CRISPR-Cas9 targeted deletion of the *C9orf72* repeat expansion mutation corrects cellular phenotypes in patient-derived iPS cells

Mochtar Pribadi[1], Zhongan Yang[1], Tanya S. Kim[1], Elliot W. Swartz[1], Alden Y. Huang[1], Jason A. Chen[1], Deepika Dokuru[1], Jaeyun Baek[1], Fuying Gao[1], Andrea T. Fua[1], Kevin Wojta[1], Qing Wang[1], Anna Karydas[2], Jamie Fong[2], Ed Lezcano[3], Stephanie Ng[3], Farid F. Chehab[3], Harry V. Vinters[4,5], Bruce L. Miller[2], Giovanni Coppola[1,4]*

Supplemental Figures

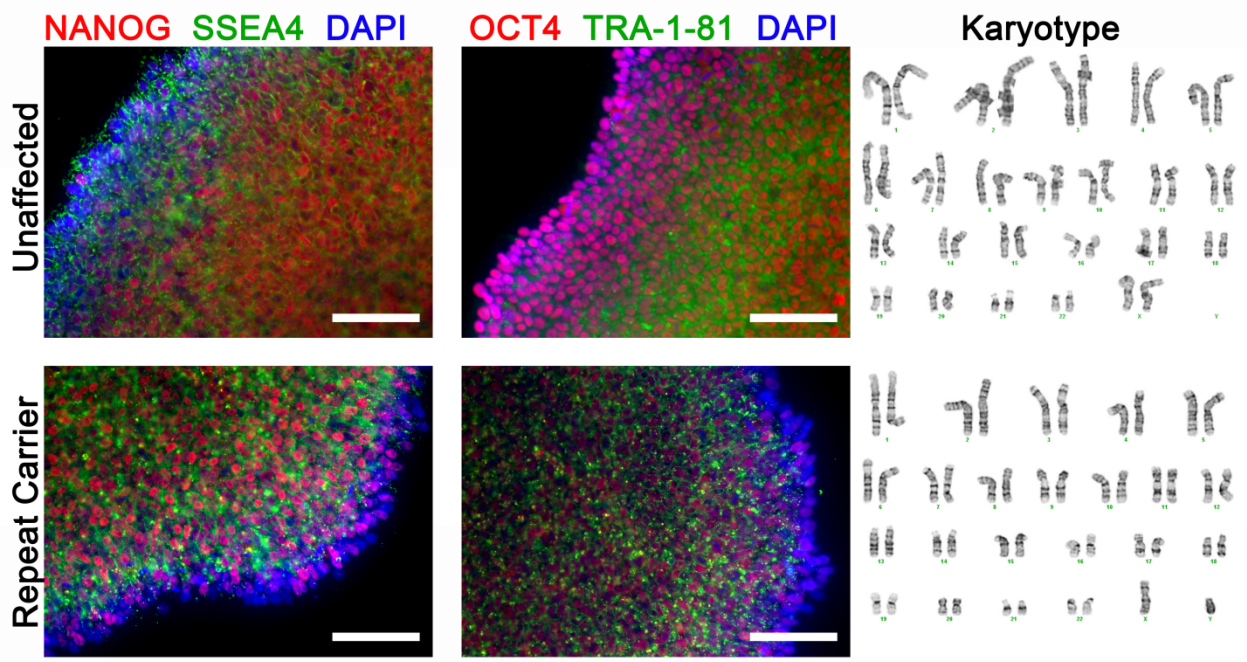

Figure S1. Normal iPS cell generation from *C9orf72* expansion carriers. Human iPS cells from *C9orf72* repeat expansion carriers and controls express pluripotency markers SSEA and TRA 1-81 (green), and the nuclear markers NANOG and OCT4 (red). Scale bar: 100um. Karyotype analyses (right panel) show no abnormalities.

