

Supplementary Material

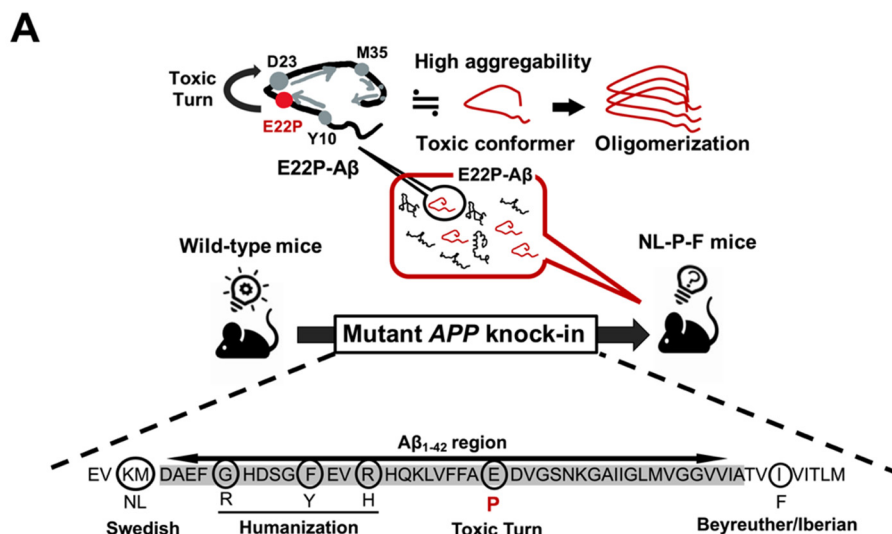
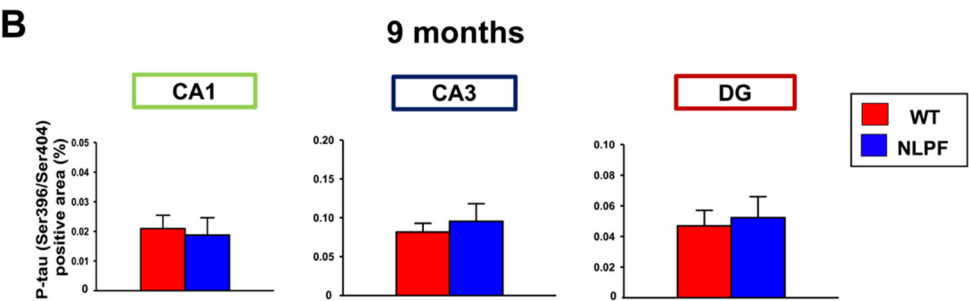
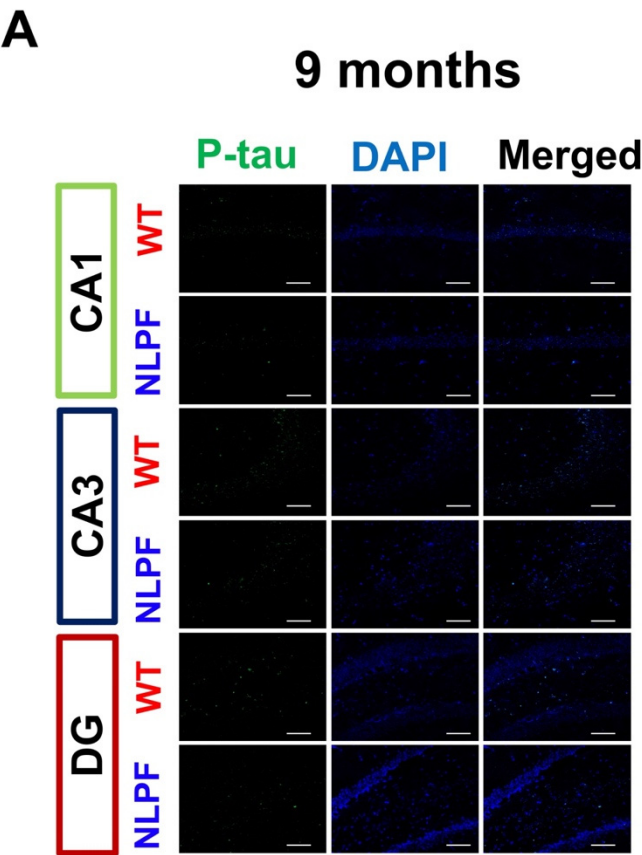


