IBRO Reports 9 (2020) 138–146

Contents lists available at ScienceDirect

IBRO Reports

journal homepage: www.elsevier.com/locate/ibror

Research Paper

Overexpression of serotonin receptor 5b expression rescues neuronal and behavioral deficits in a mouse model of Kabuki syndrome

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ARTICLE INFO

Keywords:
5-HT5B
UTX
Kabuki syndrome
Synaptic transmission
Anxiety
Memory

ABSTRACT

5-hydroxytryptamine receptor 5B (5-HT5B) is a gene coding for a G protein-coupled receptor (GPCR) that plays key roles in several neurodevelopmental disorders. Our previous study showed that disruption of 5-HT5B induced by lysine (K)-specific demethylase 6A (*Kdm6a*, also known as *Utx*) conditional knockout (cKO) in mouse hippocampus was associated with cognition deficits underlying intellectual disability in Kabuki syndrome (KS), a rare disease associated with multiple congenital and developmental abnormalities, especially neurobehavioral features. Here we show that *Utx* knockout (KO) in cultured hippocampal neurons leads to impaired neuronal excitability and calcium homeostasis. In addition, we show that 5-HT5B overexpression reverses dysregulation of neuronal excitability, intracellular calcium homeostasis, and long-term potentiation (LTP) in cultured *Utx* KO hippocampal neurons and hippocampal slices. More importantly, overexpression of 5-HT5B in Utx cKO mice results in reversal of abnormal anxiety-like behaviors and impaired spatial memory ability. Our findings therefore indicate that 5-HT5B, as a downstream target of Utx, functions to modulate electrophysiological outcomes, thereby affecting behavioral activities in KS mouse models.

Introduction

Kabuki syndrome (KS) is a rare, autosomal dominant multiple congenital anomaly (MCA) syndrome characterized by multiple congenital anomalies involving the development and function of various organ systems (Armstrong et al., 2005; Bogershausen et al., 2013). Major clinical features include moderate-to-severe mental retardation, visceral and skeletal abnormalities, postnatal growth impairment (short stature) and facial abnormalities (Armstrong et al., 2005; Niikawa et al., 1981; Caciolo et al., 2018).

Whole-exome sequencing successfully identified heterozygous mutations in the histone methyltransferase KMT2D (previously MLL2) and demethylase KDM6A gene (previously UTX) as the major cause of KS (Ng et al., 2010). UTX plays a crucial role in general chromatin remodeling (Cho et al., 2007; Lan et al., 2007) and interacts with MLL2, in a conserved SET-1-like complex which trimethylates H3K4 (Lan et al., 2007). Previous study suggests that UTX and MLL2 may have overlapping functional roles in the development of tissues and organs in KS subjects (Van Laarhoven et al., 2015). More than 90 % KS patients have intellectual disability (ID, 92 %) (Niikawa et al., 1988). In our previous report, we model its intellectual disability features in mice with the KO of *Utx* in neural progenitor cell (NPC) (*Emx1-Cre: Utx* $^{flox/flox}$). We show that forebrain KO of *Utx* results in reduced long-term potentiation and amplitude of miniature excitatory postsynaptic currents, increased anxiety-like behaviors and impaired spatial learning and memory in mice. Bioinformatics analysis reveals that signal pathways involved in serotonergic synapse formation are disrupted in *Utx* cKO mice (Tang et al., 2017a).

Serotonin (5-hydroxytryptamine, 5-HT) is a widespread neurotransmitter in the nervous systems, which regulates numerous physiological functions by activating multiple receptors in sensory-motor, autonomic and behavioral systems (Azmitia, 2001). Although many 5-

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https://doi.org/10.1016/j.ibror.2020.07.005
Received 26 April 2020; Accepted 3 July 2020

HT receptors have been extensively studied (McCorvy and Roth, 2015), 5-HT5B to date remains poorly understood (Noda et al., 2004; Hoyer et al., 1994). In human central nervous system (CNS), the coding sequence of *HTR5B* is interrupted by stop codons, leading to a non-functional *HTR5B* gene (Grailhe et al., 2001a). Previous study suggests that upregulation of 5-ht_{5b} in the dorsal raphe nuclei might partly contribute to the abnormal behaviors of *Atf7* deficient or social isolation stress mice (Maekawa et al., 2010). Recently, in *Mecp2* deficient mice, a mouse model of Rett syndrome (RS), 5-HT5B dysregulation is a key but probably not the only factor that mediates irregular breathing behavior (Vogelgesang et al., 2017, 2018).

In our previous report, *Utx* cKO mice, which recapitulate neurobehavioral phenotypes of KS, show decreased level of 5-ht_{5b} in the hippocampus (Tang et al., 2017b). Furthermore, 5-ht_{5b} overexpression in cultured newborn hippocampal neurons rescues the neuronal morphology defects by *Utx* KO, suggesting that 5-ht_{5b} is implicated in the abnormalities of the neuronal development in *Utx* cKO mice (Tang et al., 2017b). Although downregulation of 5-ht_{5b} contributes to the morphological phenotypes in our established *Utx* cKO mice, we still don't know about the physiological and functional roles of 5-HT5B in vivo. Therefore, based on our findings in which *Utx* regulates neuronal development and morphology through the functional downstream target 5-HT5B, we hypothesized 5-ht_{5b} could affect cognitive, exploratory, and anxiety-related behavior and its restoration might be enough to rescue neuronal and behavioral deficits in the mouse model of KS induced by *Utx* KO. Here we first show that neuronal excitability and intracellular Ca^{2+} decrease in the soma of *Utx* KO hippocampal neurons. Next, 5-HT5B overexpression restores dysregulation of neuronal excitability and long-term potentiation (LTP) in *Utx* cKO mice. More importantly, behavioral analyses show that 5-ht_{5b} upregulation results in reversal of anxiety-like behavior and spatial memory ability induced by *Utx* KO. These findings indicate that down-regulation of 5-ht_{5b} expression is an important factor which modulates KS-like behaviors in *Utx* cKO mice.

