SUPPLEMENTARY MATERIAL

Table S1. ***Sequences of Primers Used for Amplification of Analyzed Genes.***

Gene name	Exon examined	Forward	Reverse	number of PCR cycles
ATE1	7,8	GATTTGAGTAAGCCTCCATGTCG	AAGGGTGAACTGCAAAGGAATC	25
CAPZB	8	TCAGAAGTACGCTGAACGAGA	TGATGCAGCTGTTATGTGACC	28
CLCN1	5,6,7,8	CATCTCTCCCCAGGCTGT	GCATCCTTGTTCCACACT	30
FHL1	5	AACCGCTTCTGGCATGACAC	CGCTTGTTGGCCAGATTCAC	25
INSR	11	CCAAAGACAGACTCTCAGAT	AACATCGCCAAGGGACCTGC	26
LDB3	11	GCAAGACCCTGATGAAGAAGCTC	GACAGAAGGCCGGATGCTG	30
MAPT	2,3	AAGACCAAGAGGGTGACACG	TTTTACTGACCATGCGAGCTT	26
MBNL1	7	GCTGCCCAATACCAGGTCAAC	TGGTGGGAGAAATGCTGTATGC	25
PHKA	19	TGCACACACTTGAGCTTCATGGA	AAAGTCCACCTCCCCAGACTGGTC	28
SERCA1	22	GCTCATGGTCCTCAAGATCTCAC	AGCTCTGCCTGAAGATGTGTCAC	30
SOS1	24	CAGTACCACAGATGTTTGCAGTG	TCTGGTCGTCTTCGTGGAGGAA	25
ZNF289	8	GACTCTGATTTCTTCACAGAACAC	TGCTCACGGAGCTTCTCTGCCA	28

Table S2. The Repeat Length and Expression Levels of EGFP Transcript Ccontaining CUG or CAG Repeats in the Stable Transfectant Cell Lines

Lp.	cell line	number of repeats	relative expression level [egfp/gapdh]	splicing changes
HeLa				
1.	Hela(CUG)5	5	5.87	N
2.	Hela(CUG)5	5	13.75	N
3.	Hela(CUG)5	5	20.64	N
4.	Hela(CUG)5	5	7.46	N
5.	Hela(CUG)5	5	11.47	N
6.	Hela(CUG)30	30	2.63	N
7.	Hela(CUG)30	30	10.83	N
8.	Hela(CUG)30	30	5.67	N
9.	Hela(CUG)30	30	6.40	N
10.	Hela(CUG)74	74	2.41	N
11.	Hela(CUG)74	74	8.35	N
12.	Hela(CUG)74	74	10.54	Y
13.	Hela(CUG)74	74	5.34	Y
14.	Hela(CUG)74	74	50.22	Y
15.	Hela(CUG)74	74	4.43	Y
16.	Hela(CUG)200	200	1.16	N
17.	Hela(CUG)200	200	7.00	Y
18.	Hela(CUG)200	200	6.03	Y
19.	Hela(CUG)200	200	4.92	Y

