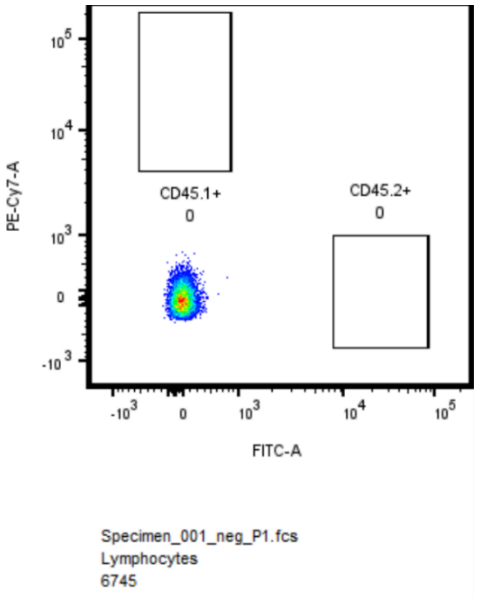
Stem Cell Reports, Volume *19*

Supplemental Information

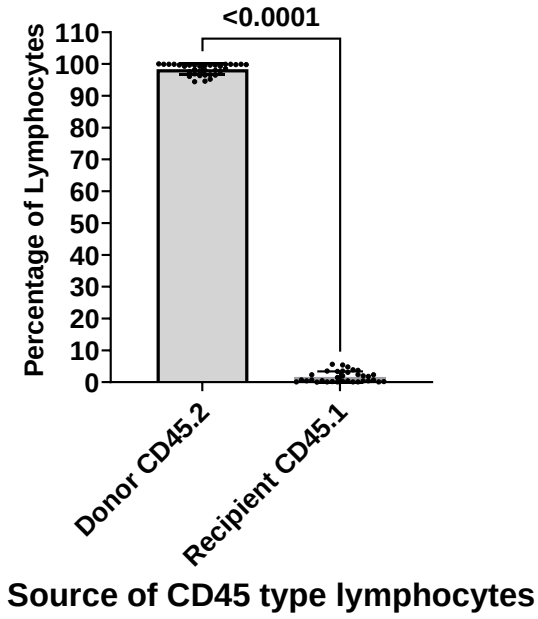
Conclusive demonstration of iatrogenic Alzheimer's disease transmission in a model of stem cell transplantation

Chaahat S.B. Singh, Kelly Marie Johns, Suresh Kari, Lonna Munro, Angela Mathews, Franz Fenninger, Cheryl G. Pfeifer, and Wilfred A. Jefferies

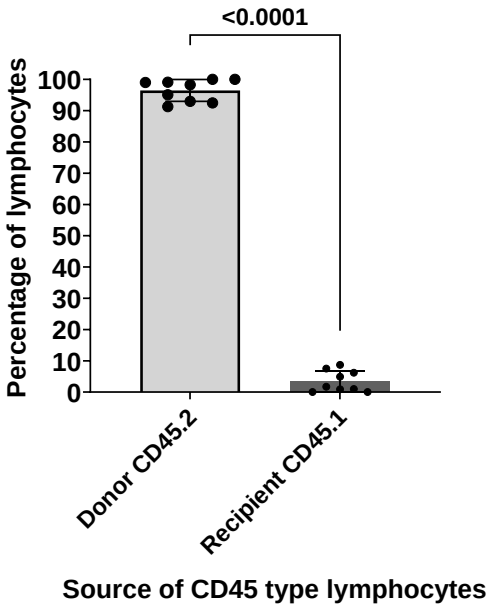


Supplemental Figure 1: Negative control for FACS analysis of successful reconstitution of donor bone marrow cell population in recipient.

a.



