

COMPOSITIONS AND METHODS FOR TREATING NEURODEGENERATIVE DISEASES

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text
5 format in lieu of a paper copy, and is hereby incorporated by reference into the
specification. The name of the text file containing the Sequence Listing is
630264_401WO_SEQUENCE_LISTING_ST25.txt. The text file is 651 KB, was
created on February 4, 2021, and is being submitted electronically via EFS-Web.

BACKGROUND

10 Ataxin-2 (ATXN2) protein is a cytoplasmic protein that is a component
of stress granules. Stress granules are thought to be transient subcellular compartments
induced by arrest of protein translation, and include a number of proteins known to be
mutated in subjects with neurodegenerative disease (Brown and Al-Chalabi, *N Engl J*
Med (2017) 377:162-172). Ataxin-2 contains a sequence of glutamine residues, known
15 as a polyglutamine repeat, that in normal individuals is ~22 amino acids in length.
Expansions of this polyglutamine repeat to a length of 34 or longer is found in
individuals with a neurodegenerative disease Spinocerebellar Ataxia-2 (SCA2). This
disease is characterized by progressive death of Purkinje neurons in the cerebellum and
other neuronal cell types. Patients with Spinocerebellar Ataxia-2 develop ataxia,
20 sensory problems, and other clinical features, which worsen over time. Moderate
expansion of Ataxin-2 polyglutamine repeat, which are longer than that observed in
most individuals but that are shorter than those typically observed in subjects with
Spinocerebellar Ataxia-2 (e.g., between 27 and 33 glutamine residues), have been
reported at a substantially elevated frequency in individuals with the motor neuron
25 disease amyotrophic lateral sclerosis (ALS) as compared to normal subjects (Elden et
al., *Nature* (2010) 466:7310). This suggests that these polyglutamine repeats of
intermediate length, *i.e.*, between those found in normal individuals and those found in

spinocerebellar ataxia-2 patients, increase risk for ALS. Currently, treatment options for SCA2 and ALS are limited.

BRIEF SUMMARY

Aspects of the disclosure relate to compositions and methods for
 5 modulating expression of genes associated with spinocerebellar ataxia-2 (SCA2),
 amyotrophic lateral sclerosis (ALS), and conditions associated with TDP-43
 proteinopathies. In particular, inhibitory nucleic acids are provided that are useful for
 inhibiting expression or activity of ataxin 2 (ATXN2). For example, inhibitory nucleic
 acids are provided that target one or more isoforms of ATXN2, e.g., a subset of ATXN2
 10 isoforms, or all ATXN2 isoforms.

In one aspect, the disclosure provides an isolated nucleic acid molecule
 comprising an expression construct encoding an inhibitory nucleic acid that inhibits
 expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide
 strand sequence comprising the nucleic acid sequence set forth in any one of SEQ ID
 15 NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48,
 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94,
 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132,
 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166,
 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200,
 20 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234,
 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268,
 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302,
 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338,
 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372,
 25 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406,
 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288,
 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209..

In some embodiments, the inhibitory nucleic acid is a siRNA duplex,
 shRNA, miRNA, or dsRNA.

In some embodiments, the inhibitory nucleic acid further comprises a passenger strand sequence, optionally wherein the passenger strand sequence is selected from Tables 1, 19, 23, and 24, or a passenger strand sequence selected from Tables 1, 19, 23, and 24, and having 1-10 insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof.

In some embodiments, the inhibitory nucleic acid is an artificial miRNA wherein the guide strand sequence is contained within a miRNA backbone sequence.

In some embodiments, the guide strand sequence and passenger strand sequence of the artificial miRNA are contained within a miRNA backbone sequence.

In some embodiments, the miRNA backbone sequence is a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-16-2 backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

In some embodiments, the inhibitory nucleic acid is a miRNA comprising the nucleic acid sequence set forth in any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

In some embodiments, the nucleic acid sequence encoding the inhibitory nucleic acid is located in an untranslated region of the expression construct. In some embodiments, the untranslated region is an intron, a 5' untranslated region (5'UTR), or a 3' untranslated region (3'UTR).

In some embodiments, the isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid further comprises a promoter. In some embodiments, the promoter is a RNA pol III promoter (e.g., U6, H1, etc.), a chicken-beta actin (CBA) promoter, a CAG promoter, a H1 promoter, a CD68 promoter, a human synapsin promoter, or a JeT promoter. In some embodiments, the promoter is an H1 promoter comprising nucleotides 113-203 of SEQ ID NO:1522, nucleotides 1798-1888 of SEQ ID NO:1521, nucleotides 113-343 of SEQ ID NO:2257, or nucleotides 244-343 of SEQ ID NO:2257.

In some embodiments, the expression construct is flanked by a 5' adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence, or variants thereof. In some embodiments, one of the ITR sequences lacks a functional terminal resolution site. In some embodiments, the ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVrh10, AAV11, and variants thereof. In some embodiments, the 5' ITR comprises nucleotides 1-106 of SEQ ID NO:2257 and the 3' ITR comprises nucleotides 2192-2358 of SEQ ID NO:2257.

In another aspect, the disclosure provides a vector comprising the isolated nucleic acid as provided in the present disclosure. In some embodiments, the vector is a plasmid or viral vector. In some embodiments, the viral vector is a recombinant adeno-associated virus (rAAV) vector or a Baculovirus vector. In some embodiments, the vector is a self-complementary rAAV vector. In some embodiments, the vector (e.g., rAAV vector) further comprises a stuffer sequence. In some embodiments, the stuffer sequence comprises nucleotides 348-2228 of SEQ ID NO:1522 or nucleotides 489-2185 of SEQ ID NO:2257. In some embodiments, the vector (e.g., rAAV vector) comprises the nucleotide sequence of any one of SEQ ID NOS:2257-2260.

In another aspect, the disclosure provides a recombinant adeno-associated (rAAV) particle comprising the isolated nucleic acid molecule or rAAV vector as provided in the present disclosure. In some embodiments, the rAAV particle comprises a capsid protein. In some embodiments, the capsid protein is capable of

crossing the blood-brain barrier. In some embodiments, the capsid protein is an AAV9 capsid protein or AAVrh.10 capsid protein. In some embodiments, the rAAV particle transduces neuronal cells and/or non-neuronal cells of the central nervous system (CNS).

5 In another aspect, the disclosure provides a pharmaceutical composition comprising the isolated nucleic acid as provided in the present disclosure, the vector as provided in the present disclosure, or the rAAV particle as provided in the present disclosure, and optionally a pharmaceutically acceptable carrier.

In another aspect, the disclosure provides a host cell comprising the
10 isolated nucleic acid as provided in the present disclosure, the vector as provided in the present disclosure, or the rAAV particle as provided in the present disclosure.

In another aspect, the disclosure provides method for treating a subject having or suspected of having a neurodegenerative disease, the method comprising administering to the subject the isolated nucleic acid molecule as provided in the
15 present disclosure, the vector as provided in the present disclosure, the rAAV particle as provided in the present disclosure, or the pharmaceutical composition as provided in the present disclosure. In some embodiments, the administration comprises direct injection to the CNS of the subject. In some embodiments, the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection subpial
20 injection, or any combination thereof. In some embodiments, the direct injection is direct injection to the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or intralumbar injection. In some embodiments, the subject is characterized as having an ATXN2 allele having at least 22 CAG trinucleotide repeats, optionally wherein the ATXN2
25 allele has at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats. In some embodiments, the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy,
30 chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal

degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

In another aspect, the disclosure provides a method of inhibiting ATXN2 expression in a cell, the method comprising delivering to the cell the isolated nucleic acid molecule as provided in the present disclosure, the vector as provided in the present disclosure, the rAAV particle as provided in the present disclosure, or the pharmaceutical composition as provided in the present disclosure. In some embodiments, the cell has an ATXN2 allele having at least 22 CAG trinucleotide repeats, optionally wherein the *ATXN2* allele has at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats. In some embodiments, the cell is a cell in the CNS, optionally a neuron, glial cell, astrocyte, or microglial cell. In some embodiments, the cell is in vitro. In some embodiments, the cell is from a subject having one or more symptoms of a neurodegenerative disease. In some embodiments, the cell is from a subject having or suspected of having a neurodegenerative disease. In some embodiments, the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

In another aspect the present disclosure provides a method of inhibiting ATXN2 expression in the central nervous system of a subject, the method comprising administering to the subject the isolated nucleic acid molecule as provided in the present disclosure, the vector as provided in the present disclosure, the rAAV particle as provided in the present disclosure, or the pharmaceutical composition as provided in the present disclosure. In some embodiments, the administration comprises direct injection to the CNS of the subject. In some embodiments, the direct injection is intracerebral

injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, or any combination thereof. In some embodiments, the direct injection is injection to the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or

5 intralumbar injection. In some embodiments, the subject has an *ATXN2* allele having at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

In another aspect, the present disclosure provides an artificial miRNA comprising a guide strand sequence and a passenger strand sequence, wherein the guide

10 strand sequence comprises any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180,

15 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352,

20 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the guide strand sequence and passenger strand

25 sequence are contained within a miR backbone sequence. In some embodiments, the miR backbone sequence is a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-16-2 backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M

30 backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence,

a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

5 In some embodiments, the artificial miRNA comprises a sequence as set forth in any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170,
10 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

In another aspect, the present disclosure provides an isolated RNA duplex comprising a guide strand sequence and a passenger strand sequence, wherein the guide strand sequence comprises the nucleic acid sequence set forth in any one of
15 SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196,
20 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368,
25 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, and 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, optionally wherein the guide strand sequence and passenger strand sequence are linked by a loop region to form a hairpin structure comprising a duplex structure and a loop region. In
30 some embodiments, the loop structure comprises from 6 to 25 nucleotides.

In another aspect, the disclosure provides a kit comprising a container housing a composition as described by the present disclosure.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 shows tuning mean squared error for mir-30 dataset (Pelossof et al., Nature Biotechnology (2017) 35:350-353). Data showing the mean squared error (MSE) for prediction performance of the shRNA prediction algorithm on a set of shRNAs targeting Kras, held out from a data set of shRNAs used to train the support vector machine model to predict shRNA performance. Mean squared error is calculated as the square of the difference between the score of the support vector machine (SVM) predictor and the label 1 or -1, corresponding to shRNAs empirically determined to yield good knockdown or poor knockdown. These squared differences are averaged across shRNAs tested. The hyperparameter c was varied and the mean squared errors calculated for each value c .

FIG. 2 shows a plot of precision vs recall for SVM model applied to held-out shRNAs targeting Trp53 gene, after training on the shRNAs targeting the other genes in the TILE dataset (Pelossof et al, Nature Biotechnology (2017) 35:350-353). Horizontal line at approximately 0.19 represents the fraction of shRNAs that are positive, i.e., yielding good knockdown, out of the total number of shRNAs, in the set of all shRNAs targeting Trp53. The precision-recall line represents, varying across values of the SVM score, the fraction of true positives that are included in the dataset ('recall'), versus the fraction of true positives relative to false positives ('precision'), at a given SVM score cutoff. Thus, at the least stringent SVM score, all true positives are included (recall = 1), but precision is low because many negative shRNAs are included.

FIG. 3 shows two curves are plotted against SVM score. In one, the cumulative fraction of positive shRNAs that are expected to be lost as the classifier score is increased is shown. This is denoted by the bold line. In the other, the percent improvement in rejection of low-performing shRNAs is shown. This is denoted by the lighter line. Vertical dashed lines, from left to right, represent the 25th percentile (light

dashed) and 50th percentile (bold dashed) of SVM scores in the dataset, the shRNAs targeting Trp53.

FIG. 4 shows jitter plots of the distribution of SVM score predictions as a function of the first base of the guide sequence of the shRNA sequences targeting ATXN2. All data points are shown; the horizontal width of the ‘violin’ is proportional to the number of points at each SVM score, which is plotted on the y axis. On the left, the score is calculated for guide sequences that are perfectly complementary to the ATXN2 sequence (guide sequence base at position 1 is A, U, C, or G). On the right, the score is calculated if the first base is converted to U (edit guide sequence base at position 1 to U if guide at position 1 does not natively begin with U). Note that guide sequences which originally begin with U will have the same score in the right plot, whereas sequences which begin with A, G, or C will have different scores. In general, the SVM score increases if the first base is U.

FIG. 5 shows a plot of ATXN2 quantigene assay values across a panel of commonly used cell lines. Signal is reported with 30 μ L (left bar) or 10 μ L (right bar) of lysate. “-” represents negative control with no cellular material. Y-axis is the assay signal. Additional horizontal line represents the minimal signal selection criterion.

FIGS. 6A-6B show a ‘Sashimi’ plot of the alternative splicing of Ataxin-2 transcript from human brain or from HepG2.5 cells. **FIG. 6A:** For brain, representative plots from two different individuals are shown. The height of the bars in the plot represents the number of reads aligning to the position in Ataxin-2, according to the diagram underneath the plot. Numbers on curved arcs represent the count of reads aligning across exon-exon junctions. In junctions where the arcs are on both top and bottom, this indicates potential alternative splicing of the transcript. Arrows point out exons subject to substantial alternative splicing, such that these exons do not appear in a substantial number of ATXN2 transcripts in human brain. The diagram at the bottom of the plot represents the structure of the transcript ENST00000377617.7, with exons as solid rectangles. The transcript is oriented from right to left, with exon 1 on

the right. **FIG. 6B:** Similar data from HepG2 cells is shown. The alignment to the transcript is not to scale.

FIG. 7 shows *ATXN2* mRNA values across tested siRNAs, at 20 nM, 1 nM, and 200 pM doses. The x-axis shows the position of the *ATXN2* sequence (SEQ ID NO: 2) that the corresponding siRNA is complementary to. *ATXN2* mRNA values represents the ratio of *ATXN2* to *GAPDH* signal from quantigene assay, normalized to mock control. 3' UTR on the X-axis shows the general position of the 3' untranslated region of the *ATXN2* transcript.

FIG. 8. Correlation plot of the *ATXN2* mRNA knockdown (ratio of *ATXN2* to *GAPDH* signal, normalized to mock transfected controls), versus the SVM score. The expected correlation is observed, indicating that high SVM scores predict good knockdown performance.

FIG. 9. Plot of *ATXN2* signal from *ATXN2* siRNA treated U2OS cells, derived from indirect immunofluorescence, for the indicated conditions. XD-ID Nos represent treatment with different siRNAs corresponding to **Table 1**, at the indicated dose (20 nM (top) or 1 nM (bottom)). Other treatments are indicated as follows: “no_primary_secondary” = during antibody staining, the Ataxin-2 primary antibody was omitted, secondary antibody was included; “no_primary_no secondary” = during antibody staining, both the Ataxin-2 primary antibody and the secondary fluorescent antibody against the Ataxin-2 antibody were omitted; “primary_no secondary” = during antibody staining, the Ataxin-2 primary antibody was included but the secondary fluorescent antibody against the Ataxin-2 antibody was omitted; “SMP” = a pool of 4 siRNAs targeting *ATXN2*, with chemically modified nucleotides, obtained from Dharmacon; “primary_secondary” = untreated cells stained with primary and secondary antibody; “NTC” = cells treated with a ‘non-targeting control’ siRNA, not expected to target any human transcripts, with chemically modified nucleotides, obtained from Dharmacon; “XD-LucControl” = an siRNA, comprised only of RNA bases as in the *ATXN2* targeting siRNAs, expected to target the luciferase gene but not to target *ATXN2*. In the plot, each point represents signal averaged across all cells in a well.

Outliers, which were excluded from calculation of mean knockdown across wells in **Tables 6 and 7**, are shown as lighter colored points.

FIGS. 10A-10B show representative images of *ATXN2* siRNA treated U2OS cells as described in FIG. 9. **FIG. 10A:** Representative images of siRNA (20 nM) treated U2OS cells. Top panels, Hoechst staining demarcates cell nuclei. Bottom panels, *ATXN2* indirect immunofluorescence. Treatment/staining procedure is shown below image panels. **FIG. 10B:** As in FIG. 10A, but for U2OS samples treated with siRNAs at 1 nM.

FIG. 11 shows a plot of normalized *ATXN2* indirect immunofluorescence signal, as a function of position along *ATXN2* transcript (SEQ ID NO: 2). The x-axis is restricted to the positions along the *ATXN2* transcript spanning the binding sites of the tested siRNAs.

FIGS. 12A-12C show dose response of various siRNAs tested. **FIG. 12A (top)** shows a plot of log IC₅₀ across siRNA IDs tested in Group 1. Bars represent span of 95% confidence interval for IC₅₀ values. **FIG. 12A (bottom)** shows representative dose response curves for siRNAs. Y-axis represents ratio of *ATXN2* to *GAPDH* signal from quantigene assay of mRNA levels, from lysates of HepG2 cells dosed with indicated concentration of siRNA. Fits represents 3-parameter logistic regression fits, with Hill slope set constant at 1. Outliers were automatically identified, excluded from curve fitting and IC₅₀ estimation. **FIG. 12B** shows a plot of log IC₅₀ across siRNA IDs tested in Group 2. Bars represent span of 95% confidence interval for IC₅₀ values. **FIG. 12C** shows a representative dose response curves for siRNAs. Y-axis represents ratio of *ATXN2* to *GAPDH* signal from quantigene assay of mRNA levels, from lysates of HepG2 cells dosed with indicated concentration of siRNA. Fits represents 3-parameter logistic regression fits, with Hill slope set constant at 1. Outliers were automatically identified, excluded from curve fitting and IC₅₀ estimation, and are indicated on graph.

FIG. 13 shows predicted folding patterns of guide sequences embedded in miRNA backbones, as created using the web-based server mfold. Multiple fold predictions are obtained; a representative fold is shown. Note the unpaired, 'bulged'

nucleotides at several positions in each miRNA in the vicinity of the guide sequence, except in the 'sealed' variant.

FIG. 14 shows fluorescence automated cell sorting data demonstrating reduction in signal intensity for a GFP (stop) – *ATXN2* reporter construct-expressing U2OS cell line by artificial miRNAs. Cells were transfected with vectors containing inserts either including the guide sequence of XD-14792 (SEQ ID NO:112), or control guide sequences, embedded in miRNA backbones. Y-axis plots the median fluorescence intensity of cells within each replicate. Replicates derive from wells of a 96-well plate containing cells that were transfected with vectors. The cells were dissociated with trypsin prior to FACS analysis.

FIG. 15 shows thresholding procedure to distinguish transduced from untransduced cells in imaging experiments using lentivirally packaged *ATXN2*-specific artificial miRNAs. Lentiviral vectors (similar to pLVX-EF1A_mCherry-miR-1-1-XD_14890-WPRE_CMV (SEQ ID NO:546)) express mCherry and so identification of mCherry expression distinguishes transduced from untransduced cells. Left panel shows histogram of signal in the fluorescence channel used to detect mCherry signal (including indirect immunofluorescence from an anti-mCherry antibody and fluorescent secondary antibody). Right panel shows histogram of signal from cells transduced with mCherry-encoding vector, with a clear bimodal distribution of signals representing untransduced cells (low signal) and transduced cells (high signal). Vertical line shows threshold used to separate mCherry positive from mCherry negative cells, placed such that no untransduced cells exceed this signal threshold and such that the large majority of the right peak of the bimodal histogram of mCherry signal in transduced cells exceeds this threshold.

FIG. 16 shows *ATXN2* signal normalization procedure for artificial miRNA high content imaging assay. Each point represents signal in the channel used to detect indirect immunofluorescence for *ATXN2*, average across cells in the well. *ATXN2* knockout cells were used to determine the background levels of indirect immunofluorescence for the *ATXN2* antibody. The different cell types and staining conditions are shown, with the y-axis normalized with 100% set to the signal from

wild-type, untransduced cells and 0% set to the signal from untransduced *ATXN2* knockout cells. The signal in the *ATXN2*-antibody stained *ATXN2* knockout cells somewhat exceeds signal from cells not stained with antibody, indicating that there is some background associated with the antibody and that using the *ATXN2* knockout can help correct for this background to improve accuracy in measuring *ATXN2* protein signal.

FIGS. 17A-17B show plots of *ATXN2* signal from wells transduced with lentiviral vectors expressing guide sequences (shown on x-axis) embedded in miRNA backbones (miR-155E - **FIG. 17A**; miR1-1 - **FIG. 17B**). Guide sequences and miRNA context sequences are listed in Table 11.

FIG. 18A-18B show representative images of Hoechst 33342 stain (top row), mCherry signal (middle row), and *ATXN2* indirect immunofluorescence signal (bottom row) from cells as quantified in FIG. 17. **FIG. 18A** shows data for guide sequences embedded in miR-155E backbone; **FIG. 18B** shows data for guide sequences embedded in miR1-1 backbone.

FIG. 19 shows a plot of *ATXN2* protein signal from miRNA-embedded anti-*ATXN2* guide sequences versus *ATXN2* mRNA signal from anti-*ATXN2* siRNA treatment. There is correlation between the mRNA and protein knockdown across conditions tested.

FIGS. 20A-20C show validation of CRISPR guide RNAs in disrupting Ataxin-2 gene and knocking out Ataxin-2 protein in U2OS cells. **FIG. 20A** shows western blot analysis of U2OS cells nucleofected with *ATXN2* – targeting CRISPR gRNAs, complexed with Cas9 protein. Treatments include no nucleofection controls, control guide RNAs targeting CD81 or expected to be non-targeting, and five unique *ATXN2* targeting guides. Immunoblots against Ataxin-2 protein and alpha-tubulin loading control are shown. **FIG. 20B** shows representative histograms and **FIG. 20C** shows median fluorescence intensity within treated wells of Ataxin-2 indirect immunofluorescence signal for cells nucleofected with indicated treatments, as in FIG. 20A.

FIGS. 21A-21B show U2OS ATXN2 knockout clones generated for assay calibration. **FIG. 21A** shows *ATXN2* U2OS knockout cell line generation scheme. **FIG. 21B** shows western blot analysis from clonal lines generated after nucleofection with indicated *ATXN2* targeting gRNA. The lane containing protein from
5 lysed material from the clone (clone 43) selected for use is indicated by the arrow.

FIG. 22 show knockdown of Ataxin-2 protein *in vivo* after AAV vectorized amiRNA delivery. AAV encoding miRNAs XD-14792 or XD-14887, embedded in the miR-1-1 backbone, or a control construct lacking a miRNA, was
10 delivered intravenously to adult wild-type mice by tail vein injection. 15 days after injection, animals were euthanized and livers were harvested and snap-frozen. GFP fluorescence, resulting from vector encoded GFP, was detected in the liver upon blue light illumination. **FIG. 22 (left)**: Liver lysate was immunoblotted for Ataxin-2, beta-actin, and GFP (not shown). Each lane is derived from a different animal. **FIG. 22**
15 **(right)**: Ataxin-2 signal was normalized to beta-actin signal. All miRNA-dosed animals had lower Ataxin-2 signal than animals dosed with control AAV vector. Each point represents ratio of Atxn2 to Beta actin signal from an individual animal.

FIGS. 23A-23B show quality metrics of pooled library screen of Atxn2-targeting miRNAs ("Deep Screen 1"). **FIG. 23A** shows a scatter plot comparing ratios
20 of high- and low- sorted samples in two replicates, showing tight correlation. **FIG. 23B** shows correlation matrix between all samples tested. Spearman correlation was calculated between guide sequence count vectors between all samples.

FIG. 24 shows ratio baseline subtraction procedure. Raw count ratios (log-base 2 transformed) are shown on x-axis, for top, *ATXN2*-targeting miRNAs, and
25 bottom, scrambled miRNAs. For subsequent calculations, the median of the ratio for the scrambled miRNAs was subtracted.

FIG. 25 shows a plot of *ATXN2* signal depletion versus cell depletion. Each point represents a library element, containing a miRNA targeting either the *ATXN2* transcript; a scrambled sequence; or a sequence targeting an essential gene and
30 expected to reduce cell proliferation and/or viability. The x-axis is the average across replicates of the ratio of sequence counts derived from cells in the high- and low-*ATXN2* FACS gate populations. The y-axis is the average across replicates of the ratio

of sequence counts derived from HeLa cells after initial transduction and after 16 days. Points falling toward the bottom of the axis represents elements that were depleted from the 16 day timepoint relative to the initial transduction timepoint.

FIG. 26 shows a plot of *ATXN2* signal depletion versus position on *ATXN2* transcript of complementarity of guide sequence. Points toward the bottom represent guide sequences with greater knockdown of *ATXN2*; points toward the top of the y-axis represent guide sequences with less knockdown of *ATXN2*.

FIG. 27 shows a similar plot as in FIG. 26, but zoomed-in on the 3' end of the *ATXN2* transcript. In black are sequences deemed part of 'hotspots' in the 3' UTR of the *ATXN2* transcript.

FIG. 28 shows the percent of reads, averaged across scrambled guide sequences, that match to a guide sequence excised from the pri-miRNA at the indicated position. The diagram above shows an example sequence, where the bold text to the left is miR backbone sequence and the regular text is the guide sequence. Arrows and numbers indicated cleavage position (for the tiled screened described here, in the miR 16-2 backbone, Droscha is the expected enzyme for this cleavage event). The seed sequence for a guide sequence cut at the expected position is shown. The position of this seed sequence will shift if the guide position is cut out of the pri-miRNA at a different position from the expected position.

FIG. 29 shows representative images used in assessing the production of motor neurons in the stem cell differentiation protocols. Upper left image shows overlay of indirect immunofluorescence signal from anti-HB9 and anti-Beta 3 tubulin (TUJ1) antibodies. Upper right shows overlay of signal from anti-ISLET1 and TUJ1 signal. Lower left shows overlay of HB9, ISLET1, and TUJ1 signal. Bottom right shows overlay of HB9, ISLET1, TUJ1, and nuclear DAPI stain. In the images, neuronal processes are clearly seen as labeled by TUJ1 antibody. Neuronal nuclei are labeled by the motor neuron markers HB9 and Islet1, with 25-35% of neurons labeled with HB9, 50-60% labeled by Islet1, and 70-80% of cells positive for TUJ1 signal.

FIG. 30A-30C show data from an experiment testing knockdown of *ATXN2* mRNA and protein after transduction of *ATXN2*-targeting amiRNAs in lentiviral format in stem-cell derived motor neurons. **FIG. 30A** is a schematic of the cassette packaged in lentiviral vectors, with an H1 promoter driving the artificial

miRNA, followed by a Pol III termination signal (6T). After this miR expression cassette, a CMV Pol II promoter drives expression of the fluorescent reporter GFP, and is followed by a WPRE element to stabilize the GFP transcript. **FIG. 30B** shows data from qPCR against ATXN2 mRNA. Each dot represents a biological replicate derived from a distinct tissue culture well of motor neurons. Data represent average signal calculated from change in qPCR threshold (CT) for ATXN2 versus either GUSB or B2M. Bars are mean of replicates, and error bars are standard deviation across replicates. ATXN2 signal is normalized to levels measured from motor neurons growing in wells not treated with vector. Data from wells treated with a control lentiviral vector with the multiple cloning site (MCS) in place of the amiRNA is shown as "MCS." Two amiRNAs were tested, with amiRNAs targeting indicated position in ATXN2 transcript (1784 or 4402) indicated; amiRs were embedded in the miR16-2 backbone. The guide sequence targeting ATXN2 position 1784 is also referred to as XD-14792. Lentiviral vectors were dosed at two concentrations. The viral dose to achieve a multiplicity of infection (MOI) of 2.5 or 4.5 was calculated based on titration in U2OS cells (FACS analysis of GFP signal, calculating % cells positive for GFP). Using these values and the number of neurons plated per well, the corresponding dose of vector to achieve MOI of 2.5 or 4.5 in the motor neuron cultures (calculated based on the U2OS infectivity) was used. Observation of GFP fluorescence in cultures confirmed that transduction was near complete, as expected if the U2OS MOI was similar to the motor neuron MOI. **FIG. 30C** shows assessment of ATXN2 protein assessment from cultures treated the same as in FIG. 30B. The top panel shows the Western blot, with clear evidence of reduction in signal in lanes with protein from wells treated with amiRNAs targeting ATXN2 versus untreated wells or wells treated with the control MCS vector. Bottom panel quantifies ATXN2 immunoblot signal, with each point representing a biological replicate, the bars representing mean across replicates and the error bars standard deviation.

FIG. 31. Data is presented from an experiment performed similarly to that shown in FIG. 30. In this experiment, the MOI (as calculated by infectivity in U2OS cells) was 3.5. Knockdown in motor neurons treated with lentiviral vectors with miR 16-2 backbone-embedded amiRNAs targeting indicated ATXN2 transcript position is shown. Horizontal dashed line represents the threshold of 80% knockdown. In this

experiment, it is apparent that the amiRNAs targeting the *ATXN2* transcript in the 3' UTR do not yield the same level of knockdown as amiRNAs targeting the *ATXN2* coding sequence. Bars show mean knockdown, normalized to wells not treated with lentiviral vector; each point is a biological replicate (neurons from an individual well), and error bars are standard deviations across replicates. As above, MCS represents a lentiviral vector with a control multiple cloning site in place of a miR cassette.

FIG. 32. 2% agarose TAE gel demonstrating truncations in miR16-2 backbone-embedded amiRNAs packaged in AAV9. AAV genomic DNA was column purified and concentration quantified by Qubit fluorometer. Equal amounts of vector genome DNA, by Qubit measurement, were loaded into gel and subject to electrophoresis. Note that the gel image shown was spliced together for clarity. Leftmost lane is a DNA size ladder, with indicated DNA sizes in kilobases shown. From left to right, samples are (all DNA derived from purified AAV vector genomes): (1) H1 promoter driving miR1-1 XD-14792 (1784), followed by CBh promoter driving GFP; (2) H1 promoter followed by a non-miR multiple cloning site, followed by stuffer sequence "AMELY_V1"; (3 – 11) From left to right, AAV with amiRNAs targeting *ATXN2* at positions 1784, 1479, 1755, 3330, 4402, 4405, 4406, 4409, and 4502. Each lane has an amiRNA targeting *ATXN2*, in the same vector genome format as lane 2 replacing the MCS with the indicated miR cassette, with miR16-2 backbone. Note in all of the material from AAV genomes with miR16-2 backbone miR cassettes the presence of both an upper band, running at the intended size, as well as a faster migrating lower band.

FIG. 33A-33B. Data from Deep Screen 2 showing replicate to replicate consistency (**FIG. 33A**) and performance across miR backbones (**FIG. 33B**). In **FIG. 33A**, each point represents the relative abundance of a library element, with position on the x-axis representing the log₂ fold change in abundance between the 10th percentile *ATXN2* sort and unsorted cells from the first screen replicate, and the y-axis the corresponding log₂ fold change from the second screen replicate. Points on the far right of the graph represent data where the denominator in the ratio of sequence counts for sorted and unsorted cells is 0, and hence undefined when log-transformed. There is good correspondence between the replicates for elements exhibiting substantial knockdown (log₂ fold change < -1), but for inactive controls (including essential gene

targeting amiRNAs, 911 controls, and scramble controls), there is more variability from replicate to replicate in this screen compared to Deep Screen 1. As a result there is some deviation from screen replicate to replicate in the negative control medians. No baseline subtraction was done because of the agreement in $\log_2$ fold change values for active amiRNAs. In **FIG. 33B**, boxplots represent the ATXN2 knockdown performance across amiRNAs embedded in various miR backbones. In each boxplot, the center line is the median, the upper and lower edges of the box represent the 75th and 25th percentiles, and the line extends beyond the box edges to either the maxima/minima or 1.5 times the interquartile range (difference between 25th and 75th percentiles), whichever is closer to the median. Overlaying points (very faint, transparent) represent the ATXN2 knockdown signal from individual miRNAs. The y-axis represents the mean $\log_2$ fold-change between the abundance of sequencing reads of elements detected in the 10th percentile of ATXN2 signal relative to the abundance of the guide in unsorted cells. In this screen, the theoretical maximum fold-change is 10-fold between the 10th percentile sort and un-sorted cells.

FIG. 34. Depletion of essential-gene targeting amiRNAs in various miR backbones at a late timepoint T₁ (18 days after transduction) versus an early timepoint T₀ (1 day after transduction). The y-axis represents the $\log_2$ fold change in abundance between the two timepoints, and was not baseline subtracted. A similar ranking between the ‘performance’ of each miR backbone in inducing guide depletion over time, when expressing essential gene-targeting amiRNAs as in this figure, versus performance of miR backbones in ATXN2 knockdown when expressing ATXN2-targeting amiRNAs, as in **FIG. 33**, can be seen.

FIG. 35. Agarose gel with purified AAV vector genomes with various miR backbones, with amiRNA targeting Atxn2 at position 4402 (first 10) embedded, or targeting position 1784 (last 2; 1784 guide sequence is same as XD-14792). Note that image is spliced for clarity (to place lane including DNA size ladder immediately adjacent to relevant lanes). Some lanes have bands that both migrate differently than others (miR122, miR1-1-4402, miR-1-1XD14792), this is likely due to differences in loading or dye binding and not true migration differences. More importantly, across miR backbones there are differences in the relative intensity of the second most intense band, migrating farther than the most intense upper band which is the presumed

intended vector genome. AAV vector genomes with miR100 and miR128 backbones in particular have a less intense faster migrating band than others.

FIG. 36. Agarose gel with AAV vector genomes derived from pools of *cis* plasmids. Each pool includes elements generated by PCR amplification from an oligonucleotide pool containing a mixture of amiRs embedded in multiple miR backbones, and the PCR primers used do not distinguish between parent and “_M” form miR backbones. Thus, the pool labeled miR-1-1 will include amiRs in backbones miR-1-1 and miR-1-1_M; the pool labeled miR-100 will contain miR-100 and miR-100_M backbones; the pool labeled miR-190a will contain miR-190a and miR-190a_M backbones; the pool miR-124 will contain miR-124 and miR-124_M backbones; the pool miR-138-2 will contain miR-138-2 and miR-138-2_M backbones. miR-155M and miR-155E, though not related to each other by the “_M” modification rules, also have high sequence similarity and therefore the pool labeled “miR-155M” likely contains a mix of miR-155M and miR-155E backbones. Each lane contains purified vector genome DNA from AAV generated with indicated plasmid pool. The last lane is derived from a mixture of the 5 micropools shown in the gel as well as micropools with miR backbones miR-124, miR-128, miR-138-2, miR-144, and miR-155M. As in FIG. 35, the AAV pool with the miR-100 backbone (dashed box) has a less intense faster migrating band than the other AAV pools.

FIG. 37. Data from Deep Screen 2, only including elements with miR-100 or miR-100_M backbones. As in FIG. 33A, each point represents the relative abundance of a library element, with position on the x-axis representing the log2 fold change in abundance between the 10th percentile ATXN2 sort and unsorted cells from the first screen replicate, and the y-axis the corresponding log2 fold change from the second screen replicate.

FIG. 38. RT-ddPCR data demonstrating knockdown of ATXN2 mRNA in stem-cell derived motor neurons 7 days after treatment with scAAV-DJ vectors expressing ATXN2-targeting amiRNAs. Each point represents a biological replicate (a well of neurons treated with AAV at indicated dose of vector genomes per cell). Indicated amiRNAs, denoted as miR backbone – Atxn2 targeting position, mark x-axis. The amiRNAs were embedded in a self-complementary vector genome, with an H1 promoter driving the amiR, and a stuffer sequence modified from PSG11, “PSG11_V5”

(nucleotides 489-2185 of SEQ ID NO:2257) 3' of the miR cassette up to the wild-type ITR. The y-axis represents RT-ddPCR signal, with copies of each transcript per unit microliter derived from percentage of positive to negative droplets for primer/probesets specific to ATXN2, GUSB, or B2M. The points represent averages of ratios of

5 ATXN2/GUSB and ATXN2/B2M ratios.

FIG. 39. This graph shows a RT-ddPCR experiment similar to that in FIG. 38, except spanning a broader range of indicated doses. Because of constraints on the number of available cells, not all amiRNAs were treated with all doses. In this experiment, the ATXN2 mRNA level is calculated by ATXN2/B2M RT-ddPCR ratios.

10 **FIG. 40.** Images of stem-cell derived motor neurons treated with scAAV-DJ vector as in FIGS. 38 and 39. Cells were treated with a dose of 1E4 vector genomes per cell. Representative images of DAPI stain (to label cell nuclei), indirect immunofluorescence signal for anti-ISL1 antibody (to label motor neurons), and TUJ1 signal, to label neuronal processes. No obvious differences were seen in neuronal

15 processes between neurons treated with an active ATXN2-targeting amiRNA (1755) and an inactive (1755_911) amiRNA in scAAV-DJ. Panels at right (top) quantify total number of cells, defined by DAPI staining, and (bottom) quantify fraction of cells that are positive for ISL1. Each point represents average quantification across fields for a given well. Asterisks indicate significant ($p < .05$) difference versus vehicle (PBS +

20 0.001% PF-68) control, calculated by one-way ANOVA followed by Dunnett's multiple comparisons test. Vectors encode amiRNAs targeting indicated ATXN2 transcript position in miR100 or miR100_M backbone (Fig. 38 and 39 show which amiRNA is in miR100 and which is in miR100_M backbones). "PBS" represents wells of motor neurons treated with vehicle (PBS + 0.001% PF-68); GFP represents the

25 amiRNA and GFP expressing vector H1-miR1-1.XD-14792-CBh-GFP packaged in scAAV-DJ.

FIGS. 41A-41C. Similar to FIG. 40, **FIG. 41A** shows representative images of neuronal morphology across stem-cell derived motor neuron treated with indicated scAAV-DJ vector encoding specified amiRNA, embedded in miR100 or

30 miR100_M backbone vector. There is no readily apparent alteration in neuronal morphology for any treatment compared to vehicle. Total number of Hoechst+ nuclei

(FIG. 41B) and the % of total nuclei that are Isl1+ (FIG. 41C) in AAV treated stem-cell derived motor neurons was quantified.

FIG. 42. Shows ‘volcano plots’ of RNAseq data, comparing gene expression in neurons treated with active amiRNA versus their inactive, ‘9-11’ control counterparts. The 911 controls do not reduce *ATXN2* levels, but differ only by 3 nucleotides (bases 9, 10 and 11) from the active amiRNAs. Off-target effects of the amiRNAs not involving bases 9, 10 and 11 may therefore be conserved with the cognate non-911 control amiRNA, and the comparison can be considered to enrich the ‘on-target’ transcriptional impact of lowering *Atxn2* levels. By far the most robust transcriptional effect observed in comparisons of miR100_1755 and miR100_2945 versus their 911 controls is *ATXN2*. In the plots, each point represents a gene (counts for different transcripts are collapsed gene-wise); the y-axis represents the nominal p value; the x-axis the log₂ fold change for gene expression between conditions. Data is derived from n = 5-6 biological replicates per treatment. Neurons were treated with a dose of 1E4 vector genomes/cell, and RNA collected for RNAseq (quantseq) 7 days later.

FIG. 43. Panel of ‘volcano plots’ comparing each indicated amiR AAV treatment, with the same treatment conditions described as in FIG. 42, to all other amiRNA treatments shown (n = 6 replicates/condition). Axes are as in FIG. 42; horizontal dashed line represents the false discovery rate threshold of 10%. Here, what are plotted are predicted off-target transcripts (with detectable expression levels in this system) for each amiR, that is transcripts with complementarity to bases 2-18 of the guide sequence with 2 or fewer mismatches. For most of the amiRNAs, none or only very few of the predicted off-targets are downregulated relative to the set of other amiRNAs, and exceed the 10% false discovery rate threshold.

FIG. 44. Plot of *Atxn2* mRNA versus biodistribution of *ATXN2* amiRNA expressing vectors (miR1-1-1784 (left) and miR100-3330 (right)) from mice dosed intrastrially with vectors expressing indicated amiRNA AAV construct. Each point represents RT-ddPCR mRNA and vector distribution data from RNA and DNA isolated from an individual striatal biopsy, taking the average of *Atxn2*/Gusb and *Atxn2*/Tbp droplet ratios, normalized to vehicle treated animals. Multiple distinct

vector formats are included, all with one version of the H1 promoter and various stuffer sequences.

FIGS. 45A-45B. Plot of Taqman qPCR data from striatal biopsies of animals dosed with indicated amiRNA AAV constructs (miR1784 – **FIG. 45A**;
 5 miR3330 – **FIG. 45B**). For each striatal biopsy assessed, two data points are shown: the y-axis plots the CT threshold difference between amplification of cDNA from an exogenous amiR and an endogenous miR, miR124; or the difference between amplification of two endogenous miRs. The x-axis shows the (log-base-2 transform of)
 10 vector distribution data, as in FIG. 44. Dashed lines are linear fits. Note that the relationship between CT and expression is of a form similar to $\text{expression} \sim 2^{\text{CT}}$, consistent with the apparent linear relationship between CT difference and $\log_2$ (vector genomes/diploid genome).

FIG. 46. qPCR data (a subset of the data shown in FIG. 45) is plotted against small RNAseq quantification of exogenous amiR expression/total miR
 15 expression, for RNA deriving from the same striatal punch biopsies. The relationship between the delta CT of exogenous amiR versus endogenous miR and small RNAseq quantification is separately fit to a linear model (linear regression) for each of the indicated amiRs. The slope of fits for the qPCR versus small RNAseq for the two
 20 amiRs are similar, and the fits are good as quantified by residuals, R^2 .

FIG. 47. This graph shows use of the linear model in FIG. 46 to derive a predicted absolute amiR expression level, as a function of total miR expression, for the remaining samples that only had amiR expression measured by qPCR. This
 25 predicted amiR expression level is plotted on the x-axis. Each point represents an individual striatal punch biopsy. The y-axis represents the RT-ddPCR quantified Atxn2 mRNA level for that biopsy, same as in FIG. 44. A loess fit is used to separately fit a curve to data from biopsies from animals dosed with miR1-1.1784 expressing AAVs (black filled circles, dashed line); or miR100.3330 expressing AAVs (open diamonds; dotted line).

FIGS. 48A-48B. Liver enzyme data, alanine transaminase (ALT)(**FIG. 48A**) and aspartate aminotransferase (AST)(**FIG. 48B**) from blood collected from the
 30 submandibular vein, at 2 or 3 weeks after intravenous dosing of AAVs expressing indicated amiRs. Naïve animals were monitored in parallel.

FIG. 49. Plot of Atxn2 mRNA knockdown and vector distribution, as in Fig. 44, in striatal biopsies from animals dosed with AAVs expressing indicated amiRNAs. Lines represent loess (locally estimated scatterplot smoothing) fits for each series, implemented in R (stats::loess).

5 **FIGS. 50A-50B.** Expression of amiRNAs in tissue from animals dosed with AAVs expressing indicated amiRNAs. Liver tissue was analyzed from animals dosed intravenously (**FIG. 50A**); striatal tissue was analyzed from animals dosed via intrastriatal injection (**FIG. 50B**). amiRNA expression is plotted as normalized to total miRNA expression.

10 **FIG. 51.** Plot showing 5' end homogeneity of processed miRNAs in striatal tissue in animals dosed intrastriatally. The y-axis (log₁₀ scale) plots cumulative sequencing reads, across all samples (n = 4/AAV), for mature amiRNAs initiating at the 'expected' position 0, 5' of the expected start site (negative numbers) or 3' of the expected start site (positive numbers). For all of these amiRs, the vast majority of
15 mature processed amiRNAs initiate at the expected start site.

FIGS. 52A-52D. (Top) Diagrams of representative predicted folding structures (mfold) of amiRNAs miR100_1755 (**FIG. 52A**), miR100_2586 (**FIG. 52B**), miR100_2945 (**FIG. 52C**), and miR100_3330 (**FIG. 52D**), embedded in miR100 backbone. Arrow indicates typical start position of processed miRNA guide strand.
20 (Bottom) Observed small RNAseq sequencing reads. On the left are observed sequences, on the right the number of observations across all samples (n = 3-4 liver, n = 6 striatal biopsy). Note that the sequence reads are DNA, and in the corresponding miRNA the sequence would be generated by substituting "U" bases for "T" in the reads. A small number of sequences were fusions between the amiR and endogenous
25 miRs, but these are considered to be artifacts of the ligation reaction during the small RNAseq procedure and were excluded. By comparing the observed sequences to the pri-miRNA sequence on top, it. An be noted that in some cases 3' modifications are occurring, such as addition of 'A' or 'U' bases ('T' in the DNA sequencing reads) at the 3' terminus of the amiRNA.

30 **FIGS. 53A-53C** show knockdown of Ataxin-2 protein *in vivo* after AAV9 vectorized miRNA delivery into cerebrospinal fluid. As in FIG. 22, AAVs encoding miRNAs XD-14792 or XD-14887, embedded in the miR-1-1 backbone, or a

control construct lacking a miRNA, were dosed, in this case injected bilaterally intracerebroventricularly (ICV) in postnatal day 0 mice, 3 microliters per hemisphere. amiRNAs were expressed either under the control of the neuron-specific Synapsin promoter (as in nucleotides 1128-1575 of SEQ ID NO:622 or nucleotides 1128-1575 of SEQ ID NO:623), or the ubiquitous CAG promoter. Brain tissue (cortex) was harvested at indicated timepoint after injection. (FIG. 53A) Diagrams are shown of the expression cassettes used. (FIG. 53B) Representative immunoblot from Western analysis, similar to FIG. 22. Immunoblotting was performed against Ataxin-2, Beta-actin and GFP. For each treatment dose administered per hemisphere is listed, calculated by qPCR titering against the GFP region in the vector genome. In FIG. 53C, quantification of signal intensity of Atxn2 protein or GFP protein, normalized to total protein signal intensity (Revert 700, Licor), are shown. Atxn2 signal is scaled to the average of CAG-MCS and SYN-MCS controls at the indicated times, and GFP signal is scaled to the GFP maximum for the 4 week timepoint or to the average GFP signal of multiple CAG-MCS vector IV dosed liver samples that were loaded onto each Western blot for the 8 week timepoint. Each point represents data from an individual cortex (from a single animal), averaging across technical replicates. Error bars show standard deviation across technical replicates. A reduction in Atxn2 levels relative to control AAV vectors (MCS) is apparent for CAG vectors expressing the XD-14792 miR at 4 and 8 week timepoints, and for the 8 week timepoint for vectors with the Synapsin promoter.

FIGs. 54A-54B show representative immunofluorescence micrographs of tissue sections of cortex and cerebellum from animals dosed i.c.v. with AAV9 control or amiRNA vectors expressing (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133), as in FIG. 53. Red corresponds to indirect immunofluorescence signal for anti-Atxn2 antibodies; Green to anti-GFP signal; and blue are nuclei (Dapi stained). In FIG. 54A, presumptive layer 5 cortical pyramidal neurons are seen, with apical dendrites projecting up in the image. Intensity from the GFP reporter is present in neurons, which are likely transduced with the AAV. On the left, GFP-expressing neurons in the animal transduced with the control amiRNA also have strong Atxn2 (red) signal, and neurons can be clearly seen with both GFP and Atxn2 signal. On the right, which corresponds to an image of tissue from an animal dosed with an ATXN2

amiRNA (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133) expressing vector, by contrast, neurons with strong GFP intensity do not also have strong Atxn2 intensity, and overall the number of neurons with strong Atxn2 signal appears to be reduced. **FIG. 54B** shows similar results as FIG. 54A, but captures Purkinje cells in the cerebellum.

5 On the right, the image shows Cerebellar tissue from an animal injected with Atxn2 amiRNA (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133) expressing vector. GFP labeled, AAV transduced Purkinje cells do not have strong Atxn2 signal, whereas Purkinje cells lacking GFP transduction have strong Atxn2 expression. By contrast, on the left, which corresponds to an image from an animal dosed with control vector, cells

10 with GFP signal also have Atxn2 signal.

DETAILED DESCRIPTION

Expansions of *ATXN2* polyglutamine repeat to a length of 34 or longer causes spinocerebellar ataxia type 2 (SCA2). Moreover, intermediate length

15 polyglutamine expansions in *ATXN2* increase risk of ALS. Reduction of *ATXN2* levels has been demonstrated to have therapeutic benefit in animal models of spinocerebellar ataxia-2 and ALS. Knocking down the *ATXN2* protein using nucleic acid based therapies alleviates the progressive neurodegeneration that occurs in animal models expressing a variant of the human *ATXN2* containing an expanded polyglutamine

20 repeat. In an animal model of ALS, which overexpresses the TDP-43 protein, a component of the most common neuropathology found in patients with ALS, animals normally develop a progressive death of motor neurons. However, breeding these animals with *ATXN2* knock out mice dramatically increased survival time (Elden et al., Nature (2010) 466:7310). Similarly, reducing *ATXN2* protein levels by introducing

25 antisense oligonucleotide nucleic acids also increased survival of TDP-43 transgenic mice. Lowering *ATXN2* levels markedly increased lifespan and improved motor function in TDP-43 transgenic mice and decreased the burden of TDP-43 inclusions. *ATXN2* may modulate toxicity by affecting the aggregation propensity of TDP-43. TDP-43 proteinopathy has also been observed in a number of neurodegenerative

30 diseases, including ALS, FTD, primary lateral sclerosis, progressive muscular atrophy,

limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, and
5 Alzheimer's disease. Thus, reducing *ATXN2* levels may be useful for treating neurodegenerative diseases where *ATXN2* is a causative agent (*e.g.*, SCA2), as well as neurodegenerative diseases where *ATXN2* is not the causative agent but modifies TDP-43 pathological aggregation.

Aspects of the invention relate to inhibitory nucleic acids (*e.g.*, siRNAs, shRNAs, miRNAs, including artificial miRNAs) that when administered to a subject
10 reduce the expression or activity of Ataxin-2 in the subject. Accordingly, compositions and methods provided in the present disclosure are useful for the treatment of neurodegenerative diseases, including spinocerebellar ataxia type 2 (SCA2), amyotrophic lateral sclerosis (ALS), Alzheimer's frontotemporal dementia (FTD),
15 parkinsonism, and conditions associated with TDP-43 proteinopathies.

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

In the present description, any concentration range, percentage range,
20 ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless
25 otherwise indicated. As used herein, the term "about" means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have"

and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

As used herein, the term "nucleic acid" or "polynucleotide" refer to any nucleic acid polymer composed of covalently linked nucleotide subunits, such as polydeoxyribonucleotides or polyribonucleotides. Examples of nucleic acids include RNA and DNA.

As used herein, "RNA" refers to a molecule comprising one or more ribonucleotides and includes double-stranded RNA, single-stranded RNA, isolated RNA, synthetic RNA, recombinant RNA, as well as modified RNA that differs from naturally-occurring RNA by the addition, deletion, substitution, and/or alternation of one or more nucleotides. Nucleotides of RNA molecules may comprise standard nucleotides or non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides.

As used herein, "DNA" refers to a molecule comprising one or more deoxyribonucleotides and includes double-stranded DNA, single-stranded DNA, isolated DNA, synthetic DNA, recombinant DNA, as well as modified DNA that differs from naturally-occurring DNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides. Nucleotides of DNA molecules may comprise standard nucleotides or non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides.

"Isolated" refers to a substance that has been isolated from its natural environment or artificially produced. As used herein with respect to a cell, "isolated" refers to a cell that has been isolated from its natural environment (e.g., from a subject, organ, tissue, or bodily fluid). As used herein with respect to a nucleic acid, "isolated" refers to a nucleic acid that has been isolated or purified from its natural environment (e.g., from a cell, cell organelle, or cytoplasm), recombinantly produced, amplified, or synthesized. In embodiments, an isolated nucleic acid includes a nucleic acid contained within a vector.

As used herein, the term "wild-type" or "non-mutant" form of a gene refers to a nucleic acid that encodes a protein associated with normal or non-pathogenic

activity (e.g., a protein lacking a mutation, such as a repeat region expansion that results in higher risk of developing, onset, or progression of a neurodegenerative disease).

As used herein, the term “mutation” refers to any change in the structure of a gene, e.g., gene sequence, resulting in an altered form of the gene, which may be passed onto subsequent generations (hereditary mutation) or not (somatic mutation). Gene mutations include the substitution, insertion, or deletion of a single base in DNA or the substitution, insertion, deletion, or rearrangement of multiple bases or larger sections of genes or chromosomes, including repeat expansions.

As used herein, the term “Ataxin 2” or “ATNX2” refers to a protein encoded by the *ATXN2* gene, which contains a polyglutamine (polyQ, CAG repeat) tract. *ATXN2* gene or transcript may refer to normal alleles of *ATXN2*, which usually have 22 or 23 repeats, or mutated alleles having intermediate (~24-32 repeats) or longer repeat expansions (~33 to >100 repeats). In some embodiments, ATNX2 refers to mammalian ATNX2, including human ATNX2. In some embodiments, wild-type ATNX2 refers to a protein sequence of Q99700.2 as set forth in SEQ ID NO:1 or naturally occurring variants thereof. In some embodiments, wild-type *ATXN2* nucleic acid refers to a nucleic acid sequence of NM_002973.3 (SEQ ID NO:2), ENST00000377617.7, ENST00000550104.5, ENST00000608853.5, or ENST00000616825.4, or naturally occurring variants thereof.

As used herein, the term “inhibitory nucleic acid” refers to a nucleic acid that comprises a guide strand sequence that hybridizes to at least a portion of a target nucleic acid, e.g., *ATXN2* RNA, mRNA, pre-mRNA, or mature mRNA, and inhibits its expression or activity. An inhibitory nucleic acid may target a protein coding region (e.g., exon) or non-coding region (e.g., 5'UTR, 3'UTR, intron, etc.) of a target nucleic acid. In some embodiments, an inhibitory nucleic acid is a single stranded or double stranded molecule. An inhibitory nucleic acid may further comprise a passenger strand sequence on a separate strand (e.g., double stranded duplex) or in the same strand (e.g., single stranded, self-annealing duplex structure). In some embodiments, an inhibitory nucleic acid is an RNA molecule, such as a siRNA, shRNA, miRNA, or dsRNA.

As used herein, a “microRNA” or “miRNA” refers to a small non-coding RNA molecule capable of mediating silencing of a target gene by cleavage of the target

mRNA, translational repression of the target mRNA, target mRNA degradation, or a combination thereof. Typically, miRNA is transcribed as a hairpin or stem-loop (e.g., having a self-complementary, single-stranded backbone) duplex structure, referred to as a primary miRNA (pri-miRNA), which is enzymatically processed (e.g., by Drosha, DGCR8, Pasha, etc.) into a pre-miRNA. Pre-miRNA is exported into the cytoplasm, where it is enzymatically processed by Dicer to produce a miRNA duplex with the passenger strand and then a single-stranded mature miRNA molecule, which is subsequently loaded into the RNA-induced silencing complex (RISC). Reference to a miRNA may include synthetic or artificial miRNAs.

As used herein, a “synthetic miRNA” or “artificial miRNA” or “amiRNA” refers to an endogenous, modified, or synthetic pri-miRNA or pre-miRNA (e.g., miRNA backbone or scaffold) in which the endogenous miRNA guide sequence and passenger sequence within the stem sequence have been replaced with a miRNA guide sequence and a miRNA passenger sequence that direct highly efficient RNA silencing of the targeted gene (*see, e.g.*, Eamens et al. (2014), *Methods Mol. Biol.* 1062:211-224). In some embodiments, the nature of the complementarity of the guide and passenger sequences (e.g., number of bases, position of mismatches, types of bulges, etc.) can be similar or different from the nature of complementarity of the guide and passenger sequences in the endogenous miRNA backbone upon which the synthetic miRNA is constructed.

As used herein, the term “microRNA backbone,” “miR backbone,” “microRNA scaffold,” or “miR scaffold” refers to a pri-miRNA or pre-miRNA scaffold, with the stem sequence replaced by a miRNA of interest, and is capable of producing a functional, mature miRNA that directs RNA silencing at the gene targeted by the miRNA of interest. A miR backbone comprises a 5' flanking region (also referred to 5' miR context, ≥ 9 nucleotides), a stem region comprising the miRNA duplex (guide strand sequence and passenger strand sequence) and basal stem (5' and 3', each about 4-13 nucleotides), at least one loop motif region including the terminal loop (≥ 10 nucleotides for terminal loop), a 3' flanking region (also referred to 3' miR context, ≥ 9 nucleotides), and optionally one or more bulges in the stem. A miR

backbone may be derived completely or partially from a wild type miRNA scaffold or be a completely artificial sequence.

As used herein, the term “antisense strand sequence” or “guide strand sequence” of an inhibitory nucleic acid refers to a sequence that is substantially complementary (e.g., at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% complementary) to a region of about 10-50 nucleotides (e.g., about 15-30, 16-25, 18-23, or 19-22 nucleotides) of the mRNA of the gene targeted for silencing. The antisense sequence is sufficiently complementary to the target mRNA sequence to direct target-specific silencing, e.g., to trigger the destruction of the target mRNA by the RNAi machinery or process. In some embodiments, the antisense sequence or guide strand sequence refers to the mature sequence remaining following cleavage by Dicer.

As used herein, the term “sense sequence” or “passenger strand sequence” of an inhibitory nucleic acid refers to a sequence that is homologous to the target mRNA and partially or completely complementary to the antisense strand sequence or guide strand sequence of an inhibitory nucleic acid. The antisense strand sequence and sense strand sequence of an inhibitory nucleic acid are hybridized to form a duplex structure (e.g., forming a double-stranded duplex or single-stranded self-annealing duplex structure). In some embodiments, the sense sequence or passenger strand sequence refers to the mature sequence remaining following cleavage by Dicer.

As used herein, a “duplex,” when used in reference to an inhibitory nucleic acid, refers to two nucleic acid strands (e.g., a guide strand and passenger strand) hybridizing together to form a duplex structure. A duplex may be formed by two separate nucleic acid strands or by a single nucleic acid strand having a region of self-complementarity (e.g., hairpin or stem-loop).

As used herein, the term “complementary” refers to the ability of polynucleotides to form base pairs with each other. Base pairs are typically formed by hydrogen bonds between nucleotide subunits in antiparallel polynucleotide strands or a single, self-annealing polynucleotide strand. Complementary polynucleotide strands can form base pairs in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any

other manner that allows for the formation of duplexes. As apparent to skilled persons in the art, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenosine. Furthermore, when a "U" is denoted in the context of the present invention, the ability to substitute a "T" is understood, unless otherwise stated. Complementarity also encompasses Watson-Crick base pairing between non-modified and modified nucleobases (e.g., 5-methyl cytosine substituted for cytosine). Full complementarity, perfect complementarity or 100% complementarity between two polynucleotide strands is where each nucleotide of one polynucleotide strand can form hydrogen bond with a nucleotide unit of a second polynucleotide strand. % complementarity refers to the number of nucleotides of a contiguous nucleotide sequence in a nucleic acid molecule that are complementary to an aligned reference sequence (e.g., a target mRNA, passenger strand), divided by the total number of nucleotides and multiplying by 100. In such an alignment, a nucleobase/nucleotide which does not form a base pair is called a mismatch. Insertions and deletions are not permitted in calculating % complementarity of a contiguous nucleotide sequence. It is understood by skilled persons in the art that in calculating complementarity, chemical modifications to nucleobases are not considered as long as the Watson-Crick base pairing capacity of the nucleobase is retained (e.g., 5-methyl cytosine is considered the same as cytosine for the purpose of calculating % complementarity).

The "percent identity" between two or more nucleic acid sequences refers to the proportion nucleotides of a contiguous nucleotide sequence in a nucleic acid molecule that are shared by a reference sequence (*i.e.*, % identity = number of identical nucleotides/total number of nucleotides in the aligned region (e.g., the contiguous nucleotide sequence) x 100). Insertions and deletions are not permitted in the calculation of % identity of a contiguous nucleotide sequence. It is understood by skilled persons in the art that in calculating identity, chemical modifications to nucleobases are not considered as long as the Watson-Crick base pairing capacity of the nucleobase is retained (e.g., 5-methyl cytosine is considered the same as cytosine for the purpose of calculating % identity).

As used herein, the term “hybridizing” or “hybridizes” refers to two nucleic acids strands forming hydrogen bonds between base pairs on antiparallel strands, thereby forming a duplex. The strength of hybridization between two nucleic acid strands may be described by the melting temperature (T_m), defined as at a given ionic strength and pH, the temperature at which 50% of a target sequence hybridizes to a complementary polynucleotide.

As used herein, “expression construct” refers to any type of genetic construct containing a nucleic acid (e.g., transgene) in which part or all of the nucleic acid encoding sequence is capable of being transcribed. In some embodiments, expression includes transcription of the nucleic acid, for example, to generate a biologically-active polypeptide product or inhibitory RNA (e.g., siRNA, shRNA, miRNA) from a transcribed gene. In some embodiments, the transgene is operably linked to expression control sequences.

As used herein, the term “transgene” refers to an exogenous nucleic acid that has been transferred naturally or by genetic engineering means into another cell and is capable of being transcribed, and optionally translated.

As used herein, the term “gene expression” refers to the process by which a nucleic acid is transcribed from a nucleic acid molecule, and often, translated into a peptide or protein. The process can include transcription, post-transcriptional control, post-transcriptional modification, translation, post-translational control, post translational modification, or any combination thereof. Reference to a measurement of “gene expression” may refer to measurement of the product of transcription (e.g., RNA or mRNA), the product of translation (e.g., peptides or proteins).

As used herein, the term “inhibit expression of a gene” means to reduce, down-regulate, suppress, block, lower, or stop expression of the gene. The expression product of a gene can be a RNA molecule transcribed from the gene (e.g., an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

As used herein, “vector” refers to a genetic construct that is capable of transporting a nucleic acid molecule (e.g., transgene encoding inhibitory nucleic acid) between cells and effecting expression of the nucleic acid molecule when operably-linked to suitable expression control sequences. Expression control sequences may include transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. The vector may be a plasmid, phage particle, transposon, cosmid, phagemid, chromosome, artificial chromosome, virus, virion, etc. Once transformed into a suitable host cell, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself.

As used herein, “host cell” refers to any cell that contains, or is capable of containing a composition of interest, e.g., an inhibitory nucleic acid. In embodiments, a host cell is a mammalian cell, such as a rodent cell, (mouse or rat) or primate cell (monkey, chimpanzee, or human). In embodiments, a host cell may be in vitro or in vivo. In embodiments, a host cell may be from an established cell line or primary cells. In embodiments, a host cell is a cell of the CNS, such as a neuron, glial cell, astrocyte, and microglial cell.

As used herein, “neurodegenerative disease” or “neurodegenerative disorder” refers to diseases or disorders that exhibit neural cell death as a pathological state. A neurodegenerative disease may exhibit chronic neurodegeneration, e.g., slow, progressive neural cell death over a period of several years, or acute neurodegeneration, e.g., sudden onset or neural cell death. Examples of chronic, neurodegenerative diseases include Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, spinocerebellar ataxia type 2 (SCA2), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). Chronic neurodegenerative diseases include diseases that feature TDP-43 proteinopathy, which is characterized by nucleus to cytoplasmic mislocalization, deposition of ubiquitinated and hyper-phosphorylated TDP-43 into

inclusion bodies, protein truncation leading to formation of toxic C-terminal TDP-43 fragments, and protein aggregation. TDP-43 proteinopathy diseases include ALS, FTD, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy
5 bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease. Acute neurodegeneration may be caused by ischemia (e.g., stroke, traumatic brain injury), axonal transection by demyelination or trauma (e.g., spinal cord injury or multiple
10 sclerosis). A neurodegenerative disease may exhibit death of mainly one type of neuron or of multiple types of neurons.

As used herein, "subject," "patient," and "individual" are used interchangeably herein and refer to living organisms (e.g., mammals) selected for treatment or therapy. Examples of subjects include human and non-human mammals,
15 such as primates (monkey, chimpanzee), cows, horses, sheep, dogs, cats, rats, mice, guinea pigs, pigs, and transgenic species thereof.

Inhibitory Nucleic Acids

In one aspect, the disclosure provides isolated inhibitory nucleic acids
20 that inhibit expression or activity of Ataxin 2 (ATXN2). The inhibitory nucleic acid is a nucleic acid that specifically binds (e.g., hybridizes to) at least a portion of the *ATXN2* nucleic acid, such as an *ATXN2* RNA, pre-mRNA, mRNA, and inhibits its expression or activity. In some embodiments, the inhibitory nucleic acid is complementary to a protein coding region or non-coding region (e.g., 5'UTR, 3'UTR, intron, etc.) of
25 *ATXN2*. In some embodiments, the inhibitory nucleic acid is complementary to a wild type ATXN2 nucleic acid or a naturally occurring variant thereof. In some embodiments, the *ATXN2* gene encodes a polypeptide identified by NCBI Reference Sequence NP_002964.4 or NP_002964.3. In some embodiments, an *ATXN2* transcript comprises the sequence set forth in SEQ ID NO:2 or encodes an amino acid sequence
30 set forth in SEQ ID NO:1. In some embodiments, the *ATXN2* allele contains

approximately 22 CAG trinucleotide repeats. In some embodiments, the *ATXN2* allele has at least 22 CAG trinucleotide repeats, at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats. In some embodiments, the inhibitory nucleic acid is
 5 single stranded or double-stranded. In some embodiments, the inhibitory nucleic acid is a siRNA, shRNA, miRNA, or dsRNA.

In some embodiments, the inhibitory nucleic acid is capable of inhibiting expression or activity of *ATXN2* by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%,
 10 at least 70%, at least 80%, at least 90% at least 95% or more in a cell compared to the expression level of *ATXN2* in a cell that has not been contacted with the inhibitory nucleic acid. In some embodiments, the inhibitory nucleic acid is capable of inhibiting expression or activity of *ATXN2* by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-95%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%,
 15 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% compared to the expression level of *ATXN2* in a cell that has not been
 20 contacted with the inhibitory nucleic acid. Methods of measuring *ATXN2* expression, e.g., levels of RNA, mRNA polypeptides, are known in the art including those described herein.

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any
 25 one of the guide sequences in Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100,
 30 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136,

138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170,
 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204,
 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238,
 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272,
 5 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306,
 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342,
 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376,
 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410,
 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827,
 10 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the inhibitory nucleic acid comprises a guide
 strand sequence comprising or consisting of the nucleic acid sequence set forth in any
 one of the guide sequences in Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one
 of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42,
 15 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88,
 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128,
 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162,
 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196,
 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230,
 20 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264,
 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298,
 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334,
 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368,
 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402,
 25 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436,
 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4,
 or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the inhibitory nucleic acid comprises a guide
 strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%,
 30 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide

sequences in Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of the guide sequences in Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065,

2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO.

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 12. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some

embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 13. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, preferably wherein

the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 19. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 23. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid

sequence set forth in any one of SEQ ID NOS:1908-2007. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some

5 embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous

10 nucleotides of a sequence of any one of SEQ ID NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ

15 ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 24. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid

20 sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314,

25 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one

30 of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211,

1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

15 In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence of Table 25. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 25 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the 30 guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID

NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that
5 the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the
10 inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%,
15 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, preferably wherein the guide strand sequence retains positions 2-7 ("seed
20 sequence") of the selected SEQ ID NO. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at
25 the variant nucleotide(s).

In some embodiments, the inhibitory nucleic acid is an isolated siRNA duplex that targets *ATXN2* mRNA to interfere with *ATXN2* expression by mRNA degradation or translational inhibition. A siRNA duplex is a short, double stranded RNA comprising a guide strand, which is complementary to the target *ATXN2* mRNA,
30 and a passenger strand, which is homologous to the target *ATXN2* mRNA. The guide

strand and passenger strand hybridize together to form a duplex structure, and the guide strand has sufficient complementarity to the *ATXN2* mRNA sequence to direct *ATXN2*-specific RNA interference. The guide strand of the siRNA duplex may be about 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides in length or 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, 22-30, 22-29, 22-28, 22-27, 22-26, 22-24, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 24-30, 24-29, 24-28, 24-27, 24-26, 25-30, 25-29, 25-28, 25-27, 26-30, 26-29, 26-28, 27-30, 27-29, 28-30 nucleotides in length. The passenger strand of the siRNA duplex may be about 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides in length or 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, 22-30, 22-29, 22-28, 22-27, 22-26, 22-24, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 24-30, 24-29, 24-28, 24-27, 24-26, 25-30, 25-29, 25-28, 25-27, 26-30, 26-29, 26-28, 27-30, 27-29, 28-30 nucleotides in length. In some embodiments, the siRNA duplex contains 2 or 3 nucleotide 3' overhangs on each strand. In some embodiments, the 3' overhangs are complementary to the *ATXN2* transcript. In some embodiments, the guide strand and passenger strand of the siRNA duplex are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 100% complementary to each other, not including any nucleotides in overhang(s).

In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88,

90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128,
 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162,
 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196,
 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230,
 5 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264,
 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298,
 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334,
 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368,
 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402,
 10 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436,
 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the siRNA duplex comprises a guide strand
 sequence comprising or consisting of the nucleic acid sequence set forth in any one of
 the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of
 15 SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42,
 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88,
 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128,
 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162,
 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196,
 20 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230,
 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264,
 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298,
 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334,
 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368,
 25 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402,
 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436,
 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4,
 or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the siRNA duplex comprises a guide strand
 30 sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%,

75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416,

418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO.