Figure S1. To construct the APP knock-in mice, the sequence for Aβ and the surrounding mutation sites related to gene mutation of familial AD were substituted. Swedish double mutations (K670N substitution and M671L substitution), Beyreuther/Iberian (I716F substitution) and Toxic Turn (E693P substitution) mutations were introduced. Furthermore, the Aβ sequence was humanized by introducing mutations leading to the substitutions G676R, F681Y, and R684H. E22P-Aβ has an intramolecular turn at positions 22–23, forming a toxic conformer. Toxic conformers are highly aggregative and promote Aβ oligomerization. Under physiological conditions, *APP^{NL-P-F/NL-P-F}* (NL-P-F) mice exhibit phenotypes characterized by toxic conformers with production of E22P-Aβ. (A) NL-P-F mice are generated by knock-in technique of the mutant *APP* gene and produce Aβ oligomers by toxic conformers.



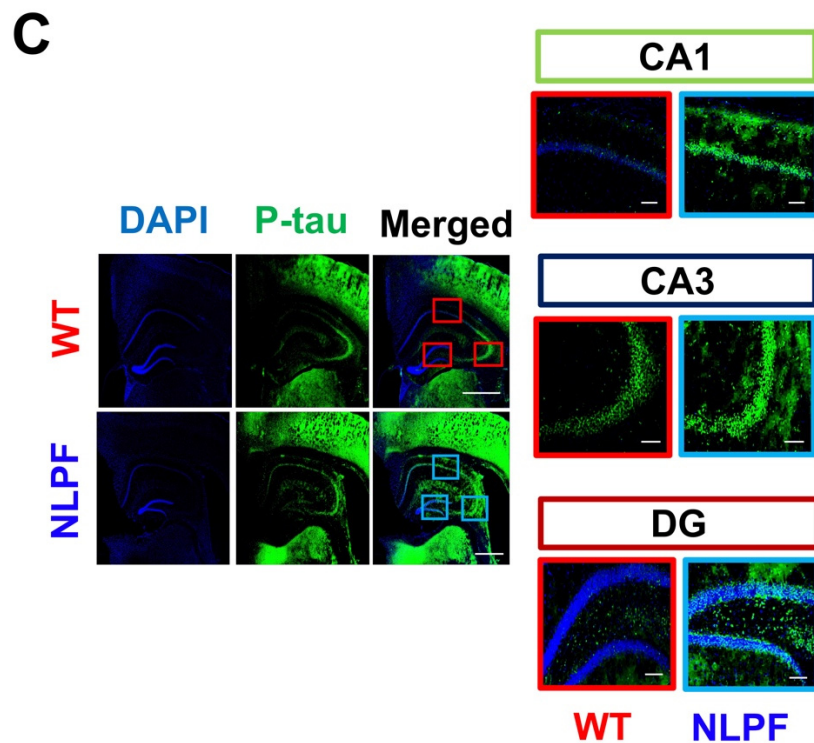


Figure S2. The phosphorylated tau-positive area in the hippocampal CA1, CA3, and DG (A–B) at nine months and (C) twelve months in wild-type (WT) and NL-P-F (NLPF) mice. (A) Representative confocal images. Scale bar indicates 50 μ m. (B) The phosphorylated tau-positive area (% of total area). (C) Representative fluorescent images. The scale bar indicates 1 mm (50 μ m in enlarged images) WT and NLPF ($n = 6$). Values indicate the mean \pm SEM.

