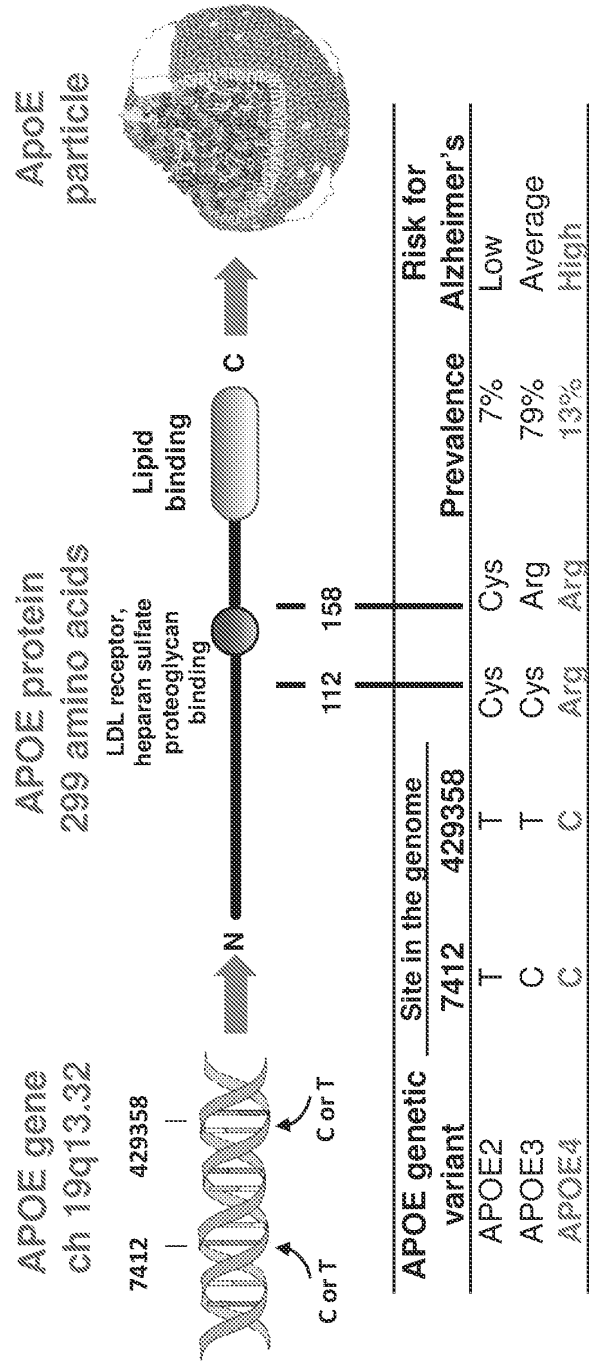
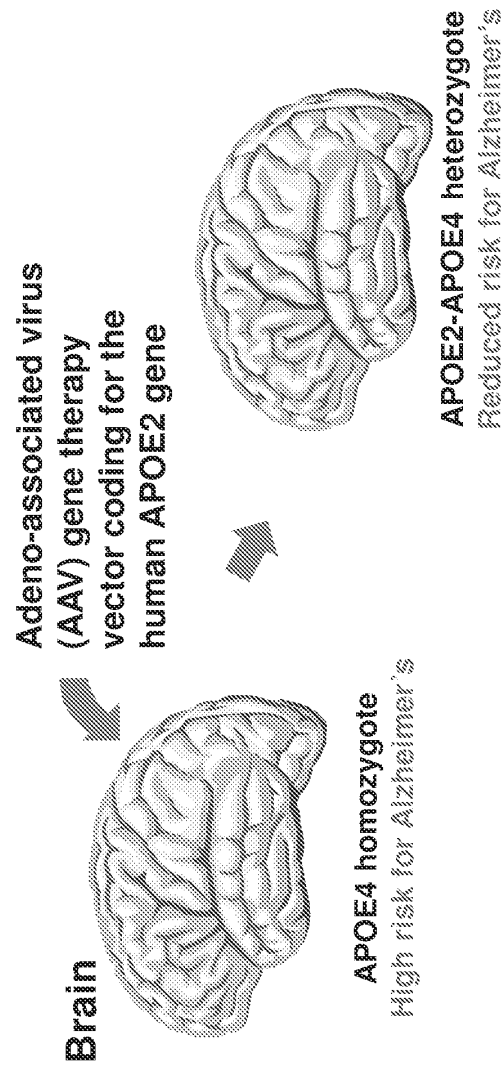


Figure 1. APOE4 Gene, Protein  
and Risk for Alzheimer's  
Disease

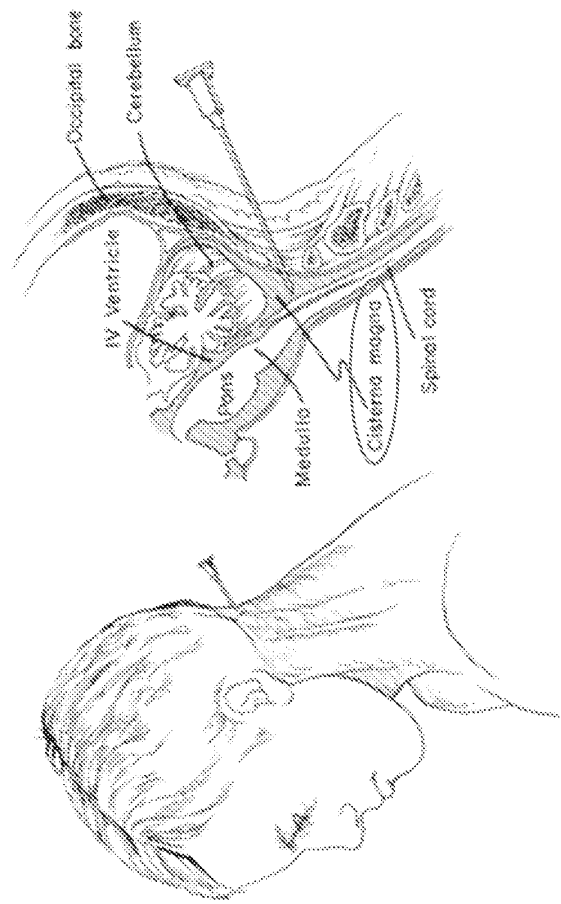


**Figure 2. APOE2 Gene Therapy to Convert the APOE4 Brain to APOE2E4**



- Adeno-associated virus delivery of the APOE2 gene to the CNS will genetically convert the E4 brain into an E2/E4 brain, thus reducing or preventing the pathology of Alzheimer's disease in APOE4 homozygotes