In some embodiments, the siRNA duplex comprises a guide strand
 5 sequence comprising or consisting of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138,
 10 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308,
 15 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions
 20 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand
 sequence of Table 12. In some embodiments, the siRNA duplex comprises a guide
 25 strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124,
 30 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362 with at least 1, 2, 3, 4,

or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand sequence of Table 13. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108,

112, 128, 166, 198, 242, 308, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand sequence of Table 19. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand sequence of Table 23. In some embodiments, the siRNA duplex comprises a guide

strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand sequence of Table 24. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822,

1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 5 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 10 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the siRNA duplex comprises a guide strand 15 sequence of Table 25. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 20 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, 25 and 314. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the 30 siRNA duplex comprises a guide strand sequence comprising or consisting of a

sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

5 In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811, with at least
10 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the siRNA duplex comprises a guide strand sequence
15 comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID
20 NOS:1185, 1816, 1213, and 1811, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments the siRNA duplex comprises a guide strand
25 sequence and passenger strand sequence of any one of siRNA duplexes provided by Tables 1, 19, 23, and 24. In some embodiments, the siRNA duplex comprises a guide strand sequence and passenger strand sequence comprising any one of: SEQ ID NOS:12 and 11; SEQ ID NOS: 14 and 13; SEQ ID NOS: 40 and 39; SEQ ID NOS: 60 and 59; SEQ ID NOS: 100 and 99; SEQ ID NOS: 104 and 103; SEQ ID NOS: 108 and 107;
30 SEQ ID NOS: 112 and 111; SEQ ID NOS: 124 and 123; SEQ ID NOS: 126 and 125;

SEQ ID NOS: 128 and 127; SEQ ID NOS: 166 and 165; SEQ ID NOS: 198 and 197; SEQ ID NOS: 220 and 219; SEQ ID NOS: 242 and 241; SEQ ID NOS: 302 and 301; SEQ ID NOS: 306 and 305; SEQ ID NOS: 308 and 307; SEQ ID NOS: 330 and 320; SEQ ID NOS: 336 and 335; and SEQ ID NOS: 362 and 361. In some embodiments,

5 the siRNA duplex comprises a guide strand sequence and passenger strand sequence comprising any one of: SEQ ID NOS: 14 and 13; SEQ ID NOS: 40 and 39; SEQ ID NOS: 100 and 99; SEQ ID NOS: 108 and 107; SEQ ID NOS: 112 and 11; SEQ ID NOS: 128 and 127; SEQ ID NOS: 166 and 165; SEQ ID NOS: 198 and 197; SEQ ID NOS: 242 and 241; SEQ ID NOS: 308 and 307; SEQ ID NOS: 336 and 335; and SEQ

10 ID NOS: 362 and 361.

Table 1: *ATXN2* Specific siRNA Duplex Sequences

duplex ID	sense sequence (passenger)	antisense sequence (guide)
XD-14738	CCUCCGCCUCAGACUGUUUUGG [SEQ ID NO:3]	AAAACAGUCUGAGGCGGAGGGA [SEQ ID NO:4]
XD-14739	CUCCGCCUCAGACUGUUUAGU [SEQ ID NO:5]	UAAAACAGUCUGAGGCGGAGGG [SEQ ID NO:6]
XD-14740	CGGCGGCAGCGGCCUUCUACG [SEQ ID NO:7]	UUAGAAGGCCGUGCCGCCGGG [SEQ ID NO:8]
XD-14741	GGACUGCCUCAGUCUACGAUUU [SEQ ID NO:9]	AUCGUAGACUGAGGCAGUCCUU [SEQ ID NO:10]
XD-14742	CUGCCUCAGUCUACGAUUUAUU [SEQ ID NO:11]	UAAAUUCGUAGACUGAGGCAGUC [SEQ ID NO:12]
XD-14743	UGCCUCAGUCUACGAUUUCUUU [SEQ ID NO:13]	AGAAAUCGUAGACUGAGGCAGU [SEQ ID NO:14]
XD-14744	CCUCAGUCUACGAUUUCUUUG [SEQ ID NO:15]	AAAGAAAUCGUAGACUGAGGCA [SEQ ID NO:16]
XD-14745	CAGUCUACGAUUUCUUUGAUG [SEQ ID NO:17]	UCAAAGAAAUCGUAGACUGAG [SEQ ID NO:18]
XD-14746	GAGGAUGGUCAUAUACUUACA [SEQ ID NO:19]	UAAGUAUAUGAACCAUCCUCAU [SEQ ID NO:20]
XD-14747	AGGAUGGUCAUAUACUUAAU [SEQ ID NO:21]	UUAAGUAUAUGAACCAUCCUCA [SEQ ID NO:22]
XD-14748	GGAUGGUCAUAUACUUACAUC [SEQ ID NO:23]	UGUAAGUAUAUGAACCAUCCUC [SEQ ID NO:24]
XD-14749	UUCAUAUACUUACAUCAGUUGU [SEQ ID NO:25]	AACUGAUGUAAGUAUAUGAACC [SEQ ID NO:26]
XD-14750	AUGAGAAAAGUACAGAAUCAAG [SEQ ID NO:27]	UGAUUCUGUACUUUCUCAUGU [SEQ ID NO:28]
XD-14751	GAGAAAAGUACAGAAUCCAAUU [SEQ ID NO:29]	UUGGAUUCUGUACUUUCUCAU [SEQ ID NO:30]
XD-14752	AAAGUACAGAAUCCAGUUCAGG [SEQ ID NO:31]	UGAACUGGAUUCUGUACUUUC [SEQ ID NO:32]
XD-14753	AAGUACAGAAUCCAGUUCGAGG [SEQ ID NO:33]	UCGAACUGGAUUCUGUACUUUU [SEQ ID NO:34]

XD-14754	GUACAGAAUCCAGUUCGGGACC [SEQ ID NO:35]	UCCCGAACUGGAUUCUGUACUU [SEQ ID NO:36]
XD-14755	UACAGAAUCCAGUUCGGGACG [SEQ ID NO:37]	UCCCGAACUGGAUUCUGUACU [SEQ ID NO:38]
XD-14756	UCAGACUUUGUUGGUACAGU [SEQ ID NO:39]	UGUACCACAACAAAGUCUGAAC [SEQ ID NO:40]
XD-14757	UUUGUUGUGGUACAGUUUAAAG [SEQ ID NO:41]	UUAAACUGUACCACAACAAAGU [SEQ ID NO:42]
XD-14758	UUGUUGUGGUACAGUUUAAAGA [SEQ ID NO:43]	UUUAAACUGUACCACAACAAAG [SEQ ID NO:44]
XD-14759	UGUGGUACAGUUUAAAGAU AUG [SEQ ID NO:45]	UAUCUUUAAACUGUACCACAAC [SEQ ID NO:46]
XD-14760	UUACUGACUCUGCUAUCAGUGC [SEQ ID NO:47]	ACUGAUAGCAGAGUCAGUAAAA [SEQ ID NO:48]
XD-14761	CUGACUCUGCUAUCAGUGC UAA [SEQ ID NO:49]	AGCACUGAUAGCAGAGUCAGUA [SEQ ID NO:50]
XD-14762	UGACUCUGCUAUCAGUGC UAAA [SEQ ID NO:51]	UAGCACUGAUAGCAGAGUCAGU [SEQ ID NO:52]
XD-14763	CUAAAGUGAAUGGCGAACAAAA [SEQ ID NO:53]	UUGUUCGCCAUUCACUUUAGCA [SEQ ID NO:54]
XD-14764	AAAGUGAAUGGCGAACACAAAG [SEQ ID NO:55]	UUGUGUUCGCCAUUCACUUUAG [SEQ ID NO:56]
XD-14765	GUGAAUGGCGAACACAAAGAGA [SEQ ID NO:57]	UCUUUGUGUUCGCCAUUCACUU [SEQ ID NO:58]
XD-14766	CUUUGGAAAAUGACGU AUCUAA [SEQ ID NO:59]	AGAUACGUCAUUUCCAAAGCC [SEQ ID NO:60]
XD-14767	UUGGAAAAUGACGU AUCUAAUG [SEQ ID NO:61]	UUAGAUACGUCAUUUCCAAAG [SEQ ID NO:62]
XD-14768	GGAAAAUGACGU AUCUAAUAGA [SEQ ID NO:63]	UAUUAGAUACGUCAUUUCCAA [SEQ ID NO:64]
XD-14769	AAAUGACGU AUCUAAUGGAUGG [SEQ ID NO:65]	AUCCAUUAGAUACGUCAUUUUC [SEQ ID NO:66]
XD-14770	AUGACGU AUCUAAUGGAUGAGA [SEQ ID NO:67]	UCAUCCAUUAGAUACGUCAUUU [SEQ ID NO:68]
XD-14771	UAAUGGAUGGGAUCCCAAUAU [SEQ ID NO:69]	UAUUGGGAUCCCAUCCAUUAGA [SEQ ID NO:70]
XD-14772	AAUGGAUGGGAUCCCAAUGAUA [SEQ ID NO:71]	UCAUUGGGAUCCCAUCCAUUAG [SEQ ID NO:72]
XD-14773	AUAUGUUUCGAUAUAAUGAAGA [SEQ ID NO:73]	UUCAUUAUUCGAAACAUUAUCA [SEQ ID NO:74]
XD-14774	AUGUUUCGAUAUAAUGAAGAAA [SEQ ID NO:75]	UCUUCAUUAUUCGAAACAUUAU [SEQ ID NO:76]
XD-14775	GUCUACGU AUGAUAGCAGUUUA [SEQ ID NO:77]	AACUGCUAUCAUACGUAGACAC [SEQ ID NO:78]
XD-14776	CGUAUACAGUGCCCUUAGAAAG [SEQ ID NO:79]	UUCUAAGGGCACUGUAUACGAA [SEQ ID NO:80]
XD-14777	UAUACAGUGCCCUUAGAAAAAG [SEQ ID NO:81]	UUUUCUAAGGGCACUGUAUACG [SEQ ID NO:82]
XD-14778	CAAGGGCAAACAGUUAGCAGA [SEQ ID NO:83]	UGCUAACUGGUUUGCCCUUGCU [SEQ ID NO:84]
XD-14779	AGGGCAAACAGUUAGCAGAAG [SEQ ID NO:85]	UCUGCUAACUGGUUUGCCCUUG [SEQ ID NO:86]
XD-14780	UGAGUCAAGUGCCCAGUACAAA [SEQ ID NO:87]	UGUACUGGGCACUUGACUCAAU [SEQ ID NO:88]
XD-14781	GAGUCAAGUGCCCAGUACAAAAG [SEQ ID NO:89]	UUGUACUGGGCACUUGACUCAAA [SEQ ID NO:90]

XD-14782	GCCAGUACAAAGCUCGAGUGG [SEQ ID NO:91]	ACUCGAGCUUUGUACUGGGCAC [SEQ ID NO:92]
XD-14783	AAGAAAAUACACAGCAGUUCA [SEQ ID NO:93]	AACUGCUGUGUAUUUUUCUCC [SEQ ID NO:94]
XD-14784	GAGGGGCACAGCAUAAACAAUA [SEQ ID NO:95]	UUGUUUAUGCUGUGCCCCUCAC [SEQ ID NO:96]
XD-14785	AGUCAUAUCCUGGGGAAGUAGG [SEQ ID NO:97]	UACUCCCCCAGGAUAUGACUUC [SEQ ID NO:98]
XD-14786	GAGACAGAAUUCACCGCUAUG [SEQ ID NO:99]	UACGCGGUGAAUUCUGUCUCC [SEQ ID NO:100]
XD-14787	AGACAGAAUUCACCGCUAUGG [SEQ ID NO:101]	AUACGCGGUGAAUUCUGUCUCC [SEQ ID NO:102]
XD-14788	GACAGAAUUCACCGCUAUGG [SEQ ID NO:103]	UAUACGCGGUGAAUUCUGUCUC [SEQ ID NO:104]
XD-14789	ACAGAAUUCACCGCUAUGAGC [SEQ ID NO:105]	UCAUACGCGGUGAAUUCUGUCU [SEQ ID NO:106]
XD-14790	ACUUCAGAUUUAACCCGAAUU [SEQ ID NO:107]	UUCGGGUUGAAUUCUGAAGUGU [SEQ ID NO:108]
XD-14791	UCAGACCAAAGAGUAGUAAUG [SEQ ID NO:109]	UUAACUACUCUUUGGUCUGAAC [SEQ ID NO:110]
XD-14792	CAGACCAAAGAGUAGUAAUGG [SEQ ID NO:111]	AUUAACUACUCUUUGGUCUGAA [SEQ ID NO:112]
XD-14793	AGACCAAAGAGUAGUAAUAGA [SEQ ID NO:113]	UAUUAACUACUCUUUGGUCUGA [SEQ ID NO:114]
XD-14794	GACCAAAGAGUAGUAAUGAAG [SEQ ID NO:115]	UCAUUAACUACUCUUUGGUCUG [SEQ ID NO:116]
XD-14795	CCAAAGAGUAGUAAUGGAAGU [SEQ ID NO:117]	UUCCAUUAACUACUCUUUGGUC [SEQ ID NO:118]
XD-14796	UGUCCCCAAAGGCCAGCGACA [SEQ ID NO:119]	UCGUGGGCCUUUGGGGACAUC [SEQ ID NO:120]
XD-14797	GCCCAGCGACAUCCUCGAAAUUC [SEQ ID NO:121]	UUUCGAGGAUGUCGUGGGCCU [SEQ ID NO:122]
XD-14798	CCCAGCGACAUCCUCGAAAUCA [SEQ ID NO:123]	AUUUCGAGGAUGUCGUGGGCC [SEQ ID NO:124]
XD-14799	CCAGCGACAUCCUCGAAAUAC [SEQ ID NO:125]	UAUUUCGAGGAUGUCGUGGGC [SEQ ID NO:126]
XD-14800	AGCGACAUCCUCGAAAUCAAAG [SEQ ID NO:127]	UUGAUUUCGAGGAUGUCGUGG [SEQ ID NO:128]
XD-14801	GCGACAUCCUCGAAAUACAGA [SEQ ID NO:129]	UGUGAUUUCGAGGAUGUCGUG [SEQ ID NO:130]
XD-14802	UGCUGGGAGGGGUCCAUAUCC [SEQ ID NO:131]	AUAUGGAACCCUCCAGCAGA [SEQ ID NO:132]
XD-14803	GCUACUCCUCCAGUAGCAAAGA [SEQ ID NO:133]	UUUGCUACUGGAGGAGUAGCUG [SEQ ID NO:134]
XD-14804	CCUCCAGUAGCAAGGACCAUUC [SEQ ID NO:135]	UUGGUCCUUGCUACUGGAGGAG [SEQ ID NO:136]
XD-14805	GUGGUCAGUGGGGUCCAAAUAU [SEQ ID NO:137]	UUUGGAACCCACUGACCACUG [SEQ ID NO:138]
XD-14806	GUGGGGUCCAAGAUUAUACCC [SEQ ID NO:139]	UGAUAAUUCUUGGAACCCACUG [SEQ ID NO:140]
XD-14807	UCCAAGAUUAUCCCUAAAACU [SEQ ID NO:141]	UUUUAGGGGAUAAUCUUGGAAC [SEQ ID NO:142]
XD-14808	CCAAGAUUAUCCCUAAAACU [SEQ ID NO:143]	UUUUAGGGGAUAAUCUUGGAA [SEQ ID NO:144]
XD-14809	CAAGAUUAUCCCUAAAACUCA [SEQ ID NO:145]	AGUUUUAGGGGAUAAUCUUGGA [SEQ ID NO:146]

XD-14810	AAGAUUAUCCCCUAAAACUAAU [SEQ ID NO:147]	UAGUUUUAGGGGAUAAUCUUGG [SEQ ID NO:148]
XD-14811	AGAUUAUCCCCUAAAACUCAUA [SEQ ID NO:149]	UGAGUUUUAGGGGAUAAUCUUG [SEQ ID NO:150]
XD-14812	GAUUAUCCCCUAAAACUCAUAG [SEQ ID NO:151]	AUGAGUUUUAGGGGAUAAUCUU [SEQ ID NO:152]
XD-14813	AUUAUCCCCUAAAACUCAUAGA [SEQ ID NO:153]	UAUGAGUUUUAGGGGAUAAUCU [SEQ ID NO:154]
XD-14814	UAUCCCCUAAAACUCAUAGACC [SEQ ID NO:155]	UCUAUGAGUUUUAGGGGAUAAU [SEQ ID NO:156]
XD-14815	AACUCAUAGACCCAGGUCUACC [SEQ ID NO:157]	UAGACCUGGGUCUAUGAGUUUU [SEQ ID NO:158]
XD-14816	UCCCCAAGCUGGUAAUUAUACA [SEQ ID NO:159]	UAAUAAUACCAGCUUGGGGAGA [SEQ ID NO:160]
XD-14817	CCCCAAGCUGGUAAUUAUCAA [SEQ ID NO:161]	UGAAUAAUACCAGCUUGGGGAG [SEQ ID NO:162]
XD-14818	CAUCUCCUACGCCUGCUAGUCC [SEQ ID NO:163]	ACUAGCAGGCGUAGGAGAUGCA [SEQ ID NO:164]
XD-14819	CCUGCUAGUCCUGCAUCGAACA [SEQ ID NO:165]	UUCGAUGCAGGACUAGCAGGCG [SEQ ID NO:166]
XD-14820	UGCUAGUCCUGCAUCGAACAGA [SEQ ID NO:167]	UGUUCGAUGCAGGACUAGCAGG [SEQ ID NO:168]
XD-14821	CUGCAUCGAACAGAGCUGUUAC [SEQ ID NO:169]	AACAGCUCUGUUCGAUGCAGGA [SEQ ID NO:170]
XD-14822	GCAUCGAACAGAGCUGUUAACC [SEQ ID NO:171]	UUAACAGCUCUGUUCGAUGCAG [SEQ ID NO:172]
XD-14823	ACCCUUCUAGUGAGGCUAAAG [SEQ ID NO:173]	UUAGCCUCACUAGAAGGGGUAA [SEQ ID NO:174]
XD-14824	CCCUUCUAGUGAGGCUAAAGA [SEQ ID NO:175]	UUUAGCCUCACUAGAAGGGGUA [SEQ ID NO:176]
XD-14825	CCUUCUAGUGAGGCUAAAGAUU [SEQ ID NO:177]	UCUUUAGCCUCACUAGAAGGGG [SEQ ID NO:178]
XD-14826	UUCUAGUGAGGCUAAAGAUUCC [SEQ ID NO:179]	AAUCUUUAGCCUCACUAGAAGG [SEQ ID NO:180]
XD-14827	AAUGAAACAUACCCUAGCUUCU [SEQ ID NO:181]	AAGCUAGGUGAUGUUUAUUGG [SEQ ID NO:182]
XD-14828	ACAUCACCUAGCUUCUCAAAG [SEQ ID NO:183]	UUUGAGAAGCUAGGUGAUGUUU [SEQ ID NO:184]
XD-14829	AUCACCUAGCUUCUAAAAACU [SEQ ID NO:185]	UUUUUGAGAAGCUAGGUGAUGU [SEQ ID NO:186]
XD-14830	CCUAGCUUCUAAAAGCUGAAA [SEQ ID NO:187]	UCAGCUUUUGAGAAGCUAGGUG [SEQ ID NO:188]
XD-14831	AAAACAAAGGUAAUACACCAGU [SEQ ID NO:189]	UGGUGAAUACCUUGUUUUUCA [SEQ ID NO:190]
XD-14832	UAAGAAUGAUUUUAGGUUAAAG [SEQ ID NO:191]	UUAACCUAAAAUCAUUCUAAA [SEQ ID NO:192]
XD-14833	AAGAAUGAUUUUAGGUUACAGC [SEQ ID NO:193]	UGUAACCUAAAAUCAUUCUAAA [SEQ ID NO:194]
XD-14834	ACUUCUGAAUCUAUGGAUCAAC [SEQ ID NO:195]	UGAUCCAUAGAUUCAGAAGUAG [SEQ ID NO:196]
XD-14835	UGAAUCUAUGGAUCAACUAAUA [SEQ ID NO:197]	UUAGUUGAUCCAUAGAUUCAGA [SEQ ID NO:198]
XD-14836	AUCUAUGGAUCAACUACUAAAC [SEQ ID NO:199]	UUAGUAGUUGAUCCAUAGAUUC [SEQ ID NO:200]
XD-14837	UCUAUGGAUCAACUACUAAACA [SEQ ID NO:201]	UUUAGUAGUUGAUCCAUAGAUU [SEQ ID NO:202]

XD-14838	UAUGGAUCAACUACUAAACAAA [SEQ ID NO:203]	UGUUUAGUAGUUGAUCCAUAGA [SEQ ID NO:204]
XD-14839	AUGGAUCAACUACUAAACAAA [SEQ ID NO:205]	UUGUUUAGUAGUUGAUCCAUAG [SEQ ID NO:206]
XD-14840	UGGAUCAACUACUAAACAAA [SEQ ID NO:207]	UUUGUUUAGUAGUUGAUCCAU [SEQ ID NO:208]
XD-14841	GGAUCAACUACUAAACAAA [SEQ ID NO:209]	UUUUGUUUAGUAGUUGAUCCAU [SEQ ID NO:210]
XD-14842	GCCGAAUAGCCCCAGCAUUUCC [SEQ ID NO:211]	AAUUGCUGGGGCUAUUCGGCUU [SEQ ID NO:212]
XD-14843	CAGCAUUUCCCCUCAAUAAU [SEQ ID NO:213]	UUAUUGAAGGGGAAUUGCUGGG [SEQ ID NO:214]
XD-14844	UUUCCCCUCAAUACUAGUAA [SEQ ID NO:215]	ACUAAGUAAUUGAAGGGGAAU [SEQ ID NO:216]
XD-14845	UUCCCCUCAAUACUAGUAA [SEQ ID NO:217]	UACUAAGUAAUUGAAGGGGAAU [SEQ ID NO:218]
XD-14846	UCCCCUCAAUACUAGUAA [SEQ ID NO:219]	UUACUAAGUAAUUGAAGGGGAA [SEQ ID NO:220]
XD-14847	CCCCUCAAUACUAGUAA [SEQ ID NO:221]	UUUACUAAGUAAUUGAAGGGGAA [SEQ ID NO:222]
XD-14848	UUCAAUACUAGUAAACGAG [SEQ ID NO:223]	UCGUGUACUAGUAAUUGAAGG [SEQ ID NO:224]
XD-14849	UCAAUACUAGUAAACGAG [SEQ ID NO:225]	UCCGUGUACUAGUAAUUGAAG [SEQ ID NO:226]
XD-14850	AUACUAGUAAACGAGCACA [SEQ ID NO:227]	UGCUCGUGUACUAGUAAUUG [SEQ ID NO:228]
XD-14851	GUCACUCCCAAGGGGUUCAGA [SEQ ID NO:229]	UGAACCCCUUGGGAAGUGACCU [SEQ ID NO:230]
XD-14852	CCAAGGGGUUCAGACUCCAGC [SEQ ID NO:231]	UGGAAGUCUGAACCCCUUGGGA [SEQ ID NO:232]
XD-14853	GACGCAGCUGAGCAAGUUAAGA [SEQ ID NO:233]	UUAACUUGCUCAGCUGCGUCU [SEQ ID NO:234]
XD-14854	CUGAGCAAGUAGGAAUCAAAC [SEQ ID NO:235]	UGAUUCCUAAUUGCUCAGCU [SEQ ID NO:236]
XD-14855	GAGCAAGUAGGAAUCAAU [SEQ ID NO:237]	UUUGAUUCCUAAUUGCUCAG [SEQ ID NO:238]
XD-14856	CCAUGCAAAGGAGUCAAACC [SEQ ID NO:239]	UUUGAACUCCUUGCAUUGGGA [SEQ ID NO:240]
XD-14857	GGAGUCAAACCCACGUCCUUC [SEQ ID NO:241]	AGGAACGUGGGUUGAACUCCU [SEQ ID NO:242]
XD-14858	UCUCAGCCAAAGCCUUCUAAU [SEQ ID NO:243]	UUAGAAGGCUUUGGCUGAGAGA [SEQ ID NO:244]
XD-14859	CAGCCAAAGCCUUCUACUACC [SEQ ID NO:245]	UUAGUAGAAGGCUUUGGCUGAG [SEQ ID NO:246]
XD-14860	AGCCAAAGCCUUCUACUACC [SEQ ID NO:247]	UGUAGUAGAAGGCUUUGGCUGA [SEQ ID NO:248]
XD-14861	GCCAAAGCCUUCUACUACC [SEQ ID NO:249]	UGGUAGUAGAAGGCUUUGGCUG [SEQ ID NO:250]
XD-14862	ACCCCAACUUCACCUCGGCAUC [SEQ ID NO:251]	UGCCGAGGUGAAGUUGGGGUAG [SEQ ID NO:252]
XD-14863	CCCCAACUUCACCUCGGCCUCA [SEQ ID NO:253]	AGGCCGAGGUGAAGUUGGGGUA [SEQ ID NO:254]
XD-14864	CCCAACUUCACCUCGGCCUAAA [SEQ ID NO:255]	UAGGCCGAGGUGAAGUUGGGGU [SEQ ID NO:256]
XD-14865	ACUUCACCUCGGCCUCAAAGAC [SEQ ID NO:257]	UCUUGAGGCCGAGGUGAAGUUG [SEQ ID NO:258]

XD-14866	ACCUCGGCCUCAAGCACAAACU [SEQ ID NO:259]	UUUGUGCUUGAGGCCGAGGUGA [SEQ ID NO:260]
XD-14867	CCUCGGCCUCAAGCACAAUA [SEQ ID NO:261]	UGUUGUGCUUGAGGCCGAGGUG [SEQ ID NO:262]
XD-14868	AGCACAACCUAGCCCAUCUAUG [SEQ ID NO:263]	UAGAUGGGCUAGGUUGUGCUUG [SEQ ID NO:264]
XD-14869	GCACAACCUAGCCCAUCUAUGG [SEQ ID NO:265]	AUAGAUGGGCUAGGUUGUGCUU [SEQ ID NO:266]
XD-14870	ACAACCUAGCCCAUCUAUGAUG [SEQ ID NO:267]	UCAUAGAUGGGCUAGGUUGUGC [SEQ ID NO:268]
XD-14871	CAACCUAGCCCAUCUAUGGUGG [SEQ ID NO:269]	ACCAUAGAUGGGCUAGGUUGUG [SEQ ID NO:270]
XD-14872	GCCCAUCUAUGGUGGGUCAUCA [SEQ ID NO:271]	AUGACCCACCAUAGAUGGGCUA [SEQ ID NO:272]
XD-14873	CCCAUCUAUGGUGGGUCAUAAA [SEQ ID NO:273]	UAUGACCCACCAUAGAUGGGCU [SEQ ID NO:274]
XD-14874	CCAUCUAUGGUGGGUCAUCAAC [SEQ ID NO:275]	UGAUGACCCACCAUAGAUGGGC [SEQ ID NO:276]
XD-14875	CAUCUAUGGUGGGUCAUCAACA [SEQ ID NO:277]	UUGAUGACCCACCAUAGAUGGG [SEQ ID NO:278]
XD-14876	GCCAAUCCAGUUUAUACUAAG [SEQ ID NO:279]	UAGUAUAAACUGGAGUUGGCUG [SEQ ID NO:280]
XD-14877	CACCAAAUAGAUGUAUCCAGU [SEQ ID NO:281]	UGGAUACAUAUUAUUGGUGCA [SEQ ID NO:282]
XD-14878	AGCCCAGGCGUGCAACCUUUUAU [SEQ ID NO:283]	AAAGGUUGCACGCCUGGGCUCA [SEQ ID NO:284]
XD-14879	UACCCAAUACCUAUGACGCACA [SEQ ID NO:285]	UGCGUCAUAGGUUAUUGGGUAUA [SEQ ID NO:286]
XD-14880	CAAUACCUAUGACGCCAUACC [SEQ ID NO:287]	UAUGGGCGUCAUAGGUUAUUGGG [SEQ ID NO:288]
XD-14881	AUACCUAUGACGCCAUAGCAAG [SEQ ID NO:289]	UGCAUGGGCGUCAUAGGUUAUUG [SEQ ID NO:290]
XD-14882	GAAUCAAGCCAAGACAUAUAGA [SEQ ID NO:291]	UAUAUGUCUUGGCUUGAUUCAC [SEQ ID NO:292]
XD-14883	AAUCAAGCCAAGACAUAUAAAG [SEQ ID NO:293]	UUAUAUGUCUUGGCUUGAUUCA [SEQ ID NO:294]
XD-14884	AUCAAGCCAAGACAUAUAGAGC [SEQ ID NO:295]	UCUAUAUGUCUUGGCUUGAUUC [SEQ ID NO:296]
XD-14885	AAGCCAAGACAUAUAGAGCAGU [SEQ ID NO:297]	UGCUCUAUAUGUCUUGGCUUGA [SEQ ID NO:298]
XD-14886	CCAAUAUGCCCCAACAGCAGC [SEQ ID NO:299]	UGCUGUUGGGGCAUAUUGGUA [SEQ ID NO:300]
XD-14887	CAAAUAUGCCCCAACAGCGACA [SEQ ID NO:301]	UCGCGUUGGGGCAUAUUGGU [SEQ ID NO:302]
XD-14888	GCAGCGGGCCACCGAUUGAAG [SEQ ID NO:303]	UCAAUCGGUGGGCCCGCUGCUG [SEQ ID NO:304]
XD-14889	ACCAGCUUACUCCACGCAAUAU [SEQ ID NO:305]	AUUGCGUGGAGUAAGCUGGUGG [SEQ ID NO:306]
XD-14890	CCAGCUUACUCCACGCAAUAUG [SEQ ID NO:307]	UAUUGCGUGGAGUAAGCUGGUG [SEQ ID NO:308]
XD-14891	AGCUUACUCCACGCAAUAUAU [SEQ ID NO:309]	UAUAUUGCGUGGAGUAAGCUGG [SEQ ID NO:310]
XD-14892	UCCACGCAAUAUGUUGCCUACA [SEQ ID NO:311]	UAGGCAACAUAUUGCGUGGAGU [SEQ ID NO:312]
XD-14893	AGUCUCAGCAUCCUAUGUAUA [SEQ ID NO:313]	UACAUGAGGAUGCUGAGACUGA [SEQ ID NO:314]

XD-14894	CAGCAUCCUCAUGUCUAUAAUC [SEQ ID NO:315]	UUAUAGACAUGAGGAUGCUGAG [SEQ ID NO:316]
XD-14895	GUCCUGUAAUACAGGGUAAUGC [SEQ ID NO:317]	AUUACCCUGUAUUACAGGACUA [SEQ ID NO:318]
XD-14896	ACAGGGUAAUGCUAGAAUGAUG [SEQ ID NO:319]	UCAUUCUAGCAUUACCCUGUAU [SEQ ID NO:320]
XD-14897	ACUCAGUACGGGGCUCAUGAGC [SEQ ID NO:321]	UCAUGAGCCCCGUACUGAGUUG [SEQ ID NO:322]
XD-14898	GAGCAGACGCAUGCGAUGUAUG [SEQ ID NO:323]	UACAUCGCAUGCGUCUGCUCAU [SEQ ID NO:324]
XD-14899	AGCAGACGCAUGCGAUGUAUGC [SEQ ID NO:325]	AUACAUCGCAUGCGUCUGCUCA [SEQ ID NO:326]
XD-14900	GCAGACGCAUGCGAUGUAUACA [SEQ ID NO:327]	UAUACAUCGCAUGCGUCUGCUC [SEQ ID NO:328]
XD-14901	CUUGCUCAGCAGUAUGCGCACC [SEQ ID NO:329]	UGCGCAUACUGCUGAGCAAGGG [SEQ ID NO:330]
XD-14902	CAGCAGUAUGCGCACCCUAACG [SEQ ID NO:331]	UUAGGGUGCGCAUACUGCUGAG [SEQ ID NO:332]
XD-14903	CAGUAUGCGCACCCUAACGAUA [SEQ ID NO:333]	UCGUUAGGGUGCGCAUACUGCU [SEQ ID NO:334]
XD-14904	AGUAUGCGCACCCUAACGCUAC [SEQ ID NO:335]	AGCGUUAGGGUGCGCAUACUGC [SEQ ID NO:336]
XD-14905	GUAUGCGCACCCUAACGCUACC [SEQ ID NO:337]	UAGCGUUAGGGUGCGCAUACUG [SEQ ID NO:338]
XD-14906	CAGCAGUCAGCCAUUUACCACG [SEQ ID NO:339]	UGGUAUAGGGUGACUGCUGCU [SEQ ID NO:340]
XD-14907	AGCCAUUUACCACGCGGGGAUU [SEQ ID NO:341]	UCCCCGCGUGGUAAUAGGCUGA [SEQ ID NO:342]
XD-14908	UCCAACACGCAGUCGCCACAGA [SEQ ID NO:343]	UGUGGCGACUGCGUGUUGGAGG [SEQ ID NO:344]
XD-14909	AACACGCAGUCGCCACAGAAUA [SEQ ID NO:345]	UUCUGUGGCGACUGCGUGUUGG [SEQ ID NO:346]
XD-14910	ACGCAGUCGCCACAGAAUAAUU [SEQ ID NO:347]	UUAUUCUGUGGCGACUGCGUGU [SEQ ID NO:348]
XD-14911	GCAGUCGCCACAGAAUAGUUUC [SEQ ID NO:349]	AACUAUUCUGUGGCGACUGCGU [SEQ ID NO:350]
XD-14912	ACAGAAUAGUUUCCAGCAACA [SEQ ID NO:351]	UUGCUGGGAAACUAUUCUGUGG [SEQ ID NO:352]
XD-14913	AGUUUCCAGCAGCACAAACAGA [SEQ ID NO:353]	UGUUGUGCUGCUGGGAAACUAU [SEQ ID NO:354]
XD-14914	ACGAUCCAUCUUCUCACGUUC [SEQ ID NO:355]	ACGUGAGAAGGAUGGAUCGUAA [SEQ ID NO:356]
XD-14915	CUCACGUUCAGCCGGCGUAUAC [SEQ ID NO:357]	AUACGCCGGCUGAACGUGAGAA [SEQ ID NO:358]
XD-14916	UCACGUUCAGCCGGCGUAUACC [SEQ ID NO:359]	UAUACGCCGGCUGAACGUGAGA [SEQ ID NO:360]
XD-14917	ACGUUCAGCCGGCGUAUACAAA [SEQ ID NO:361]	UGUAUACGCCGGCUGAACGUGA [SEQ ID NO:362]
XD-14918	CGUUCAGCCGGCGUAUACCAAC [SEQ ID NO:363]	UGGUAUACGCCGGCUGAACGUG [SEQ ID NO:364]
XD-14919	CCCACAUGGCCACGUACCUCA [SEQ ID NO:365]	AGGUACGUGGGCCAUGUGGGGU [SEQ ID NO:366]
XD-14920	CCACAUGGCCACGUACCUAAG [SEQ ID NO:367]	UAGGUACGUGGGCCAUGUGGGG [SEQ ID NO:368]
XD-14921	ACAUGGCCACGUACCUAAGC [SEQ ID NO:369]	UUGAGGUACGUGGGCCAUGUGG [SEQ ID NO:370]

XD-14922	CCAACAGCAGUUGUAAGGCUGC [SEQ ID NO:371]	AGCCUUACAACUGCUGUUGGUG [SEQ ID NO:372]
XD-14923	UCUUGUAACAUCCAAUAGGAAU [SEQ ID NO:373]	UCCUAUUGGAUGUUACAAGAAA [SEQ ID NO:374]
XD-14924	GACCGAGUAGAGGCAUUUAAGA [SEQ ID NO:375]	UUAAAUGCCUCUACUCGGUCCA [SEQ ID NO:376]
XD-14925	CGAGUAGAGGCAUUUAGGAACU [SEQ ID NO:377]	UUCCUAAAUGCCUCUACUCGGU [SEQ ID NO:378]
XD-14926	GGAACUUGGGGGCUAUUCCAUA [SEQ ID NO:379]	UGGAAUAGCCCCAAGUCCUA [SEQ ID NO:380]
XD-14927	ACUUGGGGGCUAUUCCAUAUU [SEQ ID NO:381]	UUAUGGAAUAGCCCCAAGUUC [SEQ ID NO:382]
XD-14928	UUGGGGGCUAUUCCAUAUUCC [SEQ ID NO:383]	AAUUAUGGAAUAGCCCCAAGU [SEQ ID NO:384]
XD-14929	GGGGGCUAUUCCAUAUUCAAU [SEQ ID NO:385]	UGAAUUAUGGAAUAGCCCCAA [SEQ ID NO:386]
XD-14930	GGGGGCUAUUCCAUAUUCCAUA [SEQ ID NO:387]	UGGAAUUAUGGAAUAGCCCCCA [SEQ ID NO:388]
XD-14931	CUCUGCUUGCCGAAACUGGAAG [SEQ ID NO:389]	UCCAGUUUCGGCAAGCAGAGCU [SEQ ID NO:390]
XD-14932	UUGCCGAAACUGGAAGUUUUUU [SEQ ID NO:391]	AUAACUCCAGUUUCGGCAAGC [SEQ ID NO:392]
XD-14933	AUAACCCUUGAAAGUCAUGAAC [SEQ ID NO:393]	UCAUGACUUUCAAGGGUUUAUA [SEQ ID NO:394]
XD-14934	UGAACACAUCAGCUAGCAAAAG [SEQ ID NO:395]	UUUGCUAGCUGAUGUGUUAUG [SEQ ID NO:396]
XD-14935	UGAUUCUUGCUGCUAUUACUGC [SEQ ID NO:397]	AGUAAUAGCAGCAAGAAUCACU [SEQ ID NO:398]
XD-14936	UGGAACGCCCUUUUACUAAACU [SEQ ID NO:399]	UUUAGUAAAAGGGCGUCCAAG [SEQ ID NO:400]
XD-14937	GGAACGCCCUUUUACUAAAUAU [SEQ ID NO:401]	UUUUAGUAAAAGGGCGUCCAA [SEQ ID NO:402]
XD-14938	GAACGCCCUUUUACUAAACUUG [SEQ ID NO:403]	AGUUUAGUAAAAGGGCGUCCA [SEQ ID NO:404]
XD-14939	AACGCCCUUUUACUAAACUUGA [SEQ ID NO:405]	AAGUUUAGUAAAAGGGCGUCC [SEQ ID NO:406]
XD-14940	AGUAAAUUCUACCGUCAAAACU [SEQ ID NO:407]	UUUGACGGUAAGAAUUUACUGA [SEQ ID NO:408]
XD-14941	GUAAAUUCUACCGUCAAAAUG [SEQ ID NO:409]	UUUGACGGUAAGAAUUUACUG [SEQ ID NO:410]
XD-14942	UCUUAACCGUCAAAACUGACGAU [SEQ ID NO:411]	UCGUCAGUUUGACGGUAAGAAU [SEQ ID NO:412]
XD-14943	CUUACCGUCAAAACUGACGGAUU [SEQ ID NO:413]	UCCGUCAGUUUGACGGUAAGAA [SEQ ID NO:414]
XD-14944	UUACCGUCAAAACUGACGGAUUA [SEQ ID NO:415]	AUCCGUCAGUUUGACGGUAAGA [SEQ ID NO:416]
XD-14945	ACCGUCAAAACUGACGGAUUUAU [SEQ ID NO:417]	UAAUCCGUCAGUUUGACGGUA [SEQ ID NO:418]
XD-14946	CCGUCAAACUGACGGAUUUAUA [SEQ ID NO:419]	AUAAUCCGUCAGUUUGACGGUA [SEQ ID NO:420]
XD-14947	CGUCAAAACUGACGGAUUUAUUAU [SEQ ID NO:421]	AAUAAUCCGUCAGUUUGACGGU [SEQ ID NO:422]
XD-14948	GUCAAAACUGACGGAUUUAUUAU [SEQ ID NO:423]	UAAUAAUCCGUCAGUUUGACGG [SEQ ID NO:424]
XD-14949	UCAAAACUGACGGAUUUAUUAUU [SEQ ID NO:425]	AUAAUAAUCCGUCAGUUUGACG [SEQ ID NO:426]

XD-14950	ACUGACGGAUUAUUUAUUA [SEQ ID NO:427]	AUAAAUAUAAUCCGUCAGUUU [SEQ ID NO:428]
XD-14951	CUGACGGAUUAUUUAUUA [SEQ ID NO:429]	UAUAAAUAUAAUCCGUCAGUU [SEQ ID NO:430]
XD-14952	AUGAGGUGAUCACUGUCUAAAG [SEQ ID NO:431]	UUAGACAGUGAUCACCUCAUCA [SEQ ID NO:432]
XD-14953	GAGGUGAUCACUGUCUCAAUG [SEQ ID NO:433]	UUGUAGACAGUGAUCACCUCAU [SEQ ID NO:434]
XD-14954	CUGUCUACAGUGGUCAACUUU [SEQ ID NO:435]	AGUUGAACACUGUAGACAGUG [SEQ ID NO:436]

In some embodiments, the isolated siRNA duplexes of the present disclosure, particularly when not delivered as an expression construct or within a vector, comprise at least one modified nucleotide, including a modified base, modified sugar, or modified backbone. siRNA having nucleotide modification(s) may have increased stability, increased specificity, reduced immunogenicity, or a combination thereof. Modified nucleotides may occur on either the guide strand, passenger strand, or both the guide strand and passenger strand.

Modified bases refer to nucleotide bases such as, for example, adenine, guanine, cytosine, thymine, uracil, xanthine, inosine, and queuosine that have been modified by the replacement or addition of one or more atoms or groups. Some examples of modifications on the nucleobase moieties include, but are not limited to, alkylated, halogenated, thiolated, aminated, amidated, or acetylated bases, individually or in combination. More specific examples include, for example, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N,-dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-amino)propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methoxyuridine, deazanucleotides such as 7-deazaadenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-

methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4,6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl
 5 nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Sugar modified nucleotides include, but are not limited to 2'-fluoro, 2'-amino and 2'-thio modified ribonucleotides, e.g., 2'-fluoro modified ribonucleotides.

Modified nucleotides may be modified on the sugar moiety, as well as be nucleotides having non-ribosyl sugars or analogs thereof. For example, the sugar
 10 moieties may be, or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles.

A normal "backbone," as used herein, refers to the repeatedly alternating sugar-phosphate sequences in a DNA or RNA molecule. The deoxyribose/ribose sugars are joined at both the 3'-hydroxyl and 5'-hydroxyl groups to
 15 phosphate groups in ester links, also known as "phosphodiester" bonds or linkages. One or more, or all phosphodiester linkage(s) may be modified as phosphorothioate linkages, boranophosphate linkages, amide linkages, phosphorodithioate linkages, or triazole linkages.

In some embodiments, the inhibitory nucleic acid is a shRNA. In some
 20 embodiments, the shRNA is a stem-loop duplex molecule comprising a guide strand and passenger strand of a siRNA duplex as provided herein (e.g., siRNA duplexes of Tables 1 and 19), linked by a spacer sequence, *i.e.*, loop. In some embodiments, loop sequence is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
 25 nucleotides in length or 4-25, 4-24, 4-23, 4-22, 4-21, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 5-25, 5-24, 5-23, 5-22, 5-21, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 6-25, 6-24, 6-23, 6-22, 6-21, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 7-25, 7-24, 7-23, 7-22, 7-21, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 8-25, 8-24, 8-23, 8-22, 8-21, 8-20, 8-19, 8-18, 8-11, 8-10, 9-25, 9-24, 9-23, 9-22, 9-21, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 10-25, 10-24, 10-23, 10-22, 10-21,
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10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 11-25, 11-24, 11-23, 11-22, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 12-25, 12-24, 12-23, 12-22, 12-21, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, or 12-14 nucleotides in length.

In some embodiments, the inhibitory nucleic acid is an isolated miRNA.

5 A miRNA may be a pri-miRNA, a pre-miRNA, mature miRNA, or artificial miRNA. In some embodiments, a miRNA is comprised of a guide strand and passenger strand. In some embodiments, the guide strand and passenger strand are within the same nucleic acid strand, where the guide strand and passenger strand hybridize together to form a self-annealing duplex structure. MiRNA is initially transcribed as a pri-miRNA, which
 10 is processed by nuclear nuclease (e.g., Drosha-DGCR8 complex) into pre-miRNA. A pri-miRNA is a single-stranded molecule having a stem-loop structure. In some embodiments, the pri-miRNA is about 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000 or more nucleotides in length or about 100-3000, 100-2500, 100-2000, 100-1900, 100-1800,
 15 100-1700, 100-1600, 100-1500, 100-1400, 100-1300, 100-1200, 100-1100, 100-1000, 100-900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 100-150, 150-3000, 150-2500, 150-2000, 150-1900, 150-1800, 150-1700, 150-1600, 150-1500, 150-1400, 150-1300, 150-1200, 150-1100, 150-1000, 150-900, 150-800, 150-700, 150-600, 150-500, 150-400, 150-300, 150-200, 200-3000, 200-2500, 200-2000, 200-1900,
 20 200-1800, 200-1700, 200-1600, 200-1500, 200-1400, 200-1300, 200-1200, 200-1100, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-3000, 300-2500, 300-2000, 300-1900, 300-1800, 300-1700, 300-1600, 300-1500, 300-1400, 300-1300, 300-1200, 300-1100, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-3000, 400-2500, 400-2000, 400-1900, 400-1800, 400-1700, 400-1600, 400-1500, 400-1400, 400-1300, 400-1200, 400-1100, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-3000, 500-2500, 500-2000, 500-1900, 500-1800, 500-1700, 500-1600, 500-1500, 500-1400, 500-1300, 500-1200, 500-1100, 500-1000, 500-900, 500-800, 500-700, or 500-600 nucleotides in length.

Pre-miRNA is also a single-stranded molecule having a stem-loop
 30 structure. In some embodiments, the pre-miRNA is about 40, 50, 60, 70, 80, 90, 100,

200, 300, 400, or 500 nucleotides in length, or about 40-500, 40-400, 40-300, 40-200, 40-100, 40-90, 40-80, 40-70, 40-60, 40-50, 50-500, 50-400, 50-300, 50-200, 50-100, 50-90, 50-80, 50-70, 60-500, 60-400, 60-300, 60-200, 60-100, 60-90, 60-80, 70-500, 70-400, 70-300, 70-200, 70-100, 70-90, 80-500, 80-400, 80-300, 80-200, 80-100, 90-
 5 500, 90-400, 90-300, 90-200, 100-500, 100-400, 100-300, 100-200, 200-500, 200-400, 200-300, 300-500, 300-400, or 400-500 nucleotides in length.

The pre-miRNA is transported from the nucleus to the cytoplasm by exportin-5 and further processed by Dicer to produce a mature, double-stranded miRNA duplex comprising a guide strand and a passenger strand. The mature miRNA duplex is
 10 then incorporated into the RNA inducing silencing complex (RISC), mediated by TRBP (HIV transactivating response RNA-binding protein). The passenger strand is generally released and cleaved, while the guide strand remains in RISC and binds to the target mRNA and mediates silencing. In some embodiments, a mature miRNA refers to the guide strand of a mature miRNA duplex. In some embodiments, a mature miRNA is
 15 about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length, or ranges from about 19-30 nucleotides, 19-29 nucleotides, 19-28 nucleotides, 19-27 nucleotides, 19-26 nucleotides, 19-25 nucleotides, 19-24 nucleotides, 19-23 nucleotides, 19-21 nucleotides, 20-30 nucleotides, 20-29 nucleotides, 20-28 nucleotides, 20-27 nucleotides, 20-26 nucleotides, 20-25 nucleotides, 20-24 nucleotides, 20-23
 20 nucleotides, 20-22 nucleotides, 21-30 nucleotides, 21-29 nucleotides, 21-28 nucleotides, 21-27 nucleotides, 21-26 nucleotides, 21-25 nucleotides, 21-24 nucleotides, 21-23 nucleotides, 22-30 nucleotides, 22-29 nucleotides, 22-28 nucleotides, 22-27 nucleotides, 22-26 nucleotides, 22-25 nucleotides, 22-24 nucleotides, 23-30 nucleotides, 23-29 nucleotides, 23-28 nucleotides, 23-27
 25 nucleotides, 23-26 nucleotides, 23-25 nucleotides, 24-30 nucleotides, 24-29 nucleotides, 24-28 nucleotides, 24-27 nucleotides, 24-26 nucleotides, 25-30 nucleotides, 25-29 nucleotides, 25-28 nucleotides, 25-27 nucleotides, 26-30 nucleotides, 26-29 nucleotides, 26-28 nucleotides, 27-30 nucleotides, 27-29 nucleotides, or 28-30 nucleotides in length.

Artificial miRNA refers to an endogenous, modified or synthetic pri-mRNA or pre-mRNA scaffold or backbone capable of producing a functional mature miRNA, where the guide strand sequence and passenger strand sequence of the miRNA duplex within the stem region have been replaced with a guide strand sequence and
5 passenger strand sequence of interest that directs silencing of the target mRNA of interest. Artificial miRNA design is described in Eamens et al. (2014) *Methods Mol Biol.* 1062:211-24 (incorporated by reference in its entirety). Synthetic miRNA backbones are described in U.S. Patent Publication 2008/0313773 (incorporated by reference in its entirety). In some embodiments, the artificial miRNA is about 100-200
10 nucleotides, 100-175 nucleotides 100-150 nucleotides, 125-200 nucleotides 125-175 nucleotides, or 125-150 nucleotides in length. In some embodiments, the artificial miRNA is about 100 nucleotides, about 120 nucleotides, about 130 nucleotides, about 140 nucleotides, about 150 nucleotides, about 160 nucleotides, about 170 nucleotides, about 180 nucleotides, about 190 nucleotides, or about 200 nucleotides in length.

15 In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56,
20 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240,
25 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412,

414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216,

218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250,
 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284,
 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318,
 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354,
 5 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388,
 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422,
 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152,
 2203, and 2209.

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an
 10 artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising
 or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a
 sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and
 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32,
 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78,
 15 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118,
 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154,
 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188,
 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222,
 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256,
 20 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290,
 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326,
 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360,
 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394,
 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428,
 25 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209,
 preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of
 the selected SEQ ID NO.

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an
 artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising
 30 or consisting of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12,

13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 12. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%,

99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least

5 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA

10 comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant

15 nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 13. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the

20 nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, with at

25 least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166,

30 198, 242, 308, 336, and 362. In some embodiments, the miRNA is a pri-miRNA, a pre-

mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, preferably wherein the guide strand sequence retains
5 positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant
10 nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 19. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA,
15 or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288,
20 40, 108, and 166, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1176-1288, 40,
25 108, and 166. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ
30 ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial

miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 23. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 24.

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 25. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the miRNA is a pri-miRNA, a pre-

mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, an artificial miRNA comprises a guide strand sequence according to any of the embodiments described herein, contained within a miR backbone sequence. In some embodiments, the guide strand sequence and passenger strand sequence of the artificial miRNA are contained within a miRNA backbone sequence. In some embodiments, the miRNA backbone sequence is a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190A backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-16-2 backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, miR-128 backbone sequence, a

miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

In some embodiments, the miRNA backbone sequence is a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a
 5 miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-16-2 backbone
 10 sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

In some embodiments, the miRNA backbone sequence is a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-122 backbone sequence, a miR-122_M
 15 backbone sequence, a miR-124 backbone sequence, a miR-130a backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-144 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR-190a_M backbone sequence, or a miR-190a_M backbone sequence.

In some embodiments, the miRNA backbone sequence is a miR-100
 20 backbone sequence or miR-100_M backbone sequence.

Table 2 provides examples of DNA sequences representing segments in miR-1-1, miR-100, miR-122, miR-124, miR-128, miR-130a, miR-155E, miR-155-M, and miR-138-2 backbones. Table 21 provides examples of DNA sequences representing segments in miR-1-1, miR-1-1_M, miR-100, miR-100_M, miR-122, miR-
 25 122_M, miR-124, miR-124_M, miR-128, miR-130a, miR-155E, miR-155M, miR-138-2, miR-144, miR-190a, miR-190a_M, miR-132, miR-451a, miR-223, and miR-16-2 backbones. It is understood that RNA sequences of the miR backbone segments in Tables 2 and 21 may be obtained by converting the “T” nucleotides in the sequences of Tables 2 and 21 to “U” nucleotides. Artificial miRNAs may be designed to insert
 30 desired guide and passenger sequences of the present disclosure into the miRNA

backbones as defined in Table 2 or 21, and optionally wherein the passenger sequence is designed according to the rules in Table 8. For example, an artificial miRNA with miR-100 backbone in DNA format (e.g., for insertion into a transfer plasmid) may be designed according to Table 21 comprising from 5' to 3': 5' miR context (flanking) sequence of SEQ ID NO:1529; 5' basal stem sequence of SEQ ID NO:1530; desired guide sequence; loop sequence of SEQ ID NO:1531; desired passenger sequence designed according to the rules in Table 8; 3' basal stem sequence of SEQ ID NO:1532; and 3' miR context (flanking) sequence of SEQ ID NO:1533.

Table 2: Annotation of miR Backbone Sequences

miR	5' miR context (flanking segment)	5' basal stem	5p	Terminal loop	3p	3' basal stem	3' miR context (flanking segment)
miR-1-1	catgcagactgcctgct [SEQ ID NO:491]	TGGG [SEQ ID NO:492]	passenger	TATGGACCTGCTAAGC TA [SEQ ID NO:493]	guide	CTCA [SEQ ID NO:494]	ggccgggagccctcicgccgcactgaggg gcactccacaccacggggggccg [SEQ ID NO:495]
miR-100	CCCAAAAGAGAGAAAGATA TTGAGG [SEQ ID NO:496]	CCTGTTGCCACA [SEQ ID NO:497]	guide	GTATTAGTCCG [SEQ ID NO:498]	passenger	TGTGTCTGTTA GG [SEQ ID NO:499]	CAATCTACGGACCTGGG GCTTTGCTTATATGCC [SEQ ID NO:500]
miR-122	ggctacagagttt [SEQ ID NO:501]	CCTTAGCAGAGCTG [SEQ ID NO:502]	guide	TGCTAAACTAT [SEQ ID NO:503]	passenger	TAGCTACTGCT AGGC [SEQ ID NO:504]	aatccctccicgataaalgcttggcagctgt tgctttg [SEQ ID NO:505]
miR-124	TTCCTTCCTCAGGAGAA [SEQ ID NO:506]	AGGCCTCTCTC [SEQ ID NO:507]	passenger	ATTAAATGTCCATAC AAT [SEQ ID NO:508]	guide	GAATGGGGCT G [SEQ ID NO:509]	GCTGAGCACCGTGGGTCCG GCGAGGGCCCCGCCAagga [SEQ ID NO:510]
miR-128	ATTTgcataatggcctgttcc [SEQ ID NO:511]	TGAGCTGTTGGA [SEQ ID NO:512]	passenger	GAGGTTTACATTTC [SEQ ID NO:513]	guide	TTCAGCTGCTT C [SEQ ID NO:514]	ctggctctttttactacaggtttccactgct [SEQ ID NO:515]
miR-130a	gcaggccggcagctgcctc [SEQ ID NO:516]	TGCTGCTGGCCA [SEQ ID NO:517]	passenger	CTGCTGCACCTGTCA CTAG [SEQ ID NO:518]	guide	TGGCCGTGTAG TG [SEQ ID NO:519]	Ciaccagcgtggcgcctcctcagcatt g [SEQ ID NO:520]
miR-155E	CTGGAGGCTTGCTTT [SEQ ID NO:521]	GGGCTGTATGCTG [SEQ ID NO:522]	guide	TTTTGGCCTCTGACTG A [SEQ ID NO:523]	passenger	CAGGACAAAG CCC [SEQ ID NO:524]	TTTATCAGCACCTCACATGG AACAAATGGCCACCGTG [SEQ ID NO:525]
miR-155-M	CCTGGAGGCTTGCTGA [SEQ ID NO:526]	AGGCTGTATGCTG [SEQ ID NO:527]	guide	TTTTGGCCTGACTG A [SEQ ID NO:528]	passenger	CAGGACACAA GGCC [SEQ ID NO:529]	TGTTACTAGCACTCACATG GAACAAATGGCCACC [SEQ ID NO:530]
miR-138-2	gcggcgaggtctggtat [SEQ ID NO:531]	CGTTGCTGC [SEQ ID NO:532]	guide	GACGAGCAGCGCATC CTCTTACCC [SEQ ID NO:533]	passenger	GTTGCATCA acc [SEQ ID NO:534]	taaccatctctccaggcgagccctgagg acc [SEQ ID NO:535]

In some embodiments, the terminal loop, stem, 5' flanking segment, 3' flanking segment, or any combination thereof of the miR-155 backbone sequence, miR1-1 backbone sequence, miR-100 backbone sequence, miR-190A backbone sequence, miR-124 backbone sequence, miR-16-2 backbone sequence, miR-132 backbone sequence, miR-9 backbone sequence, miR-138-2 backbone sequence, miR-122 backbone sequence, miR-130a backbone sequence, miR-128 backbone sequence, miR-144 backbone sequence, miR-451a backbone sequence, or miR-223 backbone sequence is modified (e.g., has nucleotide insertion, deletion, substitution, mismatch, wobble, or any combination thereof).

Sequence motifs that enable efficient processing of pri-miRNA backbones have previously been identified. These include an UG motif at the 5' end of the pre-miRNA, a mismatched GHG motif in the stem, and a 3' CNNC motif. In some embodiments, the miR backbone sequence has been modified to incorporate these motifs, including for example, miR-155E backbone sequence, miR-1-1_M backbone, miR-100_M backbone sequence, miR-124_M backbone sequence, and miR-122_M backbone sequence. Such modified miR backbones are labeled herein by the suffix "_M."

In some embodiments, the miRNA (pri-miRNA, a pre-miRNA, an artificial miRNA, or a mature miRNA) comprises or consists of a guide strand sequence and corresponding passenger strand sequence of any one of the duplex sequences set forth in Tables 1, 19, 23, and 24. In some embodiments, the passenger strand sequence of the miRNA comprises a sequence that is 100% complementary or perfectly complementary to the guide strand sequence. For example, a guide strand sequence may comprise or consist of a sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306,

308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, or 436 (guide sequences in Table 1), and the
 5 passenger strand sequence may comprise or consist of a sequence of SEQ ID NO: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173,
 10 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353,
 15 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, or 435 (passenger sequences in Table 1), respectively. In some embodiments, the passenger strand sequence of the miRNA is not 100% complementary or to the guide strand sequence. For example, a guide strand sequence may comprise or
 20 consist of a sequence of SEQ ID NO:1176 and the corresponding passenger strand sequence may comprise or consist of a sequence of SEQ ID NO:1289 (see, Table 19).

In some embodiments, the miRNA (pri-miRNA, a pre-miRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198,
 25 220, 242, 302, 306, 308, 330, 336, and 362, and a passenger strand sequence of comprising a sequence that is 100% complementary or perfectly complementary to the guide strand sequence. For example, a guide strand sequence may comprise or consist of a sequence of SEQ ID NO: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, or 362, and the passenger strand sequence may comprise or consist of a

sequence of SEQ ID NO: 11, 13, 39, 59, 99, 103, 107, 111, 123, 125, 127, 165, 197, 219, 241, 301, 305, 307, 329, 335, or 361, respectively.

In some embodiments, the miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, and the passenger strand sequence of the miRNA comprises or consists of a sequence that is 100% complementary or perfectly complementary to the guide strand. For example, a guide strand sequence may comprise a sequence of SEQ ID NO: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, or 362, and the passenger strand sequence may comprise a sequence of SEQ ID NO: 13, 39, 99, 107, 111, 127, 165, 197, 241, 307, 335, or 361, respectively.

In some embodiments, the miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of the guide sequences of Tables 1, 19, 23, and 24 and the passenger strand sequence comprises or consists of a corresponding passenger sequence of Tables 1, 19, 23, and 24 that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence of Tables 1, 19, 23 and 24. In some embodiments, the miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376,

378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, and a passenger strand sequence comprising or consisting a sequence of SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, respectively, wherein the passenger strand sequence has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence of SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow A$

conversion in the passenger strand sequence. In some embodiments, a mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the passenger strand sequence. In some embodiments, a wobble is a G-U wobble, wherein a C is converted to a T in the passenger strand sequence. In some embodiments, the passenger strand sequence is modified according to the rules of Table 8.

In some embodiments, the miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, and a passenger strand sequence comprising or consisting of a sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence comprising or consisting of a sequence of SEQ ID NOS: 11, 13, 39, 59, 99, 103, 107, 111, 123, 125, 127, 165, 197, 219, 241, 301, 305, 307, 329, 335, and 361, respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow A$ conversion in the passenger strand sequence. In some embodiments, a mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the passenger strand sequence. In some embodiments, a wobble is a G-U wobble, wherein a C is converted to a T in the passenger strand sequence. In some embodiments, the passenger strand sequence is modified according to the rules of Table 8.

In some embodiments, the miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, and a passenger strand sequence comprising or consisting of a sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence comprising or consisting of a sequence of SEQ ID NO: 13, 39, 99, 107, 111, 127, 165, 197, 241, 307, 335, or 361, respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow A$ conversion in the passenger strand sequence. In some embodiments, a mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the

passenger strand sequence. In some embodiments, a wobble is a G-U wobble, wherein a C is converted to a T in the passenger strand sequence. In some embodiments, the passenger strand sequence is modified according to the rules of Table 8.

In some embodiments, the miRNA is an artificial miRNA comprising a
5 guide strand sequence according to any of the embodiments described herein, contained within a miR-155 backbone sequence, miR1-1 backbone sequence, miR-100 backbone sequence, miR-124 backbone sequence, miR-138-2 backbone sequence, miR-122 backbone sequence, miR-128 backbone sequence, miR-130a backbone sequence, or miR-16-2 backbone sequence, wherein the artificial miRNA comprises a passenger strand
10 sequence that is modified according to Table 8. In some embodiments, the passenger strand sequence comprises a mismatch, wherein a mismatch is a G → C, C → G, A → T, or T → A conversion in the passenger strand sequence; a mismatch (to create a bulge with the guide strand) is a G → T, C → A, A → C, or T → G conversion in the passenger strand sequence; and a wobble is a G-U wobble, wherein a C is converted to a T in the passenger
15 strand sequence.

