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## **Supplemental Information**

### **Development of an AAV-Based MicroRNA Gene**

#### **Therapy to Treat Machado-Joseph Disease**

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## Supplemental material

Groups	Treatment	Route of injection	Number of animals (n)	Amount of injection ( $\mu$ l)
1	AAV5-miATXN3_7	ICV	3	10
2		Cisterna magna	3	10
3		Bilateral DCN	3	4 = 2+2
4	AAV5-miATXN3_9	ICV	3	10
5		Cisterna magna	3	10
6		Bilateral DCN	3	4 = 2+2
7	AAV5-miATXN3_11	ICV	3	10
8		Cisterna magna	3	10
9		Bilateral DCN	3	4 = 2+2
10	AAV5-GFP	Bilateral DCN	3	4 = 2+2
11	No treatment	Not applicable	3	0

Table S1. Treatment group design of the *in vivo* investigations. AAV5-miATXN3\_7, -9 and -11 were tested *in vivo* and delivered to the SCA3 knock-in mice by 3 different routes of administration.

Treatment	Transcript (Blast)	% coverage (Blast)	Fold Change (treatment vs PBS)	p-value (treatment vs PBS)
miATXN3_7	ATXN3	100%	-1.2	9.4E-03
miATXN3_7	GNPTAB	63%	1.1	4.2E-03
miATXN3_7	SOX5	63%	-1.1	2.6E-02
miATXN3_7	GNPTAB	63%	1.1	4.2E-03
miATXN3_7	CTSC	63%	1.3	2.2E-16
miATXN3_9	ATXN3	100%	-1.5	3.4E-16
miATXN3_9	TNFRSF6B	59%	1.3	4.4E-02
miATXN3_9	FGD6	59%	-1.2	9.1E-03
miATXN3_11	ATXN3	100%	-1.3	1.0E-06
miATXN3_11	ICA1	68%	1.3	4.8E-03
miATXN3_11	CACNA1D	63%	1.5	2.9E-02

Table S2. Prediction of off-target genes based on BLAST search and RNA sequencing. BLAST search was performed with the guide sequences of miATXN3\_7, miATXN3\_9 and miATXN3\_11. The blast results were then compared to RNA sequencing expression values obtained from human-derived frontal brain-like neurons treated with the AAV5-miATXN3 candidates or the formulation buffer. P-values >0.05 were excluded

Gene name	TPOTS value	8mer	7mer-M8	7mer-1A	6mer	Fold Change (mi7 vs PBS)	p-value (mi7 vs PBS)
WDR72	0.816	1	1	9	1	-1.4	9.5E-03
SCAI	0.615	1	0	7	0	-1.3	3.4E-05
GDA	0.547	3	0	1	2	-2.3	6.9E-03
FOXP2	0.526	2	2	1	1	-1.3	2.9E-02
DNAL1	0.525	0	1	7	0	-1.3	1.5E-02
AAK1	0.522	2	0	3	7	1.1	3.3E-02
KCNMA1	0.488	1	0	5	3	1.3	1.8E-03
ONECUT2	0.487	1	0	5	2	-1.2	6.9E-04
FBXL20	0.428	1	1	3	3	-1.2	3.6E-03
IKZF2	0.426	1	1	3	1	-1.4	3.2E-02
BMPR1B	0.426	1	1	3	1	-1.3	1.6E-04
CSRNP3	0.403	0	2	4	3	-1.3	1.4E-02
LIFR	0.367	1	2	1	2	-1.4	1.5E-03
ZNF652	0.36	1	1	2	0	-1.1	3.1E-03
LONRF2	0.36	1	0	3	5	1.2	8.9E-05
CPNE3	0.36	1	1	2	0	-1.2	7.7E-03
PHF6	0.357	1	0	3	2	-1.2	1.3E-02
C21orf91	0.357	1	0	3	2	-1.2	1.2E-02
TMEM47	0.356	1	0	3	1	-1.3	1.3E-02
ARL3	0.355	1	0	3	0	-1.1	2.0E-03
ATXN3	0.295	1	1	1	0	-1.2	9.4E-03

Table S3. Prediction of off-target genes in human by siSPOTR and RNA sequencing after AAV5-miATXN3\_7 treatment. A search was performed on siSPOTR with the seed sequence of miATXN3\_7 to check for binding within 3'UTR of human transcripts. The top 20 genes with highest tPOTS were

compared to RNA sequencing expression values in human-derived frontal brain-like neurons. RNA sequencing was performed on RNA isolated from human-derived frontal brain-like neurons treated with AAV5-miATXN3\_7 or the formulation buffer for 7 days. The genes with p-value < 0.05 were excluded.

