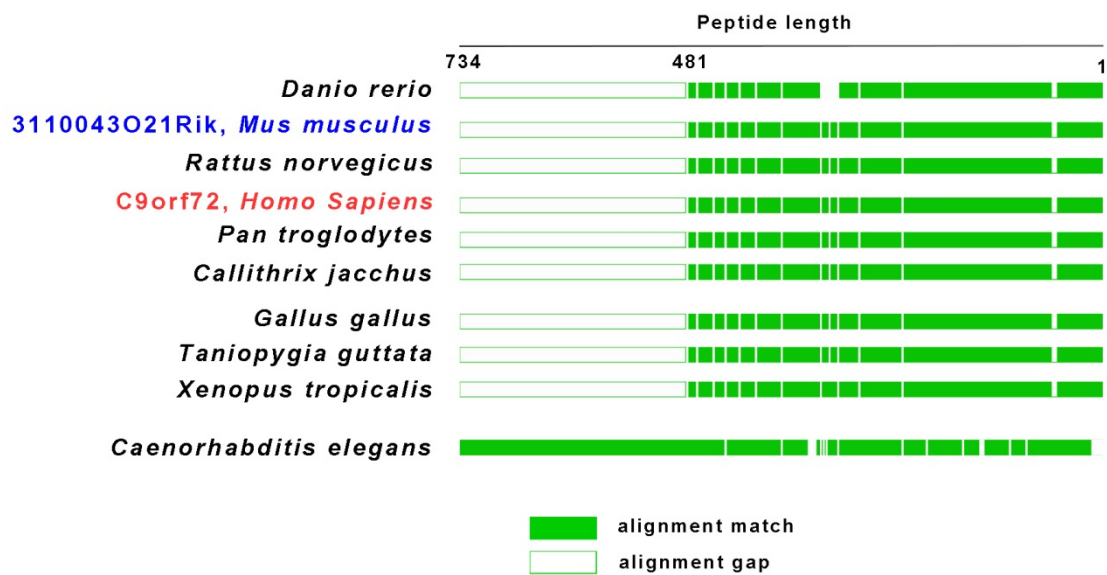


Supplementary Information to

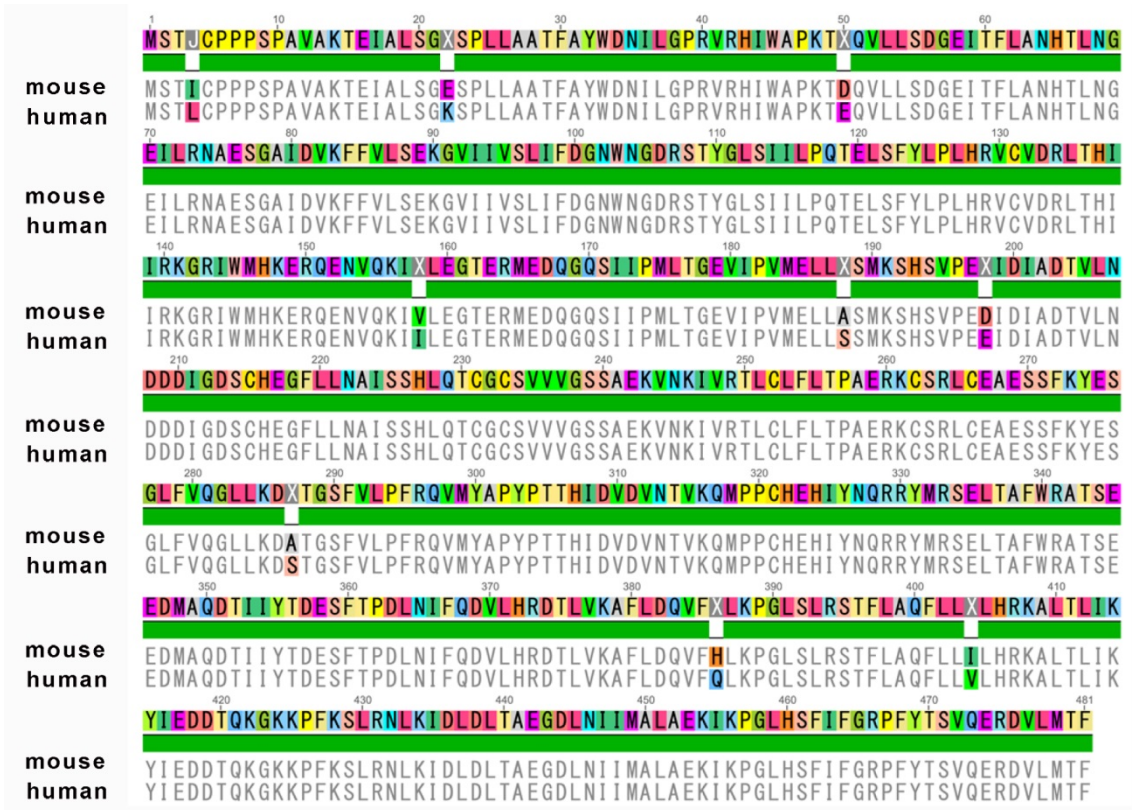
Transcriptional activity of the *C9ORF72* mouse ortholog corresponds with the selectivity of neural degeneration in ALS / FTD

