

OMTN, Volume 16

## **Supplemental Information**

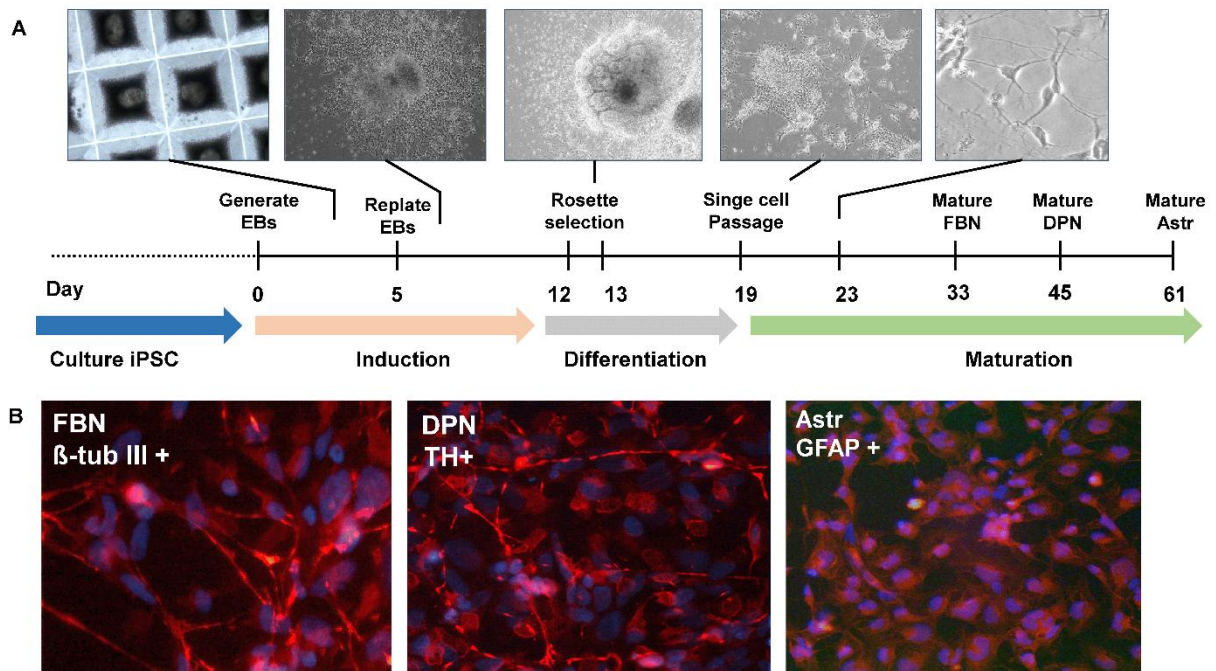
### **Targeting RNA-Mediated Toxicity**

#### **in C9orf72 ALS and/or FTD**

#### **by RNAi-Based Gene Therapy**

**Raygene Martier, Jolanda M. Liefhebber, Ana García-Osta, Jana Miniarikova, Mar Cuadrado-Tejedor, Maria Espelosin, Susana Ursua, Harald Petry, Sander J. van Deventer, Melvin M. Evers, and Pavlina Konstantinova**

## Supplementary material



**Figure S1. Differentiation and characterization of iPSC neurons.** a) iPSC cells were seeded on AggreWell800 plates and cultured in STEMdiff Neural Induction Medium until day 5 to induce embryoid bodies formation. Embryoid bodies were harvested and replated in STEMdiff Neural Induction Medium for 7 days. At day 12, Rosettes were selected with rosette selection medium and differentiated in STEMdiff Neuron Differentiation medium or STEMdiff astrocyte Differentiation medium (STEMCELL) for 5 days. The cells were then matured into mature FBN or astrocytes for one week.

