Supplemental Figures

Figure S1

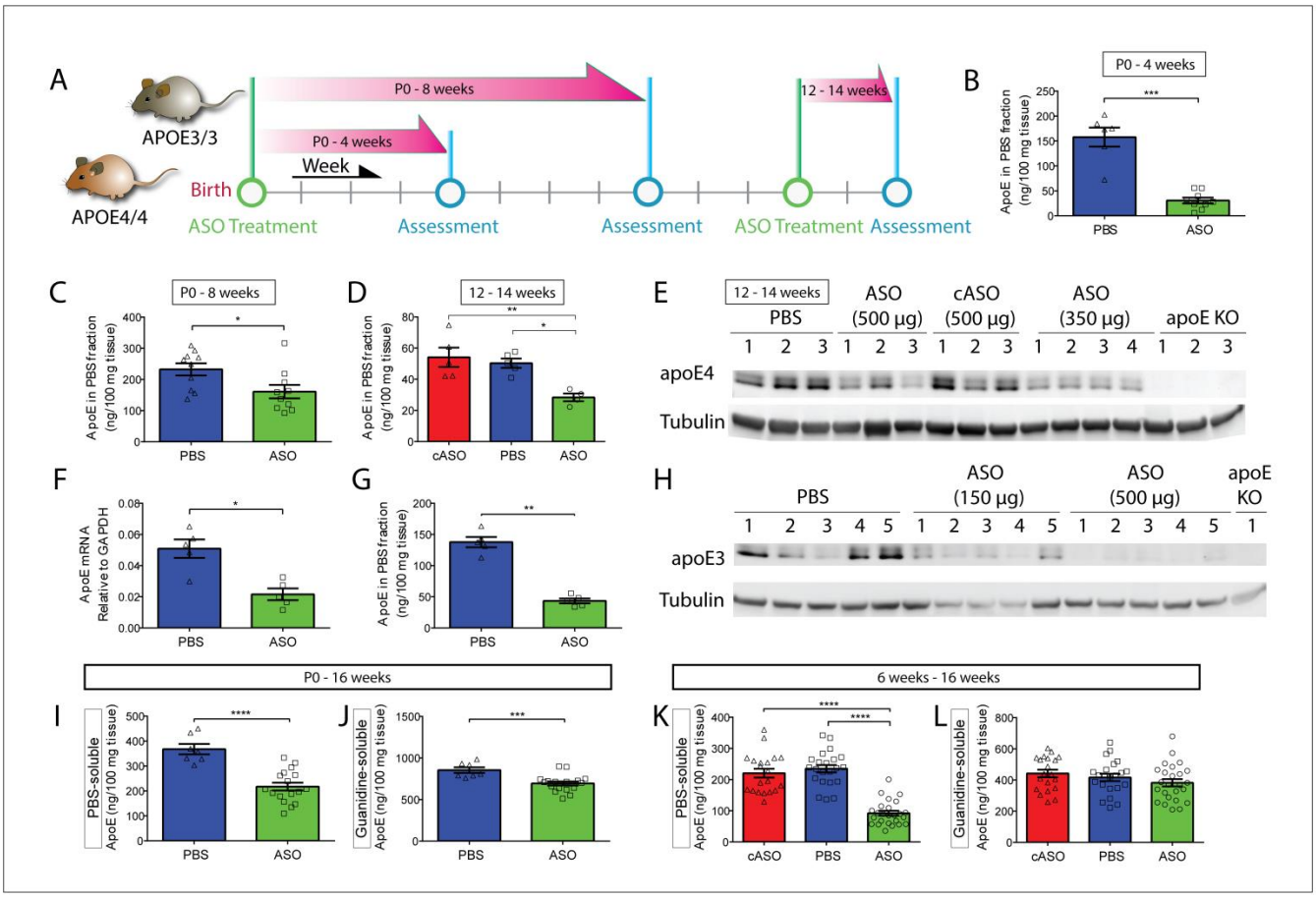


Figure S1. Related to Figure 1. ASO Treatment effectively reduces expression of apoE3 and apoE4 in mice.