Results

Overexpression of 5-HT5B rescues the decreased neuronal excitability in cultured Utx knockout hippocampal neurons

To characterize the functional role of Utx in synaptic plasticity, we first performed whole-cell patch-clamp recording (Fig. 1A) for the electrophysiological properties of the cultured hippocampal neurons by measuring resting membrane potential (RMP) (Fig. 1B), cell membrane capacitance (Cm) (Fig. 1C), and action potential (AP) (Fig. 1F). The passive membrane properties of *Utx* KO neurons exhibited negative resting membrane potential (RMP; −40 to −50 mV, Fig. 1B). The capacitance values of *Utx* KO neurons remained stable (Fig. 1C).

The ability to fire a train of repetitive APs upon membrane depolarization is a unique functional feature of mature neurons (Song et al., 2013). We firstly tested repetitive APs of the cultured P0 hippocampus neurons dissected and dissociated from WT and *Utx* KO mice. The results showed that *Utx* KO in the cultured hippocampal neurons led to the alteration of the firing pattern and firing frequency (Fig. 1D, E). In *Utx* KO group, only 30 % of the neurons fired repetitive APs, while the others had no AP or single/multiple APs (Fig. 1D, E). In contrast, the hippocampal neurons from WT mice showed repetitive normal APs (Fig. 1D, E). Because voltage-gated Na^+ channels play a major role in regulating APs firing, we further tested the change of the averaged amplitude of inward Na^+ (I_{Na}) between both groups. The results indicated that *Utx* KO resulted in a significant decrease in the amplitude of I_{Na} (Fig. 1F). Tetrodotoxin (TTX) treatment, which is a selective blocker of voltage-gated Na^+ channels, did not affect the RMP and Cm of the hippocampal neurons (Fig. 1B, C). However, the evoked inward I_{Na} was confirmed by its sensitivity to the blocking action of 100 nM TTX (Fig. 1E) and the current-voltage (I-V) relationship revealed by voltage steps from the holding potentiation of −50 mV up to +30 mV in 10 mV increments (Fig. 1I). In our previous study, 5-HT5B was downregulated in *Utx* cKO mouse hippocampus, to determine whether 5-HT5B was related to the disruption of neuronal excitability induced by *Utx* KO, we restored 5-HT5B by lentivirus-mediated gene delivery in cultured *Utx* KO hippocampal neurons. We firstly confirmed the overexpression of 5-HT5B protein by western blotting (Fig. 1G, H). The results showed that Lenti-Htr5b virus infection with 5-HT5B cDNA significantly increased 5-HT5B protein levels compared to the control Lenti-GFP infected hippocampal neurons (Fig. 1G, H). One week after lentivirus infection, whole-cell patch-clamp recording was then performed for the electrophysiological properties of the neurons. *Utx* KO neurons with 5-HT5B overexpression showed increased the AP frequency and produced more normal repetitive APs (Fig. 1D, E). Consistent with AP feature, the amplitudes of I_{Na} in *Utx* KO + *Htr5b* hippocampal neurons were also increased compared to those in *Utx* KO neurons, reaching comparable levels of control (Fig. 1F).

Overexpression of 5-HT5B rescues the decreased intracellular Ca^{2+} levels in Utx knockout hippocampal neurons

Dysregulation of Ca^{2+} homeostasis has been shown to be associated with many neurological diseases (Sepulveda-Falla et al., 2014; Blandini et al., 2009). Neuronal excitability is regulated by the changes of intracellular Ca^{2+} concentration (Sola et al., 2001). To determine whether neuronal intracellular Ca^{2+} concentration is altered in *Utx* cKO mice, we therefore performed confocal microscopic Ca^{2+} imaging for unbiased screening of the cultured hippocampal neurons. Consistent with the above findings, *Utx* KO neurons displayed significantly lower intracellular Ca^{2+} levels in the soma relative to the control neurons (Fig. 2A, C, E).

We next examined whether 5-HT5B overexpression could rescue the abnormal intracellular Ca^{2+} concentration induced by *Utx* KO. As shown in Fig. 2D and E, the intracellular Ca^{2+} concentration in *Utx* KO neurons was increased to the control level after one-week infection (Fig. 2A, B, D, E), which suggests the importance of Ca^{2+}homeostasis maintained by Utx and 5-HT5B to the functional integrity of the hippocampal neurons. These results further showed a key functional role of 5-HT5B in the mediation of delayed maturation of APs in *Utx* KO hippocampal neurons.

Overexpression of 5-HT5B rescues the decreased long-term potentiation in Utx cKO mice

Published researches demonstrate that KS mouse models show obvious hippocampal memory defects which might be related to intellectual disability (Tang et al., 2017b; Bjornsson et al., 2014). In central nervous system, LTP of synaptic transmission is the major form of activity-dependent plasticity and serves as a key synaptic model for investigating the cellular and molecular mechanisms of learning and memory (Bliss and Collingridge, 1993; Kandel, 2012; Bliss and Collingridge, 2013). In our previous work, *Utx* cKO mice display abnormalities of LTP and basal synaptic transmission in CA1 of the hippocampus (Tang et al., 2017b). Therefore, in the present study, we assessed whether 5-HT5B overexpression could reverse the decreased LTP induced by *Utx* KO (Fig. 3A). As expected, adult overexpression of 5-HT5B in the hippocampus was enough to rescue the decreased LTP induced by *Utx* KO in vivo (Fig. 3B, C). Together, our study provided significant evidence that 5-HT5B was a critical functional target of *Utx* in modulating hippocampal synaptic transmission.

