Manuscript Title

Adolescent sleep defects and dopaminergic hyperactivity in mice with a schizophrenia-linked Shank3 mutation.

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Supplemental Material List

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Supplementary Table 1.

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Supplementary Figure Legends

Supplementary Figure 1. Adolescent sleep architecture in male and female *R1117X* mice.

Sleep architecture parameters quantified from adolescent $R1117X^{+/+}$ mice and WT littermates. Male, n = 5 in WT; 6 in $R1117X^{+/+}$; female, n = 5 each. (A, F) Total amount of time in Wake, NREM and REM states over 24 h. Welch's t-test between genotypes, (A) Wake, $t = 3.14$, $df = 7.06$, $P = 0.02$; NREM, $t = 2.80$, $df = 5.57$, $P = 0.03$, (F) Wake, $t = 2.81$, $df = 7.91$, $P = 0.02$; NREM, $t = 2.64$, $df = 7.61$, $P = 0.03$, REM, $t = 3.11$, $df = 6.08$, $P = 0.02$. (B, G) Average length of Wake, NREM and REM bouts over 24 h. Welch's t-test between genotypes, (B) Wake, $t = 3.20$, $df = 8.91$, $P = 0.01$, (G) NREM, $t = 4.56$, $df = 7.45$, $P = 0.002$. (C-J) Bout number of each state during light/dark phase and the 24 h cycle. Welch's t-test between genotypes, (D) Wake, $t = 2.58$, $df = 8.78$, $P = 0.03$; NREM, $t = 2.72$, $df = 8.86$, $P = 0.02$, (E) Wake, $t = 2.91$, $df = 6.75$, $P = 0.02$; NREM, $t = 2.96$, $df = 6.44$, $P = 0.02$, (I) REM, $t = 2.89$, $df = 4.49$, $P = 0.04$. (K) Representative movement trajectories of WT and $R1117X^{+/+}$ mice in their respective home-cages during 20 min in the dark phase. (L, M) Total distance traveled (L) and moving speed (M) of WT and $R1117X^{+/+}$ mice. n = 5 each. Welch's t-test, $P > 0.05$. Data are shown as mean ± s.e.m. All tests were two-sided. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant, $P > 0.05$.

Supplementary Figure 2. Adult sleep architecture in *R1117X* mice.

(A-C) Hourly percentage of Wake (A), NREM (B) and REM (C) sleep in adult $R1117X^{+/+}$ and WT mice (male and female combined, P70 – 90) over a 24-h light-dark cycle (n = 5 in WT; 4 in $R1117X^{+/+}$). RM two-way ANOVA followed by Bonferroni's post-tests between genotypes, statistical significance as indicated on the graph. (D-L) Total time (D, G, J), average bout length (E, H, K) and bout number (F, I, L) of adult $R1117X^{+/+}$ and WT mice (n = 5 each). Welch's t-test between genotypes, (D) REM, $t = 2.49$, $df = 7.32$, * $P = 0.04$, (E) NREM, $t = 2.51$, $df = 7.98$, * $P = 0.04$, (G) Wake, $t = 3.81$, $df = 6.46$, ** $P = 0.008$; NREM, $t = 3.51$, $df = 6.50$, * $P = 0.01$; REM, $t = 5.04$, $df = 7.21$, ** $P = 0.001$, (H) NREM, $t = 4.81$, $df = 7.17$, ** $P = 0.002$, (J) Wake, $t = 5.51$, $df = 8.00$, *** $P = 0.0006$; NREM, $t = 5.35$, $df = 7.97$, *** $P = 0.0007$; REM, $t = 4.77$, $df = 7.96$, ** $P = 0.001$, (K) NREM, $t = 4.13$, $df = 7.99$, ** $P = 0.003$, (L) REM, $t = 2.52$, $df = 8.00$, * $P = 0.04$. (M-R) EEG

