GENETICS

A missense mutation in *RRM1* contributes to animal tameness

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The increased tameness to reduce avoidance of human in wild animals has been long proposed as the key step of animal domestication. The tameness is a complex behavior trait and largely determined by genetic factors. However, the underlying genetic mutations remain vague and how they influence the animal behaviors is yet to be explored. Behavior tests of a wild-domestic hybrid goat population indicate the locus under strongest artificial selection during domestication may exert a huge effect on the flight distance. Within this locus, only one missense mutation *RRM1*^{I241V} which was present in the early domestic goat ~6500 years ago. Genome editing of *RRM1*^{I241V} in mice showed increased tameness and sociability and reduced anxiety. These behavioral changes induced by *RRM1*^{I241V} were modulated by the alternation of activity of glutamatergic synapse and some other synapse-related pathways. This study established a link between *RRM1*^{I241V} and tameness, demonstrating that the complex behavioral change can be achieved by mutations under strong selection during animal domestication.

INTRODUCTION

Domestication presents an extreme behavioral and physiological stress shift for free-living animals, especially for herbivores naturally wary of humans (*1*). The tameness of animals is a complex phenotype. It includes anxiety, sociability, novelty-seeking, and learning-memory (*2*–*5*) that are regulated by multiple hormones and neurotransmitters (*6*, *7*). On the other hand, the tame animals with less fear and stress reactions tend to have less injury, higher immune and reproductive capacity in captive breeding environments (*8*, *9*). Therefore, they will be continuously selected by breeders, no matter consciously or unconsciously (*10*, *11*). Investigating the genes under artificial selection during domestication may uncover the key genes of tameness.

Our previous study revealed two loci under strong selection during goat (*Capra hircus*) domestication. One locus contains the *MUC6* gene that confers enhanced immune resistance to gastrointestinal pathogens (*12*). At that time, the function of the other locus on chromosome 15: 32.24 to 32.37 Mb was unclear. This locus shows a higher selection signal than *MUC6* and contains two protein coding genes: *STIM1* and *RRM1*. These two genes are potentially related to the function and development of the nervous system. *STIM1* is an endoplasmic reticulum calcium sensor regulating Ca^{2+} and metabolic glutamate receptor signals in the nervous system (*13*, *14*). RRM1 is one of the large and catalytic subunits of ribonucleotide reductase that converts ribonucleotides to deoxyribonucleotides and maintains a balanced deoxynucleoside triphosphate pool, which is crucial for DNA synthesis, repair, and maintenance, including mitochondrial DNA (mtDNA) (*15*). In mice, *RRM1* is related to valproic acid-induced neural tube defects (*16*). In human genome, a recent large-scale genome-wide association study (GWAS) showed that the *STIM1-RRM1* locus in the human genome (NC_000011.10, Chr11:3,855,664 - 4,138,932) was associated with childhood attention-deficit/hyperactivity disorder + disruptive behavior disorders (DBDs) (*17*). The association of *STIM1-RRM1* locus with human behavior disorders and animal domestication suggests that it may account for a common behavioral regulation mechanism in mammals.

In this study, the association between tameness and *STIM1-RRM1* locus was validated in a unique hybrid population between domestic goats and Siberian ibexes (*Capra sibirica*). Then, the key mutations of this locus were identified by population genomics of modern and ancient goats. Last, the corresponding point mutation mice were generated to perform behavioral experiments. These results jointly uncover the function of the *STIM1-RRM1* locus and reveal the key genes and mutations responsible for animal behavior.

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RESULTS

STIM1-RRM1 locus was under the strongest selection during goat domestication

Goat domestication was investigated using a comprehensive panel of genomes including 210 domestic goats worldwide and 24 bezoars which are the ancestor of goats (table S1). Eleven wild related species of Capra are also used in the following study. The worldwide domestic goat populations were compared with all 24 bezoars to identify key selective sweeps in domestication by genetic diversity (π ln-ratio bezoar/domestic goat), cross-population extended haplotype homozygosity (XP-EHH), and extreme divergence in allele frequency (F_{ST}) (Fig. 1A). As we previously reported, the region between 32.24 and 32.37 Mb on chromosome 15 belongs to the top outlier for all the selective sweep analysis (windowed F_{ST} = 0.87, π ln-ratio = 3.94, XP-EHH = 8.12).

Then, the signals of the sliding windows in this region were checked one by one. The windows with the highest signal of F_{ST}, composite likelihood ratio (CLR), and Tajima's *D* in domestic goats cover a part of the *STIM1* gene and the whole *RRM1* gene (Fig. 1B). Therefore, the top outlier locus under selection in goat domestication was referred to as the *STIM1-RRM1* locus. We also performed selection sweep analysis of four main domestic goat populations from different continents separately. *STIM1-RRM1* locus is also included in the three regions under selection among all four

