Supplementary experimental procedures

1. Respirometry monitored using metabolic cages. Oxygen consumption (VO_2) and carbon dioxide excretion (VCO_2) values were measured in 6-7 month old female mice using an O_2/CO_2 metabolism measuring system for small animals (MK-5000RQ, Muromachi Kikai). The mice were isolated in a semi-sealed cage, and the inner air was aspirated at a constant volume/min (approximately 0.65-0.70 l/min). The concentrations of O_2 and CO_2 in the aspirated air were measured per minute at intervals of 1 minute and automatically corrected using standard O_2 and CO_2 values. Scores were obtained for at least 30 hours, and the 24-hour profile of daily respiration was produced by averaging the data for mice of the same genotype.

2. Locomotive activity monitored using implanted transmitting devices. Spontaneous motor activity was measured using an activity-monitoring system (ACTIMO-100, Shin factory). The mice were undisturbed and isolated in their home cages, which were made of clear plastic, and had been implanted with transmitting devices. The infrared sensors (20 mm distance) detected any movement (except for small movements of the nose or tail) with a frequency of 2 events per second. Scores were obtained as counts per 30 minutes, and the 24 hours profile of daily activity was obtained by averaging 7 days of continuous data.

3. Y maze. All behavioral experiments were performed longitudinally. The Y maze test was performed according to the protocols developed by Ognibene *et al*. [19]. The Y maze apparatus

(Muromachi Kikai) was made of gray plastic walls of 12 cm high, consisting of three compartments (40 cm x 2 cm) connected by 2 cm x 2 cm passages. The mice were placed into one of the three arms of the maze and allowed to explore all three arms for 10 min. An arm entry was recorded when all four paws entered the compartment. After testing each mouse, the maze apparatus was thoroughly cleaned with 70% ethanol. Performance was monitored with the CompACT VAS/DV video-tracking system (Muromachi Kikai). The percentage of spontaneous alternations is the ratio of arm choices differing from the previous two choices, based on the natural habit of mice to explore novel locations.

4. Elevated plus maze. Anxiety-based activity was assessed using the elevated plus maze task [20]. The elevated plus maze apparatus (Muromachi Kikai) consists of two open arms (30 x 6 cm) and two closed arms of the same size with 14 cm-high gray opaque walls made of polyvinyl chloride. The arms and central square were made of gray opaque polyvinyl chloride and were elevated to a height of 40 cm above the floor. The mice were placed alone in the central square (6 x 6 cm) of the maze facing one of the open arms and allowed to explore freely for 10 min. The apparatus was thoroughly cleaned with 70% ethanol between trials. The counts of each arm entry and the time spent in each arm were recorded automatically with a CompACT VAS/DV Video video-tracking system.

5. Open field test. The open-field test was performed according to the protocols developed by Rustay *et al*. [21]. As in the open-field test, we used a water maze pool containing no water

(Muromachi Kikai) with a diameter of 120 cm. The entry point was the center of the field. A 10-minute spontaneous probe trial was carried out in an open area. Performance was monitored with the CompACT VAS/DV video-tracking system.

6. Statistical Analyses. All data are presented as means ± s.e.m. The differences among the four groups were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's test, and the *P* values of < 0.05 or < 0.01 between two groups on the unpaired T-test and Welch's test were considered statistically significant.

Supplementary Figures

Figure 1: Body weight of the PS2Tg2576 mice. Body weights of male (a) and female (b) transgenic mice. Statistical significance was assessed using one-way ANOVA: $*P < 0.05$ compared with all other mice of the same age. Values represent the mean ± s.e.m. (n = 7-21).

Figure 2: Metabolic analysis of PS2Tg2576 mice. (a) Food intake at 6-7 months of age. (b) Amount of drinking water consumed at 6-7 months of age. Values represent the mean ± s.e.m of 7 days of measurements from each animal (n = 18, male and female mixture). (c) Mean time course of oxygen consumption rates (VO_2) of female mice at 6-7 months of age. (d) Mean time course of respiratory quotients (RQ) of female mice at 6-7 months of age. Values represent the mean of 24 hours of measurements from each animal (n = 6, female).

Figure 3: Early accumulation of Aβ42 deposition in the brains of PS2Tg2576 mice. Amyloid plaques in the brain were immunoreacted with the polyclonal antibody against the C-terminus of Aβ42. (a) Amyloid plaques in the PS2Tg2576 mice at 2-3 months of age. (b) Amyloid deposition in the PS2Tg2576 mice at 4-5 months. (c) Typical amyloid plaques were observed in the 6-7 month-old PS2Tg2576 mice. (d) No plaques were detected in the brains of the Tg2576 mice at 6-7 months of age. The arrowheads indicate significant plaques in the PS2Tg2576 mice at 2-3 months of age. The insets show high magnification images of the boxed area. The scale bars represent 200 μm and 5 μm in the main panel and inset, respectively.

Figure 4: Early accumulation of Aβ40 depositions in the brains of PS2Tg2576 mice. Amyloid plaques in the brain were immunoreacted with the polyclonal antibody against the C-terminus of Aβ40. (a) Amyloid plaques in the PS2Tg2576 mice at 2-3 months of age. (b) Amyloid depositions in the PS2Tg2576 mice at 4-5 months. (c) Typical amyloid plaques were observed in the 6-7 month-old PS2Tg2576 mice. (d) No plaques were detected in the brains of the Tg2576 mice at 6-7 months of age. The arrowheads indicate significant plaques found in the PS2Tg2576 mice at 2-3 months of age. The insets show high magnification images of the boxed area. The scale bars represent 200 μm and 5 μm in main panel and inset, respectively.

Figure 5: Behavioral testing of PS2Tg2576 mice in their home cages. Records of continuous locomotive activity monitored using implanted transmitting devices (n = 18, male and female mixture).

Figure 6: Behavioral testing of PS2Tg2576 mice in the Y maze. The percentage of spontaneous alternations was measured during 10 minutes exploration. (n = 18-25, male and female mixture). Statistical significance was assessed using the unpaired T-test: $^*P < 0.05$ compared with the wild-type mice at the same age. Values represent the mean ± s.e.m.

Figure 7: Behavioral testing of the PS2Tg2576 mice in the elevated plus-maze. Number of open-arm entries (a) and the percentage of time spent in the open-arms (b) were measured

during 10 minutes exploration. (n = 18-25, male and female mixture). Statistical significance was assessed using the unpaired T-test: *$P < 0.05$ compared with the wild-type mice at the same age. Values represent the mean ± s.e.m.

