Supplemental Information

Materials and Methods

Colony maintenance, expression study and behavioral testing of G72/G30 Transgenic (Tg) mice

Colony maintenance

The male Tg founder mouse was mated with female B6CBAF1/J wild-type (WT) mice (The Jackson Laboratory, Bar Harbor, ME) to keep the line.

Our G72/G30 BAC transgenic mouse train has been deposited and is available at the Mutant Mouse Regional Resource center (MMRRC, www.mmrrc.org, supported by NIH. Strain name: B6;CBA-Tg(RP11-166E2)1Cliu/Mmmh. Stock number: 34680).

Animal housing

All mice were housed in same-sex groups of 3 to 5 members in standard mouse cages (30 cm × 18 cm ×16 cm), unless otherwise specified for a particular behavioral assessment. Conventional laboratory conditions – constant room temperature (22 ± 2°C), humidity level (55 ± 5%), 12 h light: 12 h dark cycle (lights on at 6 a.m.) – were maintained. Food and water were available *ad libitum*.

Mouse tail DNA extraction and PCR genotyping of G72/G30 Tg mice

Genomic DNA was extracted from mouse tail using the traditional SDS/proteinase K digestion method and used as PCR template to screen founders and transgenic mice. A pair of G72 genomic sequence specific primers, 1-2-3 F and 1-2-3 R (Supp. Table 1), was used.

RT-PCR, T-A cloning and sequencing

The quality of total RNA was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Only samples with an rRNA 28s/18s ratio >1.2 and RNA integrity number (RIN)> 8.0 were used.

Approximately 5 µg of RNA extracted from mouse tissues was reverse transcribed to cDNA in a 20 µl reaction system with Oligo-$dT_{12\text{-}18}$ primer or random hexamer primers (SuperScript First Strand synthesis system, Invitrogen, Carlsbad, CA). Several pairs of G72 and G30 specific primers, the products of which bridged exons, were used in PCR (Supp. Table 1). Advantage GC-2 PCR system (Clontech, Mountain View, CA) was used. The 50 µl PCR reaction contained 5 µl of reversed transcribed cDNA template, 3 µl of each primer at 10 µM, 5X GC 2 PCR buffer 10 µl, 50X dNTP mix (10 µM each) 1 µl, 50X Advantage GC 2 polymerase mix 1 µl, PCR grade-H_2O to 50 µl. The PCR was performed in a Peltier Thermal Cycler (PTC-225; MJ Research, Watertown, MA) under the following conditions: 94°C for 3 min, then 42 cycles of 94°C for 15 sec, 65°C for 30 sec, and 68°C for 2 min; followed by 7 min at 68°C.

PCR products were separated by 1% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. PCR bands were cut out and purified (QIA Quick gel extraction Kit, Qiagen, Valencia, CA) and cloned into the pGEM-T Easy vector system (Promega, Madison, WI) for Sanger sequencing.

For the G72 and G30 gene specific RT-PCR, G72 3'- terminal primer R2 and G30 3' terminal primer G30 R (Supp. Table 1) were used as reverse transcription primers. Total RNA from the Tg mouse cerebral cortex were used as templates for reverse transcription. Reverse transcribed cDNA was used with primers 1F2/R2 (for G72) and G30 F/G30 R (for G30).

For detection of the longest possible G72 transcript, primer P2 in the last exon of G72 and primer G30 nest R in exon 5 of G30 were used for the PCR.

G72/G30 TaqMan quantitative PCR (qPCR)

Pre-designed FAM dye-labeled TaqMan MGB 20X gene expression assays for G72 and G30 were obtained from Applied Biosystems (Foster City, CA). The G72 probe (assay ID: Hs00738456_m1) targeted sequence TCTCTCCAGCTTTTCAGGGTATAAA, which bridges exons 2 and 3 of G72. The G30 probe (assay ID: Hs00542663_m1) targeted sequence TACACTAGGTTTTGTGCATCTCTAA, which bridges exons 5 and 6 of G30. Eukaryotic 18s ribosomal RNA (part number: 4352930E, Applied Biosystems) was used as an endogenous control to normalize cDNA template quantity.

The singleplex 20 µl reaction included 10 µl 2X TaqMan Universal PCR Master Mix (No AmpErase UNG), 1 µl 20X probe, 1 µl reverse-transcribed cDNA and 8 µl RNase-free water. All reactions were performed in triplicate. The PCR thermal cycling conditions were as follows: 50°C for 2 min, then 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60 °C for 1 min. PCR was performed in a 7900 HT Fast Realtime PCR system (Applied Biosystems). Relative quantification analysis (comparative cycle threshold (C_t): $2^{-\Delta\Delta Ct}$ method) was used for gene expression level comparison.

