The microtubule regulator *ringer* functions downstream of the RNA repair/splicing pathway to promote axon regeneration

Supplemental Data

Supplemental Figure 1. Quantification of regeneration length. (*A* and *B*) Regeneration length is plotted and corresponds to Figure 2C and 2F. $N = 21$ to 62 neurons from 6 to 16 larvae. $^{**}P < 0.01$ by one-way ANOVA followed by Tukey's test (A), two-tailed unpaired Student's t-test (B).

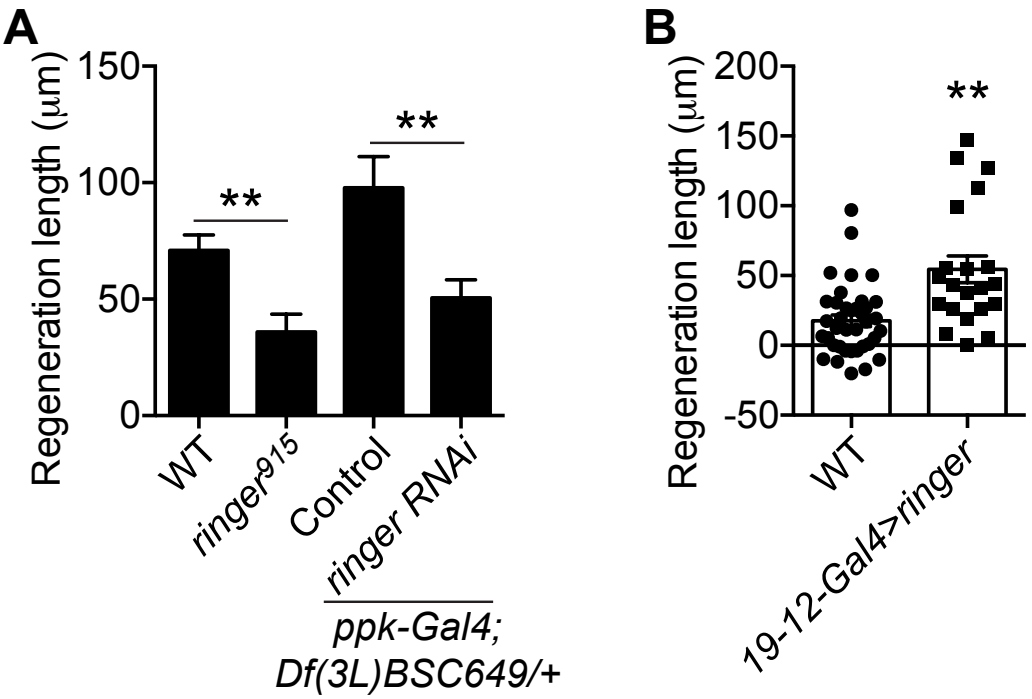
Supplemental Figure 2. Quantitative RT-PCR of *futsch* and *ringer* transcripts in various mutants. (*A*) *futsch* transcription is reduced in $ringer^{915}$ mutants. (*B*) *ringer* transcription is reduced in *HDAC6KO* and $futsch^{N94}$ mutants. $N = 3$ experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ by one-way ANOVA followed by Holm-Sidak's test.

Supplemental Figure 3. HDAC6 inhibitor tubacin promotes C3da neuron axon regeneration. (*A*) C3da neuron axons fail to regenerate in the DMSO vehicle control. Application of tubacin (50 μM) in the fly food from 0 h AEL onward promotes axon regeneration. The injury site is demarcated by the dashed circle. Arrow marks axon stalling while arrowheads show the regrowing axon tips. (*B* and *C*) Quantifications of C3da neuron axon regeneration. $N = 21$ to 24 neurons from 5 to 6 larvae. $^{*}P < 0.05$ by by Fisher's exact test (B), two-tailed unpaired Student's t-test (C). Scale bar = 20 μm.

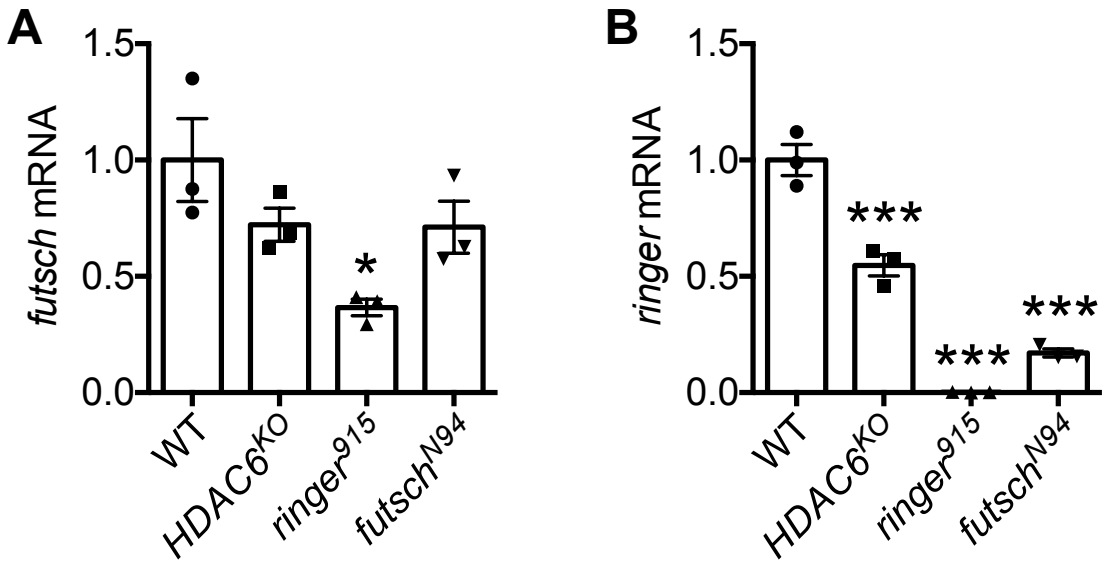
Supplemental Figure 4. Proposed model for a ringer-mediated genetic pathway regulating axon regeneration. Rtca inhibits regeneration by suppressing Xbp1 splicing, which in turn leads