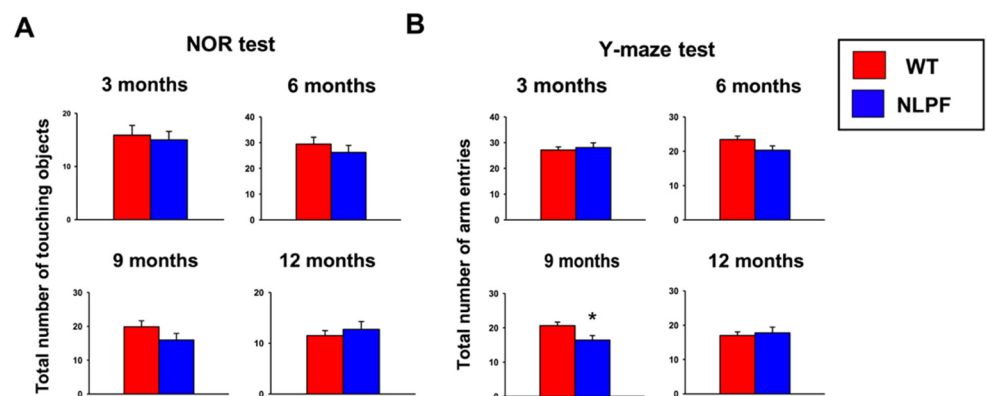


Figure S3. From three to twelve months, the total number of touches in the NOR test and entries in the Y-maze test were almost the same. (A) Total number of times mice touched familiar and novel objects in the NOR test. (B) Total number of times mice entered each arm in the Y-maze test. Values indicate the mean \pm SEM. * $P < 0.05$, compared with age-matched WT, two-way ANOVA post hoc Holm-sidak Tukey's test.

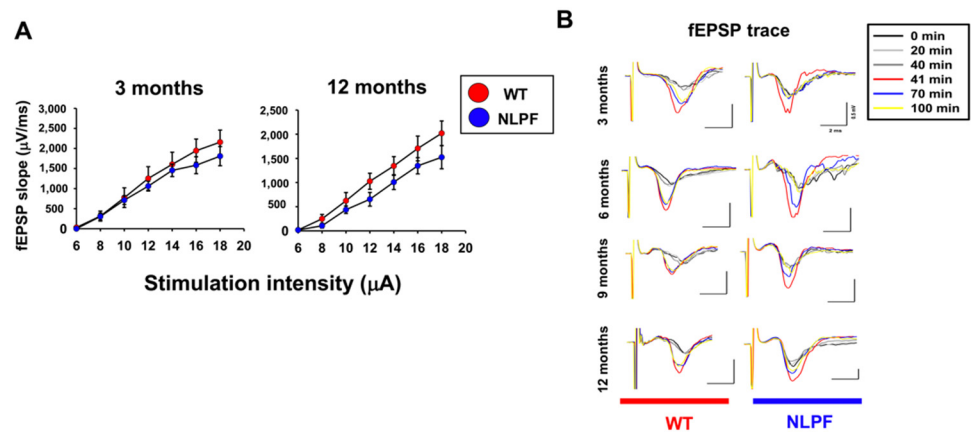


Figure S4. From three to twelve months, Input-output curves were measured at hippocampal CA1 synapses and representative fEPSP trace of LTP measurement are shown. (A) Input-Output curve. WT and NLPF ($n = 9$) at three months, WT ($n = 11$) and NLPF ($n = 10$) at twelve months. (B) Representative fEPSP traces of LTP measurement (black: 0 min, gray: 20 min, dark gray: 40 min, red: 41 min, blue: 70 min, and yellow: 100 min). Values indicate the mean \pm SEM.

