SUPPLEMENTARY INFORMATION

Single APP knockin mouse models of Alzheimer’s disease
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**Supplementary Table 1. Intrinsic problems of APP-Tg mice.**

1. APP overexpression perturbs axonal transport because APP interacts with kinesin via JIP-1.
2. Not only Aβ but also other APP fragments such as sAPP, CTF-β, CTF-α and AICD are overproduced. These fragments may possess some biological functions.
3. Cross-breeding APP-Tg mice with other mutant mice is likely to generate even more-complicated artifacts.
4. Cell type-specific alternative splicing of APP mRNA is spared.
5. Utilization of artificial promoters results in transgene expression in cells not necessarily identical to those expressing endogenous APP.
6. Artificial promoters may compete with endogenous promoters for common transcription factors.
7. The transgene is inserted into a gene locus of the host animal, often in a multi-copy manner, and destroys endogenous gene(s). In this respect, use of homozygous mice becomes irrelevant. Two loci are destroyed in double Tg mice overexpressing mutant APP and presenilin 1.
8. Expression of the transgene varies from line to line and from time to time.
9. Mouse lines to be used are generally selected in a phenotype-biased manner.
10. APP-Tg mice often die of unknown cause(s).
Supplementary Figure 1. Concept for generating new AD mouse models.

(a) Intrinsic problems of APP-Tg mice. The mice express non-physiologically high levels of APP. Because APP interacts with c-Jun N-terminal kinase interacting protein-1 (JIP-1), a mediator for kinesin-1, overexpressed APP may perturb axonal transport and generate artificial phenotype(s). APP fragments other than Aβ, i.e. sAPP, CTF-β and AICD, are also overproduced. Thus, it remains uncertain whether the various phenotypes observed in these mice are genuinely caused by Aβ deposition. See supplementary Table 1 for other problems. (b) Design of new mouse models. We humanized the mouse Aβ sequence and introduced Swedish and Beyreuther/Iberian mutations by knockin technology. We also generated mutant mice that in addition carried the Arctic mutation.
Supplementary Figure 2. Procedures to generate $APP^{NL-F/NL-F}$ mice.

(a) APP wild-type allele, targeting vector and targeted allele. The mutations introduced to exons 16 and 17 are indicated by closed triangles. Restriction enzyme cleavage sites by ApaLI, NotI and XbaI are also shown. Neo denotes the neomycin-resistance gene cassette for positive selection surrounded by the lox/FRT sequence. SA and LA denote the short and long arms of the targeting vector, respectively. The annealing positions of the 5’ and 3’ probes used in the Southern blot analysis are located outside the targeted allele. (b) Mutations introduced to exons 16 and 17. Changes in the genomic sequence
and in the amino acid sequence are depicted. Exon 16 contains the Swedish mutations and humanized sequence; exon 17 contains the Arctic (in case of APP\textsuperscript{NL-G-F/NL-G-F} mice) and Beyreuther/Iberian mutations. Numbers represent amino acid positions in the A\beta sequence. (c) Germline transmission identified by Southern blot analysis. Genomic DNA extracted from an APP\textsuperscript{NL-L-F/NL-F} mouse tail (clone #271) was digested by XbaI and ApaLI, and hybridized with the 5’ and 3’probes shown in panel A. Southern blot analysis was repeated twice to select the positive clone for microinjection. (d) Genotyping of mice by PCR. The upper band is amplified from the wild-type allele, and the lower band from the mutant allele. (e) Northern blot analysis of APP mRNA. Total RNA extracted from brains was detected using a mouse APP-specific probe. β-actin was used as an internal control. We quantified the expression levels by densitometry and found no significant difference between the wild-type and mutant mice. Data represent mean ± s.e.m (n = 3). Each set of experiments was repeated at least three times to confirm the results. The full-length image are shown in **Supplementary Figure13**.
Supplementary Figure 3. Western blot analyses and quantitative results of APP expression levels and neuroinflammation in APP\textsuperscript{NL-F} mice.