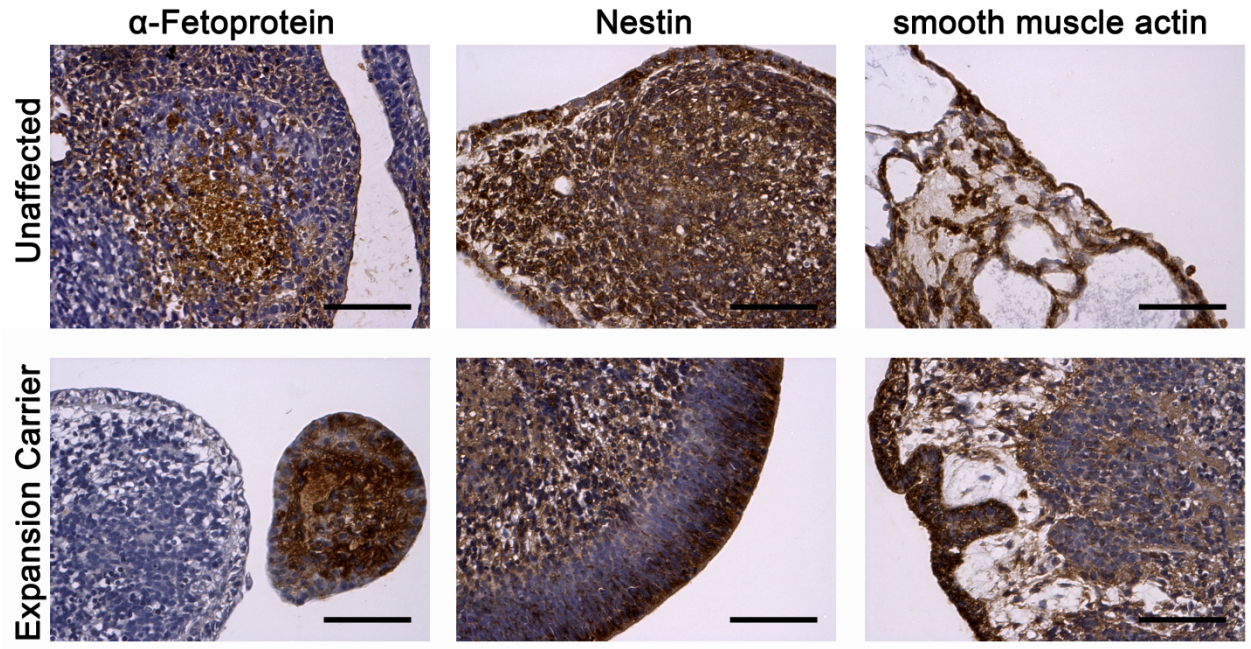

Figure S2. iPS cells are pluripotent. *In vitro* embryoid body formation of iPS cell demonstrates the capability to differentiate to the three germ layers. Embryoid bodies express Alpha-Fetoprotein, Nestin, and Smooth Muscle Actin, which are markers for the endoderm, ectoderm, and mesoderm, respectively. Scale bar: 100um.