to a reduction in ringer expression. Ringer is also inhibited by HDAC6. Ringer and futsch form a complex with tubulin, and appear to be responsible for relaying a cellular stress signal via the microtubule network. Rtca and Xbp1 may have additional downstream effectors independent of ringer, and futsch likely receive additional inputs, in parallel to ringer, during regulation of the axonal regenerative response.

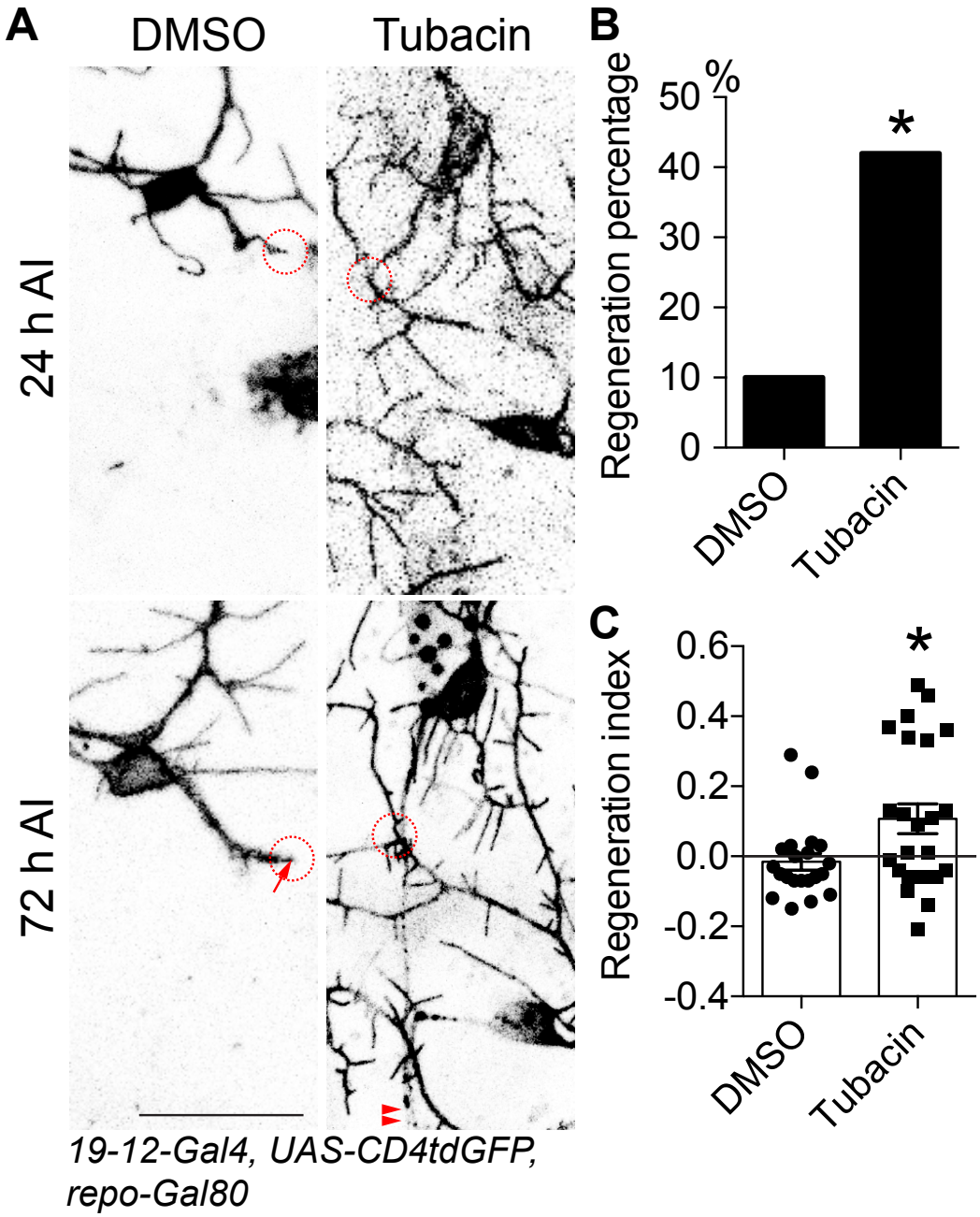
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4