(A) Timeline of various experimental approaches to test for efficacy and optimal dosing of the ASO.

(B) APOE4/4 mice were given a single bolus of ASO or PBS at P0 through ICV injection into the right hemisphere, and the PBS-soluble apoE levels in the ipsilateral cortex were assessed at one month of age. (n = 6 – 8 per group, p = 0.0004)***.***

(C) A separate cohort of APOE4/4 mice were unilaterally injected with a single bolus of either ASO or PBS at P0, and the apoE levels in the contralateral cortex were assessed at 2 mo through an ELISA. (n = 10 per group, p = 0.0354).

(D) PBS, cASO, or ASOs were injected into the right lateral ventricle of ε4/ε4 mice mice at 3 – 4 mo (*n* = 5 per group) and PBS-soluble apoE levels were measured in brain lysates from the contralateral hippocampus 2 weeks later. ($p = 0.0049$, $F = 8.963$).

(E) Western blot analysis of apoE from the same cohort of mice.

(F) 10 µl of PBS or ASOs at two different concentrations (15 or 50 µg/µl) were injected into the right lateral ventricle of APOE3/3 mice at 3 – 4 mo (*n* = 5 per group) and apoE mRNA level in the ipsilateral posterior cortex was analyzed 2 weeks later. ASO treatment effectively lowered apoE mRNA level in the PBS fraction by more than 50% in the ipsilateral posterior cortex (n = 5 per group, $p = 0.0159$).

(G) PBS-soluble ApoE levels were measured using brain lysates from the same region ($p = 0.0079$).

(H) Western blot analysis of apoE in the PBS fraction from the ipsilateral posterior cortex brain lysates using anti-apoE antibody HJ15.7. ***I,*** A cohort of APPE3 mice were unilaterally injected with a bolus of either ASO or PBS at P0, a booster bolus at 2 mo, and the apoE levels in the ipsilateral cortex were assessed at 4 mo (n = 7 – 17 per group, $p < 0.0001$).

(J) ApoE levels in the guanidine fraction was measured from the same cohort ($p = 0.0004$).

(K) A separate cohort of APPE3 mice were unilaterally injected with a bolus of either cASO, PBS, or ASO starting at 6 weeks of age, a booster bolus at 11 weeks of age, and the apoE levels in the PBS fraction from the contralateral cortex brain lysates were assessed at 4 mo (n = 20 – 25 per group, $p < 0.0001$, $F = 52.43$).

(L) ApoE levels in the guanidine fraction were measured from the same cohort ($p = 0.2099$, $F = 0.600$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. All values are reported as mean ± SEM.

