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Supplemental Information

Microtubule Acetylation Is Required for Mechanosensation in *Drosophila*

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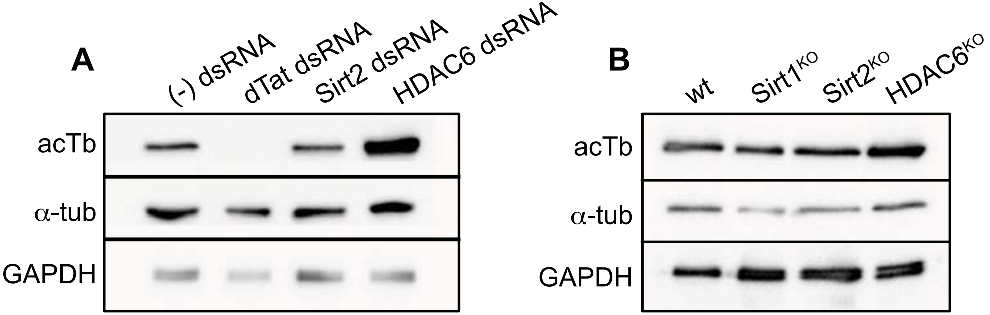


Figure S1. Related to Figure 1. HDAC6 is the major alpha tubulin deacetylase in *Drosophila*. (A) Western blots of cell lysates from S2 cells treated with dsRNA to the indicated genes showing that *HDAC6* RNAi leads to increased levels of acTb. (B) Western blots of larval lysates from the indicated genotypes showing that loss of *HDAC6* function leads to increased accumulation of acTb *in vivo*.

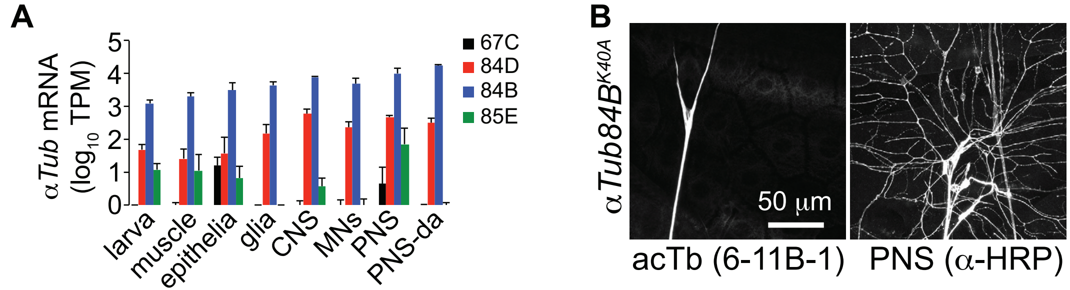


Figure S2. Related to Figure 3. αTubulin K40 is required for tubulin acetylation. (A) Larval mRNA expression of the *Drosophila αTubulin* isotypes in the indicated cell types; *αTub84B* accounts for >90% of *α Tubulin* mRNA in the PNS. (B) acTb staining in *αTub84B*^{K40A} mutant third instar larva showing that the *αTub84B* isotype accounts for the majority of acTb in larvae.

