Supporting Information

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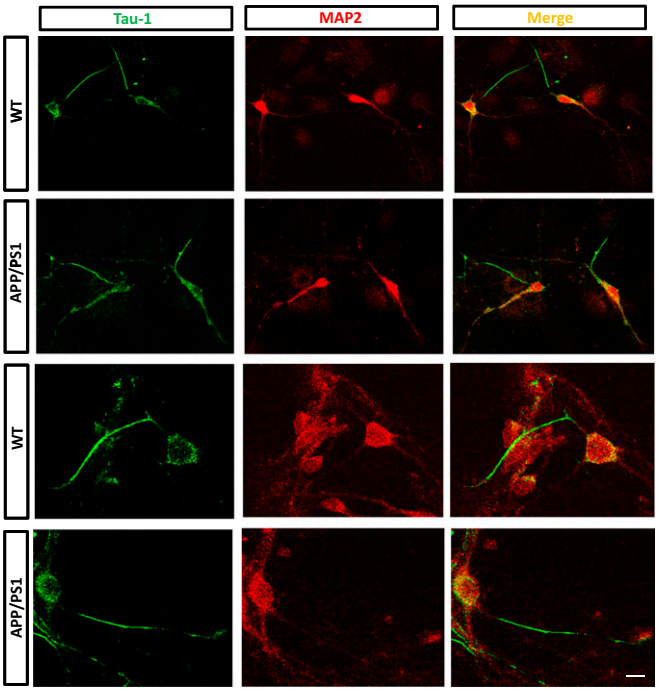

Fig. S1. Lack of MAP2 staining served as the marker for the axon. WT and APP/PS1 neurons were immunostained by Tau-1 (red) and MAP2 (green). MAP2 specifically labeled the soma and dendrites, whereas Tau-1 labeled the axons with overlap with MAP2 staining in some of the dendrites. (Scale bar: 5 μm.)

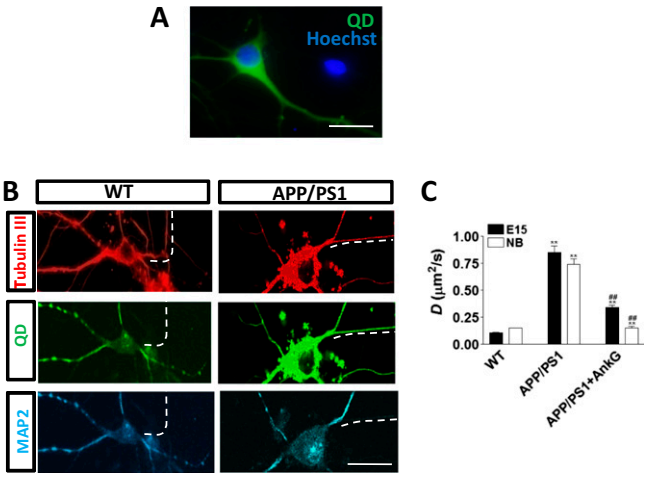

Fig. S2. Impaired filtering at the axon initial segment (AIS) altered quantum dot (QD) dynamics. (*A*) Example of a neuron loaded by microinjection with QD. (Scale bar: 20 μm.) (*B*) In WT mouse neurons, microinjected QD was located mainly at the soma and dendrites at 10 min after injection, whereas in APP/PS1 mouse neurons, QD was abnormally located at the axon. The dashed line represents the AIS. (Scale bar: 20 μm.) (*C*) The diffusion coefficient, *D*, was measured by QD in WT, APP/PS1, and APP/PS1 neurons injected with AnkG-expressing constructs. Average lateral diffusion coefficient (*D*) values were measured at the AIS by loading neurons with QD at the soma ($n = 200$ for each preparation; experiments were repeated in three independent preparations). Data are mean ± SE. $^{**}P < 0.01$ compared with WT; $^{\#\#}P < 0.01$ compared with APP/PS1.

