Supplemental Information

Supplemental Methods

Targeting vector construction: The targeting vector, diagramed below, was constructed based on a neo-loxP vector in which a neomycin-resistant gene (neo) was flanked by two loxP sites (Papaioannou, 2005), and PCR amplification using the pfu polymerase (Stratgene). Specifically, a 650 bp fragment containing *APP* exon 3 was amplified from AB2.2 ES DNA by PCR using primers:

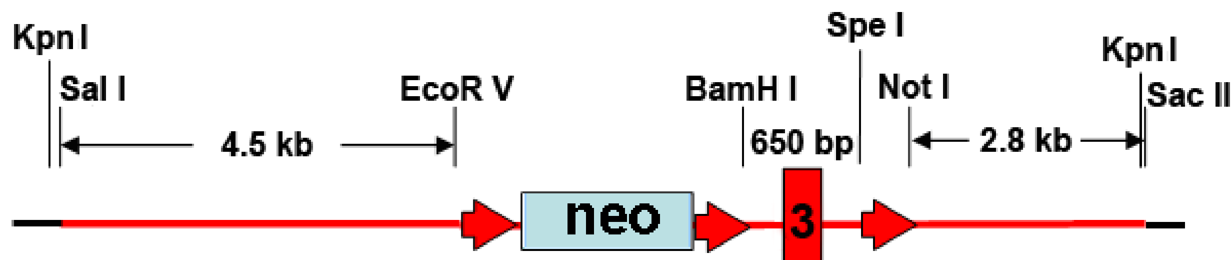
5'-CGGGATCCGCAAGTCACCTTGGAGTGGA-3' and
BamH1
5'-AAGCGGCCGCTAGGACTAGTTCGCCTCTCCTTACTTCCAA-3'
NotI SpeI

The fragment was cloned into the BamHI and NotI sites of the pKS(-) vector. A synthesized loxP oligonucleotide was introduced between the SpeI and Not I sites. The fragment was then released from the pKS vector and inserted into the BamH1 and NotI sites of the neo-loxP vector. The 4.5 kb of 5' homologous arm in intron 2 of *APP* and immediately upstream the 650 bp fragment was amplified using the following primers:

5'-ATGTCGACTCCTAGGAGTCTGATGGTGGTGTGTG-3' and
SalI
5'-AAGATATCATCGAGCTGTCTTTTCAGACACTG-3'
EcoRV

The amplified fragment cloned into the Sal I and EcoR V sites of the neo-loxP vector. The 3' arm of 2.8 kb *APP* sequences located in intron 3 was amplified using primers and cloned into the Not I and Sac II sites:

5'-AAGCGGCCGCGGCGATGTATTTGTTTCTTCA-3' and
NotI
5'-GGCCGCGG GGTACCCAACCTTTCTTGCATGATTTG-3'
SacII KpnI



Gene targeting: The targeting vector was digested with Kpn I to remove the plasmid backbone and electroporated into AB2.2 ES cells. Homologous recombination resulted in the insertion of neomycin-resistant gene (neo) and three loxP sites flanking neo and exon 3 respectively (Figure S1A). Homologous recombinants were identified following selecting and analyzing G418-resistant clones by mini-Southern analysis (Zheng, 2004). EcoRV digestion created a wild-type fragment (WT) at 8.3 kb and targeted fragment (T) at 5.4 kb due to the insertion of the EcoRV site in the vector (Figure S1B). 23 targeted clones were identified from a total of 300 G418^r clones analyzed. Three correctly targeted clones were expanded for microinjection and two resulted in germline transmission.

Cre-mediated recombination: Mice containing the *APP* targeted allele were crossed with two Cre-recombinase transgenic lines: 1) GDF9-iCre, which is a strong germline deleter leading to complete recombination and deletion of PGKneo and exon 3 (ΔE3, Figure S1A) (Lan et al., 2004); 2) CMV-Cre, line B6 (Papaioannou, 2005), a weak germline deleter which results in both compete and partial recombination including deletion of the neo cassette only, creating exon 3 floxed allele (fl, Figure S1A).

Quantitative RT-PCR: Total RNA was isolated from spinal cord or gastronomies muscle using the RNeasy Lipid Tissue Mini Kit (Invitrogen) and subjected to DNase I digestion to remove contaminating genomic DNA. Reverse transcription was performed using a SuperScript III

RNase H-reverse transcriptase (Invitrogen), and the reaction mix was subjected to quantitative real-time PCR using ABI PRISM Sequence Detection System 7000 (Applied Biosystems, Inc.). Primers were designed with Primer Express Version 2.0 software (Applied Biosystems) using sequence data from NCBI. The sets of GAPDH and HPRT primers were used as an internal control for each specific gene amplification. The relative levels of expression was quantified and analyzed by using ABI PRISM Sequence Detection System 7000 software. The real-time value for each sample was averaged and compared using the comparative CT method. The relative amount of target RNA was calculated relative to the expression of endogenous reference and relative to a calibrator which was the mean CT of control samples.

