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Expanded polyglutamine impairs normal nuclear distribution of FUS and PCBP1 in Huntington's disease

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Complete List of Authors:	Mori, Shinichirou; Kyushu University, Neuropathology Honda, Hiroyuki; Kyushu University, Department of Neuropathology Ishii, Takashi; Department of Biochemistry, Fukuoka Dental College Yoshimura, Motoi ; Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University Sasagasako, Naokazu; Neuro-Muscular Center, National Omuta Hospital, Neurology Taniwaki, Takayuki; Division of Respirology, Neurology and Rheumatology, Department of Medicine, Kurume University School of Medicine Suzuki, Satoshi; Graduate School of Medical Sciences, Kyushu University, Neuropathology Iwaki, Toru; Kyushu University, Neuropathology
Keywords:	huntington's disease, polyglutamine, ribonucleoprotein, FUS, PCBP1
Abstract:	Huntington's disease (HD) is an inherited neurodegenerative disease caused by a polyglutamine repeat expansion in the huntingtin protein. Immunohistochemistry studies using the 1C2 antibody for polyglutamine expansion have detected characteristic intranuclear inclusions in affected neurons in HD. Further, in vitro and mouse models of HD have shown that the intranuclear inclusions recruit several proteins relating to RNA splicing and translation. In the present study, we immunohistochemically investigated the association of intranuclear inclusions with various heterogeneous nuclear ribonucleoproteins in the cerebral cortex of four autopsy cases of HD. Fused in sarcoma (FUS) was colocalized with 1C2-positive nuclear inclusions in all examined cases. Localization of poly(rC)-binding protein 1(PCBP1) in 1C2-positive nuclear inclusions was also observed. Double immunofluorescence revealed complete or partial loss of the normal, diffuse nuclear distribution of FUS or PCBP1 in neurons with 1C2-positive nuclear inclusions. This maldistribution of FUS in cortical neurons suggests a severe disturbance of mRNA processing, which may be a common pathogenetic mechanism of FUS-related familial amyotrophic lateral sclerosis.

Expanded polyglutamine impairs normal nuclear distribution of FUS and PCBP1 in Huntington's disease

Shinichiro Mori, MD.[1,4] Hiroyuki Honda, MD., PhD.[1], Takashi Ishii., PhD.[2], Motoi Yoshimura, MD.[1], Naokazu Sasagasako, MD., PhD [3], Satoshi O Suzuki, MD., PhD.[1], Takayuki Taniwaki MD., PhD.[4], Toru Iwaki MD., PhD.[1]

[1] Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

[2]Department of Biochemistry, Fukuoka Dental College, Fukuoka, Japan

[3]Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Omuta, Japan

[4]Department of Neurology, Division of Respirology, Neurology and Rheumatology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

Running title: FUS and PCBP1 in Huntington's disease

Correspondence to: Dr. Toru Iwaki

Department of Neuropathology, Graduate School of Medical Sciences

Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Tel.: +81-92-642-5536; Fax: +81-92-642-5540; Email: iwaki@np.med.kyushu-u.ac.jp

ABSTRACT

Huntington's disease (HD) is an inherited neurodegenerative disease caused by a polyglutamine repeat expansion in the huntingtin protein. Immunohistochemistry studies using the 1C2 antibody for polyglutamine expansion have detected characteristic intranuclear inclusions (INIs) in affected neurons in HD. Further, *in vitro* and mouse models of HD have shown that the INIs recruit several proteins relating to RNA splicing and translation. In the present study, we immunohistochemically investigated the association of INIs with various heterogeneous nuclear ribonucleoproteins in the cerebral cortex of four autopsy cases of HD. Fused in sarcoma (FUS) was colocalized with 1C2-positive nuclear inclusions in all examined cases. Localization of poly(rC)-binding protein 1(PCBP1) in 1C2-positive nuclear inclusions was also observed. Double immunofluorescence revealed complete or partial loss of the normal, diffuse nuclear distribution of FUS or PCBP1 in neurons with 1C2-positive nuclear inclusions. This maldistribution of FUS in cortical neurons suggests a severe disturbance of mRNA processing, which may be a common pathogenetic mechanism of FUS-related familial amyotrophic lateral sclerosis.

Keywords: Huntington's disease, polyglutamine, ribonucleoprotein, FUS, PCBP1

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by clinical symptoms including chorea, cognitive impairment, and psychiatric manifestation. HD is caused by dominant CAG trinucleotide (polyQ) expansion mutations in the gene encoding huntingtin [1].The macroscopic pathological features of HD include severe atrophy of the caudate nucleus and putamen. Under microscopic examination, HD is characterized by intranuclear inclusions (INI) immunopositive for huntingtin and expanded polyglutamine[2 3].

Toxicity of the mutant protein and impairment of RNA metabolism are thought to be associated with the pathophysiology of triplet-repeat diseases, including HD[4]. Impairment of RNA metabolism is observed in several neurodegenerative diseases, especially amyotrophic lateral sclerosis (ALS)[4 5]. Further, there are HD cases that overlap pathologically with sporadic ALS, with neuronal inclusions immunoreactive for trans-activation response DNA-binding protein of 43 kDa (TARDBP or TDP43), a member of the heterogeneous nuclear ribonucleoproteins (hnRNP) family[6]. TARDBP was also frequently colocalized with huntingtin in dystrophic neurites and various intracellular inclusions in HD cases[7]. Additionally, a study using *in vitro* and mouse models of HD showed that the INI recruits several hnRNPs[8]. Thus, impairment of hnRNPs, which causes disordered RNA metabolism, is likely involved in the pathogenesis of HD. However, the relationship between huntingtin INIs and various hnRNPs in human HD cases remains unknown. Thus, in the present study we examined the immunohistochemical association of neuronal INIs and several hnRNPs in cortical neurons of HD cases.

MATERIALS AND METHODS

Case material

We examined four autopsied cases clinicopathologically diagnosed with HD and one autopsied case of Machado-Joseph disease (MJD) from National Omuta Hospital, Fukuoka, Japan. Patients' characteristics are shown in Table 1. In case 1 and case 2, genetic tests revealed pathogenetic CAG repeat expansion. Control cases were autopsied in Kyushu University Hospital, Fukuoka, Japan. Characteristics of control cases are shown in Table 2. All patients died between 1977 and 2017, and general autopsies were performed. Written informed consent for autopsy including use of tissue for research purposes was obtained. All analyses were performed in accordance with the Declaration of Helsinki.

Neuropathological examination

The brains and spinal cords were fixed in 10% buffered formalin and processed into paraffin sections. Specimens of HD included the precentral gyrus, middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, basal ganglia, thalamus, and spinal cord. We also examined pons of the MJD case. Multiple 6 μm-thick sections were stained by hematoxylin and eosin and Klüver–Barrera staining.

