Reduced amyloid deposition in mice overexpressing RTN3 is adversely affected by preformed dystrophic neurites

Qi Shi, Marguerite Prior, Wanxia He, Xiangying Tang, Xiangyou Hu and Riqiang Yan*

1Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation,
Cleveland, OH 44195,

*Correspondence should be addressed to: Riqiang Yan, Ph.D., Department of
Neurosciences, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. Tel: 216-445-2690, Fax: 216-444-7927, E-mail:
yanr@ccf.org.

Supplementary Figure 1. LI-COR Odyssey near-infrared (IR) was used to conduct quantitative analysis of proteins on Western blots. IR imaging has been widely used for quantification by direct detection of proteins on Western blots. After proteins were transferred to nitrocellulose membranes from the gel, membranes were incubated in Odyssey blocking buffer at room temperature for 60 minutes. The membranes were then incubated with primary rabbit antibody against APP-C terminus and primary mouse antibody against β-tubulin that were dissolved in Odyssey blocking buffer (0.1% Tween-20 added) at 4°C overnight. After being washed 4 times (5 minutes each), the membranes were incubated with either IRDYE800CW donkey anti-rabbit secondary antibody or IRDYE680 goat anti-mouse secondary antibody at room temperature for 60 minutes. After washed 4 times (5 minutes each), the membranes were directly scanned in the Odyssey IR imaging system at a wavelength of 800 nm for IRDYE800CW and 700 nm for IRDYE680. Protein band intensity was analyzed using Odyssey infrared imaging system application software. The ratios of APP CTF99 p or CTF99 np to total APP CTF83 were calculated based on their intensity from 4 independent experiments (P<0.05).