UNIVERSITY OF CALIFORNIA SAN DIEGO

A Role of *Prickle1* in Synapse Development and Disease

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Jingyi Wang

Committee in charge:

Professor Yimin Zou, Chair
Professor Jill Leutgeb
Professor Gentry Patrick

2019

The Thesis of Jingyi Wang is approved and it is acceptable in quality and form for publication microfilm and electronically:

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University of California San Diego

2019

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PREFACE

This thesis is submitted for the Master Degree of Science in Biology of University of California San Diego. The research described here was performed under the supervision of Professor Yimin Zou in the Department of Biological Science, University of California San Diego, 2019.

This research project was accomplished by collaborating with two postdocs and several undergraduates in Dr. Yimin Zou's lab. Dr. Ting Yu generated the Prickle 1 R104Q mice using CRISPR/Cas9. Dr. Yue Ban helped set up the whole behavioral paradigm. The behavioral tests were performed with the help from Dr. Yue Ban, Can Liu and Xiaojia Wang. Majority of the video analysis and data collection was done by Dr. Yue Ban, Can Liu and Sogoli Sadraeinouri. Tissue collection, genomic DNA extraction and PCR for genotyping was done with tremendous help from Xiaojia Wang and Sogoli Sadraeinouri.

ACKNOWLEDGEMENT

I would like to express my grateful appreciation to Professor Yimin Zou for being my committee chair and thesis advisor. I would like to thank him for allowing me to be a volunteer in the lab and start my research journal here and accepting me as a master student. Along the past few years, he has offered me great advice and help.

I would also like to thank my committee members, Professor Jill Leutgeb and Professor Gentry Patrick, who kindly accepted to be my committee members.

I would like to thank Dr. Yue Ban for all the guidance on this project, and advice on this thesis. Without her persistent help, I would not be able to reach this point. I would like to thank Dr. Sonal Thakar for being my mentor during my undergraduate period and Dr. Ting Yu for being my mentor during the beginning of my master project. I would like to thank all other lab members in Zou Lab including Dr. Keisuke Onishi, Dr. Bo Feng, Dr. Huaping Qin, Runyi Tian, Dr. Dinghong Zhang, Yeorang Lee, Xiaojia Wang, Sogoli Sadraeinouri, Can Liu for all the help and support in the past few years. Without their persistent effort, I would not be able to finish this thesis.

In the end, I would like to thank my father Yan Wang and my mother Weili Liu for all the love and support. You are always on my back. I would also like to thank my boyfriend Xiang Wang for all the company and support.

ABSTRACT OF THE THESIS

A Role of *Prickle1* in Synapse Development and Disease

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Professor Yimin Zou, Chair

The Wnt signaling pathways play important roles in many developmental processes from invertebrate to vertebrate. The Wnt/Planar cell polarity (PCP) pathway is one of the non-canonical Wnt signaling pathways that mainly regulate cytoskeleton. Without the control of PCP components, Drosophila wing epithelia would grow in random directions. Our lab previous work has shown that PCP components Celsr3, Vangl2 and Frizzled3 are responsible for neuronal migration, axon guidance and synapse formation. *Prickle* is one of the core components of PCP signaling pathway. Studies have reported that several neurological abnormalities were identified with *Prickle* mutation in human, mice, zebrafish and Drosophila. *Prickle1 R104Q* missense mutation was reported in three consanguineous families with Progressive Myoclonus Epilepsy-

Ataxia Syndrome. Here, we used CRISPR-Cas 9 gene editing to generate this human point mutation in mice. In this thesis, we performed examination of behavioral deficits in *Prickle1 R104Q* mutant mice. We found that *Prickle1 R104Q* mutant mice have normal locomotor activity in open field assay and light-dark transition assay, while they exhibited deficit in novel object recognition test. Then we used three-chamber social interaction test and found that these *Prickle1* mutant mice have abnormal sociability and normal social novelty. Using Barnes maze, we showed *Prickle1* mutant mice have normal learning ability and spatial memory. In conclusion, our work has identified behavioral deficits in *Prickle1 R104Q* mutant mice.

Introduction

The Wnt signaling pathway is an important signal transduction pathway that evolutionarily conserved through many species. It is highly involved in many developmental processes in animals like cell fate regulation, cell proliferation, structure, and polarity, etc.[1]. There are two characterized types of Wnt signaling pathways. The canonical Wnt signaling pathway requires β-catenin accumulation in the cytoplasm and later translocate to nucleus to act a factor to inhibit transcription through transcription factors leukocyte enhance factor (LEF) and T cell factor (TCF) [2]. Without the Wnt binding to the membrane receptor Frizzled (Fz), β-catenin would go to degradation instead translocate to nucleus [3]. The Wnt/Planar cell polarity pathway (PCP) is one of the β-catenin independent noncanonical Wnt pathways [4]. The Wnt/PCP pathway mainly regulates cytoskeleton development and polarization through gastrulation. The Wnt signal is transduced through the activation of Rho-associated kinase and profilin to regulate cytoskeleton [5]. Activation of JNK signaling is integrated through Rac together to regulate actin polymerization during gastrulation [5]. Altogether PCP applies to a variety of cell types to modulate cell developmental processes that include diverse cell morphologies and multiple cell behaviors [6].

PCP is expressed in many epithelial cells where they show asymmetric structure along the tissue plane. The highly conserved PCP components assemble along the distal and proximal side of the cells to form the asymmetric assembly, which could transmit and propagate global polarity signaling [6, 7]. There are total of 6 core PCP components identified including Frizzled (Fz), Dishevelled (Dsh), Diego, Prickle (Pk), Vang(l) and Flamingo (Fmi)/Celsr [8]. Studies have shown that several PCP components play important roles in axon guidance. Without PCP

signaling, commissural neurons have defective anterior to posterior guidance after midline crossing [9-11]. PCP components are also involved in glutamatergic and GABAergic synapse formation [8, 12-14]. The formation of excitatory synapse formation requires Celsr3, which is a homology form of Flamingo in Drosophila. While Vangl2 plays an opposite role as Celsr3 which could inhibit excitatory synapse formation *in vivo* [8].

Prickle is a highly conserved component of PCP signaling pathway in many species and usually remains in the cytoplasm and later becomes abundant in the proximal side of cell membrane [15]. This protein family contains a Prickle Espinas Testin (PET) domain and three Linl-1, Isl-1 and Mec-3 (LIM) domains [16, 17]. PET domain is an approximately 110 amino acid motif that is in the N-terminus of LIM domain. Study suggests that PET domain is required for membrane insertion of the protein in order to interact with Dsh at the membrane for downstream signaling. The three LIM domains have a cysteine-rich motif with two zinc fingers. This cysteine-rich motif facilitates the protein folding to its secondary structure to further modulate protein-protein interaction [17].