Gene name	TPOTS value	8mer	7mer-M8	7mer-1A	6mer	Fold Change (mi9 vs PBS)	p-value (mi9 vs PBS)
<i>PHC3</i>	0.651	2	1	4	1	-1.3	1.2E-03
<i>HIF3A</i>	0.617	3	1	1	2	-1.4	3.1E-02
<i>ONECUT2</i>	0.606	0	3	6	6	-1.2	3.4E-13
<i>UBE2R2</i>	0.515	2	0	3	0	-1.1	1.5E-04
<i>H6PD</i>	0.493	1	1	4	3	-1.2	6.0E-16
<i>RAB3B</i>	0.43	1	1	3	5	-1.3	6.3E-10
<i>PTGS1</i>	0.427	1	1	3	2	-3.4	1.3E-121
<i>KSR2</i>	0.412	0	4	2	2	1.4	2.1E-04
<i>TTC14</i>	0.391	2	1	0	1	-1.1	1.9E-05
<i>BBX</i>	0.391	2	1	0	1	-1.2	3.2E-04
<i>ZNF286B</i>	0.385	2	0	1	0	-1.2	3.4E-03
<i>SYT2</i>	0.385	2	0	1	0	-1.2	2.2E-05
<i>SYP</i>	0.385	2	0	1	0	1.3	0.0E+00
<i>THY1</i>	0.365	1	2	1	0	1.3	7.2E-03
<i>TFDP2</i>	0.365	1	2	1	0	-1.4	1.3E-17
<i>PRLR</i>	0.357	1	0	3	2	-1.2	8.0E-03
<i>FMN1</i>	0.328	0	0	5	3	2.1	2.6E-05
<i>PRX</i>	0.326	0	0	5	1	-1.2	1.1E-02
<i>NCALD</i>	0.326	0	0	5	1	-1.1	1.9E-05
<i>ATXN3</i>	0.001	0	0	0	1	-1.5	3.4E-16

Table S4. Prediction of off-target genes in human by siSPOTR and RNA sequencing for AAV5-miATXN3\_9 treatment. Performed as described in table S3 for AAV5-miATXN3\_9.

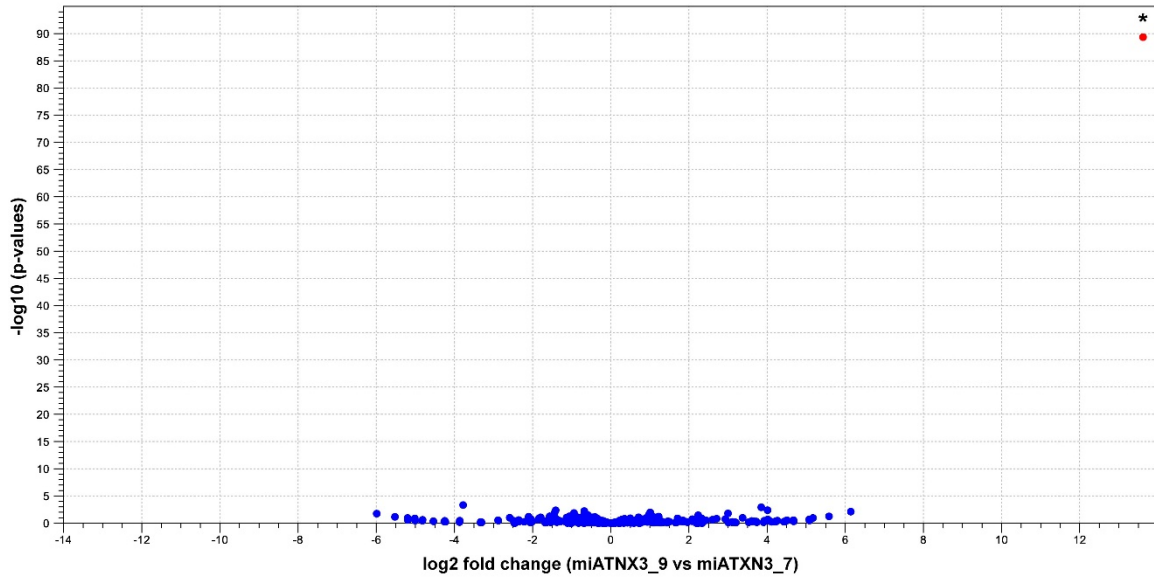
Gene name	TPOTS value	8mer	7mer-M8	7mer-1A	6mer	Fold Change (mi11 vs PBS)	p-value (mi11 vs PBS)
<i>SPTY2D1</i>	0.615	3	1	1	0	-1.1	9.3E-05
<i>ENAH</i>	0.586	2	1	3	1	1.2	3.0E-08
<i>TDRD6</i>	0.55	3	1	0	0	-1.6	2.8E-02
<i>PRKAB2</i>	0.546	3	0	1	1	1.1	9.2E-05
<i>TAB3</i>	0.525	2	2	1	0	-1.2	5.4E-04
<i>C1orf21</i>	0.518	2	0	3	3	-1.3	4.7E-57
<i>SMAD2</i>	0.517	2	0	3	2	-1.1	2.4E-03
<i>BRWD1</i>	0.515	2	0	3	0	-1.1	4.0E-17
<i>EXD2</i>	0.498	1	2	3	3	-1.2	4.1E-05
<i>NFYA</i>	0.497	1	2	3	2	-1.3	1.0E-35
<i>AAK1</i>	0.484	0	5	2	4	1.2	8.7E-06
<i>RRAGD</i>	0.456	2	1	1	1	-1.2	6.7E-03
<i>RBM43</i>	0.455	2	1	1	0	-1.4	2.3E-07
<i>ATRNL1</i>	0.455	2	1	1	0	-1.1	8.8E-04
<i>ZFH3</i>	0.451	2	0	2	1	1.1	1.5E-02
<i>LYSMD3</i>	0.451	2	0	2	1	-1.2	3.6E-02