Naoki Suzuki, Asif Maroof, Florian T Merkle, Kathryn Koszka, Atsushi Intoh, Ian Armstrong, Rob Moccia, Brandi N

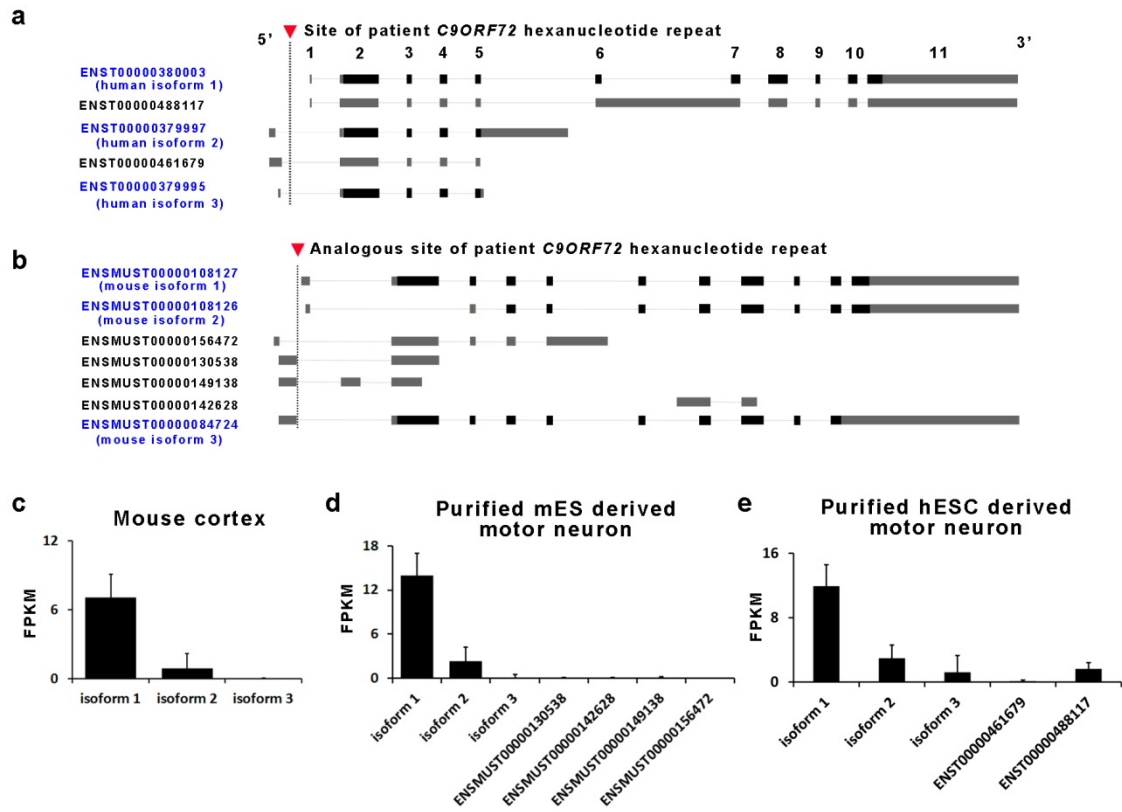
Davis-Dusenbery, and Kevin Eggan



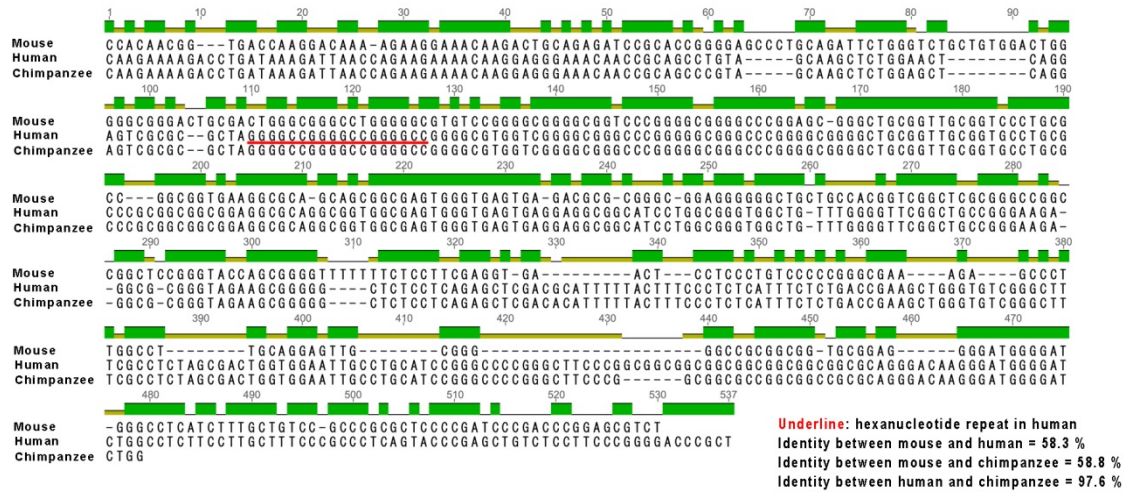
Supplementary Figure 1. Gene tree of *C9ORF72* gene and its orthologs. Note that *C9ORF72* orthologs are highly conserved among several species, especially in vertebrata. Black line represents x1 branch length, while blue line represents x10 branch length. Green box represents alignment match, while white box represents alignment gap. F18A1.6 in *C. elegans* has 23 % identity with human *C9ORF72*.