power spectrum of each state (M-O, shaded area indicates s.e.m.) and quantification of each frequency band (P-R). n = 5 each. Welch's t-test between genotypes, (Q) Delta, $t = 9.97$, $df = 7.13$, $P = 0.00002$; Alpha, $t = 12.36$, $df = 6.95$, $P = 0.000006$; Beta, $t = 5.33$, $df = 7.98$, $P = 0.0007$; Spindle range, $t = 8.43$, $df = 5.92$, $P = 0.0002$, (R) Theta, $t = 4.58$, $df = 6.81$, $P = 0.003$; Alpha, $t = 4.25$, $df = 5.69$, $P = 0.006$. Data are shown as mean ± s.e.m. All tests were two-sided. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant, $P > 0.05$.

Supplementary Figure 3. Adolescent sleep architecture in male *Nlgn3 R451C* mice.

(A-C) Hourly percentage of Wake (A), NREM (B) and REM (C) sleep in male *R451C/y* mice and WT littermates over a 24-h light-dark cycle during P37 – 40 (n = 6 each). RM two-way ANOVA followed by followed by Bonferroni's post-tests. (D-L) Total time (D, G, J), average bout length (E, H, K) and bout number (F, I, L) of male *R451C/y* and WT mice (n = 6 each). Welch's t-test between genotypes, (F) REM, $t = 2.34$, $df = 9.90$, $P = 0.04$, (K) REM, $t = 2.40$, $df = 6.83$, $P = 0.048$, (L) REM, $t = 2.48$, $df = 9.97$, $P = 0.03$. (M-R) EEG power spectrum of each state (M-O, shaded area indicates s.e.m.) and quantification of each frequency band (P-R). n = 6 each. Welch's t-test between genotypes, (R) Alpha, $t = 2.76$, $df = 8.72$ $P = 0.02$. Data are shown as mean ± s.e.m. All tests were two-sided. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant, $P > 0.05$.

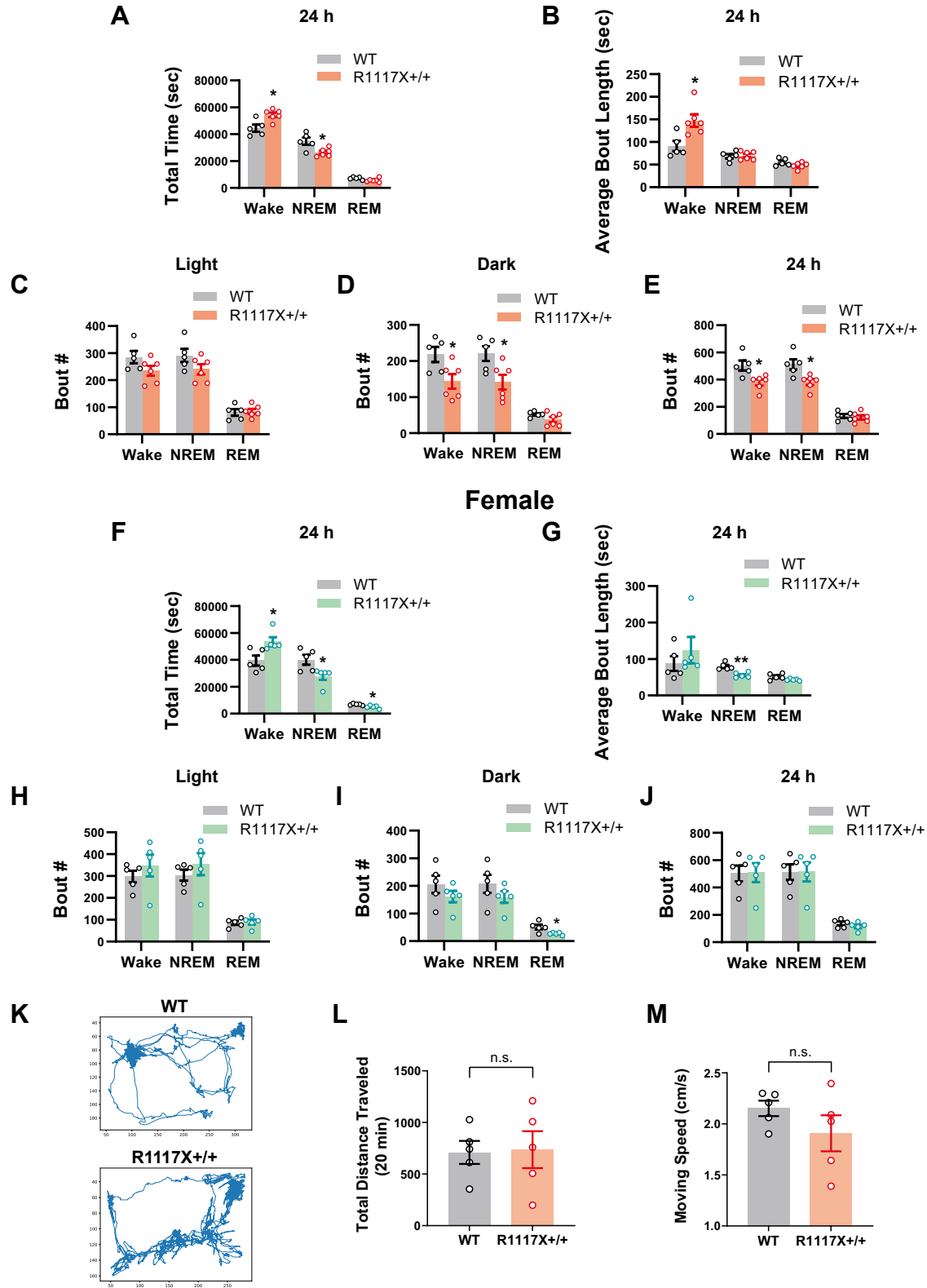**Supplementary Figure 4. Adolescent sleep architecture in male *16p11.2* deletion mice.**