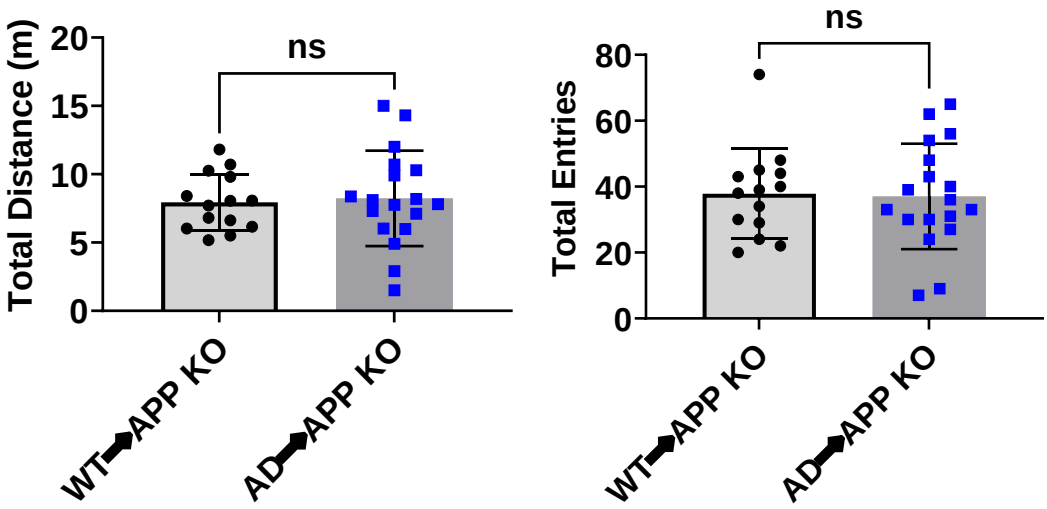
b.



Supplemental Figure 2: Summary statistics for percentage of CD45.1+ and CD45.2+ cells for all recipient animals at 2m post-transplantation

- **a.** Percentage of lymphocytes from donors and recipients. The percentage of donor lymphocytes is higher than the threshold set for a successful transplant (90%) *indicating a successful reconstitution of AD or WT donor bone marrow cells in irradiated APP KO recipients.*
- **b.** Percentage of lymphocytes from donors and recipients. The percentage of donor lymphocytes is higher than the threshold set for a successful transplant (90%) *indicating a successful reconstitution of AD donor bone marrow cells in irradiated WT recipients.*

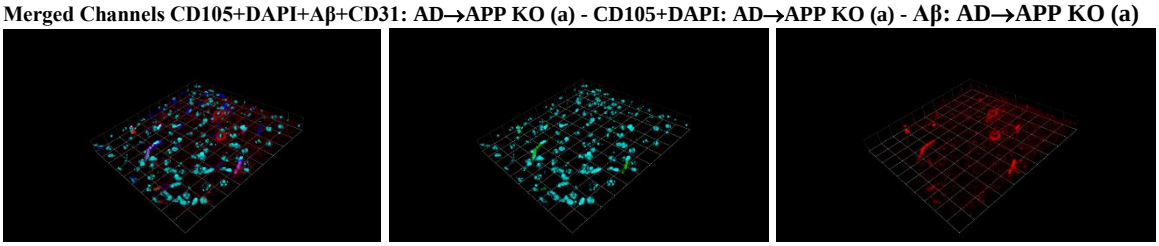
Data represented as the mean± standard deviation. Statistical significance was calculated using unpaired t-test, $p \leq 0.05$.



