Supplementary Materials for Blundell et. al.,

"Neuroligin 1 deletion results in impaired spatial memory and increased repetitive behavior"

SUPPLEMENTARY METHODS

Anxiety-like Behavior

The dark/light box, elevated plus maze, and open field behavioral tasks were performed essentially as described (Powell et al., 2004; Tabuchi et al., 2007). In the dark/light box, one side was kept dark (room light entry limited) while a light built into the top lit the other side (1700 lux, each chamber 25 cm X 26 cm). Mice were placed in the dark side and allowed to freely explore the light and dark sides for 10 minutes. Anxiety-like behavior was measured using latency to enter the light side, time spent in the light side, number of crosses to the light side and time spent in the dark side. In the elevated plus maze, mice were placed in the center of a black, Plexiglas, elevated plus maze with white floors (each arm 33 cm long and 5 cm wide with 25 cm high walls on closed arms) in a dimly lit room for 5 min and videotracking software from Noldus (Ethovision 2.3.19) recorded time spent and frequency in the open and closed arms. The open field test was performed for 20 min in a brightly lit (~800 lux), 48 X 48 X 48 cm white plastic arena using video tracking software from Noldus (Ethovision 2.3.19). Time spent in the center zone (15 x 15 cm) and frequency of entries into the center were recorded. Locomotor activity was also measured during the open field test, elevated plus maze and dark/light box. All behavior was analyzed with a 2-way ANOVA.

Pre-pulse Inhibition and Startle Amplitude Tests.

Pre-pulse inhibition and startle threshold protocol was performed as described (Kwon et al., 2006). Data were analyzed with a 2-way ANOVA or a 3-way mixed ANOVA.

Locomotor Activity and Accelerating Rotarod

To assess locomotor activity, mice were placed for 2 hours in a fresh home cage with minimal bedding. Horizontal activity was monitored using photo beams linked to computer data acquisition software (San Diego Instruments) and averaged in 5 min bins. Data were analyzed with a mixed ANOVA. An accelerating rotarod designed for mice (IITC Life Sciences) was used essentially as described (Powell et al., 2004) except that except that 3 sets of 3 trials were performed per day over 3 days. Briefly, the rotarod was activated after placing a mouse on the motionless rod. The rod accelerated from 0 to 45 revolutions per min in 60 s. Time to fall off the rod or to turn one full revolution was measured. Data were analyzed with a 3-way mixed ANOVA.

SUPPLEMENTARY REFERENCES

Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF (2006) Pten regulates neuronal arborization and social interaction in mice. Neuron 50:377-388.

Powell CM, Schoch S, Monteggia L, Barrot M, Matos MF, Feldmann N, Sudhof TC, Nestler EJ (2004) The presynaptic active zone protein RIM1alpha is critical for normal learning and memory. Neuron 42:143-153.

Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC (2007) A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318:71-76.

SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1. NL1 KO Mice Show Normal Anxiety-Like Behavior

(A-B) Elevated plus maze (EPM) test. Anxiety-like behavior does not differ between WT and NL1 KO mice as measured by frequency to enter the open arms divided by frequency to enter all arms (A, 2-way ANOVA: genotype: $p=0.99$, sex: $p=0.62$, genotype x sex interaction: $p<0.021$, but no significant comparisons with Tukey post hoc test for the interaction) or as measured by the time spent in the open arms divided by time in all arms (B, 2-way ANOVA: genotype: $p=0.34$, sex: $p=0.23$, genotype x sex interaction: $p<0.039$, but no significant comparisons with Tukey post hoc test for the interaction). N = 23 littermate pairs. Legend in A applies to all other panels. **(C-D)** Dark/Light Box. The latency to enter the light side (C, 2-way ANOVA: genotype: $p=0.32$, sex: $p=0.79$, genotype x sex interaction: $p<0.035$, but no significant comparisons with Tukey post hoc test for the interaction) and the total time spent in the light side (D, 2-way ANOVA: genotype: $p=0.077$, sex: $p=0.72$, genotype x sex interaction: $p=0.59$) did not differ across genotype. N = 23 littermate pairs. **(E-F)** Open Field Task. The frequency of entrances into the center (E, 2-way ANOVA: genotype: $p=0.47$, sex: $p=0.05$, genotype x sex interaction: $p=0.40$) and the time spent in the center divided by time in the periphery (F, 2-way ANOVA: genotype: $p=0.47$, sex: $p=0.17$, genotype x sex interaction: $p=0.29$) was similar in NL1 KO and WT mice. N = 23 littermate pairs. Data represent means +/- SEM.