(A-C) Hourly percentage of Wake (A), NREM (B) and REM (C) sleep in male *16p11.2del/+* mice and WT littermates over a 24-h light-dark cycle during P37 – 40 (n = 6 in WT; 7 in *del/+*). RM two-way ANOVA followed by followed by Bonferroni's post-tests. (D-L) Total time (D, G, J), average bout length (E, H, K) and bout number (F, I, L) of male *R451C/y* and WT mice (n = 6 in WT; 7 in *del/+*). Welch's t-test between genotypes, all n.s. (M-R) EEG power spectrum of each state (M-O, shaded area indicates s.e.m.) and quantification of each frequency band (P-R). n = 6 in WT; 7 in *del/+*. Welch's t-test between genotypes, (P) Low Delta, $t = 2.78$, $df = 10.74$ $P = 0.02$. Data are shown as mean ± s.e.m. All tests were two-sided. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant, $P > 0.05$.

Supplementary Figure 5. EEG power in multiple frequency bands during adolescent NREM sleep correlates with adult sociability.

(A) Linear regression between $PI_{Sociability}$ in adult three-chamber test and relative EEG power in each frequency band during adolescent NREM sleep (n = 18). (B) Linear correlations of adolescent NREM EEG power in the spindle range (10 – 15 Hz) with adult $PI_{Sociability}$ or PI_{SN} (n = 18).