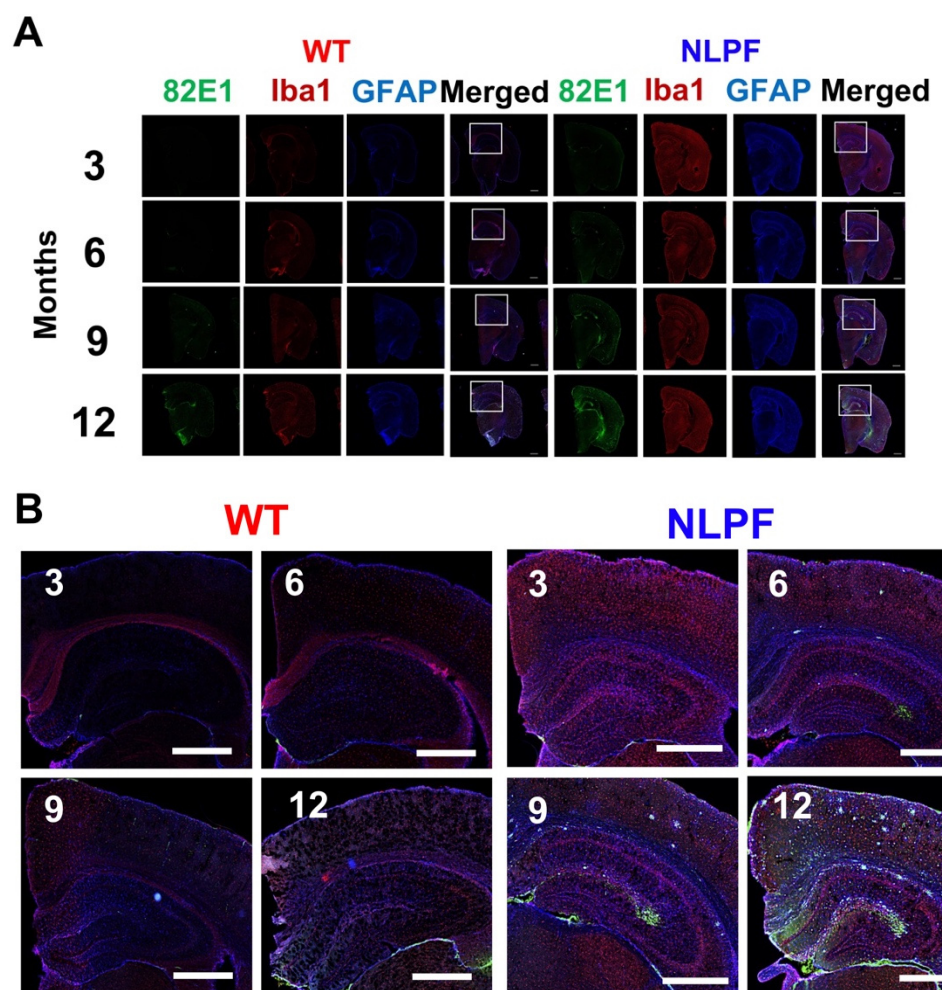


Figure S5. Triple stained images of coronal brain sections including the hippocampal region. Increased Iba1 and GFAP fluorescence was detected in NL-P-F mice from three to twelve months. (A) A β deposition and glial cell activation in NL-P-F mice. Microglia and astrocytes were immunostained with antibodies for Iba1 and GFAP, respectively. A β deposition was detected by

82E1. (B) Enlarged images of the square surrounded by the white line in (A). Scale bar indicates 1 mm.

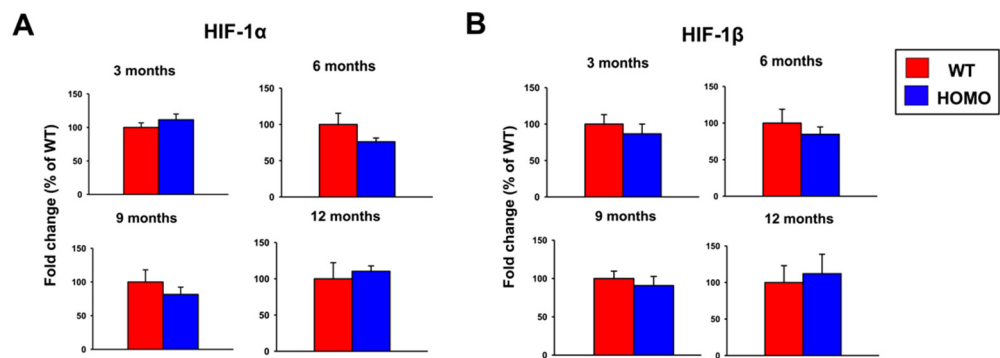


Figure S6. Relative gene expression levels of (A) HIF-1 α and (B) HIF-1 β of hippocampal tissue of NL-P-F mice from three to twelve months. Gene expression levels were not significantly different between wild-type and NL-P-F mice. (A) WT ($n = 5-6$), NLPF ($n = 6$). (B) WT ($n = 5-6$), NLPF ($n = 6$). Values indicate the mean \pm SEM.

