

Table S1 Statistical significance value of differences in PC0 between genotypes at 2- and 20-DAE age. n≥20. Significance level was established by one way ANOVA post-hoc Scheffe test.

Day 1										
	PC1	yw	UAS-Tau	UAS-Tau ^{R406W}	UAS-Tau, nmnat+/-	UAS-Tau ^{R406W} , nmnat+/-	UAS-Tau, UAS-NMNAT	UAS-Tau, UAS-WR	UAS-Tau ^{R406W} , UAS-NMNAT	UAS-Tau ^{R406W} , UAS-WR
Day 20	yw	-	0.339	0.278	0.015	0.004	0.405	0.772	0.884	0.201
	UAS-Tau	<0.0001	-	0.844	0.125	0.024	0.104	0.552	0.490	0.712
	UAS-Tau ^{R406W}	<0.0001	0.913	-	0.155	0.028	0.066	0.416	0.369	0.863
	UAS-Tau, nmnat+/-	<0.0001	0.841	0.757	-	0.387	0.007	0.037	0.041	0.185
	UAS-Tau ^{R406W} , nmnat+/-	<0.0001	0.593	0.538	0.687	-	0.001	0.006	0.008	0.032
	UAS-Tau, UAS-NMNAT	0.003	<0.0001	<0.0001	<0.0001	<0.0001	-	0.268	0.343	0.042
	UAS-Tau, UAS-WR	0.008	<0.0001	<0.0001	<0.0001	<0.0001	0.836	-	0.895	0.314
	UAS-Tau ^{R406W} , UAS-NMNAT	0.009	<0.0001	<0.0001	<0.0001	<0.0001	0.660	0.819	-	0.279
	UAS-Tau ^{R406W} , UAS-WR	0.014	<0.0001	0.0002	<0.0001	<0.0001	0.899	0.777	0.624	-
	Day 20									

Day 1

Table S2. Statistical significance of differences in PC6 between genotypes at 2 and 20 DAE. $n \geq 20$. Significance level was established by one way ANOVA post-hoc Scheffe test.

Day 1										
Day 20	PC6	<i>yw</i>	UAS-Tau	UAS-Tau ^{R406W}	UAS-Tau, <i>nmnat+/-</i>	UAS-Tau ^{R406W} , <i>nmnat+/-</i>	UAS-Tau, UAS-NMNAT	UAS-Tau, UAS-WR	UAS-Tau ^{R406W} , UAS-NMNAT	UAS-Tau ^{R406W} , UAS-WR
	<i>yw</i>	-	0.196	0.105	0.0002	<0.0001	0.531	0.813	0.878	0.487
	UAS-Tau	<0.0001	-	0.628	0.369	0.0004	0.062	0.279	0.374	0.650
	UAS-Tau ^{R406W}	<0.0001	0.953	-	0.009	0.001	0.039	0.146	0.224	0.387
	UAS-Tau, <i>nmnat+/-</i>	<0.0001	0.574	0.452	-	0.386	0.0001	0.0002	0.001	0.001
	UAS-Tau ^{R406W} , <i>nmnat+/-</i>	<0.0001	0.247	0.146	0.416	-	<0.0001	<0.0001	0.0003	0.0002
	UAS-Tau, UAS-NMNAT	0.003	<0.0001	<0.0001	<0.0001	<0.0001	-	0.389	0.528	0.237
	UAS-Tau, UAS-WR	0.002	<0.0001	<0.0001	<0.0001	<0.0001	0.956	-	0.971	0.614
	UAS-Tau ^{R406W} , UAS-NMNAT	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.688	0.672	-	0.654
	UAS-Tau ^{R406W} , UAS-WR	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	0.478	0.468	0.756	-
Day 20										

Day 1

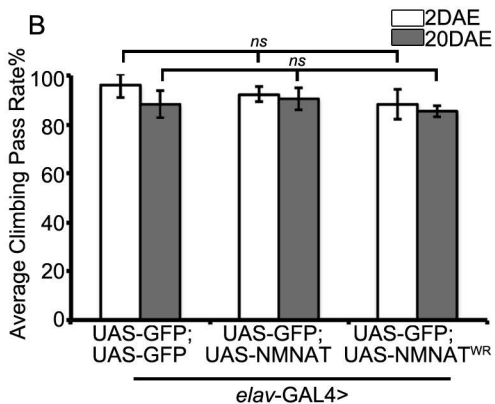
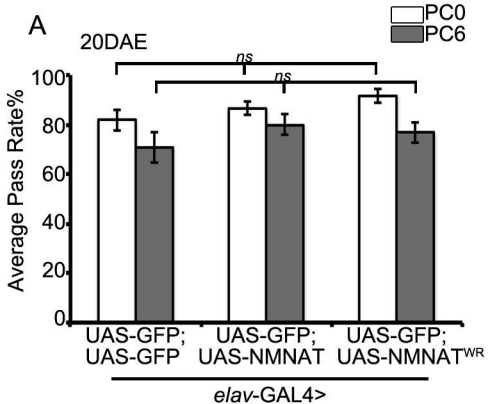
Supplementary Figure Legends:

Figure S1. Neuronal overexpression of NMNAT or NMNAT^{WR} does not affect cognitive or motor performance. (A) Learning (PC0) and memory (PC6) were assayed in flies overexpressing either UAS-GFP;UAS-GFP, or UAS-GFP;UAS-NMNAT, or UAS-GFP;UAS-NMNAT^{WR} using a pan-neuronal driver. There was no significant difference in the learning or memory index between any genotypes. (B) Climbing performance was measured using negative geotaxis assay in the same flies in (A). There was no significant difference in motor performance between any groups at either DAE2 or DAE20.