Supplementary Figure 1

Male

A 24 h

B 24 h

C Light

D Dark

E 24 h

Female

F 24 h

G 24 h

H Light

I Dark

J 24 h
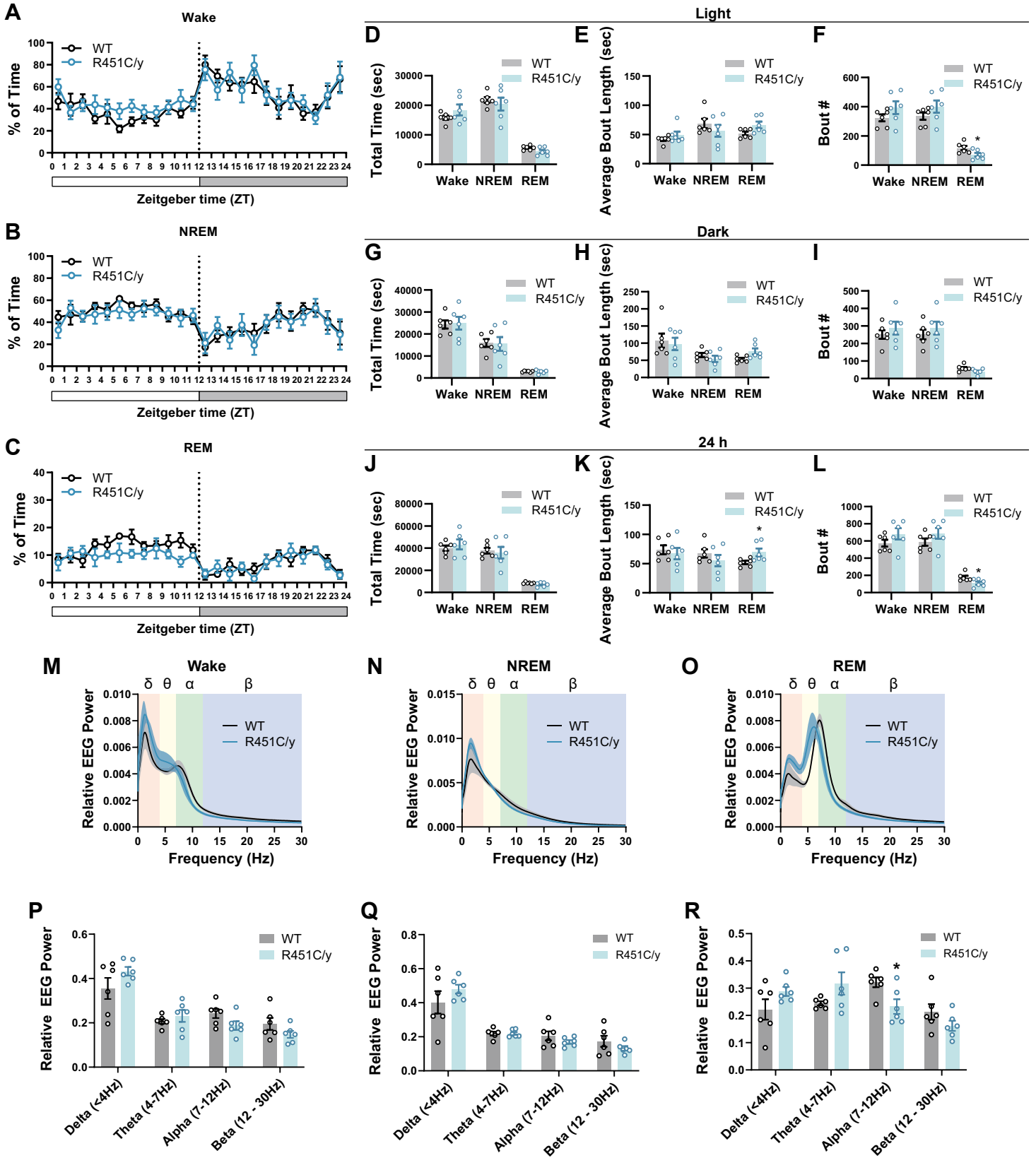
K WT

R1117X+/+

L n.s.

M n.s.

