

Supplemental material

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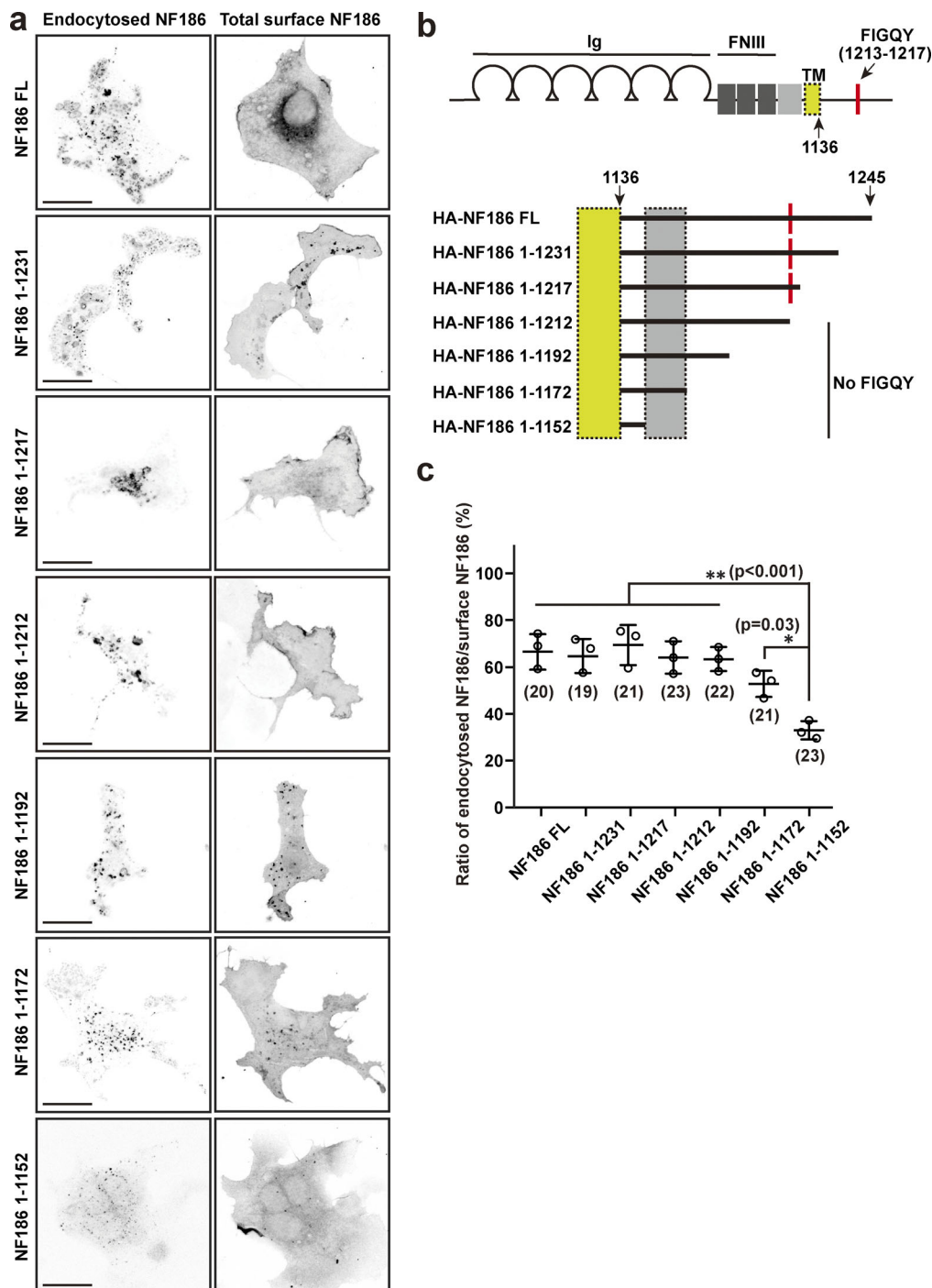