Immunohistochemistry

Immunohistochemistry was performed using primary antibodies against polyQ-expansion (1C2; monoclonal, mouse, 1:10,000; Millipore, Burlington, MA, USA), huntingtin (monoclonal, rabbit, 1:500; Abcam, Cambridge, UK), TARDBP (polyclonal, rabbit, 1:2000; Protein Tech Group Inc., Chicago, IL, USA), phosphorylated TARDBP

(p-TDP43; polyclonal, rabbit, 1:5000; Cosmo Bio, Tokyo, Japan), hnRNPA1 (monoclonal, rabbit, 1:200; Abcam), hnRNPA2B1 (monoclonal, mouse, 1:500; Abcam), hnRNPD (polyclonal, rabbit, 1:500; Atlas Antibodies, Stockholm, Sweden), PCBP1 (polyclonal, rabbit, 1:100; ab74794 Abcam)(Fig. 1), FUS (polyclonal, rabbit, 1:200; NB100-2599 Novus Biologicals, LLC, CO, USA) [9, 10], and sequestosome 1 (p62; polyclonal, rabbit, 1:500; MBL, Nagoya, Japan). Sections were deparaffinized in xylene and rehydrated in an ethanol gradient. Tissues were pretreated for antigen retrieval by autoclaving in 0.01 M citrate buffer (pH 6.0) at 121°C for 10 min. Sections were then incubated with primary antibodies overnight at 4°C. After rinsing, immunoreaction products were detected using the polymer immunocomplex method (Envision system; DakoCytomation, Glostrup, Denmark). Immunoreactivity was visualized using 3, 3′-diaminobenzidine (Dojindo, Kumamoto, Japan), and specimens were lightly counterstained with hematoxylin.

Immunofluorescence

We performed double immunofluorescence with the following combinations of antibodies: anti-polyQ-expansion antibody with anti-huntingtin antibody, anti-hnRNPA1 antibody, anti-hnRNPD antibody, anti-PCBP1 antibody, anti-FUS antibody, or anti-p62 antibody. Alexa 488-labeled anti-mouse IgG (Invitrogen, Carlsbad, CA, USA) and Alexa 546-labeled anti-rabbit IgG (Invitrogen) were used as secondary antibodies (1:50 dilution). Sections were deparaffinized and rehydrated, and then autoclaved to unmask the epitope as described above. Sections were then incubated with the primary antibodies overnight at 4°C, rinsed, incubated with appropriate combinations of secondary antibodies for 1 h at room temperature, and then

counterstained with 4′,6-diamidino-2-phenylindole (Invitrogen). Specimens were observed using a ZEISS LSM 700 Laser Scanning Microscope (ZEISS, Oberkochen, Germany).

Antibody conjugation kits (ReadiLink; Bio-Rad) were used for double immunofluorescence labeling of polyQ and hnRNPA2B1. According to the manufacturer instructions, the monoclonal mouse anti-polyQ antibody was conjugated with iFlur555, and the monoclonal mouse anti-hnRNPA2B1 antibody was conjugated with iFlur492. Sections were incubated with the conjugated antibodies and then processed as described above.

RESULTS

All four cases showed polyQ-expansion (1C2)-positive INIs in numerous cortical neurons (Fig. 2A). INIs were also immunopositive for huntingtin (Fig. 2B), p62 (Fig. 2C) and FUS (Fig. 3B), occasionally positive for PCBP1(Fig.4B). In the cortical neurons of the normal control, immunohistochemistry for TDP43, hnRNP A2B1, hnRNP D (Fig.4) and FUS (Fig. 3A) revealed intense, nuclear immunoreactivity. Immunohistochemistry for PCBP1 (Fig. 4A) shows immunoreactivity of both the nuclei and cytoplasm.

Double immunostaining for polyQ-expansion (1C2) and huntingtin showed their colocalization in INIs of cortical neurons (Fig.2D-I). Almost all the 1C2-positive intranuclear inclusions colocalized with huntingtin and p62. In double immunofluorescence for polyQ-expansion (1C2) and FUS, cortical neurons without polyQ-expansion (1C2)-positive INIs retained nuclear FUS immunoreactivity. In all examined cases including the MJD case, double immunofluorescence for polyQ-

expansion (1C2) and FUS revealed colocalization in the INIs (Fig. 3C–H, Fig. 6A–C), while diffuse nuclear distribution of FUS disappeared. Some neurons with nuclear inclusions double-positive for 1C2 and FUS showed a preserved diffuse nuclear distribution of FUS (Fig. 3C–E), while other neurons were immunopositive for FUS restricted to the polyQ-expansion (1C2)-positive INI (Fig. 3F–H). In Case 1, colocalization of FUS was observed in 51% (51/100) of cortical neurons with polyQ-expansion (1C2)-positive INIs. Immunopositive staining for FUS restricted to the polyQ-expansion (1C2)-positive INIs was observed in 33% (33/100) of neurons. Eighteen percent (18/100) of neurons with nuclear inclusions double-positive for polyQ-expansion (1C2) and FUS showed a preserved diffuse nuclear distribution of FUS.

Colocalization of polyQ-expansion (1C2) and PCBP1 was observed in three HD cases and one MJD case (Fig. 4C–E, Fig. 6D–F, Table 3). However, the frequency of colocalization was very low (4%: 2/50) compared with that of 1C2 and FUS colocalization in case1. Cortical neurons without polyQ-expansion (1C2)-positive INIs retained diffuse nuclear immunoreactivity for PCBP1. When PCBP1 was colocalized with the INIs, the normal diffuse nuclear distribution of PCBP1 was always missing (Fig. 4C–E, Fig. 6D–F). Colocalization of 1C2 with hnRNPA1, hnRNPA2B1, hnRNPD, TARDBP and p-TDP43 was not observed (Fig. 6G-O, Fig. 7, Table 3). The normal nuclear localization of these proteins was preserved even in neurons with 1C2-positive nuclear inclusions. For TARDBP immunostaining, a skein-like inclusion was only observed in one case (case 1), and neurons with a skein-like inclusion lost nuclear TARDBP staining (data not shown).

DISCUSSION

The main findings of the present study were that FUS and PCBP1 were colocalized with huntingtin INIs, and that their normal nuclear distribution was frequently altered, in all HD cases. Abnormal deposition of TARDBP was observed only in one case, and there was no colocalization of the inclusions with other hnRNPs. Thus, FUS and PCBP1 are more closely associated with the pathogenesis of HD than for other hnRNPs, including TDP43.