SYBR Green qPCR validation of genes with differential expression between Tg and WT mice

The expression levels of four genes in Tg and WT mouse cerebral cortex were validated using SYBR Green qPCR. These genes, including *Dlg4* (Mus musculus discs, large homolog 4 (Drosophila)), *Kcne1* (Mus musculus potassium voltage-gated channel, Isk-related subfamily, member 1), *Pcdh8* (Mus musculus protocadherin 8, transcript variant 2) and *Psap* (Mus musculus prosaposin, transcript variant 2), showed differential expression between Tg and WT mice in the mouse whole genome expression study.

Single-stranded cDNAs were synthesized from 2 µg of total RNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). Fifty ng of cDNA template was subjected to PCR amplification with primer sets specific to the differentially expressed genes using a *Power* SYBR Green PCR Master Mix (Applied Biosystems). The expression level of each target gene was then normalized to RNA content for each sample using mouse housekeeping gene *Arbp* (Acidic ribosomal phosphoprotein PO, synonym: *36B4*) as an endogenous control. PCR analysis was performed under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60 °C for 1 min. Amplified products were detected with the 7900 HT Fast Realtime PCR system. Immediately after the PCR run, a melt curve was generated to confirm the absence of nonspecific amplification including primer-dimer formation. Relative quantification analysis (comparative cycle threshold (C_t): $2^{-\Delta\Delta Ct}$ method) was used for gene expression level comparison.

Sequences of primer pairs used for SYBR Green qPCR were as follows:

5'-TCAGACGGTCACGATCATCGCT-3' and 5'-GTTGCTTCGCAGAGATGCAGTC-3' for *Dlg4*
5'-ACCATTTAGCTACCTCTGCAC-3' and 5'-AGAACAGTCGTGGAATTGGG-3' for *Kcne1*
5'-ACAGTGACTCGGACATCAGCGG-3' and 5'-GCAGCGATCAGAATGACCTAGG-3' for *Pcdh8*
5'-GTCTGATGTCCAGACTGCTGTG-3' and 5'-CTGGACACAGACCTCGGAATAC-3' for *Psap*
5'-GCTTCGTGTTCACCAAGGAGGA-3' and 5'-GTCCTAGACCAGTGTTCTGAGC-3' for *Arbp*.

All primer pairs were designed to amplify an exon junction in the corresponding cDNA targets.

Mouse behavior testing

Animal usage and care The Tg and WT mice of both sexes (2 - 4 months old) were used in behavioral experiments. The experimenter was blind to the genotypes of the mice used. All of the behavioral tests were conducted in a well-designed behavioral room, which was soundproof, located in the animal facility and close to animal colonies. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Chicago. All animal studies were conducted in accordance with NIH guidelines for the use of animals.

Open field test An automatic recording open field working station (Opto-Varimex unit with ATM3 Auto-Track system, Columbus Instruments, Columbus, OH) was used (1;2). The test field (43 × 43 × 30 cm high) was divided into 16 identical squares with a grid of infrared photocells around the arena and illuminated with a dim light (20 lux). During the test, mice were individually released into the center of the box and allowed to explore the field freely for 60 minutes. The general locomotor activities and stereotypic behaviors were recorded by light beam interruptions at a sampling rate of every 100-ms. Data were collected automatically by the computer sampling system. The following measurements were analyzed: ambulatory distance, ambulatory time, resting time, time spent in central area/peripheral area, and time spent in stereotypic behaviors (small movements such as scratching, grooming, or digging that repeatedly interrupt only a single optical beam).

To validate the fewer stereotypic behaviors phenotype observed in Tg mice in the above experimental setting, we used a new batch of Tg mice with controls in another open field tracking system (VersaMax animal activity monitoring system, AccuScan Instruments, Columbus, Ohio) as described in (3;4). The box size was 42 cm (length) X 42 cm (width) X 30 cm (height). Three sets of 16 pulse-modulated infrared photobeams were placed on opposite walls to record *x–y* ambulatory movements. The mice were individually placed into the chamber and activity was recorded for 30 minutes. If the animal broke the same beam repeatedly, the system considered that the animal was exhibiting stereotypy, which typically happens during grooming, head bobbing, *etc*. Stereotypy count, the number of beam breaks that occured during the period of stereotypic activity, was used as measurement of stereotypic behaviors. Other measures of general locomotor activity included ambulatory distance, ambulatory time, resting time, and time spent in central area/peripheral area.