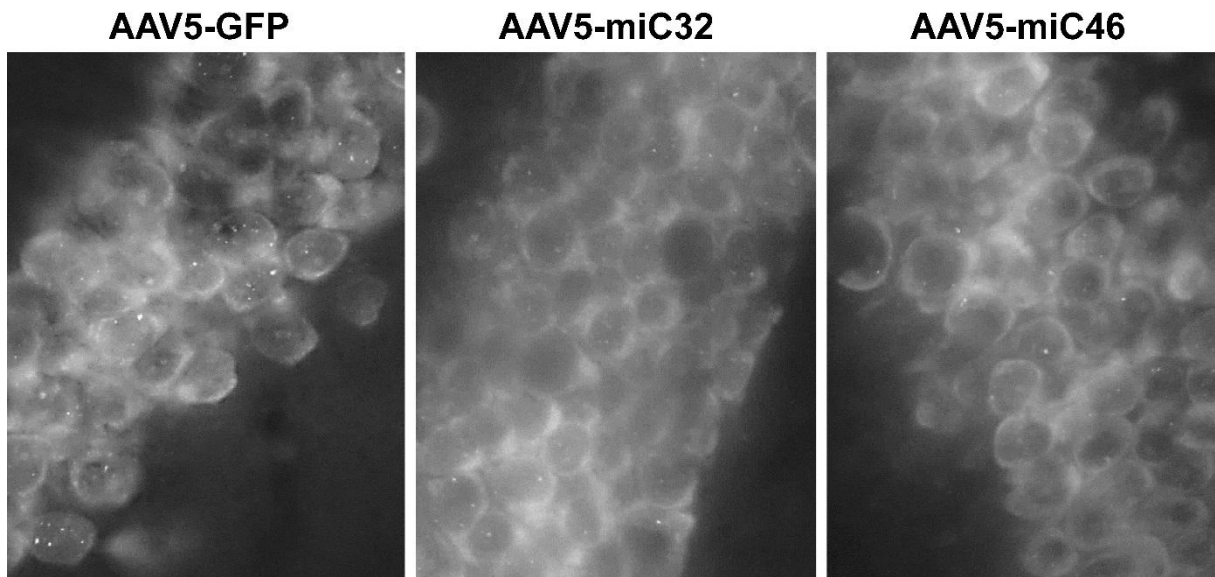


Figure S2. Reduction of RNA foci in hippocampus of Tg(*C9orf72\_3*) line 112 mice.

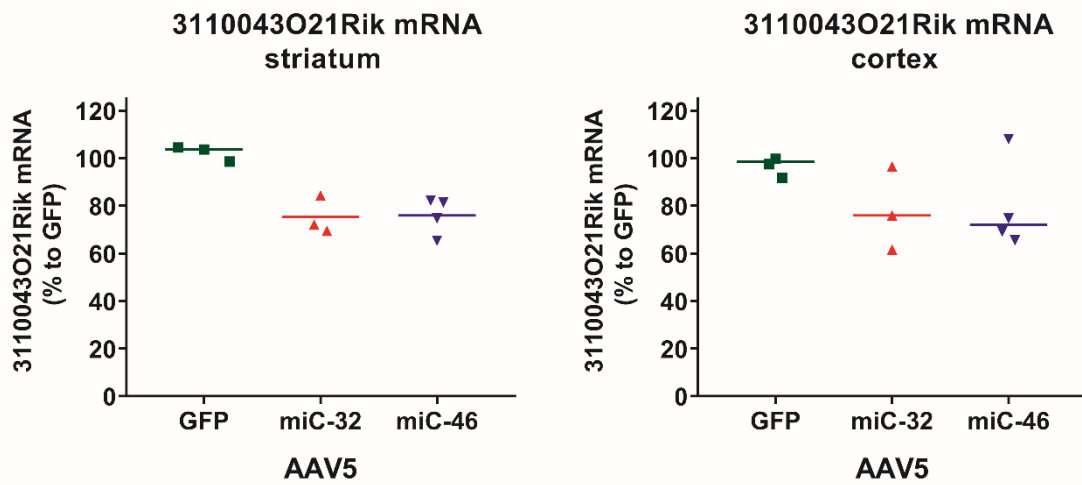


Figure S3. Reduction of the mouse *C9orf72* in striatum and cortex of C9BAC mice. Total RNA was isolated from the striatum and cortex of mice treated with AAV5-miC32 and AAV5-miC46. RT-qPCR was performed using primers to detect the mouse *C9orf72* ortholog (3110043021Rik). RNA input levels were normalized to GAPDH and set relative to AAV-GFP treated mice.