20.	Hela(CAG)5	5	2.24	N
21.	Hela(CAG)5	5	5.60	N
22.	Hela(CAG)5	5	10.05	N
23.	Hela(CAG)5	5	3.05	N
24.	Hela(CAG)30	30	8.13	N
25.	Hela(CAG)30	30	8.35	N
26.	Hela(CAG)30	30	4.67	N
27.	Hela(CAG)30	30	6.82	Y
28.	Hela(CAG)74	74	2.23	N
29.	Hela(CAG)74	74	1.60	N
30.	Hela(CAG)74	74	20.01	Y
31.	Hela(CAG)74	74	3.86	Y
32.	Hela(CAG)74	74	10.15	Y
33.	Hela(CAG)200	200	2.84	N
34.	Hela(CAG)200	200	10.36	Y
35.	Hela(CAG)200	200	3.45	Y
SK-N-MC				
36.	SK-N-MC(CUG)5	5	10.65	N
37.	SK-N-MC(CUG)5	5	7.38	N
38.	SK-N-MC(CUG)5	5	10.60	N
39.	SK-N-MC(CUG)5	5	2.72	N
40.	SK-N-MC(CUG)30	30	2.68	N
41.	SK-N-MC(CUG)30	30	20.72	N
42.	SK-N-MC(CUG)30	30	6.90	Y
43.	SK-N-MC(CUG)74	74	7.99	N
44.	SK-N-MC(CUG)74	74	4.51	Y
45.	SK-N-MC(CUG)74	74	10.43	Y
46.	SK-N-MC(CUG)74	74	9.48	Y
47.	SK-N-MC(CUG)74	74	10.67	Y
48.	SK-N-MC(CUG)200	200	4.51	N
49.	SK-N-MC(CUG)200	200	10.32	Y
50.	SK-N-MC(CAG)5	5	12.31	N
51.	SK-N-MC(CAG)5	5	6.48	N
52.	SK-N-MC(CAG)5	5	10.67	N
53.	SK-N-MC(CAG)5	5	60.40	N
54.	SK-N-MC(CAG)30	30	9.83	N
55.	SK-N-MC(CAG)30	30	2.82	N
56.	SK-N-MC(CAG)30	30	7.95	Y
57.	SK-N-MC(CAG)74	74	6.83	N
58.	SK-N-MC(CAG)74	74	24.784	N
59.	SK-N-MC(CAG)74	74	11.6009	Y
60.	SK-N-MC(CAG)74	74	4.51	Y
61.	SK-N-MC(CAG)74	74	10.32	Y
62.	SK-N-MC(CAG)74	74	40.39	Y
63.	SK-N-MC(CAG)200	200	7.45	Y
64.	SK-N-MC(CAG)200	200	5.89	Y

N - no change, Y- presence of splicing defects

Table S3. MLPA Sense and Antisense.

Provided as separate excel file.

Figure S1. ***Steady-State Levels of CUGBP1 and MBNL1 in HeLa, SK-N-MC Cells and Human Fibroblasts are Similar.***

Relative expression levels of CUGBP1 and MBNL1 were determined using western blot analysis and shown as bar graphs which represent mean values (±SD) of three independent experiments normalized to GAPDH level.

Figure S2. ***Similar Aberrations in Alternative Splicing are Observed in SK-N-MC Cells Expressing Untranslated CUG and CAG Repeats.***

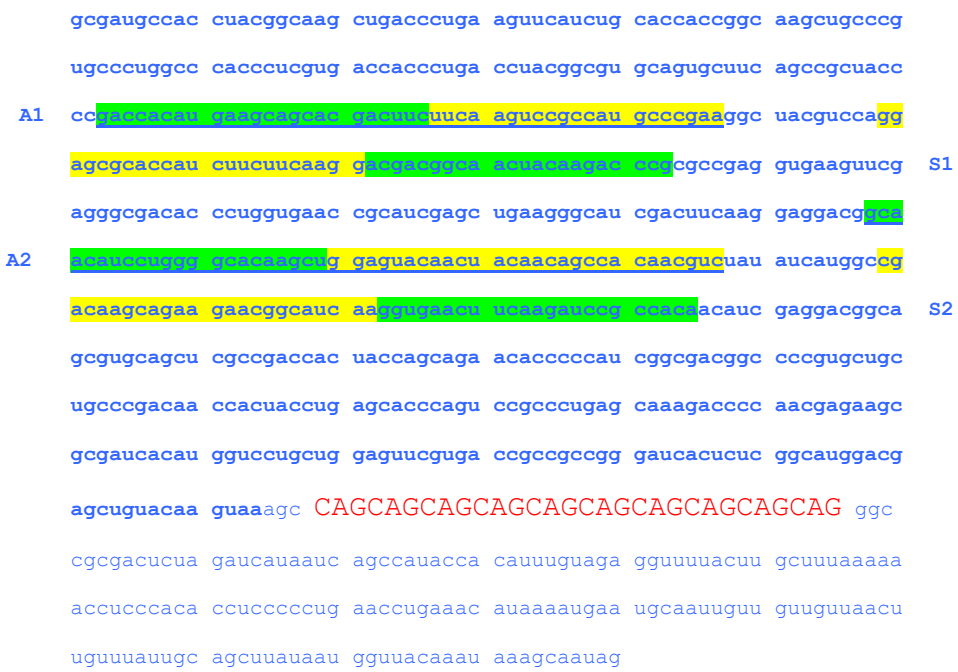
Total RNA isolated from clonal lines of SK-N-MC cells was subjected to RT-PCR using *SERCA1, CLCN1, MAPT, CAPZB,* and *ATE1* specific primers. SK-N-MC cells with siRNA downregulated MBNL1 protein (siMBNL1) were used as a positive control. The experiments were carried out in triplicate and representative gels are shown. The fraction of exon inclusion/exclusion (±SD) shown on bar diagrams was calculated by dividing the amount of the band corresponding to the inclusion/exclusion splice product by the total amount of splice products. **P* =<0.05; 0.001>, ***P* < 0.001 compared with the control.