Supplemental Figure 2. NL1 KO Mice Exhibit Normal Locomotor Activity, Motor Coordination, and Auditory Startle Responses. (A-B) Locomotor activity during the

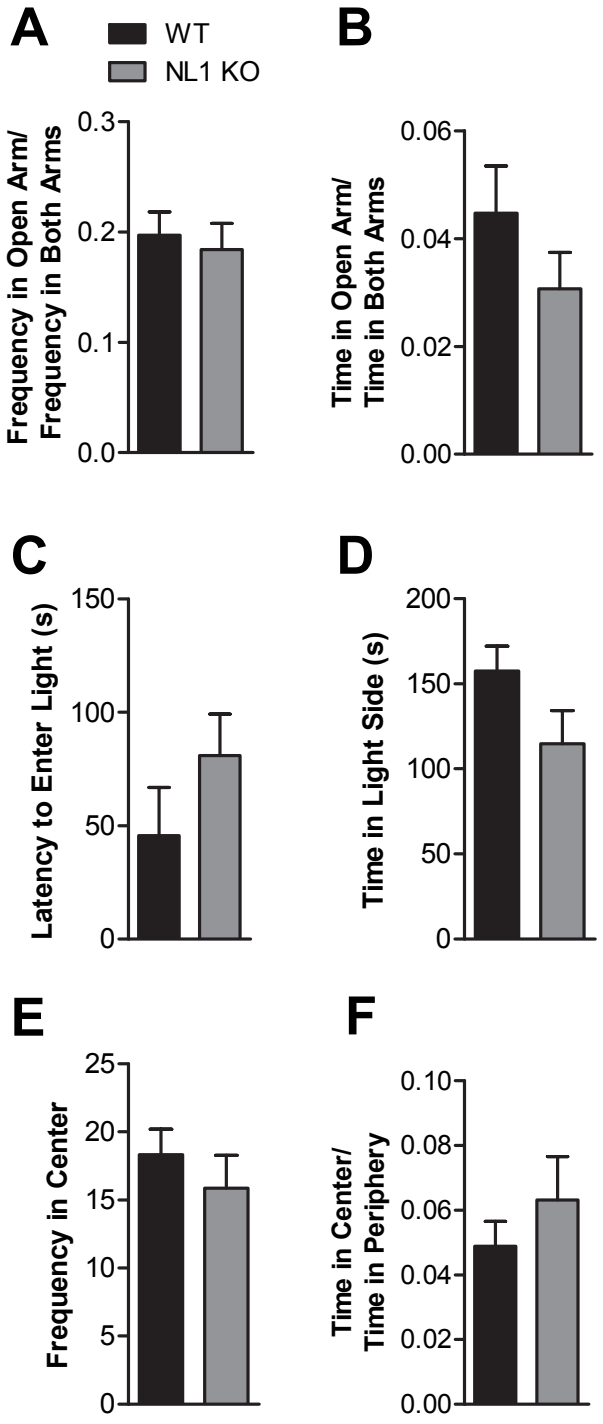
open field test. The total distance travelled (A) and the average velocities (B) during the open field test were similar between WT and KO mice (for both measures, 2-way ANOVA: genotype: p=0.21, sex: p=0.68, genotype x sex interaction: p=0.70). N = 23 littermate pairs. Legend in A applies to Panels A-D. **(C)** Locomotor activity during the elevated plus maze (EPM). The total distance moved during the EPM was slightly less for NL1 KO mice compared to WT mice (2-way ANOVA: genotype: $p<0.049$, sex: p=0.41, genotype x sex interaction: p=0.52). N = 23 littermate pairs. **(D)** Locomotor activity during the Dark/Light Box task. The total locomotor activity (as measured by the total number of photobeam breaks) was the same across genotype in the dark/light box (2-way ANOVA: genotype: p=0.073, sex: p=0.92, genotype x sex interaction: p=0.73). N = 23 littermate pairs. **(E)** Locomotor activity (as measured by the total number of photobeam breaks) in a novel home cage was measured in 5 min bins over a total of 2 hrs. Locomotor habituation was similar in WT and NL1 KO mice (3-way mixed ANOVA, genotype: p=0.84, sex: $p<0.016$, trial: $p<0.00001$, no genotype x sex interaction: p=0.90, no genotype x trial interaction: p=0.94, no sex x trial interaction: p=0.99, no genotype x sex x trial interaction: p=0.62). N = 23 littermate pairs. Legend in E applies to Panel F. **(F)** The time to fall off an accelerating rotarod was measured over 3 days. Each day, mice were tested in 3 sets of 3 trials. NL1 KO and WT mice displayed similar motor coordination and motor learning in the rotarod task (3-way mixed ANOVA, genotype: p=0.69, sex: $p<0.033$, trial: $p<0.00001$, no genotype x sex interaction: p=0.56, no genotype x trial interaction: p=0.78, no sex x trial interaction: p=0.59, no genotype x sex x trial interaction: p=0.96). N = 23 littermate pairs. **(G)** Prepulse Inhibition. Data represent the percent inhibition of the mean startle response to a 120