Overexpression of 5-HT5B ameliorates anxiety-like behaviors and spatial learning and memory deficits in Utx cKO mice

Finally, we performed the behavioral tests to determine if 5-HT5B overexpression could reverse the behavioral abnormalities induced by

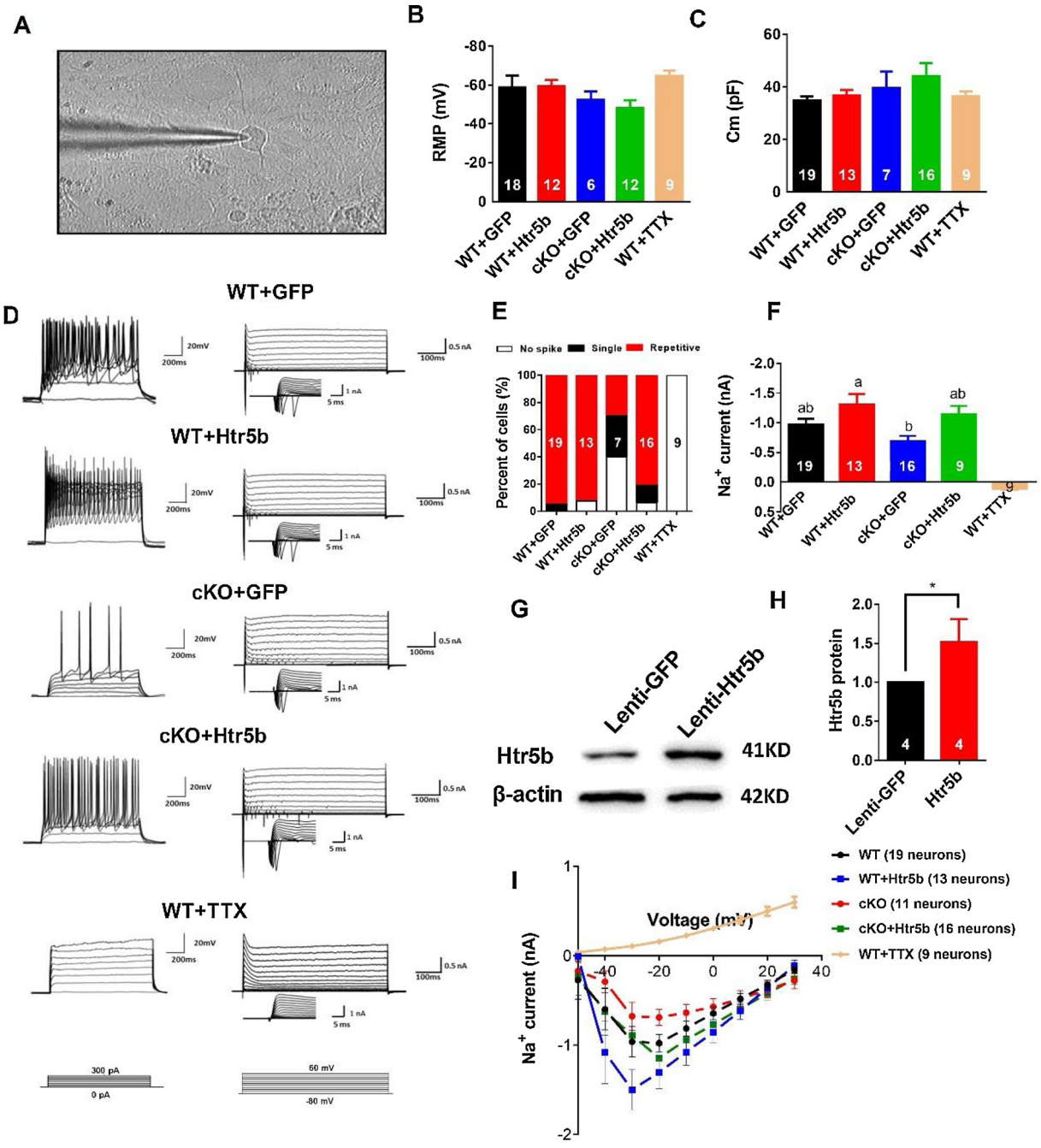

Fig. 1. Overexpression of 5-HT5B rescues decreased neuronal excitability in *Utx* knockout hippocampal neurons.
(A) Representative images of the soma of primary hippocampal neuron with a patch pipette on the surface. (B) Whole-cell patch clamp recording of resting membrane potential (RMP) in DIV10-15 hippocampal neurons. (C) Whole-cell patch clamp recording of cell membrane capacitance (Cm) in DIV10-15 hippocampal neurons. (D) Representative whole-cell recording of action potential from wild-type and *Utx* knockout neurons infected with lenti-virus expressing either GFP or Htr5b (GFP represents lenti-GFP virus infection, Htr5b represents lenti-Htr5b virus infection) (E) The effect of 5-HT5B on the induction of action potentiation (AP). (F) The pure inward Na^+ (I_{Na}) was evoked by voltage steps from -50 to +30 mV. Different letters above the bar mean significant difference ($p < 0.05$). ((G) Representative images of western blotting showing an increase of 5-HT5B in the Lentivirus-infected hippocampal neurons. Lenti-GFP and Lenti-Htr5b represents control and Htr5b overexpression, respectively. β-actin was used as loading control. (H) Quantification of western blotting analysis for infected lenti-Htr5b in the hippocampal neurons. (I) Comparison of mean voltage-current relationships for voltage-gated sodium currents in wild-type and Utx knockout neurons infected with lenti-virus expressing either GFP or Htr5b. The number of cells recorded in each case were shown in the figures. Lenti-GFP virus injected into WT (*Utx* $^{flox/flox}$) and cKO (*Emx1-Cre: Utx* $^{flox/flox}$) mice were labeled WT + GFP and cKO + GFP, respectively. Lenti-Htr5b virus injected into WT (*Utx* $^{flox/flox}$) and cKO (*Emx1-Cre: Utx* $^{flox/flox}$) mice were labeled WT + Htr5b and cKO + Htr5b, respectively.