Supplemental References

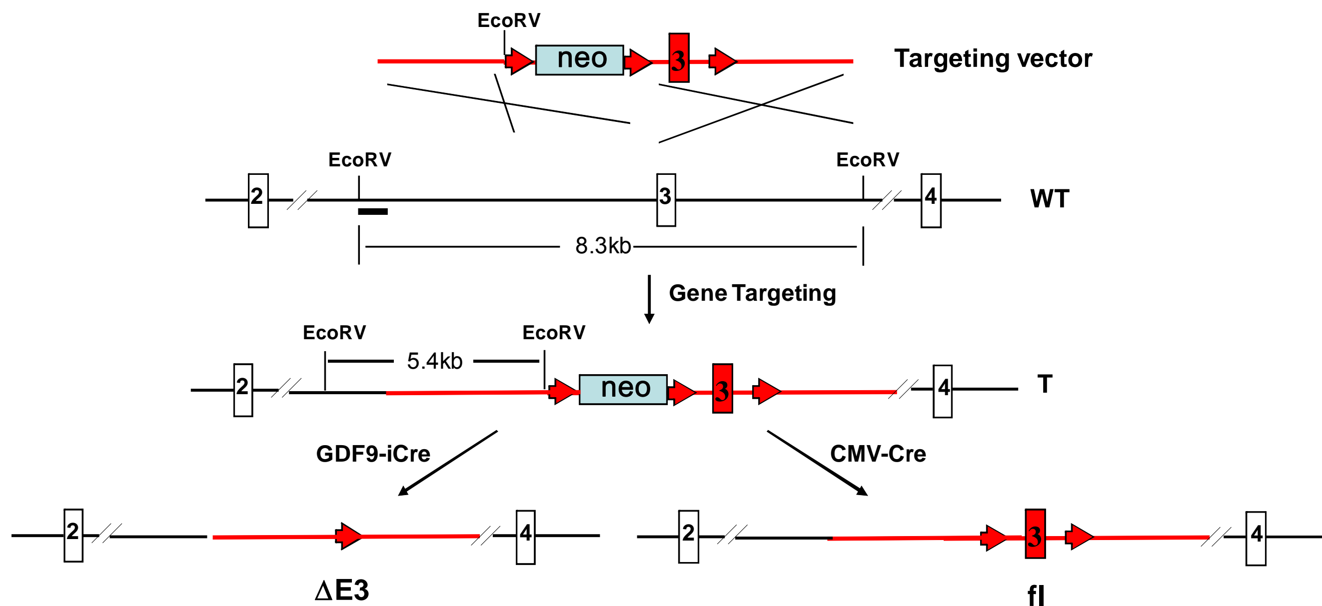
Lan ZJ, Xu X, Cooney AJ (2004) Differential oocyte-specific expression of Cre recombinase activity in GDF-9-iCre, Zp3cre, and Msx2Cre transgenic mice. Biol Reprod 71:1469-1474. Epub 2004 Jun 1423.

Papaioannou VE, Boehringer R (2005) Mouse phenotypes: a handbook of mutation analysis: Cold Spring Harbor Laboratory Press.

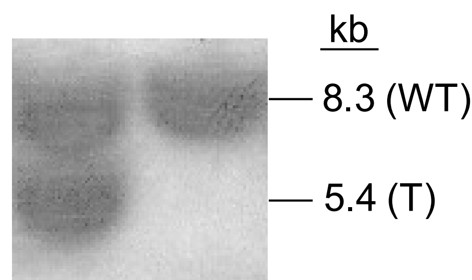
Zheng H (2004) Generation of amyloid precursor protein knockout mice. In: Amyloid precursor protein: A practical approach. (Xia W, Xu, H., ed), pp 201-218. London, UK: CRC Press.