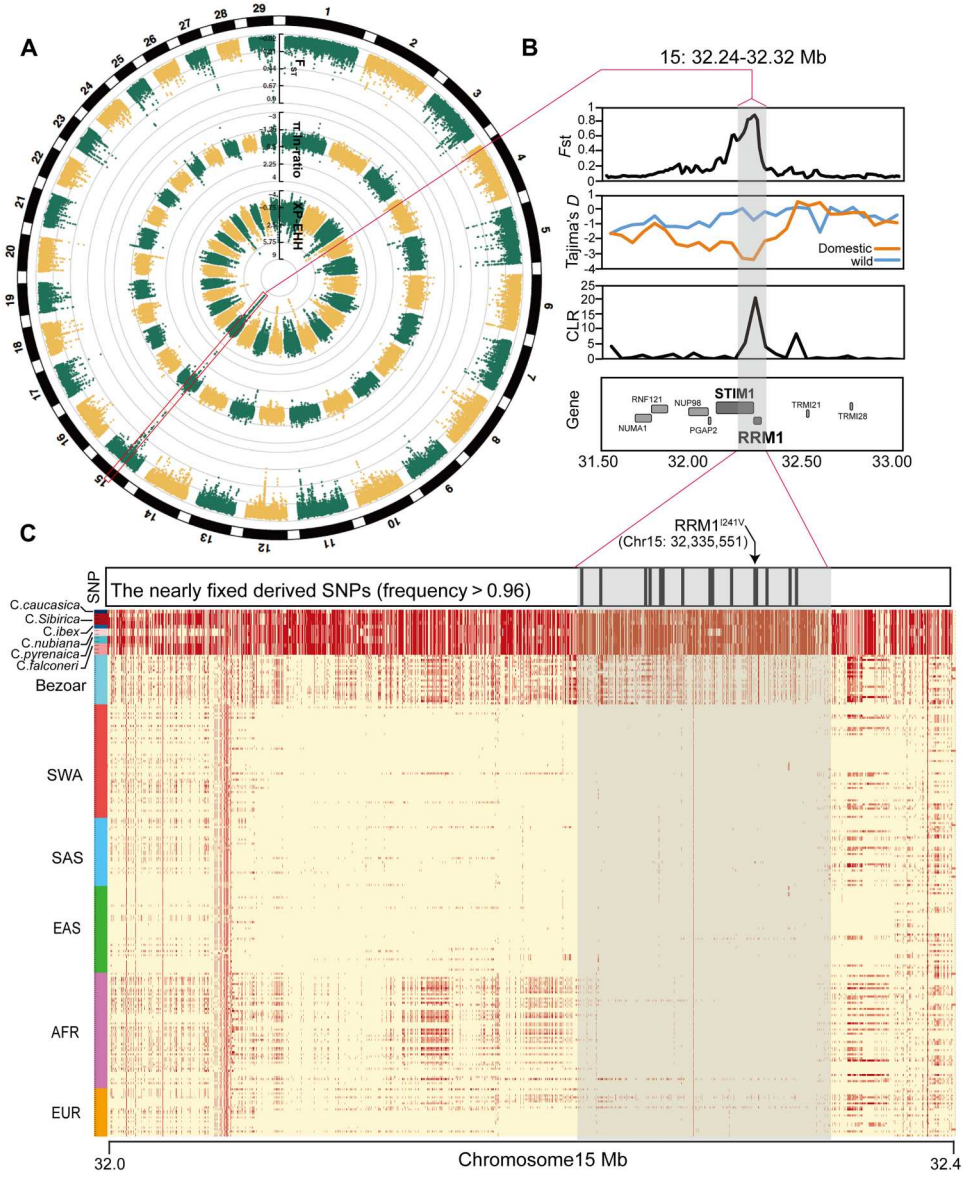

Fig. 1. The selection signals of goat genomes and *STIM1-RRM1* locus. (**A**) Selection sweep analysis (F_{ST}, π ln-ratio, and XP-EHH) using whole genomes of bezoars and domestic goats. Sliding window = 50 kb, step = 20 kb. (**B**) F_{ST}, Tajima's *D*, and CLR test at the *STIM1-RRM1* locus. (**C**) The haplotypes of *STIM1-RRM1* locus in goat populations and related species. The alleles of domestic and nondomestic samples are indicated by light yellow and red, respectively. Bezoar (*n* = 24), *Capra_caucasica* (*n* = 1), *Capra_Sibirica* (*n* = 3), *Capra_ibex* (*n* = 1), *Capra_nubiana* (*n* = 2), *Capra_pyrenaica* (*n* = 2), *Capra_falconeri* (*n* = 3), EUP: European (*n* = 24), AFR: African (*n* = 56), SWA-SAS: Southwest-South Asia (*n* = 88), and EAS: East Asia (*n* = 42). The nearly fixed derived SNPs are marked at the top.

different groups (fig. S1), indicating that this locus represents a function with broad significance.

The haplotype of *STIM1-RRM1* locus in domestic goats (referred to as *STIM1-RRM1*^{dom}) is distinct from the haplotype of bezoars and other wild species (referred to as *STIM1-RRM1*^{wild}; Fig. 1C). In *STIM1-RRM1*^{dom}, we found 15 nearly fixed derived single-nucleotide polymorphism (SNPs), which are defined as absent in bezoar and other wild species and with >0.96 frequency in domestic goats (Fig. 1C and table S2). Only one of the nearly fixed SNPs is a missense mutation, located at Chr15:32,335,551, causing conversion of the 241st amino acid of RRM1 from Ile to Val (referred to as *RRM1*^{I241V}). The other 13 of them are located in the intron, and the left one is a synonymous mutation of *STIM1*.

Goats with homozygous *STIM1-RRM1*^{dom} showed less fear response to human

To investigate the effect of *STIM1-RRM1*^{dom} and *STIM1-RRM1*^{wild} during domestication, we established a backcross (BC) segregating population generated by Siberian ibex × domestic goat (see Materials and Methods). Siberian ibexes are wild relatives of domestic goats, and their hybrid offspring are fertile. We measured the flight distance (FD) of the hybrids. FD is the distance an animal fleeing from an approaching human (*18*, *19*) (Fig. 2A). FD test mimics the fear response of animals when facing direct human management which is a key challenge in domestication (*1*).

To recruit more individuals of the same age and sex in the test, all individuals in this hybrid population were genotyped using polymerase chain reaction (PCR) and Sanger sequencing. Considering age, sex, and STIM1-RRM1 locus genotyping, we selected five heterozygous (*STIM1-RRM1*$^{dom/wild}$ BC) and eight homozygous (*STIM1-RRM1*$^{dom/dom}$ BC) for the FD test. All experimental individuals were 4- to 5-year-old females. In addition to veterinary examination, blood routine tests (table S4) were also used to ensure the health of all experimental individuals. The *STIM1-RRM1*$^{dom/wild}$ BC individuals showed a longer FD than the *STIM1-RRM1*$^{dom/dom}$ BC ones [$P = 5.56 \times 10^{-7}$, one-way analysis of variance (ANOVA)] (Fig. 2B), indicating that they are more afraid of humans. The FD test preliminarily proved the correlation between *STIM1-RRM1* locus and tameness based on genomic selection signal, laying the foundation for further screening and verification of functional mutations.

Mutations may affect gene expression and protein function. To compare the expression differences of *STIM1* and *RRM1* in the nervous system, two *STIM1-RRM1*$^{dom/wild}$ hybrids were sacrificed. RNA sequencing was performed using tissues of the nervous system including thalamus, hypothalamus, prefrontal, and pituitary. The expression level of the goat and ibex alleles of the same individual was quantified and compared. Totally 1436 allele-specific expression (ASE) genes (table S5) were identified, representing the functional divergence between goat and ibex. The expression of *STIM1* and *RRM1* are not significantly different between *STIM1-RRM1*^{dom} and *STIM1-RRM1*^{wild} (table S6) in the ibex-goat hybrids. This result raises the possibility that protein coding is the main functional mutation of the *STIM1-RRM1* locus. Compared with ibex, the direct ancestor bezoar has fewer divergent cis-regulatory elements with domestic goat and is less likely to change gene expression. Therefore, the only missense mutation *RRM1*^{I241V} is targeted as the key genetic variant within the locus for further validation.