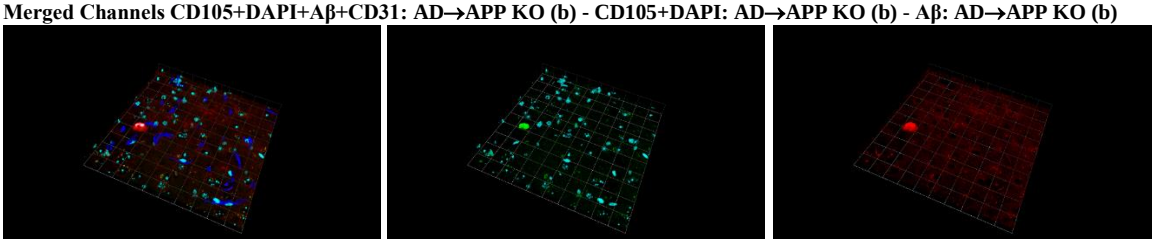
Supplemental figure 3: Total distance travelled and total number of entries made by mice in the Y-maze

No significant difference is seen in total distance travelled in the Y-maze and entries made between AD→APP KO mice and WT→APP KO mice. Data represented as the mean± standard deviation. Statistical significance was calculated using unpaired t-test, $p \leq 0.05$.

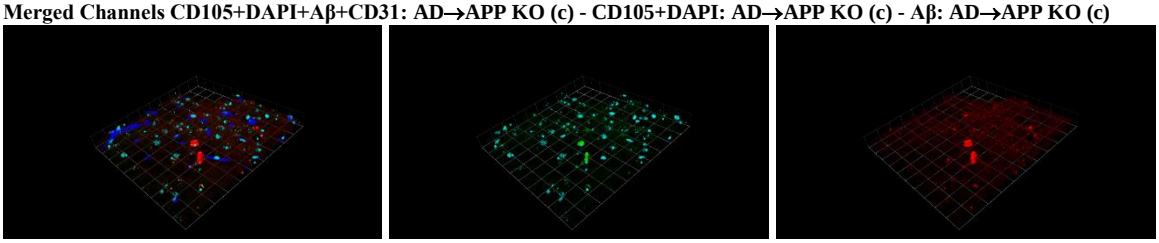
Merged Channels CD105+DAPI+Aβ+CD31: AD→APP KO (a) - CD105+DAPI: AD→APP KO (a) - Aβ: AD→APP KO (a)



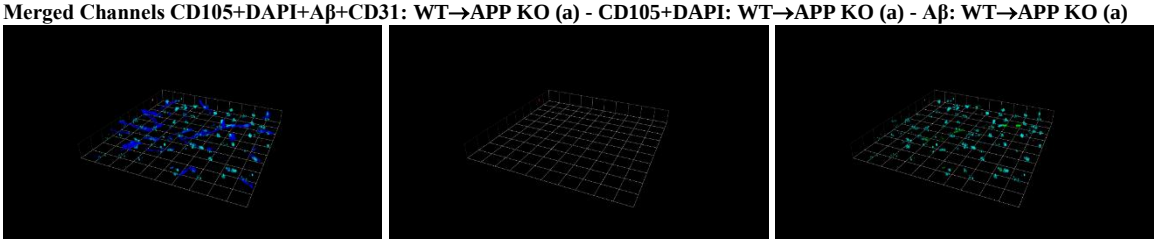
Merged Channels CD105+DAPI+Aβ+CD31: AD→APP KO (b) - CD105+DAPI: AD→APP KO (b) - Aβ: AD→APP KO (b)



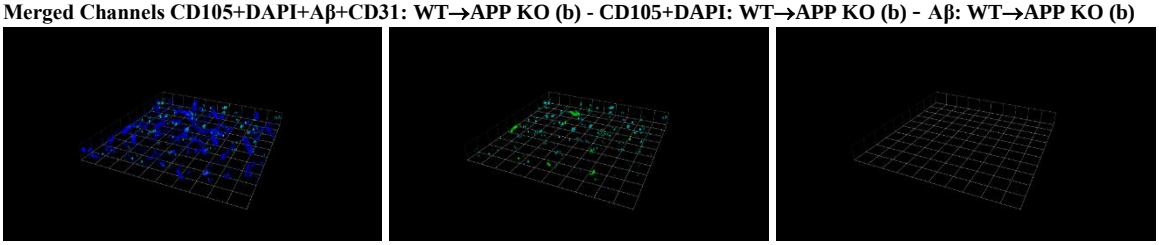
Merged Channels CD105+DAPI+Aβ+CD31: AD→APP KO (c) - CD105+DAPI: AD→APP KO (c) - Aβ: AD→APP KO (c)