Supplemental Figures

A



B



C

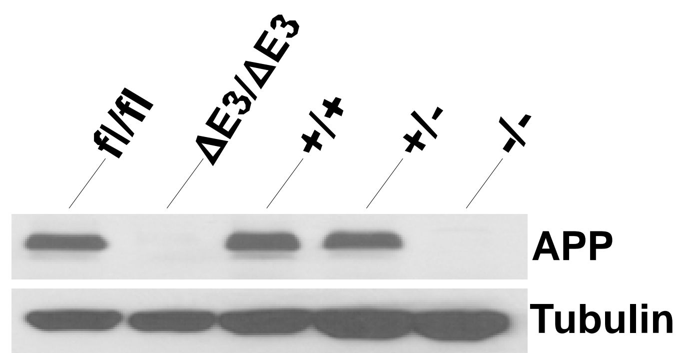


Figure S1. Gene targeting in ES cells and Cre-mediated recombination in mice. A. Gene targeting strategy for creating the *APP* targeted allele. The targeting vector (not drawn to scale) consists of a 4.5 Kb and a 2.8 Kb of 5’ (left red line) and 3’ (right red line) homologous sequences respectively. The neomycin-resistant gene (neo) was flanked by the loxP sites (red

arrows) with an additional loxP sequence inserted into 3' of exon 3. Gene targeting resulted in insertion of the neo and loxP sequences into the *APP* locus. The wild-type (WT) and targeted (T) alleles were distinguished by Southern blot analysis upon EcoRV digestion and hybridization with the 5'-probe (black bar underneath WT), which yielded a wild-type fragment (WT) at 8.3 kb and a targeted fragment (T) at 5.4 kb due to the insertion of the EcoRV site in the vector (examples shown in B). The clones were also confirmed with a 3' probe (not shown). Mice containing the targeted allele were crossed with GDF9-iCre and CMV-Cre (line B6) to create exon 3 deleted (ΔE3) and exon 3 floxed (fl) alleles, respectively, resulting from complete or partial Cre-mediated recombination. C. Western blot analysis of APP expression in the brains of homozygous *APP* floxed (fl/fl), ΔE3/ΔE3 mice, documenting that the loxP sequences in the fl allele did not affect APP expression, whereas deletion of exon 3 created a null allele. Wild-type (WT), *APP* heterozygous (+/-) and *APP* homozygous null (-/-) were used as APP expression controls. Tubulin was used as a loading control.

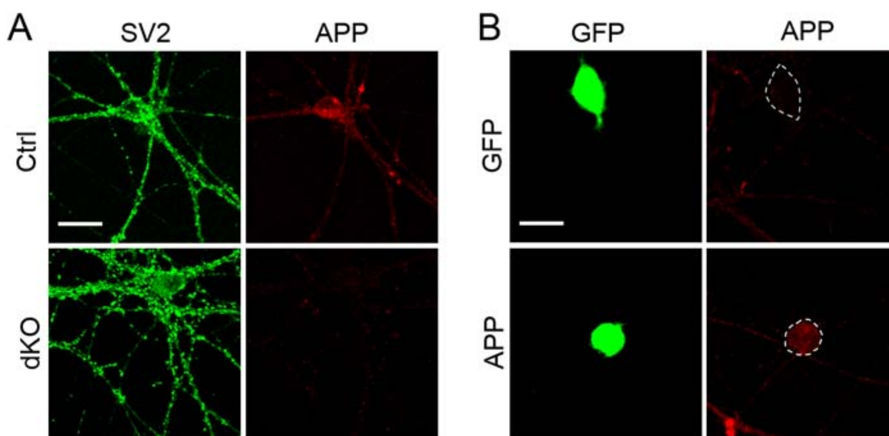


Figure S2. Characterization of the rabbit monoclonal anti-APP antibody (Y188). A. Immunofluorescent staining of primary *APLP2* null control (Ctrl) and *APP/APLP2* double knockout (dKO) hippocampal neurons with an anti-SV2 antibody (1:1000, Green) and the Y188 anti-APP antibody (1:300, Red), showing positive APP staining in both the cell body and processes in the control neuron but minimum staining in dKO neuron. B. Immunostaining of GFP transfected and APP/GFP co-transfected HEK293 cells using the Y188 antibody, demonstrating positive immunoreactivity in APP transfected, but not GFP transfected HEK293 cells (circled). Scale bar: 20 μM.

