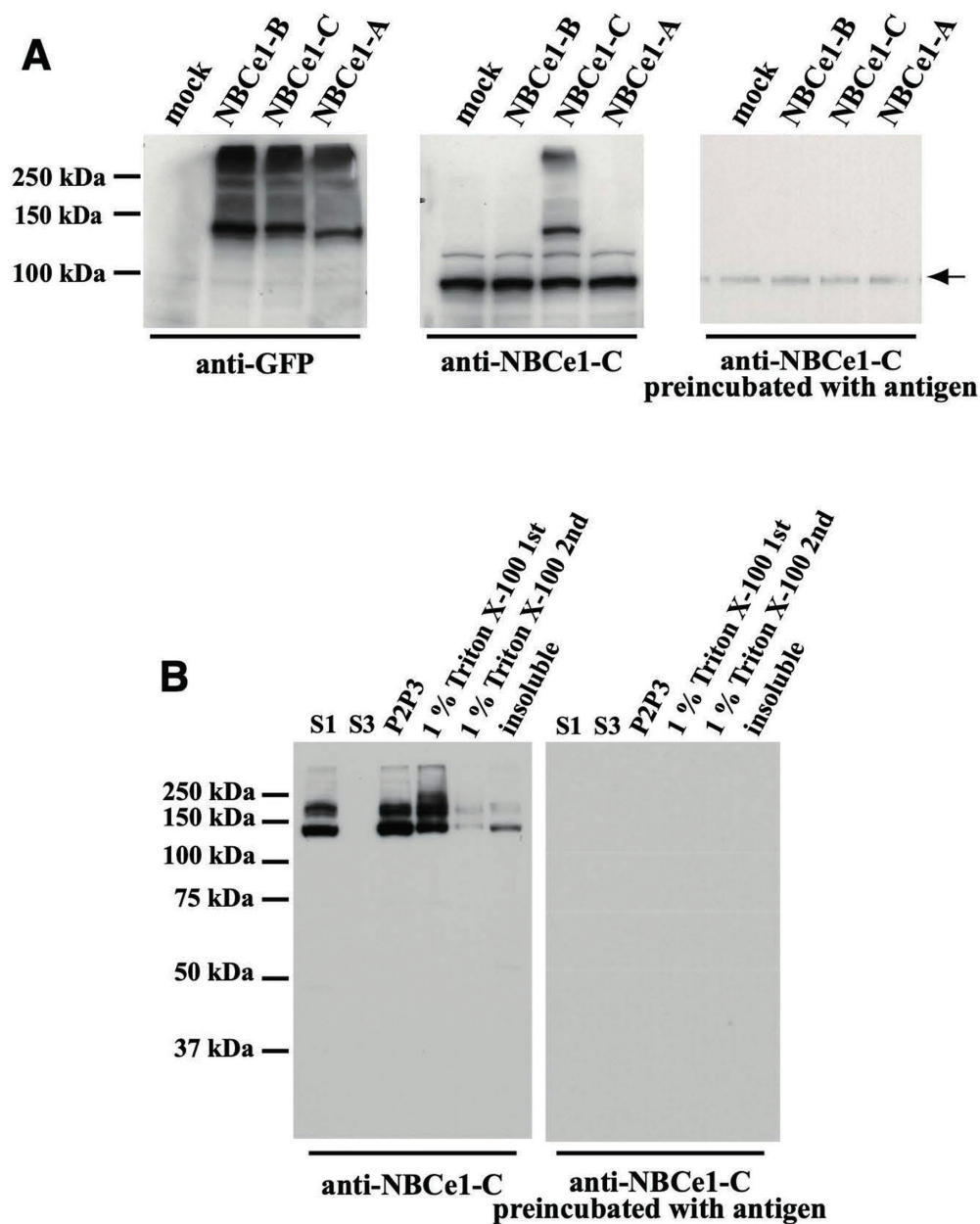
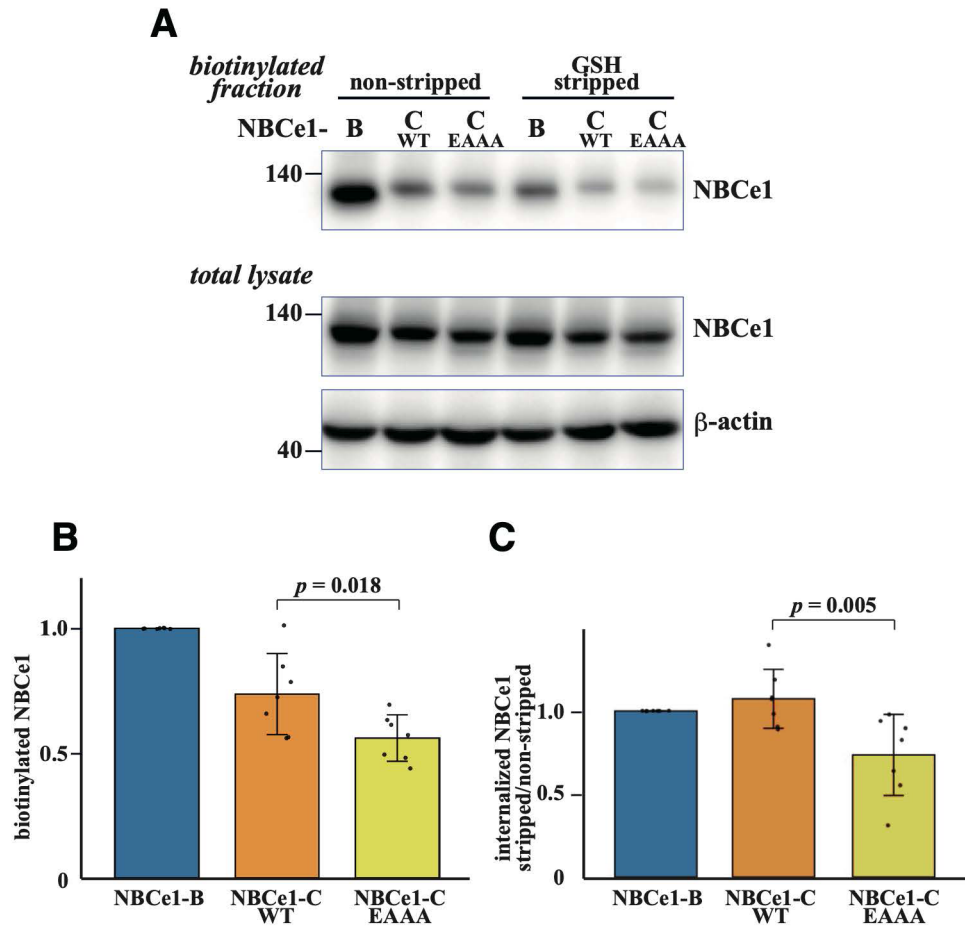




