Characterization of Social and Repetitive Behaviors of *Mllt11/Af1q/TcF7c* Conditional Knockout Mice

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Running Title: Sex Differences in *Mllt11* Conditional Knockout Mice

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Abstract

Mllt11 (myeloid/lymphoid or mixed-lineage leukemia translocated to chromosome 11; also known as *Af1q/TcF7c*) has been identified as a novel regulator of neural development, playing a role in the migration and outgrowth of cortical projection neurons. We previously reported that the conditional inactivation of the *Mllt11* gene in the mouse superficial cortex resulted in reduced connectivity of the corpus callosum and white fiber tracts, resulting in reduced cortical thickness. However, the behavioral consequences of *Mllt11* loss are unknown. Callosal abnormalities are thought to be present in 3-5% of all neurodevelopmental disorders and reduced corpus callosum volume correlates with core symptoms of autism spectrum disorder (ASD) in humans. Cortical thickness dysregulation is likewise shared among various neurodevelopmental disorders including ASD. We therefore investigated the behavioral consequences of conditional knockout of *Mllt11* in upper cortical layer 2/3 projection neurons using transgenic *Cux2*^{iresCre} mice. Utilizing tasks designed to reflect core ASD symptoms, we examined the behaviors of both male and female conditional knockout animals. These tests included olfaction habituation/dishabituation, three-chambered social approach, marble burying, and nestlet shredding. We found sex-dependent disruptions in social preference, and nestlet shredding in animals lacking *Mllt11*, with the female mice presenting with more disruptions than the males. Understanding the behavioral phenotype associated with genes of interest specifically in the context of sex differences is crucial to individualized treatment for neurodevelopmental disorders.

Introduction

The genesis of the mammalian brain is dependent upon the regulation of cytoskeletal proteins that promote the migration of neurons, extension of axonal growth cones and dendrites, and ultimately functional, synaptically-coupled networks[1,2]. Specifically, axons and dendrites are underlain by a structural network of microtubules (MTs), actin, intermediate filaments, and associating proteins that change dynamically to promote neurite outgrowth and synaptogenesis[1]. It is therefore not surprising that mutations in MTs and associated proteins are implicated in neurodevelopmental disorders (NDDs). Our lab has been investigating a neuronally-restricted cytoskeletal-associated protein called Mllt11 (myeloid/lymphoid or mixed-lineage leukemia translocated to chromosome 11; also known as Af1q, or Tcf7c). A previous report identified a novel copy number variant (CNV) in *Mllt11* in an individual with ASD. The affected chromosomal region included a deletion of MLLT11 among other genes, although at the time, as the authors mentioned "the function..[of MLLT11]..is either unrelated to brain function or poorly characterized"[3]. Our investigations have since provided evidence for the role of Mllt11 in neural development, regulating both migration of cortical neurons, and neuritogenesis, which is crucially required for the establishment of neuronal connectivity. We found that *Mllt11* is enriched in developing neurons during the formation of the upper layers (UL) 2/3. Thus, we generated a conditional knockout (cKO) mouse that inactivated *Mllt11* in the UL 2/3 of the cerebral cortex using the *Cux2*^{iresCre} driver mouse line[4-6,7]. We found that *Cux2*^{iresCre}; *Mllt11*$^{flox/flox}$ cKO animals displayed a thinning of the cortex, reduced complexity of superficial CPN arborization patterns, and reduced axonal projections across the corpus callosum[8]; consistent with anatomical abnormalities reported in various NDDs including ASD and SZ[9-15]. Although divided into distinct disorders, NDDs converge on various levels in symptoms, behavior, biomarkers, and genetic risk[16] and are often co-diagnosed[17-20]. Two such convergences are the development of the corpus callosum and altered cortical thickness[21]. Two hundred million callosal axons integrate and transfer information between the two hemispheres in the human brain[22,23]. This communication is integral for the various functions of the cerebral cortex including management of social and emotional stimuli; as such, abnormalities in the corpus callosum are thought to be present in 3-5% of all neurodevelopmental disorders[24,25]. Differences in cortical thickness, likewise, are shared among at least 6 common NDDs[21,26,27]. *Mllt11* has also been found to be dysregulated in fetal mice following maternal Valproic Acid (VPA) administration - a common mouse model used to study ASD[28,29]. Given that *Mllt11* cKO mouse brains share histological abnormalities with individuals with NDDs, its dysregulation following VPA, and the CNV of the region containing *Mllt11* in an ASD patient, we hypothesized that Mllt11 loss of function in our *Cux2*^{iresCre}-driven *Mllt11* cKO mice would display ASD

behavioral symptomology. To investigate this, we selected behavioral tasks which reflect the core human ASD symptomology[30]: communication via the olfactory habituation dishabituation task; sociability via the three-chamber social preference task; and examination of repetitive/compulsive behaviors via marble burying, nestlet shredding and scoring of behaviors such as digging, and grooming during other tasks.

Materials and Methods

Animals

All experiments described herein were performed according to approved IACUC protocols at Dalhousie University. Mice were generated as previously described[8]. Briefly, *Mllt1*$^{flox/flox}$; *Rosa26*^{TdTomato} *mice* were crossed with *Mllt11*$^{flox/+}$;*Cux2*^{iresCre};*Rosa26*^{TdTomato} mice to generate the following mice for testing: littermate Control animals – *Mllt11*$^{flox/+;}$*Rosa26*^{TdTomato}, Heterozygous animals – *Mllt11*$^{flox/+}$; *Cux2*^{iresCre};*Rosa26*^{TdTomato}, and finally conditional knockouts (cKO) – *Mllt11*$^{flox/flox}$;*Cux2*^{IRESCre};*Rosa26*^{TdTomato}. As these crosses did not allow for littermate controls that express Cre alone, Cre control animals were generated by crossing *Rosa26*$^{TdTomato+/-}$ mice with *Cux2*^{iresCre};*Rosa26*$^{TdTomato+/-}$ mice. These crosses generated the following mice for testing: WT - *Mllt11*$^{+/+}$;*Rosa26*^{TdTomato} and Cre – *Mllt11*$^{+/+}$;*Cux2*^{iresCre};*Rosa26*^{TdTomato}. Both male and female mice of the correct genotype were used for each experiment. Animals were housed with sex matched littermates. Animals were ear clipped on P8 and ear clips were used for genotyping. The offspring produced were all genotyped via PCR as described previously[8].

Behavioral Analysis

All behavioral experiments took place during the light cycle between (7am-7pm). A full timeline of the experiments performed can be seen in **Figure. 1**. Animals were weaned on postnatal day (P) 23. One week prior to initiation of testing, animals were brought daily to the testing room for habituation to the room and handling. First, the animals were left for 10 minutes to habituate to the testing room. After 10 minutes, the animals were gently handled, weighed, and then returned to their home cage in the animal colony room.

Olfaction Habituation/Dishabituation

This test was performed on P30 and utilized the procedures outlined by Yang and Crawley [31]. Animals were placed into a clean, empty cage with corncob bedding and brought to the testing room. A

20-minute period of habituation was initiated by insertion of a clean, dry cotton swab into the cage. Following habituation, sequences of three swabs dipped in identical odors were inserted into the cage for 2 minutes each with 1-minute intertrial intervals. The odors are as follows: (1) distilled water, (2) vanilla extract 1:100, (3) lemon extract 1:100, (4) swabs soaked in distilled water and run in a zig-zag pattern along the bottom of a three-day old unfamiliar, dirty mouse cage of each sex acquired fresh the day of testing. Presentation of the odors was done in the following order: three distilled water, three non-social odor 1, three non-social odor 2, three social odor 1, and three social odor 2, and the order of specific odors within this paradigm was randomized and balanced across sexes and genotypes. Every subject was exposed to novel social odor from two different dirty cage sources. An experimenter blinded to the genotypes scored time spent sniffing the swab with a stopwatch. Video recordings were acquired and scored for repetitive behaviors (see below).

Three-Chamber Social Approach

This test was performed on P37. Our protocol was based upon a protocol from Kaidanovich-Beilin, Lipina, Vukobradovic, Roder and Woodgett [32]. Briefly, animals were first placed in a clean empty cage with corncob bedding and brought to the testing room. The mouse being tested was then placed in the center of the three-chambered apparatus with the two openings closed for 5 minutes (Habituation A). After 5 minutes, the doors to each side of the chamber were opened and the mouse was able to freely roam the entire arena for an additional 5 minutes (Habituation B). The right and left side of the chamber contained empty upside-down wire mesh pencil cups in the center. After 5 minutes, the doors to each peripheral chamber were closed when the testing mouse returned to the center chamber. A novel age and sex matched mouse was then placed inside the pencil cup in one of the adjacent chambers. (All novel mice were habituated to the enclosures the day before testing. Briefly described, the novel mice were placed in the pencil cup for 10 minutes each, then returned to the home cage in the testing room for 10 minutes, then returned to the pencil cup for 10 minutes. This was done until each novel mouse completed three 10-minute cycles in the enclosure.) Both doors of the chamber were then opened, and the testing mouse was able to freely explore the apparatus for 10 minutes. At the end of 10 minutes the doors to each chamber were closed once the mouse returned to the center chamber. A second novel mouse was then placed inside the previously empty pencil cup. The doors were then removed, and the mouse was able to freely roam the entire arena for an additional 10 minutes. Video recordings were acquired, and time spent sniffing the pencil cups, and time spent in each area of the apparatus were recorded for each segment of the experiment by an experimenter blind to the mouse genotype.

Marble Burying and Nestlet Shredding

This test was performed on P51. Our protocol was based upon a previously published protocol from Angoa-Perez *et al.*[33]. The mice were brought to the testing room as described previously and placed into corncob lined clean cages with a single pre-weighed cotton nestlet square in the center of the cage. Mice were left for 30 minutes and the amount of unshredded nestlet was weighed. The mice were then returned to their home cage in the colony room for 1 hour. During this time, standard rat cages were filled with 5 cm of fresh corn cob bedding. Glass marbles were placed in a grid pattern with 5 rows of 4 marbles evenly spaced. After 1 hour, mice were returned to the testing room and placed in the prepared rat cage and allowed to freely roam and interact with the marbles for 30 minutes. The number of buried marbles was recorded by an experimenter blind to the genotypes at the end of 30 minutes.

