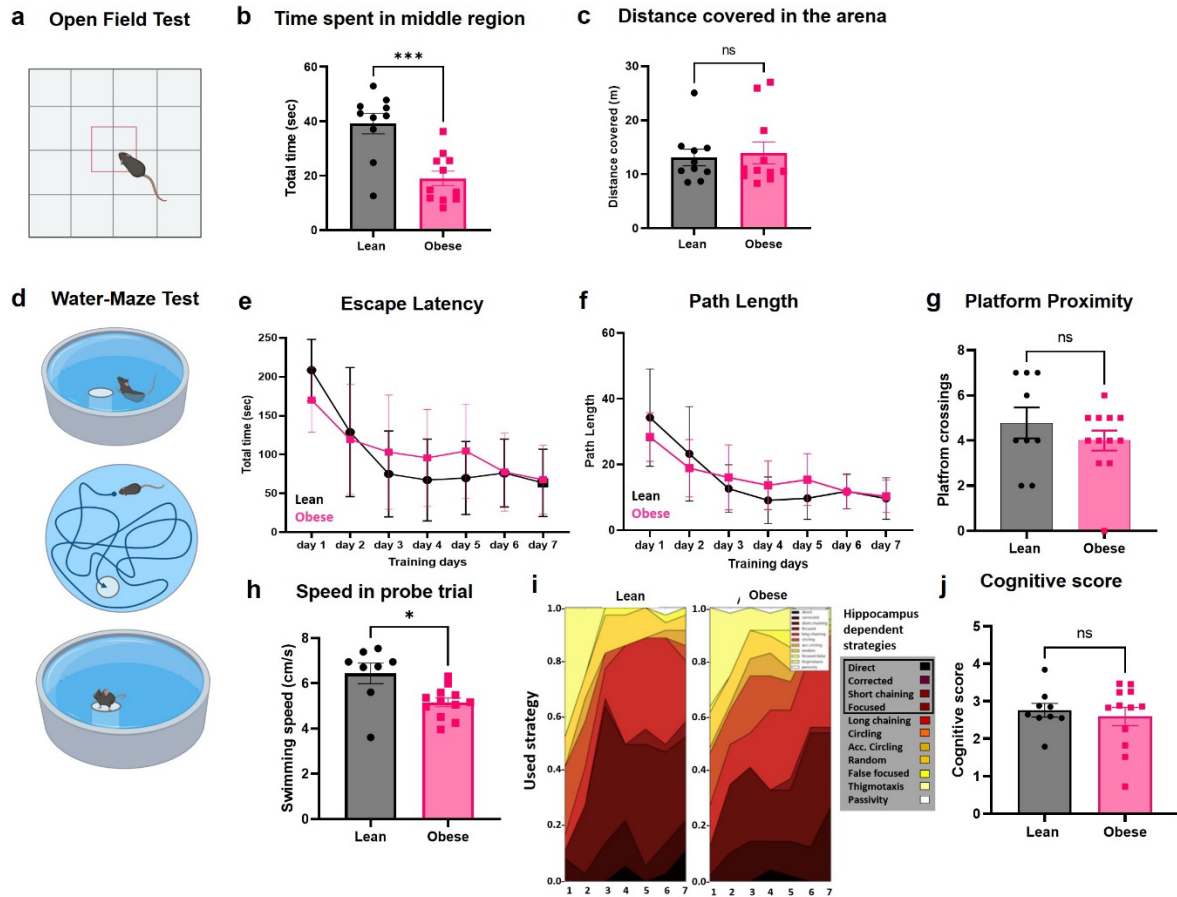# Supplementary Results

6-month old mice
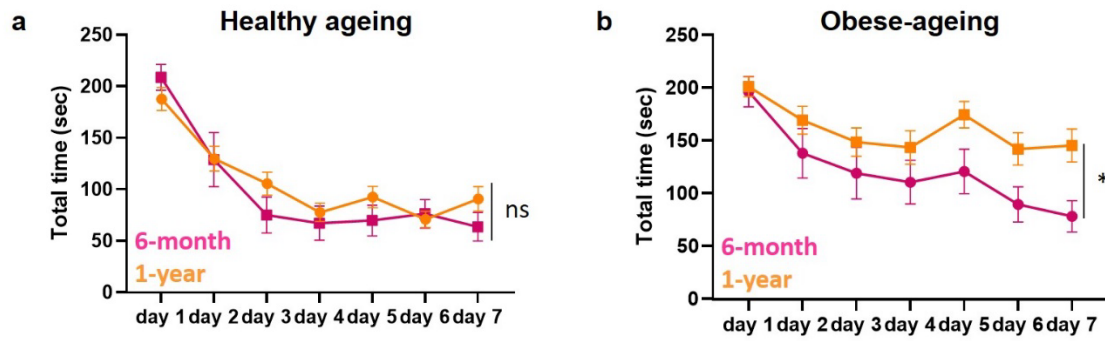

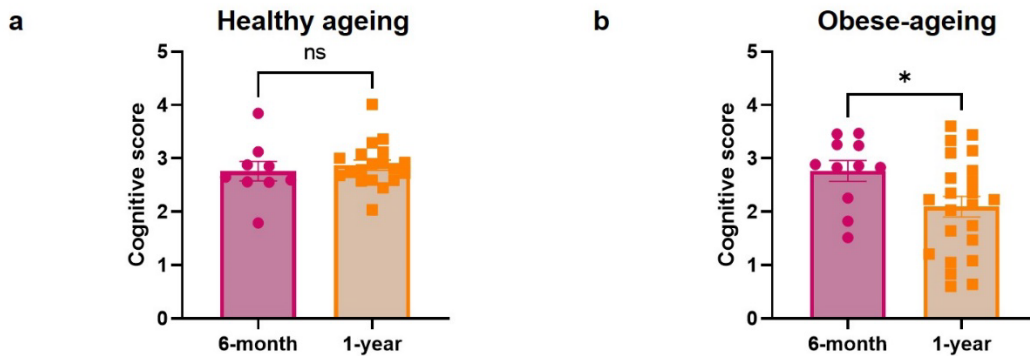***Suppl. Figure 1. Analysis of activity of 6-months-old lean and obese mice in an open field***. *(a)Schematic representation of open field test arena (b). Total time spent by 6-months-old lean and obese mice in the center of the arena. The average time spent in the center by wildtype mice was significantly more as compared to obese mice, which leads to the interpretation that lean mice were more willing to explore. (c). The average distance covered by the two groups of mice was similar.* **Morris Water-maze test in 6-month old mice**. *(d). Schematic representation of Morris water-maze test arena. (e). Escape latency during the water-maze training days comparing 6-month old lean and obese mice. The time taken to find the platform significantly reduced over the training days for lean and obese mice. The escape latency of*