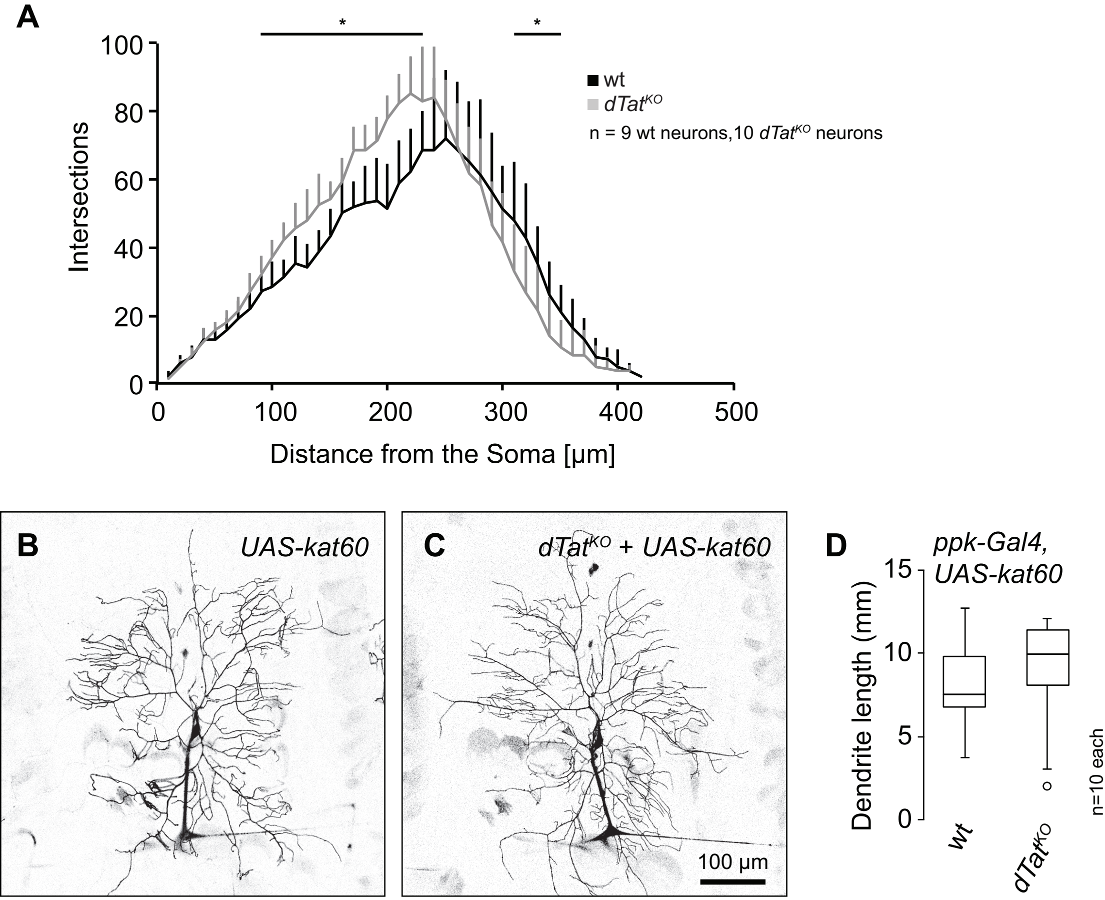
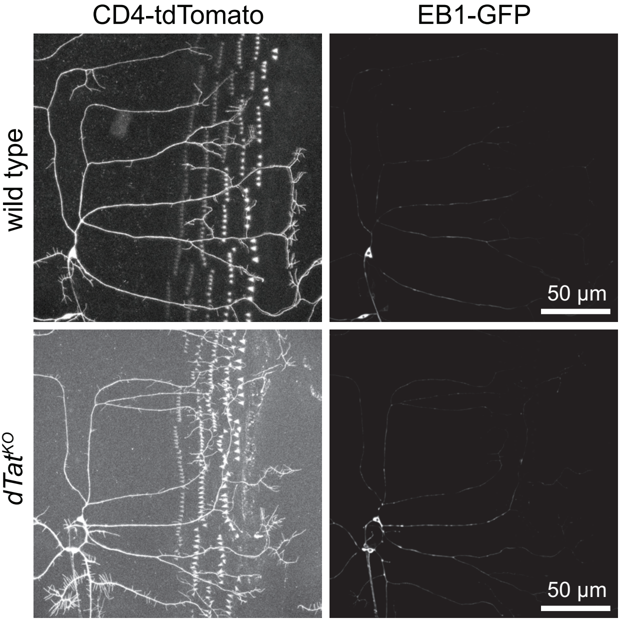


Figure S3. Related to Figure 4. (A) *dTat* has a minor effect on dendrite branch distribution. Sholl analysis depicting mean number and standard deviation of dendrite intersections as a function of distance from soma for 9 wt C4da neurons and 10 *dTat*^{KO} mutant C4da neurons. *P < 0.05 compared to wt controls, Welch's t-test. (B-D) *dTat* has no effect on *katanin*-induced remodeling of c4da dendrite arbors. Representative images of control larvae (B) or *dTat*^{KO} mutant larvae (C) overexpressing *katanin* (*UAS-kat60*) in c4da neurons under the control of *ppk-Gal4*. Dendrites were labeled with the c4da-specific marker *ppk-CD4-tdTomato* visualized via live confocal imaging. (D) Quantification of total dendrite length for the indicated genotypes. Boxes mark 1st and 3rd quartiles, bands mark medians, whiskers mark 1.5 x IQR, and outliers are shown as points. Welch's test of unequal variance revealed no significant difference was detected between the two groups.