Statistical Analysis

Analysis was performed using Prism 10 software. Two-way ANOVAs were performed when comparing male or female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO animals on the cumulative olfaction habituation/dishabituation task and the three-chamber social task. One-way ANOVAs were performed when comparing male or female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO animals marble burying, nestlet shredding, grooming, digging, rearing, and total freezing. For Cre control cohorts, two-way ANOVAs were performed when comparing male or female WT vs Cre animals on the olfaction habituation/dishabituation task and the three-chamber social task. Unpaired T-Tests were performed with comparing male or female WT vs Cre animals marble burying, nestlet shredding, grooming, digging, rearing, and total freezing. For multiple comparisons, Sidak's test was used to explore main effects and Tukey's test was used to explore interactions found between two or more groups when the number of comparisons per column family was greater than one and Uncorrected Fisher's LSD was used to explore interactions found between two groups when the number of comparisons per column family was one. For all graphs, error bars reflect the standard error of the mean (SEM) and ns= not significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Results

Female cKO animals display reduced time spent with social odors

Olfactory ability and interest in social odors were assessed by evaluating habituation/dishabituation and time spent investigating social and non-social odors among the various groups. All three groups

(*Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO) in males and females displayed habituation to each of the tested odors as consecutive presentations of odors resulted in reduced interest **(Fig. 2a-b).** Furthermore, all three groups dishabituated as there was an increase in investigation time with each presentation of a novel odor **(Fig. 2a-b)**. We then evaluated the interest in social odors by examining the cumulative time spent investigating the social odors in each group **(Fig. 2c-d)**. Two-way ANOVA revealed no significant interaction of group and odor between the three male groups with the time spent with social or non-social odors ($F(2,31) = 0.002992$; $p=0.9970$; **Fig. 2c**). There was a significant effect of odor ($F(1,31) = 50.89$; $p < 0.0001$) and Sidak's multiple comparisons revealed all three groups spent significantly more time investigating the social odors (*Mllt11*$^{flox/+}$ $p=0.0005$; *Mllt11*$^{flox/+}$*;Cre*: $p=0.0018$; cKO $p=0.0005$; **Fig 2c**). However, two-way ANOVA within the females revealed a significant interaction ($F(2,34) = 3.881$; $p=0.0303$) and Tukey's multiple comparisons found that the female cKO animals spent significantly less time investigating the social odors than the *Mllt11*$^{flox/+}$ ($p=0.0474$) and Mllt11$^{flox/+}$;Cre ($p=0.0007$) animals **(Fig. 2d)**. As with the male mice, all three female groups spent significantly more time investigating the social odors than the nonsocial odors (*Mllt11*$^{flox/+}$ $p<0.0001$; *Mllt11*$^{flox/+}$*;Cre*: $p<0.0001$; cKO $p=0.0342$; **Fig. 2d**).

To ensure that the effects in the *Mllt11*$^{flox/+}$*;Cre* and cKO animals were not due to Cre expression, we performed the same tests in our Cre control groups **(Fig 2e-h)**. Beginning with males, two-way ANOVA did not reveal a significant interaction ($F(1,13)=3.282$; $p=0.0932$; **Fig. 2g**) but did reveal an effect of odor ($F(1,13) = 45.37$; $p<0.0001$). Sidak's multiple comparisons revealed that both groups spent significantly more cumulative time investigating the social odors (WT $p<0.0001$; Cre $p = 0.0100$). Similarly, between the females, a two-way ANOVA did not reveal a significant interaction ($F(1,16)=4.478$; $p=0.0504$) between groups **(Fig. 2h)**. There was an effect of odor ($F(1,16)=31.64$; $p<0.0001$). Sidak's multiple comparisons revealed that although both groups sniffed the social odor more, the Cre group but not the WT group spent significantly more cumulative time with the social odors (WT $p=0.0624$; Cre $p<0.0001$; **Fig. 2h**). This suggests that cKO of *Mllt11* in females but not males resulted in reduced interest in social odors and that this difference is due to loss of both *Mllt11* copies and not simply expression of Cre as the effects of Cre expression alone were not identical to those of the *Mllt11* cKO groups.

Sex specific disruptions in social preference, novelty, and social anxiety

We next evaluated the social preference of the mice using the three-chamber social task. Beginning with the males, two-way ANOVA revealed a significant interaction between group and time spent in either chamber ($F(2,32)=3.868$; $p=0.0313$; **Fig. 3a**). Sidak's multiple comparisons showed that the

Mllt11$^{flox/+}$*;Cre* males and the cKO males both did not display a significant preference for the chamber containing the stranger mouse (*Mllt11*$^{flox/+}$*;Cre* p =0.5972; cKO p=0.1499; **Fig. 3a**). The *Mllt11*$^{flox/+}$ males however did display a significant preference for the chamber containing the stranger mouse (p=0.0001; **Fig. 3a**). As the previous test found no differences in investigation of social odors between the males, we next evaluated the investigation behavior of the males (defined as sniffing the pencil cup) (**Fig. 3b)**. In alignment with the social odor presentation test, two-way ANOVA revealed no significant interaction between groups ($F(2,32)=2.785$; p=0.0768) but did reveal a significant effect of chamber side ($F(1,32) = 78.68$; p<0.0001; **Fig. 3b**). Sidak's Multiple comparisons revealed that all three groups spent significantly more time investigating the pencil cup containing the stranger than the empty pencil cup (*Mllt11*$^{flox/+}$ p<0.0001; *Mllt11*$^{flox/+}$*;Cre*: p=0.0023; cKO p =0.0002; **Fig. 3b**). Demonstrating that all three groups distinguished and attended to the presence of the stranger mouse **(Fig. 3b)**.

Among the females, two-way ANOVA found no interaction between groups ($F(2,31)=1.951$;p=0.1591) but a significant effect among groups in the time spent in either peripheral chamber ($F(1,31)=53.64$;p<0.0001; **Fig. 3c**). Sidak's multiple comparisons test showed that the *Mllt11*$^{flox/+}$*;Cre*, and cKO mice all displayed a significant preference for the chamber containing the stranger (*Mllt11*$^{flox/+}$*;Cre* p<0.0001; cKO p<0.0001) while the *Mllt11*$^{flox/+}$ did not (p=0.0825; **Fig. 3c**). Evaluation of investigation behavior in the females revealed no significant interaction between groups ($F(2,31)=1.253$; p=0.2997) but did reveal a significant effect of chamber side within groups ($F(1,31)=156.4$; p<0.0001; **Fig. 3d**). Sidak's multiple comparisons revealed similarly to the males that all three female groups spent significantly more time investigating the pencil cup containing the stranger (Mllt11$^{flox/+}$ p<0.0001; *Mllt11*$^{flox/+}$*;Cre*: p<0.0001; cKO p<0.0001) once again demonstrating that, while they did not display social preference, they did display preferential investigation of the presence of the stranger mouse **(Fig. 3d)**.

To exclude any effects of Cre, we performed the same experimentation on cohorts of Cre controls. Neither male group displayed a significant preference for the chamber containing the stranger ($F(1,12)=0.002267$; p=0.9628; **Fig. 3e**). As this lack of preference mirrors that of the male *Mllt11*$^{flox/+}$*;Cre* males and the cKO, we believe the lack of preference cannot be explained by the lack of one or both copies of *Mllt11* alone. Examination of investigation behavior in the males revealed a significant effect of chamber side within groups ($F(1,12)=42.56$; p<0.0001) but no interaction between groups ($F(1,12)=2.599$; p=0.1329). Sidak's multiple comparisons showed that both the WT and the Cre animals spent more time investigating the pencil cup containing the stranger (WT p =0.0002; Cre p=0.0092; **Fig. 3f**). Within the female Cre control cohort, there was a significant interaction between group and chamber

side (F(1,13)=20.38; p=0.0006). Uncorrected Fisher's LSD multiple comparisons revealed that the WT animals displayed a significant preference for the empty chamber (p=0.0351) while the Cre females displayed a significant preference for the chamber containing the stranger (p=0.0009; **Fig. 3g)**. We then examined the amount of time the WT/Cre females spent investigating each pencil cup. We found that the Cre group, but not the WT group, spent significantly more time investigating the pencil cup containing the stranger (p<0.0001) and that the Cre animals spent more time than the WT animals investigating the pencil cup containing the stranger (p=0.0002; **Fig. 3h**). As these results are not what we saw in our three female Mllt11 groups, the effects seen in our Mllt11 groups can be attributed to *Mllt11* loss.

Examination of the novelty stage of the task identified some interesting results. In the male Mllt11 groups, two-way ANOVA found no significant interaction between group and chamber side (F(2,32) = 0.7323; p=0.4887) but did find a significant effect of chamber side within groups (F(1,32)=12.95;p=0.0011). Sidak's multiple comparisons showed that only the *Mllt11*$^{flox/+}$*;Cre* males spent significantly more time within the novel stranger side (p=0.0301; **Fig. 4a**). However, examination of investigation times showed that while there was no significant interaction between group and investigation choice (F(2,32)=1.695;p=0.1997) there was a significant effect of investigation choice within groups (F(1,32)=44.49;p<0.0001) that indicated via Sidak's multiple comparisons that only the *Mllt11*$^{flox/+}$*;Cre* and cKO spent significantly more time investigating the pencil cup with the novel stranger (*Mllt11*$^{flox/+}$*;Cre* p=0.0002; cKO p=0.0002; **Fig. 4b**).

In the females, there was not a significant interaction between groups and chamber side (F(2,31)=0.2531;p=0.7780) and no significant effect within groups and chamber side (F(1,31)=1.227;p=0.2765). Examination of investigation time found no significant interaction between groups (F(2,31)=0.2749;p=0.7615) but did find an effect within groups (F(1,31)=12.94;p=0.0011) however Sidak's multiple comparisons revealed that there were no differences in time spent investigating the novel vs familiar mouse in any of the three groups (*Mllt11*$^{flox/+}$ p=0.0631; *Mllt11*$^{flox/+}$*;Cre* p=0.2393; cKO p=0.1553; **Fig. 4d**).