Both hnRNPs (FUS, PCBP1, etc.) and TDP43 have RNA binding domain, and they play multiple roles especially in regulating gene expression including alternative splicing[11, 12]. TDP43 and other hnRNPs are also known to be associated with several neurodegenerative pathologies. Neumann et al. reported the absence of nuclear hnRNPs in neurons with TDP43-positive inclusions in affected brain regions in FTLD cases with TDP43 proteinopathy[13]. Nuclear hnRNPA1 immunoreactivity was lost in the nucleus of ALS motor neurons[14]. Recent studies demonstrate that some hnRNPs expression level modulates TDP43 function in human neuronal cells[15, 16]. Thus, several hnRNP family interact with TDP43 and play an important role in neurodegenerative diseases including HD.

In all examined cases, FUS was frequently colocalized with the INIs in neurons. FUS is a ribonucleotide protein associated with transcription, processing, and regulation of translation. FUS is also involved in a diverse range of diseases including familial ALS and frontotemporal lobar degeneration[17, 18]. In addition, some studies revealed that FUS binds to INIs in the human brains from patients with polyglutamine diseases [19, 20].

For example, FUS was shown to be a major components of nuclear polyQ aggregate-interacting proteins in an *in vitro* HD model, and interacted with neuronal INIs in HD

model mice[8, 19, 21]. Further, recent studies demonstrated that huntingtin forms a complex with several RNA binding proteins and translation factors in the process of aggregation,[8] and forms a complex with its own mRNA[22]. Kino et al. also demonstrated that HD model mice with heterozygous FUS deletion have a shortened life span compared with HD model mice without the deletion[21]. In that study, degradation of FUS worsened the phenotypes of HD. Further, the authors suggested that the exploitation of FUS by the huntingtin INI causes functional impairment of FUS. Thus, an altered distribution of FUS is likely associated with progression of neurodegeneration in HD. FUS has also been reported to form fibrils by the self-assembly and phase separation of its low-complexity domains[23]. Thus, we suggest that FUS itself is important for the formation and growth of INIs. By contrast, huntingtin inclusions were reported to prevent neuronal apoptosis, but induce slow cell death by necrosis[8]. Further, Tauffenberger et al. revealed that FUS reduced polyglutamine toxicity in HD models[24]. Thus, the potential roles of FUS in HD pathology remains to be clarified.

In the present study, we also found colocalization of PCBP1 with the INI in three cases. Normal nuclear staining of PCBP1 was reduced in neurons with INIs. PCBP1 (also termed hnRNPE1) participates in mRNA binding and stabilization, translational activation or silencing[25], and iron chaperone function[26]. There are limited reports of a role of PCBP1 in neurodegenerative diseases. For example, PCBP1 was reported to interact with the HSPB1-P182L mutant, as well as other genes causing hereditary neurological disorders[27]. However, to our knowledge there are no reports suggesting a role of PCBP1 in HD. Nevertheless, PCBP1, an iron chaperon for ferritin, may be involved in ferroptosis in HD. Ferroptosis is a new form of cell death caused by intracellular iron and reactive oxygen species (ROS) accumulation[28 29], and ferroptosis

is associated with HD pathophysiology.[30] Further, in the present study, we observed colocalization of PCBP1 with INIs in HD patients. Although this may relate to secondary aggregation, the normal distribution of PCBP1 in the nucleus was also disturbed, suggesting that PCBP1 is involved in HD pathogenesis. Other hnRNPs including hnRNPA1, hnRNPA2, hnRNPD, and TARDBP showed no colocalization with the INIs and no alterations in their localization, further suggesting specific roles of FUS and PCBP1 in HD pathogenesis.

We have extended our observation to the other polyglutamine disease: MJD. MJD is caused by a heterozygous (CAG)n trinucleotide repeat expansion encoding glutamine repeats in the ataxin-3 gene. Normal individuals have up to 44 glutamine repeats, and MJD patients have between 52 and 86 glutamine repeats[31]. As the result, INIs of MJD also showed similar immunostaining profiles as to FUS and PCBP1. We considered that the common findings are important in expanded CAG repeat diseases.

HD is caused by expansions of CAG repeats encoding polyglutamine, and is one of the triplet repeat diseases. In myotonic dystrophy caused by CUG-repeat expansions, accumulation of the RNA-binding protein muscleblind-like 1 (MBNL1) in INIs containing mutant repeat RNA leads to loss of MBNL1 function[32]. Thus, functional impairment of MBNL1 causes several disorders in MD patients[32]. As such, the abnormal distribution and loss of RNA binding proteins is thought to cause their dysfunction. Based on our findings, we propose that the aggregation and maldistribution of hnRNPs, especially FUS, may be an important factor for neuronal degeneration in HD. Our data support the involvement of abnormal RNA metabolism in the pathogenesis of HD. Further functional studies are required to elucidate the specific role of hnRNPs in HD pathogenesis.

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DISCLOSURE

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Tables

Table 1 Demographic characteristics of human cases of the diseases

Case number	1 (HD)	2 (HD)	3 (HD)	4 (HD)	5 (MJD)
Sex	M	M	F	F	M
Age at death, years	60	73	65	52	66
Disease duration, years	21	11	26	17	25
Family history	+	+	+	+	+
Expanded CAG repeat	+	+	not examined	not examined	+

F, female; HD, Huntington's disease; M, male; MJD, Machado-Joseph disease

Table 2 Demographic characteristics of human control cases

Case number	1	2	3
Sex	F	M	F
Age at death, years	76	66	67
Neuropathological findings	(No significant finding)	Old lacunar infarction	(No significant finding)

F, female; M, male

Table 3 Heterogeneous nuclear ribonucleoproteins (hnRNPs) colocalized with 1C2-positive nuclear inclusions

Case number	1 (HD)	2 (HD)	3 (HD)	4 (HD)	5 (MJD)
FUS	+	+	+	+	+
PCBP1	+	+	+	−	+
hnRNP A1	−	−	−	−	−
hnRNP A2B1	−	−	−	−	−
hnRNP D	−	−	−	−	−
TDP43	−	−	−	−	−

FUS, Fused in sarcoma; HD, Huntington's disease; MJD, Machado-Joseph disease; PCBP1, poly(rC)-binding protein 1; TARDBP, trans-activation response DNA-binding protein of 43.

Figure legends

Fig. 1 Western blotting with the anti-PCBP1 polyclonal antibodies (ab74794 (Abcam)) to confirm the specificity. For this purpose, we prepare cell extracts from HeLa S3, PCBP1 and PCBP2 knock out cell lines [33] using SDS sample buffer (60 mM Tris·HCl [pH 6.8], 2% SDS and 10% glycerol). Recombinant His-PCBP1 protein is expressed in E. coli and purified using His affinity column. The cell extracts (containing 7.5 μg protein each) and purified proteins (containing 3.75 ng protein each) are applied to SDS-PAGE and examine for specificity by Western blotting using the PCBP1 antibody.