Supplementary Figure 2

Adult R1117X (P70 - 90)
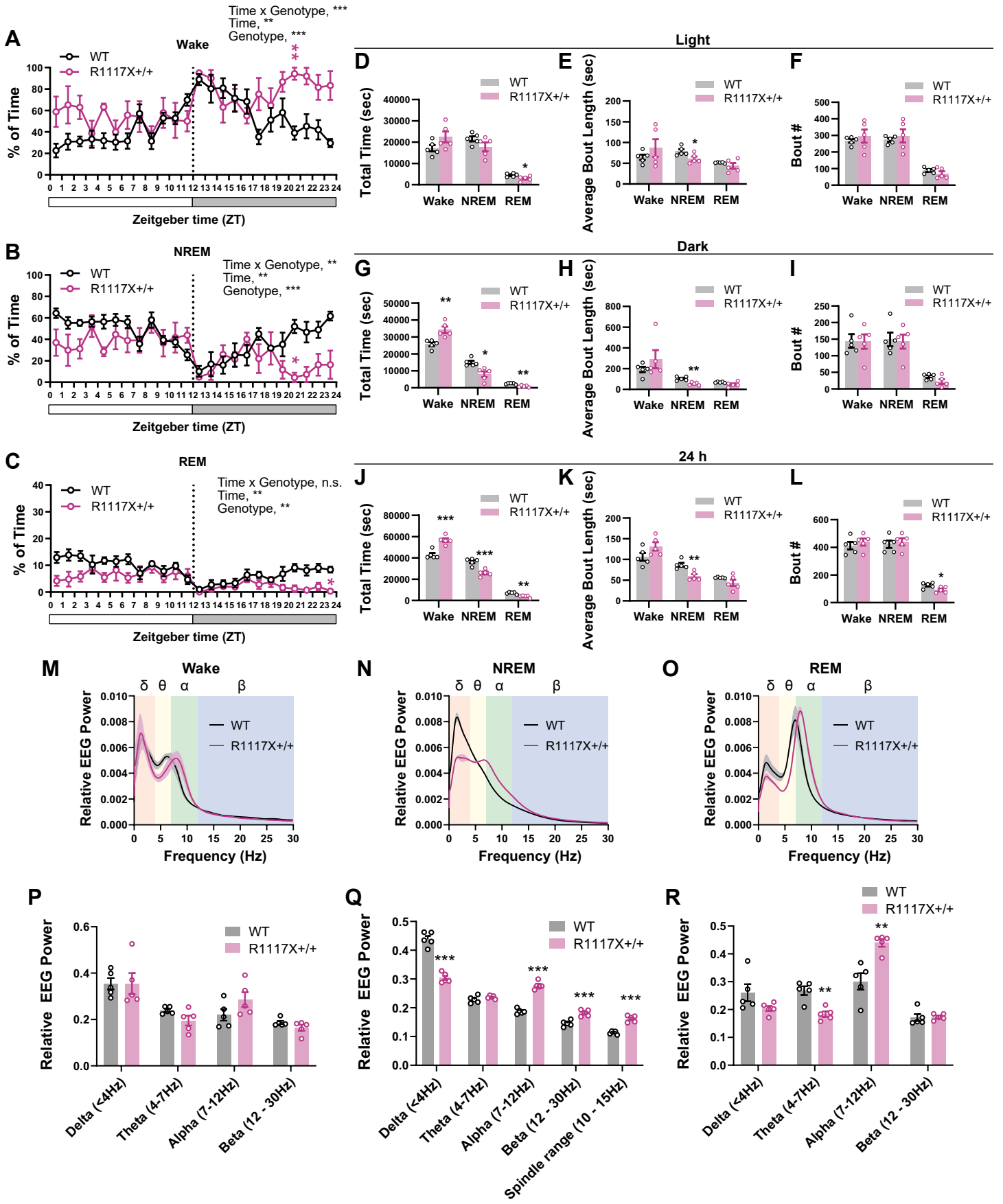
Supplementary Figure 3

Supplementary Figure 4

Supplementary Figure 5

A

Delta (<4Hz), $R^2 = 0.32$, $P = 0.01*$
Theta (4-7Hz), $R^2 = 0.17$, $P = 0.09$
Alpha (7-12Hz), $R^2 = 0.29$, $P = 0.02*$
Beta (12-30Hz), $R^2 = 0.31$, $P = 0.02*$