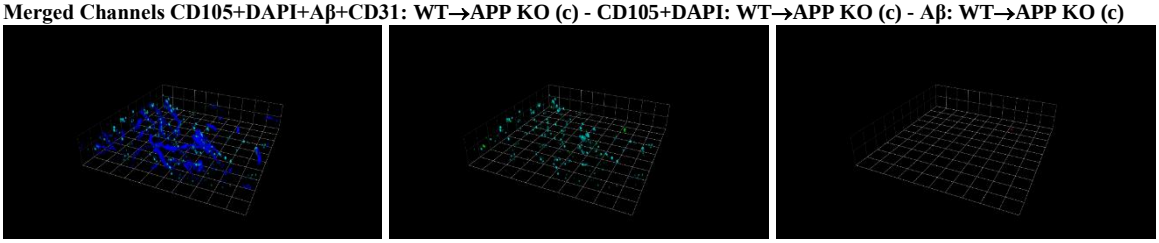
Merged Channels CD105+DAPI+Aβ+CD31: WT→APP KO (a) - CD105+DAPI: WT→APP KO (a) - Aβ: WT→APP KO (a)



Merged Channels CD105+DAPI+Aβ+CD31: WT→APP KO (b) - CD105+DAPI: WT→APP KO (b) - Aβ: WT→APP KO (b)

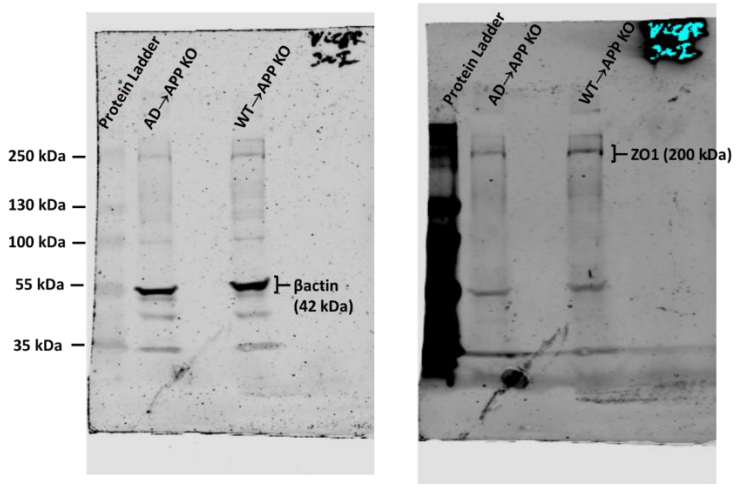


Merged Channels CD105+DAPI+Aβ+CD31: WT→APP KO (c) - CD105+DAPI: WT→APP KO (c) - Aβ: WT→APP KO (c)