We performed the same tests in our male and female WT/Cre animals to determine if the effects we saw were due specifically to the loss of *Mllt11* as opposed to Cre expression in cortical projection neurons. First, WT/Cre males showed no significant effect between (F(1,12)=2.171;p=0.1663) or within (F(1,12)=2.126;p=0.1705) groups in regard to time spent in chamber sides **(Fig. 4e)**. Similarly, there were no effects between (F(1,12)=2.171;p=0.1143) or within (F(1,12)=3.182; p=0.0997) groups in regard to investigation times **(Fig. 4f)**. As the effects seen in the Mllt11 groups were not replicated in the WT/Cre

groups, this indicates that the effects in the Mllt11 males are due to loss of *Mllt11* and not Cre expression. Examination of our WT/Cre females likewise found no significant effects between ($F(1,13)=1.506$;$p=0.2415$) or within ($F(1,13)=0.4029$;$p=0.5366$) groups and time spent on either chamber side **Fig. 4g**. When examining the investigation times, there was a significant interaction between groups ($F(1,13)=6.327$;$p=0.0258$) and within groups ($F(1,13)=5.085$;$p=0.0420$; **Fig. 4h**). Uncorrected Fisher's LSD multiple comparisons found that the WT females did not spend a significantly different amount of time investigating the novel mouse vs the familiar ($p=0.8692$) but the Cre females did spend significantly more time investigating the novel mouse than the familiar ($p=0.0023$; **Fig. 4h**). Furthermore, the investigation time towards the novel mouse was found to be significantly higher in the Cre group than the WT group ($p=0.2727$; **Fig. 4h**). These effects were absent in the *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre,* and cKO cohorts indicating that the results were not impacted by Cre expression alone.

We then compared freezing times between groups as a measure of social context-specific anxiety. One-way ANOVA revealed no significant difference between the males total freezing time across the entire session ($F(2,31)=0.9782$; $p = 0.0.3869$**; Fig. 5a).** To visualize whether the amount of freezing would correlate in any way to the addition of a stranger mouse, we visualized freezing specifically within each portion of the P37 test. Two-way ANOVA did not find a significant interaction ($F(6,96)=1.223$;$p=0.3011$) but did find an effect within groups ($F(2.395, 76.62)$;$p=0.0251$). Tukey's multiple comparisons showed that the male cKOs spent significantly less time freezing in Habituation B than Habituation A **(Fig. 5b)**. There was however a significant difference between the females ($F(2,31)=3.642$; $p=0.0380$) with Tukey's multiple comparisons revealing that the cKO females spent significantly more time freezing during testing than the *Mllt11*$^{flox/+}$*;Cre* animals and though not significant, they also spent more time freezing than the *Mllt11*$^{flox/+}$ animals as well **(Fig. 5c)**. We then visualized freezing specifically within each portion of the P37 test and found that, while the two-way ANOVA revealed there were no significant differences between the female groups ($F(6, 93)=1.594$;$p=0.1576$) or within groups at any one portion of the test ($F(1.168,36.20)=2.447$;$p=0.1224$), there was a large increase in freezing time in the cKO females with the introduction of the first stranger mouse potentially indicating a social context specific anxiety response **(Fig. 5d)**. There were no significant differences between the total freezing time of the WT/Cre male ($p=0.0687$; **Fig. 5e**). There were some significant differences when examining freezing at each portion of the test, however the differences showed Cre animals freezing less than WT animals during the Approach test portion ($p=0.0224$) a result which was not seen in our *Mllt11* heterozygous or cKO males **(Fig. 5f)**. Additionally, there was not a significant difference in the total freezing among the female WT/Cre control groups ($p=0.5076$; **Fig. 5g**) and no significant difference between groups at each portion

of the test (F(3,39)=0.7293;p=0.5407; **Fig. 5h**) indicating that the effect on freezing we saw in our *Mllt11* cKO females was not due to the presence of Cre alone.

Given the differences in freezing, we then investigated the exploratory locomotion of the mice via entry counts into any portion of the chamber (**Fig. 6)**.We did not find any significant differences between groups in the total number of entries made during any portion of the task for the males (F(4,64) = 1.721;p=0.1563) and all three male groups were more exploratory during the social approach (*Mllt11*$^{flox/+}$ p<0.0001; *Mllt11*$^{flox/+}$*;Cre* p=0.0007 cKO p<0.0001) and novelty (*Mllt11*$^{flox/+}$ p=0.0008; *Mllt11*$^{flox/+}$*;Cre* p=0.0004; cKO p<0.0001) portion of the task than during the habituation (**Fig. 6a)**. Similarly, we did not find any significant differences between the three female groups (F(4, 62) = 0.8274; p=0.5128), and all three female groups were more exploratory during the social approach (*Mllt11*$^{flox/+}$ p=0.0007; *Mllt11*$^{flox/+}$*;Cre* <0.0001; cKO p=0.0015) and novelty (*Mllt11*$^{flox/+}$ p=0.0022; *Mllt11*$^{flox/+}$*;Cre* p=0.0002; cKO p=0.0016) portion of the task than during the habituation (**Fig. 6b)**. The same was found to be true of the WT/Cre males (F(2,24)=2.852;p=0.0774; Approach: WT p=0.0044, Cre p=0.0005; Novelty: WT p=0.0231, Cre p=0.0038; **Fig. 6c**). For the Cre females, in addition to the increased exploratory behavior during approach (WT p=0.0002; Cre p=0.0003) and novelty (WT p=0.0205; Cre p=0.0029) for both groups (**Fig. 6d**), there was a significant difference between groups (F(2,26)=4.747;p=0.0174) with multiple comparisons revealing that the WT females were more exploratory than the Cre females during the approach portion (p=0.0229; **Fig 6d**). We then binned the entries data for the approach portion of the task for the WT/Cre females to understand this difference. The WT females made significantly more entries than the Cre females into the empty (p=0.0142) and the center (p=0.0223), but there were no differences in the number of entries to the stranger side (p=0.0780; **Fig. 6e**). This correlates with our previous results showing the WT females spending more time in the empty side than with the stranger and indicates that as their number of entries into the stranger side did not differ between groups that the differences in time spent in the stranger chamber are due to time alone and not lack of exploration into that chamber by the WT animals. In addition, these results indicate that the differences in freezing we found, particularly in *Mllt11* cKO females, did not impact the overall exploratory behaviors of the animals; finally, the differences in the WT/Cre animals were also not replicated in the *Mllt11* heterozygous and homozygous groups. Thus, we can conclude that the differences in preference and investigation times were not due to any generalized lack of ambulatory exploration or any effect of Cre on exploratory behaviors.

Female cKO animals display reduced nestlet shredding

On P51, we performed both a marble burying task and a nestlet shredding task. One-way ANOVAs found no significant differences in number of marbles buried between the male groups (*Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO) (F(2,31)=1.178;p=0.3213; **Fig. 7a**). After the animals returned to their home cage in the animal colony for one hour, we performed a nestlet shredding task. The males did not display any difference in percent of nestlet shredded (F(2,31)=0.3958;p=0.6765; **Fig. 7b**). Similarly we found no significant differences in number of marbles buried between the female groups (*Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO; F(2,29)=0.7560;p=0.4786; **Fig. 7c**). For the nestlet portion however, one-way ANOVA revealed a significant effect in the females (F(2,31)=5.279;p=0.0106) and Tukey's multiple comparisons revealed that the female cKO animals shredded significantly less nestlet than the *Mllt11*$^{flox/+}$ females (p=0.0089; **Fig. 7d**). We then confirmed that the effects we saw were not due to Cre expression alone via analysis of the WT/Cre groups. Similarly, we found no significant differences in the number of marbles buried by the male (p=0.9364; **Fig. 7e**) or the amount of nestlet shredded (p=0.2127; **Fig. 7f**). Between the WT/Cre female control groups, we found no significant difference in number of marbles buried (p=0.7041; **Fig. 7g**) and we confirmed that the reduction in nestlet shredding was not due to Cre expression as the female WT and Cre animals did not display any significant differences in the percentage of nestlet shredded (p=0.2435; **Fig. 7h**).

Mllt11 loss did not result in presentation of repetitive behaviors

As repetitive and stereotypic behaviors are a core phenotype of ASD, we desired to examine our mice for presentation of any repetitive behaviors. We measured the amount of time spent grooming, digging, and rearing within the P30 task. There were no significant differences for the *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO males in grooming (F(2,31)=0.4023;p=0.6722; **Fig. 8a**), digging (F(2,31)=0.4928;p=0.6156; **Fig. 8b**); or rearing (F(2,31)=1.648;p=0.2089; **Fig. 8c**). Likewise, within the *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO females there were no significant differences in grooming (F(2,34)=1.892;p=0.1663; **Fig. 8d**), digging (F(2,34)=1.647;p=0.2076; **Fig. 8e**), or rearing (F(2,34)=0.4441;p=0.6451; **Fig. 8f**). While there was no difference in male WT/Cre grooming (p=0.3482;**Fig. 8g**), or digging (p=0.2980; **Fig 8h**), the male Cre controls did rear significantly more than the WTs (p=0.0302;**Fig. 8i**). Among the female WT/Cre groups, there were no significant differences between groups for digging (p=0.3084;**Fig. 8j**) but there was a significant difference in digging as the Cre females spending significantly more time digging than the WT females (p=0.0131; **Fig.8k**). Finally, there was not a significant difference between the Cre/WT females and the number of rears (p=0.5781; **Fig. 78**).

