SUPPLEMENTARY INFORMATION

APP intracellular domain/WAVE1 pathway reduces amyloid β production

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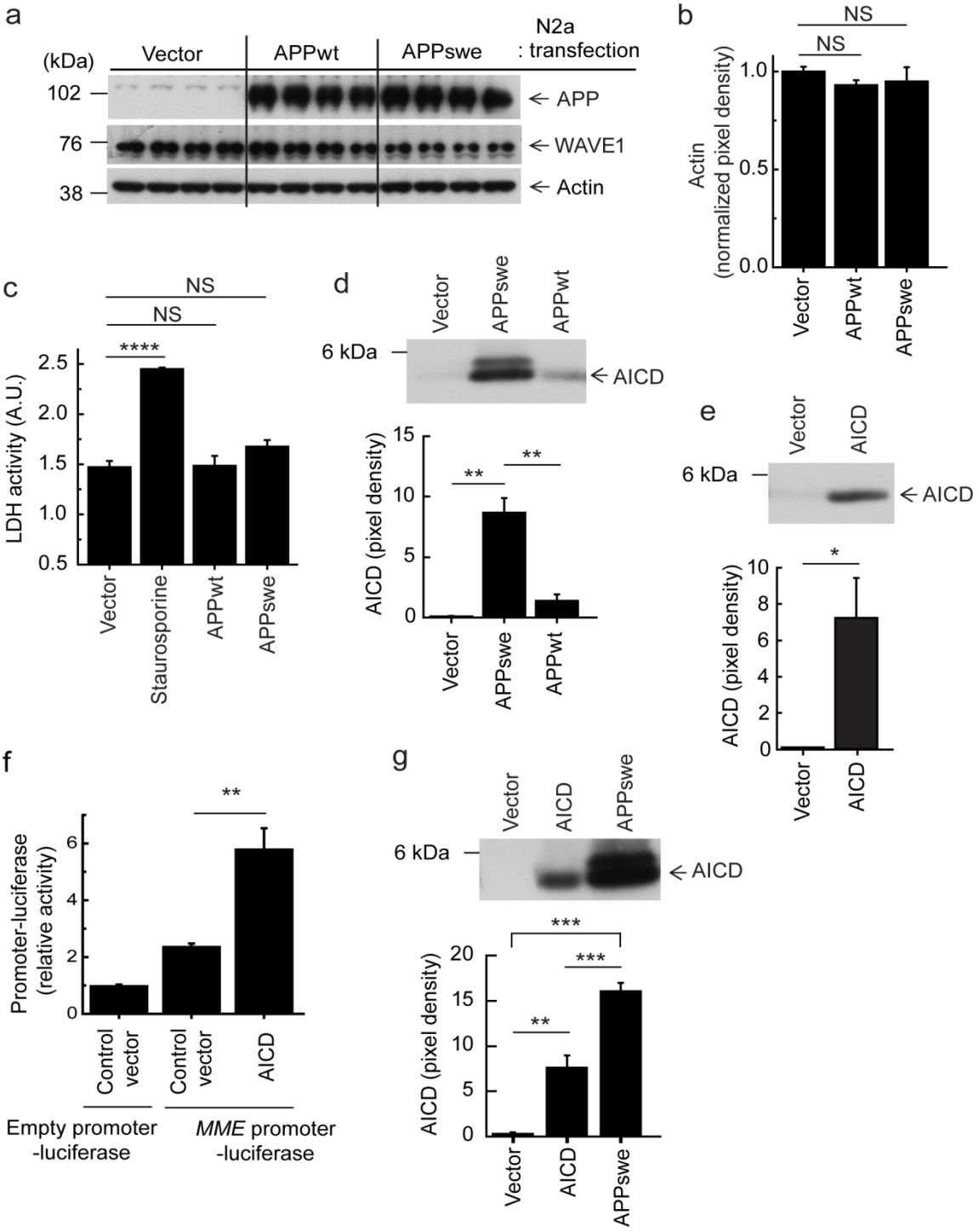
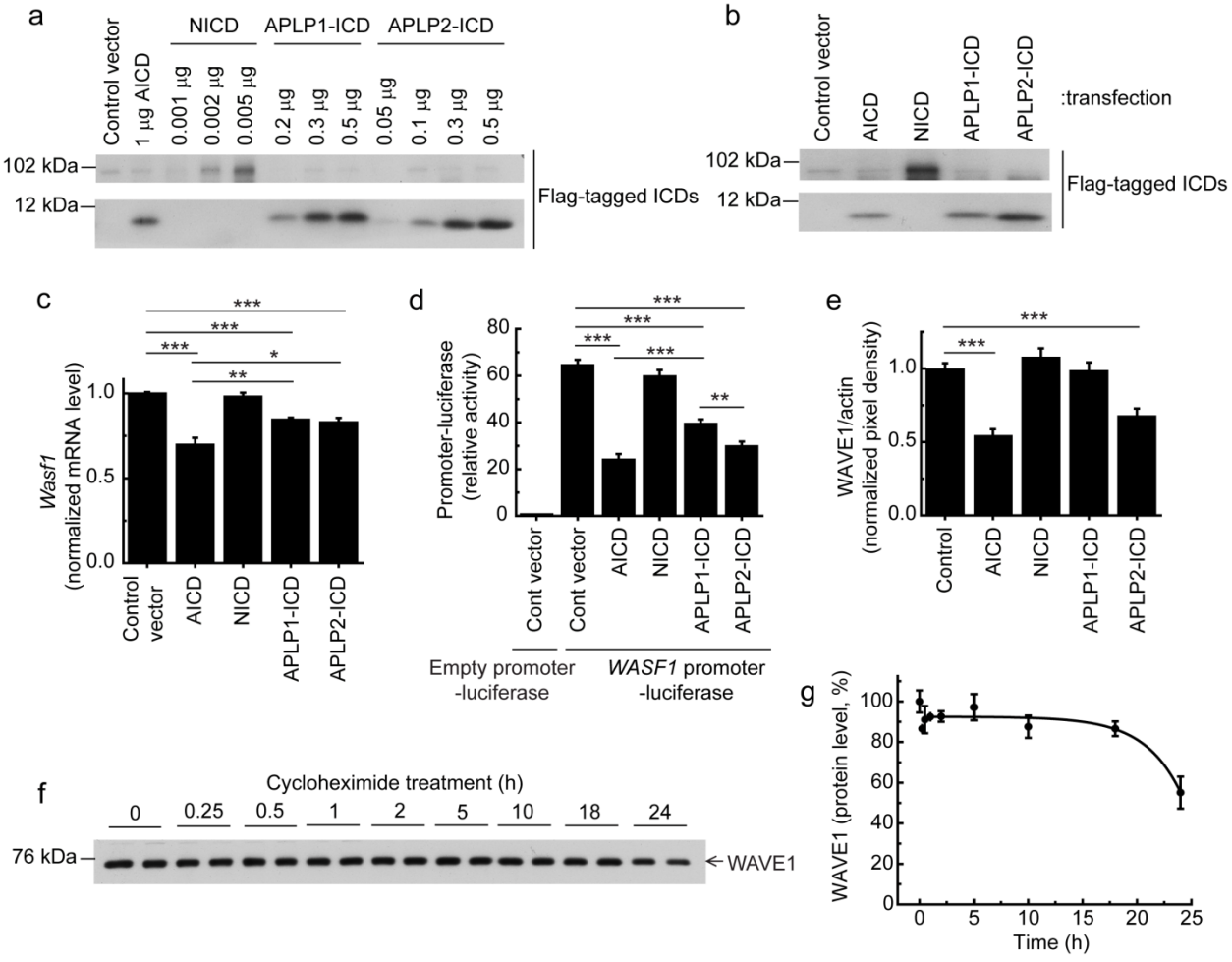
Supplementary Figure 1