In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in any one of Tables 3, 9, 11, 19, 23, 24, and 25. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011,
20 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

Table 3: *ATXN2* Specific amiRNAs

Parent Guide Sequence	Parent Duplex ID	miR Backbone	Category	amiRNA Sequence
AGGAACGUGGGUUGAACUCCUU [SEQ ID NO:242]	XD-14857	miR-1-1	911 Control	CAUCAGACUGCUGCUGGUAUGGAGGAGUUAAGGACGUCGCC UUAUGGACCUGUAAGCUAAGGAACGUCCUUGAACUCCUUCU CAGCGGGGACCUCUCUCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:437]
AGGAACGUGGGUUGAACUCCUU [SEQ ID NO:242]	XD-14857	miR-155E	911 Control	CUGGAGGCUUGCUUUUGGGCUGUAUGCUGAGGAACGUCCUUGA ACUCCUUUUUUGGCCUCUGACUGAAAGGAGUUAAGGACGUCC UCAGGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAAUAGGC CACCGUG [SEQ ID NO:438]
UUCGGGUUGAAAUCUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-155E	911 Control	CUGGAGGCUUGCUUUUGGGCUGUAUGCUGGUGGUGUUCUUAUCU GAAGUUUUUUGGCCUCUGACUGAACACUCAAUAGAACC CGA ACAGGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAAUAGGC CACCGUG [SEQ ID NO:439]
UUCGGGUUGAAAUCUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-1-1	911 Control	CAUCAGACUGCUGCUGCUGGAGAGACUUCAGAUAGAACC CGAGA AUAUGGACCUGUAAGCUAUUCGGGUUCUUAUCUGAAGUGUCU CAGCGGGGACCUCUCUCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:440]
UUGAUUUCGAGGAUGUCGUGG [SEQ ID NO:128]	XD-14800	miR-155E	911 Control	CUGGAGGCUUGCUUUUGGGCUGUAUGCUGUUAUUCUCCUGAUG UCGUGGUUUUGGCCUCUGACUGACCGACGACUCGGGAAUUA ACAGGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAAUAGGC CACCGUG [SEQ ID NO:441]
UUGAUUUCGAGGAUGUCGUGG [SEQ ID NO:128]	XD-14800	miR-1-1	911 Control	CAUCAGACUGCUGCUGCUGGAGAGAGCAUCAGGAGAAACGCA AUAUGGACCUGUAAGCUAUUAUUAUUCUGAUGUCGUGGCU CAGCGGGGACCUCUCUCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:442]
AGAAAUUCGUAGACUGAGGCAGU [SEQ ID NO:14]	XD-14743	miR-1-1	Atxn2 targeting	CAUCAGACUGCUGCUGCUGGAGUGCCUCAGUCUACGAUCGUC UUAUGGACCUGCUAAGCUAAGAAAUUCGUAGACUGAGGCAGUCU

					CAGGCCGGACCCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:443]
AGAAUACGUAGACUGAGGCAGU [SEQ ID NO:14]	XD-14743	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUGAGAAAUCGUAGACUG AGGCAGUUUUUGGCCUCUGACUGAACUGCCUCGUCACGAUUUC UCAGGACAAGGCCCUUUUCAGCACUCACAUUGGAACAAAUGGC CACCGUG [SEQ ID NO:444]
AGAUACGUCAUUUUCCAAAGCC [SEQ ID NO:60]	XD-14766	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGGCCUUUGAAAAAUGACGUCCUC UUUUGGACCUGCUAAGCUAAGAUACGUCAUUUUCCAAAGCCCU CAGGCCGGACCCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:445]
AGAUACGUCAUUUUCCAAAGCC [SEQ ID NO:60]	XD-14766	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUGAGAUACGUCAUUUC CAAAGCCUUUGGCCUCUGACUGAGGCUUUGGAAAGACGUAUC UCAGGACAAGGCCCUUUUCAGCACUCACAUUGGAACAAAUGGC CACCGUG [SEQ ID NO:446]
AGCGUUAGGGUGCGCAUACUGC [SEQ ID NO:336]	XD-14904	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUGAGCGUUAGGGUGCGC AUACUGCUUUUGGCCUCUGACUGAGCAGUAUGGCACCUAACGC UCAGGACAAGGCCCUUUUCAGCACUCACAUUGGAACAAAUGGC CACCGUG [SEQ ID NO:447]
AGCGUUAGGGUGCGCAUACUGC [SEQ ID NO:336]	XD-14904	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGGAGUAUGCGCACCCCUAAGAGC UUUUGGACCUGCUAAGCUAAGCGUUAGGGUGCGCAUACUGCCU CAGGCCGGACCCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:448]
AGGAACGUGGGUUGAACUCCUU [SEQ ID NO:242]	XD-14857	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUGAGGAACGUGGGUUGA ACUCCUUUUUGGCCUCUGACUGAGGAAAGGAGUUAAACCGUUC UCAGGACAAGGCCCUUUUCAGCACUCACAUUGGAACAAAUGGC CACCGUG [SEQ ID NO:449]
AGGAACGUGGGUUGAACUCCUU [SEQ ID NO:242]	XD-14857	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGAUGGAGUUCAACCCACGUCGCC UUUUGGACCUGCUAAGCUAAGGAACGUGGGUUGAACCUUCU CAGGCCGGACCCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC

AUAAUAAUCCGUCAGUUUGACG [SEQ ID NO:426]	XD-14949	miR-155E	Atxn2 targeting	[SEQ ID NO:450]	CUGAGGCUUGCUUUGGGCUGUAUGCUGAUAAUAAUCCGUCAG UUUGACGUUUUGGCCUCUGACUGACGUCAAACGAGUAUUA UCAGGACAAGGCCCUUUAUCAGCACUCACAUAGGAACAAUUGGC CACCGUG [SEQ ID NO:451]
AUAAUAAUCCGUCAGUUUGACG [SEQ ID NO:426]	XD-14949	miR-1-1	Atxn2 targeting	[SEQ ID NO:452]	CAUGCAGACUGCCUCUUGGGCCUCAAACUCAGCGAUUACGU UUAUGGACCUGUAAGCUAAUAAUAAUCCGUCAGUUUGACGU CAGCCGGGACCCUCUCUGCCGCCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:453]
AUACGGGGUGAAUUCUGUCUCC [SEQ ID NO:102]	XD-14787	miR-155E	Atxn2 targeting	[SEQ ID NO:454]	CUGAGGCUUGCUUUGGGCUGUAUGCUGAUACGCGGUGAAUUC UGUCUCCUUUUGGCCUCUGACUGAGGACAGAUUACCGCGUA UCAGGACAAGGCCCUUUAUCAGCACUCACAUAGGAACAAUUGGC CACCGUG [SEQ ID NO:455]
AUACGGGGUGAAUUCUGUCUCC [SEQ ID NO:102]	XD-14787	miR-1-1	Atxn2 targeting	[SEQ ID NO:456]	CAUGCAGACUGCCUCUUGGGGCAGACAGAAUUCACCGCCUUA UUAUGGACCUGUAAGCUAAUAAUACGCGGUGAAUUCUGUCUCCU CAGCCGGGACCCUCUCUGCCGCCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:457]
AUUAACUACUCUUGGUCUGAA [SEQ ID NO:112]	XD-14792	miR-1-1	Atxn2 targeting	[SEQ ID NO:458]	CAUGCAGACUGCCUCUUGGGUACAGACCAAGAGUAGUCGAA UUAUGGACCUGUAAGCUAAUAAUACUACUCUUGGUCUGAACU CAGCCGGGACCCUCUCUGCCGCCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:459]
AUUAACUACUCUUGGUCUGAA [SEQ ID NO:112]	XD-14792	miR-155E	Atxn2 targeting	[SEQ ID NO:460]	CUGAGGCUUGCUUUGGGCUGUAUGCUGAUAAUACUACUCUUUG GUCUGAAUUUUGGCCUCUGACUGAUUACAGACCAGGAGUUA UCAGGACAAGGCCCUUUAUCAGCACUCACAUAGGAACAAUUGGC CACCGUG [SEQ ID NO:461]
AUUGCGGUGGAGUAGCUGGUGG [SEQ ID NO:306]	XD-14889	miR-155E	Atxn2 targeting	[SEQ ID NO:462]	CUGAGGCUUGCUUUGGGCUGUAUGCUGAUUGCGUGGAGUAAG CUGGUGUUUUGGCCUCUGACUGACCACCAAGCACCACGAA UCAGGACAAGGCCCUUUAUCAGCAUCACAUAGGAACAAUUGGC CACCGUG [SEQ ID NO:463]

AUUGCGUGGAGUAAGCUGGGUGG [SEQ ID NO: 306]	XD-14889	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUCUUGGGCGACGCUUACUCACGGAAA UUAUGGACCUGUAAGCUAAUUGCGUGGAGUAAGCUGGUGGU CAGCGCGGACCUUCUCUGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 458]
AUUUCGAGGAUGUCGUGGGGCC [SEQ ID NO: 124]	XD-14798	miR-155E	Atxn2 targeting	CUGAGGCUUUGGCUUUGGGGCUUAUGCUAAUUCGAGGAUGUGG CUGGGCCUUUGGCCUCUGACUGAGGCCAGCACACUCGAAA UCAGGACAAGGCCUUUAUCAGCACUCACAUAGGAACAAAUGGC CACCGUG [SEQ ID NO: 459]
AUUUCGAGGAUGUCGUGGGGCC [SEQ ID NO: 124]	XD-14798	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUCUUGGGCCCCAGCGACAUCUCGCGCAA UUAUGGACCUGUAAGCUAAUUCGAGGAUGUCGUGGGCCCU CAGCGCGGACCUUCUCUGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 460]
UAAAUUCGUAGACUGAGGCAGUC [SEQ ID NO: 12]	XD-14742	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUCUUGGGGUCUCUCAGUCUACGACGUU AUAUGGACCUGUAAGCUAAAUUCGUAACUGAGGCAGUCU CAGCGCGGACCUUCUCUGCGGCACUCAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 461]
UAAAUUCGUAGACUGAGGCAGUC [SEQ ID NO: 12]	XD-14742	miR-155E	Atxn2 targeting	CUGGAGGCUUUGCUUUGGGGCUUAUGCUAAAUUCGUAACUGA GGCAGUCUUUGGCCUCUGACUGAGACUGCCUAGUUACGAUU ACAGGACAAGGCCUUUAUCAGCACUCACAUAGGAACAAAUGGC CACCGUG [SEQ ID NO: 462]
UACGCGGUGAAUUCUGUCUCCC [SEQ ID NO: 100]	XD-14786	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUCUUGGGCGAGACAGAAUUCACCGGAGU AUAUGGACCUGUAAGCUAACGCGGUGAAUUCUGUCUCCC CAGCGCGGACCUUCUCUGCGGCACUCAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 463]
UACGCGGUGAAUUCUGUCUCCC [SEQ ID NO: 100]	XD-14786	miR-155E	Atxn2 targeting	CUGAGGCUUUGCUUUGGGGCUUAUGCUACGCGGUGAAUUCU GUUCCUUUGGCCUCUGACUGAGGGGACAAUUCACCGCGU ACAGGACAAGGCCUUUAUCAGCACUCACAUAGGAACAAAUGGC CACCGUG [SEQ ID NO: 464]
UAAUACGCGGUGAAUUCUGUCUC [SEQ ID NO: 104]	XD-14788	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUCUUGGGGUGACAGAAUUCACCGCGCAU AUAUGGACCUGCUAAAGCUAAUACGCGGUGAAUUCUGUCUCCU

					CAGCGCGGACCCUCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGCC [SEQ ID NO:465]
UAUACGGGUGAAUUCUGUCUC [SEQ ID NO:104]	XD-14788	miR-155E	Atxn2 targeting		CUGAGGCUUGCUUUGGGCUGUAUGCUUAUACGGGUGAAU CUGUCUUUUGGCCUCUGACUGAGAGACAGAUUCCGCGU ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAUAGGC CACCGUG [SEQ ID NO:466]
UAUUGCGUGGAGUAAGCUGGUG [SEQ ID NO:308]	XD-14890	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGCUCACGCUUACUCCAGCCCCAU AUAUGGACCUGUAAGCUAUAUUGCGUGAGUAAGCUGGUGCU CAGCGGGACCCUCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGCC [SEQ ID NO:467]
UAUUGCGUGGAGUAAGCUGGUG [SEQ ID NO:308]	XD-14890	miR-155E	Atxn2 targeting		CUGAGGCUUGCUUUGGGCUGUAUGCUUAUUGCGUGGAGUAA GCUGUGUUUUGGCCUCUGACUACACGCUACUCCAGCAAU ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAUAGGC CACCGUG [SEQ ID NO:468]
UAUUUCGAGGAUGUCGCGGGC [SEQ ID NO:126]	XD-14799	miR-155E	Atxn2 targeting		CUGAGGCUUGCUUUGGGCUGUAUGCUUAUUCGAGGAUGUC GCUGGCUUUUGGCCUCUGACUGAGCCAGCGCAUCUCGAAU ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAUAGGC CACCGUG [SEQ ID NO:469]
UAUUUCGAGGAUGUCGCGGGC [SEQ ID NO:126]	XD-14799	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGGCCAGGACAUCCUGGACCAU AUAUGGACCUGUAAGCUAUAUUCGAGGAUGUCGUGGGCCU CAGCGGGACCCUCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGCC [SEQ ID NO:470]
UCGCUUGGGGCAUAUUUGGU [SEQ ID NO:302]	XD-14887	miR-1-1	Atxn2 targeting		CAUGCAGACUGCCUGCUUGGGAGCAAAUAGCCCCACACUCG AUAUGGACCUGUAAGCUAUGCUGUUGGGCAUAUUGGUCU CAGCGGGACCCUCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGCC [SEQ ID NO:471]
UCGCUUGGGGCAUAUUUGGU [SEQ ID NO:302]	XD-14887	miR-155E	Atxn2 targeting		CUGAGGCUUGCUUUGGGCUGUAUGCUUGCGUGUUGGGCAU AUUUGUUUUGGCCUCUGACUGAACCAAAUAGCCCAACAGCG ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAUAGGC CACCGUG

UGCGCAUACUGCUGAGCAAGGG [SEQ ID NO: 330]	XD-14901	miR-1-1	Atxn2 targeting	[SEQ ID NO: 472] CAUGCAGACUGCUGCUGGGCGCUUGCUCAGCAGUAUUGGAGC AUAUGGACCUGUAAGCUAUGCGCAUACUGCUGAGCAAGGGCU CAGCGGGGACCUCUCUGCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 473] CUGGAGGCUUGCUUUGGGCUGUAUGCUGGCGCAUACUGCUGA GCAAGGGUUUGGCCUCUGACUGACCCUUGCUAGCGUAUGCGC ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAAUUGGC CACCGUG [SEQ ID NO: 474] CAUGCAGACUGCUGCUGGGGAUCAGACUUUUGUGGGCGAC AUAUGGACCUGUAAGCUAUGUACCAACAAAGUCUGAACCU CAGCGGGGACCUCUCUGCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 475] CUGGAGGCUUGCUUUGGGCUGUAUGCUGUACCAACAAAG UCUGAACUUUGGCCUCUGACUGAGUUUCAGACUUUGUGGUAC ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAAUUGGC CACCGUG [SEQ ID NO: 476] CAUGCAGACUGCUGCUGGGGUGAGUUACGCCGGCGUACGAC AUAUGGACCUGUAAGCUAUGUAUACGCCGGCUGAACGUGACU CAGCGGGGACCUCUCUGCGCGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO: 477] CUGGAGGCUUGCUUUGGGCUGUAUGCUGUUAUACGCCGGCUG AACUGAUUUUGGCCUCUGACUGAUCAGUUUCGCCGCUAUAC ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAAUUGGC CACCGUG [SEQ ID NO: 478] CUGGAGGCUUGCUUUGGGCUGUAUGCUGUUAUACAAUUGAA GGGAAAUUUUGGCCUCUGACUGAUUUUCCCUCAAACUAGUA ACAGACAAGGCCUUUAUCAGCACUCACAUGGAACAAAUUGGC CACCGUG [SEQ ID NO: 479]
UGCGCAUACUGCUGAGCAAGGG [SEQ ID NO: 330]	XD-14901	miR-155E	Atxn2 targeting	
UGUACCAACAACAAAGUCUGAAC [SEQ ID NO: 40]	XD-14756	miR-1-1	Atxn2 targeting	
UGUACCAACAACAAAGUCUGAAC [SEQ ID NO: 40]	XD-14756	miR-155E	Atxn2 targeting	
UGUAUACGCCGGCUGAACGUGA [SEQ ID NO: 362]	XD-14917	miR-1-1	Atxn2 targeting	
UGUAUACGCCGGCUGAACGUGA [SEQ ID NO: 362]	XD-14917	miR-155E	Atxn2 targeting	
UUACUAAAGUAUUGAGGGGAAA [SEQ ID NO: 220]	XD-14846	miR-155E	Atxn2 targeting	

UUACUAAGUAUUGAAGGGAAA [SEQ ID NO:220]	XD-14846	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUGCUUGGGUAUCCCUUCAAUACUUAUUA AUUGGACCUUGUAAGCUAUUACUAAUAUUGAAGGGAAACU CAGCCGGGACCUUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:480]
UUAGUUGAUCCAUAGAUUCAGA [SEQ ID NO:198]	XD-14835	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUGCUUGGGUGUGAAUUAUGGAUCAAAGAU AUUGGACCUUGUAAGCUAUUAGUUGAUCAUAGAUUUCAGACU CAGCCGGGACCUUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:481]
UUAGUUGAUCCAUAGAUUCAGA [SEQ ID NO:198]	XD-14835	miR-155E	Atxn2 targeting	CUGAGGCUUGCUUUUGGGCUGUAUGCUUUAGUUGAUCCAUAG AUUCAGAUUUUGGCCUCUGACUGACUGAAUUAUGAUCAAUA ACAGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAUUGGC CACCGUG [SEQ ID NO:482]
UUCGAUGCAGGACUAGCAGGCG [SEQ ID NO:166]	XD-14819	miR-155E	Atxn2 targeting	CUGAGGCUUGCUUUUGGGCUGUAUGCUUUUGGAUGCAGGACUA GCAGCGUUUUGGCCUCUGACUGACGCGCUGCUGCUGCAUCGA ACAGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAUUGGC CACCGUG [SEQ ID NO:483]
UUCGAUGCAGGACUAGCAGGCG [SEQ ID NO:166]	XD-14819	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUGCUUGGGCCCCCUGUAGUCCUGCAUGAGA AUUGGACCUUGUAAGCUAUUUGAUGAGGACUAGAGGCGCU CAGCCGGGACCUUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:484]
UUCGGGUUGAAAUUCUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-155E	Atxn2 targeting	CUGAGGCUUGCUUUUGGGCUGUAUGCUUUUGGGUUGAAUUCU GAAGUUUUUGGCCUCUGACUGAACACUUAUUAUCAAACCCGA ACAGACAAGGCCUUUAUCAGCACUCACAUUGGAACAAUUGGC CACCGUG [SEQ ID NO:485]
UUCGGGUUGAAAUUCUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUGCUUGGGAGACUUCAGAUUUAACCGAGA AUUGGACCUUGUAAGCUAUUUGGGUUGAAUUAUGAUGUGUCU CAGCCGGGACCUUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:486]
UUGAUUUCGAGGAUGUCGUGG [SEQ ID NO:128]	XD-14800	miR-1-1	Atxn2 targeting	CAUGCAGACUGCGUGCUUGGGGAGCGACAUCCUGAAACGCA AUUGGACCUUGUAAGCUAUUUGAUGAGGGAUUGCUGUGCU

					CAGCGCGGACCUCUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:487]
UUGAUUUCGAGGAUGUCGUGG [SEQ ID NO:128]	XD-14800	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUUUGAUUUCGAGGAUG UCGUGGUUUUGGCCUCUGACUGACCCAGCGACUCCGAAAUCA ACAGGACAAGGCCCUUUAUCAGCACUCACAUGGAACAAAUAGGC CACCGUG [SEQ ID NO:488]
UUGUACUGGGCACUUGACUCAA [SEQ ID NO:90]	XD-14781	miR-155E	Atxn2 targeting		CUGGAGGCUUGCUUUGGGCUGUAUGCUUUGAUUUCUGGGCACUU GACUCAAUUUGGCCUCUGACUGAUUGAGUCAGUGCCAGUACA ACAGGACAAGGCCCUUUAUCAGCACUCACAUGGAACAAAUAGGC CACCGUG [SEQ ID NO:489]
UUGUACUGGGCACUUGACUCAA [SEQ ID NO:90]	XD-14781	miR-1-1	Atxn2 targeting		CAUGCAGACUGCGUGCUUGGGUAGAGUCAAUGGCCAGUCCCA AUUGGACCUUGUAAGCUAUUUAUCUGGGCACUUGACUCAACU CAGCGCGGACCUUCUCGCGGCACUGAGGGGCACUCCACACC ACGGGGGCC [SEQ ID NO:490]

In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 3. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:443-490.

5 In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 9. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1109-1111, and 1114.

In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 11. In some embodiments, an artificial miRNA
10 comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1121-1168.

In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 19. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1405-
15 1520.

In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 23. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1908-2007.

20 In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence set forth in Table 24. In some embodiments, an artificial miRNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1908-1934, 1936-1977, 1979-1982, 1984-1994, 1997, 1998, 2000, 2001, 2005-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067,
25 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

In some embodiments, an artificial miRNA comprises or consists of a
30 nucleic acid sequence set forth in Table 25. In some embodiments, an artificial miRNA

comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1915, 1982, 1965, 1937, 1985, 1921, and 2021.

Expression Constructs

5 In another aspect, the present disclosure provides an isolated nucleic acid comprising an expression construct or expression cassette encoding any one of the inhibitory nucleic acids (e.g., siRNA, shRNA, dsRNA, miRNA, amiRNA, etc.) that inhibit the expression or activity of ATXN2 as described herein.

 In some embodiments, the isolated nucleic acid comprises an expression
10 construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54,
15 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238,
20 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410,
25 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

 In some embodiments, the isolated nucleic acid comprises an expression
construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence
30 comprising or consisting of the nucleic acid sequence set forth in any one of the guide

sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356,

358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12,
10 13, 19, 23, 24, and 25 e.g., SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184,
15 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356,
20 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO.

25 In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20,
30 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66,

68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

15 In some embodiments, the isolated nucleic acid molecule comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 12. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 200, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 200, 242, 302, 306, 308, 330, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%,

70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 13. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the

expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some

5 embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362,

10 preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40,

15 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression

20 construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 19. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic

25 acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 1176-1288, 40, 108, and 166 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated

30 nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that

inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 23. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand

sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 24. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an

inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the inhibitory nucleic acid comprises a guide strand sequence of Table 25. In some embodiments, the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of ATXN2, wherein the

inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide

strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an inhibitory nucleic acid that inhibits the expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that targets *ATXN2* mRNA to interfere with *ATXN2* expression by mRNA degradation or translational inhibition. In some embodiments, the guide strand of the siRNA duplex may be about 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides in length or 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 21-30, 21-29, 21-28, 21-27, 21-26,

21-25, 21-24, 21-23, 22-30, 22-29, 22-28, 22-27, 22-26, 22-24, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 24-30, 24-29, 24-28, 24-27, 24-26, 25-30, 25-29, 25-28, 25-27, 26-30, 26-29, 26-28, 27-30, 27-29, 28-30 nucleotides in length. In some embodiments, the passenger strand of the siRNA duplex may be about 18 nucleotides, 19 nucleotides, 20
5 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides in length or 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 21-30, 21-29, 21-28, 21-27, 21-26,
10 21-25, 21-24, 21-23, 22-30, 22-29, 22-28, 22-27, 22-26, 22-24, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 24-30, 24-29, 24-28, 24-27, 24-26, 25-30, 25-29, 25-28, 25-27, 26-30, 26-29, 26-28, 27-30, 27-29, 28-30 nucleotides in length. In some embodiments, the siRNA duplex contains 2 or 3 nucleotide 3' overhangs on each strand. In some embodiments, the 3' overhangs are complementary to the *ATXN2* transcript. In some
15 embodiments, the guide strand and passenger strand of the siRNA duplex are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 100% complementary to each other, not including any nucleotides in overhang(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising
20 or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, and 24, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138,
25 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308,
30 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344,

346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, and 24, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 10 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 15 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 20 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence sequence 25 comprising or consisting of a sequence that at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, and 24, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 30 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144,

146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178,
180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212,
214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246,
248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280,
5 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314,
316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350,
352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384,
386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418,
420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083,
10 2152, 2203, and 2209.

In some embodiments, the isolated nucleic acid comprises an expression
construct encoding a siRNA duplex that comprises a guide strand sequence comprising
or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a
sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, and 24,
15 e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34,
36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80,
82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120,
124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156,
158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190,
20 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224,
226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258,
260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292,
294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328,
330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362,
25 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396,
398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430,
432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209,
preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of
the selected SEQ ID NO.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, and 24, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence of Table 12. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence.

In some embodiments, the siRNA duplex comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence of Table 13. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%,

80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

15 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence of Table 19. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1176-1288, 40, 108, and 166 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18,

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- 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a
- 5 siRNA duplex that comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).
- 10 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence of Table 23. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007.
- 15 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007 with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a
- 20 siRNA duplex that comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising a guide strand sequence comprising
- 25 or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a
- 30 sequence of any one of SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at

positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression
5 construct encoding a siRNA duplex that comprises a guide strand sequence of Table 24. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224,
10 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822,
15 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to
20 any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising a guide strand sequence comprising or consisting of at least 15, 16, 17, 18,
25 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an
30 expression construct encoding a siRNA duplex that comprises a guide strand sequence

comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that
 5 the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence of Table 25. In some embodiments, the isolated nucleic acid comprises an expression construct
 10 encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of the nucleic acid
 15 sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%,
 20 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a
 25 sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a siRNA duplex that comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID
 30 NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4

nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments the isolated nucleic acid comprises an expression
5 construct encoding a siRNA duplex comprising a guide strand sequence and passenger strand sequence of any one of siRNA duplexes provided in Tables 1, 19, 23, and 24. In some embodiments the isolated nucleic acid comprises an expression construct encoding a siRNA duplex comprising a guide strand sequence and passenger strand sequence, comprising or consisting of any one of: SEQ ID NOS:12 and 11; SEQ ID
10 NOS: 14 and 13; SEQ ID NOS: 40 and 39; SEQ ID NOS: 60 and 59; SEQ ID NOS: 100 and 99; SEQ ID NOS: 104 and 103; SEQ ID NOS: 108 and 107; SEQ ID NOS: 112 and 111; SEQ ID NOS: 124 and 123; SEQ ID NOS: 126 and 125; SEQ ID NOS: 128 and 127; SEQ ID NOS: 166 and 165; SEQ ID NOS: 198 and 197; SEQ ID NOS: 220 and 219; SEQ ID NOS: 242 and 241; SEQ ID NOS: 302 and 301; SEQ ID NOS: 306 and 305; SEQ ID NOS: 308 and 307; SEQ ID NOS: 330 and 320; SEQ ID NOS: 336 and 335; and SEQ ID NOS: 362 and 361. In some embodiments the isolated nucleic acid comprises an expression construct encoding a siRNA duplex comprising a guide strand sequence and passenger strand sequence comprising or consisting of any one of: SEQ ID NOS:14 and 13; SEQ ID NOS: 40 and 39; SEQ ID NOS: 100 and 99; SEQ ID
20 NOS: 108 and 107; SEQ ID NOS: 112 and 111; SEQ ID NOS: 128 and 127; SEQ ID NOS: 166 and 165; SEQ ID NOS: 198 and 197; SEQ ID NOS: 242 and 241; SEQ ID NOS: 308 and 307; SEQ ID NOS: 336 and 335; and SEQ ID NOS: 362 and 361.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a shRNA comprising a guide strand and passenger strand of a
25 siRNA duplex as provided herein, linked by a short spacer sequence, *i.e.*, loop. In some embodiments, loop sequence is 4, 5, 6, 7, 8, 9, or 10 nucleotides in length or 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 6-9, 6-8, 7-10, 7-9, or 8-10 nucleotides in length.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA,
30 or a mature miRNA, wherein the miRNA comprises a guide strand sequence

comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374,

376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence.

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of the guide sequences of Tables 1, 3, 9,
 10 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178,
 15 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350,
 20 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

In some embodiments, the isolated nucleic acid comprises an expression
 25 construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22,
 30 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68,

70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 5 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 10 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 (“seed sequence”) of the selected SEQ ID NO.

In some embodiments, the isolated nucleic acid comprises an expression
15 construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence of any one of the guide sequences of Tables 1, 3, 9, 11, 12, 13, 19, 23, 24, and 25, e.g., any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 20 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 25 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 30 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-

1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 12. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA
10 comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the
15 miRNA comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a
20 pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362. In some embodiments, the
25 isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306,
30 308, 330, 336, and 362, preferably wherein the guide strand sequence retains positions

2-7 (“seed sequence”) of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of

5 SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)) such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

10 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 13. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA,

15 comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand

20 sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA wherein the miRNA comprises a guide strand sequence comprising or

25 consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a

30 guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or

22 contiguous nucleotides of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 19. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS: 1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS: 1176-1288, 40, 108, and 166. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous

nucleotides of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1176-1288, 40, 108, and 166, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

10 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 23. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA,

15 comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of the nucleic acid

20 sequence set forth in any one of SEQ ID NOS:1908-2007, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100%

25 identical to any one of SEQ ID NOS:1908-2007. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID

30 NOS:1908-2007, preferably wherein the guide strand sequence retains positions 2-7

(“seed sequence”) of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of
5 SEQ ID NOS:1908-2007, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression
10 construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 24. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, comprising a guide strand sequence comprising or consisting of the nucleic acid
15 sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a
20 guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an
25 artificial miRNA, or a mature miRNA wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209. In
30 some embodiments, the isolated nucleic acid comprises an expression construct

encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA comprising a guide strand sequence of Table 25. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA wherein the miRNA comprises a guide strand sequence comprising or consisting of a

nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, comprising a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811, with at least 1, 2, 3, 4, or 5 mismatches to the target *ATXN2* mRNA sequence. In some embodiments, the miRNA is a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA wherein the miRNA comprises a guide strand sequence comprising or consisting of a nucleic acid sequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100% identical to any one of SEQ ID NOS:1185, 1816, 1213, and 1811. In some embodiments, the isolated

nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of at least 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleotides of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, preferably wherein the guide strand sequence retains positions 2-7 ("seed sequence") of the selected SEQ ID NO. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA, such as a pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA, wherein the miRNA comprises a guide strand sequence comprising or consisting of a sequence of any one of SEQ ID NOS:1185, 1816, 1213, and 1811, wherein 1, 2, 3, or 4 nucleotides at positions 19-22 differ from the selected SEQ ID NO (variant nucleotide(s)), such that the guide strand sequence is no longer complementary to the *ATXN2* target sequence at the variant nucleotide(s).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA comprising a guide strand sequence according to any of the embodiments described herein, contained within a miR backbone sequence. In some embodiments, the guide strand sequence and passenger strand sequence of the artificial miRNA are contained within a miRNA backbone sequence. In some embodiments, the miRNA backbone sequence is contained within a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190A backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-16-2 backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence. In some embodiments, the terminal loop, stem, 5' flanking segment, 3' flanking segment, or any combination thereof of the miR-155 backbone sequence, miR1-1 backbone sequence, miR-100 backbone sequence, miR-190A backbone sequence, miR-124 backbone

sequence, miR-16-2 backbone sequence, miR-132 backbone sequence, miR-9 backbone sequence, miR-138-2 backbone sequence, miR-122 backbone sequence, miR-130a backbone sequence, miR-128 backbone sequence, miR-144 backbone sequence, miR-451a backbone sequence, or miR-223 backbone sequence is modified (e.g., nucleotide
 5 insertion, deletion, substitution, mismatch, wobble, or any combination thereof).

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprising a guide strand sequence and corresponding passenger strand sequence comprising or consisting of any one of the duplex sequences set forth
 10 in Tables 1, 19, 23, and 24. In some embodiments, the passenger strand sequence of the miRNA comprises a sequence that is 100% complementary or perfectly complementary to the guide strand sequence. For example, the encoded guide strand sequence may comprise or consist of a sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70,
 15 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250,
 20 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422,
 25 424, 426, 428, 430, 432, 434, or 436 (guide sequences in Table 1), and the encoded passenger strand sequence may comprise or consist of a sequence of SEQ ID NO: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133,
 30 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167,

169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 5 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, or 435, respectively (passenger sequences in Table 1). In some embodiments, the passenger strand sequence of the miRNA is not 100% complementary or to the guide strand sequence. For example, a guide strand sequence may comprise or consist of a sequence of SEQ ID NO:1176 and the corresponding passenger strand sequence may comprise or consist of a sequence of SEQ ID NO:1289 (see, Table 19).

In some embodiments, the isolated nucleic acid comprises an expression 15 construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) comprising a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, and a passenger strand sequence of comprising a sequence that is 100% complementary or perfectly complementary to the guide strand 20 sequence. For example, the encoded guide strand sequence may comprise or consist of a sequence of SEQ ID NO: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, or 362, and the encoded passenger strand sequence may comprise or consist of a sequence of SEQ ID NO: 11, 13, 39, 59, 99, 103, 107, 111, 123, 125, 127, 165, 197, 219, 241, 301, 305, 307, 329, 335, or 361, respectively.

25 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) wherein the miRNA comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, and a passenger strand sequence comprising a sequence that is 100% 30 complementary or perfectly complementary to the guide strand. For example, the

encoded guide strand sequence may comprise or consist of a sequence of SEQ ID NO: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, or 362, and the encoded passenger strand sequence may comprise or consisting of a sequence of SEQ ID NO: 13, 39, 99, 107, 111, 127, 165, 197, 241, 307, 335, or 361, respectively.

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA), wherein the miRNA comprises a guide strand sequence comprising or consisting of any one of the guide sequences of Tables 1, 19, 23, and 24, and the passenger strand sequence comprises or consists of a corresponding passenger sequence
10 of Tables 1, 19, 23, and 24 that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence of Tables 1, 19, 23, and 24. In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA), wherein the miRNA
15 comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168,
20 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340,
25 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, and a passenger strand sequence comprising or consisting of a sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any
30 combination thereof relative to the corresponding passenger strand sequence of SEQ ID

NOS: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47,
 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93,
 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129,
 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163,
 5 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197,
 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231,
 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265,
 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299,
 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333,
 10 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367,
 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401,
 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435
 respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow$
 A conversion in the encoded passenger strand sequence. In some embodiments, a
 15 mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow$
 G conversion in the encoded passenger strand sequence. In some embodiments, a
 wobble is a G-U wobble, wherein a C is converted to a T in the encoded passenger
 strand sequence. In some embodiments, the passenger strand sequence is modified
 according to the rules of Table 8.
 20 In some embodiments, the isolated nucleic acid comprises an expression
 construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a
 mature miRNA) wherein the miRNA comprises a guide strand sequence comprising or
 consisting of any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126,
 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362, and a passenger strand
 25 sequence comprising or consisting of a sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or
 more insertions, deletions, substitutions, mismatches, wobbles, or any combination
 thereof, relative to the passenger strand sequence comprising or consisting of SEQ ID
 NOS: 11, 13, 39, 59, 99, 103, 107, 111, 123, 125, 127, 165, 197, 219, 241, 301, 305, 307,
 329, 335, and 361, respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow$
 30 G, $A \rightarrow T$, or $T \rightarrow A$ conversion in the passenger strand sequence. In some

embodiments, a mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the passenger strand sequence. In some embodiments, a wobble is a G-U wobble, wherein a C is converted to a T in the passenger strand sequence. In some embodiments, the passenger strand sequence is modified according to the rules of Table 8.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding a miRNA (pri-miRNA, a pre-mRNA, an artificial miRNA, or a mature miRNA) wherein the miRNA comprises a guide strand sequence comprising or consisting of any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362, and a passenger strand sequence comprising a sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof relative to the passenger strand sequence comprising or consisting of SEQ ID NOS: 13, 39, 99, 107, 111, 127, 165, 197, 241, 307, 335, and 361, respectively. In some embodiments, a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow A$ conversion in the encoded passenger strand sequence. In some embodiments, a mismatch (to create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the encoded passenger strand sequence. In some embodiments, a wobble is a G-U wobble, wherein a C is converted to a T in the encoded passenger strand sequence. In some embodiments, the passenger strand sequence is modified according to the rules of Table 8.

In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA comprising a guide strand sequence according to any of the embodiments described herein, contained within a miR-155M backbone sequence, miR-155E backbone sequence, miR1-1 backbone sequence, miR-100 backbone sequence, miR-124 backbone sequence, miR-138-2 backbone sequence, miR-122 backbone sequence, miR-128 backbone sequence, miR-130a backbone sequence, or miR-16-2 backbone sequence, wherein the artificial miRNA comprises a passenger strand sequence that is modified according to Table 8. In some embodiments, the passenger strand sequence comprises a mismatch, wherein a mismatch is a $G \rightarrow C$, $C \rightarrow G$, $A \rightarrow T$, or $T \rightarrow A$ conversion in the passenger strand sequence; a mismatch (to

create a bulge with the guide strand) is a $G \rightarrow T$, $C \rightarrow A$, $A \rightarrow C$, or $T \rightarrow G$ conversion in the passenger strand sequence; and a wobble is a G-U wobble, wherein a C is converted to a T in the passenger strand sequence.

In some embodiments, the isolated nucleic acid comprises an expression
5 construct encoding an artificial miRNA comprising or consisting of a nucleic acid sequence set forth in any one of Tables 3, 9, 11, 1923, 24, and 25. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA comprising or consisting of any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011, 2017, 2021, 2025, 2027, 2031,
10 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267..

In some embodiments, the isolated nucleic acid comprises an expression
15 construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 3. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:443-490.

In some embodiments, the isolated nucleic acid comprises an expression
20 construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 9. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1109-1111, and 1114.

In some embodiments, the isolated nucleic acid comprises an expression
25 construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 11. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1121-1168.

In some embodiments, the isolated nucleic acid comprises an expression
30 construct encoding an artificial miRNA that comprises or consists of a nucleic acid

sequence set forth in Table 19. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1405-1520.

5 In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 23. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1908-2007.

10 In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 24. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1908-1934, 1936-1977, 1979-1982, 1984-1994, 1997, 1998, 2000, 2001, 2005-2007, 2011, 2017, 2021, 2025, 15 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

20 In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence set forth in Table 25. In some embodiments, the isolated nucleic acid comprises an expression construct encoding an artificial miRNA that comprises or consists of a nucleic acid sequence of any one of SEQ ID NOS:1915, 1982, 1965, 1937, 1985, 1921, and 2021.

25 In some embodiments, expression constructs encoding the inhibitory nucleic acids that target ATXN2 mRNA comprises or consists of any of the guide strand sequences or artificial miRNA sequences disclosed in DNA format. For example, Tables 9, 11, 23, and 24 provide amiRNA sequences in DNA format, which DNA sequence may be inserted into expression constructs. Alternatively, amiRNA

sequences provided herein can be converted to DNA format by replacing each “U” nucleotide with a “T” nucleotide.

In some embodiments, the expression construct encodes two or more inhibitory nucleic acids that target an *ATXN2* mRNA transcript described herein. In some embodiments, the expression construct encodes an inhibitory nucleic acid that targets *ATXN2* transcript and an inhibitory nucleic acid that targets a second target transcript other than *ATXN2*. In some embodiments, the second target transcript is C9ORF72. Examples of inhibitory nucleic acids targeting C9ORF72 are described in US Patent Publication US2019/0316126 (incorporated by reference in its entirety). In some embodiments, the expression construct encodes an inhibitory nucleic acid that targets *ATXN2* transcript and encodes a therapeutic polypeptide or protein.

In some embodiments, the expression construct is monocistronic. In some embodiments, the expression construct is polycistronic (e.g., expression construct encodes two or more peptides or polypeptides). In some embodiments, a nucleic acid sequence encoding a first gene product (e.g., inhibitory nucleic acid targeting *ATXN2* mRNA) and a nucleic acid sequence encoding a second gene product within an expression construct are separated by an internal ribosome entry site (IRES), furin cleavage site, or viral 2A peptide. In some embodiments, a viral 2A peptide is a porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), *B. mori* cytoplasmic polyhedrosis virus (BmCPV 2A), *B. mori* flacherie virus (BmIFV 2A), or variant thereof.

In some embodiments, the expression construct further comprises one or more expression control sequences (regulatory sequences) operably linked with the transgene (e.g., nucleic acid encoding an artificial miRNA). “Operably linked” sequences include expression control sequences that are contiguous with the transgene or act in trans or at a distance from the transgene to control its expression. Examples of expression control sequences include transcription initiation sequences, termination sequences, promoter sequences, enhancer sequences, repressor sequences, splice site sequences, polyadenylation (polyA) signal sequences, or any combination thereof.

In some embodiments, a promoter is an endogenous promoter, synthetic promoter, constitutive promoter, inducible promoter, tissue-specific promoter (e.g., CNS-specific), or cell-specific promoter (neurons, glial cells, or astrocytes). Examples of constitutive promoters include, Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), cytomegalovirus (CMV) promoter (optionally with the CMV enhancer), SV40 promoter, and dihydrofolate reductase promoter. Examples of inducible promoters include zinc-inducible sheep metallothioneine (MT) promoter, dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, T7 polymerase promoter system, the ecdysone insect promoter, tetracycline-repressible system, tetracycline-inducible system, RU486-inducible system, and the rapamycin-inducible system. Further examples of promoters that may be used include, for example, chicken beta-actin promoter (CBA promoter), a CAG promoter, a H1 promoter, a CD68 promoter, a JeT promoter, synapsin promoter, RNA pol II promoter, or a RNA pol III promoter (e.g., U6, H1, etc.). In some embodiments, the promoter is a tissue-specific RNA pol II promoter. In some embodiments, the tissue-specific RNA pol II promoter is derived from a gene that exhibits neuron-specific expression. In some embodiments, the neuron-specific promoter is a synapsin 1 promoter or synapsin 2 promoter.

In some embodiments, the promoter is an H1 promoter comprising or consisting of the sequence set forth in nucleotides 113-203 of SEQ ID NO:1522. In some embodiments, the promoter is an H1 promoter comprising or consisting of the sequence set forth in nucleotides 1798-1888 of SEQ ID NO:1521. In some embodiments, the promoter is an H1 promoter comprising or consisting of the sequence set forth in nucleotides 113-343 of any one of SEQ ID NOS:2257-2260. In some embodiments, the promoter is an H1 promoter comprising or consisting of the sequence set forth in nucleotides 244-343 of any one of SEQ ID NOS:2257-2260.

In some embodiments, the sequence encoding the inhibitory nucleic acid of the present disclosure is positioned in an untranslated region of an expression construct. In some embodiments, the sequence encoding the inhibitory nucleic acid of the present disclosure is positioned in an intron, a 5' untranslated region (5'UTR), or a

3' untranslated region (3'UTR) of the expression construct. In some embodiments, the sequence encoding the inhibitory nucleic acid of the present disclosure is positioned in an intron downstream of the promoter and upstream of an expressed gene.

In some embodiments, the isolated nucleic acid comprises an expression
 5 construct encoding an inhibitory nucleic, flanked by two AAV inverted terminal repeats (ITRs) (e.g., 5' ITR and 3' ITR). In some embodiments, each AAV ITR is a full length ITR (e.g., approximately 145 bp in length, and containing functional Rep binding site (RBS) and terminal resolution site (trs)). In some embodiments, one of the ITRs is truncated (e.g., shortened or not full-length). In some embodiments, a truncated ITR
 10 lacks a functional terminal resolution site (trs) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a truncated ITR is a truncated version of AAV2 ITR referred to as AITR (D-sequence and TRS are deleted). In some embodiments, the ITRs are selected from AAV serotypes of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14),
 15 AAV10, AAV11, AAV 12, AAVrh8, AAVrh10, AAV-DJ8, AAV-DJ, AAV- PHP.A, AAV-PHP.B, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B- SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT,
 20 AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B- STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A 15/G2A3, AAVG2B4, AAVG2B5, and variants thereof.

In some embodiments, the isolated nucleic acid molecule comprising an
 25 expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* comprises the nucleotide sequence set forth in any one of SEQ ID NOS:2257-2260. In some embodiments, the isolated nucleic acid molecule comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* comprises the nucleotide sequence set forth in SEQ ID NO:2257. In
 30 some embodiments, the isolated nucleic acid molecule comprising an expression

construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* comprises the nucleotide sequence set forth in SEQ ID NO:2258. In some embodiments, the isolated nucleic acid molecule comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* comprises the nucleotide sequence set forth in SEQ ID NO:2259. In some embodiments, the isolated nucleic acid molecule comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* comprises the nucleotide sequence set forth in SEQ ID NO:2260.

Additional isolated nucleic acid molecules comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2* may be constructed using the nucleotide sequence set forth in any one of SEQ ID NOS:2257-2260, by substituting the desired inhibitory nucleic acid sequence (e.g., artificial miRNA cassette) of the present disclosure into nucleotide positions 344-481 of any one of SEQ ID NOS:2257-2260.

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Vectors and Host Cells

Inhibitory nucleic acid molecules (siRNAs, shRNAs, miRNAs) described herein can be encoded by vectors. The use of vectors, e.g., AAV, for expressing inhibitory nucleic acids of the present disclosure may allow for continual or controlled expression of inhibitory nucleic acid in the subject, rather than multiple doses of isolated inhibitory nucleic acids to the subject. The present disclosure provides a vector comprising an isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid described herein. A vector can be a plasmid, cosmid, phagemid, bacterial artificial chromosome (BAC) or viral vector. Examples of viral vectors include herpesvirus (HSV) vectors, retroviral vectors, adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, baculoviral vectors, and the like. In some embodiments, a retroviral vector is a mouse stem cell virus, murine leukemia virus (e.g., Moloney murine leukemia virus vector), feline leukemia virus, feline sarcoma virus, or avian reticuloendotheliosis virus vector. In some embodiments, a lentiviral vector is a HIV (human immunodeficiency virus, including

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HIV type 1 and HIV type 2, equine infectious anemia virus, feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus (SIV), equine infectious anemia virus, or Maedi-Visna viral vector.

In some embodiments, the vector is an AAV (AAV) vector, such as a
5 recombinant AAV (rAAV) vector, which is produced by recombinant methods. AAV is a single-stranded, non-enveloped DNA virus having a genome that encodes proteins for replication (rep) and the capsid (Cap), flanked by two ITRs, which serve as the origin of replication of the viral genome. AAV also contains a packaging sequence, allowing packaging of the viral genome into an AAV capsid. A recombinant AAV
10 vector (rAAV) may be obtained from the wild type genome of AAV by using molecular methods to remove the all or part of the wild type genome (e.g., Rep, Cap) from the AAV, and replacing with a non-native nucleic acid, such as a heterologous nucleic acid sequence (e.g., a nucleic acid molecule encoding an inhibitory nucleic acid). Typically, for AAV one or both inverted terminal repeat (ITR) sequences are retained in the AAV
15 vector. In some embodiments, the rAAV vector comprises an expression construct encoding an inhibitory nucleic acid of the present disclosure flanked by two cis-acting AAV ITRs (5' ITR and 3' ITR). Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV viral particle. Thus, an AAV vector is defined herein to include at least those sequences required in cis for replication and
20 packaging (e.g., functional ITRs) of the virus. In some embodiments, each AAV ITR is a full length ITR (e.g., approximately 145 bp in length, and containing functional Rep binding site (RBS) and terminal resolution site (trs)). In some embodiments, one or both of the ITRs is modified, e.g., by insertion, deletion, or substitution, provided that the ITRs provide for functional rescue, replication, and packaging. In some embodiments,
25 a modified ITR lacks a functional terminal resolution site (trs) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a modified ITR is a truncated version of AAV2 ITR referred to as AITR (D-sequence and TRS are deleted).

In some embodiments, the AAV vector comprises a 5' ITR comprising
30 or consisting of nucleotides 1-106 of any one of SEQ ID NOS:2257-2260. In some

embodiments, the AAV vector comprises a 3' ITR comprising or consisting of nucleotides 2192-2358 of any one of SEQ ID NOS:2257-2260. In some embodiments, the AAV vector comprises: a 5' ITR comprising or consisting of nucleotides 1-106 of SEQ ID NO:2257 and a 3' ITR comprising or consisting of nucleotides 2192-2358 of SEQ ID NO:2257; a 5' ITR comprising or consisting of nucleotides 1-106 of SEQ ID NO:2258 and a 3' ITR comprising or consisting of nucleotides 2192-2358 of SEQ ID NO:2258; a 5' ITR comprising or consisting of nucleotides 1-106 of SEQ ID NO:2259 and a 3' ITR comprising or consisting of nucleotides 2192-2358 of SEQ ID NO:2259; or a 5' ITR comprising or consisting of nucleotides 1-106 of SEQ ID NO:2260 and a 3' ITR comprising or consisting of nucleotides 2192-2358 of SEQ ID NO:2260.

In some embodiments, the rAAV vector is a mammalian serotype AAV vector (e.g., AAV genome and ITRs derived from mammalian serotype AAV), including a primate serotype AAV vector or human serotype AAV vector. In some embodiments, the AAV vector is a chimeric AAV vector. In some embodiments, the ITRs are selected from AAV serotypes of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV 12, AAVrh8, AAVrh10, AAV-DJ8, AAV-DJ, AAV- PHP.A, AAV-PHP.B, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B- EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B- SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B- STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A 15/G2A3, AAVG2B4, AAVG2B5, and variants thereof.

Other expression control sequences may be present in the rAAV vector operably linked to the inhibitory nucleic acid, including one or more of transcription initiation sequences, termination sequences, promoter sequences, enhancer sequences, repressor sequences, splice site sequences, polyadenylation (polyA) signal sequences, or any combination thereof.

AAV preferentially packages a full-length genome, *i.e.*, one that is approximately the same size as the native genome, and is not too big or too small. However, expression cassettes encoding inhibitory nucleic acid sequences are rather small. To avoid packaging of fragmented genomes, a stuffer sequence may be linked to an expression construct encoding inhibitory nucleic acids of the present disclosure and flanked by the 5' ITR and 3' ITR to expand the packagable genome, resulted in a genome whose size was near-normal in length between the ITRs. In some embodiments, the rAAV vector comprising a stuffer sequence and expression cassette encoding an inhibitory nucleic acid sequence of the present disclosure has a total length of about 4.7 kb between the 5' ITR and 3' ITR. In some embodiments, the rAAV vector is a self-complementary rAAV vector comprising a stuffer sequence and expression cassette encoding an inhibitory nucleic acid sequence of the present disclosure and has a total length of about 2.4 kb between the 5' ITR and 3' ITR. An exemplary stuffer sequence for use in the rAAV vectors of the present disclosure includes a sequence comprising or consisting of nucleotides 348-2228 of SEQ ID NO:1522 and a sequence comprising or consisting of nucleotides 489-2185 of any one of SEQ ID NOS:2257-2260.

rAAV vectors may have one or more AAV wild type genes deleted in whole or in part. In some embodiments the rAAV vector is replication defective. In some embodiments, the rAAV vector lacks a functional Rep protein and/or capsid protein. In some embodiments, the rAAV vector is a self-complementary AAV (scAAV) vector.

In some embodiments, the rAAV vector comprises from 5' ITR to 3' ITR the nucleotide sequence set forth in any one of SEQ ID NOS:2257-2260. In some embodiments, the rAAV vector comprises from 5' ITR to 3' ITR the nucleotide sequence set forth in SEQ ID NO:2257. In some embodiments, the rAAV vector comprises from 5' ITR to 3' ITR the nucleotide sequence set forth in SEQ ID NO:2258. In some embodiments, the rAAV vector comprises from 5' ITR to 3' ITR the nucleotide sequence set forth in SEQ ID NO:2259. In some embodiments, the rAAV vector comprises the nucleotide sequence set forth in SEQ ID NO:2260.

Recombinant AAV vectors of the present disclosure may be encapsidated by one or more AAV capsid proteins to form a rAAV particle. A “rAAV particle” or “rAAV virion” refers to an infectious, replication-defective virus including an AAV protein shell, encapsidating a rAAV vector comprising a transgene of interest, which is flanked on each side by a 5’ AAV ITR and 3’ AAV ITR. A rAAV particle is produced in a suitable host cell which has had sequences specifying a rAAV vector, AAV helper functions and accessory functions introduced therein to render the host cell capable of encoding AAV polypeptides that are required for packaging the rAAV vector (containing the transgene sequence of interest) into infectious rAAV particles for subsequent gene delivery.

Methods of packaging recombinant AAV vector into AAV capsid proteins using host cell culture are known in the art. In some embodiments, one or more of the required components for packaging the rAAV vector, (e.g., Rep sequence, cap sequence, and/or accessory functions) may be provided by a stable host cell that has been engineered to contain the one or more required components (e.g., by a vector). Expression of the required components for AAV packaging may be under control of an inducible or constitutive promoter in the host packaging cell. AAV helper vectors are commonly used to provide transient expression of AAV rep and/or cap genes, which function in *trans*, to complement missing AAV functions that are necessary for AAV replication. In some embodiments, AAV helper vectors lack AAV ITRs and can neither replicate nor package themselves. AAV helper vectors can be in the form of a plasmid, phage, transposon, cosmid, virus, or virion.

In some embodiments, rAAV particles may be produced using the triple transfection method (see, e.g., U.S. Patent No. 6,001,650, incorporated herein by reference in its entirety). In this approach, the rAAV particles are produced by transfecting a host cell with a rAAV vector (comprising a transgene) to be packaged into rAAV particles, an AAV helper vector, and an accessory function vector. In some embodiments, the AAV helper function vector supports efficient AAV vector production without generating any detectable wild-type AAV virions (e.g., AAV virions containing functional rep and cap genes). The accessory function vector

encodes nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication (e.g., “accessory functions”). The accessory functions include those functions required for AAV replication, including, without limitation, those moieties involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses such as adenovirus, herpesvirus (other than herpes simplex virus type-1), and vaccinia virus. In some embodiments, a double transfection method, wherein the AAV helper function and accessory function are cloned on a single vector, which is used to generate rAAV particles.

The AAV capsid is an important element in determining these tissue-specificity of the rAAV particle. Thus, a rAAV particle having a capsid tissue specificity can be selected. In some embodiments, the rAAV particle comprises a capsid protein selected from a AAV serotype of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV 12, AAVrh8, AAVrh10, AAV-DJ8, AAV-DJ, AAV- PHP.A, AAV-PHP.B, AAVPHP.B2, AAVPHP.B3, AAVPHP.N/PHP.B-DGT, AAVPHP.B- EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B- SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B- STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A 15/G2A3, AAVG2B4, AAVG2B5, and variants thereof. In some embodiments, the AAV capsid is selected from a serotype that is capable of crossing the blood-brain barrier, e.g., AAV9, AAVrh.10, AAV-PHP-B, or a variant thereof. In some embodiments, the AAV capsid is a chimeric AAV capsid. In some embodiments, the AAV particle is a pseudotyped AAV, having capsid and genome from different AAV serotypes.

In some embodiments, the rAAV particle is capable of transducing cells of the CNS. In some embodiments, the rAAV particle is capable of transducing non-

neuronal cells or neuronal cells of the CNS. In some embodiments, the CNS cell is a neuron, glial cell, astrocyte, or microglial cell.

In another aspect, the present disclosure provides host cells transfected with the rAAV comprising the inhibitory nucleic acids or vectors described herein. In some embodiments, the host cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the host cell is a mammalian cell (e.g., HEK293T, COS cells, HeLa cells, KB cells), bacterial cell (E. coli), yeast cell, insect cell (Sf9, Sf21, Drosophila, mosquito), etc.

10 Pharmaceutical Compositions

In some aspects, the disclosure provides pharmaceutical compositions comprising an inhibitory nucleic acid, isolated nucleic acid comprising an expression construct, or vector as described herein and a pharmaceutically acceptable carrier. As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with cells and/or tissues without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the cell or tissue being contacted. Additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, weight, body surface area, age, the level of expression of inhibitory RNA expression required to achieve a therapeutic effect, stability of the inhibitory nucleic acid, specific disease being treated, stage of disease, sex, time and route of administration, general health, and other drugs being administered concurrently. In some embodiments, a rAAV particle as described herein is administered to a subject in an amount of about 1×10^6 VG (viral genomes) to about 1×10^{16} VG per subject, or about 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 2.1×10^{11} , 2.2×10^{11} , 2.3×10^{11} , 2.4×10^{11} , 2.5×10^{11} , 2.6×10^{11} , 2.7×10^{11} , 2.8×10^{11} , 2.9×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 7.1×10^{11} , 7.2×10^{11} , 7.3×10^{11} , 7.4×10^{11} , 7.5×10^{11} , 7.6×10^{11} , 7.7×10^{11} , 7.8×10^{11} , 7.9×10^{11} , 8×10^{11} , 9×10^{11} , 1×10^{12} , 1.1×10^{12} , 1.2×10^{12} , 1.3×10^{12} , 1.4×10^{12} , 1.5×10^{12} , 1.6×10^{12} , 1.7×10^{12} , 1.8×10^{12} , 1.9×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 4.1×10^{12} , 4.2×10^{12} , 4.3×10^{12} , 4.4×10^{12} , 4.5×10^{12} , 4.6×10^{12} , 4.7×10^{12} , 4.8×10^{12} , 4.9×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , 8.1×10^{12} , 8.2×10^{12} , 8.3×10^{12} , 8.4×10^{12} , 8.5×10^{12} , 8.6×10^{12} , 8.7×10^{12} , 8.8×10^{12} , 8.9×10^{12} , 9×10^{12} , 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 6.7×10^{13} , 7×10^{13} , 8×10^{13} , 9×10^{13} , 1×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , 9×10^{14} , 1×10^{15} , 2×10^{15} , 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , 9×10^{15} , or 1×10^{16} VG/subject. In some embodiments, a rAAV particle as described herein is administered to a subject in an amount of about 1×10^6 VG/kg to about 1×10^{16} VG/kg, or about 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 2.1×10^{11} , 2.2×10^{11} , 2.3×10^{11} , 2.4×10^{11} , 2.5×10^{11} , 2.6×10^{11} , 2.7×10^{11} , 2.8×10^{11} , 2.9×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 7.1×10^{11} , 7.2×10^{11} , 7.3×10^{11} , 7.4×10^{11} , 7.5×10^{11} , 7.6×10^{11} , 7.7×10^{11} , 7.8×10^{11} , 7.9×10^{11} , 8×10^{11} , 9×10^{11} , 1×10^{12} ,

1.1×10^{12} , 1.2×10^{12} , 1.3×10^{12} , 1.4×10^{12} , 1.5×10^{12} , 1.6×10^{12} , 1.7×10^{12} , 1.8×10^{12} ,
 1.9×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 4.1×10^{12} , 4.2×10^{12} , 4.3×10^{12} , 4.4×10^{12} , 4.5×10^{12} ,
 4.6×10^{12} , 4.7×10^{12} , 4.8×10^{12} , 4.9×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , 8.1×10^{12} ,
 8.2×10^{12} , 8.3×10^{12} , 8.4×10^{12} , 8.5×10^{12} , 8.6×10^{12} , 8.7×10^{12} , 8.8×10^{12} , 8.9×10^{12} , 9×10^{12} ,
5 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 6.7×10^{13} , 7×10^{13} , 8×10^{13} , 9×10^{13} ,
 1×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , 9×10^{14} , 1×10^{15} , 2×10^{15} ,
 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , 9×10^{15} , or 1×10^{16} VG/kg.

Pharmaceutical compositions may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose and a suitable duration and frequency of administration of the compositions will be determined by such factors as the health condition of the patient, size of the patient (*i.e.*, weight, mass, or body area), the type and severity of the patient's disease, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provide the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (such as described herein, including an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity). For prophylactic use, a dose should be sufficient to prevent, delay the onset of, or diminish the severity of a disease associated with disease or disorder. Prophylactic benefit of the compositions administered according to the methods described herein can be determined by performing pre-clinical (including *in vitro* and *in vivo* animal studies) and clinical studies and analyzing data obtained therefrom by appropriate statistical, biological, and clinical methods and techniques, all of which can readily be practiced by a person skilled in the art.

Compositions (e.g., pharmaceutical compositions) may be administered by any route, including enteral (e.g., oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subpial, intraparenchymal, intrastriatal, intracranial, intracisternal, intra-cerebral, intracerebral ventricular, intraocular, intraventricular, intralumbar, subcutaneous, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal,

nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (e.g., its stability in the environment of the gastrointestinal tract),
5 and/or the condition of the subject. In some embodiments, compositions are directly injected into the CNS of the subject. In some embodiments, direct injection into the CNS is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastratial injection, subpial injection, or any combination thereof. In some
10 embodiments, direct injection into the CNS is direct injection into the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or intralumbar injection.

In some embodiments, pharmaceutical compositions comprising rAAV particles are formulated to reduce aggregation of rAAV particles, particularly where high rAAV particle concentrations are present (e.g., $\sim 10^{13}$ VG/ml or more). Methods
15 for reducing aggregation of rAAV particles are well known in the art and, include, for example, addition of surfactants, pH adjustment, salt concentration adjustment, etc. (See, e.g., Wright F R, et al., Molecular Therapy (2005) 12:171-178, incorporated herein by reference in its entirety).

Kits

20 In some embodiments, the compositions provided herein may be assembled into pharmaceutical or research kits to facilitate their use in therapeutic or research use. A kit may include one or more containers comprising: (a) inhibitory nucleic acid, isolated nucleic acid comprising an expression construct, or vector as described herein; (b) instructions for use; and optionally (c) reagents for transducing the
25 kit component (a) into a host cell. In some embodiments, the kit component (a) may be in a pharmaceutical formulation and dosage suitable for a particular use and mode of administration. For example, the kit component (a) may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. The components of the kit may require mixing one or more components prior to use or may be prepared in a premixed
30 state. The components of the kit may be in liquid or solid form, and may require addition of a solvent or further dilution. The components of the kit may be sterile. The

instructions may be in written or electronic form and may be associated with the kit (e.g., written insert, CD, DVD) or provided via internet or web-based communication. The kit may be shipped and stored at a refrigerated or frozen temperature.

5 Methods of Treatment

In another aspect, the present disclosure provides methods for inhibiting the expression or activity of *ATXN2* in a cell, comprising administering a composition of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle,
10 pharmaceutical composition) to a cell, thereby inhibiting the expression or activity of *ATXN2* in the cell. In some embodiments, the cell is a CNS cell. In some embodiments, the cell is a non-neuronal cell or neuronal cell of the CNS. In some embodiments, the non-neuronal cell of the CNS is a glial cell, astrocyte, or microglial cell. In some embodiments, the cell is in vitro. In some embodiments, the cell is from a
15 subject having one or more symptoms of a neurodegenerative disease or suspected of having a neurodegenerative disease. In some embodiments, the cell expresses an *ATXN2* having at least 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or more CAG trinucleotide (polyglutamine) repeats. In some embodiments, the cell expresses an *ATXN2* having about 22 or 23 repeats, 24-32 repeats, or 33-100 or more repeats.

20 In another aspect, the present disclosure provides methods for inhibiting the expression or activity of *ATXN2* in the central nervous system of a subject, comprising administering a composition of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle, pharmaceutical composition) to the
25 subject, thereby inhibiting the expression or activity of *ATXN2* in the subject.

In another aspect, the present disclosure provides methods for treating a subject having or suspected of having a neurodegenerative disease, comprising administering a composition of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic
30 acid, vector, rAAV particle, pharmaceutical composition) to the subject, thereby

treating the subject. As used herein, the term "treat" refers to preventing or delaying onset of neurodegenerative disease (e.g., ALS/FTD, Alzheimer's disease, Parkinson's disease, etc.); reducing severity of neurodegenerative disease; reducing or preventing development of symptoms characteristic of neurodegenerative disease; preventing
5 worsening of symptoms characteristic of neurodegenerative disease, or any combination thereof.

Neurodegenerative diseases that may be treated in a subject using the compositions of the present disclosure include neurodegenerative diseases where *ATXN2* is a causative agent (e.g., SCA2), as well as neurodegenerative diseases where
10 *ATXN2* is not the causative agent but modifies TDP-43 pathological aggregation. Neurodegenerative diseases associated with TDP-43 proteinopathy include ALS, FTD, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia
15 Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, and Alzheimer's disease.

In some embodiments, the subject is characterized as having an *ATXN2* allele having at least 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or more CAG trinucleotide (polyglutamine) repeats. In some embodiments, the subject is
20 characterized as having an *ATXN2* allele having about 22 or 23 repeats, 24-32 repeats, or 33-100 or more repeats.

In some embodiments, the methods for treatment of the present disclosure reduces, prevents, or slows development or progression of one or more symptom characteristic of a neurodegenerative disease. Examples of symptoms
25 characteristic of neurodegenerative disease include motor dysfunction, cognitive dysfunction, emotional/behavioral dysfunction, or any combination thereof. Paralysis, shaking, unsteadiness, rigidity, twitching, muscle weakness, muscle cramping, muscle stiffness, muscle atrophy, difficulty swallowing, difficulty breathing, speech and language difficulties (e.g., slurred speech), slowness of movement, difficulty with
30 walking, dementia, depression, anxiety, or any combination thereof.

In some embodiments, the methods for treatment of the present disclosure of the present disclosure comprise administration as a monotherapy or in combination with one or more additional therapies for the treatment of the neurodegenerative disease. Combination therapy may mean administration of the compositions of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle, pharmaceutical composition) to the subject concurrently, prior to, subsequent to one or more additional therapies. Concurrent administration of combination therapy may mean that the the compositions of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle, pharmaceutical composition) and additional therapy are formulated for administration in the same dosage form or administered in separate dosage forms.

In some embodiments, the one or additional therapies that may be used in combination with the inhibitory nucleic acids of the present disclosure include: inhibitory nucleic acids or antisense oligonucleotides that target neurodegenerative disease related genes or transcripts (e.g., C9ORF72), gene editing agents (e.g., CRISPR, TALEN, ZFN based systems) that target neurodegenerative related genes (e.g., C9ORF72), agents that reduce oxidative stress, such as free radical scavengers (e.g., Radicava (edaravone), bromocriptine); antiglutamate agents (e.g., Riluzole, Topiramate, Lamotrigine, Dextromethorphan, Gabapentin and AMPA receptor antagonist (e.g., Talampanel)); Anti-apoptosis agents (e.g., Minocycline, Sodium phenylbutyrate and Arimoclomol); Anti-inflammatory agents (e.g., ganglioside, Celecoxib, Cyclosporine, Nimesulide, Azathioprine, Cyclophosphamide, Plasmapheresis, Glatiramer acetate and thalidomide); Beta-lactam antibiotics (penicillin and its derivatives, ceftriaxone, and cephalosporin); Dopamine agonists (Pramipexole, Dexpramipexole); and neurotrophic factors (e.g., IGF-1, GDNF, BDNF, CTNF, VEGF, Colivelin, Xaliproden, Thyrotrophin-releasing hormone and ADNF).

In some embodiments, an inhibitory nucleic acid of the present disclosure is administered in combination with an additional therapy targeting

C9ORF72. In some embodiments, the additional therapy targetin C9ORF72 comprises an inhibitory nucleic acid targeting C9ORF72 transcript, a C9ORF72 specific antisense oligonucleotide, or a C9ORF72 specific gene editing agent. Examples of C9ORF72 specific therapies are described in US Patent No. 9,963,699 (antisense
5 oligonucleotides); PCT Publication No. WO2019/032612 (antisense oligonucleotides); US Patent No. 10,221,414 (antisense oligonucleotides); US Patent No. 10,407,678 (antisense oligonucleotides); US Patent No. 9,963,699 (antisense oligonucleotides); US Patent Publication US2019/0316126 (inhibitory nucleic acids); US Patent Publication No. 2019/0167815 (gene editing); PCT Publication No. WO2017/109757 (gene
10 editing), each of which is incorporated by reference in its entirety.

In some embodiments, a subject treated in any of the methods described herein is a mammal (e.g., mouse, rat), preferably a primate (e.g., monkey, chimpanzee), or human.

In any of the methods of treatment described herein, a composition of
15 the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle, pharmaceutical composition) may be administered to the subject by intrathecal, subpial, intraparenchymal, intrastriatal, intracranial, intracisternal, intra-cerebral, intracerebral ventricular, intraocular, intraventricular, intralumbar administration, or any combination
20 thereof.

In some embodiments, a composition of the present disclosure (e.g., inhibitory nucleic acid, isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid, vector, rAAV particle, pharmaceutical composition) is directly injected into the CNS of the subject. In some embodiments,
25 direct injection into the CNS is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, or any combination thereof. In some embodiments, direct injection into the CNS is direct injection into the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, intralumbar injection, or any
30 combination thereof.

In some embodiments, the methods of the present disclosure reduces *ATXN2* expression or activity in a cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95% or more in a cell compared to the expression level of *ATXN2* in a cell that has not been contacted with the inhibitory nucleic acid. In some embodiments, the methods of the present disclosure reduces *ATXN2* expression or activity in a cell by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-95%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% compared to the expression level of *ATXN2* in a cell that has not been contacted with the inhibitory nucleic acid.

In some embodiments, the methods of the present disclosure reduces *ATXN2* expression or activity in the CNS of a subject by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95% or more in the CNS compared to the expression level of *ATXN2* in the CNS of an untreated subject. In some embodiments, the methods of the present disclosure reduces *ATXN2* expression or activity in the CNS of a subject by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-95%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% compared to the expression level of *ATXN2* in the CNS of an untreated subject.

30

EXAMPLES

EXAMPLE 1: DESIGN AND TESTING OF siRNA SEQUENCES TO KNOCK DOWN HUMAN ATAXIN-2

A number of criteria were used to select and design siRNA sequences to knock down *ATXN2*. The potential siRNA sequences that were initially considered included all possible 22-nucleotide RNAs complementary to ENST00000377617.7 (*ATXN2*-201). Human transcripts encoding for human Ataxin-2 were first examined. Only sequences found in all five of *ATXN2* transcripts, NM_002973.3 (SEQ ID NO:2), ENST00000377617.7, ENST00000550104.5 (), ENST00000608853.5 (), and ENST00000616825.4 (), were selected.

The set of sequences was then filtered by cross-reactivity to the orthologous *ATXN2* gene in rhesus and cynomolgous monkey. This allows the sequences to be tested in these species if needed to establish the activity and safety of gene therapies containing these inhibitory nucleic acid sequences prior to therapeutic use in humans. Thus, the sequence was also required to be in rhesus (*Macaca Mulatta*) *ATXN2* (NCBI Reference Sequences: XM_015152804.1, XM_015152805.1, XM_015152806.1, XM_015152807.1, XM_015152809.1, XM_015152810.1, XM_015152811.1, XM_015152812.1, XM_015152814.1, (Ensemble ID:) ENSMMUT00000062319, and ENSMMUT00000074794) and cynomolgous monkeys (*Macaca fascicularis*) *ATXN2* (NCBI Reference Sequences: XM_005572266.2, XM_005572267.2, XM_015431532.1, XM_015431533.1, XM_015431534.1, XM_015431535.1, XM_015431536.1, XM_015431537.1, XM_015431538.1, XM_015431539.1, XM_015431540.1, XM_015431541.1, XM_015431542.1, XM_015431543.1, XM_015431544.1, XM_015431546.1, XM_015431547.1, XM_015431548.1, XM_015431549.1, XM_015431550.1, ENSMFAT00000019903.1). The *ATXN2* transcript XM_015152813.1 of rhesus was also examined. This transcript was observed to be lacking a component of exon 1 and exon 2 (by comparison to human *ATXN2* sequence SEQ ID NO:2). As described above for rhesus sequences, the following *Macaca ATXN2* transcripts were identified to lack upstream sequence in exon 1: XM_015431551.1 and ENSMFAT00000019905.1. For these sequences, the exon 1 sequence was added back

from human (SEQ ID NO:2) so as not to filter out that sequence. The nucleotide sequence in the *ATXN2* gene encoding for the poly-glutamine repeat contains elements likely found elsewhere in the genome in other poly-glutamine repeat sequences. It is possible that automated transcript assignment algorithms, relying on alignment of RNAseq data, would mis-align sequencing reads overlapping with the poly-glutamine-encoding stretch (CAG repeating sequence) elsewhere in the genome, undercounting this sequence. These sequences in the upstream part of *ATXN2* were therefore not excluded, except due to non-conservation from human to primate sequences.

Based on an analysis of brain RNAseq, exon 12 skipping is about 3% frequency, so this was not filtered out despite some alternative splice isoforms not including this isoform.

After defining the sequences expected to be present in human *ATXN2* and key toxicology species, siRNAs were further selected based on criteria to reduce likelihood of off-target effects and to improve likelihood of strong *ATXN2* knockdown. The seed sequences of both the antisense and sense strands of siRNAs, that is, bases 2 – 7 of the sequences which are known to be key determinants of activity of endogenous microRNAs, were examined for conservation in endogenous miRNAs expressed in human, mouse and rat. Antisense sequences present in any human endogenous miRNA were excluded, as were all sequences that were conserved in both mouse and rat. Sense sequences were excluded if seed regions were conserved in endogenous miRNAs present in more than 2 species out of human, mouse and rat.

A predicted knockdown ranking was calculated by adapting a version of an algorithm published in Pelossof et al. (Nature Biotechnology (2017) 35:350-353). Essentially, a support vector machine was trained on tiled sequencing data, provided in the publication. To generate the points in the space in which the support vector machine attempts to separate training examples which are labeled positive and negative, for good and bad knockdown respectively, features were selected as a weighted degree kernel. Features input to the support vector machine classifier were essentially the same as in Pelossof et al. For the SVM model, the “LibSVM” function from the Shogun module (version 6.1.3, Python version 2.7) was used instead of “SVMlite.”

The training set included 18,421 shRNA sequences from the genes PCNA, Trp53, Hras, Rpa3, Mcl1, hMyc, Myc, Bcl2, and Kras, all from the 'TILE' data set included in Pelosof et al.. The TILE dataset empirically tests the performance of unbiased libraries of shRNAs covering sequences in the 9 genes described. The cost function c was assessed across a range of values training the SVM classifier on all genes except one of the nine left out, and calculating mean squared error on predictions for performance on data from the held-out gene. An example with Kras as the held out gene is shown (**FIG. 1**). A value of $c = 4$ was selected which minimized the mean square error among values of c tested.

To further assess the performance of the classifier, knockdown data from another gene in the data set (Trp53) was held out after training the classifier on the other 8 genes. **FIG. 2** shows a precision – recall curve for the classifier, as trained on data not including the Trp53 shRNAs, predicting performance of shRNA knockdown in the Trp53 targeting shRNAs. That is, after filtering shRNAs by a given classifier score, the fraction of of true positives identified by the classifier (recall) is plotted as a function of the number of true positives versus false positives (precision) (**FIG. 2**). Additionally, the anticipated cumulative fraction of 'positive' shRNAs (high performing) shRNAs that are expected to be lost as the classifier score was increased in stringency was plotted (**FIG. 3**), alongside the percent improvement in rejection of low-performing shRNAs. A separation in the curves was noted between scores of approximately -1.5 to -0.8, going from roughly the 25th to 50th percentiles of scores for Trp53 targeting shRNAs.

Next, siRNA sequences were triaged by specificity considerations, then ranked by the score from the above classifier. In addition to conservation of the seed sequences with endogenous miRNAs, as described above, metrics of specificity were: (a) comparison of seed sequences (guide bases 2 – 7) to a published data set of transfected siRNA seed sequences versus cell proliferation (Gao et al. Nature Communications (2018) 9:4504), excluding sequences with a > 70% reduction of cell proliferation in the published assay; (b) the number of transcripts complementary to the first 19 nucleotides of the guide sequence, with 2 or fewer mismatches, was required to

be less than 15; and (c) other considerations such as an internal algorithm of specificity were also factored in but triaged fewer samples than the criteria of (a) and (b).

Following filtering by specificity, sequences in the most common *ATXN2* transcript, were ranked by SVM score and top-ranked candidate sequences
5 selected. In calculating the SVM classifier score for shRNAs, however, it was found that the classifier score significantly increased for shRNAs beginning with U (**FIG. 4**). This was consistent with prior prediction algorithms (e.g., Vert et al., BMC Bioinformatics (2006) BMC Bioinformatics 7:520) and literature suggesting that the argonaute 2 binding pocket interacts best with this base, although guide base 1 does not
10 base pair with the target mRNA (Boland et al., EMBO Reports (2010) 11:522-527). Therefore, for shRNA design, if the base was a 'G' or 'C,' based on complementarity to the target mRNA sequence, that base was replaced with a 'U' and the corresponding performance score calculated. The top 93 sequences beginning with A or U (SVM score > -0.8) and 34 sequences edited from a shRNA beginning with G or C, with a
15 more stringent filter (SVM score > 0.4).

Additional sequences were included for testing based on other criteria, including: (a) cross-reactivity with *ATXN2L*. *ATXN2L* shares considerable amino acid sequence similarity with *ATXN2*. Homologous genes often execute similar functions in a cell, and it is possible that knockdown of *ATXN2L* may serve similar therapeutic
20 functions as knocking down *ATXN2*. Sequences which match both *ATXN2* and *ATXN2L* may therefore have additional therapeutic benefit, and thus, 10 sequences were selected with potential to target both *ATXN2* and *ATXN2L*; (b) sequences meeting a stringent off-target match criteria, with 2 or fewer transcripts matching at 2 or fewer positions in the first 19 nucleotides of the siRNA guide sequence (10 siRNAs), but
25 ignoring SVM-based efficacy prediction; (c) sequences with perfect match or single mismatch to mouse *ATXN2* in the first 19 nucleotides of the guide sequence. 'Single mismatch' guide sequences were defined as those where only one mismatch occurs between bases 12 and 19 nts against the mouse sequence, and none in bases 1 – 11. For guide sequences perfect-matching or single-mismatching mouse, the specificity criteria

were relaxed, with guide sequences accepted with fewer than 50 complementary transcripts with 2 or fewer mismatches.

Selection of cell line to screen siRNA candidates

5 Following selection of siRNAs for testing, an *in vitro* cell system was established to assess knockdown of *ATXN2* by siRNAs. *ATXN2* levels were assessed by quantigene assay (Thermo Fisher), across a panel of cell lines (**FIG. 5**). The cell lines HepG2, KB, HT-29, LNCAP, C4-2 and Panc-1 all showed robust *ATXN2* expression. To see if the splice patterns of cells were similar to that of *ATXN2* in
10 relevant target tissues, including in neurodegenerative disease states, RNAseq of postmortem human brain (Mayo Clinic Alzheimer's Disease Genetics Studies**;
accessed via the synapse.org platform) was examined for splice patterns of *ATXN2* and compared to data from cell lines (National Cancer Institute GDC Legacy Archive). In **FIG. 6A**, alternatively spliced exons were identified by reads crossing genomic regions
15 that skip over the alternatively spliced exons. Exons 10, 21, and 24 in brain are frequently alternatively spliced. Examining alternative splicing in cell lines, HepG2 were similar to human brain (**FIG. 6B**). This line was selected for *ATXN2* siRNA studies because of the high level of *ATXN2* expression relative to background and consistent alternative splice patterns.