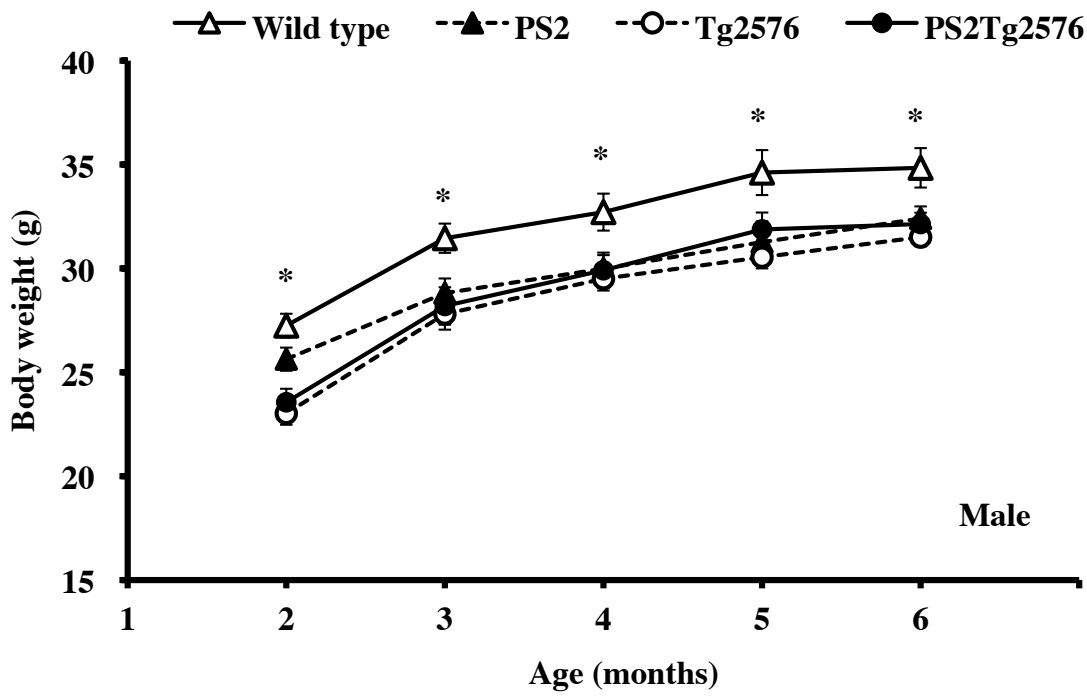
Figure 8: Behavioral testing of the PS2Tg2576 mice in the open-field test. Total locomotive distance was measured at 6-7 months of age during a 10-minute period. Representative moving paths are shown under the graph. (n = 3-5, male and female mixture). Values represent the mean ± s.e.m.

References in supplemental materials

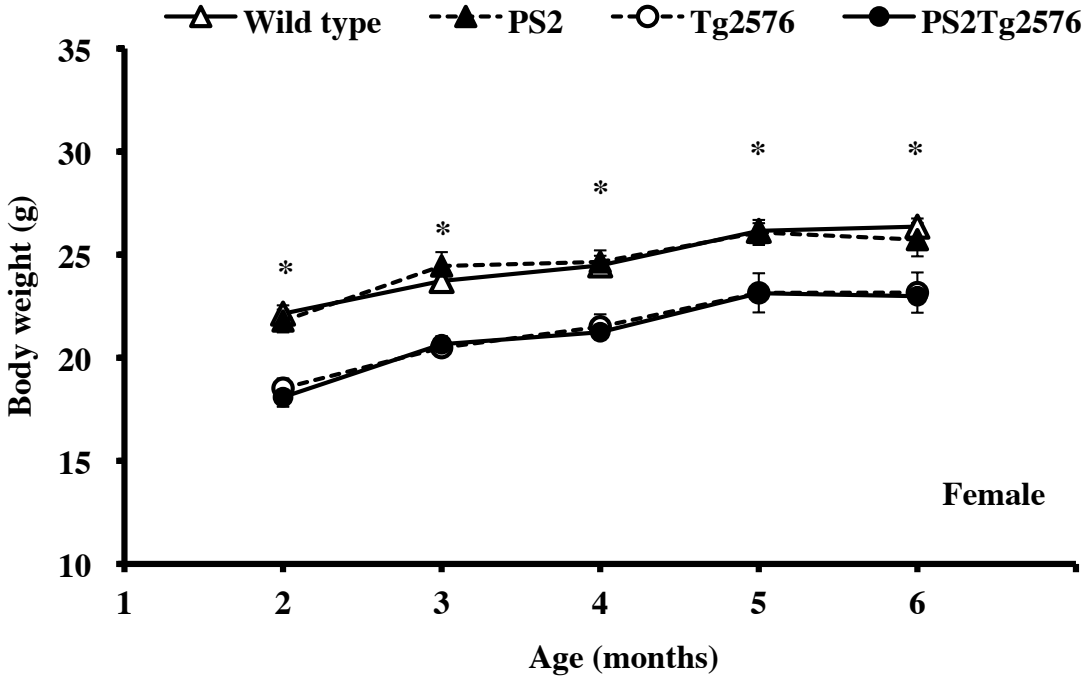
[19] E. Ognibene, S. Middei, S. Daniele *et al*., "Aspects of spatial memory and behavioral disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease," *Behav Brain Res*, vol. 156, no. 2, pp. 225-232, 2005.

[20] M. Tabuchi, T. Yamaguchi, S. Iizuka, S. Imamura, Y. Ikarashi, andY. Kase, "Ameliorative effects of yokukansan, a traditional Japanese medicine, on learning and non-cognitive disturbances in the Tg2576 mouse model of Alzheimer's disease," *J Ethnopharmacol*, vol. 122, no. 1, pp. 157-162, 2009.

[21] N. R. Rustay, E. A. Cronin, P. Curzon *et al*., "Mice expressing the Swedish APP mutation on a 129 genetic background demonstrate consistent behavioral deficits and pathological markers of Alzheimer's disease," *Brain Res*, vol. 1311, pp. 136-147, 2010.