Supplementary Figure 1 Actin levels, cytotoxicity, AICD levels, and measurement of AICD activity using *MME* promoter-luciferase assay. (**a**–**d**) N2a cells were transiently transfected with vector plasmid, WT (APPwt) or Swedish mutant (APPswe) APP695. Representative immunoblotting images of indicated proteins (**a**). The quantified protein level of actin (**b**, $n = 8$). Cytotoxicity was assessed by lactate dehydrogenase (LDH) assay (**c**, $n = 6$ for vector, n=4 for other groups). Incubation with 100 nM staurosporine for 24 h was used as a positive control. Representative immunoblotting image of AICD (upper panel) and quantification of AICD protein levels (**d**, $n = 4$). (**e**) N2a cells were transiently transfected with vector plasmid or AICD. Representative immunoblotting image of AICD (upper panel) and quantification of AICD protein levels ($n = 4$). (**f**) N2a cells were transiently co-transfected with empty promoter-luciferase vector plus control vector, or luciferase reporter gene conjugated to the human *neprilysin* promoter (*MME* promoter-luciferase) plus control vector or AICD. Luciferase activity is shown ($n = 6$). (**g**) AICD levels were measured in N2a cells transiently transfected with control vector, AICD or APPswe. Representative immunoblotting image of AICD (upper panel) and quantification of AICD protein levels ($n = 4$). Data represent means ± SEM. NS (non-significant), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$, two-tailed *t*-test.