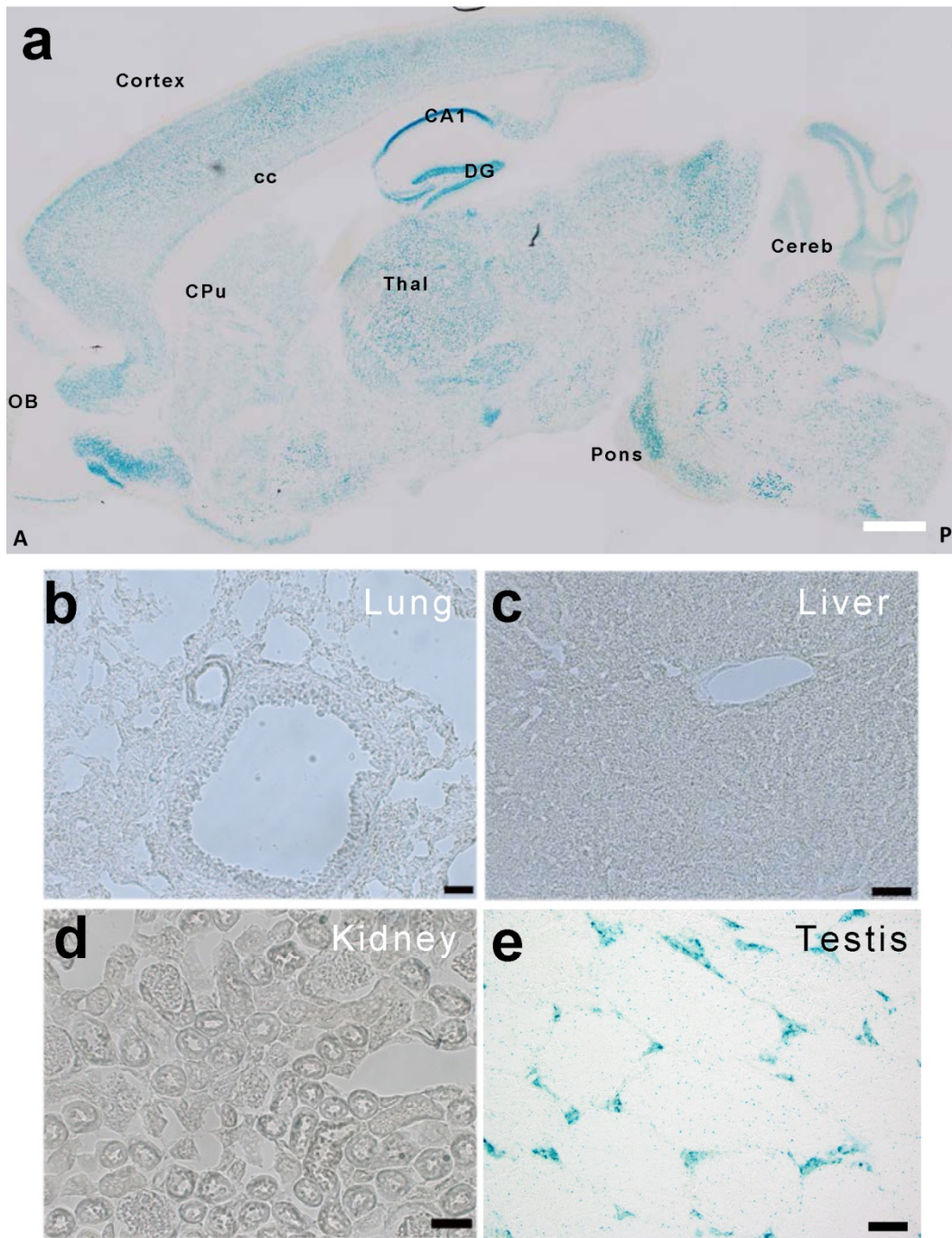
Supplementary Figure 2. Amino acid sequence of human C9ORF72 and mouse 3110043O21Rik gene. Upper and lower lane represent human and mouse ortholog respectively.



