SUPPLEMENTAL INFORMATION

Dysregulation of FUS Disrupts Synaptic Homeostasis

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Immunostaining. Mice (ages P18-P25) were anesthetized and were transcardially perfused with 4% paraformaldehyde (PFA, wt/vol) dissolved in 1X phosphate-buffered saline (PBS) and post-fixed in 4% PFA (wt/vol) overnight at 4°C. Tissues were washed extensively in 1X PBS, dehydrated in 30% sucrose (wt/vol) and frozen in OCT. Tissue sections of 15 μM were immunostained with primary antibodies GFAP (Millipore, AB5541), IBA1 (Wako, 019-19741), GFP (Aves 1020), human FUS (B327D) FUS (Santa Cruz, sc-47711), FUS (Sigma, HPA008784), Ubiquitin (Abcam, Ab7780) or To-Pro3 (LifeTechnologies T3605) followed by Alexa Fluor®-conjugated secondary antibodies (Invitrogen). Primary antibodies were incubated overnight at 4°C and Alexa Fluor®-conjugated secondary antibodies (Invitrogen) were incubated for 2 hours at room temperature.

Whole mounts of triangularis sterni muscles of mice (P18-P25) were fixed in 2% PFA (wt/vol) in 0.1 M phosphate buffer (pH 7.3) for 1 hr at room temperature. The samples were blocked in dilution buffer (500 mM NaCl, 0.01 M phosphate buffer, 3% bovine serum albumin (wt/vol) and 0.01% thimerosal, then incubated for 30 min with Alexa Fluor® 647 α-bungarotoxin (Invitrogen) followed by overnight incubation at 4°C with antibodies: GFP (Aves 1020), Syntaxin 1, or S100 (Dako, Z0311). After extensive washes, muscle whole mounts were incubated with Alexa Fluor®-conjugated secondary antibody (Invitrogen). Samples were then washed with 1X PBS and mounted in VECTASHIELD mounting medium (Vector Laboratories). Images were acquired using a Zeiss LSM 510 confocal microscope. Three animals from each genotype (n=3) were analyzed and a minimum of 400 NMJs per genotype were assessed.

Western blot analysis. Tissues were homogenized in lysis buffer (10mM HEPES, pH 7.4, 4M Urea, 1% LDS (wt/vol), 1X protease cocktail inhibitor (Roche) in lysing matrix D tubes, using the FastPrep homogenizer (Millipore). Lysates were clarified by centrifugation at 20,000 x g for 30 min at 4°C. Protein concentration was determined by BCA assay (Themo Scientific), and equivalent amounts were resolved by SDS-PAGE and immunoblotted by a standard protocol. GAPDH (Sigma, G9545), FUS (Sigma, HPA008784), FUS (Santa Cruz (SC), sc-47711) and GFP (Aves, 1020) were used as primary antibodies. Quantification of western blots by densitometry was done using the NIH ImageJ software. Each sample was normalized to GAPDH. Affinity purified Human FUS antibodies were a gift from Hongxia Zhou at Thomas Jefferson University (1). Human FUS peptide antibodies B327D (SYGQPQSGSYSQQPS) were generated in rabbits as previously described (2). Note that although we were able to visually estimate the amounts of human and mouse FUS in the transgenic animals by using larger SDS-PAGE gels (Figure 1D), we were unable to accurately quantify human FUS level

due to the similar molecular weights between human FUS (526 amino acids) and mouse FUS (518 amino acids) (Figure S1D).

Li-Cor Odyssey. Equal protein lysates were resolved by SDS-PAGE and transferred to Immobilon® FL PVDF membrane (Millipore IPFL00010). Blots were then rinsed with MiliQ water and blocked using Odyssey blocking buffer (Li-Cor P/N: 927-40000). Blots were probed with following primary antibodies in Odyssey blocking buffer: Arc (Synaptic Systems, 156003), CamKII (Santa Cruz, sc-5391), GluR1 (Millipore, MAB2263), and Psd-95 (Thermo, MA1-0145). Following primary antibody incubation, blots were probed with IR Dye 800CW goat anti-rabbit (Li-Cor P/N: 827-08365), IR Dye 800CW donkey anti-goat (Li-Cor P/N: 926-32214), IR Dye 800CW goat anti-mouse (Li-Cor P/N: 827-08364) and IR Dye 680RD goat anti-mouse (Li-Cor P/N: 926-68170) respectively. Blots were imaged using Li-Cor Odyssey imaging system and quantified using Li-Cor Image Studio software.

Golgi staining for the analysis of dendrites in cortical neurons and cervical spinal motor neurons. Both male and female CAG-FUS^{WT} and CAG-FUS^{R521G} transgenic mice and their littermate controls were used for this analysis. Golgi staining on brains and cervical spinal cords from postnatal day 18 (P18) wild-type and CAG-FUS^{WT} or CAG-FUS^{R521G} littermate mice was performed using the Rapid GolgiStain Kit (FD Neurotechnologies) following the manufacturer's instructions. Briefly, brains and spinal cords from P18 mice were removed and immersed in solutions A and B in the dark for 2 weeks at room temperature. Brains were then transferred into solution C for at least 48 h at 4°C, sectioned at 100 μm thickness using a cryostat, mounted onto 3% gelatin-coated slides (wt/vol) and developed following the manufacturer's protocol (3).

Dendritic tracing was performed using Neurolucida software (MicroBrightField, Williston, VA) with Olympus BX51 and a 60X objective. Neurons were traced with the center of the soma as a focal point. Three animals from each genotype (n=3) were analyzed, with 12 cervical spinal motor neurons (from ventral horn region) and 10 cortical neurons (from layers IV-V in the sensorimotor cortex) randomly selected and analyzed from regions of interest. Neurolucida Explorer 10 software (MicroBrightField, Williston, VA) was used to perform Sholl analysis to determine the number of intersections, cumulative surface area and to generate representative Golgi-tracing neurons (3).

Counting of the dendritic spines in the apical dendrites of the cortical motor neurons was performed using Neurolucida and analyzed with NeuroExplorer software (3). Briefly, beginning with a radius of 30 μm away from the center of the soma, a total distance of 100 μm from the primary apical dendrite was traced and analyzed. The entire length of the immediate secondary apical dendrite attached to the primary dendrite was also traced and analyzed for the study. Three animals from each genotype (n=3) were analyzed, with 10 primary and secondary branches from each animal traced and analyzed.

ChAT staining of spinal cord and quantification of spinal motor neurons. Tissues were fixed with 4% PFA (wt/vol) and sectioned at 40 μm thickness and free-floated in 1X PBS. Free-floating sections were treated with antigen retrieval solution (10 mM sodium citrate buffer) at 95°C for 10 min, washed three times in 1X TBS, and

then incubated in blocking solution (5% goat serum (wt/vol), 0.1% Triton X-100 in 1X TBS) for 1 hour at room temperature. DAB staining of the floating sections was then performed. The following antibodies and reagents were used: anti-choline acetyltransferase (ChAT)(Millipore), biotinylated rabbit anti-goat IgG antibody (Vector Labs); VECTASTAIN Elite ABC Kit (Vector Labs). DAB stained sections were mounted onto slides with Permount. Bright-field images of the ventral horns were captured using a 10X objective on an Olympus BX53 and on an Olympus DP72 digital camera. ChAT positive cells were quantified in each image field of the ventral horn region, and a minimum of 12 images for each animal were examined. CAG-FUS^{R521G} (n=3) and CAG-FUS^{WT} (n=4) transgenic mice and their littermate controls (n=3-4) were analyzed.

