Supplemental figures and legends

Figure S1. Alignment of the protein sequences of Nmnat enzymes from various species. The amino acid sequence of Nmnat1 (mouse), Nmnat3 (mouse), dNmnat (*Drosophila melanogaster* Nmnat1B), yNmnat (*Saccharomyces cerevisiae* Nmnat2), and mjNmnat (*Methanocaldococcus jannaschii* Nmnat) were aligned using VectorNTI (Informax). Mutated residues are indicated as red arrow head (H24) and red asterisk (W170). Conserved residues are highlighted in blue.

Figure S2. Axonal protection is a conserved function of Nmnat proteins. *A*, Nmnat proteins from mouse (Nmnat1), *Drosophila melanogaster* (dNmnat), *Saccharomyces cerevisiae* (yNmnat), and *Methanocaldococcus jannaschii* (mjNmnat) were expressed in HEK293T cells. Immunocytochemistry using anti-6xHis antibody demonstrated that these Nmnat proteins were distributed predominantly in the cytoplasm. *B*, Lysates of HEK293T cells expressing Nmnat1, dNmnat, yNmnat, or mjNmnat were analyzed by Western blotting with anti-6xHis antibody. The calculated molecular masses of each protein were 32 kDa (Nmnat1), 34 kDa (dNmnat), 45 kDa (yNmnat), and 21 kDa (mjNmnat). *C*, DRG neurons were infected with lentivirus expressing EGFP, Nmnat1, dNmnat, yNmnat, or mjNmnat. Representative images of axons at the indicated time after transection are shown. Axonal degeneration was significantly delayed in DRG neurons expressing each of the Nmnat proteins when compared to EGFP-expressing neurons.