**Figure 3. AAVrh.10APOE2 Is Administered by  
the Intracisternal Route Directly to  
Brain Cerebral Spinal Fluid**



- Administration to the cisterna magna is an outpatient procedure

# Figure 4. APOE3 ChristChurch Alzheimer's Disease-Protective APOE mutation

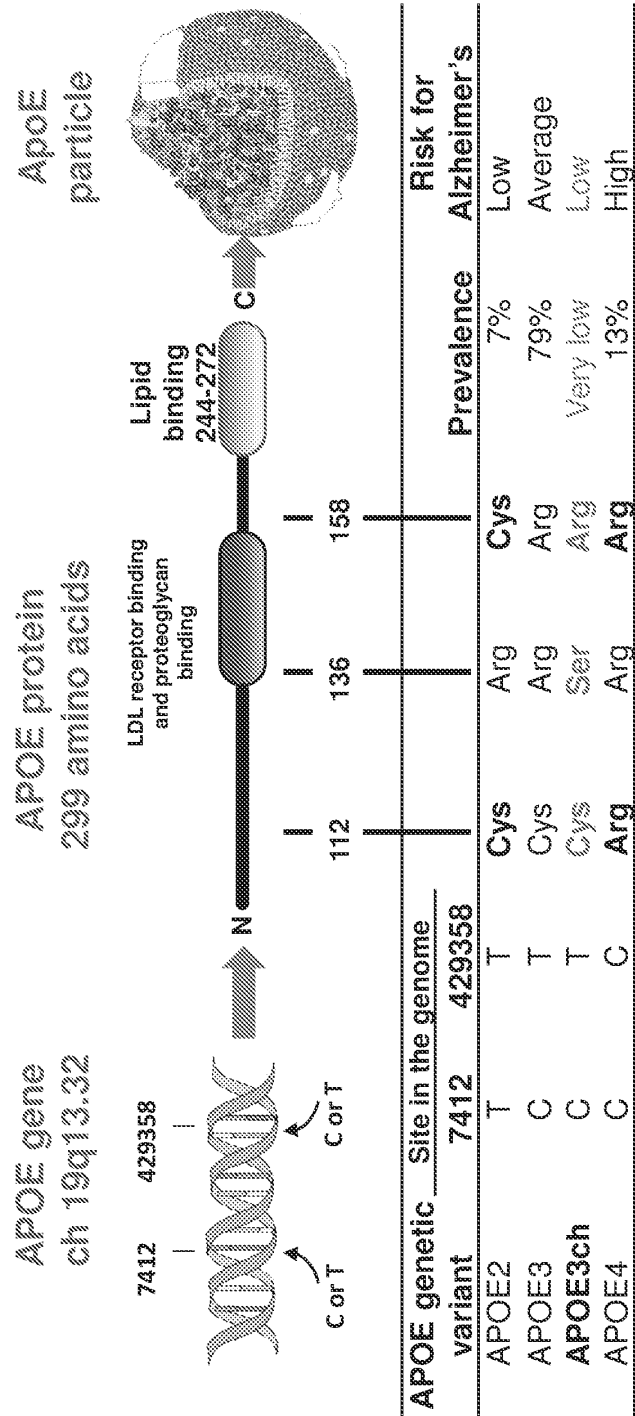
| APOE genetic<br>variant | Amino acid |     |     | Prevalence | Risk for Alzheimer's |
|-------------------------|------------|-----|-----|------------|----------------------|
|                         | 112        | 136 | 158 |            |                      |
| APOE2                   | Cys        | Arg | Cys | 7%         | Low                  |
| APOE3                   | Cys        | Arg | Arg | 79%        | Average              |
| APOE3Chc                | Cys        | Ser | Arg | Very low   | Low                  |
| APOE4                   | Arg        | Arg | Arg | 13%        | High                 |

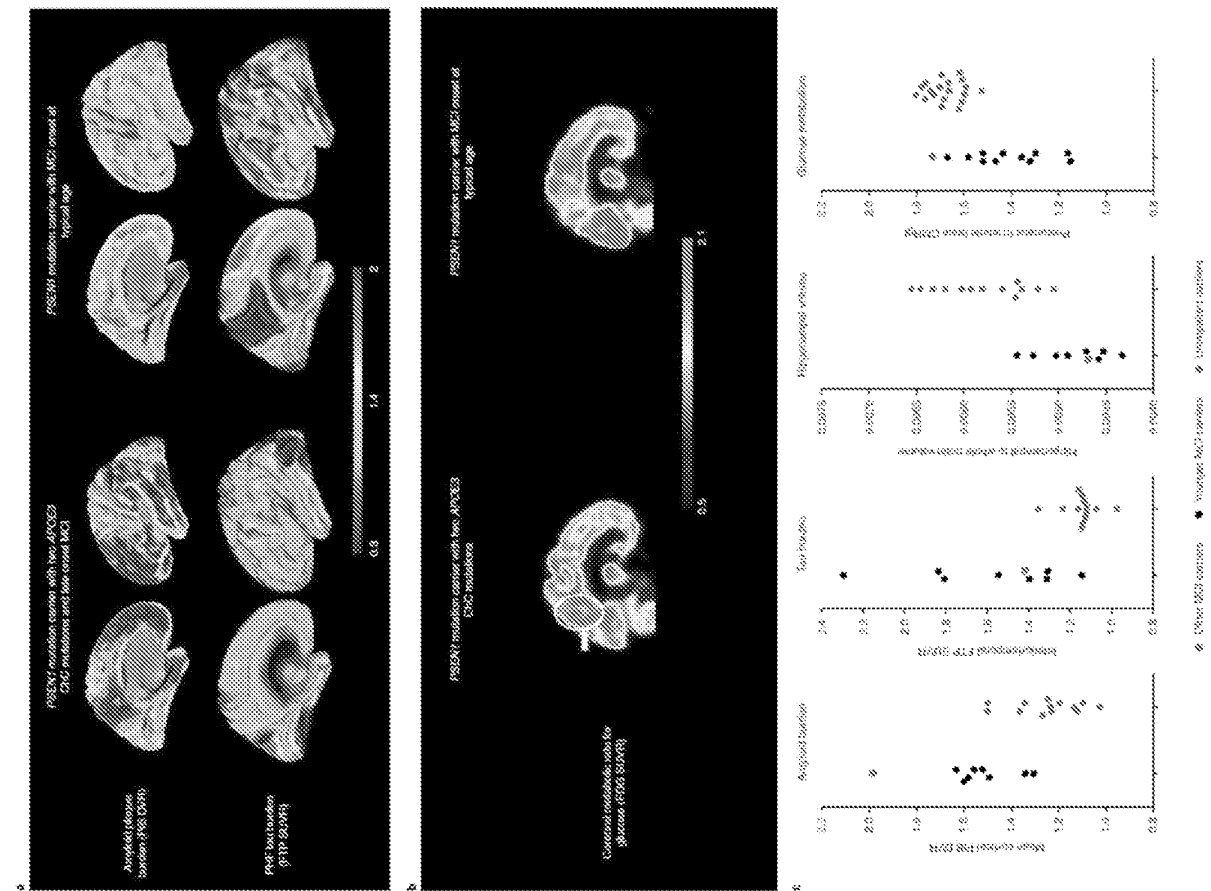
[Arboleda-Velasquez et al 2019](#) Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. Nature Medicine