dB pulse with 3 different prepulse/pulse pairings (Prepulses at 4, 8, or 16 dB above the background noise level of 70 dB). Prepulse Inhibition in NL1 KO mice is normal, regardless of the decibel level of the prepulse (3-way mixed ANOVA: genotype: $p=0.80$, sex: $p=0.069$, decibel: $p<0.000001$, genotype x sex interaction: $p=0.36$, genotype x decibel interaction: $p=0.71$, sex x decibel interaction: $p=0.14$, genotype x sex x decibel interaction: $p=0.56$). N = 23 littermate pairs. Legend in G also applies to Panel H. **(H)** NL1 KO mice and WT exhibited similar baseline startle responses, as measured by the mean startle amplitude to 6 presentations of a 120 dB auditory pulse (2-way ANOVA: genotype: $p=0.36$, sex: $p=0.35$, genotype x sex interaction: $p=0.76$). N = 23 littermate pairs. **(I)** Visible water maze task. Data represent the latency to find a visible platform in a pool of water. NL1 WT and KO mice exhibited similar levels of performance in this task (3-way Mixed ANOVA; sex: $p=0.38$; genotype: $p=0.91$; trial: $p<0.000001$; sex x genotype interaction: $p=0.95$; sex x trial interaction: $p=0.89$; genotype x trial interaction: $p=0.14$; sex x genotype x trial interaction: $p=0.51$). N = 11 littermate pairs. Data represent means +/- SEM.

