

Targeting angiotensin like-2 positive senescent cells improves cognitive impairment in adult male but not female atherosclerotic LDLR^{-/-};hApoB₁₀₀^{+/+} mice

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Supplemental data

Fig. S1: Impact of sh-*angptl2* on body weight over the 3 months of treatment.

Fig. S2: Cognition in the MWM test at 6-month of age, before the treatment was started.

Fig. S3: Impact of sh-*angptl2* treatment on atherosclerotic lesion, blood flow index, carotid compliance and cerebral endothelial dilatory function.

Fig. S4: Impact of sh-*angptl2* treatment on cognition in the MWM test in mice selected for single-nuclei RNA sequencing analysis.

Table S1: Sequence of sh-*angptl2* or sh-SCR

Table S2: Sequence of primers for AAV1-pU6-ITR-sh (BGH-poly A) titration

Table S3: List of primers used for real-time quantitative PCR

Table S4: Impact of sh-*angptl2* or sh-SCR treatment on blood markers

Table S5: Proportion (%) of the identified cell types in male and female hippocampal region

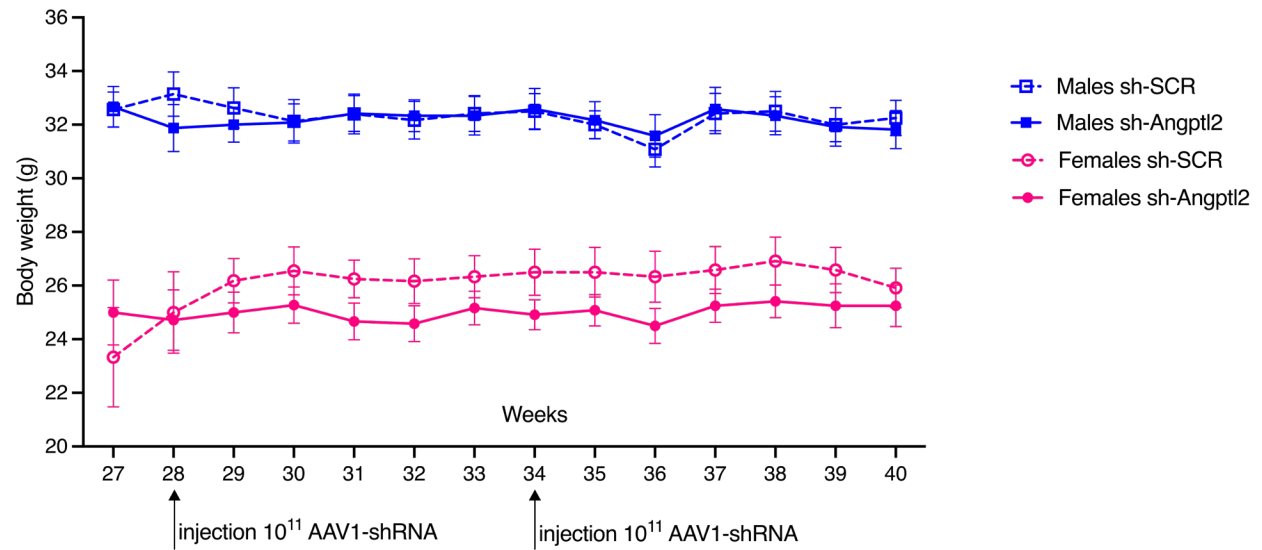


Figure Supplemental 1: Impact of *sh-angptl2* on body weight over the 3 months of treatment. Data are expressed as mean $\pm$ SEM of n mice (n=12 male sh-SCR, n=12 male sh-*angptl2*, n=13 female sh-SCR and n=11 female sh-*angptl2* mice).