20 With regard to the synapse.org platform, study data were provided by the following sources: The Mayo Clinic Alzheimer's Disease Genetic Studies, led by Dr. Nilüfer Ertekin-Taner and Dr. Steven G. Younkin, Mayo Clinic, Jacksonville, FL using samples from the Mayo Clinic Study of Aging, the Mayo Clinic Alzheimer's Disease Research Center, and the Mayo Clinic Brain Bank. Data collection was supported
25 through funding by NIA grants P50 AG016574, R01 AG032990, U01 AG046139, R01 AG018023, U01 AG006576, U01 AG006786, R01 AG025711, R01 AG017216, R01 AG003949, NINDS grant R01 NS080820, CurePSP Foundation, and support from Mayo Foundation. The following publications are applicable:

[1] Carrasquillo et. al., Nat Genet. (2009) 41:192-8.

[2] Zou et. al. PLoS Genet. (2012) 8(6):e1002707. [3] Allen et al. Sci Data.

(2016) 3:160089.

5 Synthesis and testing of siRNAs

siRNAs were synthesized as 22 nucleotide RNAs, with 20 bp of complementarity (complementarity from positions 1 – 20, of guide and passenger strands). Here, guide strand refers to the sequence complementary to, or antisense to, the *ATXN2* target mRNA, and passenger strand refers to the strand complementary to
10 guide strand. Guide and passenger strands, also referred to as antisense and sense strand RNAs, are shown in **Table 1**. Sequences were synthesized as guide and passenger strands. All but 6 of the sequences met the following criteria: single strands within .05% of calculated mass (by LC/MS). At least 85% of full-length oligonucleotide purity (by HPLC). After annealing guide and passenger strands, duplex
15 purity of >90% by non-denaturing HPLC. Oligonucleotides not meeting these criteria are noted as “FAIL,” but data are included for completeness.

Annealed siRNAs were reverse transfected, adding 20,000 cells per well of a 96-well plate, on top of a solution of lipofectamine 2000 with siRNA to yield a final siRNA concentration in the diluted culture media as noted below, in a volume of
20 0.5 microliters of transfection solution per well. siRNAs were tested in quadruplicate wells and incubated for 24 hours. *ATXN2* and *GAPDH* levels were assayed in cell lysates by Quantigene assay using *ATXN2* and *GAPDH* probes (Thermo Fisher). The ratio of *ATXN2* mRNA levels to levels of the housekeeping gene *GAPDH* was calculated, and values were normalized to *ATXN2/GAPDH* ratios obtained for cells
25 mock-treated with lipofectamine not containing siRNA.

All siRNAs were tested at doses of 20 nM or 1 nM (final calculated concentration of siRNA in cell culture media) for level of *ATXN2* following knockdown (**Table 4**). A significant correlation, as assessed by a linear model fit, was observed plotting the predicted SVM score classifier against the 20 nM siRNA
30 knockdown data (**FIG. 8**) ($p < 10^{-8}$, $R^2 = 0.15$). Subsequently, the top ranked 100 siRNAs, by *ATXN2* knockdown from 1 nM siRNA dosing data, were rescreened at 200

pM (Table 5). FIG. 7 plots the knockdown of *ATXN2* mRNA for siRNAs as a function for position along the *ATXN2* transcript that they transcript.

Table 4: siRNA Single Point Testing Data 20 nM – knockdown of *ATXN2* mRNA

siRNA Duplex ID	20nM_mean	20nM_SD	1nM_mean	1nM_SD
XD-14738	84.1	6.8	91.1	3.6
XD-14739	88.6	4.7	96.8	6.9
XD-14740	87.1	3.4	82.9	3.4
XD-14741	56.4	2.3	54.6	3.2
XD-14742	44.5	2.5	44.7	3.5
XD-14743	44.9	2	48.1	3.5
XD-14744	62	2.9	65.7	2.1
XD-14745	42.5	3.9	47.3	3.5
XD-14746	65.4	3.9	62.5	2
XD-14747	43.2	2.4	41.2	1.8
XD-14748	52.4	1.6	49.3	1.9
XD-14749	60.2	3	57.4	1.3
XD-14750	52.3	3.3	53.5	1.2
XD-14751	47.7	4	54.6	1.1
XD-14752	78.4	3.3	76.4	6.4
XD-14753	47.7	2.4	55	1.1
XD-14754	47.5	8.4	50.6	2.8
XD-14755	79	3.6	73.3	3.9
XD-14756	37.6	5.2	46.8	7.8
XD-14757	32.5	1	39.7	0.4
XD-14758	29.6	0.9	35.3	5.2
XD-14759	43.6	4.3	51.8	5.5
XD-14760	45	1.3	52.3	3.6
XD-14761	40.2	1.8	54.7	4.1
XD-14762	39.4	2.5	47.8	2.3
XD-14763	39.2	3	50.6	1.7
XD-14764	80.9	4.4	81.2	8.7
XD-14765	43.1	2.9	49.5	5.1
XD-14766	30.3	0.7	33.1	3.6
XD-14767	43.6	5.6	38.5	2.5
XD-14768	35.9	3.3	40.9	2
XD-14769	72	7.6	78.7	2
XD-14770	50.7	7.6	56.9	3.1
XD-14771	59.8	6.5	77.4	17.7
XD-14772	81.1	2.1	80.2	1.1
XD-14773	44.2	3.1	52.1	5.2

XD-14774	81	7.1	74.8	12.1
XD-14775	42.3	4.3	44.3	2.6
XD-14776	50.3	2.2	45.7	2.7
XD-14777	51	5.4	53.2	1.9
XD-14778	71.2	5.2	69.2	6.1
XD-14779	36.5	2.8	34.7	4.3
XD-14780	121.4	8.7	97.2	6
XD-14781	52.7	5.9	69.7	3.3
XD-14782	46.3	2	61.1	3.2
XD-14783	44.6	2.1	56.9	2.8
XD-14784	49.8	1.3	64.4	2.7
XD-14785	48.1	8.7	61.4	4.5
XD-14786	45.1	2.5	44.7	6.5
XD-14787	43.3	7.4	48.2	2.8
XD-14788	34.8	1.4	41.7	4.7
XD-14789	44.6	0.8	62.3	2.8
XD-14790	31.3	0.8	36.7	2.6
XD-14791	29.5	1.5	38.9	2.9
XD-14792	31.2	2.6	38	0.9
XD-14793	38.3	1.7	47.9	2.9
XD-14794	34.7	1.4	38.6	1.3
XD-14795	36.4	5.4	47.9	4.1
XD-14796	91.9	7	91.1	8.3
XD-14797	61.9	1.2	74.9	17.3
XD-14798	31.2	2.3	33.9	2
XD-14799	32.5	2.1	39.8	3.3
XD-14800	30.4	1.4	43.3	6
XD-14801	32.8	3.4	36.2	0.9
XD-14802	56.3	1	70.5	2.4
XD-14803	45.2	1.3	56.8	5.2
XD-14804	74.3	3.6	76.2	5
XD-14805	47.2	3.5	50.1	3.5
XD-14806	71.4	5.8	75	4.4
XD-14807	42.4	2.4	52.7	3.6
XD-14808	42.8	2.6	53.1	5
XD-14809	47.4	3.3	49	2.8
XD-14810	39.7	1.7	48	1.4
XD-14811	38.9	4.8	46.3	0.9
XD-14812	40.8	4.9	47.1	2.3
XD-14813	57.8	5.9	59.5	2.3
XD-14814	103.2	29.6	81.5	2.9
XD-14815	54.8	4.4	48.6	7

XD-14816	35.9	2.8	40.8	3.3
XD-14817	64	11.2	66.3	1.4
XD-14818	49.4	2.8	49.5	1
XD-14819	41.9	4	40.8	1.5
XD-14820	42.8	4.3	46	1.3
XD-14821	47.9	3.4	63	4.3
XD-14822	37.3	3.1	45.5	2.9
XD-14823	51.7	4.6	66.4	0.4
XD-14824	38.6	1.3	45.5	2.3
XD-14825	35.9	2.7	41.3	0.9
XD-14826	39.2	1.5	46.1	3
XD-14827	54.3	2.7	62.3	1.4
XD-14828	63.8	1.9	77.1	3.5
XD-14829	40.1	0.6	38.2	8.1
XD-14830	36.7	0.7	46	0.9
XD-14831	42.5	2.9	61.8	3.4
XD-14832	71.3	2	95.6	1.5
XD-14833	56.5	2.8	75.1	2.3
XD-14834	38.3	1.6	47	2.6
XD-14835	30.6	2.4	38.5	1.6
XD-14836	44.5	3.2	58	2.7
XD-14837	29.2	3.4	36.2	0.5
XD-14838	36.5	2	46.1	1.2
XD-14839	32	2.9	42.5	4
XD-14840	29.3	2.3	34.1	7.6
XD-14841	32.9	2.5	41.2	3.7
XD-14842	38.8	3.4	45.6	2.5
XD-14843	32.9	1.2	40	1.6
XD-14844	88.5	4.9	84.6	4.8
XD-14845	72.5	9.4	67.6	1.9
XD-14846	29	1.7	34.6	3.4
XD-14847	34.1	1.2	38.6	2.5
XD-14848	40.4	0.5	46.3	3
XD-14849	58.5	2.1	67	1.4
XD-14850	34.7	0.6	38.7	0.7
XD-14851	46.6	3.4	47	0.9
XD-14852	61.4	2.4	55.9	2.6
XD-14853	47	3.7	42	2.4
XD-14854	43.3	5.3	40.8	4.9
XD-14855	45.5	1.6	43.7	2.1
XD-14856	43.9	3.3	40.7	5
XD-14857	39.5	5.3	36.1	2.3

XD-14858	63.8	4.7	54.1	1.1
XD-14859	39	3.6	40.3	1
XD-14860	35.5	3.2	36.2	1.6
XD-14861	35.8	2.9	42.3	2.2
XD-14862	85.3	2.6	84.2	7
XD-14863	56.3	6.3	52.6	3.4
XD-14864	46.3	3.1	47.2	2.3
XD-14865	77.5	2.6	79.6	4
XD-14866	52.6	4.4	53.2	7.4
XD-14867	70.6	4.6	56.7	5.4
XD-14868	79.8	3.4	72.5	3.8
XD-14869	93.1	3	89.4	3.7
XD-14870	60.9	0.9	65.1	4.2
XD-14871	86.1	1.6	94	8.6
XD-14872	93.1	3.6	91.8	5.5
XD-14873	94.4	2.3	90.2	4.6
XD-14874	60.7	4.3	56.9	7.5
XD-14875	50	1.3	49.5	2.7
XD-14876	53.5	15.9	51.7	4.1
XD-14877	55	4	61	1.4
XD-14878	49.6	1.5	47.4	2.2
XD-14879	65	2.2	64.9	2.3
XD-14880	47	1	44.5	1.8
XD-14881	90.3	4.7	90.6	2
XD-14882	39.7	1.5	40.1	5.1
XD-14883	58	1.4	65.9	5
XD-14884	62.7	2.2	67.9	6.1
XD-14885	46.6	3.3	53.8	6.6
XD-14886	39.5	1.4	44.4	7.5
XD-14887	38.2	0.4	41.7	5.1
XD-14888	61.4	2.1	68.9	4.9
XD-14889	31.2	8.8	40.7	2.7
XD-14890	36.3	1.9	37.3	2.7
XD-14891	45.2	4	46.8	2.1
XD-14892	49.1	2.8	62.5	1.7
XD-14893	41.7	2.3	49.7	6
XD-14894	47	2.7	64.9	3
XD-14895	53.3	4	67.2	2.3
XD-14896	45.1	1.3	66.8	2.4
XD-14897	74.3	8.9	87.7	4.6
XD-14898	68.4	5.8	73	4.8
XD-14899	44.3	6.4	55	1.2

XD-14900	45.6	2.8	47.8	1.6
XD-14901	47.9	12.1	41.4	1.7
XD-14902	56.1	6.3	52.8	2.5
XD-14903	52.1	4.2	46.6	2.7
XD-14904	47	3.7	36.6	8.4
XD-14905	40.6	2	40.6	3.2
XD-14906	47.1	4.6	50.7	3.3
XD-14907	57	4.1	51.1	2.7
XD-14908	58.8	6.3	51.1	4
XD-14909	47.1	2.8	50	2.6
XD-14910	46.9	2.8	53.5	9.2
XD-14911	61.7	3.6	55	4.3
XD-14912	58	3.8	59.3	3.9
XD-14913	72.1	5.5	73.3	6.4
XD-14914	68.9	4.7	66.3	5.7
XD-14915	47.4	5.1	59.1	9.5
XD-14916	43.1	5.7	55.6	9.4
XD-14917	43.4	4.6	42.7	4.3
XD-14918	64.2	2.3	67.4	5.7
XD-14919	62.5	6.6	61.8	3.8
XD-14920	76.1	1.8	67.6	4.2
XD-14921	64.4	3.9	73.9	7
XD-14922	52.2	6.9	66.7	7.5
XD-14923	48.7	2.6	56.3	3.8
XD-14924	47.6	1	51.8	2.1
XD-14925	51.7	3.7	56.5	6.2
XD-14926	43	3.9	54.9	3.2
XD-14927	53.3	6.2	64.3	8.2
XD-14928	61.7	1.7	70.4	3.8
XD-14929	54.7	3.9	64.4	2.8
XD-14930	50.1	3	63.6	5.5
XD-14931	52.9	3.9	62.4	4.9
XD-14932	62.6	3.7	69	3.8
XD-14933	62.2	5	66.7	3.3
XD-14934	59.7	7.6	62	4.8
XD-14935	60.5	4.1	65.9	7.5
XD-14936	54.4	2.6	71.3	4.7
XD-14937	61.2	5.5	74.1	2.9
XD-14938	65.8	6.6	71.9	3.7
XD-14939	61.9	7.8	73.8	2.6
XD-14940	68.8	6.8	79.6	2.2
XD-14941	57.8	4.1	69.7	3.1

XD-14942	75.3	9.3	85.4	4.4
XD-14943	69.8	4.2	77.7	6.7
XD-14944	84.1	4.3	84.2	8.5
XD-14945	53.5	3	55.2	2
XD-14946	58	1.8	56.9	3.7
XD-14947	59.2	2.2	59.2	1.3
XD-14948	56.8	4.4	53.4	2.4
XD-14949	51.7	2.5	52.4	2.3
XD-14950	63.2	2.2	69.6	4.3
XD-14951	53.9	2.7	62.6	3.8
XD-14952	40.3	0.9	51.3	6.2
XD-14953	46.6	2.5	56.1	4.1
XD-14954	56.1	8.6	55.9	6.6

Table 5: siRNA Single Point Testing Data 200pM

siRNA Duplex ID	0.2nM_mean	0.2nM_SD
XD-14742	55.2	5.4
XD-14743	49.9	3.2
XD-14745	68.9	2.5
XD-14747	59.3	5.8
XD-14748	63.9	3.8
XD-14754	75.9	3.2
XD-14756	52	8.9
XD-14757	57.2	6.7
XD-14758	60.8	3.3
XD-14759	60.3	3.4
XD-14760	65.8	4.2
XD-14762	61.7	7.7
XD-14763	58.1	5.1
XD-14765	72.6	6
XD-14766	59.5	4.1
XD-14767	66.2	3.5
XD-14768	56.5	3.7
XD-14773	73.1	4.5
XD-14775	69.1	5.1
XD-14776	62.5	1.8
XD-14779	58.2	1.3
XD-14786	51.1	1.4
XD-14787	61.7	1.5
XD-14788	58.1	4.7
XD-14790	49.7	1

XD-14791	51.2	4.3
XD-14792	41.9	4.6
XD-14793	62.2	8.4
XD-14794	60.6	5.3
XD-14795	75.9	7.3
XD-14798	53.1	2.4
XD-14799	60.4	2.2
XD-14800	54.1	4
XD-14801	59.7	13.7
XD-14805	56.7	2.4
XD-14807	66.9	7
XD-14809	48.4	2.9
XD-14810	54.5	4.6
XD-14811	51.1	3.5
XD-14812	62.3	6.6
XD-14815	76.1	2
XD-14816	71.8	1.7
XD-14818	75.9	1.9
XD-14819	57.2	2.6
XD-14820	68.9	2.7
XD-14822	63.7	0.7
XD-14824	69.8	3.6
XD-14825	52.5	2.2
XD-14826	60.6	3.6
XD-14829	67.7	4.5
XD-14830	59	5.7
XD-14834	67.7	2.8
XD-14835	51.7	12.4
XD-14837	63.4	10.1
XD-14838	73.2	4
XD-14839	65.7	1.9
XD-14840	60.7	2.7
XD-14841	65.5	2.2
XD-14842	67.7	2
XD-14843	76.7	9.6
XD-14846	54.9	6.5
XD-14847	69.6	1.7
XD-14848	77.5	1.1
XD-14850	74.4	3.2
XD-14851	89.7	2.4
XD-14853	77	3.2
XD-14854	73	3.6

XD-14855	82.4	4.2
XD-14856	72.7	4.7
XD-14857	54.4	3.6
XD-14859	63.8	3.2
XD-14860	52	2.3
XD-14861	63.2	3.5
XD-14863	72.3	3.1
XD-14864	63.9	2.2
XD-14875	63.1	2.9
XD-14876	62.9	2.5
XD-14878	66.1	3.8
XD-14880	64.5	4.5
XD-14882	52.7	2.8
XD-14886	67.8	4.4
XD-14887	63.1	2.3
XD-14889	51.5	3.5
XD-14890	52.3	0.6
XD-14891	67.7	5.1
XD-14893	51.7	2.4
XD-14900	66.2	2.8
XD-14901	55.3	1
XD-14902	72.5	2.1
XD-14903	69.5	2.5
XD-14904	62.2	2.2
XD-14905	63.9	3.9
XD-14906	76.6	4.8
XD-14907	77.2	8.1
XD-14908	76.5	6.7
XD-14909	75.1	7.7
XD-14917	59.8	7.9
XD-14924	68	5.5
XD-14949	75.9	7.4
XD-14952	75.8	5.2

Overall, the siRNA treatment data shows successful *ATXN2* mRNA knockdown.

Confirmation of ATXN2 protein level reduction by siRNA treatment

To assess whether ATXN2 protein levels were also reduced by the informatically predicted siRNAs, 56 siRNAs were resynthesized (44 top ranked siRNAs by knockdown at 200 pM; 2 additional siRNAs near the top ranked, but having ATXN2L cross-reactivity (XD-14776) or mouse cross-reactivity (XD-14887) as characteristics which merited their re-testing; additional 10 siRNAs selected by a joint assessment of the ranking by knockdown at 20 nM dosed siRNA (from the top 55 ranked by knockdown), and also taking into account an informatic prediction of off-target likelihood. These siRNAs were synthesized to a reported purity of 80-85% (Dharmacon). As before, siRNAs were synthesized as 22 nucleotide guide and passenger strands, with a 20 nucleotide complementary sequence between guide base 1 – 20 and passenger bases 1 – 20, with 2 nucleotide 3' overhangs on each strand, and introduced by transient transfection. Three additional controls were included. A non-targeting control (NTC) (Dharmacon, ON-Target plus Control Non-Targeting siRNA #1, D-001810-01-05) and a sequence targeting luciferase controlled for any nonspecific effects of siRNA treatment, including transfection reagents, on ATXN2 signal. For the luciferase control, sense sequence: GGAATTATAATGCTTATCTATA (SEQ ID NO:536); antisense sequence: TAGATAAGCATTATAATTCCTA (SEQ ID NO:537). A 'SMARTPool' (SMP), a combination of 4 siRNAs targeting *ATXN2* (Dharmacon; ON-TARGETplus Human *ATXN2* siRNA SMARTPool, L-011772-00-0005) was used as a positive control for specific targeting of *ATXN2*. Both the NTC and SMARTPool siRNAs are chemically modified to limit off-target effects.

An imaging based assay used indirect immunofluorescence signal by antibodies against ATXN2 to quantify ATXN2 levels. For these experiments U2OS cells were selected because of their large and uniform cell bodies, which permit good visualization of Ataxin-2 levels in the cytoplasm. siRNAs were introduced by transient transfection, and then 3 days later cells were fixed in paraformaldehyde, and then blocked and immunostained for Ataxin-2 and counterstained with Hoechst dye 33342 to identify cell nuclei.

Images were segmented using custom pipelines developed in Cell Profiler. First, cell nuclei are identified and outlined based on Hoechst 33342 signal. Subsequently, the nuclei outline is expanded to generate a ring. Within this ring, for each cell, the signal from the indirect immunofluorescence channel corresponding to a fluorescent secondary antibody binding to anti-Ataxin-2 is quantified. To calculate the ATXN2 signal for a well, the mean across cells in the well (typically 1000-3500 cells imaged/well) of cellular ATXN2 signal was calculated. The upper quartile ATXN2 signal within the cytoplasmic region was used. By taking the upper quartile of signal, this avoids the influence of signal from segmented regions of the image that may inadvertently not contain cells.

Cells were dosed with 20 or 1 nM siRNA in 96-well format, across multiple plates with controls in each plate. Background was subtracted by, within each imaging plate, wells stained with secondary antibody but not primary antibody, and not transfected. This reflects background intensity due to nonspecific binding of the secondary antibody. Ataxin-2 intensity values were normalized to those from wells transfected with non-targeting control ('NTC'). From this, normalized ATXN2 signal represents a proxy for degree of protein level knockdown. Importantly, ATXN2 signal was similar for wells treated with luciferase targeting siRNA as with cells treated with NTC control. Note that the 'NTC' control (Dharmacon) chemistry is modified to reduce off-target effects whereas all *ATXN2*-targeting and luciferase-targeting siRNAs tested were unmodified. **FIG. 9** quantifies knockdown of *ATXN2* signal for siRNAs at 20 and 1 nM dose levels. **FIGS. 10A and 10B** show representative images from the knockdown experiments, with evidence of clear reduction of Ataxin-2 intensity from the indicated siRNAs. **FIG. 11** plots the siRNAs protein knockdown data, at either 20 or 1 nM siRNA, as a function of *ATXN2* transcript position. Almost all of these top siRNAs yielded substantial knockdown of siRNA at the protein level. At 1 nM, all of these top siRNAs exceeded the knockdown performance of the SMARTPool siRNA. **Tables 6 and 7** display the mean and standard deviation of *ATXN2* signal across wells. Sequences of the siRNAs from Tables 6 and 7 are provided in **Table 1**. For mean and SD calculations, outliers were excluded (outliers defined as wells where value deviated

from the median value across wells by more than 1.5 standard deviations and by greater than 10% normalized *ATXN2* signal). Outlier wells are highlighted in **FIG. 9**.

Table 6: ATXN2 protein knockdown, measured by high content imaging, after

5 **siRNA treatment at 20 nM**

Treatment/ Immunostain	Dose in nM	Mean ATXN2 Signal	Standard Deviation ATXN2 Signal	N
no_primary_no_ secondary	20	-0.7	2.2	23
no_primary_secon da ry	20	0	1.3	24
NTC	20	100	6.1	64
primary_no_secon da ry	20	1.7	1.9	24
primary_secondary	20	92.5	7.2	46
SMP	20	23.4	5.8	61
XD-14742	20	13.6	3.5	4
XD-14743	20	20.7	4.9	4
XD-14747	20	11.4	1.9	3
XD-14756	20	6.9	3	3
XD-14757	20	18.2	6.9	4
XD-14758	20	14.6	2.3	4
XD-14759	20	11.1	3.2	4
XD-14762	20	13.7	5.9	4
XD-14763	20	7.4	5.7	4
XD-14766	20	15.7	5	4
XD-14768	20	11.6	3.9	4
XD-14775	20	14.1	3.3	4
XD-14776	20	15.9	1.7	4
XD-14779	20	13.2	1.5	3
XD-14786	20	19.8	4.9	3
XD-14787	20	12.7	6.7	4
XD-14788	20	16.7	7.4	4
XD-14790	20	17.9	8.1	4
XD-14791	20	15.6	5.4	4
XD-14792	20	12.5	9.1	4
XD-14793	20	17.4	4.4	4
XD-14794	20	14.5	4.2	4
XD-14798	20	15.9	4.7	3
XD-14799	20	14.5	1.8	4

XD-14800	20	13.1	5.7	3
XD-14801	20	9	6.1	4
XD-14805	20	22.2	4.3	4
XD-14809	20	44.2	4.9	4
XD-14810	20	24.8	6.4	4
XD-14811	20	29	6.3	4
XD-14819	20	27.1	9.1	4
XD-14822	20	29.4	4.6	4
XD-14825	20	19.9	5.2	4
XD-14826	20	22.2	7.5	4
XD-14830	20	11.7	5.4	4
XD-14834	20	7.5	4.3	4
XD-14835	20	6.2	3.5	3
XD-14837	20	13.3	4.8	4
XD-14839	20	23.4	0.9	4
XD-14840	20	19.7	4.2	4
XD-14841	20	25.3	2.7	4
XD-14843	20	16.8	2.8	4
XD-14846	20	11.2	2	3
XD-14847	20	8.9	2.8	4
XD-14850	20	21.6	3.1	4
XD-14857	20	16.8	7.4	4
XD-14860	20	8.7	4	3
XD-14882	20	14.2	1.3	3
XD-14887	20	19.2	4.2	4
XD-14889	20	16.2	2.6	4
XD-14890	20	13.8	4.9	4
XD-14893	20	24.7	12.4	4
XD-14901	20	17.7	4	4
XD-14904	20	15.9	5.8	4
XD-14905	20	13.6	5.7	3
XD-14917	20	18.7	5.9	4
XD-LucControl	20	101	4.8	3

Table 7: ATXN2 protein knockdown, measured by high content imaging, after siRNA treatment at 1 nM

Treatment/ Immunostain	Dose in nM	Mean ATXN2 Signal	Standard Deviation ATXN2 Signal	N
no_primary_no_secondary	1	-1.3	2.8	29
no_primary_secondary	1	0	2.7	30

NTC	1	99	17.1	78
primary_no_secondary	1	1.4	2.8	28
primary_secondary	1	97.9	10	62
SMP	1	50.1	7.1	78
XD-14742	1	27.2	5.5	5
XD-14743	1	18.1	3.3	4
XD-14747	1	21.7	3.8	5
XD-14756	1	18.2	5.5	4
XD-14757	1	26.7	3.6	5
XD-14758	1	19.8	5.2	5
XD-14759	1	16	3.2	5
XD-14762	1	18.3	2.3	5
XD-14763	1	20.7	3.8	5
XD-14766	1	19.7	2.9	5
XD-14768	1	25.5	7.6	5
XD-14775	1	28	2.5	4
XD-14776	1	25.4	4.7	5
XD-14779	1	26.8	4.8	5
XD-14786	1	24.4	4	4
XD-14787	1	23.2	4.6	5
XD-14788	1	25.7	5.7	4
XD-14790	1	22.9	5.6	5
XD-14791	1	25.5	5.9	5
XD-14792	1	18.6	5.7	5
XD-14793	1	37.6	4	5
XD-14794	1	23.4	4.7	5
XD-14798	1	20.2	6.1	4
XD-14799	1	29.5	3.7	5
XD-14800	1	22.6	1.8	4
XD-14801	1	16.2	4.5	4
XD-14805	1	35.4	2.5	5
XD-14809	1	40	8.5	5
XD-14810	1	40.7	4.3	5
XD-14811	1	42	14.5	4
XD-14819	1	36.6	0.7	4
XD-14822	1	31.5	5.6	5
XD-14825	1	28.2	3.4	5
XD-14826	1	32.7	6.5	4
XD-14830	1	26.6	4.6	5
XD-14834	1	19.7	4.9	5
XD-14835	1	20.3	4.9	5
XD-14837	1	19	4.6	5

XD-14839	1	21.6	3.1	4
XD-14840	1	20.7	5.4	5
XD-14841	1	29.8	4.9	5
XD-14843	1	26.3	2.5	4
XD-14846	1	23.1	5.8	5
XD-14847	1	15.1	2.9	5
XD-14850	1	26.4	8.2	5
XD-14857	1	26.1	3.8	5
XD-14860	1	18.5	4	5
XD-14882	1	23.5	4.9	5
XD-14887	1	22.3	4.2	5
XD-14889	1	24.4	7	5
XD-14890	1	21.6	2.4	4
XD-14893	1	17.3	2	3
XD-14901	1	25.6	2.9	4
XD-14904	1	19.9	2.1	5
XD-14905	1	26.5	2.5	5
XD-14917	1	19.2	2.7	5
XD-LucControl	1	114.6	12.1	5

Remarkably, 53 out of 56 of the *ATXN2*-targeting sequences achieved greater than 60% *ATXN2* signal knockdown by this assay. In this assay, nonspecific antibody signal was not corrected. In subsequent assays (see below), *ATXN2* knockout cells were used as controls demonstrating that some *ATXN2* antibody background is present. Therefore, the *ATXN2* protein level knockdown values here may underestimate the amount of protein knockdown caused by the *ATXN2*-targeting siRNA treatments.

Selection of Top-Ranked Sequences for Evaluation in siRNA dose response and in miRNA Backbones

To assess the potency of guide sequences targeting *ATXN2*, dose-response profiling of siRNAs and testing of guide sequences in miRNA format of 22 top sequences was conducted. To select top sequences for this detailed profiling, rankings of RNA knockdown for siRNAs at 20 nM and 200 pM were first assessed. In addition to this ranking of RNA knockdown, a method for predicting the number of off-target transcripts that would be influenced by the guide sequence was used, generating a probability of off-targeting score (POTS).

<https://sispotr.icts.uiowa.edu/sispotr/tools/lookup/evaluate.html>) (Boudreau et al., Nucleic Acids Research 2013 41(1):e9). This score considers the seed sequence of the siRNA, and as such is supplementary to the initial assessment of off-target prediction based on the number of transcripts with 2 or fewer mismatches to the first 19 nucleotides of the guide sequence. Going down the knockdown ranks of siRNAs, sequences with increasingly stringent POTS score were favored. Additional criteria evaluated were: proximity to the region of *ATXN2* complementarity for other guide sequences; re-examination of the number of transcripts closely complementary to nucleotides 2 – 19 were taken into account and resulted in the exclusion of two other sequences. The specific predicted off-targets were not examined for the selection of sequences for these experiments.

In addition to top-ranked sequences, two low-performing siRNAs (XD-14781 and XD-14949) that had low mRNA knockdown when assessed as siRNAs at 20 nM or 1 nM, were included to confirm the range and sensitivity of downstream assays.

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siRNA dose response versus ATXN2 mRNA knockdown testing

Dose response profiling was performed by testing dilution series of siRNAs transfected into HepG2 cells (**FIG. 12**), as described above for single-dose experiments. As expected, sequences XD-14781 (guide SEQ ID NO:90; passenger SEQ ID NO:89) and XD-14949 (guide SEQ ID NO:426; passenger SEQ ID NO:425), which had poor performance when assessed at 20 and 1 nM, had low potency and reduced maximal knockdown when assessed in dose response. IC50s of all other top-ranked siRNAs separated from these values. Two batches of testing were performed. Top sequences from one of the batches were estimated to have concentrations achieving half-maximal knockdown of < 10 pM, indicating that the top-ranked siRNAs are highly potent. Performances of siRNAs had some dissimilarities between the batches but this was not investigated further, and the sequences were advanced into further testing in miRNA format. This miRNA testing, discussed below, showed that the lowest performing sequences from each batch were separated from the highest performing sequences in efficacy of *ATXN2* protein lowering, but that the performance of top

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siRNAs from the two batches were similar. The miRNA testing is therefore regarded as more relevant for precise ranking of sequences.

Design and production of ATXN2-targeting sequences in miRNA backbones

5 Following identification of active siRNA sequences, siRNAs were embedded in miRNAs for expression from DNA vectors. The miR-155 and miR-1-1 backbones were considered.

 The miR-155 was originally identified as a promising scaffold for construction of RNA polymerase II-based miRNA vectors due to its location within a conserved non-coding RNA⁸. After initial identification and design of miR-155 shRNA, subsequent sequence improvements increased microprocessor cleavage³. Many groups took the miR-155 scaffold to preclinical use in mice^{10,11}, sheep¹² and non-human primates¹³, enabling gene therapy approaches in genetically-driven human disease.

 Initial experiments were conducted using a version of the miR-155 scaffold that, in one previous report, was engineered into an artificial mRNA and used in a mouse *in vivo* proof of concept study to knockdown HTT¹⁰. Small RNA sequencing had demonstrated high strand bias by this miRNA backbone¹⁰. *ATXN2* targeting guide sequences and controls were incorporated into this scaffold sequence, which was termed “mR-155M” and assayed for protein knockdown after transfection of U2OS cells.

 To rationally improve miR-155, human genomic sequence was examined, and the span of flanking miR-155 sequence to be used was defined by the region surrounding miR-155 with high evolutionary conservation across similar species. That is, a plot of sequence conservation versus position was visualized, and the genomic position from the endogenous miR-155 at which this sequence conservation dropped off was used to determine how much flanking context around the miR-155 stem structure should be included. Next, the miR-155 loop was examined for features which might impact the use of this miR in different expression systems. A homotetrameric UUUU in the miR-155 loop was noted. UUUU sequences have been reported to induce Polymerase III termination¹⁴, which would lead to aberrantly

truncated miRNAs which do not undergo stem pairing. To interrupt this homotetrameric UUUU, an apical UGU motif within the miR-155 loop was added. This motif additionally has been reported to enhance miRNA processing.^{1,2} In addition to previously engineered UG and CNNC motifs³, a basal stem mismatched GHG motif² was added to improve precise processing.

To expand the number of amiRNA scaffolds beyond the miR-155 backbones, backbones from endogenous miRNAs reported to have high processing precision were prioritized. The miR-1-1 backbone ranks among the highest in processing precision according to reference:¹⁵ has high strand bias by small RNAseq⁵, and the guide strand is on the 3 prime arm of the miRNA stem, which may improve processing accuracy compared to 5 prime-arm positioned guide strands¹⁶. Natively integrated favorable sequence motifs include a basal mismatched GHG motif and downstream CNNC motif. It also has a short context for sequencing and has been successfully engineered for artificial miRNA expression in drosophila models¹⁷.

Additional miRNA scaffolds that may be considered for the amiRNAs of the present disclosure include:

- miR-100 and miR-190a – high throughput screen identified high on-target/off-target ratio¹⁵.
- miR-124 and miR-132 – both motor-neuron expressed miRNAs do not change expression in an ALS rat model¹⁸. The cell-type specific expression and consistent levels throughout ALS disease course are favorable miRNA characteristics. Neuronal specificity has been confirmed in a sRNAseq cross-tissue expression database¹⁹ (<https://ccb-web.cs.uni-saarland.de/tissueatlas/>).
- miR-9 – neuron-specific expression²⁰.
- miR-138-2, miR-122, miR-130a, and miR-128 were selected to be naturally asymmetric (either exclusively 5' or 3' strand is observed in small RNAseq datasets), highly homogeneous (i.e. high “5' homogeneity score”¹⁵), not reported to undergo post-transcriptional regulation (e.g.

which occurs for clustered miRNAs), are consensus miRNAs on miRBase, have flexible loop structure and simple duplex stem.

To further mimic the miRNA backbones, bulges and mismatches can be inserted into the guide:passenger strand duplex in a manner to replicate the bulge pattern observed in endogenous miRNAs, but applied to artificial miRNAs targeting *ATXN2*. The modifications that can be done to the passenger strand to introduce these native-miRNA mimicking structures are provided in Table 8.

Table 8: Design Rules for Exemplary miRNA formats

miR	Modifications to passenger sequence
miR-1-1	Mismatch base 2 Bulge mismatch transversion base 19 Insert a base between bases 18 and 19, either G or C but not complementary to the guide
miR155 (M or E)	Delete bases 9 and 13 of passenger
miR100	Bulge mismatch transversion base 11 Bulge mismatch transition bases 7, 18 add GU wobble base 17 Mismatch base 22
miR124	Mismatch base 3 Bulge mismatch transition bases 2, 17 Bulge mismatch transversion 12, 16
miR138-2	Mismatch bases 5, 21 Bulge mismatch bases 6 Add GU wobble base 13 delete bases 7, 22
miR122	Bulge mismatch transition base 12 Bulge mismatch transversion bases 20, 21
miR-128	Truncate guide to 21 bp Mismatch bases 1,2,12 Bulge mismatch transversion bases 3, 11, 13 Insert 2 bases between bases 20 and 21, not complementary to the guide add GU wobbles bases 5, 7
miR130a	Mismatch base 2 Add GU wobbles bases 18, 19 bulge mismatch transversion base 11 Bulge mismatch transition base 22
miR 16 -2	Bulge mismatch transversion bases 11, 12 Mismatch base 22

10

Note: For the above, 'passenger' sequence refers to a sequence complementary to the 22 nucleotides of the guide sequence. This is not the same as passenger sequences as used in describing siRNA duplexes. Mismatch refers to the following substitution rule: G → C, C → G, A → T, T → A. Bulge mismatch transition refers to the rule: T → C, C → A, A → G, G → T. Bulge mismatch transversion refers to the rule: G → T, C → A, A → C, T → G. Add GU wobble refers to the rule: If base is C, then convert to T.

15

Initial Testing of ATXN2 targeting guide sequences in miR155-M and miR1-1
backbones

As an initial test of the ability of the Atxn2 targeting siRNAs to knock
down Atxn2 when embedded in a miRNA context, the guide sequence of XD-14792
5 (SEQ ID NO:112), which had the highest ranked *ATXN2* mRNA knockdown when
dosed at 200 pM as an siRNA, was embedded in several miRNA contexts as shown in
Table 9. The amiRNA DNA sequences are provided in Table 9 as SEQ ID NOS:538-
543. The corresponding amiRNA RNA sequences are provided in Table 9 as SEQ ID
NOS:1109-1114, respectively.

10

Table 9: XD-14792 sequences embedded in amiRNAs

Name	Target/ Type	Guide Sequence	Variation	miR Backbone	Artificial miRNA (DNA Sequence)	Artificial miRNA (RNA Sequence)
XD-14792_miR1-1	ATXN2	AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]		1-1	CATGCAGACTGCCTGCTTGGGTA CAGACCAAGAGTAGTCGAATTA TGGACCTGCTAAGCTAATTAAT ACTCTTGGTCTGAACCTCAGGCC GGGACCTCTCTCGCCGCACTGAG GGGCATCTCCACACCACGGGGCC [SEQ ID NO:538]	CAUGCAGACUGCCUGCUUGGGU ACAGACCAAGAGUAGUCGAAU UAUGGACCUAGCUAAGCUAAUUA ACUACUCUUUGGUCUGAACUCA GGCCGGGACCUUCUCGCCGCAC UGAGGGGACUCCACACCACGG GGGCC [SEQ ID NO:1109]
XD-14792_miR1-1 enhanced	ATXN2	AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]	E	1-1	CATGCAGACTGCCTGCTTGGGTA CAGACCAAGAGTAGTCGAATTA TGGACCTGCTAAGCTAATTAAT ACTCTTGGTCTGAACCTCAGGCC GGGACCTCTCTCGCCGCACTGAG GGGCATCTCCACACCACGGGGCC [SEQ ID NO:539]	CAUGCAGACUGCCUGCUUGGGU ACAGACCAAGAGUAGUCGAAU UAUGGACCUAGCUAAGCUAAUUA ACUACUCUUUGGUCUGAACUCA GGCCGGGACCUUCUCGCCGCAC UGAGGGGACUCCACACCACGG GGGCC [SEQ ID NO:1110]
XD-14792_miR155	ATXN2	AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]		155	CCTGGAGGCTTGTGAAGGCTGT ATGCTGATTAAGTACTCTTTGGTC TGAATTTGGCCACTGACTGATTC AGACCAAGGTAGTTAATCAGGAC ACAAGGCTGTACTAGCACTCA CATGGAAACAAATGGCCACCGG [SEQ ID NO:540]	CCUGGAGCUUGGCUAAGGCUG UAUGCUGAUUAACUACUCUUUG GUCUGAAUUUUGCCACUGACU GAUUCAGACCAAGGUAGUUAU CAGGACACAAGGCCUGUUAACUA GCACUACAUGGAACAAUUGGC CACCGG [SEQ ID NO:1111]
XD14792_911_miR155	ATXN2 911 Control	AUUAACUAGAGUU UGGUCUGAA [SEQ ID NO:544]		155	CCTGGAGGCTTGTGAAGGCTGT ATGCTGATTAAGTACTCTTTGGT CTGAATTTGGCCACTGACT GATTCAGACCAACCTAGTTAATC AGGACACAAGGCTGTACTAGC ACTACATGGAACAAATGGC CACCGG [SEQ ID NO:541]	CCUGGAGCUUGGCUAAGGCUG UAUGCUGAUUAACUAGAGUUUG GUCUGAAUUUUGCCACUGACU GAUUCAGACCAACCUAGUUAU CAGGACACAAGGCCUGUUAACUA GCACUACAUGGAACAAUUGGC CACCGG [SEQ ID NO:1112]
XD-14792_SScr miR155	ATXN2 SScr	AUUAACUAGAGUU CGGUCUCUU [SEQ ID NO:545]		155	CCTGGAGGCTTGTGAAGGCTGT ATGCTGATTAAGTACTCTTTGGT CTCTTTTGGCCACTGACTGAAA	CCUGGAGCUUGGCUAAGGCUG UAUGCUGAUUAACUAGAGUUCG GUCUCUUUUUUGCCACUGACU

						GAGACCATATTAGTTAATCAGGA CACAAAGGCTGTTACTAGCACTC ACATGGAACAAATGGCCACCGG [SEQ ID NO:542]	GAAAGAGACCAUUAUAGUUAU CAGGACACAAAGGCCUGUUAUA GCACUCACAUGGAACAAUAGGC CACCGG [SEQ ID NO:1113]
XD-14792_miR155 sealed	ATXN2	AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]	S	155		CCTGGAGGCTTGCTGAAGGCTGT ATGCTGATTAACCTACTCTTTGGTC TGAATTTGGCCACTGACTGATTTC AGACCAAAGAGTAGTTAATCAGG ACACAAGGCTGTTACTAGCACT CACATGGAAACAAATGGCCACC [SEQ ID NO:543]	CCUGGAGGCUUGCUGAAGGCUG UAUGCUGAUUAACUACUCUUUG GUCUGAAUUUUGGCCACUGACU GAUUCAGACCAAGAGAGUUA AUCAGGACACAAGGCCUGUUA UAGCACUCACAUGGAACAAUAG GCCACC [SEQ ID NO:1114]

In the variation column: "E" refers to "enhanced," "S" refers to "sealed"

In Table 9, the guide sequences (including the guide sequence, any variants, as well as the parental guide sequence from which they are derived) are shown in RNA form, and the artificial miR sequence is provided in both RNA format, and for when embedded in the vector is shown in DNA form. The miR backbones used include: (a) miR155, preserving a bulge format reported in (Fowler et al., Nucleic Acids Res. (2015) 44:e48); (b) miR155, with no sequence bulges, yielding a perfectly complementary stem (“sealed”); (c) miR1-1, preserving a native bulge format as in the endogenous miRNA; and (d) miR1-1 with the “Enhanced” variation, including a modification in the 3’ arm that in other miRNAs was previously reported to enhance processing (Auyeung et al., Cell 2013). **FIG. 13** shows one of the predicted RNA folds of the miRNA stems of several of the constructs, using the web server mfold. Bulges in the stem in the region including or apposed to the guide sequence are apparent, which are designed to mimic the native mismatches of the endogenous forms of the microRNAs from which derive the surrounding context for the guide sequence. As controls (“911 controls”), bases 9, 10, and 11 of XD-14792 guide sequence were modified to be the complementary bases (that is, substituting A -> T, T -> A, C -> G, or G -> C); or (“SScr”), in which all bases except bases 1 – 7 were scrambled. In both cases, any seed-mediated off-target activity (deriving from bases 1 – 7) should be preserved, whereas the on-target Atxn2 slicing activity should be blocked.

pLVX-EF1A_mCherry-miR-1-1-XD_14890-WPRE_CMV (SEQ ID NO:546) is a representative lentiviral vector that can be used for expressing these artificial microRNAs. Nucleotides 4275-4412 of SEQ ID NO:546 (XD-14890 guide sequence in a miR-1-1 backbone) can be substituted with another artificial miRNA of interest. In this lentiviral vector suitable for packaging into lentivirus, an EF1-alpha promoter drives expression of a mCherry protein. After a stop codon, the amiRNA stem is expressed downstream within a 3’ UTR. Downstream of that a WPRE element (Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element) enhances the stability of the transcript. Adapters may be included upstream or downstream of the artificial miRNA construct to facilitate cloning and downstream detection of the sequences, but these adapters are not expected to influence the performance of the microRNA. A CMV promoter (as in sequence shown), or a PGK promoter (as in

plasmids transfected for data shown **FIG. 14**), downstream, drives expression of the puromycin resistance protein for puromycin selection in mammalian cells. This is a similar design to the vector used in (Kampmann et al., *PNAS* 2015).

pcDNA3.1 NEGFP STOP ATXN2 3'UTR.gb (SEQ ID NO:547)

- 5 represents a plasmid used to generate a GFP-ATXN2 reporter line. A CMV promoter was used to drive the expression of a transcript encoding enhanced green fluorescent protein (EGFP). A stop codon at the end of the EGFP open reading frame was followed by the *ATXN2* sequence, but removing the initial ATG such that the sequence is expected to not be translated. A separate SV40 promoter downstream drives the
- 10 expression of the NeoR/KanR protein product which enabled selection of U2OS cells stably integrating the plasmid by G418 selection. EGFP fluorescence was bright and diffuse, and not restricted to the cytoplasm as expected if the ATXN2 protein was translated and fused to the EGFP. Several lines were generated by single-cell cloning after G418 selection, and one line ultimately selected based on uniform fluorescent
- 15 signal distribution by FACS as well as a larger differential between control-transfected (siNTC) and ATXN2 siRNA-transfected cells.

- Constructs with the artificial miRNAs noted above were transfected into U2OS cells stably expressing the GFP – Atxn2 reporter by transient transfection (lipofectamine 3000). Four days later, GFP – ATXN2 levels were quantified by
- 20 fluorescence automated cell sorting (FACS), gating cells by the expression of the mCherry encoded on the miRNA vector to isolate cells expressing the artificial miRNA construct. **FIG. 14** shows median fluorescence intensity signal of GFP intensity. XD-14792 sequences embedded in artificial miRNA backbones miR-155 or miR-1-1 considerably reduced ATXN2 GFP reporter intensity relative to cells expressing control
- 25 constructs (XD-14792 911 and XD-14792 Sscr, embedded in the miR-155 stem backbone). A 'sealed' XD-14792 construct in a miR-155 backbone, in which the stem is perfectly complementary (**FIG. 14**) did not reduce the ATXN2 GFP reporter signal as much as did XD-14792 when embedded in either miR-155 or miR-1-1 with bulged residues.

Expanded screening of ATXN2 targeting sequences in artificial microRNA vectors in lentiviral format

Given the encouraging results with the knockdown of the ATXN2 GFP reporter, a set of *ATXN2* targeting sequences was cloned into the artificial microRNA expressing vector described above (SEQ ID NO:546). The same set of *ATXN2* targeting sequences as were tested in dose-response testing for mRNA knockdown were incorporated into plasmids to enable lentiviral packaging. Vectors were packaged into lentivirus (see methods below) and transduced into unmodified U2OS cells or U2OS cells deficient for ATXN2 (described below) in a 96-well format, across multiple plates. Each plate had controls to enable plate-wise signal normalization. 3.5 days after transduction, cells were fixed with paraformaldehyde, blocked and stained with anti-ATXN2 antibodies, anti-mCherry antibodies, and Hoechst dye (33342) to demarcate cellular nuclei, and ATXN2 signal was quantified by image segmentation and signal intensity measurement as described above. Transduced and untransduced cells were differentiated by anti-mCherry signal. **FIG. 15** shows histograms of the expected mCherry signal for untransduced cells as well as wildtype transduced cells. The threshold was set such that the signal from untransduced wild-type cells did not exceed this threshold, but most of the cells in the right peak of the bimodally distributed signals (right panel, wild-type transduced cells) were considered positive.

ATXN2 signal was subtracted for background measured in U2OS cells with the *ATXN2* gene disrupted by CRISPR and in which ATXN2 protein had been verified to be eliminated by Western analysis. **FIGS. 20 – 21** show the data for the knockout generation process. **FIG. 20** shows Western and FACS analysis of Ataxin-2 signal in cells nucleofected with Cas9 – gRNA complexes targeting Ataxin-2 or control targets. Robust reduction of Ataxin-2 protein is seen with multiple guides, consistent with editing and disruption of the Ataxin-2 gene. **FIG. 21A** shows the workflow to generate clonal ATXN2 knockout cells, and **FIG. 21B** shows Western analysis of single-cell clones derived from Cas9 – gRNA nucleofected cells, from which clone 43 was confirmed to be null for Ataxin-2 and selected for further use. The clone was

sequenced by Sanger sequencing, and using the ICE tool (Synthego), a mixture of disrupting mutations consistent with disruption of the ATXN2 alleles was confirmed.

As shown in **FIG. 16**, the signal in ATXN2 deleted cells was slightly increased relative to wild-type cells that were treated with secondary antibody but not primary anti-Ataxin-2 antibody, suggesting some nonspecific, background binding of the ATXN2 antibody. These cells were not transduced with virus. After background subtraction, signal was normalized relative to *ATXN2* signal in untransduced wild-type cells.

FIG. 17 shows well-level quantification of ATXN2 signal intensities across artificial microRNA constructs, with representative images shown in **FIG. 18**. Transduced cells were identified by anti-mCherry levels exceeding the threshold defined above. A median of 3355 cells per well were mCherry positive and included for ATXN2 signal calculation, with a range of 2469 – 4582 cells and standard deviation of 391 cells per well.

Table 10 shows mean and standard deviations of ATXN2 signals, normalized as above, for sequences, embedded either in the enhanced miR-155 backbone or the miR1-1 backbone (sequences provided in Table 11). In general, for most but not all sequences, ATXN2 knockdown performance was superior when the guide sequence was embedded in the miR1-1 backbone. None of the 911 controls, where the artificial microRNA was engineered such that guide bases 9, 10 and 11 were complemented (A → T, T → A, C → G, or G → C), exhibited knockdown, indicating that the reduction in ATXN2 signal is dependent on the direct RNA interference activity of the microRNAs on the endogenous *ATXN2* transcript. Additionally, protein level knockdown across guide sequences, when examined in the miR-1-1 backbone, correlated with mRNA knockdown in HepG2 cells after 200 pM siRNA treatment. (linear model $p < .001$; $R^2 = 0.5$; **FIG. 19**).

Table 10: ATXN2 protein levels following amiRNA treatment

Duplex ID/Category	miR Backbone	Mean normalized ATXN2 Signal	Standard Deviation
XD-14790 911	miR-1-1	111.7	15.9
XD-14790 911	miR-155E	116.8	5.4

XD-14800 911	miR-1-1	113.3	10.1
XD-14800 911	miR-155E	111.1	8
XD-14857 911	miR-1-1	112.7	11.5
XD-14857 911	miR-155E	116.7	10.1
XD-14742	miR-1-1	54.4	1.3
XD-14742	miR-155E	89.8	10.7
XD-14743	miR-1-1	39.8	2
XD-14743	miR-155E	89.9	8.1
XD-14756	miR-1-1	44.9	5.4
XD-14756	miR-155E	73.2	7.3
XD-14766	miR-1-1	55.4	1.2
XD-14766	miR-155E	53.3	5.1
XD-14781	miR-1-1	79.2	10.3
XD-14781	miR-155E	100.8	14.4
XD-14786	miR-1-1	45	1.7
XD-14786	miR-155E	77	11.4
XD-14787	miR-1-1	83.8	6.9
XD-14787	miR-155E	103.4	12
XD-14788	miR-1-1	69.7	9.8
XD-14788	miR-155E	98	3.8
XD-14790	miR-1-1	43.2	7.6
XD-14790	miR-155E	56.3	7.6
XD-14792	miR-1-1	37.9	4.8
XD-14792	miR-155E	51.5	9.9
XD-14798	miR-1-1	60.8	2.6
XD-14798	miR-155E	66.8	3
XD-14799	miR-1-1	73.5	6.7
XD-14799	miR-155E	84.9	8.9
XD-14800	miR-1-1	41	6.4
XD-14800	miR-155E	78.1	5.7
XD-14819	miR-1-1	46.6	7.1
XD-14819	miR-155E	63	7.5
XD-14835	miR-1-1	41.3	7.4
XD-14835	miR-155E	67.3	21.5
XD-14846	miR-1-1	69.3	9.3
XD-14846	miR-155E	97.5	5.6
XD-14857	miR-1-1	41.2	6.3
XD-14857	miR-155E	65.3	8.9
XD-14887	miR-1-1	71.8	11.7
XD-14887	miR-155E	87.8	16.3
XD-14889	miR-1-1	62.5	8.8
XD-14889	miR-155E	62.6	3.7

XD-14890	miR-1-1	24.8	7.2
XD-14890	miR-155E	83.8	9.2
XD-14901	miR-1-1	61.8	8.3
XD-14901	miR-155E	84	4.7
XD-14904	miR-1-1	44.2	8.3
XD-14904	miR-155E	55.2	9.9
XD-14917	miR-1-1	38.5	8.1
XD-14917	miR-155E	67.2	3.8
XD-14949	miR-1-1	106.8	9.7
XD-14949	miR-155E	55.2	6.7

Table 11 provides the parent guide RNA sequences, amiRNA sequences, and amiRNA DNA sequences as embedded in microRNA backbone-expressing vectors of both active guide sequences as well as a small set of control sequences. The guide sequence anticipated to be produced in cells is described in RNA form, and the sequence encoding the guide sequence (embedded in miRNA) is provided in DNA form.

Table 11: amiRNA Sequences

Parent Guide ID	miR Backbone	Category	amiRNA DNA Sequence	amiRNA RNA Sequence
AGGAACGUGGGUU GAACUCCUU [SEQ ID NO:242]	miR-1-1	911 Control	CATGCAGACTGCCTGCTTGGGATG	CAUGCAGACUGCCUGCUUGGGAU
			GAGTTCAAGGACGTCGCCCTTATG	GGAGUUAAGGAGGUGCCUUA
XD-14857	miR-155E	911 Control	GACCTGCTAAGCTAAGGAACGTCC	UGGACCUUGCUAAGCUAAGGAACG
			CTTGAACTCCTTCTCAGGCCGGGA	UCCCUUGAACUCCUUCACAGGCCG
AGGAACGUGGGUU GAACUCCUU [SEQ ID NO:242]	miR-155E	911 Control	CCTCTCGCCGCACTGAGGGGCA	GGACCUUCUGCCGCGACUGAGG
			CTCCACACCACGGGGGCC	GGCACUCCACACCGGGGGCC
XD-14857	miR-155E	911 Control	[SEQ ID NO:548]	[SEQ ID NO:1115]
			CTGAGGCTTGTCTTTGGGCTGTAT	CUGAGGCUUGCUUUUGGGCUGUA
XD-14790	miR-155E	911 Control	GCTGAGGAACGTCCTTGAACCTCC	UGCUGAGGAACGUCUUGAACU
			TTTTTTGGCCTCTGACTGAAAGGA	CCUUUUUUGGCCUCUGACUGAAA
UUCGGGUUGAAAU CUGAAGUGU [SEQ ID NO:108]	miR-155E	911 Control	GTTAAGGACGTTCTCAGGACAAG	GGAGUUAAAGACGUUCCUCAGGA
			GCCCTTTATCAGCACTCATATGGA	CAAGGCCUUUAUCAGCACUCAC
XD-14790	miR-155E	911 Control	ACAAATGGCCACCGTG	AUGGAACAAAUUGGCCACCGUG
			[SEQ ID NO:549]	[SEQ ID NO:1116]
UUCGGGUUGAAAU CUGAAGUGU [SEQ ID NO:108]	miR-155E	911 Control	CTGAGGCTTGTCTTTGGGCTGTAT	CUGAGGCUUGCUUUUGGGCUGUA
			GCTGTTCCGGGTTCTTATCTGAAGTG	UGCUGUUCGGGUUUCUUAUCUGAA
XD-14790	miR-1-1	911 Control	TTTTTTGGCCTCTGACTGAACACTTC	GUGUUUUUUGCCUCUGACUGAAC
			AATAGAACCCGACACAGGACAAG	ACUUCAAUAGAACCCGAAACAGGA
UUCGGGUUGAAAU CUGAAGUGU [SEQ ID NO:108]	miR-1-1	911 Control	CCCTTTATCAGCACTCATATGGA	CAAGGCCUUUAUCAGCACUCAC
			CAAATGGCCACCGTG	AUGGAACAAAUUGGCCACCGUG
XD-14790	miR-155E	911 Control	[SEQ ID NO:550]	[SEQ ID NO:1117]
			CATGCAGACTGCCTGCTTGGGAGA	CAUGCAGACUGCCUGCUUGGGAG
UUCGGGUUGAAAU CUGAAGUGU [SEQ ID NO:108]	miR-1-1	911 Control	CTTCAGATAAGAACCCGAGATATG	ACUUCAGAUAAAGAACCGAGAAUA
			GACCTGCTAAGCTATTGGGTTCTT	UGGACCUUGCUAAGCUAUUCGGGU
XD-14800	miR-155E	911 Control	ATCTGAAGTGTCTCAGGCCGGGAC	UCUUUUCUGAAAGUGUCUAGGCC
			CTCTCTGCCGCACTGAGGGGCAC	GGGACCUUCUCGCCGCAUCUGAG
UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	miR-155E	911 Control	TCCACACCACGGGGGCC	GGGCACUCCACACCGGGGGCC
			[SEQ ID NO:551]	[SEQ ID NO:1118]
XD-14800	miR-155E	911 Control	CTGAGGCTTGTCTTTGGGCTGTAT	CUGAGGCUUGCUUUUGGGCUGUA
			GCTGTTGATTTCCTCGATGTCGCTG	UGCUGUUGAUUUCUUCGAGUCG
XD-14800	miR-155E	911 Control	GTTTGGCCTCTGACTGACCAAGCG	CUGGUUUUUGCCUCUGACUGACC
			ACTCGGGAAATCAACAGGACAAG	AGCGACUCGGGAAAUCAACAGGA

UUAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14800	miR-1-1	911 Control	GCCCTTTATCAGCACTCACATGGA ACAAATGGCCACCGTG [SEQ ID NO:552]	CAAGGCCUUUAUCAGACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1119]
AGAAUUCGUAGAC UGAGGCAGU [SEQ ID NO:14]	XD-14743	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGCGA GCGACATCGAGGAACGCAATATG GACCTGCTAAGCTATTGATTCTC GATGCTGCTGGCTCAGGCCGGGAC CTCTCGCCGCACTGAGGGGCGAC TCCACACCACGGGGGCC [SEQ ID NO:553]	CAUGCAGACUGCCUGCUUGGGCG AGCGACUACGAGGAACGCAUA UGGACCUAGCUAAGCUAUAUU CCUCGAUGUCGUGGCUAGGCC GGGACCUUCUCGCGCCACUGAG GGGACUCCACACCCACGGGGGCC [SEQ ID NO:1120]
AGAAUUCGUAGAC UGAGGCAGU [SEQ ID NO:14]	XD-14743	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGCTGAT GCCTCAGTCTACGATCGTCTTATGG ACCTGCTAAGCTAAGAAATCGTAG ACTGAGGCAGTCTCAGGCCGGGAC CTCTCGCCGCACTGAGGGGCGAC TCCACACCACGGGGGCC [SEQ ID NO:554]	CAUGCAGACUGCCUGCUUGGGGAG UGCCUCAGUCUACGAUCGUCUUA UGGACCUAGCUAAGCUAUAUU GUAGACUGAGGAGUCUCAGGCC GGGACCUUCUCGCGCCACUGAG GGGACUCCACACCCACGGGGGCC [SEQ ID NO:1121]
AGAUACGUCAUUU UCCAAAGCC [SEQ ID NO:60]	XD-14766	miR-1-1	Akx2 targeting	CTGGAGGCTTGTCTTGGGCTGTAT GCTGAGAAATCGTAGACTGAGGCA GTTTTTGGCTCTGACTGAACCTGCC TCGTCACGATTCTCAGGACAAAG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:555]	CUGGAGCUUGCUUUGGGCUGUA UGCUGAGAAAUUGUAGACUGAGG CAGUUUUUGGCUUCUGACUGAAC UGCCUCGUCACGAUUUCUCAGGA CAAGGCCUUUAUCAGACACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1122]
AGAUACGUCAUUU UCCAAAGCC [SEQ ID NO:60]	XD-14766	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGCGCC TTTGGAATAAGCTCCTCTTATG GACCTGCTAAGCTAAGATACGTCA TTTTCCAAAGCCCTCAGGCCGGGA CCCTCTCGCCGCACTGAGGGGCA CTCCACACCACGGGGGCC [SEQ ID NO:556]	CAUGCAGACUGCCUGCUUGGGGC CUUUGGAAAUUGACGUCCUCUUA UGGACCUAGCUAAGCUAUAUU UCAUUUCCAAAGCCUCAGGCC GGGACCUUCUCGCGCCACUGAG GGGACUCCACACCCACGGGGGCC [SEQ ID NO:1123]

AGCGUUAGGGUGC GCAUACUGC [SEQ ID NO:336]	XD-14904	miR-155E	Akx2 targeting	CCCTTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:557]	CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1124]
AGCGUUAGGGUGC GCAUACUGC [SEQ ID NO:336]	XD-14904	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGAGCGTTAGGTGCGCATACT GCTTTTGGCTCTGACTGAGCAGT ATGGCACCTAACGCTCAGGACAAG GCCCTTATCAGCACTCACATGGA ACAAATGGCCACCGTG [SEQ ID NO:558]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGAGCGUUAAGGUGCGCAUA UGCUUUUGGCCUCUCAGUCAGC AGUAUGGCACCUAACGUCAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1125]
AGCGUUAGGGUGC GCAUACUGC [SEQ ID NO:336]	XD-14904	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTTGGGGGA GTATGCGCACCCCTAAGAGCTTATG GACCTGCTAAGCTAAGCGTTAGGG TGCGCATACTGCTCAGGCCGGGA CCTCTCGCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:559]	CAUGCAGACUGCCUUGGGGGG AGUAUGCGACCCUAAAGGCUUA UGGACCUUGCUAAGCUAAGCGUUA GGUGCGCAUACUGCCUAGGCC GGGACCUUCUCGCCGACUCAG GGGACUCCACACACCGGGGGC [SEQ ID NO:1126]
AGGAACGUGGGUU GAACUCCUU [SEQ ID NO:242]	XD-14857	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGAGAACGTTGGTTGAACCTCC TTTTTTGGCTCTGACTGAAAGGA GTTAACCAACGTTCTCAGGACAAG GCCCTTATCAGCACTCACATGGA ACAAATGGCCACCGTG [SEQ ID NO:560]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGAGGAACGUGGUUGAACU CCUUUUUGGCCUCUCAGUGAAA GGAGUUAAACACGUCUCCAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1127]
AGGAACGUGGGUU GAACUCCUU [SEQ ID NO:242]	XD-14857	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTTGGGATG GAGTTCAACCCACGTCGCCCTATG GACCTGCTAAGCTAAGGAACGTGG GTTGAACCTCTCTCAGGCCGGGA CCTCTCGCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:561]	CAUGCAGACUGCCUUGGGGAU GGAGUUAACCCACGUCGCCUUA UGGACCUUGCUAAGCUAAGGAACG UGGUUGAACUCCUUCAGGCC GGGACCUUCUCGCCGACUCAG GGGACUCCACACACCGGGGGC [SEQ ID NO:1128]
AUAAUAAUCCGUC AGUUAGACG [SEQ ID NO:426]	XD-14949	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGATAATAATCCGTCAGTTTGA CGTTTTGGCTCTGACTGACGTCAA ACGACGATTATTATCAGGACAAGG	CUGGAGGCUUGCUUUGGGCUGUA UGCUGAUAAUAAUCCGUCAGUUU GACGUUUUGGCCUCUGACGACG UCAACGACGAUUAUUAUCAGGA

AUAAUAAUCCGUC AGUUGACG [SEQ ID NO:426]	XD-14949	miR-1-1	Akn2 targeting	CCCTTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:562]	CAAGGCCUUUAUCAGACACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1129]
AUACGCGGUGAAU UCUGUCUCC [SEQ ID NO:102]	XD-14787	miR-155E	Akn2 targeting	CATGCAGACTGCCTGCTTGGGCTTAT GCTGATACGCGGTGAATTCTGTCT CCTTTGGCTCTGACTGAGGAGA CAGATTACCGCTATCAGGACAAG GCCCTTATCAGCACTCACATGGA ACAAATGGCCACCGTG [SEQ ID NO:563]	CAUGCAGACUGCCUUGGUGGCC UCAACUCAGCGGAUACGUAUUA UGGACCUUUAAGCUAAUAAUAA UCCGUCAGUUUAGCCUCAGGCC GGGACCUUCUCGCGCCACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1130]
AUACGCGGUGAAU UCUGUCUCC [SEQ ID NO:102]	XD-14787	miR-1-1	Akn2 targeting	CATGCAGACTGCCTGCTTGGGGA GACAGAAATTCACCGCTTATTATG GACCTGCTAAGCTAATACGCGGTG AATTCTGCTCCCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGA CTCCACACACCGGGGCC [SEQ ID NO:564]	CUGGAGGUUGCUUUGGGCUGUA UGCUGAUACGCGGUAUUCUGU CUCCUUUGGCCUCUGACUGAGG AGACAGAUUACCGCUAUCAGGA CAAGGCCUUUAUCAGACACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1131]
AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]	XD-14792	miR-1-1	Akn2 targeting	CATGCAGACTGCCTGCTTGGGTAC AGACCAAGAGTAGTCGAATTATG GACCTGCTAAGCTAATTAATACT CTTTGGTCTGAATCAGGCCGGGA CCTCTCGCCGCACTGAGGGGA CTCCACACACCGGGGCC [SEQ ID NO:565]	CAUGCAGACUGCCUUGGUGUA CAGACCAAGAGUAAGUCGAAUUA UGGACCUUUAAGCUAAUAAUUA ACUCUUUGGUCUGAACUCAGGCC GGGACCUUCUCGCGCCACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1132]
AUUAACUACUCUU UGGUCUGAA [SEQ ID NO:112]	XD-14792	miR-155E	Akn2 targeting	CTGGAGGCTTGTCTTGGGCTGTAT GCTGATTAACACTCTTGGTCTGA ATTTGGCTCTGACTGATTCAGAC CAAGGTAGTTAATCAGGACAAGGC	CUGGAGGUUGCUUUGGGCUGUA UGCUGAUUAAUACUCUUUGGUC UGAAUUUGGCCUCUGACUGAUU CAGACCAAGGUAUUAUUCAGGA [SEQ ID NO:1133]

AUUGCUGGAGUA AGCUGGUGG [SEQ ID NO:306]	XD-14889	miR-155E	Akx2 targeting	CCTTTATCAGCACTCACATGGAAC AAATGGCCACCGTG [SEQ ID NO:566]	CAAGGCCUUUAUCAGCACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1134]
AUUGCUGGAGUA AGCUGGUGG [SEQ ID NO:306]	XD-14889	miR-1-1	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGATTGCTGGAGTAAGCTGGT GGTTTGGCTCTGACTGACCACC AGTACCCACGCAATCAGGACAAG GCCCTTATCAGCACTCACATGGA ACAAATGGCCACCGTG [SEQ ID NO:567]	CUGGAGCUUUGCUUUGGGCUGUA UGCUGAUUGGUGGAGUAAGCUG GUGUUUUGGCUUCUGACUGACC ACCAGUACCCACGCAAUACAGGAC AAGGCCUUUAUCAGCACUCACA UGGAACAAAUUGGCCACCGUG [SEQ ID NO:1135]
AUUGCUGGAGUA AGCUGGUGG [SEQ ID NO:306]	XD-14889	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGCGA CCAGCTTACTCCACGGAAATTATG GACCTGCTAAGCTAATTGCCGTGGA GTAAGCTGGTGGCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:568]	CAUGCAGACUGCCUUGGCGCG ACCAGCUUACUCCACGGAUAUA UGGACCUAGUAAGCUAAUUGCGU GGAGUAGCUGUGGUCAGGCC GGACCUUCUCGCGCACUCAG GGCACUCCACACACCGGGGCC [SEQ ID NO:1136]
AUUUCGAGGAGUA CGCUGGGCC [SEQ ID NO:124]	XD-14798	miR-155E	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGATTGAGGATGCTGCTGGG CCTTTGGCTCTGACTGAGGCCCA GCACACCTCGAAATCAGGACAAGG CCCTTATCAGCACTCACATGGA CAAATGGCCACCGTG [SEQ ID NO:569]	CUGGAGCUUUGCUUUGGGCUGUA UGCUGAUUUGGAGUAUGUCGUG GGCUUUUGGCUUCUGACUGAGG CCCAGCACACCUCCGAAUACAGGAC AAGGCCUUUAUCAGCACUCACA UGGAACAAAUUGGCCACCGUG [SEQ ID NO:1137]
AUUUCGAGGAGUA CGCUGGGCC [SEQ ID NO:124]	XD-14798	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGCC CCAGCAGATCCTCGCCAAATTATG GACCTGCTAAGCTAATTTCGAGGA TGTCGCTGGGCCCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:570]	CAUGCAGACUGCCUUGGCGCG CCCAGCAGAUCCUCCGCAUUAU GGACCUAGUAAGCUAAUUCGAG GAUUGCUGGGCCUUCAGGCCG GGACCUUCUCGCGCACUCAGG GGCACUCCACACCGGGGCC [SEQ ID NO:1138]
UAAAUUCGAGACU GAGGCAGUC [SEQ ID NO:12]	XD-14742	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGTC TGCTCAGTCTAGCAGCTTATATG GACCTGCTAAGCTATAAATCGTAG ACTGAGGCAGTCTCAGGCCGGGA	CAUGCAGACUGCCUUGGCGGU CUGCCUCAGUCUACGACGUAUA UGGACCUAGUAAGCUAAUUCG UAGACUGAGGACGUCUCCAGGCC

