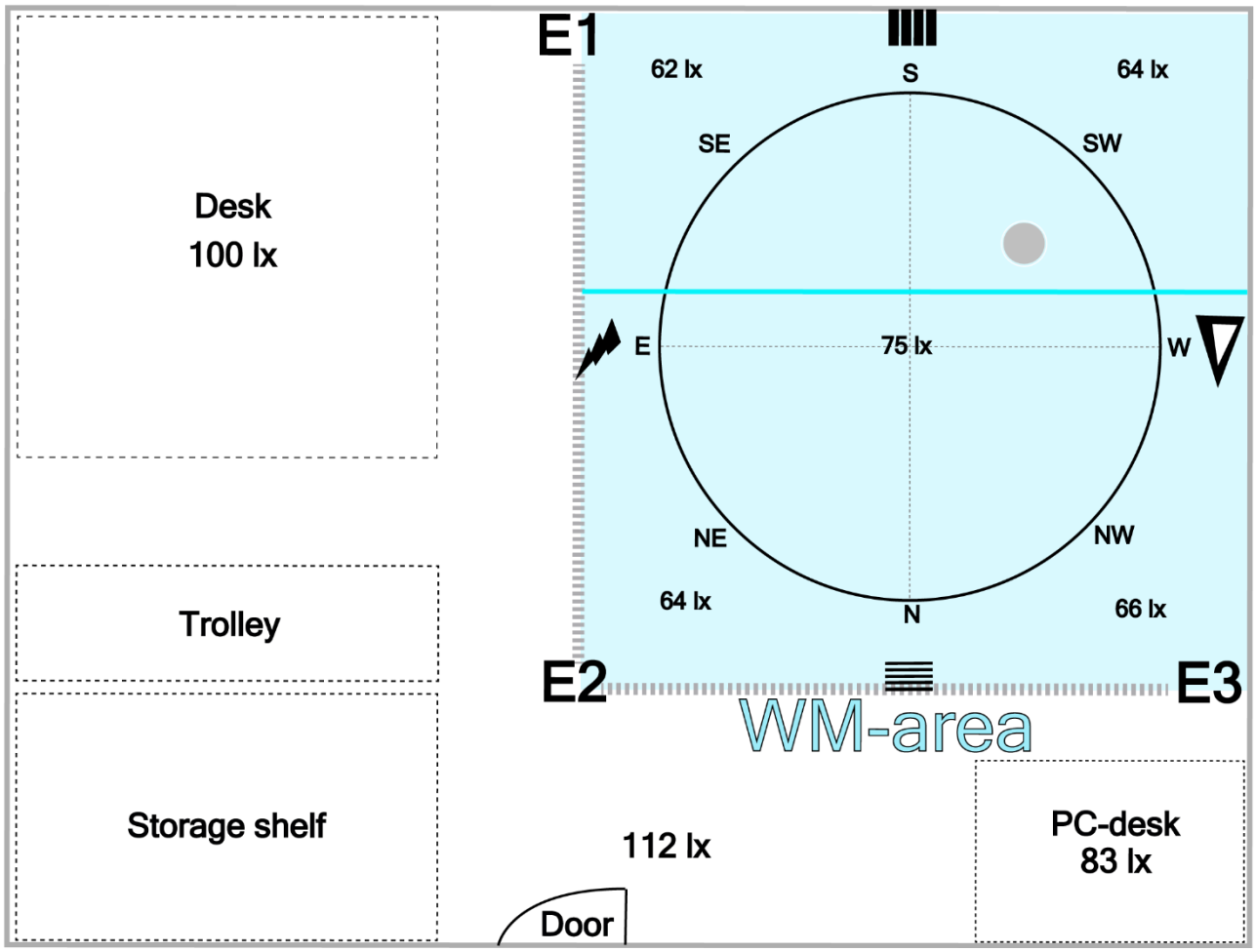
Supplementary Material

Detection and prediction of mild cognitive impairment in Alzheimer's disease mice