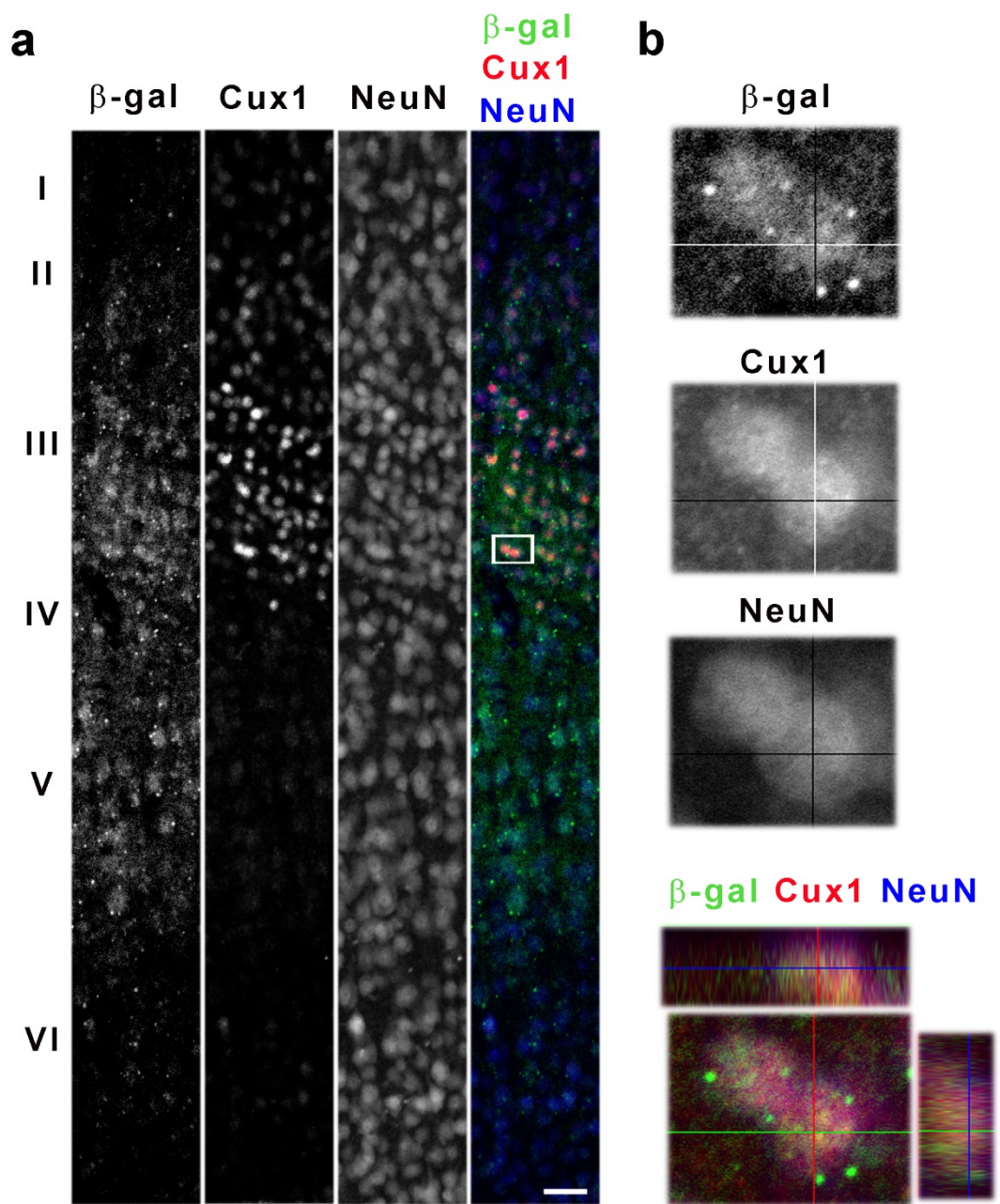
Supplementary Figure 3. Comparison of splicing isoforms between human *C9ORF72* (a) and mouse 3110043021Rik gene (b). Black boxes represent translated exons and grey boxes represent predicted non-coding exons. Blue font represents translated isoforms. Red arrowhead and dashed line represent the location of hexanucleotide repeat expansion in human patients with ALS/FTD (a) and analogous site of patient *C9ORF72* hexanucleotide repeat (b). RNA sequence data (c-e). Isoform 1 is highly expressed in mouse cortex (c), mouse purified ES derived motor neuron (d), and human purified ES derived motor neuron (e). FPKM, Fragments Per Kilobase of exon per Million mapped fragments. Error bars indicate s.d. n=2 for (c), n=3 for (d) and (e).



