Supplementary Materials: Balan et al.

Experimental Procedures

NAD^+/NADH level Quantification: The nicotinamide nucleotide concentrations were determined using an NAD^+/NADH Quantification kit purchased from BioVision (Mountain View, CA). For each point, 10 decapitated 5 days old flies were pooled and homogenized in 400 µl of extraction buffer (supplied with the kit) and clarified of debris by centrifugation. Samples containing 5 to 50 µg of protein were used for the assay according to the manufacturer's protocol. Nucleotide concentrations were assayed in the linear range of the calibration curve and calculated based on standards provided with the kit.

Figure legends

Supplementary Fig. 1: Alignment of *Drosophila* D-NAAM (accession no. NP_732446; TPA accession no. BK005756), *Sacharomyces cerevisie* PNC1 (accession no. AY558481) and *Pyrococcus horikoshii* PH999 (accession no. BAA30096) using the ESPript program. Identities are highlighted and conserved residues are boxed. Residues described as important for nicotinamidase catalytic activity (36) are underscored with triangles.

Supplementary Fig. 2: Expression of V5-D-NAAM protein in pUAST-D-NAAM male and female transgenic lines in tubulin-Gal4 or *elav*-Gal4 driver backgrounds was analyzed using V5 immunoblotting (top panel). Equal protein loading was verified using tubulin immunoblotting (bottom panel).

Supplementary Table 1

Effect of D-NAAM overexpression on NAD+/NADH levels

Genotype	Gender	[total NAD]	[NADH]	NAD^+/NADH
W^{1118}	M	2.71 ± 0.06	1.81 ± 0.03	0.50 ± 0.06
UAS-D-$NAAM^{42}$/tub-Gal4	M	2.36 ± 0.11	1.29 ± 0.02	0.83 ± 0.11
W^{1118}	F	5.43 ± 0.31	0.86 ± 0.03	5.34 ± 0.58
UAS-D-$NAAM^{42}$/tub-Gal4	F	5.56 ± 0.12	0.73 ± 0.01	6.66 ± 0.23
		pg/ug protein	pg/ug protein	

The experiment was performed in triplicates and is representative of 3 independent experiments