RRM1^{I241V} was the candidate function mutation

We next try to identify the functional SNPs in *STIM1-RRM1*^{dom} from ancient genomes and comparative genomics (table S3). We collected 70 published ancient goat genomes (*12*, *20*–*22*) mainly from the Fertile Crescent, the center of goat domestication (fig. S3A). The 15 nearly fixed SNPs in *STIM1-RRM1*^{dom} were screened in ancient goat genomes. Compared to other SNPs, *RRM1*^{I241V} was the latest discovered in ancient goats, which was first detected at ~6500 years ago (Fig. 3A). Since then, the *STIM1-RRM1*^{dom} haplotype with *RRM1*^{I241V} expanded rapidly in the subsequent ancient goats and has become almost fixed in all modern goats (fig. S3). This time of *RRM1*^{I241V} expansion is also consistent with the other domestication gene *MUC6* (fig. S3) (*12*).

RRM1 is essential for the conversion of ribonucleotides into deoxyribonucleotides, and key point mutations of this gene have been proven to cause early embryonic lethality in mice (*RRM1*^{W684G}) (*23*) and an mtDNA maintenance disorder (*RRM1*^{N427K}) (*15*). Comparative genomics showed that the *RRM1*^{I241V} is conserved in tetrapods (Fig. 3B). The RRM1-I241 is located on the alpha helix of the R1 dimer interface (Fig. 3C and fig. S4) (*24*). And the prediction of hydrophobicity showed that the I241V mutation increased the hydrophobicity of amino acids in RRM1 from position 236 to 246. (Fig. 3D and table S7). The mutation in the dimer

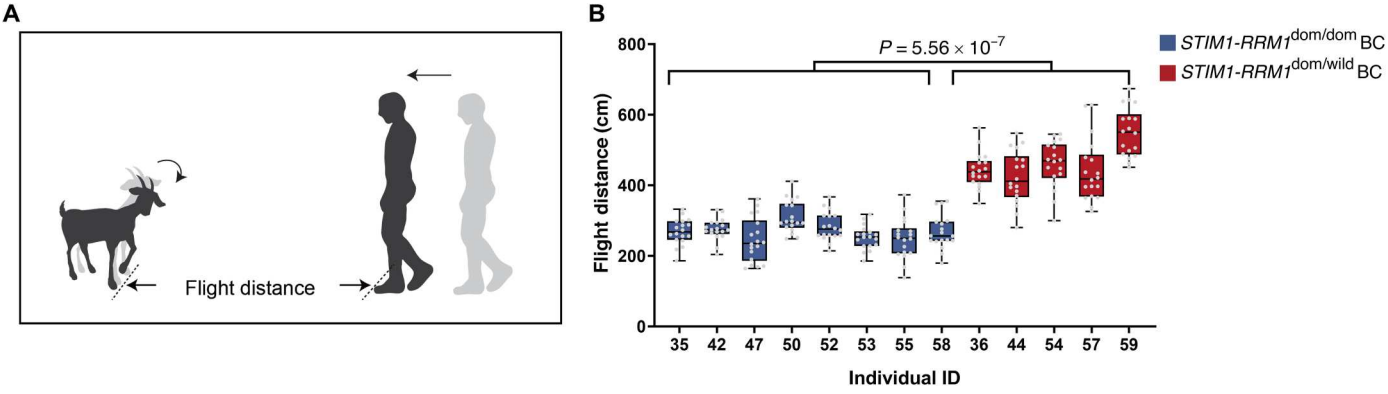

Fig. 2. The association between the *STIM1-RRM1* locus and tameness. (**A**) Schematic representation of FD test. The FD was measured when a standing idle goat started to move away from an approaching individual. (**B**) The FD of the ibex-goat hybrid individuals. The *P* value between the two groups was calculated by one-way ANOVA.