lean and obese mice each day was almost similar. (f). The path length covered to find the platform each day was significantly reduced over the training days, however, the distance covered by the lean and obese mice was similar over the days. (g). Number of visits to platform quadrant during the probe test were similar for lean and obese mice. (h) Swimming speed during the probe test. (i). Analysis of different strategies used for searching the platform by the two groups. Lean and obese mice notably used more of hippocampus dependent strategies over the training days indicating better learning and memory for both groups. (j). Cognitive scores are computed on the basis of (scores assigned to each) of hippocampus dependent strategies used during the training days to navigate toward the platform. The graph indicates that the cognitive abilities of lean and obese mice were equally good. Lean mice, n= 10 and obese mice, n=12. Welch's T test and one-way ANOVA were applied to the data and represented as mean with SEM. Whereas, Brown-Forsythe ANOVA test was applied to data sets shown in (e) and (f) and the graphical representations show mean with SEM. 1a and 1d were created with BioRender.com
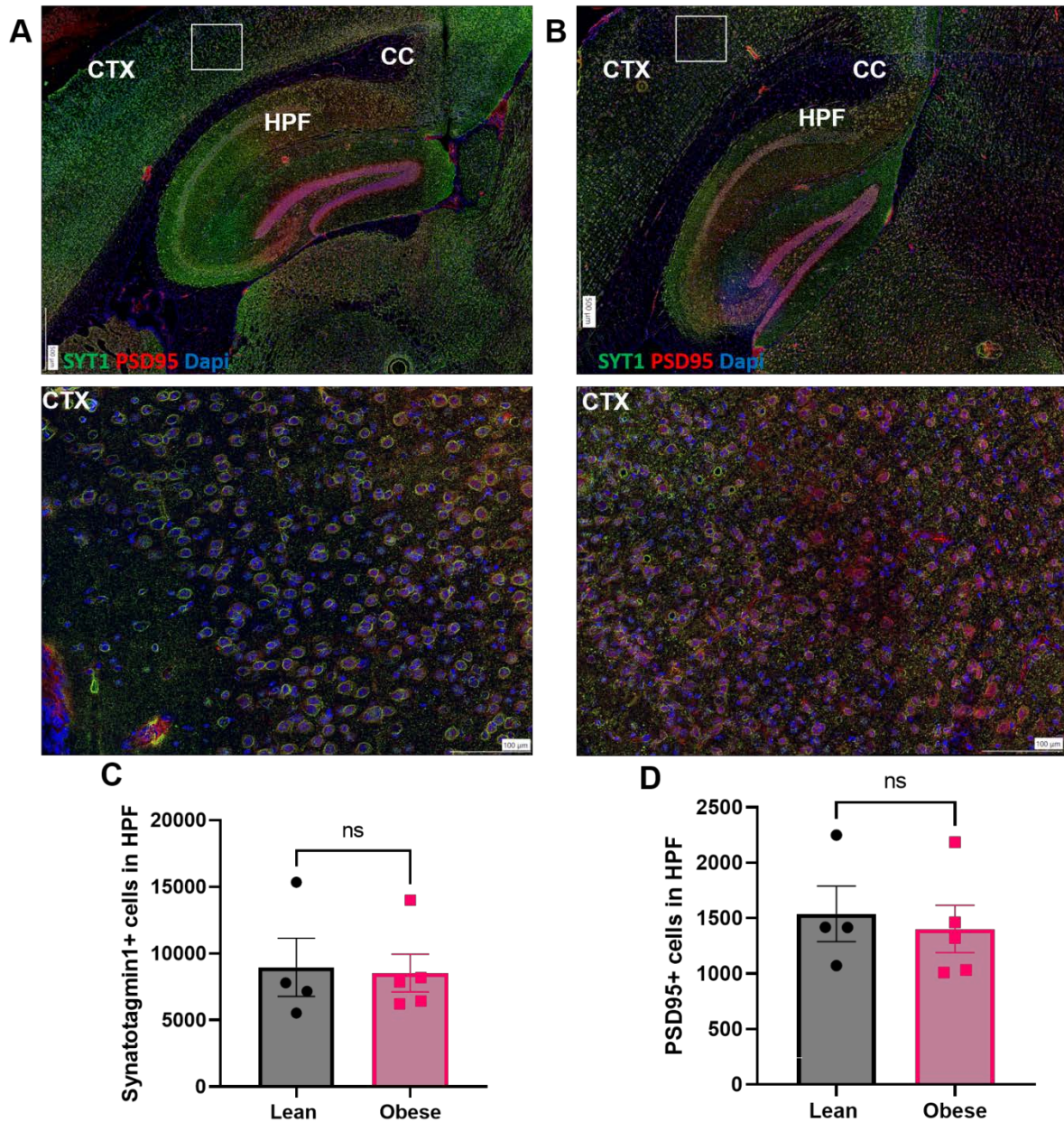
A. Escape Latency during Water-maze test