A. Raw images from Figure 7D-G



B. Raw images from Figure 7I-K

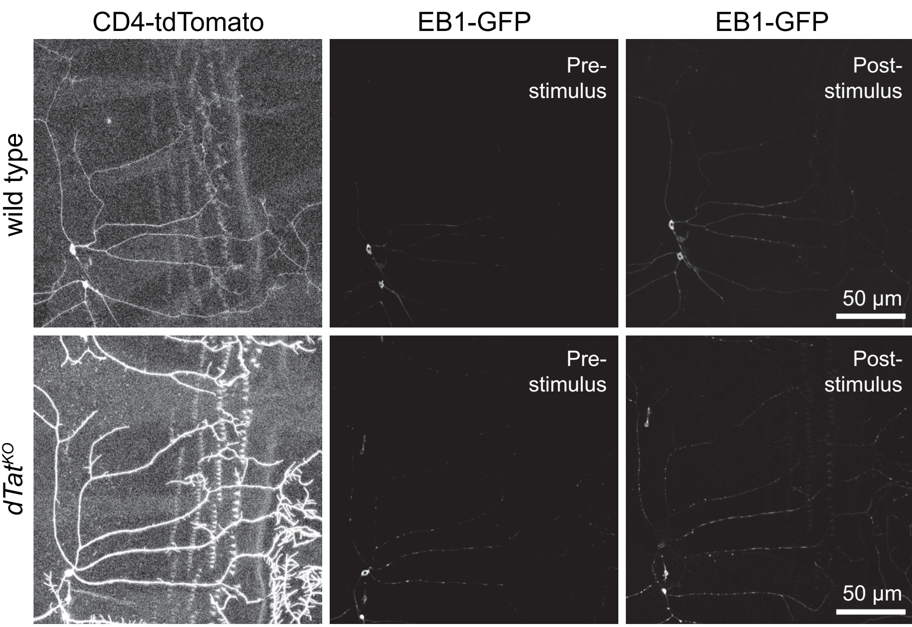


Figure S4. Related to Figure 7. Raw images of c3da neurons expressing EB1-GFP to label microtubule plus ends and the membrane marker CD4-tdTomato from (A) untreated wild type or *dTat*^{KO} mutant larvae and (B) wild type and *dTat*^{KO} mutant larvae that were subjected to mechanical stimulus.

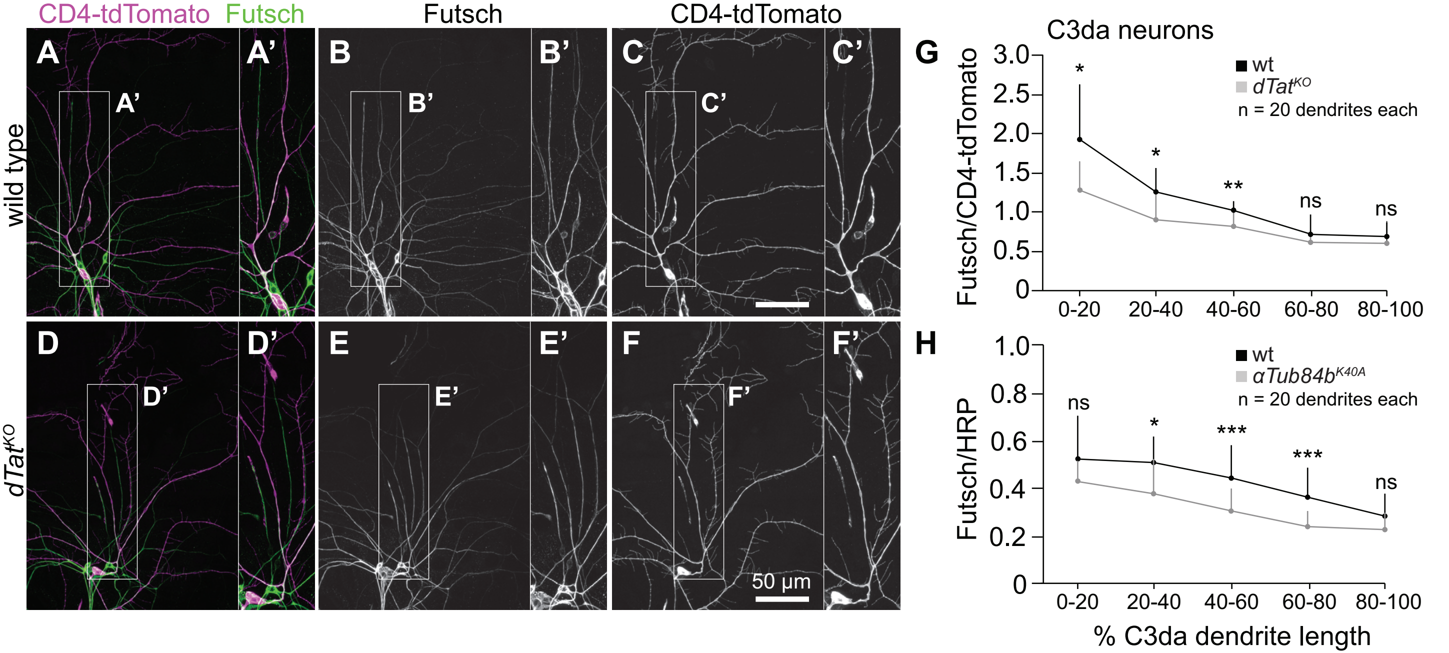


Figure S5. Related to Figure 7. Loss of acTb alters composition of the microtubule cytoskeleton in c3da neurons. Representative images of da neuron arbors immunostained for CD4-tdTomato (A, D), Futsch (B, E), or both (C, F) are shown for wild type (A-C) and *dTat*^{KO} mutant (D-F) third instar larvae expressing the membrane marker CD4-tdTomato in c3da neurons (*nompC-Gal4, UAS-CD4tdTomato*). (G) Quantification of Futsch intensity (means ± s.d.) in c3da neurons is shown for wild type and *dTat*^{KO} mutants. Futsch levels were measured along c3da dendrites (0-100% dendrite length, originating at the soma) and normalized to CD4-tomato levels. (H) Quantification of Futsch intensity (means ± s.d.) in c3da neurons is shown for wild type and *αTub84B*^{K40A} mutants with Futsch intensity normalized to HRP levels. ns, not significant, *P<0.05, ***P<0.001 compared to wt controls, unpaired t-test with Welch's correction.