(a)



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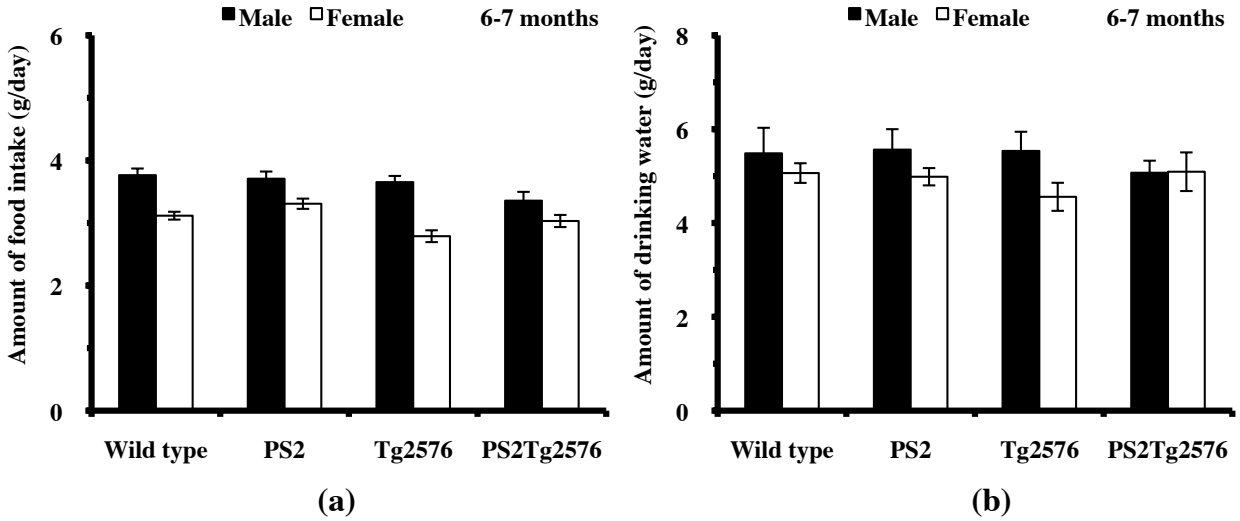
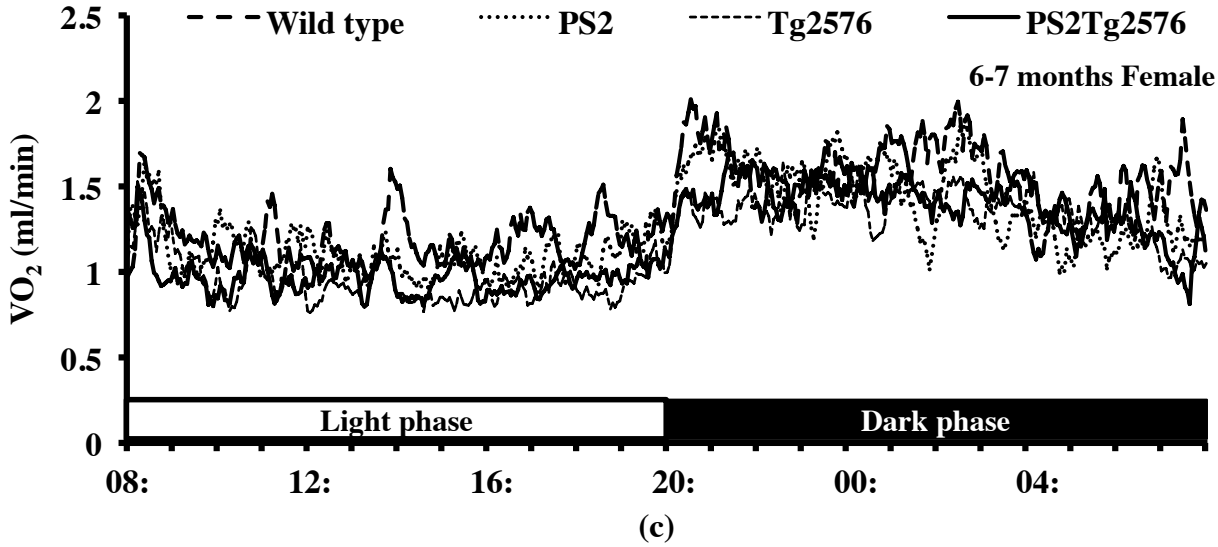
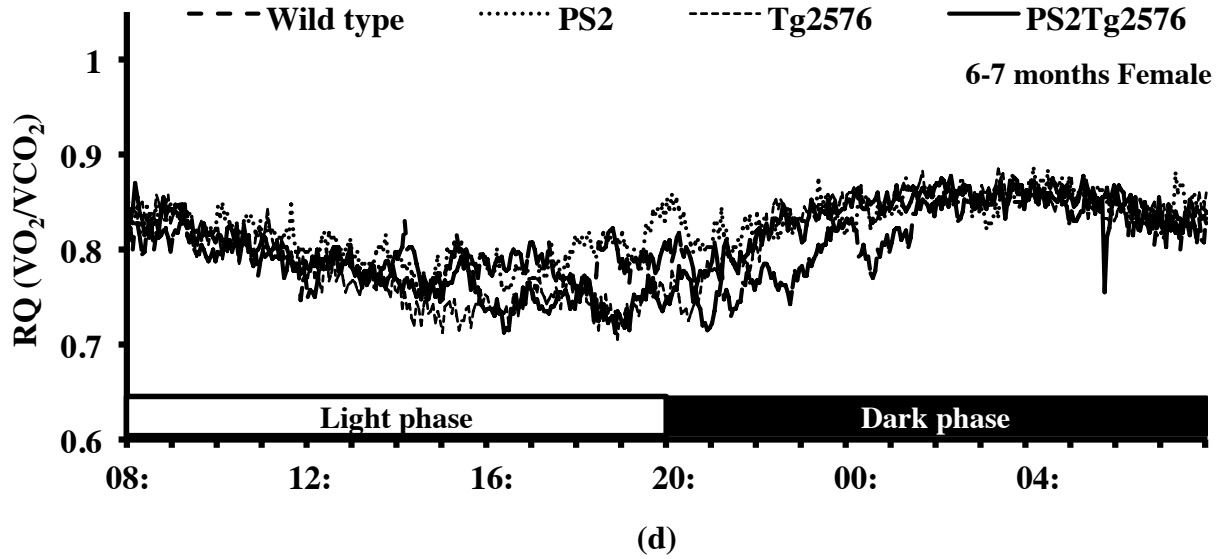
Supplementary Figure 1: **T. Toda *et al.***