[Zalocusky et al 2019](#) An Alzheimer's-disease-protective APOE mutation, Nature Medicine

[Reiman et al 2019](#) Exceptionally low likelihood of Alzheimer's dementia in APOE2 homozygotes. medRxiv

Figure 5. APOE3ChristChurch Gene and Protein



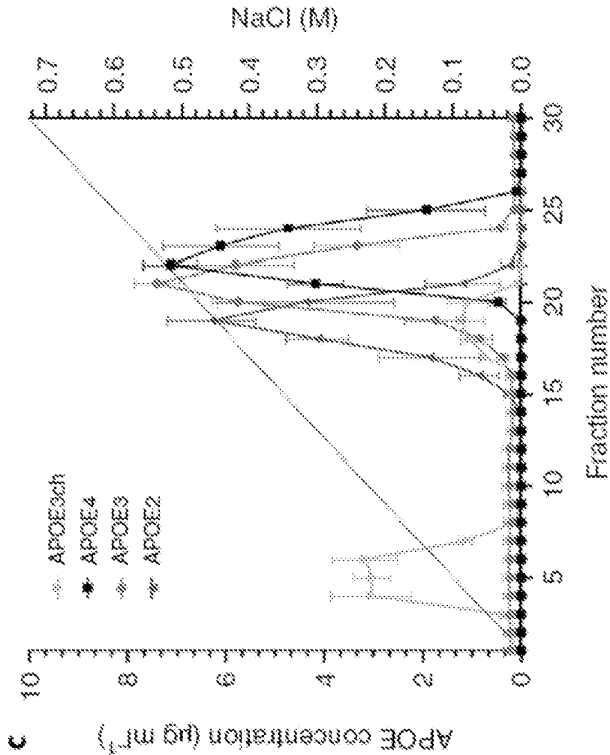


## Figure 7. Impact of the APOE3ch Mutation

- The Colombian woman inherited two copies of this ApoE3 variant, known as the Christchurch mutation
- The Christchurch mutation appears to be recessive. Four other Paisa mutation carriers in the Colombian kindred inherited a single copy, and all developed cognitive impairment at the expected age
- The ApoEch mutation seems to block secondary tau pathology (latest Reiman study shows that this is also the case for ApoE2 homozygotes, as seen from autopsy samples from 24 ApoE2 homozygotes)
- This protective effect is similar to, but greater than, that of ApoE2
- *In vitro* experiments showed that ApoE3ch behaves like the ApoE2 allele; both isoforms poorly bind heparin, corresponding to the extracellular matrix molecules implicated in the propagation and uptake of toxic forms of tau
- Binding to heparin: E4>E3.E2>E3ch
- Protection therefore may correlate with lost binding to HSPGs, believed to propagate tangles

# Figure 8. Low Affinity of ApoE3ch Binding to Heparin

- HSPGs have been implicated in the buildup of amyloid plaques, as well as in promoting the microglial response to amyloid
- HSPGs have also been linked to tau pathology and suggested that these sticky receptors allow tau fibrils to get attached to neurons, facilitating uptake and propagation
- ApoE3 bound with an Ab to the aa 130- 143 (which includes the region of the Christchurch mutation) converts ApoE3 to ApoEch as measured by heparin binding



ApoE3ch was eluted from the heparin column with the lowest NaCl concentration, revealing impaired heparin binding compared to other ApoE isoforms.

## Figure 9

- ApoE could be facilitating tau binding to HSPGs which in turn may play a role in tau pathology
- Since the ChristChurch variant's weaker affinity for HSPG binding disrupts the facilitation of tau pathology it may represent a design for therapy
- Alternatively, any interventions that weakens ApoE-HSPG binding might have therapeutic potential

## Figure 10

- Gene therapy with AAV-APOE3ch
- Gene therapy with AAV-APOE2ch
- Target ApoE-HSPG binding with an antibody delivered by AAV
- Use an siRNA to silence ApoE4 in combo with AAV-APOE3ch delivery in same construct
- Dyslipidemia is unlikely because the gene therapy is mostly confined to the CNS; any dyslipidemia can be treated with current lipid therapies

Figure 11. ApoE Protein Mutations that Result in Lipid Disorders May Alter Alzheimer's Predilection