Utx cKO. Four weeks later after hippocampus virus injection, we firstly conducted open field test and found that no difference in total distance moved between groups (Fig. 4A, C, E). 5-HT5B upregulation in WT littermates did not affect entries into the center of the arena, whereas such treatment in *Utx* cKO mice markedly increased the entry times (Fig. 4B, D, F). The buffering effect of 5-HT5B on the anxiety-like behavior of *Utx* cKO mice was then confirmed in the light-dark box test (Fig. 4G–M). 5-HT5B overexpression in control mice had no effects on the entry, time spent, and distance in the light box (Fig. 4H, K, L, M). However, *Utx* cKO mice with the 5-HT5B overexpression substantially

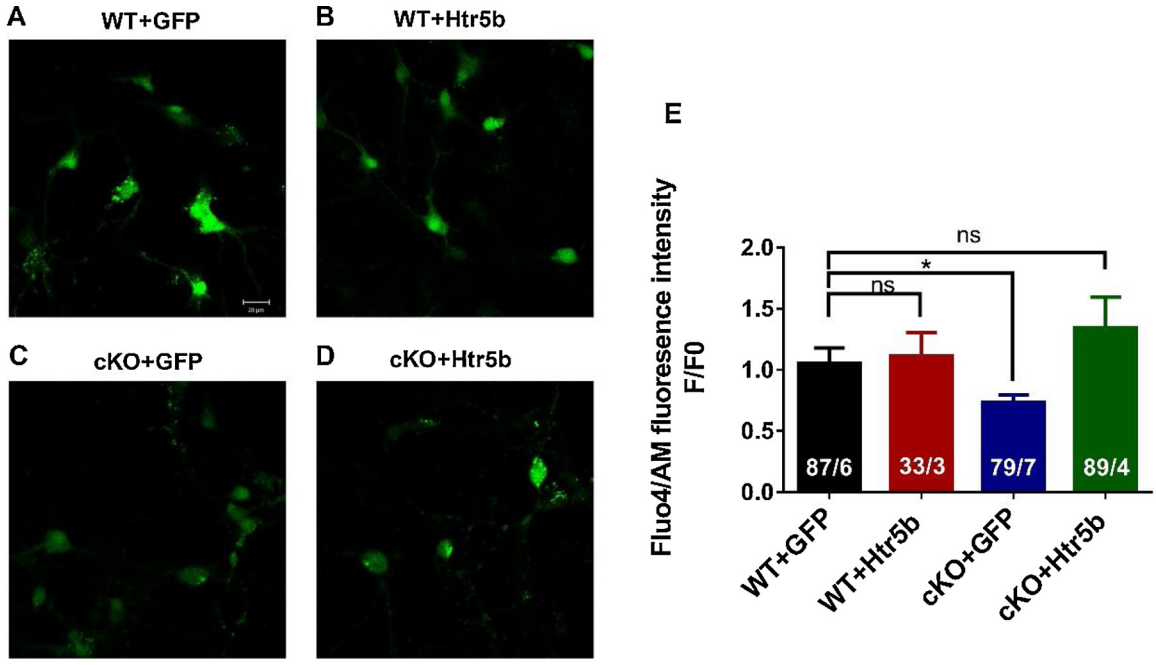

Fig. 2. Overexpression of 5-HT5B rescues decreased intracellular [Ca^{2+}] in *Utx* knockout hippocampal neurons.
(A-D) Representative confocal images of fluorescent intracellular [Ca^{2+}] in the DIV10-12 hippocampal neurons. Scale bars, 20 μm. (E) Effect of 5-HT5B on average [Ca^{2+}] in the DIV10-12 hippocampal neurons from three preparations. Lenti-GFP virus injected into WT (Utx $^{flox/flox}$) and cKO (Emx1-Cre: Utx $^{flox/flox}$) mice were labeled WT + GFP and cKO + GFP, respectively. Lenti-Htr5b virus injected into WT (Utx $^{flox/flox}$) and cKO (Emx1-Cre: Utx $^{flox/flox}$) mice were labeled WT + Htr5b and cKO + Htr5b, respectively. The number of cells recorded are shown on the columns. (*) $p < 0.05$.

increased entries, time spent, and distance in the light box (Fig. 4J, K, L, M). These behavioral tests clearly demonstrated that the overexpression of 5-HT5B could ameliorate anxiety-like behaviors in *Utx* cKO mice.

Next, we evaluated whether 5-HT5B overexpression could rescue spatial learning and memory deficits in *Utx* cKO mice by using Morris water maze test. During the training period, we did not observe any difference of latency (Time to find the submerged platform) between control mice with or without 5-HT5B overexpression (Fig. 4N, O, R). However, in *Utx* cKO mice, the overexpression of 5-HT5B resulted in the decreased latency (Fig. 4P, Q, R). Furthermore, these mice took a shorter time to locate the hidden platform in the training trials and spent more time in the platform zone during the probe test, compared with *Utx* cKO mice (Fig. 4R). Finally, *Utx* cKO mice with 5-HT5B overexpression displayed comparable times of target cross to control mice during the probe test (Fig. 4N, Q, S). These findings indicated that 5-HT5B played a critical role in the regulation *Utx* KO-mediated spatial memory deficits.

Discussion

Among all 5-HT receptors identified in rodents, the 5-HT_5 receptor family is composed by two subtypes named 5-ht_{5a} and 5-HT5B which have low sequence homology (less than 50 %) with the other 5-HT receptors (Humphrey, 1997). In rodent species, 5-HT5B is expressed exclusively in hippocampal pyramidal neurons and in the medial habenula (Grailhe et al., 2001a; Meneses, 1999). Human 5-HT5B was suggested to have loss of function due to stop codons existing in the first exon (Grailhe et al., 2001b). In addition, selective agonist or antagonist are still no available for 5-HT_{5B}. Therefore, up to now, a very limited number of studies were conducted to address the function of 5-ht_{5b} in CNS (Maekawa et al., 2010; Vogelgesang et al., 2017, 2018; Niebert et al., 2017).