Supplementary Table 2
D-NAAM overexpression extends fly lifespan

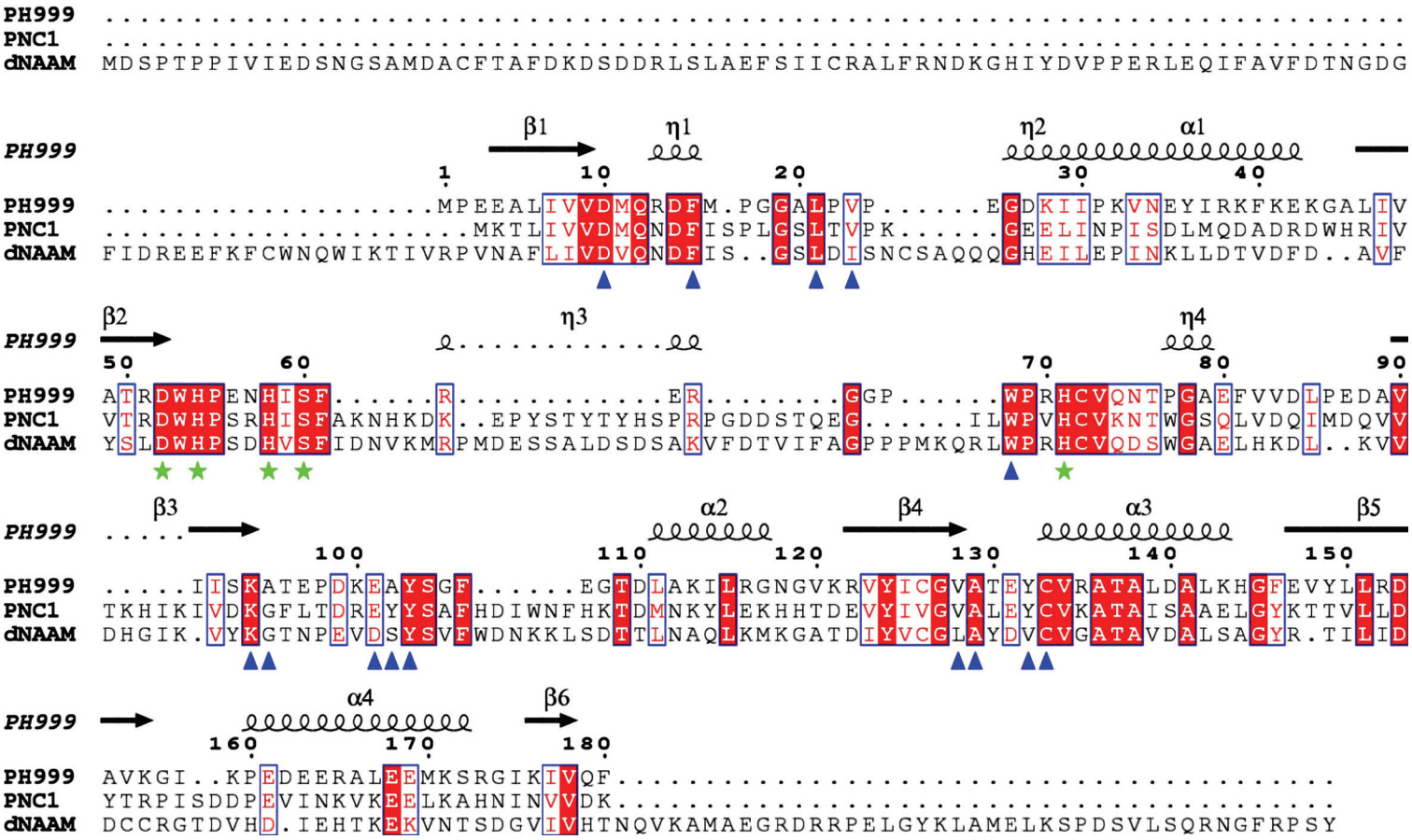
Females

Genotype	Mean lifespan control flies	Mean lifespan test flies	%Change (* p<0.001)	Max lifespan control flies	Max lifespan test flies	%Change (* p<0.001)
UAS-D-NAAM^{33}/tub-Gal4	39.05	43.48	11	62.73	69.81	11 (*)
UAS-D-NAAM^{31}/tub-Gal4	45.75	50.05	9	65.54	77.02	18 (*)
UAS-D-NAAM^{42}/tub-Gal4	48.25	52.16	8	68.17	80.13	18 (*)
UAS-D-NAAM^{33}/elav-Gal4	47.77	51.04	7	66.35	72.83	10 (*)
UAS-D-NAAM^{31}/elav-Gal4(chromosome II)	41.07	48.23	17 (*)	59.38	71.67	21 (*)
UAS-D-NAAM^{31}/elav-Gal4(chromosome III)	47.11	57.61	22 (*)	69.67	77.39	14 (*)
UAS-D-NAAM^{42}/elav-Gal4	47.51	61.26	29 (*)	66.04	80.56	22 (*)

Males

Genotype	Mean lifespan control flies	Mean lifespan test flies	%Change (* p<0.001)	Max lifespan control flies	Max lifespan test flies	%Change (* p<0.001)
UAS-D-NAAM^{33}/tub-Gal4	38.99	43.88	13	58.81	64.97	10 (*)
UAS-D-NAAM^{31}/tub-Gal4	39.97	50.41	26 (*)	57.18	68.23	19 (*)
UAS-D-NAAM^{42}/tub-Gal4	42.05	46.83	11	61.51	69.05	12 (*)
UAS-D-NAAM^{33}/elav-Gal4	43.56	50.26	15 (*)	61.84	67.63	9 (*)
UAS-D-NAAM^{31}/elav-Gal4(chromosome II)	37.49	44.63	19 (*)	48.76	59.79	23 (*)
UAS-D-NAAM^{31}/elav-Gal4(chromosome III)	43.71	57.01	30 (*)	59.14	71.03	20 (*)
UAS-D-NAAM^{42}/elav-Gal4	35.12	42.71	22 (*)	49.19	56.64	15 (*)

Supplementary figure 1



Supplementary figure 2