Figure S2

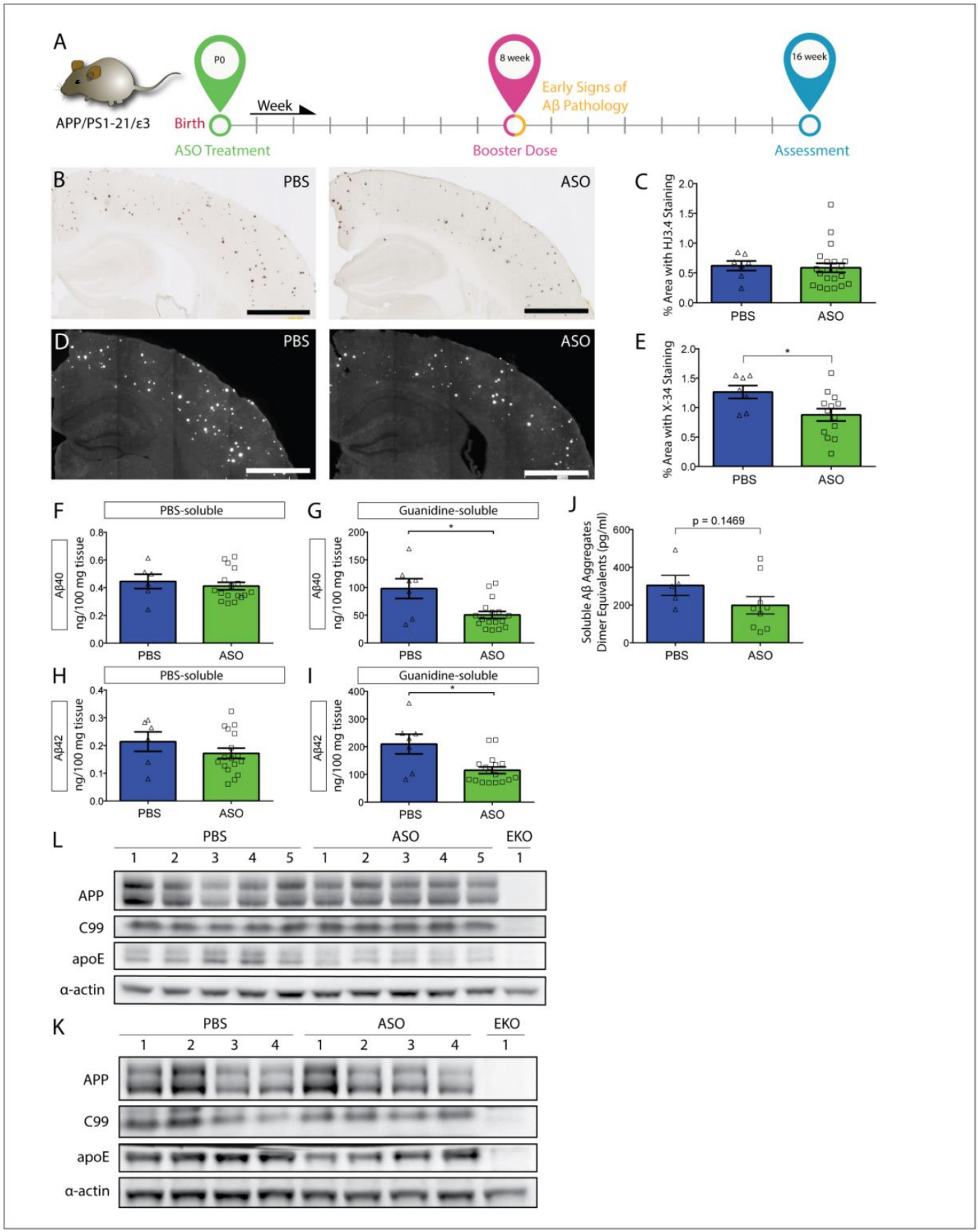

A
APP/PS1-21/ε3
P0
Birth
ASO Treatment
Week
8 week
Early Signs of Aβ Pathology
Booster Dose
16 week
Assessment
B
PBS
ASO
C
% Area with HJ3.4 Staining
PBS
ASO
D
PBS
ASO
E
% Area with X-34 Staining
*
PBS
ASO
F
PBS-soluble
Aβ40 ng/100 mg tissue
PBS
ASO
G
Guanidine-soluble
*
Aβ40 ng/100 mg tissue
PBS
ASO
J
p = 0.1469
Soluble Aβ Aggregates Dimer Equivalents (pg/ml)
PBS
ASO
H
PBS-soluble
Aβ42 ng/100 mg tissue
PBS
ASO
I
Guanidine-soluble
*
Aβ42 ng/100 mg tissue
PBS
ASO
L
PBS
ASO
EKO
1 2 3 4 5 1 2 3 4 5 1
APP
C99
apoE
α-actin
K
PBS
ASO
EKO
1 2 3 4 1 2 3 4 1
APP
C99
apoE
α-actin

Figure S2. Related to Figure 2. ASO treatment at P0 significantly reduces insoluble Aβ in APPE3 mice.

(A) Experimental timeline for P0 cohort of APPE3 mice.