Prepulse inhibition (PPI) Acoustic startle and PPI response were measured in a startle chamber (San Diego Instruments, San Diego, CA) as described elsewhere (5). Startle chambers were Plexiglas cylinders resting on a Plexiglas platform in a ventilated chamber (San Diego Instruments, San Diego, CA) as described elsewhere (6). Mice were subjected to five different trial types: a 40-ms broadband 120 dB burst (PULSE ALONE); three different PREPULSE+PULSE trials in which 20 ms long 3 dB (pp3p120), 6 dB (pp6p120) or 12 dB (pp12p120) stimuli above a 65 dB background preceded the 120 dB pulse by 100 ms (onset to onset), and a NO STIMULUS trial, in which only the background noise was presented. The test session began with a 5 min acclimation period followed by four consecutive blocks of test trials. Blocks one and four consisted of six consecutive PULSE ALONE trials, while blocks two and three each contained PULSE ALONE trials, NO STIMULUS trials and pp3p120, pp6p120, and pp12p120 trials. Trials were presented in a pseudo-random order. An average of 15 seconds (range: 7–23 s) separated total 62 trials. Whole-body startle responses of the mouse were recorded for each stimulus (7). The %PPI was calculated for each prepulse intensity using the formula: %PPI = 100 × [(pulse-alone) – (prepulse + pulse score)]/pulse-alone score, where the pulse-alone score is the average of pulse-alone values from Blocks 2 and 3. Average startle magnitude for six pulse-alone trials comprising Block 1 measured startle reactivity. The average startle magnitude of pulse-alone from each block was used as a measure of habituation, a simple form of non-associative learning which refers to the exponential decrement from block 1 to 4 in response to repeated presentations of an initially novel and intense stimulus in rodents and humans (8;9).

Sucrose preference test Sucrose preference was assessed using a home cage two-bottle choice test, as described previously (10;11). Mice were singly housed with *ad libitum* access to standard chow and two identical water bottles. One bottle contained water, while the other bottle contained sucrose solution of increasing concentrations over the course of the experiment (0%, 0.2%, 2%, 5%, 10% and 15%). The habituation phase (0% sucrose, water only) lasted for six days; sucrose solutions at each concentration were then provided for four days. The positions of the bottles were rotated daily to counter-balance potential position preferences. The bottles were weighed daily and the amount consumed was recorded. Preference was calculated as a percentage by dividing the amount consumed from the sucrose bottle by the total amount consumed from both bottles.

Morris water maze Spatial learning and memory was examined with a Morris water-maze as described previously (12). The training was carried out for six continuous days (one session/day) and each session consisted of four trials. For each trial, the mouse was released from the tank wall, while facing the wall, into the water and was allowed to search for, find and stand on the platform for 10 seconds. The order of the quadrants in which mice were released into the water tank was randomly designated. If a mouse did not find the platform in 1 min, the mouse was placed on the platform for 10 seconds. The navigation of the mice was tracked by a video camera. The task performance data, including swimming paths, speed, time spent in each quadrant, and escape latency to the platform, were collected by EthoVision 2.1 software (Noldus Information

Technology, Wageningen, Netherlands).

A probe test was conducted 24 hours after the last day of training. During the probe test, the platform was removed from the pool and the mice were allowed to swim for 60 s. The time spent in each quadrant and the time mice took to cross the platform site was analyzed to determine memory retention.

Forced swimming test (FST) The test conducted was a modification of the traditional rat FST (13), and was performed as described previously (4;14). The apparatus used was plastic buckets (23 cm deep and 19 cm in diameter) filled with 23–25°C tap water. On the first day, mice were individually placed into the water for 6 min. After 24 hours, the mice were exposed to the water again for 6 minutes. Both swim sessions were videotaped from a tripod-mounted camera positioned directly above the swim buckets. The swim sessions were analyzed by a blind scorer using a time sampling technique to rate the predominant behavior (swimming, immobility, climbing) over a 5 second interval (15). Only the last 4 minutes of the second day test were scored.

Statistical analysis Analysis of Variance (ANOVA) was applied to each measure of behavior with two main factors, genotype and sex. Significant interactions were resolved using Newman-Keuls *post-hoc* tests for between subject factors. No significant gender effects on G72/G30 expression and behavior measurements were confirmed by *post hoc* tests.

For the Morris water maze test, a repeated measures ANOVA was applied for the escape latency of the mice. For the FST, animals with mean immobility values greater than 2 standard deviations from the group mean were considered to be outliers and were removed from the analysis.