UAAUUCGUAGACU GAGGCAGUC [SEQ ID NO:12]	XD-14742	miR-155E	Akx2 targeting	CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:571]	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGTAATCGTAGACTGAGGCAG TCTTTTGGCTCTGACTGAGACTGC CTAGTTACGATTACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:572]	GGGACCUUCUCGCCGACACUGAG GGGCACUCCACACACCGGGGCC [SEQ ID NO:1139]
UACGCGGUGAAUU CUGUCUCCC [SEQ ID NO:100]	XD-14786	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTTGGGGCG AGACAGAATTCACCGGAGTATATG GACCTGCTAAGCTATACGCGGTGA ATTCTGCTCCCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:573]	CATGCAGACTGCCTGCTTTGGGGCG AGACAGAATTCACCGGAGTATATG UGGACCUUCUAAAGCUAUACGCGG UGAAUUCUCUCCCCCAGGCC GGGACCUUCUCGCCGACACUGAG GGGCACUCCACACACCGGGGCC [SEQ ID NO:1141]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUAAAUCGAGACUGAGGC AGUCUUUUGGCUUCUGACUGAGA CUGCCUAGUUAAGAUUACAGGA CAAGGCCUUUAUCAGACACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1140]
UACGCGGUGAAUU CUGUCUCCC [SEQ ID NO:100]	XD-14786	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGTACGCGGTGAATTCTGTCTCC CTTTTGGCTCTGACTGAGGGAGA CAAATCACCGCGTACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:574]	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGTACGCGGTGAATTCTGTCTCC UCCUUUUGGCUUCUGACUGAGG GAGACAAUACACCGCGUACAGGA CAAGGCCUUUAUCAGACACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1142]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUACGCGGUGAAUUCUGUC UCCUUUUGGCUUCUGACUGAGG GAGACAAUACACCGCGUACAGGA CAAGGCCUUUAUCAGACACAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1141]
UAUACGCGGUGAA UUCUGUCUC [SEQ ID NO:104]	XD-14788	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTTGGGGTG ACAGAAATTCACCGCGCATATATG GACCTGCTAAGCTATATACGCGGT GAATTCTGTCTCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:575]	CATGCAGACTGCCTGCTTTGGGGTG ACAGAAATTCACCGCGCATATATG UGGACCUUCUAAAGCUAUACGCG GGUGAAUUCUGUCUCCUACAGGCC GGGACCUUCUCGCCGACACUGAG GGGCACUCCACACACCGGGGCC [SEQ ID NO:1143]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUAAUACGCGGUGAAUUCUG UCCUUUUGGCUUCUGACUGAGA GACAGAUUCCCGCGUAUACAGGA
UAUACGCGGUGAA UUCUGUCUC [SEQ ID NO:104]	XD-14788	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGTATACGCGGTGAATTCTGTCT CTTTTGGCTCTGACTGAGAGACA GATTCGCGGTATACAGGACAAGG	CTGGAGGCTTGTCTTTGGGCTGTAT GCTGTATACGCGGTGAATTCTGTCT CTTTTGGCTCTGACTGAGAGACA GATTCGCGGTATACAGGACAAGG	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUAAUACGCGGUGAAUUCUG UCCUUUUGGCUUCUGACUGAGA GACAGAUUCCCGCGUAUACAGGA

UAUUGCGUGGAGU AAGCUGGUG [SEQ ID NO:308]	XD-14890	miR-1-1	Akx2 targeting	CCCTTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:576]	CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1144]
UAUUGCGUGGAGU AAGCUGGUG [SEQ ID NO:308]	XD-14890	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGCTC CAGCTTACTCCAGCCCATATATG GACCTGCTAAGCTATATTGGGTGG AGTAAGCTGGTCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:577]	CAUGCAGACUGCCUUGGCGCU CCAGCUUACUCCAGCCCAUUAU GGACUGCUAAGCUAUUUGCGU GGAGUAGCUGGUGUCAGGCCG GGACCUUCUGCCGACACUGAGG GGCACUCCACACCGGGGGCC [SEQ ID NO:1145]
UAUUGCGUGGAGU AAGCUGGUG [SEQ ID NO:308]	XD-14799	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTGGGCTGTAT GCTGTATTGCTGGAGTAAGCTGG TGTTTGGCTCTGACTGACACCCAG CTACTCAGCAATACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:578]	CUGGAGCUUUGCUUUGGCGUGA UGCUGUAUUGCGUGGAGUAAGCU GGUUUUUGGCUUGACUGACA CCAGCUACUCACGCAUACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1146]
UAUUGCGAGGAGU UCGCGGGC [SEQ ID NO:126]	XD-14799	miR-155E	Akx2 targeting	CTGGAGGCTTGTCTTGGGCTGTAT GCTGTATTGCTGGAGTGTGCTGG GCTTTGGCTCTGACTGAGCCCA GCGCATCTCGAAATACAGGACAAG GCCCTTATCAGCACTCACATGGAA ACAAATGGCCACCGTG [SEQ ID NO:579]	CUGGAGCUUUGCUUUGGCGUGA UGCUGUAUUGCGAGGAGUCGCU GGCUUUUUGGCUUGACUGAGC CCAGCGCAUCUCGAAUACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1147]
UAUUGCGAGGAGU UCGCGGGC [SEQ ID NO:126]	XD-14799	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGGC CAGCGACATCTCGACCATATATG GACCTGCTAAGCTATATTTCGAGG ATGCTGCTGGGCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:580]	CAUGCAGACUGCCUUGGCGGG CCAGCGCAUCUCCGACCAUUA UGGACCUUUAAGCUAUUUCG AGGAUGUCUGGGCCUCAGGCC GGGACCUUCUGCCGACACUGAG GGGACUCCACACCGGGGGCC [SEQ ID NO:1148]
UCGCGUUGGGGC AUUUUGGU [SEQ ID NO:302]	XD-14887	miR-1-1	Akx2 targeting	CATGCAGACTGCCTGCTTGGGAGC AAATATGCCCCAACCTCGATATG GACCTGCTAAGCTATCGCTGTGG GGCATAATTGGTCTCAGGCCGGGA	CAUGCAGACUGCCUUGGCGAG CAAAUUGCCCCAACACUCGUA UGGACCUUUAAGCUAUUUGCGU UGGGGCAUUAUUGGUCUCAGGCC

UGCGUUAUUGGGG AUAUUUGGU [SEQ ID NO:302]	XD-14887	miR-155E	Akx2 targeting	CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:381]	GGGACCUUCUCGCCGACACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1149]
UGCGCAUACUGCUG AGCAAGGG [SEQ ID NO:330]	XD-14901	miR-1-1	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGCTGGCTGTGGGGCATATTG GTTTGTGGCTCTGACTGAACCAA ATAGCCCAACAGCGACAGGACAA GGCCCTTTATCAGCACTCAGCATGG AACAAATGGCCACCGTG [SEQ ID NO:382]	CUGGAGGUUGCUUUGGGCUGUA UGCUGUCGCUUUGGGCAUAUU UGUUUUUUGGCUUCUGACUGAAC CAAAUAGCCCAACAGCGACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1150]
UGCGCAUACUGCUG AGCAAGGG [SEQ ID NO:330]	XD-14901	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGCG TTGCTCAGCAGTATGGAGCATATG GACCTGCTAAGCTATGCCGCTACT GCTGAGCAAGGCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:383]	CAUGCAGACUGCCUUGGGCGG CUUGCUCAGCAGUAUUGGAGCAUA UGGACCUUGCUAAGCUAUGCGCAU ACUGCUGAGCAAGGCUACAGGCC GGGACCUUCUCGCCGACACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1151]
UGUACCACAAACAAA GUCUGAAC [SEQ ID NO:40]	XD-14756	miR-1-1	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTGCGCATACTGCTGAGCAAG GGTTTGGGCTCTGACTGACCCCTTG CTAGCGTATGCGCACAGGACAAAG CCCTTATCAGCACTCAGCATGGAA CAAATGGCCACCGTG [SEQ ID NO:384]	CUGGAGGUUGCUUUGGGCUGUA UGCUGUCGCAUACUGCUGAGCA AGGUUUUUGGCUUCUGACUGACC CUUGCUGCUAUGGCGACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1152]
UGUACCACAAACAAA GUCUGAAC [SEQ ID NO:40]	XD-14756	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGAT CAGACTTTGTTGTGGGACATATG GACCTGCTAAGCTATGTACCAAA CAAAGTCTGAACCTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:385]	CAUGCAGACUGCCUUGGGGGA UCAGACUUUUGUUGGGGACAUUA UGGACCUUGCUAAGCUAUGUACCA CAACAAAGUCUCGAAACCUACGGCC GGGACCUUCUCGCCGACACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1153]

					CCCTTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:586]	CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1154]
UGUAUACGCCGGCU [SEQ ID NO:362]	XD-14917	miR-1-1	Atxn2 targeting		CATGCAGACTGCCTGCTTGGGTGA CGTTACGCCGGCTAGCACATATG GACCTGCTAAGCTATGTATACGCC GGCTGAACGTGACTCAGGCCGGGA CCTCTCGCCGCACTGAGGGGCA CTCCACACCAGGGGGCC [SEQ ID NO:587]	CAUGCAGACUGCCUGUUGGGUG ACGUUCAGCCGGCGUACGACAU UGGACCUUGAAGCUAUUAUAC GCCGGCUAAGCUAGUCAGGCC GGGACCUUCUCGCCGACACUGAG GGGCAUCCACACCAACCGGGGCC [SEQ ID NO:1155]
UGUAUACGCCGGCU [SEQ ID NO:362]	XD-14917	miR-155E	Atxn2 targeting		CTGGAGGCTTGCTTTGGGCTGTAT GCTGTGTATACGCCGGCTGAACGT GATTTGGCTCTGACTGATCACGT TCGCCGCTATACACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:588]	CUGGAGGCUUGCUUUGGGCUGA UGCUGUUAUACGCCGGCUGAAC GUGAUUUUGCCUCUGACUGAUC ACGUUCGCCGCUUACACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1156]
UUUACUAAAGUAUUG [SEQ ID NO:220]	XD-14846	miR-155E	Atxn2 targeting		CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTACTAAGTATTGAAGGGGA AATTTGGCTCTGACTGATTTCCC CTCAAACCTTAGTAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:589]	CUGGAGGCUUGCUUUGGGCUGA UGCUGUUAUAAAGUAUUGAAGG GAAUUUUGCCUCUGACUGAUC UCCCCUAAACUUAAGUAACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAUUGGCCACCGUG [SEQ ID NO:1157]
UUUACUAAAGUAUUG [SEQ ID NO:220]	XD-14846	miR-1-1	Atxn2 targeting		CATGCAGACTGCCTGCTTGGGTAT CCCCTTCAATACTTACTTAATATGG ACCTGCTAAGCTATTACTAAGTATT GAAGGGAAACTCAGGCCGGGAC CTCTCTGCCGCACTGAGGGGCAC TCCACACCAGGGGGCC [SEQ ID NO:590]	CAUGCAGACUGCCUGUUGGGUA UCCCCUCAAUACGUACUUAUA UGGACCUUGAAGCUAUUAUAC GUUUGAAGGGGAAACUCAGGCC GGGACCUUCUCGCCGACACUGAG GGGCAUCCACACCAACCGGGGCC [SEQ ID NO:1158]
UUUAGUUGAUCCAU [SEQ ID NO:198]	XD-14835	miR-1-1	Atxn2 targeting		CATGCAGACTGCCTGCTTGGGTGT GAATCTATGGATCAAGATAATATG GACCTGCTAAGCTATTAGTTGATC CATAGATTACAGCTCAGGCCGGGA	CAUGCAGACUGCCUGUUGGGUG UGAAUCUUAUGGAUCAAAGUA UGGACCUUGAAGCUAUUAUUG AUCCAUAGAUUICAGATCAGGCC

UUAGUUGAUCCAU AGAUUCAGA [SEQ ID NO:198]	XD-14835	miR-155E	Akx2 targeting	CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:591]	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTAGTTGATCCATAGATTCA GATTTTGGCCTCTGACTGATCTGAA TCATGATCAACTAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:592]	GGGACCUUCUCGCCGACUCGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1159]
UUCGAUGCAGGAC UAGCAGGCG [SEQ ID NO:166]	XD-14819	miR-155E	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTGATGCAAGGACTAGCAGG CGTTTGGCCTCTGACTGACGCTG CTGTCTGCATCGAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:593]	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTGATGCAAGGACTAGCAGG CGTTTGGCCTCTGACTGACGCTG CTGTCTGCATCGAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:593]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUUCGAUGCAGGACUAGCA GGCUGUUGGCUUCUGACUGACG CCUGCUGUCGACUACGAAACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1160]
UUCGAUGCAGGAC UAGCAGGCG [SEQ ID NO:166]	XD-14819	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGGCC CTGCTAGTCTGCTGATGAAATATG GACCTGCTAAGCTATTGATGACAG GACTAGCAGGCGCTCAGGCCGGGA CCTCTCTGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:594]	CATGCAGACTGCCTGCTTGGGGCC CTGCTAGTCTGCTGATGAAATATG GACCTGCTAAGCTATTGATGACAG GACTAGCAGGCGCTCAGGCCGGGA CCTCTCTGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:594]	CAUGCAGACUGCCUUGGGGCC CCUGCUAGUCCUGCAUGAGAAUA UGGACCUUGCUAAGCUAUUCGAUG CAGGACUAGCAGGCGCUCAGGCC GGGACCUUCUCGCCGACUCGAG GGGACUCCACACCGGGGCC [SEQ ID NO:1161]
UUCGGGUUGAAAAU CUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-155E	Akx2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTGCGGTTGAAATCTGAAGT GTTTGGCCTCTGACTGAACACTT CAATTCAACCCGAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:595]	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTTGCGGTTGAAATCTGAAGT GTTTGGCCTCTGACTGAACACTT CAATTCAACCCGAACAGGACAAGG CCCTTATCAGCACTCACATGGAA CAAATGGCCACCGTG [SEQ ID NO:595]	CUGGAGGCUUGCUUUGGGCUGUA UGCUGUUCGGGUUGAAUUCUGAA GUGUUUUUGGCUUCUGACUGAAC ACUUCAAUUAUACCCGAAACAGGA CAAGGCCUUUAUCAGCACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1162]
UUCGGGUUGAAAAU CUGAAGUGU [SEQ ID NO:108]	XD-14790	miR-155E	Akx2 targeting	CATGCAGACTGCCTGCTTGGGAGA CTTCAGATTTCACCGAATATG GACCTGCTAAGCTATTGCGGTTGA AATCTGAAGTGTCTCAGGCCGGGA	CATGCAGACTGCCTGCTTGGGAGA CTTCAGATTTCACCGAATATG GACCTGCTAAGCTATTGCGGTTGA AATCTGAAGTGTCTCAGGCCGGGA	CAUGCAGACUGCCUUGGGGAG ACUUCAGAUUUCAAACCGAGAAUA UGGACCUUGCUAAGCUAUUCGGGU UGAAAUUCUGAAUGUCUCAGGCC [SEQ ID NO:1163]

UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14800	miR-1-1	Akn2 targeting	CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:596]	GGGACCUUCUCGCCGACACUGAG GGGACUCCACACACCGGGGCC [SEQ ID NO:1164]
UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14800	miR-155E	Akn2 targeting	CATGCAGACTGCCTGCTTGGGGCA GCGACATCTCGAAACGCAATATG GACCTGCTAAGCTATTGATTCGA GGATGTCGCTGGCTCAGGCCGGGA CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:597]	CAUGCAGACUGCCUUCUUGGGCG AGCGACUCCUCGAAACGCAUA UGGACCUAGCUAAGCUAUUGAUUU CGAGGAUGUCUGGUCAGGCC GGGACCUUCUCGCCGACACUGAG GGGACUCCACACCGGGGCC [SEQ ID NO:1165]
UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14781	miR-155E	Akn2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTGATTTCGAGGATGTCGCT GGTTTGGGCTCTGACTGACCAAG GACTCCCGAAATCAACAGGACAAG GCCCTTATCAGCACTCATGGA ACAAATGGCCACCGTG [SEQ ID NO:598]	CUGGAGGUUGCUUUGGGCUGUA UGCUGUUGAUUUCGAGGAUGUCG CUGGUUUUGGCUUGACUGACC AGCGACUCCCGAAAUCAACAGGA CAAGGCCUUUAUCAGACACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1166]
UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14781	miR-155E	Akn2 targeting	CTGGAGGCTTGCTTTGGGCTGTAT GCTGTGATTTCGAGGACTTGACTC AATTTGGGCTCTGACTGATTGAGT CAGTGCCAGTACAACAGGACAAGG CCCTTATCAGCACTCATGGA CAAATGGCCACCGTG [SEQ ID NO:599]	CUGGAGGUUGCUUUGGGCUGUA UGCUGUUGAUUUGGCGACUUGAC UCAUUUUUGGCUUGACUGAUUU GAGUCAGUGCCAGUACAACAGGA CAAGGCCUUUAUCAGACACUCAC AUGGAACAAAUUGGCCACCGUG [SEQ ID NO:1167]
UUGAUUUCGAGGA UGUCGUGG [SEQ ID NO:128]	XD-14781	miR-1-1	Akn2 targeting	CATGCAGACTGCCTGCTTGGGTAG AGTCAAGTGCCAGTCCCAATATG GACCTGCTAAGCTATTGACTGGG CACTTGACTCAACTCAGGCCGGGA CCTCTCTCGCCGCACTGAGGGGCA CTCCACACACGGGGGCC [SEQ ID NO:600]	CAUGCAGACUGCCUUCUUGGGUA GAGUCAAGUGGCCAGUCCCAUA UGGACCUAGCUAAGCUAUUGUACU GGGACUUGACUACAACAGGCC GGGACCUUCUCGCCGACACUGAG GGGACUCCACACCGGGGCC [SEQ ID NO:1168]

ATXN2-targeting miRNA guide sequences having at least 25% *ATXN2* immunofluorescence signal knockdown are shown in **Table 12** (both RNA and DNA versions). *ATXN2*-targeting miRNA guide sequences having at least 50% *ATXN2* immunofluorescence signal knockdown are shown in **Table 13** (both RNA and DNA versions).

Table 12: miRNA guide sequences with at least 25% knockdown of *ATXN2*

siRNA duplex ID	Guide (antisense sequence) (RNA)	Guide (antisense sequence) (DNA)
XD-14742	UAAAUCGUAGACUGAGGCA GUC [SEQ ID NO:12]	TAAATCGTAGACTGAGGCA GTC [SEQ ID NO:601]
XD-14743	AGAAAUCGUAGACUGAGGC AGU [SEQ ID NO:14]	AGAAATCGTAGACTGAGGC AGT [SEQ ID NO:602]
XD-14756	UGUACCACAACAAAGUCUG AAC [SEQ ID NO:40]	TGTACCACAACAAAGTCTGA AC [SEQ ID NO:603]
XD-14766	AGAUACGUCAUUUCCAAAG GCC [SEQ ID NO:60]	AGATACGTCATTTCCAAAG CC [SEQ ID NO:604]
XD-14786	UACGCGGUGAAUUCUGUCU CCC [SEQ ID NO:100]	TACGCGGTGAATTCTGTCTC CC [SEQ ID NO:605]
XD-14788	UAUACGCGGUGAAUUCUGU CUC [SEQ ID NO:104]	TATACGCGGTGAATTCTGTCTC TC [SEQ ID NO:606]
XD-14790	UUCGGGUUGAAAUCUGAAG UGU [SEQ ID NO:108]	TTCGGGTTGAAATCTGAAGT GT [SEQ ID NO:607]
XD-14792	AUUAACUACUCUUUGGUCU GAA [SEQ ID NO:112]	ATTAAC TACTCTTTGGTCTG AA [SEQ ID NO:608]
XD-14798	AUUUCGAGGAUGUCGCGG GCC [SEQ ID NO:124]	ATTTTCGAGGATGTCGCTGGG CC [SEQ ID NO:609]
XD-14799	UAUUUCGAGGAUGUCGCGG GGC [SEQ ID NO:126]	TATTTTCGAGGATGTCGCTGG GC [SEQ ID NO:610]
XD-14800	UGAUUUUCGAGGAUGUCGC UGG [SEQ ID NO:128]	TTGATTTTCGAGGATGTCGCT GG [SEQ ID NO:611]
XD-14819	UUCGAUGCAGGACUAGCAG GCG [SEQ ID NO:166]	TTCGATGCAGGACTAGCAGG CG [SEQ ID NO:612]

XD-14835	UUAGUUGAUCCAUAGAUUC AGA [SEQ ID NO:198]	TTAGTTGATCCATAGATTCA GA [SEQ ID NO:613]
XD-14846	UUACUAAGUAUUGAAGGGG AAA [SEQ ID NO:220]	TTACTAAGTATTGAAGGGGA AA [SEQ ID NO:614]
XD-14857	AGGAACGUGGGUUGAACUC CUU [SEQ ID NO:242]	AGGAACGTGGGTTGAACTCC TT [SEQ ID NO:615]
XD-14887	UCGCUGUUGGGCAUAAUU GGU [SEQ ID NO:302]	TCGCTGTTGGGGCATATTTG GT [SEQ ID NO:616]
XD-14889	AUUGCGUGGAGUAAGCUGG UGG [SEQ ID NO:306]	ATTGCGTGGAGTAAGCTGGT GG [SEQ ID NO:617]
XD-14890	UAUUGCGUGGAGUAAGCUG GUG [SEQ ID NO:308]	TATTGCGTGGAGTAAGCTGG TG [SEQ ID NO:618]
XD-14901	UGCGCAUACUGCUGAGCAA GGG [SEQ ID NO:330]	TGCGCATACTGCTGAGCAAG GG [SEQ ID NO:619]
XD-14904	AGCGUUAGGGUGCGCAUAC UGC [SEQ ID NO:336]	AGCGTTAGGGTGCGCATACT GC [SEQ ID NO:620]
XD-14917	UGUAUACGCCGGCUGAACG UGA [SEQ ID NO:362]	TGTATACGCCGGCTGAACGT GA [SEQ ID NO:621]

Table 13: miRNA sequences with at least 50% knockdown of ATXN2

siRNA duplex ID	Guide (antisense sequence) (RNA)	Guide (antisense sequence) (DNA)
XD-14743	AGAAAUCGUAGACUGAGGC AGU [SEQ ID NO:14]	AGAAATCGTAGACTGAGGC AGT [SEQ ID NO:602]
XD-14756	UGUACCACAACAAAGUCUG AAC [SEQ ID NO:40]	TGTACCACAACAAAGTCTGA AC [SEQ ID NO:603]
XD-14786	UACGCGGUGAAUUCUGUCU CCC [SEQ ID NO:100]	TACGCGGTGAATTCTGTCTC CC [SEQ ID NO:605]
XD-14790	UUCGGGUUGAAAUCUGAAG UGU [SEQ ID NO:108]	TTCGGGTTGAAATCTGAAGT GT [SEQ ID NO:607]
XD-14792	AUUAACUACUCUUUGGUCU GAA [SEQ ID NO:112]	ATTAATACTCTTTGGTCTG AA [SEQ ID NO:608]
XD-14800	UUGAUUUCGAGGAUGUCGC UGG [SEQ ID NO:128]	TTGATTTCGAGGATGTCGCT GG [SEQ ID NO:611]

XD-14819	UUCGAUGCAGGACUAGCAG GCG [SEQ ID NO:166]	TTCGATGCAGGACTAGCAGG CG [SEQ ID NO:612]
XD-14835	UUAGUUGAUCCAUAAGAUUC AGA [SEQ ID NO:198]	TTAGTTGATCCATAGATTCA GA [SEQ ID NO:613]
XD-14857	AGGAACGUGGGUUGAACUC CUU [SEQ ID NO:242]	AGGAACGTGGGTGAACTCC TT [SEQ ID NO:615]
XD-14890	UAUUGCGUGGAGUAAGCUG GUG [SEQ ID NO:308]	TATTGCGTGGAGTAAGCTGG TG [SEQ ID NO:618]
XD-14904	AGCGUUAGGGUGCGCAUAC UGC [SEQ ID NO:336]	AGCGTTAGGGTGCGCATACT GC [SEQ ID NO:620]
XD-14917	UGUAUACGCCGGCUGAACG UGA [SEQ ID NO:362]	TGTATACGCCGGCTGAACGT GA [SEQ ID NO:621]

Embedding of miRNAs in AAV plasmids

miRNA sequences such as the above are envisioned to have a therapeutic benefit for patients with neurodegenerative disease when expressed from an AAV genome. Therefore, miRNA sequences were inserted into AAV *cis*-plasmids, flanked by AAV2 inverted terminal repeats (ITRs). miRNAs were inserted in an intron, then followed by an exon expressing green fluorescent protein (GFP). After a stop codon, a SV40 poly adenylation sequence was inserted to ensure robust polyadenylation. The miRNA-encoding transcript was inserted downstream of either a CAG or human Synapsin promoter, as Polymerase-II promoters. The sequence was also inserted into a vector downstream of an H1 promoter, with a CBh promoter controlling the expression of GFP downstream of the H1 miRNA insert. Sequences scAAV.Syn.miR1-1.XD14792.GFP.SV40 (SEQ ID NO:622), scAAV.Syn.miR1-1.XD-14887.GFP.SV40 (SEQ ID NO:623), ssAAV.CAG.miR1-1.XD-14792.GFP.SV40pA (SEQ ID NO:624), ssAAV.CAG.miR-1-1.XD-14887.GFP.SV40pA (SEQ ID NO:625) show representative *cis*-plasmids with miRNA XD-14792 or XD-147887 inserted. For clinical constructs, GFP sequence are replaced by inert sequence, derived from portions of the genome expected to have no effect if expressed. For Synapsin or H1-promoter containing vectors, the insert was flanked by one full-length ITR and one ITR with a truncated terminal resolution site.

AAV plasmids were generated by conventional large-scale DNA preparation and the integrity of ITRs verified by digestion with the restriction endonuclease SmaI, with the expected banding pattern observed. Plasmids were used to package genomes containing the miRNAs into AAV9-capsid encapsidated viruses (Vector Biolabs). AAVs were titrated by qPCR with primers against GFP to calculate genome counts per mL.

AAV Tail Vein Injection

Guide sequences of XD-14792 (SEQ ID NO:112) and XD-14887 (SEQ ID NO:302) are complementary to the mouse *ATXN2* transcript, with one base pair mismatch at base 22 of XD-14792. Wu et al. (PLoS One (2011) 6:e28580) and Ohnishi et al. (Biochem Biophys Res Commun (2005) 329:516-21) suggest that these 3' mismatches do not impair knockdown.

In order to assess the ability of these viruses to knockdown *ATXN2* *in vivo*, concentrated AAV was diluted to a concentration of 3×10^{11} genome counts per 200 microliters in 0.001% Pluronic F-68 (Gibco # 24040-032) in PBS (VWR #K812-500ML). 2 month old C57Bl/6 male mice were each injected intravenously (IV) into the tail vein with 200 microliters of virus (3×10^{11} GC total injected for viruses with CAG promoters; 2×10^{11} GC injected for viruses with Synapsin promoters). Fifteen days after injection, mouse tissue was processed for analysis. Following carbon dioxide-induced euthanasia and transcardial perfusion with PBS, tissues were immediately snap-frozen in liquid nitrogen. Samples were subsequently stored at -80°C .

Western Analysis of *ATXN2* Levels:

Protein extraction was performed by cutting approximately 50 mg of right medial liver tissue samples on dry ice, placing each into 500 microliters RIPA buffer (TEKNOVA #50-843-016) supplemented with protease and phosphatase inhibitor tablet (Pierce #A32959), Halt protease inhibitor cocktail (Thermo #1861279) and PMSF (Cell Signaling Technology #8553S). Tissues were disrupted in a Precellys Evolution Homogenizer (tubes = 0.5 mL CK14, protocol = 3x45s 5000rpm, 15s break, 0°C). Samples were incubated on ice for 30min, centrifuged for 15min at $17,000 \times g$ at

4°C, and supernatant was transferred to a fresh tube and stored at -80°C. Protein lysates were quantitated (Pierce, 23225), resulting in approximately 8µg/µl protein per sample.

The NuPage system (Thermo) was used for gel electrophoresis. 20 µg of each sample was loaded onto 4-12% Bis-Tris protein gels (Thermo, NP0321BOX) and run at constant 200V for 1hr. Revert 700 (Licor, 926-11010) was used to assay for protein loading. Proteins were transferred onto PVDF membrane (EMD Millipore, IPFL00005) overnight at 4°C using constant 30V and 90mA. Membranes were blocked for 1hr at RT (Rockland, MB-070). Primary antibody incubation was performed overnight rocking at 4°C, including anti-Atn2 (1:1000, BD, 611378), anti-GFP (1:2000, CST, 2956) and beta-actin (1:2000, CST, 4970). Washing was performed 4x 5min with TBS + 0.1% tween-20, and secondary antibodies were incubated for 1hr rocking at RT (1:15,000 each of 800CW goat anti-mouse and 680RD donkey anti-rabbit, Licor). Membranes were washed again and imaging was performed on an Odyssey Fc Imaging system (Licor). Signal quantitation was by Licor image-studio lite.

FIG. 22 (left panel) shows Western analysis of tissues from animals dosed with CAG-promoter containing viruses. Liver tissue from animals dosed with viruses expressing miRNA XD-14792 miR1-1 (SEQ ID NO:1133) or XD-14887 miR1-1 (SEQ ID NO:1149) showed a substantial reduction in ATXN2 signal, as quantified by the ratio of ATXN2 immunoblot signal to Beta-actin signal, relative to a control virus lacking a miRNA (**FIG. 22 (right panel)**). As expected, since expression from the synapsin promoter is CNS-enriched, AAV with a synapsin promoter expressing the same miRNAs showed much less GFP expression, and did not reduce ATXN2 protein levels (data not shown). Therefore, AAV-mediated delivery of *ATXN2* targeting miRNAs can modulate ATXN2 protein levels *in vivo*, consistent with the therapeutic objective.

To assess whether ATXN2-targeting amiRNAs expressed from AAV dosed into the cerebrospinal fluid could lower ATXN2 levels, neonatal mice were dosed via the intracerebroventricular route (i.c.v.) at postnatal day 0 with AAV-amiRNAs with either CAG or Synapsin promoters (**FIG. 53A**). AAV expressing XD-14792 in miR1-1 backbone (SEQ ID NO:1133) or XD-14887 in miR1-1 backbone

(SEQ ID NO:1149) were used. As in the intravenous dosing experiment, the vectors also included GFP reporters to allow for identification of transduced cells. Cortex tissue was harvested after either 4 or 8 weeks, and ATXN2 protein levels assessed by Western along with GFP levels (**FIGS. 53B-53C**). Decreased levels of ATXN2 protein were observed relative to tissue from animals dosed with control, non-amiRNA vectors (MCS) at both 4 and 8 weeks with CAG vectors, for XD-14792 amiRNAs, and at 8 weeks with Synapsin promoter vectors.

To verify that the cell types that experienced knockdown included the therapeutically intended target cell types, *i.e.*, neurons, fixed cortex from i.c.v. dosed animals was subject to immunofluorescence analysis with antibodies against Atxn2 and GFP. Clear evidence of reduced anti-ATXN2 immunofluorescence signal was seen in the brain of animals dosed with ATXN2 amiRNAs versus animals dosed with control vector. Within individual tissue sections, transduced and untransduced cells can be distinguished by the expression of the GFP reporter. **FIG. 54A** shows immunofluorescence of cortex; in tissue from animals dosed with ATXN2 amiRNA (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133) expressing AAVs, comparing neurons expressing GFP with neurons without GFP shows a clear reduction in Axn2 signal in GFP expressing neurons, which will also express the active amiRNA, versus neurons without the GFP. In slices from animals treated with vector without an active amiRNA, there is not an apparent difference in Atxn2 expression level between GFP expressing and non-GFP expressing neurons. Similarly, **FIG. 54B** shows sections of the cerebellum from animals treated with Atxn2 miRNA (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133) expressing AAV or control virus. In animals treated with Atxn2 miRNA (XD-14792 in miR-1-1 backbone, SEQ ID NO:1133), GFP expressing neurons (which will also express the Atxn2 miRNA) have lower levels of Atxn2 signal.

Materials and Methods

ATXN2 siRNA transfection for immunostaining

U2OS cells (unmodified; wildtype) were seeded at 5,000 cells/well 1 day prior to siRNA transfection in 96-well Flat Clear Bottom Black Polystyrene TC-treated

microplates (Corning, P/N 3094). After siRNAs were diluted from stock solutions into Opti-MEM I Reduced Serum Medium (Gibco, P/N 31985-062), transfection mixtures were generated using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen P/N 56532). Transfection mixtures were then aliquoted onto U2OS cells using the Apricot S-PIPETTE S2 and placed into the tissue culture incubator at 37C/ 5% CO₂/ 20% O₂.

ATXN2 siRNA immunostaining and imaging protocol

Three days after transfection, cells were fixed (4% paraformaldehyde/4% sucrose final), followed by washing (PBS), blocking and permeabilization (IF buffer: 0.5% BSA, 0.2% saponin, 5% goat serum). Primary antibody (BD 611378) was applied to the cells at 1:200 in IF buffer in an overnight incubation. Following PBS washing, cells were incubated in secondary antibody (Life Technologies, Alexa Fluor Plus 488) followed by a DNA stain (Hoechst 33342). After final PBS washing, cells were incubated overnight at 4C followed by imaging the next day. Using the Thermo Scientific Invitrogen EVOS FL Auto 2 Imaging System with a 20x objective, images were captured by autofocusing on the nuclear DNA stain, capturing the DNA stain, then auto-repositioning to capture the ATXN2 signal with a total of 9 fields imaged per well.

Artificial miRNA Construct development

Oligonucleotides (Twist) containing Atxn2 targeting shRNAs embedded within miR-1-1 and miR-155E backbones were PCR amplified using regions common to all oligonucleotides (Forward: TAAGCCTGCAGGAATTGCCTAG (SEQ ID NO:626), Reverse: CATGTCTCGACCTGGCTTACTAG (SEQ ID NO:627)). Following amplification, PCR products were verified for the correct sized product by gel electrophoresis. Diluted PCR products were then inserted into a XbaI and EcoRI-digested pLVX EF1alpha > mCherry CMV > Puro construct, similar to SEQ ID NO:546 using NEB HiFi DNA Assembly Master Mix (NEB P/N M5520AA). A portion of the reaction mixture was then incubated with NEB Stable Competent E. coli cells (NEB P/N C3040H) on ice, heat shocked at 42°C, allowed to recovery on ice, followed

by addition of S.O.C. media and incubated at 30°C. The bacterial culture was then applied to LB agar plates with the antibiotic Carbenicillin and grown overnight at 30°C. Individual bacterial colonies were sanger sequence verified (Primer: CATAGCGTAAAAGGAGCAACA (SEQ ID NO:628)). After verifying the correct insert based on the Sanger sequencing, bacterial cultures were grown and the plasmid DNA purified and quantified.

Virus production

With sequence-verified constructs, lentivirus was produced using Lenti-X 293T cells (Takara) and the pc-Pack2 Plasmid Mix (Cellecta P/N CPCP-K2A). Using the Lipofectamine 3000 Transfection Kit (Invitrogen, P/N L3000-008), Lenti-X 293Ts were transfected with individual pLVX EF1a > mCherry miR insert CMV > Puro constructs and the pc-Pack2 Plasmid Mix. The transfection-containing media was aspirated and replaced with viral product media (VPM; 293T media + 20 mM HEPES (gibco, P/N 15630-08)). VPM was collected 48 hours later and aliquoted into 96-well 2.0 mL Deepwell plates (Thermo, P/N 4222) and frozen at -80°C.

Viral transduction

Prior to adding the VPM to cells, U2OS wildtype (unmodified) and ATXN2 knockout (C43) were seeded at 5,000 cells/well 8 hours prior in 96-well Flat Clear Bottom Black Polystyrene TC-treated microplates (Corning, P/N 3094). After adding polybrene (8 µg/ml final, Cellecta, P/N LTDR1), thawed VPM was added using Apricot S-PIPETTE S2. The cells were then placed into the tissue culture incubator at 37°C/5%CO₂/20%O₂. The media on the cells containing the VPM and polybrene was removed 12 hours later and replace with fresh media (U2OS media only) and placed into the tissue culture incubator at 37°C/5%CO₂/20%O₂.

ATXN2 pLVX EF1a > mCherry miR insert CMV > Puro immunostaining and imaging protocol

Three days after changing the media (3.5 days since after the VPM), cells were fixed (4% paraformaldehyde/4% sucrose final), followed by washing (PBS),
 5 blocking and permeabilization (IF buffer: 0.5% BSA, 0.2% saponin, 5% goat serum). Primary antibodies (Atxn2; BD 611378, 1:200 dilution, mCherry; ab205402, 1:1000 dilution) were applied to the cells in IF buffer in an overnight incubation. Following PBS washing, cells were incubated in secondary antibody (Life Technologies, Alexa Fluor Plus 488 and Alexa Fluor Plus 647) followed by a DNA stain (Hoechst 33342).
 10 After final PBS washing, cells were incubated overnight at 4°C followed by imaging the next day. Using the PerkinElmer Operetta CLS High-Content Analysis System with a 20x objective, non-confocal images were captured by autofocusing the bottom of the plate, then capturing the DNA signal, the ATXN2 signal and the mCherry signal with a total of 9 fields imaged per well.

15

miR-155 and miR-1-1 transfection and ATXN2 western blot

A version of the miR-155 scaffold was engineered into an artificial miRNA and used in a mouse *in vivo* proof of concept study to knockdown HTT¹⁰. ATXN2-targeting guide sequences and controls were incorporated into this scaffold
 20 sequence, which we term “miR-155M,” and assayed for protein knockdown after transfection of U2OS cells.

The “miR-1-1E,” where “E” signifies “enhanced,” is taking the human miR-1-1 scaffold and simply introducing a downstream CNNC motif.

To perform the transfection, U2OS cells were plated at 90,000 cells/well
 25 in a 12-well dish, 24 hours later, transfected 2 micrograms/well of the 8 EF1alpha>mCherry constructs (7 with inserts, 1 control) with Lipofectamine 3000 (ThermoFisher). Specifically, each transfection used 2 µL enhancer reagent, 1.5 µL lipofectamine reagent; diluted samples in water to uniform amounts).

Following day imaging with Evos, a good number of mCherry cells
 30 observed. Much higher expression level observed in the control vector without insert.

Wells were aspirated and replaced with 1ml of media with 1 microgram/mL puromycin. Selection occurred over the weekend and then puromycin was removed for recovery.

Three days post-selection, many dead cells were observed. Imaging of mCherry indicated there remained a good number of bright, surviving cells, however. Aspirated media and replaced with prewarmed media containing 200 ng/mL puromycin (a 5-fold dilution).

Two days later (7 days post-transfection), cells were lysed in RIPA buffer with Pierce phosphatase and protease inhibitor tablet.

Western blot was performed and imaged on Odyssey Fc (Licor).

Quantitation of ATXN2 band and control α -tubulin signal intensity was performed with ImageStudio software (LiCor).

Generation of ATXN2 knockout in U2OS cells

ATXN2 knockout cells in U2OS cells was performed using a Cas9-gRNA RNP nucleofection approach. In brief, crRNA and tracrRNA (IDT) were duplexed at equimolar ratios and complexed with recombinant Cas9 (IDT v3) and nucleofected using SF buffer and CM130 program (Lonza 4D nucleofector).

CRISPR guide RNAs were selected from two CRISPR library sources. Three CRISPR guide RNAs (gATXN2_1, gATXN2_2, gATXN2_3) were chosen from the Collecta CRISPR cutting library (one was not selected due to its upstream position before the 2nd ATG). Two additional guides (gATXN2_4 & gATXN2_5) were chosen from the another CRISPR cutting library reported by Bassik et al.²⁶. Additionally, a non-targeting control guide was chosen from the Collecta library. CRISPR guide RNA sequences as well as DNA format are provided in Table 14.

Table 14: CRISPR Guide RNA Sequences for Targeting ATXN2

Name	Guide DNA Sequence	Guide RNA Sequence
gATXN2_1	AAGTTACTCACCGTAGACTG [SEQ ID NO:629]	AAGUUACUCACCGUAGACUG [SEQ ID NO:1169]

gATXN2_2	AATAGAGAAGTCATATCCTG [SEQ ID NO:630]	AAUAGAGAAGUCAUAUCCUG [SEQ ID NO:1170]
gATXN2_3	GTATTGGAAATACCCCCAGT [SEQ ID NO:631]	GUAUUGGAAAUACCCCCAGU [SEQ ID NO:1171]
gNTC	GTAGCGAACGTGTCCGGCGT [SEQ ID NO:632]	GUAGCGAACGUGUCCGGCGU [SEQ ID NO:1172]
gATXN2_4	GTGAGTTCACCTGCATCCCA [SEQ ID NO:633]	GUGAGUUCACCUGCAUCCCA [SEQ ID NO:1173]
gATXN2_5	GCTATCAGTGCTAAAGTGAA [SEQ ID NO:634]	GCUAUCAGUGCUAAAAGUGAA [SEQ ID NO:1174]
gCD81 (cutting control that cuts the CD81 gene)	GTTGGCTTCCTGGGCTGCTA [SEQ ID NO:635]	GUUGGCUUCCUGGGCUGCUA [SEQ ID NO:1175]

Post nucleofection, bulk population of cells were allowed to recover for 5 days and lysed for western blot analysis.

U2OS clone selection

- 5 The bulk population of cells were also single cell sorted into 96-well plates for clonal expansion. Because guides gATXN2_1 and gATXN2_5 had the most decrease in ATXN2 protein signal by western blot (~90% reduction), we proceeded with these cells for single cell cloning. After trypsinization and single cell suspension, a SONY SH800S was used to gate for singlet cells and to sort directly into U2OS growth
- 10 media. Cells were allowed to grow for ~2-3 weeks and lysed for genomic DNA extraction for Sanger sequencing and protein extraction for western blotting (10µg of protein used per lane in this setting)

ICE confirmation of clones

Genomic DNA was extracted using a Qiagen Blood and Tissue Kit.

Genomic primers were designed to amplify the genomic region surrounding the guide RNA cut site with the goal of sequencing the cut site by Sanger sequencing and

5 validating an out-of-frame indel pattern consistent with a single clone.

Primer Blast (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) was used with the following settings: For guide 1, we turned off repeat filter and low complexity filter due to the repetitive nature of ATXN2, but otherwise kept the default settings. The import function of Snapgene was used to import “6311” from NCBI. 500
10 upstream and 500 downstream bases from the protospacer sequence was used to as input for primer blast. Product size was set for 400-1000 and 2 distinct primer pairs were selected (**Table 15**).

Table 15: ATXN2 PCR primers

Primer Name	Primer Sequence
Forward primer (ICE_gATXN2_1_set1_Fwd)	AAGTTTCCTGAGGCCTCCCC [SEQ ID NO:636]
Reverse primer (ICE_gATXN2_1_set1_Rev)	TCCAGGCAAGCAGTGCATAG [SEQ ID NO:637]
Product length 522	
Forward primer (ICE_gATXN2_1_set2_Fwd)	AGTTTCCTGAGGCCTCCCC [SEQ ID NO:638]
Reverse primer (ICE_gATXN2_1_set2_Rev)	CCCTACCTGTTGTGGGTCTC [SEQ ID NO:639]
Product length 539	

15

Furthermore, amplicon internal sequencing primers were designed for Sanger sequencing in both forward and reverse directions to read the cut site (**Table 16**). The primer(+) algorithm (<http://www.biology.wustl.edu/gcg/prime.html>) was used to design the sequencing primers on this web interface

20 (<https://www.eurofinsgenomics.eu/en/ecom/tools/sequencing-primer-design/>).

Table 16: ATXN2 Sequencing Primers

Primer Name	Primer Sequence
ICE_gATXN2_1_Rseq	AGAGCCATCCTTAGGTAGCC [SEQ ID NO:640]

ICE_gATXN2_1_Fseq	GAGGTTTAATTGACTCATGCTCTG [SEQ ID NO:641]
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For guide 5, we turned off repeat filter but turned on the low complexity filter, but otherwise kept the default settings. 500 upstream and 500 downstream bases from the protospacer sequence was used to as input for primer blast. Product size was set for 400-1000 and 2 distinct primer pairs were selected (**Table 17**).

Table 17: ATXN2 Primer Sequences

Primer Name	Primer Sequence
Forward primer (ICE_gATXN2_5_set1_Fwd)	AAAACACACCGGCATTTC [SEQ ID NO:642]
Reverse primer (ICE_gATXN2_5_set1_Rev)	CCTGGGCAACAGAACGAGAC [SEQ ID NO:643]
Product length 853	
Forward primer (ICE_gATXN2_5_set2_Fwd)	AGCAAAACACACCGGCATT [SEQ ID NO:644]
Reverse primer (ICE_gATXN2_5_set2_Rev)	ATCACGCCACTGCATTCCA [SEQ ID NO:645]
Product length	

Internal sequencing primers were designed by the primer(+) algorithm (Table 18).

Table 18: ATXN2 Sequencing Primers

Primer Name	Primer Sequence
ICE_gATXN2_5_Fseq	TCATGAGCATCCACAAGAACAG [SEQ ID NO:646]
ICE_gATXN2_5_Rseq	ACGTGTGTGAGTGTAAGTATTGC [SEQ ID NO:647]

PCR was performed with NEBNext Ultra II Q5 Master Mix (NEB, M0544S) with gDNA and primer pairs indicated above. Amplified products were visualized by agarose gel and correctly sized amplicons were gel purified and submitted for Sanger sequencing with forward and reverse sequencing primers. Chromatogram
5 (.abi files) results were uploaded to the Inference of CRISPR Editing (ICE) tool²⁷ <https://ice.synthego.com/#/> for deconvolution of Sanger reads to identify indels.

Clone 43 from guide 5 nucleofection, which verified both by western and Sanger sequencing as a bona fide knock-out clone, was carried forth for further studies.

10

Ataxin-2 western blot

To prepare lysates, 1x RIPA (Teknova, Tris-HCl 50 mM, NaCl 150 mM, 1% Triton X-100, Sodium Deoxycholate 1%, SDS 0.1%, EDTA 2 mM, pH 7.5) was supplemented with Pierce protease/phosphatase tablet (Thermo, A32959) and incubated
15 15min on ice, spun down at 17,000xg at 4°C for 15min.

Pierce BCA kit (Thermo Scientific, 23225) was used for protein quantitation and 20µg of protein was loaded per lane in SDS-PAGE gel electrophoresis (NuPage Bis Tris, Thermo Scientific).

Samples were prepared with 10 or 20 micrograms of protein, 4x LDS
20 loading buffer (NP0007), 10x sample reducing agent (NP0004), water to 20µl. Samples were heated at 70°C for 10 min.

Protein size ladders were Precision plus protein dual color standard (Bio-rad, 1610374) or Chameleon® Duo Pre-stained Protein Ladder (Licor, 928-60000).

Samples were loaded onto 1.0mm x 10well 4-12% Bis-Tris protein gel
25 (NP0301PK2) and gel electrophoresis was run with MOPS SDS running buffer (NP0001) for 1hr 20min at constant 200V to resolve higher molecular weight bands.

Tris/glycine transfer buffer was used (Bio-rad, 1610734) without methanol. All components including sponges, filter paper, gel, and membrane were equilibrated at least 15min with transfer buffer. The PVDF membrane was dipped in
30 methanol for 15 seconds prior to equilibration with transfer buffer. Wet transfer was

performed in a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-rad, 1703930) overnight at 4°C at constant 30V, 90mA.

After overnight transfer, membranes were air dried for 1hr at RT.

Membranes were rinsed with 1x TBS (no tween) and blocked in Odyssey blocking
5 buffer (LI-COR) at room temperature rocking for 30min-1hr.

Membranes were incubated with primary antibodies overnight at 4°C at 1:1000 dilutions in Odyssey blocking buffer with 0.1% Tween-20. The mouse ATXN2 antibody (BD, 611378) and Rabbit α -Tubulin antibody (CST, 2144S) was used as a loading control.

10 Membranes were washed 4x5min with TBS-0.1%Tween-20.

Membranes were treated with two secondary antibodies for 1hr rocking at RT at 1:20,000 dilutions in Odyssey blocking buffer with 0.1% Tween-20 and 0.01% SDS.

The secondary antibodies were IRDye 800CW Goat anti-mouse IgG,
15 (Li-cor, 926-32210) and IRDye 680RD Donkey anti-rabbit IgG (Li-cor, 926-68073). Membranes were washed 4x5min with TBS-0.1%Tween-20 and rinsed with TBS (no Tween) before imaging on a LI-COR Odyssey scanner (Fc) with both 700 and 800 channels.

20 *Ataxin-2 FACS*

Cells were trypsinized, transferred to a 96-well v-bottom format, each treatment assayed in triplicate, and washed in wash buffer (PBS/0.5% BSA (no EDTA)) and fixed with ice-cold methanol dropwise, incubated on ice for 10min, then 200 μ l of PBS were added and cells were rocked overnight at 4°C.

25 Cells were spun down at 1000xg, 5min cold and washed twice with cold FACS wash buffer (PBS/0.5%BSA/2mM EDTA/0.2%saponin). Primary antibody (BD 611378) was applied at 1:100 and incubated for 1hr, rocking in 4°C. The buffer was supplemented with 5% goat serum to reduce non-specific binding. Cells were washed twice in cold FACS wash buffer. Cells were incubated in 1:100 secondary antibody
30 (PE/Cy7 Biolegend clone RMG1-1) with cells resuspended in cold FACS wash buffer

with 5% goat serum and incubated for 1hr on ice. Cells were washed twice and resuspended in cold FACS wash buffer and sampled on an Attune (Thermo Scientific).

Intracerebroventricular injections

- 5 For intracerebroventricular injections, postnatal day 0 pups were cryo-anesthetized and injected at a depth of 2 mm using Hamilton syringes, delivering a maximum volume of 3 uL per each ventricle.

Immunofluorescence analysis

- 10 Animals dosed i.c.v with rAAV were euthanized 4 weeks after dosing with rAAV, fixed overnight in 4% paraformaldehyde. Tissue was then cryopreserved in cold 30% sucrose, then embedded in OCT media and frozen. 5 micrometer frozen sections were prepared on a cryostat. For staining, sections were thawed and dried, washed twice in PBS, heated in 95C antigen retrieval solution (citra antigen retrieval, pH 6.0,
- 15 Vector Labs #H-3300-250) for 10 minutes, then cooled for 30 minutes at room temperature. Sections were then washed 5 minutes each in water, PBS, and PBS-0.25% Triton-X-100, and 10 minutes in PBS. Sections were then blocked with 5% goat serum in PBS for 30 minutes in humidified chambers. Sections were treated with primary antibody solution in PBS + 1% BSA, including: Mouse anti-ATXN2 antibody (BD
- 20 #611378), 1:50; Rabbit anti-GFP antibody (Cell Signaling Technologies #25555), 1:2000 overnight at 4C. After 3x washes in PBS, sections were incubated with secondary antibody solutions in PBS + 1% BSA, including: goat anti-mouse Alexa Fluor 555 (Thermo Scientific #A21424) 1:250, Goat anti-Rabbit Alexa Fluor 488 (Thermo Scientific #A11008), 1:250 for 30 minutes at room temperature. Sections
- 25 were then washed, and mounted in VectaShield PLUS with Dapi (H-2000-10). Images were collected with a Revolve microscope (Discover Echo).

EXAMPLE 2: IDENTIFICATION OF HIGH PERFORMING AMiRNAs BY TILED SCREEN OF ATXN2 TARGETING MiRNAs IN LENTIVIRAL FORMAT

As an alternative approach to siRNA screening followed by embedding of the associated guide sequences in miRNA backbones and testing one-by-one, pooled screening of *ATXN2*-targeting miRNAs was conducted (“Deep Screen 1”).

ATXN2 target sequences

Homo sapiens *ATXN2* mRNA (NM_002973, transcript variant 1, SEQ ID NO:2) was used to identify target sequences for the artificial miRNAs. All human and primate cross-reactive sequences were identified and 22-nt guide sequences were designed taking into consideration criteria for effective shRNA and miRNA sequences, including the preference for A or U at guide position 1. Therefore, taking into consideration the 22 nucleotide antisense sequences complementary to the Ataxin-2 construct, if the first guide base was G or C this was converted to a ‘U’, whereas sequences that began with A or U were not changed from the base complementary to the corresponding position on the *ATXN2* transcript. As above, U bases are encoded as T in the lentiviral expression construct. In total 2,381 *ATXN2*-targeting sequences were introduced into a modified variant of the miR-16-2 backbone. Passenger sequences (the sequence on the opposite side of the miRNA stem from the guide sequence) were generated following the rules in **Table 8** for this backbone.

Toxicity controls

By examining the abundance of elements of the library in cells that had been allowed to grow for lengthy periods of time versus initially transduced cells, the pooled screen can identify elements that alter cellular proliferation or viability. To calibrate the dynamic range of the assay, additional toxic elements were added to the library. Ten essential genes were selected with ten shRNAs each (removing 2 sequences that had polyT sequences deemed problematic because they may serve as termination signals for PolIII). To identify the “essential” gene list, genetic dropout screens performed in parallel with shRNA and CRISPR guide RNAs in the K562 cancer cell line²¹ were examined. Across both screens, genes were rank ordered by shRNA lethality, specifically genes that scored highly in the K562 shRNA dropout by

combined Castle score (negative is more lethal). Since toxicity screen was performed in HeLa cells, the K562 top genes were intersected and identified the top 10 genes that also scored highly (bayesian factor >100) in a HeLa CRISPR cutting dropout screen²². The essential genes selected were: COPB1, COPB2, DHX15, EIF3A, EIF4A3, NUP93,
 5 PRPF8, PSMB6, PSMD1, and SF3B2.

To select 10 shRNA targeting each gene, the 25 shRNA/gene in a previously published shRNA library were considered and rank ordered by their performance in the dropout screen¹⁵. Specifically, the shRNAs were rank-ordered by the dropout metric (read counts in replicate 1 and replicate 2 divided by plasmid reads),
 10 and the top performing shRNAs that had at least one count across all replicates were selected.

GFP controls

GFP controls (n=50) were designed to target two different GFP reporter
 15 systems. The first system involved tagging endogenous ATXN2 with the 11th beta strand of GFP (GFP₁₁) in conjunction with overexpression of GFP₁₋₁₀ to constitute a self-complementary GFP system²³, and the second is a GFP-stop-ATXN2 overexpression reporter. The 11th beta strand of GFP was targeted by entirely tiling the transcript with 28 individual 21nt shRNA, adding an A at guide position 1 to form 22nt
 20 oligomer sequences. Additional shGFP (n=22) were selected to target GFP₁₋₁₀ using the Design portal of the Broad Institute Genetic Perturbation Platform (<https://portals.broadinstitute.org/gpp/public/seq/search>), using the GFP₁₋₁₀ sequence as input. Although the split GFP system was not ultimately used to read out ATXN2 levels, the 50 shGFP still target the GFP-stop-ATXN2 reporter.

ATXN2 transcript scrambled controls

Neutral controls were designed that should not have any effect in both the efficacy and toxicity screens. These elements can be used for baseline normalization. The guide sequences targeting *ATXN2* were scrambled and 974 of these scrambled guide sequences used to construct amiRNAs as before. After scrambling, the
 30 same rules for the first base as with targeting sequence were imposed. Following this correction step, the GC content was adjusted by converting one of the guide bases 2 –

22 that were A or T, randomly selected, to G or C, randomly chosen, such that overall this set of scrambled controls maintains similar GC content relative to the *ATXN2*-targeting sequences.

Promoter selection

5 The H1 promoter, an RNA polymerase III promoter, was selected to drive artificial miRNA expression as many groups have used it to achieve robust target knockdown.

Pooled library cloning

10 The oligonucleotide pool was synthesized on chip (oligo length 172bp, Agilent), PCR amplified, and cloned into the pRSICPH1 vector (Cellecta) by BplI restriction digestion and T4 ligase ligation. Each individual miRNA cassette was expressed under the control of an H1 promoter and subsequently followed by a short constant region and 17bp barcode sequence that uniquely tags each miRNA. The elements were designed to contain both miRNA and barcode tags to enable multiple
15 ways to amplify and sequence the constructs to readout the pooled screens. For instance, if PCR amplification bias were to confound the representation of high GC content sequences²⁴, comparison of the abundance of amplicons containing the guide sequence versus the abundance of amplicons containing the FREE barcode would resolve any discrepancies. FREE barcodes were used as they are indel-correcting and
20 robust to DNA synthesis and NGS errors²⁵. The library was checked by Sanger sequencing and next-generation sequence (Illumina) to verify lack of synthesis errors, >99% amiRNA and FREE barcode were correctly paired, and the fold-representation between the top and bottom amiRNAs were within four fold-change.

25 Viral production

 Lenti-X 293T (Takara) cells were used to produce lentivirus by transfection of 4th generation packaging plasmids (Lenti-X Packaging single shots, Takara) followed by viral concentration with Lenti-X concentrator and resuspension in PBS. Virus was titered in U2OS and Hela cells by infection and antibiotic selection
30 followed with estimation of viral units and multiplicity of infection (MOI) by Cell-Titer-Glo (Promega).

Cell culture and transfections

U2OS cells and the GFP-ATXN2 reporter cell line were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin/ glutamine. Hela cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin/glutamine.

Efficacy screen

Two pooled lentiviral miRNA screens for on-target efficacy were performed to identify miRNA that diminish ATXN2 protein signal, reading out ATXN2 levels by 1) an exogenous GFP-stop- ATXN2 reporter or 2) endogenous ataxin-2 antibody in a FACS assay. Cells were infected with the pooled lentiviral library at a multiplicity of infection (MOI) of 0.1 into ($\sim 5 \times 10^7$ cells) with polybrene (8 $\mu\text{g/ml}$, EMD Millipore) and distributed across four T225 flasks. Two days post-infection, U2OS cells were selected with puromycin at 2 $\mu\text{g/ml}$. The MOI was confirmed by cell-titer-glo at day 5 (3 days after selection) in a 96 well format. An unsorted fraction (7×10^6 cells) was collected at day 7 as a reference control. The remaining cells were washed in wash buffer (PBS/0.5% BSA (no EDTA)) and fixed with ice-cold methanol dropwise while vortexing on day 7, at a ratio of 1ml methanol/ 2×10^6 cells, incubated on ice for 10min, then 10x volumes of PBS were added and cells were rocked overnight at 4°C.

Cells were spun down at 1000xg, 5 min cold (Corning 500ml centrifuge tubes, 431123) and resuspended in cold FACS wash buffer (PBS/0.5%BSA/2mM EDTA/0.2%saponin). Cells were counted and resuspended in $2 \times 10^6/\text{ml}$ in cold FACS wash buffer.

Primary antibody (BD 611378) was applied at 1:200 and incubated for 1hr, rocking in 4°C. The buffer was supplemented with 5% goat serum to reduce non-specific binding.

Cells were washed twice in cold FACS wash buffer. Cells were incubated in 1:200 secondary antibody (PE/Cy7 Biolegend clone RMG1-1) with cells resuspend in $2 \times 10^6/\text{ml}$ cold FACS wash buffer with 5% goat serum and incubated for 1hr on ice. Cells were washed twice in and resuspended in cold FACS wash buffer at 4-

5x10⁶/ml to achieve 1000-2000 events per second on the Sony SH800S (approximately the maximal stable cell velocity on the instrument). Samples were filtered through a cell strainer directly into FACS tubes (FALCON 352235). Sorted cells were collected in 3 mL PBS/10% FBS in 15ml conicals.

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Dropout screen

A pooled lentiviral miRNA screen for off-target toxicity was additionally performed, by identifying miRNA dropout between an early and late timepoint. HeLa cells were infected with polybrene (8µg/ml, EMD Millipore) at a multiplicity of infection of 0.1 at 1000x representation (that is, the number of cells was >10,000x the number of library elements). Two days post-infection, HeLa cells were selected with puromycin at 0.5 micrograms/mL. Cells were passaged for a total of 10 doublings (~16 days). The screen was performed in triplicate (3 separate infections).

DNA processing

15 Genomic DNA was extracted from each sample using the Machery Nagel Blood L kit (FACS collections; early and late collection timepoints). A two-step PCR was conducted. In a first PCR reaction, an amplicon spanning both the guide and passenger sequences, and downstream past the FREE barcode, was generated. In a second PCR reaction, a nested amplicon was generated spanning either the guide and passenger sequence, or the FREE barcode. The second PCR was designed to incorporate Illumina binding sequences (P5 and P7) and sample index barcodes to enable demultiplexing on Illumina sequencing platforms. Each distinct sample (that is, FACS collection, or timepoint) was given a distinct index. Specifically, the guide and passenger amplicon was single-indexed, with an i7 sequence included upstream of the 6nt sample barcode and P7 sequence. In contrast, the FREE barcode amplicon was single-indexed on the P5 end and no i7 sequence was included on the P7 end. Samples were sequenced on an Illumina MiSeq such that guide and passenger sequences can be matched in paired reads, with read 1 using a custom primer reading the 22nt guide sequence, and read 2 being the standard Illumina primer reading the passenger sequence. FREE barcodes were also separately amplified and sequenced, with read 1 being a custom primer reading the 17nt FREE barcode, and read 2 being a custom

primer reading the 6nt sample barcode. In general, calculations of abundances were highly similar for FREE barcode derived amplicons and guide/passenger sequence containing amplicons (using a lookup table of the association between FREE barcodes and guide/passenger sequences). Analyses below focused on counts of the guide
5 sequences.

Computational Analysis

Occurrences of each guide sequence were counted, without tolerating sequencing or other errors (that is, no mismatches to the library input guide sequences were tolerated), in read 1 sequences, which directly sequences amiRNA guide
10 sequences. To estimate ATXN2 knockdown efficiency, the abundance of guide sequence counts in the ATXN2 high FACS collection was divided by the abundance of guide sequence counts in the ATXN2 low FACS collection. Sequences that effectively knock down ATXN2 are enriched in the ATXN2 low FACS collection.

15 To assess whether the guide sequence influences cytotoxicity or reduces proliferation, the ratio of counts of each guide sequence for a pool of cells collected 16 days after library transduction, versus the ratio of counts for the library collected 18 hours after library transduction, were measured.

Data was highly consistent across replicates. **FIG. 23A** plots the
20 high/low count ratios for two independent replicates against one another. Most points fall along $y = x$, indicating good correlation. **FIG. 23B** plots the matrix of Spearman correlation coefficients for count values for each condition against all others. The replicates are hierarchically clustered, and clustered blocks represent similar conditions. Note the strong anticorrelation between low and high conditions, as expected given that
25 guides that deplete ATXN2 are expected to be differentially present in the low and high conditions. Note also that conditions where ATXN2 signal was visualized by antibody staining against endogenous Ataxin-2 protein, and conditions where the signal was visualized by fluorescence of the ATXN2 GFP reporter, correlate.

Following the calculation of count ratios, a normalization procedure was
30 taken to rank ATXN2 targeting sequences by their ability to deplete ATXN2 signal. In

FIG. 24, histograms for the distribution of high and low condition guide sequence counts for ATXN2 targeting guides, top trellis, and scrambled sequences, bottom trellis, are shown. The ATXN2 scrambled sequences exhibit a sharp, unimodal distribution of ratios of counts in the high and low ATXN2 FACS conditions. The median ratio from this distribution was taken to be no-effect, and the ATXN2 depleting effect of ATXN2 targeting miRNAs was therefore calculated by subtracting this (log base 2 – transformed) value.

The ability of guide sequences to knock down ATXN2 and the presence of any altered proliferation or cytotoxicity were examined. **FIG. 25** shows a plots of three classes of guide sequences in this experiment: *ATXN2* targeting sequences, *ATXN2* scrambled sequences, and amiRNAs targeting essential genes (predicted to be toxic). As expected, the log base-2 ATXN2 signal depletion (the scramble-baseline-corrected ATXN2 depletion in counts from high to low ATXN2 FACS conditions) was centered around 0 (no effect). However, many of these sequences exhibited remarkable shifts in abundance at a late collection timepoint, 16 days after transduction, versus an early timepoint after transduction. This is consistent with the reported essentiality for these sequences and demonstrates that this system can elicit cellular toxicity or proliferation impairment.

ATXN2 targeting guide sequences fall along a much wider spectrum along the axis of ATXN2 signal depletion compared to amiRNAs targeting essential genes or scrambled sequences, with targeting sequences exceeding 5 logs (base 2), corresponding to approximately 32-fold depletion of cells expressing these amiRNAs in high ATXN2 FACS collections versus low ATXN2 FACS collections.

The near complete tiling of the *ATXN2* transcript enables the detection of ‘hotspots’ of Ataxin-2 targeting guide sequences, defined by the proximity of their complementary regions of the Ataxin-2 transcript. **FIG. 26** shows a plot of the knockdown efficacy, as measured by the depletion of counts for a given guide from the high ATXN2 FACS collection versus low ATXN2 FACS collection. Across the transcript, multiple regions where adjacent *ATXN2* targeting guide sequences exhibit strong ATXN2 knockdown are noted. **FIG. 27** shows a ‘zoom-in’ of regions within the

3' UTR of *ATXN2*, and highlights guide sequences (as dark points) with unusually high *ATXN2* lowering, as measured by the count reduction.

Small RNAseq confirmation of pri-miRNA processing precision in the pooled screen

5 Guide sequences are excised from a miRNA stem by successive Drosha and Dicer processing. Each enzyme cuts the RNA. In the case of the miR backbone used for this tiled screen of *ATXN2*, the guide sequence from the corresponding endogenous miRNA (miR 16-2) is excised from the upstream, 5' prime arm, and therefore the guide sequence is cleaved from the parent stem at the 5' side by Drosha.
10 Because the position of the 5' cut site determines the composition of the seed sequence, bases 2 – 7 counting from the 5' nucleotide, the cutting position is important in determining both on- and off-target activity of the resulting guide sequence. Therefore, small RNAseq was conducted to assess the position of this cut.

The tiling library, in packaged lentiviral form, was transduced at high
15 multiplicity of infection into U2OS cells. After selection by puromycin to eliminate untransduced cells (the library vector contains a puromycin selection cassette), RNA was extracted by standard methods, and small RNA was purified and ligated with adapters to enable small RNA sequencing using the Nextflex small RNAseq kit v3. After PCR amplification, the resulting library was subject to next-generation
20 sequencing on an Illumina MiSeq. A high proportion of reads had sequences of length 21, 22, and 23 nucleotides, with a peak at 22 nucleotides, consistent with the detection of processed miRNAs (guide and passenger sequences). To examine the precision of 5' processing, the number of observations of 22-mer sequences matching several models of processed guide sequences were calculated. In one model, the guide sequence was
25 assumed to be correctly processed. In other models, the guide sequence was assumed to be processed either upstream or downstream of the expected nucleotide. If the guide sequence is cut upstream of the intended nucleotide, then the expected upstream bases are incorporated from the miRNA backbone sequence. If the guide sequence is cut downstream of the intended nucleotide, then the first base of the resulting guide
30 sequence is downstream of expected. Because the scrambled sequences in the library

do not generally overlap from one another, for example, lowering the likelihood of ‘collisions’ where a guide sequence processed by excision from the stem at a nucleotide one downstream of the intended first nucleotide is the same as a guide sequence aligning to a position in the *ATXN2* transcript one bp shifted, the processing position across all scrambling sequences was analyzed and averaged to estimate the most probable cutting position. **FIG. 28** plots the percent of reads of the guide sequence with cut position at each nucleotide relative to the intended first nucleotide, and shows a very high proportion of reads begin at the intended position.

10 Additional *ATXN2* targeting sequences from pooled screen

By examination of the knockdown efficacy against *ATXN2* (as measured by depletion from the high versus low *ATXN2* FACS collections) across the positions of complementarity to the *ATXN2* transcript, several regions of interest were noted where clusters of high performing *ATXN2*-targeting guide sequences were observed. **Table 19** lists these guide sequences, the targeting position of the guide sequences relative to the *ATXN2* transcript (SEQ ID NO:2), the guide sequences inserted into the miRNA16-2 backbone (which are also the highest probability sequence that will be generated in the cell according to the above small RNAseq experiments), and the passenger sequences generated for the miR16-2 backbone. The guide sequences, miRNA16-2 formatted passenger sequences, and amiRNA sequences are provided in Table 19 in RNA format and DNA format (e.g., for insertion into a plasmid for AAV). Exemplary passenger RNA sequences (e.g., not modified for a specific miRNA backbone) are also provided in Table 19 in both RNA and DNA format. Efficacy of *ATXN2* knockdown is represented by the signal depletion column. Altogether, sequences with high efficacy and low potential for dropout may represent good candidates to incorporate into therapeutic vectors targeting *ATXN2*.