Pk1 is expressed in multiple regions of mouse brain including hippocampus, cerebral cortex, thalamus and cerebellum as early from embryonic development [18, 19]. However, the expression of Pk1 is specific in neurons but not in glia [19]. In mouse hippocampal neuron primary culture, Pk1 is colocalized with Syn I [20]. The interaction between Pk1 and Syn I falls under the mutation domain of Syn I with ASD and epilepsy [20]. According to a study, Syn I is involved in synaptogenesis, synapse development and neurotransmitter release in both vertebrates and invertebrates [21]. *Pk1* is expressed in mouse embryo in a dynamic expression [18]. Early embryonic death at E8.5 is observed in mPk1 -/- null mice due to the impaired

anterior-posterior guidance and abnormal mesoderm formation [22]. mPk1 +/- mice exhibit abnormal social behavior resemble ASD-like behaviors. Specifically mPk1 +/- mutant mice have deficiency in social behavior in free moving social interaction assay, as well as altered circadian rhythms in home cage monitoring system [19]. A single shared *Prickle 1* missense mutation R104Q which lies in the PET domain of human *Prickle1* gene, which was identified in three consanguineous families with Progressive Myoclonus Epilepsy (PME). *Pk1* R104Q mutation disrupts the interaction between Pk1 and REST (Re1-Silencing Transcription Factor) *in vitro.* It suggests that *Pk1* R104Q mutation could prevent the translocation of REST into cytoplasm and further prevent silencing downstream genes [19]. Study has shown that REST is involved in ion channel and neurotransmitter [23]. However, the specific role of *Prickle* mutation in PME is not yet well understood.

Autism spectrum disorder (ASD) is a neurodevelopmental disease that becomes more prevalent in recent years. The prevalence nowadays is estimated to approximately 1.5% in human population worldwide [24]. ASD is characterized by a series of symptom before the age of 3, which includes: abnormal social behavior, impaired social communication and interaction, restricted interest and repetitive behaviors [24-26]. Genetic and environmental factors are both considered to affect ASD. The heritability of ASD is as high as 0.9 based on twins' study. Besides, the risk factor of ASD in population is around 25-fold higher with the presence of autistic siblings [25, 27, 28]. There is also a 5%-38% prevalence of epilepsy in autism patients [29, 30]. Several genes mutations are suggested to related to both epilepsy and autism from studies including Synapsin I (Syn I), FMR1 etc. [28, 30, 31].

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures that impairs normal brain function. It is defined by a history of at least of one seizure, consistent change in brain and associated cognitive and behavioral impairments [32]. There are approximately 39.2 million people have epilepsy generally worldwide in 2015 [33]. Epilepsy can be caused by genetic factor or result of brain damage, stroke, brain tumor, etc. which would affect multiple aspects in patients including consciousness, motor, sensory and autonomic functions, emotion and cognitive abilities etc. [32, 34]. Epileptic seizure is thought to cause by abnormal neuron firing from inhibition to excitation together with electric discharge in brain cells [31]. Studies have shown that the co-occurring of epilepsy and autism. However, the specific pathology of ASD with epilepsy is not fully understood [32].

In order to understand the function of *Pk1* in depth, our lab generated CRISPR-Cas9 mediated mice carrying human mutation *Pk1 R104Q* to test if these animals can act as a model to study this disorder. Therefore, we hypothesize that *Pk1 R104Q* mutant mice exhibit ASD-like behavioral deficits.

Materials and Methods

All animal experiments performed here were approved by University of California San Diego Institutional Animal Care and Use committee. Experimenters were trained to handle mice properly and gently. Animals were housed in an uncrowded environment with sufficient food and water to minimize the uncomfortableness and stress. All behavioral animals were littermates at the age of 8-10 weeks. Prior to the start of behavioral experiments, animals were transferred to the waiting area outside the experiment room for at least 30 min. Experimenter were double-blind when performing behavioral tests and data analysis.

Generation and genotyping of Prickle1 and Prickle2 point mutation mice

The *Prickle1 R104Q* missense mutation mice were generated using CRISPR-Cas 9 from former postdoc Ting Yu in UCSD Transgenic core. The Cas 9 protein, sgRNA and ssODN repair template were injected into C57BL/6 mouse embryo pronucleus at E0.5 day. Then the embryos were implanted into the fallopian tube of recipient ICR female mouse. Pups were born 21 days after implantation. After genotyping, *Pk1* mutant mice were backcrossed with C57BL/6 animals. All animals tested in behavioral experiments were at least 8^{th} generation or above. Experimental animals were weaned at postnatal 21 days and then performed genotype. The genotyping of *Pk1 R104Q* mutant mice contained two sets: wildtype allele genotype contained one common reverse primer, and one wildtype forward primer; mutant allele genotype contained one common reverse primer and one mutant forward primer. Pk1 WT forward primer sequence: 5'-TGG GGA GAG GAA CCA TCA AAC TC -3'. Pk1 *R104Q* forward primer sequence: 5'- CTG GGG AGA GGA ACT ATT AAG TTG -3'. Pk1 common reverse primer sequence: 5'- ACC GAG GCT TGA GCA GTT CAG -3'. The band size of *Pk1 R104Q* wildtype allele is 717bp. The band size of *Pk1*

R104Q mutant allele is 718bp. The polymerase chain reaction (PCR) was performed by the following protocol: 95℃ for 3 min; 35 cycles of 95℃ for 30s, 60℃ for 30s, 72℃ for 1 min; and 72℃ for 10 min.

Open field assay

Open field chamber (40cm wide x 40cm deep x 34cm tall) (Stoelting Co.) was used here to assess the locomotor activity. Both male and female mice were tested. Animals were introduced into the same location of the open field chamber and allowed to freely explore the chamber for 10 min under dim light. Total distance traveled was quantified and reported here.

Light-dark transition assay

Light-dark transition assay was performed here to assess rodent locomotor activity. The light-dark transition apparatus (40cm width x 40cm depth x 35cm height) (Stoelting Co.) contained a closed dark enclosure next to an open-lid light enclosure, which were connected through a small open door. Both male and female animals were tested. Behavior was assessed for 10 min. The total transition times, duration spent in the light chamber were recorded.

Novel object recognition

Novel object recognition test was used to assess rodent learning ability. Modified open field chamber (40cm wide x 25cm deep x 34cm tall) (Stoelting Co.) was used here. Both male and female animals were tested. On day 1 (Habituation day), animals were habituated for 30 min in the apparatus under dim light. On the second day (Testing day), animals were first introduced to two identical objects placed diagonally in the chamber and explored for 5 min (training

phase). After one-hour interval, animals were introduced to two different objects for 5 min, one familiar object and one novel object (testing phase). Interaction was defined as direct sniffing of animals to the object within a 2cm radius. Time interacting with two identical objects during training phase and time interacting with familial object and novel object during testing phase were reported here.