Figure S3. ***Binding Sites of MLPA Probes***.

ccggacucag aucucgagcu caagcuucga auucugcagu cgacgguacc gcgggcccgg

gauccaucgc cacc**auggug agcaagggcg aggagcuguu caccggggug gugcccaucc**

uggucgagcu ggacggcgac guaaacggcc acaaguucag cguguccggc gagggcgagg



mRNA sequence encoded by pEGFP-N3 containing CAG repeats; bolded- EGFP coding sequence; A1, A2- sequence of antisense-specific probes; S1, S2- sequence complementary to sense probes; green 3' probes, yellow- 5' probes.

Figure S4. ***Level of Transcript and Protein Expressed from pEGFP-mutATXN3 is Comparable.***

(A) The level of transcript expressed from pEGFP-mutATXN3 was determined by RT-PCR analysis in three established clonal SK-N-MC cell lines. Position of bands representing transgene mRNA and endogenous mRNA of *ATXN3* are indicated. **(B)** Western blot analysis of EGFP-mutant Ataxin 3 fusion protein was carried out with anti-ATXN3 antibody. GAPDH staining was used as a loading control. The bar graph depicts quantification results from three western blots.

Figure S5. ***Splicing pattern in clonal SK-N-MC cell lines overexpressing ATXN3 wild type***

Total RNA isolated from clonal lines of SK-N-MC cells was subjected to RT-PCR using *SERCA1, , MAPT,* and *ATE1* specific primers. The experiments were carried out in triplicate and representative gels are shown. The fraction of exon inclusion/exclusion (±SD) shown on bar diagrams was calculated by dividing the amount of the band corresponding to the inclusion/exclusion splice product by the total amount of splice products

Figure S6. ***Expression of CAG and CUG RNA Repeats does not Affect CUGBP1 Upregulation.***

The CUGBP1 steady-state levels in cells expressing CTG and CAG repeats of indicated lengths were analyzed by western blotting. As shown, in both cell lines the protein levels remain unchanged in the presence of repeat containing transcripts regardless of their length when compared with control cells. GAPDH was used as a loading control.