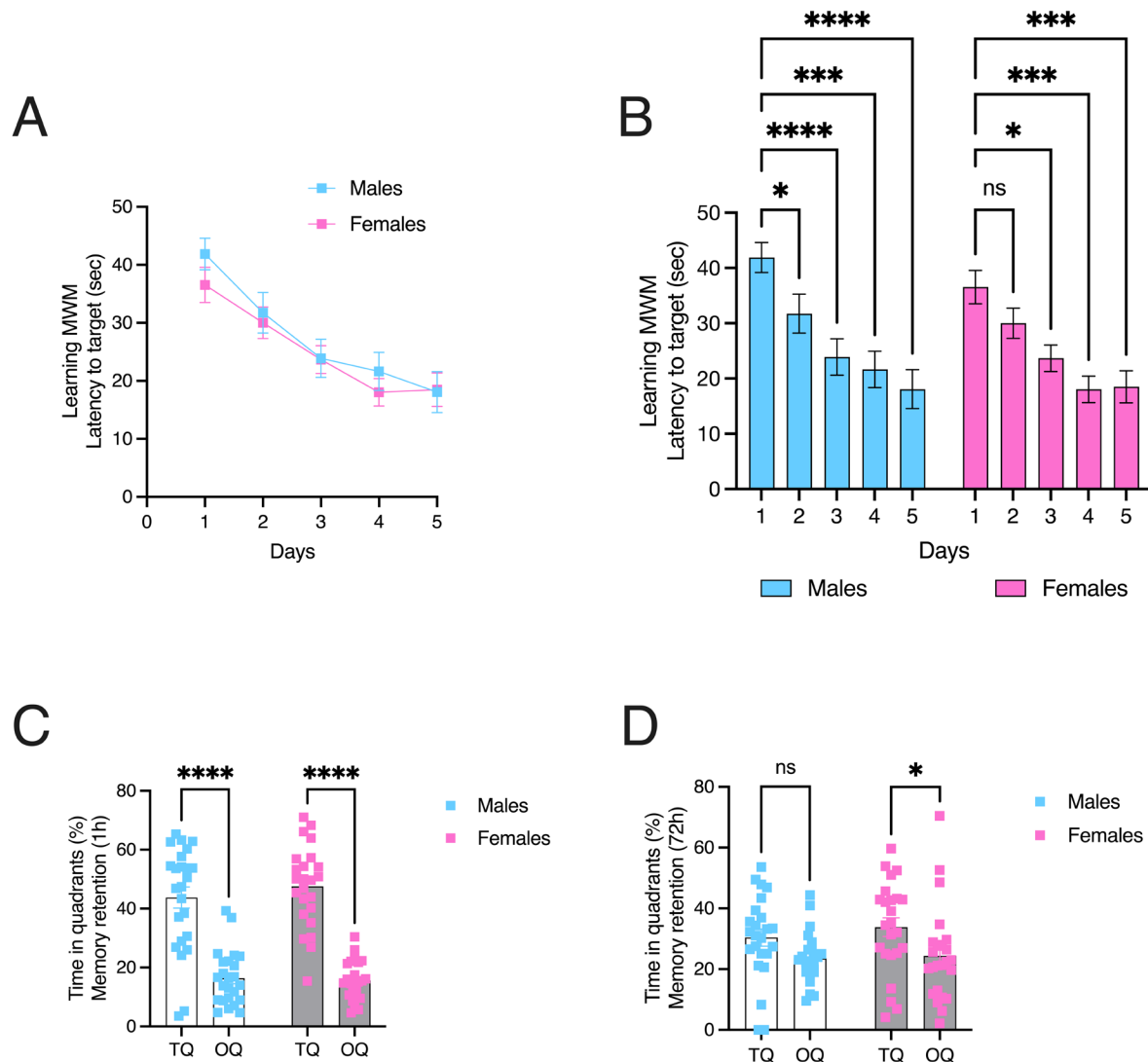


Figure Supplemental 2: Cognition in the MWM test at 6-month of age, before the treatment was started (A-B) Latency to find the hidden platform from day 1 to day 5 in the acquisition phase (learning memory) of the Morris water maze (MWM) test of male and female ATX mice. Each value is a mean of 4 trials performed by each mouse, per day of learning. Latency to target was also analyzed to determine in each group at what day the mice started to remember the localization of the hidden platform in male and female mice: * $p < 0.05$ vs. Day1, Two-way ANOVA with repeated measures (Days x Treatment) and Dunnett's multiple comparisons test. **(C-D)** Percentage of time spent in the target quadrant (TQ, quadrant in which the platform was hidden) and the opposite quadrant (OQ) by male and female mice during the probe test. This probe test was performed on day 5, 1h after the last acquisition of the learning phase (short-term memory retention, **C**) and 72h after the end of the learning phase (delayed memory retention, **D**). *: $p < 0.05$

vs. TQ, Two-way ANOVA (Sex x Treatment) and Sidak's multiple comparisons test. N=24 male and n=24 female mice. Data are expressed as mean $\pm$ SEM of n mice. *: $p<0.05$; ****: $p<0.0001$.