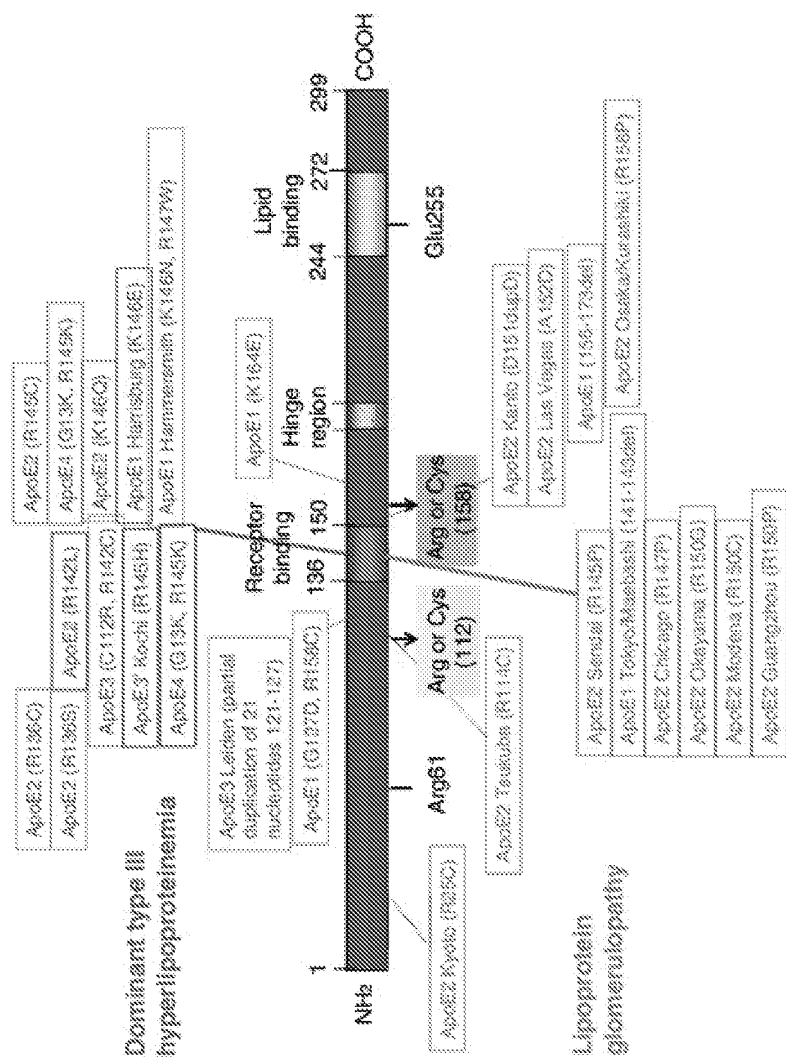


FIG. 11

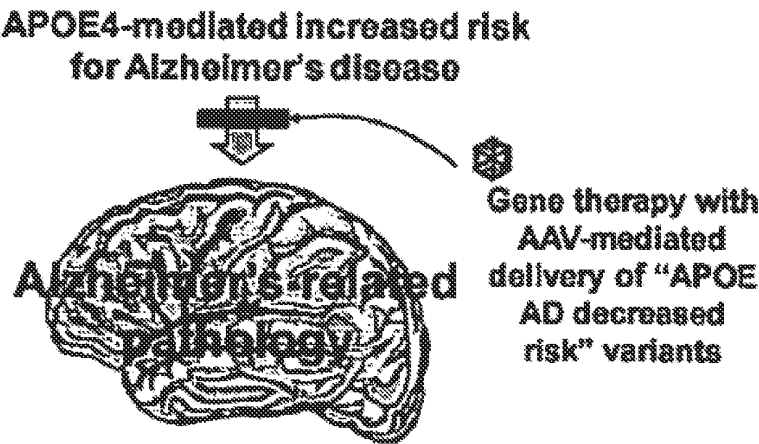


Figure 12

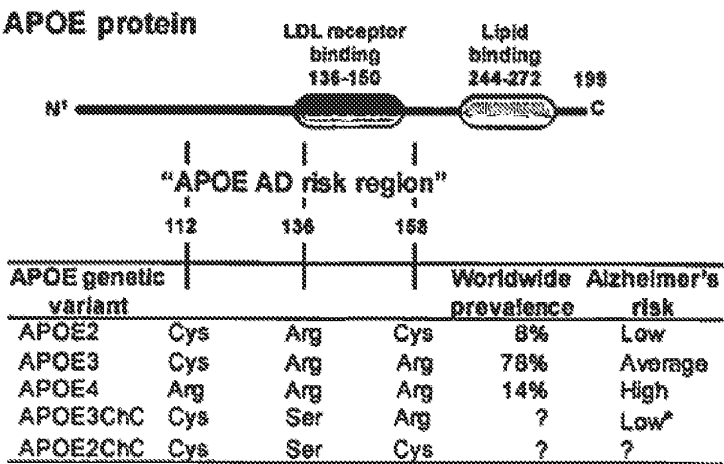


Figure 13

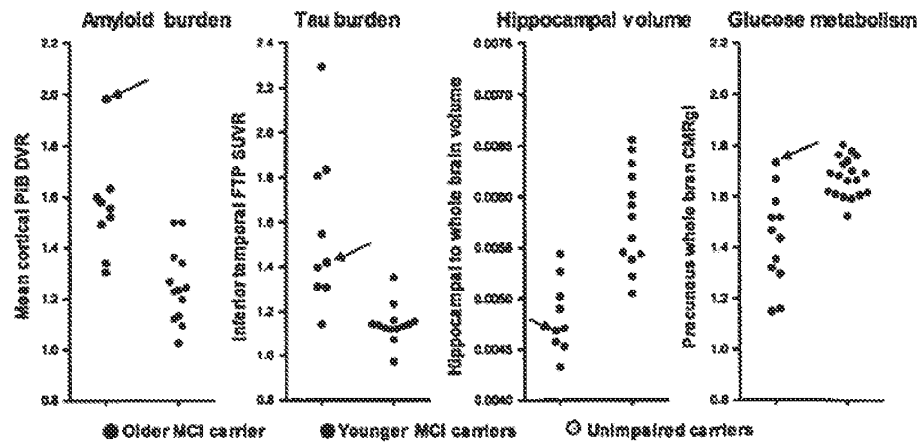


Figure 14

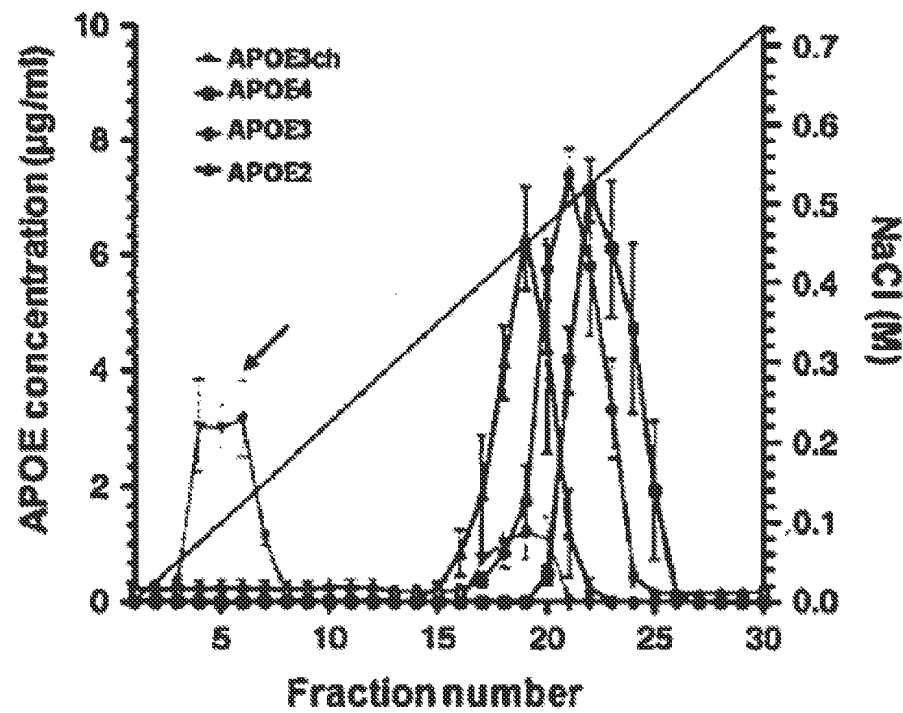


Figure 15

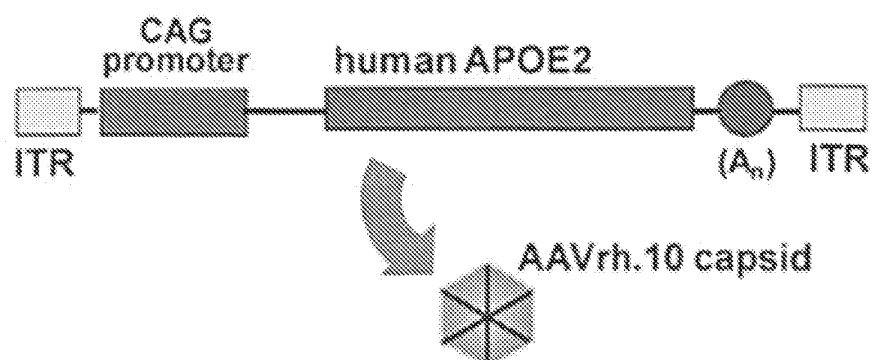