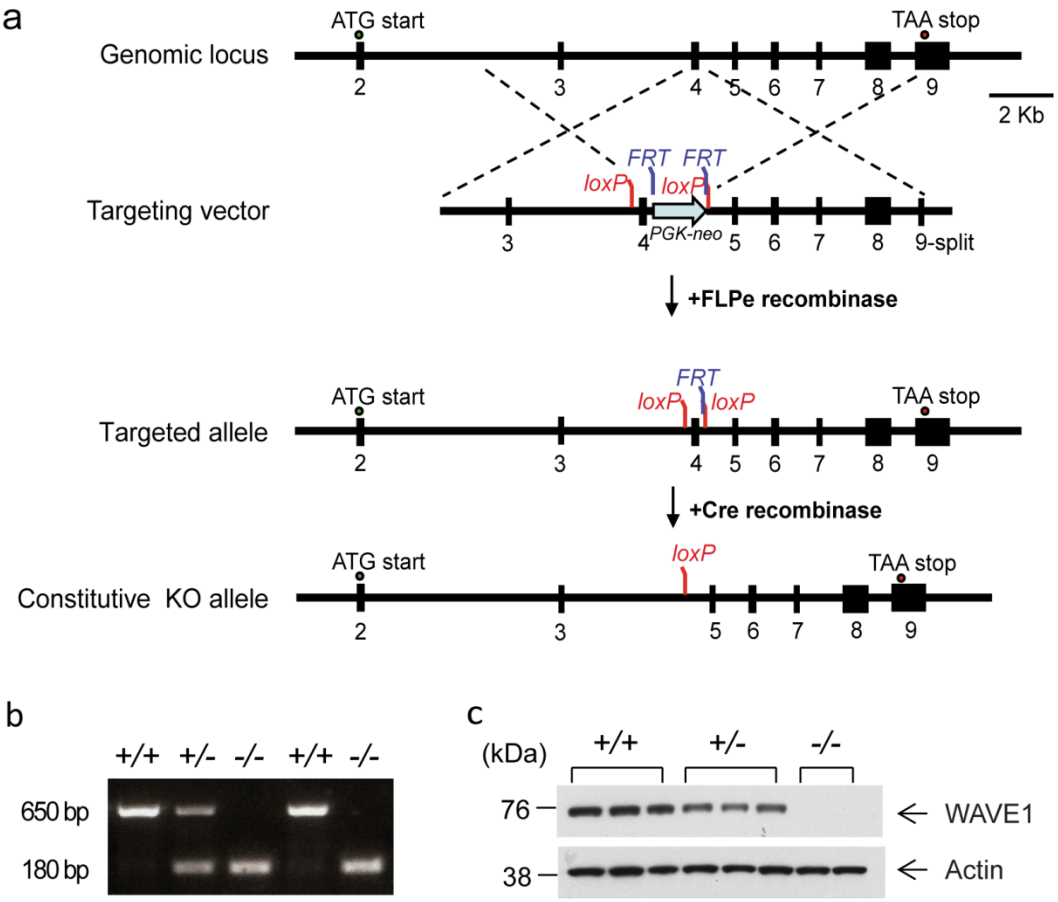
Supplementary Figure 2



Supplementary Figure 2 Downregulation of WAVE1 by AICD, APLP1-ICD and APLP2-ICD but not by NICD. (**a**) N2a cells were transiently transfected with 1 μg of control vector or 1 μg of plasmid vector for AICD-3xFlag, or various doses of plasmid vectors for NICD-3xFlag, APLP1-ICD-3xFlag or APLP2-ICD-3xFlag. Flag-tagged ICDs were detected by immunoprecipitation followed by immunoblotting with anti-Flag antibody. Comparable plasmid dosages were determined based on the expression levels of ICDs. (**b**–**e**) N2a cells were transiently transfected with total 1 μg of plasmids (control vector, 1 μg; AICD-3xFlag, 1 μg; NICD-3xFlag, 0.005 μg; APLP1-ICD-3xFlag, 0.3 μg; APLP2-ICD-3xFlag, 0.3 μg). Control vector was added to NICD,