Three chamber social interaction

Three chamber social interaction assay was used to assess rodent sociability and social novelty. Both female and male mice were tested. The three-chamber social interaction apparatus (60cm wide x 40cm deep x 22cm tall) (Ugo Basile) was used here. The apparatus was divided into three chambers, each was 20cm wide x 40cm deep x 20cm tall. There were two slide doors (5cm x 8cm) on the two side chambers that allowed access. Subject animals were first introduced to the center chamber with two side doors closed for 10min. Followed by opening up the two slide doors and animals freely explored the entire three chambers for 10min. After this, the first unfamiliar non-littermate target mouse with same gender and similar age was introduced to one side of the chamber under a chrome wire cage (Chrome Galaxy Pencil Holder, Spectrum Diversified) during the sociability phase. An identical chrome wire cage with no animal was placed to the other side of the chamber. Two cages were placed diagonally. Subject mice were allowed to freely explore whole three chambers for 10min. Subject animals were transferred back to home cage for 1 hour. Followed by the social novelty phase, which a second unfamiliar non-littermate target mouse was introduced to the other side of the chamber under the chrome wire cage while the first target was placed in its original place. Subject animals were allowed to explore for 10min. Sociability was reported here through direct sniffing time to the chrome wire

cage containing either no mouse or one target mouse within 2cm radius during sociability phase. Social novelty was reported here by direct sniffing time to the cage containing familiar target mouse or the novel target mouse during social novelty phase.

Barnes maze

Barnes maze was used here to assess rodents learning ability and spatial memory. Both female and male mice were tested. Barnes maze apparatus (ePlastics, San Diego CA) was a circular table with diameter of 90cm and 56.2cm tall. And there were 20 circular holes with a diameter of 5cm evenly located around the perimeter of the table. During testing phase, 19 holes were inserted with false escapes box underneath the holes, while one hole was inserted with a real escapes box (15cm length x 6cm width x 6.6cm depth) and allowed animals to fully enter. The location of the real escapes box was set different among animals and remain constant from day 1 to day 4 (acquisition training phase), and real escapes box location was 180-degree reverse and remain unchanged during day 6 to day 9 (reversal training phase). 4 different shaped and colored visual cues were applied to the 4 sides of the table. One day before the start of acquisition training phase, animals were habituated in a 2L glass beaker above the real escapes box with designated location for 5 min. All visual cues were blocked during this habituation. On day 1 to day 4 of acquisition training phase, animals were released in an open-lid start chamber in the center of the table with a 4000 Hz buzzer turned on. The animals were allowed to explore the table for up to 3min. The end of each trial was defined as either the animal entering the real escape box voluntarily or after 3min trial time animal was gently guided to the real escapes box. Animals were allowed to stay inside the real escapes box for 1 min with buzzer turned off and then transferred back to its home cage. Each animal was tested for 2 trials per day with a 15min

between-trial interval time for 4 days. On day 5 (acquisition probe day), the designed real escapes box location was replaced with a false escapes box and each animal was allowed to explore the table for 3 min then transfer back to its home cage. On day 6 to day 9 (reversal training phase), each animal was tested for 2 trials per day with a 15min between-trial interval time for 4 days. On day 10 (reversal probe day), each animal was allowed to explore the table for 3 min with buzzer turned on and then transferred back to its home cage. The latency to locate and enter the real escapes box during acquisition training and reversal training phases were reported here. On acquisition probe day and reversal probe day, the table was evenly divided into four quadrants. The duration that animals spent in each quadrant during day 5 and day 9 was reported here.

Results

Pk1 disrupted mice showed normal locomotor activity

In order to study if the animals' locomotor activities as a behavioral factor, the open field assay was used. The test animals were set to explore the open field chamber for 10 minutes. The total travel distance was reported and analyzed. *Pk1 R104Q/+* and *Prickle1 R104Q/R104Q* mutant mice spent similar time exploring the chamber relatively to their wildtype littermates, which there was no significant difference in the total travel distance across wildtype mice, *Pk1* heterozygous and homozygous mutant mice (Figure 2A). This indicates *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* mutant mice have normal locomotor activity relatively to wildtype mice.

The total transition times in the light-dark transition test is also a measurement for animals' locomotor activity. Study has shown that the transition times in light-dark transition assay are more relevant to locomotor activity than anxiety-link behavior [35]. There is no significant difference in the total transition time among wildtype mice, *Pk1* heterozygous and homozygous mutant mice (Figure 2B), indicating locomotor activity is not affected in *Pk1* disrupted mice.

Novel object recognition deficit was exhibited in Pk1 R104Q/R104Q mice

Novel object recognition test is used to study animals' learning and memory without external stimuli. This assay is mainly based on rodents' innate exploratory instinct [36]. During the first 5 minutes training phase when animal was exposed to two identical objects in the modified open field chamber, all three kinds of animals including wildtype mice, *Pk1*

heterozygous and *Pk1* homozygous mice showed no significant preference exploring object 1 and object 2 (Figure 3A). However, only *Pk1 R104Q/R104Q* mice showed no significant discrimination in terms of preference to the novel and familiar objects during the testing phase (Figure 3B). While wildtype and *Pk1 R104Q/+* mice showed significant preference to the novel object relative to the familiar object (Figure 3B).

Pk1 disrupted mice exhibited abnormal sociability but normal social novelty

Three chamber social test was used here to assess the sociability and social novelty in *Pk1* mutant mice and wildtype littermates' control. During the social interaction phase, when one target mouse was introduced to one side of the chamber, only the wildtype mice showed significant preference to the target animals rather than the empty cage on the other side of the chamber (Figure 4A). *Pk1* heterozygous and homozygous mutant mice showed no significant preference to the target mouse and empty cage suggesting that *Pk1* mutant mice have an abnormal sociability (Figure 4A). During the social novelty phase, all three types of animal showed a stronger preference to the novel target mouse when they were exposed to one familiar and one novel target mice on two side chambers (Figure 4B). It suggests that these animals have normal social novelty.