(a) Western blot analyses of APP and APP-derived fragments in APP\textsuperscript{NL-F/NL-F} mice. Antibodies to the N-terminus of APP (22C11), to the human sequence of A\textbeta{} (6E10), and to the C-terminus of APP were used. The full-length image are shown in...
**Supplementary Figure 14a.** (b) Quantification of APP and its fragments in the brains of APP\(^{NL}\) and APP\(^{NL-F}\) KI mice. Intensities of immunoreactive bands on Western blots in a were quantified by densitometric analysis. We found no significant difference in the quantities of 22C11-reactive APP or AICD between the wild-type and the mutant mice (\(n = 6\) of APP\(^{wt/wt}\), 6 of APP\(^{NL-F/wt}\), 6 of APP\(^{NL-F/NL-F}\), 4 of APP\(^{NL/wt}\) and 4 of APP\(^{NL/NL}\), one-way ANOVA). Human A\(\beta\) sequence-containing (6E10-reactive) APP and CTF-\(\beta\) signals increased in a manner dependent on the Swedish mutation gene dose; CTF-\(\alpha\) signals decreased. The levels of CTF-\(\beta\) and CTF-\(\alpha\) in APP\(^{NL-F/NL-F}\) mice were similar to those of APP\(^{NL/NL}\) mice (\(n = 6\) of APP\(^{wt/wt}\), 6 of APP\(^{NL-F/wt}\), 6 of APP\(^{NL-F/NL-F}\), 4 of APP\(^{NL/wt}\) and 4 of APP\(^{NL/NL}\), one-way ANOVA with Sheffe’s F test). Data represent mean ± s.e.m. (c) Cortical immunoreactivities of Iba1 and GFAP signals in 18-month-old APP\(^{wt/wt}\), APP\(^{NL/NL}\) and APP\(^{NL-F/NL-F}\) mice (Fig. 1d) were quantified. Data represent mean ± s.e.m. (\(n = 4\) of APP\(^{wt/wt}\), 6 of APP\(^{NL-F/NL-F}\) and 4 of APP\(^{NL/NL}\), one-way ANOVA with Sheffe’s F test, **\(P < 0.01\).
Supplementary Figure 4. Levels of APP, APP-derived fragments and Aβ in 2 month old APP<sup>NL</sup>, APP<sup>NL-F</sup> and APP23 mice.

(a) Western blot analyses of APP and APP-derived fragments in APP<sup>NL-F/NL-F</sup> and APP23 mice. See Supplementary Figure 3a legend for details. The full-length image are shown in Supplementary Figure 14b. (b–e) Steady-state levels of Aβ<sub>40</sub> and Aβ<sub>42</sub> and ratios of Aβ<sub>42</sub>/Aβ<sub>40</sub> in the mouse brains. Panels B and C show the amounts of TS-soluble and
GuHCl-extractable Aβ, respectively. Panels D and E show the ratio of Aβ_{42}/Aβ_{40} in the TS and GuHCl fractions, respectively. Data represent mean ± s.e.m. (n = 5 of APP_{wt/wt}, 4 of APP^{NL-F/wt}, 5 of APP^{NL-F/NL-F}, 4 of APP^{NL/wt} and 4 of APP^{NL/NL} and 3 of APP23, one-way ANOVA with Scheffe’s F test, **P < 0.01).
Supplementary Figure 5. Initial deposition of Aβ in APP<sup>NL-F/NL-F</sup> mice.

(a) Immunohistchemical staining of Aβ plaques in APP<sup>NL-F/NL-F</sup> mice. Brain sections
from 6-month-old $APP^{NL-F/NL-F}$ mice ($n = 4$) were immunostained using the anti-Aβ antibody, 6E10. The right panel shows an enlarged image of the area indicated by a square in the left panel. We detected approximately 1 to 2 plaques in an $APP^{NL-F/NL-F}$ cortical slice at this age. (b) N- and C-terminal structures of Aβ species in $APP^{NL-F/NL-F}$ brains. Brain sections from 6-month-old $APP^{NL-F/NL-F}$ mice ($n = 4$) were double stained using 4G8 antibody combined with antibodies to Aβ₁₋₄₀, to Aβ₃pE₋₄₀, to Aβₓ₋₄₀ and to Aβₓ₋₄₂. Scale bars represent 100 μm (a, left panel), 25 μm (a, right panel) and 50 μm (b), respectively.
Supplementary Figure 6. Aβ deposition in APP23 mice.

(a) Quantification of Aβ40 and Aβ42 in APP23 mouse brains. The amounts of
GuHCl-extractable Aβ species at different ages are shown. (b) Aβ deposition in APP23 brain. Brain sections from 9-, 12- and 15-month-old APP23 mice were immunostained with the anti-Aβ antibody, 4G8. Immunoreactive Aβ deposition was detected from 12 months of age (n = 4/time point). Although the knockin mice start accumulating Aβ in cortex like in humans, APP23 mice simultaneously in cortex and hippocampus under the control of Thy-1 promoter. (c) N- and C-terminal structures of Aβ species in APP23 mouse brains. Serial sections from 18-month-old APP23 mouse brains (n = 4) were immunostained with antibodies to Aβ1–X, to Aβ3pE–X, to AβX–40 and to AβX–42. Scale bars represent 500 μm (b) and 100 μm (c).
Supplementary Figure 7. Aβ amyloidosis in heterozygous APP<sub>NL-F/wt</sub> mice.