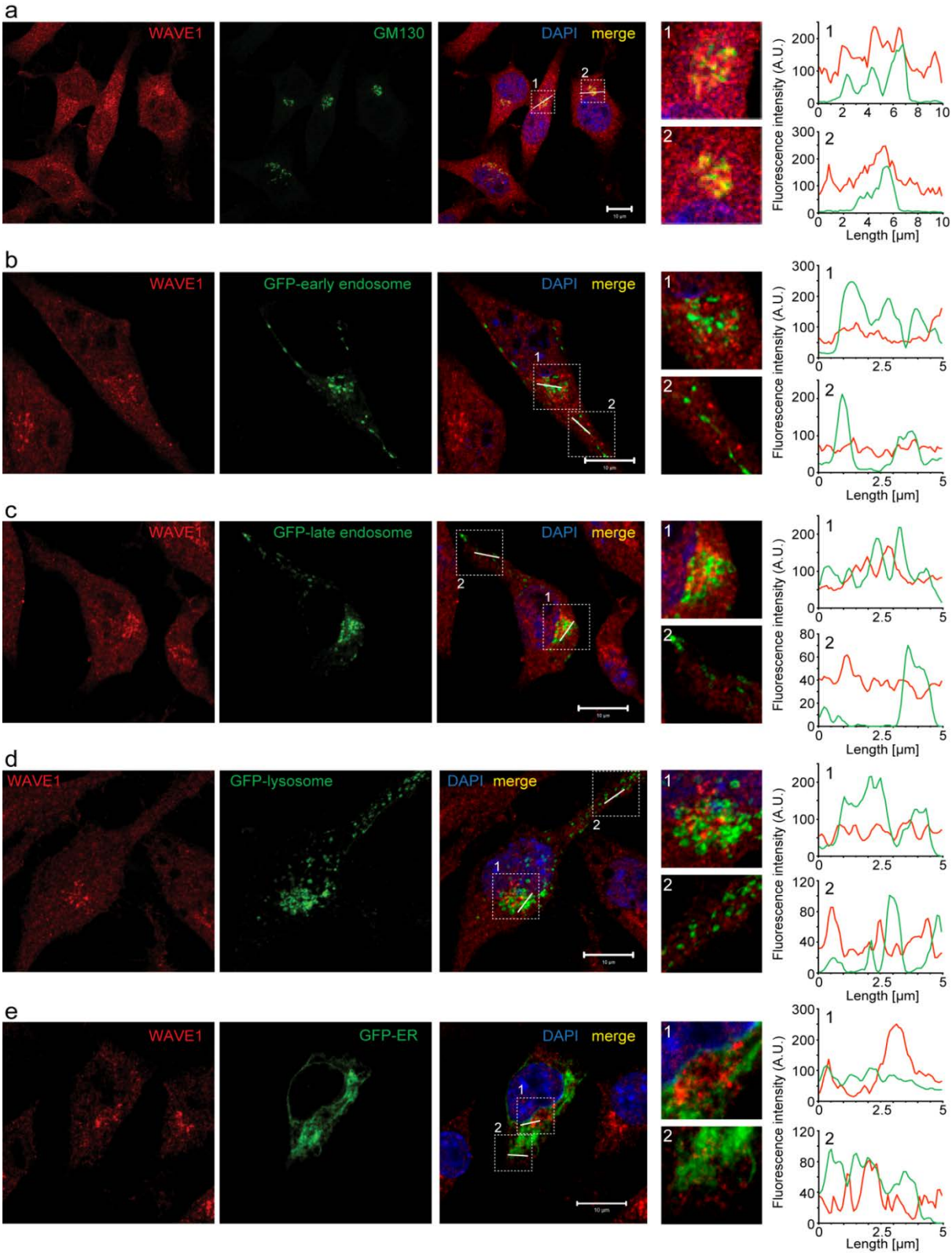
APLP1-ICD and APLP2-ICD to give a total of 1 µg plasmid for each transfection. Immunoblotting with anti-Flag antibody indicated comparable expression of ICDs (**b**). The quantified mRNA levels (**c**, $n = 6$), *WASF1* promoter-luciferase activities (**d**, $n = 7$ for APLP1-ICD and APLP2-ICD, $n = 8$ for other groups) and WAVE1 protein levels (**e**, $n = 6$) are shown. Data represent means ± SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, two-tailed *t*-test. (**f, g**) N2a cells were treated with a protein synthesis inhibitor, cycloheximide (25 µg/ml) for the indicated times to assess the stability of WAVE1 protein. Immunoblotting of WAVE1 (**f**) and quantification of duplicates (**g**, $n = 2$) are shown. Data represent means ± SEM.

Supplementary Figure 3



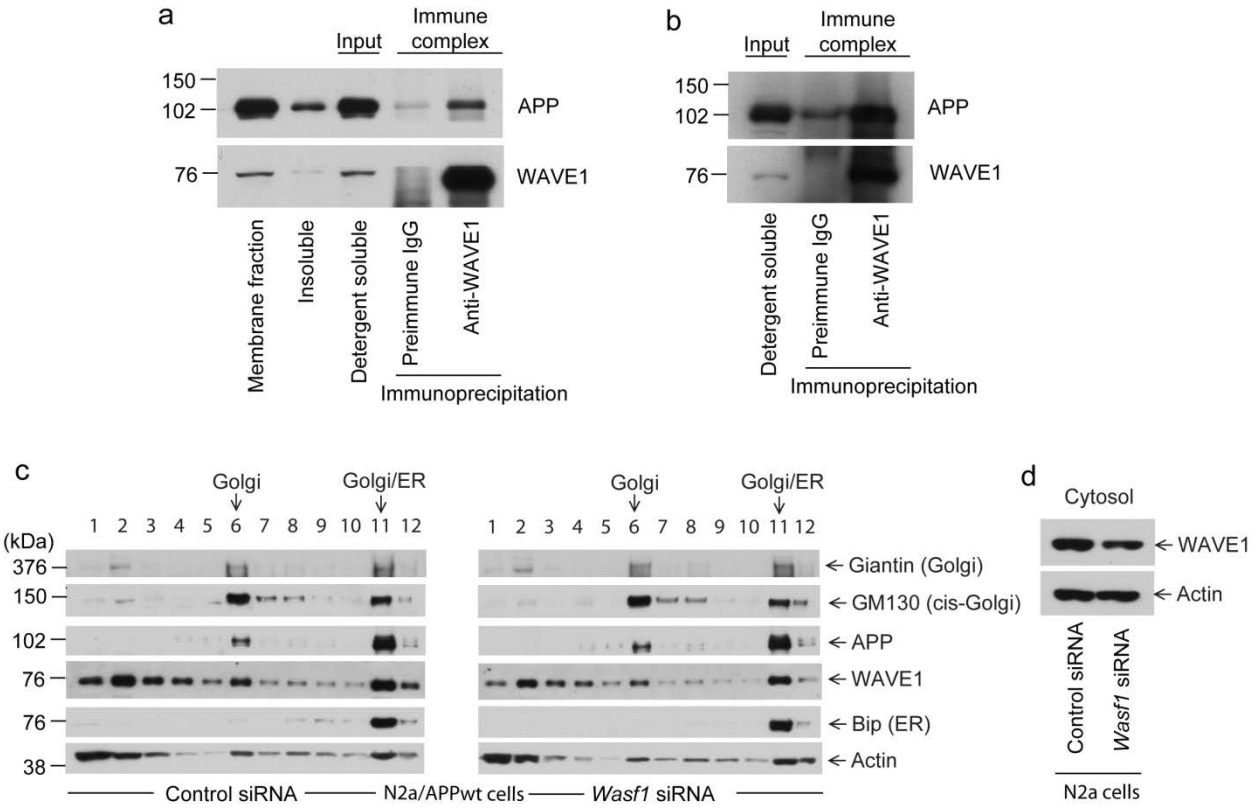
Supplementary Figure 3 Generation of *Wasf1* KO mice. (**a**) Schematic for *Wasf1* gene KO strategy. In the targeting vector, exon 4 was flanked by *loxP* sites. A selection marker (*PGK-neo*) flanked by *FRT* sites was inserted downstream of exon 4. The selection marker in the targeted allele was deleted after breeding with FPL mice expressing FLPe recombinase. The constitutive *Wasf1* KO mouse line was established after breeding with CRE deleter mice. Deletion of exon 4 creates a frame shift in downstream exons and generates an early STOP codon. (**b**) Forward primer upstream of exon 4 and reverse primer downstream of exon 4 were designed to genotype WT (650 bp, PCR product) and deleted (180 bp) alleles from tail DNA samples. (**c**) Western blot analysis of WAVE1 and actin in the hippocampus of $Wasf1^{+/+}$, $Wasf1^{+/-}$ and $Wasf1^{-/-}$ mice.