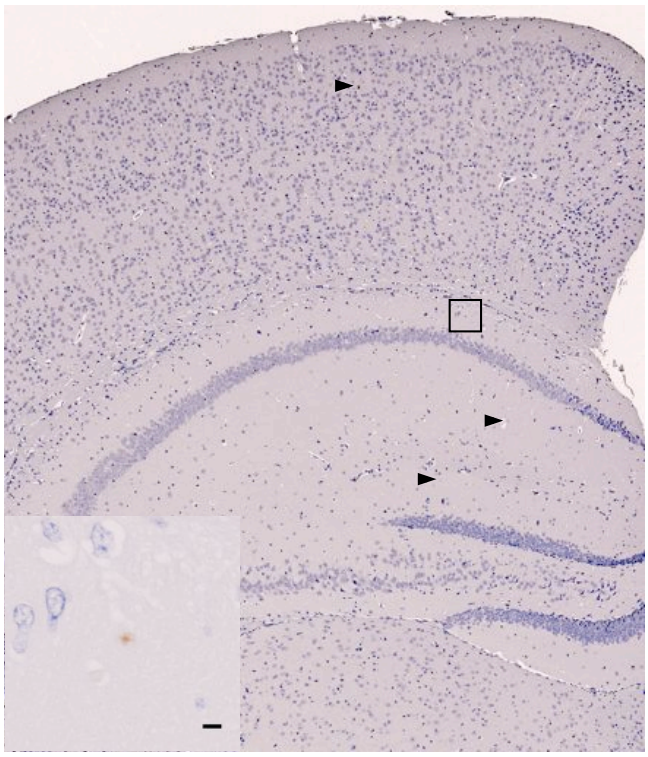






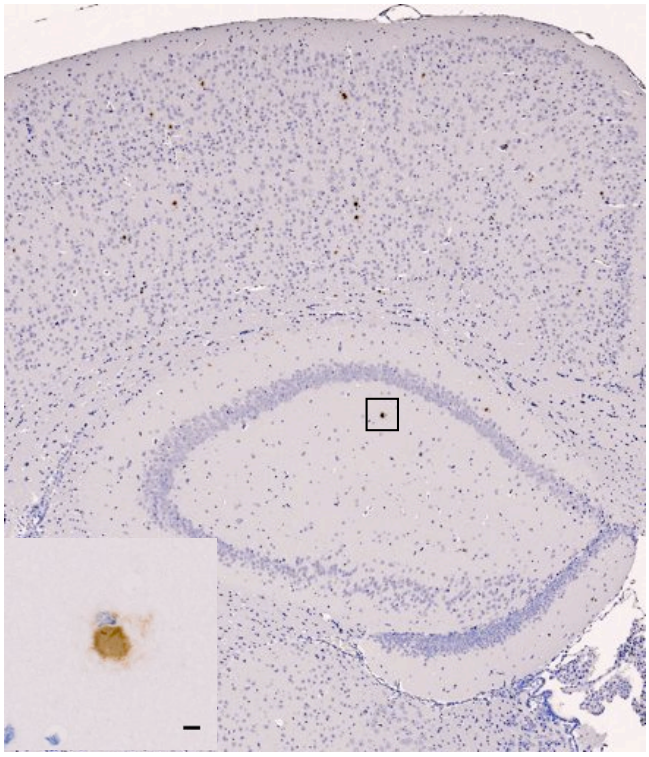
Supplementary Figure 2: **T. Toda *et al.***

2-3 months
PS2Tg2576



(a)

4-5 months
PS2Tg2576



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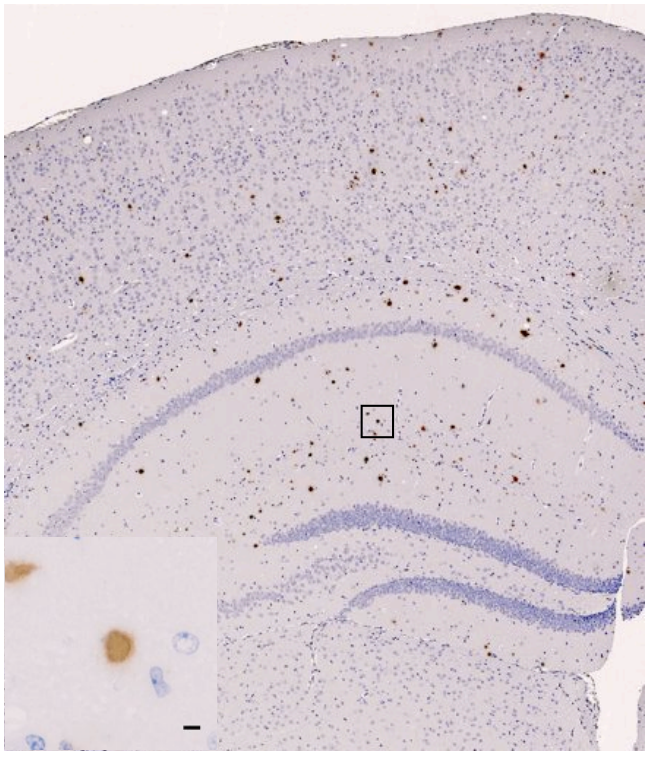
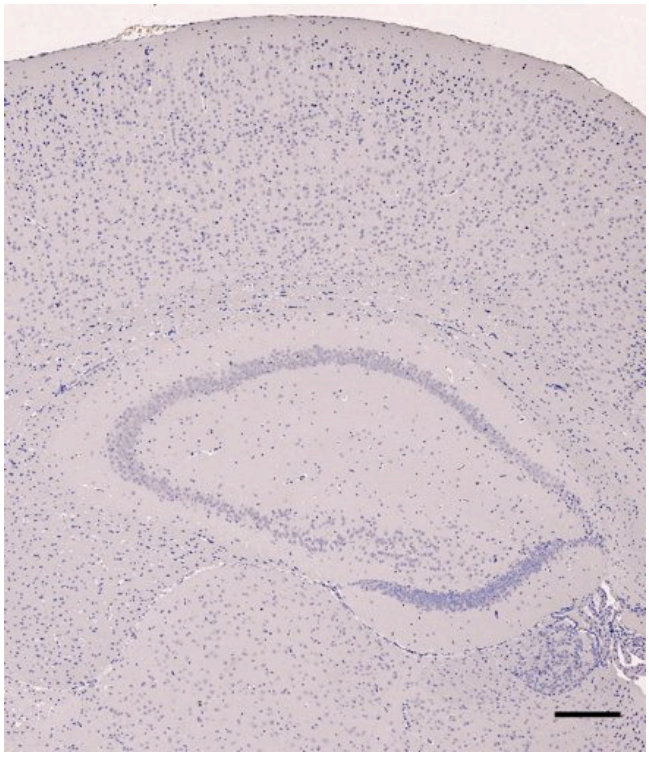
6-7 months
PS2Tg2576