Discussion

The current study revealed that *Mllt11* loss in mice resulted in sex-dependent behavioral alterations. Specifically, we found the female *Mllt11* cKO mice had reduced interest in social odors, however, when in the presence of social cues elicited by the physical presence of another mouse in the three-chamber task, the cKO females displayed the expected preference for the social side of the chamber as well as investigation of the stranger mouse. Interestingly, they then did not show a preference for social novelty in the final portion of the three-chamber task. The same effects were not seen in the Cre control female cohort, indicating these effects were specifically due to the loss of *Mllt11*. *Mllt11* cKO males, on the other hand, showed no disruption in social interest during the olfaction test, as well as normal social approach. However, only those lacking one or both copies of *Mllt11* investigated the novel mouse significantly more than the familiar mouse. Future studies will need to perform novel object recognition tasks to investigate the perception and preference for novelty in both the male and the female mice.

While the females did not show any significant differences in the marble burying task or in any measures of behaviors such as grooming, digging, or rearing, the female cKOs did shred significantly less nestlet. In the literature, excessive shredding is an indicator of repetitive behavior; however, reduced nestlet shredding has been viewed as restricted interest in novel objects[34]. In combination with the lack of preference for social novelty, we believe this is best interpreted as others as restricted interest in novel objects or decreased responsivity to novelty[34]. This would align with a symptom seen in children with ASD as some have been found to not show attentional preferences for novel stimuli under certain conditions[35]. Novel object recognition tasks should be performed in future studies to identify whether this interpretation is correct in our *Mllt11* cKO animals.

Finally, the higher total amount of freezing exhibited by the female cKO animals during the three chamber-chamber task with no increase in marble burying may be indicative of social context-specific anxiety. An increase in marble burying as well would be expected if there were a broad underlying higher baseline anxiety – thus we hypothesize that the increased freezing seen in the cKO animals may be increased anxiety specifically in the social context. This would correlate with what is seen in humans as higher social anxiety is seen in human females as opposed to human males with ASD[36].

Mechanisms and Sex Differences

Sex differences in NDD presentation are well documented[37]. For example, in ASD, females experience more severe internal symptoms such as anxiety and depression and, while both males and females have

disrupted social and communication skills. It is also suggested that females 'camouflage' their social deficits more than their male counterparts[38]. For this reason, we find it particularly intriguing that the female cKO displayed reduced interest in social odors but no differences in social preference or investigation time in a more social condition like the three-chamber social approach task. We hypothesize that the strength of the available social cues could contribute to these differences. The saliency of a swab from a dirty cage is expected to be lower than that of the physical presence of another mouse. As such, if the social disturbances are mild, one might expect the presence of more salient social cues to override any deficits. Indeed, in humans with ASD, it has been shown that the saliency of social cues is perceptible and affects attention despite an overall deficit in social attention[39]. Future studies should investigate ultrasonic vocalizations of these mice to better understand what degree of social deficits may exist.

In regard to mechanism, the impact of Mllt11 on maintenance of UL2/3-specific markers may provide a possible mechanism through which it may play a role in modulation of behavior. Previous work by our lab found that the loss of *Mllt11* resulted in decreased expression of Satb2 as well as CDP/Cux1 in the superficial somatosensory cortex[8]. First, CDP/Cux1 has been shown to play a role in regulating both neurite outgrowth and overall neuronal morphology, and its loss gives rise to neurons with shorter, less arborescent neurites[40,41]. Neuronal morphology altered in this manner has been established as a hallmark feature of both human and mouse ASD or ASD-like pathology[42,43]. Indeed, we previously reported that *Mllt11* cKO results in reduced callosal crossing fibers and less complex arborization patterns[8]. There is some evidence in the literature from animal models that indicates sex-dependent disruptions of Cux1 expression following prenatal exposure to bisphenol A (BPA)[44], an implicated risk factor for NDDs[45]. Second, Satb2 mutation in humans gives rise to SATB2 syndrome, an NDD characterized by intellectual disability, language and communication deficits, some ASD-like behaviors such as repetitive or restrictive movements and interests, as well as hyperactivity and aggression[46]. However, the effects of *Mllt11* cKO were mainly seen in our female mice and to date no sex-differences have been reported in Satb2 syndrome[47,48]. Future studies should examine whether the expression of Satb2 and CDP/Cux1 are reduced following *Mllt11* loss in a sex-dependent manner, as our previous work did not consider sex as a variable.

Another hypothesis is that the sex-specific effects may be due to two potential Mllt11 interactions, including Wnt signaling and non-muscle myosin NMIIA/B interactions. To begin, Mllt11 is known to activate Wnt signaling and is specifically implicated in the activation of the β-catenin-mediated canonical Wnt pathway[49]. It is hypothesized that the level of Wnt/β-catenin signaling could contribute to the phenotypical heterogeneity of ASD[50]. Research on the activation of the Wnt pathway in other NDDs,

supports this hypothesis. For example, in individuals with Schizophrenia, examination of the expression of DEK, a chromatin-remodeling phosphoprotein which, like Mllt11, is an oncogene[51] and activator of the Wnt pathway[52]. O'Donovan et al.[52] found that DEK protein depletion down-regulated the Wnt pathway and was a marker of cognitive impairment. However, these effects were sex-specific such that lower DEK, hypothetically less down-regulation of the Wnt pathway, in females was associated with more severe cognitive impairment, whereas in males, higher levels of DEK were associated with severity of cognitive impairment. As Mllt11 is known to activate Wnt signaling[49], we hypothesize that loss of *Mllt11* may alter Wnt/β-catenin signaling and differentially alter behavior thus contributing to the phenotypical heterogeneity of ASD[50].

Previous work from our lab has shown that Mllt11 interacts with two non-muscle myosin II (NMII) paralogs, NMIIA and NMIIB[10]. Furthermore, we found that *Mllt11* cKO resulted in increased expression of NMIIB [10]. Intriguingly, NMIIB overexpression is known to inhibit the Wnt/β-catenin pathway[53] while NMIIA activates Wnt/β-catenin signaling by interacting with β-catenin and controlling β-catenin transcriptional activity[54]. In mice, NMIIB was found to be differentially expressed in the brains of male and female mice and is a known ASD risk gene[55]. With this being the case, we hypothesize that the combined differential expression of NMIIA/B and its differential contribution on the Wnt/β-catenin pathway could contribute to the sex-specific disruptions. Future experiments should determine if *Mllt11* loss differentially regulates the Wnt/β-catenin pathway, whether the increased expression of NMIIB is found in both males and females, and whether these alterations are present consistently across the lifespan of the mice.

Cux2^{iresCre} knowns/unknowns

To our knowledge, we are the first to report behavioral effects due to *Cux2*^{iresCre} expression. Our Cre control groups displayed several significant differences that were unexpected. Our male Cre mice did not spend a significantly greater amount of time in the chamber or investigating the stranger mouse as opposed to the empty pencil cup. As we saw a similar lack of significance in our *Mllt11*$^{flox/+}$*;Cre*, and cKO males, this suggests that these particular effects were not solely due to the loss of *Mllt11*. The female Cre mice displayed a preference for the stranger mouse, both in time spent in the chamber and investigation time. However, the WT females displayed a preference for the empty chamber and did not investigate the stranger more than the empty pencil cup. These results are perplexing, and we included an additional measurement of freezing to see if this may have been the source of the effect. However, we did not see an increase in freezing behavior of the WT females, suggesting this lack of preference was

not due to anxiety. Further testing will be required to parse apart the source of this effect. However, since we did not see a similar effect in our female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO group, we believe this is a difference that does not impact the results of our study. We also found that the female Cre mice spent significantly more time digging; however, this result was not reflected in our female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups. Cre lines are frequently utilized with the assumption that the expression of the Cre gene results in either minimal or no phenotypic behavioral changes. Unfortunately, this has been shown to not be the case by many groups and within various Cre mice including ChAT-Cre lines[56,57], DAT-Cre [58,59], Nestin-Cre[60]. Others have also found sex-specific effects in other Cre mice[61]. Specifically, Baghdadi et al.[61] reported that when examining male and female Syn1Cre mice, they found a male-specific increase in anxiety-like behaviors and reduced body weight[61]. Interestingly, in the liver of mice and rats, Cux2 is expressed at significantly higher levels in females than in males[62] but whether sex-differences in Cux2 expression are present in the brain is unknown. For future studies, we believe it is important to perform more extensive behavioral testing of the *Cux2*^{iresCre} mice to determine if replication of these differences is possible and to investigate the contribution of Cre expression to these differences.

Another consideration when interpreting the behavioral phenotypes of mutant mouse models of NDDs is the background of the strain in which the mutation is maintained. Our line was maintained in a mixed C57Bl/6J/FVB/NJ background. C57Bl/6 mice have been shown, in some cases, to display various autism-like behaviors under wild type conditions. This phenomenon is likely due to the level of inbreeding required in maintaining the genetic purity characteristic of this strain[63]. Alternatively, genetic outbreeding and the genetic variability enhanced when crossing strains may also be a source of variability in behaviors. As the mice used in the experiments reported herein were derived from outbred C57Bl/6/FVB hybrid crosses, this requires careful consideration when parsing the impact of *Mllt11* loss from other potential sources of variation. To try to mitigate the effects of this variability, littermates of all genotypes were used as behavioral controls. For this reason, we believe that the littermate *Mllt11*$^{flox/+}$ mice are a better control than the WT mice which were not littermates.

Conclusion

In conclusion, we found that our female *Mllt11* cKO mice display more ASD-like behavioral deficits than our male *Mllt11* cKO mice, including reduced interest in social odors and reduced nestlet shredding. As 80% of those diagnosed with autism are male children, pre-clinical investigations typically focus specifically on male mice. This focus can have, as Murta et al[64] have argued, "detrimental consequences on our understanding of ASD etiology and pathophysiology." We therefore believe that the presentation

of a mouse model with ASD-like behavioral deficits in the females but not the males is intriguing and necessary to utilize in future investigations. Future studies are needed to identify the depth of the communication deficits as well as the perception of novelty as well as identifying the molecular contributions to the sex-differences we observed.

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Figure Legends:

Figure 1: Timeline of Experiments

Timeline of behavioral experiments described in detail within the methods section.