Supplementary Figure 4



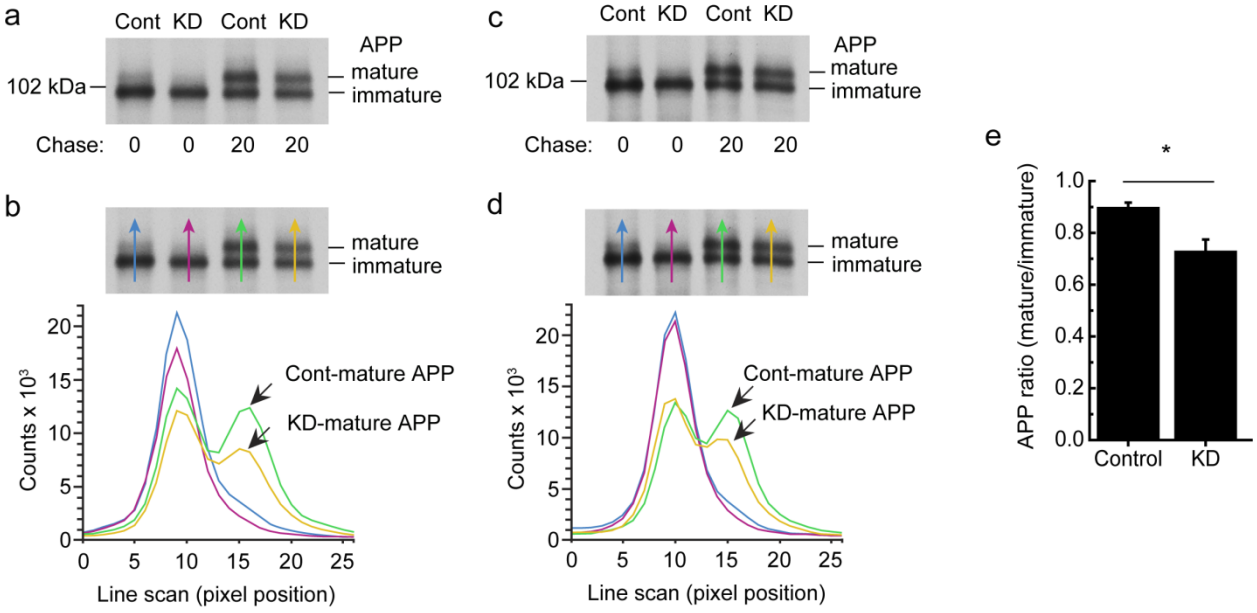
Supplementary Figure 4 WAVE1 is highly colocalized with a Golgi marker but rarely detected in early and late endosomes, lysosomes and ER. (**a**–**e**) Confocal images of immunocytochemistry of WAVE1, and GM130 (**a**, Golgi maker), in N2a/APPswe.PS1ΔE9 cells infected with viral vectors expressing GFP-fused marker proteins (**b**–**e**) as indicated. High magnification images and line scans (white lines) of the rectangular areas in the merged images are shown right. Line scans show the fluorescence intensity signals for WAVE1 and GM130 or other organelle markers plotted against distance in μm. DAPI counterstaining was used to show the nucleus (blue). Scale bars, 10 μm.