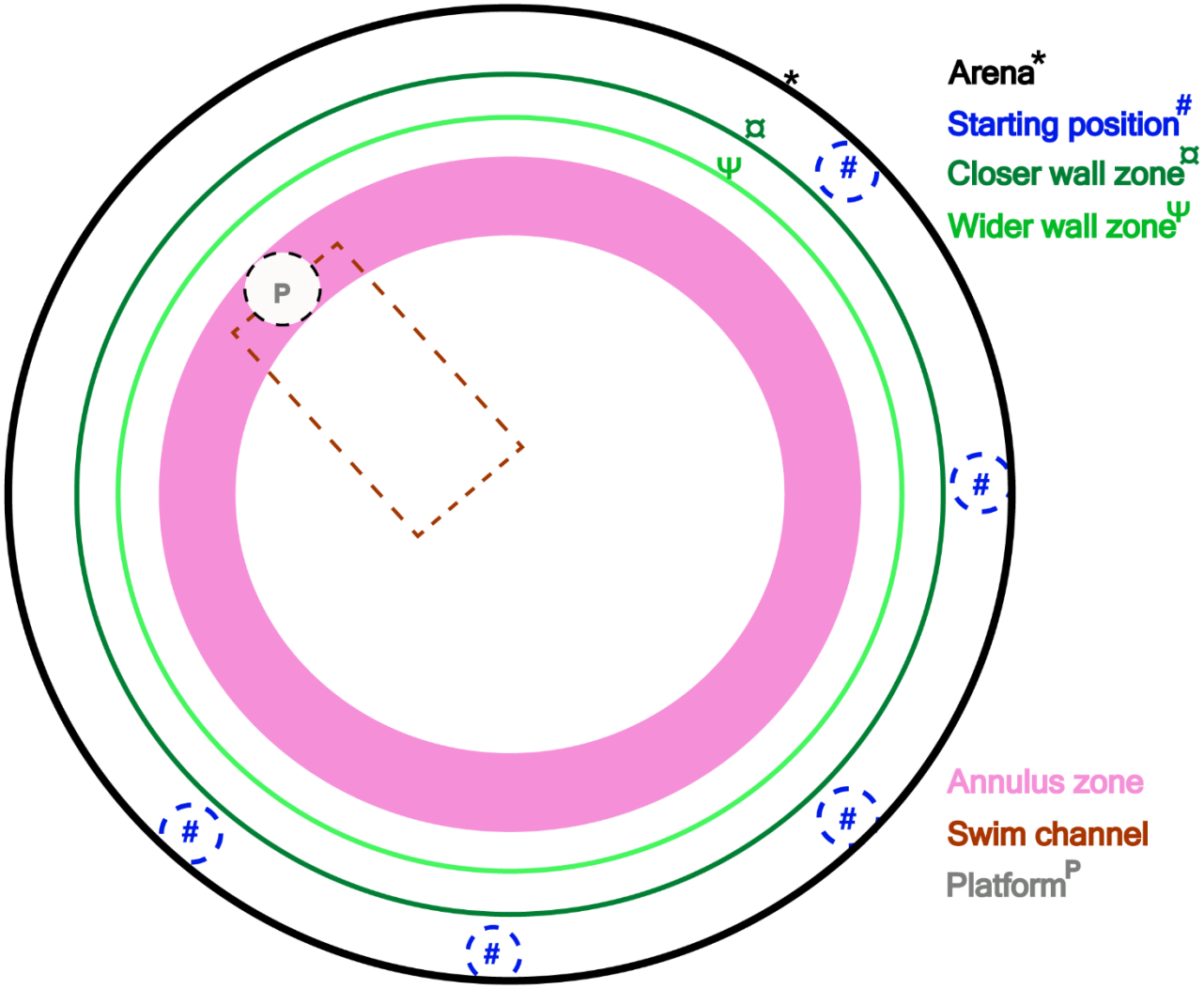
Supplementary Table 1. WM start positions for training trials, probe trials, and visual cue trials, respectively. The sequence of training trials is shown in a light blue background, probe trials are shown in green background, and visual cue trials are shown in light orange background. Once a mouse completed a task for a day, it would be transferred back to normal housing cage. A housing cage was changed on day 1, day 4, and day 8 after completion of a WM task. Water was changed on day 5 after completion of the task.

Day	Probe trial	Trial A	Trial B	Trial C	Trial D	Comment
1		N (1)	E (2)	NW (3)	SE (4)	Change cage
2		SE (5)	N (6)	E (7)	NW (8)	
3		NW (9)	SE (10)	N (11)	E (12)	
4	NE	E (13)	NW (14)	SE (15)	N (16)	Change cage
5		N (17)	SE (18)	NW (19)	E (20)	Change water
6	NE	NW (21)	E (22)	N (23)	SE (24)	
7		SE (25)	N (26)	E (27)	NW (28)	
8		E (29)	NW (30)	SE (31)	N (32)	Change cage
9	NE	Visual cue trials				Clean tank