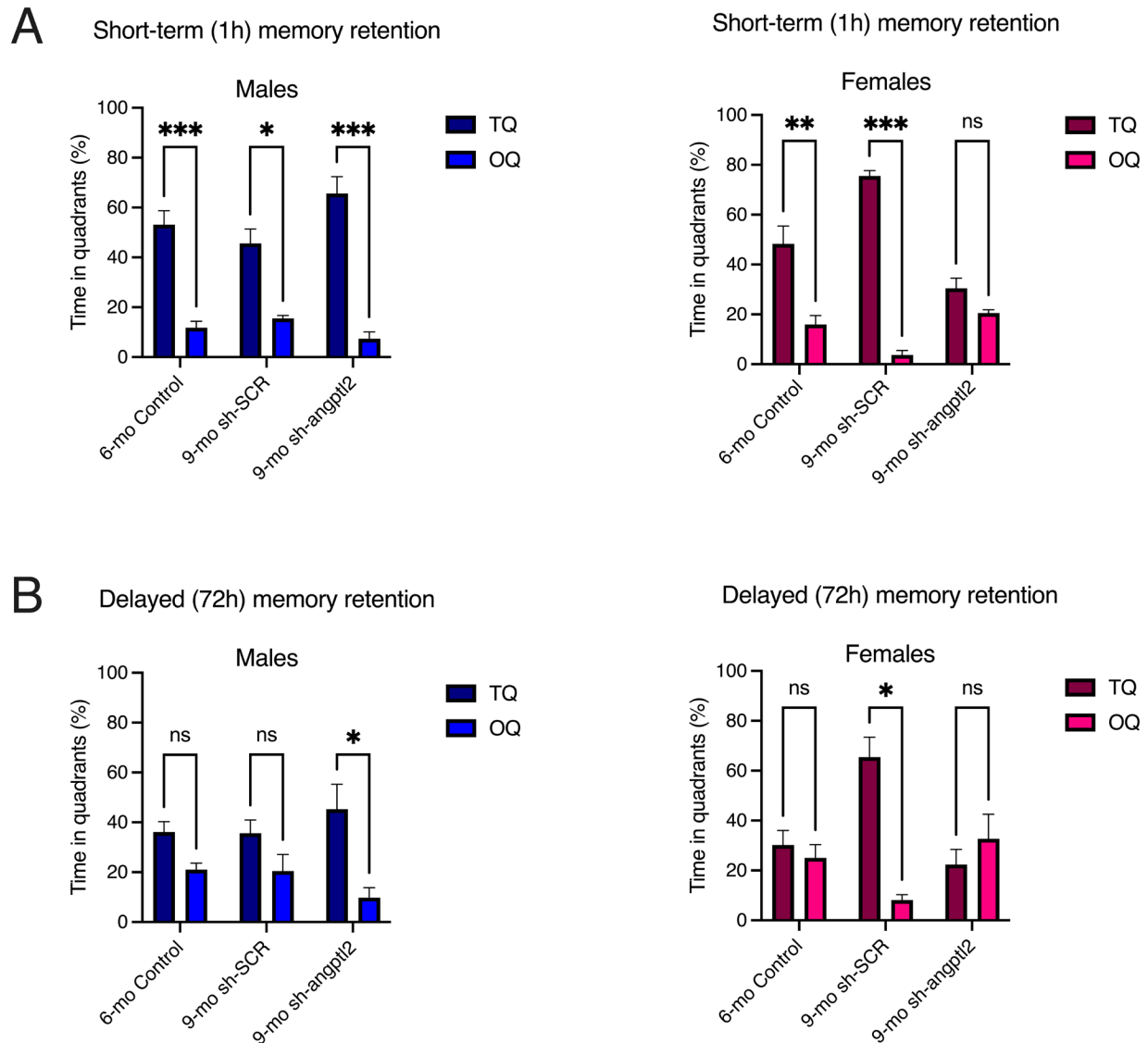


Figure Supplemental 3: Impact of *sh-angptl2* treatment on cognition in the MWM test in mice selected for single-nuclei RNA sequencing analysis. Percentage of time spent in the target quadrant (TQ, quadrant in which the platform was hidden) and the opposite quadrant (OQ) by male and female mice after treatment with sh-SCR or sh-*angptl2* during the probe tests. These probe tests were performed on day 5, 1h after the last acquisition of the learning phase (short-term memory retention, **A**) and 72h after the end of the learning phase (delayed memory retention, **B**). *: $p < 0.05$ vs. TQ. Two-way ANOVA (Sex x Treatment) and Sidak's multiple comparisons test. N=6 mice at 6-month of age and n=3 at 9-month of age, per group. Data are expressed as mean $\pm$ SEM of n mice. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table S1: Sequence of sh-*angptl2* or sh-SCR

sh- <i>angptl2</i>	GCAGAGTCTTCCAATCAGTTAATCAAGAGTTAACTGATTGGAAGACTCTGC
sh- SCR	CCTAAGGTAAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG

Table S2 : Sequence of primers for AAV1-pU6-ITR-sh (BGH-poly A) titration

BGH-Forward	5'TGCCTTCCTTGACCCT
BGH-Reverse	5'CCTTGCTGTCCTGCCC

Table S3: List of primers used for real-time quantitative PCR

Target gene	Primers	Sequences
<i>Angptl2</i>	Forward	GATCCAGAGTGACCAGAATC
	Reverse	TCTCAGGCTTCACCAGGTAG
<i>CycloA</i>	Forward	CCGATGACGAGCCCTTGG
	Reverse	GCCGCCAGTGCCATTATG