Acute cortical tissue slices, treatment with DHPG and synaptoneurosome isolation. Treatment of acute cortical tissue slices and isolation of synaptoneurosomes (SNs) from P18 mice were performed similar to previously reported (4). P18 mice were anesthetized with pentobarbital, whole brains were dissected out and immersed into ice cold oxygenated dissection buffer (110 mM choline Cl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM D-glucose, 3.1 Na pyruvate, 11.6 Na ascorbate, 14 mM $MgCl_2$, 0.5 mM $CaCl_2$). Acute slices of neocortex were taken at 400 μm thickness and recovered in normal artificial cerebrospinal fluid (ACSF; 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM dextrose, 2 mM $MgSO_4$-anhydrous, 2 mM $CaCl_2$-2H2O) for 35 minutes in a 35°C, oxygenated water bath. Slices were then transferred to SN recovery buffer containing AMPA (20 μM DNQX) and NMDA (5 μM CPP) inhibitors and pretreated for 30 minutes before stimulation with DHPG (100 μM, 10 minutes) (Tocris, Biosciences, US). Cortical slices were then transferred to 1 ml ice-cold homogenization buffer (10 mM HEPES, 1 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 1X Roche Protease inhibitors) and homogenized using a Dounce homogenizer (10 strokes with A and 10 strokes with B). Total cell lysates (TLC) were collected and the remainder was passed through two 100 μm filters followed by one 10 μm filter. SNs were pelleted after a 10 min centrifugation at 1000 x g. TCL, Supernatants, and SNs were lysed in lysis buffer as previously described (5). Experimental replicates (n=4) were analyzed for each genotype CAG-FUS^{WT}, CAG-FUS^{R521G}.

Synaptoneurosome isolation, *in vitro* treatment with DHPG. Brain cortices from P16 mice were removed, washed in ice-cold gradient medium (GM buffer: 0.25 M sucrose, 5 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA), transferred to a glass Dounce homogenizer containing ice cold GM buffer, and gently homogenized with ten strokes of the loose pestle followed by ten strokes of the tight pestle. Cellular debris and nuclei were pelleted from the homogenate by centrifugation at 1000 x g for 10 min at 4°C. The supernatant was applied to percoll gradients (layers 2 ml each of 23%, 15%, 10%, and 3% isosmotic percoll, vol/vol) and spun at speed (32,500 x g) for 5 min at 4°C. The third band from the top of the gradient (the 23%/15% interface) containing intact SNs was removed and pooled for the experiments. The salt concentration of the SNs was adjusted by adding one-tenth volume of 10X stimulation buffer (100 mM Tris-HCl, pH 7.5, 5 mM Na2HPO4, 4mM KH2PO4, 40 mM NaHCO3, 800 mM NaCl). To suppress nonspecific excitation, tetrodotoxin (Tocris, Biosciences, US) to 1 μM was added. SNs were equilibrated to room temperature by rotation on a nutator mixer for 10 minutes, samples

were then placed at 37°C and stimulated with DHPG (100 μM) for the times indicated. All samples were incubated at 37°C for the same total time. Pretreatment of DMSO (vehicle) or 25 μM MG132 (Tocris, Biosciences, US) occurred at room temperature for 10 minutes prior to DHPG stimulation.

Toluidine blue staining. Mice were anesthetized and transcardially perfused with 4% PFA (wt/vol) and 1% glutaraldehyde (wt/vol) dissolved in 0.1M cacodylate, pH7.4. Tissues were post-fixed in 2.5% glutaraldehyde dissolved (wt/vol) in 0.1M cacodylate, pH7.4. Tissues were then post-fixed in buffered 1% osmium tetroxide (wt/vol) for 2 changes of 90 minutes each. Tissues were rinsed with dH_2O, en bloc stained in 4% uranyl acetate (vol/vol) in 50% ethanol, dehydrated with a graded series of ethanol, and embedded in EMbed-812 resin. 1 μm semi-thin sections of the L4 spinal cord and dorsal and ventral roots were taken and stained with 1% toluidine blue (wt/vol).

Counting Alpha Motor Neurons. Alpha motor neurons were counted in spinal cord sections prepared from mice after perfusion with 4% PFA (wt/vol). Samples were paraffin-embedded, sectioned serially (10 μm) onto 10 slides, and stained with cresyl violet. Motor neurons were counted in every 10th section through each population examined. Alpha motor neurons were chosen based on the criteria: 1) located in the ventral horns (right and left) of the spinal cord; 2) 80-100 μm in size; 3) containing large soma; 4) containing a clear nucleus with intact nuclear membrane; and 5) having at least one clump of nucleolar material.

Juvenile Social Interaction. Adult mice were placed into a clean, empty mouse cage for approximately 15 min to habituate to the cage. A novel juvenile mouse (3-4 weeks, same sex as the test mouse) was then introduced into the cage and the total time that the adult mouse interacted with the juvenile was recorded. Trial duration was 2 min. All tests were conducted under red light in order to minimize any stress and anxiety. CAG-FUS^{R521G} (Tg/+;Cre/+, n=18) and littermate controls (Tg/+;+/+, n=19 and +/+;Cre/+, n=19) were tested at 2, 4, 6 and 8 months of age.

Ladder Walking Test. This task was used to evaluate fine motor skills involved in performing accurate stepping behavior (6, 7). The task apparatus and scoring system were adapted from Farr et al. (2006) and Tennant & Jones (2009). The horizontal ladder (Plexiglas walls, 81 cm long, 15 cm tall, elevated 25 cm from ground) was composed of 0.15 cm diameter metal rungs spaced evenly 1.5 cm apart. Animals performed 3 trials (crossings) on a single test day (inter-trial interval at least 10 min). Video was analyzed frame-by-frame for step quality according to a 0-6 point scale. Scores of 0-2 indicated varying severity of slips, with scores of 3-5 indicating lesser types of missteps, and a score of 6 indicating an ideal paw placement. Two values were derived from this analysis: a step score (average of all scored steps) and an error rate (count of steps scored 0-2 divided by total step count). Forelimb and hindlimb scores were tallied separately; scores from right/left limbs were pooled. CAG-FUS^{R521G} (Tg/+;Cre/+, n=10) and littermate controls (Tg/+;+/+, n=9 and +/+;Cre/+, n=11) were tested at 4 months of age.

Water Y-maze. Mice were tested in a Y-shaped maze (arms 34 cm long and 10 cm wide) filled with water (21°C) and a small amount of white paint. The submerged (1 cm) escape platform was located at one end of the arms of the maze. The location of the platform was alternated between cages. Mice were given 5 blocks of trials to learn the platform location. Each block consisted of 5 trials separated by approximately 30 sec – 2 min. Each block was separated by approximately 1 hr. 24 hours after the training, mice were given another 2 blocks of trials with the platform in the same location to assess whether they had learned the location. Mice that did not score 80% or better were excluded from analysis. 24 hrs. after the test the platform was moved to the arm opposite the location they were trained and the mice were given another 5 blocks of trials to learn this new location. Once the mouse entered an arm, the data were scored as either correct (the arm which contained the platform) or an error (the arm which did not contain the platform). CAG-FUS^{R521G} (Tg/+;Cre/+, n=18) and littermate controls (Tg/+;+/+, n=19 and +/+;Cre/+, n=19) were tested at 2 months of age.

Olfactory Discrimination Test. Mice were placed individually into a clean mouse cage with bedding and allowed to habituate for 15-45 min. During this time a dry, long-handled cotton-tipped applicator was placed through the lid into the center of the cage and lowered to the height of the mice. For the test, the cage was moved into a quiet, dimly lit room to minimize any anxiety. The applicator was replaced by new cotton tipped applicator that had been dipped into water. The time that the mouse sniffed this applicator during a 2 min period was recorded. This process was repeated a total of three times with a new applicator used for each test. The test was then repeated with an applicator which had been run through the dirty bedding of another mouse cage. This test was also repeated 3 times with a new applicator dipped into the dirty bedding. Sniffing was defined as the mouse's nose pointed in the direction of the applicator and within approximately 1 cm. CAG-FUS^{R521G} (Tg/+;Cre/+, n=18) and littermate controls (Tg/+;+/+, n=19 and +/+;Cre/+, n=19) were tested at 4 months of age.

All behaviour testing were performed on CAG-FUS^{WT}, CAG-FUS^{R521G} and their littermate controls. There were no sex differences observed for any behavioral tests performed and sexes were evenly distributed for each genotype tested.