Figure 2: Olfaction Habituation/Dishabituation

Graphical representation of the recorded Time spent sniffing the swab during the olfactory habituation/dishabituation test. **a)** Time spent sniffing swabs in the male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups. **b)** Time spent sniffing swabs in the female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre,* and cKO groups. **c)** Cumulative time spent sniffing the two nonsocial odors vs the two social odors was compared in the male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups. **d)** Cumulative time spent sniffing the two nonsocial odors vs the two social odors was compared in the female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups. **e)** Time spent sniffing swabs in the male WT and Cre groups. **f)** Time spent sniffing swabs in the female WT and Cre groups. **g)** Cumulative time spent sniffing the two nonsocial odors vs the two social odors was compared in the male WT and Cre groups. **h)** Cumulative time spent sniffing the two nonsocial odors vs the two social odors was compared in the male WT and Cre groups. (For all graphs, ### = All three groups cumulative non-social time was significantly less than cumulative social time. ## = All two groups cumulative non-social time was significantly less than cumulative social time. $p<0.05$; * = $p<0.05$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 3: Three-Chamber Social Approach

Graphical representation of the data recorded during the Social Approach portion of the three-chamber task. This is the stage in which one side of the chamber remains empty while the other side contains the first stranger mouse. The time spent by the mice in either the 'Empty' chamber which contained just an empty pencil cup or in the 'Stranger' chamber which contained the stranger mouse within a pencil cup was recorded and compared between the **a)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **c)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **e)** male WT and Cre groups, **g)** female WT and Cre groups. As a measure of investigation, the time spent by the mice actively sniffing either the 'Empty pencil cup or the pencil cup containing the 'Stranger' was recorded and compared between the **b)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **d)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **f)** male WT and Cre groups, **h)** female WT and Cre groups. (For all graphs * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 4: Three-Chamber Social Novelty

Graphical representation of the data recorded during the Social Novelty portion of the three-chamber task. This is the stage in which a second Stranger mouse – the 'novel' mouse is added to the pencil cup in the previously empty side of the chamber. The time spent by the mice in either the 'Familiar' chamber, which contained the same 'Stranger' mouse from the Social Approach portion contained in in a pencil cup, or in the 'Novel' chamber which contained the 'Novel' mouse within a pencil cup, was recorded and compared between the **a)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **c)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **e)** male WT and Cre groups, **g)** female WT and Cre groups. As a measure of investigation, the time spent by the mice actively sniffing either the pencil cup containing the 'Familiar' mouse or the pencil cup containing the 'Novel' mouse was recorded and compared between the **b)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **d)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre,* and cKO groups, **f)** male WT and Cre groups, **h)** female WT and Cre groups. (For all graphs ns= not significant * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 5: Three-Chamber Freezing

Graphical representation of the time spent freezing during the various stages of the three-chamber task. The total time across the entire three-chamber task was recorded and compared between the **a)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **c)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **e)** male WT and Cre groups, **g)** female WT and Cre groups. To determine identify differential freezing behaviors across stages of the three-chamber task, the freezing in each stage of the test was presented independently. These sections were as follows: Habituation A: the time during which the mouse freely explored the center chamber while the doors to either side were closed; Habituation B: the time during which the mouse freely explored all three chambers; Approach: when the mouse could freely explore all three chambers, one of which now contained the 'Stranger'; Novelty: when the mouse could freely explore all three chambers, one side of which contained the previous 'Stranger' mouse now referred to as the 'Familiar' mouse, and the other side of the chamber now contained the 'Novel' mouse. The time spent freezing was recorded and compared between the **b)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **d)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **f)** male WT and Cre groups, **h)** female WT and Cre groups. (For all graphs ns= not significant * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 6: Three-Chamber Entries

Representation of the number of entries during the portions of the three-chamber task in which the doors were open. **a)** Total entries compared between the male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups during Habituation B, Social Approach, and Social Novelty. (### - indicates that all three groups are significantly different from their values in Habituation B) **b)** Total entries compared between the female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups during Habituation B, Social Approach, and Social Novelty. (### - indicates that all three groups are significantly different from their values in Habituation B) **c)** Total entries compared between the male WT and Cre groups during Habituation B, Social Approach, and Social Novelty. (## - indicates that all two groups are significantly different from their values in Habituation B) **d)** Total entries compared between the female WT and Cre groups during Habituation B, Social Approach, and Social Novelty. (## - indicates that all two groups are significantly different from their values in Habituation B) **e)** Binned entries into the empty, center, or stranger side during the Social Approach portion of the task for the WT and Cre females. (For all graphs ns= not significant * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 7: Marbles and Nestlet Shredding

Graphical representation of the tests performed on P55 which included the buried marble test followed by the nestlet shredding task. The number of marbles buried following 30 minutes in the rat cage was recorded and compared between the **a)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **c)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **e)** male WT and Cre groups, **g)** female WT and Cre groups. The percentage of the nestlet square was recorded following 30 minutes and compared between the the **b)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **d)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre,* and cKO groups, **f)** male WT and Cre groups, **h)** female WT and Cre groups. (For all graphs ns= not significant * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

Figure 8: Repetitive Behaviors

Graphical representation of the repetitive behaviors scored via video that occurred during the Olfaction Habituation/Dishabituation task performed on P30. These included grooming, digging, and rearing. **a-c)** male *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **d-f)** female *Mllt11*$^{flox/+}$, *Mllt11*$^{flox/+}$*;Cre*, and cKO groups, **g-i)** male WT and Cre groups, **j-l)** female WT and Cre groups. (For all graphs ns= not significant * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$)

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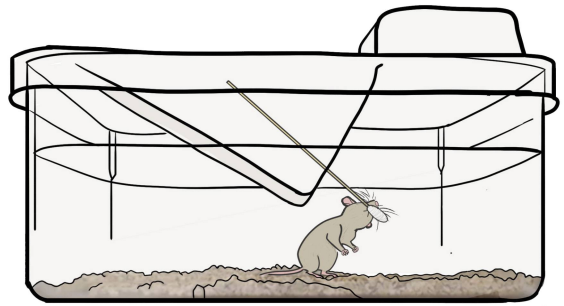
Timeline

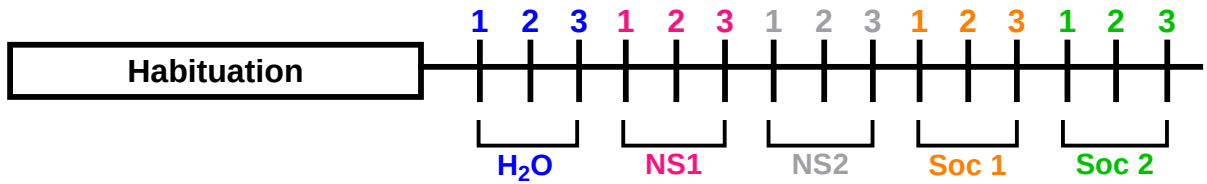
Habituation
7 days

P30

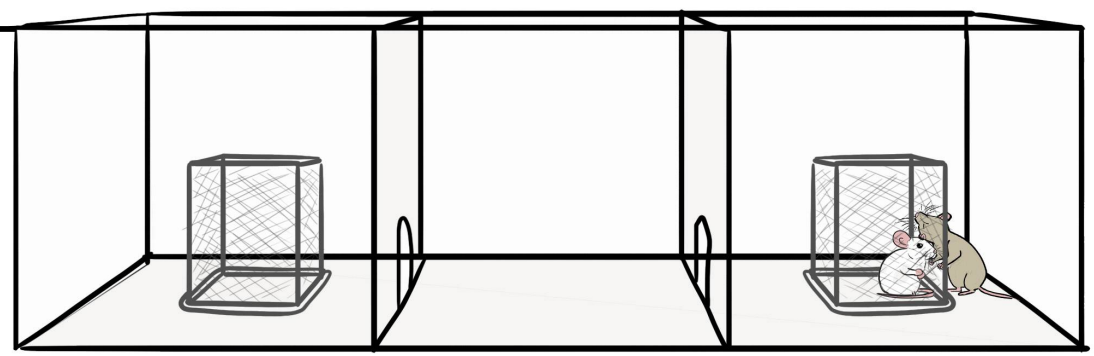
Habituation: 20 min
Scent Presentation: 2 min each
Interval Between Scents: 1 min

Odors Presented via Swab:
Nonsocial (NS) - Vanilla, Lemon
Social (Soc) - Dirty cage 1, Dirty cage 2





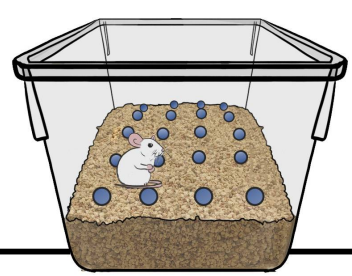
P37



Habituation A — 5 min:center
Habituation B — 5 min: doors open
Social Approach — 10 min: doors open
Social Novelty — 10 min: doors open