(B, C) Brain sections from 4-mo APPE3 mice unilaterally injected with PBS or ASO starting at P0 were immunostained for Aβ with anti-Aβ antibody ***(B)*** and the extent of Aβ deposition was quantified from the ipsilateral cortex ***(C)*** (Scale bar = 1mm).

(D, E) Brain sections from the same cohort of mice were stained with X-34 dye that recognizes only fibrillar plaques and the fibrillar plaque load was quantified from the cortex ***(E)*** (Scale bar = 1 mm).

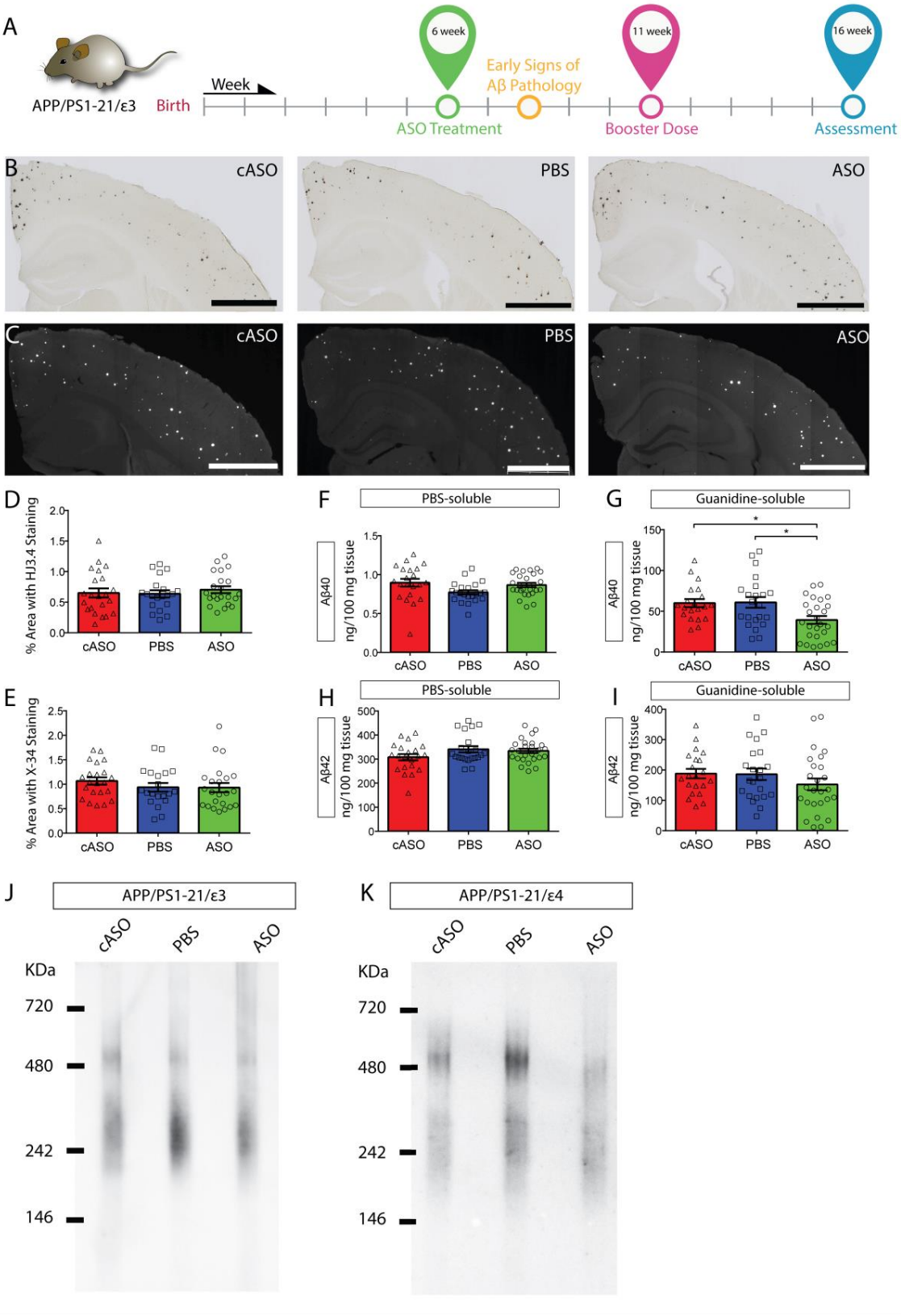
(F, H) PBS-soluble Aβ40 ***(F)*** and $A\beta_{42}$ levels ***(H)*** from the contralateral posterior cortex were measured from the brains of 4-mo APPE3 mice following unilateral treatment with ASO or controls starting at P0 and a booster dose at 2 mo ($p = 0.4722$ and 0.3191, respectively).