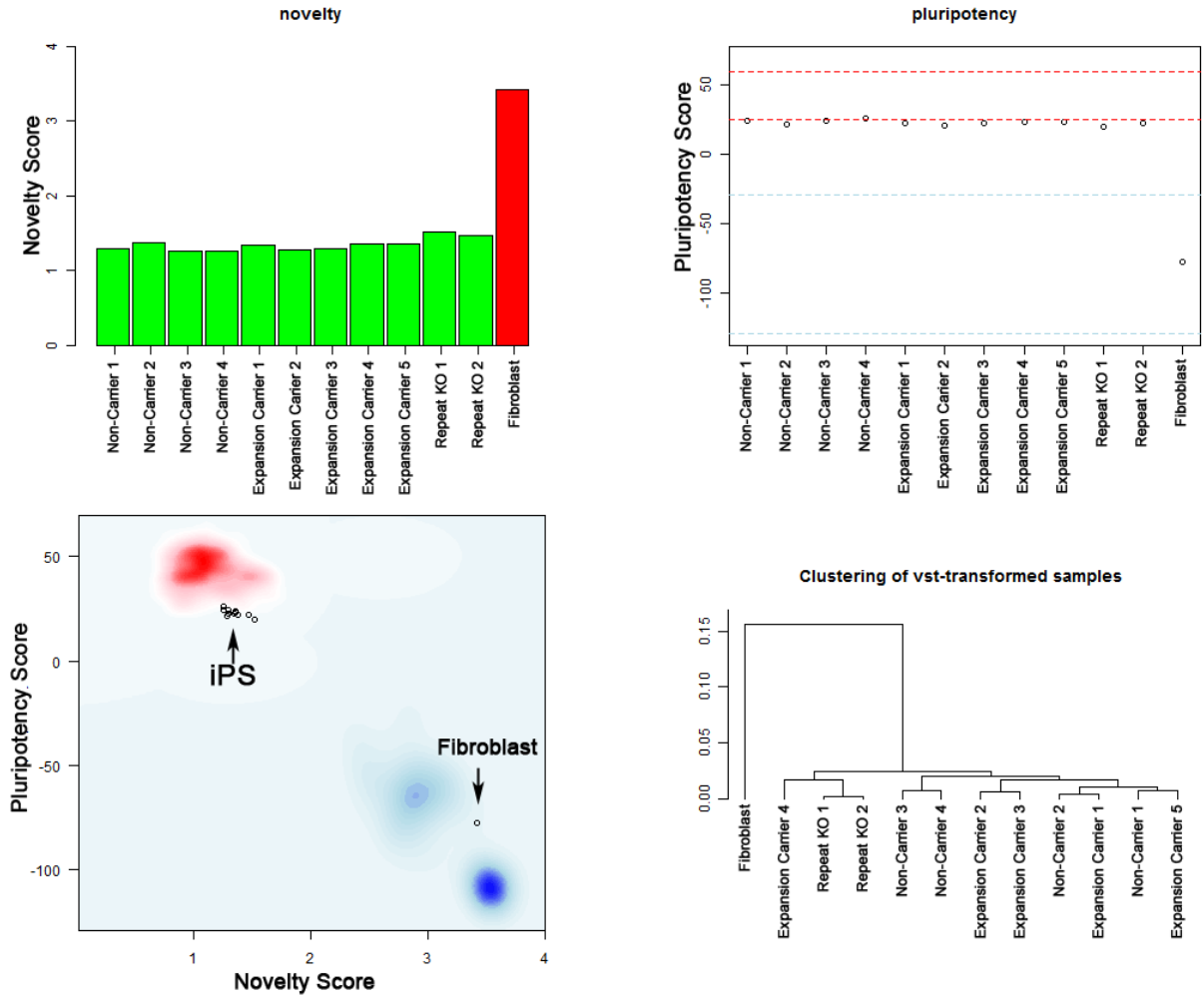

Figure S3. All iPS cells pass the Pluritest assay. Representative web-based Pluritest assay showing iPS cells presenting a low novelty score (a measure of variation), a high pluripotency score (which is based on well-characterized human pluripotent cells), and clustering separately from a fibroblast cell line (bottom-right panel).

.