Fig. 2 Anti-polyQ-expansion(1C2) antibody-labelled (A), anti-huntingtin antibody-labelled (B), and anti-p62 antibody-labelled (C) neuronal intranuclear inclusions(INI) in the frontal cortex of HD patients. Double immunofluorescence for polyQ-expansion and huntingtin showing colocalization of 1C2-positive nuclear inclusions and p62 (D-F). Double immunofluorescence for polyQ-expansion and huntingtin showing colocalization of 1C2-positive nuclear inclusions and huntingtin (G-I). Scale bars = 20 μm.

Fig. 3 Expression of FUS in cortex neuron of the control case (A). FUS antibody-labelled (B) neuronal INIs(the arrow pointed) and loss of nuclear staining outside the INI in the frontal cortex of HD patients.

The distribution of FUS is restricted to the 1C2-positive nuclear inclusions, and its normal nuclear distribution is lost (C-E). In other neurons, the nuclear distribution of FUS is only partly lost (F-H). Scale bars = 20 μm.

Fig. 4 Expression of poly(rC)-binding protein 1(PCBP1) in cortex neuron of the control case (A). PCBP1 antibody labelled neuronal INIs (arrow) and loss of nuclear staining outside the INI in the frontal cortex of HD patients(B).
Double immunofluorescence for polyQ-expansion and PCBP1 in cortical neurons of HD cases. Double immunofluorescence microscopy showing colocalization of 1C2-positive nuclear inclusions and PCBP1 (C-E). Scale bars = 20 μm.

Fig. 5 Immunohistochemistry for hnRNP A1(A), hnRNPA2(B), hnRNP D(C) and TDP43(D) in cortex neuron of the control cases. Scale bar = 20 μm.

Fig. 6 Double immunofluorescence for polyQ-expansions, FUS, PCBP1, hnRNP A1, hnRNPD and TDP43 in pontine neurons of the MJD case.
FUS and PCBP1 are colocalized with 1C2-positive INIs (A-F). hnRNPA1, hnRNPD and TDP43 are not colocalized with 1C2 (G-O).
Scale bar = 20 μm.

Fig. 7 Double immunofluorescence for polyQ-expansions, heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), hnRNPA2, hnRNPD, trans-activation response DNA-binding protein of 43 (TDP43) and phosphorylated TDP43 in cortical neurons of HD cases. hnRNPA1, hnRNPA2, hnRNPD, TDP43 and p-TDP43 were not colocalized

with 1C2 and they showed normal distribution in neurons with 1C2-positive nuclear inclusions (A-O). Scale bar = 20 μm.

REFERENCES

1 Group THsDCR. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993; **72**: 971-83.

2 Herndon ES, Hladik CL, Shang P, Burns DK, Raisanen J, White CL, 3rd. Neuroanatomic profile of polyglutamine immunoreactivity in Huntington disease brains. *J Neuropathol Exp Neurol.* 2009; **68**: 250-61.

3 DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP*, et al.* Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*. 1997; **277**: 1990-3.

4 Chan HY. RNA-mediated pathogenic mechanisms in polyglutamine diseases and amyotrophic lateral sclerosis. *Front Cell Neurosci.* 2014; **8**: 431.

5 Butti Z, Patten SA. RNA Dysregulation in Amyotrophic Lateral Sclerosis. *Fronti Genet*. 2018; **9**: 712.

6 Tada M, Coon EA, Osmand AP, Kirby PA, Martin W, Wieler M*, et al.* Coexistence of Huntington's disease and amyotrophic lateral sclerosis: a clinicopathologic study. *Acta neuropathol*. 2012; **124**: 749-60.

7 Schwab C, Arai T, Hasegawa M, Yu S, McGeer PL. Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of

Huntington disease. *J Neuropathol Exp Neurol.* 2008; **67**: 1159-65.

8 Ramdzan YM, Trubetskov MM, Ormsby AR, Newcombe EA, Sui X, Tobin MJ*, et al.* Huntingtin Inclusions Trigger Cellular Quiescence, Deactivate Apoptosis, and Lead to Delayed Necrosis. *Cell Rep*. 2017; **19**: 919-27.

9 Matsuoka T, Fujii N, Kondo A, Iwaki A, Hokonohara T, Honda H*, et al.* An autopsied case of sporadic adult-onset amyotrophic lateral sclerosis with FUS-positive basophilic inclusions. *Neuropathology*. 2011; **31**: 71-6.

10 Tateishi T, Hokonohara T, Yamasaki R, Miura S, Kikuchi H, Iwaki A*, et al.* Multiple system degeneration with basophilic inclusions in Japanese ALS patients with FUS mutation. *Acta neuropathologica*. 2010; **119**: 355-64.

11 Geuens T, Bouhy D, Timmerman V. The hnRNP family: insights into their role in health and disease. *Hum Genet.* 2016; **135**: 851-67.

12 Sephton CF, Cenik B, Cenik BK, Herz J, Yu G. TDP-43 in central nervous system development and function: clues to TDP-43-associated neurodegeneration. *Biol Chem.* 2012; **393**: 589-94.

13 Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT*, et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006; **314**: 130-3.

14 Honda H, Hamasaki H, Wakamiya T, Koyama S, Suzuki SO, Fujii N*, et al.* Loss of hnRNPA1 in ALS spinal cord motor neurons with TDP-43-positive inclusions. *Neuropathology*. 2015; **35**: 37-43.

15 D'Ambrogio A, Buratti E, Stuani C, Guarnaccia C, Romano M, Ayala YM*, et al.* Functional mapping of the interaction between TDP-43 and hnRNP A2 in vivo. *Nucleic Acids Res.* 2009; **37**: 4116-26.

16 Appocher C, Mohagheghi F, Cappelli S, Stuani C, Romano M, Feiguin F*, et al.* Major hnRNP proteins act as general TDP-43 functional modifiers both in Drosophila and human neuronal cells. *Nucleic Acids Res.* 2017; **45**: 8026-45.

17 Kwiatkowski TJ, Jr., Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C*, et al.* Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*. 2009; **323**: 1205-8.

18 Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J*, et al.* Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*. 2009; **323**: 1208-11.

19 Doi H, Koyano S, Suzuki Y, Nukina N, Kuroiwa Y. The RNA-binding protein FUS/TLS is a common aggregate-interacting protein in polyglutamine diseases. *Neurosci Res.* 2010; **66**: 131-3.