miC precursor	mature miRNA (Guide strand)	length	reads	% reads	mature miRNA (passenger strand)	nt length	reads	% reads
<b>C9BAC mouse striatum 333 (AAV5_miC32)</b>								
Total expression values: 454435								
5' u c u g g c passenger c g u c u a	1 C T T A C T C T A G G A C C A A G A A	20	317977	70,0%	1 C T T G G T C C T A G A G T A A C G G	19	17591	3,9%
g c c u u c u u g g u c c u g a g a a g g a c u	2 C T T A C T C T A G G A C C A A G A A	19	22720	5,0%	2 C T T G G T C C T A G A G T A A C G G A	20	11244	2,5%
c e g a a g a a c c a g g a t c u c a u u c c u g	3 C T T A C T C T A G G A C C A A G A A T	20	9351	2,1%	3 C T T G G T C C T A G A G T A A C G G A C	21	9383	2,1%
a u a g g a guide a a a u c			total	77,0%			total	8,4%
3' a a a u c								
<b>C9BAC mouse striatum 334 (AAV5_miC32)</b>								
Total expression values: 394961								
5' u c u g g c passenger c g u c u a	1 C T T A C T C T A G G A C C A A G A A	20	255155	64,6%	1 C T T G G T C C T A G A G T A A C G G	19	25219	6,4%
g c c u u c u u g g u c c u g a g a a g g a c u	2 C T T A C T C T A G G A C C A A G A A	19	18480	4,7%	2 C T T G G T C C T A G A G T A A C G G A	18	14210	3,6%
c e g a a g a a c c a g g a t c u c a u u c c u g	3 C T T A C T C T A G G A C C A A G A A T	20	7553	1,9%	3 C T T G G T C C T A G A G T A A C G G A	21	13098	3,3%
a u a g g a guide a a a u c			total	71,2%			total	13,3%
3' a a a u c								
<b>C9BAC mouse striatum 341 (AAV5_miC32)</b>								
Total expression values: 257715								
5' u c u g g c passenger c g u c u a	1 C T T A C T C T A G G A C C A A G A A	20	169312	65,7%	1 C T T G G T C C T A G A G T A A C G G	19	15032	5,8%
g c c u u c u u g g u c c u g a g a a g g a c u	2 C T T A C T C T A G G A C C A A G A A	19	12731	4,9%	2 C T T G G T C C T A G A G T A A C G G A	20	9099	3,5%
c e g a a g a a c c a g g a t c u c a u u c c u g	3 C T T A C T C T A G G A C C A A G A A T	20	4820	1,9%	3 C T T G G T C C T A G A G T A A C G G	18	8177	3,2%
a u a g g a guide a a a u c			total	72,5%			total	12,5%
3' a a a u c								
<b>C9BAC mouse striatum 342 (AAV5_miC32)</b>								
Total expression values: 423128								
5' u c u g g c passenger c g u c u a	1 C T T A C T C T A G G A C C A A G A A	20	283816	67,1%	1 C T T G G T C C T A G A G T A A C G G	19	19759	4,7%
g c c u u c u u g g u c c u g a g a a g g a c u	2 C T T A C T C T A G G A C C A A G A A	19	22210	5,2%	2 C T T G G T C C T A G A G T A A C G G A	20	12406	2,9%
c e g a a g a a c c a g g a t c u c a u u c c u g	3 C T T A C T C T A G G A C C A A G A A T	20	8896	2,1%	3 C T T G G T C C T A G A G T A A C G G A C	18	11793	2,8%
a u a g g a guide a a a u c			total	74,4%			total	10,4%
3' a a a u c								
<b>C9BAC mouse striatum 323 (AAV5_miC46)</b>								
Total expression values: 22153								
5' u c u g g c passenger u g u c u a	1 T A T C T T C A G G T T C C G A A G A G	20	870	3,9%	1 C T T O G G A A C C T G A A G A T T G A C	21	16507	74,5%