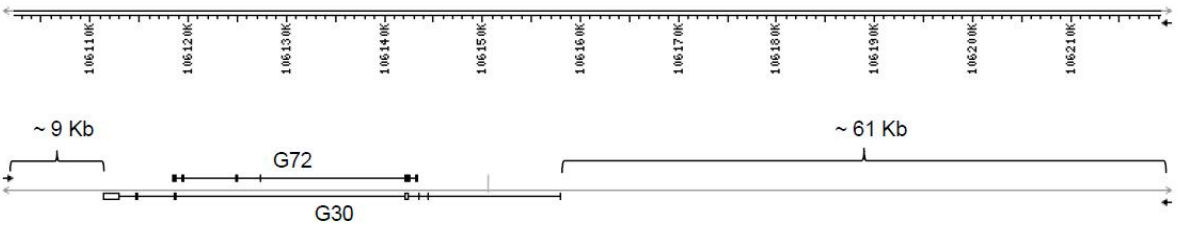
Expression study of G72/G30 in human tissues

To better understand the extent to which the Tg mouse can model the G72/G30 gene in human, we also assayed G72/G30 expression in multiple human tissues and brain regions.

Human RNA/cDNA samples, RT-PCR and T-A cloning

The following human RNA/cDNA samples were used: Human MTC Panel I and II (Clontech, Mountain View, CA) include cDNA from 16 human tissues (heart, adult whole brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocyte). Human adult whole brain total RNA, human brain whole Marathon ready cDNA, human fetal brain QUICK-Clone cDNA and human brain QUICK-Clone cDNAs from 5 subregions (amygdala, substantia nigra, thalamus, cerebral cortex, hippocampus) were from Clontech. Human fetal brain total RNA was obtained from Stratagene (La Jolla, CA).

Approximately 5 µg of human brain total RNA was reverse transcribed to cDNA using SuperScript First Strand synthesis system. Due to the very low abundance of G72 and G30 in human tissues reported previously (16;17), different PCR systems and kits were tried. The PCR conditions were optimized by testing different G72/G30 primer pairs and by changing the parameters including cDNA template quantity, primer concentration, annealing temperature, PCR cycles, *etc.* The PCR system and specifics used are described in detail above in the mouse RT-PCR section.



Supp. Figure 1. Schematic representation of the BAC clone (RP11-166E2 BAC, 117 kb, gray line under the scale bar) used for the generation of transgenic mice. Scale bar displays the chromosome 13 region (106,102,180 nt to 106,219,247 nt). Upstream (~ 9 Kb) and downstream (~ 61 Kb) "blank" regions of G72/G30 locus are also indicated.

Supp. Table 1: The primers used in G72/G30 PCR.

G72 gene specific primers:

Primer name	Sequence
P1	5' TGCTGAATGGAAAGCCAGAAAGTAGAGTG 3'
P2	5' GATTCTCCCAGTCACACAGGC 3'
1F2	5' TTAGCTGGGAGGACCCAAAATGCTG 3'
R2	5' ACACAGGCCAGGTGGGTGCATAGAAAT 3'
1F	5' TCATCTCTGCTTCACAATGCC 3'
Regu-R2	5' AGGTGGGTGCATAGAAATGG 3'
1-2-3 F	5' GGAGACAGAAGAAGGAAGAGAGACGGTA 3'
R	5' GGTCCCAGACACAGAGTCCGTCAGA 3'
1-2-3 R	5' GGGCTGAGGAAGGTAAGAGACCCAAG 3'

G30 gene-specific primers:

Primer name	Sequence
G30 F	5' GCCGAGCTGGAACCAATCTCACTGTTT 3'
G30 R	5' CCCAAAAGGAGCCCTGAACAAGTCAT 3'
G30 F2	5' GTTTGAGGCTGGAGAGATGTGCTACCC 3'
G30 R2	5' CCATCATGCTCAGCTCCAGACACTACC 3'
G30 nest F	5' CACCGGAGTCAAGTGTCATCACCTTTC 3'
G30 nest R	5' TGCTGCTAAGTCACTGGGAAGATCAGG 3'

Results

Mouse expression study results

Supp. Table 2. G72/G30 mRNA expression in Tg mouse. TaqMan qPCR indicated that G72 and G30 mRNA was expressed at the highest level in the testis, followed by the brain, and it was very low or undetectable in other tissues.