20 Mori F, Toyoshima Y, Tanji K, Kakita A, Takahashi H, Wakabayashi K. FUS colocalizes with polyglutamine, but not with TDP-43 in neuronal intranuclear inclusions in spinocerebellar ataxia type 2. *Neuropathol Appl Neurobiol.* 2014; **40**: 351-5.

21 Kino Y, Washizu C, Kurosawa M, Yamada M, Doi H, Takumi T*, et al.* FUS/TLS acts as an aggregation-dependent modifier of polyglutamine disease model mice. *Sci Rep.* 2016; **6**: 35236.

22 Culver BP, DeClercq J, Dolgalev I, Yu MS, Ma B, Heguy A*, et al.* Huntington's Disease Protein Huntingtin Associates with its own mRNA. *J Huntingtons Dis*. 2016; **5**: 39-51.

23 Murray DT, Kato M, Lin Y, Thurber KR, Hung I, McKnight SL*, et al.* Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains. *Cell*. 2017; **171**: 615-27 e16.

24 Tauffenberger A, Chitramuthu BP, Bateman A, Bennett HP, Parker JA. Reduction of polyglutamine toxicity by TDP-43, FUS and progranulin in Huntington's disease models. *Hum Mol Genet*. 2013; **22**: 782-94.

25 Chaudhury A, Chander P, Howe PH. Heterogeneous nuclear ribonucleoproteins (hnRNPs) in cellular processes: Focus on hnRNP E1's multifunctional regulatory roles. *RNA*. 2010; **16**: 1449-62.

26 Shi H, Bencze KZ, Stemmler TL, Philpott CC. A cytosolic iron chaperone that delivers iron to ferritin. *Science (New York, NY)*. 2008; **320**: 1207-10.

27 Geuens T, De Winter V, Rajan N, Achsel T, Mateiu L, Almeida-Souza L*, et al.* Mutant HSPB1 causes loss of translational repression by binding to PCBP1, an RNA binding protein with a possible role in neurodegenerative disease. *Acta Neuropathol Commun*. 2017; **5**: 5.

28 Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE*, et al.* Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012; **149**: 1060-72.

29 Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X*, et al.* Ferroptosis: process and function. *Cell Death Differ.* 2016; **23**: 369-79.

30 Mi Y, Gao X, Xu H, Cui Y, Zhang Y, Gou X. The Emerging Roles of Ferroptosis in Huntington's Disease. *Neuromolecular Med.* 2019.

31 Todd PK, Paulson HL. RNA-mediated neurodegeneration in repeat expansion disorders. *Ann Neurol.* 2010; **67**: 291-300.

32 Kino Y, Washizu C, Kurosawa M, Oma Y, Hattori N, Ishiura S*, et al.* Nuclear localization of MBNL1: splicing-mediated autoregulation and repression of repeat-derived aberrant proteins. *Hum Mol Genet*. 2015; **24**: 740-56.

33 Ishii T, Hayakawa H, Igawa T, Sekiguchi T, Sekiguchi M. Specific binding of PCBP1 to heavily oxidized RNA to induce cell death. *Proc Natl Acad Sci U S A.* 2018; **115**: 6715-20.

Fig. 1

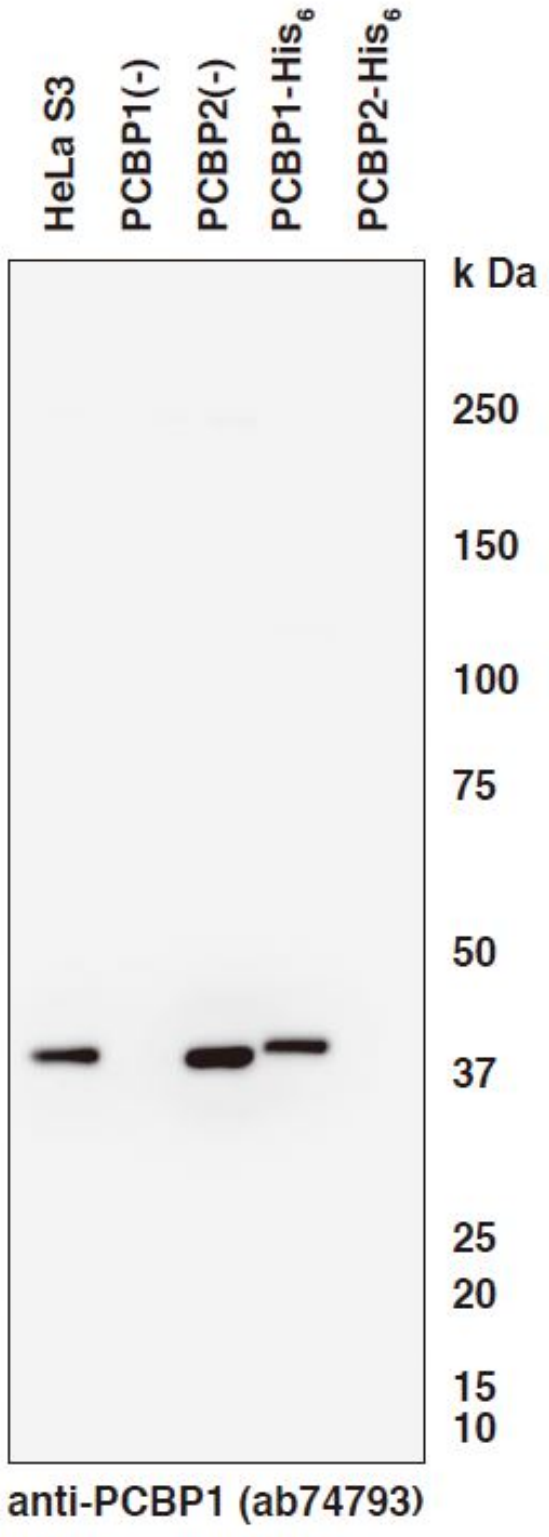

HeLa S3
PCBP1(-)
PCBP2(-)
PCBP1-His6
PCBP2-His6
k Da
250
150
100
75
50
37
25
20
15
10
anti-PCBP1 (ab74793)