**AAVrh.10hAPOE2vector**

Figure 16

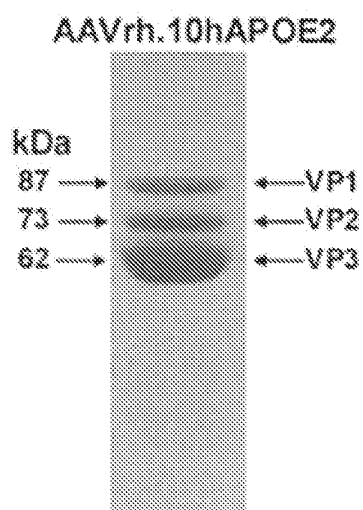


Figure 17

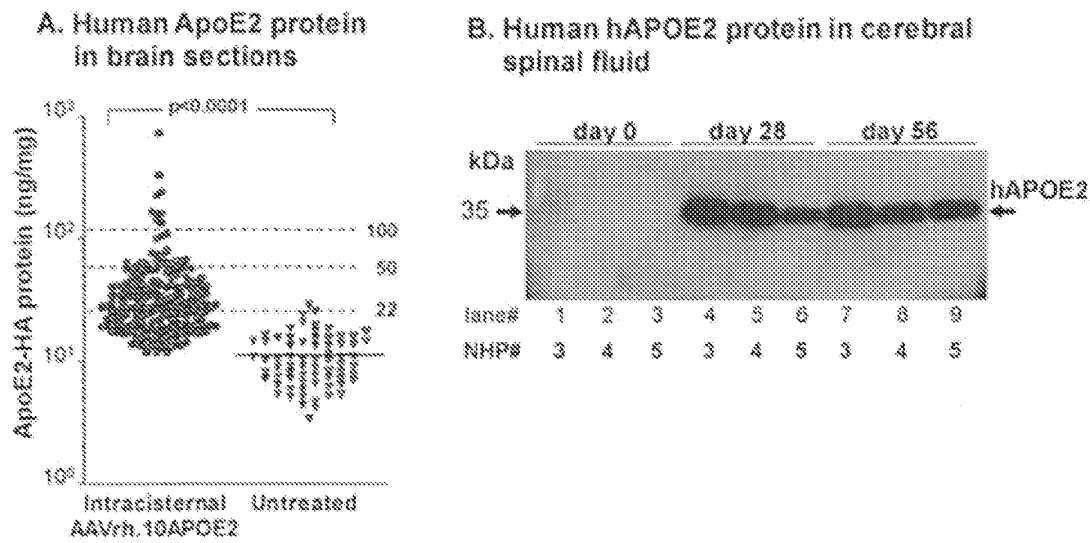


Figure 18

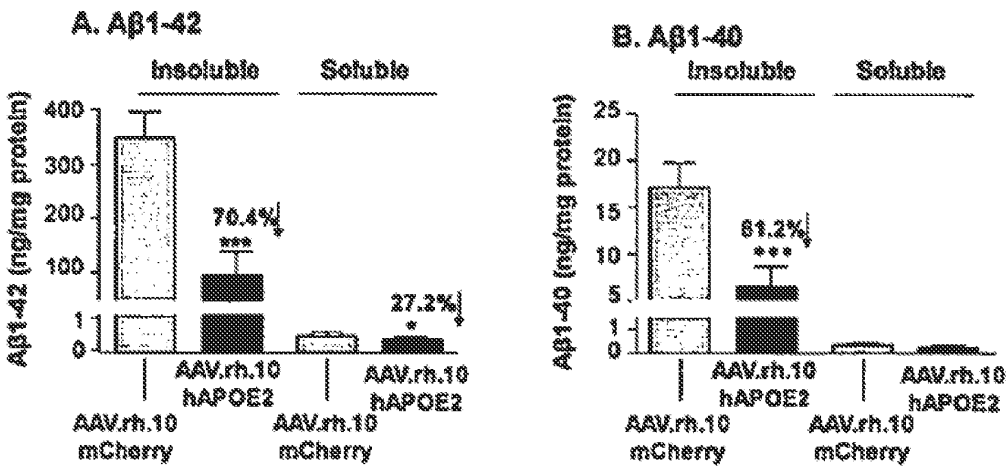


Figure 19

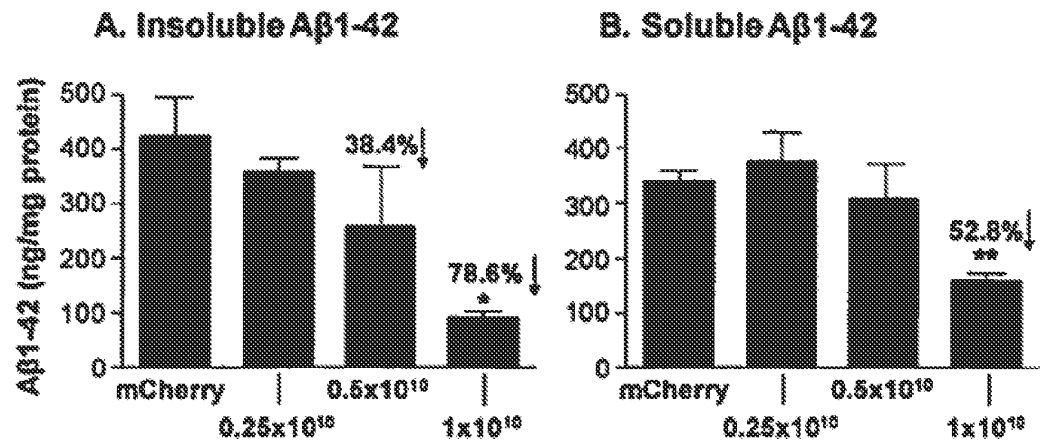


Figure 20