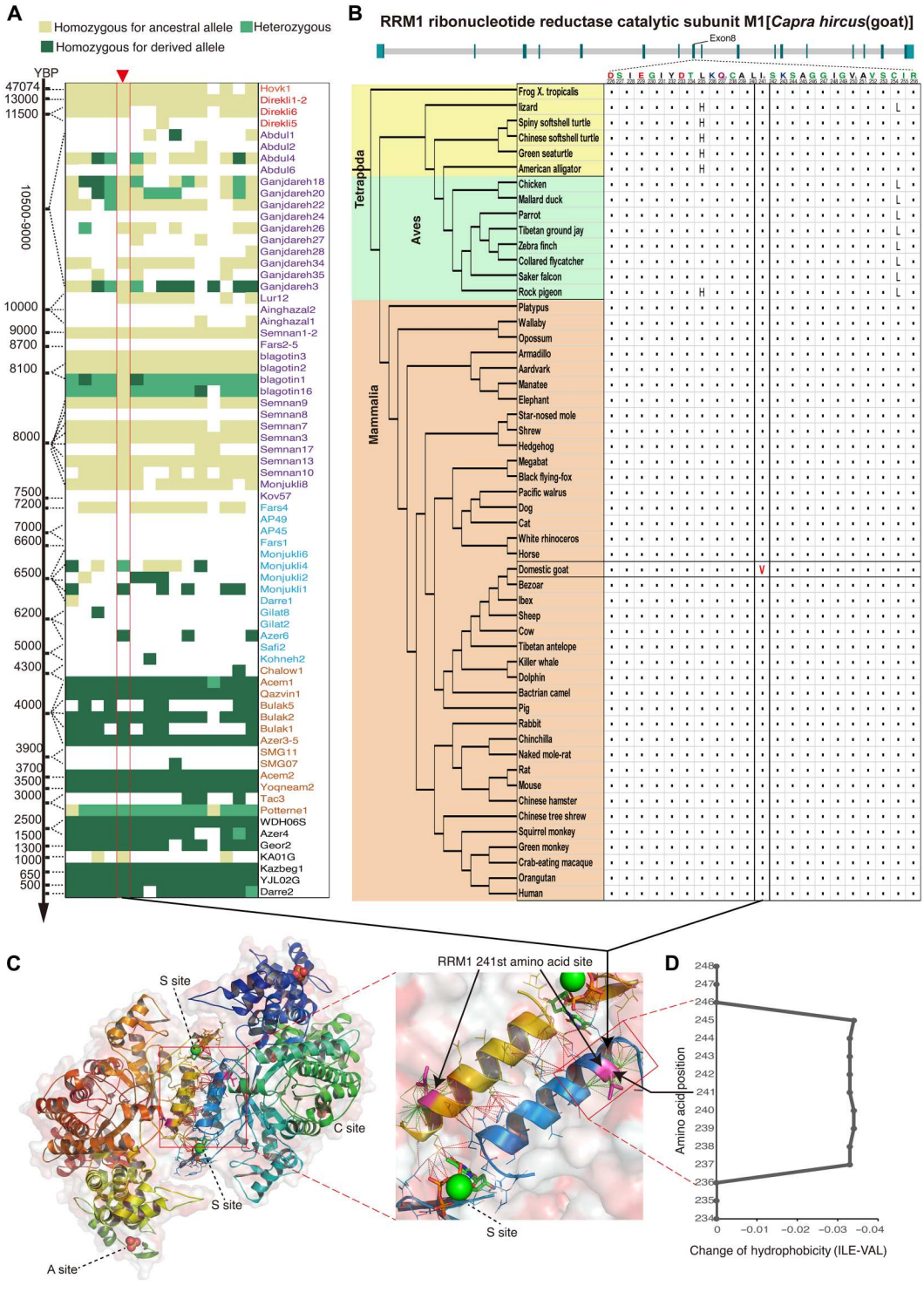

Fig. 3. The character of *RRM1*^{I241V} in ancient genome, conservation, and protein structure analysis. (**A**) Haplotype of *STIM1-RRM1* locus in the ancient goats. The red triangle indicates the *RRM1*^{I241V}. Colors of sample names represent different periods. YBP, years before present. (**B**) Conservative analysis of RRM1 241st amino acid. (**C**) Predicted three-dimensional structures for RRM1 protein. The outermost layer is hydrophobic, and the color from red to white represents the hydrophobicity from low to high. The catalytic site (C site), specificity site (S site), and activity site (A site) of RRM1 were labeled separately. (**D**) The hydrophobicity prediction of RRM1. Amino acid substitution (ILE to VAL) at position 241 of the RRM1 reduces the hydrophobicity from amino acids 236 to 246.

interface affects the overall stability and enzyme activity of the protein (*25*). Therefore, *RRM1*^{I241V} may be a mild mutation affecting the hydrophobic-induced dimerization and enzyme activity.

RRM1$^{I241V/I241V}$ mice showed reluctance to avoid human and expression changes in glutamatergic synapse pathway

To investigate the function of *RRM1*^{I241V} in vivo, the point mutation was introduced into C57BL/6J mice using CRISPR-Cas9. *RRM1*$^{I241V/I241V}$ mice were viable and fertile and showed no significant differences in weight, morphology, and brain weight from birth to adulthood from wild-type (WT) mice (fig. S5).

The tameness of *RRM1*^{I241V} mice was quantified by behavioral tests for active tameness (motivation to approach humans), passive tameness (reluctance to avoid humans), and the willingness to stay on a human hand. In the active tame test, the *RRM1*$^{I241V/I241V}$ mice spent more time in heading or contacting human hands than WT ($P < 0.001$) (Fig. 4A). In the passive tame test, *RRM1*$^{I241V/I241V}$ mice spent more time in heading toward the human hand ($P = 0.003$) (Fig. 4B). In the stay-on-hand test, the *RRM1*$^{+/+}$ mice barely accepted this greater simulation. In contrast, mutant mice were more acceptable showing a significantly longer staying time on the hand ($P < 0.001$) (Fig. 4C). There was no significant difference in locomotion between the two groups of mice throughout the tame test, suggesting that the *RRM1*^{I241V} mutation did not affect the mice's movement ability.

Comparisons of the brain transcriptome of *RRM1*$^{I241V/I241V}$ and *RRM1*$^{+/+}$ mice revealed a total of 164 up-regulated and 213 down-regulated differentially expressed genes (DEGs) (table S8). The expression of *STIM1* and *RRM1* between the two strains was not significantly different, similar to that observed in the ASEs analysis of hybrid goats. Enrichment analysis of the DEGs evidenced that the Glutamatergic synapse is the most significantly enriched pathway (Fig. 4D). Some other synapse-related signaling pathways are also significantly enriched, including gonadotropin-releasing hormone signaling, dopaminergic synapse, serotonergic synapse, and γ-aminobutyric acid–releasing synapse pathways (table S9).

RRM1$^{I241V/I241V}$ mice showed increased social interaction with larger mice and reduced anxiety-like behaviors

To test the sociability of the *RRM1*$^{I241V/I241V}$ mice, modified multibox social experiments were performed (Fig. 5A). One intruder adult male C57BL/6J mouse was placed in a specially designed box to have interactions with the residents in different regions. Because of the social hierarchy of mice, adult males will attack immature mice (region 2) competing for social dominance with an adult male of the same breeds (region 3) (*26*, *27*) and are subjected to social defeat by the larger and more aggressive ICR mouse (region 4) (*28*). The social interaction scores were estimated on the basis of the time duration of mice spent in each interaction region. *RRM1*$^{I241V/I241V}$ and *RRM1*$^{+/+}$ mice showed no difference in both region 2 and region 3, suggesting that *RRM1*^{I241V} does not affect social competition or aggressive behaviors. The *RRM1*$^{I241V/I241V}$ mice spent more time in region4 than *RRM1*$^{+/+}$ mice ($P < 0.001$; Fig. 5B and fig. S6), indicating that they are more adaptable to stress from superior breeds or species.

Then the anxiety-like behaviors of *RRM1*$^{I241V/I241V}$ mice were quantified. In the open field test (OFT), *RRM1*$^{I241V/I241V}$ mice spent more time in the inner area compared to *RRM1*$^{+/+}$ (Fig. 5, C and D), suggesting less anxiety-like behaviors under the open-field–induced stress. Next, we used the elevated plus maze (EPM) test to examine the anxiety-like behaviors under a highly stressful condition. *RRM1*$^{I241V/I241V}$ spent more time running along the open arms than *RRM1*$^{+/+}$ (Fig. 5, E and F). The collective results suggest that *RRM1*^{I241V} mutation reduces anxiety-like behaviors under stressful conditions. Meanwhile, *RRM1*$^{I241V/I241V}$ and *RRM1*$^{+/+}$ mice showed no significant difference in the Morris Water Maze, indicating that *RRM1*^{I241V} did not affect learning-memory ability (fig. S8).

DISCUSSION

In this study, we first used population genetics to reveal that the *STIM1-RRM1* locus was under the highest selection in goat domestication. Behavioral test in hybrid goats initially validated that goat with *STMI1-RRM1*^{dom} show less fear of human. Because the limitation of hybrid animal sample size, we next try to narrow down the functional mutations in *STMI1-RRM1*^{dom} for further experiments. The region with top selection signals includes 15 linked SNPs, which are nearly fixed in worldwide goat populations and absent in bezoar and ibex. All these SNPs and other mutations under selection show potential function in domestication through multiple genetic pathways. *RRM1*^{I241V} was selected for further validation based on the following evidence. First, *RRM1*^{I241V} is the only missense mutation, highly conserved in tetrapods, and potentially altered the protein structure. Second, the expression of *STIM1* and *RRM1* were not significantly different between goats and ibex alleles. Third, in ancient goat genomes, after the detection of *RRM1*^{I241V} at ~6500 years ago, the *STMI1-RRM1*^{dom} haplotype with *RRM1*^{I241V} rapidly spread throughout the domestic goat population.