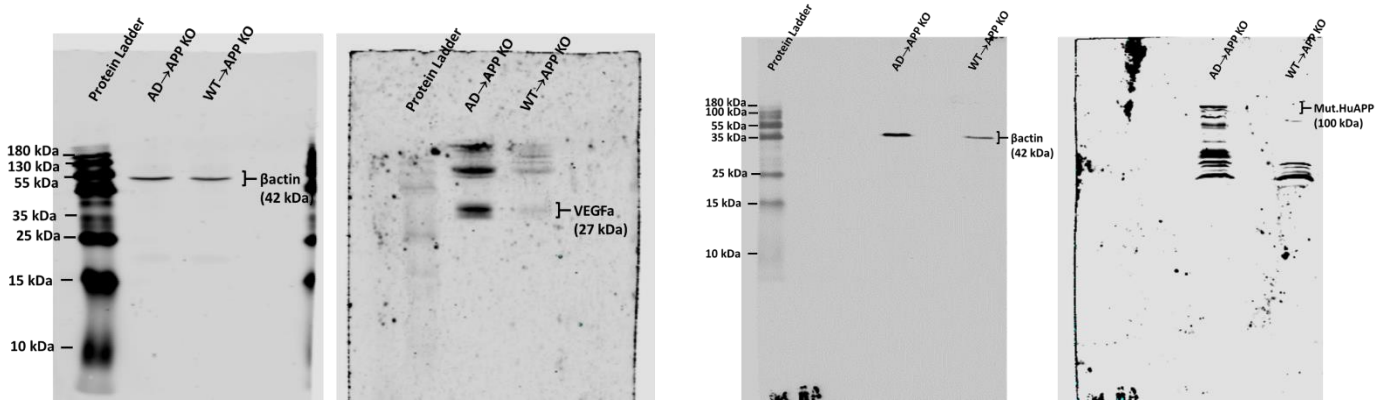
Supplemental figure 4: IHC images showing presence of proteins implicated in AD pathology in the cortical region of mouse brains; Aβ in Tg2576.BM→APP.KO and absence of it in B6/SJL.BM→APP.KO brains. Scale is 100 µm

a.



b.

c.



Supplemental Figure 5: Full representative blots for presence of or reduction of proteins of interest implicated in AD pathology in AD→APP KO mice.

a. Blot shows the lower ZO 1 expression in AD→APP KO mice compared to WT →APP KO mice.
b. Blot shows the higher VEGFa (pro-angiogenic protein) in AD→APP KO mice compared to WT→APP KO mice.
c. Blot indicates the presence of the mutant human APP protein in AD→APP KO mice and the absence of it in WT→APP KO mice.

Star Procedures

Mice

The Tg2576 AD model mouse expresses the K670N/M671L Swedish mutation of the amyloid precursor protein (Hsiao et al., 1996; Hsiao et al., 1995) under control of the hamster prion protein promoter. The promoter is active predominantly in the nervous system and other tissues (Asante et al., 2002), but is also expressed in the haemopoietic lineage that give rise to platelets (Starke et al., 2005). Mice were maintained on hybrid C57Bl/B-SJL (B6/SJL) background by mating heterozygous Tg2576 males to B6/SJL F1 females. Genotyping was performed for all mice by PCR as described by Hsiao *et al.* (Hsiao et al., 1995). Briefly, two parallel PCR reactions were performed to distinguish heterozygote from wildtype. The PrP-APP fusion DNA (corresponding to the heterozygote) was amplified using primers 1502 (hamster PrP promoter, 5'-GTGGATAACCCCTCCCCCAGCCTAGACCA-3') and 1503 (human APP, 5'-CTGACCACTCGACCAGGTTCTGGGT-3'). The primer combination 1502 and 1501 (mouse PrP, 5'-AAGCGGCCAAAGCCTGGAGGGTGGAACA-3') was used as a positive control for the reaction. Tg2576 mice are always heterozygous for the transgene as the homozygous offspring is not viable.

Behavioral Testing

Open Field Test

The Open Field Test used a plexiglass chamber measuring 50 cm (length) x 50 cm (width) x 38 cm (height) with dark coloured walls and a light source focused in the center. All animals were tested separately. The floor of the chamber was demarcated into central and peripheral regions and this field was calibrated in the computer software, so the camera could create physical distance data from pixel-based information. The system was connected to a black and white analog tracking camera with an RTV24 Digitizer that was placed overhead of the open field. The path travelled and the time spent in either region of the field was tracked and recorded for a total of 5 minutes using the computer tracking system (ANY-maze, Stoelting). The Open Field Test exploits the innate behaviour of 'thigmotaxis' where the mice tend to stay towards the shaded edges of an open field and keep away from the brighter center. This implies

that mice that have intact cognition and awareness of the potential danger in the environment will spend less time in the center of the field. This test assesses an animal's anxiety, locomotion and exploration of a novel environment.

