APP amyloidogenic processing is enhanced in the brains of Alcadein α-deficient mice

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Running title: *Alcadein α deficiency enhances APP amyloidogenic processing*

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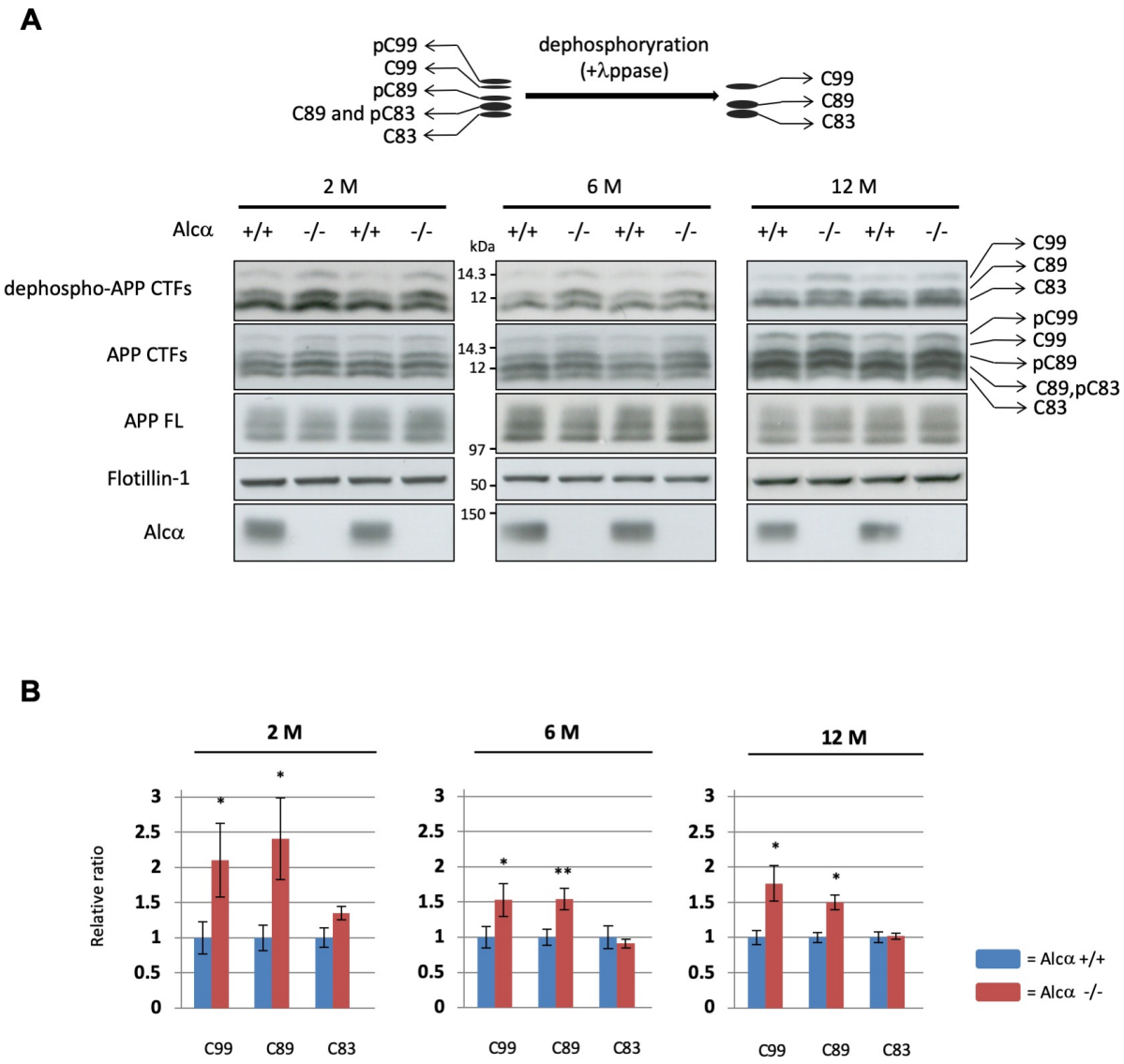
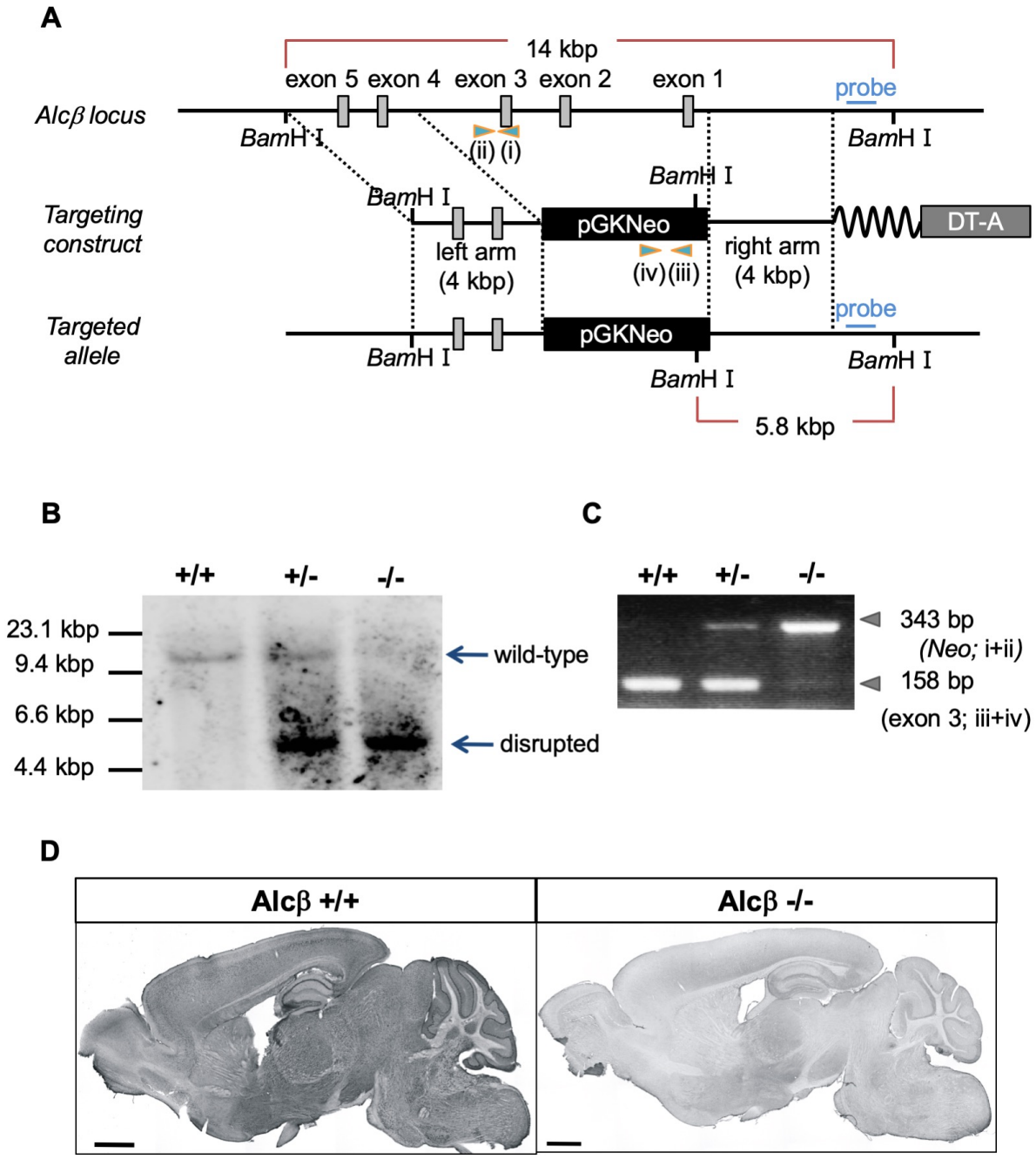
List of material included:
Supplemental Figure 1 to 5 and the figure legends