Brain sections from 24- and 30-month-old mice were immunostained with anti-Aβ<sub>1-42</sub> antibody (n = 6). Aβ plaques were detected in the cortex of APP<sub>NL-F/wet</sub> mice over 24 months of age, whereas no plaques were detected in APP<sub>wt/wt</sub> and APP<sub>NL/NL</sub> mice (data not shown). Scale bars represent 200 μm.
Supplementary Figure 8. Aβ amyloidosis in sporadic AD brains.

(a) N- and C-terminal structures of Aβ species in sporadic AD brains. Serial cortical sections from sporadic AD patients (n = 4) were immunostained with the antibodies to Aβ1-40, to Aβ3pE-40, to AβX-40 and to AβX-42. (b) Neuroinflammation in sporadic AD brains. Inflammatory responses were detected by triple staining using FSB, anti-GFAP antibody and anti-Iba1 antibody as markers of cored Aβ plaques, astrocytosis and
microgliosis, respectively. (c) Presynaptic alteration in sporadic AD. Brain sections were double-stained with 4G8 antibody and anti-synaptophysin antibody. (d) Postsynaptic alteration in sporadic AD. Brain sections were double-stained with 4G8 antibody and anti-PSD95 antibody. We observed synaptic disruptions at cored plaques but not at diffuse plaques. Scale bars represent 50 μm (a), 20 μm (b) and 10 μm (c, d), respectively.
Supplementary Figure 9. Phenotypes of calpastatin-deficient \textit{APP}\textsuperscript{NL-F/NL-F} mice.

(a) Effect of calpastatin deficiency on the survival of \textit{APP}\textsuperscript{NL-F/NL-F}, \textit{APP}\textsuperscript{NL/NL} and APP-Tg mice over 60 weeks of age. All the mouse lines were housed under identical conditions. (\textit{n} = 22 of \textit{APP}\textsuperscript{NL-F/NL-F}, 24 of \textit{APP}\textsuperscript{NL/NL}, 25 of \textit{APP}\textsuperscript{NL-F/NL-F} x \textit{Cast}\textsuperscript{−/−} and 20 of \textit{APP}\textsuperscript{NL/NL} x \textit{Cast}\textsuperscript{−/−}) (b) Acceleration of Aβ deposition in \textit{APP}\textsuperscript{NL-F/NL-F} x \textit{Cast}\textsuperscript{−/−} brains. Brain sections from 15-month-old mice were immunostained using antibodies to different N-terminal structures of Aβ as indicated. Scale bars represent 500 μm. (c) Increased neuroinflammation in \textit{APP}\textsuperscript{NL-F/NL-F} x \textit{Cast}\textsuperscript{−/−} brains. Brain sections from 12-month-old mice were double-stained using anti-Iba1 and anti-GFAP antibodies. Cortical immunoreactivities were quantified as shown in the graph (\textit{n} = 3, one-way ANOVA with Sheffe’s \textit{F} test, *\textit{P} < 0.05 and **\textit{P} < 0.01). Scale bars represent 100 μm. (d) Biochemical quantities of Aβ in \textit{APP}\textsuperscript{NL-F/NL-F} x \textit{Cast}\textsuperscript{−/−} brains. The levels of Aβ\textsubscript{40} and
Aβ42 in the TS and GuHCl fractions of cortex from 15-month-old mice were quantified by sandwich ELISA. Data represent mean ± s.e.m. (n = 5 of APP<sup>NL/NL</sup>, 5 of APP<sup>NL/NL</sup> x Cast<sup>−/−</sup>, 6 of APP<sup>NL-F/NL-F</sup> and 4 of APP<sup>NL-F/NL-F</sup> x Cast<sup>−/−</sup>, Student-t test, *P < 0.05 and **P < 0.01). (e) Memory impairment in APP<sup>NL-F/NL-F</sup> x Cast<sup>−/−</sup> mice. The Y-maze test was performed using 15-month-old mice. Data represent mean ± s.e.m. (n = 9 of APP<sup>NL/NL</sup>, 10 of APP<sup>NL/NL</sup> x Cast<sup>−/−</sup>, 9 of APP<sup>NL-F/NL-F</sup> and 11 of APP<sup>NL-F/NL-F</sup> x Cast<sup>−/−</sup>, one-way ANOVA with Sheffe’s F test, *P < 0.05).
**Supplementary Figure 10. Western blot analyses and quantitative results of APP expression levels and neuroinflammation in APP\textsuperscript{NL-G-F} mice.**