Pk2 disrupted mice exhibited normal learning ability and spatial memory in Barnes maze test

Barnes maze is a behavioral test that used to assess rodents' spatial memory and learning ability. *Pk1* heterozygous and homozygous mutant mice showed similar learning curve to locate the escape hole and enter escapes hole without statistical significance as the wildtype mice

during the acquisition training phase (Day 1 – Day 4) (Figure 5A-5B). At acquisition probe day (Day 5), *Pk1* heterozygous and homozygous mutant mice spent similar time with wildtype littermates in all four quadrants including: the target quadrant, opposite quadrant, target quadrant to the left and target quadrant to the right (Figure 5C). But all three types of animals spent significantly more time in the target quadrant than the other three quadrants which indicating that *Pk1* disrupted mice have normal spatial memory as the wildtype mice (Figure 5C). During reverse training phase (Day 6 – Day 9), *Pk1* mutant mice resembled wildtype littermates in the learning curve of locating and entering the opposite escapes box without any significant discrimination (Figure 5D-5E). On the reverse probe day (Day 10), *Pk1* heterozygous and homozygous mutant mice spent similar time with wildtype littermates within all four quadrants (Figure 5F). All three types of animals spent significantly more time in the target quadrant than the other three quadrants which indicating that *Pk1* disrupted mice have normal spatial memory as the wildtype mice (Figure 5F).

Discussion

Study has shown that human *Prickl1 R104Q* missense mutation was found and shared in patients with progressive myoclonus epilepsy-ataxia syndrome among three unrelated families, whereas this mutation was not previously discovered [34]. This evolutionary highly conserved missense mutation is located inside the protein interaction PET domain and preventing REST binding for further downregulation of the gene, whereas the targets of REST gene are ion channel and neurotransmitter etc. that could further involve in PME-Ataxia [34]. Although the role of human *Pk1* in PME-Ataxia pathogenesis is not well understood yet, the study of *Pk1* in animal model could further help to understand this gene better. In addition, *Pk1* +/- mutant mice showed altered social behavioral, irregular circadian rhythm and repetitive behaviors that resemble ASD patients phenotypes [18]. *Pk1* also interacts with Synapsin I D Domain, which is a known region that related to both epilepsy and autism [18,31]. Due to the high cooccurrence of epilepsy in ASD patients, *Pk1 R104Q* missense mutation may also play a role in ASD [29-30].

Here, we have found that *Pk1 R104Q* heterozygous and homozygous mutant mice have normal locomotor activity from open field assay and light-dark transition test. But *Pk1 R104Q* homozygous mutant mice have a novel object deficit that animals were not able to distinguished between the familiar and novel objects. In the three-chamber social test, *Pk1 R104Q* heterozygous and homozygous mutant mice showed abnormal sociability but normal social novelty. *Pk1* mutant mice showed similar learning abilities and normal spatial memory relatively to wildtype littermates control suggesting that *Pk1* disrupted animals have normal learning ability and spatial memory.

Our data has shown that *Pk1 R104Q* mutant mice have abnormal social interaction ability that associated with ASD phenotype and alter novel object recognition. Further behavioral assays including elevated plus maze and ultrasonic vocalization are needed to assess their anxiety level and further sociability. Since *Pk1 R104Q* missense mutation is first discovered in patients with PME-Ataxia patients, epilepsy phenotypes related behavioral assay including induced seizure threshold test is needed for better understanding of *Pk1*. In conclusion, we demonstrated here that *Pk1 R104Q* mutant mice have normal locomotor activity, learning ability and spatial memory but novel object recognition deficit and abnormal social interaction that associated with ASD phenotypes.

Figures

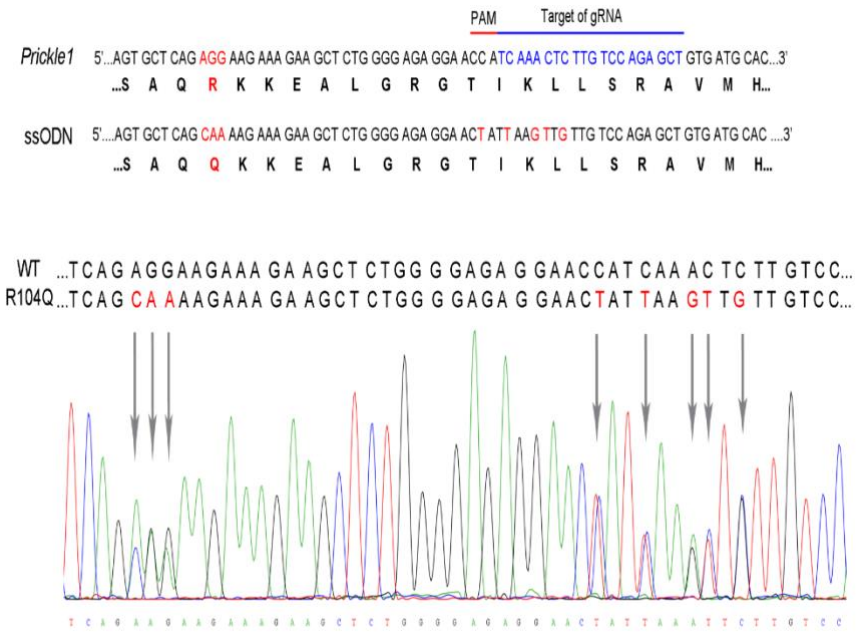


Figure 1. *Prickle1 R104Q* CRISPR design and sequencing data. The original sequence of *Prickle1* with corresponding guide RNA and PAM site are shown here. AGG (Arginine) was mutated to CAA (Glutamine) at locate 104. Sequencing result of *Pk1 R104Q* heterozygous mutant mice confirmed the expected missense mutation in the mutant allele.