(G, I) Guanidine-soluble Aβ40 ***(G)*** and Aβ42 levels ***(I)*** were measured from the same cohort of mice ($p = 0.0195$ and 0.0106, respectively). $n = 9 – 10$ per group.

(J) PBS-soluble oligomeric Aβ levels were measured from the PBS-soluble brain lysates ($n = 5 – 9$ per group, $p = 0.1469$)***.***

(K) Western blot analysis of APP, C99, and apoE from PBS-soluble brain homogenates of P0-treated APPE3 mice, α-actin was used as a loading control. ***L,*** Similar analyses were done on APPE4 mice. *$p < 0.05$. All values are reported as mean ± SEM.

Figure S3


A
APP/PS1-21/ε3
Birth
Week
6 week
11 week
16 week
Early Signs of Aβ Pathology
ASO Treatment
Booster Dose
Assessment
B
cASO
PBS
ASO
C
cASO
PBS
ASO
D
% Area with HJ3.4 Staining
cASO
PBS
ASO
E
% Area with X-34 Staining
cASO
PBS
ASO
F
PBS-soluble
Aβ40
ng/100 mg tissue
cASO
PBS
ASO
G
Guanidine-soluble
Aβ40
ng/100 mg tissue
cASO
PBS
ASO
H
PBS-soluble
Aβ42
ng/100 mg tissue
cASO
PBS
ASO
I
Guanidine-soluble
Aβ42
ng/100 mg tissue
cASO
PBS
ASO
J
APP/PS1-21/ε3
cASO
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ASO
KDa
720
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242
146
K
APP/PS1-21/ε4
cASO
PBS
ASO
KDa
720
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242
146

Figure S3. Related to Figure 3. Reduction of apoE expression starting at 6 weeks of age did not significantly alter total Aβ levels in APPE3 mice.

(*A)* Experimental timeline for 6wk cohort of APPE3 mice. PBS, cASO, or ASOs were injected into the right lateral ventricle of APPE3 mice starting at 6 weeks of age, and the brains were harvested at 4 mo.

(B, D) Brain sections were immunostained with an anti-Aβ antibody **(B)**, and the extent of Aβ deposition was quantified from the ipsilateral cortex ***(D)*** ($p = 0.7417$, $F = 0.3003$).

(***C, E)*** Brain sections from the same cohort of mice were stained with X-34 dye that recognizes only fibrillar plaques ***(C)*** and the fibrillar plaque load was quantified from the cortex ***(E)*** ($p = 0.4559$, $F = 0.7955$).

(F, H) PBS-soluble $A\beta_{40}$ ***(F)*** and $A\beta_{42}$ levels ***(H)*** were measured from the contralateral posterior cortex of the same cohort of mice ($n = 20 – 25$ per group, $p = 0.0531$, $F = 3.070$ and $p = 0.1289$, $F = 2.114$, respectively).

(G, I) Guanidine-soluble $A\beta_{40}$ ***(G)*** and $A\beta_{42}$ levels ***(I)*** were measured from the same cohort of mice ($p = 0.0074$, $F = 5.297$ and $p = 0.2918$, $F = 1.255$, respectively).

(J) Native gel analysis of CSF samples from APPE3 mice treated with either cASO, PBS, or ASO.