Relative EEG Power

$PI_{Sociability}$

B

Sociability, $R^2 = 0.33$, $P = 0.01*$
SN, $R^2 = 0.009$, $P = 0.71$

NREM power in spindle range
(10 - 15 Hz)

PI
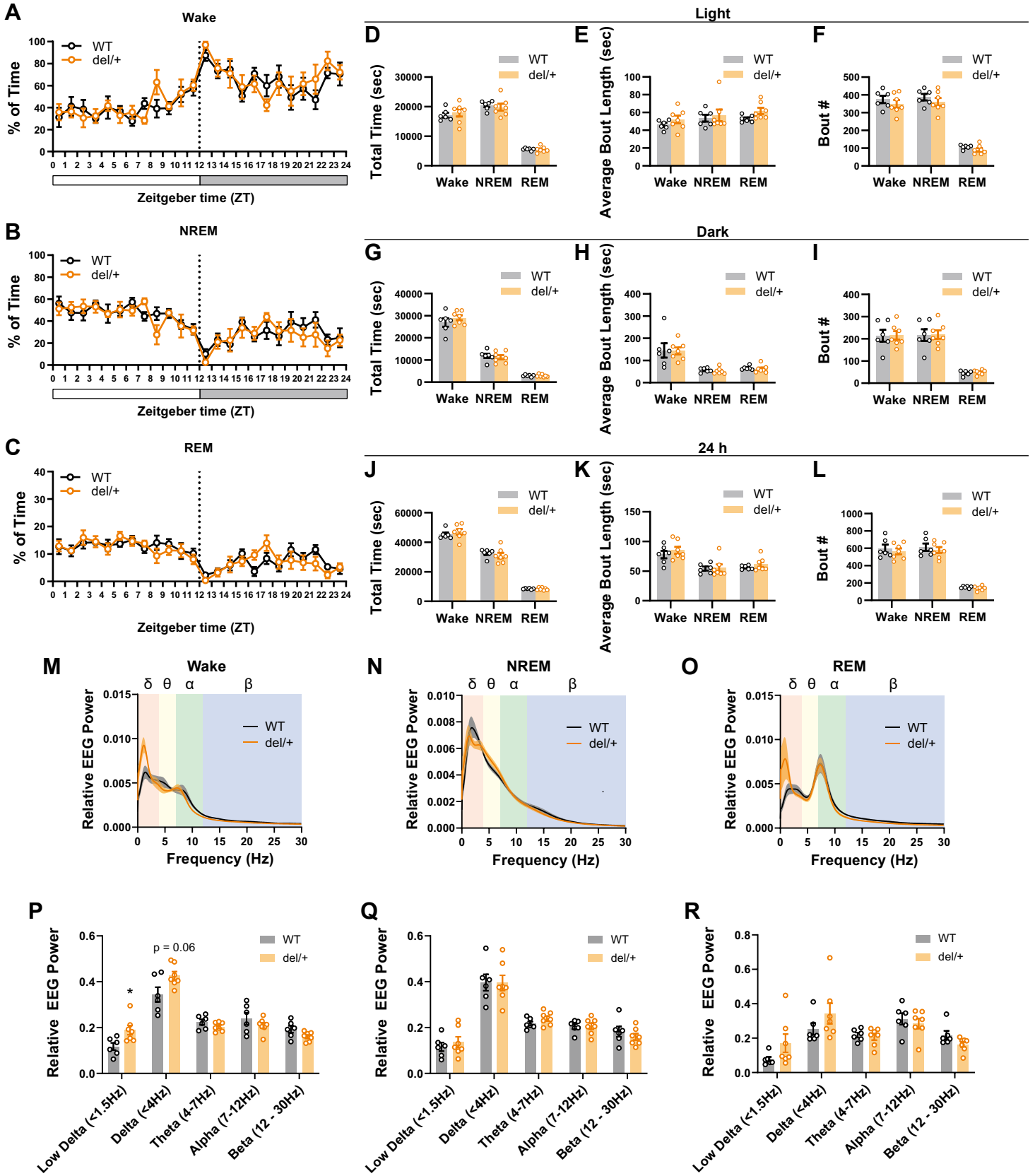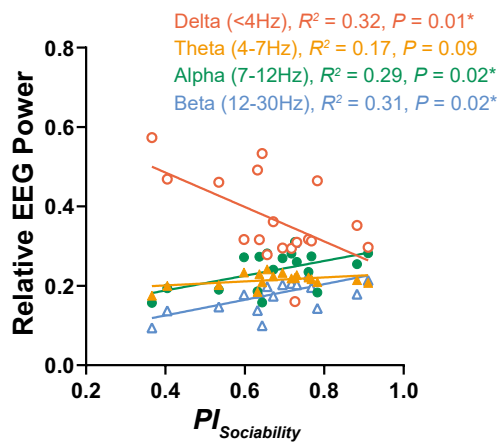
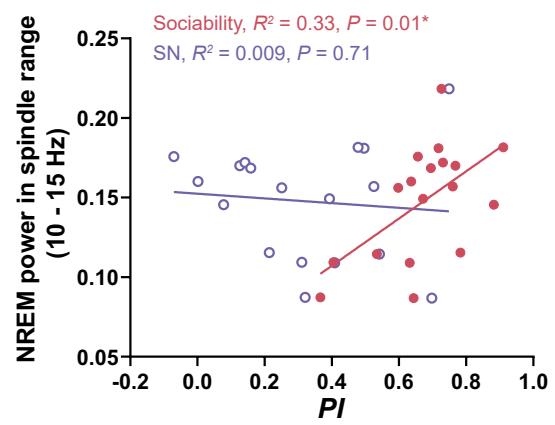**Supplementary Table 1. Summary of linear regressions between adult social preferences and adolescent sleep parameters**