Fig. 2

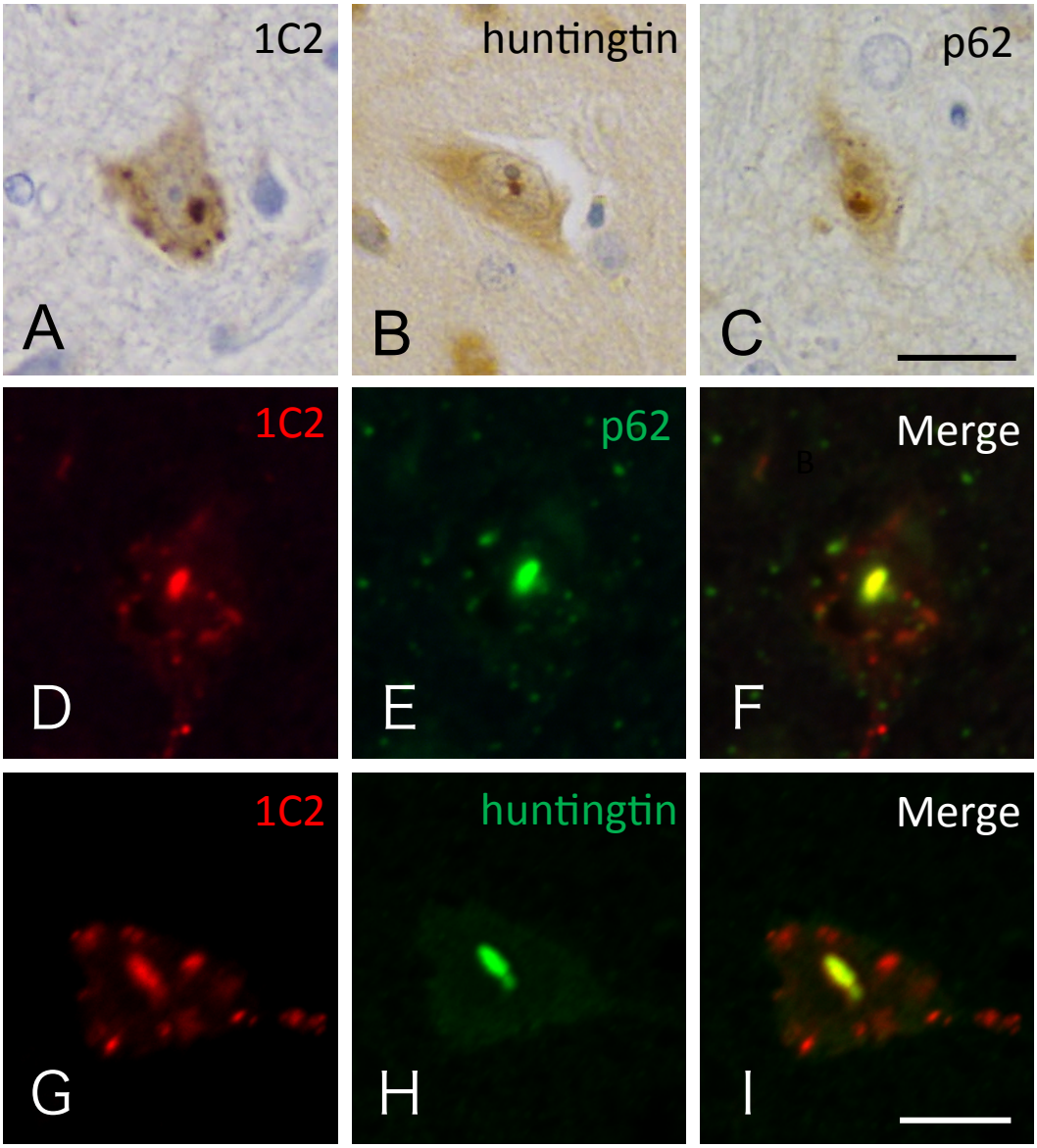

1C2
huntingtin
p62
A
B
C
1C2
p62
Merge
D
E
F
1C2
huntingtin
Merge
G
H
I

Fig. 3