(K) Similar analyses were done on CSF samples from APPE4 mice. $^*p < 0.05$. Scale bars = 1 mm. All values are reported as mean ± SEM.

Figure S4

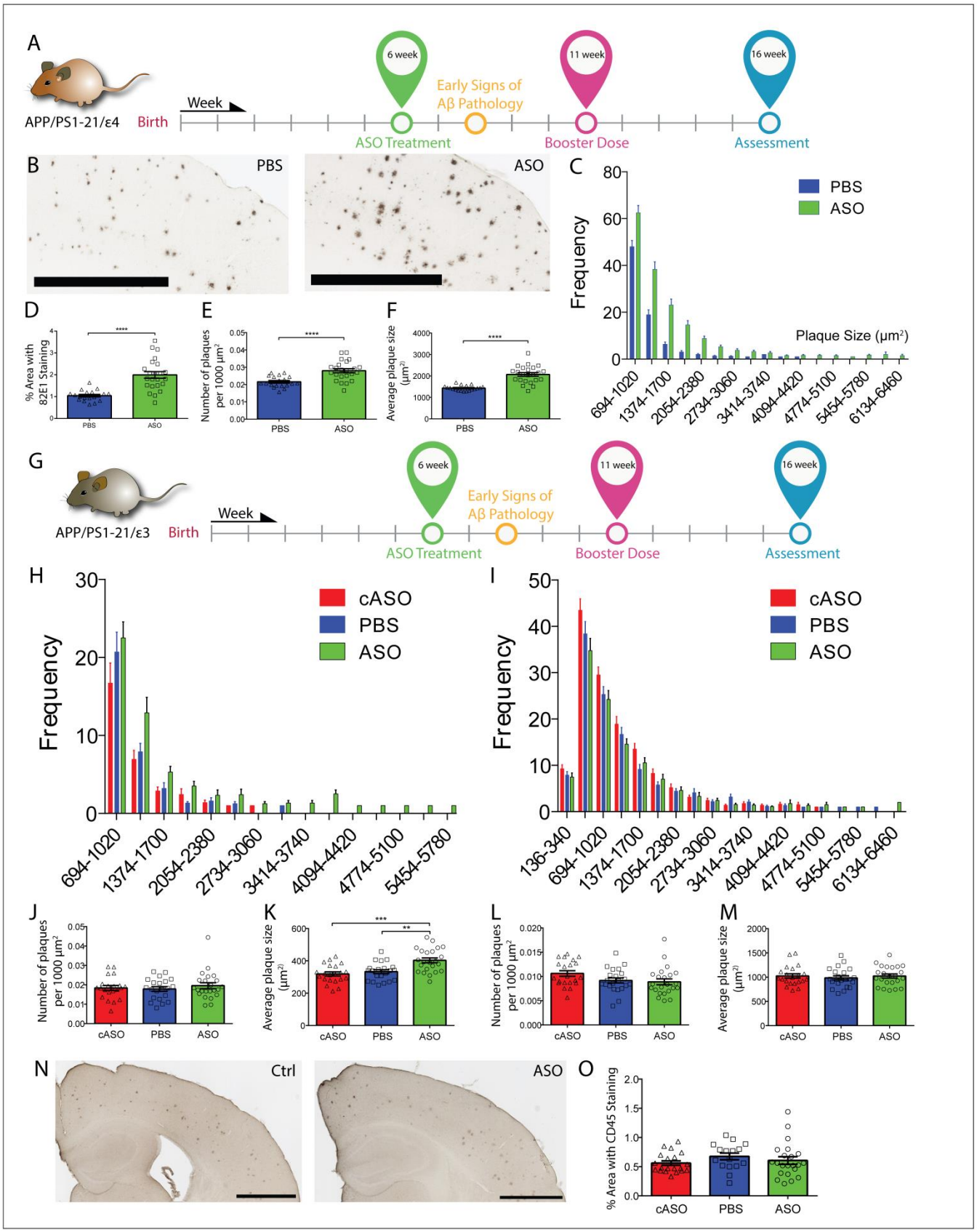
A
APP/PS1-21/ε4
Birth
Week
6 week
11 week
16 week
Early Signs of Aβ Pathology
ASO Treatment
Booster Dose
Assessment
B
PBS
ASO
C
Frequency
PBS
ASO
Plaque Size (μm²)
694-1020
1374-1700
2054-2380
2734-3060
3414-3740
4094-4420
4774-5100
5454-5780
6134-6460
D
% Area with 82E1 Staining
E
Number of plaques per 1000 μm²
F
Average plaque size (μm²)
G
APP/PS1-21/ε3
H
cASO
PBS
ASO
I
136-340
J
K
L
M
N
Ctrl
ASO
O
% Area with CD45 Staining