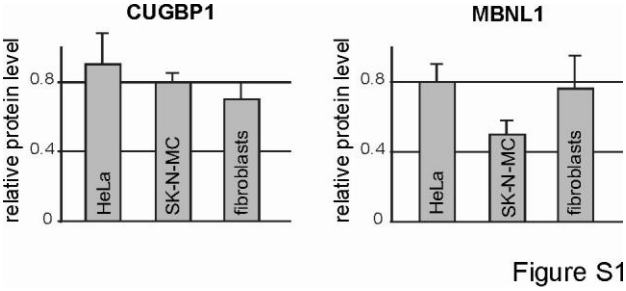

CUGBP1
MBNL1
relative protein level
0.8
0.4
0
HeLa
SK-N-MC
fibroblasts

Figure S1

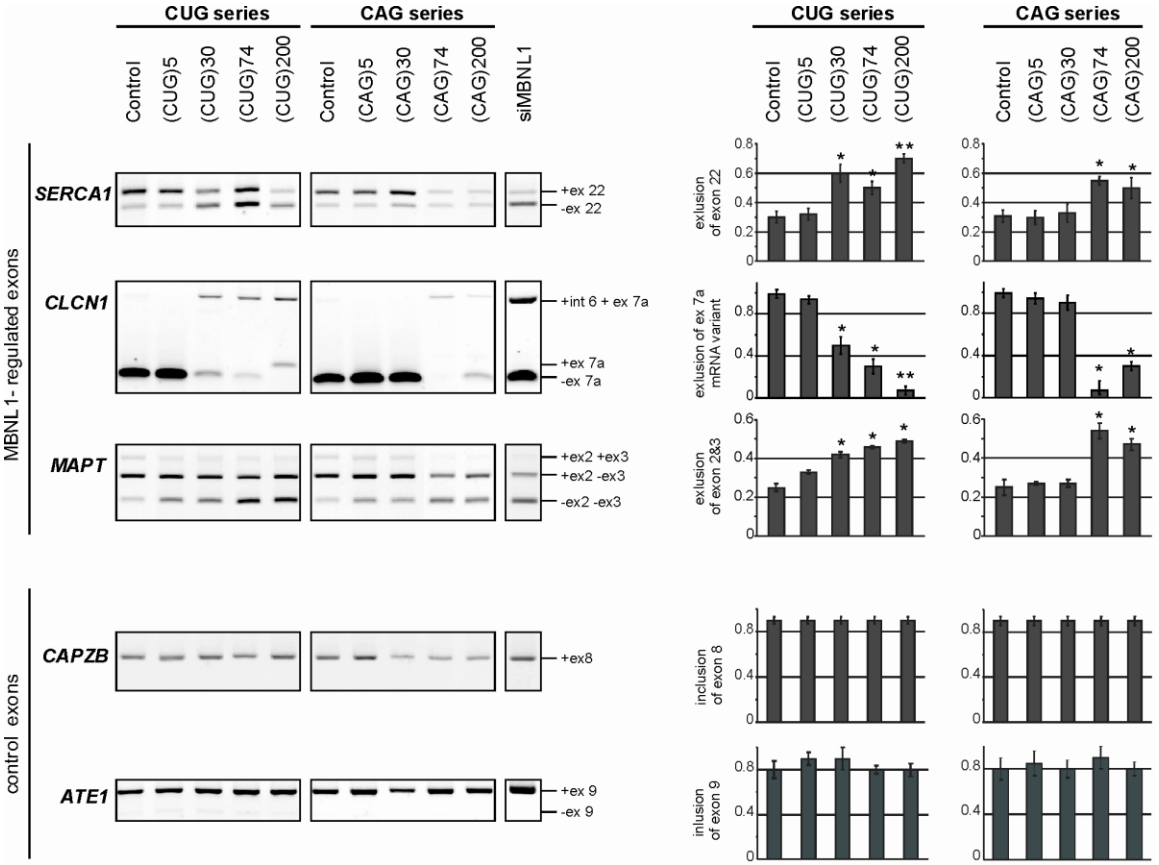

CUG series
CAG series
siMBNL1
Control
(CUG)5
(CUG)30
(CUG)74
(CUG)200
(CAG)5
(CAG)30
(CAG)74
(CAG)200
MBNL1- regulated exons
SERCA1
+ex 22
-ex 22
CLCN1
+int 6 + ex 7a
+ex 7a
-ex 7a
MAPT
+ex2 +ex3
+ex2 -ex3
-ex2 -ex3
control exons
CAPZB
+ex8
ATE1
+ex 9
-ex 9
exlusion of exon 22
exlusion of ex 7a mRNA variant
exlusion of exon 2&3
inclusion of exon 8
inlusion of exon 9