Growing evidence indicates that 5-HT receptors mediate excitatory responses in the brain and are closely associated with cognitive behaviors (Meneses, 1999; Bockaert et al., 1992; Chapin et al., 2002; Xiang and Prince, 2003; Zhang, 2003; Tada et al., 2004; Davies et al., 1987). Our previous work presents that *Utx* KO leads to impairments in neuronal development and synaptic plasticity and consequently contributes to cognitive deficits in *Utx* cKO mice (Tang et al., 2017b). *Utx* knockout disrupts *Htr5b* expression and 5-HT5B overexpression in newborn hippocampal neurons rescues *Utx* KO-mediated morphological defects, suggesting that 5-HT5B is a functional downstream target of *Utx* in modulating neuronal growth and morphology (Tang et al., 2017b). Therefore, we aimed to further investigate whether 5-HT5B functions to regulate neuronal excitability in vitro and synaptic transmission in vivo. Firstly, we found that *Utx* KO resulted in decreased Na^+ current and decreased APs frequency in the cultured hippocampal neurons. Moreover, 5-HT5B overexpression restored dysregulation of Na^+ current and firing frequency induced by *Utx* KO. Our study provided clear and strong evidence that 5-ht_{5b} could modulate neuronal excitability of the hippocampal neurons, serving as a functional downstream target of *Utx*. 5-HT5B specifically interacts with 5-ht_{1a}, resulting in reduction of *Htr1a* surface expression (Vogelgesang et al., 2017). 5-ht_{1a} exerts inhibitory effects on the firing activity of hippocampus CA1 pyramidal neurons (Tada et al., 2004). It will be interesting to investigate whether 5-HT5B -induced firing impairments are dependent on reduction of *Htr1a* receptor surface expression in future.

Neuronal firing is associated with Ca^{2+} influx (Markram et al., 1995; Abel et al., 2004). APs firing and synaptic inputs can therefore be assessed, sometimes quantitatively, by measuring changes in concentration of intracellular Ca^{2+} (Yasuda et al., 2004). In our previous study, *Utx* KO in the hippocampus resulted in dysregulated expression of several genes (*Gng4, Unc13c, Cacna1g, Cacna1i, Actn2, Pvalb, Sytl5*) which encode either calcium channel or calcium binding protein, indicating a key role of *Utx* in the regulation of calcium homeostasis (Tang et al., 2017b). Our present work indicated that the concentration of intracellular Ca^{2+} was dramatically decreased in *Utx* KO neurons. In line with the necessary role of 5-HT5B in neuronal firing, upregulation of 5-HT5B in *Utx* loss neurons could rescue the lower intracellular Ca^{2+} levels to the control levels. Since many cellular functions are Ca^{2+}-dependent, the regulation of 5-HT5B on intracellular Ca^{2+} may have an important physiological meaning.

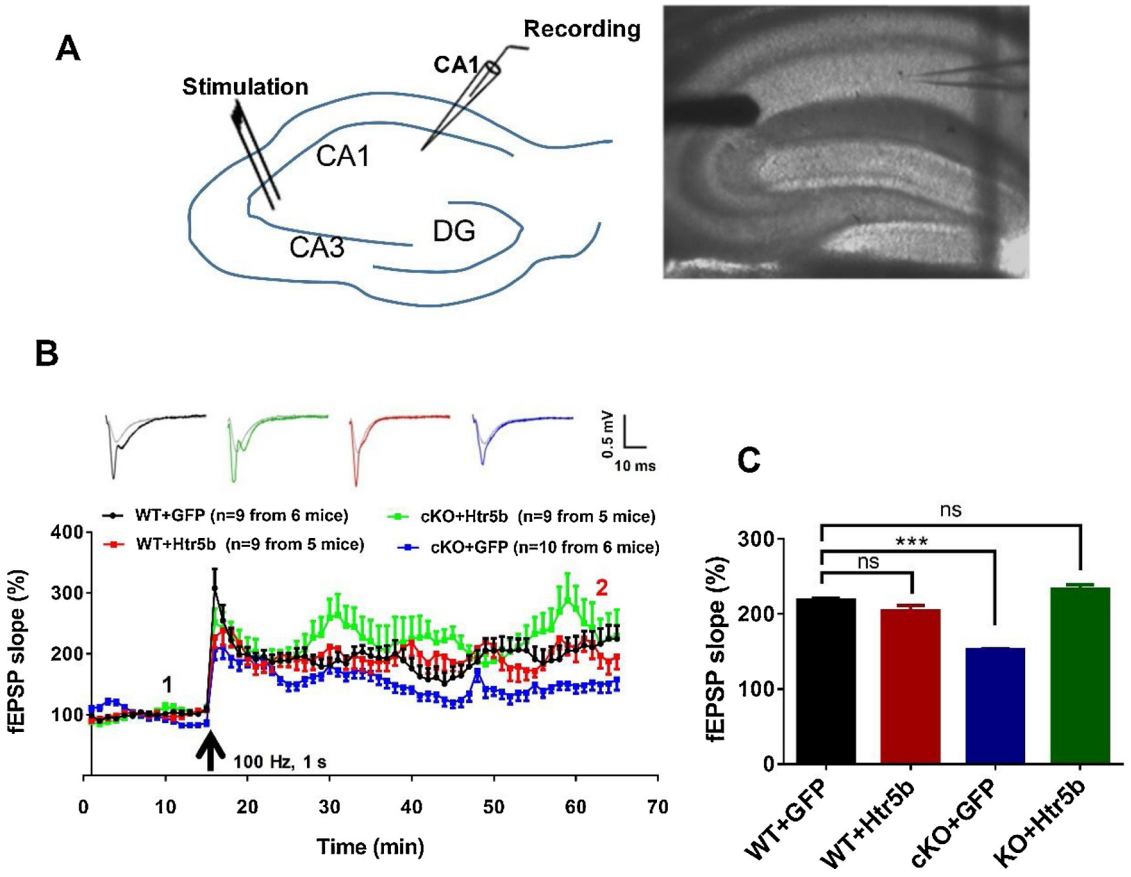

Fig. 3. Overexpression of 5-HT5B rescues decreased long-term potentiation in *Utx cKO* mice.
(A)Schematic overview of the electrophysiological protocol for acute hippocampal slice recording. (B) Typical experiment showing time course of CA1 LTP for a single recording from WT and cKO mice injected with lenti-virus expressing either GFP or 5-HT5B. fEPSP traces before (1, black) and after (2, red) are shown in the inset above. WT: n = 9 slices from 6 mice; WT + *Htr5b*: n = 9 slices from 5 mice; KO: n = 10 slices from 6 mice; KO + *Htr5b*: n = 9 slices from 5 mice. (C) Effect of 5-HT5B expression on LTP amplitude measured at 45 − 50 min post-induction. (***) $p < 0.001$.