Table 19: Guide sequences in 'hot spots' targeting *ATXN2* from tiled screen and corresponding passenger and miRNA sequences

Guide Sequence (DNA)	Guide Sequence (RNA)	16_2_format_ passenger (DNA)	16_2_format_ passenger (RNA)	Mean Atxn2 Log2 Signal Depletion	Atxn2 Position	amiRNA Sequence (DNA)	amiRNA Sequence (RNA)
TACCACAAACAA GTCTGAACAT [SEQ ID NO:648]	UACCACAAACAA GUCUGAACAU [SEQ ID NO:1176]	ATGTTTCAGACCC TGTGTGTGGTT [SEQ ID NO:761]	AUGUUCAGACCC UGUUGUGGU [SEQ ID NO:1289]	-2.75633	1157	ACCGGACATACTTGT CCACTCTACCAACA AAGTCTGAACATTAGT GAAATATATATTTAAA TGTTTCAGACCCCTGTTG TGGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO:877]	ACCGGACAUACUUGUU CCACUCUACCAACA AAGUCUGAACAUUAGU GAAUUAUUAUUAUAA UGUUCAGACCCUUGU UGGUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO:1405]
TTACCACAAACAA AGTCTGAACA [SEQ ID NO:649]	UUACCACAAACAA AGUCUGAACAA [SEQ ID NO:1177]	TGTTTCAGACTCC GTTGTGGTAT [SEQ ID NO:762]	UGUUCAGACUCC GUUGUGGUU [SEQ ID NO:1290]	-2.02091	1158	ACCGGACATACTTGT CCACTCTTACCAAC AAAGTCTGAACATTAGT GAAATATATATTTAAA GTTTCAGACTCCGTTGT GGTATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO:878]	ACCGGACAUACUUGUU CCACUCUACCAACA AAAGUCUGAACAUUAGU GAAUUAUUAUUAUAA GUUCAGACCCUUGU GGUUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO:1406]
TGTACCACAAAC AAGTCTGAAC [SEQ ID NO:603]	UGUACCACAAAC AAGUCUGAAC [SEQ ID NO:40] (same as guide in XD- 14756)	GTTTCAGACTTCT TTGTGGTACT [SEQ ID NO:763]	GUUCAGACUUCU UUGUGGUACU [SEQ ID NO:1291]	-3.6909	1159	ACCGGACATACTTGT CCACTCTTACCAACA CAAAGTCTGAACATTAGT GAAATATATTTAAA TTCAGACTTCTTGTG GTACTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO:879]	ACCGGACAUACUUGUU CCACUCUACCAACA CAAAGUCUGAACUAGU GAAUUAUUAUUAUAA UUCAGACUUCUUGUG GUACUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO:1407]
TTGTACCACAAAC AAAGTCTGAA [SEQ ID NO:650]	UUGUACCACAAAC AAAGUCUGAAC [SEQ ID NO:1178]	TTTCAGACTTTTC TGTGGTACAT [SEQ ID NO:764]	UUCAGACUUCUUC UGUGGUACAU [SEQ ID NO:1292]	-1.52769	1160	ACCGGACATACTTGT CCACTCTTACCAACA ACAAAGTCTGAATAGT GAAATATATTTAAA TCAGACTTTCTGTGG TACATTAGTGTGACAG [SEQ ID NO:879]	ACCGGACAUACUUGUU CCACUCUUGUACCAACA ACAAAGUCUGAACUAGU GAAUUAUUAUUAUAA UACAGACUUCUUGUG UACAUUAGUGUGACAG [SEQ ID NO:1407]

ACTGTACCACAA CAAACTCTGA [SEQ ID No: 651]	ACUGUACCACAA CAAGUCUGA [SEQ ID No: 1179]	TCAGACTTTGCC GTGGTACAGA [SEQ ID No: 765]	UCAGACUUUGCC GUGGUACAGA [SEQ ID No: 1293]	UCAGACUUUGUU GUGGUACA [SEQ ID No: 997]	-3.46564	1161	GGATACAGCAACTATT TTATCAA [SEQ ID No: 880]	GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1408]
AACTGTACCACA ACAAAGTCTG [SEQ ID No: 652]	AAUGUACCACA ACAAAGUCUG [SEQ ID No: 1180]	CAGACTTTGTCT TGGTACAGTA [SEQ ID No: 766]	CAGACUUUGUCU UGGUACAGUA [SEQ ID No: 1294]	CAGACUUUGUU UGGUACAG [SEQ ID No: 998]	-3.89476	1162	ACCGGACATACCTTGT CCACTCAACTGTACCA CAACAAAGTCTGTAGT GAAATATATATTAAAC AGACTTTGTCTTGSTA CAGTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 881]	ACCGGACAUACUUGUU CCACUCAAACUGUACCA CAACAAAGUCUGUAGU GAAUAUAUAUUAAC AGACUUUGUCUUGGUA CAGUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1409]
AACTGTACCAC AACAAAGTCT [SEQ ID No: 653]	AAACUGUACCAC AACAAAGUCU [SEQ ID No: 1181]	AGACTTTGTTTC GGTACAGTTA [SEQ ID No: 767]	AGACUUUGUUUC GGUACAGUA [SEQ ID No: 1295]	AGACUUUGUUGU GGUACAGU [SEQ ID No: 999]	-2.62277	1163	ACCGGACATACCTTGT CCACTCAACTGTACC ACAAAGTCTGTAGT GAAATATATATTAAAC GACTTTGTCTCGGTAC AGTTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 883]	ACCGGACAUACUUGUU CCACUCAAACUGUACC ACAAAGUCUUGUAGU GAAUAUAUAUUAAC GACUUUGUUUGGUA AGUUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1411]
TTAACTGTACC ACAAAGT [SEQ ID No: 654]	UUAACUGUACC ACAAAGUAGU [SEQ ID No: 1182] (same as guide in XD- 14757)	ACTTTGTTGTTT TACAGTTTAT [SEQ ID No: 768]	ACUUUGUUGUUU UACAGUUUAU [SEQ ID No: 1296]	ACUUUGUUGUGG UACAGUUU [SEQ ID No: 1000]	-0.49686	1165	ACCGGACATACCTTGT CCACTCTTAAACGTGA CCACAAAGTGTAGT GAAATATATATTAAAC CTTTGTTGTTTACAG TTTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 884]	ACCGGACAUACUUGUU CCACUCAAACUGUUA CCACAAAGUUGUAGU GAAUAUAUAUUAAC CUUUGUUUUUACAG UUUUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1412]
TTGCTAACTGGT TTGCCCTTGC	UUGCUAACUGGU UUGCCCUUGC	GCAAGGGCAAGA CAGTTAGCAT	GCAAGGGCAAGA CAGUAGCAU	GCAAGGGCAAC CAGUAGC	-3.48781	1479	ACCGGACATACCTTGT CCACTCTTGTCTAACTG	ACCGGACAUACUUGUU CCACUCAAACUGUUA CCACAAAGUUGUAGU GAAUAUAUAUUAAC CUUUGUUUUUACAG UUUUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1412]

[SEQ ID NO: 655]	[SEQ ID NO: 1183]	[SEQ ID NO: 769]	[SEQ ID NO: 1297]	[SEQ ID NO: 1001]				GTTTCCCTTGCTAGT GAAATATATATTAAAG CAAGGCAAGACAGTT AGCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 885]	GUUUGCCCUUGCUAGU GAAAUUAUUAUAAAG CAAGGCAAGACAGUU AGCAUUAUUAUAAAU GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1413]
TGGGTTGAAATC TGAAGTGTGA [SEQ ID NO: 656]	UGGGUUGAAAU UGAAGUGUGA [SEQ ID NO: 1184]	TCACACTTCATG TTTCAACCCCT [SEQ ID NO: 770]	UCACACUUCAG UUUCAACCCU [SEQ ID NO: 1298]	UCACACUUCAGA UUUCAACC [SEQ ID NO: 1002]	1754	-1.9344		ACCGGACATACCTGTT CCACTCTGGGTTGAAA TCTGAAGTGTGATAGT GAAATATATATTAAAT CACACTTCATGTTTCA ACCCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 886]	ACCGGACAUACUUGUU CCACUCUGGUGUAAA UCUGAAGUGUGAUAGU GAAAUUAUUAUAAAU CACACUUCAGUUUUA ACCCUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1414]
TCGGGTTGAAAT CTGAAGTGTG [SEQ ID NO: 657]	UCGGGUUGAAAU CUGAAGUGUG [SEQ ID NO: 1185]	CACACTTCAGGC TTCAACCCCGT [SEQ ID NO: 771]	CACACUUCAGGC UUCAACCCGU [SEQ ID NO: 1299]	CACACUUCAGAU UUCAACCC [SEQ ID NO: 1003]	1755	-4.71279		ACCGGACATACCTGTT CCACTCTGGGTTGAA ATCTGAAGTGTGATAGT GAAATATATATTAAAC ACACTTCAGGCTTCAA CCGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 887]	ACCGGACAUACUUGUU CCACUCUGGUGUAAA AUCUGAAGUGUGUAGU GAAAUUAUUAUAAAC ACACUUCAGGCUUCAA CCCGUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1415]
TTGGGTTGAAA TCTGAAGTGT [SEQ ID NO: 607]	UUCGGGUUGAAA UCUGAAGUGU [SEQ ID NO: 108] (same as guide in XD-14790)	ACACTTCAGACC TCAACCCGAT [SEQ ID NO: 772]	ACACUUCAGACC UCAACCCGAU [SEQ ID NO: 1300]	ACACUUCAGAUU UCAACCCG [SEQ ID NO: 1004]	1756	-3.7055		ACCGGACATACCTGTT CCACTCTGGGTTGA AATCTGAAGTGTGATAGT GAAATATATATTAAAA CACTTCAGACCTCAAC CCGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 888]	ACCGGACAUACUUGUU CCACUUCUGGUGUAGA AAUCUGAAGUGUAGU GAAAUUAUUAUAAAA CACUUCAGACCCUAA CCGUAUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1416]
TGATCAGGACT AGCAGGCGTA [SEQ ID NO: 658]	UGAUGCAGGACU AGCAGGCGUA [SEQ ID NO: 1186]	TACGCCCTGCTGT TCCCTGCATCT [SEQ ID NO: 773]	UACGCCUGGCUU UCCUGCAUCU [SEQ ID NO: 1301]	UACGCCUGCUAG UCCUGCAU [SEQ ID NO: 1005]	2351	-1.91676		ACCGGACATACCTGTT CCACTCTGATGCAGGA CTAGCAGGCGTATAGT GAAATATATATTAAAT ACGCCTGCTGTTCCCTG CATCTTAGTGTGACAG	ACCGGACAUACUUGUU CCACUUCUGGUGUAGA CUAGCAGGCGUUAAGU GAAAUUAUUAUAAAU ACGCCUGGCUUCCUG CAUCUUAUAGUGUGACAG

TCGATGCAGGAC TAGCAGGCGT [SEQ ID No: 659]	UUGAUGCAGGAC UAGCAGGCGU [SEQ ID No: 1187]	ACGCTGCTATC CCTGCATCGT [SEQ ID No: 774]	AGCCUGCUAUC CCUGCAUCGU [SEQ ID No: 1302]	ACGCCUGCUAGUC CUGCAUCG [SEQ ID No: 1006]	-2.27165	2352	GGATACAGCAACTATT TTATCAA [SEQ ID No: 889]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1417]
TTTCGATGCAGGA CTAGCAGGCG [SEQ ID No: 612]	UUGAUGCAGGGA CUAGCAGGCG [SEQ ID No: 1166] (same as guide in XD- 14819)	CGCTGCTAGCA CTGCATCGAT [SEQ ID No: 775]	CGCCUGCUAGCA CUGCAUCGAU [SEQ ID No: 1303]	CGCCUGCUAGUC CUGCAUCG [SEQ ID No: 1007]	-4.78943	2353	ACCGACATACCTTGT CCACTCTTCGATGCGAG GACTAGCAGGCGTAGT GAAATATATATTAAAC GCCTGCTAGCACTGCA TCGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 890]	ACCGACATACCTTGT CCACTCTTCGATGCGAG GACTAGCAGGCGTAGT GAAATATATATTAAAC GCCTGCTAGCACTGCA TCGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1418]
TTTCGATGCAGG ACTAGCAGGC [SEQ ID No: 660]	UUUGAUGCAGG ACUAGCAGGC [SEQ ID No: 1188]	GCTGCTAGTAA TGCATCGAAT [SEQ ID No: 776]	GCCUGCUAGUAA UGCAUCGAAU [SEQ ID No: 1304]	GCCUGCUAGUCC UGCAUCGA [SEQ ID No: 1008]	-3.98959	2354	ACCGACATACCTTGT CCACTCTTCGATGCA GGACTAGCAGGCGTAGT GAAATATATATTAAAG CCTGCTAGTAAATGCAT CGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 891]	ACCGACATACCTTGT CCACTCTTCGATGCA GGACTAGCAGGCGTAGT GAAATATATATTAAAG CCTGCTAGTAAATGCAT CGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1419]
TGTTTCGATGCAG GACTAGCAGG [SEQ ID No: 661]	UGUUGAUGCAG GACUAGCAGG [SEQ ID No: 1189] (same as guide in XD- 14820)	CCTGCTAGTCAC GCATCGAACT [SEQ ID No: 777]	CCUGCUAGUCAC GCAUCGAAU [SEQ ID No: 1305]	CCUGCUAGUCCU GCAUCGAA [SEQ ID No: 1009]	-3.78415	2355	ACCGACATACCTTGT CCACTCTTCGATGCG AGGACTAGCAGGTTAGT GAAATATATATTAAAC CTGCTAGTCAGGCGATC GAACTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 892]	ACCGACATACCTTGT CCACTCTTCGATGCG AGGACTAGCAGGTTAGT GAAATATATATTAAAC CTGCTAGTCAGGCGATC GAACTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1420]
TTGTTTCGATGCA GGACTAGCAG	UUGUUGAUGCA GGACUAGCAG	CTGCTAGTCCCT CATCGAAT	CUGCUAGUCCU CAUCGAAU	CUGCUAGUCCUG CAUCGAAC	-2.10173	2356	ACCGACATACCTTGT CCACTCTTCGATGCG [SEQ ID No: 893]	ACCGACATACCTTGT CCACTCTTCGATGCG [SEQ ID No: 1421]

[SEQ ID NO: 662]	TCTGTTCGATGC AGGACTAGCA [SEQ ID NO: 663]	[SEQ ID NO: 1190]	[SEQ ID NO: 778]	[SEQ ID NO: 1306]	[SEQ ID NO: 1010]			CAGGACTAGCAGTAGT GAAATATATATTAAAC TGCTAGTCCTCTCATCG AACATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 894]	CAGGACUAGCAGUAGU GAAAUUAUUAUAAAC UGCUAGUCCCUCAUCG AACAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1422]
	UUCUGUUGAUG AGGACUAGCA [SEQ ID NO: 1191]	TGCTAGTCCTTA ATCGAACAGT [SEQ ID NO: 779]	UGCUAGUCCUUA AUCGAACAGU [SEQ ID NO: 1307]	UGUAGUCCUGC AUCGAACA [SEQ ID NO: 1011]	-1.98657	2357		ACCGACATACCTTGT CCACTCTCTGTTGAT GCAGGACTAGCATAGT GAAATATATATTAAAT GCTAGTCCTTAATCGA ACAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 895]	ACCGGACAUACUUGU CCACUCUCUGUUCGAGU GCAGGACUAGCAUAGU GAAAUUAUUAUAAAU GCUAGUCCUUAUUCGA ACAGUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1423]
	TTCTGTTCGATG CAGGACTAGC [SEQ ID NO: 664]	UUCUGUUGAUG CAGGACUAGC [SEQ ID NO: 1192]	GCTAGTCCTGAG TCGAACAGAT [SEQ ID NO: 780]	GCUAGUCCUGAG UCGAACAGU [SEQ ID NO: 1308]	GCUAGUCCUGCA UCGAACAG [SEQ ID NO: 1012]	-4.1672	2358	ACCGACATACCTTGT CCACTCTCTGTTGCGA TGCAGGACTAGCTAGT GAAATATATATTAAAG CTAGTCCTGAGTCGAA CAGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 896]	ACCGGACAUACUUGU CCACUCUCUGUUCGAGU UGCAGGACUAGCAUAGU GAAAUUAUUAUAAAG CUAGUCCUGAGUCGAA CAGAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1424]
	TCTGTTCGATG GCAGGACTAG [SEQ ID NO: 665]	UCUGUGUUGAU GCAGGACUAG [SEQ ID NO: 1193]	CTAGTCCTGCGC CGAACAGAT [SEQ ID NO: 781]	CUAGUCCUGCGC CGAACAGAGU [SEQ ID NO: 1309]	CUAGUCCUGCAU CGAACAGA [SEQ ID NO: 1013]	-1.7173	2359	ACCGACATACCTTGT CCACTCTCTGTTGCG ATGCAGGACTAGTAGT GAAATATATATTAAAC TAGTCCTGCGCCGAA AGAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 897]	ACCGGACAUACUUGU CCACUCUCUGUUCG AUGCAGGACUAGUAGU GAAAUUAUUAUAAAC UAGUCCUGCGCCGAA AGAGUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1425]
	TGAGAGAAGGAA CGTGGGTTGA [SEQ ID NO: 666]	UGAGAGAAGGAA CGUGGGUUGA [SEQ ID NO: 1194]	TCAACCCACGCC CCTTCTCTCT [SEQ ID NO: 782]	UCAACCCACGCC CCUUCUCU [SEQ ID NO: 1310]	UCAACCCACGUU CCUUCUCU [SEQ ID NO: 1014]	-0.42433	2926	ACCGACATACCTTGT CCACTCTGAGAGAAGG AACGTGGGTTGATAGT GAAATATATATTAAAT CAACCCACGCCCTTC TCTCTAGTGTGACAG	ACCGGACAUACUUGU CCACUCUGAGAGAAGG AACUGGGUUGAUAGU GAAAUUAUUAUAAAU CAACCCACGCCCUUC UCUCUAGUGUGACAG

TTGAGAGAGGA ACGTGGGTG [SEQ ID No: 667]	UUGAGAGAGGA ACGUGGGUG [SEQ ID No: 1195]	CAACCCACGTCA CTTCTCTCAT [SEQ ID No: 783]	CAACCCACGUCA CUUCUCU [SEQ ID No: 1311]	CAACCCACGUUC CUUCUC [SEQ ID No: 1015]	-3.38604	2927	GGATACAGCAACTATT TTATCAA [SEQ ID No: 898]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1426]
TCTGAGAGAGG AACGTGGGT [SEQ ID No: 668]	UCUGAGAGAGG AACGUGGGU [SEQ ID No: 1196]	AACCCACGTAA TTCTCTCAGT [SEQ ID No: 784]	AACCCACGUUA UUCUCAGU [SEQ ID No: 1312]	AACCCACGUUC UUCUCUA [SEQ ID No: 1016]	-3.5546	2928	ACCGACATACCTTGT CCACTCTCTGAGAA GGAACGTGGGTAGT GAAATATATATTA ACCCACGTAAATCTC TCAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 899]	ACCGACATACCTTGT CCACTCTCTGAGAA GGAACGTGGGTAGT GAAATATATTA ACCCACGTAAATCTC TCAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1427]
TGCTGAGAGAG GAACGTGGT [SEQ ID No: 669]	UGCUGAGAGAG GAACGUGGG [SEQ ID No: 1197]	ACCCACGTTCAC TCTCTCAGCT [SEQ ID No: 785]	ACCCACGUUAC UCUCUCAGU [SEQ ID No: 1313]	ACCCACGUUCU UCUCUCAG [SEQ ID No: 1017]	-2.96935	2929	ACCGACATACCTTGT CCACTCTCTGAGAA AGGAACGTGGGTAGT GAAATATATTA CCACGTTTCACTCTCT CAGCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 901]	ACCGACATACCTTGT CCACTCTCTGAGAA AGGAACGTGGGTAGT GAAATATATTA CCACGTTTCACTCTCT CAGCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1428]
TGGCTGAGAGAA GGAACGTGGG [SEQ ID No: 670]	UGGUGAGAGAA GGAACGUGGG [SEQ ID No: 1198]	CCACGTTTCCCC CTCTCAGCCT [SEQ ID No: 786]	CCACGUUCCCC CUCUCAGCU [SEQ ID No: 1314]	CCACGUUCCUU CUCUCAGC [SEQ ID No: 1018]	-1.84629	2930	ACCGACATACCTTGT CCACTCTCTGAGAA AAGGAACGTGGGTAGT GAAATATATTA CCACGTTTCCCCCTCTC AGCCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 902]	ACCGACATACCTTGT CCACTCTCTGAGAA AAGGAACGTGGGTAGT GAAATATATTA CCACGTTTCCCCCTCTC AGCCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1430]
TTGGCTGAGAGA AGGAACGTGG	UUGGUGAGAGAG AGGAACGUGG	CCACGTTTCTCTCA TCTCAGCCT	CCACGUUCCUCA UCUCAGCAU	CCACGUUCCUUC UCUCAGCC	-4.19621	2931	ACCGACATACCTTGT CCACTCTCTGAGAA	ACCGACATACCTTGT CCACTCTCTGAGAA

[SEQ ID NO: 671]	[SEQ ID NO: 1199]	[SEQ ID NO: 787]	[SEQ ID NO: 1315]	[SEQ ID NO: 1019]			GAAGGAACGTGGTAGT GAAATATATATTAAAC CACGTTCTCATCTCA GCCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 903]	GAAGGAACGTGGTAGT GAAATATATATTAAAC CACGTTCTCATCTCA GCCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1431]
TTTGGCTGAGAG AAGGAACGTG [SEQ ID NO: 672]	UUUGGCGAGAG AAGGAACGUG [SEQ ID NO: 1200]	CAGGTTCTTAC CTCAGCCAAT [SEQ ID NO: 788]	CAGUCCUUCAC CUCAGCAAU [SEQ ID NO: 1316]	CAGUCCUUCUC CUCAGCAA [SEQ ID NO: 1020]	-3.26413	2932	ACCGGACATCTTGT CCACTCTTTGGCTGAG AGAAGGAACGTGTAGT GAAATATATATTAAAC ACGTTCTTACCTCAG CCAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 904]	ACCGGACATCTTGT CCACTCTTTGGCTGAG AGAAGGAACGTGTAGT GAAATATATATTAAAC ACGTTCTTACCTCAG CCAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1432]
TTTTGGCTGAG GAAGGAACGT [SEQ ID NO: 673]	UUUUGGCGAGAG GAAGGAACGUG [SEQ ID NO: 1201]	AGGTTCTTCTCA TCAGCCAAT [SEQ ID NO: 789]	AGUCCUUCUCC UCAGCCAA [SEQ ID NO: 1317]	AGUCCUUCUC UCAGCCAA [SEQ ID NO: 1021]	-0.25972	2933	ACCGGACATCTTGT CCACTCTTTGGCTGAG GAGAAGGAACGTGTAGT GAAATATATATTAAAC CGTTCTTCTCAGC CAAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 905]	ACCGGACATCTTGT CCACTCTTTGGCTGAG GAGAAGGAACGTGTAGT GAAATATATATTAAAC CGTTCTTCTCAGC CAAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1433]
TCCTTTGGCTGAG AGAAGGAACG [SEQ ID NO: 674]	UCUUUGGCGAG AGAAGGAACG [SEQ ID NO: 1202]	CGTTCTTCTCA CAGCCAAAGT [SEQ ID NO: 790]	CGUCCUUCUCC CAGCCAAAGU [SEQ ID NO: 1318]	CGUCCUUCUC CAGCCAA [SEQ ID NO: 1022]	-0.75797	2934	ACCGGACATCTTGT CCACTCTTTGGCTGAG AGAAGGAACGTGTAGT GAAATATATATTAAAC GTTCTTCTCAGCC AAAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 906]	ACCGGACATCTTGT CCACTCTTTGGCTGAG AGAAGGAACGTGTAGT GAAATATATATTAAAC GTTCTTCTCAGCC AAAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1434]
TGCTTTGGCTGA GAGAAGGAAC [SEQ ID NO: 675]	UGUUUGGCGUGA GAGAAGGAAC [SEQ ID NO: 1203]	GTTCTTCTCTCA AGCCAAAGCT [SEQ ID NO: 791]	GUUCCUUCUCCA AGCCAAAGCU [SEQ ID NO: 1319]	GUUCCUUCUC AGCCAAAG [SEQ ID NO: 1023]	-1.45481	2935	ACCGGACATCTTGT CCACTCTTTGGCTGAG GAGAAGGAACGTGTAGT GAAATATATATTAAAG TTCCTTCTCAGCCCA AAGCTTAGTGTGACAG	ACCGGACATCTTGT CCACTCTTTGGCTGAG GAGAAGGAACGTGTAGT GAAATATATATTAAAG TTCCTTCTCAGCCCA AAGCTTAGTGTGACAG

AGGCTTTGGCTG AGAGAAGGAA [SEQ ID No: 676]	AGGCUUUGGCG AGAGAAGGAA [SEQ ID No: 1204]	TTCTCTCTCTAG GCCAAGCCA [SEQ ID No: 792]	UCCUUCUCUAG GCCAAGCCA [SEQ ID No: 1320]	UCCUUCUCUCA GCCAAGCC [SEQ ID No: 1024]	-0.85197	2936	GGATACAGCAACTATT TTATCAA [SEQ ID No: 907]	GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1435]
AAGGCTTTGGCT GAGAGAAGGA [SEQ ID No: 677]	AAGGCUUUGGCG GAGAGAAGGA [SEQ ID No: 1205]	TCCTTCTCTCTG CCAAAGCCTA [SEQ ID No: 793]	UCCUUCUCUCU CCAAAGCCUA [SEQ ID No: 1321]	UCCUUCUCUCAG CCAAAGCC [SEQ ID No: 1025]	-3.87114	2937	ACCGACATACCTTGT CCACTCAAGGCTTTGG CTGAGAGAAGGATAGT GAAATATATATTAAAT CCCTTCTCTGTCCTAAA GCCATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 909]	ACCGACATACCTTGT CCACTCAAGGCTTTGG CTGAGAGAAGGATAGT GAAATATATATTAAAT CCCTTCTCTGTCCTAAA GCCATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1437]
TAAGGCTTTGGC TGAGAGAAGG [SEQ ID No: 678]	UAGGCUUUGGCG UGAGAAGG [SEQ ID No: 1206]	CCTTCTCTCTATA CAAAGCCTTT [SEQ ID No: 794]	CUUCUCUCUAA CAAAGCCUUU [SEQ ID No: 1322]	CUUCUCUCUAGC CAAAGCCU [SEQ ID No: 1026]	-0.34984	2938	ACCGACATACCTTGT CCACTCAAGGCTTTG GCTGAGAGAAGGATAGT GAAATATATATTAAAC CTTCTCTATACAAAG CCTTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 910]	ACCGACATACCTTGT CCACTCAAGGCTTTG GCTGAGAGAAGGATAGT GAAATATATATTAAAC CUUCUCUAAUAAAC CUUCUCUAAUAAAC CUUCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1438]
AGAAGGCTTTGG CTGAGAGAAG [SEQ ID No: 679]	AGAAGGCUUUGG CUGAGAAG [SEQ ID No: 1207]	CTTCTCTCAGAA AAAGCCTTCA [SEQ ID No: 795]	CUUCUCUCAGAA AAAGCCUUA [SEQ ID No: 1323]	CUUCUCUCAGCC AAAGCCUU [SEQ ID No: 1027]	-2.37082	2939	ACCGACATACCTTGT CCACTCAGAAAGGCTTT GGCTGAGAGAAGGATAGT GAAATATATATTAAAC TTCTCTCAGAAAAGC CTTCTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 911]	ACCGACATACCTTGT CCACTCAGAAAGGCTTT GGCTGAGAGAAGGATAGT GAAATATATATTAAAC UUCUCUAGAAAAGC CUUCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1439]
TAGAAGGCTTTG GCTGAGAGAA	UAGAAGGCUUUG GCUGAGAGAA	TTCTCTCTCAGCAG AAGCCTTCTT	UUCUCUCAGCAG AAGCCUUCU	UUCUCUCAGCCA AAGCCUUC	-0.15876	2940	ACCGACATACCTTGT CCACTCTAAGGCTTT	ACCGACATACCTTGT CCACTCTAAGGCTTT

[SEQ ID NO: 680]	[SEQ ID NO: 1208]	[SEQ ID NO: 796]	[SEQ ID NO: 1324]	[SEQ ID NO: 1028]				TGGCTGAGAGATAGT GAAATATATATTAAAT TCTCTCAGCAGAAGCC TTCTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 912]	UUGGCUAGAGAAUAGU GAAAUUAUUAUAAU UUCUCAGCAGAAGCC UUCUUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1440]
TTAGAGGCTTT GGCTGAGAGA [SEQ ID NO: 681]	UUAAGAAGGCUU GGCUGAGAGA [SEQ ID NO: 1209] (same as guide in XD- 14858)	TCTCTCAGCCGG AGCCTTCTAT [SEQ ID NO: 797]	UCUCAGCCGG AGCCUUAU [SEQ ID NO: 1325]	UCUCAGCCAA AGCCUUCU [SEQ ID NO: 1029]	2941	-0.48849		ACCGACATATTGTT CCACTCTTAGAAGCT TTGGCTGAGAGATAGT GAAATATATATTAAAT CTCTCAGCCGAGCCT TCTATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 913]	ACCGGACAUACUUGUU CCACUCUAGAGAGGU UUGGCUAGAGAAUAGU GAAAUUAUUAUAAU CUCUCAGCCGAGCCU UCUUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1441]
AGTAGAAGGCTT TGGCTGAGAG [SEQ ID NO: 682]	AGUAGAAGGCUU UGGUGAGAG [SEQ ID NO: 1210]	CTCTCAGCCAGG GCCTTCTACA [SEQ ID NO: 798]	CUCUCAGCCAGG GCCUUAU [SEQ ID NO: 1326]	CUCUCAGCCAAA GCCUUCUA [SEQ ID NO: 1030]	2942	-2.61597		ACCGACATATTGTT CCACTCAGTAGAAGGC TTTGGCTGAGAGTAGT GAAATATATATTAAAC TCTCAGCCAGGCGCTT CTACATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 914]	ACCGGACAUACUUGUU CCACUCUAGAGAGGC UUGGCUAGAGAAUAGU GAAAUUAUUAUAAU UUCUCAGCCGAGCCU CUACUUAUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1442]
TAGTAGAAGGCT TTGGCTGAGA [SEQ ID NO: 683]	UAGUAGAAGGCU UUGGUGAGAG [SEQ ID NO: 1211]	TCTCAGCCAAAT CCTTCTACTT [SEQ ID NO: 799]	UCUCAGCCAAAGU CCUUCUACU [SEQ ID NO: 1327]	UCUCAGCCAAAG CCUUCUAC [SEQ ID NO: 1031]	2943	-2.42796		ACCGACATATTGTT CCACTCTAGTAGAAGG CTTTGGCTGAGAGTAGT GAAATATATATTAAAT CTCAGCCCAAGTCCCTC TACTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 915]	ACCGGACAUACUUGUU CCACUCUAGAGAGG CUUUGGCUAGAGAAU GAAAUUAUUAUAAU CUCAGCCCAAGCCUUC UACUUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1443]
TTAGTAGAAGGC TTTGGCTGAG [SEQ ID NO: 684]	UUAGUAGAAGGC UUUGGUGAG [SEQ ID NO: 1212]	CTCAGCCAAATA CTTCTACTAT [SEQ ID NO: 800]	CUCAGCCAAUA CUUCUACU [SEQ ID NO: 1328]	CUCAGCCAAAGC CUUCUACU [SEQ ID NO: 1032]	2944	-1.95956		ACCGACATATTGTT CCACTCTTAGTAGAAG GCTTTGGCTGAGTAGT GAAATATATATTAAAC TCAGCCCAATACTTCT ACTATTAGTGTGACAG	ACCGGACAUACUUGUU CCACUCUAGAGAGG GCUUUGGCUAGAGAAU GAAAUUAUUAUAAU UCAGCCCAAAUACUUCU ACUUAUAGUGUGACAG

TGTTAGTGAAGG CTTTGGCTGA [SEQ ID NO: 685]	(same as guide in XD- 14859)	UGUAGUAGAAGG CUUUGGCUGA [SEQ ID NO: 1213]	TCAGCCAAAGAA TTCTACTACT [SEQ ID NO: 801]	UCAGCCAAAGAA UUCUACUACU [SEQ ID NO: 1329]	UCAGCCAAAGCC UUCUACUA [SEQ ID NO: 1033]	-4.21076	2945	GGATACAGCAACTATT TTATCAA [SEQ ID NO: 916]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1444]
TGTTAGTGAAGG GCTTTGGCTG [SEQ ID NO: 686]	(same as guide in XD- 14860)	UGGUAGUAGAAG GCUUUGGCUG [SEQ ID NO: 1214]	CAGCCAAAGCAC TCTACTACTCT [SEQ ID NO: 802]	CAGCCAAAGCAC UUCUACUACU [SEQ ID NO: 1330]	CAGCCAAAGCCU UCUACUAC [SEQ ID NO: 1034]	-2.92638	2946	ACCGGACATACCTTGT CCACTCTGGTAGAGA AGGCTTTGGCTGTAGT GAAATATATATTAAAC AGCCAAAGCACTCTAC TACCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 917]	ACCGGACAUACUUGUU CCACUCUGGAGUAGA AGGCUUUGGCUGAGU GAAUUAUUAUUAAC AGCCAAAGCACUCUAC UACCUUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1445]
TGAACAAGGGG TGATTTGGGA [SEQ ID NO: 687]	(same as guide in XD- 14861)	UGAACAAAGGGG UGAUUUGGGA [SEQ ID NO: 1215]	TCCCAAATCATA CCCTTGTCT [SEQ ID NO: 803]	UCCCAAUACAUA CCCUUGUUCU [SEQ ID NO: 1331]	UCCCAAUACAGC CCCUUGUU [SEQ ID NO: 1035]	-4.19616	3301	ACCGGACATACCTTGT CCACTCTGAACAAGG GCTGATTTGGGATAGT GAAATATATATTAAAT CCCAAATCATACCTT GTTCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 918]	ACCGGACAUACUUGUU CCACUCUGAACAAGG GCUGAUUUGGAGUAGU GAAUUAUUAUUAAC CCCAAUACAACCCUU GUUCUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1446]
TTGAACAAGGG CTGATTTGGG [SEQ ID NO: 688]	UGAACAAAGGG CUGAUUUGGG [SEQ ID NO: 1216]	UUGAACAAAGGG CCTTGTTCAT [SEQ ID NO: 804]	CCCAAATCAGAA CCTTGTTCAT [SEQ ID NO: 804]	CCCAAUACAGAA CCUUGUUCU [SEQ ID NO: 1332]	CCCAAUACAGCC CCUUGUUC [SEQ ID NO: 1036]	-4.85857	3302	ACCGGACATACCTTGT CCACTCTGAACAAGG GGCTGATTTGGGATAGT GAAATATATATTAAAC CCAAATCAGAACCTTG TTCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 919]	ACCGGACAUACUUGUU CCACUCUGAACAAGG GGCUGAUUUGGAGUAGU GAAUUAUUAUUAAC CCAAUACAGAACCCUUG UUCUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1447]
TCTGAACAAGGG GCTGATTTGG	UCUGAACAAAGGG GCUGAUUUGG	CCAAATCAGCAA CTTGTTCAGT	CCAAUACAGCAA CUUGUUCAGU	CCAAUACAGCAA CUUGUUCAGU	CCAAUACAGCC CUUGUUCA	-2.36325	3303	ACCGGACATACCTTGT CCACTCTGTGAACAAG	ACCGGACAUACUUGUU CCACUCUGAACAAGG GGCUGAUUUGGAGUAGU GAAUUAUUAUUAAC CCAAUACAGAACCCUUG UUCUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1448]

[SEQ ID NO: 689]	[SEQ ID NO: 1217]	[SEQ ID NO: 805]	[SEQ ID NO: 1333]	[SEQ ID NO: 1037]			GGCTGATTGGTAGT GAAATATATATTAAAC CAATCAGCAACTTGT TCAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 921]	GGGCAGAUUUGGUAGU GAAAUUAUUAUAAAC CAAUUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1449]
TGTGGCACATGC TGAACAAGGG [SEQ ID NO: 690]	UGUGGCAUAGC UGAACAAAGGG [SEQ ID NO: 1218]	CCCTTGTTCATA ATGTGCCACT [SEQ ID NO: 806]	CCUUGUUAUA AUGGCCACU [SEQ ID NO: 1334]	CCUUGUUAAGC AUGGCCA [SEQ ID NO: 1038]	-2.10246	3313	ACCGACATACCTTGT CCACTCTGTGGCACAT GCTGAACAAGGGTAGT GAAATATATATTAAAC CCTTGTTCATAATGTG CCACTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 922]	ACCGGACAUACUUGUU CCACUCUGUGGCACAU GCUGAACAAAGGUAGU GAAUUAUUAUUAAC CCUUGUUAUUAUGUG CCACUUAUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1450]
TTGCCATCATTC TAGCATTACC [SEQ ID NO: 691]	UUGGCAUUAUC UAGCAUUAACC [SEQ ID NO: 1219]	GGTAATGCTATG ATGATGGCAT [SEQ ID NO: 807]	GGUAAUGCUAUG AUGAUGGCAU [SEQ ID NO: 1335]	GGUAAUGCUAGA AUGAUGGC [SEQ ID NO: 1039]	-0.43513	3378	ACCGACATACCTTGT CCACTCTGTGGCACAT TCTAGCAATACCTTAGT GAAATATATATTAAAG GTAATGCTATGATGAT GGCATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 923]	ACCGGACAUACUUGUU CCACUCUGUGGCACAU UCUAGCAUUAUUAAG GAAUUAUUAUUAAC GUAUUGCUAUGAUGAU GGCAUUAUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1451]
TTGCTGGGAAAC TATTCTGTGG [SEQ ID NO: 692]	UUGCUGGGAAC UAUUCUGUGG [SEQ ID NO: 1220] (same as guide in XD-14861)	CCACAGAATATC TTCCCAGCAT [SEQ ID NO: 808]	CCACAGAAUATC UUCCCAGCAU [SEQ ID NO: 1336]	CCACAGAAUAGU UUCCCAGC [SEQ ID NO: 1040]	-2.70652	3804	ACCGACATACCTTGT CCACTCTGTGGGAA ACTATTCTGTGGTAGT GAAATATATATTAAAC CACAGAATATCTTCCC AGCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 924]	ACCGGACAUACUUGUU CCACUCUGUGGGGAA ACUUAUCUGUGGUAGU GAAUUAUUAUUAAC CACAGAAUUAUCUCCC AGCAUUAUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1452]
TCTGTGGGAAA CTATTCTGTG [SEQ ID NO: 693]	UCUGCUGGAAA CUAUUCUGUG [SEQ ID NO: 1221]	CACAGAATAGCC TCCCAGCAGT [SEQ ID NO: 809]	CACAGAAUAGCC UCCCAGCAGU [SEQ ID NO: 1337]	CACAGAAUAGUU UCCCAGCA [SEQ ID NO: 1041]	-4.91212	3805	ACCGACATACCTTGT CCACTCTGTGGGAA AATATTCTGTGGTAGT GAAATATATATTAAAC ACAGAATAGCCTCCCA GCAGTTAGTGTGACAG	ACCGGACAUACUUGUU CCACUCUGUGGGGAA AACUUAUCUGUGUAGU GAAUUAUUAUUAAC ACAGAAUAGCCUCCCA GCAGUUAUGUGGACAG

TGCTGCTGGGAA ACTATTCTGT [SEQ ID No: 694]	UGUGUCUGGGAA ACUAUUCUGU [SEQ ID No: 1222]	ACAGATAGTCC CCAGCAGCT [SEQ ID No: 810]	ACAGAAUAGUCC CCCAGAGCU [SEQ ID No: 1338]	ACAGAAUAGUU CCCAGAG [SEQ ID No: 1042]	-3.54976	3806	GGATACAGCAACTATT TTATCAA [SEQ ID No: 925]	GGAAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1453]
TGAACGTGAGAA GGATGGATCG [SEQ ID No: 695]	UGAACGUGAGAA GGAUGGAUCG [SEQ ID No: 1223]	CGATCCATCCCC CTCAGGTTCT [SEQ ID No: 811]	CGAUCCAUCUCC CUCACGUU [SEQ ID No: 1339]	CGAUCCAUCUU CUCACGUU [SEQ ID No: 1043]	-3.0586	3844	ACCGACATACCTTGT CCACTCTGAACGTGAG AAGGATGGATCGTAGT GAAATATATATTAAAC GATCCATCCCCCTCAC GTTCTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 926]	ACCGACATACCTTGT CCCGACAUACUUGUU AAGGATGGATCGTAGT GAAUUAUUAUUAAC GAUCCAUCCCCUCAC GUUCUAGUGUGACAG GGAAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1454]
TTGAACGTGAGA AGGATGGATC [SEQ ID No: 696]	UUGAACGUGAGAA AGGAUGGAUC [SEQ ID No: 1224]	GATCCATCCTCA TCACGTTTCT [SEQ ID No: 812]	GAUCCAUCUCCA UCACGUUCAU [SEQ ID No: 1340]	GAUCCAUCUUC UCACGUUC [SEQ ID No: 1044]	-4.40112	3845	ACCGACATACCTTGT CCACTCTGAACGTGAG GAGGATGGATCGTAGT GAAATATATATTAAAG ATCCATCCTCATCACG TTCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 927]	ACCGACATACCTTGT CCCGACAUACUUGUU GAGGATGGATCGTAGT GAAUUAUUAUUAAG AUCCAUCCUACUACAG UUCAUUAGUGUGACAG GGAAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1455]
TCTGAACGTGAG AAGGATGGAT [SEQ ID No: 697]	UCUGAACGUGAG AAGGAUGGAU [SEQ ID No: 1225]	ATCCATCCTTAC CACGTTTCTAGT [SEQ ID No: 813]	AUCCAUCUUAAC CACGUUCAU [SEQ ID No: 1341]	AUCCAUCUUCU CAGGUUCA [SEQ ID No: 1045]	-2.54571	3846	ACCGACATACCTTGT CCACTCTGAACGTGAG AGAAGGATGGATTAGT GAAATATATATTAAAG TCCATCCTTACCAAGT TCAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 928]	ACCGACATACCTTGT CCCGACAUACUUGUU CAGGATGGATCGTAGT GAAUUAUUAUUAAG UCCAUCCUACCAAGT UCAGUAGUGUGACAG GGAAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1456]
AACTGTTAGCAT TCTTATTGGA	AACUGUUAAGCAU UCCUAUUGGA	TCCAATAGGAGC GCTAACAGTA	UCCAAUAGGAGC GCUAACAGUA	UCCAAUAGGAU GCUAACAG	-3.8378	4235	ACCGACATACCTTGT CCACTCAACTGTTAGC [SEQ ID No: 929]	ACCGACATACCTTGT CCCGACAUACUUGUU AAGGATGGATCGTAGT GAAUUAUUAUUAAG UCCAUCCUACCAAGT UCAGUAGUGUGACAG GGAAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1457]

[SEQ ID NO: 698] TAACGTGTTAGC TTCTTATTGG [SEQ ID NO: 699]	[SEQ ID NO: 1226] UACUGUUAGCA UUCUUAUUGG [SEQ ID NO: 1227]	[SEQ ID NO: 814] CCAATAGGAAC CTAACAGTTT [SEQ ID NO: 815]	[SEQ ID NO: 1342] CCAUAGGAACU CUAACAGUU [SEQ ID NO: 1343]	[SEQ ID NO: 1046] CCAUAAGGAUG CUAACAGU [SEQ ID NO: 1047]		4236 -2.52035	ATTCTATTGGATAGT GAAATATATTTAAAT CCAATAGGAGCGCTAA CAGTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 930] [SEQ ID NO: 1458]	AUCCUAUUGGAUAGU GAAUAUAUAUUAUU CCAUAAGAGCGCUAA CAGUAUAGUGUGACAG GGUAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1459]
[SEQ ID NO: 700] TGAACTGTTAGC ATTCTATTGG [SEQ ID NO: 701]	[SEQ ID NO: 1228] UGAACUGUUAGC AUUCCUAUUG [SEQ ID NO: 1229]	[SEQ ID NO: 816] CAATAGGAATTA TAACAGTTCT [SEQ ID NO: 817]	[SEQ ID NO: 1344] CAUAGGAUUA UAACAGUUCU [SEQ ID NO: 1345]	[SEQ ID NO: 1048] CAUAGGAUUGC UAACAGUUC [SEQ ID NO: 1049]		4237 -4.90789	ACCGACATCTTGT CCACTCTGAACCTGTTA GCATTCTTATTGTAGT GAAATATATTTAAAC AATAGGAATTTAAACA GTTCTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 931] [SEQ ID NO: 1460]	ACCGACAUACUUGUU CCACUCUAGACUGUU GCAUCCUAUUGUAGU GAAUAUAUAUUAUU AAUAGGAUUAUUAACA GUUCUUAUGUGUGACAG GGUAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1461]
[SEQ ID NO: 702] TTGAACTGTTAG CATTCTCTAT [SEQ ID NO: 703]	[SEQ ID NO: 1230] UGAACUGUUUA CAUCCUAUUG [SEQ ID NO: 1231]	[SEQ ID NO: 818] AATAGGAATGCCG ACAGTTTACA [SEQ ID NO: 819]	[SEQ ID NO: 1346] AAUAGGAUUGCG AACAGUUCU [SEQ ID NO: 1347]	[SEQ ID NO: 1050] AAUAGGAUUGCU AACAGUUC [SEQ ID NO: 1051]		4238 -3.95764	ACCGACATCTTGT CCACTCTTGAACCTGTT AGCATTCCTATTGTAGT GAAATATATTTAAAC AATAGGAATGACACAG TTCAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 932] [SEQ ID NO: 1462]	ACCGACAUACUUGUU CCACUCUAGACUGUU AGCAUCCUAUUGUAGU GAAUAUAUAUUAUU AAUAGGAUUAUUAACA GUUCUUAUGUGUGACAG GGUAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1463]
[SEQ ID NO: 704] AGTGAACCTGTTA GCATTCTCTAT [SEQ ID NO: 705]	[SEQ ID NO: 1232] AGUAAACUGUUUA GCAUCCUAUUG [SEQ ID NO: 1233]	[SEQ ID NO: 820] ATAGGAATGCCG ACAGTTTACA [SEQ ID NO: 821]	[SEQ ID NO: 1348] AAUAGGAUUGCG AACAGUUCU [SEQ ID NO: 1349]	[SEQ ID NO: 1052] AAUAGGAUUGCU AACAGUUC [SEQ ID NO: 1053]		4239 -4.39189	ACCGACATCTTGT CCACTCTTGAACCTGTT TAGCATTCCTATTGTAGT GAAATATATTTAAAC TAGGAATGCCGACAGT TCACATAGTGTGACAG	ACCGACAUACUUGUU CCACUCUAGACUGUU UAGCAUCCUAUUGUAGU GAAUAUAUAUUAUU UAGGAUUGCCGACAGU UCACAUAGUGUGACAG

AAGTGAAGTGT AGCATTCCTA [SEQ ID No: 703]	AAGUGAACUGUU AGCAUUCUA [SEQ ID No: 1231]	TAGGAATGCTGG CAGTTTCACTA [SEQ ID No: 819]	UAGGAUUGCUGG CAGUUCACUA [SEQ ID No: 1347]	UAGGAUUGCUAA CAGUUCAC [SEQ ID No: 1051]	-4.80102	4240	GGATACAGCAACTATT TTATCAA [SEQ ID No: 934]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1462]
TAAGTGAAGTGT TAGCATTCCT [SEQ ID No: 704]	UAAGUGAACUGU UAGCAUUCU [SEQ ID No: 1232]	AGGAATGCTAGA AGTTCACTTT [SEQ ID No: 820]	AGGAUUGCUAGA AGUUCACUU [SEQ ID No: 1348]	AGGAUUGCUAAC AGUUCACU [SEQ ID No: 1052]	-2.45702	4241	ACCGACATACCTTGT CCACTCTAAGTGAAC GTTAGCAITCCTTAGT GAAATATATATATAA GGAATGCTAGAGTTC ACTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 935]	ACCGACATACCTTGT CCACTCTAAGTGAAC GTTAGCAITCCTTAGT GAAATATATATAA GGAATGCTAGAGTTC ACTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1463]
TCAAGTGAAGTGT TTAGCATTC [SEQ ID No: 705]	UCAAGUGAACUG UAGCAUUC [SEQ ID No: 1233]	GGAATGCTAAG GTTCACTTGT [SEQ ID No: 821]	GGAUUGCUAAG GUUCACUUGU [SEQ ID No: 1349]	GGAUUGCUAACA GUUCACUU [SEQ ID No: 1053]	-4.855	4242	ACCGACATACCTTGT CCACTCTAAGTGAAC TGTAGCATTCCTTAGT GAAATATATATAA GGAATGCTAAGGTTCA CTTGTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 937]	ACCGACATACCTTGT CCACTCTAAGTGAAC TGTAGCATTCCTTAGT GAAATATATATAA GGAATGCTAAGGTTCA CTTGTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1464]
TGCAAGTGAAGTGT GTTAGCATTC [SEQ ID No: 706]	UGCAAGUGAACU GUUAGCAUUC [SEQ ID No: 1234]	GAATGCTAAGCT TTCACCTTGT [SEQ ID No: 822]	GAAUGCUAAGU UUCACUUGU [SEQ ID No: 1350]	GAAUGCUAACAG UUCACUUG [SEQ ID No: 1054]	-4.05556	4243	ACCGACATACCTTGT CCACTCTAAGTGAAC CTGTAGCATTCCTTAGT GAAATATATATAA AATGCTAAGCTTTCAC TTGCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 938]	ACCGACATACCTTGT CCACTCTAAGTGAAC CTGTAGCATTCCTTAGT GAAATATATATAA AATGCTAAGCTTTCAC TTGCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1465]
TTGCAAGTGAAG TTGTTAGCATTC	UUGCAAGUGAAC UGUAGCAUU	AATGCTAAGCATC TTCACCTTGCAT	AAUGCUAACAU UACUUGCAU	AAUGCUAACAGU UACUUGC	-3.92834	4244	ACCGACATACCTTGT CCACTCTTTCAGAGTGA [SEQ ID No: 1466]	ACCGACATACCTTGT CCACTCTTTCAGAGTGA [SEQ ID No: 1466]

[SEQ ID NO: 707] ACTGCAAGTGA CTGTTAGCAT [SEQ ID NO: 708]	[SEQ ID NO: 1235] ACUGCAAGUGAA CUGUAGCAU [SEQ ID NO: 1236]	[SEQ ID NO: 823] ATGCTAACAGCC CACTTGACAGA [SEQ ID NO: 824]	[SEQ ID NO: 1351] AUGCUAACAGCC CACUUGCAGA [SEQ ID NO: 1352]	[SEQ ID NO: 1055] AUGCUAACAGUU CACUUGCA [SEQ ID NO: 1056]	-4.32689	4245	ACTGTTAGCATTTAGT GAAATATATATTAAAA ATGCTAACATCTCACT TGCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 939]	ACUGUAGCAUUUAGU GAAAUUAUUUUAAAA AUGCUAACAUUCACU UGCAUUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID NO: 1467]
[SEQ ID NO: 709] TACTGCAAGTGA ACTGTTAGCA [SEQ ID NO: 710]	[SEQ ID NO: 1237] UACUGCAAGUGA ACUGUAGCA [SEQ ID NO: 1238]	[SEQ ID NO: 825] TGCTAACAGTCA ACTTGCAAGT [SEQ ID NO: 826]	[SEQ ID NO: 1353] UGCUAACAGUGA ACUUGCAGUU [SEQ ID NO: 1354]	[SEQ ID NO: 1057] UGCUAACAGUUC ACUUGCAG [SEQ ID NO: 1058]	-4.77129	4246	ACCGGACATACCTGTT CCACTCTACTGCAAGT GAACTGTTAGCATAGT GAAATATATATTAAAA GCTAACAGTCAACTTG CAGTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 941]	ACCGGACAUACUUGUU CCACUCUACUGCAAGU GAAUUAUUUUAAAA GCUAACAGUCAACUUG CAGUUUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID NO: 1468]
[SEQ ID NO: 711] TCACTGCAAGTG AACTGTTAGC [SEQ ID NO: 712]	[SEQ ID NO: 1239] UCACUGCAAGUG AACUGUAGC [SEQ ID NO: 1240]	[SEQ ID NO: 827] GCTAACAGTTAG CTTGCAAGTGT [SEQ ID NO: 828]	[SEQ ID NO: 1355] GCUAACAGUUAG CUUGCAGUGU [SEQ ID NO: 1356]	[SEQ ID NO: 1059] GCUAACAGUUCAC CUUGCAGUG [SEQ ID NO: 1060]	-3.83727	4247	ACCGGACATACCTGTT CCACTCTACTGCAAG TGAACTGTTAGTGTAGT GAAATATATATTAAAA CTAACAGTTAGCTTGC AGTGTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 942]	ACCGGACAUACUUGUU CCACUCUACUGCAAG UGAACUGUUAGCUAGU GAAAUUAUUUUAAAA CUAACAGUUAGCUUGC AGUUUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID NO: 1470]
[SEQ ID NO: 713] TCCACTGCAAGT GAACTGTTAG [SEQ ID NO: 714]	[SEQ ID NO: 1241] UCCACUGCAAGU GAACUGUAG [SEQ ID NO: 1242]	[SEQ ID NO: 829] CTAACAGTTTGA TTGCAAGTGGT [SEQ ID NO: 830]	[SEQ ID NO: 1357] CUAACAGUUCGA UUGCAGUGGU [SEQ ID NO: 1358]	[SEQ ID NO: 1061] CUAACAGUUCAC UUGCAGUG [SEQ ID NO: 1062]	-4.9039	4248	ACCGGACATACCTGTT CCACTCTACTGCAAG GTGAACCTGTTAGTGTAGT GAAATATATATTAAAA TACAGTTGATTGCA GTGGTTAGTGTGACAG	ACCGGACAUACUUGUU CCACUCUACUGCAAG GUGAACUGUUAGUAGU GAAAUUAUUUUAAAA UACAGUUCGAAUUGCA GUGGUUAGUGUGACAG

TTCCACTGCAAG TGAAGTGTGA [SEQ ID No: 712]	UCCACUGCAAG UGAACUGUUA [SEQ ID No: 1240]	TAACAGTTCAAC TGCAAGTGGAT [SEQ ID No: 828]	UACAGUUCAC UGCAGUGGAU [SEQ ID No: 1356]	UACAGUUCACU UGCAGUGG [SEQ ID No: 1060]	-3.84653	4249	GGATACAGCAACTATT TTATCAA [SEQ ID No: 943]	GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1471]
TTTCCACTGCAA GTGAAGTGT [SEQ ID No: 713]	UUUCCACUGCAA GUGAACUGUU [SEQ ID No: 1241]	AACAGTTCAACC GCAGTGGAAAT [SEQ ID No: 829]	AACAGUUCACCC GCAGUGGAU [SEQ ID No: 1357]	AACAGUUCACUU GCAGUGGA [SEQ ID No: 1061]	-3.95941	4250	ACCGACATACCTTGT CCACTCTTCCACTGC AAGTGAAGTGTGTAGT GAAATATATATATAAA ACAGTTCAACCCGAGT GGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 944]	ACCGACATACCTTGT CCACTCTTCCACTGC AAGTGAAGTGTGTAGT GAAATATATATAAA ACAGTTCAACCCGAGT GGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1472]
TCTTCCACTGCA AGTGAAGTGT [SEQ ID No: 714]	UCUCCACUGCA AGUGAACUGU [SEQ ID No: 1242]	ACAGTTCACTCT CAGTGGAGT [SEQ ID No: 830]	ACAGUUCACUU CAGUGGAAGU [SEQ ID No: 1358]	ACAGUUCACUUG CAGUGGA [SEQ ID No: 1062]	-4.68439	4251	ACCGACATACCTTGT CCACTCTTCCACTGC CAGTGAAGTGTGTAGT GAAATATATATAAA CAGTTCACTCTCAGT GAAGTGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 945]	ACCGACATACCTTGT CCACTCTTCCACTGC CAGTGAAGTGTGTAGT GAAATATATATAAA CAGTTCACTCTCAGT GAAGTGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1473]
ATCTTCCACTGC AAGTGAAGTGT [SEQ ID No: 715]	AUCUCCACUGC AAGUGAACUG [SEQ ID No: 1243]	CAGTTCACTTTTA AGTGGAGAA [SEQ ID No: 831]	CAGUUCACUUUA AGUGGAAGAA [SEQ ID No: 1359]	CAGUUCACUUGC AGUGGAAG [SEQ ID No: 1063]	-2.98094	4252	ACCGACATACCTTGT CCACTCTTCCACTGC CAGTGAAGTGTGTAGT GAAATATATATAAA AGTTCACTTTAAGTGG AAGAAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 946]	ACCGACATACCTTGT CCACTCTTCCACTGC CAGTGAAGTGTGTAGT GAAATATATATAAA AGTTCACTTTAAGTGG AAGAAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1474]
TATCTTCCACTG CAAGTGAAGT	UAUGUCCACUG CAAGUGAACU	AGTTCACTTTGAG GTGGAGAGATT	AGUUCACUUGAG GUGGAAGAU	AGUUCACUUGCA GUGGAAGA	-3.46197	4253	ACCGACATACCTTGT CCACTCTTCCACTGC [SEQ ID No: 947]	ACCGACATACCTTGT CCACTCTTCCACTGC [SEQ ID No: 1475]

[SEQ ID NO: 716] TGGAAGCAGAG CTGGGTACC [SEQ ID NO: 717]	[SEQ ID NO: 1244] UGGAAGCAGAG CUGGGUACC [SEQ ID NO: 1245]	[SEQ ID NO: 832] GGTACCCAGAC CTGCTTGCT [SEQ ID NO: 833]	[SEQ ID NO: 1360] GGUACCCAGAC CUGUUGCCU [SEQ ID NO: 1361]	[SEQ ID NO: 1064] GGUACCCAGCUC CUGUUGCC [SEQ ID NO: 1065]		4348		TGCAAGTGAACCTTAGT GAAATATATATTA GTTCACTTGAAGTGA AGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 948] ACCGACATACCTTGT CCACTCTGGCAAGCAG AGCTGGGTACCTTAGT GAAATATATATTAAG GTACCCAGACCTGCT TGCCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 949]	UACAAGUAGCUUAGU GAAAUUAUUAUAAA GUUCACUUGAGGUGA AGAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1476] ACCGACAUACUUGUU CCACUCUGGCAAGCAG AGCUGGGUACCUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1477]
[SEQ ID NO: 718] TCGGCAAGCAGA GCTGGGTAC [SEQ ID NO: 719]	[SEQ ID NO: 1246] UCGGCAAGCAGA GCUUGGGUAC [SEQ ID NO: 1247]	[SEQ ID NO: 834] GTACCCAGACCA TGCTTGCCGT [SEQ ID NO: 835]	[SEQ ID NO: 1362] GUACCCAGCCG UGCUUGCCG [SEQ ID NO: 1363]	[SEQ ID NO: 1066] GUACCCAGCUC UGCUUGCC [SEQ ID NO: 1067]		4349		ACCGACATACCTTGT CCACTCTGGCAAGCAG GAGCTGGGTACTTAGT GAAATATATATTAAG TACCCAGACCTGCT GCCGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 950] ACCGACATACCTTGT CCACTCTGGCAAGCAG AGAGCTGGGTACTAGT GAAATATATATTAAG ACCCAGCTACGCTTG CCGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 951]	UACAAGUAGCUUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1478] ACCGACAUACUUGUU CCACUCUGGCAAGCAG AGCUGGGUACCUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1479]
[SEQ ID NO: 720] TTTCGGCAAGCA GAGCTGGGT [SEQ ID NO: 721]	[SEQ ID NO: 1248] UUUCGGCAAGCA GAGCUGGGGU [SEQ ID NO: 1249]	[SEQ ID NO: 836] ACCCAGCTCCT CTTGCCGAAT [SEQ ID NO: 837]	[SEQ ID NO: 1364] ACCCAGCUCCU CUUGCCGAU [SEQ ID NO: 1365]	[SEQ ID NO: 1068] ACCCAGCUCUG CUUGCCGA [SEQ ID NO: 1069]		4351		ACCGACATACCTTGT CCACTCTGGCAAGCAG CAGAGCTGGGTACTAGT GAAATATATATTAAG CCAGCTCCTCTTGC CGAATTAGTGTGACAG [SEQ ID NO: 1480] ACCGACAUACUUGUU CCACUCUGGCAAGCAG AGCUGGGUACCUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1481]	UACAAGUAGCUUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1482] ACCGACAUACUUGUU CCACUCUGGCAAGCAG AGCUGGGUACCUAGU GAAAUUAUUAUAAA GUACCCAGACCUUGU UGCCUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1483]

TTTTTCGGCAAGC AGAGCTGGGG [SEQ ID No: 721]	UUUUCGGCAAGC AGAGCUGGGG [SEQ ID No: 1249]	CCGAGCTCTTAA TTGCCGAAAT [SEQ ID No: 837]	CCCAGCUCUUA UUGCCGAAAU [SEQ ID No: 1365]	CCCAGCUCUGC UUGCCGAA [SEQ ID No: 1069]	-2.43883	4352	GGATACAGCAACTATT TTATCAA [SEQ ID No: 952]	GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1480]
AGTTTCGGCAAG CAGAGCTGGG [SEQ ID No: 722]	AGUUUCGGCAAG CAGAGCUGGG [SEQ ID No: 1250]	CCGAGCTCTGAC TGCCGAAACA [SEQ ID No: 838]	CCCAGCUCUGAC UUGCCGAAACA [SEQ ID No: 1366]	CCCAGCUCUGCU UGCCGAAA [SEQ ID No: 1070]	-3.70458	4353	ACCGGACATACCTTGT CCACTCAGTTTCGGCA AGCAGAGCTGGGTAGT GAAATATATATTAAAC CCAGCTCTGACTGCCG AAACATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 953]	ACCGGACAUACUUGUU CCACUCAGUUUCGGCA AGCAGAGCUGGUAGU GAAUUAUUAUUAAC CCAGCUCUGACUGCCG AAACUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1481]
TAGTTTCGGCAA GCAGAGCTGG [SEQ ID No: 723]	UAGUUUCGGCAA GCAGAGCUGG [SEQ ID No: 1251]	CCAGCTCTGCCC GCCGAAACTT [SEQ ID No: 839]	CCAGCUCUGCCC GCCGAAACUU [SEQ ID No: 1367]	CCAGCUCUGCUU GCCGAAAC [SEQ ID No: 1071]	-2.834	4354	ACCGGACATACCTTGT CCACTCAGTTTCGGC AAGCAGAGCTGGGTAGT GAAATATATATTAAAC CAGCTCTGCCGCCGA AACTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 954]	ACCGGACAUACUUGUU CCACUCAGUUUCGGC AAGCAGAGCUGGUAGU GAAUUAUUAUUAAC CAGCUCUGCCGCCGA AACUUUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1482]
TCAGTTTCGGCA AGCAGAGCTG [SEQ ID No: 724]	UCAGUUUCGGCA AGCAGAGCUG [SEQ ID No: 1252]	CAGCTCTGCTCT CCGAAACTGT [SEQ ID No: 840]	CAGCUCUGCUU CCGAAACUGU [SEQ ID No: 1368]	CAGCUCUGCUUG CCGAAACU [SEQ ID No: 1072]	-4.92603	4355	ACCGGACATACCTTGT CCACTCAGTTTCGG CAGCAGAGCTGTAGT GAAATATATATTAAAC AGCTCTGCTCTCCGAA ACTGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 955]	ACCGGACAUACUUGUU CCACUCAGUUUCGG CAAGCAGAGCUGUAGU GAAUUAUUAUUAAC AGCUCUGCUCUCCGAA ACUGUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1483]
TCCAGTTTCGGC AAGCAGAGCT	UCCAGUUUCGGC AAGCAGAGCU	AGCTCTGCTTTA CGAAACTGGT	AGCUCUGCUUUA CGAAACUGGU	AGCUCUGCUUGC CGAAACUG	-4.91921	4356	ACCGGACATACCTTGT CCACTCTCCAGTTTCG	ACCGGACAUACUUGUU CCACUCAGUUUCGG CCAGCAGAGCUGUAGU GAAUUAUUAUUAAC AGCUCUGCUCUCCGAA ACUGUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1484]

[SEQ ID NO: 725]	[SEQ ID NO: 1253] (same as guide in XD-14861)	[SEQ ID NO: 841]	[SEQ ID NO: 1369]	[SEQ ID NO: 1073]			GCAAGCAGAGCTTAGT GAAATATATATTAAAA GCTCTGCTTTACGAAA CTGGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 957] [SEQ ID NO: 1485]	GAAAGCAGAGCUUAGU GAAAUUAUUUAAAA GCUCUGCUUACGAAA CUGGUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1486]
TTCCAGTTTCGG CAAGCAGAGC [SEQ ID NO: 726]	UUCAGUUUCGG CAAGCAGAGC [SEQ ID NO: 1254]	GCTCTGCTTGAA GAAACTGGAT [SEQ ID NO: 842]	GCUCUGCUUGAA GAAACUGGAU [SEQ ID NO: 1370]	GCUCUGCUUGCC GAAACUGG [SEQ ID NO: 1074]	-4.12374	4357	ACCGGACATACCTGTT CCACTCTTCCAGTTTC GGCAAGCAGAGCTAGT GAAATATATATTAAAG CTCTGCTTGAAGAAAC TGGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 958] [SEQ ID NO: 1486]	ACCGGACAUACUUGUU CCACUCUUCGAGUUUC GGCAAGCAGAGCUAGU GAAAUUAUUUAAAA CUCUGCUUGAAGAAAC UGGAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1487]
TTTCCAGTTTCG GCAAGCAGAG [SEQ ID NO: 727]	UUUCCAGUUUCG GCAAGCAGAG [SEQ ID NO: 1255]	CTCTGCTTGCAAT AAACTGGAAT [SEQ ID NO: 843]	CUCUGCUUGCAU AAACUGGAU [SEQ ID NO: 1371]	CUCUGCUUGCCG AAACUGGA [SEQ ID NO: 1075]	-4.13551	4358	ACCGGACATACCTGTT CCACTCTTCCAGTTTC CGGCAAGCAGAGTAGT GAAATATATATTAAAC TCTGCTTGCAATAACT GGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 959] [SEQ ID NO: 1487]	ACCGGACAUACUUGUU CCACUCUUCGAGUUUC CGGCAAGCAGAGUAGU GAAAUUAUUUAAAA CUCUGCUUGAAGAAAC UGGAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1488]
ACTTCCAGTTTC GGCAAGCAGAG [SEQ ID NO: 728]	ACUCCAGUUUC GGCAAGCAGAG [SEQ ID NO: 1256]	TCTGCTTGCTGCTG AACTGGAAGA [SEQ ID NO: 844]	UCUGCUUGCCUG AACUGGAAGA [SEQ ID NO: 1372]	UCUGCUUGCCGA AACUGGAA [SEQ ID NO: 1076]	-4.43988	4359	ACCGGACATACCTGTT CCACTCACTTCCAGTT TCGGCAAGCAGAGTAGT GAAATATATATTAAAT CTGCTTGCTGAACTG GAAGATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 960] [SEQ ID NO: 1488]	ACCGGACAUACUUGUU CCACUCUUCGAGUUUC UCGGCAAGCAGAGUAGU GAAAUUAUUUAAAA CUCUGCUUGAAGAAAC UGGAUUAGUGUGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1489]
AATTCCAGTTT CGGCAAGCAG [SEQ ID NO: 729]	AACUCCAGUUU CGGCAAGCAG [SEQ ID NO: 1257]	CTGCTTGCTGCTG AACTGGAAGTA [SEQ ID NO: 845]	CUGCUUGCCGGG ACUGGAAGUA [SEQ ID NO: 1373]	CUGCUUGCCGAA ACUGGAAG [SEQ ID NO: 1077]	-3.57411	4360	ACCGGACATACCTGTT CCACTCACTTCCAGTT TCGGCAAGCAGAGTAGT GAAATATATATTAAAC TGCTTGCTGCTGCTG AAAGTATAGTGTGACAG [SEQ ID NO: 961] [SEQ ID NO: 1490]	ACCGGACAUACUUGUU CCACUCUUCGAGUUUC UUCGGCAAGCAGUAGU GAAAUUAUUUAAAA UGCUUGCCGGGACUGG AAGTATAGTGTGACAG [SEQ ID NO: 1491]

TAATCTCCAGTT TCGGCAAGCA [SEQ ID No: 730]	UAAUUCAGUU UCGGCAAGCA [SEQ ID No: 1258]	TGCTTGCCGAGG CTGGAAGTTT [SEQ ID No: 846]	UGUUGCCGAGG CUGGAAGUU [SEQ ID No: 1374]	UGUUGCCGAAA CUGGAAGU [SEQ ID No: 1078]	-3.33142	4361	GGATACAGCAACTATT TTATCAA [SEQ ID No: 961]	GGAUACAGCAACUAAU UUAUCAA [SEQ ID No: 1489]
ATAACTTCCAGT TTGGCAAGC [SEQ ID No: 731]	AUAACUUCAGU UUCGCAAGC [SEQ ID No: 1259] (same as guide in XD- 14932)	GCTTGCCGAGG TGGAAGTTAA [SEQ ID No: 847]	GCUUGCCGAGG UGGAAGUAA [SEQ ID No: 1375]	GCUUGCCGAAA UGGAAGUU [SEQ ID No: 1079]	-3.93535	4362	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 963]	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1491]
ATAACTTCCAG TTTCGGCAAG [SEQ ID No: 732]	AAUAAUUCAG UUUCGGCAAG [SEQ ID No: 1260]	CTTGCCGAGG GGAAGTTATA [SEQ ID No: 848]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1376]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1080]	-3.56666	4363	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 964]	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1492]
ATAACTTCCAG GTTTCGGCAAG [SEQ ID No: 733]	AAUAAUUCAG UUUCGGCAAG [SEQ ID No: 1261]	CTTGCCGAGG GGAAGTTATA [SEQ ID No: 849]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1377]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1081]	-2.9553	4364	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 965]	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1493]
ATAACTTCCAG GTTTCGGCAAG [SEQ ID No: 734]	AAUAAUUCAG UUUCGGCAAG [SEQ ID No: 1262]	CTTGCCGAGG GGAAGTTATA [SEQ ID No: 850]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1378]	CUGCCGAGG GGAAGUAA [SEQ ID No: 1082]	-3.3862	4365	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 966]	ACCGACATACCTTGT CCACTCAATACCTTCCA GTTTCGGCAAGCTAGT GAAATATATATTAAAG CTTGCCGAGGATGGA GTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1494]