Supplemental Figure 1. β-site cleavages of APP in the brains of Alcα-deficient mice with age.
(**A**) Immunoblot analyses of APP and APP CTFs with schematic representation of electrophoresis patterns of naïve and dephosphorylated APP CTFs. Membrane fraction (5 μg) or 15 μg (for CTFs) of the hippocampus and cerebral cortex of wild-type (+/+) and homozygous mutant (-/-) mice of indicated ages were analyzed in 8% or 15% (for CTFs) resolving gels with anti-APP, anti-Alcα, and anti-Flotillin-1 antibodies.
(**B**) Band densities for wild-type (blue columns) and Alcα-deficient (red columns) mice were standardized to the density of Flotillin-1, and the value of wild-type was assigned as a reference value of 1.0. C99, CTFβ; C89, CTFβ'; C83, CTFα of APP CTFs (n = 5 mice per group, except 12 months-old Alcα-deficient mice (4 mice); unpaired t-test, *, $p<0.05$; **, $p<0.01$). The error bars indicate S.E.



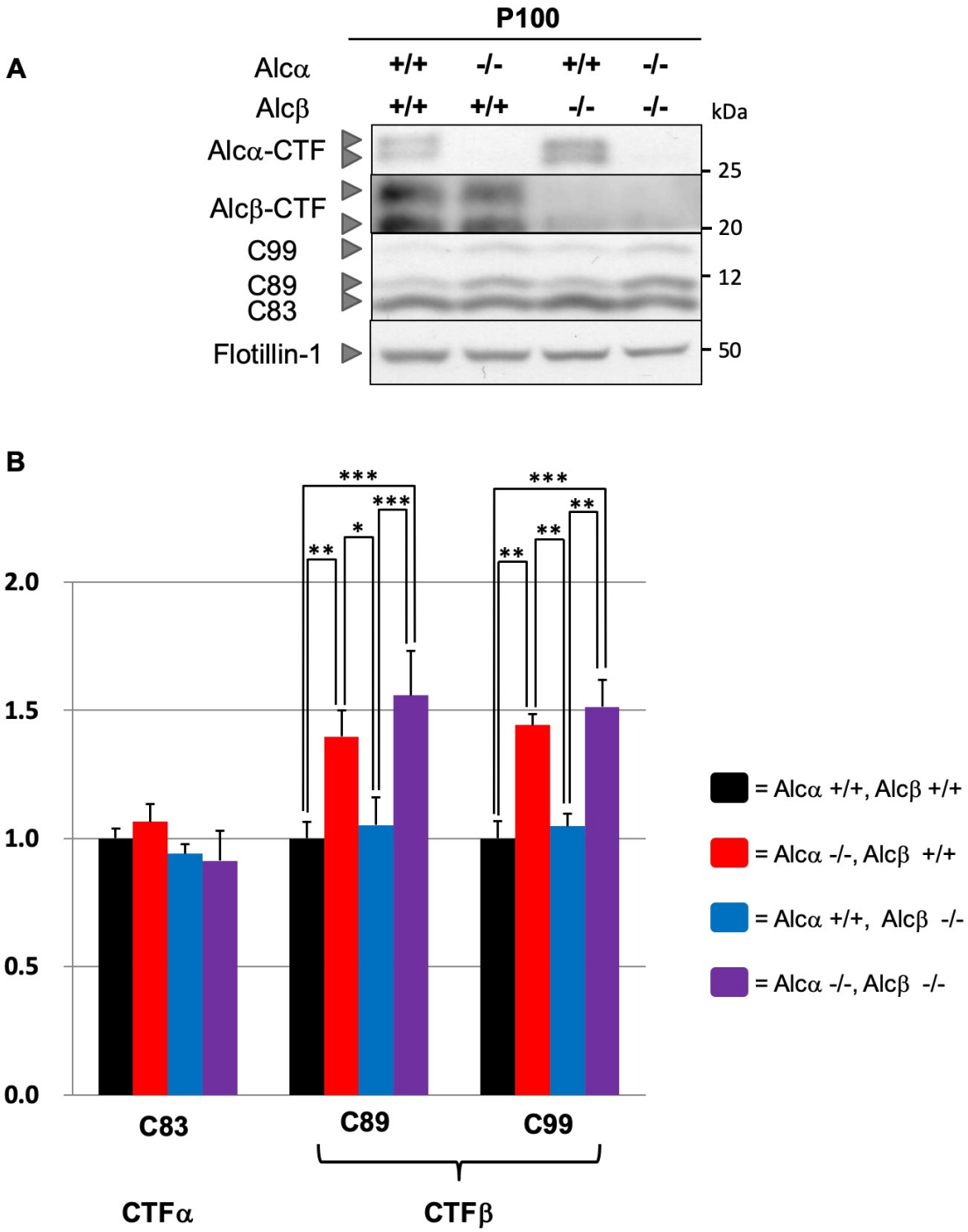
Supplemental Figure 2. Generation of Alcβ-deficient mice.

(**A**) Gene-targeting procedure. Schematic of the structure of the Alcβ gene including exon 1-5, targeting construct, and targeted allele.