5-HT is a potent neuromodulator of hippocampal circuitry (Bickmeyer et al., 2002) and involved in synaptic plasticity (Bliss and Collingridge, 1993; Kirkwood, 2000). Several 5-HT receptors are implicated in the regulation of LTP (Lee et al., 2018; Hashemi-Firouzi et al., 2017), however, the roles of different types of 5-HT receptors in LTP induction seem to be variable and even to be contradictory (Lee et al., 2018). 5-ht$_{5b}$ is expressed in hippocampal CA1 cells and, to a lesser extent, in dentate granule cells, subiculum and entorhinal cortex (Wisden et al., 1993). These regions are viewed as an excitatory circuit loop, 5-HT5B may therefore be involved in tonically modulating the general tone of this circuit (Wisden et al., 1993). Previously, we reported that loss of *Utx* in the hippocampus leads to impaired neuronal development and reduced LTP. Overexpression of 5-HT5B expression in newborn hippocampal neurons could rescue the defects of neuronal morphology induced by *Utx* ablation. In this study, upregulation of 5-ht$_{5b}$ had no effect on LTP in control mice, while its overexpression in Utx cKO mice restored the lower LTP, comparable to the levels in control mice. These results provided a clear evidence that 5-ht$_{5b}$ may act as a powerful regulator of LTP induction in the hippocampus.

Numerous studies suggested extended roles of 5-ht$_{5a}$ in the regulation of behaviors (Noda et al., 2004). However, there are only two studies that report the possible roles of 5-ht$_{5b}$ in the regulation of animal behavior. 5-HT5B has been found to be dramatically upregulated in mice undergoing social stress (Maekawa et al., 2010) and in mouse models of Rett syndrome (RTT) (Vogelgesang et al., 2018; Niebert et al., 2017), a severe neurodevelopmental disorder caused by mutations in the transcription factor MeCP2. The role of 5-HT5B in the brain remains to be determined. Previously, we reported that *Utx* KO resulted in increased anxiety-like behaviors and impaired spatial learning and memory in mice (Tang et al., 2017b). Here we found that 5-HT5B overexpression in in the hippocampal neurons of *Utx* cKO mice was enough to restore anxiety-like behaviors and spatial memory deficits induced by *Utx* KO. To our knowledge, this is the first study demonstrating a clear role of 5-HT5B in the regulation emotional behavior, learning and memory.

Materials and methods

Mice

All experiments involving mice complied with the animal protocol approved by the Institutional Animal Care and Use Committee at The Institute of Zoology, Chinese Academy of Sciences. Mice were kept in a temperature- and humidity-controlled 12 h light-dark cycle and had free access to water and standardized pellet food. *Utx* $^{flox/flox}$ (stock number 021926) and *Emx1-IRES-Cre* transgenic mice (stock number 005628) were obtained from Jackson Lab. The conditional *Utx* knockout mice were generated by breeding *Utx* $^{flox/flox}$ mice with *Emx1-IRES-Cre* transgenic mice.

DNA constructs

The mouse *Htr5b* (1,110 base pairs) was amplified by polymerase chain reaction (PCR) from mouse brain cDNA. PCR products were cloned into pCDH-CMV-MCS-EF1-copGFP (System Biosciences).