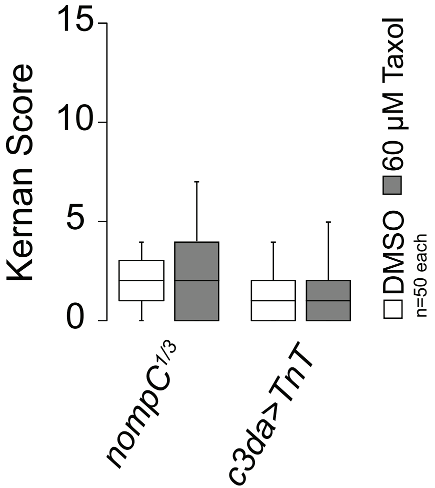


Figure S6. Related to Figure 7. Taxol enhancement of gentle touch responses depends on activity of NOMPC and c3da neurons. Boxplots depict behavioral responses of third instar larvae of the indicated gentoypes to gentle touch stimulus at 96 h after egg laying (AEL). Mutation of *nompC* or blocking synaptic transmission by expressing Tetanus toxin in c3da neurons (*c3da>TnT*) rendered taxol-fed larvae insensitive to gentle touch.

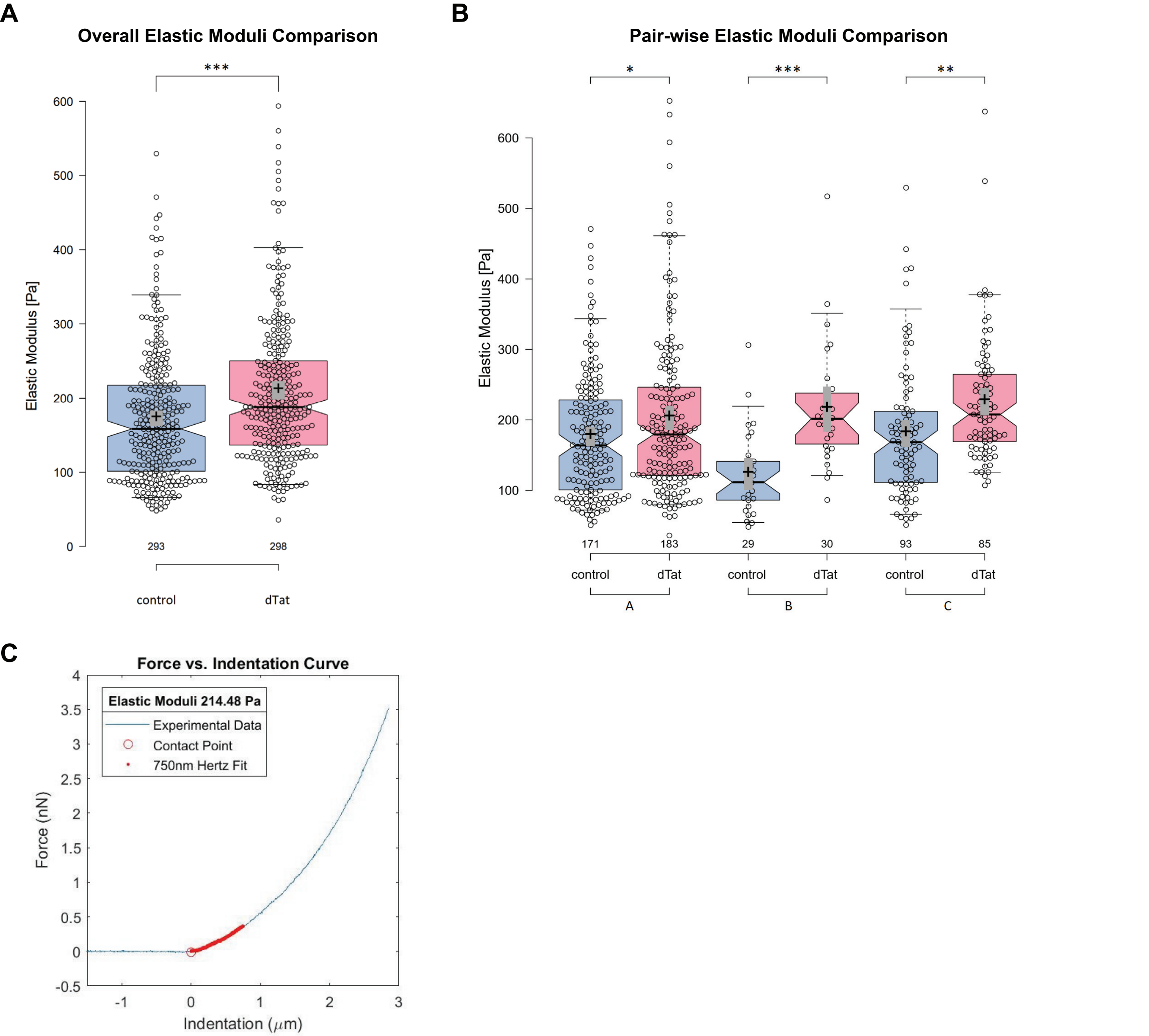


Figure S7. Related to Figure 7. dTAT regulates cellular rigidity in S2 cells. (A) Plots show the pattern of stiffness data for a combined 3 days of experiments, with the median (central bar) and mean (+ sign) shown. Means of 172 Pa for control and 211 Pa for *dTat* RNAi are significantly different to $p < 0.0001$. The medians of each were control: 158.5 Pa; *dTat* RNAi: 187.9 Pa, and the Mann Whitney comparison was also significant > 0.0001. (B) The paired comparisons of the cellular stiffness data shown in (A). The waist of the boxplots indicates the median and the height of the notches represents the 95% confidence interval of the median. The mean is denoted by the plus sign and the grey box represents the 95% confidence interval of the mean. The whiskers extend from the 5th to the 95th percentile. Statistical significance is marked as follows: "***" for a p-value < 0.001, "**" for a p-value between 0.001 and 0.005, and "*" for a p-value < 0.05. Significance levels for each pair, based on the student's t-test, assuming unequal variance, are indicated in Table S2. Each pair was