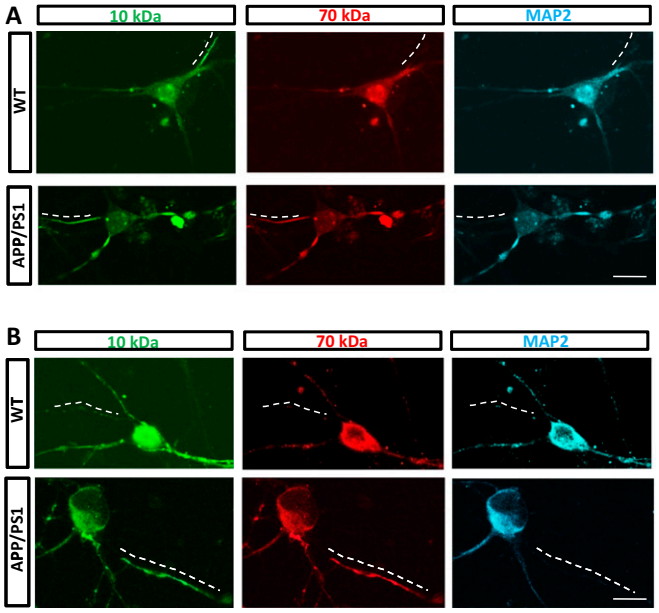

Fig. S3. Impaired AIS filtering was not related to developmental delay in APP/PS1 neurons. (*A*) Examples of axonal diffusion of 10-kDa and 70-kDa dextran loaded in the soma of newborn APP/PS1 mouse hippocampal neurons at 5 DIV. (*B*) Examples of axonal diffusion of 10-kDa and 70-kDa dextran loaded in the soma of E14 APP/PS1 mouse hippocampal neurons at 14 DIV. The dashed line represents the AIS. (Scale bars: 10 μm.)

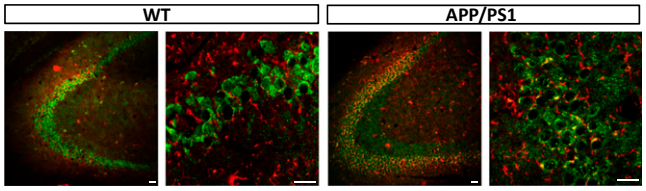

Fig. S4. Downregulation of AnkG induced an impaired AIS filtering in APP/PS1 neurons. In 3-mo-old WT and APP/PS1 mouse hippocampal tissues, immunostaining showed that AnkG (red) overlapped more with NR2B (green) in the axon in APP/PS1 tissue than in WT tissue, indicating impaired AIS filtering in APP/PS1 neurons. (Scale bars: 20 μm.)