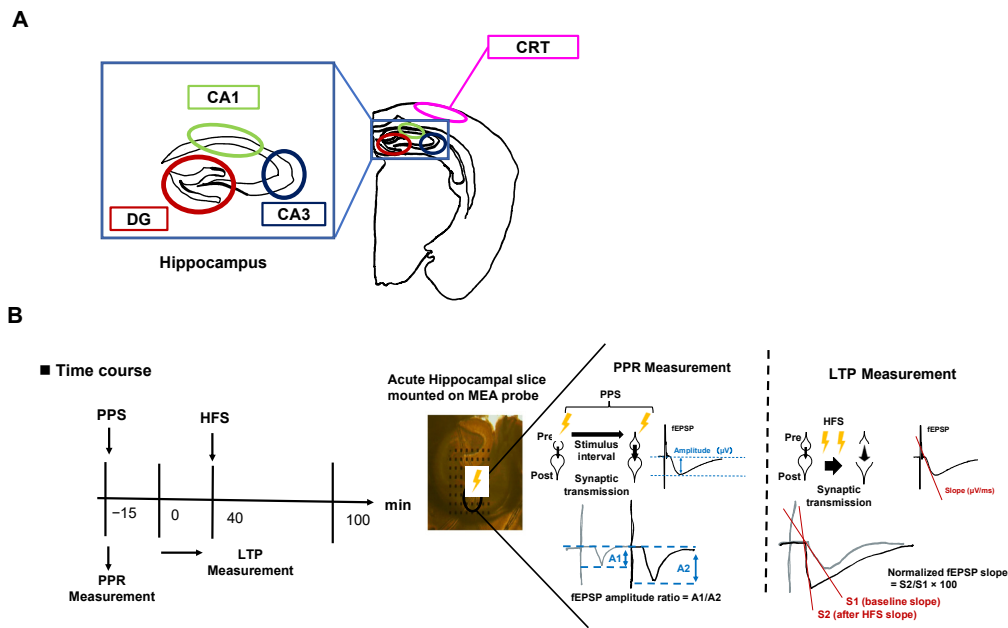


Figure S7. (A) Staining area of coronal sections of brain hemisphere were shown. Hippocampal CA1, CA3, DG, and cortex (CRT) were analyzed by Immunohistochemistry. (B) Time course and method to calculate PPR and LTP of the electrophysiological recording.

Table S1. Detailed information on statistical analysis.

Fig.	F value	P-value	Statistical method
1A	Genotype: (1, 29) = 216.755 Month: (3, 29) = 30.330 Genotype x Month: (3, 29) = 30.469	Genotype: < 0.001 Month: < 0.001 Genotype x Month: < 0.001 NLPF vs. WT 3 months: 0.004 6 months: 0.002 9 months: < 0.001 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
1B	Genotype: (1, 28) = 38.430	Genotype: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test

	Month: (3, 28) = 13.397 Genotype x Month: (3, 28) = 13.395	Month: < 0.001 Genotype x Month: < 0.001 3 months: 0.932 6 months: 0.661 9 months: < 0.001 12 months: < 0.001	
2B-CA1	Genotype: (1, 48) = 37.473 Month: (3, 48) = 18.341 Genotype x Month: (1, 48) = 18.378	Genotype: < 0.001 Month: < 0.001 Genotype x Month: < 0.001 NLPF vs. WT 3 months: 0.982 6 months: 0.647 9 months: 0.014 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
2B-DG	Genotype: (1, 48) = 46.633 Month: (3, 48) = 22.669 Genotype x Month: (3, 48) = 22.843	Genotype: < 0.001 Month: < 0.001 Genotype x Month: < 0.001 NLPF vs. WT 3 months: 0.973 6 months: 0.534 9 months: 0.009 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
2B-CRT	Genotype: (1, 48) = 27.959 Month: (3, 48) = 8.718 Genotype x Month: (3, 48) = 8.243	Genotype: < 0.001 Month: < 0.001 Genotype x Month: < 0.001 NLPF vs. WT 3 months: 0.999 6 months: 0.460 9 months: < 0.001 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
2E		CA1: 0.015 CA3: 0.002 DG: 0.002	Mann-Whitney rank sum test
2H		CA1: 0.835 CA3: 0.615	Mann-Whitney rank sum test
3B	Genotype: (1, 98) = 120.307 Month: (3, 98) = 8.682 Genotype x Month: (3, 98) = 6.288	Genotype: < 0.001 Month: < 0.001 Genotype x Month: < 0.001 NLPF vs. WT 3 months: 0.195 6 months: < 0.001 9 months: < 0.001 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
3C	Genotype: (1, 98) = 61.971 Month: (3, 98) = 2.461 Genotype x Month: (3, 98) =	Genotype: < 0.001 Month: 0.067 Genotype x Month:	2 way ANOVA post hoc Holm-sidak Tukey's test