[SEQ ID NO: 734]	[SEQ ID NO: 1262]	[SEQ ID NO: 850]	[SEQ ID NO: 1378]	[SEQ ID NO: 1082]			TCCAGTTTCGGCTAGT GAAATATATATTAAAG CCGAAACTGTGAGTTA TTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 966]	TCCAGTTTCGGCTAGT GAAATATATATTAAAG CCGAAACTGTGAGTTA TTTAATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1494]
AAATAATAACTT CCAGTTTCGG [SEQ ID NO: 735]	AAUAAUAAACUU CCAGUUCGG [SEQ ID NO: 1263]	CCGAAACTGGGG GTATTATTATA [SEQ ID NO: 851]	CCGAAACUGGGG GUUUUUUA [SEQ ID NO: 1379]	CCGAAACUGGAA GUUUUUUA [SEQ ID NO: 1083]	-3.45626	4367	ACCGGACATACCTTGT CCACTCAATAATAAC TTCAGTTTCGGTAGT GAAATATATATTAAAC CGAACTGGGGTTAT TTATATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 967]	ACCGGACATACCTTGT CCACTCAATAATAAC TTCAGTTTCGGTAGT GAAATATATATTAAAC CGAACTGGGGTTAT TTATATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1495]
AAATAATAACT TCCAGTTTCG [SEQ ID NO: 736]	AAUAAUAAACU UCCAGUUCG [SEQ ID NO: 1264]	GAAACTGGAGT TTATTATTATA [SEQ ID NO: 852]	CGAAACUGGAGU UUUUUUUA [SEQ ID NO: 1380]	CGAAACUGGAAG UUUUUUUA [SEQ ID NO: 1084]	-1.76663	4368	ACCGGACATACCTTGT CCACTCAATAATAAA CTTCAGTTTCGGTAGT GAAATATATATTAAAC GAACTGGAGTTTATT TATTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 968]	ACCGGACATACCTTGT CCACTCAATAATAAA CTTCAGTTTCGGTAGT GAAATATATATTAAAC GAACTGGAGTTTATT TATTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1496]
AAAAATAATAAC TTCAGTTTC [SEQ ID NO: 737]	AAAAUAAAUAA UCCAGUUC [SEQ ID NO: 1265]	GAAACTGGAATC TATTATTATA [SEQ ID NO: 853]	GAAACUGGAUUC UUUUUUUA [SEQ ID NO: 1381]	GAAACUGGAAGU UUUUUUUA [SEQ ID NO: 1085]	-0.22922	4369	ACCGGACATACCTTGT CCACTCAATAATAA ACTTCAGTTTCCTAGT GAAATATATATTAAAG AACTGGATCTATT ATTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 969]	ACCGGACATACCTTGT CCACTCAATAATAA ACTTCAGTTTCCTAGT GAAATATATATTAAAG AACTGGATCTATT ATTATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 1497]
AAAAATAATAA CTTCAGTTT [SEQ ID NO: 738]	AAAAUAAAUAA CUCCAGUUU [SEQ ID NO: 1266]	AAACTGGAAGCC ATTATTATTATA [SEQ ID NO: 854]	AAACUGGAAGCC AUUUUUUA [SEQ ID NO: 1382]	AAACUGGAAGUU AUUUUUUA [SEQ ID NO: 1086]	-0.00312	4370	ACCGGACATACCTTGT CCACTCAATAATAA AACTTCAGTTTTCCTAGT GAAATATATATTAAAG AACTGGAGCCATTATA TTTATAGTGTGACAG	ACCGGACATACCTTGT CCACTCAATAATAA AACTTCAGTTTTCCTAGT GAAATATATATTAAAG AACTGGAGCCATTATA TTTATAGTGTGACAG

ATGACTTTCAAG GGTTATTAAA [SEQ ID No: 739]	AUGACUUUCAAG GGUUAUUAAA [SEQ ID No: 1267]	TTTAATAACCCAC TGAAAAGTCAA [SEQ ID No: 855]	UUUAUAACCCAC UGAAAAGUC [SEQ ID No: 1383]	UUUAUAACCCUU UGAAAAGUC [SEQ ID No: 1087]	-2.77085	4390	GGATACAGCAACTATT TTATCAA [SEQ ID No: 970]	GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1498]
TATGACTTTTCAA GGGTTATTAA [SEQ ID No: 740]	UAUGACUUUCA GGGUUAUUAA [SEQ ID No: 1268]	TTTAATAACCCCC GAAAGTCATT [SEQ ID No: 856]	UUUAUAACCCCC GAAAGUCAU [SEQ ID No: 1384]	UUUAUAACCCUU GAAAGUCA [SEQ ID No: 1088]	-3.75832	4391	ACCGGACATACCTTGT CCACTCTATGACTTTC AAGGGTTATTAAAGT GAAATATATATTAAAT TAATAACCCCGGAAAG TCATTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 971]	ACCGGACAUACUUGUU CCACUCUACUACUUC AAGGGUUAUUAAUAGU GAAUAUAUUUUAAA UAAUAACCCCGGAAAG UCAUUUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1499]
TCATGACTTTCA AGGGTTATTA [SEQ ID No: 741]	UCAUGACUUUCA AGGGUUAUUA [SEQ ID No: 1269] (same as guide in XD- 14933)	TAATAACCCCTCT AAAGTCATGT [SEQ ID No: 857]	UUUAUAACCCCU AAAGUCAU [SEQ ID No: 1385]	UUUAUAACCCUUG AAAGUCAU [SEQ ID No: 1089]	-4.61593	4392	ACCGGACATACCTTGT CCACTCTATGACTTTC CAAGGGTTATTAAAGT GAAATATATATTAAAT AATAACCCCTCTAAAGT CATGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 973]	ACCGGACAUACUUGUU CCACUCUACUACUUC CAAGGGUUAUUAAUAGU GAAUAUAUUUUAAA AATAACCCCGUAAAGU CAUGUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1501]
TTTCATGACTTTC AAGGGTTATT [SEQ ID No: 742]	UUCAUGACUUUC AAGGGUUAU [SEQ ID No: 1270]	AATAACCCCTTGT AAGTCATGAT [SEQ ID No: 858]	AAUAACCCUUUG AAGUCAUG [SEQ ID No: 1386]	AAUAACCCUUGA AAGUCAUG [SEQ ID No: 1090]	-3.88921	4393	ACCGGACATACCTTGT CCACTCTATGACTTTC TCAAGGGTTATTAAAGT GAAATATATATTAAAT ATAACCCCTTGAAGTC ATGATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 974]	ACCGGACAUACUUGUU CCACUCUACUACUUC UCAAGGGUUAUUAAUAGU GAAUAUAUUUUAAA AUAACCCUUUAAAGUC AUGAUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1502]
TTTCATGACTTTC CAAGGGTTAT	UUCAUGACUUU CAAGGGUUAU	ATAACCCCTTGGG AGTCATGAAT	AUAACCCUUGGG AGUCAUAAU	AUAACCCUUGAA AGUCAUGA	-3.57278	4394	ACCGGACATACCTTGT CCACTCTTTCATGACT	ACCGGACAUACUUGUU CCACUCUACUACUUC UCAAGGGUUAUUAAUAGU GAAUAUAUUUUAAA AUAACCCUUUAAAGUC AUGAUAGUGUGACAG GGAUACAGCAACUAAU UUUAUCAA [SEQ ID No: 1502]

[SEQ ID NO: 743]	[SEQ ID NO: 1271]	[SEQ ID NO: 859]	[SEQ ID NO: 1387]	[SEQ ID NO: 1091]			TTCAAGGGTTATTAGT GAAATATATATTAAAA TAACCCCTTGGGAGTCA TGAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 975]	UUAUCAA [SEQ ID NO: 1503]	UUAUCAA [SEQ ID NO: 1503]
TGTTTCATGACTT TCAAGGGTTA [SEQ ID NO: 744]	UGUUCAGACUU UCAAGGGUUA [SEQ ID NO: 1272]	TAAACCTTGAGG GTATGAACT [SEQ ID NO: 860]	UAACCCUUGAGG GUCAUGAACU [SEQ ID NO: 1388]	UAACCCUUGAAA GUCAUGAA [SEQ ID NO: 1092]	-4.31654	4395	ACCGGACATACCTTGT CCACTCTGTTCATGAC TTTCAAGGGTTATAGT GAAATATATATTAAAT AACCTTGAAGGGTCAT GAACCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 976]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1504]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1505]
TGTTTCATGACT TTCAAGGGTT [SEQ ID NO: 745]	UGUUCAGACUU UCAAGGGUUA [SEQ ID NO: 1273]	AACCCCTTGAACT TCATGAACAT [SEQ ID NO: 861]	AACCCUUGAAAU UCAUGAACU [SEQ ID NO: 1389]	AACCCUUGAAAG UCAUGAAC [SEQ ID NO: 1093]	-3.76092	4396	ACCGGACATACCTTGT CCACTCTGTTCATGAC CTTTCAAGGGTTATAGT GAAATATATATTAAAA ACCCUUGAGGGTCATG AACCTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 977]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1506]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1507]
TGTTTCATGACT TTTCAAGGGT [SEQ ID NO: 746]	UGUGUUCAGAC UUUCAAGGGU [SEQ ID NO: 1274]	ACCCCTTGAAATC CATGAACACT [SEQ ID NO: 862]	ACCCUUGAAAU CAUGAACACU [SEQ ID NO: 1390]	ACCCUUGAAAGU CAUGAAC [SEQ ID NO: 1094]	-4.07971	4397	ACCGGACATACCTTGT CCACTCTGTTCATGAC ACTTTCAAGGGTTATAGT GAAATATATATTAAAA CCCTTGAATCCATGA ACACTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 978]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1508]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1509]
ATGTTTCATGA CTTTCAAGGG [SEQ ID NO: 747]	AUGUGUUCAGAC CUUUCAGGGG [SEQ ID NO: 1275]	CCCTTGAAAGCA ATGAACACAA [SEQ ID NO: 863]	CCCUUGAAAGCA AUGAACACAA [SEQ ID NO: 1391]	CCCUUGAAAGUC AUGAACAC [SEQ ID NO: 1095]	-4.76135	4398	ACCGGACATACCTTGT CCACTCTGTTCATGAC GACTTTCAAGGGTTATAGT GAAATATATATTAAAC CCCTTGAAGCAATGAA CACAATAGTGTGACAG	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1510]	ACCGGACAUACUUGUU CCACUCUGUUCAGAC UUUCAAGGGUUUAGU GAAAUUAUUAUAAAA AACCCUUGAGGGUCAU GAACUUAUGUGGACAG GGAUACAGCAACUAAU UUAUCAA [SEQ ID NO: 1511]

TATGTGTTTCATG ACTTTCAAG [SEQ ID No: 748]	UAUGUGUUAUG ACUUUCAAGG [SEQ ID No: 1276]	CCTTGAAGTAG TGAACACATT [SEQ ID No: 864]	CCUUGAAAGUAG UGAACACAUU [SEQ ID No: 1392]	CCUUGAAAGUCA UGAACACA [SEQ ID No: 1096]	-4.33797	4399	GGATACAGCAACTATT TTATCAA [SEQ ID No: 979]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID No: 1507]
TGATGTGTTTCAT GACTTTCAAG [SEQ ID No: 749]	UGAUGUGUUAUG GACUUUCAAG [SEQ ID No: 1277]	CTTGAAGTCGC GAACACATCT [SEQ ID No: 865]	CUUGAAAGUCGC GAACACAUU [SEQ ID No: 1393]	CUUGAAAGUCAU GAACACAU [SEQ ID No: 1097]	-4.13084	4400	ACCGGACATACCTTGT CCACTCTGATGTGTC ATGACTTTCAAGTAGT GAAATATATATTAAAC TTGAAAGTCGCGAACA CATCTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 980]	ACCGGACATACCTTGT CCACTCTGATGTGTC ATGACTTTCAAGTAGT GAAATATATATTAAAC TTGAAAGTCGCGAACA CATCTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1508]
TTGATGTGTTTCA TGACTTTTCAA [SEQ ID No: 750]	UUGAUGUGUUAUG UGACUUUCA [SEQ ID No: 1278]	TTGAAAGTCATT AACACATCAT [SEQ ID No: 866]	UUGAAAGUCACU AACACAUU [SEQ ID No: 1394]	UUGAAAGUCAUG AACACAU [SEQ ID No: 1098]	-4.24964	4401	ACCGGACATACCTTGT CCACTCTGATGTGTT CATGACTTTCAATAGT GAAATATATATTAAAT TGAAGTCACTAACAC ATCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 981]	ACCGGACATACCTTGT CCACTCTGATGTGTT CATGACTTTCAATAGT GAAATATATATTAAAT TGAAGTCACTAACAC ATCATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1509]
TCTGATGTGTTTC ATGACTTTTCA [SEQ ID No: 751]	UCUGAUGUGUUC AUGACUUUCA [SEQ ID No: 1279]	TGAAAGTCATTG ACACATCAGT [SEQ ID No: 867]	UGAAAGUCAUUG ACACAUU [SEQ ID No: 1395]	UGAAAGUCAUGA ACACAU [SEQ ID No: 1099]	-4.95673	4402	ACCGGACATACCTTGT CCACTCTGATGTGTT TCATGACTTTTCAATAGT GAAATATATATTAAAT GAAAGTCACTAACAC TCAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 982]	ACCGGACATACCTTGT CCACTCTGATGTGTT TCATGACTTTTCAATAGT GAAATATATATTAAAT GAAAGTCACTAACAC TCAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No: 1510]
AGCTGATGTGTT CATGACTTTTC	AGCUGAUGUGUU CAUGACUUUC	GAAAGTCATGGG CACATCAGCA	GAAAGUCAUGGG CACAUCA	GAAAGUCAUGAA CACAUCA	-3.87865	4403	ACCGGACATACCTTGT CCACTGATGTGTTG	ACCGGACATACCTTGT CCACTGATGTGTTG

[SEQ ID NO: 752]	[SEQ ID NO: 1280]	[SEQ ID NO: 868]	[SEQ ID NO: 1396]	[SEQ ID NO: 1100]				TTTCATGACTTTCTAGT GAAATATATATTAAAG AAAGTCATGGGCACAT CAGCATAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 984]	UUC AUGACUUUCUAGU GAAAUUAUUAUAAAG AAAGUCAUGGCACAU CAGCAUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1512]
TAGCTGATGTGT TCATGACTTT [SEQ ID NO: 753]	UAGCUGAUGUGU UCAUGACUUU [SEQ ID NO: 1281]	AAAGTCATGAGA ACATCAGCTT [SEQ ID NO: 869]	AAAGUCAUGAGA ACAUCAGCUU [SEQ ID NO: 1397]	AAAGUCAUGAAC ACAUCAGC [SEQ ID NO: 1101]	4404	-3.03908		ACCGGACATACCTTGT CCACTCTAGCTGTGT GTTTCATGACTTTTGT GAAATATATATTAAAA AAGTCATGAGAACATC AGCTTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 985]	ACCGGACAUACUUGUU CCACUCUAGCUGAUGU GUUCAUGACUUUUAAGU GAAAUUAUUAUAAAA AAGUCAUGAGAACAU AGCUUAGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1513]
TTAGCTGATGTG TTTCATGACTT [SEQ ID NO: 754]	UUAGCUGAUGUG UUC AUGACUU [SEQ ID NO: 1282]	AAGTCATGAAAG CATCAGCTAT [SEQ ID NO: 870]	AAGUCAUGAAAG CAUCAGCUU [SEQ ID NO: 1398]	AAGUCAUGAAC CAUCAGCU [SEQ ID NO: 1102]	4405	-4.75357		ACCGGACATACCTTGT CCACTCTAGCTGTGT TGTTTCATGACTTTGT GAAATATATATTAAAA AGTCATGAAAGCATCA GCTATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 986]	ACCGGACAUACUUGUU CCACUCUAGCUGAUGU UGUCAUGACUUUUAAGU GAAAUUAUUAUAAAA AGUCAUGAAAGCAUCA GCUUUAUGUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1514]
TCTAGCTGATGT GTTTCATGACT [SEQ ID NO: 755]	UCUAGCUGAUGU GUUCAUGACU [SEQ ID NO: 1283]	AGTCATGAACGA ATCAGCTAGT [SEQ ID NO: 871]	AGUCAUGAACGA AUCAGCUU [SEQ ID NO: 1399]	AGUCAUGAACAC AUCAGCU [SEQ ID NO: 1103]	4406	-5.05901		ACCGGACATACCTTGT CCACTCTAGCTGTGT GTGTTTCATGACTTAGT GAAATATATATTAAAA GTCTATGAACGAATCAG CTAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO: 987]	ACCGGACAUACUUGUU CCACUCUAGCUGAUGU GUGUCAUGACUUUAAGU GAAAUUAUUAUAAAA GUCAUGAACGAACU CUAGUUAUGUGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID NO: 1515]
TGCTAGCTGATG TGTTTCATGAC [SEQ ID NO: 756]	UGCUAGCUGAUG UGUUAUGAC [SEQ ID NO: 1284]	GTCATGAACAAG TCAGCTAGCT [SEQ ID NO: 872]	GUCAUGAACAAAG UCAGCUAGCU [SEQ ID NO: 1400]	GUCAUGAACACA UCAGCUAG [SEQ ID NO: 1104]	4407	-4.47567		ACCGGACATACCTTGT CCACTCTGCTAGCTGA TGTTTCATGACTTAGT GAAATATATATTAAAG TCATGAACAAGTCAGC TAGCTTAGTGTGACAG	ACCGGACAUACUUGUU CCACUCUAGCUGAUGU UGUGUCAUGACUUAAGU GAAAUUAUUAUAAAG UCAUGAACAGUCAGC UAGCUUAGUGUGACAG

TTTGCTAGCTGAT GTGTTTCATGA [SEQ ID No.: 757]	UUGCUAGCUGAU GUGUUCUAGA [SEQ ID No.: 1285]	TCATGAACACGC CAGCTAGCAT [SEQ ID No.: 873]	UCAUGAACACGC CAGCUAGCAU [SEQ ID No.: 1401]	UCAUGAACACAU CAGCUAGC [SEQ ID No.: 1105]	-4.42302	4408	GGATACAGCAACTATT TTATCAA [SEQ ID No.: 988]	GGAUACAGCAACUAUU UUAUCAA [SEQ ID No.: 1516]
TTTGCTAGCTGA TGTGTTTCATG [SEQ ID No.: 758]	UUUGCUAGCUGA UGUGUUCUAG [SEQ ID No.: 1286] (same as guide in XD- 14934)	CATGAACACACA AGCTAGCAAT [SEQ ID No.: 874]	CAUGAACACACA AGCUAGCAU [SEQ ID No.: 1402]	CAUGAACACAU AGCUAGCA [SEQ ID No.: 1106]	-5.35102	4409	ACCGGACATACCTGTT CCACTCTTTGCTAGCT GATGTGTTTCATGATG GAAATATATATTAAAC ATGAACACACAAGCTA GCAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No.: 990]	ACCGGACAUACUUGUU CCACUCUUGCUAGCU GAUGUUCUAGUAGU GAAUUAUUAUUAAC AUGAACACACAAGCUA GCAUUAUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID No.: 1518]
TTTTGCTAGCTG ATGTGTTTCAT [SEQ ID No.: 759]	UUUGCUAGCUG AUGUGUUCAU [SEQ ID No.: 1287]	ATGAACACATAG GCTAGCAAT [SEQ ID No.: 875]	AUGAACACAUAG GCUAGCAAU [SEQ ID No.: 1403]	AUGAACACAUCA GCUAGCAA [SEQ ID No.: 1107]	-0.73644	4410	ACCGGACATACCTGTT CCACTCTTTGCTAGC TGATGTGTTTCATGAT GAAATATATATTAAAC TGAACACATAGGCTAG CAAATTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No.: 991]	ACCGGACAUACUUGUU CCACUCUUGCUAGC UGAUGUGUUCUAGU GAAUUAUUAUUAAC UGAACACAUAGGCUAG CAAUUAUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID No.: 1519]
TCCTTTGCTAGC TGATGTGTTTC [SEQ ID No.: 760]	UCUUUUGCUAGC UGAUGUGUUC [SEQ ID No.: 1288]	GAACACATCATTA TAGCAAAAGT [SEQ ID No.: 876]	GAACACAUCAUA UAGCAAAAGU [SEQ ID No.: 1404]	GAACACAUAGC UAGCAAAA [SEQ ID No.: 1108]	-1.29476	4412	ACCGGACATACCTGTT CCACTCTCTTTTGCTA GCTGATGTGTTCTAGT GAAATATATATTAAAG AACACATCATATAGCA AAAGTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID No.: 992]	ACCGGACAUACUUGUU CCACUCUUCUUGCUA GCUGAUGUGUUCUAGU GAAUUAUUAUUAAC AACACAUCAUUAAGCA AAAGUAGUGUGGACAG GGAUACAGCAACUAUU UUAUCAA [SEQ ID No.: 1520]
TGTCAGGTTTAG TAAAGGGCG		CGCCCTTTTAAC AAACTTGACT		CGCCCUUUUACU AAACUUGA	-4.72	4502	ACCGGACATACCTGTT CCACTCTGTCAAGTTT	

[SEQ ID NO:2269]		[SEQ ID NO:2270]		[SEQ ID NO:2271]			AGTAAAAGGCGTAGT GAAATATATATTAAAC GCCCTTTTAACAAACT TGACTTAGTGTGACAG GGATACAGCAACTATT TTATCAA [SEQ ID NO:2272]	
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EXAMPLE 3: TESTING OF TOP HITS FROM POOLED SCREEN IN LENTIVIRAL TRANSDUCTION OF HUMAN NEURONS

Several top hits from pooled Deep Screen 1 (Example 2) were cloned into lentiviral vectors, packaged, and tested in stem-cell derived motor neuron cultures for knockdown of *ATXN2* mRNA and protein. An example lentiviral vector is given in H1-miR-16-2_1755-AMELY_V1_CMV_GFP_lenti (SEQ ID NO:1521) which contains a amiRNA targeting position 1755 of *ATXN2* transcript embedded in a miR-16-2 backbone, or the other vectors described here. The amiRNA sequence in the vector (e.g., nucleotides 1889-2020 of SEQ ID NO:1521) may be replaced with the corresponding amiR or control non-miRNA sequence (MCS) but the rest of the vector is left unchanged.) Characterization of motor neurons (**FIG. 29**) shows that cultures (differentiation protocol described in below methods) generated cultures enriched for motor neurons, with elaborated neuronal processes. amiRNAs were embedded in lentiviral vectors (**FIG. 30A**) with an H1 promoter as well as a GFP expression cassette. In a first experiment, two amiRNAs, targeting *ATXN2* at position 1784 (guide sequence SEQ ID NO:112) in the coding sequence or *ATXN2* at position 4402 (guide sequence SEQ ID NO:1279) having miR-16-2 backbones were tested at two different doses. Strong knockdown of *ATXN2* mRNA and protein was detected by qPCR analysis of mRNA and Western analysis of protein, respectively (**FIGS. 30B-30C**). Protein levels as measured in this assay showed a greater fractional reduction of protein levels than mRNA levels, indicating that measurements of mRNA may represent at least the amount of *ATXN2* protein reduced by a given amiRNA. Surprisingly, the amiRNA targeting the *ATXN2* coding sequence (1784) yielded greater knockdown than the amiRNA targeting the 3' UTR (4402), which is different than the relative performance of those amiRNAs in Deep Screen 1.

As a further investigation of amiRNA targeting the coding region versus the 3' UTR, a second experiment was done (**FIG. 31**). In this case, all neurons were treated at a dose intermediate between the two levels tested in the first human neuron lentiviral dosing experiment. As before, amiRNAs targeting the coding sequence (1755 (guide sequence SEQ ID NO:1185), 1784 (guide sequence SEQ ID NO:112), 3302

(guide sequence SEQ ID NO:1216), 3330 (guide sequence SEQ ID NO:1811), and 3805 (guide sequence SEQ ID NO:1221) yielded stronger knockdown than amiRNAs targeting the 3' UTR (4402 (guide sequence SEQ ID NO:1279), 4242 (guide sequence SEQ ID NO:1233), and 4502) in these neuronal cultures. The amount of mRNA reduction exceeded 75% for some amiRNAs, such as 1755 (guide sequence SEQ ID NO:1185), 1784 (guide sequence SEQ ID NO:112) and 3330 (guide sequence SEQ ID NO:1811).

10 **Methods**

Motor neuron production

Induced pluripotent stem cells (GM25256, Coriell Institute) were cultured in feeder-free conditions, in mTeSR1 media on Matrigel coated plates, according to standard procedures. To begin differentiation, iPSC colonies grown in 6-well dishes were dissociated with 500 μ L ReLeSR, incubating 3 minutes at 37C, and gently agitated. 1 mL of complete mTeSR1 media is added to stop dissociation. Cell suspension was collected, ReLeSR removed and cells resuspended in N2B27 differentiation media: 50 mL of 50% mTeSR1 and 50% NB27 differentiation media (50% DMEM-F12, 50% Neurobasal medium, 1X N-2 supplement, 1X B-27 supplement, XenoFree, 0.5X penicillin-streptomycin, 1X 2-mercaptoethanol, 20 μ M L-ascorbic acid). Rock Inhibitor Y-27632 (5 micromolar), LDN (200 nM), SB 431542 (40 micromolar), and Chir 99021 (3 micromolar) were added. Cell suspension was then transferred to a 75 cm² ultra low attachment U-flask for 24 hours. Cells then aggregated into small spheroids.

Media changes were then performed on days 2, 4, 6, 9, and 12. Media included (all based in N2B27 differentiation media): Day 2: Retinoic acid (1 micromolar), SAG (1 micromolar), LDN-193189 (0.2 micromolar), SB 431542 hydrate (40 micromolar), CHIR 99021 (3 micromolar). Day 4: Retinoic acid (1 micromolar), SAG (1 micromolar), LDN-193189 (0.2 micromolar). Day 6: Retinoic acid (1 micromolar); SAG (1 micromolar). Day 9: Retinoic acid (1 micromolar), SAG (1

micromolar), DAPT (10 micromolar). Day 12: DAPT (10 micromolar). By day 14, neuronal spheroids were present and were dissociated to plate motor neurons.

Neuronal spheroids were then dissociated with a papain:DNase solution and triturated 4-5x. Cell suspensions were then divided into wells of 6-well plates; and after a 15 minute incubation, further triturated. Following this dissociation, enzyme was inactivated with a DMEM and knockout serum replacement (KOSR) mix, centrifuged, washed again in 90% DMEM/10% KOSR, centrifuged, and resuspended in complete neurobasal media: Neurobasal medium, 1X N-2 supplement, 1X B-27 supplement, XenoFree, 0.5X penicillin-streptomycin, 20 uM L-ascorbic acid, 1% KOSR, Rock Inhibitor Y-27632 (5 micromolar), GDNF (10ng/mL), BDNF (20ng/mL), CNTF (10ng/mL), DAPT (5 micromolar). Cells were then centrifuged again, resuspended in complete neurobasal media, passed through a 40 micron cell strainer, counted via trypan blue staining and a hemocytometer, then diluted to 20K/well (96-well format) or 200K/well (24-well format) for plating in PDL/Laminin coated plates. Cells were cultured in a volume of neurobasal media: 200uL/well (96-well format) or 1mL/well (24-well format).

The PDL/Laminin coating was done by treating plates with a 100 microgram/mL solution of poly-D-lysine in PBS overnight at 4C; washing 3 times with PBS; then treating plates overnight at 4C with a 50 microgram/mL solution of laminin in PBS.

48 hours after plating, 50% of media was replaced with neuron maintenance media (Neurobasal, with 1X N-2 supplement, 1X Xeno-Free B-27 supplement, 0.5X penicillin-streptomycin, 20 micromolar L-ascorbic acid, with 10 ng/mL GDNF, 10 ng/mL BDNF, 10 ng/mL CNTF), including DAPT (5 micromolar). Thereafter, 50% of media was replaced 3 times per week, not including DAPT.

References relevant to the above protocol include: (Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling (Chambers et al., Nat Biotechnol (2009) 27:275-280) and (Maury et al., Nat Biotechnol (2014) 33:89-96).

30

Reagents and equipment for iPSC embryoid body formation

Reagent	Vendor	Cat. No.
DMEM/F-12	ThermoFisher	11320-033
Neurobasal Medium		21103049
N-2 Supplement (100X)		17502-048
B-27 Supplement (50X), XenoFree		A1486701
Phosphate-Buffered Saline (PBS) pH 7.4		10010023
Penicillin-Streptomycin 100x		15140122
2-Mercaptoethanol 1000x		21985023
L-Ascorbic Acid	Millipore Sigma	A4403-100MG
Rock Inhibitor (Y-27632), 5MG		Y0503-5MG
SB 431542 hydrate		S4317-5MG
Retinoic Acid		R2625-50MG
DAPT		D5942-5MG
SAG, SHH agonist		566660-5MG
MTesR1 cGMP, feeder-free maintenance medium kit	StemCell Technologies, Inc	85850
ReLeSR		05872
LDN-193189 in solution	Fisher Scientific	NC0689818
CHIR 99021 10MG	Tocris	4423

Equipment/ Supply	Vendor	Cat. No.
Ultra-Low Attachment 75cm ² U-Flask	Corning	3814
6-well plate	Corning	353046
Nalgene Rapid-Flow 500mL Filter Units	VWR (Nalgene)	73520-984

5 **Reagents for embryoid body dissociation and motor neuron culture:**

Reagent	Vendor	Cat. No.
Neurobasal Medium	ThermoFisher	21103049
DMEM/F-12		11320-033
N-2 Supplement (100X)		17502-048
B-27 Supplement (50X), XenoFree		A1486701
2-Mercaptoethanol 1000x		21985023
Phosphate-Buffered Saline (PBS) pH 7.4		10010-049
Penicillin-Streptomycin 100x		15140122
KnockOut Serum Replacement		10828010
Laminin Mouse Protein, 1mg in solution	Millipore Sigma	23017015
Rock Inhibitor (Y-27632), 5mg		Y0503-5MG
DAPT		D5942-5MG

L-Ascorbic Acid		A4403-100MG
Poly-D-Lysine solution, 1mg/mL		A-003-E
Recombinant Human GDNF, 50µg		212-GD-050
Recombinant Human BDNF, 50µg	R&D Systems	248-BDB-050
Recombinant Human CNTF, 50µg		257-NT-050
Papain, ≥100 units per vial	Worthington	LK003178
DNase, ≥1,000 Kunitz units per vial		LK003172

Equipment for embryoid body dissociation and motor neuron culture

Equipment/ Supply	Vendor	Cat. No.
6-well Clear TC-treated Plates	Corning	353046
12-well Clear TC-treated Plates	Corning	353043
24-well Clear TC-treated Plates	Corning	353047
96-well optic clear bottom, black wall microplate	PerkinElmer	6005550
Nalgene Rapid-Flow 500mL Filter Units	VWR (Nalgene)	73520-984

Lentiviral Production

- 5 To test the efficacy of miR16-2 embedded guides in stem-cell derived motor neurons, amiRNAs were expressed from an H1 promoter embedded within a lentiviral construct as described above. Lentivirus was generated with Lenti-X 293T (Takara, 632180) cells transfected with psPAX2 (Cellecta, P/N CPCP-PAX2) and pMD2.2 (Cellecta, CPCP-PM2G) using Lipofectamine LTX and PLUS Reagent
- 10 (Thermo, P/N 15338-100). The following day after transfection media was changed to include ViralBoost Reagent (Alstem, P/N VB100) and then 2 days later the viral production media was filtered and concentrated using Lenti-X Concentrator (Takara, P/N 631232) and resuspended in N2B27 media.

15 qPCR Analysis

- Stem-cell derived motor neurons were transduced, and 7 days post-transduction, media was removed, washed with PBS and cells lysed with Buffer RLT supplemented with beta-Mercaptoethanol. RNA was purified using Qiagen RNeasy Plus Mini Kit (Qiagen, P/N 74134) and reverse-transcribed using SuperScript VILO
- 20 cDNA Synthesis Kit (Thermo, P/N 11754250). Using TaqMan Fast Advanced Master Mix (Thermo, P/N 4444556) and QuantStudio 6 Flex Real-Time PCR System

(Thermo), Ct values were calculated using primer/probe sets to *ATXN2* (Thermo, Hs01002847_m1), *GUSB* (Thermo, Hs00939627_m1), and *B2M* (Thermo, Hs00187842_m1). The average Ct across 4 replicates was calculated, and using the delta-delta Ct method, the delta Ct was calculated for *ATXN2* to each internal control, then the delta-delta Ct was calculated to the average of the untreated conditions. The mean of the normalized values to untreated conditions were calculated and graphed as shown.

10 *Western Analysis of ATXN2 Levels from neurons treated with ATXN2 amiRNA expressing lentiviruses*

Protein extraction was performed by placing plates on ice, aspirating media, and adding 50-100 microliters cold RIPA buffer (TEKNOVA #50-843-016) supplemented with protease and phosphatase inhibitor tablet (Pierce #A32959), Halt protease inhibitor cocktail (Thermo #1861279) and PMSF (Cell Signaling Technology #8553S). Individual cell lifters were used to scrape each well thoroughly, plates were tilted and lysates were harvested and incubated on ice for an additional 30min. Samples were centrifuged for 15min at 17,000xg at 4°C, and supernatant was transferred to a fresh tube and stored at -80°C. Protein lysates were quantitated (Pierce, 23225), resulting in approximately 40µg total protein per sample.

20 The NuPage system (Thermo) was used for gel electrophoresis. Five µg of each sample was loaded onto 4-12% Bis-Tris protein gels (Thermo, NP0321BOX) and run at constant 200V for 1hr. Revert 700 (Licor, 926-11010) was used to assay for protein loading. Proteins were transferred onto PVDF membrane (EMD Millipore, IPFL00005) overnight at 4°C using constant 30V and 90mA. Membranes were blocked for 1hr at RT (Rockland, MB-070). Primary antibody incubation was performed overnight rocking at 4°C, including anti-Atxn2 (1:1000, BD, 611378), anti-GFP (1:2000, CST, 2956) and beta-actin (1:2000, CST, 4970). Washing was performed 4x 5min with TBS + 0.1% tween-20, and secondary antibodies were incubated for 1hr rocking at RT (1:15,000 each of 800CW goat anti-mouse and 680RD donkey anti-rabbit, Licor). Membranes were washed again and imaging was performed on an Odyssey Fc Imaging system (Licor). Signal quantitation was by Licor image-studio lite.

EXAMPLE 4: EMBEDDING OF TOP HITS FROM POOLED SCREEN IN AAV CIS-PLASMIDS AND AAV PRODUCTION

To test the ability of top performing amiRNAs identified from the pooled screen to knock down ATXN2 when embedded in AAV, 10 top miRNAs were cloned downstream of a H1 promoter (nucleotides 113-203 of SEQ ID NO:1522) in a cis plasmid (transfer plasmid) for AAV production. An example of a plasmid sequence (5' ITR to 3' ITR) (scAAV_AMELY_V1_H1_micropool_ITR_to_ITR) comprises the nucleotide sequence of SEQ ID NO:1522; where the desired amiRNA embedded in a miRNA backbone is inserted in nucleotides 204-341 of SEQ ID NO:1522. After AAV9 production by triple transfection of HEK293T cells with the cis-plasmid and helper plasmids and harvest of encapsidated AAV, vector genome DNA was extracted with Quick-DNA Viral Kit (Zymo, P/N D3015) to assess vector integrity. Purified vector was quantified using Qubit dsDNA HS Assay Kit (Thermo, P/N Q32854) and vector genome size was assessed by agarose gel electrophoresis and stained SyberSafe for visualization. Vector genome size was assessed by agarose gel electrophoresis (FIG. 32). Surprisingly, two bands were observed. The upper band migrated at the expected size 2284 bp, whereas the lower band migrated farther than the calculated vector size, or smaller in length than the full-length vector. Extraction of the band representing the full-length vector and subsequent Sanger sequencing with a primer amplifying towards the embedded aimRNA resulted in successful sequencing of the expected amiRNA. Whereas extraction of the smaller DNA product and sequencing failed to sequence through the embedded aimRNA, suggesting that the lower band might represent a vector truncation centered around the artificial miRNA, as noted in (Xie et al., Molecular Therapy (2013) 28:422-430). Calculation of predicted DNA secondary structure for miRs in the miR16-2 backbone using mfold (Zuker Nucleic Acids Research (2003) 31:3406-15) showed this sequence to form strong secondary structure, with Gibbs free binding energy of -26.78.

Using ImageJ, the individual vector genome lanes of an image gathered with the SyberSafe stained DNA gel were selected, the intensity of the lane plotted, and peaks quantified. Using the calculated lengths of the full-length and miR-centered truncated vector genomes of 2284 and 2077 bp respectively, the relative staining-

intensity-derived molarity of each was calculated. With these values, the percentage full-length vector was calculated as the percentage of full-length divided by the combined amount of full-length and miR-centered truncated vector genomes (**Table 20**)

5 **Table 20: Percentage of full-length vector genome**

H1 AMELY V1 guide	percentage full-length vector genome
miR16-2-XD-14792	68.3
miR16-2-1479	56.7
miR16-2-1755	70.3
miR16-2-3330	62.5
miR16-2-4402	64.1
miR16-2-4405	67.8
miR16-2-4406	71.0
miR16-2-4409	67.2
miR16-2-4502	64.9

EXAMPLE 5: SECOND POOLED MIRNA SCREEN

Given the truncation observed in AAV vectors expressing miR16-2 embedded amiRNAs, a second pooled amiRNA screen was devised to embed the guide
 10 sequences from the top ATXN2 miRNA hits from the first pooled screen into a diverse set of 20 miRNA backbones.

ATXN2 targeting sequence selection for DS2

ATXN2 targeting sequences presumed to be efficacious and safe were
 15 selected from Deep Screen 1 to enter “Deep Screen 2.” Sequences that were enriched in the low ATXN2 signal FACS bin and demonstrated low dropout (minimal change in representation comparing an early to a late timepoint) were prioritized. To calibrate the dynamic range of the assay, some sequences with high dropout were additionally included. Since there may be biological variability in the processing precision of the
 20 mature guide strand, guides bracketing efficacious guides (by position along the *ATXN2* transcript) were additionally entered into Deep Screen 2.

Essential gene control miRNA selection

A subset of the essential gene targeting amiRNAs with either ‘high’ or ‘medium’ dropout, with respect to other essential-gene targeting amiRNAs, were selected for Deep Screen 2 based on performance in Deep Screen 1.

5 911 controls

A subset of sequences targeting *ATXN2* were paired with their cognate 911 controls. In a 911 control, bases 9, 10, and 11 of the guide strand are complemented, along with corresponding change in the passenger strand, such that the resulting mature miRNA does not slice the target mRNA of the original guide. Because
10 many aspects of amiRNA ‘off-target’ activity are presumed to occur through binding interactions with the seed region (bases 2 – 8), these 911 controls should in principle display a similar off-target profile as the original miRNA and should help distinguish on- and off-target activity.

ATXN2 scramble controls

15 A subset of the miRNA scramble controls from Deep Screen 1 was carried over into Deep Screen 2. These were considered for mean centering the data.

ATXN2 backbone selection, processing enhancement motifs, and passenger variations

MicroRNA backbones were selected for naturally exhibiting high processing precision, high guide to passenger ratio, and efficient target knockdown as
20 an artificial miRNA. Both miRNA performance in functional screens and 5' guide processing homogeneity were considered¹⁻⁴.

Primary miRNA transcript sequence was identified in miRbase. The extended sequence contexts around the miRNAs were ascertained in EntrezGene. Surrounding 5' and 3' sequence with high mammalian conservation were used to define
25 final 138nt miRNA-embedded fragments that would be inserted into the pooled library.

Mfold and RNAfold were used to examine folding patterns and to consider Gibbs free energy, as there is evidence that high Gibbs free energy derived from extensive secondary structure in the miRNA may produce miR-centered truncations when later cloned and produced into AAV.

The basal stem, loop, and guide and passenger sequences were defined by stem loop folding predictions on miRbase and Mfold. The rules for passenger variations such as bulges and other asymmetries were chosen to mimic non-complementary base pairing in the endogenous hairpin stem and incorporated into the library construction algorithms.

Sequence motifs that enable efficient processing of pri-miRNA backbones have previously been identified. These include an UG motif at the 5' end of the pre-miRNA, a mismatched GHG motif in the stem, and a 3' CNNC motif. Many of the primary miRNA transcripts selected naturally contain these motifs. Some of these motifs were artificially incorporated into five backbones, and these resulting miRNA backbones are denoted by "_M" (e.g., "miR-1-1_M"). **Table 21** provides miRNA backbone sequences (in DNA format) used in Deep Screen 2. The RNA sequences of the miRNA backbone are provided by converting the "T" nucleotides in the sequences of Table 21 to "U" nucleotides.

Table 21: miRNA backbone sequences used in Deep Screen 2

miR_with_ suffix	5' miR context (flanking)	5' basal stem	5p	loop	3p	3' basal stem	3' miR context (flanking)
miR-1-l	catgcagactgcctgct [SEQ ID NO:1523]	TGGG	passenger	TATGGACCTGCTAA GCTA [SEQ ID NO:1524]	guide	CTCA	ggccgggacactctctgcgcg cactgaggggacactccaca ccacggggggcg [SEQ ID NO:1525]
miR-1-l_M	catgcagactgcctgct [SEQ ID NO:1526]	TGGG	passenger	TATGGACCTGCTAA GCTA [SEQ ID NO:1527]	guide	CTCA	ggccgggacactctctgcgcg cactgaggggacactccaca ccacggggggcg [SEQ ID NO:1528]
miR-100	CCCAAAAGAGA GAAAGATATTGA GG [SEQ ID NO:1529]	CCTGTTGCCAC A [SEQ ID NO:1530]	guide	GTATTAGTCCG [SEQ ID NO:1531]	passenger	TGTGTCTGTTA GG [SEQ ID NO:1532]	CAATCTCACGGA CCTGGGGCTTTGC TTATATGCC [SEQ ID NO:1533]
miR-100_M	CCCAAAAGAGA GAAAGATATTGA G [SEQ ID NO:1534]	CCTGTTGCCAC A [SEQ ID NO:1535]	guide	GTATTAGTCCG [SEQ ID NO:1536]	passenger	TGTGTCTGTTA GG [SEQ ID NO:1537]	ClATeCACGGACC TGGGGCTTTGCTT ATATGCC [SEQ ID NO:1538]
miR-122	ggctacagagtt [SEQ ID NO:1539]	CCTTAGCAGAG CTG [SEQ ID NO:1540]	guide	TGTCTAAACTAT [SEQ ID NO:1541]	passenger	TAGCTACTGCT AGGC [SEQ ID NO:1542]	aatcctccctcgataaagtc ttggcatcggttgcttgg [SEQ ID NO:1543]
miR-122_M	ggctacagagtt [SEQ ID NO:1544]	GCTTAGCAGAG CTG [SEQ ID NO:1545]	guide	TGTCTAAACTAT [SEQ ID NO:1546]	passenger	TAGCTACTGCT AGGC [SEQ ID NO:1547]	catcctccctcgataaagtc ttggcatcggttgcttgg [SEQ ID NO:1548]
miR-124	TTCCTTCCTCAG GAGAA [SEQ ID NO:1549]	AGGCCTCTCTC [SEQ ID NO:1550]	passenger	ATTAAATGTCCAT ACAAT [SEQ ID NO:1551]	guide	GAATGGGGCTG [SEQ ID NO:1552]	GCTGAGCACCGT GGGTGGCGGAGG GCCCCCAagg [SEQ ID NO:1553]
miR-124_M	TTCCTTCCTCAG GAGAA [SEQ ID NO:1554]	tGGCCTCTCTC [SEQ ID NO:1555]	passenger	ATTAAATGTCCAT ACAAT [SEQ ID NO:1556]	guide	GAATGGGGCTt [SEQ ID NO:1557]	aCTGccgaCTGG GTCGGCGAGGGC CCGCCAagg [SEQ ID NO:1558]

miR-128	ATTtTgcaataatggc ctgttcc [SEQ ID NO:1559]	TGAGCTGTGTGG A [SEQ ID NO:1560]	passenger	GAGGTTTACATTTC [SEQ ID NO:1561]	guide	TTCAGCTGCTTC [SEQ ID NO:1562]	ctggctctttttactcagggtt ccactgct [SEQ ID NO:1563]
miR-130a	gcaggccggcgatgcct c [SEQ ID NO:1564]	TGCTGTGTGGCC A [SEQ ID NO:1565]	passenger	CTGTCTGCACCTGTCTC ACTAG [SEQ ID NO:1566]	guide	TGGCCGTGTAG TG [SEQ ID NO:1567]	ctaccagcgctggcgctgct cctcagcatg [SEQ ID NO:1568]
miR-155E	CTGGAGGCTTG CTTT [SEQ ID NO:1569]	GGGCTGTATGC TG [SEQ ID NO:1570]	guide	TTTTGGCCTCTGACT GA [SEQ ID NO:1571]	passenger	CAGGACAAAGC CC [SEQ ID NO:1572]	TTTATCAGCACTC ACATGGAACAAA TGGCCACCGTG [SEQ ID NO:1573]
miR-155M	CCTGGAGGCTT GCTGA [SEQ ID NO:1574]	AGGCTGTATGC TG [SEQ ID NO:1575]	guide	TTTTGGCCTCTGACT GA [SEQ ID NO:1576]	passenger	CAGGACAAAG GCC [SEQ ID NO:1577]	TGTTACTAGCACT CACATGGAACAA ATGGCCACC [SEQ ID NO:1578]
miR-138-2	gccggcgaggtctggt t [SEQ ID NO:1579]	CGTTGCTGC	guide	GACGAGCAGCGCAT CCTCTTACCC [SEQ ID NO:1580]	passenger	GTTGCATCA	taccatctctccaggcga gccctggggaccGG [SEQ ID NO:1581]
miR-144	TCAAGCCATGC TTCCTGTGCCCC CAG [SEQ ID NO:1582]	TGGGGCCCTGG CT [SEQ ID NO:1583]	passenger	AGTTTGCATGAGA CAC [SEQ ID NO:1584]	guide	AGTCCGGGCAC CCCC [SEQ ID NO:1585]	AGTCTTGAGCC TGACAAAGGAggaca [SEQ ID NO:1586]
miR-190a	GAGCTCAGTCA AACCTGGATGC CTTTTC [SEQ ID NO:1587]	TGCAGGCCTCT GTG [SEQ ID NO:1588]	guide	TGTTATTTAATCCA [SEQ ID NO:1589]	passenger	CTACAGTGTCT TGCC [SEQ ID NO:1590]	CTGTCTCCGGGG GTTCTTAATAAA G [SEQ ID NO:1591]
miR-190a_M	GAGCTCAGTCA AACCTGGATGC CTTTTC [SEQ ID NO:1592]	TGCAGGCgTCT GTG [SEQ ID NO:1593]	guide	TGTTATTTAATCCA [SEQ ID NO:1594]	passenger	CTACAGTcTCTT GCC [SEQ ID NO:1595]	CTGTCTCCGGGG GTTCTTAATAAA G [SEQ ID NO:1596]
miR-132	GCCGTCCGCGC GCC [SEQ ID NO:1597]	CCGCCCCCGG TCTCCAGGG [SEQ ID NO:1598]	Passenger	CTGTGGGAACCTGGA GG [SEQ ID NO:1599]	guide	CCCCGCAGCAC GCCACGCGC [SEQ ID NO:1600]	CGGCCACGCCG CGCCCCGAGCC [SEQ ID NO:1601]

miR-451a	GCTCTCTGCTC AGCCTGTCACA ACCTACTGACT GCCAGGGCA [SEQ ID NO:1602]	CTTGGGAATGG CAAGG [SEQ ID NO:1603]	guide	None	passenger	TCTTGCTATACC CAGA [SEQ ID NO:1604]	AAACGTGCCAGG AAGAGAACTCAG [SEQ ID NO:1605]
miR-223	TCCCCACAGAA GCTCTTGG [SEQ ID NO:1606]	CCTGGCCTCCT GCAGTGCCACG CT [SEQ ID NO:1607]	passenger	CTCCATGTGGTAGA G [SEQ ID NO:1608]	guide	AGTGGGCACA TGCTTACCAG [SEQ ID NO:1609]	CTCTAGGCC
miR-16-2	TTATGTTTGGAT GAACTGACATA CTT [SEQ ID NO:1610]	GTCCCACTC	guide	TAGTGAAATATATA TTAAA [SEQ ID NO:1611]	passenger	TAGTGTGAC	AGGATACAGCA ACTATTTTATCAA TTGTTT [SEQ ID NO:1612]

Oligonucleotides were designed that embedded the guide sequences described in Table 19 into miRNA backbones, using flanking sequences as defined in Table 21, and with passenger sequences defined by the rules in Table 8. For example, an artificial miRNA with miR-100 backbone in DNA format for insertion into a transfer plasmid may be designed comprising from 5' to 3': 5' miR context (flanking) sequence of SEQ ID NO:1529; 5' basal stem sequence of SEQ ID NO:1530; desired guide sequence; loop sequence of SEQ ID NO:1531; desired passenger sequence designed according to the rules in Table 8; 3' basal stem sequence of SEQ ID NO:1532; and 3' miR context (flanking) sequence of SEQ ID NO:1533. The artificial miRNA in RNA format may be obtained by converting the "T" nucleotides in these sequences to "U" nucleotides. The pooled library oligonucleotides were cloned into a lentiviral plasmid pLVX-EF1A-miR-CMV-Puro (5' LTR to 3' LTR sequence comprises the nucleotide sequence of SEQ ID:1613) with an EF1alpha promoter to express the amiR, and a CMV promoter to express a PuroR selection marker. The artificial miRNA oligonucleotide may be inserted at nucleotides 3126-3263 of SEQ ID NO:1613. After packaging the library in the plasmid, library composition was assessed by sequencing, and it was noted that the abundance of miRs embedded in the miR-16-2 backbone was in general substantially less than other backbones. One potential explanation would be that during library amplification – when all library elements undergo PCR amplification – elements including the miR-16-2 backbone are amplified less efficiently than other backbones. This could perhaps be because of the strong DNA hairpin that forms with the miR-16-2 backbone. Due to the low number of miR-16-2 backbone elements remaining in the library, counts of miR-16-2 containing guides were low and therefore noisy, and not included in further analyses.

After cloning, packaging, and execution of screen (see methods), sequencing data were analyzed essentially as for Deep Screen 1. Abundance of library elements were calculated by number of sequencing reads exactly matching input library elements. In this screen no baseline subtraction was done for either ATXN2 levels or for dropout. **FIG. 33A** shows a scatterplot plotting the correspondence in the ATXN2 knockdown metric for two screen replicates against each other. In this case what is

plotted is the ratio of abundance of sequence reads for guide elements in the 10% low-ATXN2 signal sort bin versus an unsorted sort bin. There is good correspondence for elements that have low ratios for unsorted/10% low-ATXN2 signal – that is, elements that induce ATXN2 depletion – but there is less correspondence for elements with

5 similar abundance in the unsorted and 10% unsorted bin.

FIG. 33B shows boxplots of knockdown performance of miRs embedded in the shown backbones; **Table 22** shows the median and 95th percentiles of performance. By this metric, some of the top performing miRs, as measured by median performing miRNA, were miR-1-1_M, miR1-1, miR-130a, miR-100, and miR-100_M.

10 It is noted, however, that there were top miRNAs in each of these backbones that, as measured by this assay (ratio of counts of guides in a low-Atxn2 sorted pool versus guides in unsorted cells), performed similarly across miR backbones. Therefore, this assay made available multiple miR backbones with strong performance. This was

likely due to good processing of the artificial pri-miRNA by the microprocessor and

15 dicer complexes.

Table 22: Performance of miRNAs Across miRNA Backbones

	miR with suffix	median	ninety fifth
1	miR-1-1_M	-0.6772802	-2.2788937
2	miR-1-1	-0.5968477	-2.1875722
3	miR-130a	-0.5111669	-2.157525
4	miR-100	-0.2912209	-2.0887075
5	miR-100_M	-0.2094112	-1.9758731
6	miR-155E	-0.1742495	-1.8818528
7	miR-132	-0.1144013	-2.0204587
8	miR-190a	-0.030798	-2.0813007
9	miR-190a_M	0.04198442	-2.1250904
10	miR-122	0.05806267	-1.9039473
11	miR-122_M	0.17311752	-1.851009
12	miR-155M	0.50609504	-1.11192
13	miR-124_M	0.55091372	-0.9016042
14	miR-124	0.57242321	-0.95078
15	miR-144	0.71869783	-1.3372836
16	miR-138-2	0.77876566	-0.8666664

17	miR-223	1.04743176	0.46242634
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The depletion of elements targeting essential genes was also used as an orthogonal evaluation of miR backbone performance. **FIG. 34** shows boxplots of the depletion of elements from the 18-day timepoint versus the 1-day post transduction timepoint. There is a similar ranking of ‘performance’ of the various miR backbones by this metric compared to the ATXN2 knockdown metric. This may be because of the ranking of miR backbones in processing to yield mature amiRNAs.

Table 23 lists the top 100 amiRNAs, ranked by mean enrichment in the ATXN2 low signal sorted cells. The miR backbone, guide sequence, targeting position within the complementary *ATXN2* transcript sequence, passenger sequence, and the amiRNA sequence (including the miR backbone, loop, *ATXN2* targeting guide and passenger), are provided in both RNA and DNA format. The ‘passenger’ sequence refers to sequence complementary to the guide sequence, but including bulges and mismatches designed according to the rules set forth in Table 8 to mimic endogenous miRNA structure. Note that after processing of the pri-miRNA, the passenger strand will likely initiate 1-3 nt downstream of the nucleotide shown in the table, and include 1-3 nt beyond the last nucleotide listed, derived from the miR cassette. **Table 24** lists the top 10 amiRNAs for each miR backbone, excluding low performing backbones. Top amiRNAs were ranked by mean enrichment of sequence counts of the given amiR constructs in the ATXN2 low signal sorted cells. The miR backbone, guide sequence, targeting position within the complementary *ATXN2* sequence, passenger sequence, and the amiRNA sequence are provided in RNA and DNA format.

Table 23: Top 100 amiRNAs

Atxn2 Targeting Position	miR Backbone	Atxn2 low/unsort log2 FC	T1/T0 log2 FC	Guide Sequence (DNA)	Passenger Sequence (DNA)	miR Cassette (DNA)	Guide Sequence (RNA)	Passenger Sequence (RNA)	miR Cassette (RNA)
2945	miR-1-1_M	-2.89804	0.152222	TGTAAGTAG AAGGCTTTG GCTGA [SEQ ID NO:685]	TGAGCCAA AGCTTCTA CCGACA [SEQ ID NO:1633]	CATGCAGACTGC CTGCTTGGGTGA GCCAAAGCCTTC TACCGACATATG GACCTGCTAAGC TATGTAGTAGAA GGCTTTGGCTGA CTCAGGCCGGG ACCTTTCCGCC GCACTGAGGG CACTCCACACCA CGGGGGCC [SEQ ID NO:1711]	UGUAGUAG AAGCUUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UGAGCCAA AGCCUUU ACCGACA [SEQ ID NO:1828]	CAUGCAGACUG CCUGCUUGGU GAGCCAAAGCC UUCUACCGACA UAUGGACCUCC UAAGCUAUGUA GUAGAAAGGCUU UGGCUGACUCA GGCCGGGACCU CUUCCGCCGCA CUGAGGGGCAC UCCACACACG GGGGCC [SEQ ID NO:1908]
3330	miR-190a	-2.86183	0.46905	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCATTATCA GTCTCAGCA CC [SEQ ID NO:1634]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTGTATGC TGAGACTGATA ATGTGGTGTAT TTAATCCACCAT TATCAGTCTCAG CACCTACAGTG TCTTGCCTGTC TCCGGGGGTTC TAATAAG [SEQ ID NO:1712]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CCAUUAUC AGUCUCAG CACC [SEQ ID NO:1829]	GAGCUCAGUCA AACUGGAUGC UUUUUCUGCAG GCCUCUGUGUA UGCUGAGACUG AUAAUGUGGUG UUUUUUAAUCC ACCAUUAUCAG UUCAGCACCC UACAGUGUCU GCCUGUCUCC GGGGGUUCCUA AUAAAG [SEQ ID NO:1909]
3043	miR-144	-2.80802	0.325474	TTTGGTGCA AAACAAAC AGGCT	CCGGTTTGT TTATGCACC AAA	TCAAGCCATGCT TCCTGTGCCCC AGTGGGGCCT GGCTCCGGTTTG	UUUGGUGC AAAACAA CAGGCU	CCGGUUUG UUUAUGCA CCAAA	UCAAGCCAUGC UUCUGUGCCC CCAGUGGGCC CUGGCUCCGGU

2602	miR-144	-2.76068	-0.25422	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	TCGATGGAT CATACTACT AAA [SEQ ID NO:1636]	TTTATGCACCAA AAGTTTGGCATG AGACACTTTGGT GCAAAACAAAC AGGAGTCCGG CACCCCAAGCTC TGGAGCCTGAC AAGGAGGACA [SEQ ID NO:1713]	[SEQ ID NO:1812] UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202] (Same guide as XD-14837)	[SEQ ID NO:1830] UCGAUGGA UCAUACUA CUAAA [SEQ ID NO:1831]	[SEQ ID NO:1910] UCAAGCAUGC UUCUGUGCCC CCAGUGGGCC CUGCUUCGAU GGAUCAUACUA CUAAAAGUUUG CGAUGAGACAC UUUAGUAGUUG AUCCAUAGAAG UCCGGGCACCC CCAGCUCUGGA GCCUGACAAGG AGGACA
3341	miR-1-1_M	-2.70967	0.193529	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATCCTCATG TGATAT [SEQ ID NO:1637]	CATGCAGACTGC CTGCTTGGGGAC TCAGCATCCTCA TGTGATATTATG GACCTGCTAAGC TAATAGACATG AGGATGCTGAG ACCTCAGGCCG GGACCTCTTCCG CCGACTTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1715]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUCCUCAU GUGAUU [SEQ ID NO:1832]	[SEQ ID NO:1911] CAUGCAGACUG CCUGCUUGGGG ACUCAGCAUCC UCAUGUGAUU UAUGGACCUGC UAAGCUAAUAG ACAUGAGGAUG CUGAGACCUCA GGCCGGGACCU CUUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC

3302	miR-100	-2.68521	-0.3818	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAAACC AGACCCCTTG CTCAT [SEQ ID NO:1638]	CCCAAAAGAGA GAAGATATTGA GGCTGTTGCCA CATTGAACAAG GGGCTGATTGG GGTATTAGTCCG CCCAAAACCAGA CCCTTGCTCAT GTGTCGTAGG CAATCTCACGGA CCTGGGGCTTGG CTTATATGCC [SEQ ID NO:1716]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAAAACC AGACCCUU GCUCAU [SEQ ID NO:1833]	CCCAAAAGAGA GAAGAUUUGA GGCCUGUUGCC ACAUUGAACAA GGGCGUAUUU GGGUUAUAGU CCGCCCAAACC AGACCCUUGCU CAUUGUGUCUG UUAGGCAUUCU CACGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1913]
3043	miR-100	-2.67985	-0.0584	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCTGTCTT GGTTTGCAA CAAT [SEQ ID NO:1639]	CCCAAAAGAGA GAAGATATTGA GGCTGTTGCCA CATTGTTGCAA AACAAACAGGC TGTATTAGTCCG AGCTGTCTTGGT TTGCAACAATTG TGTCTGTTAGGC AATCTCACGGAC CTGGGGCTTTGC TTATATGCC [SEQ ID NO:1717]	UUUGGUGC AAAACAAA CAGGCU [SEQ ID NO:1812]	AGCCUGCU UGGUUUGC AACAAU [SEQ ID NO:1834]	CCCAAAAGAGA GAAGAUUUGA GGCCUGUUGCC ACAUUUGGUGC AAAACAAACAG GCUGAUUAGU CCGAGCCUGCU UGGUUUGCAAC AAUUGUGUCUG UUAGGCAUUCU CACGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1914]
1755	miR-100	-2.66915	-0.04161	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTCCA GCTTTCAAA CCGT [SEQ ID NO:1640]	CCCAAAAGAGA GAAGATATTGA GGCTGTTGCCA CATCGGTTGAA ATCTGAAGTGTG GTATTAGTCCG ACACTCCAGCTT TCAAAACCGTTGT GTCTGTTAGGCA ATCTCACGGACC TGGGGCTTTGCT TATATGCC	UCGGGUUG AAUUCUGA AGUGUG [SEQ ID NO:1185]	CACACUCC AGCUUUC AACCGU [SEQ ID NO:1835]	CCCAAAAGAGA GAAGAUUUGA GGCCUGUUGCC ACAUCCGGUUG AAUUCUGAAGU GUGGUUAUAGU CCGCACACUCC AGCUUUCAAAC CGUUGUGUCUG UUAGGCAUUCU CACGGACCUGG

						[SEQ ID NO:1718]			
3272	miR-100	-2.65898	-0.1606	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATACG TTTCTTACA ATCCA [SEQ ID NO:1641]	CCCAAAAGAGA GAAGATATTGA GGCTGTGGCA CAAGGACTGTA GGCAACATATTG CGTATTAGTCCG GCAATACGTTTC CTACAATCCATG TGTCGTAGGC AATCTCAGGAC CTGGGGCTTTGC TTATATGCC [SEQ ID NO:1719]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAAUACG UUUCCUAC AAUCCA [SEQ ID NO:1836]	GGCUUUGCUUA UAUGCC [SEQ ID NO:1915]
967	miR-190a_M	-2.64307	0.113083	ACTGATGTA AGTATATG AACCA [SEQ ID NO:1619]	TGGCATATA CTTACATCA AG [SEQ ID NO:1642]	GAGCTCAGTCA AACCTGGATGCC TTTTCTGCAGGC GTCTGTGACTGA TGTAAGTATATG AACCATGTTATT TAATCCATGGCA TATACTTACATC AAGCTACAGTCT CTTGCCCTGTCT CCGGGGGTTCCT AATAAAG [SEQ ID NO:1720]	ACUGAUGU AAGUAUAU GAACCA [SEQ ID NO:1815]	UGGCAUAU ACUUAACU CAAG [SEQ ID NO:1837]	GAGCUCAGUCA AACCUUGAUGC CUUUUCUGCAG GCGUCUGUGAC UGAUGUAAGUA UAUGAACCAUG UUUUUAAUCC AUGGCAUAUAC UUACAUCACAGC UACAGUCUCUU GCCUCUCUCC GGGGGUUCCUA AUAAAG [SEQ ID NO:1917]
3302	miR-190a	-2.64186	0.124524	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCATCAGC CCCTTGTTCC CC [SEQ ID NO:1643]	GAGCTCAGTCA AACCTGGATGCC TTTTCTGCAGGC CTCTGTGTTGAA CAAGGGGCTGA TTTGGGTGTTAT TTAATCCACCCA TCAGCCCCTTGT TCCCCTACAGTG	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAUCAG CCCCUUGU UCCC [SEQ ID NO:1838]	GAGCUCAGUCA AACCUUGAUGC CUUUUCUGCAG GCCUCUGU GAACAAAGGGC UGAUUUUGGUG UUUUUAAUCC ACCAUCAGCC CCUUUGUCCCC

3302	miR-130a	-2.62809	0.136636	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CGCAAATC AGACCTTG TTCAC [SEQ ID NO:1644]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCACGCA AATCAGACCTT GTTACCTGTCT GCACCTGTCACT AGTTGAACAAG GGGCTGATTGG GTGGCCGTGTAG TGCTACCCAGG CTGGCTGCCTCC TCAGCATTG [SEQ ID NO:1722]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CGCAAATC AGACCTTU GUUCAC [SEQ ID NO:1839]	GCAGGGCCGGC AUGCCUCUGU GUGGGCCACGC AAUUCAGACCC UUGUUCACCU UCUGCACCUU CACUAGUUGAA CAAGGGGCUA UUUGGGUGGCC GUGUAGUGCUA CCCAGGCGUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1919]	UACAGUGUCUU GCCUGUCUCC GGGGGUUCCUA AUAAAG [SEQ ID NO:1918]
1755	miR-1-l_M	-2.62482	0.169485	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CTCACTTCA GATTTCAAC GACGA [SEQ ID NO:1645]	CATGCAGACTGC CTGCTTGGGCTC ACTTCAGATTTC AACGACGATAT GGACCTGCTAA GCTATCGGGTTG AAATCTGAAGT GTGCTCAGGCCG GGACCTTTCGG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1723]	UCGGGUUG AAUUCUGA AGUGUG [SEQ ID NO:1185]	CUCACUUC AGAUUUC ACGACGA [SEQ ID NO:1840]	CAUGCAGACUG CCUGCUUGGGC UCACUUCAGAU UUCACACGACGA UAUGGACCUCC UAAGCUAUCGG GUUGAAUUCUG AAGUGUGCUCA GGCCGGGACCU CUUCGCGCGCA CUGAGGGGCAC UCCACACCCAG GGGGCC [SEQ ID NO:1920]	CAUGCAGACUG CCUGCUUGGGC UCACUUCAGAU UUCACACGACGA UAUGGACCUCC UAAGCUAUCGG GUUGAAUUCUG AAGUGUGCUCA GGCCGGGACCU CUUCGCGCGCA CUGAGGGGCAC UCCACACCCAG GGGGCC [SEQ ID NO:1920]
3302	miR-100_M	-2.62238	-0.19177	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAACC AGACCTTG CTCAT [SEQ ID NO:1638]	CCCAAAAGAGA GAAGATATTGAT GCCTGTTGCCAC ATTGAACAAGG GGCTGATTGGG GTATTAGTCCGC CCAAACCAGAC	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAAACC AGACCTTU GCUCAU [SEQ ID NO:1833]	CCCAAAAGAGA GAAGAUUUGA UGCCUGUUGCC ACAUUGAACAA GGGGCUGAUUU GGGUUAUAGU CCGCCCAAAC	CCCAAAAGAGA GAAGAUUUGA UGCCUGUUGCC ACAUUGAACAA GGGGCUGAUUU GGGUUAUAGU CCGCCCAAAC

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								TATTGAACAAG GGGCTGATTGG GCTCAGGCCGG GACCTCTTCGC CGACTGAGGG GCACTCCACACC ACGGGGGCC [SEQ ID NO:1727]			UAGCUAUUGA ACAAGGGCUG AUUUGGCUCA GGCCGGGACCU CUCCGGCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1924]
1755	miR-130a	-2.56989	0.011195	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CTCACTTCA GCTTTCAAT TCGC [SEQ ID NO:1648]	GCAGGGCCGGC ATGCCTCTGCTG CTGGCCACTCAC TTCAGCTTTCAA TTCGCCTGTCTG CACCTGTCACTA GTCGGGTTGAA ATCTGAAGTGTG TGGCCGTGTAGT GCTACCCAGCGC TGGCTGCCTCT CAGCATTG [SEQ ID NO:1728]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CUCACUUC AGCUUUA AUUCGC [SEQ ID NO:1843]		GCAGGGCCGGC AUGCCUCUGU GCGGCCACUC ACUUCAGCUUU CAAUUCGCUG UCUGCACCGU CACUAGUCGGG UUGAAUUCUGA AGUGUGGCGC GUGUAGUGCUA CCCAGCGCUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1925]	
3272	miR-100_M	-2.56927	-0.01914	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATACG TTTCTTACA ATCCA [SEQ ID NO:1641]	CCCAAAAAGAGA GAAGATAATTGAT GCCTGTGGCCAC AAGGACTGTAG GCAACATATTGC GTATTAGTCCGG CAATACGTTTCC TACAATCCATGT GTCTGTAGGCT ATTCCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1729]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAAUACG UUUCCUAC AAUCCA [SEQ ID NO:1836]		CCCAAAAAGAGA GAAGAUAUUGA UGCCUGUUGCC ACAAGGACUGU AGGCAACAUAU UGCGUAUUAU CCGGCAUAACG UUUCCUACAAU CCAUGUGUCUG UUAGGCUAUUC CAGGACCCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1926]	
3301	miR-130a	-2.55451	-0.07529	TGAACAAG GGGCTGATT TGGGA	TGCCAAATC ATCCCCCTTG TTCC	GCAGGGCCGGC ATGCCTCTGCTG CTGGCCATGCCA	UGAACAAAG GGGCUGAU UUGGGA	UGCCAAAU CAUCCCUU UGUUCG		GCAGGGCCGGC AUGCCUCUGU GCUGCCAUGC	

2943	miR-1-1	-2.54936	0.148183	TAGTAGAA GGCTTTGGC TGAGA [SEQ ID NO:683] TAGTAGAA GGCTTTGGC TGAGA [SEQ ID NO:687] TGTGAGCCA AAGCCTTCT CCCTA [SEQ ID NO:1650]	AATCATCCCTT GTTCCCTGTCTG CACCTGTCACTA GTGAACAAGGG GCTGATTTGGGA TGGCCGTGTAGT GCTACCCAGCGC TGGCTGCCTCCT CAGCATTG [SEQ ID NO:1730]	UAGUAGAA GGCUUUGG CUGAGA [SEQ ID NO:1211]	UGUCAGCC AAAGCCUU CUCCCUA [SEQ ID NO:1845]	CAAAUCAUCCC CUUGUUCUCCUG UCUGCACCUGU CACUAGUGAAC AAGGGGCUGAU UUGGAUGGCC GUGUAGUGCUA CCCAGCGCUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1927]
3338	miR-1-1	-2.52716	-0.2585	TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TGAGTCTCA GCATCCTCA CGGTA [SEQ ID NO:1651]	UACAUGAG GAUGCUA GACUGA [SEQ ID NO:314]	UGAGUCUC AGCAUCCU CACGGUA [SEQ ID NO:1846]	CAUGCAGACUG CCUGCUUGGGU GAGUCACGCA UCCUCACGGUA UAUGGACCUGC UAAGCUAUACA UGAGGAUGCUG AGACUGACUCA GGCCGGGACCU CUCUCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1928]