Spontaneous alternation (Y-maze):

The test for novelty exploration using spatial and working memory was conducted using a symmetrical Y-maze with a grey steel bottom plate and grey Perspex® walls (Stoelting Co, Wood Dale, IL). Each arm of the Y-maze was 35 cm long, 5 cm wide, and 10 cm high, and the wall at the end of each arm was identified by a different colour: white, blue or red. The spatial acquisition phase comprised a one-day trial with the mice tracked while moving freely through the three arms of the Y maze during an 8-minute session. The movements were tracked by a computer tracking system (ANY-maze, Stoelting)(Miedel et al., 2017). The performance was gauged by the percentage of alternations that was calculated as the total number of alternations × 100/ (total number of arm entries − 2). Alternation was defined as successive entries into the three arms on overlapping triplet sets. A high percentage of alternation was indicative of sustained cognition, as the animals must remember which arm was entered last to avoid re-entering it(Miedel et al., 2017).The minimum number of arm entries needed for a mouse to be included in the analysis was 5 entries (Consortium).

Contextual Fear Conditioning:

The Contextual Fear Conditioning apparatus consisted of a transparent chamber inside an enclosure with an opening in the ceiling to allow video recordings(Curzon P, 2009).The chamber had a steel grid floor connected to a shock generator scrambler. The test encompassed two sessions: conditioning and a context test. On the conditioning day, the mice were individually placed in the chamber and allowed to explore freely for 5 minutes during which, at the 180th second, they received a foot shock of 0.50–0.80 mA for 3 seconds through the bars of the floor. The amplitude of the shocks delivered was determined based on

previous studies performed(Singh et al., 2021). 24 hours after conditioning, the mice were individually placed back in the chamber for 4 minutes, this time with no noxious stimuli. The mice were monitored for movement and freezing behaviour was recorded using computer software (Limelight, ActiMetrics, Wilmette, IL, USA). Exclusion criteria were set for freezing events less than 2 seconds. This test was used to determine associative working memory. We explored the animal's ability to associate an environment with a noxious event that it experienced there. When the animal is returned to the same environment, it generally will demonstrate a freezing response if it remembers and associates that environment with the shock. Freezing is a species-specific response to fear, which is defined as "the absence of movement except for respiration"(Curzon P, 2009). This may last for seconds to minutes depending on the strength of the aversive stimulus and whether the subject is able to recall the shock.

Radial arm water maze (RAWM):

The RAWM consists of eight swim paths (arms) extending out of an open central area, with an escape platform located at the end of any of four alternate arms called the 'goal arms'(Penley et al., 2013). The starting position and the goal arms were fixed throughout the duration of the study. The mice were individually placed facing the wall of an arm that was selected and maintained throughout the study as the 'start position' of the maze. The observer remained stationary at this position once the subject was placed in the maze. This was the visual cue for the mice to orient themselves in the maze. Each subject was given 60 seconds to locate one of the 4 escape platforms. With each trial, the platform that was used to escape was removed and not placed back into the maze until the end of that test day. The test was repeated until only one platform was left. Once the animal found the last platform it marked the end of the test for that day. In between each trial, the animal was removed and placed back in its heated home cage for 90 seconds to avoid hypothermia. These trials were conducted daily for a total of 5 days. The latency to reach the platform for each trial and the arm entries were recorded manually. Performance of memory and learning was gauged each day based on the average time taken to find the escape platforms and the total

number of errors, i.e. Reference memory errors + Working memory errors. A Reference memory error is defined as the entry into an arm which never had an escape platform and Working memory error is defined as the subsequent entry into an arm where the platform had been removed in the previous trial(Penley et al., 2013).