The point editing *RRM1*$^{I241V/I241V}$ mice further revealed the role of *RRM1*^{I241V} in tameness, social ability, and fear response, similar to *STMI1-RRM1*^{dom} in goats. In human genetics study, the *STIM1-RRM1* locus is also related to mental disorder (*17*). The p.R381 mutation in human RRM1 adjacent to the S-site affects the binding of effectors and impaired dimerization, which leads to mtDNA maintenance disorder (*15*). The normal maintenance and metabolism of mtDNA are essential for the development and function of the nervous system (*29*). However, because of the complexity of nervous systems, more research on the functional consequences of the *RRM1*^{I241V} is needed. As a natural and safe mutation, *RRM1*^{I241V} could be a candidate target to improve the temperament traits of other domestic animals. Furthermore, the common function of *STMI1-RRM1* in goat and mouse will encourage deep mining of this locus in human population. These researches may uncover the functional mutations of DBDs which did not precisely define in GWAS (*17*). *RRM1*$^{I241V/I241V}$ mouse can also be a tantalizing disease model, especially because the *RRM1* knockout mouse is nonviable.

In conclusion, our studies uncovered that *STMI1-RRM1*^{dom} haplotype and *RRM1*^{I241V} are associated with elevated tameness in domestic goat and mouse. These results demonstrated a method to uncover the cross specials major effect genes of complex traits. However, the function of other variants on this haplotype and the detailed cellular mechanisms involved in the behavior changes are still unknown, which are potential limitations that need to be addressed in future research.

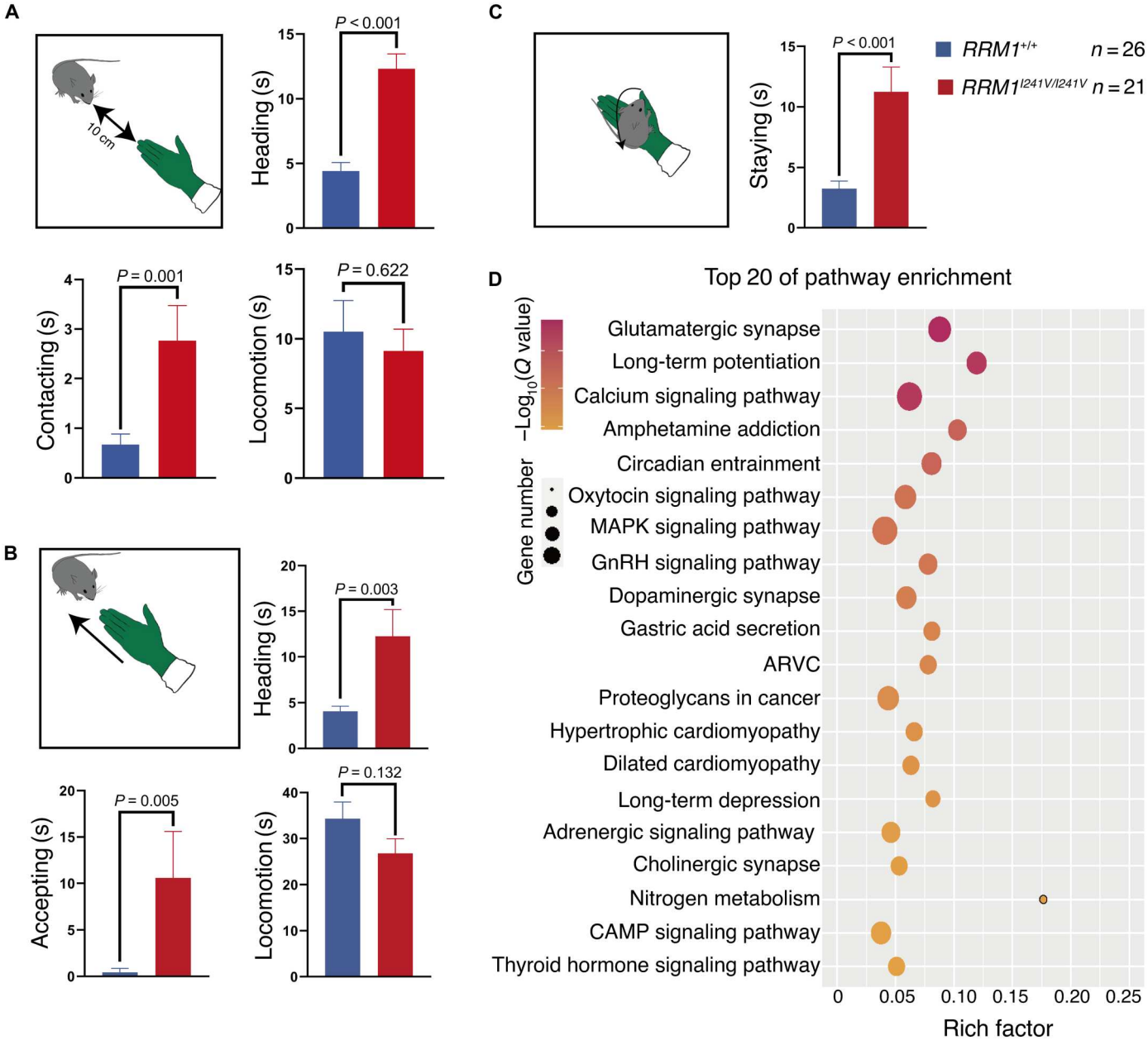

Fig. 4. *RRM1*$^{I241V/I241V}$ mice domestication characterization. The schematic diagram and behavioral difference between *RRM1*$^{+/+}$ mice and *RRM1*$^{I241V/I241V}$ mice in (**A**) active tame test, (**B**) passive tame test, (**C**) staying on hand test. (A) Comparison of heading toward the hand, locomotion, and contacting the hand in the active tame test among the two strains. (B) Comparison of heading toward the hand, locomotion, and accepting touching by the hand in the passive tame test among the two strains. (C) Comparison of staying time in the stay-on-hand test among the two strains. The time spent in the three tame tests were used to quantify the tameness between *RRM1*$^{+/+}$ and *RRM1*$^{I241V/I241V}$ mice. Error bars in graphs represent means ± SEM. (**D**) Top 20 Kyoto Encyclopedia of Genes and Genomes pathways enriched in DGEs. Bubble color and size correspond to the *Q* value (<0.05) and gene number enriched in the pathway. MAPK, mitogen-activated protein kinase; GnRH, gonadotropin-releasing hormone; ARVC, arrhythmogenic right ventricular cardiomyopathy; CAMP, cyclic adenosine monophosphate.