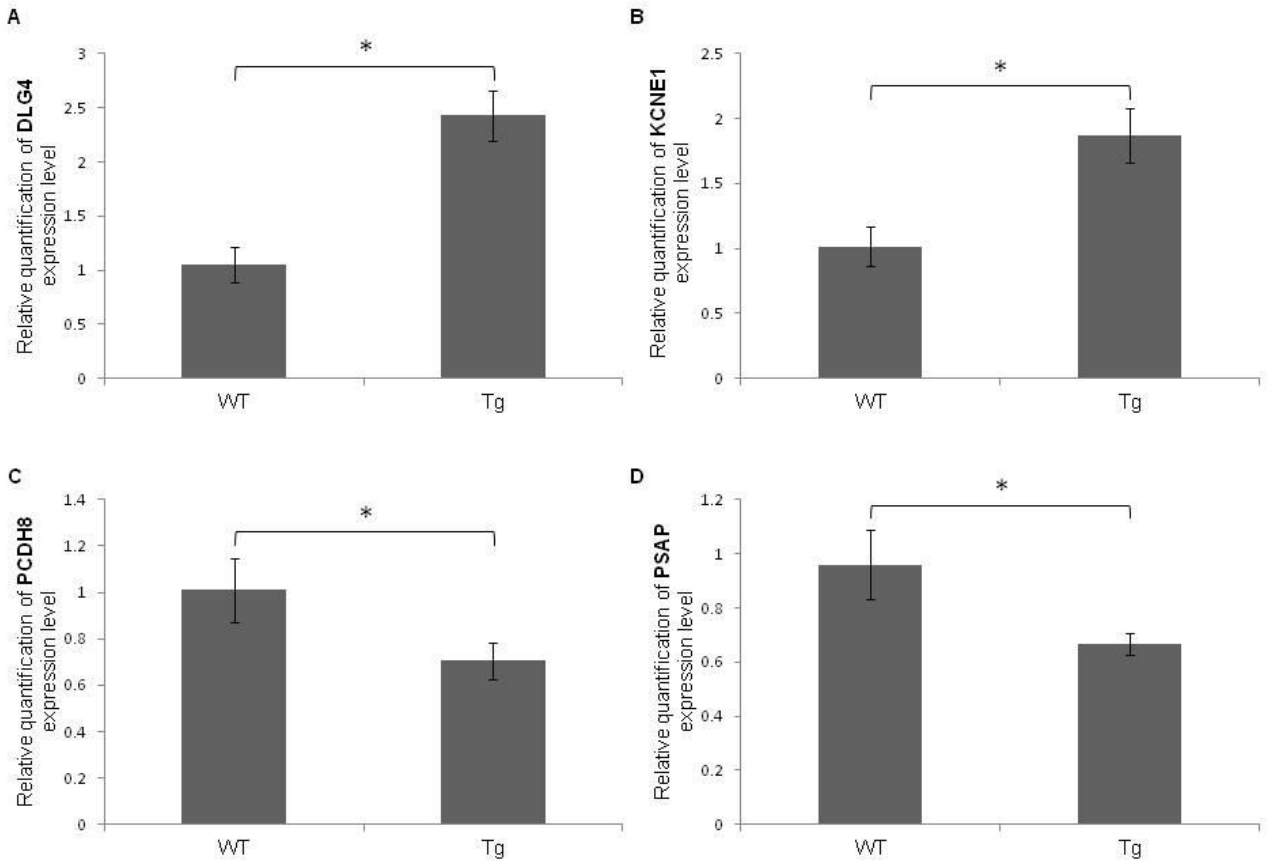
Gene	Testis	Transgenic mouse brain regions				Lung	Heart	Other tissues (Kidney, spleen, liver and muscle)
		Cerebral cortex	Hippocampus	Striatum	cerebellum			
G72	++++	+++	++	++	+/-	+/-	+/-	-
G30	++++	+++	++	+	-	+	-	-

Expression level scale: ++++ highest +++ high ++ medium + low +/- very low - not detectable

Supp. Table 3. The whole genome expression profile study in mouse cerebral cortex revealed that 361 genes showed significantly differentially expression between Tg and WT mice (FDR q≤ 0.05). The table is attached as a separate file.

Supp. Table 4. Function and disease analysis of 361 significantly differentially-expressed genes (q ≤ 0.05) in the cerebral cortex between G72/G30 Tg and WT mice. The functional analysis annotation tool in Ingenuity Pathway Analysis (IPA) software was used. The top five categories are listed here, with *p* value and number of molecules involved.