Supplementary Figure 5



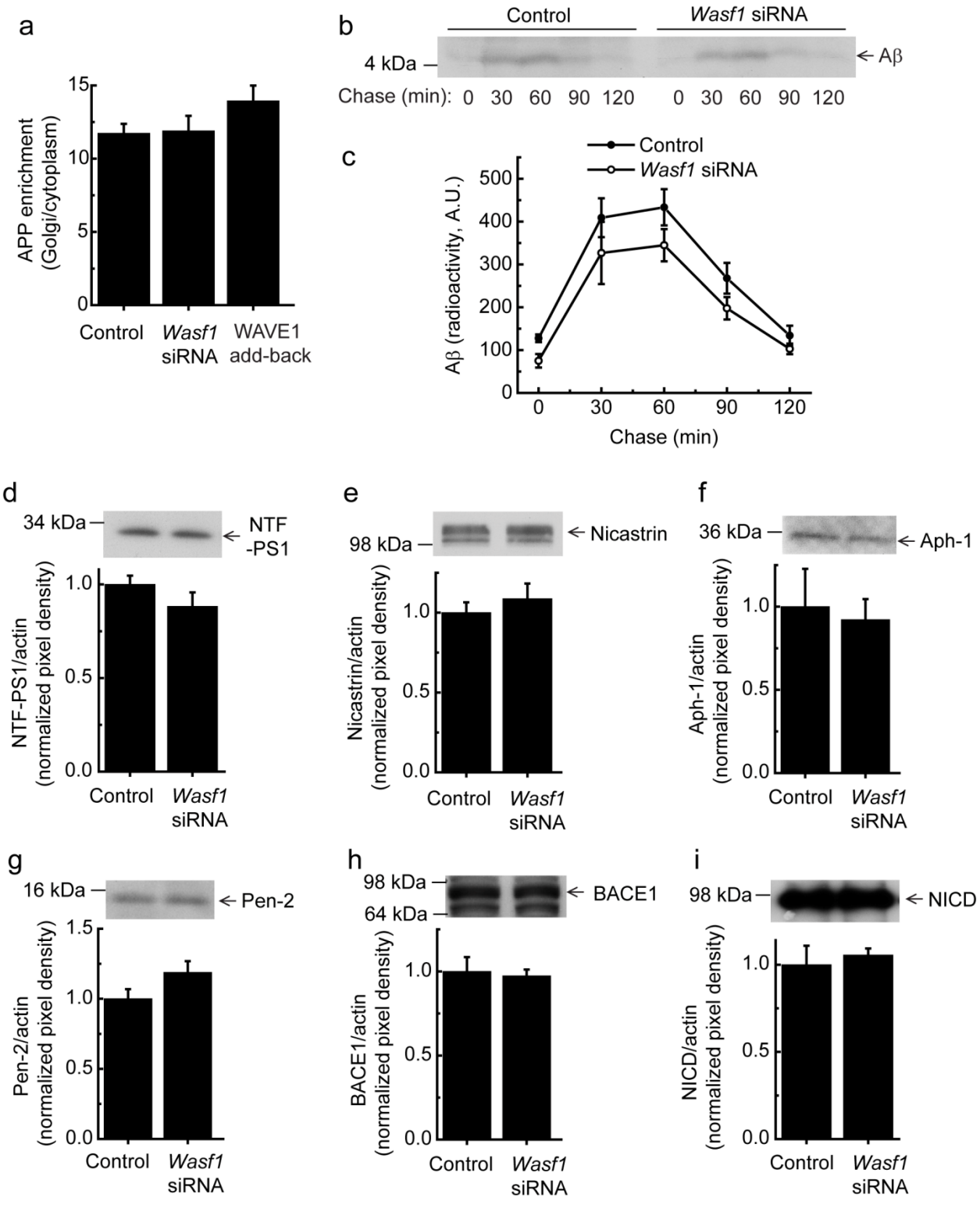
Supplementary Figure 5 Additional co-immunoprecipitation data, and preparation of Golgi membrane and cytosolic fractions for *in vitro* budding assay. (**a**, **b**) A detergent soluble membrane fraction from N2a/APPwt cells was used for immunoprecipitation with preimmune IgG or anti-WAVE1 antibody. WAVE1 co-precipitated with APP. (**c**) N2a/APPwt cells were transiently transfected with control siRNA or *Wasf1* siRNA. Cells were homogenized and the homogenates were subjected to sucrose gradient centrifugation. Fractions of sucrose gradients were analyzed by immunoblotting for markers for Golgi and ER, APP, WAVE1 and actin. (**d**) N2a cells were transiently transfected with control siRNA or *Wasf1* siRNA. Cytosolic fractions were prepared and analyzed by immunoblotting for WAVE1 and actin.