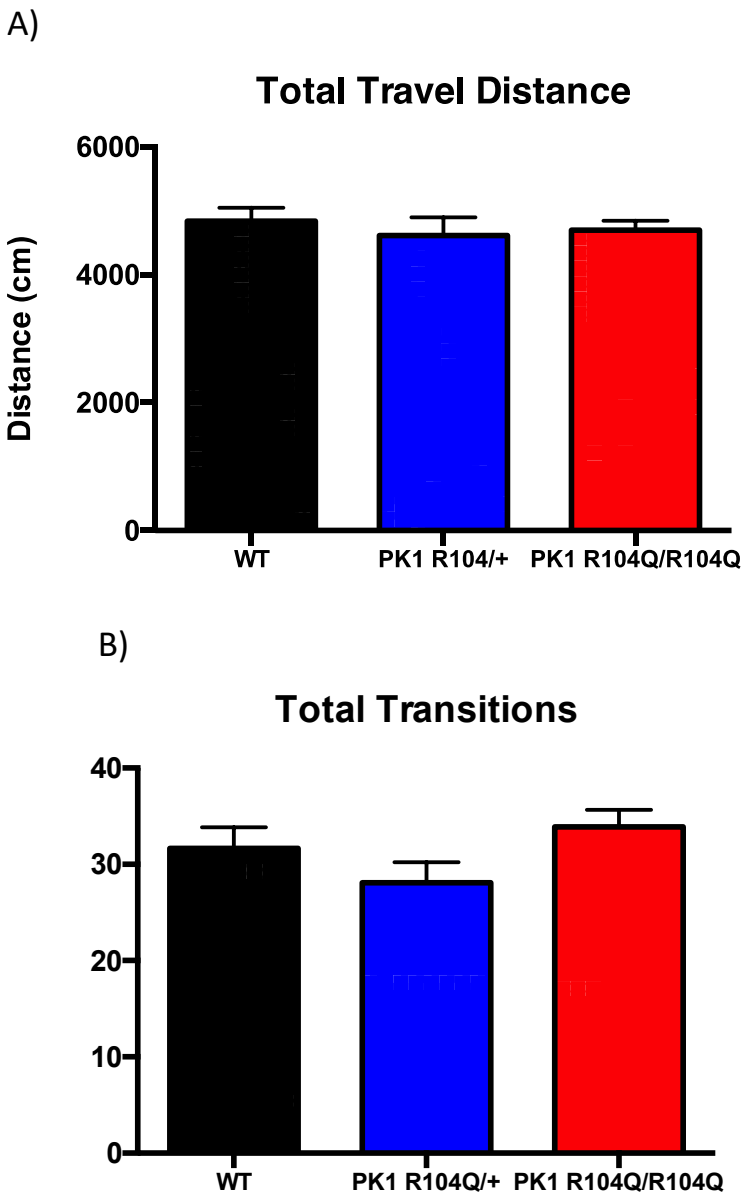


Figure 2. *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* showed normal locomotor activity. A) Open field assay. Total traveled distance in centimeter. B) Light-dark transition assay. Total transition time between light enclosure and dark enclosure. A) Wildtype (in black), *Pk1 R104Q/+* (in blue) and *Pk1 R104Q/R104Q* (in red) traveled similar amount of distance (Wildtype, n = 16, *Pk1 R104Q/+* n = 14, *Pk1 R104Q/R104Q* n = 14, both female and male). B) Wildtype (in black), *Pk1 R104Q/+* (in blue) and *Pk1 R104Q/R104Q* (in red) have no significant discrimination in total transition time (Wildtype, n = 17; *Pk1 R104Q/+*, p = 0.2602, n = 14; *Pk1 R104Q/R104Q*, p = 0.4306, n = 17; both female and male). Error bars represent s.e.m.

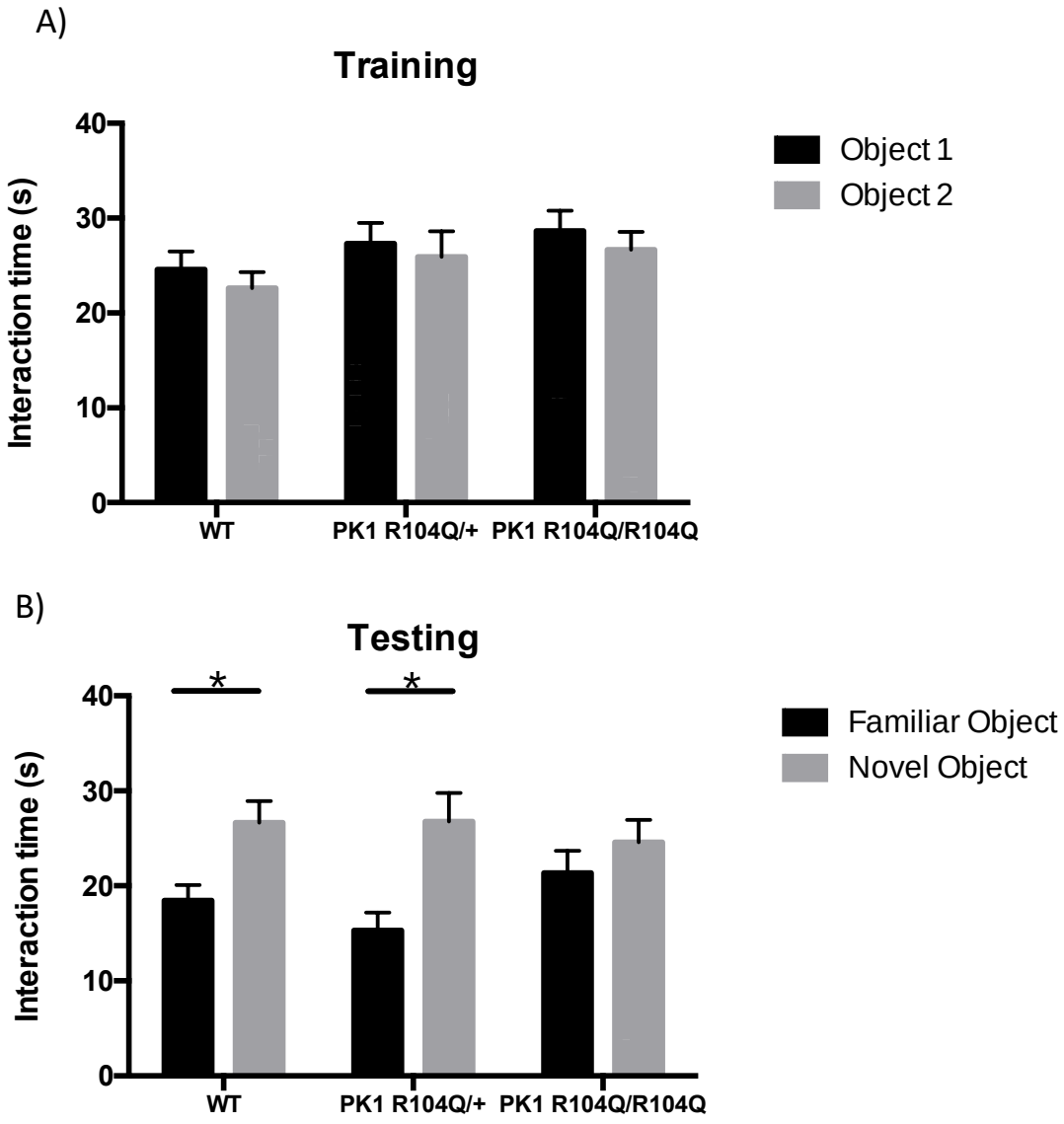


Figure 3. *Pk1 R104Q/R104Q* mice exhibited novel object recognition deficit. A) Novel object recognition training phase. B) Novel object recognition testing phase. Direct interaction time with each object. A) Wildtype, *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* spent similar amount of time interacting with the identical object 1 and object 2 (Wildtype, n = 14, *Pk1 R104Q/+*, n=14, *Pk1 R104Q/R104Q*, n = 14, both female and male). B) Wildtype (*p = 0.0064) and *Pk1 R104Q/+* (*p = 0.0033) spent significant more time interacting with the novel object than the familiar object during testing phase. While *Pk1 R104Q/R104Q* (p = 0.3381) spent similar amount of time exploring the two objects. Error bars represent s.e.m.