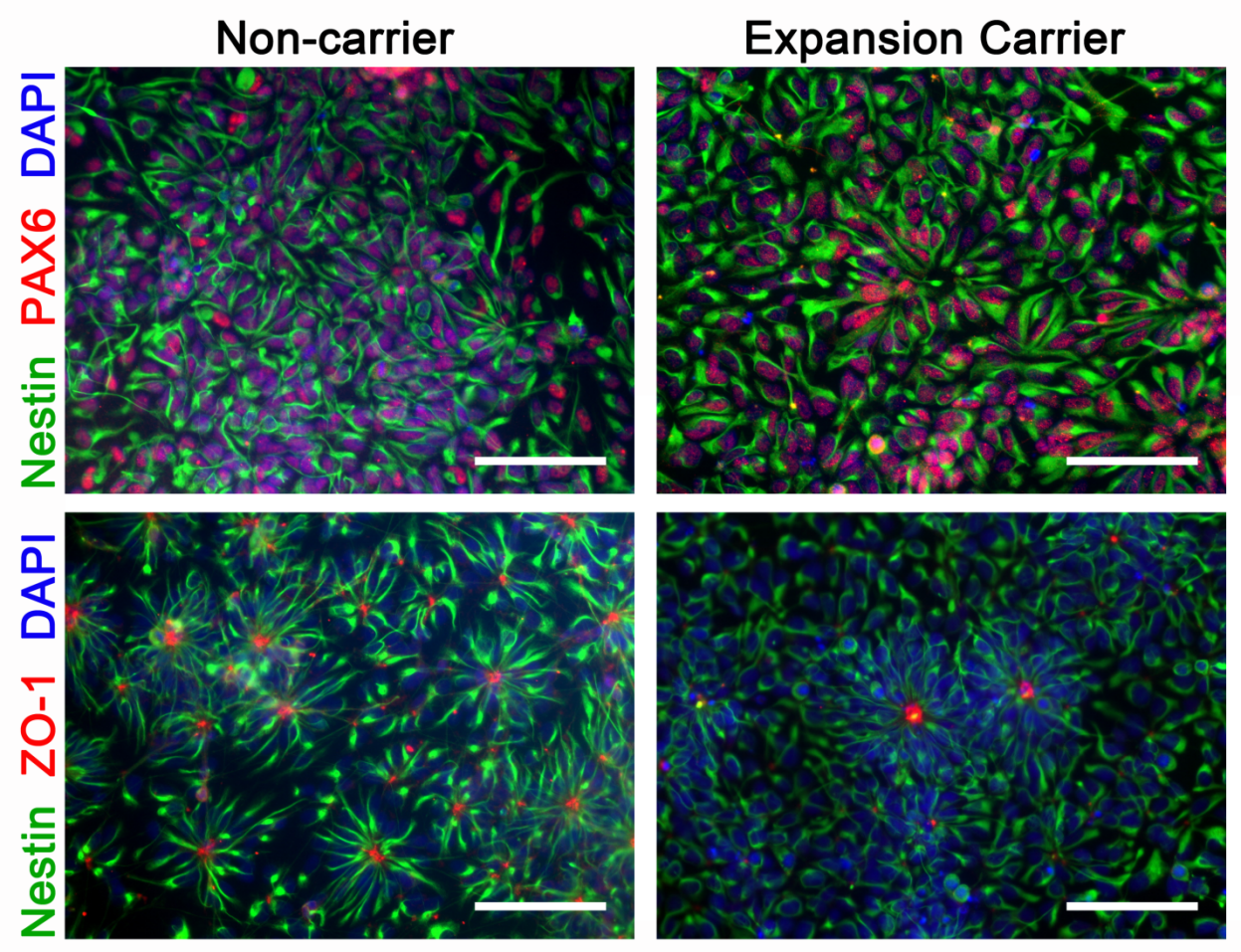

Figure S4. Efficient generation of neural progenitor cells from iPS generated from C9orf72 expansion carriers. Directed neuronal differentiation from iPS cells yields neural progenitor cells positive for Nestin (green) and PAX6 (red). Rosettes were visualized with ZO-1 (red), a marker for tight junctions. Scale bar: 100um.

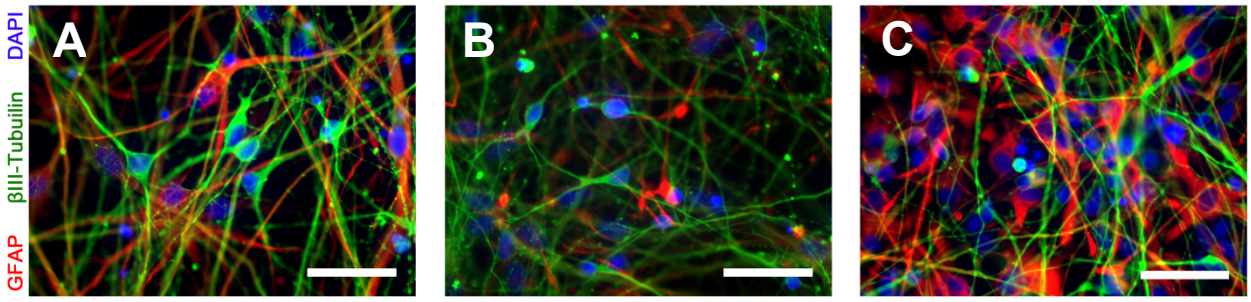

Figure S5. Directed neuronal differentiation. Directed differentiation from control (A), repeat carrier (B), and repeat KO (C) NPCs efficiently produces βIII-tubulin-positive neurons (green) and GFAP-positive glia (red). Scale bar: 50um.