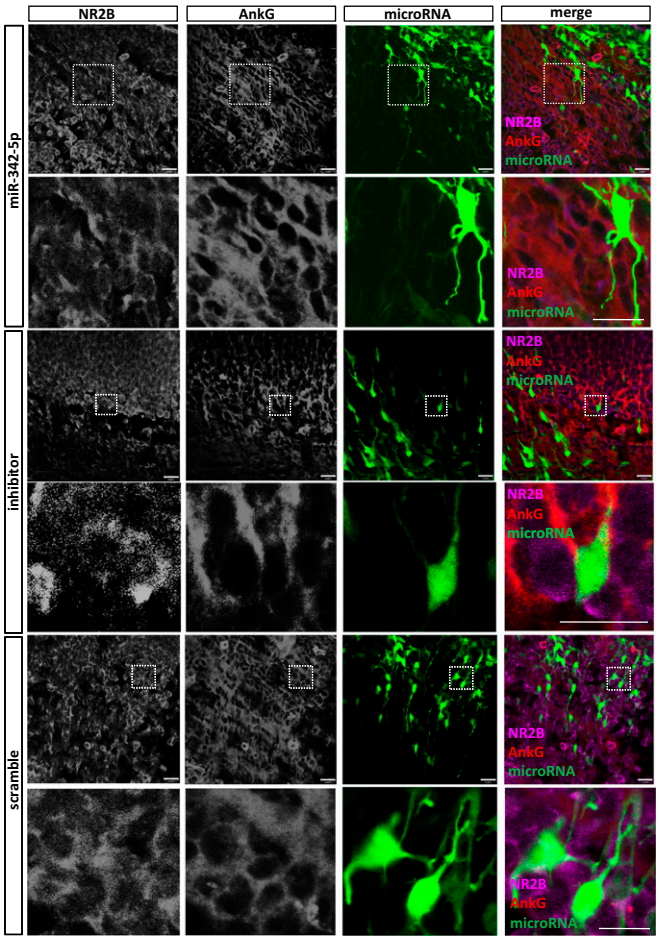

Fig. S5. Overexpression of miR-342–5p impaired AIS filtering. MiR-342–5p, its inhibitor, or scramble control miRNA was transduced into the cortical layers II-IV of E14.5 WT mouse embryos by in utero electroporation with cytomegalovirus early enhancer/chicken β-actin promoter-driven EGFP. After electroporation, the embryos were returned and left to develop to P0. The brain slices were labeled with NR2B and AnkG antibodies. The data show that in WT neurons, miR-342–5p induced colocalization of NR2B with AnkG in the axon, whereas inhibitor or scramble did not. In the merge images. Purple, NR2B; red, AnkG; green, miRNAs. (Scale bars: 20 μm.)

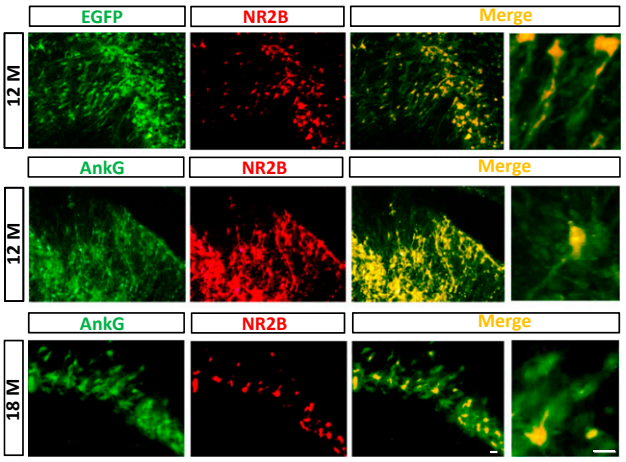

Fig. S6. Overexpression of AnkG rescued impaired AIS filtering in 12-mo-old and 18-mo-old APP/PS1 mouse brain tissues. AnkG-EGFP and NR2B-RFP were transduced into the CA1 region of the hippocampus of APP/PS1 mice by adenovirus-mediated infection. In the control EGFP-infected 12-mo-old APP/PS1 mice, NR2B entered into the axons (*Top*). In both 12-mo-old and 18-mo-old APP/PS1 mice, AnkG-EGFP could block NR2B from entering into the axons (*Middle* and *Bottom*, respectively). (Scale bars: 20 μm.)

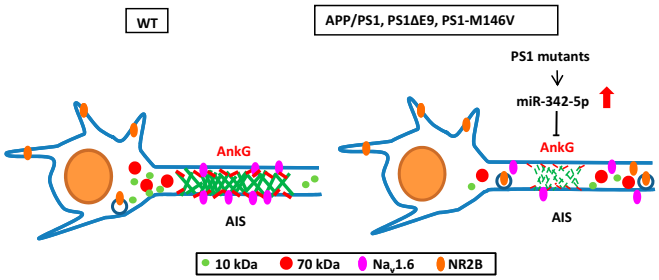

Fig. S7. Schematic drawing illustrating how miR-342–5p regulates the protein level of AnkG and alters the structure of the filtering machinery at the AIS. In WT neurons, AnkG as one of the major components forms a selective filter at the AIS of the axon. The selective filter regulates protein trafficking in the axon and prevents some proteins from entering into the distal area of the axon. In APP/PS1, PS1ΔE9, and PS1-M146V neurons, PS1 mutations lead to up-regulation of miR-342. An increased level of parent miR-342 results in an enhanced level of the dicing product miR-342–5p. One target of miR-342–5p is AnkG mRNA 3′ UTR; thus, in APP/PS1, PS1ΔE9, and PS1-M146V neurons, AnkG level is down-regulated, which leads to impaired selective filtration at the AIS. The impaired AIS filtering in APP/PS1, PS1ΔE9, and PS1-M146V neurons might induce mislocalization of action potential-generating proteins such as Na_V 1.6 and neurotransmitter receptors such as NR2B, thereby affecting neuronal functions.