Supplementary Information

Deletions within its subcellular targeting domain enhance the axon protective capacity of Nmnat2 *in vivo*

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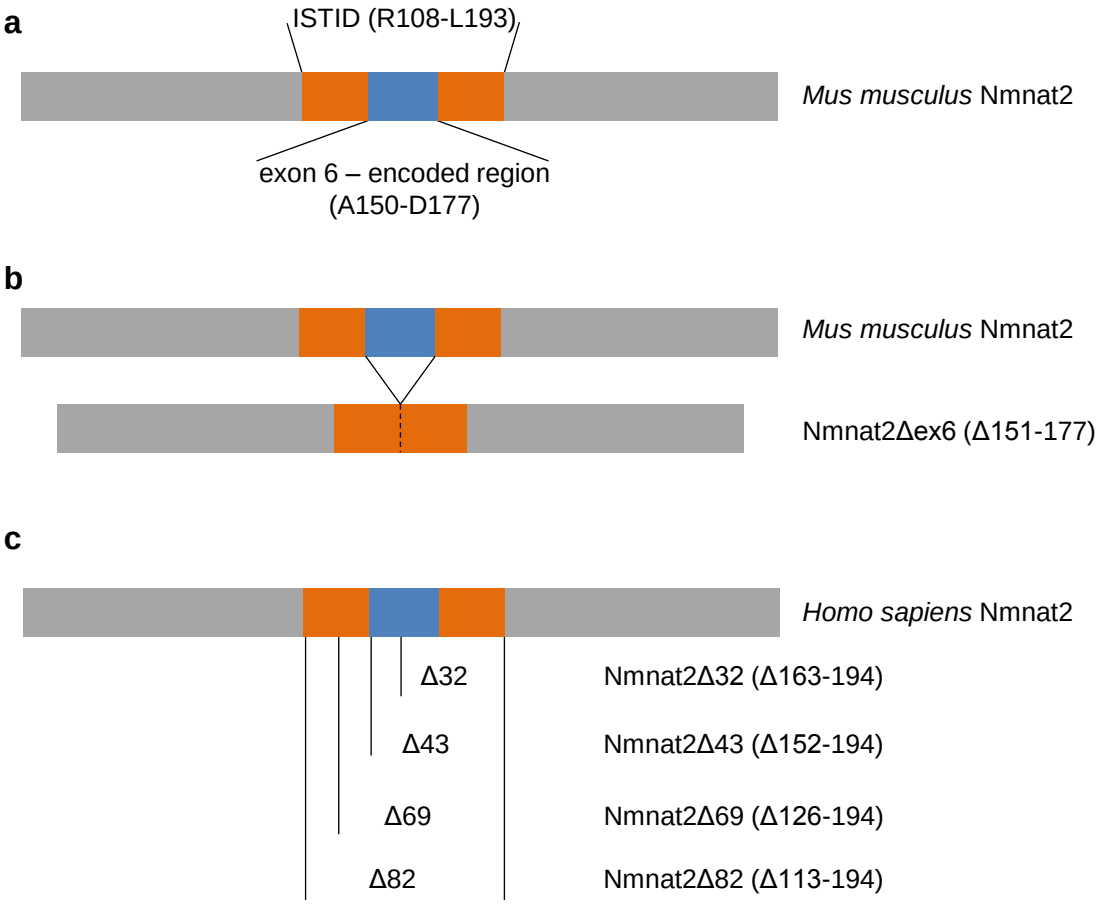
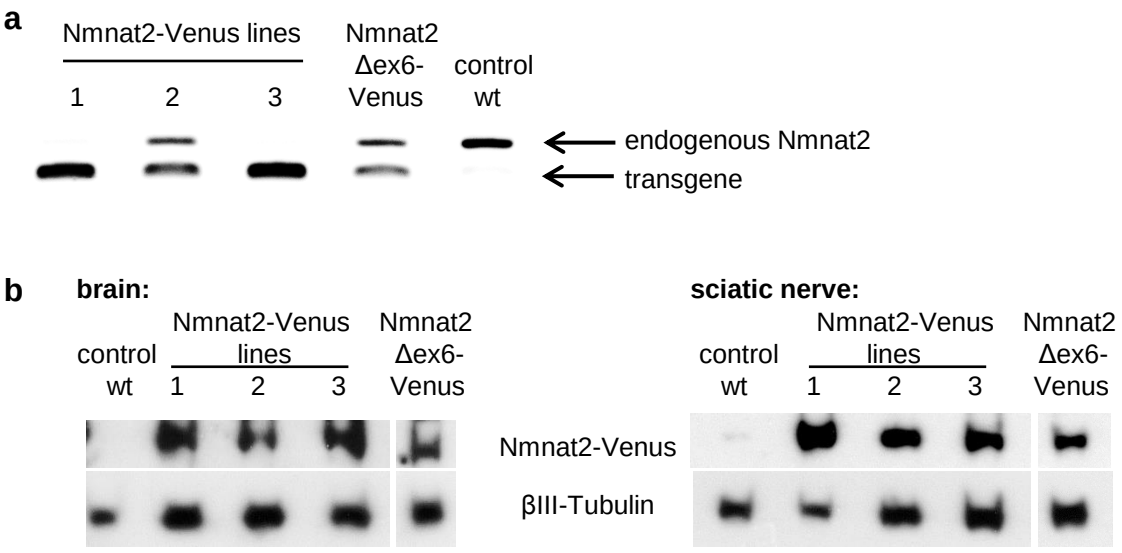
Supplementary Figures