derived from cells separately grown and maintained; no wells were re-used. (C) Characteristic Force vs. Indentation curve fit to the Hertz model. The plot shows the entire force-indentation curve in blue, a red circle for the contact point, and the Hertz model fit for 750 nm indent in red. Within the Hertz model, force increases as the 3/2 power of indentation. Here this fitting resulted in a cell modulus of 214 Pa.

Table S1. Related to Figure 5. Expression levels of mechanosensory channels in different cell types.

	PNS	da neurons	motoneurons
CG46121	56.91	7.10	0
iav	3.26	0	4.59
nan	0	0.02	0.19
nompC	87.84	22.92	0
pain	6.23	4.08	0.51
Piezo	16.93	100.48	4.00
ppk	272.00	398.80	0
ppk26	628.29	650.44	0
rpk	0	0	0
trp	0	0	0
Trpgamma	0	0	0.02
trpl	20.83	0.03	0
wtrw	0.25	0	0

Mean mRNA expression levels (tpm, transcripts per million) are shown for mechanosensory channel genes in each of the indicated cell types. N = 4 independent samples for PNS and da neurons; 7 independent samples for motoneurons.

Table S2. Related to STAR Methods. Means, medians and significance values for AFM measurements.

	Pair A		Pair B		Pair C	
Mean [Pa]	176.8	203.0	123.2	215.3	180.7	226.1
Median [Pa]	163.8	179.3	111.5	201.6	168.4	207.7
p (same mean)	0.016		1.08E-05		9.88E-04	
	*		***		**	

Significance levels for each pair were calculated with the Student's t-test assuming unequal variance. Statistical significance is marked as follows: "***" for a p-value < 0.001, "**" for a p-value between 0.001 and 0.005, and "*" for a p-value < 0.05.

Table S3. Related to Figures 1-7. Experimental genotypes used in this study.