(c)

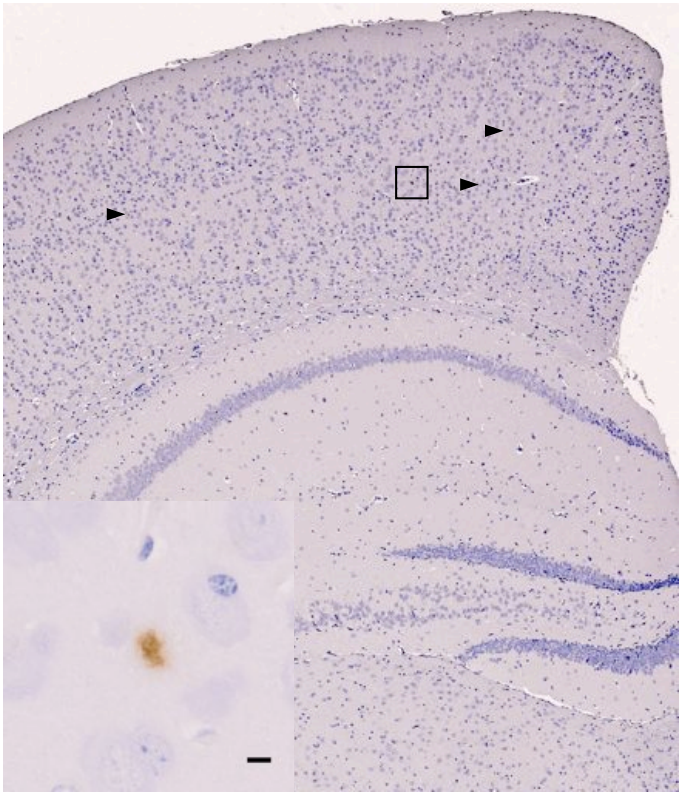
6-7 months
Tg2576



(d)

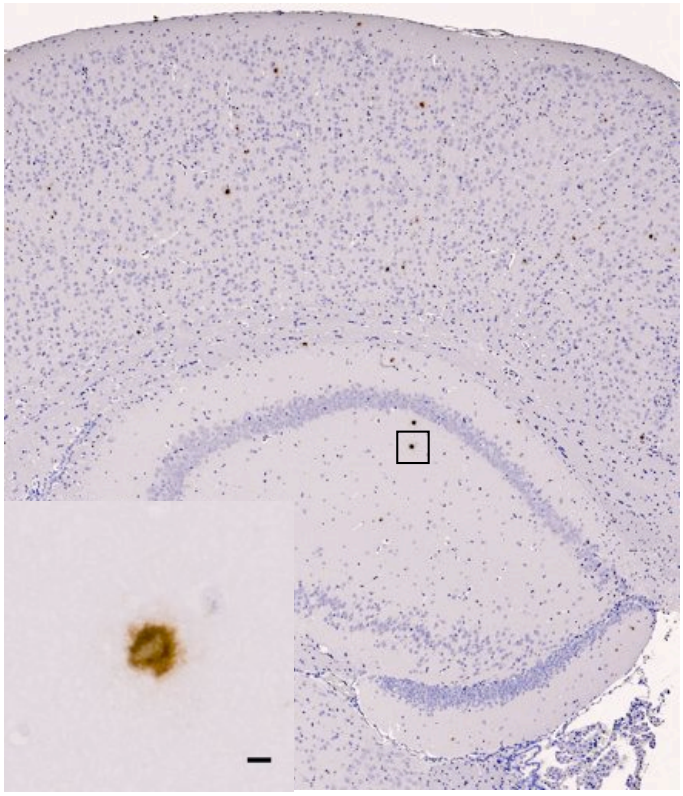
Supplementary Figure 3: **T. Toda *et al.***

2-3 months
PS2Tg2576



(a)