B. Cognitive scores based on strategies used during Water-maze test

Suppl. Figure 2. (A) escape latencies of (a) lean mice at the age of 6 months compared to 1 year was statistically not significant while (b) escape latencies of 6-month-old obese mice was significantly different from 1-year-old obese mice. Brown-Forsythe ANOVA test was applied to these data sets and the graphical representations show mean with SEM. (B) cognitive scores of (a) 6-month-old lean mice versus 1-year-old lean mice were similar while (b) cognitive scores of 6-month-old obese mice were significantly higher than that of 1-year-old obese mice. One-way ANOVA was applied to these data sets and the graphical representations show mean with SEM.

Suppl. Figure 3. Immunofluorescence staining *on brain cryo-sections with* ***Synaptotagmin*** ***1*** *and* ***PSD95*** *in the 1-year-old mice****.*** *(A). Lean mouse brain showing double-immunofluorescence staining with Synaptotagmin 1 and PSD95,* <mark>*scale bar- 500µm at magnification of 10X. Inlet showing a magnified image of cortical region, scale bar- 100µm at magnification of 40X.*</mark> *(B). Obese mouse brain showing double-immunofluorescence staining with Synaptotagmin 1 and PSD95,* <mark>*scale bar- 500µm at magnification of 10X. Inlet showing a magnified image of cortical region, scale bar- 100µm at magnification of 40X.*</mark> *(C). Graph*

representing Synaptotagmin 1 positive cells in the cortex of lean and obese mice. (D). Graph representing PSD95 positive cells in the cortex of lean and obese mice. <mark>Lean and obese, n=5; at least 2 sections per brain were analyzed.</mark>

CTX- Cerebral cortex; CC- Corpus callosum; HPF- Hippocampal formation.

Quantification of expression of candidate genes

Genes	Hippocampus		Cortex	
	1-year	2-years	1-year	2-years
Spp1	↑ *	↑ *	N.S.	↑ **
Hbb-bs	↑ *	↑ *	N.S.	↑ **
Ptgds	↑ *	↑ *	↓ ***	N.S.
Fmod	↑ *	N.S.	↓ *	N.S.
Prg4	N.S.	N.S.	N.S.	N.S.

Suppl. Figure 4. *Quantification by RT-PCR of relative gene expression of candidate genes in cohorts of obese and lean mice. The relative expression was tested in hippocampus and cortex samples. The table shows changes observed in obese mice. Spp1, Secretory phosphoprotein 1 (Spp1 = osteopontin); Hbb-bs, hemoglobin beta-s adult chain; Ptgds, prostaglandin D2 synthase; Fmod, fibromodulin; Prg4, proteoglycan 4.*