Paired-end RNA-seq. Spinal cords were dissected from control and transgenic mice at postnatal day P20 and stored at -80°C until total RNA was extracted using RNA Stat 60 reagent (Amsbio). Selected mice were between a health score of 1-2 as described in the material and methods in the main text. The mice were carefully selected to be phenotypically similar. Additionally, each paired-end RNA-Seq library was generated using equal amounts of RNA pooled from 3 animals, to take into account phenotypic variability. Quality of RNA was assessed with a Bioanalyzer using a nanochip. RNA samples with RIN (RNA integrity number) > 7 were used for RNA-Seq and qRT-PCR. Paired-end RNA-Seq libraries were generated for CAG-FUS^{WT} (n=2), CAG-FUS^{R521G} (n=2) and their littermate controls (n=2) using the Illumina TruSeq RNA Sample Preparation Kit v2 (RS-122-2001). A total of ~630 million paired-end RNA-Seq reads (2 x 100nt) were obtained using the Illumina HiSeq GAII sequencing platform. Reads were mapped to reference mouse genome (mm10) using TopHat (8-

10) (v 2.0.4) with default parameters (read alignment with up to 2 mismatches allowed, using a known mouse reference annotation (UCSC genes), etc.). Post read-mapping DESeq (11) was implemented to identify the differentially expressed genes (DEG).

Properly paired mapped reads were used to identify differentially expressed genes using read DESeq (11). DESeq (11) is an R/Bioconductor package based method which employs a negative binomial distribution method to quantify differential gene expression between transgenic samples and control samples, using count data from mapped RNA-Seq reads. HTSeq (12) (a python based tool) was used to generate the count data for each condition. DESeq (11) identified differentially expressed genes (with adjusted P-value < 0.05) which were assessed for functional annotation using the DAVID (13) functional annotation tool.

Statistical Analysis. Results are expressed as the mean ± SEM (standard error of the mean) or ± SD (standard deviation) where indicated. Three-way statistical comparisons use one-way ANOVA (GraphPad Prism version 6). We utilized a two-tailed, unpaired Student's t-test for all pair-wise comparisons (GraphPad Prism version 6). *P* values less than 0.05 were considered significant.

Genotyping. Genomic DNA from ear biopsies were lysed in Quick Lysis Buffer (50 mM NaCl, 10 mM Tris-HCl pH 8.3, 0.2% Tween 20 and 0.4 mg/ml proteinase K) at 55ºC for 1 hour and then 95ºC for 10 min. The PCR contained genomic DNA, genotyping primers (listed below) and standard Taq buffer supplemented with 1 M betaine, 3.3% DMSO (vol/vol), 1.5 mM $MgCl_2$, 0.1 mg/ml BSA, 0.2 mM deoxynucleoside triphosphates and 1.25 units of *Taq* polymerase (New England Biolabs, NEB). After enzymatic amplification for 35 cycles, the PCR products were resolved on 2% agarose gel (wt/vol) in 1X Tris acetate-EDTA buffer.

Genotyping Primers:

Gene symbol	Forward primer	Reverse primer
Cre	GCATAACCAGTGAAACAGCATTGC	GGACATGTTCAGGGATCGCCAGGC
GFP	CTGACCCTGAAGTTCATCTGCACC	TGGCTGTTGTAGTTGTACTCCAGC
Human FUS	GACCAGGTGGCTCTCACATG	GTCGCTACAGACGTTGTTTGTC
Internal control (Pin1)	ATCATCCTGCGCACAGAATG	TCAATTCCTCCAGAAGGAGC

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. (A,B) Genotyping results from Meox2Cre crosses from three founders from the *CAG-Z-FUS^{WT}-EGFP* (lines: 629 and 638) and *CAG-Z-FUS^{R521G}-EGFP* (lines: 673 and 682) transgenic lines. PCR products using primers for FUS, GFP, Cre and Pin1 (internal control) are shown. (C) FISH (fluorescent in situ hybridization) of chromosomes isolated from CAG-FUS^{WT} (629) and CAG-FUS^{R521G} (673) MEF cells. CAG-FUS^{WT} (629) and CAG-FUS^{R521G} (673) founders show single insertion of transgenes. (D) Immunoblot of HeLa total cell lysates (human) and whole mouse brain lysates (mouse) showing molecular weight differences for human and mouse FUS protein. (E,F) Immunostaining for GFP (green) shows specific staining in brain and spinal cord of CAG-FUS^{WT} and CAG-FUS^{R521G} mice (P0) and increased staining for FUS (red, Sigma,

HPA008784). Spinal cord sections from end-stage CAG-FUS^{WT} and CAG-FUS^{R521G} mice are co-stained with (G) anti-hFUS (red) and anti-GFP (green) or (H) Ubiquitin (red) and FUS (green, Santa Cruz). No mislocalization or ubiquitination of human FUS^{WT} or FUS^{R521G} are observed. (G,H) Shown is the ventral horn of the lumbar region of the spinal cord.

Figure S2. (A-B) Body weights of CAG-FUS^{WT} (638) and CAG-FUS^{R521G} (682) mice from P0-P20. (C-D) Body weights of female and male CAG-FUS^{R521G} (682) mice from 5-14 weeks of age. (E) Grip test of CAG-FUS^{R521G} (682) mice, postnatal stages (P14-30), n=17 litters. Red circles (**o**) indicate CAG-FUS^{R521G} mice that had loss of motor function and early lethality. (A-E) Error bars represent SD of the mean.

Figure S3. ImageJ quantification of integrated density of GFAP and Iba1 staining in (A) CAG-FUS^{WT} and (B) CAG-FUS^{R521G} mice. Immunofluorescence staining of CAG-FUS^{R521G} mice that escape early lethality in the CA3 region of the hippocampus (C) and of the ventral horn of the spinal cord (D) for Iba1 (microglia;red) and GFAP (astrocytes;green) showing no neuroinflammation. (A,B) Quantification of microglia and astrocytes a, $P < 0.05$; b, $P < 0.01$; c. $P < 0.005$ (Student *t* test). Error bars represent SEM of the mean.

Figure S4. H&E staining of the hippocampus and cortex (A) showing no loss of cells. Toluidine blue staining of dorsal and ventral roots (L4-5) (B), dorsal cortical (DCST) and lateral spinal tracts (LST) (C) of CAG-FUS^{WT} and CAG-FUS^{R521G} mice (P20-23) showing no changes in myelinated axons. (D) Cresyl violet (top panel) and H&E (bottom panel) staining of cervical spinal cord from aged CAG-FUS^{R521G} mice (2 years old). (E) Quantification of cervical motor neurons from CAG-FUS^{R521G} mice (2 years old). Student *t* test shows no significant differences between groups. ns, not significant. Error bars represent SEM of the mean.

Figure S5. (A) Neuromuscular junctions (NMJ) from CAG-FUS^{WT} and CAG-FUS^{R521G} mice at end-stage are costained for presynaptic terminals (nerve;red) and bungarotoxin for postsynaptic terminals (AchR;green) showing abnormal morphology compared with littermate controls. (B,C) NMJs in CAG-FUS^{WT} and CAG-FUS^{R521G} mice (P20) stain positive for terminal myelinating Schwann cells (S100B;red), GFP;green, and bungarotoxin for postsynaptic terminals (AchR;blue), although their morphology is not typical of the pretzel shape observed in control (CTL) mice.

Figure S6. MA plot showing differentially expressed genes (DEG) in (A) CAG-FUS^{WT} (638) transgenic mice against control wild-type mice, and (B) CAG-FUS^{R521G} (682) transgenic mice against control wild-type mice. All genes are shown in grey and DEG are shown in black. CAG-FUS^{WT} transgenic mice show more genes affected compared to CAG-FUS^{R521G}.

Figure S7. (A) Open field test from 2 and 4 month old CAG-FUS^{R521G} mice and littermates show no differences in total distance travelled. (B) Total daily food intake and (C) food intake per body weight of 2 month old CAG-FUS^{R521G} (682) mice during running wheel testing. (D) Digigait trace for a control animal showing parameters that are measured for gait analysis. Ladder walking test shows the forepaws have a lower step score (E) and more errors per step (F). Hindpaws show no deficits (G,H). Social interactions of CAG-FUS^{R521G} mice were

reduced with juveniles at 2 months and significantly decreased by 4 months of age (I). All mice performed equally in a Y-test which measures learning and decision making (J). Shown are the results from reversal testing that measures the ability of the mice to find the platform in the opposite arm to which they were entrained (J). Olfaction testing showing no alterations in CAG-FUS^{R521G} mice (K). Studies were conducted with littermate controls (+/+;Cre/+ and Tg/+;+/+), which showed no statistical impairments. (A,E-K) Statistical comparisons uses one-way ANOVA. a, $P < 0.05$; b, $P < 0.01$. (‘ compares +/+;Cre/+ with Tg/+;Cre/+). (B,C) Uses Student *t* test. ns, not significant. Error bars represent SEM of the mean.