Figure 1E	
wt	w^{1118}
$dTat^{KO}$	w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$
Figure 2A	
Larva	w^{1118}
Muscle	w^{1118}; *MHC-Gal4* / *UAS-nls-GFP*
Epi.	w^{1118}; *UAS-nls-GFP* / + ; *A58-Gal4* / +
Glia	w^{1118}; *UAS-nls-GFP* / + ; *repo-Gal4* / +
CNS	w^{1118}, *elav-Gal4* / + ; *UAS-nls-GFP* / +
MNs	w^{1118}; *UAS-nls-GFP* / + ; *Vglut-Gal4* / +
PNS	w^{1118}, *elav-Gal4* / + ; *UAS-nls-GFP* / +
PNS-da	w^{1118}; *21-7-Gal4* / *UAS-nls-GFP*
Figure 2B-2C	
wild type	w^{1118}
Figure 2D-2G	
$dTat^{GFP}$	w^{1118}; +; $dTat^{GFP}$ / $dTat^{GFP}$
Figure 2H-2K	
wild type	w^{1118}
$dTat^{KO}$	w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$
Figure 2L-2O	
$dTat^{KO}$ + *19-12>GFP-dTat*	w^{1118}; *UAS-GFP-dTat-L* / +; *19-12-Gal4*, $dTat^{KO}$ / $dTat^{KO}$
Figure 3A	
wt	w^{1118}
$dTat^{KO}$	w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$
$dTat^{KO / Df}$	w^{1118}; +; $dTat^{KO}$ / *Df(3L)BSC113*
$\alpha Tub84B^{K40A}$	w^{1118}; +; $\alpha Tub84B^{K40A}$ / $\alpha Tub84B^{KO}$
$\alpha Tub84B^{K40R}$	w^{1118}; +; $\alpha Tub84B^{K40R}$ / $\alpha Tub84B^{KO}$
$dTat^{KO}$, $\alpha Tub84B^{K40A}$	w^{1118}; +; $dTat^{KO}$, $\alpha Tub84B^{K40A}$ / $dTat^{KO}$, $\alpha Tub84B^{K40A}$
Figure 3B	
c3da-Gal4 / +	w^{1118}; *nompC-Gal4* / +
UAS-dTat / +	w^{1118}; *UAS-GFP-dTat-L* / +
$dTat^{KO}$ + *c3da>dTat*	w^{1118}; *nompC-Gal4* / *UAS-GFP-dTat-L*; $dTat^{KO}$ / $dTat^{KO}$
c3da>dTat	w^{1118}; *nompC-Gal4* / *UAS-GFP-dTat-L*
$HDAC6^{KO}$	y^{1}, w^{1118}, $HDAC6^{KO}$
$\alpha Tub84B^{K40R}$ + *c3da>dTat*	w^{1118}; *nompC-Gal4* / *UAS-GFP-dTat-L*; $\alpha Tub84B^{K40R}$ / $\alpha Tub84B^{K40R}$
Figure 3C	
wt	w^{1118}
$dTat^{KO}$	w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$
Figure 3D	
wt	w^{1118}
c4da>Kir	w^{1118}; +; *ppk-Gal4* / *UAS-KIR2.1*
$dTat^{KO}$	w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$

αTub84B^{K40A}	w^{1118}; +; αTub84B^{K40A} / αTub84B^{KO}
dTat^{KO} + c4da>dTat	w^{1118}; UAS-GFP-dTat-L / +; ppk-Gal4, dTat^{KO} / dTat^{KO}
Figure 3E-3F	
wt	w^{1118}
dTat^{KO}	w^{1118}; +; dTat^{KO} / dTat^{KO}
αTub84B^{K40A}	w^{1118}; +; αTub84B^{K40A} / αTub84B^{KO}
dTat^{KO} + cho>dTat	w^{1118}; UAS-GFP-dTat-L / nompC-Gal4; dTat^{KO} / dTat^{KO}
Figure 3G	
wt	w^{1118}
c4da>Kir	w^{1118}; +; ppk-Gal4 / UAS-KIR2.1
dTat^{KO}	w^{1118}; +; dTat^{KO} / dTat^{KO}
αTub84B^{K40A}	w^{1118}; +; αTub84B^{K40A} / αTub84B^{KO}
Figure 3H	
wt	w^{1118}
dTat^{KO}	w^{1118}; +; dTat^{KO} / dTat^{KO}
αTub84B^{K40A}	w^{1118}; +; αTub84B^{K40A} / αTub84B^{KO}
Figure 4A-4D	
wild type	w^{1118}; nompC-Gal4, UAS-CD4-tdGFP / +
dTat^{KO}	w^{1118}; nompC-Gal4, UAS-CD4-tdGFP / +; dTat^{KO} / dTat^{KO}
Figure 4E-4G	
wt	w^{1118}
dTat^{KO}	w^{1118}; +; dTat^{KO} / dTat^{KO}
Figure 4H-4L	
wild type	w^{1118}, ppk-mCD8-GFP
dTat^{KO}	w^{1118}, ppk-mCD8-GFP; +; dTat^{KO} / dTat^{KO}
Figure 5A-5C	
wt	w^{1118}; nompC-Gal4 / UAS-GCaMP6s
dTat^{KO}	w^{1118}; nompC-Gal4 / UAS-GCaMP6s; dTat^{KO} / dTat^{KO}
αTub84B^{K40A}	w^{1118}; nompC-Gal4 / UAS-GCaMP6s; αTub84B^{K40A} / αTub84B^{K40A}
Figure 5D	
dTat^{KO}	w^{1118}; +; dTat^{KO} / dTat^{KO}
nompC$^{1/3}$	w^{1118}; nompC^{1}/ nompC^{3}
c3da>TnT	w^{1118}; nompC-Gal4 / UAS-Tetanus Toxin
nompC$^{1/3}$; dTat^{KO}	w^{1118}; nompC^{1}/ nompC^{3}; dTat^{KO} / dTat^{KO}
Figure 5F-5G	
Vglut>GFP	w^{1118}; OK377-Gal4, UAS-mCD8-GFP / +
Vglut>nompC	w^{1118}, UAS-GFP-nompC; OK377-Gal4, UAS-mCD8-GFP / +
Vglut>nompC; dTat^{KO}	w^{1118}, UAS-GFP-nompC; OK377-Gal4, UAS-mCD8-GFP / +; dTat^{KO} / dTat^{KO}
Figure 6A	
wild type	w^{1118}, UAS-GFP-nompC; nompC-Gal4, UAS-mCD4-tdTomato / +
dTat^{KO}	w^{1118}, UAS-GFP-nompC; nompC-Gal4, UAS-mCD4-tdTomato / +; dTat^{KO} / dTat^{KO}
Figure 7A-7H	