(a) Western blot analyses of APP and APP-derived fragments in APP\textsuperscript{NL-G-F/NL-G-F} mice. See Supplementary Figure 3a legend for details. The full-length image are shown in **Supplementary Figure 14c.**

(b) Quantification of APP and its fragments in the brain of
*APP*<sup>NL-G-F</sup> KI mice. Intensities of immunoreactive bands on Western blots shown in a were quantified by densitometric analysis. We found no significant difference in the quantities of 22C11-reactive APP or AICD between the wild-type and the mutant mice (*n* = 4, one-way ANOVA). Human Aβ sequence-containing (6E10-reactive) APP and CTF-β signals increased in a manner dependent on the Swedish mutation gene dose; CTF-α signals decreased. The levels of CTF-β and CTF-α in *APP*<sup>NL-G-F/NL-G-F</sup> mice were similar to those in *APP*<sup>NL-F/NL-F</sup> mice (*n* = 4, one-way ANOVA with Sheffe’s *F* test). Consistent with the data presented in **Supplementary Fig. 11d**, the 6E10 immunoreactivity of APP in *APP*<sup>NL-G-F</sup> mice was smaller than that in *APP*<sup>NL-F</sup> mice (*n* = 4, one-way ANOVA with Sheffe’s *F* test, **P** < 0.01). Data represent mean ± s.e.m. (c) Cortical immunoreactivities of Iba1 and GFAP signals from 6-month-old *APP*<sup>wt/wt</sup>, *APP*<sup>NL-F/NL-F</sup>, *APP*<sup>NL-G-F/NL-G-F</sup> and 18-month-old *APP*<sup>NL-F/NL-F</sup> mice (**Fig. 2d**) were quantified. Data represent mean ± s.e.m. (*n* = 3 of *APP*<sup>wt/wt</sup>, 4 of *APP*<sup>NL-F/NL-F</sup> (6-month-old), 6 of *APP*<sup>NL-G-F/NL-G-F</sup> and 5 of *APP*<sup>NL-F/NL-F</sup> (18-month-old), one-way ANOVA with Sheffe’s *F* test, *P* < 0.05, **P** < 0.01).
Supplementary Figure 11. Reactivity of antibodies to Arctic Aβ in \(APP^{NL-G-F/NL-G-F} \) mice.

(a) Epitope map of anti-Aβ antibodies. (b) Quantification of Arctic Aβ species using
BNT77 as a capture antibody. BNT77 binds to the mid-portion of Aβ. A sandwich ELISA kit (Wako, Japan) was used. (c) Quantification of Arctic Aβ species using BAN50 as a capture antibody. BAN50 binds to the N-terminal region of Aβ. BNT77 and BAN50 captured Arctic Aβ more weakly than wild-type Aβ. (d) Immunohistochemistry using various anti-Aβ antibodies. Brain sections of 24-month-old APP<sup>NL-F/NL-F</sup> mice were immunostained using antibodies with different epitopes after antigen retrieval as indicated (upper panels); those of 9-month-old APP<sup>NL-G-F/NL-G-F</sup> mice were similarly immunostained (lower panels).
**Supplementary Figure 12. Aβ deposition in heterozygous APP^{NL-G-F/wt} mice.**

(a) Quantification of Aβ_{40} and Aβ_{42} in heterozygous APP^{NL-G-F/wt} mouse brains. Aβ_{40} and Aβ_{42} in the TS and GuHCl fractions of cortex from 2–12-month-old mice were quantified by ELISA using Arctic Aβ-based standard curves. BNT77 was used as a capture antibody. Data represent mean ± s.e.m. (n = 3, 4, 4, 4 and 4/indicated time point). (b) Aβ deposition in APP^{NL-G-F/wt} brains. Brain sections from 2-, 4-, 6-, 9- and...
12-month-old \( \text{APP}^{\text{NL-G-F/wt}} \) mice were immunostained with anti-\( \text{A}\beta_{40-42} \) antibody. Scale bars represent 500 \( \mu \text{m} \). Plaque areas were quantified in a manner identical to that described in Figures 1 and 2. Data represent mean \( \pm \) s.e.m. (\( n = 3, 4, 4, 4 \) and 4/indicated time point).
Supplementary Figure 13. The full-length images of Northern blots in Supplementary Figure 2e.
Supplementary Figure 14. The full-length images of Western blots.

(a) The full-length blot for Supplementary Figure 3a. (b) The full-length blot for Supplementary Figure 4a. (c) The full-length blot for Supplementary Figure 10a.