Figure S4. Related to Figure 4. ASO treatment significantly alters plaque distribution in APPE4 mice.

(A) Experimental timeline for 6wk cohort of APPE4 mice.

(B) Brain sections from APPE4 mice treated with either PBS or ASO stained with anti-Aβ antibody 82E1 are shown (Scale bars = 1 mm).

(C, D) Analysis of the plaque distribution was done by stratifying total plaque coverage based on size, and the frequency of occurrence was plotted on the Y-axis ***(C)***. Three brain sections, 300 µm apart, from each mouse are included in the analysis, and only plaques larger than 694 μm^2 are shown for clarity. The extent of Aβ deposition was quantified from the ipsilateral cortex as percent coverage ***(D).***

(E, F) The density of Aβ antibody-stained plaques ***(E)*** and average plaque size ***(F)*** was analyzed in the same cohort of mice (n = 20 – 25 per group, $p < 0.0001$ and $p < 0.0001$, respectively).

(G) Experimental timeline for 6wk cohort of APPE3 mice.

(H) Brain sections from APPE3 mice treated with either ASO or controls starting at 6 weeks of age were stained with anti-Aβ antibody HJ3.4 and the plaque size distribution was analyzed by stratifying the total plaque coverage based on individual plaque size. The frequency distribution of only plaques larger than 694 μm^2 are shown for clarity. Three sections, 300 µm apart, from each mouse was included in the analysis. A two-sample K-S test was used to compare the frequency of cumulative distribution of plaque sizes between the groups, and significant differences were found between cASO and ASO groups (p = 1.0614E-10), as well as between PBS and ASO groups (p = 2.1456E-05), but not between the cASO and PBS groups (p = 0.05507).

(I) Analysis of the plaque size distribution was done on brain sections from the same cohort of mice stained with X-34 dye using a similar approach as in ***(H)***. A two-sample K-S test was used to compare the frequency of cumulative distribution of plaque sizes between the groups, and a significant shift in plaque size distribution were found between the ASO and PBS groups (p = 0.03457), but not between the ASO and cASO groups (p = 0.94044) or between the cASO and PBS groups (p = 0.20289).

(J, K) The density of Aβ antibody-stained plaques ***(J)*** and average plaque size ***(K)*** were analyzed in the same cohort of mice (n = 20 – 25 per group, p = 0.6544, F = 0.4269 and p < 0.0001, F = 11.55, respectively).

(L, M) The density of X-34-stained plaques ***(L)*** and average plaque size ***(M)*** were analyzed in the same cohort of mice (n = 20 – 25 per group, p = 0.0866, F = 2.544 and p = 0.7580, F = 0.2783, respectively).

(N, O) Brain sections from the same cohort of APPE3 mice were immunostained with an antibody to CD45 that identifies activated microglia (Scale bars = 1 mm). Due to space constraints, only the representative images of cASO and ASO treatment groups were shown. There was no statistical significance between the PBS and cASO treatment groups. ***(O)*** The percentage of areas covered by CD45 staining was quantified from the ipsilateral cortex of APPE3 mice (n = 20 – 25 per group, p = 0.3777, F = 0.9912).**p < 0.01, ***p < 0.001, ****p < 0.0001. All values are reported as mean ± SEM.