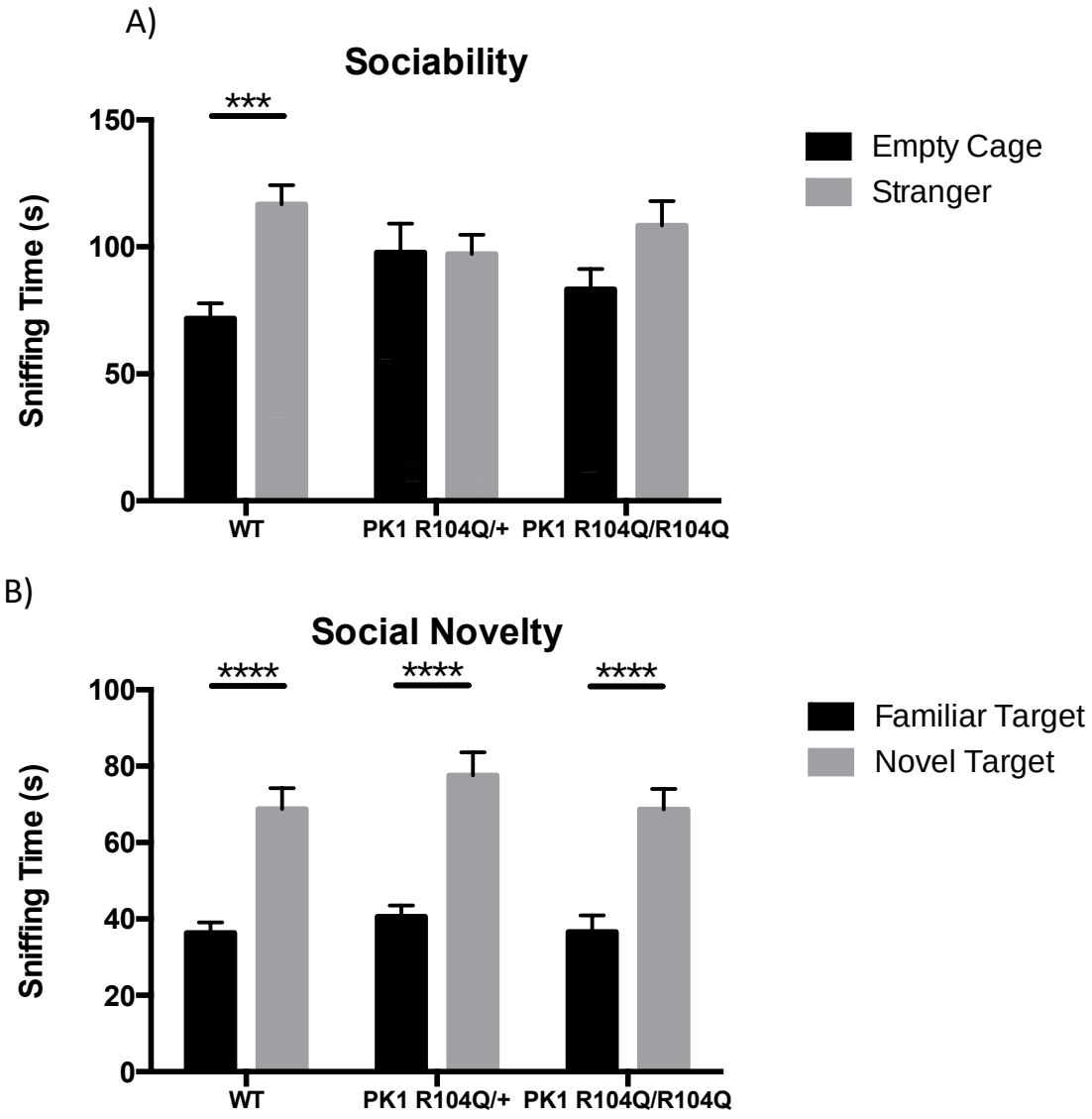
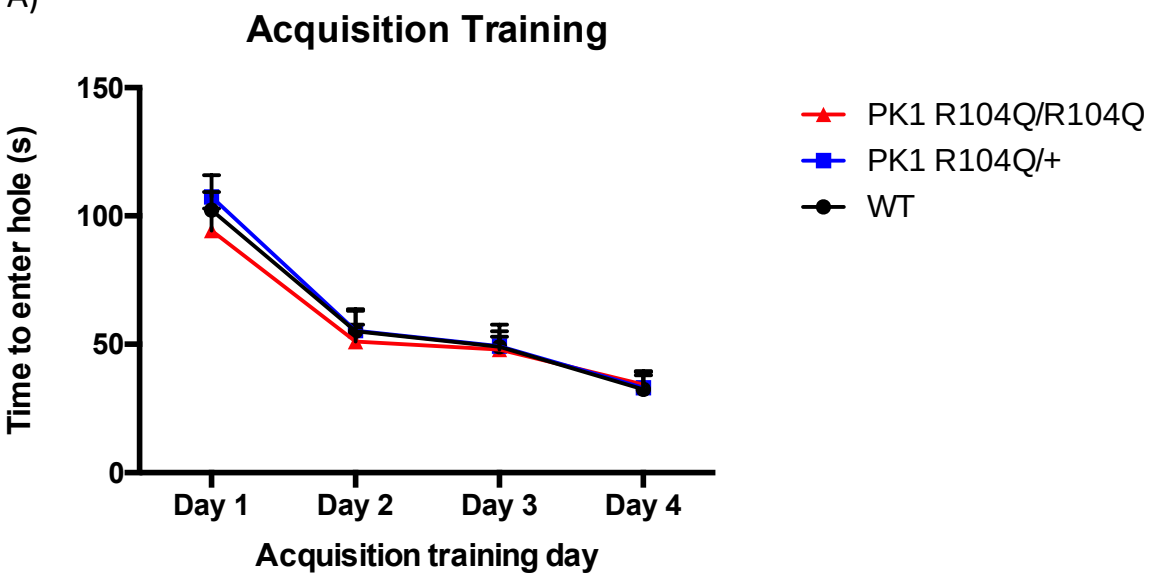


Figure 4. *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* show abnormal sociability but normal social novelty. A) Sociability phase. B) Social novelty phase. Duration of sniffing time to empty or cage containing target mouse. A) Wildtype (*** p = 0.0000618, *Pk1* +/+, n = 15) spent significant more time sniff the conspecific mouse than empty cage. While *Pk1 R104Q/+* (n = 13) and *Pk1 R104Q/R104Q* (n = 16) spent similar amount of time interacting with empty and stranger mouse. B) Wildtype (**** p = 0.0000074. *Pk1* +/+, n = 18), *Pk1 R104Q/+* (**** p = 0.0000063, n = 15) and *Pk1 R104Q/R104Q* (**** p = 0.0000521, n = 18) all spent significantly more time sniffing the novel target mouse than the familiar target mouse. Error bars represent s.e.m.

