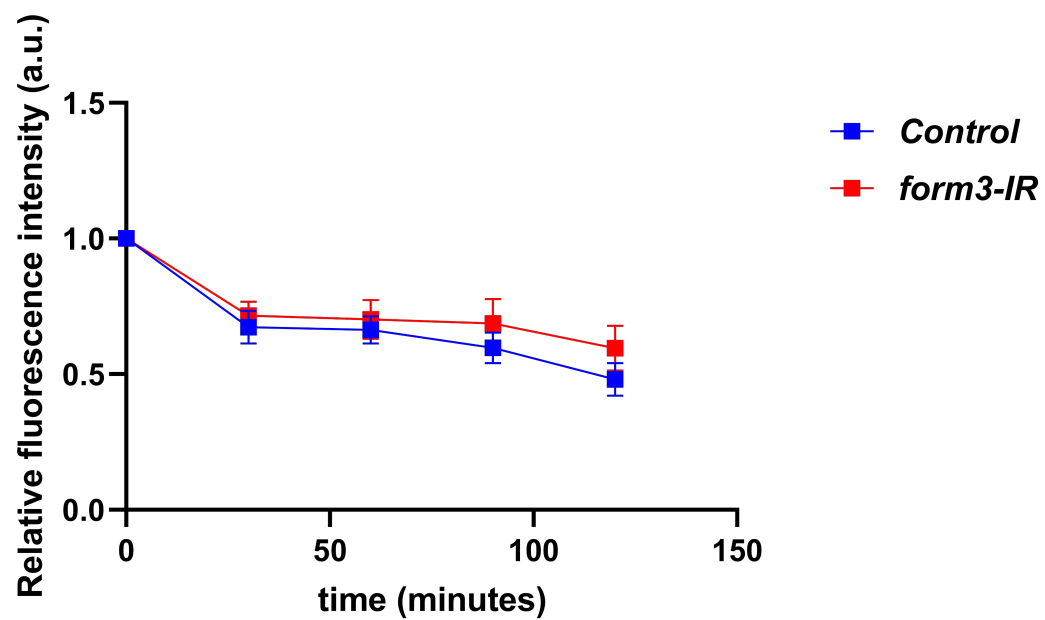


Fig. S5. *form3* does not affect MT turnover rate for the subpopulation of MTs that remain in *form3*-depleted CIV neurons. Quantitation of time-lapse imaging of MT stability over 120 minutes (see also Figure 5C-F). Statistical test performed: Unpaired t-test at 0, 60, and 120 minutes, not significant $p > 0.05$. Error bars indicate SEM.