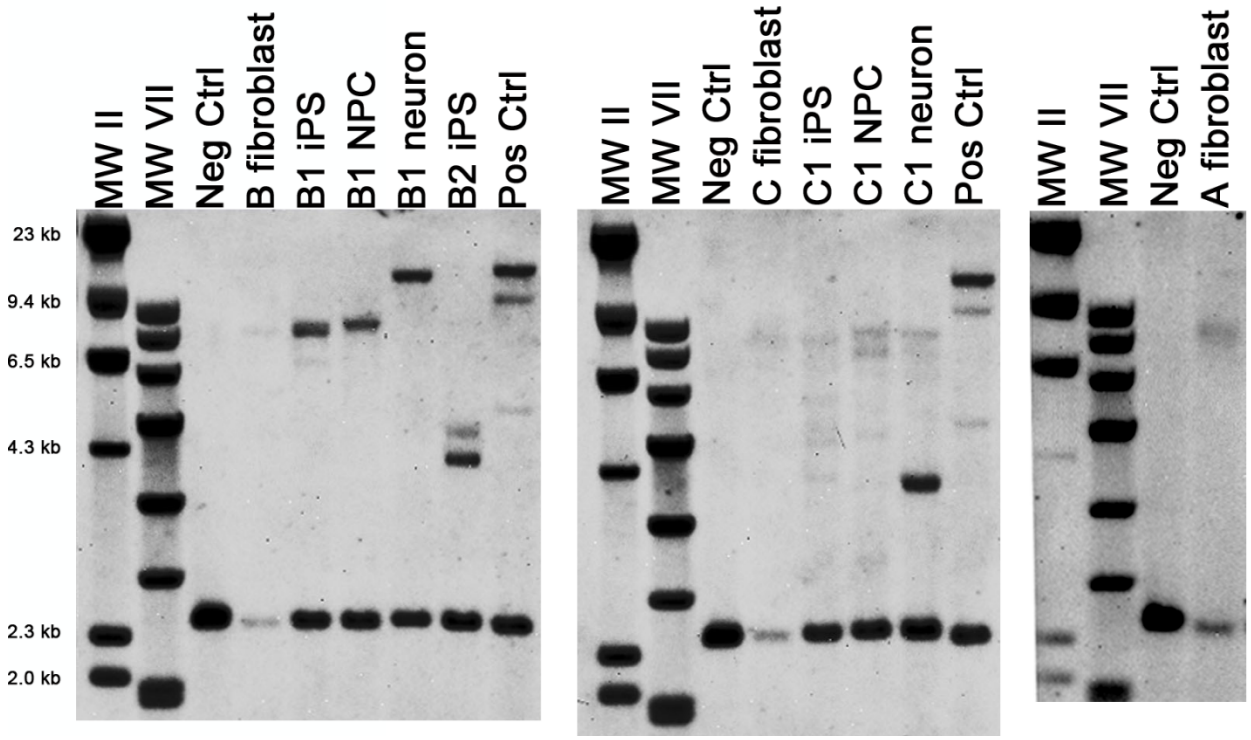

Figure S6. Southern blot of iPS and differentiated cells shows instability in repeat size. Parent lines A, B, and C fibroblasts carry an expanded repeat size of around 8kb, corresponding to a size of about 1000 repeats. Reprogramming of line B to iPS yielded lines with varying repeat sizes; B1 having the same repeat size, while the expansion in B2 decreased to two populations, a major population with 300 repeats and a minor population of 400 repeats. B1 was used for the CRISPR treatment described in this paper. Further differentiation of line B1 to NPCs and neurons expanded the repeat to between 1200-3500 repeats. Line C1 iPS cells were mosaic for the expansion with sizes ranging between 300-1000 repeats. Further differentiation to neurons produced a major population of about 300 repeats.