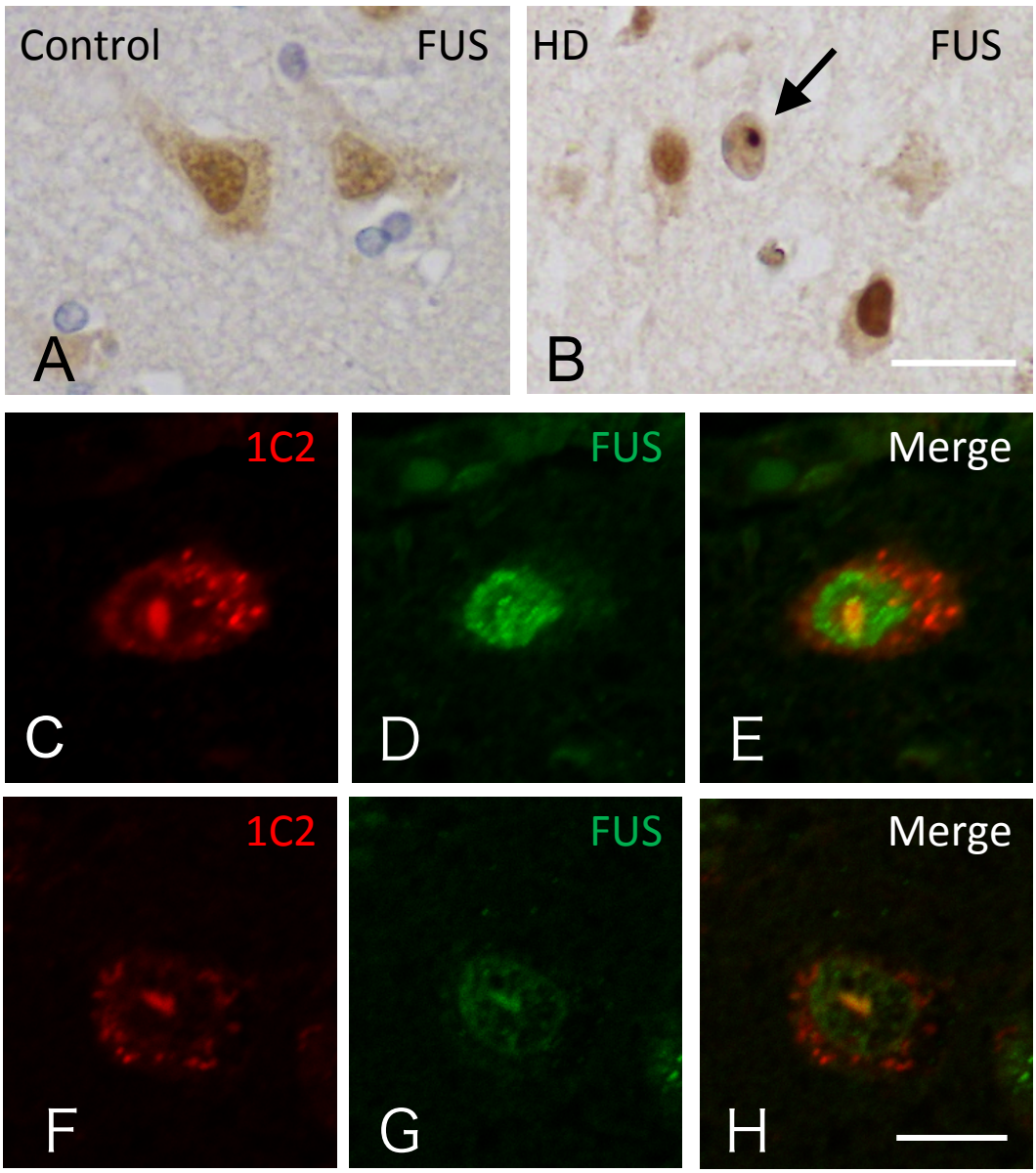


Fig. 4

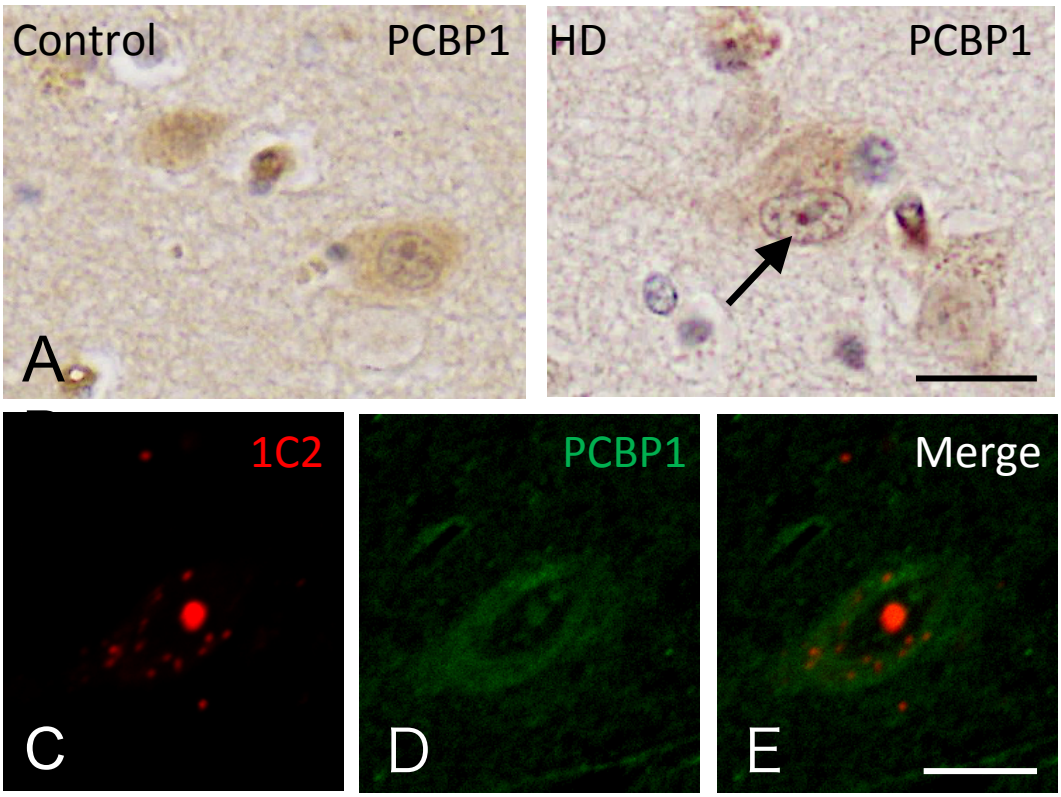
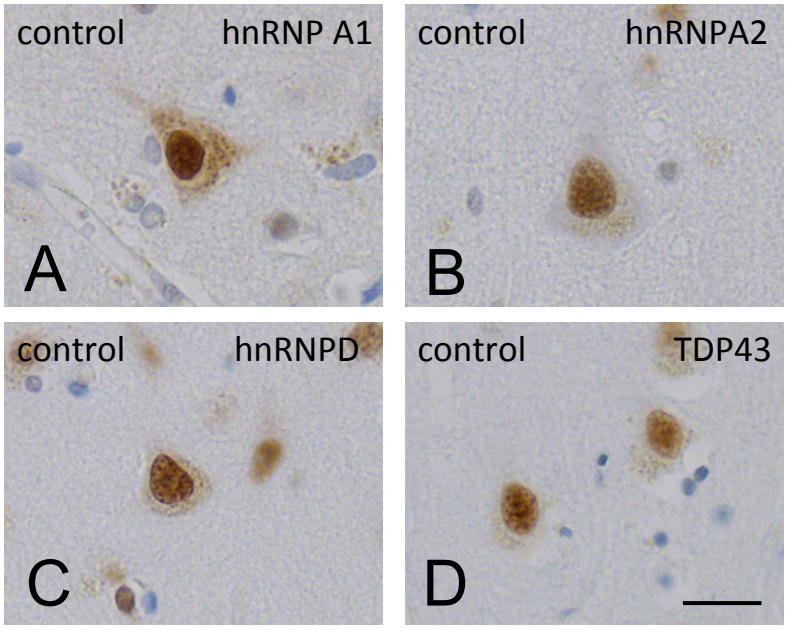