MATERIALS AND METHODS

Genome data collection

We collected a total of 315 modern and ancient goat genomes, including 245 modern and 70 ancient genomes from the Nextgen project (http://projects.ensembl.org/nextgen) and other previous studies (*12*, *20*–*22*, *30*, *31*). Details of the modern and ancient genomes used in this study are given in tables S1 and S3. In addition, the hybrid goats whole-genome data and multitissue transcriptome data were obtained from our previous study (*32*).

Read alignment and variant calling

Modern goats

The read alignment analysis was applied as described before (*12*). We used Trimmomatic (*33*) to filter low-quality reads, and high-

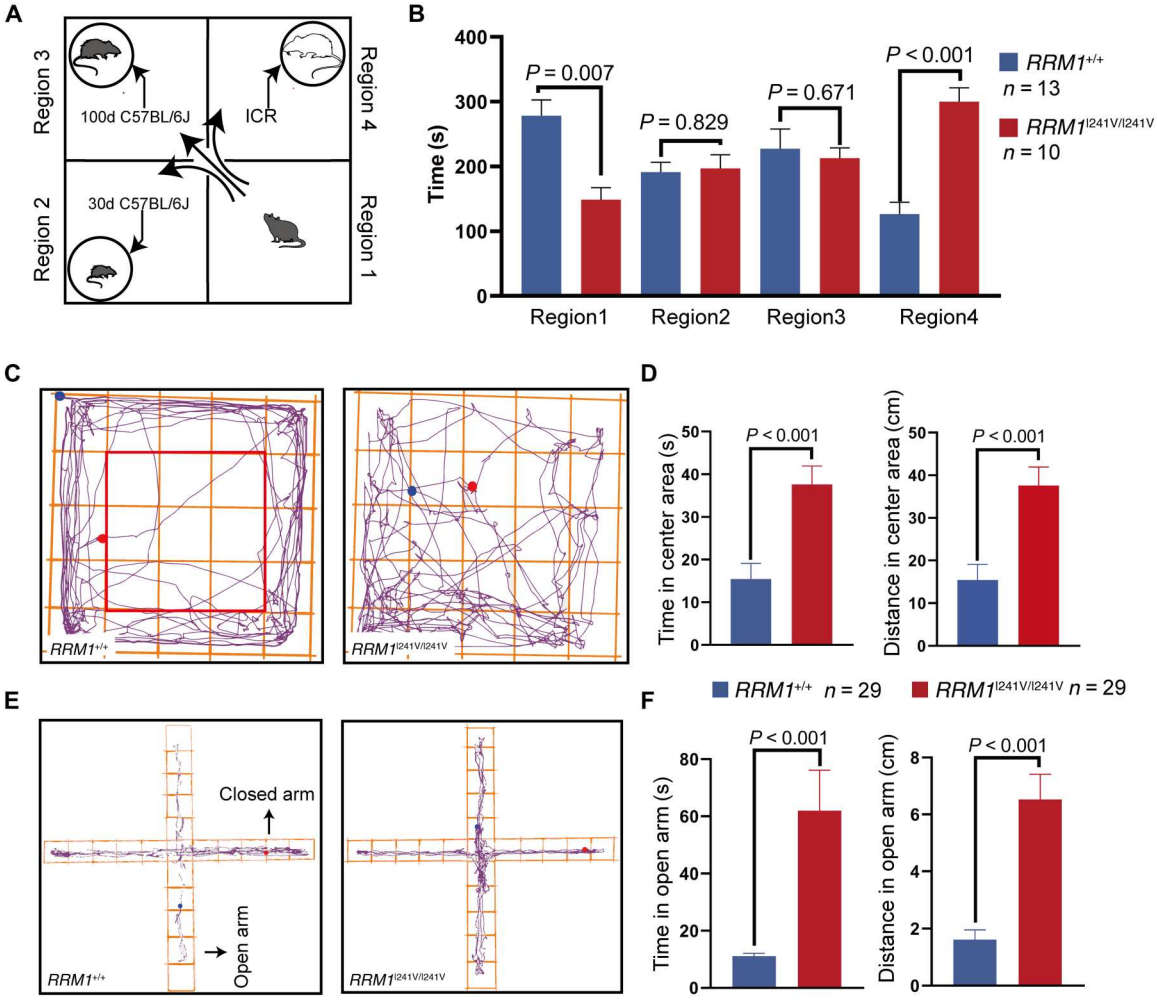

Fig. 5. Sociability and anxiety test of *RRM1*$^{I241V/I241V}$ mice. (**A**) Diagram showing the sociability test. (**B**) The time test mice spent in different regions. (**C**) Representative traces of *RRM1*$^{+/+}$ and *RRM1*$^{I241V/I241V}$ mice in the open field test (OFT). (**D**) Total time and distance traveled in the center area of the OFT. (**E**) Representative traces of *RRM1*$^{+/+}$ and *RRM1*$^{I241V/I241V}$ mice in the open and closed arms of the elevated plus maze (EPM). (**F**) Total time and distance traveled in the open arms of the EPM. Error bars in graphs represent means ± SEM.

quality paired reads were aligned the using BWA-MEM (*34*). Then, Picard (http://broadinstitute.github.io/picard/) was used to merge same samples' read groups, sort read alignments, and filter PCR duplication.

Ancient goats

For row fastq data, adapters were trimmed, reads were filtered using AdapterRemoval2 (*35*), and collapsed reads were then aligned to the goat reference genome (GCF_001704415.1). PCR duplicates were then removed using the software SAMtools and Picard Tools. After indel realignment using GATK (*36*), mapDamage2 (*37*) was used to characterize the DNA damage pattern and rescale the base quality of ancient samples.

Variant calling and filtration

We used SAMtools model implemented in ANGSD (*38*) to call SNPs with "-only_proper_pairs 1 -uniqueOnly 1 -remove_bads 1 -minQ 20 -minMapQ 30 -C 50 -doMaf 1 -doMajorMinor 1 -GL 1 -setMinDepth [Total_read_depth/3] -setMaxDepth [Total_read_depth*3] -doCounts 1 -dosnpstat 1 -SNP_pval 1". VCFtools (*39*) was used to filter SNPs with a filtering expression: "--min-alleles 2 --max-alleles 2 --max-missing 0.9". Then, all sample sets of filtered variant calls were used for imputation and phasing using BEAGLE (*40*). Last, all the variations are annotated by ANNOVAR (*41*).

Selective sweep analysis

The selective sweep analysis was performed as described before (*12*). The nucleotide diversity (π) and population genetic differentiation (F_{ST}) were calculated using a sliding window approach with windows of 50 kb and a step of 20 kb between bezoars and domestic goats. We also detected cross-population XP-EHH using selscan (*42*) with same sliding window approach. In addition, to further verify the selective sweep, Tajima's *D* and maximum likelihood test were performed using VCFtools and SweeD (*43*).

