

## SUPPORTING INFORMATION

A proteolytic C-terminal fragment of Nogo-A (reticulon-4A) is released in exosomes and potentially inhibits axon regeneration

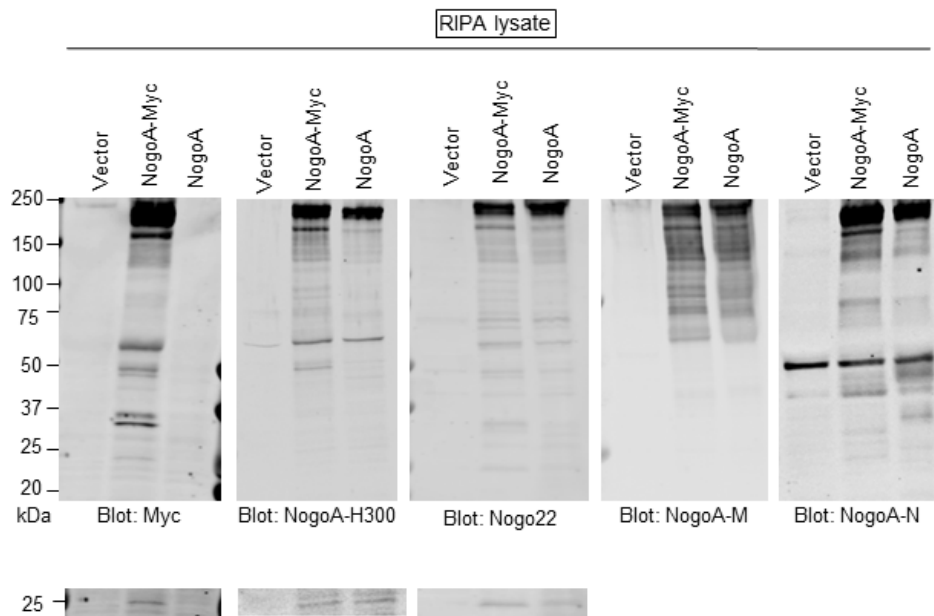
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### Material included:

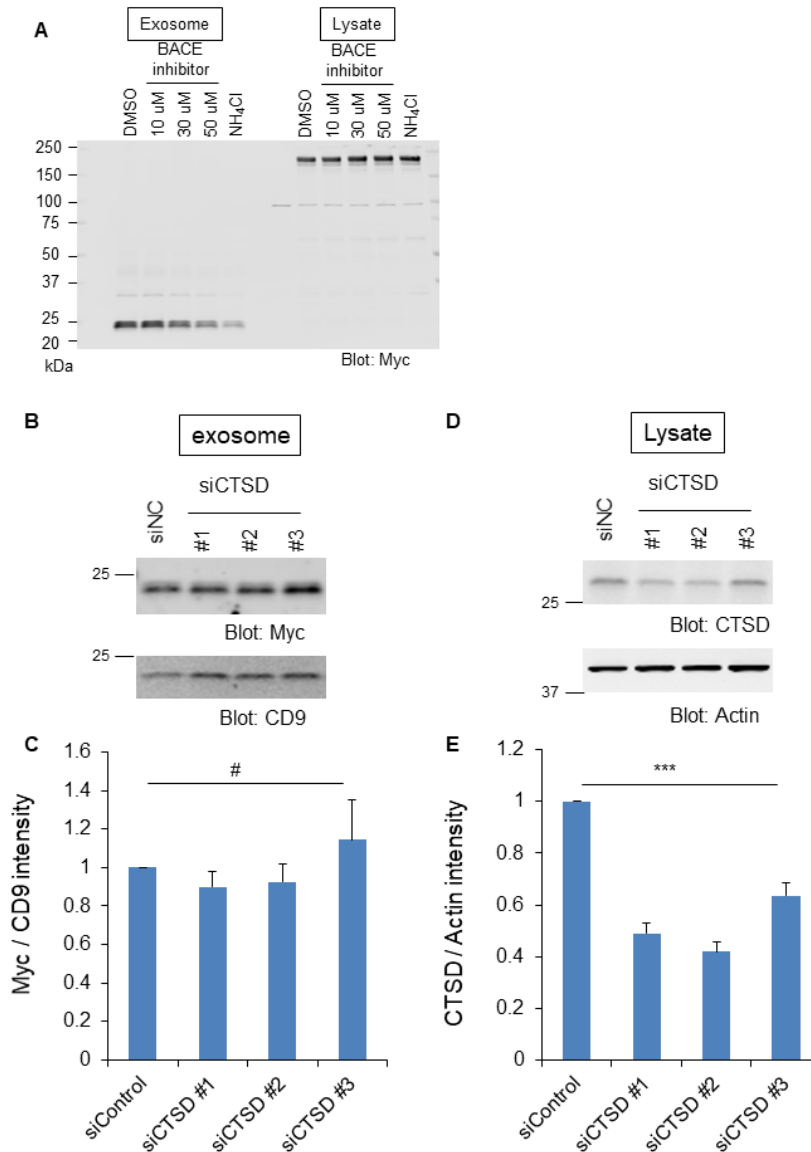
*Supplementary Figure 1*

*Supplementary Figure 2*

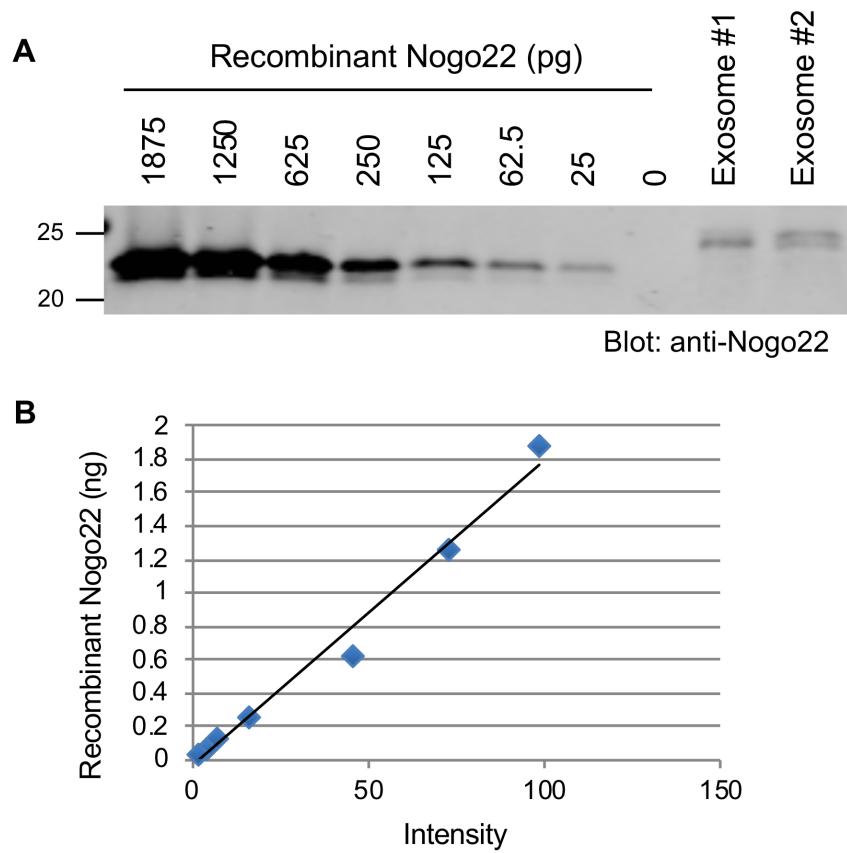
*Supplementary Figure 3*



**Supplementary Figure 1. Overexpression of Myc-tagged Nogo-A in HEK293T cells does not alter the detection of endogenous Nogo-A full-length or fragment protein expression.** HEK293T cells were transfected with vector (-), Nogo-A-Myc, or untagged Nogo-A. At 48 h after transfection, cell lysates were immunoblotted with anti-Myc, anti-NogoA-H300, anti-Nogo22, anti-NogoA-M, and anti-NogoA-N antibodies. Higher exposure images are shown below the full blots.



**Supplementary Figure 2. Effect of CTSD knock down on Nogo-A fragment level in exosome.** *A*, HEK293T cells were transfected with Nogo-A-Myc. At 24 h after transfection, media were changed, and DMSO and the indicated amount of BACE inhibitors and NH<sub>4</sub>Cl (20 mM) were added and cultured for 12 h. Then, culture supernatants and exosome fractions were isolated and immunoblotted with an anti-Myc antibody. *B*, HEK293T cells were transfected with Nogo-A-Myc and siNC, siCTSD #1, #2 or #3. Exosomes were purified at 36 h after transfection, and immunoblotted with anti-Myc and anti-CD9 antibodies. *C*, Quantification of Myc divided CD9 intensity normalized to siNC. Mean±SE, N=4 independent experiments. #, not significant. *D*, HEK293T cells were transfected with Nogo-A-Myc and siNC, siCTSD #1, #2 or #3. At 36 h after transfection, cells were lysed and immunoblotted with anti-CTSD and anti-Actin antibodies. *E*, Quantification of CTSD divided Actin intensity normalized to siNC. Mean±SE, N=4 independent experiments. \*\*\*P<0.005, one-way ANOVA followed by Dunnett's test.



**Supplementary Figure 3. Titration of Nogo-A C-terminal exosome** *A*, Indicated amount of recombinant Nogo22 and exosomes from Nogo-A transfected HEK293T cells were immunoblotted with anti-Nogo22 antibody. *B*, Nogo22 amount (ng) and Nogo22 intensity were plotted. Nogo-A C-terminal fragment amount in exosome was calculated using this graph.