Immunofluorescence and Confocal imaging

The PFA fixed hemispheres were embedded in a 4% agarose block and 50µm thick sections were cut using a Leica VT1000S Vibratome (Leica Biosystems Inc., Germany), and then further processed for immunofluorescent microscopy. . Antigen retrieval was done by placing brain sections in a sodium citrate solution (10mM pH8) that was prewarmed at 80˚C in a water bath for 30 minutes. The sections were allowed to come to room temperature and then washed thrice with PBS. The sections were then blocked with buffer (3% skimmed milk in PBS; 0.1% Tween-20) for 1 hour at room temperature followed by overnight incubation at 4°C with primary antibodies against the various proteins of interest: CD105 (R&D Systems; 15 µg/mL, AF1097); Aβ,1-16 (1µg/mL, BioLegend; 803004); tight junction protein, Occludin (1:200, abcam; ab31721); and CD-31 (1:50, abcam; ab28364). For co-localization experiments, multiple antibodies were used on the sections simultaneously. The fluorophore conjugated secondary antibody incubation was done at room temperature for 1 hour at a concentration of 1:500. DAPI, at a concentration of 0.1 µg/mL, was used for nuclear counterstaining for 5 minutes before the first wash following the secondary antibody incubation. Sections were then washed using PBS with 0.1% Tween-20 before being cover-slipped with Fluoromount-G. Slides were allowed to air-dry overnight in the dark.

The cortical region of the mouse brain was analyzed for markers of interest involved in AD

pathology. The image acquisition was done using a Olympus FV-10i confocal microscope with a high- resolution Olympus 60 X/1.4 oil-immersion objective lens (Olympus, Tokyo, Japan). For 3D image data set acquisition, the excitation beam was first focused at the maximum signal intensity focal position within the brain tissue sample and the appropriate exposure times were selected to avoid pixel saturation. A series of 2D images (Z stack) were taken at a step size of 1µm. The beginning and end of the 3D stack were set based on the signal level degradation. The series images taken get saved in the Olympus software. The Volocity software (PerkinElmer) was then used to process the series of images that were taken and generate a 3D reconstruction of the tissue.

Asante, E.A., Gowland, I., Linehan, J.M., Mahal, S.P., and Collinge, J. (2002). Expression pattern of a mini human PrP gene promoter in transgenic mice. Neurobiol Dis *10*, 1-7.

Curzon P, R.N., Browman KE (2009). Cued and Contextual Fear Conditioning for Rodents. In Methods of Behavior Analysis in Neuroscience, B. JJ, ed. (CRC Press/Taylor & Francis).

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science *274*, 99-102.

Hsiao, K.K., Borchelt, D.R., Olson, K., Johannsdottir, R., Kitt, C., Yunis, W., Xu, S., Eckman, C., Younkin, S., Price, D*., et al.* (1995). Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. Neuron *15*, 1203-1218.

Miedel, C.J., Patton, J.M., Miedel, A.N., Miedel, E.S., and Levenson, J.M. (2017). Assessment of Spontaneous Alternation, Novel Object Recognition and Limb Clasping in Transgenic Mouse Models of Amyloid-β and Tau Neuropathology. Journal of visualized experiments : JoVE, 55523.

Penley, S.C., Gaudet, C.M., and Threlkeld, S.W. (2013). Use of an eight-arm radial water maze to assess working and reference memory following neonatal brain injury. Journal of visualized experiments : JoVE, 50940-50940.

Singh, C.S.B., Choi, K.B., Munro, L., Wang, H.Y., Pfeifer, C.G., and Jefferies, W.A. (2021). Reversing pathology in a preclinical model of Alzheimer's disease by hacking cerebrovascular neoangiogenesis with advanced cancer therapeutics. EBioMedicine *71*, 103503.

Starke, R., Harrison, P., Mackie, I., Wang, G., Erusalimsky, J.D., Gale, R., Masse, J.M., Cramer, E., Pizzey, A., Biggerstaff, J*., et al.* (2005). The expression of prion protein (PrP(C)) in the megakaryocyte lineage. J Thromb Haemost *3*, 1266-1273.