wild type	*w^{1118}; nompC-Gal4, UAS-mCD4-tdTomato / UAS-EB1-GFP*
$dTat^{KO}$	*w^{1118}; nompC-Gal4, UAS-mCD4-tdTomato / UAS-EB1-GFP; $dTat^{KO}$ / $dTat^{KO}$*
Figure 7I	
wild type	*w^{1118}*
Figure 7J-7M	
wt	*w^{1118}*
$dTat^{KO}$	*w^{1118}; +; $dTat^{KO}$ / $dTat^{KO}$*
$\alpha Tub84B^{K40A}$	*w^{1118}; +; $\alpha Tub84B^{K40A}$ / $\alpha Tub84B^{KO}$*
Supplemental Figure S1B	
wt	*w^{1118}*
$Sirt1^{KO}$	*w^{1118}; $Sirt1^{2A-7-11}$*
$Sirt2^{KO}$	*w^{1118}; $Sirt2^{5B-2-35}$*
$HDAC6^{KO}$	*y^1, w^{1118}, $HDAC6^{KO}$*
Supplemental Figure S2a	
Larva	*w^{1118}*
Muscle	*w^{1118}; MHC-Gal4 / UAS-nls-GFP*
Epi.	*w^{1118}; UAS-nls-GFP / + ; A58-Gal4 / +*
Glia	*w^{1118}; UAS-nls-GFP / + ; repo-Gal4 / +*
CNS	*w^{1118}, elav-Gal4 / + ; UAS-nls-GFP / +*
MNs	*w^{1118}; UAS-nls-GFP / + ; Vglut-Gal4 / +*
PNS	*w^{1118}, elav-Gal4 / + ; UAS-nls-GFP / +*
PNS-da	*w^{1118}; 21-7-Gal4 / UAS-nls-GFP*
Supplemental Figure S2b	
$\alpha Tub84B^{K40A}$	*w^{1118}; +; $\alpha Tub84B^{K40A}$ / $\alpha Tub84B^{KO}$*
Supplemental Figure S3	
UAS-kat-60	*w^{1118}; ppk-mCD4-tdTomato, ppk-Gal4 / UAS-kat60*
UAS-kat-60 + $dTat^{KO}$	*w^{1118}; ppk-mCD4-tdTomato, ppk-Gal4 / UAS-kat60; $dTat^{KO}$ / $dTat^{KO}$*
Supplemental Figure S4	
$nompC^{1/3}$	*w^{1118}; $nompC^1$ / $nompC^3$*
c3da>TnT	*w^{1118}; nompC-Gal4 / UAS-Tetanus Toxin*