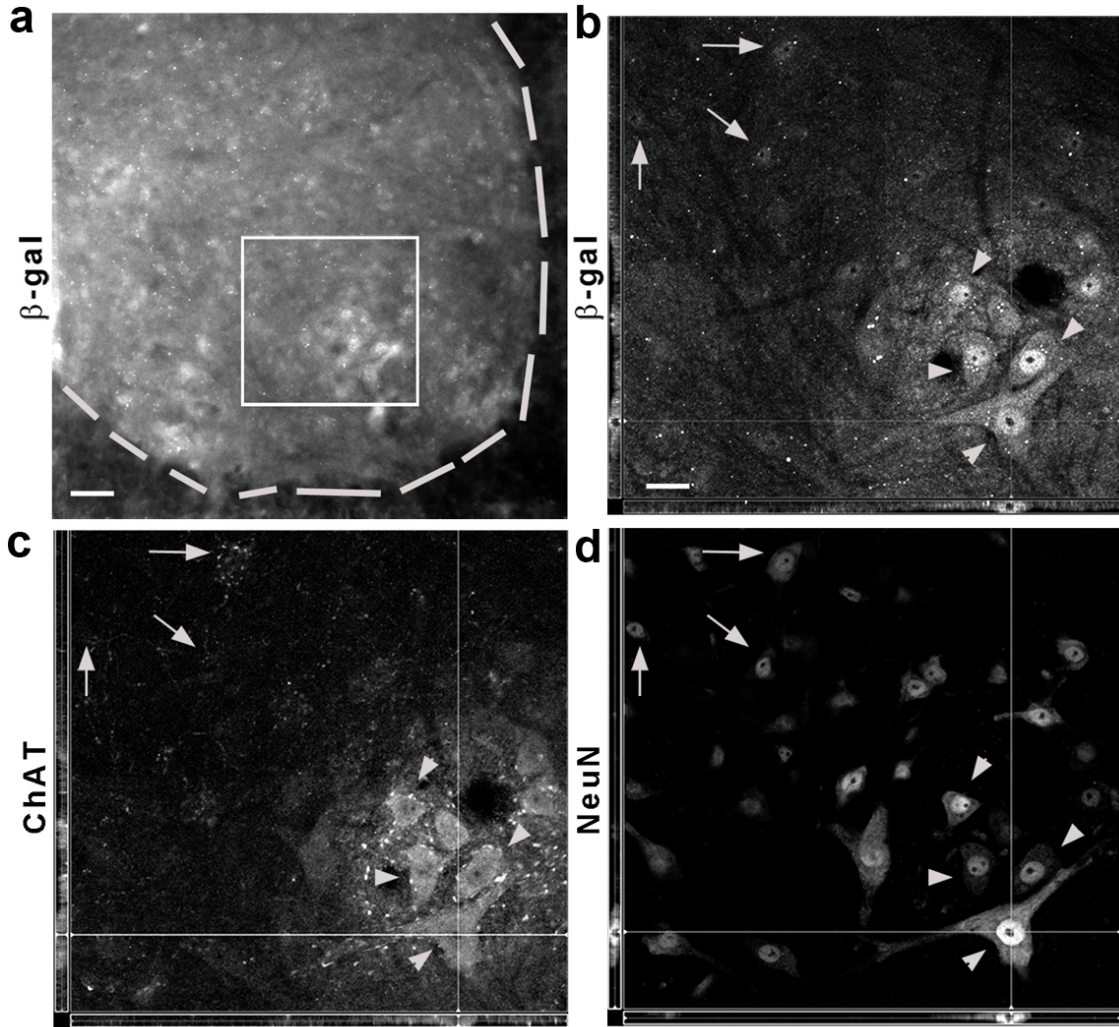
Supplementary Figure 4. Comparison of sequence between human C9ORF72, mouse 3110043O21Rik and chimpanzee LOC465031 genes around the sequence of hexanucleotide repeats in human. 58.3 % identities are found between human and mouse. Note that GC rich region in both sequences.