4-5 months
PS2Tg2576



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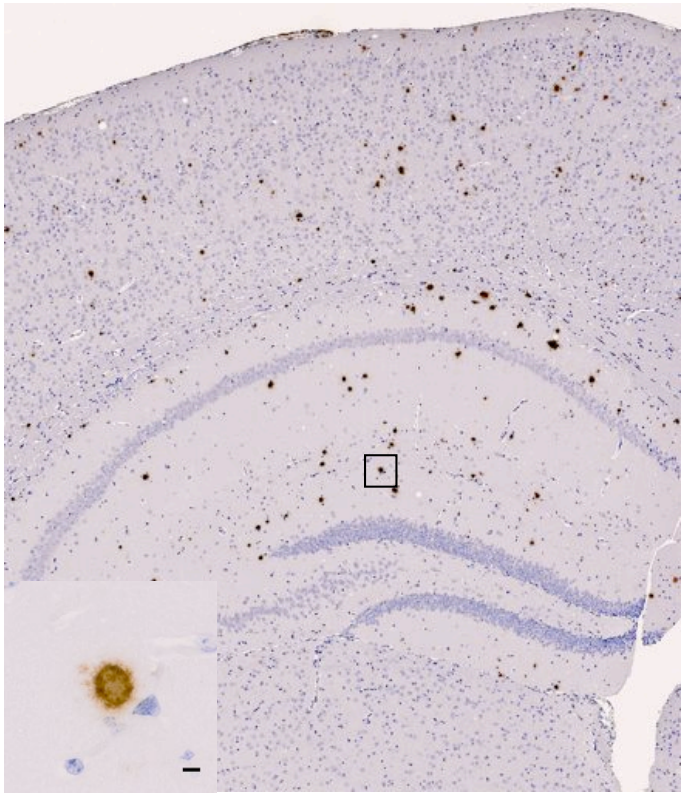
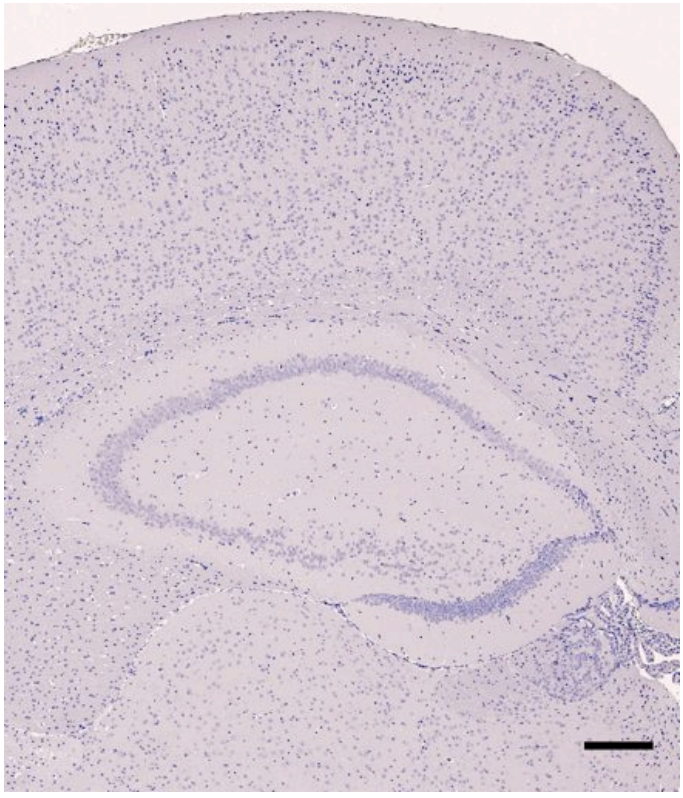
6-7 months
PS2Tg2576



(c)

6-7 months
Tg2576



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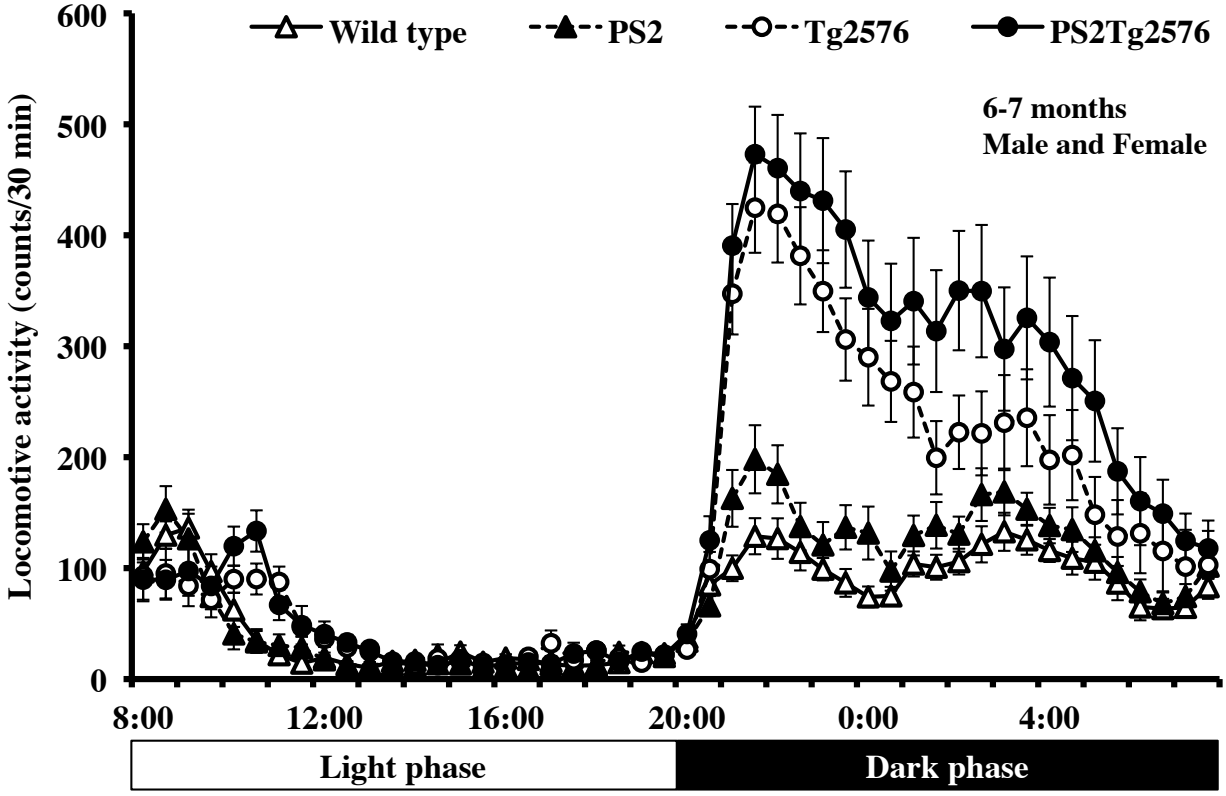