Table S4. Related to all figures. Oligonucleotide primers used in this study.	
Primers used to generate dsRNAs.	
T7-GCN5-f	TAATACGACTCACTATAGGTGAGAATTTGGATGACCTGCCTGCGGATGTAGTAATGCGCG
T7-GCN5-r	TAATACGACTCACTATAGGCAGCTATGAACTGCGTGTTCACAATGCTTGGATGCAGC
T7-NAT10-f	TAATACGACTCACTATAGGTCATGGCAAAAAGCGAGCAAAGAAGATTGCTGTGGGC
T7-NAT10-r	TAATACGACTCACTATAGGATTCACTGGCTCCACGTTGATGGTCTTGGAGCTAAGTGGC
T7-ELP3-f	TAATACGACTCACTATAGGTTCCGGCGTGGAACACGGAAATCTCCGTGAACTGGCGC
T7-ELP3-r	TAATACGACTCACTATAGGCTATATATTAATGTATATATTTAAGAGAGTAGTTTTAAG
T7-ARD1-f	TAATACGACTCACTATAGGATGAACATCCGCTGCGCAAAACCGGAAGACCTAATGACC
T7-ARD1-r	TAATACGACTCACTATAGGTCAGCAACAATGGCCATCGTGACCGCTGTGGTTGTGCG
T7-Nat1-f	TAATACGACTCACTATAGGATGCCTTCTAGCGATCCCCTGCCGCCCAAGGAGGGCGCGC
T7-Nat1-r	TAATACGACTCACTATAGGGGCCCTGGTATTTGGTCAGGTGGTCCACGGCCTGCTGC
T7-CG3967/dTAT-f	TAATACGACTCACTATAGGATGGTGGAATTCCGCTTCGATATTAAGCCGCTGTTCGCGC
T7-CG3967/dTAT-r	TAATACGACTCACTATAGGGCACGAAATTGTTCGCTTGCGGAATGATTCGCTTTAATCC
T7-CG17003-f	TAATACGACTCACTATAGGATGGTGGAGTTCGCCTTTGACATTAAGCACCTCTTTCCGC
T7-CG17003-r	TAATACGACTCACTATAGGCCCTGCGGAATGGTGCGGACCAGGCCATAGTGTTTGGC
T7-CAT-control-f	TAATACGACTCACTATAGGATCCCAATGGCATCGTAAAGAACATTTTGAGGC
T7-CAT-control-r	TAATACGACTCACTATAGGGGGCGAAGAAGTTGTCCATATTGGCCA
T7-Sirt2-fwd	TAATACGACTCACTATAGGATGGATAAGGTTCGACGCTTCTTTGCAAACACTCTACATC
T7-Sirt2-rev	TAATACGACTCACTATAGGCCATGTCGTACTCCTTGCGGCACTTAATGC
T7-HDAC6-f	TAATACGACTCACTATAGGATACAGTGCGTCGATTGCTG
T7-HDAC6-r	TAATACGACTCACTATAGGCTTGGGATTGGACTCGAAAA
Primers used to generate recombinant dTAT	
Bam-dTATalt	CCTATAGGATCCATGGTAGAGTTTCGCTTTGACATAAAACCCCTATTTGCTCAACC
dTATalt-196-Not	GGTATAGCGGCCGCTCACCCATTGCCTCCCCCGTTTCCGGACTCTCCGTCATTGAAAAAC
NheI-dTAT	GGTATAGCTAGCTATAATGGTGGAATTCCGCTTCGATATTAAG
dTAT-196-XhoI	GGTATACTCGAGATTCCCCGATTCCCCATCGTTAAAGAATCCCTCGTAGAGCACGAAATT

Primers used to generate UAS-GFP-dTat constructs	
KpnI-eGFP	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
EcoRI-eGFP	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
GFP-dTATalt-s	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
GFP-dTATalt-as	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTATalt-L-Apa	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTATalt-S-ApaI	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTATalt-L-NotI	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTATalt-S-NotI	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTatalt-G133W-G135-W-sense	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
dTatalt-G133W-G135-W-asense	CCTATAGGTACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG TGGTGCCC
Primers used for dTat-KO gRNA expression constructs.	
gRNA1F	CTTCAAGAACAATCGGGGTGCAGC
gRNA1R	AAACGCTGCACCCCGATTGTTCTT
gRNA2F	CTTCGAGCAGCGGATTGGGAGCAC
gRNA2R	AAACGTGCTCCCAATCCGCTGCTC
Primers used for constructing the dTat-KO repair template.	
dTatGFP gRNA1 S	CTTCGGGGTCCAAAAGAACAATCG
dTatGFP gRNA1 AS	AAACCGATTGTTCTTTTGGACCCC
dTatGFP gRNA2 S	CTTCGCAAGAGGTATATTTCCTACT
dTatGFP gRNA2 AS	AAACAGTAGGAAATATACCTCTTGC
Primers used for GFP-dTat gRNA expression constructs.	
dTatGFP gRNA1 S	CTTCGGGGTCCAAAAGAACAATCG
dTatGFP gRNA1 AS	AAACCGATTGTTCTTTTGGACCCC
dTatGFP gRNA2 S	CTTCGCAAGAGGTATATTTCCTACT
dTatGFP gRNA2 AS	AAACAGTAGGAAATATACCTCTTGC
Primers used for constructing the GFP-dTat repair template	
dTatGFP 5'arm F	CCCTTCGCTGAAGCAGGTGGGGTTGTGCGACTACCACTC
dTatGFP 5'arm R	CTCCTTTACTCATTATGCTGCATCCCGATTG
dTatGFP GFP F	GGGATGCAGCATAATGAGTAAAGGAGAAGAACTTTTC
dTatGFP GFP R	GGAATTCCACCATTTTGTATAGTTCATCCATGC
dTatGFP start F	TGAACTATACAAAATGGTGGAATTCCGCTTC
dTatGFP start R	ATGTCCGCGGCCGCTAGCATGCAAGCATTACTTACTTGTGACCCGC
dTatGFP 3'arm F	TAGGCCTTCTGCAGCGCAAGTAGGAAATATACCTC
dTatGFP 3'arm R	GATTGACGGAAGAGCCTCATTGGTTAACAGTTAGC