Stranger 1 Added
Stranger 2 Added

P51



Marble Burying

1 hour

Nestlet Shredding

Fig. 1

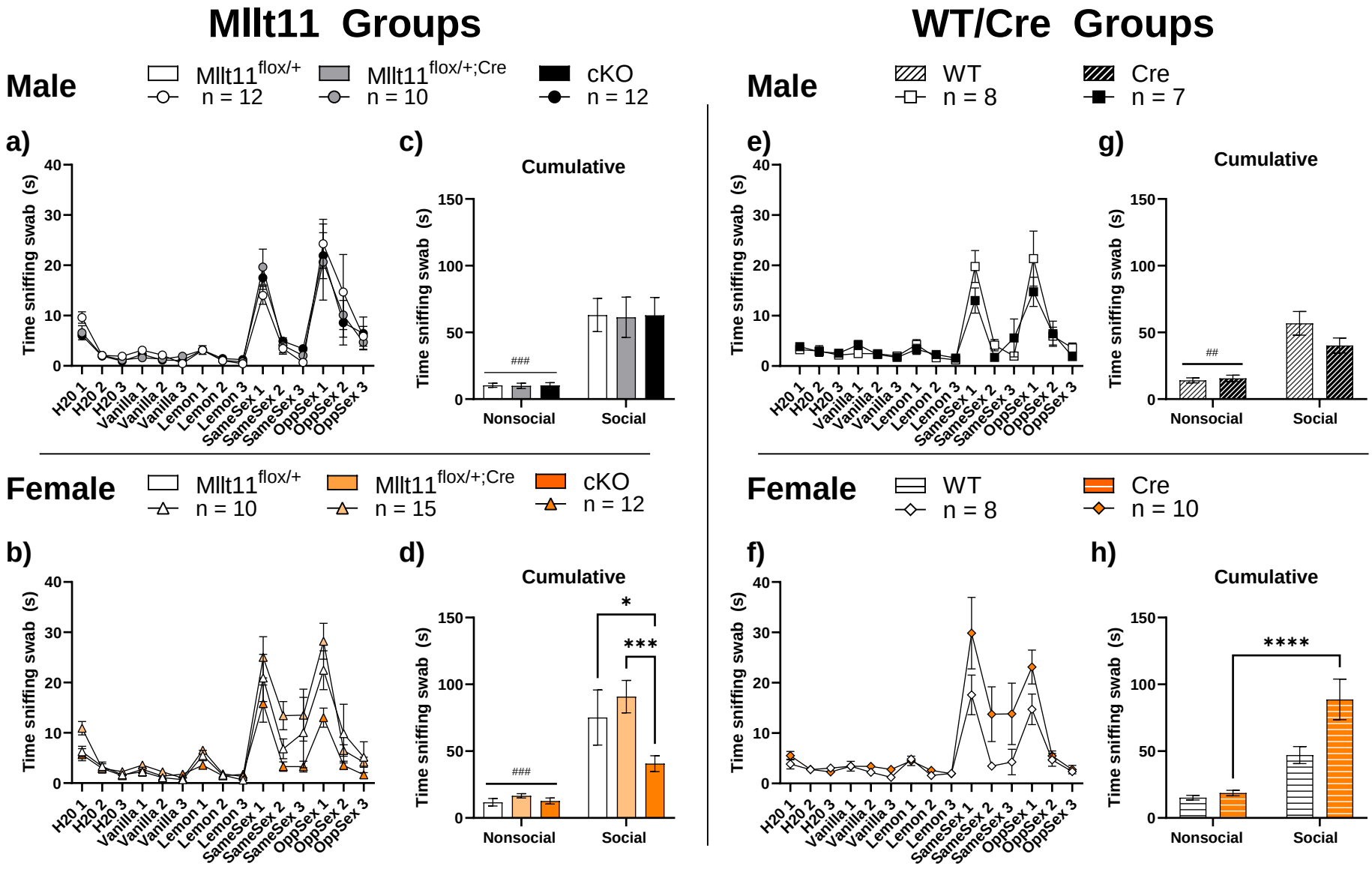

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 10
cKO n = 12
WT n = 8
Cre n = 7
Female
Mllt11flox/+ n = 10
Mllt11flox/+;Cre n = 15
cKO n = 12
WT n = 8
Cre n = 10
a)
b)
c)
d)
e)
f)
g)
h)
Time sniffing swab (s)
H20 1
H20 2
H20 3
Vanilla 1
Vanilla 2
Vanilla 3
Lemon 1
Lemon 2
Lemon 3
SameSex 1
SameSex 2
SameSex 3
OppSex 1
OppSex 2
OppSex 3
Cumulative
###
##
*
***
****
Nonsocial
Social

Fig. 2

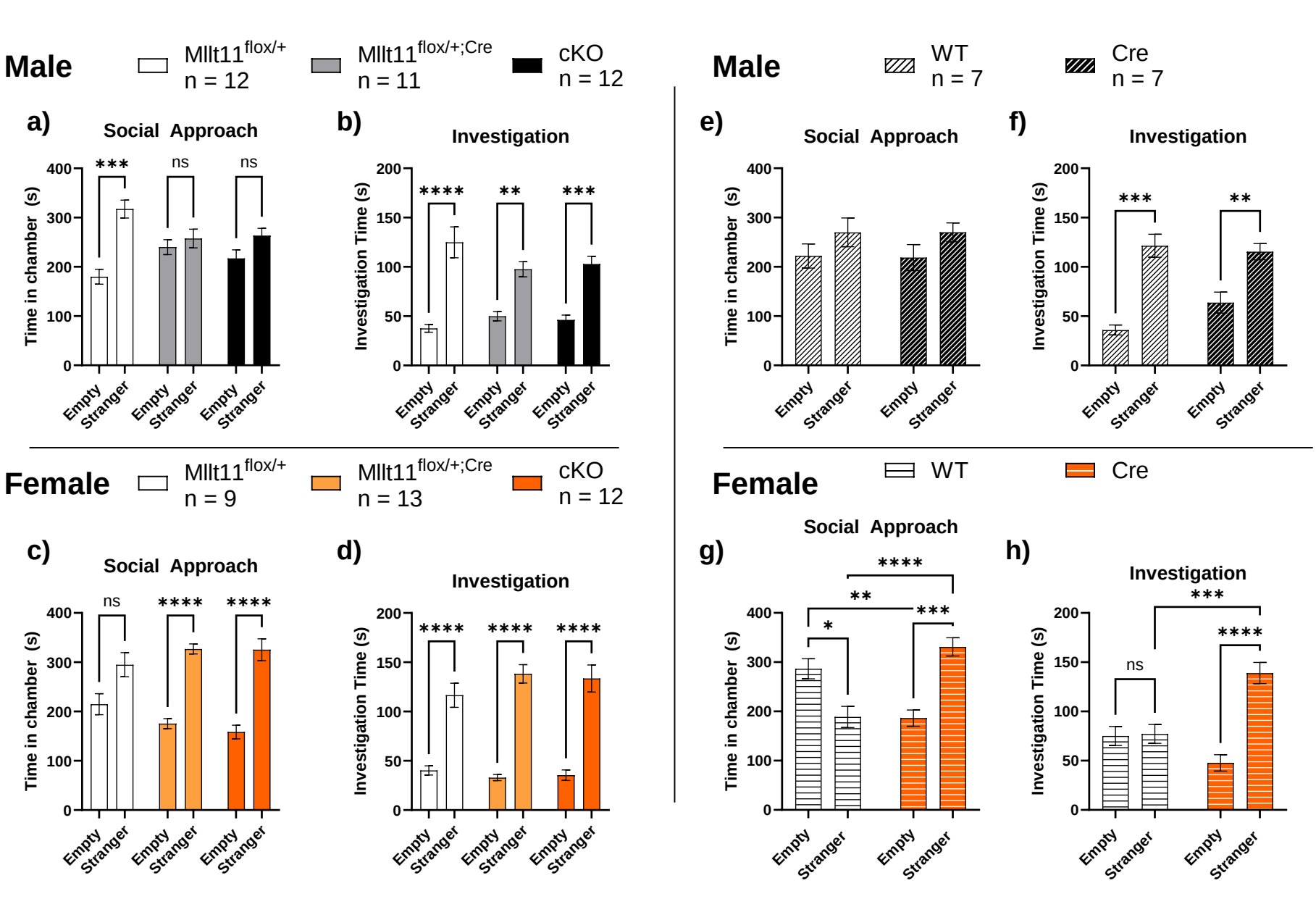

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 11
cKO n = 12
WT n = 7
Cre n = 7
a)
Social Approach
Time in chamber (s)
***
ns
ns
b)
Investigation
Investigation Time (s)
****
**
***
e)
Social Approach
Time in chamber (s)
f)
Investigation
Investigation Time (s)
***
**
Female
Mllt11flox/+ n = 9
Mllt11flox/+;Cre n = 13
cKO n = 12
WT
Cre
c)
Social Approach
Time in chamber (s)
ns
****
****
d)
Investigation
Investigation Time (s)
****
****
****
g)
Social Approach
Time in chamber (s)
*
**
***
****
h)
Investigation
Investigation Time (s)
ns
***
****
Empty
Stranger