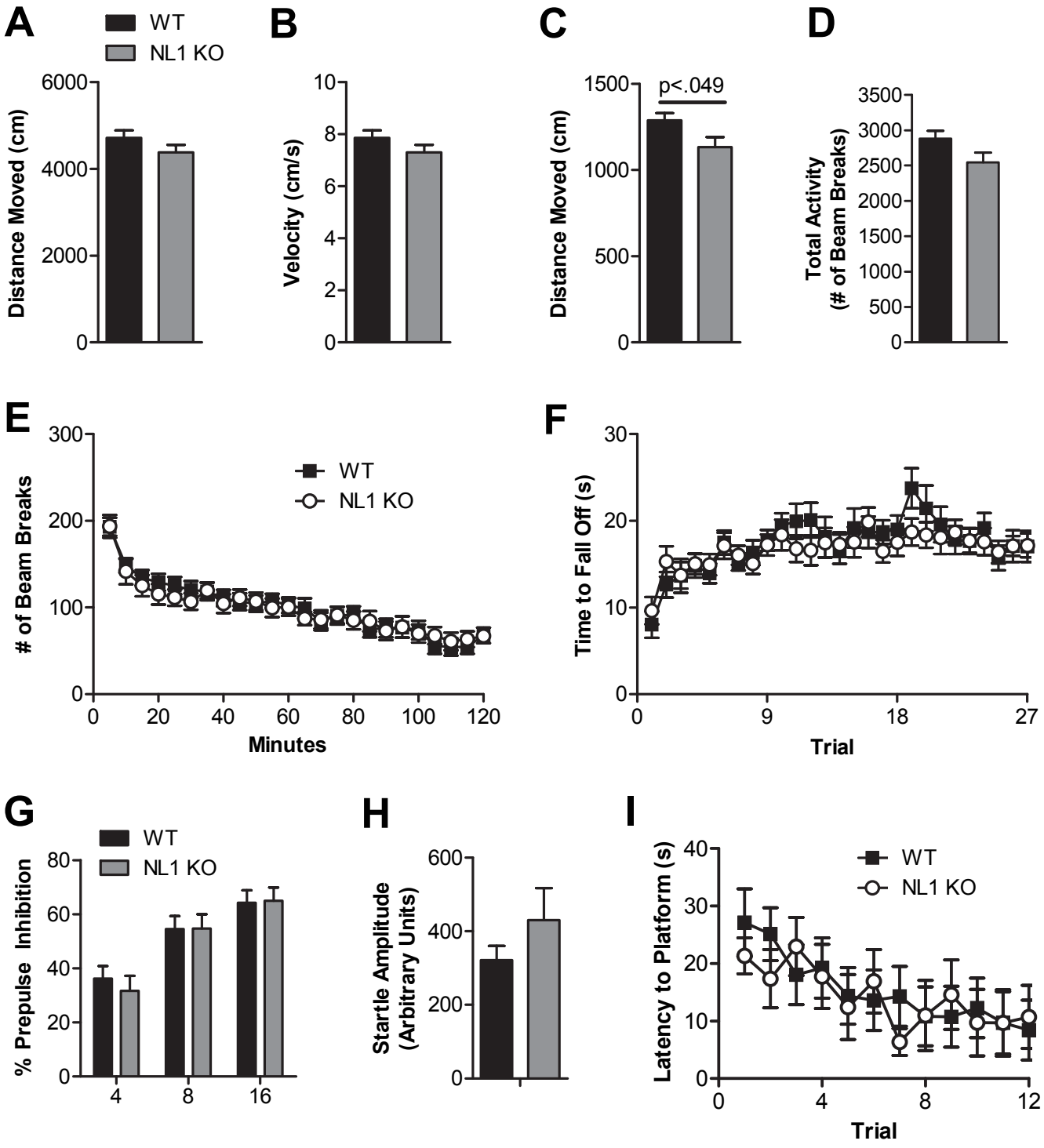
Supplemental Figure 3. Synaptic density and size are normal in NL1 KO Mice. (A-B) Representative confocal images of WT (A) and NL1 KO (B) CA1 region of hippocampus labeled with anti-synaptophysin antibodies. Scale bar = 50 µm. **(C-D)** Representative confocal images of WT (C) and NL1 KO (D) CA3 region of hippocampus labeled with anti-synaptophysin antibodies. Scale bar = 50 µm. **(E-F)** Representative confocal images of WT (E) and NL1 KO (F) CA1 region of hippocampus labeled with anti-synaptophysin antibodies. Scale bar = 10 µm. **(G-H)** Representative confocal

images of WT (G) and NL1 KO (H) CA3 region of hippocampus labeled with anti-synaptophysin antibodies. Scale bar = 10 µm. **(I-J)** Number (I) and size (J) of synaptophysin-positive puncta are normal in NL1 KO neurons compared to WT. Number and size of synaptophysin positive puncta in mutant neurons are normalized to WT littermate control. n = 3 per genotype.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3