Figure S2. Neuronal overexpression of either NMNAT or NMNAT^{WR} with GFP does not cause any morphological changes in the CNS. Whole-mount adult brains were stained for GFP (green, A,E), NMNAT (red, B, F) and DAPI (blue, C,G). Scale bar = 20µm.

Figure S3. NMNAT overexpression does not affect the levels and activity of any major kinases that phosphorylate Tau or protein phosphatase 2 (PP2) level. Cos7 cells were transfected with a vector overexpressing either mCherry or mCherry-*Dm*NMNAT and the lysates were probed for p38 MAPK and activated p38 MAPK (pThr180/pTyr182), ERK and activated ERK (pThr 202/pTyr 204), JNK and activated JNK (pThr 183/Tyr 185), GSK3β and activated GSK3β (pSer9), MARK1 and activated MARK, and PP2.

Figure S4. pTau^{Ser262} is detected in higher Tau oligomers. Fly brain lysates overexpressing either wild type Tau with GFP, or mutant Tau^{R406W} with GFP, NMNAT or NMNAT^{WR} were immunoprecipitated with a total human Tau antibody and probed for Tau phosphorylated at Ser262. Overexpression of either NMNAT or NMNAT^{WR} caused an increase in the levels of higher Tau oligomers that were probed positively with the pTau^{Ser262} antibody.



20DAE

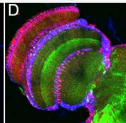
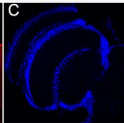
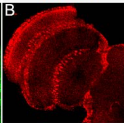
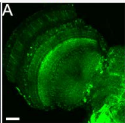
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NMNAT

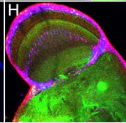
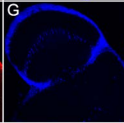
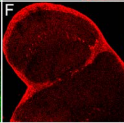
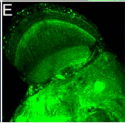
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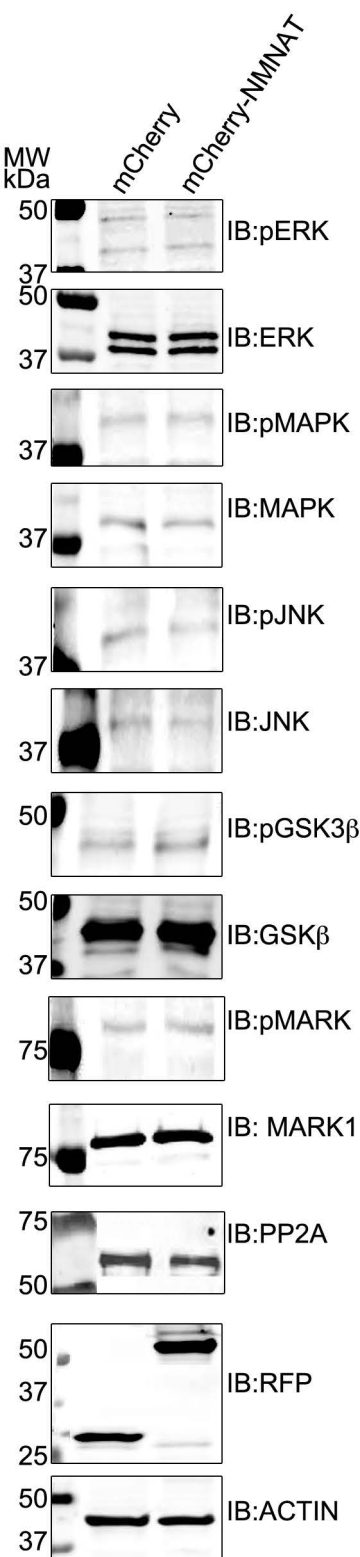
Merge

UAS-GFP;
UAS-NMNAT



UAS-GFP;
UAS-NMNAT^{TWR}





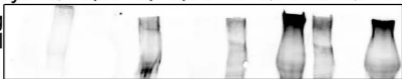
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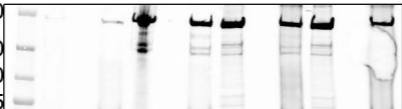
Input IgG + Input IgG + Input IgG + Input IgG +

Stacking
Gel



MW
kDa

250
150
100
75



IB: Tau^{pSer262}