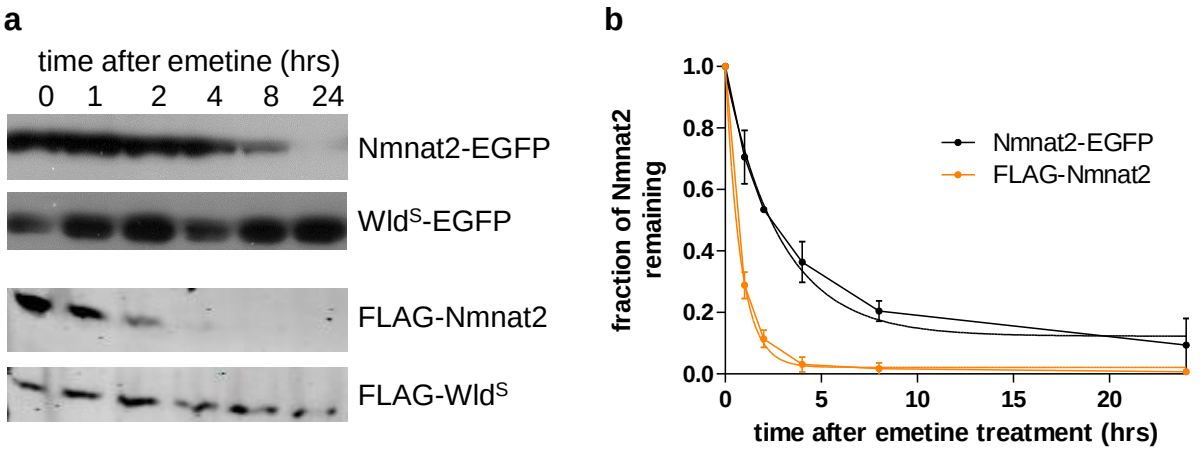
Supplementary Figure 1. Relevant Nmnat2 regions and deletion mutants.
a) Cartoon outlining the position of the isoform-specific targeting and interaction domain (ISTID) and the exon 6-encoded central ISTID region within the mouse Nmnat2 protein.
b) Illustration of the deletion of central, exon 6-encoded ISTID sequences in Nmnat2Δex6.
c) Illustration of deleted parts of the ISTID region in the Nmnat2Δ32, Δ43, Δ69 and Δ82 deletion mutants. These deletion mutants are based on human Nmnat2 which carries the following primary structure deviations relative to mouse Nmnat2: H131N, N133T, T134P and P144A.



Supplementary Figure 2. PCR genotyping and Western Blots of Nmnat2-Venus and Nmnat2Δex6-Venus mice.

a) Representative image of 2% agarose gel showing genotyping PCR products. The presence of the transgene (Nmnat2-Venus or Nmnat2Δex6-Venus) is indicated by the lower gel band. The differences between lines in the relative amplification of endogenous Nmnat2 (upper band) compared to transgene most likely reflect differences in copy number of the transgene, with higher copy numbers resulting in absence of the endogenous Nmnat2 band due to amplification dynamics in the PCR reaction.

b) Representative Western Blots (cropped) showing specific expression of Nmnat2-Venus in transgenic mouse brains and sciatic nerves by detection of the YFP Venus tag. βIII-Tubulin was used as loading control.



Supplementary Figure 3. EGFP tag stabilises Nmnat2 *in vitro*.

a) Representative Western blots (cropped) of HEK293 cells co-transfected with Wld^S-EGFP and Nmnat2-EGFP (top) or FLAG-Wld^S and FLAG-Nmnat2 (bottom). Twenty-four hours after transfection, cells were treated with 10 µM emetine for the amount of time indicated after which samples were processed for SDS-PAGE and Western blot using anti-FLAG or anti-EGFP antibody.

b) Quantification of Nmnat2 turnover after emetine treatment. For each sample and time point, the amount of Nmnat2 remaining was normalised to Wld^S as an internal control. Error bars indicate SEM. Half-lives are significantly different ($p<0.001$; non-linear curve fit; t½ FLAG-Nmnat2: 0.6 hrs; t½ Nmnat2-EGFP: 2.6 hrs).

Supplementary Movie 1. Axonal transport of Nmnat2-Venus and Nmnat2Δex6-Venus.

Live imaging of individual sciatic nerve axons from Nmnat2-Venus (wt, top) and Nmnat2Δex6-Venus mice (Δex6, bottom). Scale bar: 5 μm. Acquisition rate: 2 frames per second (fps). Playback rate: 20 fps. Total length: 240 frames (2 min).