Diseases and disorders	***p*-value**	**# Molecules**
Neurological Disease	3.11E-07 - 1.91E-02	114
Schizophrenia	2.45E-03	23
Bipolar disorder	8.61E-03	35
Genetic Disorder	2.67E-06 - 1.91E-02	184
Schizophrenia	2.45E-03	23
Bipolar disorder	8.61E-03	35
Psychological Disorders	2.67E-06 - 1.59E-02	48
Schizophrenia	2.45E-03	23
Bipolar disorder	8.61E-03	35
Gastrointestinal Disease	3.15E-06 - 1.48E-02	42
Reproductive System Disease	2.50E-05 - 1.91E-02	40



Supp. Figure 2. The expression levels of four genes in Tg and WT mouse cerebral cortex were validated using SYBR Green qPCR. **(A)** *Dlg4* expression was significantly higher in Tg mice than in WT mice [$F(1, 16) = 20.75$; $p = 0.0003$]. **(B)** *Kcne1* expression was significantly higher in Tg mice than in WT mice [$F(1, 16) = 5.71$; $p = 0.03$]. **(C)** *Pcdh8* expression was significantly lower in Tg mice than in WT mice [$F(1, 16) = 5.83$; $p = 0.028$]. **(D)** *Psap* expression was significantly lower in Tg mice than in WT mice [$F(1, 16) = 8.47$; $p = 0.01$]. Number of animals: WT, n=5; Tg, n=13.