Figure 5. *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* showed normal learning ability and spatial memory. A, B and C) Barnes maze. Acquisition of escapes hole. A) Latency to enter escapes box. B) Latency to first locate escapes box. A and B) *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* mice acquire the escapes hole normally as wildtype. C) Acquisition probe test. TA: target area, OP: opposite area, AL: target area to the left, AR: target area to the right. Wildtype (*** p = 0.0004), *Pk1 R104Q/+* (* p = 0.0245) and *Pk1 R104Q/R104Q* (** p = 0.0039) spent significantly more time in the target quadrant than other three quadrants. *Pk1 R104Q/+ and Pk1 R104Q/R104Q* spent similar time in each quadrant relatively to wildtype mice. D, E and F) Reverse learning phase. D and E) *Pk1 R104Q/+* and *Pk1 R104Q/R104Q* mice acquire the reversed escapes hole normally as wildtype. F) Reverse probe test. Wildtype (*** p = 0.0008), *Pk1 R104Q/+*(* p = 0.0453) and *Pk1 R104Q/R104Q* (** p = 0.0071) spent significantly more time in the target quadrant than other three quadrants. *Pk1 R104Q/+ and Pk1 R104Q/R104Q* spent similar time in each quadrant relatively to wildtype mice. (Wildtype, *Pk1 +/+*, n = 19; *Pk1 R104Q/+*, n = 19; *Pk1 R104Q/R104Q*, n = 19). Error bars represent s.e.m.

A)


Acquisition Training
Time to enter hole (s)
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Acquisition training day
PK1 R104Q/R104Q
PK1 R104Q/+
WT

B)

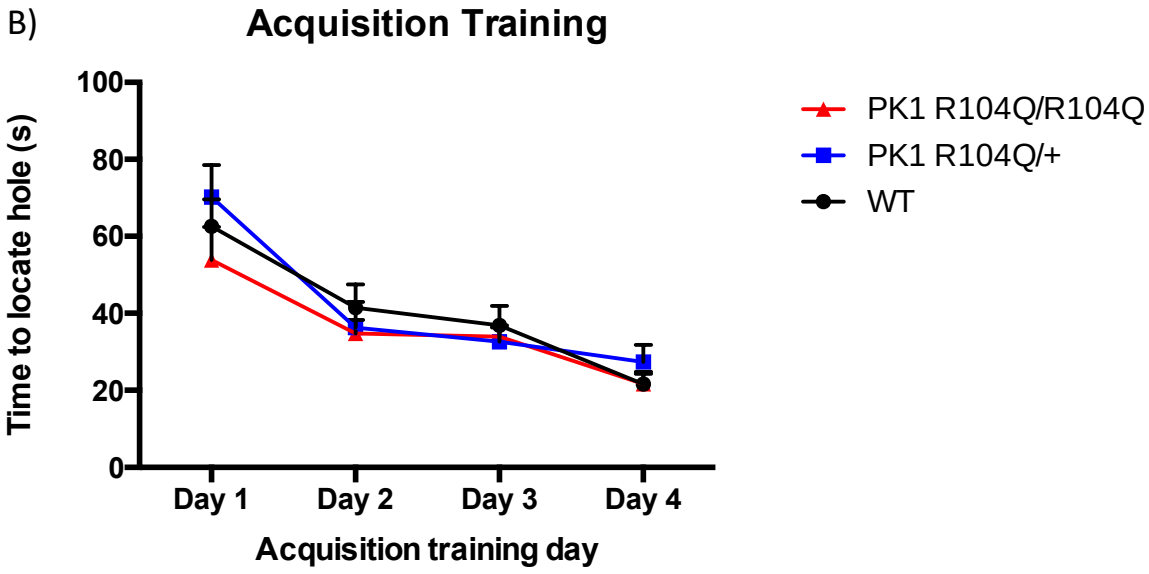
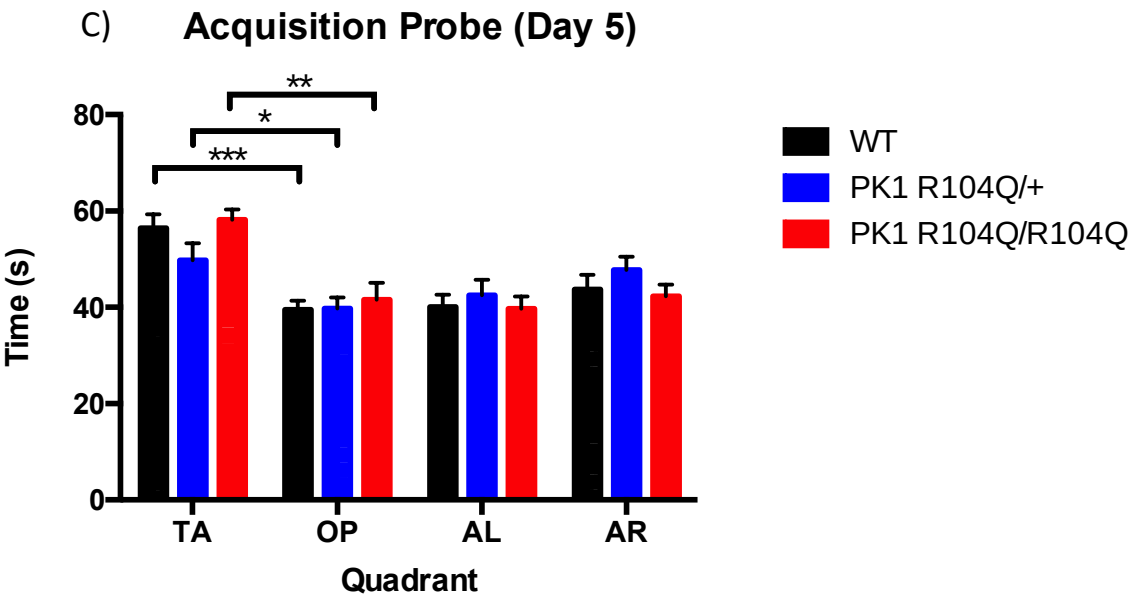

Acquisition Training
Time to locate hole (s)
100
80
60
40
20
0
Day 1
Day 2
Day 3
Day 4
Acquisition training day
PK1 R104Q/R104Q
PK1 R104Q/+
WT

