METHODS

Retina sections and transmission electron microscopy. Flies of different ages were dissected and fixed at 4 °C in 2% paraformaldehyde, 2% glutaraldehyde, 0.1 M sodium cacodylate, 0.005% $CaCl_2$ (pH 7.2) and post-fixed in 2% OsO_4. Thick sections (200 nm) of retina were stained with 1% toludine blue O, 1% sodium tetraborate (Electron Microscopy Sciences). Thin sections (50 nm) were stained with 4% uranyl acetate and 2.5% lead nitrate. Morphological features were scored double-blind by several observers.

Ataxin-1 aggregation assay in cultured cells. The ataxin-1 aggregation assay was performed as described[17] with the following modifications. Briefly, Cos7 cells were doubly transfected with either pEGFP-*hAtx-1[82Q]* and pCMV-*nmnat* or pEGFP-*hAtx-1[82Q]* and pCMV-*hsp70* using Lipofectamine (Invitrogen). After 48 h, cells were fixed and immunolabelled for NMNAT or Hsp70. Cell nuclei were labelled with TOTO3 (Molecular Probes).

Confocal image acquisition and quantification. Confocal microscopy was performed with a Zeiss LSM 510 confocal Axiovert 200M microscope equipped with Lasos HeNe 633, HeNe 543, argon ion 488 and Coherent UV Enterprise 351/364 nm laser lines and four identical photomultiplier tubes for blue, green, orange and near infrared fluorescence. Fluorescence analysis was performed with MetaMorph 5.07 (Molecular Devices/Universal Imaging Corp.), Amira 3.0 (TGS, Inc.) and Adobe Photoshop 7.0.

Protein fractionation and western blot analysis. To separate proteins into soluble or insoluble extracts, proteins from cultured Cos7 cells were extracted with RIPA buffer (50 mM Tris (pH 8), 150 mM NaCl, 1 mM EDTA, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF) and Complete protease inhibitor cocktail (Roche). The detergent-soluble fraction was defined as the supernatant of the cell lysates after centrifugation at 120,000*g* for 15 min. After removal of the supernatant, the pellets were washed with RIPA buffer and then suspended in 8 M urea, 4% SDS, 0.125 M Tris (pH 6.8), 12 mM EDTA and 3% β-mercaptoethanol. Western blot analysis was performed with infrared dye conjugated secondary antibodies, IR700 and IR800 (LI-COR Biosciences); blots were imaged and processed on an Odyssey® Infrared Imaging system.

***In vivo* luciferase folding assay.** The luciferase folding assay was performed as described[21,30]. Briefly, *Drosophila* S2 cells were triply transfected with pAC-*actin-GAL4*, pUAST-*luciferase* and one of the following plasmids: pUAST vector, pUAST-*Mash1*, pUAST-*nmnat*, pUAST-*nmnat-WR*, pUAST-*nmnat-H30A*, pUAST-*hsAT3*, pUAST-*hsp70* or pUAST-*hsp83*. At 24 h after transfection, the protein synthesis inhibitor cycloheximide was added and cells were subjected to a 15 min heat shock in an air incubator at 45 °C (which induced efficient unfolding of luciferase without killing the cells) and then allowed to recover at room temperature. Luciferase activity was measured with the Luciferase Assay System (Promega).

Aggregation measurements. Aggregation measurements were done as described[22]. Briefly, substrate proteins citrate synthase (Sigma) or insulin (Sigma) were mixed with either BSA (Sigma), egg white lysozyme (Sigma) or affinity-purified recombinant NMNAT, NMNAT-WR, NMNAT-H30, hsNMNAT3 or Hsp70 proteins in HEPES (pH 7.4) buffer. The aggregation of denatured citrate synthase was induced by incubation at 43 °C. The aggregation of insulin was induced by adding reducing agent DTT. Aggregation was monitored by measuring the apparent absorption at 360 nm, because of the Rayleigh scattering of citrate synthase or insulin homo-aggregates versus time. We used a FluoStar Optima plate reader (BMG Labtech) for measurements. The relative chaperone activity of NMNAT was calculated as the scattering of citrate synthase aggregates with time versus NMNAT concentration.

Immunocytochemistry of fly brains. Adult brains were fixed in PBS with 3.5% formaldehyde for 15 min and washed in PBS with 0.4% Triton X-100. We used the following antibody dilutions: NMNAT 1:1000; ataxin (gift from Huda Zoghbi, and from NeuroMabs Facility): 1:700; and secondary antibodies conjugated to Cy3, Cy5 or Alexa 488 (Jackson ImmunoResearch; Molecular Probes) were used at 1:250. All antibodies were incubated at 4 °C overnight in the presence of 5% normal goat serum.

Three-dimensional structure alignment. The search for similar protein structure was done on DALI sever[24] (http://www.ebi.ac.uk/dali/). The three-dimensional structure superposition was done with PyMol structure analysis software.