Fig. 5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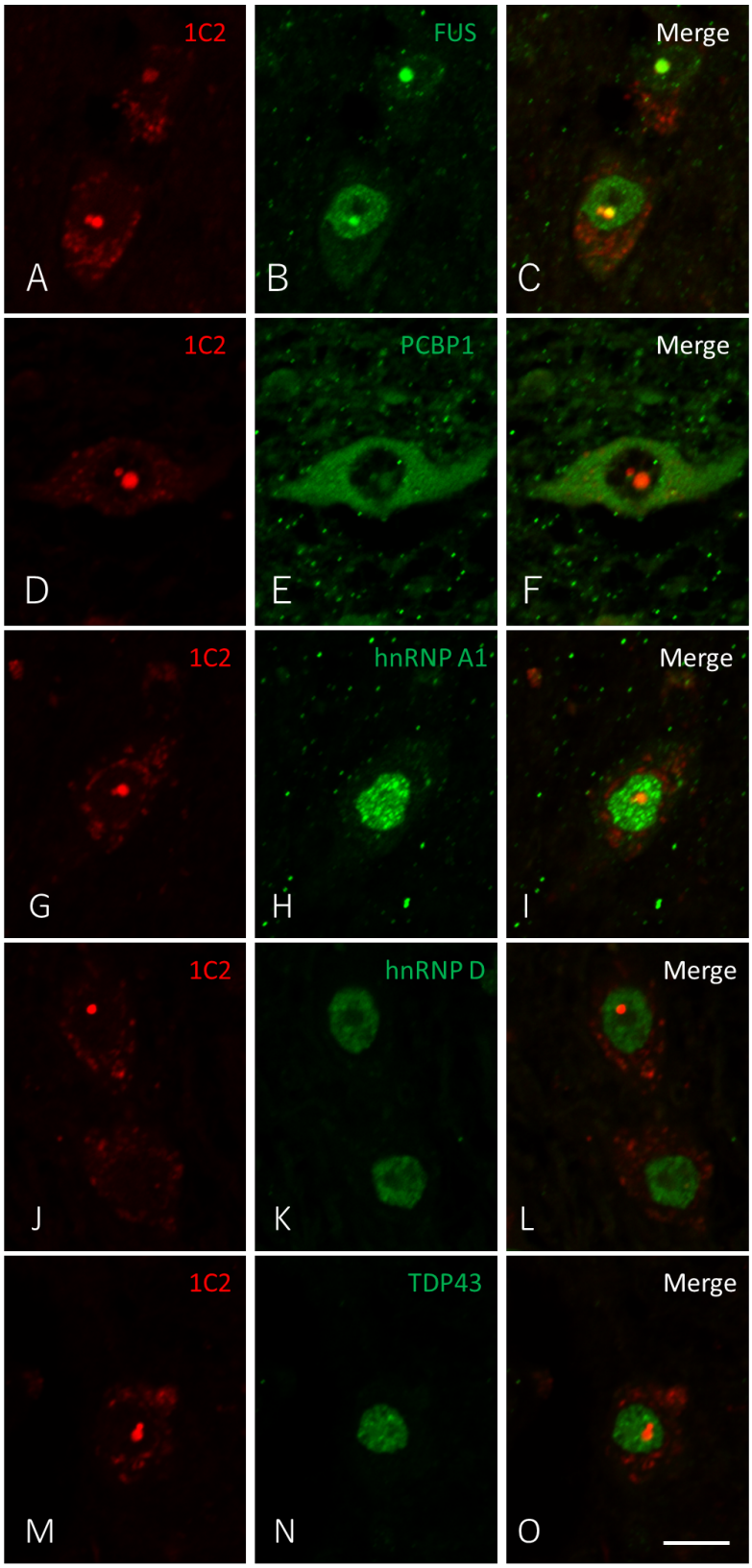
A
control
hnRNPA2
B
control
hnRNPD
C
control
TDP43
D

Fig. 6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FUS
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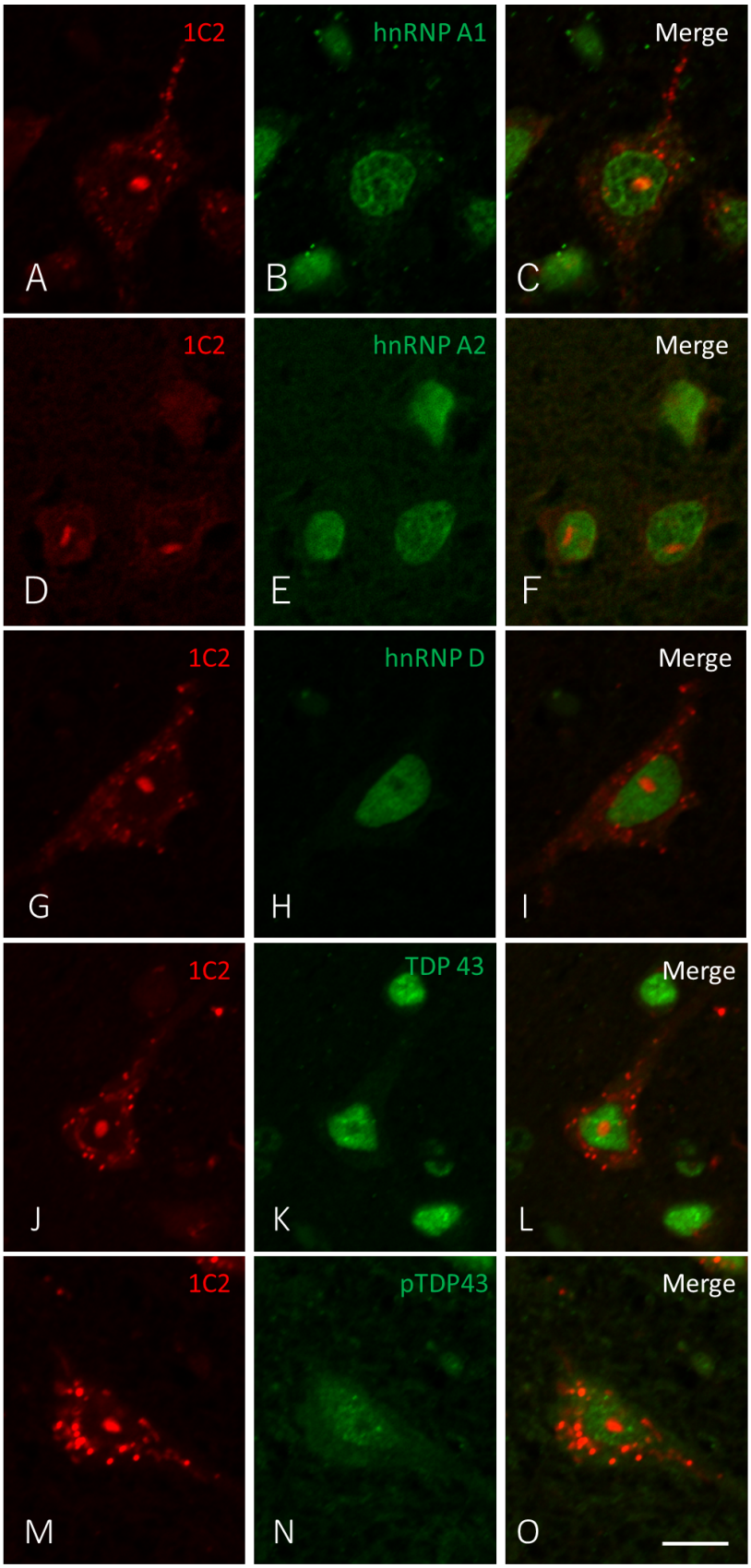
B
C
1C2
PCBP1
Merge
D
E
F
1C2
hnRNP A1
Merge
G
H
I
1C2
hnRNP D
Merge
J
K
L
1C2
TDP43
Merge
M
N
O

Fig. 7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Merge
A
B
C
1C2
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Merge
D
E
F
1C2
hnRNP D
Merge
G
H
I
1C2
TDP 43
Merge
J
K
L
1C2
pTDP43
Merge
M
N
O