Behavioral experiments related to the *STIM1-RRM1* locus

The hybrid population

All procedures involving sample collection and experiments were approved by the Northwest A&F University Animal Care

Committee (permit number: NWAFAC1019). Two male Siberian ibexes (*C. sibirica*) were crossed with female Cashmere goats (*C. hircus*) to produce male F1 hybrids. Then, the male F1 hybrids were backcrossed to female Cashmere goats to create a BC segregating population (n = 60). This population was reared at Karamay, China and subjected to the same husbandry procedures.

Kinship identification

We constructed the pedigree of the hybrids according to the completely divergent sites (F_{ST} = 1) between domestic goats and ibexes. In general, whether two hybrids come from the same family lineage is calculated on the basis of the status of the divergence sites. The higher the similarity, the closer the genetic relationship. Then, by comparing the respective mitochondrial information to determine whether from the same mother, Kinship2 [CRAN-Package kinship2 (r-project.org)] was used for visualization.

Genotyping of the STIM1-RRM1 locus

The variants at the *STIM1-RRM1* locus for the segregating population were genotyped using PCR and Sanger sequencing. The genomic segments surrounding the selected putative variants were amplified using approximately 50 ng of genomic DNA. All primers (forward primer: 5-AATGGGAGACAGTTGGTAA-3; reverse primer: 5-GAATTGTGGGAATGAAGAT-3) were designed by PRIMER3 v4.1.0 (*44*).

Selection of experimental individuals

On the basis of the results of the *STIM1-RRM1* locus genotyping, we selected five heterozygous (*STIM1-RRM1*$^{dom/wild}$ BC) and eight homozygous (*STIM1-RRM1*$^{dom/dom}$ BC) for an FD test. All individuals are under unified management and have been inspected and quarantined every year to ensure their health. All experimental individuals were healthy, 4- to 5-year-old females (most abundant age and sex groups) and had no lambing recently. Routine blood and veterinary tests were performed to ensure that all individuals were healthy and physiologically consistent.

Since all female goats in this crossbred herd needed to be used for reproduction, only male goats that cannot be used for reproduction were selected for slaughter and tissue sample collection. After slaughter, we collected the whole blood, brain tissue (including hippocampus, thalamus, hypothalamus, pituitary, prefrontal, medulla oblongata, pineal, and cerebellum), esophagus, reticulum, spleen, thigh muscle, liver, rumen, omasum, calf muscle, abomasum, lung, and kidney and sampled two male hybrids (11 and 22) immediately frozen in liquid nitrogen for RNA extraction.

FD test

An FD test was used in a segregating population to examine the relationship between vigilant behavior and the vigilant locus. Longer FD is related to increased vigilance (*45*), and a short FD is one of the pre-adaptive behavioral characteristics in animal domestication (*46*). This test was carried out as previously described (*47*, *48*) with slight modifications. Before the test, the subjected individuals were reared in an isolated home pen (4.4 m by 18.9 m) with access to a large playground (16.4 m by 18.9 m) for 3 weeks and experienced the usual frequency of contact with the same staff. Then, the test was performed in an adjacent enclosed home pen in which the animals had never been held. To increase the reliability of the data, FD measurements were carried out by two experimenters, both unfamiliar with the animals, in a double-blinded controlled manner for three continuous days in the middle of March 2018. The experimenters wore the same outer clothing (white lab coat) for doing each test. The order in which the animals were tested was randomly allocated each day. The subject animal was removed from the group and taken to the home pen. It was confined for 1 min, and the experimenter stood 7 m away. Then, one experimenter approached from the front toward the animal's head, keeping eye contact with the animal, looking toward the feet of the animal to avoid interference due to poor eyesight, and keeping arms and hands close to the body. When the subject animal withdrew at least two steps or escaped from either of the two sides, the distance between the animal's foreleg and the foot of the experimenter was measured as the FD using a measuring tape. Then, the subject animal was brought back to another playground, and the next animal was taken to the home pen for testing. The measurements were repeated six times for each animal in 1 day. For each animal, after removing the maximum and minimum values, the left four measurements were used for the subsequent analysis.

RNA sequencing and data analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocols. Genomic DNA contamination was removed using RNA-free deoxyribonuclease I, and RNA quality was measured using a bioanalyzer (Agilent, USA). The RNA integrity number was required to be greater than 7. mRNA was isolated from total RNA using a NEBNext UltraTM RNA Library PrepKit for Illumina (New England Biolabs, USA) according to the manufacturer's recommendations. cDNA fragments (300 to 500 bp) were used to construct the library. The Illumina X Ten platform was used to sequence the DNA library and generate 150-bp paired-end reads. The quality of reads was evaluated using FastQC (*49*) and using Trimmomatic (*33*) to remove adapters and low-quality reads. All high-quality reads were then aligned to GCF_001704415.1 using STAR (*50*) and Hisat2 (*51*) (recompare reads that STAR did not compare to). Expression levels were quantified using Stringtie (*52*). DEGs were obtained using Deseq2 (*53*), and Kyoto Encyclopedia of Genes and Genomes enrichment analysis was performed using KOBAS (*54*).

Identification of ASE genes

The allele analysis was applied as described before (*32*). We defined the fixed divergent sites between domestic goats and ibexes using a threshold of FST equal to 1 and removing the sites with more than two genotypes in the hybrids. Genes with ASE were identified by comparing read counts between the two alleles. The statistical significance of imbalance was calculated using a binomial test and Benjamin-Hochberg false discovery rate (FDR) correction (threshold of 0.05). Allele ratio (>0.65 or <0.35) and FDR (<0.05) cutoff criteria were used to measure ASE genes (ASEGs), as in a previous study (*55*). ASEGs were annotated with ANNOVAR and used to mark the origin of each read and separate the whole transcriptome into three categories, i.e., ibex, goat, and unknown. The separated ibex and goat transcriptomes were used to calculate gene expression levels.

Conservative analysis of *RRM1*

We conducted conservation analysis among species for the candidate SNPs. The orthologous sequences of RRM1 and the phylogenetic tree file were downloaded from the UCSC genome browser (http://hgdownload.cse.ucsc.edu/goldenPath/hg38/multiz100way). Multiple sequence alignment was performed using MEGA7 (*56*).

The online tool iTOL (https://itol.embl.de/) was applied to display the topological structure.

Protein structure prediction of RRM1

We submitted the amino acid sequence of two-types RRM1 (WT and *RRM1*^{I241V}) to SWISS-MODEL for protein structure prediction (https://swissmodel.expasy.org/interactive) (*57*). We then used PyMOL (https://pymol.org/2/) (*58*) to visualize three-dimensional structure. Furthermore, we used ProtScale (https://web.expasy.org/protscale/) to analyze the hydrophobicity of mutant SNP and adjacent amino acids. In addition, we also analyzed the conserved domain of this site through CDD (www.ncbi.nlm.nih.gov/cdd) with default parameters.