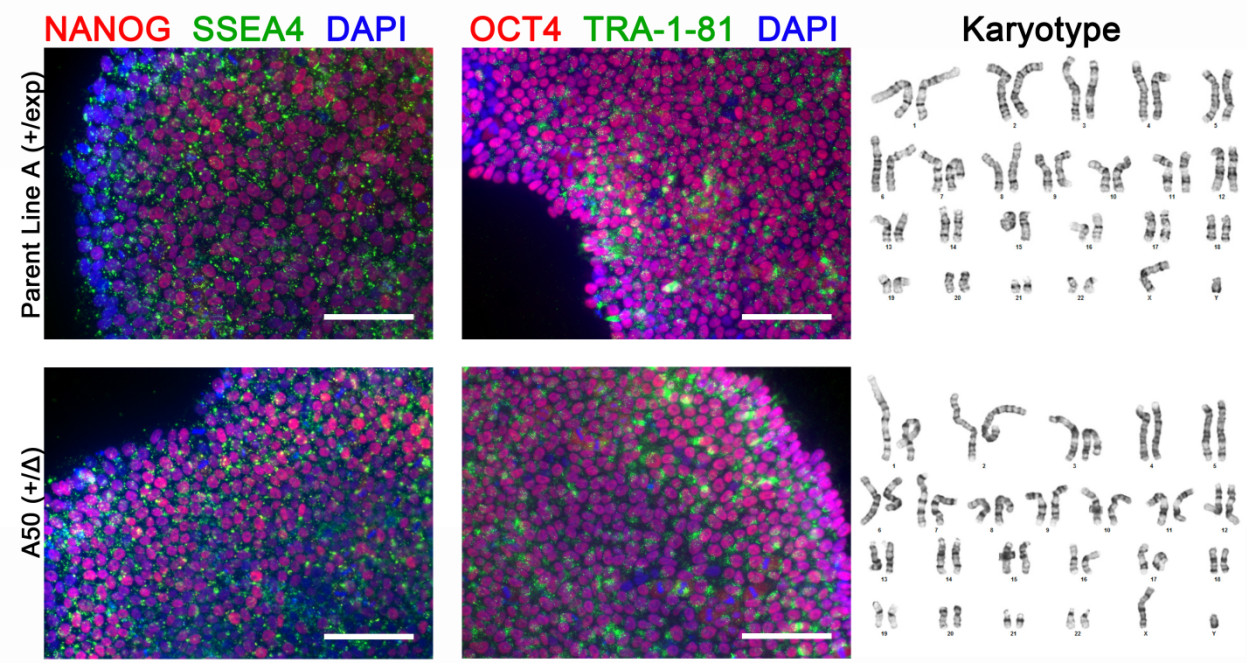

Figure S7. Pluripotency is maintained with CRISPR-mediated deletion of the *C9orf72* repeat. Human iPS cells from a *C9orf72* repeat expansion carrier (+/exp) and a repeat KO line derived from it (+/Δ) are positively labeled with the pluripotency markers SSEA and TRA 1-81 (green), and the nuclear markers NANOG and OCT4 (red). Scale bar: 100um. Karyotype analyses (right panel) showed no abnormalities.

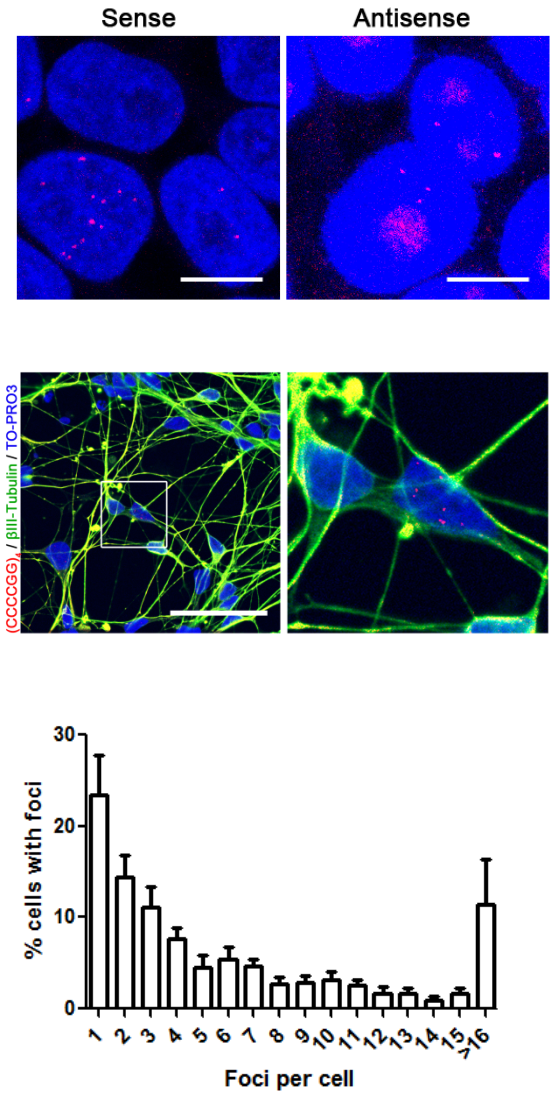

Figure S8. Repeat Expansion carrier cells express RNA foci. Top: patient-derived iPS cells form RNA foci from both the sense and antisense direction. βIII-tubulin-positive neurons (green) derived from these cells continue to exhibit nuclear RNA foci. Scale bar: 10um. Quantification of these foci in iPS cells (bottom) shows the frequency of cells with foci decreases as more foci are expressed per cell.