Supplementary Figure 6



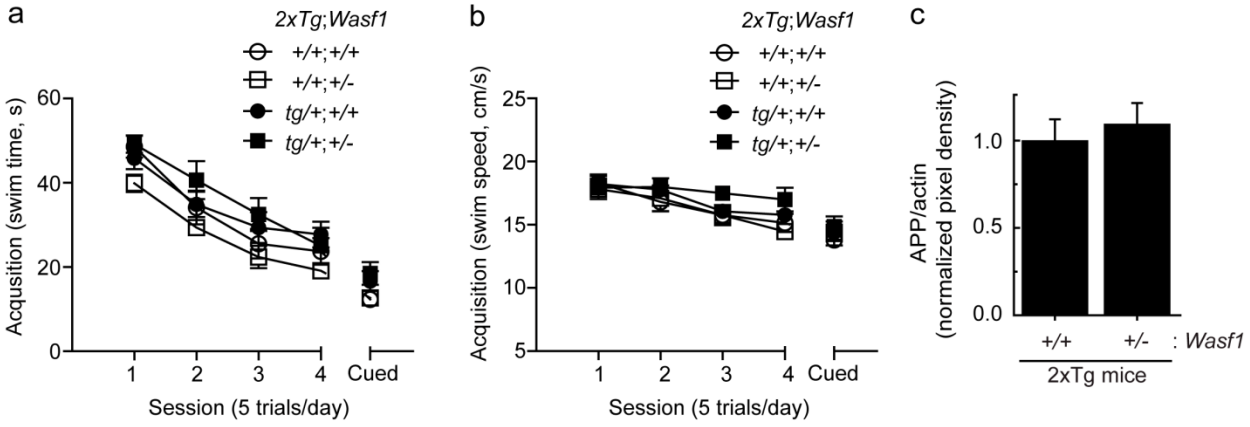
Supplementary Figure 6 Lowering WAVE1 expression reduces the ratio of mature APP to immature APP. N2a/APPswe.PS1ΔE9 cells were transfected with control siRNA (Cont) or *Wasf1* siRNA (KD). Cells were serum-starved for 30 min, pulse-labeled with [^{35}S]methionine for 15 min, and then chased with serum-containing normal medium for 20 min. APP was immunoprecipitated with anti-APP antibody. The immune complex was separated by 8% SDS-PAGE, and the separated proteins were transferred to nitrocellulose membrane. Immature and mature forms of APP were detected by autoradiogram and scanned by Typhoon scanner for quantification. (**a**, **c**) Two representative images of autoradiogram are shown. (**b**, **d**) Line scans (colored arrows) were made across immature and mature forms of APP. Black arrows indicate significant reduction of the mature form of APP by lowering WAVE1 expression. (**e**) The ratio of mature to immature APP (at 20 min after chase) was calculated with radioactivity counts obtained by line scan (n = 4). Data represent means ± SEM. *P < 0.05, two-tailed t-test.

Supplementary Figure 7



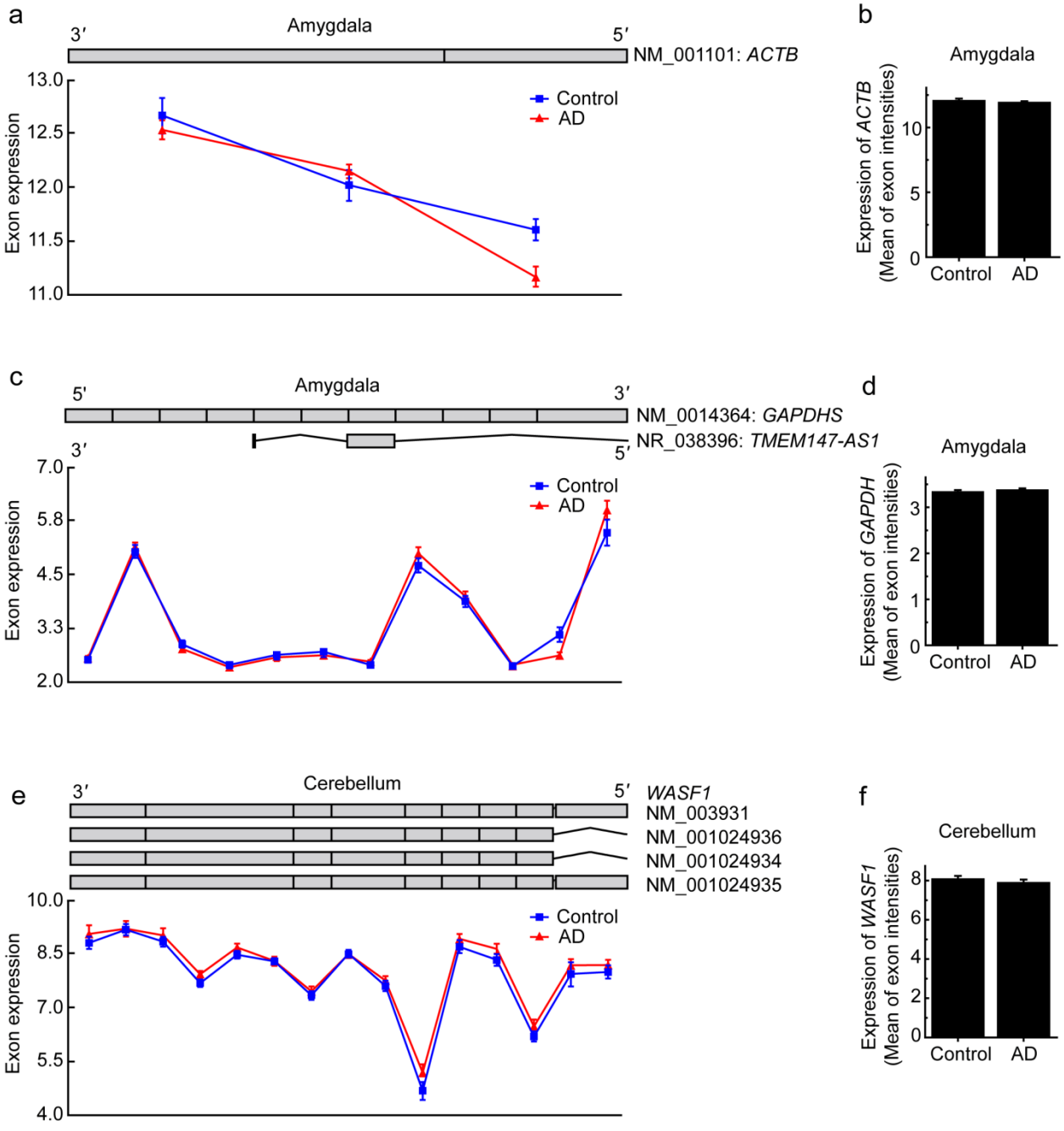
Supplementary Figure 7 Lowering WAVE1 expression does not alter APP enrichment in the Golgi, turnover rate of Aβ, the protein levels of γ-secretase complex and BACE1, and the activity of γ-secretase. (**a**) N2a/APPwt cells were transfected with control siRNA plus control plasmid (Control), *Wasf1* siRNA plus control plasmid (WAVE1 siRNA), or *Wasf1* siRNA plus siRNA-resistant plasmid for WAVE1 (WAVE1 add-back). Immunocytochemistry of APP and giantin (a marker for the Golgi apparatus) was performed. The level of APP in the Golgi compared to its level in the cytoplasm was calculated as APP enrichment (control, $n = 72$; *Wasf1* siRNA, $n = 46$; WAVE1 add-back, $n = 41$). Data represent means ± SEM. (**b**, **c**) To examine the rate of Aβ breakdown, N2a/APPswe.PS1ΔE9 cells were transfected with control siRNA (Cont) or *Wasf1* siRNA (KD). Cells were serum-starved for 30 min, then pulse-labeled with [^{35}S]methionine for 15 min, and then chased with serum-containing normal medium for the indicated times. Aβ was immunoprecipitated with 4G8 antibody. The immune complex was separated in 10-20% Tricine gel and the separated proteins were transferred to PVDF membrane. A representative autoradiogram is shown (**b**) and the radioactivity of Aβ bands was quantified using a Typhoon scanner and an associated quantification software (**c**, $n = 4$ per group). Data represent means ± SEM from four independent experiments. (**d**–**h**) N2a/APPswe.PS1ΔE9 cells were transfected with control siRNA or *Wasf1* siRNA. Immunoblotting of N-terminal fragment of presenilin 1 (NTF-PS1) (**d**), nicastrin (**e**), Aph-1 (**f**), Pen-2 (**g**) and BACE1 (**h**) was performed. Data represent means ± SEM ($n = 6$). (**i**) N2a/APPswe.PS1ΔE9 cells were co-transfected with mNotchΔE (truncated Notch-1, lacking most of the Notch extracellular domain) plus control siRNA or *Wasf1* siRNA. NICD was detected with cleaved Notch1 (Val1744) antibody to assess γ-secretase activity. Data represent means ± SEM ($n = 4$).