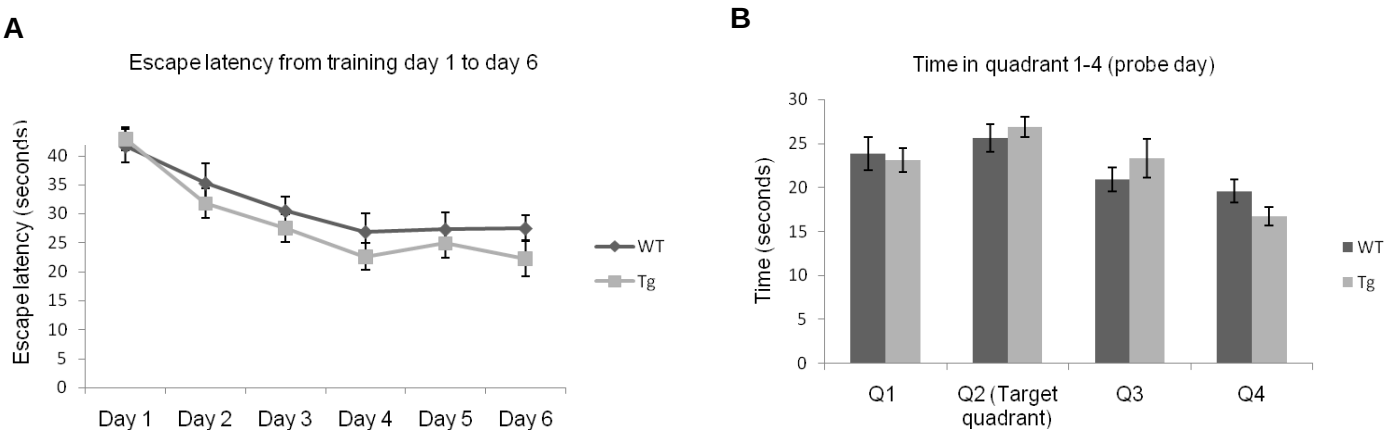
Mouse behavioral test results

Tg mice displayed normal spatial learning and memory in Morris water maze

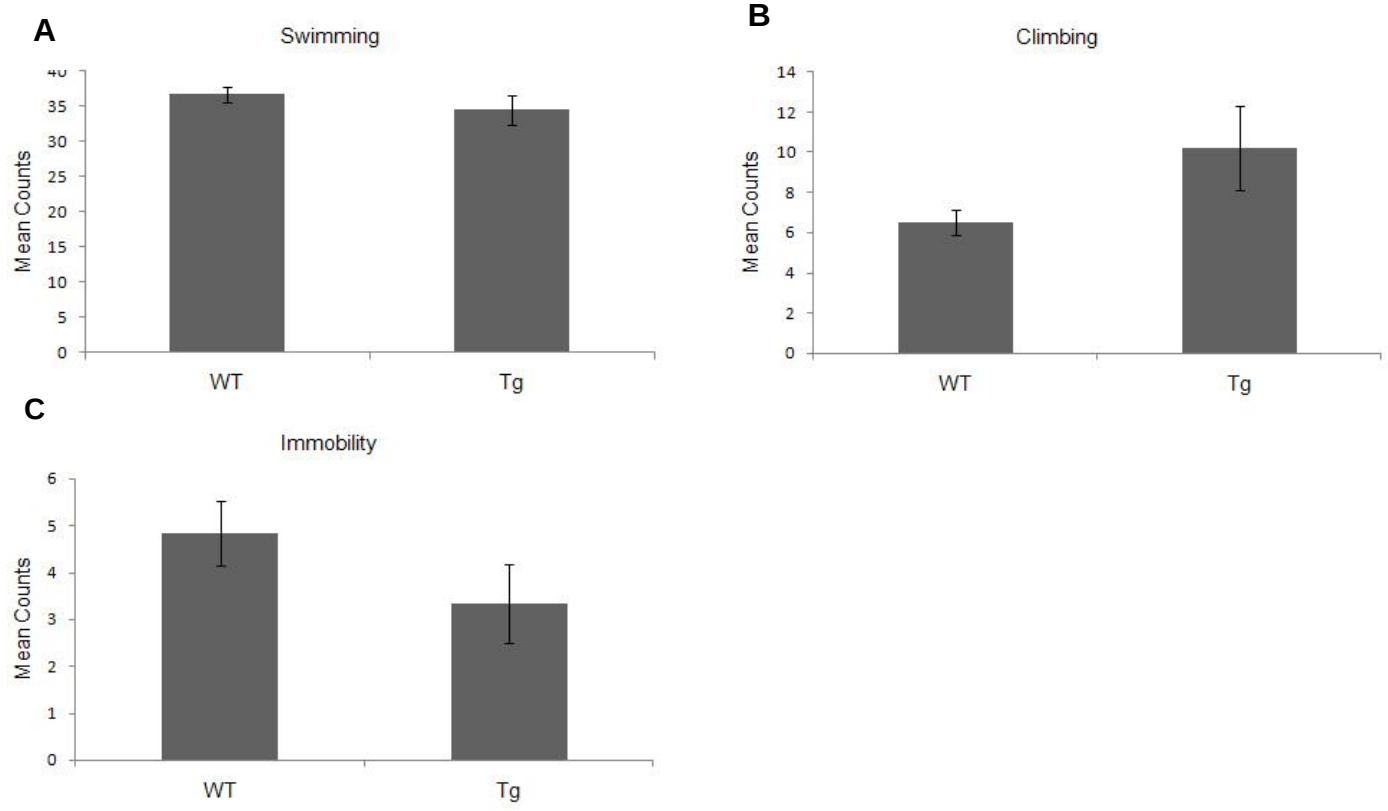
To test for spatial learning and memory, we trained mice in a Morris water maze to find a fixed, hidden platform by using distal cues. As shown in Supp. Figure 3A, the escape latency to the platform in both WT and Tg mice decreased significantly following the training sessions from day 1 to day 6 [$F(5, 200) = 19.73$; $P_{\text{Day1-6}} < 0.0001$]. There was no significant group difference throughout sessions [$F(1, 40) = 1.08$; $p = 0.31$], indicating that spatial learning in Tg mice was similar to controls. On the probe day, both groups showed place preference for the target quadrant [$F(3, 120) = 7.32$; $P_{\text{quadrant 1-4}} < 0.001$]. In comparison to controls, Tg mice exhibited a similar preference for the targeted quadrant in which the platform was previously located [$F(1, 40) = 0.64$; $P_{\textit{quadrant 1-4}} = 0.43$] (Supp. Figure 3B). Thus, G72/G30 Tg mice perform normally, similar to WT mice, and did not display deficits in spatial memory in this spatial task.

No depression-related behavioral phenotypes in G72/G30 Tg mice

In the forced swimming test, ANOVA revealed that, relative to controls, G72 Tg mice groups did not exhibit significantly decreased or increased swimming [$F(1, 19) = 0.4$; $p = 0.53$ (Supp. Figure 4A), climbing [$F(1, 19) = 1.19$; $p = 0.29$] (Supp. Figure 4B), or immobility [$F(1, 19) = 1.14$; $p = 0.3$] (Supp. Figure 4C). No main effects or interactions of genotype and gender were found.



Supp. Figure 3. G72/G30 Tg mice displayed normal spatial learning and memory in the Morris water maze test. **(A)** The escape latency to the platform for both WT and Tg mice decreased significantly following the training sessions from day 1 to day 6 [$F(5, 200) = 19.73$; $P_{Day1\text{-}6}<0.0001$]. There was no significant group difference throughout sessions [$F(1, 40) = 1.08$; $p = 0.31$]. **(B)** On the probe day, both groups showed place preference for the target quadrant [$F(3, 120) = 7.32$; $P_{quadrant\ 1\text{-}4}<0.001$]. Compared to controls, Tg mice exhibited a similar preference for the targeted quadrant in which the platform was previously located [$F(1, 40) = 0.64$; $P_{quadrant\ 1\text{-}4} =0.43$]. Number of animals: WT, n=19; Tg, n=23.