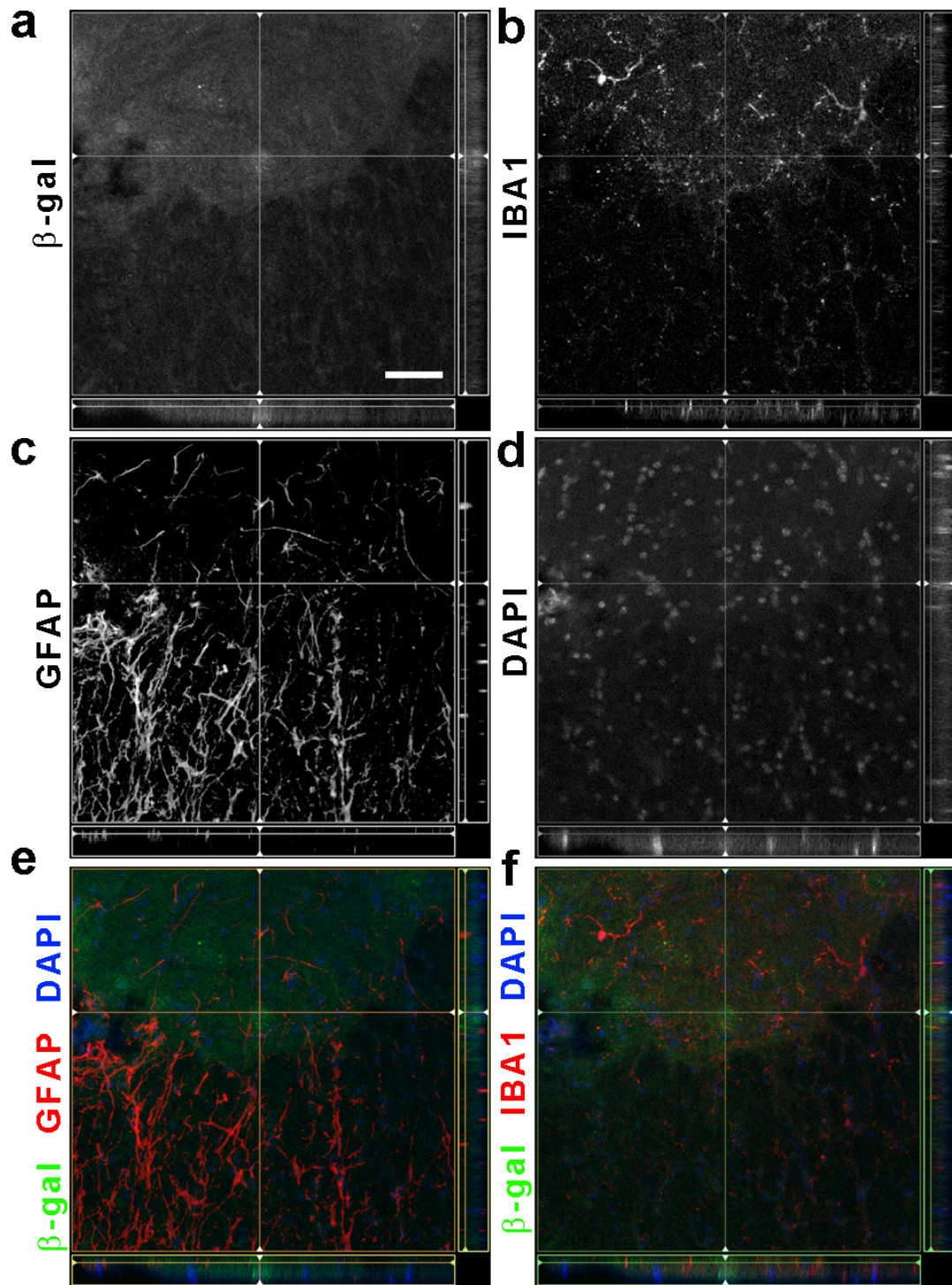
Supplementary Figure 5. X-gal staining in the brain and several organs of C9ORF72-ortholog knockin mice. (a) 0.6 mm from the midline. A, anterior; P, posterior; OB, olfactory bulb; cc, corpus callosum; CPu, caudate putamen (striatum); DC, granular dentate gyrus; thal, thalamus; cereb, cerebellum. Lung (b), liver (c), and kidney (d) are negative for X-gal staining. Testis (e) is shown as positive control. Bar, 1 mm (a) and 50 μ m (b-e).



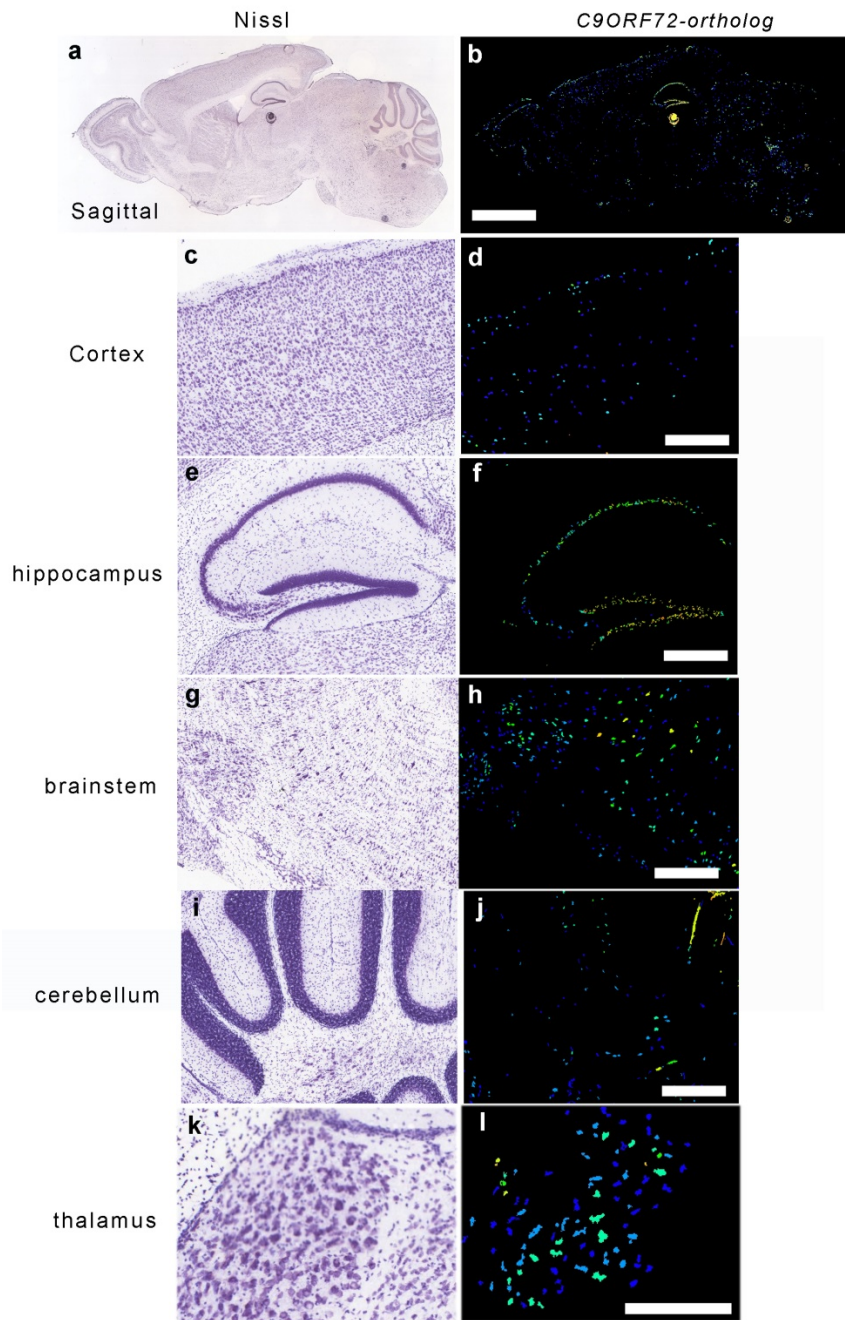
Supplementary Figure 6. Co-localization of β -gal, Cux1, and NeuN is shown (a). β -gal positive cells are also located in superficial layers (b). (b) shows Z-stack and orthogonal images of white rectangle in (a). Bar, 50 μ m (a) and 20 μ m (b).