Figure S8. Li-Cor Odyssey quantification for synaptic proteins Arc, CamKII, GluR1 and Psd-95 from (A,C) total cell lysates (TCL) and (B,D) synaptoneurosomes (SN). The graphs represent the average of 3-5 independent experiments. Student’s *t* test shows no statistical differences in protein expression between CAG-FUS^{WT} and CAG-FUS^{R521G} (682) compared to their littermate controls (CTL). Error bars represent SEM of the mean.

Movie S1. Video recording of a control resident mouse from the 8-month resident-intruder test. The video clip is representative of the mean interaction of the control groups. The control resident “test” mouse (#3935) shows normal social behaviors towards the “novel” intruder mouse. The video shown is taken 4 min after the intruder mouse is introduced into the home cage.

Movie S2. Video recording of a CAG-FUS^{R521G} resident mouse from the 8-month resident-intruder test. The video clip is representative of the mean interaction of the CAG-FUS^{R521G} group. The CAG-FUS^{R521G} resident “test” mouse (#3890) spends less time chasing the “novel” intruder mouse and displays less active social behavior overall. The video shown is taken 4 min after the intruder mouse is introduced into the home cage.

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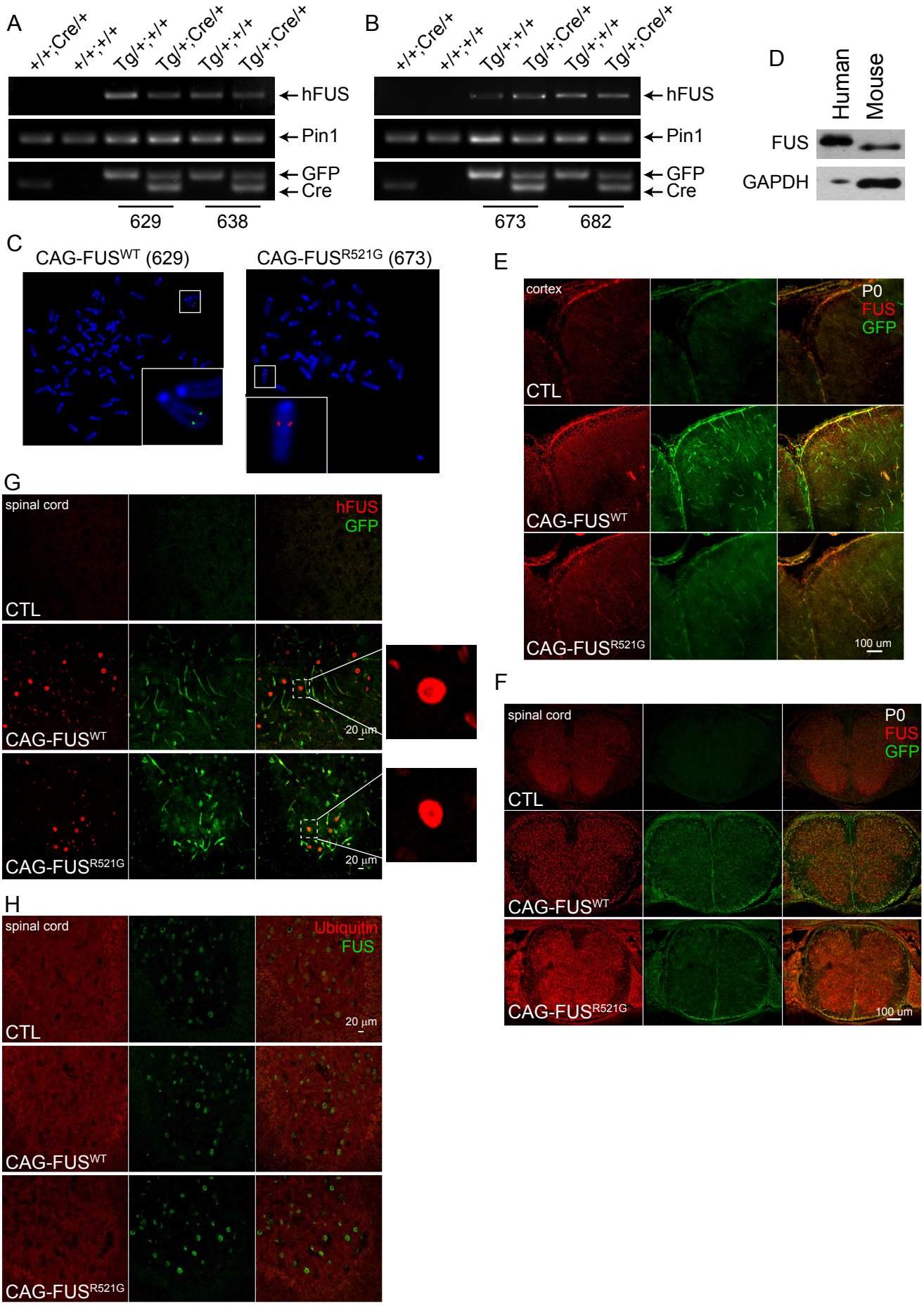
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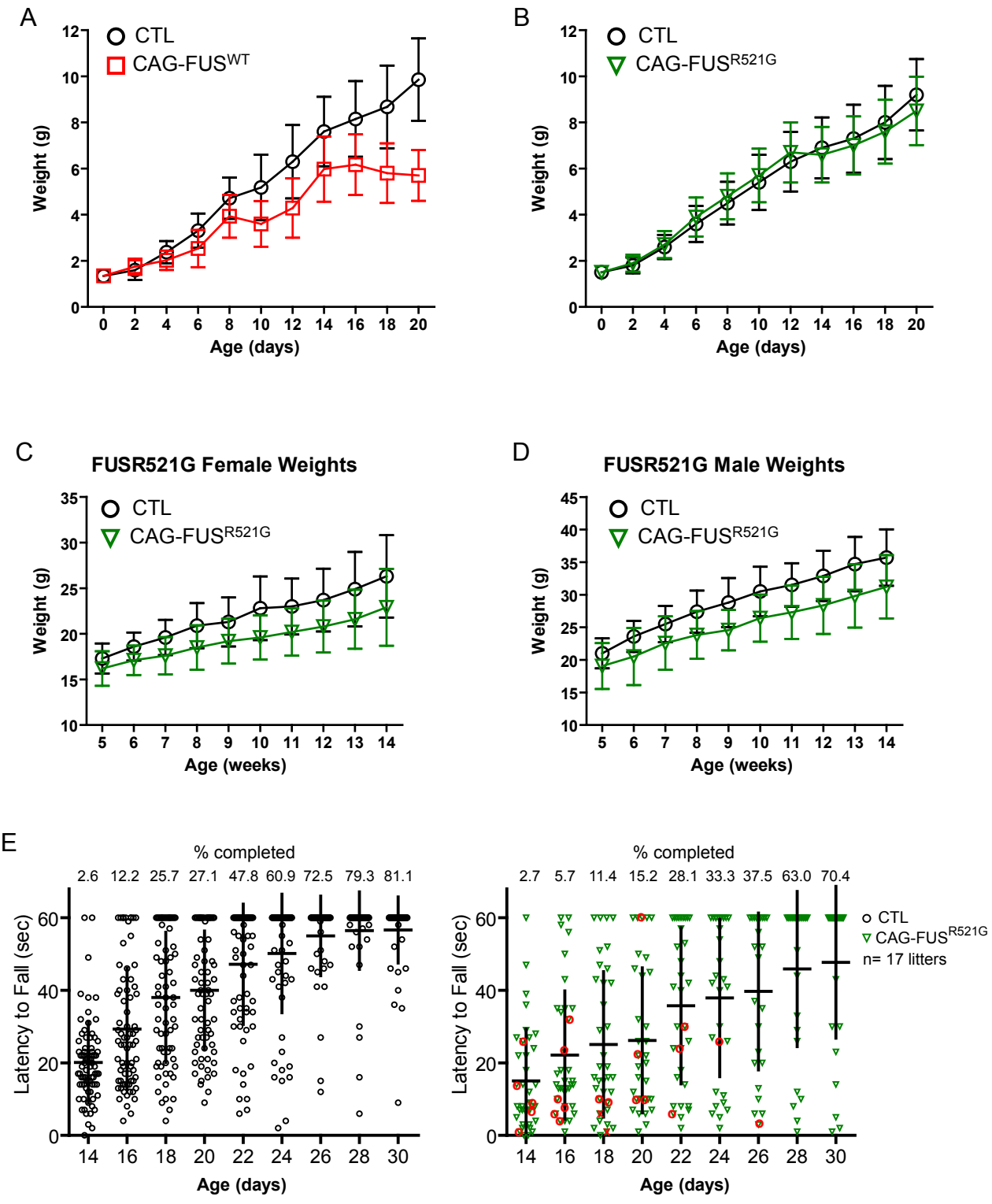
Figure S1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2.6 12.2 25.7 27.1 47.8 60.9 72.5 79.3 81.1
Latency to Fall (sec)
Age (days)
% completed
2.7 5.7 11.4 15.2 28.1 33.3 37.5 63.0 70.4
Latency to Fall (sec)
Age (days)
CTL
CAG-FUSR521G
n= 17 litters