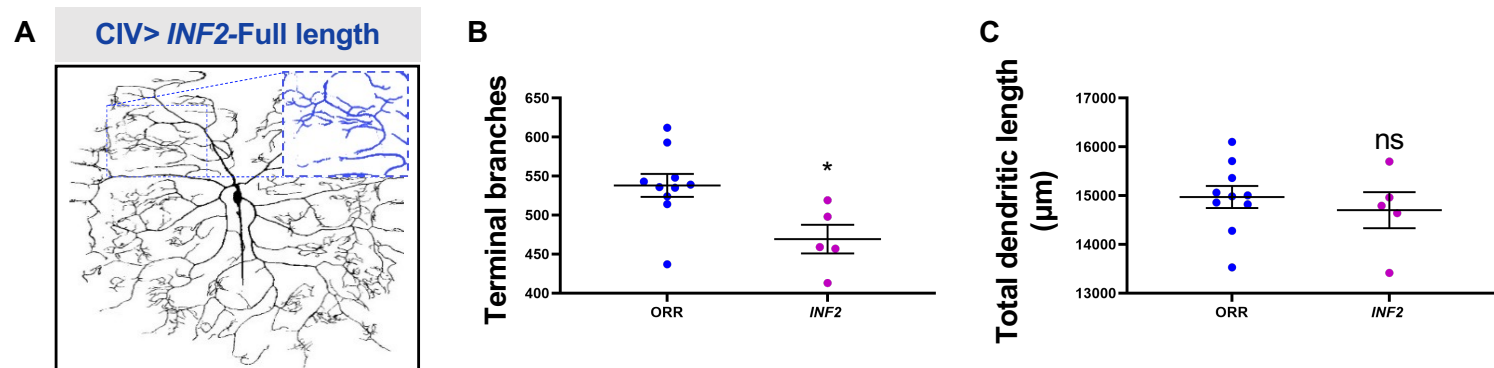
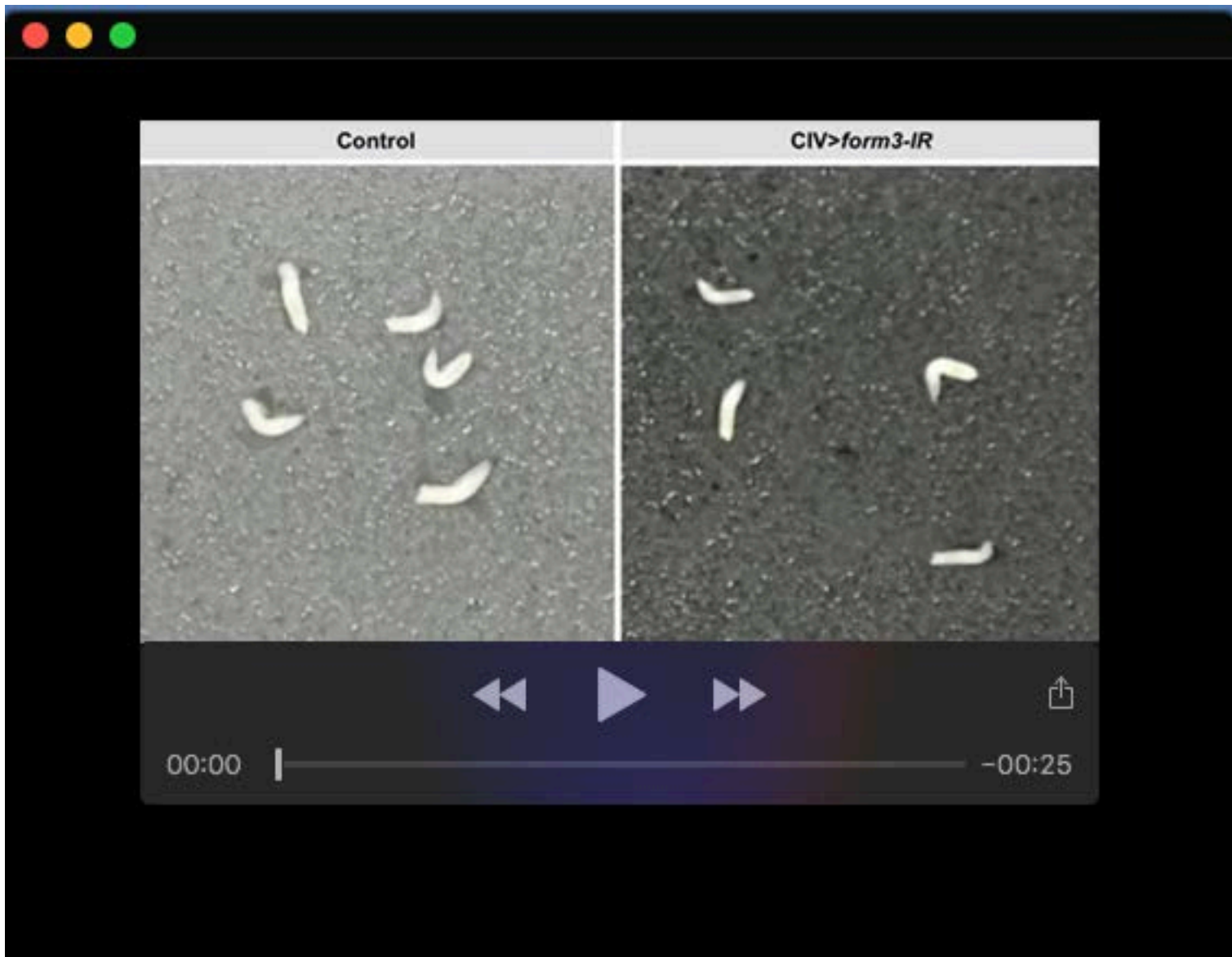
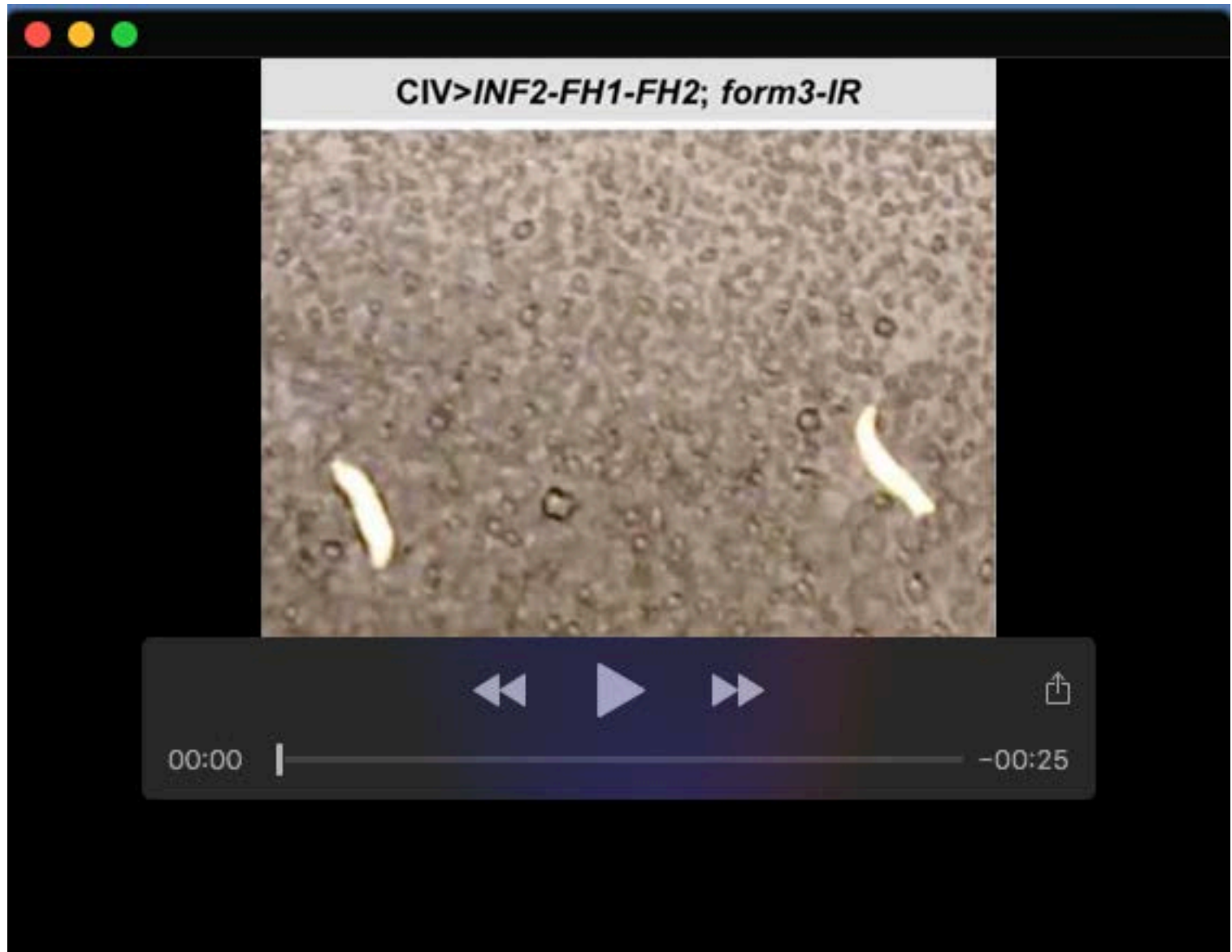


Fig. S6. Quantitative analysis of *INF2* full length and *INF2-FH1-FH2* overexpression on dendritic morphology. (A) Representative image of CIV-specific expression of full length *INF2*. (B,C) Quantitative analyses of the terminal branches and total dendritic length of control (N=10) and *INF2* (N=5). Statistical test performed: (B) unpaired t-test, $p=0.0152$; (C) unpaired t-test, ns represents not significant $p>0.05$.



Movie 1: form3 disruption in CIV nociceptive neurons severely impairs heat-evoked rolling behavior.



Movie 2. INF2-FH1-FH2 rescue of form3 impaired heat-evoked nociceptive rolling behavior.

Supplemental Materials and Methods

Synthetic Form3-RNAi resistant coding sequence (FLAG tag @C-terminus)- corresponding to Form3-PA isoform

Start Methionine

Codon modified 54bp sequence highlighted in yellow, with native nucleotides marked in red and silent mutation nucleotides marked in blue

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