<b>LOX</b>	0.451	2	0	2	1	1.8	1.6E-12
<b>LPP</b>	0.446	1	4	0	6	1.2	1.1E-02
<b>PAPOLG</b>	0.432	1	2	2	2	-1.1	2.9E-02
<b>C21orf91</b>	0.426	1	1	3	1	-1.2	4.9E-02
<b>ATXN3</b>	0	0	0	0	0	-1.3	9.96E-07

Table S5. Prediction of off-target genes in human by siSPOTR and RNA sequencing for AAV5-miATXN3\_11 treatment. Performed as described in table S3 for AAV5-miATXN3\_11.

<b>Gene name</b>	<b>TPOTS value</b>	<b>8mer</b>	<b>7mer-M8</b>	<b>7mer-1A</b>	<b>6mer</b>	<b>Fold Change (mi9 vs untreated)</b>	<b>p-value (mi9 vs untreated)</b>
<i>Ncl</i>	0.657	2	2	3	2	1.3	8.3E-03
<i>Aak1</i>	0.51	1	4	1	5	-1.6	5.0E-05
<i>Onecut2</i>	0.497	1	2	3	2	-1.2	1.3E-03
<i>Nhs12</i>	0.461	0	1	6	1	-1.5	1.5E-04
<i>Cacna1e</i>	0.458	2	1	1	3	-1.0	5.0E-05
<i>Nav1</i>	0.435	1	2	2	5	-1.4	5.0E-05
<i>Ttc14</i>	0.422	1	0	4	2	-1.6	5.0E-05
<i>Fzd3</i>	0.408	0	3	3	3	-1.3	5.0E-05
<i>Srgap3</i>	0.396	0	1	5	1	-0.8	3.0E-04
<i>Pde5a</i>	0.391	2	1	0	1	-1.0	2.0E-03
<i>Lonrf2</i>	0.385	2	0	1	0	-1.3	5.0E-05
<i>Zfp704</i>	0.37	1	2	1	5	-1.1	5.0E-05
<i>Mecp2</i>	0.368	1	2	1	3	-1.5	5.0E-05
<i>Cldn12</i>	0.362	1	1	2	2	-1.3	5.0E-05
<i>Dnajc18</i>	0.361	1	1	2	1	-0.6	1.2E-02
<i>0610030E20Rik</i>	0.361	1	1	2	1	-1.2	2.0E-04
<i>Dhx33</i>	0.36	1	1	2	0	-0.7	5.8E-03
<i>Pdzd2</i>	0.356	1	0	3	1	-1.2	5.0E-05
<i>D430041D05Rik</i>	0.355	1	0	3	0	-1.4	5.0E-05
<i>Atxn3</i>	0.16	1	0	0	0	-1.7	5.0E-05

Table S6. Prediction of off-target genes in mice by siSPOTR and RNA sequencing for AAV5-miATXN3\_9 treatment. Performed as described in table S3. siSPOTR search was performed against mouse transcripts. RNA sequencing was performed on RNA isolated from brain stem of SCA3 knock-in mice treated with AAV5-miATXN3\_9 or control (untreated) by injection in the cisterna magna.



**Figure S1: Volcano plot of miRNA expression levels between miATXN3\_09 vs miATXN3\_07 transduced neurons.** IPSC-derived neurons were treated with AAV5-miATXN3 candidates, after which small RNA sequencing was performed. Differential expression analysis on miRNAs revealed miATXN3\_9 as the only significantly altered miRNA, whereas endogenous miRNAs were at comparable levels. \* = FDR <0.05. n = 2 per treatment condition, total of 787 individual miRNAs per sample.