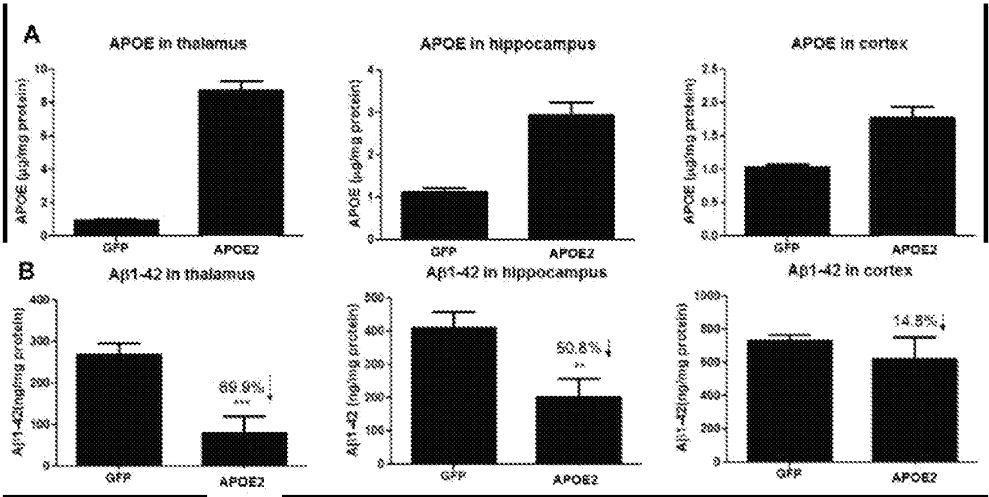


Figure 21

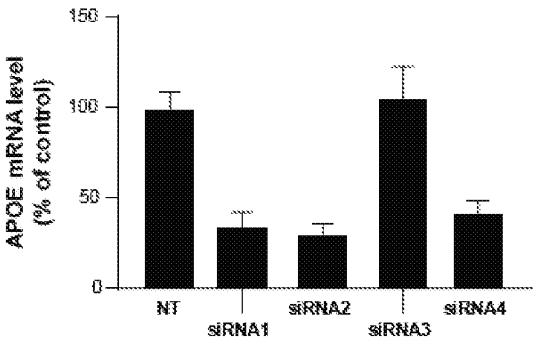


Figure 22

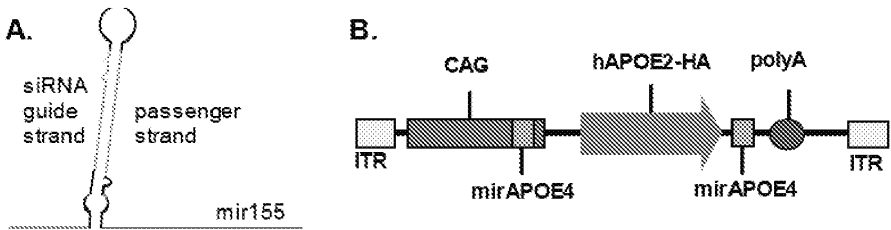


Figure 23

# Figure 24. 293T Transfection

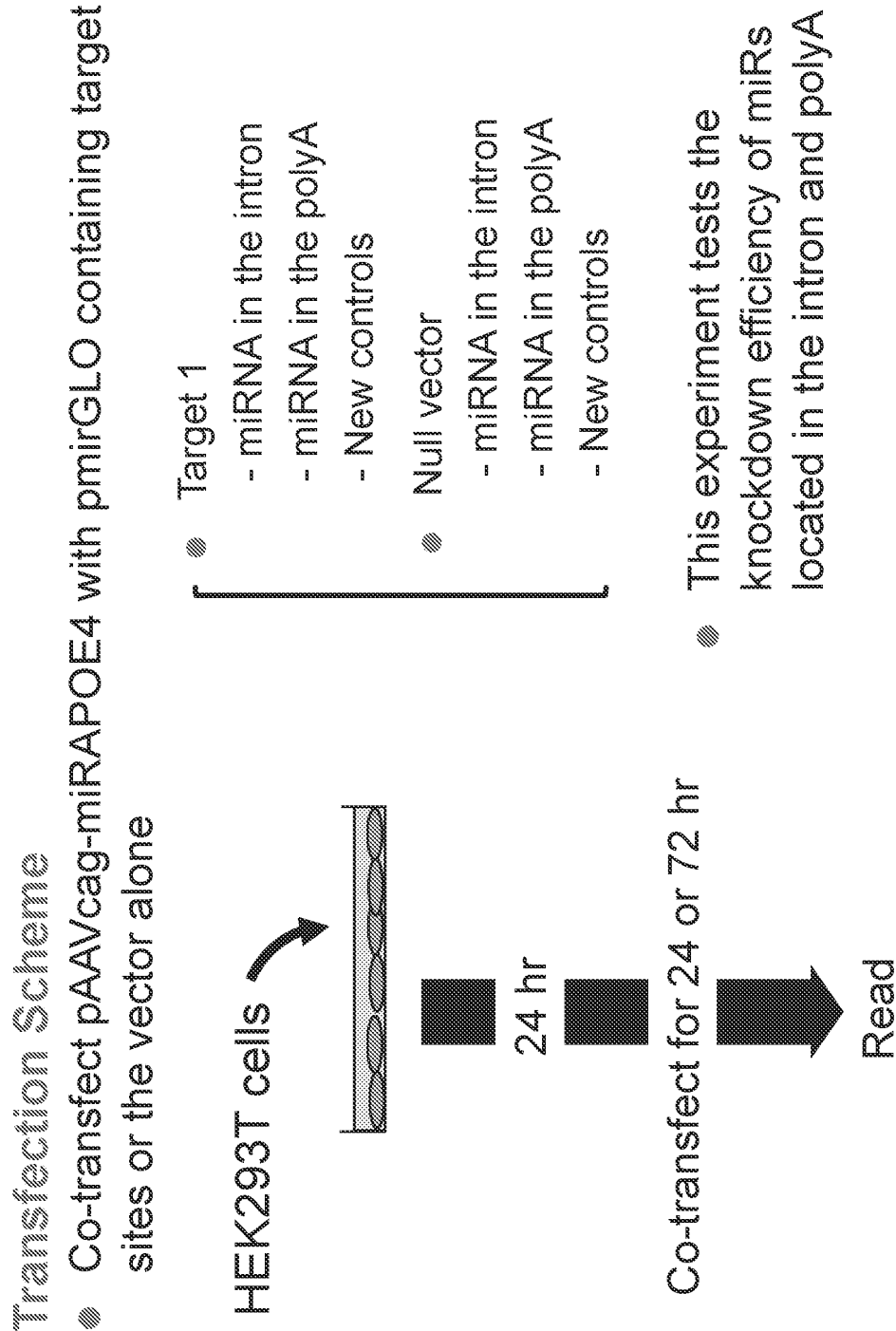


Figure 25. Luciferase Results

- Conclusions
- miR2A in the intron or polyA appears to exhibit greater knockdown as compared to miR2B
- Comments on Controls
- The empty vector controls were removed since the target site may decrease luciferase expression
  - Another assay was set up to test luciferase expression with the pmirGLO containing target site, alone.