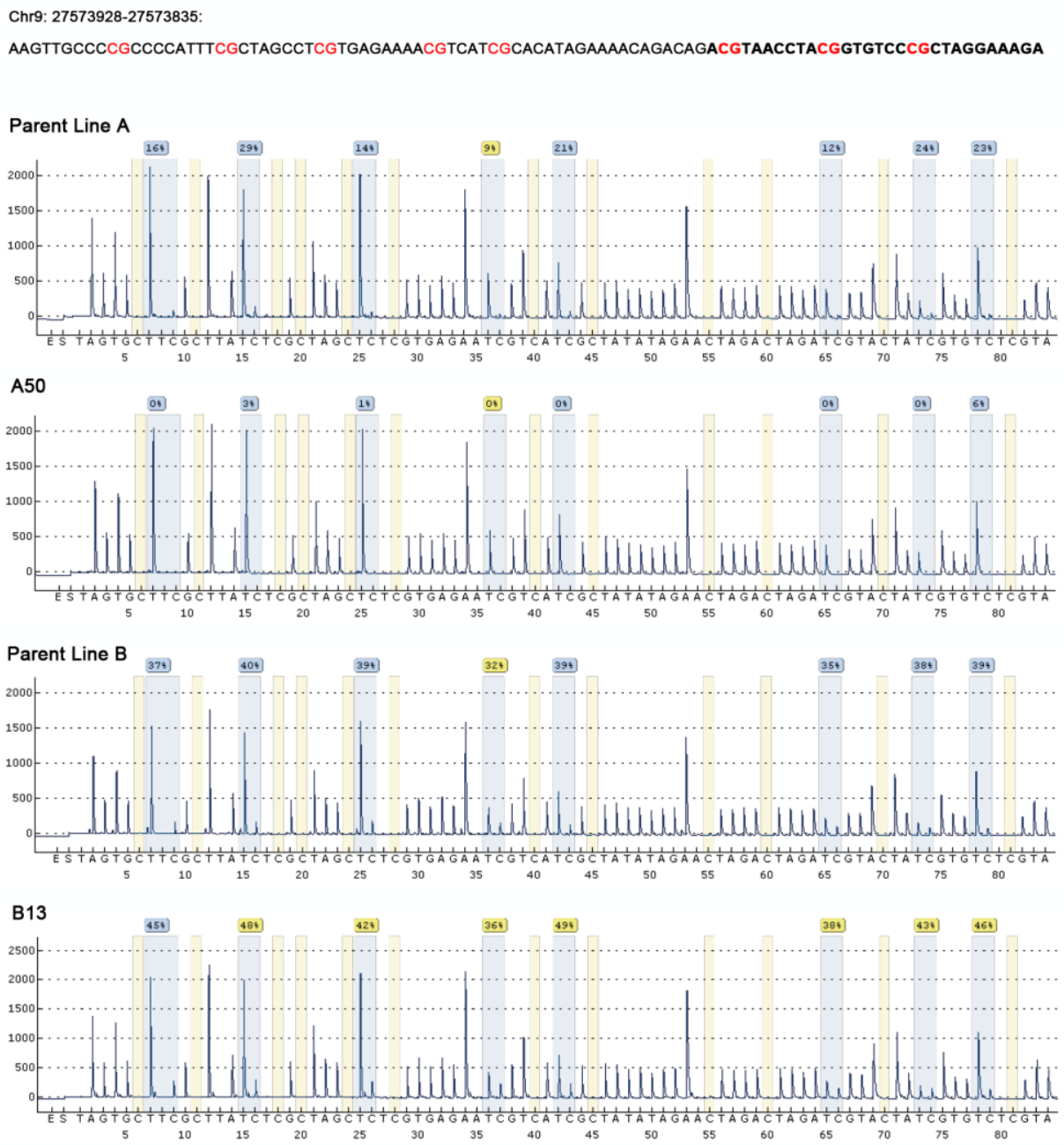

Figure S9. Pyrosequencing quantification of CpG island methylation. Sample pyrograms showing the quantification of the CpG methylation. Both expansion carrier parent lines are highly methylated. Repeat deletion in line A50 reduces methylation levels. Deletion of the normal allele in line B13 does not rescue the hypermethylation.