Fig. 3

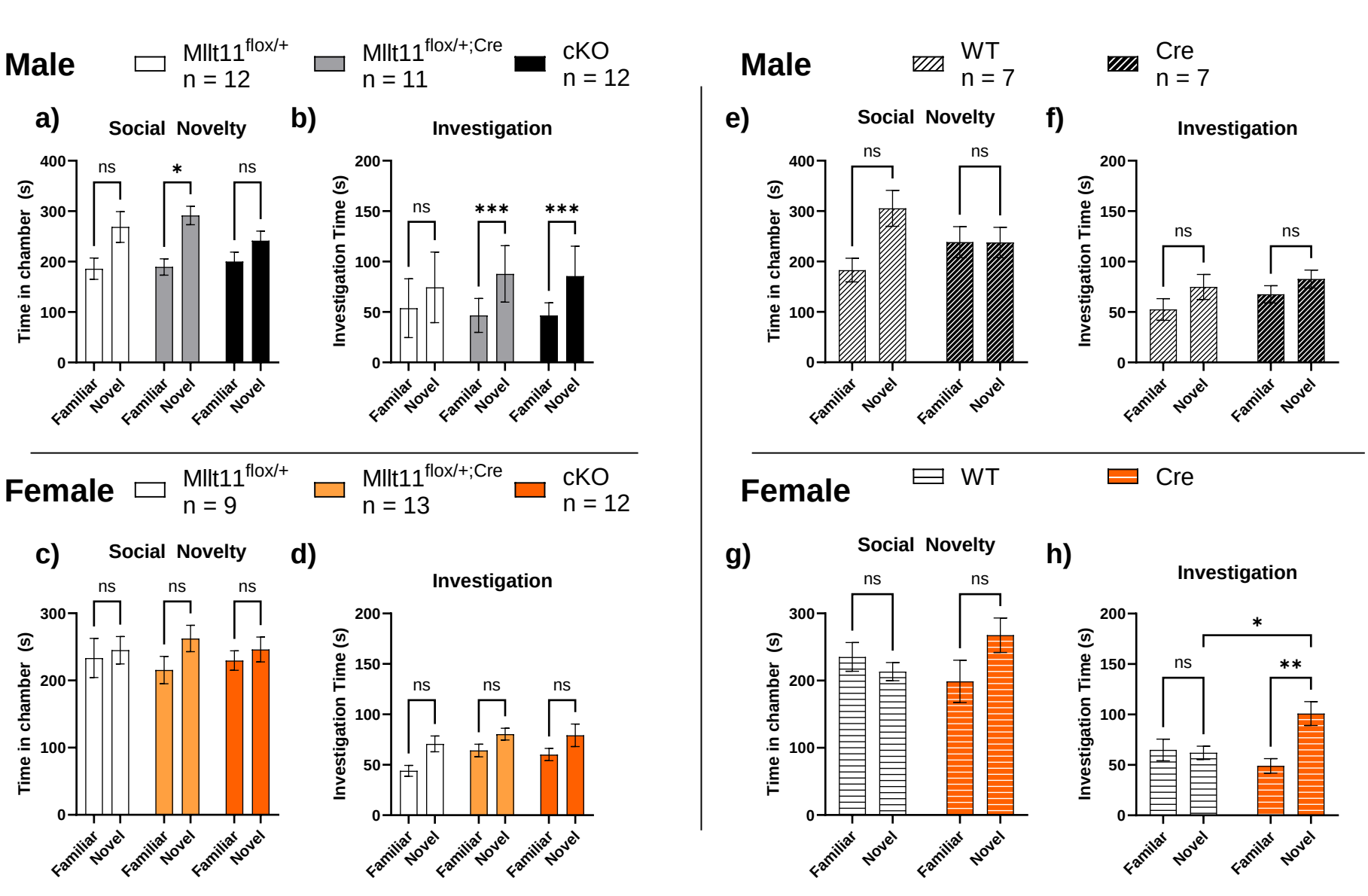

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 11
cKO n = 12
WT n = 7
Cre n = 7
a)
Social Novelty
b)
Investigation
e)
Social Novelty
f)
Investigation
Time in chamber (s)
Investigation Time (s)
ns
*
***
Familiar
Novel
Female
Mllt11flox/+ n = 9
Mllt11flox/+;Cre n = 13
cKO n = 12
WT
Cre
c)
Social Novelty
d)
Investigation
g)
Social Novelty
h)
Investigation
**

Fig. 4

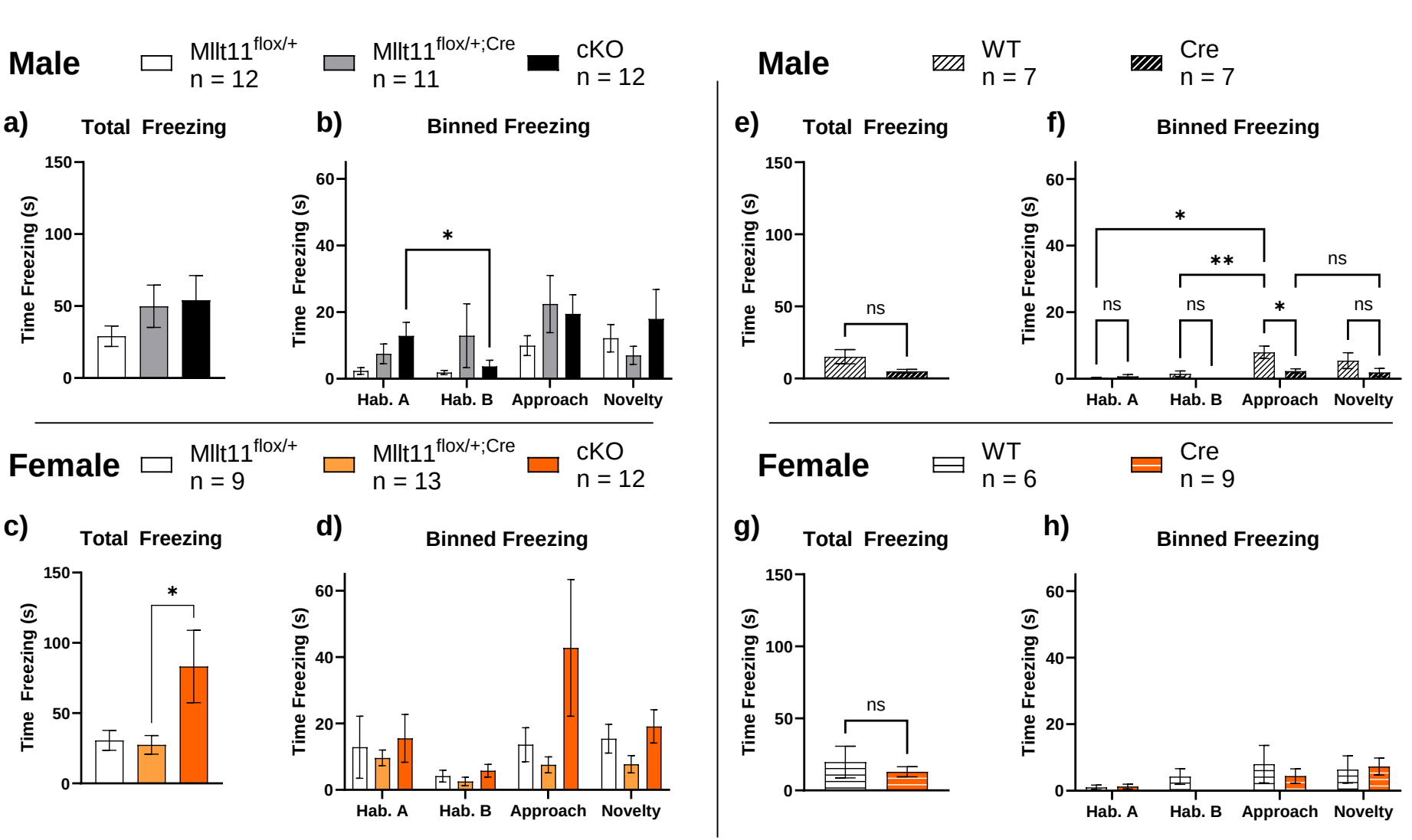

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 11
cKO n = 12
WT n = 7
Cre n = 7
a)
Total Freezing
b)
Binned Freezing
e)
Total Freezing
f)
Binned Freezing
Time Freezing (s)
Hab. A
Hab. B
Approach
Novelty
ns
*
**
Female
Mllt11flox/+ n = 9
Mllt11flox/+;Cre n = 13
cKO n = 12
WT n = 6
Cre n = 9
c)
Total Freezing
d)
Binned Freezing
g)
Total Freezing
h)
Binned Freezing

Fig. 5

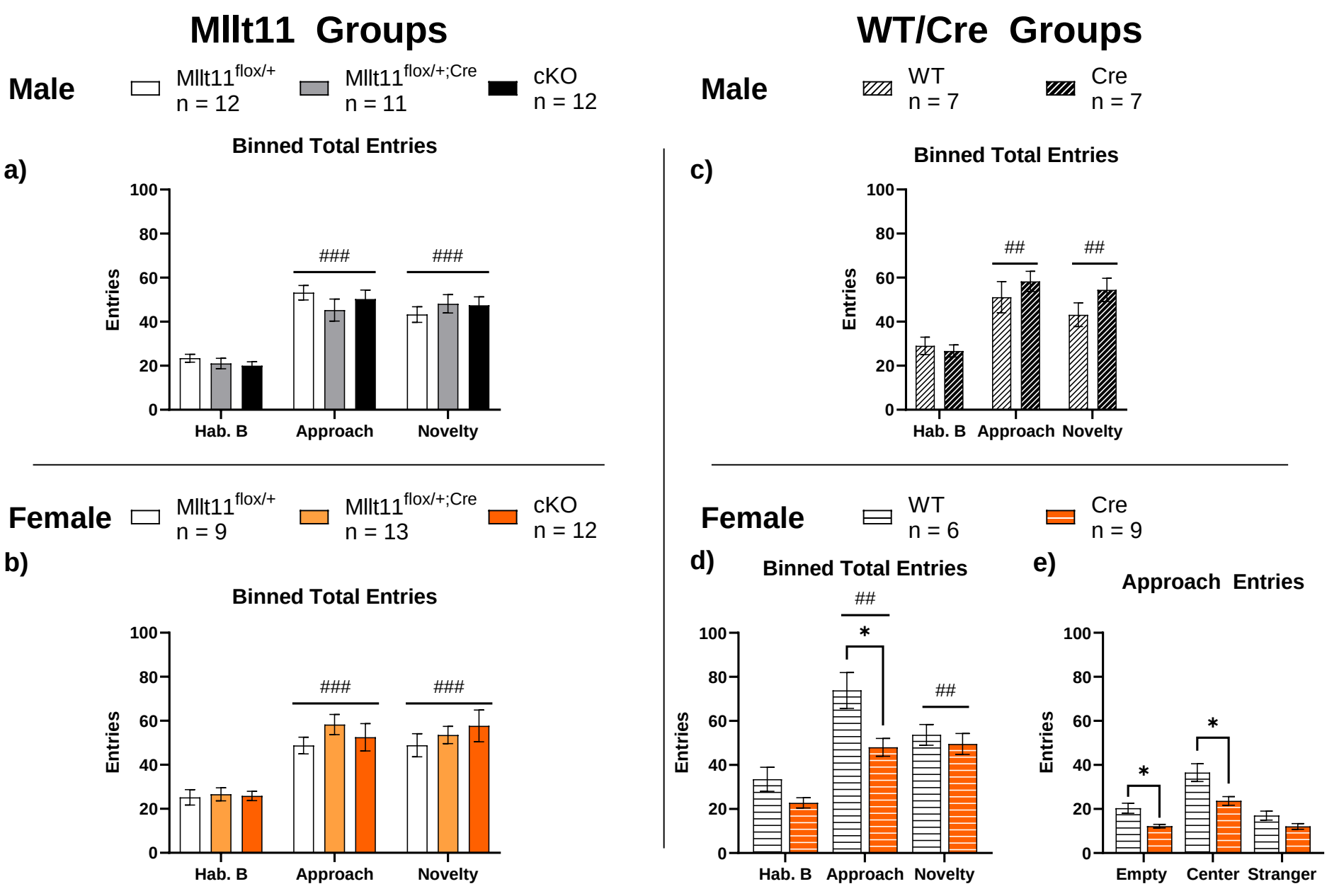

Mllt11 Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 11
cKO n = 12
a)
Binned Total Entries
Entries
100
80
60
40
20
0
###
###
Hab. B
Approach
Novelty
Female
Mllt11flox/+ n = 9
Mllt11flox/+;Cre n = 13
cKO n = 12
b)
Binned Total Entries
Entries
100
80
60
40
20
0
###
###
Hab. B
Approach
Novelty
WT/Cre Groups
Male
WT n = 7
Cre n = 7
c)
Binned Total Entries
Entries
100
80
60
40
20
0
##
##
Hab. B
Approach
Novelty
Female
WT n = 6
Cre n = 9
d)
Binned Total Entries
Entries
100
80
60
40
20
0
##
*
##
Hab. B
Approach
Novelty
e)
Approach Entries
Entries
100
80
60
40
20
0
*
*
Empty
Center
Stranger