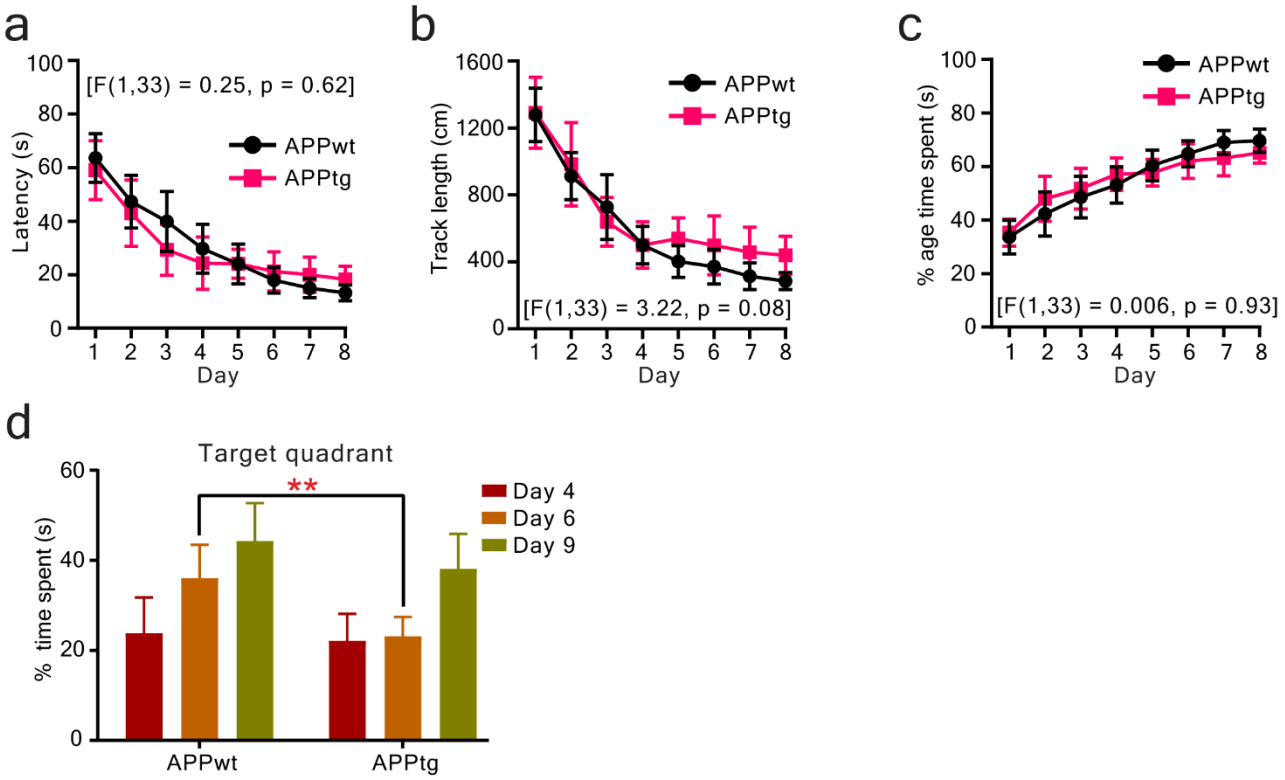
Platform position	NW (33)	SE (34)	NW (35)	SE (36)
Mouse start position	E	SW	S	NE