Supp. Figure 4. In the forced swimming test, G72 Tg mice groups did not exhibit significant decreased or increased swimming [$F(1, 19) = 0.4$; $p = 0.53$] (**A**), climbing [$F(1, 19) = 1.19$; $p = 0.29$], (**B**) or immobility [$F(1, 19) = 1.14$; $p = 0.3$] (**C**) compared to controls. Number of animals: WT, n=6; Tg, n=15.

Expression of G72/G30 in human tissues

G72 is expressed at minimal levels in human testis and brain with novel splicing isoforms

In sixteen human tissues, gel electrophoresis of the PCR products of primers 1-F2/1-2-3R, 1-2-3F/R and F2/R2 revealed weak bands of G72 in testis tissue only. PCR with another primer pair 1F2/R2 produced weak bands in human testis and brain tissue. T-A cloning from these weak bands and further sequencing identified two novel G72 splicing forms in human testis (GenBank: DQ386869, DQ386870) and one novel splicing form in human adult brain (GenBank: DQ357223) (Figure 1 and Supp. Table 5). We also detected a very weak band of G72 in human fetal brain by using primer pair 1-2-3F/R. In fourteen other human tissues, no G72 expression was detected despite the wide range of PCR conditions tried.

To examine G72 expression in human brain regions, the primer pair P1/P2 was used for PCR with cDNA from five brain subregions. Only one weak band was visualized in the substantia nigra and amygdala, in which further sequencing identified a new G72 splicing form (GenBank: DQ343761, Figure 1 and Supp. Table 5). No G72 product band(s) were detected in the cerebral cortex, hippocampus or thalamus.

Due to minimal G72 expression levels and detection sensitivity limitations, qPCR failed to detect G72 expression in human brain cDNA samples.

Minimal G30 expression in human tissues

Only primer pair G30 F2/G30 R was able to detect low expression levels of G30 through PCR in cDNA from testis tissue and human brain Whole Marathon Ready cDNA. No G30 was detected in other human tissues, or in five individual brain regions using multiple methods (Supp. Table 5). Similarly, qPCR failed to detect G30 expression in human samples.

To summarize, neither Taqman qPCR nor Northern or dot blot gave G72 signals in RNA extracted from multiple human tissues (data not shown). This result is consistent with Benzel *et al.*'s study, in which they were unable to detect G72 mRNA using either SYBR Green/TaqMan qPCR or Northern blot in 13 human brain regions (18), indicating that G72 level in the brain regions investigated are below the detection limit of any of these techniques. After further attempts, we were able to identify four novel G72 splicing forms exclusively in human testis and brain tissues; however, identification occurred only through PCR under highly specific conditions and followed by cloning and sequencing. Surprisingly, in view of the Chumakov paper (16), we were unable to detect LG72 and SG72 variants in human tissues, suggesting that the endogenous expression level of LG72/SG72 in human must be extremely low, if they are present at all. Low expression levels may explain the variability as to which splicing forms are detected in different studies under different PCR conditions (16;17). Hence, it is difficult to assess which form(s) are the most dominant *in vivo*.

Supp. Table 5. Summary of expression profile of G72/G30 variants in human multiple tissues and brain regions. We were only able to detect four novel G72 splicing forms in the testis and brain tissue exclusively, only through PCR under specific conditions and followed by cloning and sequencing. Extremely low G72 expression was detected in the adult brain (DQ357223), fetal brain, and human testis (DQ386869, DQ386870). In five brain regions, only one G72 form (DQ343761) was detected at a low level in the substantia nigra and amygdala. Expression of other forms (LG72, SG72, Variant 1-4) was described in previous reports (16;17). G30 was expressed at low levels in the testis and in brain tissue. No G30 expression was detected in five brain regions or 14 other tissues.

Gene	Adult brain	Fetal brain	Human brain regions					Human testis	14 other tissues
			Cerebral cortex	Hippocampus	Thalamus	Substantia nigra	Amygdala		
G72	+/- DQ357223 Variant 4	+/- Variant 4	-	-	-	+/- DQ343761 *In amy*: LG72, variant 4		+/- DQ386869 DQ386870 LG72 SG72 Variant 1-3	-
G30	+/-	-	-	-	-	-		+/- LG30	-

Expression level scale: +/- very low expression; - not detectable

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