Sociability (X)				
Inverse correlated				
Sleep parameters (Y)	**Slope**	**R^2**	**R**	**P**
NREM EEG Power - Delta	-0.4336	0.3218	0.567274184	0.0141*
Total amount in 24h (s) - NREM	-28512	0.2296	0.479165942	0.0442*
Average Bout Length in 24h (s) - NREM	-38.45	0.1917	0.437835586	0.0692
REM EEG Power - Delta	-0.1684	0.1617	0.402119385	0.098
Average Bout Length in 24h (s) - REM	-18.72	0.1304	0.361109402	0.1409
Total amount in 24h (s) - REM	-3322	0.1237	0.351710108	0.1523
REM EEG Power - Theta	-0.1272	0.1178	0.343220046	0.1632
Wake EEG Power - Alpha	-0.1065	0.03844	0.196061215	0.4356
Correlated				
Sleep parameters (Y)	**Slope**	**R^2**	**R**	**P**
NREM EEG Power - Spindle	0.1483	0.3278	0.572538208	0.013*
NREM EEG Power - Beta	0.197	0.3066	0.553714728	0.0171*
NREM EEG Power - Alpha	0.1861	0.2934	0.541664103	0.0202*
Total amount in 24h (s) - Wake	31834	0.2342	0.483942145	0.0419*
REM EEG Power - Beta	0.1857	0.2096	0.457820926	0.0561
NREM EEG Power - Theta	0.05046	0.1726	0.415451562	0.0864
DA transient score - NREM	0.07452	0.2172	0.466047208	0.1267
Average Bout Length in 24h (s) - Wake	134.7	0.114	0.33763886	0.1706
REM EEG Power - Alpha	0.1098	0.09507	0.308334234	0.2132
DA transient score - All states	0.04577	0.1453	0.381182371	0.2214
DA transient score - REM	0.1725	0.1077	0.328176782	0.2977
DA transient score - Wake	0.01928	0.05542	0.235414528	0.4614
Wake EEG Power - Delta	0.07462	0.008301	0.091109824	0.7192
Wake EEG Power - Beta	0.02399	0.005303	0.0728217	0.774