	2.210	0.092 NLPF vs. WT 3 months: 0.130 6 months: < 0.001 9 months: < 0.001 12 months: < 0.001	
4A-3 months		25 ms: 0.319 50 ms: 0.610 100 ms: 0.584 200 ms: 0.537	Student's t-test
4A-6 months		25 ms: 0.440 50 ms: 0.195 100 ms: 0.689 200 ms: 0.482	Student's t-test
4A-9 months		25 ms: 0.355 50 ms: 0.226 100 ms: 0.306 200 ms: 0.820	Student's t-test
4A-12 months		25 ms: 0.630 50 ms: 0.856 100 ms: 0.645 200 ms: 0.862	Student's t-test
4C	Genotype: (1, 63) = 20.639 Month: (1, 63) = 1.381 Genotype x Month: (1, 63) = 0.378	Genotype: < 0.001 Month: 0.257 Genotype x Month: 0.769 NLPF vs. WT 1.5 months: 0.901 3 months: 0.028 6 months: 0.003 9 months: 0.161 12 months: 0.020	2 way ANOVA post hoc holm-sidak Tukey's test
5B-CA1	Genotype: (1, 48) = 43.228 Month: (3, 48) = 0.356 Genotype x Month: (3, 48) = 0.918	Genotype: < 0.001 Month: 0.785 Genotype x Month: 0.440 NLPF vs. WT 3 months: < 0.001 6 months: 0.079 9 months: < 0.001 12 months: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test
5B-DG	Genotype: (1, 47) = 24.976 Month: (3, 47) = 0.374 Genotype x Month: (3, 47) = 0.227	Genotype: < 0.001 Month: 0.772 Genotype x Month: 0.877 NLPF vs. WT 3 months: 0.003 6 months: 0.044 9 months: 0.034 12 months: 0.014	2 way ANOVA post hoc holm-sidak Tukey's test
5B-CRT	Genotype: (1, 48) = 40.545	Genotype: < 0.001	2 way ANOVA post hoc holm-sidak Tukey's test

	Month: (3, 48) = 0.330 Genotype x Month: (3, 48) = 0.264	Month: 0.804 Genotype x Month: 0.851 NLPF vs. WT 3 months: 0.001 6 months: 0.022 9 months: 0.002 12 months: 0.001	
5C-CA1	Genotype: (1, 48) = 25.489 Month: (3, 48) = 0.406 Genotype x Month: (3, 48) = 0.327	Genotype: < 0.001 Month: 0.749 Genotype x Month: 0.806 NLPF vs. WT 3 months: 0.029 6 months: 0.019 9 months: 0.001 12 months: 0.048	2 way ANOVA post hoc holm-sidak Tukey's test
5C-DG	Genotype: (1, 47) = 16.071 Month: (3, 47) = 0.531 Genotype x Month: (3, 47) = 0.989	Genotype: < 0.001 Month: 0.664 Genotype x Month: 0.406 NLPF vs. WT 3 months: 0.022 6 months: 0.062 9 months: 0.003 12 months: 0.499	2 way ANOVA post hoc holm-sidak Tukey's test
5C-CRT	Genotype: (1, 48) = 27.778 Month: (3, 48) = 0.542 Genotype x Month: (3, 48) = 1.599	Genotype: < 0.001 Month: 0.656 Genotype x Month: 0.202 NLPF vs. WT 3 months: 0.241 6 months: 0.049 9 months: < 0.001 12 months: 0.002	2 way ANOVA post hoc holm-sidak Tukey's test
6A		3 months: 0.925 6 months: 0.029 9 months: 0.486 12 months: 0.340	3 months: Student's t-test 6 months: Mann-Whitney rank sum test 9 months: Mann-Whitney rank sum test 12 months: Student's t-test
6B		3 months: 0.593 6 months: 0.026 9 months: 0.025 12 months: 0.792	3 months: Student's t-test 6 months: Mann-Whitney rank sum test 9 months: Student's t-test 12 months: Mann-Whitney rank sum test