Figure e-1. Repeat-primed PCR suggested the presence of a *C9orf72* repeat expansion (>50 repeats) in all tissues of 9686.

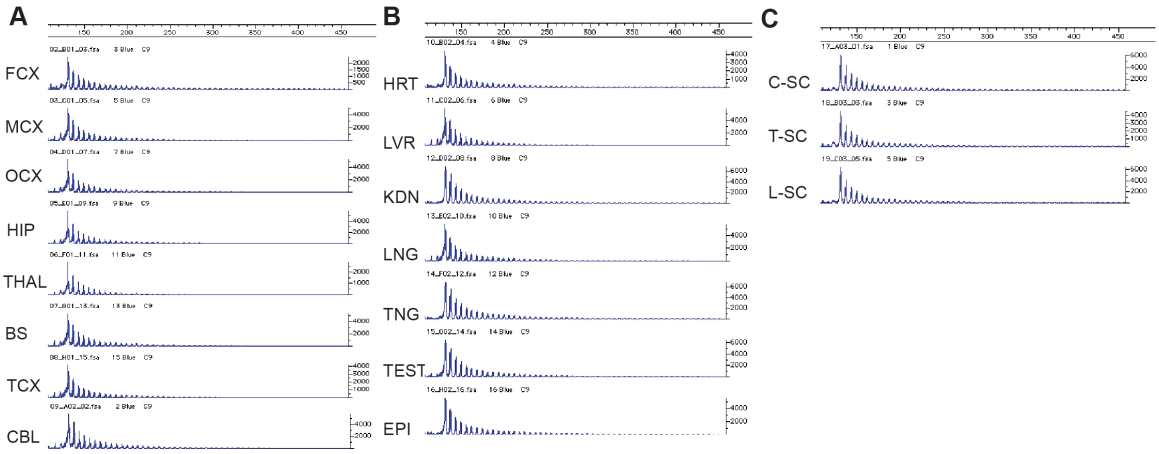

Figure e-2. Blood *C9orf72* gene expression of small expansion carrier (9686) is upregulated compared to his offspring with a large expansion (9548) and wild-type alleles (9697) (**p<0.01). Subject 9548 has less *C9orf72* expression than 9697 in blood (*p<0.05). Data are mean ± SEM.

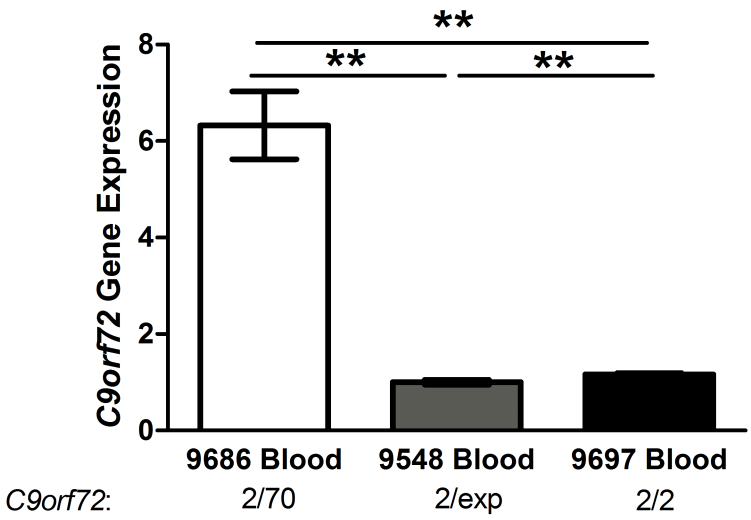

Figure e-3. Numbers of foci per nuclei are similar between 9686 and 9548. The number of RNA foci per nuclei were counted for sense and antisense foci, and expressed as a percentage of total foci, in the Purkinje cell layer of the cerebellum (CBL-PKC; A, E), frontal cortex (FCX; B, F), motor cortex (MCX; C, G) and hippocampus (HIP; D, H). A similar pattern of expression was seen for both 9686 and 9548. n=3. Data are mean ± SEM. Notably, the motor cortex of 9548 (daughter) has significantly less nuclei containing one RNA foci but more nuclei containing two RNA foci than 9686 (father); however, the number of nuclei containing >2 RNA foci per nuclei is similar for both 9686 and 9548. If RNA foci were driving toxicity then we might expect 9548 to have more foci per cell as well as a greater total number of foci, which is not the case.