g c c c u c u u c g g a a c c u g a a g a u g a c	2 T A T C T T C A G G T T C C G A A	17	343	1,5%	2 C T T O G G A A C C T G A A G A T T G A	20	1310	5,9%
c e g g a g a a c c u u g g a c u u c u a u u g	3 T A T C T T C A G G T T C C G A	16	277	1,3%	3 C T T O G G A A C C T G A A G A T T G A C G	22	460	2,1%
a u a g g a guide a a a u c			total	6,7%			total	82,5%
3' a a a u c								
<b>C9BAC mouse striatum 324 (AAV5_miC46)</b>								
Total expression values: 14529								
5' u c u g g c passenger u g u c u a	1 T A T C T T C A G G T T C C G A A G A G	20	1873	12,9%	1 C T T O G G A A C C T G A A G A T T G A C	21	9167	63,1%
g c c c u c u u c g g a a c c u g a a g a u g a c	2 T A T C T T C A G G T T C C G A A	17	580	4,0%	2 C T T O G G A A C C T G A A G A T T G A	20	737	3,3%
c e g g a g a a c c u u g g a c u u c u a u u g	3 T A T C T T C A G G T T C C G A A G A	19	196	1,3%	3 C T T O G G A A C C T G A A G A T T G A C G	22	301	1,4%
a u a g g a guide a a a u c			total	18,2%			total	67,8%
3' a a a u c								
<b>C9BAC mouse striatum 353 (AAV5_miC46)</b>								
Total expression values: 20523								
5' u c u g g c passenger u g u c u a	1 T A T C T T C A G G T T C C G A A G A G	20	2751	13,4%	1 C T T O G G A A C C T G A A G A T T G A C	21	11058	53,9%
g c c c u c u u c g g a a c c u g a a g a u g a c	2 T A T C T T C A G G T T C C G A A	17	469	2,3%	2 C T T O G G A A C C T G A A G A T T G A	20	1297	6,3%
c e g g a g a a c c u u g g a c u u c u a u u g	3 T C A G G T T C C G A A G A G	15	239	1,2%	3 T C G G A A C C T G A A G A T T G A C	19	1012	4,9%
a u a g g a guide a a a u c			total	16,9%			total	65,1%
3' a a a u c								
<b>C9BAC mouse striatum 358 (AAV5_miC46)</b>								
Total expression values: 7758								
5' u c u g g c passenger u g u c u a	1 T A T C T T C A G G T T C C G A A G A G	20	1386	17,9%	1 C T T O G G A A C C T G A A G A T T G A C	21	4871	62,8%
g c c c u c u u c g g a a c c u g a a g a u g a c	2 T A T C T T C A G G T T C C G A A	17	153	2,0%	2 C T T O G G A A C C T G A A G A T T G A	20	280	3,6%
c e g g a g a a c c u u g g a c u u c u a u u g	3 T A T C T T C A G G T T C C G A A G A	19	132	1,7%	3 C T T O G G A A C C T G A A G A T T G A C G	22	135	1,7%
a u a g g a guide a a a u c			total	21,5%			total	68,1%
3' a a a u c								

**Table S1. miC processing in mice brain.** Sequence distribution (%) of guide- and passenger strands of reads mapping to miC32 and miC46. RNA was isolated from striatum of mice that were injected with AAV5-miC32 and AAV5-miC46 and small RNA NGS was performed. The scaffold is shown in the first column. Based on miRBase, the predicted guide and passenger strand sequences of the cellular pri-miRNA scaffolds are indicated in red and blue, respectively. The 5' and 3' flanking nucleotides are indicated in black. The three most abundant guide and passenger strand sequences are shown.