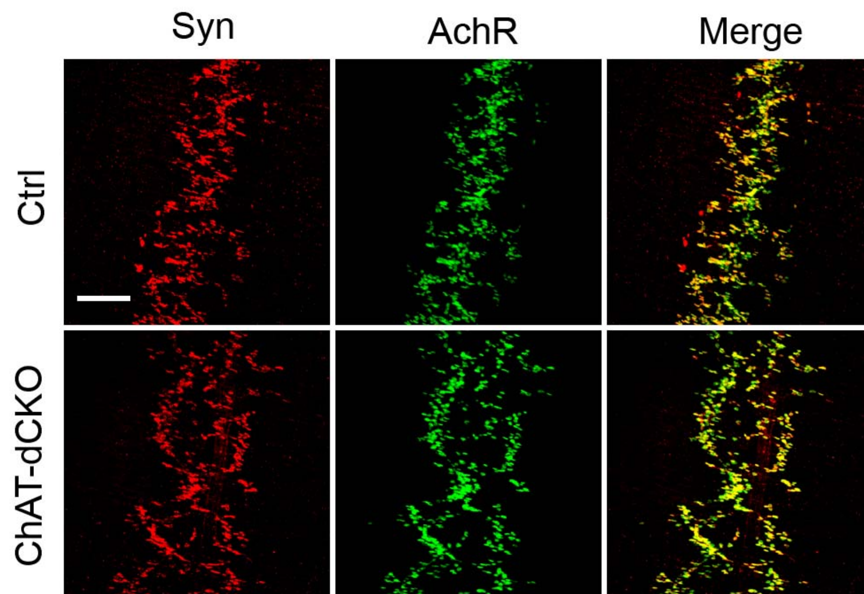


Figure S3. Neuromuscular synapse defects in ChAT-Cre; $APP^{fl/fl}$; $APLP2^{-/-}$ (ChAT-dCKO) animals. Whole mount double staining P0 diaphragm muscles with anti-synaptophysin antibody (Syn) and α-BTX (AchR). Merge: Overlap of Syn and AchR images. Scale bar: 100 μM.

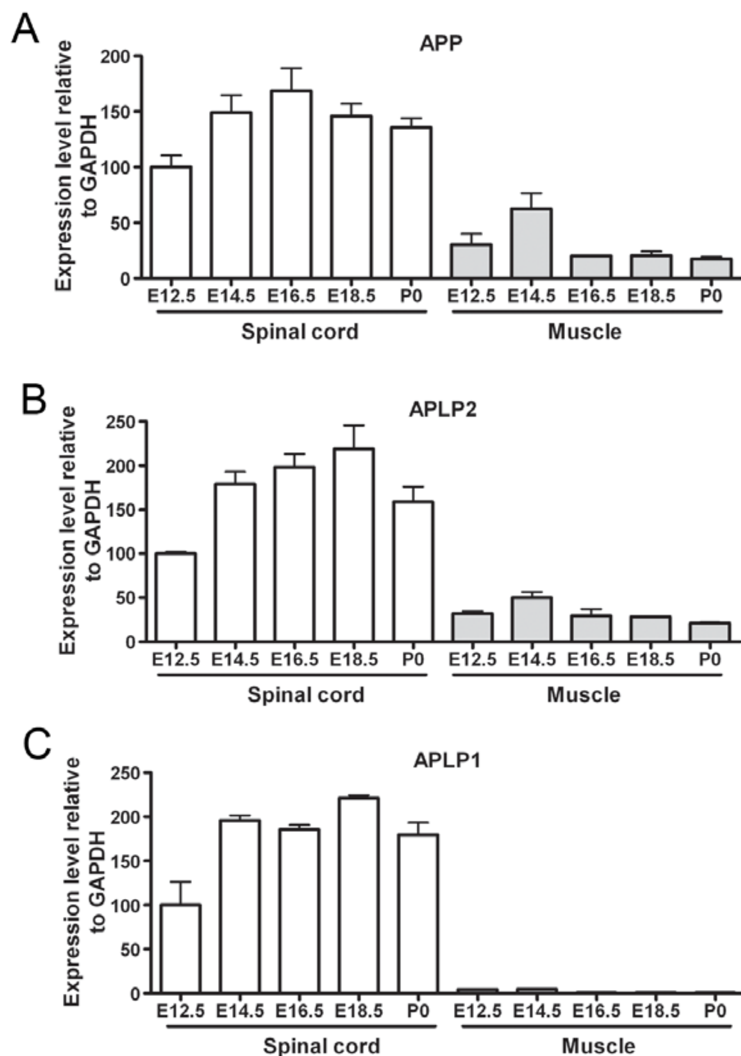


Figure S4. Quantitative RT-PCR (qRT-PCR) analysis of APP (A), APLP2 (B) and APLP1 (C) in the spinal cord and muscle of E12.5, E14.5, E16.6 and E18.5 embryos and newborn pups (P0). The real-time value for each sample was averaged from 3 animals/genotype/age and normalized to the GAPDH control. The neuronal expression at E12.5 was set at 100%.