Generation of *RRM1*^{I241V} mutation mice

All mice used for experiments were bred and raised under the standard conditions in the investigator′s colony at the Animal Core Facility of Northwest A&F University (Shanxi, P. R. China, certificate no. SCXK [SHAAN] 2017-003). All experimental procedures were approved by Northwest A&F University and performed according to the guidelines of the National Institutes of Health for laboratory animals (www.nc3rs.org.uk/arrive-guidelines).

RRM1^{I241V} point-mutant mice were generated using the CRISPR-Cas9–mediated genome editing program at the Zhaoqing Huaxia Kaiqi Biotechnology Co. Ltd. (Guangdong, P. R. China). Briefly, the fragment for *RRM1* guide RNA (gRNA) was inserted into the gRNA cloning vector. The *RRM1*^{I241V} mutation site in the donor oligo (M-Rrm1-AtG-oligo: gatgacagcattgaaggaatttatgatactctgaagcagtgtgccttg**G**tttctaagtccgctgggggaattggtgttgctgtgattgtattc) was designed and synthesized. Then, Cas9 mRNA and gRNA generated by in vitro transcription and donor oligo were coinjected into fertilized eggs for *RRM1*^{I241V} mouse production. All of these mice were kept the same C57BL/6J genetic background and were genotyped by PCR with the following primers: forward primer (5′- GAGTTCAGTTCCCAGCAACC-3′), reverse primer (5′-CCTCCTACTCACACCAACAGG-3′), melting temperature = 60°C, PCR product length of 548 bp. The positive F0 generation male mice were mated with healthy WT female C57BL/6J mice which were purchased from the Laboratory Animals Center of Xi′an Jiaotong University (Xi′an, China), to obtain a positive F1 generation mouse model that can be stably inherited. The resulting F1 offspring were intercrossed to generate the F2, and the resulting progeny were then genotyped by PCR (homozygous with *RRM1*^{I241V} mutation mice: *RRM1*$^{I241V/I241V}$; heterozygous mutant mice: *RRM1*$^{+/I241V}$; homozygous without *RRM1*^{I241V} mutation mice: *RRM1*$^{+/+}$), and mouse models of *RRM1*^{I241V} mutations were successfully constructed. All subsequent experiments were carried out using mutant and WT mice (unmutated homozygous littermates) in the same litter. All mice were homozygous and older than 3 months male mice.

Behavioral assays

Identification of tameness

Three different acclimation tests: active taming test, passive taming test, and hand test were established to measure the acclimation of mice as previous study (*5*). All tests were performed in a box (40 cm by 40 cm by 40 cm) under uniform illumination to monitor animals by video recording. In active tame test, the experimenter placed his hand at the bottom of the test field and tried to keep his hand about 10 cm away from the mouse with moving fingers slightly. The durations of three behavioral characteristics were measured: toward hand, contacting hand, and jumping. Passive taming test aims to evaluate the passive response of animals to human hands was carried out directly after the active acclimation test. The experimenter tries to touch the mouse for as long as possible, and each test measurement index is the same as the active acclimation test. Hand tests were used to quantify behavioral responses to human-forced stimuli and carried out after the passive acclimation test. The mouse was placed in hand three times and touches the back of the mouse gently. The time that the mice remained on the hand in three trials from each film was calculated, and the average was used as characteristic data.

After tame test, we sampled six test mice (three *RRM1*$^{+/+}$ mice and three *RRM1*$^{I241V/I241V}$ mice) brain tissues and then extracted RNA for transcriptome sequencing. RNA extraction and transcriptome sequencing and analysis processes are consistent with the "RNA sequencing and data analysis" section.

Social interaction test

We set up a 40 cm by 40 cm by 40 cm box that contains four separate regions and can pass each other. Each experimental mouse was placed into region 1, which also served as a control region. The region 2 was placed with a 30-day-old C57 BL/6J, the region 3 was placed with an over 100-day-old C57 BL/6J, and the region 4 was placed with an adult bigger and more aggressive ICR mouse. The equipment for shooting the video is set above the box to record the video. ANY-maze (ANY-Maze, USA) behavior tracking software was used to process and analyze the videos. Before the experiment, the tested mice were placed in the device to habituate the environment for 5 min, and the other three areas were empty. After habituation, the tested mouse was moved away, and the resident mice were placed in the three different regions, and then the tested mice were placed back to the device for testing the social ability.

Open-field test

OFT measures the exploratory activity and anxiety behavior of mice in a novel environment. The test mice were placed individually in the center of an open field box (50 cm by 50 cm by 30 cm) with inner and external areas. The box was divided into 25 equal squares including 9 cells in the center and the remaining 16 cells in the outer area. Each session lasted 5 min, and distance and time traveled in the inner area were recorded in videos and were analyzed by ANY-maze. The box was wiped with 75% ethanol every time after each trail to avoid the influence of the residual odor on the experiment.

Elevated plus maze

The EPM device is 70 cm high above the ground, consists of two open arms (dimensions: 35 cm by 6 cm), two closed arms (dimensions: 35 cm by 6 cm by 14 cm), and a central region (dimensions: 6 cm by 6 cm). The equipment was confirmed to be clean and taste-free and wiped with 75% ethanol every time after testing a mouse. Put the animal in the center of the equipment with its head facing the open arm. The duration of the test was 5 min. During the test, the time and distance in open arms were recorded.

Morris water maze

Morris water maze was performed to test the learning and memory ability of animals in spatial locations. The water maze was divided into four quadrants, and the quadrant with hidden platform was named as the target quadrant. The first 6 days were directional

navigation experiments, requiring animals to find a platform below the water surface in 60 s. Animals entered the platform from each quadrant every day and explored the platform four times in total. The space exploration experiment was arranged on the seventh day, and we observe the swimming trajectory of animals in the water with removing the platform. The swimming trajectory of the animal was recorded by a camera and analyzed by the ANY-maze video tracking digital system (ANY-Maze, USA). The detection indexes are times of crossing platform, target quadrant residence, and platform residence time.

Quantification and statistical analysis

All data are shown as mean ± SEM unless otherwise specified. Statistical analyses were done with Prism 8.1 (GraphPad). Comparisons were conducted with Student's *t* test to compare Gaussian distributions, while Mann-Whitney tests were used for non-Gaussian distributions. Results were considered statistically significant when the P value < 0.05.

Supplementary Materials

This PDF file includes:

Figs. S1 to S8
Tables S1 to S4, S6, and S7
Legends for tables S5, S8 to S14
Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

Tables S5, S8 to S14
Movies S1 and S2

View/request a protocol for this paper from *Bio-protocol*.

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