Supplemental Figure 1. Characterization of the NBCe1-C-specific antibody.

A, GFP-tagged NBCe1-A, NBCe1-B, and NBCe1-C were expressed in HEK293T cells. Whole-cell lysates were analyzed by western blotting using an anti-GFP antibody (left), the NBCe1-C-specific antibody (middle), or the NBCe1-C-specific antibody preincubated with 350 μ g/mL antigenic protein (right). The approximately 110-kDa bands detected with the NBCe1-C-specific antibody were nonspecific.

B, Mouse cerebellum was biochemically fractionated into S1 (crude lysate), S3 (cytosolic fraction), P2/P3 (crude microsomal fraction), 1% Triton X-100-soluble fraction, and 1% Triton X-100-insoluble crude postsynaptic density fraction. Each fraction was analyzed by western blotting using the NBCe1-C-specific antibody (left) or the antibody preincubated with 350 μ g/mL antigenic protein (right). No prominent nonspecific bands were detected in mouse cerebellar fractions.



Supplemental Figure 2. Cell-surface biotinylation and endocytosis of NBCe1-C.

A, HeLa cells expressing the indicated NBCe1 constructs were labeled at the cell surface with the membrane-impermeable, cleavable biotin reagent sulfo-NHS-SS-biotin. Whole-cell lysates were prepared, and biotinylated proteins were isolated using NeutrAvidin agarose. NBCe1 in the whole-cell lysates and biotinylated fractions was detected by western blotting using an anti-NBCe1 antibody (non-stripped samples). To assess endocytosis, surface-biotinylated cells were incubated in complete medium for 30 min at 37 °C to allow internalization. Endocytosis was terminated by rapid cooling, and biotin remaining on proteins at the cell surface was removed by incubation with 50 mM reduced glutathione for 30 min on ice. Biotinylated proteins protected from glutathione stripping were subsequently isolated and analyzed by western blotting (GSH-stripped samples).

B, Cell-surface expression was quantified as the ratio of biotinylated NBCe1 to total NBCe1 in the whole-cell lysate. Values were normalized to that of NBCe1-B, which was set to 1.0.

C, Internalization was quantified as the ratio of biotinylated NBCe1 remaining after glutathione stripping to total surface-biotinylated NBCe1 in the corresponding non-stripped sample. Values were normalized to that of NBCe1-B, which was set to 1.0. Data were obtained from seven independent experiments. Statistical significance was evaluated using an unpaired two-tailed Student's *t*-test.