Fig. 6

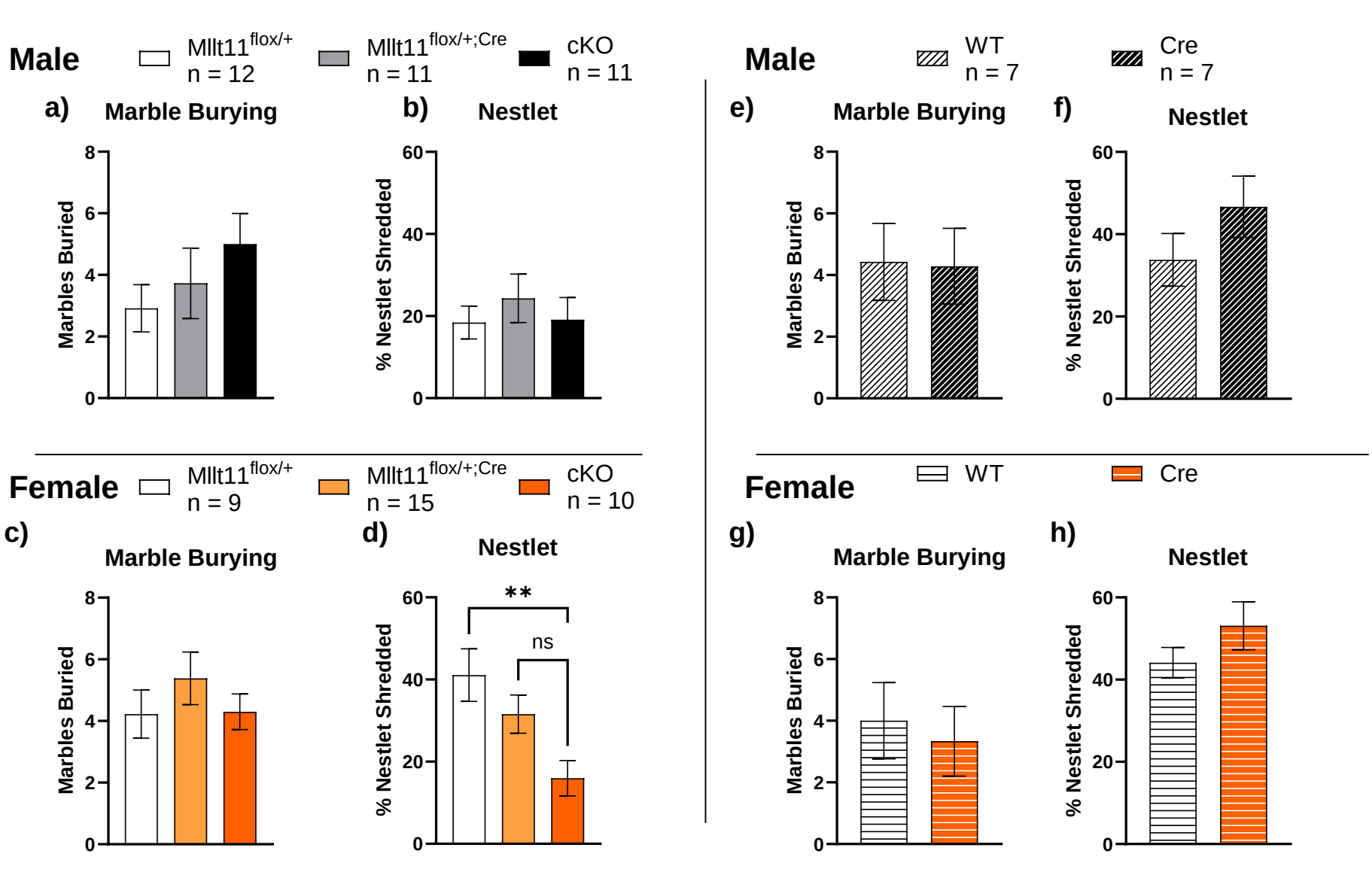

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+
n = 12
Mllt11flox/+;Cre
n = 11
cKO
n = 11
WT
n = 7
Cre
n = 7
a)
Marble Burying
Marbles Buried
b)
Nestlet
% Nestlet Shredded
e)
Marble Burying
f)
Nestlet
Female
Mllt11flox/+
n = 9
Mllt11flox/+;Cre
n = 15
cKO
n = 10
WT
Cre
c)
Marble Burying
d)
Nestlet
**
ns
g)
Marble Burying
h)
Nestlet

Fig. 7

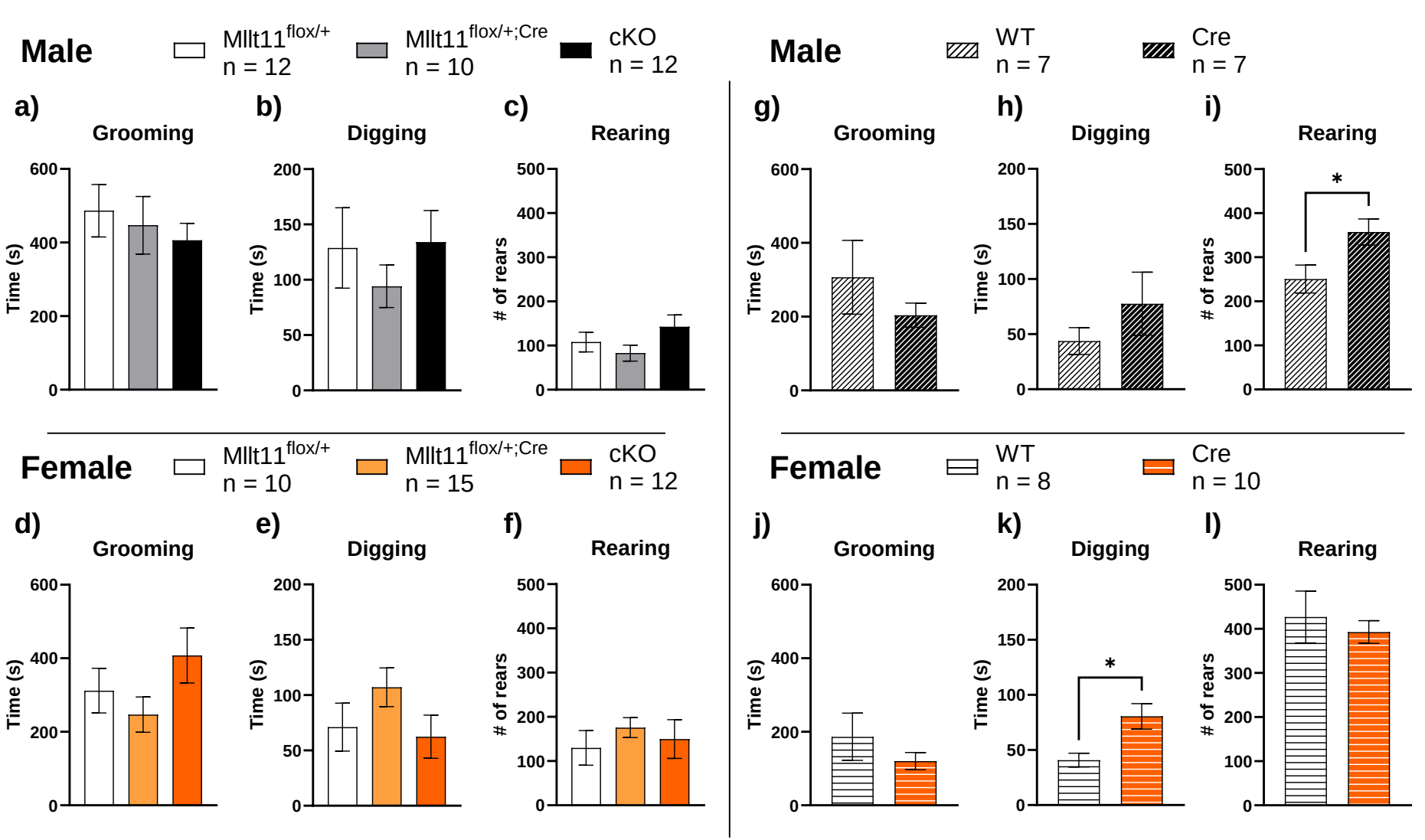

Mllt11 Groups
WT/Cre Groups
Male
Mllt11flox/+ n = 12
Mllt11flox/+;Cre n = 10
cKO n = 12
WT n = 7
Cre n = 7
a)
Grooming
b)
Digging
c)
Rearing
g)
h)
i)
Time (s)
# of rears
*
Female
Mllt11flox/+ n = 10
Mllt11flox/+;Cre n = 15
cKO n = 12
WT n = 8
Cre n = 10
d)
e)
f)
j)
k)
l)

Fig. 8