(**B**) Southern blot analysis. Probes indicated in (**A**) were used to detect wild-type (14 kbp) and targeted (5.8 kbp) fragments.

(**C**) PCR products specific to the wild-type allele (+/+) generated with primers (i plus ii, 416 bp) and to the targeted allele (-/-) generated with primers (ii plus iii, 1224 bp) were analyzed by agarose gel electrophoresis.

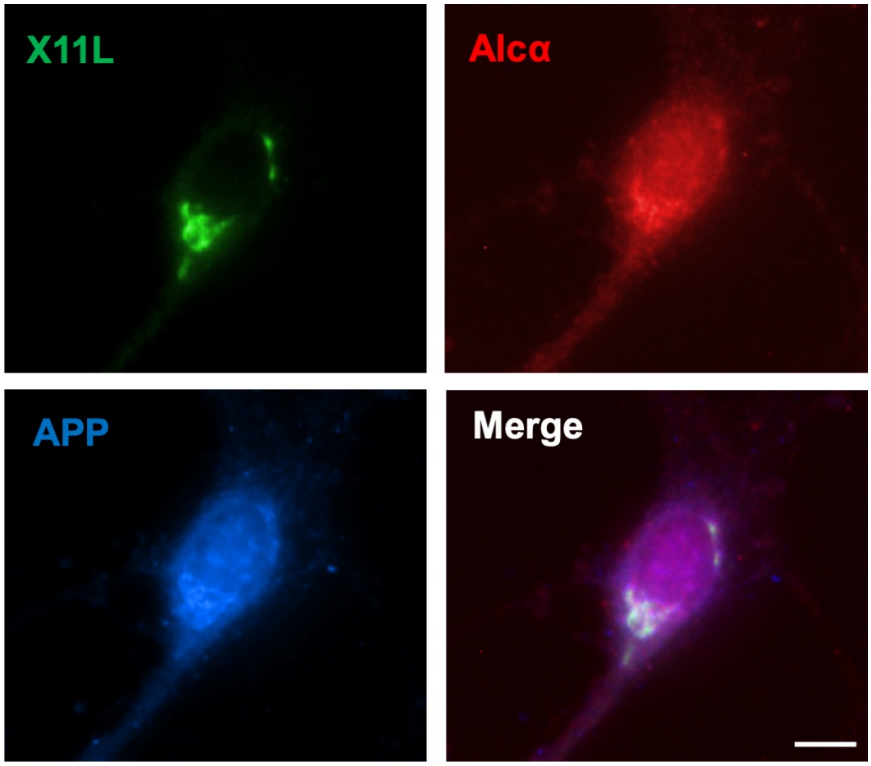
(**D**) Immunostaining of sagittal sections of wild-type (+/+) and homozygous mutant (-/-) mouse (2-3 months old) brains with anti-Alcβ antibody. Scale bar indicates 1 mm.