Figure S2

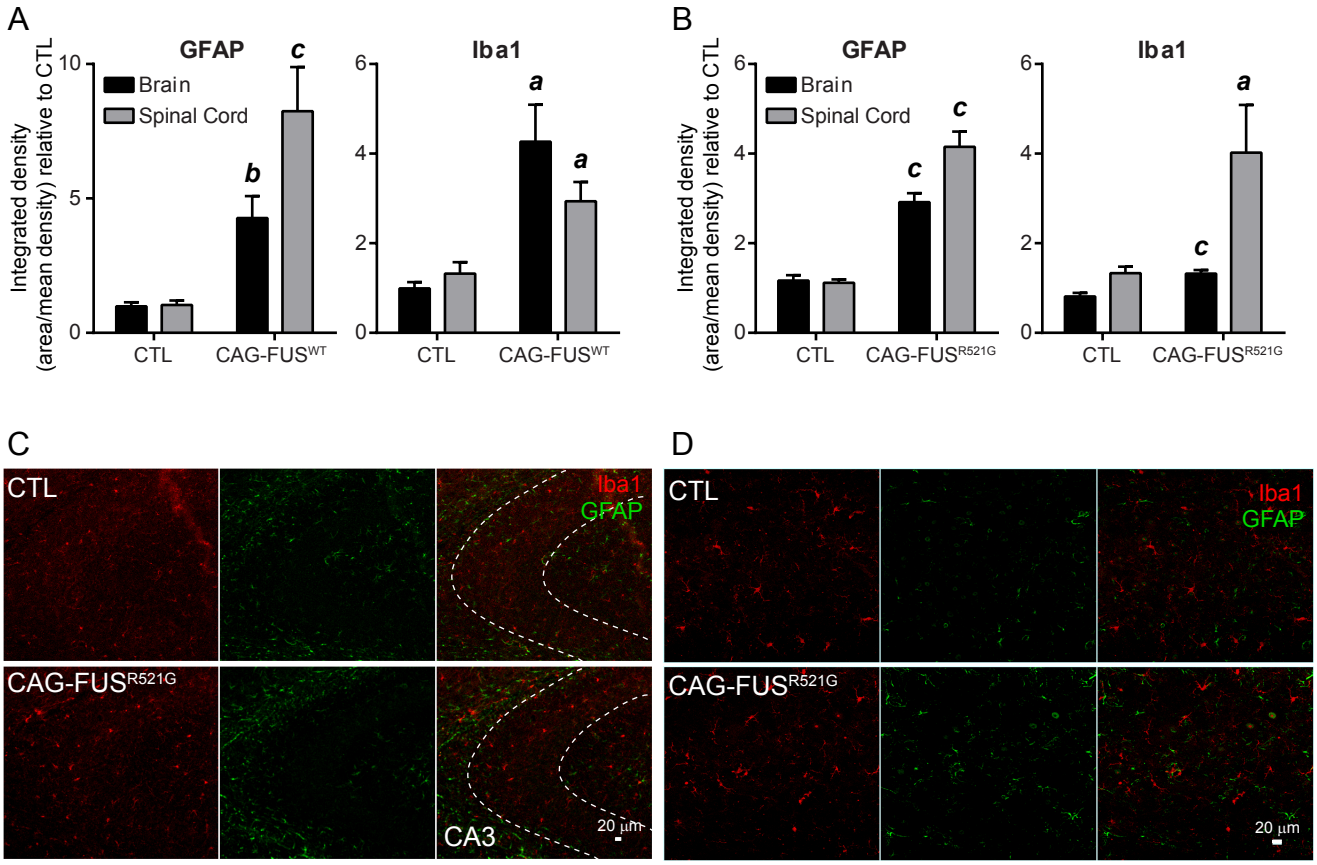
A
GFAP
Iba1
Brain
Spinal Cord
Integrated density (area/mean density) relative to CTL
CTL
CAG-FUSWT
B
CAG-FUSR521G
C
D
Iba1
GFAP
CTL
CAG-FUSR521G
CA3
20 µm

Figure S3

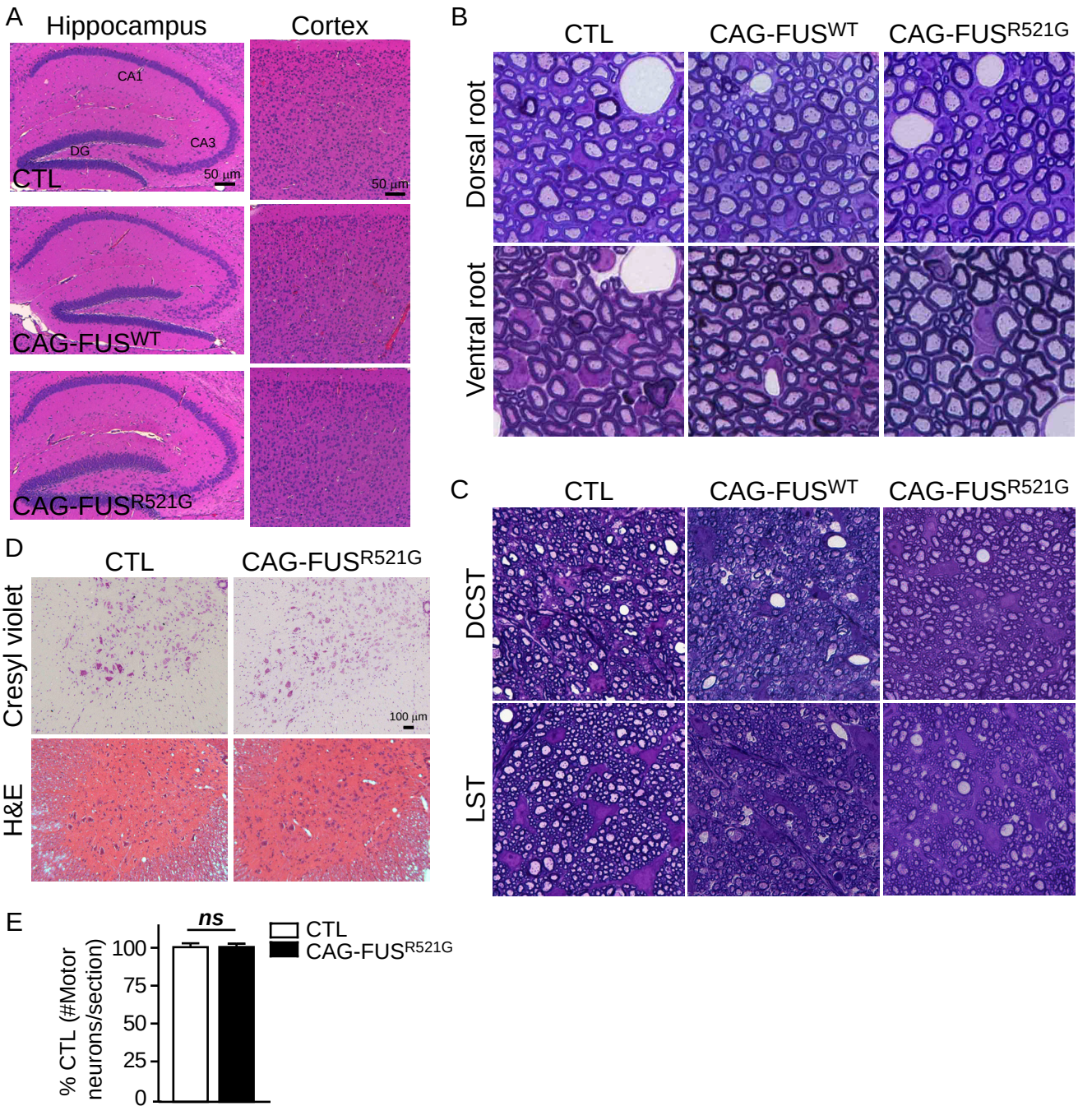
A
Hippocampus
Cortex
CA1
DG
CA3
CTL
50 μm
50 μm
CAG-FUSWT
CAG-FUSR521G
B
CTL
CAG-FUSWT
CAG-FUSR521G
Dorsal root
Ventral root
C
CTL
CAG-FUSWT
CAG-FUSR521G
DCST
LST
D
CTL
CAG-FUSR521G
Cresyl violet
H&E
100 μm
E
ns
CTL
CAG-FUSR521G
% CTL (#Motor neurons/section)
100
75
50
25
0