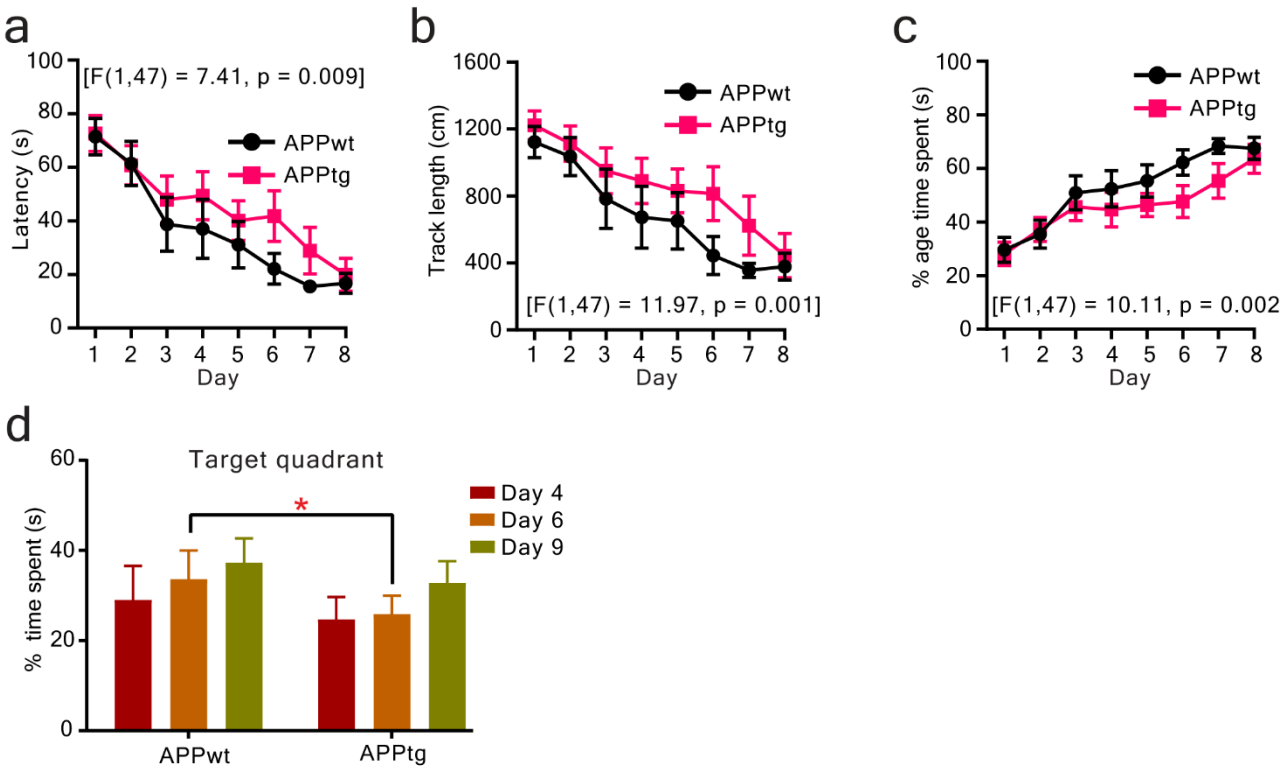
Supplementary Figure 1. Graphical layout of the WM room including WM area. Desk and computer were in the north-west (NW) corner. In the northeastern (NE) corner, a storage shelf and trolley were located. In the southeastern (SE) corner, a desk was permanently located. The WM area measured 146 cm by 146 cm was curtained at north (N) and east (E) sides. The WM pool (116 cm diameter and 30 cm high) was located approximately 30 cm away from the walls in the center of the WM area. The reflection of light on the water surface was prevented by covering the light source with white paper. Light intensities at various locations inside the room were measured. The WM pool was filled with water so that the water level was approximately 0.5 cm to 1 cm above the platform. The circular platform itself was made of plexiglass and 15 cm high and 10 cm in diameter. Within the white pool, the transparent plexiglass platform was hidden even without extra coloration of the water. The water temperature was maintained at 22.5 ± 1.0 °C throughout the study period. Four unique geometrical shapes were used as distal cues and were fixed at 115 cm above the floor (10 cm above the pool's rim). To minimize experimenter-induced spatial bias during mouse navigation, three different entry/exit positions were defined: E1 is the entry/exit for start positions S and SE; E2 is the entry/exit for start positions E, NE, and N; E3 is the entry/exit for start positions SW, W, and NW.



Supplementary Figure 2. Video analysis. Within the area of the water tank, two concentric circular zones were defined: a) The closer wall zone covering the outmost 10 cm of the tank and b) the wider wall zone covering the outmost 16 cm of the tank. Now, a 7.5 cm grid was drawn over the whole arena to monitor movements of a mouse. The size of a mouse, floating threshold, and video recordings were predefined at 140 pixels area (10 pixels = 1.96 cm^2), 5 cm s^{-1}, and 25 frames s^{-1} respectively. The camera was fixed 95 cm above the water surface so that the given value is empirically calibrated for the given camera settings. Multiple regions of interests (ROIs) as starting positions were drawn around the periphery of the tank. Starting positions were defined as trigger zones that automatically initialize recording and tracing of the movement once the mouse leaves one of the zones. A 10 cm circular ROI was drawn to label the position of the hidden platform. It defines the trigger zone that stops recording once the mouse is detected for a total of 10 s within the area. The swimming channel was configured to be 18 cm wide and termed “goal corridor”. The goal corridor was adapted automatically to extend directly to the starting position of each trial.



Supplementary Figure 3. MCI detection using additional measures (latency, track-length, and % time spent in target quadrant) in long-ITI (spaced trials) protocol. Mice were trained for eight consecutive days in which each mouse was allowed to swim four trials each day with an ITI of 15-40 min. Similarly, three independent probe trials were conducted on day 4, day 6, and day 9 respectively. a-c) Training trials. c) Probe trials. ANOVA of a mixed model repeated measure analysis were conducted for training trials whereas two-tailed unpaired t-test was performed for probe trial. Values are shown as mean ± 95% CI (confidence interval). **p < 0.01; APPwt N = 20 and APPtg N = 15 animals.



Supplementary Figure 4. MCI detection using additional measures (latency, track-length, and % time spent in target quadrant) in short-ITI (massed trials) protocol. Mice were trained for eight consecutive days in which each mouse was allowed to swim four trials each day with an ITI of 1 min. Similarly, three independent probe trials were conducted on day 4, day 6, and day 9 respectively. a-c) Training trials. c) Probe trials. ANOVA of a mixed model repeated measure analysis were conducted for training trials whereas two-tailed unpaired t-test was performed for probe trial. Values are shown as mean ± 95% CI (confidence interval). *$p < 0.05$; APPwt N = 23 and APPtg N = 26 animals.