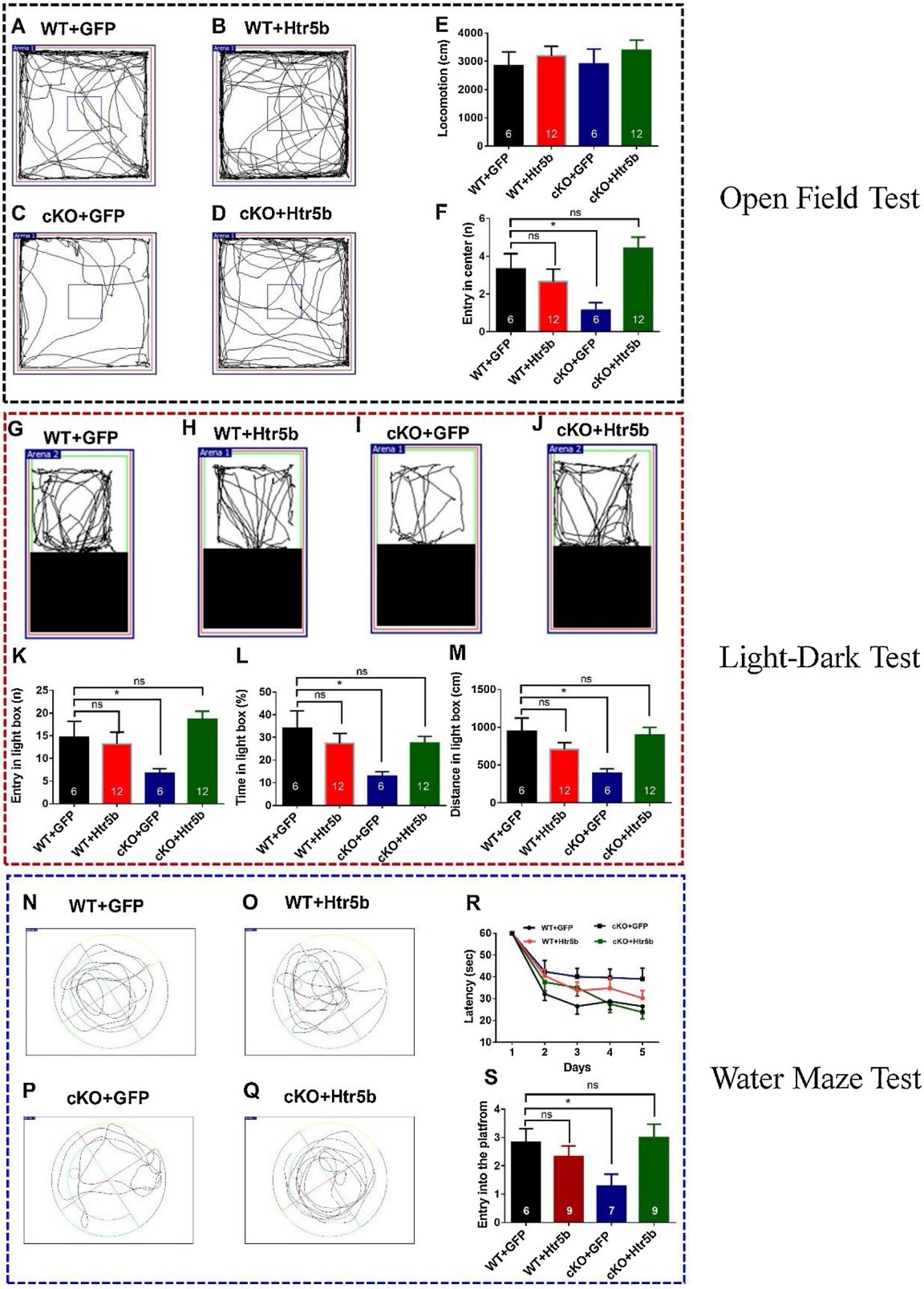

Fig. 4. Overexpression of 5-HT5B ameliorates anxiety-like behaviors and rescues spatial learning and memory deficits in *Utx* cKO mice.
(A–D) Representative locomotor patterns in an open field test. (E) Locomotivity analysis in open field test over a 5-min period. (F) Entry into the center zone during 5-min open field test. (G–J) Representative locomotor patterns in a light-dark field test. (K) Entry into the light box during 5-min light-dark test. (L) Time spent in the light box over a 5-min light-dark test. (M) Distance in the light box over a 5-min light-dark test. (N–Q) Representative locomotor patterns in water maze test. (R) Latency to target the platform during 5-day training in Morris water maze test. (S) Platform crossing in Morris water maze test. (*) $p < 0.05$.

Western blotting

Cultured neurons were lysed in buffer containing 25 mM HEPES at pH7.9, 150 mM NaCl, 1 mM PMSF, 20 mM NaF, 1 mM DTT, 0.1 % NP40, and proteinase inhibitor cocktails (Roche). 10 μg of the protein was separated on 8–12 % SDS-PAGE gels (Bio-Rad) and transferred to PVDF membranes (Millipore). The membranes were blocked in 5% BSA in TBS-T with 0.05 % Tween-20 and incubated with primary antibodies at 4 °C overnight. Dilutions of primary antibodies were 1:1000 for 5-ht_{5b} (TA315779, OriGene) and 1:10000 for β-actin antibody (Sigma). HRP-linked goat anti-mouse or HRP-Linked goat anti-rabbit were used as secondary antibodies. Dilutions of secondary antibodies were 1:1000. Enhanced chemoluminescence (ECL, Pierce) was used for detection. Quantification of the blots was determined with the use of a Quantity One Ver.4.4.0 (BioRad, USA).

Stereotactic injection

Stereotactic injections into the hippocampus (stereotaxic coordinates from Bregma: 2.0 mm caudal, 1.2 mm lateral, 2.0 mm ventral; 2.8 mm caudal, 2.0 mm lateral, 1.7 mm ventral;) were performed as follows: 8-week-old male mice were bilaterally injected with 1 μL Lentivirus at a rate of 0.125 μL/min. Lenti-GFP virus injected into WT (*Utx* $^{flox/flox}$) and cKO (*Emx1-Cre: Utx* $^{flox/flox}$) mice were labeled WT + GFP and cKO + GFP, respectively. Lenti-Htr5b virus injected into WT (*Utx* $^{flox/flox}$) and cKO (*Emx1-Cre: Utx* $^{flox/flox}$) mice were labeled WT + Htr5b and cKO + Htr5b, respectively.

Neuronal culture and transfection

The experiments were carried out according to experimental procedures described previously (Tang et al., 2017b). Briefly, the male pups (P0) control and *Utx* cKO mice were sacrificed and the brains were kept in an ice-cold PBS. The dissected hippocampus tissue was digested with TrypLE Express for 5 min at 37 °C. The tissue was then washed three times with DMEM plus 10 % FBS and were triturated by means of repetitive aspirations though a sterile and fire-polished Pasteur pipette. Neurons were grown in Neurobasal medium (Invitrogen) supplemented with 2% B27 (Invitrogen), 2 mM GlutaMAX (Invitrogen) and penicillin/streptomycin. Neuronal transfections were performed on Day7 in vitro (DIV7).

[Ca^{2+}] measurement

The [Ca^{2+}] measurements were accomplished with Fluo-4/AM by scanning laser confocal microscopy (Leica, Heidelberg, Germany). Hippocampal neurons (DIV10 − 12) were incubated with 5 μM Fluo-4/AM at 37 °C and 5% CO_2 for 30 min in the dark and then washed to remove extracellular Fluo-4/AM dye. Then, laser scanning began to obtain time series of images over a short time period (5 min) at excitation and emission wavelengths of 488 nm and 526 nm, respectively. The obtained images were quantitatively analyzed for changes in fluorescence intensities within cells. The data were expressed as the relative fluorescence intensity. Changes in [Ca^{2+}] are given as F/F0, where F0 is the baseline fluorescence level and F is determined by the relative change from the base level.