Figure S4

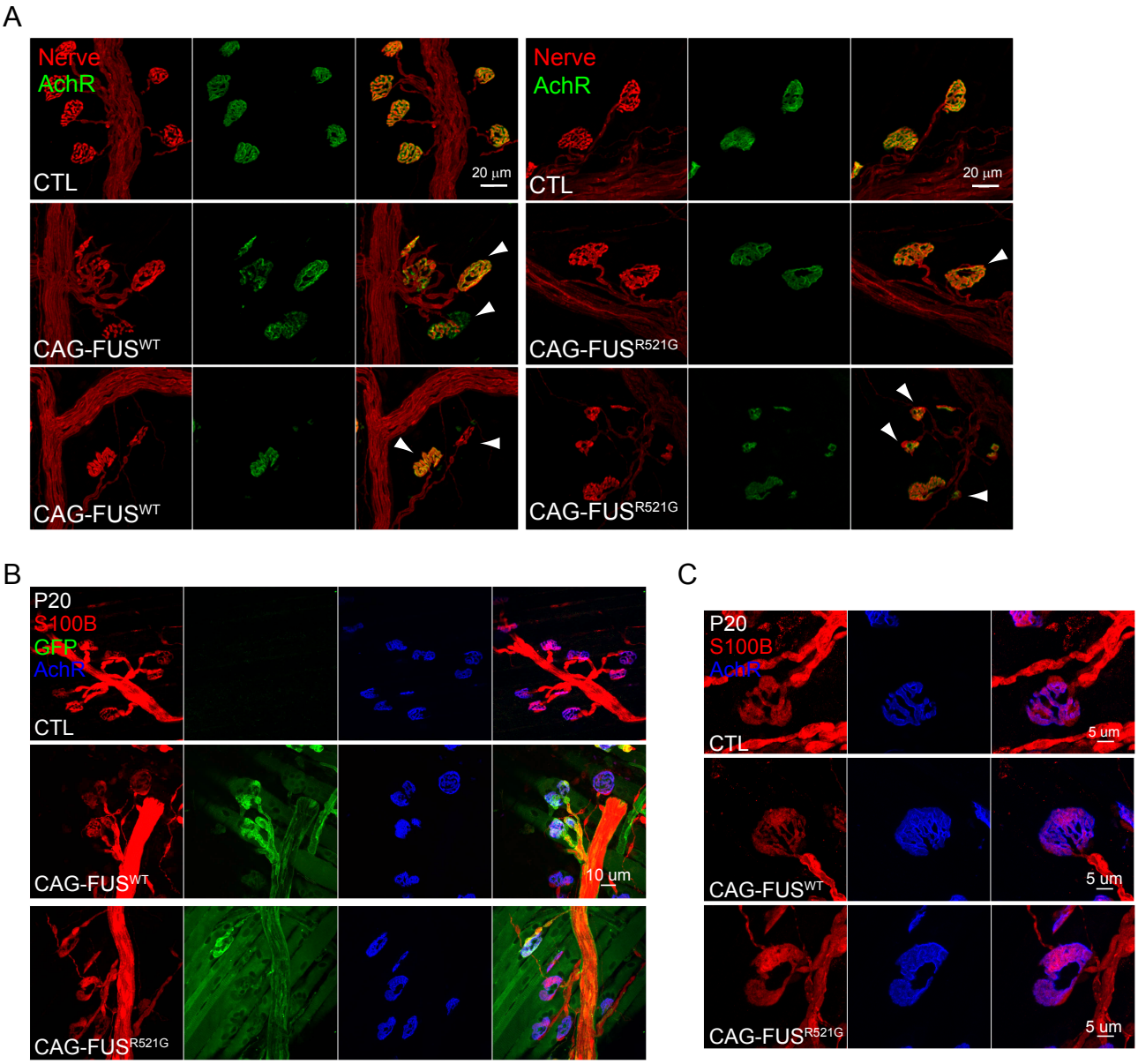
A
Nerve
AchR
CTL
20 μm
Nerve
AchR
CTL
20 μm
CAG-FUSWT
CAG-FUSR521G
CAG-FUSWT
CAG-FUSR521G
B
P20
S100B
GFP
AchR
CTL
CAG-FUSWT
10 um
CAG-FUSR521G
C
P20
S100B
AchR
CTL
5 um
CAG-FUSWT
5 um
CAG-FUSR521G
5 um

Figure S5

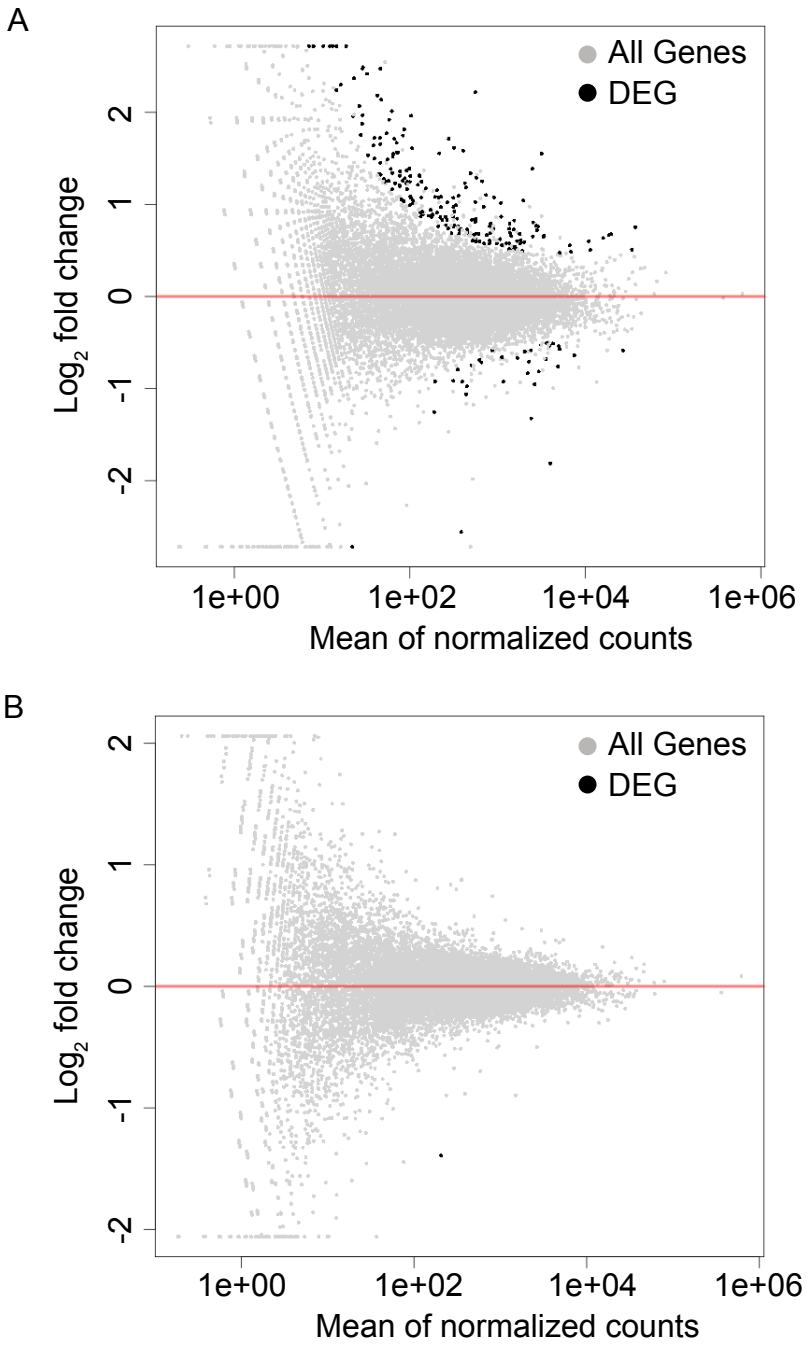
A
All Genes
DEG
Log2 fold change
2
1
0
-1
-2
1e+00
1e+02
1e+04
1e+06
Mean of normalized counts
B
All Genes
DEG
Log2 fold change
2
1
0
-1
-2
1e+00
1e+02
1e+04
1e+06
Mean of normalized counts