<i>HPRT</i>	Forward	GGTTAAGCAGTACAGCCCCA
	Reverse	GGCCTGTATCCAACACTTCG
<i>BM2</i>	Forward	TCTCACTGACCGGCCTGTAT
	Reverse	GATTTCAATGTGAGGCGGGTG
<i>PPIA</i>	Forward	CCACCGTGTTCTTCGACATC
	Reverse	CTGGCACATGAATCCTGGAA

Table S4: Impact of sh-*angptl2* or sh-SCR treatment on blood markers

	Males- sh-SCR	Males- sh-<i>angptl2</i>	Females- sh-SCR	Females- sh-<i>angptl2</i>
Glucose (mmol/L)	12.7±1.3 (8)	13.4±1.1 (6)	11.9±1.0 (8)	10.9±0.6 (6)
Cholesterol (mmol/L)	27.6±1.0 (8)	26.0±1.6 (7)	27.2±1.4 (8)	30.7±0.9 (7)
Triglycerides (mmol/L)	8.6±0.5 (8)	7.5±0.8 (7)	5.4±0.3 (8) #	5.4±0.3 (7)
Urea (mmol/L)	9.0±1.1 (8)	6.3±0.3 (7)	7.5±0.4 (8)	6.7±0.6 (7)
AST (U/L)	269.4±63.2 (8)	340.8±163.3 (6)	176.9±14.3 (8)	207.9±22.1 (7)
Phosphatase alkaline (U/L)	70.0±13.0 (5)	58.8±1.3 (4)	91.9±7.1 (8)	95.7±4.6 (7)

Data are mean ± SEM of (n) mice; 2-way ANOVA, (Sex x Treatment) and Tukey's multiple comparisons test. #: p<0.05 vs. Males (within the same treatment).

Table S5: Proportion (%) of the identified cell types in male and female hippocampal region

	Male % sh-SCR	Male sh-<i>angptl2</i>	Female sh-SCR	Female sh-<i>angptl2</i>
Neurons	58.12	74.35	54.06	56.95
Oligodendrocytes	21.98	10.07	24.73	23.22
Astrocytes	11.3	8.38	12.70	10.76
OPCs	3.16	2.77	3.13	2.48
ECs	2.16	1.39	1.73	2.75
Immune cells	1.78	0.96	1.95	2.41
Epithelial cells	1.49	2.10	1.71	1.43

Data are average proportion of cells from n=3 mice (hippocampi) per group.