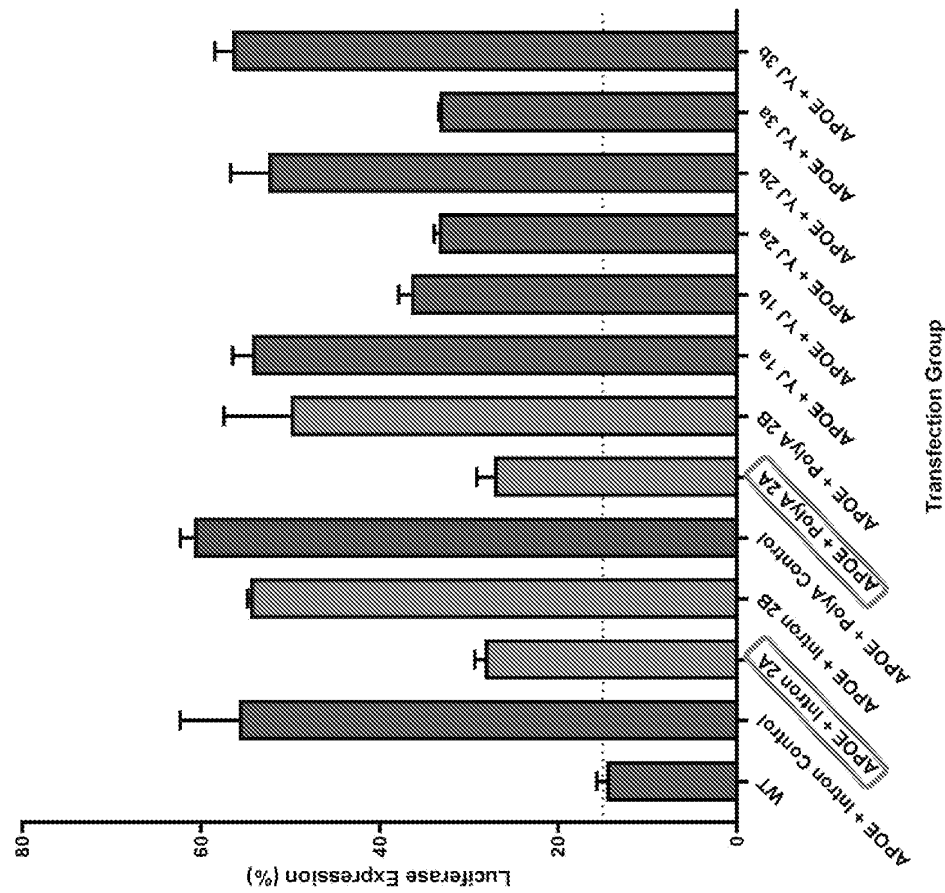


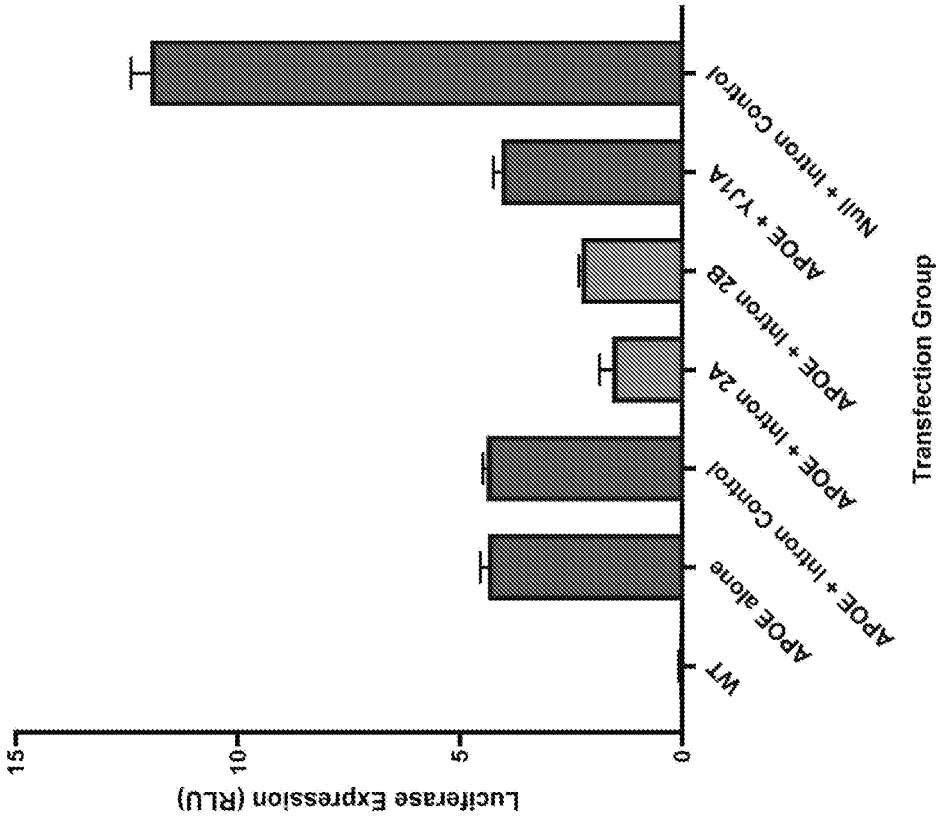
Figure 26. Testing APOE Target Site Only Control

Rationale

- If the control pmirGLO containing target site alone decreases luciferase expression, then pmirGLOuc (null) vector cannot be used as a negative control.
- The target site may affect the stability of the mRNA, may contain cryptic binding site or may interact with translation machinery.

Conclusions

- miR2A in the intron or polyA appears to exhibit greater knockdown as compared to miR2B

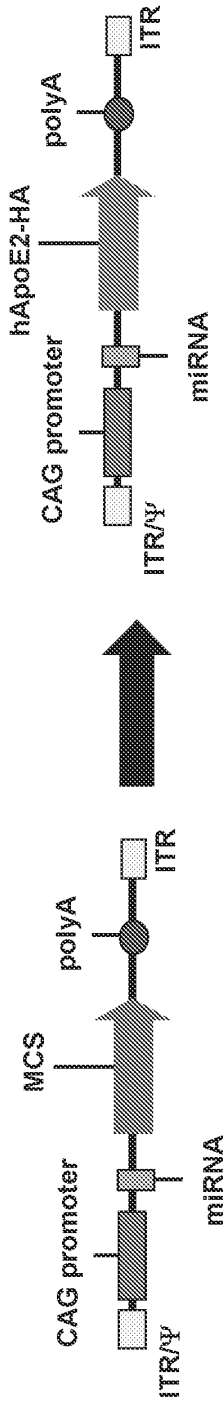




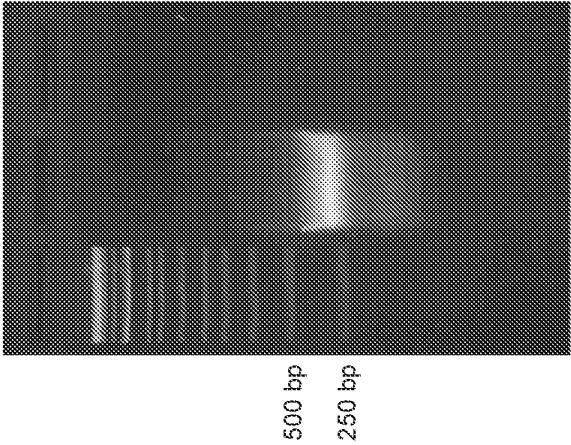
# Figure 28. Insertion of hApoE2-HA

## Strategy for Generating pAAV-miRNA-APOE-HA

- Clone APOE2 into the pAAV with miRAPOE4



- Bsal-BmgBI fragment successfully PCR amplified (~250 bp)
- Bsal-BmgBI is cloned into the pAAV-miRNA with the strongest knockdown



# Figure 29. In Vivo Studies

- Breeding P301S (Jax) mice with TRE4 mice
- AAV9 preparation with confirmed miRs

Table 1. Mouse Proof of Principle Study

| Mouse model (genotype) <sup>2</sup> | Vector                | Administration dose and route                      | Parameters to be evaluated                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------------------|-----------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P301S/E4                            | AAV9-hAPOE2           | 10 <sup>11</sup> gc, via intracisternal (IC) route | <ul style="list-style-type: none"><li>• Behavior and neurologic assessment (monthly)</li><li>• Brain expression of hAPOE4 and hAPOE2</li><li>• Soluble and insoluble A<math>\beta</math> levels</li><li>• Assessment of phosphorylated tau and total tau levels in brain homogenates</li><li>• CNS assessment of neurofibrillary tangles</li><li>• Microglial activation</li><li>• Pathology of hippocampus and cortex</li></ul> |
|                                     | AAV9-hAPOE2-mirAPOE4  |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                     | AAV9-mCherry-mirAPOE4 |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                     | AAV9-null             |                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|                                     | No therapy (PBS)      | None (PBS, IC)                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| C57Bl/6 (controls)                  | No therapy (PBS)      | None (PBS, IC)                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 2 strains                           | 5 vectors             | 1 route                                            | Necropsy @ 3 and 9 months of age                                                                                                                                                                                                                                                                                                                                                                                                 |

Figure 30. All Controls