Supplementary Figure 8



Supplementary Figure 8 Performance during the spatial acquisition sessions and non-spatial cued session in a Morris water maze task, and APP level in the hippocampus of 2xTg mice harboring *Wasf1*$^{+/+}$ or *Wasf1*$^{+/-}$. (**a, b**) Performance during the spatial acquisition sessions (Sessions 1-4; hidden platform) and non-spatial cued session (visible platform) was assessed by measuring swim time (**a**) and swim speed (**b**). Data represent means ± SEM. All sessions occurred once daily and consisted of 5 trials per day. The cued session occurred two days after Session 4. There were no significant differences between the groups for swim time (**a**) or swim speed (**b**) during the spatial acquisition sessions, as all groups learned to locate the hidden platform with similar efficiency (swim time, $P < 0.0001$; swim speed, $P < 0.0001$, three-way ANOVA test). There were also no significant differences between the groups during the cued session when the platform was visible. The number of animals analyzed is the same as shown in the legend of **Figure 4a, b**. (**c**) The level of APP was not altered in the hippocampus from 2xTg mice harboring *Wasf1*$^{+/+}$ or *Wasf1*$^{+/-}$ (7 month-old males). The protein level of APP was normalized to actin. Means ± SEM ($n = 7$ per group).

Supplementary Figure 9



Supplementary Figure 9 View of exon expression profiles and gene expression data. (**a**, **c**, **e**) The expression of exons for *ACTB* (**a**) and *GAPDH* (**c**) from the amygdala, and *WASF1* from the cerebellum (**e**) in 19 AD cases (red triangles) and 10 controls (blue squares) were analyzed with

various exon-specific probes. Each red triangle or blue square represents least squares mean expression of an exon in AD or control tissues, respectively. Error bars, SEM. The upper part shows the structure of the gene (isoforms) retrieved from the UCSC browser. (**b**, **d**, **f**) *ACTB* (**b**) and *GAPDH* (**d**) gene expression in the amygdala, and *WASF1* gene expression in the cerebellum (**f**) were represented by the mean value of exon intensities. Means ± SEM (control, $n = 10$; AD, $n = 19$).