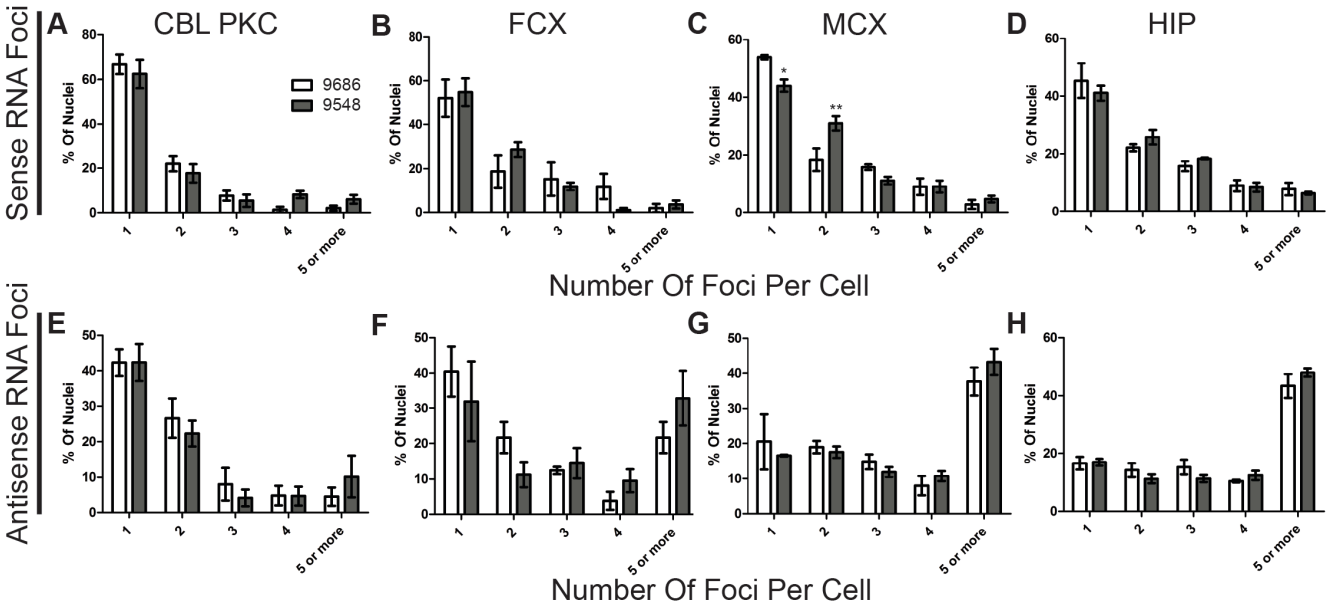

Figure e-4. Similar levels of RNA-foci positive motor neurons in the spinal cords of 9686 and 9548. Representative images of sense (A) and antisense (B) RNA foci in the spinal cord. Quantification of sense (C) and antisense RNA foci (D) did not reveal a significant difference between 9686 and 9548 in cervical or lumbar spinal cord. n=3. Data are mean ± SEM.

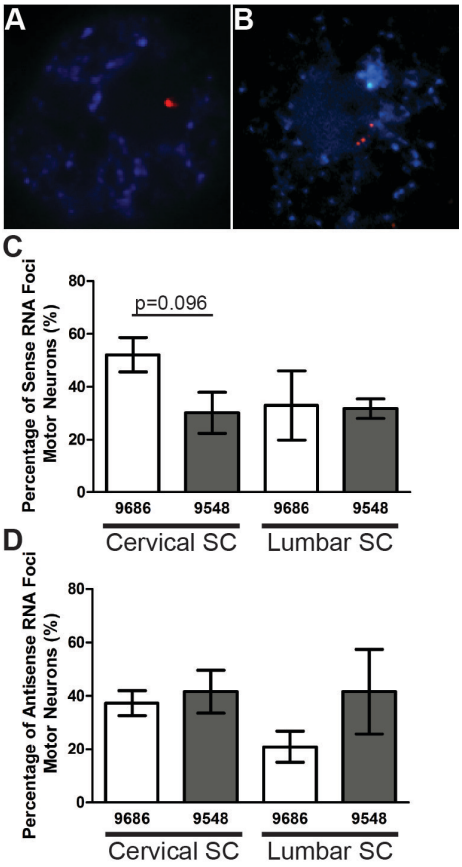

Figure e-5: DPR burden was increased in the cerebellum of 9686 but similar to 9548 in other brain regions.