Figure S2

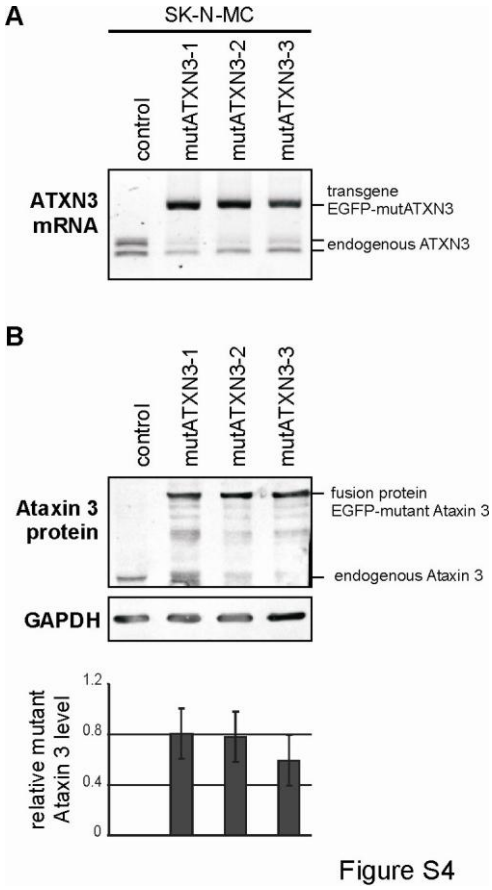

A
SK-N-MC
control
mutATXN3-1
mutATXN3-2
mutATXN3-3
ATXN3 mRNA
transgene EGFP-mutATXN3
endogenous ATXN3
B
control
mutATXN3-1
mutATXN3-2
mutATXN3-3
Ataxin 3 protein
fusion protein EGFP-mutant Ataxin 3
endogenous Ataxin 3
GAPDH
relative mutant Ataxin 3 level
1.2
0.8
0.4
0