Figure 5. continued



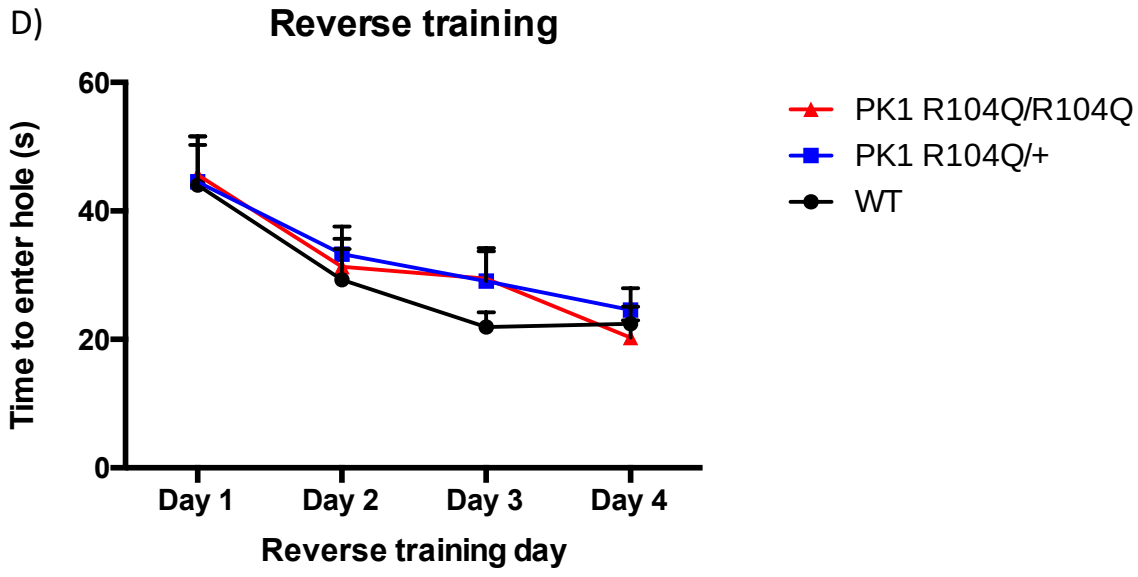
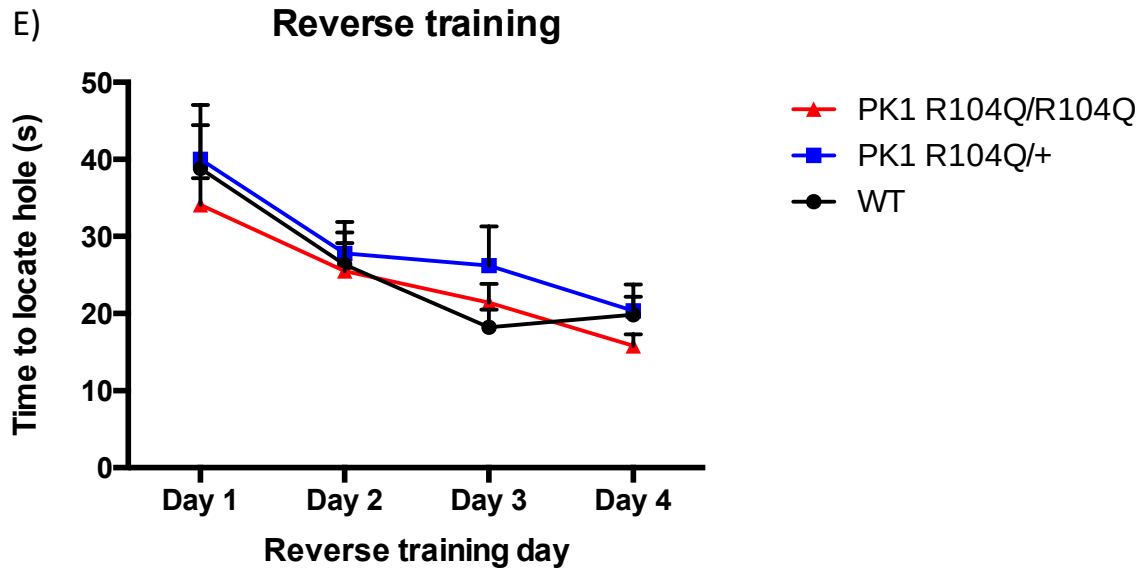
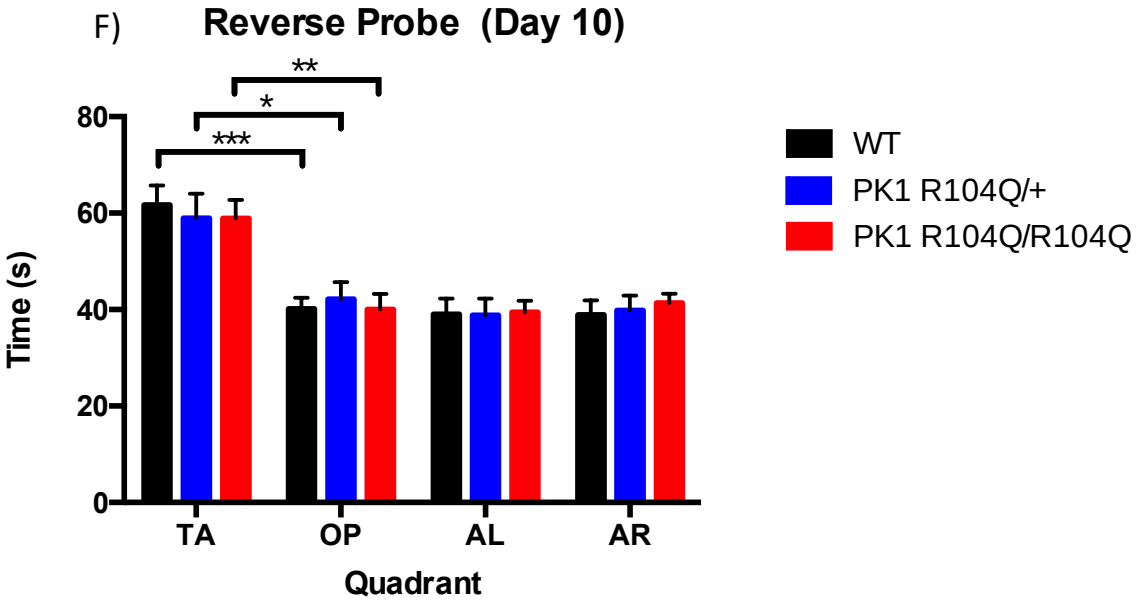


Figure 5. continued





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