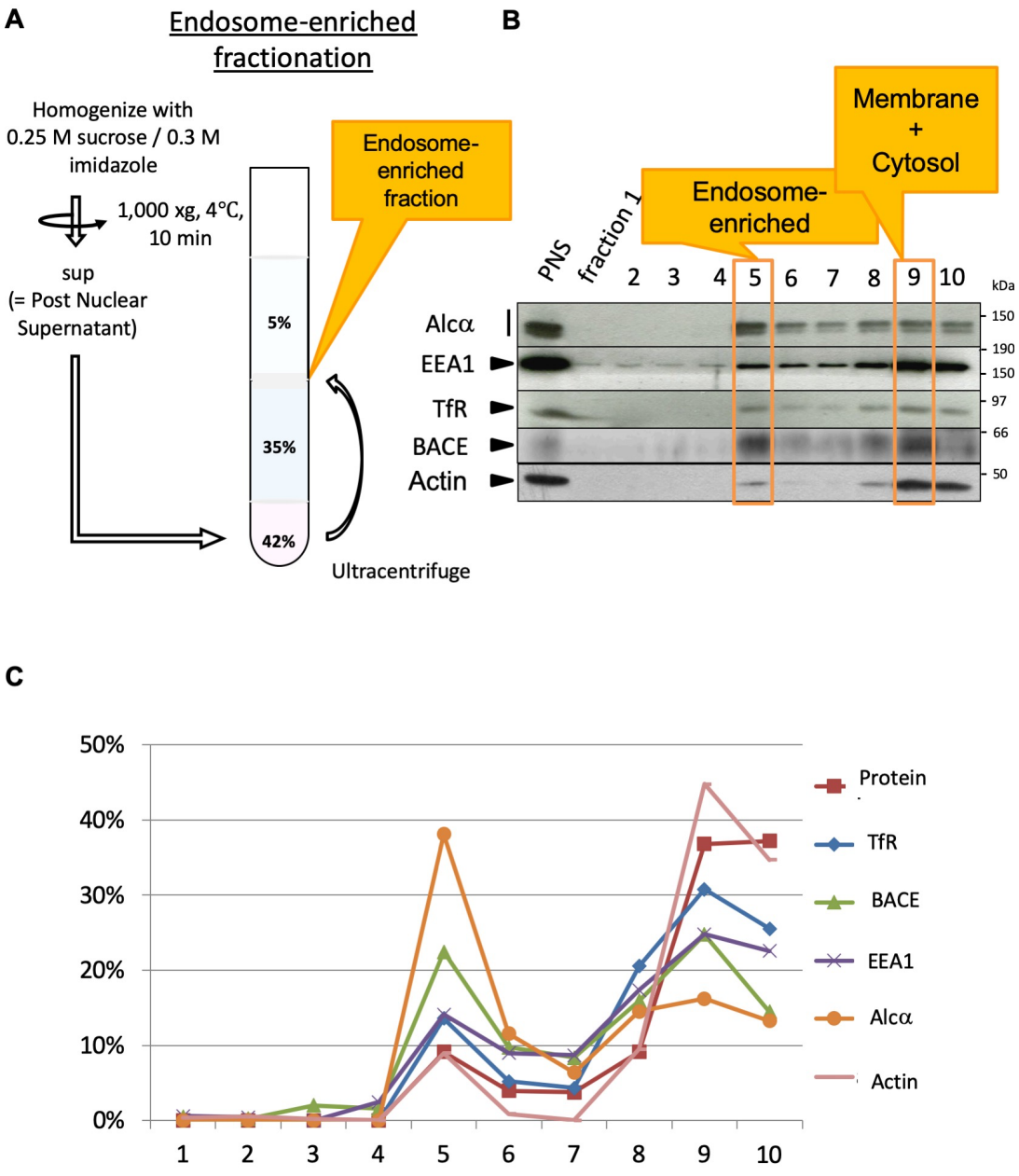
Supplemental Figure 3. Alcα-, but not Alcβ-deficient mice showed significant alterations in amyloidogenic processing of APP.

(**A**) Immunoblot analysis of APP CTFs in Alcα- or Alcβ-deficient mice. A total of 15 μg of membrane fraction of the hippocampus and cerebral cortex of wild-type (+/+) and homozygous mutant (-/-) mice (12 months old) were analyzed in 15% resolving gel with anti-APP, anti-Alcα, and anti-flotillin antibodies. Alcα CTF exhibited double bands in these electrophoresis conditions.

(**B**) Band densities of APP CTFs for wild-type (black columns) and Alcα and/or Alcβ-deficient (colored columns) 12-month-old mice were standardized to the density of flotillin-1, and the value of wild-type was assigned a reference value of 1.0. C99, CTFβ; C89, CTFβ'; C83, CTFα of APP CTFs (n = 4 mice; 2-way ANOVA, Tukey's posthoc test, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.). The error bars indicate S.E.



Supplemental Figure 4. Subcellular localizations of X11L, APP, and Alca in a primary cultured cortical neuron.
Fixed mouse primary cultured cortical neurons were simultaneously labeled with mouse anti-X11L, rabbit anti-APP, and guinea pig anti-Alcα antibodies. Their co-localization is observed especially around peri-nuclear structures. Scale bar: 10μm.



Supplemental Figure 5. Fractionation of post-nuclear supernatants of mouse brains.
(**A**) Preparation of endosome-enriched membranes. Light membranes containing early endosomes with Golgi and other membranes accumulated underneath the interface between 5% and 35% sucrose layers after ultracentrifugation. Very light membrane largely composed of late endosomes enter the 5% sucrose layer, and heavy membrane with cytosolic proteins containing plasma membrane and rough endoplasmic reticulum membrane reside in the 42.5% sucrose layer.
(**B**) Typical isolation profile of wild-type mouse brain homogemate.
(**C**) Distribution of proteins in the fractionation ~10% of proteins are in the endosome-enriched light membrane fraction with EEA1 and BACE1.