Social Novelty (X)				
Inverse correlated				
Sleep parameters (Y)	**Slope**	**R^2**	**R**	**P**
Total amount in 24h (s) - Wake	-27930	0.4929	0.702068373	0.0012**
Average Bout Length in 24h (s) - Wake	-132.9	0.3032	0.550635996	0.0179*
DA transient score - REM	-0.4457	0.4442	0.666483308	0.0179*
DA transient score - All states	-0.08518	0.3111	0.557763391	0.0595
REM EEG Power - Alpha	-0.08139	0.1957	0.442379927	0.066

DA transient score - Wake	-0.0523	0.2521	0.502095608	0.0963
REM EEG Power - Delta	-0.09416	0.1421	0.376961536	0.1231
NREM EEG Power - Theta	-0.02765	0.1417	0.376430604	0.1236
Wake EEG Power - Alpha	-0.1135	0.1193	0.345398321	0.1604
DA transient score - NREM	-0.07321	0.1295	0.359861084	0.2505
NREM EEG Power - Alpha	-0.04923	0.05611	0.236875495	0.3439
NREM EEG Power - Spindle	-0.0148	0.008921	0.094451046	0.7093
Correlated				
Sleep parameters (Y)	**Slope**	**R^2**	**R**	**P**
Total amount in 24h (s) - NREM	25297	0.4943	0.70306472	0.0011**
Wake EEG Power - Theta	0.09947	0.4676	0.68381284	0.0018**
REM EEG Power - Theta	0.1047	0.2993	0.547083175	0.0188*
Total amount in 24h (s) - REM	2632	0.2124	0.460868745	0.0542
Average Bout Length in 24h (s) - NREM	18.34	0.1192	0.34525353	0.1605
REM EEG Power - Beta	0.07086	0.09024	0.300399734	0.2258
Average Bout Length in 24h (s) - REM	4.173	0.01772	0.13311649	0.5985
Wake EEG Power - Beta	0.02466	0.01532	0.123773988	0.6247
NREM EEG Power - Delta	0.05546	0.0144	0.12	0.6353
NREM EEG Power - Beta	0.02141	0.009904	0.099518842	0.6944

Supplementary Table 2. Comparison of mouse models examined in this study.

Model	Shank3 R1117X	Neuroligin-3 R451C	16p11.2 deletion
Mutation	Non-sense mutation in Shank3 exon 21 changing an arginine to stop codon (R1117X) (Zhou et al., 2016)	Missense mutation in Neuroligin-3 changing arginine to cysteine (R451C) (Jamain et al., 2003)	Deletion of the gene locus on chromosome 16, position 11.2 (16p11.2) (Rein and Yan, 2020)
Adolescent sleep architecture	Reduced NREM and REM sleep; Deceased NREM-Delta and REM-Theta powers, increase of higher frequency powers in NREM and REM (this study).	No major defects (this study).	No major defects (this study).
Adult sleep architecture	Similar defects as in adolescence (this study)	Generally normal sleep/wake pattern, slightly altered NREM power spectrum (Liu et al., 2017)	Reduced NREM and REM sleep, decreased REM/NREM ratio, REM fragmentation, altered REM power spectrum (Angelakos et al., 2017; Lu et al., 2019)
Other behavioral abnormalities	Increased anxiety level, defects in social interaction, allogrooming and dominance-like Behavior (Zhou et al., 2016)	Abnormal social interaction, stereotyped behavior, and enhanced spatial learning (Uchigashima et al., 2021)	Hyperactivity (Angelakos et al., 2017)
Synaptic defects	Reduced synaptic transmission in striatum and prefrontal cortex (Zhou et al., 2016)	Altered synaptic transmission in hippocampus, cerebellum, and somatosensory cortex (Uchigashima et al., 2021)	Increased synaptic transmission in somatosensory cortex and nucleus accumbens, reduced transmission in prefrontal cortex (Rein and Yan, 2020)

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