Representative images of 9686 (left panels) and 9548 (right panels) cerebellum stained with GA (A, B), GP (C, D) and GR (E, F) antibodies. All five DPRs were also detected by immunohistochemistry in the cerebellum, frontal cortex, motor cortex and hippocampus, with representative images of GA (G), GP (H), GR (I), PA (J) and PR (K) inclusions. Quantification of DPR burden in the granule cell layer of the cerebellum (CBL-GC) revealed significantly more inclusions per field in 9686 vs. 9548: GA (205.8±11.62 vs. 30.33±4.13; $^{***}p<0.001$), GP (49.60±6.64 vs. 30.50±2.40; $^{**}p<0.01$) and GR (7.48±0.66 vs. 1.00±0.22; $^{***}p<0.001$). DPR burden was similar in frontal cortex (FCX; M) and hippocampus (HIP; N). Although the number of GA-inclusions was significantly lower in motor cortex (MCX; O) of 9686 vs. 9548 (3.70±1.77 vs. 5.53±2.36; $^{***}p<0.001$), this was not found for other DPRs. Data are mean ± SEM. Notably, there are significantly more poly-GA DPR inclusions in the motor cortex of 9548 (daughter) compared to 9686 (unaffected father). However, there was a normal population of Betz cells in the primary motor cortex of 9548, and there were no significant differences in other DPRs in this region. The role of the GA DPR in disease pathogenesis is unclear. Poly-GA are often found to be less toxic than the arginine-rich DPRs (GR and PR) [4]; and autopsy reports have failed to find any correlation between different types of DPRs (including poly-GA) and neurodegeneration [5,6].