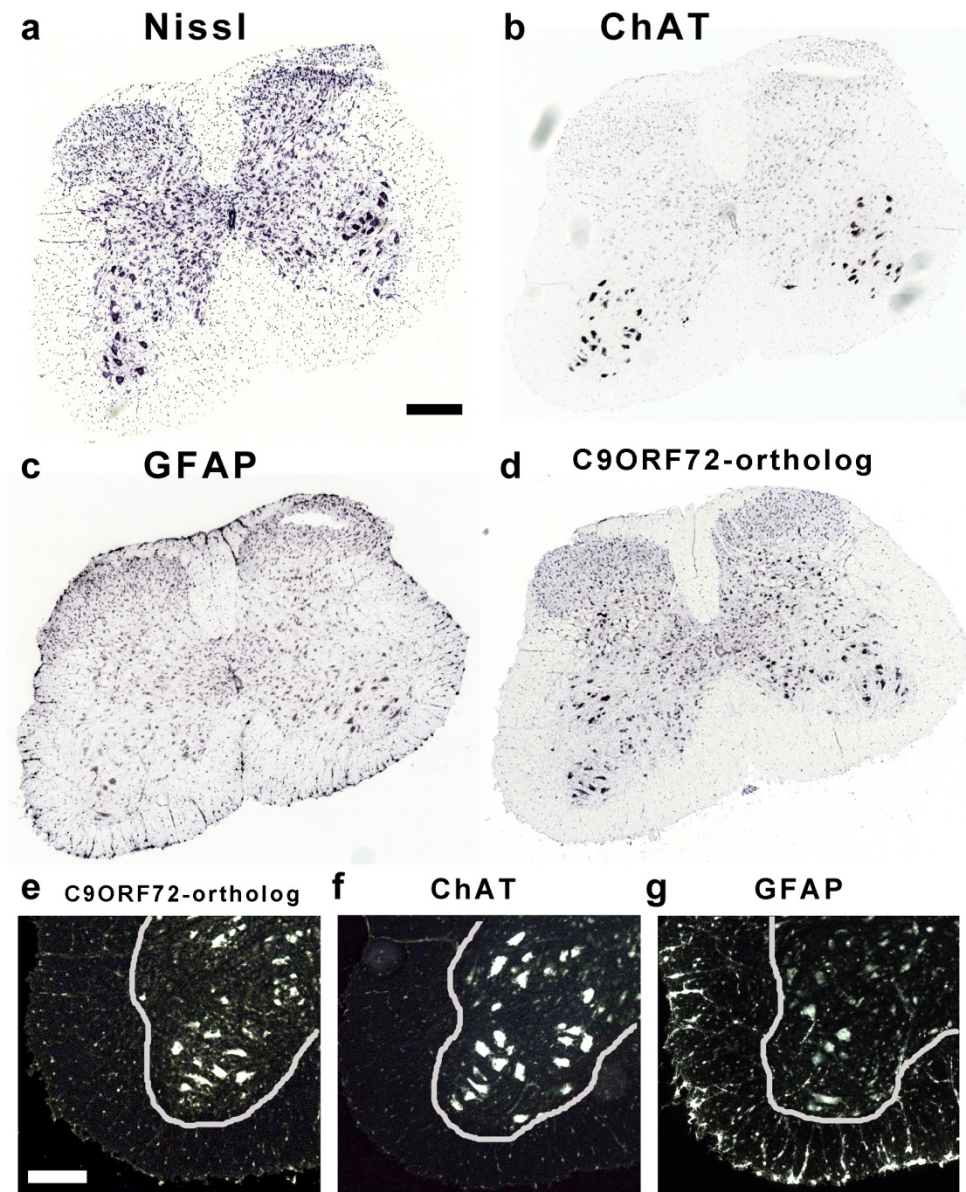
Supplementary Figure 7. Co-localization of β -gal, ChAT, and NeuN in ventral horn of spinal cord. (b-d) shows Z-stack and orthogonal images of white rectangle in (a). Arrowheads show ChAT positive motor neurons and arrows are ChAT negative cells. Bar, 50 μ m (a) and 20 μ m (b-d).