Figure S6

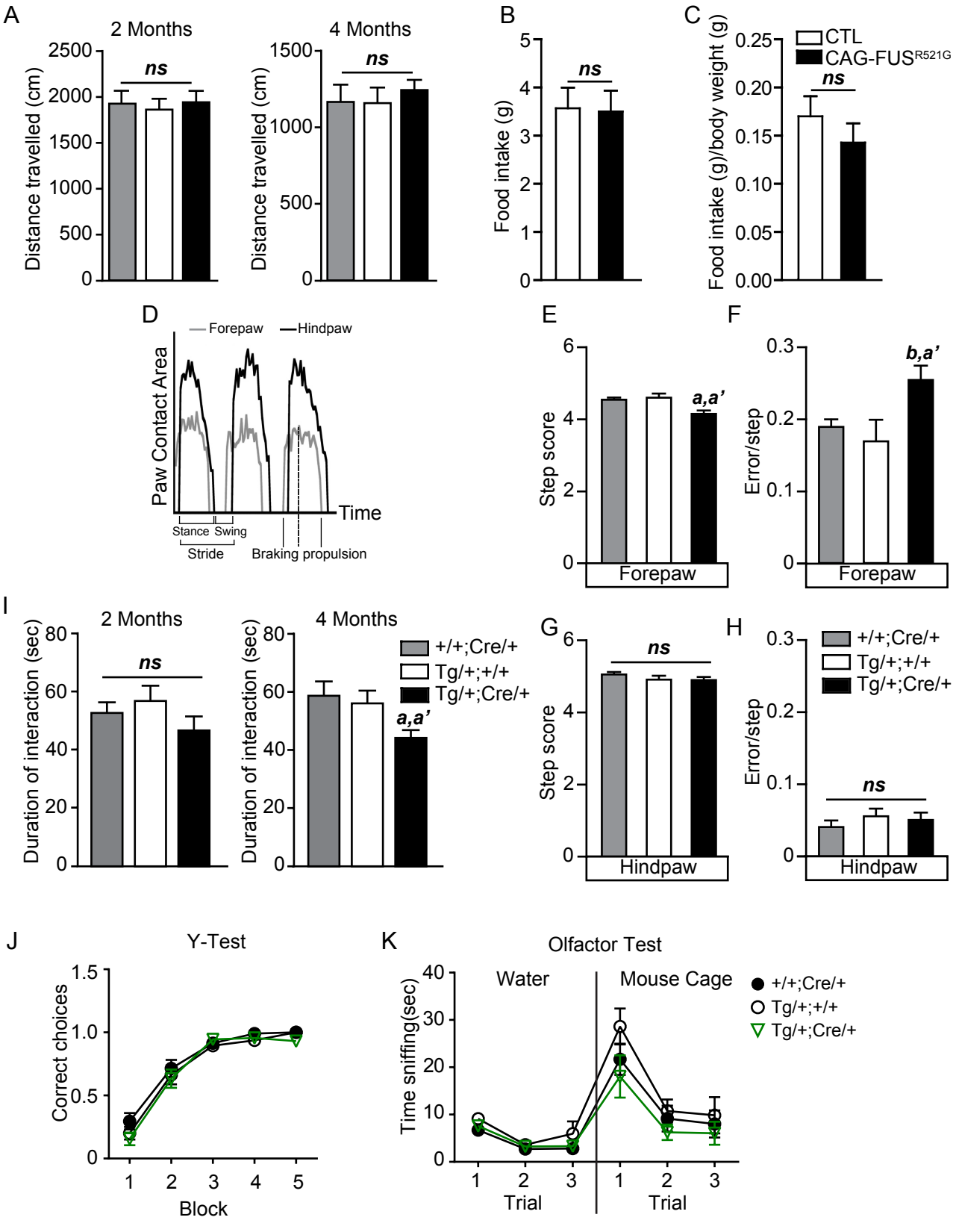
A
2 Months
4 Months
Distance travelled (cm)
ns
B
Food intake (g)
C
Food intake (g)/body weight (g)
CTL
CAG-FUS R521G
D
Forepaw
Hindpaw
Paw Contact Area
Time
Stance
Swing
Stride
Braking propulsion
E
Step score
a,a'
Forepaw
F
Error/step
b,a'
I
Duration of interaction (sec)
+/+;Cre/+
Tg/+;+/+
Tg/+;Cre/+
G
Hindpaw
H
J
Y-Test
Correct choices
Block
K
Olfactor Test
Water
Mouse Cage
Time sniffing(sec)
Trial