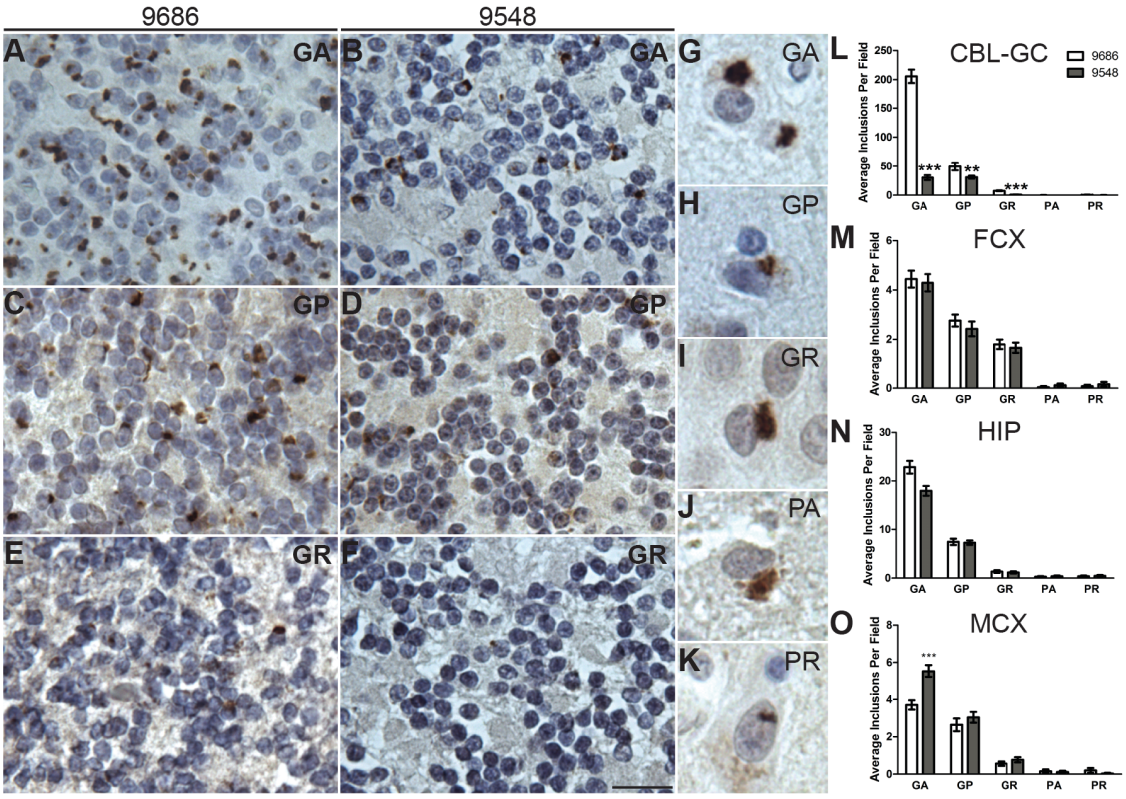

Figure e-6. The diagram showed the potential pathogenic mechanism of normal, large expanded or mosaic C9orf72 repeats in CNS tissues. The C9orf72 large expansions produced RNA foci and DPRs in CNS tissues, as well as C9orf72 haploinsufficiency that cause TDP-43 pathology and the development of ALS/FTLD. The mosaic C9orf72 repeat expansions in 9686 still produce RNA foci and DPRs, but the level of *C9orf72* haploinsufficiency was mitigated, which prevents TDP-43 pathology and neurodegenerative disease.

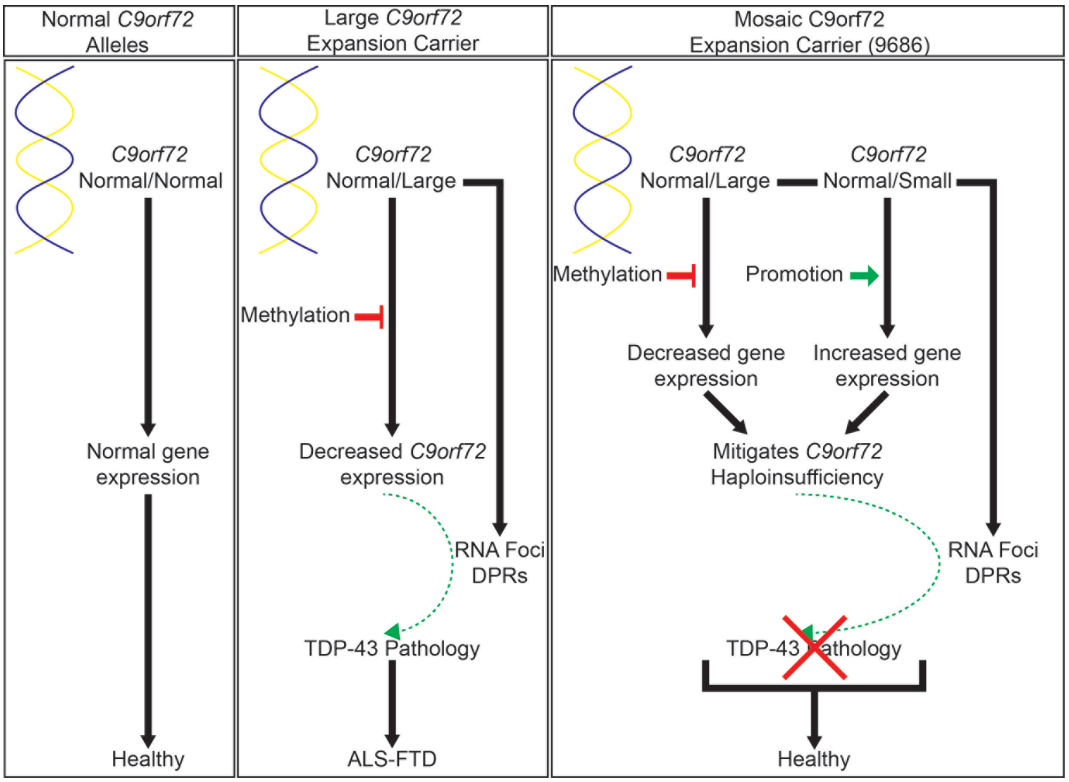

Table e-1. The genotyping results of known *C9orf72* genetic modifiers (*TMEM106B* rs1990622, T*MEM106B* rs3173615 and *ATXN2* CAG repeats).

Sample	TMEM106B rs1990622	TMEM106B rs3173615	ATXN2 CAG repeats
9548	AA	CC	22/22
9686	AG	CG	22/22

Note: Intermediate *ATXN2* alleles (27–33 CAG-repeats) were reported as modifiers in *C9orf72* carriers, rendering susceptibility to ALS [7]. The homozygosity for the minor allele (G) of rs3173615 in *TMEM106B* was reported to protect against developing FTD in *C9orf72* patients [8]. The major allele (A) of rs1990622 (conferring risk for developing FTLD) was associated to later age of onset and age of death in *C9orf72* patients [9]. These results were not able to explain the phenotype difference between 9548 and 9686.