Supplementary Figure 8. β -gal positive cell does not co-localize with IbaI and GFAP in anterior horn of spinal cord (a-f). Images are taken from the same slice. Z-stack images are also shown. Green- β -gal, red-IbaI (e) or GFAP (f) and blue-DAPI. Bar, 50 μ m (a-f).



Supplementary Figure 9. Brain expression pattern of C9ORF72 ortholog from Allen Brain Atlas. (a-l) Expression pattern of C9ORF72-ortholog of brain from Allen Brain Atlas database. Allen Brain Atlas used probes from exon 4 through exon 11. In situ hybridization of C9ORF72-ortholog in sagittal section of mice brain at P56: Nissl staining (a) and expression image (b) from similar slices. Higher magnification of cortex (c, d), hippocampus (e, f), brainstem (g, h), cerebellum (i, j), and thalamus (k, l) from similar slices. Nissl staining (a, c, e, g, i, k) and expression of C9ORF72-ortholog (b, d, f, h, j, l). Note that hippocampus has higher expression. Bar, 1 mm (a, b) and 300 μ m (c-l). Note that each image is a distinct section.



Supplementary Figure 10. Spinal cord expression pattern of C9ORF72 ortholog from Allen Brain Atlas. (a-g) Expression pattern of C9ORF72-ortholog in spinal cord from Allen Brain Atlas database. Allen Brain Atlas used probes from exon 4 through exon 11. In situ hybridization of C9ORF72-ortholog in sagittal section of mouse brain at P56: Nissl staining (a) and expression image (b-g) from similar slices. Lower (a-d) and higher (e-g) magnification are presented. Expression pattern of C9ORF72-ortholog (d, e), ChAT (b, f) and GFAP (c, g) are shown. Note that C9ORF72-ortholog transcript distributes mainly in the grey matter and displays a pattern similar to, but modestly broader than ChAT. White line shows the border between grey and white matter (e-g). Bar, 200 μm (a-d), 100 μm (e-g). Note that each image is from a distinct section.

Supplementary Table 1. Distribution of C9ORF72-ortholog in CNS

		Expression
Olfactory system	Olfactory epithelium	-
	Olfactory bulb	-
	Anterior olfactory nucleus	+
	Olfactory tubercle	+
Basal ganglia	Caudate putamen (striatum)	+
	Core of accumbens nucleus	+
	Shell of accumbens nucleus	+
	Globus pallidus	+
Thalamus		+
Hypothalamus		+
Hippocampal formation	Lacunosum moleculare layer of hippocampus	-
	Stratum radiatum of hippocampus	-
	Oriens layer of hippocampus	-
	CA1 pyramidal cell layer of hippocampus	+++
	CA1 stratum radiatum of hippocampus	-
	CA2 pyramidal cell layer of hippocampus	++
	CA2 stratum radiatum of hippocampus	-
	CA3 pyramidal cell layer of hippocampus	++
	CA3 stratum radiatum of hippocampus	-
	Molecular layer of dentate gyrus	-
	Granular layer of dentate gyrus	+
	Polymorph layer of dentate gyrus	-
	entorhinal cortex	+
	Amygdala	+
Cortex	Layer I	-
	Layer II	+
	Layer III	+
	Layer IV	+/-
	Layer V	+++
	Layer VI	+
Fiber tracts	Corpus callosum	-
	Anterior commissure	-
	Fornix	-
	Stria medullaris	-
	Internal capsule	-
	Optic tract	-
	Medial lemniscus	-
	Superior colliculus	+
Midbrain pons	Inferior colliculus	+
	Pontine nuclei	++
	Ventral tegmental area	+ / ++
	Reticular part of substantia nigra	+
	Compact part of substantia nigra	+
	Nigrostriatal bundle	-
	Red Nucleus	+
	Locus Ceruleus	+
	Facial nerve nucleus	++
	Cerebellum	Molecular layer
Purkinje cell layer		- / +
Granular layer		+
White matter		-
Spinal cord	Anterior horn	++
	Posterior horn	+
	Anterior and lateral CST	-
	Gracile fasciculus	-
	Cuneate fasciculus	-

Supplementary Table 2. X-gal expression during embryonic and postnatal stages in CNS. N/A, not applicable.

Age	Expression	Region
E9.5	–	N/A
E12.5	–	N/A
E14.5	–	N/A
E15.5	–	N/A
E18.5	–	N/A
P1	+	Brainstem, Spinal cord
P3	+	Cortex, Brainstem, Spinal cord
P14	++	Cortex, Brainstem, Spinal cord
P60	+++	Cortex, Striatum, Cerebellum, Brainstem, Spinal cord