Figure S7

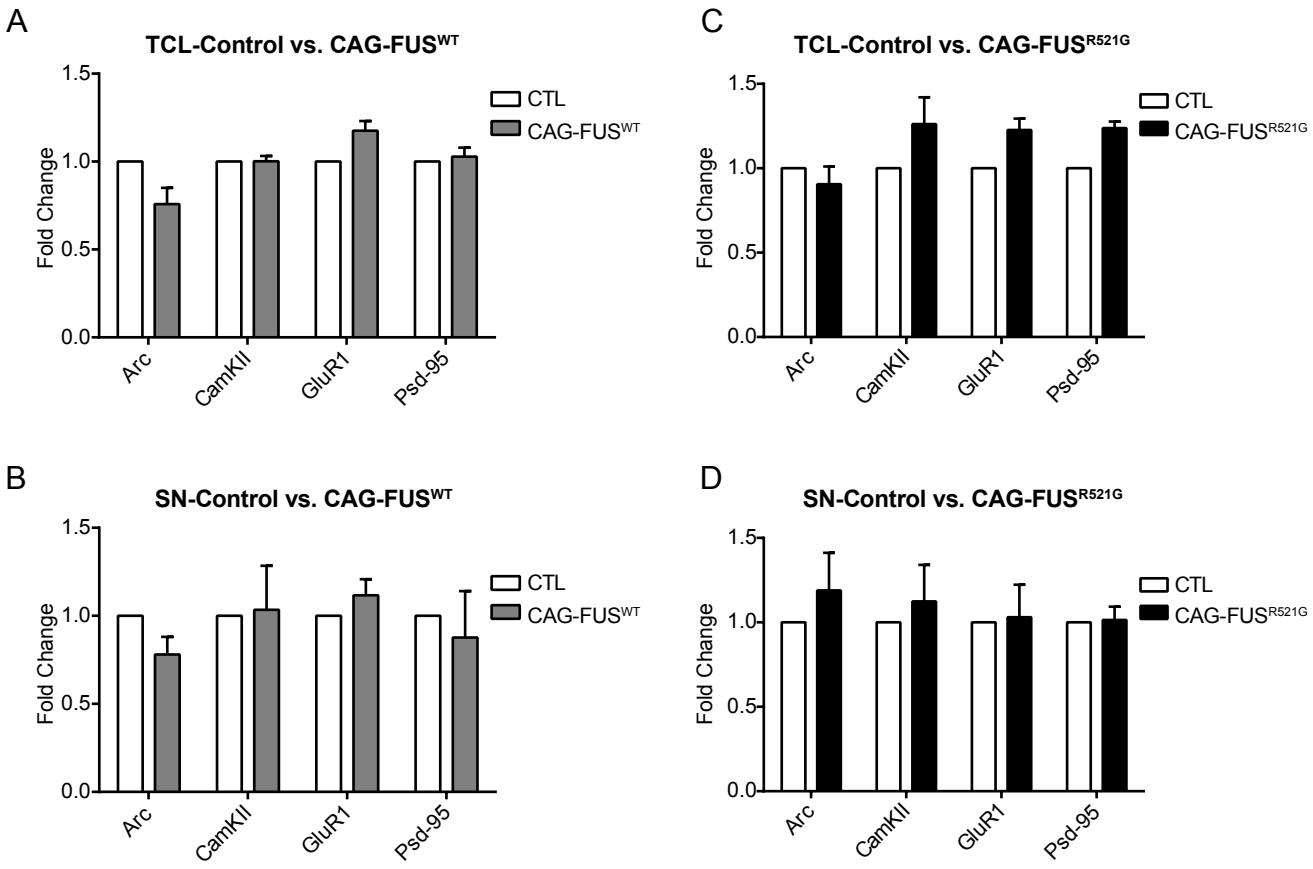
A
TCL-Control vs. CAG-FUSWT
Fold Change
1.5
1.0
0.5
0.0
CTL
CAG-FUSWT
Arc
CamKII
GluR1
Psd-95
C
TCL-Control vs. CAG-FUSR521G
Fold Change
1.5
1.0
0.5
0.0
CTL
CAG-FUSR521G
Arc
CamKII
GluR1
Psd-95
B
SN-Control vs. CAG-FUSWT
Fold Change
1.5
1.0
0.5
0.0
CTL
CAG-FUSWT
Arc
CamKII
GluR1
Psd-95
D
SN-Control vs. CAG-FUSR521G
Fold Change
1.5
1.0
0.5
0.0
CTL
CAG-FUSR521G
Arc
CamKII
GluR1
Psd-95

Figure S8

Table S1. Genotypes of P0 offspring from Tg/+;+/+ x Meox2Cre intercrosses

CAG-FUS^{WT}					
Line:	**+/+;Cre/+**	**+/+;+/+**	**Tg/+;+/+**	**Tg/+;Cre/+**	**Total**
629	24%	24%	25%	26%	95
638	23%	22%	21%	34%	120
CAG-FUS^{R521G}					
Line:	**+/+;Cre/+**	**+/+;+/+**	**Tg/+;+/+**	**Tg/+;Cre/+**	**Total**
673	24%	25%	22%	28%	99
682	27%	17%	29%	26%	66
Expected ratio:	25%	25%	25%	25%	

Table S2. Grip test of CAG-FUS^{WT} and CAG-FUS^{R521G} mice

Age (days)	CTL		CAG-FUS^{WT}	
	Mean (sec)	% Complete	Mean (sec)	% Complete
14	24.9 ± 4.0	3.1	3.8 ± 1.1 ***d***	0.0
16	39.2 ± 4.6	28.8	1.6 ± 0.5 ***d***	0.0
18	45.8 ± 4.0	47.9	0.6 ± 0.2 ***d***	0.0
20	50.8 ± 3.6	61.5	0.4 ± 0.2 ***d***	0.0

Age (days)	CTL		CAG-FUS^{R521G}	
	Mean (sec)	% Complete	Mean (sec)	% Complete
14	20.1 ± 2.7	2.6	15.1 ± 3.3	2.9
18	38.0 ± 4.2	25.7	27.4 ± 4.6 ***b***	12.9
22	47.2 ± 3.5	47.8	37.4 ± 4.6 ***a***	31.0
26	55.0 ± 2.2	72.5	41.3 ± 4.1 ***d***	39.1
30	56.6 ± 1.7	81.1	47.7 ± 3.9 ***b***	70.4

Student's *t*-test, ***a***,p<0.05; ***b***,p<0.01; ***d***,p<0.001
± SE (standard error of mean)

Table S3. Paired-end RNA-Seq reads summary

Samples	Properly paired reads	Quality passed reads	Total reads
FUS WT CTL1	55803368	74643906	85838586
FUS WT CTL2	28519390	37892168	86599378
FUS WT TG1	45656532	60879824	79388726
FUS WT TG2	67191840	90452989	67018964
FUS R521G CTL1	55028504	74358495	89150552
FUS R521G CTL2	57491368	75476514	44661408
FUS R521G TG1	52537060	68914387	72153918
FUS R521G TG2	42963068	58682073	106147744

Note: For two samples, the number of properly paired reads/quality passed reads is larger than total reads for those samples. This is due to the fact that Tophat allows reads to map to more than one place in the genome (multihits) as its default parameter, which causes such reads to be counted more than once, leading to increased number of reads in the BAM file after mapping compared to total reads in the FASTQ file.

Table S4. Forepaw gait measurements of CAG-FUS^{R521G} mice

	+/+;Cre/+ (n=19)	**Tg/+;+/+ (n=19)**	**Tg/+;Cre/+ (n=18)**
Paramenter	Mean	Mean	Mean
Swing Stride (%)	38.4 ± 0.5	38.9 ± 0.5	37.5 ± 0.7
Brake Stride (%)	30.1 ± 1.4	29.3 ± 1.5	34.8 ± 1.2 ***a,b'***
Propel Stride (%)	31.4 ± 1.5	31.8 ± 1.6	27.7 ± 1.4
Brake Stance (%)	49.1 ± 2.3	48.1 ± 2.6	55.8 ± 2.1 ***b,ns'***
Propel Stance (%)	50.9 ± 2.3	51.9 ± 2.6	44.2 ± 2.1
Stride Length (cm)	5.72 ± 0.10	5.82 ± 0.05	5.65 ± 0.08
Stance Width (cm)	1.46 ± 0.05	1.51 ± 0.05	1.46 ± 0.05
Midline Distance (cm)	-2.08 ± 0.09	-2.20 ± 0.09	-2.17 ± 0.09

One-way ANOVA, a,$p<0.05$; b,$p<0.01$; ns,not significant, ' compares +/+;Cre/+ with Tg/+;Cre/+
± SE (standard error of mean)

Table S5. Hindpaw gait measurements of CAG-FUS^{R521G} mice

	+/+;Cre/+ (n=19)	**Tg/+;+/+ (n=19)**	**Tg/+;Cre/+ (n=18)**
Paramenter	Mean	Mean	Mean
Swing Stride (%)	35.4 ± 0.6	34.9 ± 0.5	32.8 ± 0.8 ***a,b'***
Brake Stride (%)	18.1 ± 0.8	18.8 ± 0.8	17.8 ± 0.8
Propel Stride (%)	46.4 ± 0.8	46.3 ± 0.8	49.4 ± 1.2
Brake Stance (%)	28.0 ± 1.2	28.1 ± 1.1	26.6 ± 1.3
Propel Stance (%)	72.0 ± 1.2	71.2 ± 1.1	73.4 ± 1.3
Stride Length (cm)	5.74 ± 0.10	5.86 ± 0.06	5.73 ± 0.09
Stance Width (cm)	2.66 ± 0.05	2.55 ± 0.06	2.62 ± 0.05
Midline Distance (cm)	1.62 ± 0.04	1.61 ± 0.06	1.42 ± 0.07 ***a,a'***

One-way ANOVA, a,p<0.05; b,p<0.01, ' compares +/+;Cre/+ with Tg/+;Cre/+
± SE (standard error of mean)