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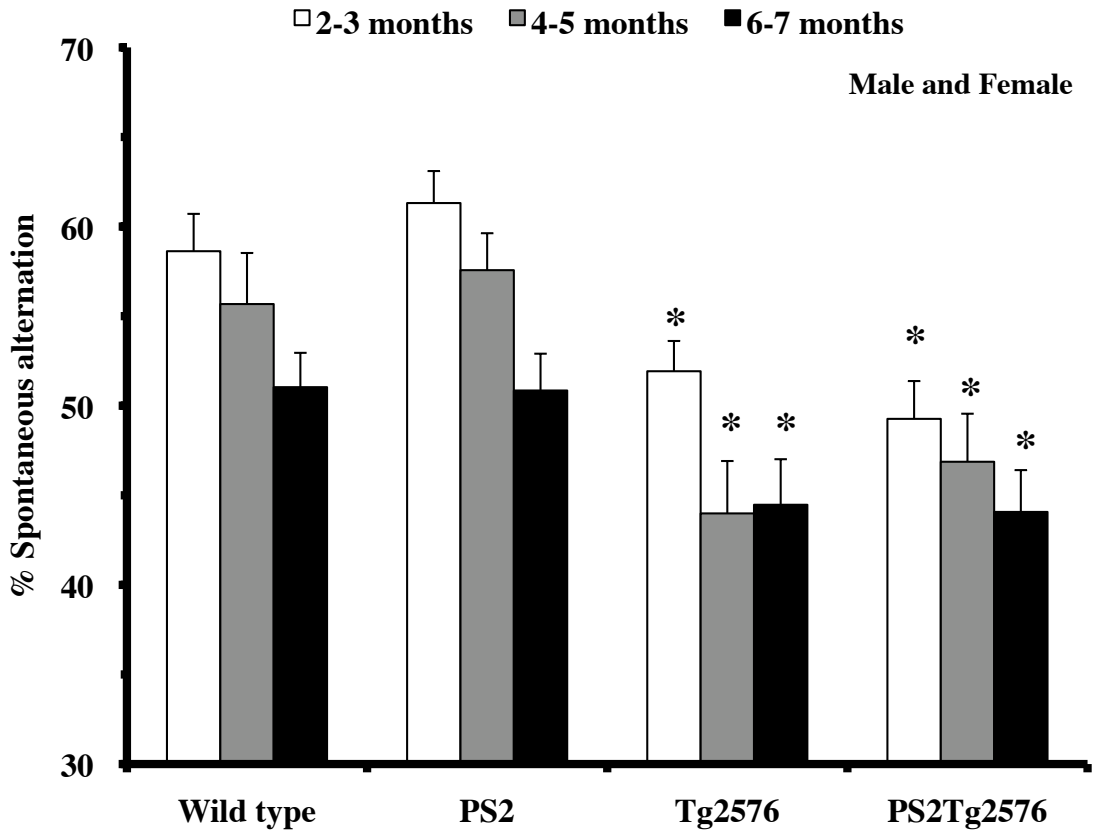