Table S1. Pyrosequencing quantification of the *C9orf72* CpG island

	ADS3233-FS1		ADS3232-FS1								Overall Region			
From TSS	-254	-215	-55	-45	-36	-26	-20	2	11	19	-254 to +19			
GRCh37/hg19, Chr9	27574117	27574078	27573918	27573908	27573899	27573889	27573883	27573862	27573853	27573845	Chr9:27574117-27573845			
	CpG#-1	**CpG#-2**	**CpG#-11**	**CpG#-12**	**CpG#-13**	**CpG#-14**	**CpG#-15**	**CpG#-16**	**CpG#-17**	**CpG#-18**	**Mean**	**St Dev**	**Min**	**Max**
Non-carrier HDF	0.0	3.3	1.2	2.2	6.8	0.0	0.0	0.0	0.0	0.0	1.4	2.2	0.0	6.8
Parent A HDF	3.2	17.4	1.0	4.1	1.5	1.1	1.2	3.3	2.7	3.1	3.9	4.9	1.0	17.4
Non-carrier iPS	1.4	3.8	0.0	2.1	1.2	0.0	0.0	0.0	0.0	1.9	1.0	1.3	0.0	3.8
Parent Line A iPS	32.8	33.0	16.3	28.7	14.1	8.9	20.8	11.6	24.4	23.2	21.4	8.6	8.9	33.0
A50 iPS	0.0	4.2	0.0	2.5	1.4	0.0	0.0	0.0	0.0	5.8	1.4	2.1	0.0	5.8
A51 iPS	0.0	5.6	0.9	2.6	1.2	1.1	1.2	0.0	0.0	2.3	1.5	1.7	0.0	5.6
A65 iPS	1.2	3.2	0.8	2.8	1.5	1.1	1.5	1.4	0.0	1.8	1.5	0.9	0.0	3.2
A80 iPS	1.7	2.6	0.8	2.0	1.7	0.9	1.0	1.2	0.0	1.9	1.4	0.7	0.0	2.6
A06 iPS	56.1	43.6	28.3	47.9	46.0	30.0	43.8	34.9	44.8	45.9	42.1	8.6	28.3	56.1
Parent Line B iPS	40.9	34.5	37.0	40.3	38.9	31.6	38.9	34.6	38.3	38.9	37.4	2.9	31.6	40.9
B13 iPS	37.2	31.5	44.9	48.4	41.6	36.2	49.3	37.5	43.5	46.1	41.6	5.8	31.5	49.3
Parent Line A neuron	47.7	40.0	30.3	51.2	41.0	38.5	48.9	42.9	49.3	45.7	43.5	6.4	30.3	51.2
A50 neuron	0.0	3.4	1.0	2.8	1.8	1.6	1.6	2.1	1.3	2.1	1.8	0.9	0.0	3.4
A51 neuron	0.9	3.3	0.0	2.3	2.0	1.1	0.0	1.6	0.0	0.0	1.1	1.2	0.0	3.3
A65 neuron	0.0	3.9	1.4	2.7	2.9	1.6	0.0	0.0	0.0	2.5	1.5	1.5	0.0	3.9
Methylation Controls	1.6	7.3	1.8	12.2	4.0	2.7	8.3	3.3	2.8	2.4	4.6	3.5	1.6	12.2
Methylation Controls	62.5	41.9	54.5	62.4	64.7	47.0	61.7	48.9	58.6	56.9	55.9	7.7	41.9	64.7
Methylation Controls	90.8	70.7	89.8	96.4	99.8	78.1	98.5	76.0	90.5	91.9	88.2	10.0	70.7	99.8

Table S1. Quantification of *C9orf72* promoter CpG sites by pyrosequencing. Both carrier and non-carrier patient-derived fibroblasts (HDF) have relatively little methylation. Reprogramming to iPS cells generates high methylation levels in expansion carriers (Parent Line A and Line B). Repeat knockout lines (A50, A51, A65, A80) have low methylation levels similar to non-carriers. Line B13, which has the normal allele deletion, and line A06, which is a clone isolated from line A without any deletion, have high levels of methylation. This methylation pattern is preserved when differentiating to neurons.