Figure S4

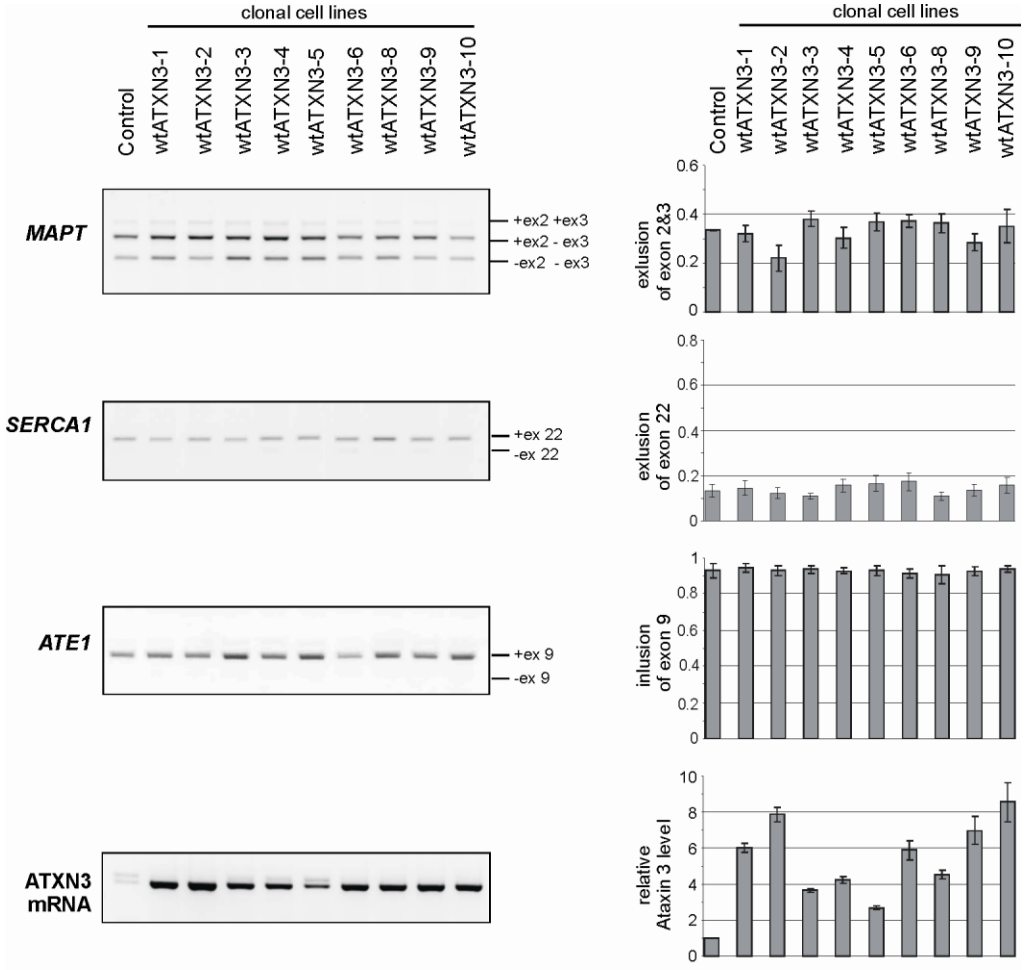

clonal cell lines
Control
wtATXN3-1
wtATXN3-2
wtATXN3-3
wtATXN3-4
wtATXN3-5
wtATXN3-6
wtATXN3-8
wtATXN3-9
wtATXN3-10
MAPT
+ex2 +ex3
+ex2 - ex3
-ex2 - ex3
SERCA1
+ex 22
-ex 22
ATE1
+ex 9
-ex 9
ATXN3 mRNA
clonal cell lines
exlusion of exon 2&3
exlusion of exon 22
inlusion of exon 9
relative Ataxin 3 level

Figure S5

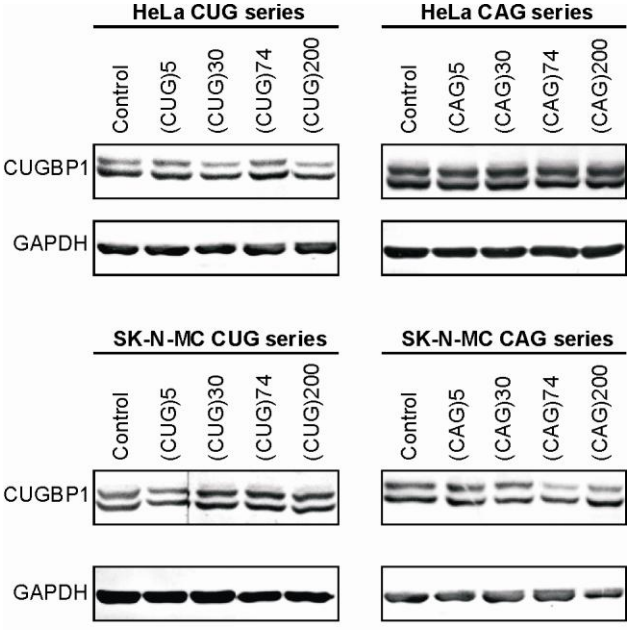

HeLa CUG series
HeLa CAG series
Control
(CUG)5
(CUG)30
(CUG)74
(CUG)200
Control
(CAG)5
(CAG)30
(CAG)74
(CAG)200
CUGBP1
GAPDH
SK-N-MC CUG series
SK-N-MC CAG series
Control
(CUG)5
(CUG)30
(CUG)74
(CUG)200
Control
(CAG)5
(CAG)30
(CAG)74
(CAG)200
CUGBP1
GAPDH

Figure S6