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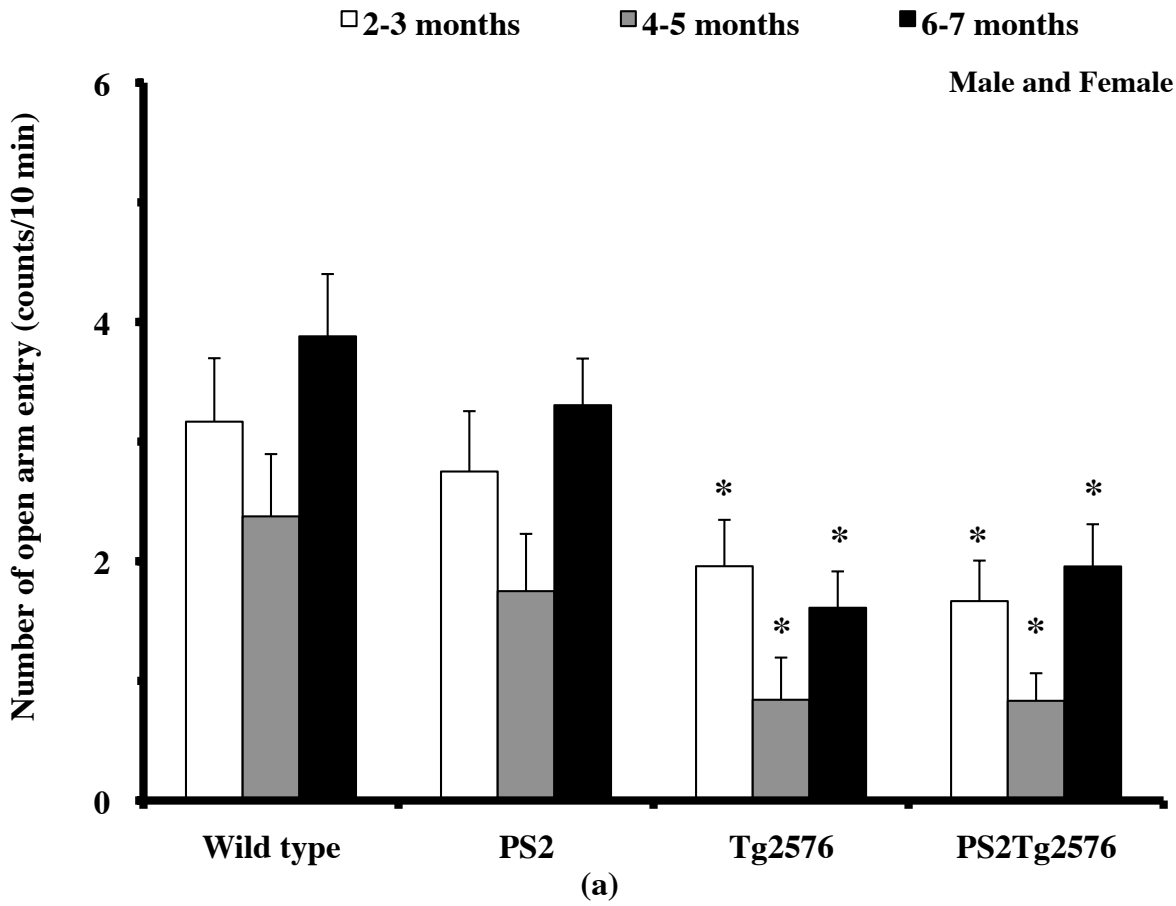
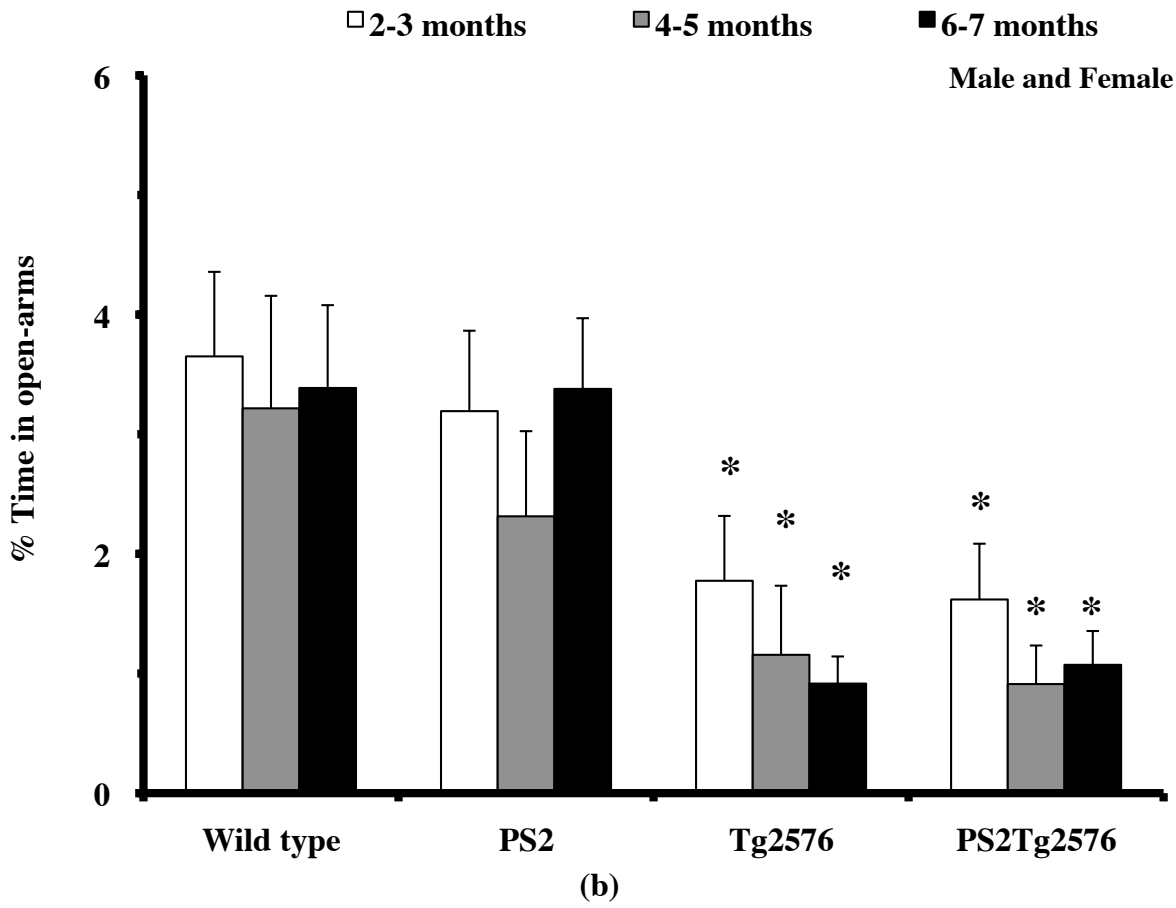
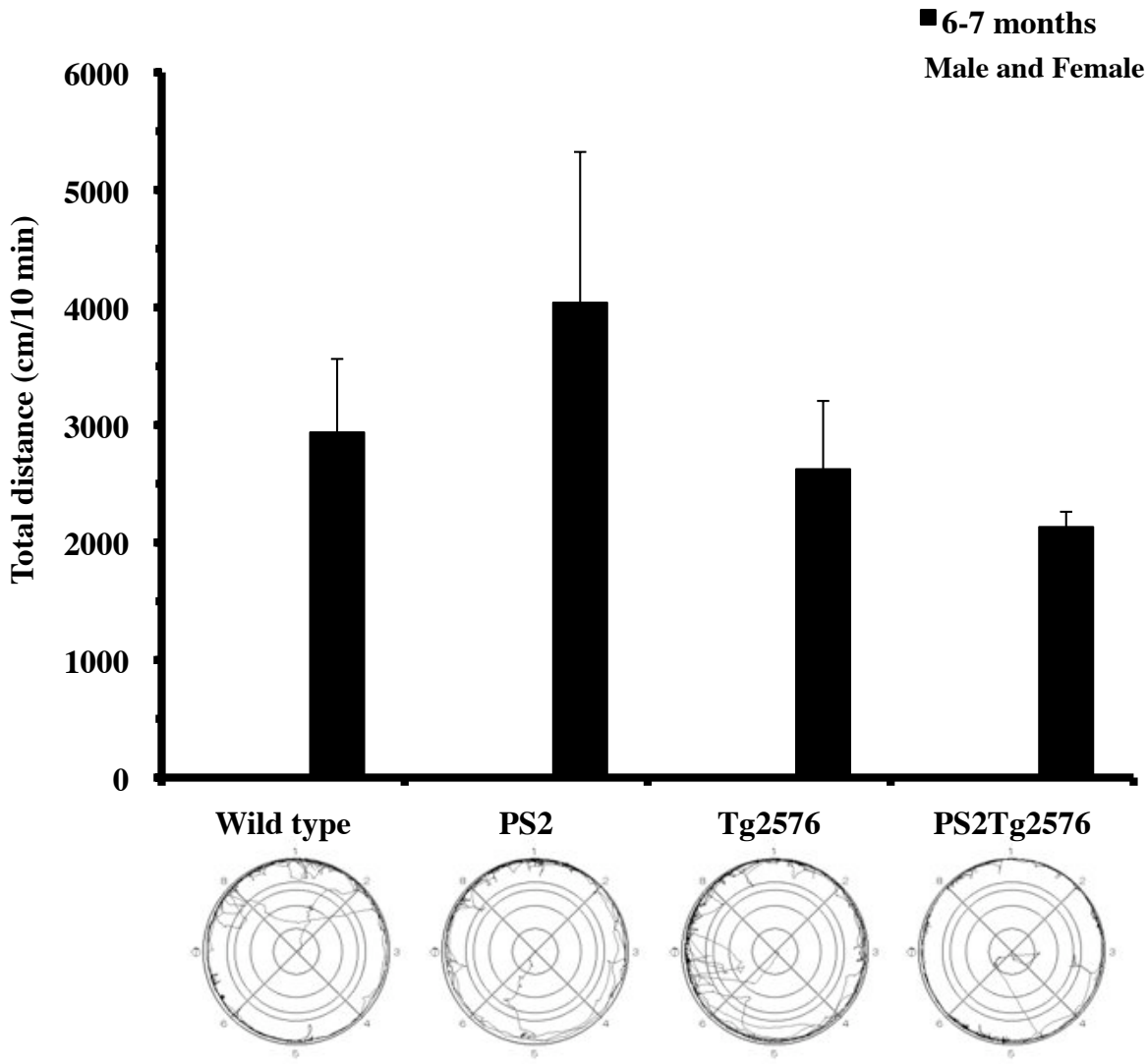




Supplementary Figure 7: T. Toda *et al.*



Supplementary Figure 8: **T. Toda *et al.***