Electrophysiology

Acute hippocampal slice preparation

Acute hippocampal slices were prepared from 8-week-old male *Utx* cKO mice and their *Utx* $^{flox/flox}$ littermates as control. Briefly, mice were deeply anaesthetized with isoflurane and decapitated. Brain hemispheres were rapidly isolated and placed in ice-cold standard solution containing (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 D-Glucose, 2 $CaCl_2$, and 1.5 $MgCl_2$ saturated with 95 % O_2 and 5 % CO_2 to pH: 7.4). Hippocampal slices of 300 μm thickness was prepared using a vibratome (Campden Instruments, Loughborough, UK) and immediately transferred to an incubation chamber containing the same solution at 32 °C (pH 7.3). The prepared slices were incubated in oxygenated aCSF at room temperature at least for 1 h, and then individual slices were transferred to a recording chamber, which was perfused with oxygenated aCSF.

Cultured neuron electrophysiological recording

Hippocampal neurons (DIV0) were placed on the glass coverslips for electrophysiological detection. Ten days later (DIV10), whole-cell patch-clamp recordings in either voltage- or current-clamp mode were conducted to measure the voltage-activated sodium/potassium currents or action potentiation, which were recorded using an Axopatch 700B amplifier (Molecular Devices). Patch pipettes (4–6 MΩ) were pulled from borosilicate glass capillaries (GB 150F-8 P) with a micropipette puller (Sutter instrument, USA). The internal pipette solution contained: (in mM) 135 K-gluconate, 10 HEPES, 2 $MgCl_2$, 10 EGTA, 0.3 MgGTP, 0.5 Na_2ATP (pH 7.3 with KOH). Series resistances and cell capacitance compensation were carried out prior to recording. Only the recordings for which a high-resistance seal (> 1 GΩ) and a series resistance < 25 MΩ was maintained through the experiment were included in this study. TTX (100 nM) was used in the bath solution for the detection of action potentiation.

Field excitatory postsynaptic potentiations (fEPSPs) recordings

The experiments were performed according to experimental procedures described previously (Tang et al., 2017b). fEPSP recording were made in artificial cerebrospinal fluid in a submersion chamber at 30 °C by stimulating Schaffer collaterals and recording in stratum radiatum of hippocampal CA1. fEPSPs in CA1 were induced by stimuli at 0.033 Hz with an intensity that elicited a fEPSP amplitude of 40–50 % of the maximum. After establishment of stable baseline recordings for at least 15 min, LTP was induced by a high-frequency stimulation (HFS) consisting of one train of 100 Hz stimulation for one second at baseline stimulation intensity. The fEPSP signals were digitized using Digidata1440A interface board. The data were sampled at 10 kHz and filtered at 2 kHz. Recordings were analyzed using the Clampfit 10.6 (Axon Instruments, Foster City, CA).

Behavioral tests

All behavioral tests were performed during the light phase of the cycle between 09:00 and 17:00. Male mice at 8–10 weeks of age were used for all the behavioral tests. The number of mice per group is indicated in the figures. All the videos were analyzed by Smart software (Pan lab, Harvard Apparatus).

Open field test

Mice were placed in the center of open-field arena (72cm × 72cm × 36 cm) and allowed to explore for 5 min. The animals were monitored from above by a video camera. To evaluate anxiety-like behavior, the numbers of entries into the center zone (18cm × 18cm) verse the periphery was compared.

Light-dark test

Light-dark test was performed according to standard protocol (Costall et al., 1993). An apparatus (45 × 27 × 27 cm) consisting of two chambers, a black chamber (18 × 27 cm) and a light chamber (27 × 27 cm), was used for the light/dark exploration test. Mice were placed in the center of the white chamber facing the opening and allowed to move freely between the two chambers for 5 min. Measurements were taken of the total number of transitions between the light and dark chambers and the total amount of time spent in the light

chamber.

Morris water maze

Morris water maze test was performed as described previously (Vorhees and Williams, 2006). A 120 cm diameter, 45 cm deep Morris water maze was filled with water to a depth of 25 cm. Target escape platform (diameter 13 cm) was hidden 1.5 cm beneath the surface of the water at the center of a given quadrant of the water tank. Four extra-maze cues, in different shapes, colors, and sizes, were uniformly located on the wall surrounding the water tank. The water temperature was adjusted to 21 ± 1°C. During training, mice received training in the Morris water maze for five successive days, and each session consisted of four trials. The mice were allowed to swim for up to 1 min to locate the platform. If it failed to locate the platform within that time, escape was assisted. Mice were introduced gently to the hidden platform and allowed to rest on the platform for 15 s. A probe test was conducted 24 h after completion of the training. During the probe test, the platform was removed from the pool, and the task performances were recorded for 60 s. Time spent and entry in each of the four quadrants (target, opposite, left and right) were analyzed.

Statistical analysis

Data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality of variance using the Kolmogorov-Smirnov test. Group differences were calculated using two-tailed *t*-tests when comparing two groups, or one-way or two-way ANOVA with multiple comparisons when appropriate for comparing four groups. Turkey *post hoc* tests were used following one-way ANOVA as appropriate. Group differences of latency to platform during training period in water maze test were assessed by repeated measures ANOVA. Data are expressed as mean ± SEM. A *P*-value of 0.05 or less was considered to represent significant differences. The *P*-values were displayed in the figures as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Author contributions

GBT, J G and CML, conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; G.B.T., T.W.M, M.L.S., H.Z.D, Z.Q.T., collection and assembly of data.

Compliance with ethical standards

All experiments involving mice complied with the animal protocol approved by the Institutional Animal Care and Use Committee at The Institute of Zoology, Chinese Academy of Sciences. We certify that formal approval to conduct the experiments described has been obtained from the animal subjects review board of their institution and could be provided upon request. We attest that we made all efforts to minimize the number of animals used and their suffering.

Conflicts of Interest

The authors declared no conflict of interest with respect to the research, authorship, and publication of this article.

Acknowledgements

This work was supported by grants from the National Key Research and Development Program of China Project (2016YFA0101402) and the National Science Foundation of China (91753140, 81771224), the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA16010302).

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