Figure S1. **NF186 deletion constructs reveal domains important for its endocytosis.** (a) Endocytosis assay in COS-7 cells using transfected HA-tagged NF186 full-length and truncated mutants (HA-NF186; 1–1231 aa, 1–1217 aa, 1–1212 aa, 1–1192 aa, 1–1172 aa, and 1–1152 aa). Cells were immunostained with anti-HA antibody (endocytosed NF186) and anti-NF186 antibody (total surface NF186). Scale bars = 10 μ m. (b) NF186 domain structure and truncation mutants. The transmembrane domain is located in the yellow boxes, while the critical region regulating endocytosis is contained in the gray boxes. FNIII, fibronectin type III domain; Ig, immunoglobulin domain, TM, transmembrane domain. (c) Ratio of endocytosed NF186/surface NF186 for full-length and deletion constructs. Mean \pm SEM is shown (NF186 FL vs. NF186 1–1152, $P = 0.0003$; NF186 1–1231 vs. NF186 1–1152, $P = 0.0006$; NF186 1–1217 vs. NF186 1–1152, $P = 0.0001$; NF186 1212 vs. NF186 1–1152, $P = 0.0007$; NF186 1192 vs. NF186 1–1152, $P = 0.0009$; NF186 1–1172 vs. NF186 1–1152, $P = 0.03$; **, $P < 0.001$; *, $P = 0.03$; $n = 3$ independent experiments, ordinary one-way ANOVA).

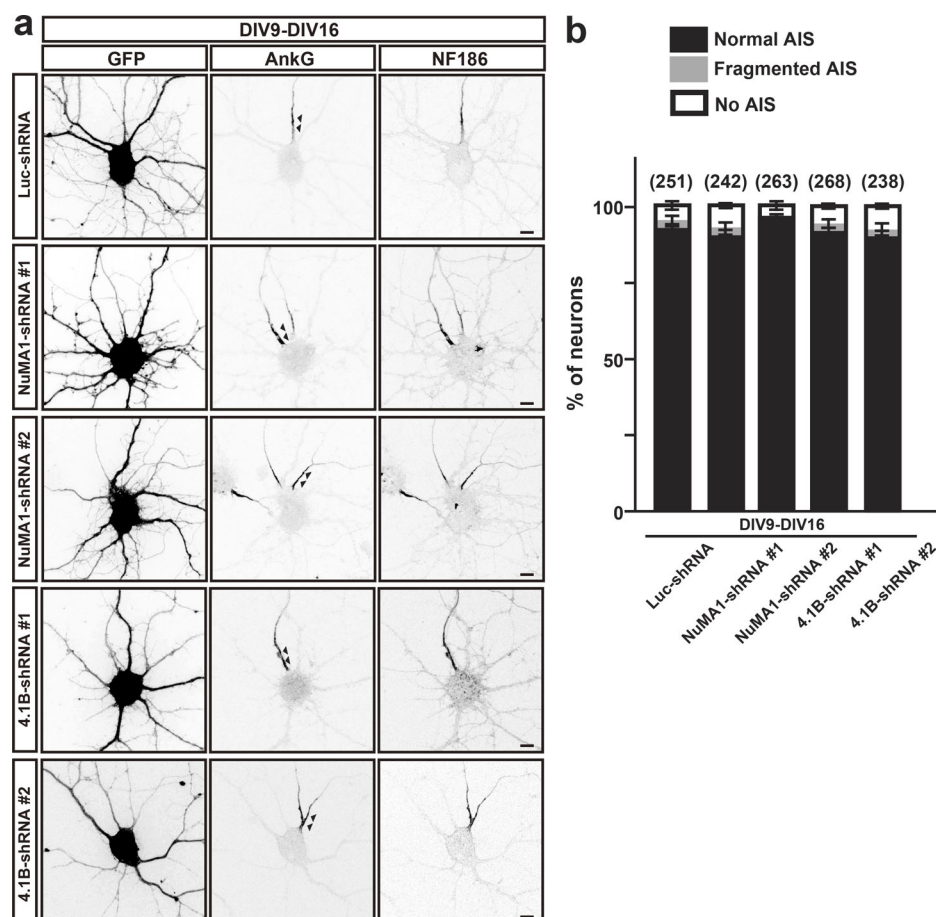


Figure S2. **Silencing expression of NuMA1 or 4.1B does not affect AIS maintenance in hippocampal neurons.** (a) Hippocampal neurons were transfected with Luc-shRNA, NuMA1-shRNA #1, NuMA1-shRNA #2, 4.1B-shRNA #1, or 4.1B-shRNA #2 at DIV9 and immunostained at DIV16 for GFP, AnkG, and NF186. Arrowheads in the AnkG-labeled panels indicate AIS. Scale bars = 5 μ m. (b) Quantification of the staining pattern for AnkG in shRNA-transfected neurons. Mean \pm SEM is shown (the number of neurons analyzed is indicated). No statistical comparison was performed.

Dataset S1 includes the PSM counts for the differential proteomics experiments shown in Fig. 1.