3302	miR-190a_M	-2.52395	0.249656	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCATCAGC CCCTTGTTCC CC [SEQ ID NO:1643]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC GTCTGTGTGAA CAAGGGGTGA TTTGGGTGTAT TTAATCCACCCA TCAGCCCTTGT TCCCCTACAGTC TCTTGCCTGTC TCCGGGGGTTC TAATAAG [SEQ ID NO:1733]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAUCAG CCCCUUGU UCCC [SEQ ID NO:1838]	GAGCUCAGUCA AACGUGAUGC CUUUUCUGCAG GGUCUCUGUU GAACAAGGGGC UGAUUUGGGUG UUUUUUAAUCC ACCAUCAGCC CCUUGUUCGCC UACAGUCUCUU GCCUGUUCUC GGGGGUUCCUA AUAAAG [SEQ ID NO:1930]
3302	miR-155E	-2.52291	0.018122	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAATC GCCCTTGT CAA [SEQ ID NO:1652]	CTGGAGGCTTGC TTTGGGCTGTAT GCTGTGAACAA GGGCTGATTG GGTTTGGCTC TGACTGACCCAA ATCGCCCTTGT CAACAGGACAA GGCCCTTATCA GCACTACATGG AACAAATGGCC ACCGTGG [SEQ ID NO:1734]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAAUUC GCCCUUGU UCAA [SEQ ID NO:1847]	CUGGAGGCUUG CUUUGGCGUGU AUGCUGUUGAA CAAGGGGUGA UUUGGCUUUUG GCCUCUGACUG ACCCAAUCCG CCUUGUUCAC AGGACAAGGCC CUUUAUCAGCA CUCACAUGGAA CAAUUGGCCAC CGUGGG [SEQ ID NO:1931]
3302	miR-1-1	-2.51939	-0.06675	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CGCAATC AGCCCTTG TCGCA [SEQ ID NO:1647]	CATGCAGACTGC CTGCTTGGGCG AAATCAGCCCT TGTCGCAATATG GACCTGCTAAGC TATTGAACAA GGGCTGATTGG GCTAGGCCGG GACCTCTCGC CGCACTGAGG GCACTCCACACC ACGGGGGCC	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CGCAAUUC AGCCCUU GUCGCA [SEQ ID NO:1842]	CAUGCAGACUG CCUGCUUGGGC GCAAUUCAGCC CCUUGUCGCA UAUGGACCCUG UAAGCUAUUGA ACAAGGGGCGU AUUUGGCGUCA GGCCGGGACCU CUCUCGCCCA CUGAGGGGCAC

2586	miR-155E	-2.5179	-0.10172	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAGTTCA CTCTGAATC TA [SEQ ID NO:1653]	CTGGAGGCTTGC TTTGGGCTGTAT GCTGTAGATTCA GAAGTAGAAT TGGTTTGGCCT CTGACTGACCAA GTTCACTCTGAA TCTACAGGACA AGGCCCTTATC AGCACTCACATG GAACAAATGCG CACCGTGGG [SEQ ID NO:1736]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAAGUUC ACUCUGAA UCUA [SEQ ID NO:1848]	UCCACACCACG GGGGC [SEQ ID NO:1932]
3341	miR-1-1	-2.50291	-0.11115	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATGCTCATG TGATAT [SEQ ID NO:1637]	CATGCAGACTGC CTGCTTGGGAC TCAGCATCCTCA TGTGATATTATG GACCTGCTAAGC TAATAGACATG AGGATGCTGAG ACCTCAGGCG GGACCTCTCTCG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1737]	AUAGACAU GAGGAGGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUCUCU GUGAUU [SEQ ID NO:1832]	CAUGCAGACUG CCUGCUUGGG ACUCAGCAUCC UCAUGUGAUU UAUGGACCUGC UAAGCUAAUAG ACAUAGGAGU CUGAGACCUCA GGCCGGGACCU CUCUCGCCGA CUGAGGGGCAC UCCACACCACG GGGGC [SEQ ID NO:1933]
1580	miR-190a	-2.50168	-0.06321	ACTGGAATT TCTCTGAAC TGCT [SEQ ID NO:1622]	AGCTTCAG AGAAATTC CAAG [SEQ ID NO:1654]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTGACTGG AATTTCTCTGAA CTGCTTGTATT TAATCCAAGCTT CAGAGAAATTC CAAGCTACAGT	ACUGGAAU UUCUCUGA ACUGCU [SEQ ID NO:1817]	AGCUUCAG AGAAAUUC CAAG [SEQ ID NO:1849]	GAGCUCAGUCA AACUCGGAUC CUUUUCUGCAG GCCUCUGUGAC UGGAUUUUCUC UGAACUCGCUU UUUUUUAAUCC AAGCUUCAGAG AAAUUCCAAGC [SEQ ID NO:1934]

3332	miR-122	-2.50104	-0.209	AGGATGCT GAGACTGA TAATGT [SEQ ID NO: 1623]	ACATTATCA GTATCAGC ATAAT [SEQ ID NO: 1655]	GGCTACAGAGTT TCCTTAGCAGAG CTGAGGATGCTG AGACTGATAAT GTTGTCTAAACT ATACATTATCAG TATCAGCATAAT TAGTACTGCTA GGCAATCCTTCC CTCGATAAATGT CTTGGCATCGTT TGCTTTG [SEQ ID NO: 1739]	AGGAUGCU GAGACUGA UAAUGU [SEQ ID NO: 1818]	ACAUUAUC AGUAUCAG CAUAAU [SEQ ID NO: 1850]	UACAGUGUCUU GCCCUGUCUC GGGGGUCCUA AUAAAG [SEQ ID NO: 1935]
3133	miR-100_M	-2.49525	0.238806	TATGTCCTG GCTTGATTC ACTG [SEQ ID NO: 1624]	CAGTGACTC ACGCCAAG CCATT [SEQ ID NO: 1656]	CCCAAAAGAGA GAAGATATTGAT GCCTGTTGCCAC ATATGTCCTGGC TTGATTCACCTGG TATTAGTCCGCA GTGACTCAGCC AAGCCATTGTG TCTGTTAGGCTA TCCACGGACCT GGGGCTTTGCTT ATATGCC [SEQ ID NO: 1740]	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO: 1819]	CAGUGACU CACGCCAA GCCAUU [SEQ ID NO: 1851]	CCCAAAAGAGA GAAGAUUUGA UGCCUGUUGCC ACAUUGUCUU GGCUUGAUCA CUGGUUUAGU CCGCAGUGACU CACGCCAAGCC AUUUGUCUG UUAGGCUAUUC CAGGACCCUG GGCUUUGCUA UAUGCC [SEQ ID NO: 1936]
3341	miR-122	-2.4857	0.187025	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO: 1617]	GTCTCAGCA TCATCATGT CGCT [SEQ ID NO: 1657]	GGCTACAGAGTT TCCTTAGCAGAG CTGATAGACATG AGGATGCTGAG ACTGTCTAAACT ATGTCTCAGCAT CATCATGTCGCT	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO: 1813]	GUCUACGC AUCAUCAU GUCCGU [SEQ ID NO: 1852]	GGCUACAGAGU UUCCUUAGCAG AGCUGAUAGAC AUAGGAUGCU GAGACUGUCU AACUAUGUCU AGCAUCAUCAU [SEQ ID NO: 1937]

3341	miR-132	-2.48196	0.14613	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GCCTCAGC ATCATAATG TCTAT [SEQ ID NO:1658]	TAGTACTGCTA GGCAATCCTTCC CTCGATAAATGT CTTGGCATCGTT TGCTTTG [SEQ ID NO:1741]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GCCUCAGC AUCAUAAU GUCUAAU [SEQ ID NO:1853]	GUCGCUUAGCU ACUGCUAGGCA AUCGUUCCUC GAUAAAUGUCU UGGCAUCGUUU GCUUUG [SEQ ID NO:1938]
3330	miR-1-1_M	-2.47751	0.392579	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CGACATTAT CAGTCTCAG GAATA [SEQ ID NO:1659]	CATGCAGACTGC CTGTTGGGCGA CATTATCAGTCT CAGGAATATAT GGACCTGCTAA GCTATATGCTGA GACTGATAATGT GGCTCAGCCG GGACCTCTCCG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1743]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CGACAUUA UCAGUCUC AGGAUA [SEQ ID NO:1854]	CAUGCAGACUG CCUGCUUGGC GACAUUAUCAG UCUCAGGAUA UAUGGACCUCG UAAGCUUAUUG CUGAGACUGAU AAUGUGGUCUA GGCCGGGACCU CUUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1940]
3255	miR-144	-2.47679	-0.08595	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	ACAAGCTT ACTGCCAC GCAAT [SEQ ID NO:1660]	TCAAGCCATGCT TCCTGTGCCCC AGTGGGGCCCT GGCTACAAGCTT ACTGCCACGCA	AUUGCUG GAGUAAGC UGGUGG [SEQ ID NO:306]	ACAAGCUU ACUGCCAC GCAAU [SEQ ID NO:1855]	UCAAGCCAU UUCUGUGCCC CCAGUGGGGCC CUGGCUACAAG CUUACUGCCAC

								ATAGTTTGCAT GAGACATTTG CGTGGAGTAAG CTGTTAGTCCGG GCACCCCAAGT CTGGAGCCTGAC AAGGAGGACA [SEQ ID NO:1744]	(Same guide as XD-14889)			GCAAUAGUUUG CGAUGAGACAC AUUGCUGGAG UAGCUGGUAG UCCGGGCACC CCAGCUCUGGA GCCUGACAAGG AGGACA [SEQ ID NO:1941]
1755	miR-100_M	-2.4723	-0.07055	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTCCA GCTTTCAAA CCGT [SEQ ID NO:1640]	CCCAAAAGAGA GAAGATATTGAT GCCTGTGCCAC ATCGGTTGAA ATCTGAAGTGTG GTATTAGTCCGC ACACTCCAGCTT TCAAACCGTTGT GTCTGTTAGGCT ATTCCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1745]	UCGGGUUG AAAUUCUGA AGUGUG [SEQ ID NO:1185]	CACACUCC AGCUUUA AACCGU [SEQ ID NO:1835]		CCCAAAAGAGA GAAGAUUUGA UGCCUGUGCC ACAUCCGGUUG AAUUCUGAAGU GUGGUUUUAGU CCGCACACUCC AGCUUUAACAC CGUUGUGUCUG UUAGGCUAUUC CACGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1942]		
2586	miR-190a_M	-2.46486	0.014777	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCATTCTAC TTCTGAATC CC [SEQ ID NO:1661]	GAGCTCAGTCA AACCTGGATGCC TTTTTGCAGGC GTCTGTGTAGAT TCAGAAAGTAGA ACTTGGTGTAT TTAATCCACCAT TCTACTTCTGAA TCCCCTACAGTC TCTTGCCCTGTC TCCGGGGGTTCC TAATAAAG [SEQ ID NO:1746]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAUUCUA CUUCUGAA UCCC [SEQ ID NO:1856]		GAGCUCAGUCA AACCCUGGAGC CUUUUCUGCAG GCGUCUGUGUA GAUUCAGAAU AGAACUUGGUG UUUUUUAAUCC ACCAUUCUACU UCUGAAUCCCC UACAGUCUCUU GCCUGUCUCC GGGGUUCUUA AUAAAG [SEQ ID NO:1943]		
2586	miR-1-l_M	-2.46317	0.179187	TAGATTGAG AAGTAGAA CTTGG	CGAAGTTCT ACTTCTGAA CGCTA	CATGCAGACTGC CTGCTTGGCGA AGTTCTACTTCT	UAGAUUCA GAAGUAGA ACUUGG	CGAAGUUC UACUUCUG AACGCA		CAUGCAGACUG CCUGCUUGGGC GAAGUUCUACU		

3272	miR-1-1_M	-2.45517	0.050153		[SEQ ID NO:1621]	[SEQ ID NO:1662]	GAACGCTATATG GACCTGCTAAGC TATAGATTTCAGA AGTAGAACTTG GCTCAGGCCGG GACCTTTCCGC CGACTGAGGG GCACTCCACAC ACGGGGGCC [SEQ ID NO:1747]	[SEQ ID NO:1816]	[SEQ ID NO:1857]	UCUGAACGCUA UAUGGACCUGC UAAGCUAAGA UUCAGAAGUAG AAUUGGCUCA GGCCGGGACCU CUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1944]
					AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GGAATATG TTGCTTACA GCGCCT [SEQ ID NO:1663]	CATGCAGACTGC CTGCTTGGGGG ATATGTTGCTA CAGCGCTTATG GACCTGCTAAGC TAAGGACTGTA GGCAACATATTG CCTCAGGCCGG GACCTCTCCGC CGACTGAGGG GCACTCCACAC ACGGGGGCC [SEQ ID NO:1748]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GGAAUAUG UUGCCUAC AGCGCCU [SEQ ID NO:1858]	CAUGCAGACUG CCUGCUUGGG GAAUAUUGUC CUACAGGCCU UAUGGACCUGC UAAGCUAAGGA CUGAGGCAAC AUUUGCCUCA GGCCGGGACCU CUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1945]
2943	miR-1-1_M	-2.45216	0.338577		TAGTAGAA GGCTTTGGC TGAGA [SEQ ID NO:683]	TGTCAGCCA AAGCCTTCT CCCTA [SEQ ID NO:1650]	CATGCAGACTGC CTGCTTGGGTGT CAGCCAAAGCC TTCTCCCTATAT GGACTGCTAA GCTATAGTAGA AGGCTTTGGCTG AGACTCAGGCC GGGACCTCTTCC GCCGACTGAG GGGCACTCCAC ACCAAGGGGGC C [SEQ ID NO:1749]	UAGUAGAA GGCUUUGG CUGAGA [SEQ ID NO:1211]	UGUCAGCC AAAGCCUU CUCCCUA [SEQ ID NO:1845]	CAUGCAGACUG CCUGCUUGGU GUCAGCCAAAG CCUUCUCCCUA UAUGGACCUGC UAAGCUAAGU AGAAGGCUUUG GCUAGACUCA GGCCGGGACCU CUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1946]

3043	miR-190a_M	-2.44458	0.289334	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCGTTTGT TTTGCACCA CC [SEQ ID NO:1664]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC GTCTGTGTTGG TGCAAAACAAA CAGGCTTGTAT TTAATCCAAGCG TTTGTTTGCAC CACCTACAGTC TCTTGCCCTGTC TCCGGGGGTTCC TAATAAG [SEQ ID NO:1750]	UUUGGUGC AAACAAA CAGGCU [SEQ ID NO:1812]	AGCGUUUG UUUUGCAC CACC [SEQ ID NO:1859]	GAGCUCAGUCA AACGUGAUGC CUUUUCUGCAG GGCUCUGUUU UGGUGCAAAAC AAACAGGCUUG UUAUUUAUCC AAGCGUUUGUU UUGCACACCC UACAGUCUUU GCCUGUCC GGGGGUUCCUA AUAAAG [SEQ ID NO:1947]
1578	miR-100	-2.43871	-0.43493	TGGAATTTC TCTGAACTG CTGT [SEQ ID NO:1626]	ACAGCAAT TCCGAGAA ACTCCT [SEQ ID NO:1665]	CCCAAAAGAGA GAAAGATATTGA GGCTGTTGCCA CATGGAATTCT CTGAACTGCTGT GTATTAGTCCGA CAGCAATCCGA GAAACTCCTGT GTCTGTAGGCA ATCTCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1751]	UGGAAUUU CUCUGAAC UGCUGU [SEQ ID NO:1820]	ACAGCAAU UCCGAGAA ACUCCU [SEQ ID NO:1860]	CCCAAAAGAGA GAAAGAUUUGA GGCUGUUGCC ACAUUGAAUUU CUCUGAACUGC UGUGUAUUAGU CCGACAGCAAU UCCGAGAAACU CCUUGUGUCUG UUAGGCAUUCU CACGGACCCUG GGCUUUGCUUA UAUGCC [SEQ ID NO:1948]
3133	miR-122_M	-2.43848	0.197681	TATGTCTTG GCTTGATTG ACTG [SEQ ID NO:1624]	CAGTGAAT CAAACCAA GACCGA [SEQ ID NO:1666]	GGCTACAGAGTT TGCTTAGCAGAG CTGTATGTCTTG GCTTGATTCAT GTGTCTAAACTA TCAGTGAATCAA ACCAAGACCGA TAGCTACTGCTA GGCCATCCTTCC CTCGATAAATGT CTTGGCATCGTT TGCTTTG	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO:1819]	CAGUGAAU CAAACCAA GACCGA [SEQ ID NO:1861]	GGCUACAGAGU UUGCUUAGCAG AGCUGUAUGUC UUGGCUUGAUU CACUGUGUCUA AACUAUCAGUG AAUCAAACCAA GACCGAUAGCU ACUGCUAGGCC AUCCUCCUCC GAUAAAUGUCU

3341	miR-130a	-2.43802	0.12379	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATACTCATG TTTAC [SEQ ID NO:1667]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCAGATC AGCATACTCATG TTTACCTGTCTG CACCTGTCACTA GATAGACATGA GGATGTGAGA CTGGCCGTGTAG TGCTACCCAGCG CTGGCTGCCCTCC TCAGCATTG [SEQ ID NO:1753]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUACUCAU GUUAC [SEQ ID NO:1862]	UGGCAUCGUUU GCUUUG [SEQ ID NO:1949]
3330	miR-130a	-2.43606	0.221874	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CGACATTAT CCGTCTCAG TATC [SEQ ID NO:1668]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCACGAC ATTATCCGTCTC AGTATCCTGTCT GCACCTGTCACT AGTATGCTGAG ACTGATAATGTG GTGGCCGTGTAG TGCTACCCAGCG CTGGCTGCCCTCC TCAGCATTG [SEQ ID NO:1754]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CGACAUUA UCCGUCUC AGUAUC [SEQ ID NO:1863]	GCAGGGCCGGC AUGCCUCUCU GUGGCCACGA CAUAUCCGUC UCAGUAUCCUG UCUGCACCCUGU CACUAUAUGC UGAGACUGAUA AUGUGGUGGCC GUGUAGUGCUA CCCAGGCGUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1950]
3255	miR-130a	-2.43262	0.01754	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CGACCAGC TTCTCTCCAC GTAAC [SEQ ID NO:1669]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCACGAC AGCTTCTCCAC GTAACCTGTCTG CACCTGTCACTA GATTGCGTGA GTAAGCTGGTG GTGGCCGTGTAG	AUUGCUG GAGUAAGC UGGUGG [SEQ ID NO:306] (Same guide as XD-14889)	CGACCAGC UCCUCCA CGUAAC [SEQ ID NO:1864]	GCAGGGCCGGC AUGCCUCUGCU GUGGCCACGA CCAGCUCCUC CACGUAACCCUG UCUGCACCCUGU CACUAUAUGC GUGGAGUAAGC UGGUGGUGGCC

						TGCTACCCAGCG CTGGCTGCTCC TCAGCATTG [SEQ ID NO:1755]					
3302	miR-132	-2.43028	0.018802	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CACAAATC AGCACATT GTTCAA [SEQ ID NO:1670]	GCCGTCCGGCG CCCCGCCCGC GTCTCCAGGCA CAAATCAGCAC ATTGTTCAACTG TGGGAACCTGA GGTTGAACAAG GGCTGATTGG GCCCCGACGA CGCCACGCGC GCGCCACGCG CGCCCCGAGCC [SEQ ID NO:1756]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CACAAAU AGCACAU GUUCAA [SEQ ID NO:1865]	GCCGUCGCG CCCCGCCCG GCUCUCACAG GCACAAU CACAUU ACUGUGGAAC UGGAGGUUGAA CAAGGGGCUA UUUGGGCCCG CAGCACGCCA CGCGCGCGCC ACGCCGCGCC CGAGCC [SEQ ID NO:1953]	GUGUAGUCUA CCCAGGCGUG CUGCCUCCUA GCAUUG [SEQ ID NO:1952]	
3133	miR-130a	-2.42664	0.412413	TATGTCTTG GCTTGATTC ACTG [SEQ ID NO:1624]	CTGTGAATC ACGCCAAG ATATC [SEQ ID NO:1671]	GCAGGGCCGGC ATGCCTCTGTG CTGGCCACTGTG AATCACGCCAA GATATCTGTCT GCACCTGTCACT AGTATGTCTTG CTTGATTCACG TGGCCGTGTAGT GCTACCCAGCGC TGGCTGCCTCT CAGCATTG [SEQ ID NO:1757]	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO:1819]	CUGUGAAU CAGCCAA GAUAUC [SEQ ID NO:1866]	GCAGGGCCGGC AUGCCUCUGU CUGGGCCACUG UGAAUACGCC AAGAUUCCUG UCUGCACCCUG CACUAGUAGU CUUGGCUUGAU UCACUGGGCC GUGUAGUGUA CCCAGGCGUG CUGCCUCCUA GCAUUG [SEQ ID NO:1954]		
3302	miR155-M	-2.42428	0.285147	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAATC GCCCTTGTT CAA [SEQ ID NO:1652]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTTGAACA AGGGCTGATTT GGTTTTGGCCA CTGACTGACCCA AATCGCCCTGTG	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAAUC GCCCUUGU UCAA [SEQ ID NO:1847]	CCUGGAGGCUU GCUGAAGGCGU UAUGCGUUGUA ACAAGGGCGUG AUUUGGUUUU GGCCACUGACU GACCCAAUUCG		

2586	miR155-M	-2.4228	-0.13209				TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CAAAGTTCA CTCTGAATC TA [SEQ ID NO:1653]		TCAACAGGACA CAAGGCTGTTA CTAGCACTCACA TGAACAAATG GCCACCGG [SEQ ID NO:1758]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAAGUUC ACUCUGAA UCUA [SEQ ID NO:1848]	CCCUGUUCAA CAGGACACAAG GCCUGUUCUA GCACUCACAUG GAACAAUUGGC CACCGG [SEQ ID NO:1955]
1755	miR-122	-2.42198	0.034447				TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTTCA GACTTCAAC CATA [SEQ ID NO:1646]		GGCTACAGAGTT TCCTTAGCAGAG CTGTGGGTGA AATCTGAAGTGT GTGTCTAACTA TCACACTTCAGA CTTCAACCATAT AGCTACTGCTAG GCAATCCTTCCC TCGATAAATGTC TTGGCATCGTTT GCTTTG [SEQ ID NO:1760]	UCGGGUUG AAUUCUGA AGUGUG [SEQ ID NO:1185]	CACACUUC AGACUUC ACCAUA [SEQ ID NO:1841]	GGCUACAGAGU UUCUUAGCAG AGCUGUCGGU UGAAUUCUGAA GUGUGUGUCA AACUAUCACAC UUCAGACUUA ACCAUUAAGCU ACUGCUAGGCA AUCCUCCUCUC GAUAAUUGUCU UGGCAUCGUUU GCUUUG [SEQ ID NO:1956]
2945	miR-1-l	-2.42094	-0.06694				TGTAGTAG AAGGCTTTG GCTGA [SEQ ID NO:685]	TGAGCCAA AGCCTTCTA CCGACA [SEQ ID NO:1633]		CATGCAGACTGC CTGCTTGGGTGA GCCAAAGCCTTC TACCGACATATG GACCTGCTAAGC [SEQ ID NO:1757]	UGUAGUAG AAGGCUUU GGCUGA [SEQ ID NO:1213]	UGAGCCAA AGCCUUCU ACCGACA [SEQ ID NO:1828]	CAUGCAGACUG CCUGCUUGGU GAGCCAAAGCC UUCUACCGACA UAUGGACCUGC [SEQ ID NO:1957]

								TATGTAGTAGAA GGCTTTGGCTGA CTCAGGCCGGG ACCTCTCTCGCC GCACTGAGGGG CACTCCACACCA CGGGGGCC [SEQ ID NO:1761]	(Same guide as XD-14860)			UAGCUAUGAA GUAGAAGGCUU UGGUGACUCA GGCCGGGACCU CUCUGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1958]
1755	miR-132	-2.41844	0.196175	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CCCACTTCA GAGTGCAA CCCGA [SEQ ID NO:1672]	GCCGTCCGCGCG CCCCGCCCCCG GTCTCCAGGGCC CACTTCAGAGTG CAACCCGACTGT GGGAACTGGAG GTCTGGGTTGAA ATCTGAAAGTGTG CCCCGACGAC GCCACGCGCC GCGCCACGCGG CGCCCCGAGCC [SEQ ID NO:1762]	UCGGGUUG AAAUUCUGA AGUGUG [SEQ ID NO:1185]	CCCACUUC AGAGUGCA ACCCGA [SEQ ID NO:1867]	GCCGUCGCGC CCCCGCCCCC GCGUCUCCAG GCCCAUUCAG AGUGCAACCCG ACUGUGGGAAC UGGAGGUCCGG UUGAAUUCUGA AGUGUGCCCCG CAGCACGCCA CGCGCCGCGCC ACGCCGCGCCC CGAGCC [SEQ ID NO:1959]			
3301	miR-1-1	-2.41082	0.051034	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TGCCAAATC AGCCCTTG CGTCA [SEQ ID NO:1673]	CATGCAGACTGC CTGCTTGGGTGC CAAATCAGCCCC TTGCGTCATATG GACCTGCTAAGC TATGAACAAGG GGCTGATTGGG ACTCAGGCCGG GACCTCTCTCGC CGCACTGAGGG GCACTCCACACC ACGGGGGCC [SEQ ID NO:1763]	UGAACAAG GGGCUGAU UUGGGA [SEQ ID NO:1215]	UGCCAAAU CAGCCCUU UGCGUCA [SEQ ID NO:1868]	CAUGCAGACUG CCUGCUUGGU GCCAAAUACAGC CCCUUGCGUCA UAUGGACCUGC UAAGCUAUGAA CAAGGGGCUGA UUUGGACUCA GGCCGGGACCU CUCUCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1960]			
2602	miR-132	-2.40987	-0.18915	TTTAGTAGT TGATCCATA GATT	ACTCTATGG ATAACCTAC TAAA	GCCGTCCGCGCG CCCCGCCCCCG GTCTCCAGGAC GCTCTCCAGGAC						

					[SEQ ID NO:1674]	TCTATGGATAAC CTACTAACTGT GGGAACGGAG GTTAGTAGTTG ATCCATAGATTG CCCGCAGCAG CCACGCGCCG GCCACGCCCG CCCCGAGCC [SEQ ID NO:1764]	[SEQ ID NO:202] (Same guide as XD-14837)	CCCAAAUC AGCACCUU GUUACA [SEQ ID NO:1870]	GACUCUAUGGA UAACCUACUAA ACUGUGGGAAC UGGAGGUUUAG UAGUUGAUCCA UAGAUUCCCCG CAGCAGGCCA CGCGCCGCC ACCGCGGCC CGAGCC [SEQ ID NO:1961]
3302	miR-122_M	-2.40308	-0.14782	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAAATC AGCACCTTG TTACA [SEQ ID NO:1675]	GGCTACAGAGTT TGCTTAGCAGAG CTGTGAAACAAG GGGCTGATTGG GTGTCTAACTA TCCCAAATCAGC ACCTTGTACAT AGCTACTGCTAG GCCATCCTTCCC TCGATAAATGTC TTGGCATCGTTT GCTTTG [SEQ ID NO:1765]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAAAUC AGCACCUU GUUACA [SEQ ID NO:1870]	GGCUACAGAGU UUGCUUAGCAG AGCUGUUGAAC AAGGGCUGAU UUGGUGUCUA AACUAUCCCA AUCAGACCCU GUUACAUAGCU ACUGCUAGGCC AUCCUCCCCC GAUAAUUGUCU UGGCAUCGUUU GCUUUG [SEQ ID NO:1962]
1755	miR-155E	-2.39604	-0.03915	TCGGGTGA AAATCGAA GTGTG [SEQ ID NO:657]	CACACTCG ATTCAACCC GA [SEQ ID NO:1676]	CTGGAGGCTTGC TTTGGGCTGTAT GCTGTCGGGTG AAATCTGAAGT GTGTTTGGCCT CTGACTGACACA CTTCGATTCAAC CCGACAGGACA AGGCCCTTATC AGCACTCACATG GAACAAATGGC CACCGTGGG [SEQ ID NO:1766]	UCGGGUUG AAUUCUGA AGUGUG [SEQ ID NO:1185]	CACACUUC GAUUC AAC CCGA [SEQ ID NO:1871]	CUGGAGGCUUG CUUUGGGCUGU AUGCUGUCGGG UUGAAUUCUGA AGUGUUUUUG GCCUCUGACUG ACACACUUCGA UUC AACCCGAC AGGACAAAGCC CUUUAUCAGCA CUCACAUUGGA CAAAU'GGCCAC CGUGGG [SEQ ID NO:1963]

3842	miR-130a	-2.39495	-0.55009	AACGTGAG AAGGATGG ATCGTA [SEQ ID NO:1625]	TTCGATCCA TACTTCTCA TGTC [SEQ ID NO:1677]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCATTCGA TCCATCTTCTC ATGTCCTGCTG CACCTGTCACTA GAACGTGAGAA GGATGGATCGT ATGGCCGTGTAG TGCTACCCAGCG CTGGCTGCCTCC TCAGCAITG [SEQ ID NO:1767]	AACGUGAG AAGGAGG AUCGUA [SEQ ID NO:1824]	UUCGAUCC AUACUUCU CAUGC [SEQ ID NO:1872]	GCAGGGCCGGC AUGCCUCUGCU GUGGCCAUUC GAUCCAACUU CUCAUGUCCUG UCUGCACCCUGU CACUAGAACGU GAGAAGGAUGG AUCGU AUGGCC GUGUAGUGCUA CCCAGCGCUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1964]
2945	miR-100	-2.38747	-0.10088	TGTAGTAG AAGGCTTTG GCTGA [SEQ ID NO:685]	TCAGCCCA AGACTTCTA ATACT [SEQ ID NO:1678]	CCCCAAAGAGA GAAGATATTGA GGCTGTGGCCA CATGTAGTAGA AGGCTTTGGCTG AGTATTAGTCCG TCAGCCCAAGA CTTCTAATACTT GTGCTGTTAGG CAATCTCACGGA CCTGGGGCTTTG CTTATATGCC [SEQ ID NO:1768]	UGUAGUAG AAGGCUUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UCAGCCCA AGACUUCU AAUACU [SEQ ID NO:1873]	CCCCAAAGAGA GAAGAUAUUGA GGCUGUUGCC ACAUAGUAG AAGGCUUUGGC UGAGUAUAGU CCGUCAGCCCA AGACUUCUAAU ACUUGUGUCUG UUAGGCAUUCU CACGGACCCUG GGCUUUGCUUA UAUGCC [SEQ ID NO:1965]
2586	miR-130a	-2.38521	0.042149	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CGAAGTTCT AATTCTGAA TCTC [SEQ ID NO:1679]	GCAGGGCCGGC ATGCTCTGCTG CTGGCCACGAA GTTCTAATTCTG AATCTCCTGTCT GCACCTGTCACT AGTAGATTGAG AAGTAGAACTT GTTGGCCGTGTA GTGCTACCCAGC GCTGGCTGCCTC CTCAGCAITG	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CGAAGUUC UAAUUCUG AAUUC [SEQ ID NO:1874]	GCAGGGCCGGC AUGCCUCUGCU GUGGCCACGA AGUUCUAAUUC UGAAUCUCCUG UCUGCACCCUGU CACUAGAAGAU UCAGAAAGUAGA ACUUGGUGGCC GUGUAGUGCUA CCCAGCGCUGG

3301	miR-1-1_M	-2.38494	0.327128	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TGCCAAATC AGCCCTTG CGTCA [SEQ ID NO:1673]	CATGCAGACTGC CTGCTTGGTGC CAAATCAGCCC TTGCGTCATATG GACCTGCTAAGC TATGAACAAGG GGCTGATTGGG ACTCAGGCCGG GACCTTTCGC CGCACTGAGGG GCACTCCACAC ACGGGGGCC [SEQ ID NO:1770]	UGAACAAAG GGGCUAGU UUGGGA [SEQ ID NO:1215]	UGCCAAAU CAGCCCU UGCUGCA [SEQ ID NO:1868]	CUGCCUCCUCA GCAUUG [SEQ ID NO:1966]
1755	miR-190a	-2.38113	0.329455	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACTTCAGA TTTCAACCC AC [SEQ ID NO:1680]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTCTCGG TTGAAATCTGAA GTGTGTGTATT TAATCCACACTT CAGATTTCACAC CACCTACAGTGT CTTGCCCTGTCT CCGGGGTTCCT AATAAAG [SEQ ID NO:1771]	UCGGGUUG AAACUGA AGUGUG [SEQ ID NO:1185]	CACUUCAG AUUUCAC CCAC [SEQ ID NO:1875]	GAGCUCAGUCA AACCUUGAUC CUUUUCGCAG GCCUCUGUGUC GGGUUGAAUUC UGAAGUGUGUG UUUUUUAUCC ACACUUCAGAU UUCACCCACC UACAGUGUCUU GCCUGUCC GGGGUUCUUA AUAAAG [SEQ ID NO:1968]
2602	miR-1-1_M	-2.37919	0.040602	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ATTCTATGG ATCAACTAC CGAAA [SEQ ID NO:1681]	CATGCAGACTGC CTGCTTGGGATT CTATGGATCAAC TACCGAAATATG GACCTGCTAAGC TATTAGTAGTT GATCCATAGATT CTCAGGCCGGG ACCTCTTCCGCC	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202] (Same guide as XD-14837)	AUUCUAUG GAUCAAACU ACCGAAA [SEQ ID NO:1876]	CAUGCAGACUG CCUGCUUGGA UUCUAUGGAUC AAUACCGAAA UAGGACCGUC UAAGCUAUUA GUAGUUGAUCC AUAGAUUCUA GGCCGGGACCU

1231	miR-100_M	-2.37228	-0.26919	TTCACCTTA GCACTGAT AGCAG [SEQ ID NO:1627]	CTGCTACCA GGGCTAAA ATGAT [SEQ ID NO:1682]	GCACTGAGGG CACTCCACACCA CGGGGGC [SEQ ID NO:1772]	UUCACUUU AGCACUGA UAGCAG [SEQ ID NO:1825]	CUGCUACC AGGGCUAA AAUGAU [SEQ ID NO:1877]	CUUCGCGCGCA CUGAGGGGCAC UCCACACCACG GGGGC [SEQ ID NO:1969]
3273	miR-155E	-2.36984	-0.0801	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATATGTG CCACAGTCC TA [SEQ ID NO:1683]	CTGGAGGCTTGC TTTGGGCTGTAT GCTGTAGGACTG TAGGCAACATAT TGTTTGGCCTC TGACTGACAATA TGTGCCACAGTC CTACAGGACAA GGCCCTTTATCA GCACTCACATGG AACAAATGGCC ACCGTGGG [SEQ ID NO:1774]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAUAUUGU GCCACAGU CCUA [SEQ ID NO:1878]	CUGGAGGCUUG CUUUGGCGUG AUGCUGUAGGA CUGUAGGCAAC AUUUGUUUUG GCCUCUGACUG ACAAUUGUGC CACAGUCCUAC AGGACAAGGCC CUUUAUCAGCA CUCACAUGGAA CAAUUGGCCAC CGUGGG [SEQ ID NO:1971]
3301	miR-190a	-2.36785	0.195332	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCAATCAG CCCCTTGT AC [SEQ ID NO:1684]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTGTGAAC AAGGGGCTGAT TTGGGATGTTAT TTAATCCATCCA	UGAACAAAG GGGCUAGU UUGGGA [SEQ ID NO:1215]	UCCAAUCA GCCCUUG UUAC [SEQ ID NO:1879]	GAGCUCAGUCA AACCTGGAGUC CUUUUCUGCAG GCCUCUGUGUG AACAAAGGGCU GAUUUGGGAUG UUUUUUAAUCC

3043	miR-100_M	-2.35911	0.045827	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCTTGCTT GGTTTGCAA CAAT [SEQ ID NO:1639]	CCCAAAAGAGA GAAGATATTGAT GCTGTGGCCAC ATTTGGTGCAA ACAAACAGGCT GTATTAGTCCGA GCCTGCTTGGTT TGCAACAATTGT GTCTGTTAGGCT ATTCCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1776]	ATCAGCCCCTTG TTACCTACAGTG TCTTGCCCTGTC TCCGGGGGTTCC TAATAAAG [SEQ ID NO:1775]	UUUGGUGC AAAACAAA CAGGCU [SEQ ID NO:1812]	AGCCUGCU UGGUUUGC AACAAU [SEQ ID NO:1834]	AUCAAUCAGC CCCUUGUACC UACAGUGUCUU GCCUGUCUCC GGGGUUCUUA AUAAAG [SEQ ID NO:1972]
2586	miR-122	-2.35707	0.153236	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAAGTTCT ACCTCTGAA TAGA [SEQ ID NO:1685]	GGCTACAGAGTT TCCTTAGCAGAG CTGTAGATTGAG AAGTAGAACTT GGTGTCTAACT ATCCAAGTTCTA CCTCTGAATAGA TAGCTACTGCTA GGCAATCCTTCC CTCGATAAATGT CTTGGCATCGTT TGCTTTG [SEQ ID NO:1777]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAAGUUC UACCUCUG AAUAGA [SEQ ID NO:1880]	GGCUACAGAGU UUCUUAGCAG AGCUGUAGAUU CAGAAGUAGAA CUUGGUGUCUA AACUAUCCAAG UUCUACCUCUG AAUAGAUAGCU ACUGCUAGGCA AUCCUUCUCCUC GAUAAAUGUCU UGGCAUCGUUU GCUUUG [SEQ ID NO:1973]	
1755	miR-190a_M	-2.34216	0.272299	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACCTCAGA TTTCAACCC AC [SEQ ID NO:1680]	GAGCTCAGTCA AACCTGGATGCC TTTTCTGCAGGC GTCTGTGTCGGG TTGAAATCTGAA [SEQ ID NO:1777]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CACUUCAG AUUUCAC CCAC [SEQ ID NO:1875]	GAGCUCAGUCA AACCGGAUGC CUUUUCUGCAG GCGUCUGUGC GGGUUGAAAUUC [SEQ ID NO:1974]	

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				[SEQ ID NO:603]	[SEQ ID NO:1688]	TGGCGACATATG GACCTGCTAAGC TATGTACACAA CAAAGTCTGAA CCTCAGGCCG GACCTCTTCGC CGCACTGAGG GCACTCCACAC ACGGGGGCC [SEQ ID NO:1781]	[SEQ ID NO:40] (Same guide as XD-14756)	[SEQ ID NO:1883]	UUGGGCACA UAUGGACCUGC UAAGCUAUGUA CCACAACAAG UCUGAACCUCA GGCCGGGACCU CUUCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1978]
3269	miR-190a_M	-2.3297	0.037293	ACTGTAGG CAACATATT GCGTG [SEQ ID NO:1629]	CACAATAT GTTGCCTAC AAG [SEQ ID NO:1689]	GAGCTCAGTCA AACCTGGATGCC TTTTCTGCAGGC GTCTGTGACTGT AGGCAACATATT GCGTGTGTATT TAATCCACACAA TATGTTGCCTAC AAGCTACAGTCT CTTGCCCTGTCT CCGGGGGTTCCT AATAAG [SEQ ID NO:1782]	ACUGUAGG CAACAUAU UGCGUG [SEQ ID NO:1822]	CACAAUUAU GUUGCCUA CAAG [SEQ ID NO:1884]	GAGCUACUGA GACCUUGAUCA UUUUUCUGCAG GCGUCUGUGAC UGUAGGCAACA UAUUGCGUGUG UUAUUUAUCC ACACAUAUGU UGCCUACAAGC UACAGUCUCUU GCCUGUCUCC GGGGGUUCCUA AUAAAG [SEQ ID NO:1979]
3043	miR-1-l	-2.32683	0.097086	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	ACCTGTTT GTTTGCAC GAAAA [SEQ ID NO:1690]	CATGCAGACTGC CTGCTTGGGACC CTGTTTGTTTG CAGCAAAATAT GGACCTGCTAA GCTATTGTGTG AAAACAAACAG GCTCTCAGGCCG GGACCTCTCTG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1783]	UUUGGUGC AAAAACAA CAGGCU [SEQ ID NO:1812]	ACCCUGUU UGUUUUGC ACGAAAA [SEQ ID NO:1885]	CAUGCAGACUG CCUGCUUGGGA CCUGUUUGUU UUGCACGAAAA UAUGGACCUGC UAAGCUAUUUG GUGCAAAACA ACAGGCUUCA GGCCGGGACCU CUCUGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1980]

3273	miR-1-1	-2.32464	-0.13929	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CTATATGTT GCCTACAGT GACTA [SEQ ID NO:1691]	CATGCAGACTGC CTGCTTGGGCTA TATGTTGCTAC AGTACTATATG GACCTGCTAAGC TATAGGACTGTA GGCAACATATTG CTCAGGCCGGG ACCTCTCTGCC GCACTGAGGGG CACTCCACACCA CGGGGGCC [SEQ ID NO:1784]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CUAUUAGU UGCCUACA GUGACUA [SEQ ID NO:1886]	CAUGCAGACUG CCUGCUUGGG UAUUGUUGCC UACAGUGACUA UAUGGACCUG UAAGCUAUAGG ACUGUAGGCA CAUUAUUCUCA GGCCGGGACCU CUCUCGCCCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1981]
2586	miR-100	-2.32164	-0.18294	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAAGTCCT AATTCTGAC TCTT [SEQ ID NO:1692]	CCCAAAAGAGA GAAGATATTGA GGCTGTTGCCA CATAGATTGAGA AGTAGAACTTG GCTATTAGTCCG CCAAAGTCCTAAT TCTGACTCTTG TGCTGTTAGGC AATCTCAGGAC CTGGGGCTTTGC TTATATGCC [SEQ ID NO:1785]	UAGAUUCA GAAGUAGA ACUUG [SEQ ID NO:1816]	CCAAGUCC UAAUUCUG ACUCUU [SEQ ID NO:1887]	CCCAAAAGAGA GAAGAUUUGA GGCCUGUUGCC ACAUAGAUUCA GAAGUAGAACU UAGGUUUAAGU CCGCCAAGUCC UAAUUCUGACU CUUUGUGUCUG UUAGGCAUUCU CACGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1982]
2585	miR-1-1_M	-2.31178	0.12336	AGATTGAG AAGTAGAA CTTGGC [SEQ ID NO:1630]	GGCAAGTT CTACTTCTG ACCTCT [SEQ ID NO:1693]	CATGCAGACTGC CTGCTTGGGGG AAGTTCTACTTC TGACCTCTTATG GACCTGCTAAGC TAAGATTGAGA AGTAGAACTTG GCCTCAGGCCG GGACCTCTCCG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC	AGAUUCAG AAGUAGAA CUUGG [SEQ ID NO:1823]	GGCAAGUU CUACUUCU GACCUU [SEQ ID NO:1888]	CAUGCAGACUG CCUGCUUGGG GCAAGUUCUAC UUCUGACCUCU UAUGGACCUG UAAGCUAAGAU UCAGAAAGUAGA ACUUGGCCUCA GGCCGGGACCU CUUCCGCCCA CUGAGGGGCAC

3273	miR-122	-2.30907	-0.11515	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATATGTT GCATACAG TCAGA [SEQ ID NO:1694]	GGTACAGAGTT TCCTTAGCAGAG CTGTAGGACTGT AGGCAACATATT GTGTCTAAACTA TCAATATGTTGC ATACAGTCAGAT AGCTACTGCTAG GCAATCCTTCCC TCGATAAATGTC TTGGCATCGTTT GCTTTG [SEQ ID NO:1787]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAUAUUG UGCAUACA GUCAGA [SEQ ID NO:1889]	UCCACACCAG GGGGC [SEQ ID NO:1983]
3301	miR-100_M	-2.30882	-0.2279	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCCAACTC ATGCCCTTA TTCT [SEQ ID NO:1695]	CCCAAAAGAGA GAAGATAATTGAT GCCTGTTGCCAC ATGAACAAGGG GCTGATTTGGGA GTATTAGTCCGT CCCAACTCATCC CCTTATTCTTGT GTCTGTTAGGCT ATTCCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1788]	UGAACAAAG GGGUGAU UUGGGA [SEQ ID NO:1215]	UCCCAACU CAUCCCU UAUUCU [SEQ ID NO:1890]	CCCAAAAGAGA GAAGAUUUGA UGCCUGUUGCC ACAUGAACAAAG GGGUGAUUUG GGAGUAUUGU CCGUCCCAACU CAUCCCUUUAU UCUUGUGUCUG UUAGGCUAUUC CAGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:1985]
2602	miR-130a	-2.30701	-0.14983	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ATTCTATGG AGCAACTA TTAAC [SEQ ID NO:1696]	GCAGGGCCGGC ATGCCTCTGCTG CTGGCCAAATTCT ATGGAGCAACT ATTAACCTGTCT GCACCTGTCACT AGTTTAGTAGTT GATCCATAGATT TGGCCGTGTAGT	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202]	AUUCUAUG GAGCAACU AUUAAAC [SEQ ID NO:1891]	GCAGGGCCGGC AUGCCUCUGCU GCUGGCCAAUU CUAUGGAGCAA CUAUUAACCU UCUGCACCUGU CACUAGUUUAG UAGUUGAUCCA UAGAUUUGGCC

						GCTACCCAGCGC TGGCTGCTCTCT CAGCATTG [SEQ ID NO:1789]		AUUGGGUG GAGUAAAGC UGGUGG [SEQ ID NO:306] (Same guide as XD-14889)	CCAAGCUU ACUCCACG CACG [SEQ ID NO:1892]	GUGUAGUGCUA CCCAGCGCUGG CUGCCUCCUCA GCAUUG [SEQ ID NO:1986]
3255	miR-190a_M	-2.30348	0.418205	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CCAAGCTTA CTCCACGCA CG [SEQ ID NO:1697]	GAGCTCAGTCA AACTTGGATGCC TTTTCTGCAGGC GTCTGTGATTGC GTGGAGTAAGC TGGTGGTGTAT TTAATCCACCAA GCTTACTCCACG CACGCTACAGTC TCTTGCCCTGTC TCCGGGGGTTC TAATAAAG [SEQ ID NO:1790]				GAGCUACGUA AACCUGGAUGC UUUUUCUGCAG GCUUCUGAU UUCGUGGAGUA AGCUGGUGGUG UUUUUAAUCC ACCAAGCUUAC UCCACGACACG UACAGUCUCUU GCCUGUCUCC GGGGUUCCUA AUAAAG [SEQ ID NO:1987]
3338	miR-132	-2.2984	-0.03531	TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TAACTCTCA GCCTACTCA TGTA [SEQ ID NO:1698]	GCCGTCCGCGG CCCCGCCCGC GTCTCCAGGTA AGTCTCAGCCTA CTCATGTACTGT GGGAAGTGGAG GTACATGAGGA TGCTGAGACTGA CCCCGCAGCAC GCCACGCGCC GGCCACGCGC CGCCCGAGCC [SEQ ID NO:1791]	UACAUGAG GAUGCUGA GACUGA [SEQ ID NO:314] (Same guide as XD-14893)	UAAAGUCUC AGCCUACU CAUGUA [SEQ ID NO:1893]	GCCGUCGCGC GCCCCGCCCG GCGUCUCCAG GUAAGUCUCAG CCUACUCAUG ACUGGGGAAC UGGAGGUACAU GAGGAUGCUGA GACUGACCCCG CAGCACGCCCA CGCGCCGCGCC ACGCCGCGCC CGAGCC [SEQ ID NO:1988]	
3842	miR-1-l	-2.27963	-0.68707	AACGTGAG AAGGATGG ATCGTA [SEQ ID NO:1625]	TTCGATCCA TCCTTCTCA GAGTT [SEQ ID NO:1699]	CATGCAGACTGC CTGTTGGGTTC GATCCATCCTTC TCAGAGTTTATG GACCTGTAAAGC TAAACGTGAGA AGGATGGATCG	AACGUGAG AAGGAUGG AUCGUA [SEQ ID NO:1824]	UUCGAUCC AUCCUUCU CAGAGUU [SEQ ID NO:1894]	CAUGCAGACUG CCUGCUUGGU UCGAUCCAUC UUUCAGAGUU UAUGGACCUG UAAGCUAAACG UGAGAAGGAUG	

3330	miR-155E	-2.26554	0.033188				TATCTAGGCGG GACCTCTCTGC CGCACTGAGG GCACTCCACAC ACGGGGCC [SEQ ID NO:1792]	UAGUCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CCCAUUA CAGCUCAG CAUA [SEQ ID NO:1895]	GAUCGUACUCA GGCCGGGACCU CUCGCGCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1989]
1162	miR-122	-2.2647	-0.33024				TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCACATTAC AGCTCAGC ATA [SEQ ID NO:1700]		CUGGAGGCUUG CUUUGGGCUGU AUGCUGAUGC UGAGACUGAUA AUGUGGUUUUG GCCUCUGACUG ACCACAUUACA GCUCAGCAUAC AGGACAAGGCC CUUUAUCAGCA CUCACAUUGAA CAAUUGGCCAC CGUGGG [SEQ ID NO:1990]
3301	miR-122_M	-2.26423	0.053866				AACTGTACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTG TTATGGTAC ATGT [SEQ ID NO:1701]		GGCUACAGAGU UUCUUAGCAG AGCUGAACUGU ACCACAACAAA GUCUGUGUCUA AACUAUCAGAC UUUGUUUUGGU ACAUUUUAGCU ACUGCUAGGCA AUCCUUCCUC GAUAAAUGUCU UGGCAUCGUUU GCUUUG [SEQ ID NO:1991]
							TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCCAAATC AGACCCCTTG TGAA [SEQ ID NO:1702]	UGAACAAAG GGGUGAU UUGGGA [SEQ ID NO:1215]	GGCUACAGAGU UUUUUAGCAG AGCUGUGAACA AGGGGUGAUU UGGGUUGUCUA

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3845	miR-1-1_M	-2.26073	-0.20695			TTGAACGTG AGAAGGAT GGATC [SEQ ID NO:696]	GTTCCATCC TTCTCACGT CGCAA [SEQ ID NO:1705]	ACCGAGAATAT GGACCTGCTAA GCTATTCTGGGTT GAAATCTGAAG TGTTCTCAGGCCG GGACTCTTCCG CCGCACTGAGG GGCACTCCACAC CACGGGGGCC [SEQ ID NO:1798]	[SEQ ID NO:108] (Same guide as XD-14790)	[SEQ ID NO:1907]
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1162	miR-155E	-2.26036	-0.20831	AACTGTACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTT TGGGTACA GTT [SEQ ID NO:1706]	CTGAGGCTTGC TTTGGGCTGTAT GCTGAACCTGTAC CACAACAAAGT CTGTTTGGCCT CTGACTGACAG ACTTTTGGGTA CAGTTCAGGAC AAGGCCCTTAT CAGCACTCACAT GGAACAAATGG CCACCGTGG [SEQ ID NO:1801]	AACUGUAC CACACAA AGUCUG [SEQ ID NO:1180]	CAGACUUU UUGGGUAC AGUU [SEQ ID NO:1905]	CUGAGGCUUG CUUUGGGCUGU AUGCUGAACUG UACCACAAAC AGUCUGUUUG GCCUCUGACUG ACAGACUUUUU GGUACAGUUC AGGACAAGGCC CUUAUCAGCA CUCACAUAGAA CAAUUGGCCAC CGUGGG [SEQ ID NO:1998]
3273	miR-1-1_M	-2.24883	0.09481	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CTATATGTT GCCTACAGT GACTA [SEQ ID NO:1691]	CATGCAGACTGC CTGCTTGGGCTA TATGTTGGCTAC AGTGACTATATG GACCTGCTAAGC TATAGGACTGTA GGCAACATATTG CTCAGGCCGGG ACCTCTTCCGCC GCACTGAGGGG CACTCCACACCA CGGGGGCC [SEQ ID NO:1802]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CUAUUUGU UGCCUACA GUGACUA [SEQ ID NO:1886]	CAUGCAGACUG CCUGCUUGGGC UAUUGUUGCC UACAGUGACUA UAUGGACCUGC UAAGCUAUAGG ACUGUAGGCAA CAUUAUUGCUA GGCCGGGACCU CUUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:1999]
2586	miR-190a	-2.24846	0.172174	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCATTCTAC TTCTGAATC CC [SEQ ID NO:1661]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTGTAGAT TCAGAAAGTAGA ACTTGGTGTAT TTAATCCACCAT TCTACTTCTGAA TCCCCTACAGTG TCTTGCCTGTG TCCGGGGGTTC TAATAAAG	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAUUCUA CUUCUGAA UCCC [SEQ ID NO:1856]	GAGCUCAGUCA AACCUUGAUGC CUUUCUGCAG GCCUCUGUGUA GAUUCAGAAGU AGAACUUGGUG UUAUUUAUCC ACCAUUCUACU UCUGAAUCCCC UACAGUGUCUU GCCUCUGUCC

3132	miR-122_M	-2.24518	0.049515	ATGTCCTGG CTTGATTCA CTGG [SEQ ID NO:1631]	CCAGTGAA TCACGCCA AGAACT [SEQ ID NO:1707]	GGTACAGAGTT TGCTTAGCAGAG CTGATGCTTGG CTTGATTCACGT GTGTCTAAACTA TCCAGTGAATCA CGCCAAGAACTT AGCTACTGCTAG GCCATCCTTCCC TCGATAAATGTC TTGGCATCGTTT GCTTTG [SEQ ID NO:1804]	AUGUCUUG GCUUGAUU CACUGG [SEQ ID NO:1826]	CCAGUGAA UCACGCCA AGAACT [SEQ ID NO:1904]	GGGGGUUCCUA AUAAAG [SEQ ID NO:2000]
3842	miR-1-1_M	-2.24315	-0.38269	AACGTGAG AAGGATGG ATCGTA [SEQ ID NO:1625]	TTCGATCCA TGCTTCTCA GAGTT [SEQ ID NO:1699]	CATGCAGACTGC CTGCTTGGGTTT GATCCATCCTTC TCAGAGTTTATG GACCTGCTAAGC TAAACGTGAGA AGGATGGATCG TACTCAGGCCGG GACCTTTCCTGC CGCACTGAGG GCACTCCACACC ACGGGGGCC [SEQ ID NO:1805]	AACGUGAG AAGGAUGG AUCGUA [SEQ ID NO:1824]	UUCGAUCC AUCCUUCU CAGAGUU [SEQ ID NO:1894]	CAUGCAGACUG CCUGCUUGGGU UCGAUCCAUC UUCUCAGAGUU UAUGGACCUGC UAAAGCUAAACG UGAGAAGGAGU GAUCGUACUCA GGCCGGGACCU CUCCGCCGCCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:2001]
2928	miR-1-1	-2.24149	0.19907	TCTGAGAG AAGGAACG TGGGTT [SEQ ID NO:668]	ATCCCACGT TCCTTCTCT GAAGA [SEQ ID NO:1708]	CATGCAGACTGC CTGCTTGGGATC CCACGTTCTTC TCTGAAGATATG GACCTGCTAAGC TATCTGAGAGA AGGAACGTGGG TTCTCAGGCCGG GACCTCTCTCGC	UCUGAGAG AAGGAACG UGGGUU [SEQ ID NO:1196]	AUCCACG UUCUUCU CUGAAGA [SEQ ID NO:1902]	CAUGCAGACUG CCUGCUUGGGA UCCACGUUCC UUCUCUGAAGA UAUGGACCUGC UAAGCUAUCUG AGAGAAGGAAC GUGGGUUCUCA GGCCGGGACCU

1231	miR-1-1_M	-2.24102	0.10817	TTCACCTTA GCACTGAT AGCAG [SEQ ID NO:1627]	CAGCTATCA GTGCTAAA GCGGAA [SEQ ID NO:1709]	CATGCAGACTGC CTGCTTGGCAG CTATCAGTGCTA AAGCGGAATAT GGACCTGCTAA GCTATTCACCTT AGCACTGATAG CAGCTCAGGCC GGGACCTCTTC GCCGACTGAG GGCACTCCAC ACCACGGGGC C [SEQ ID NO:1807]	UGGAAUUU AGCAGAAU UAGCUA [SEQ ID NO:1825]	CAGCUAUC AGUGCUAA AGCGGAA [SEQ ID NO:1903]	CAUGCAGACUG CCUGCUUGGC AGCUAUCAGUG CUAAAGCGGAA UAUGGACCUGC UAAGCUAUCA CUUAGCACUG AUAGCAGCUA GGCCGGGACCU CUUCCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:2004]	CUCUCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:2003]
1578	miR-100_M	-2.24051	-0.33444	TGGAATTTC TCTGAACTG CTGT [SEQ ID NO:1626]	ACAGCAAT TCCGAGAA ACTCCT [SEQ ID NO:1665]	CCCAAAAGAGA GAAGATAATTGAT GCCTGTTGCCAC ATGGAATTTCCT TGAAGTCTGTG TATTAGTCCGAC AGCAATCCGA GAAACTCCTTGT GTCTGTTAGGCT ATTCCACGGACC TGGGGCTTTGCT TATATGCC [SEQ ID NO:1808]	UGGAAUUU AGCAGAAU UAGCUA [SEQ ID NO:1820]	ACAGCAAU UCCGAGAA ACUCCU [SEQ ID NO:1860]	CCCAAAAGAGA GAAGAAUUGA UGCCUGUUGCC ACAUGGAAUUU CUCUGAACUGC UGUGUAUUAU CCGACAGCAAU UCCGAGAAACU CCUUGUGUCUG UUAGGCUAUUC CAGGACCUGG GGCUUUGCUUA UAUGCC [SEQ ID NO:2005]	CUCUCGCCGCA CUGAGGGGCAC UCCACACCACG GGGGCC [SEQ ID NO:2003]
967	miR-190a	-2.23671	-0.08895	ACTGATGTA AGTATATG AACCA [SEQ ID NO:1619]	TGGCATATA CTTACATCA AG [SEQ ID NO:1642]	GAGCTCAGTCA AACCTGGATGCC TTTCTGCAGGC CTCTGTGACTGA TGTAAGTATATG AACCATGTTATT TAATCCATGGCA	ACUGAUGU AAGUAUUAU GAACCA [SEQ ID NO:1815]	UGGCAUUAU ACUUAUUAU CAAG [SEQ ID NO:1837]	GAGCUCAGUCA AACCTGGAGUC CUUUUCUGCAG GCCUCUGUGAC UGAUGUAAGUA UAUGAACCAUG UUUUUUAAUCC	GAGCUCAGUCA AACCTGGAGUC CUUUUCUGCAG GCCUCUGUGAC UGAUGUAAGUA UAUGAACCAUG UUUUUUAAUCC

1436	miR155-M	-2.22884	0.141256	TGAGTTATC TCTTTCTAA GGGC [SEQ ID NO:1632]	GCCCTTAGA AGGATAAC TCA [SEQ ID NO:1710]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTGAGTTA TCTCTTTCTAAG GGCTTTTGCCA CTGACTGAGCCC TTAGAAGGATA ACTCACAGGAC ACAAAGGCTGTT ACTAGCACTCAC ATGGAACAAAT GGCCACCGG [SEQ ID NO:1810]	UGAGUUUAU CUCUUUCU AAGGGC [SEQ ID NO:1827]	GCCCUUAG AAGGAUAA CUCA [SEQ ID NO:1900]	AUGCAUAUAC UUACAUAAGC UACAGUGUCUU GCCUGUCUCC GGGGUUCUUA AUAAAG [SEQ ID NO:2006]
						CTGAGGAGGCUU GCUGAAGGCUG UAUGCUGUGAG UUUUCUCUUC UAAAGGCUUUU GGCCACUGACU GAGCCCUUAGA AGGAUAACUA CAGGACACAAG GCCUGUUACUA GCACUCACAUG GAACAAUUGGC CACCGG [SEQ ID NO:2007]			

Table 24: Top 10 miRNAs for each miR backbone

miR Backbone	Atxn2 Targeting Position	Atxn2 low/unsort log2 FC	T1/T0 log2 FC	Guide Sequence (DNA)	Passenger Sequence (DNA)	miR Cassette (DNA)	Guide Sequence (RNA)	Passenger Sequence (RNA)	miR Cassette (RNA)
miR-1-1	1755	-2.5734	-0.10352	TCGGGTGA AATCTGAA GTGTG [SEQ ID NO:657]	CTCACTTCA GATTCAAC GACGA [SEQ ID NO:1645]	CATGCAGACTGC CTGCTTGGCTCA CTTCAGATTCAA CGACGATATGGA CCTGCTAAGCTAT CGGTTGAAATC TGAAGTGTGCTC AGCCGGGACCT CTCTCGCGCACT GAGGGCACTCC ACACCACGGGG CC [SEQ ID NO:1726]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CUCACUUC AGAUUUC ACGACGA [SEQ ID NO:1840]	CAUGCAGACUGCC UGCUGGGUCAC UUCAGAUUCAAC GACGAUUGGACC UGCUAAGCUAUCG GGUUGAAUUCG AAGUGGUCAG GCCGGGACCUUC UCGCCGACUGAG GGGACUCCACAC CACGGGGCC [SEQ ID NO:1923]
miR-1-1	2943	-2.54936	0.148183	TAGTAGAA GGCTTTGGC TGAGA [SEQ ID NO:683]	TGTCAGCCA AAGCCTTCT CCCTA [SEQ ID NO:1650]	CATGCAGACTGC CTGCTTGGGTGTC AGCCAAAGCCTT CTCCCTATATGGA CCTGCTAAGCTAT AGTAGAAGGCTT TGGCTGAGACTC AGCCGGGACCT CTCTCGCGCACT GAGGGCACTCC ACACCACGGGG CC [SEQ ID NO:1731]	UAGUAGAA GGCUUUG CUGAGA [SEQ ID NO:1211]	UGUCAGCC AAAGCCUU CUCCCUA [SEQ ID NO:1845]	CAUGCAGACUGCC UGCUGGGUC AGCCAAAGCCUUC UCCCUAUUGGAC CUGCUAAGCUAUA GUAGAAGGCUU GGUGAGACUCAG GCCGGGACCUUC UCGCCGACUGAG GGGACUCCACAC CACGGGGCC [SEQ ID NO:1928]
miR-1-1	3338	-2.52716	-0.2585	TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TGAGTCTCA GCATCCTCA CGGTA [SEQ ID NO:1651]	CATGCAGACTGC CTGCTTGGGTGA GTCTCAGCATCCT CACGGTATATGG ACCTGCTAAGCT ATACATGAGGAT GCTGAGACTGAC TCAGGCCGGGAC	UACAUGAG GAUGCUGA GACUGA [SEQ ID NO:314]	UGAGUCUC AGCAUCCU CACGGUA [SEQ ID NO:1846]	CAUGCAGACUGCC UGCUGGGUGAG UUCUAGCAUCCUC ACGGUAUUGGA CCUGCUAAGCUAU ACAUGAGGAUC UGAGACUGACUCA GGCCGGGACCUUC

miR-1-1	3302	-2.51939	-0.06675	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CGCAATC AGCCCTTG TCGCAA [SEQ ID NO:1647]	CTCTCTGCCGCA CTGAGGGGCACT CCACACCACGGG GGCC [SEQ ID NO:1732]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CGAAAU AGCCCUU GUCGAA [SEQ ID NO:1842]	CAUGCAGACUGCC UGCUUGGGCGCAA AUCAGCCCUUGU CGCAUAUUGGACC UGCUAAGCUAU GAACAAGGGGU GAUUUGGGCUA GGCCGGGACUCU CUCGCCGACUGA GGGCACUCCACA CCACGGGGGCC [SEQ ID NO:1932]	CUCGCCGACUGA GGGCACUCCACA CCACGGGGGCC [SEQ ID NO:1929]
miR-1-1	3341	-2.50291	-0.1115	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATCCTCATG TGATAT [SEQ ID NO:1637]	CATGCAGACTGC CTGCTTGGGACT CAGCATCTCATG TGATATTATGGAC CTGCTAAGCTAAT AGACATGAGGAT GCTGAGACCTCA GGCCGGGACCTC TCTGCCGCACTG AGGGCACTCCA CACCACGGGGC C [SEQ ID NO:1735]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUCCUCAU GUGAUU [SEQ ID NO:1832]	CAUGCAGACUGCC UGCUUGGGGACUC AGCAUCCUUAUGU GAUUAUUGGAC CUGCUAAGCUAAU AGACAUGAGGAU GCUAGACCUACAG GCCGGGACCUUC UCGCCGACUGAG GGGCACUCCACAC CACGGGGGCC [SEQ ID NO:1934]	CAUGCAGACUGCC UGCUUGGGUGAG CCAAAGCCUUCUA CCGACAUUUGGAC CUGCUAAGCUAUG UAGUAGAAGGU UUGGCUACUCAG GCCGGGACCUUC UCGCCGACUGAG
miR-1-1	2945	-2.42094	-0.06694	TGTAGTAG AAGGCTTIG GCTGA [SEQ ID NO:685]	TGAGCCAA AGCCTTCTA CCGACA [SEQ ID NO:1633]	CATGCAGACTGC CTGCTTGGGTGA GCCAAAGCCTTCT ACCGACATATGG ACCTGCTAAGCT ATGTAGTAGAAG GCTTTGGCTGACT CAGGCCGGGACC TCTCTGCCGCGAC	UGUAGUAG AAGGCUU GGCUGA [SEQ ID NO:1213]	UGAGCCAA AGCCUUCU ACCGACA [SEQ ID NO:1828]	(Same guide as XD-14860)	

miR-1-1	3301	-2.41082	0.051034	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TGCCAAATC AGCCCTTG CGTCA [SEQ ID NO:1673]	TGAGGGCACTC CACACCAGGGG GCC [SEQ ID NO:1761]	UGAACAAAG GGGCGAU UUGGA [SEQ ID NO:1215]	UGCCAAAU CAGCCCCU UGCGUCA [SEQ ID NO:1868]	GGGACUCCACAC CACGGGGCC [SEQ ID NO:1958]
miR-1-1	3043	-2.32683	0.097086	TTTGGTGCA AAACAAC AGGCT [SEQ ID NO:1615]	ACCTGTGT GTTTGCAC GAAAA [SEQ ID NO:1690]	CATGCAGACTGC CTGCTTGGGACCC TGTTTGTGTTGCA CGAAATATGGA CCTGCTAAGCTAT TTGTTGCAAAAC AAACAGGCTCTC AGGCCGGACCT CTCTCGCGCACT GAGGGCACTCC ACACCAGGGGG CC [SEQ ID NO:1763]	UUUGGUGC AAACAAA CAGGCU [SEQ ID NO:1812]	ACCCUGU UGUUUUGC ACGAAAA [SEQ ID NO:1885]	CAUGCAGACUGCC UGCUUGGACCCU GUUUUUUGCA CGAAAAUUGGA CCUGCUAAGCUAU UUGGUGCAAAAC AAACAGGUCUCA GGCCGGACCCUCU CUCGCCGACUGA GGGCAACUCCACA CCACGGGGGCC [SEQ ID NO:1980]
miR-1-1	3273	-2.32464	-0.13929	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CTATATGTT GCCTACAGT GACTA [SEQ ID NO:1691]	CATGCAGACTGC CTGCTTGGGCTAT ATGTTGCCTACAG TGACTATATGGA CCTGCTAAGCTAT AGGACTGTAGGC AACATATTGCTCA GGCCGGGACCTC TCTCGCGCACTG AGGGGCACTCCA [SEQ ID NO:1783]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CUAUUGU UGCCUACA GUGACUA [SEQ ID NO:1886]	CAUGCAGACUGCC UGCUUGGCUAU AUGUUGCUACAG UGACUUAUGGA CCUGCUAAGCUAU AGGACUGAGGC AACAUUUGCUCA GGCCGGACCCUCU CUCGCCGACUGA

miR-1-1	3842	-2.27963	-0.68707	AACGTGAG AAGGATGG ATCGTA [SEQ ID NO:1625]	TTCGATCCA TCCTTCTCA GAGTT [SEQ ID NO:1699]	CACACGGGGG C [SEQ ID NO:1784]	AACGUGAG AAGGAUGG AUCGUA [SEQ ID NO:1824]	UUCGAUCC AUCUUUCU CAGAGUU [SEQ ID NO:1894]	GGGCACUCCACA CCACGGGGGCC [SEQ ID NO:1981]
miR-1-1_M	2945	-2.89804	0.152222	TGTAGTAG AAGGCTTTG GCTGA [SEQ ID NO:685]	TGAGCCAA AGCCTTCTA CCGACA [SEQ ID NO:1633]	CATGCAGACTGC CTGCTTGGGTGA GCCAAAGCCTTCT ACCGACATATGG ACCTGCTAAGCT ATGTAGTAGAAG GCTTTGGCTGACT CAGCCGGGACC TCTTCGCCGCAC TGAGGGGCACTC CACACCACGGGG GCC [SEQ ID NO:1792]	UGUAGUAG AAGGCUUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UGAGCCAA AGCCUUCU ACCGACA [SEQ ID NO:1828]	CAUGCAGACUGCC UGCUUGGUGAG CCAAAGCCUUCUA CCGACAUUUGGAC CUGCUAAGCUAUG UAGUAGAAAGGU UUGGUGACUCAG GCCGGGACCUUU CCGCCGACUGAG GGGCACUCCACAC CACGGGGGCC [SEQ ID NO:1908]
miR-1-1_M	3341	-2.70967	0.193529	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATCCTCATG TGATAT [SEQ ID NO:1637]	CATGCAGACTGC CTGCTTGGGACT CAGCATCCTCATG TGATATTATGGAC CTGCTAAGCTAAT AGACATGAGGAT GCTGAGACCTCA GGCCGGGACCTC TTCCGCCCACTG AGGGGCACTCCA [SEQ ID NO:1711]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUCUUCU GUGAUU [SEQ ID NO:1832]	CAUGCAGACUGCC UGCUUGGAGACUC AGCAUCCUACUGU GAUUAUUGGAC CUGCUAAGCUAAU AGACAUAGAGAU GCUAGACCUACAG GCCGGGACCUUU CCGCCGACUGAG GGGCACUCCACAC CACGGGGGCC

miR-1-l_M	1755	-2.62482	0.169485	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CTCACTTCA GATTTC AAC GACGA [SEQ ID NO:1645]	CACACACGGGGG C [SEQ ID NO:1715]	UUGGCUUG AAAUUGA AGUGG [SEQ ID NO:1185]	CUCACUUC AGAUUUA ACGACGA [SEQ ID NO:1840]	CAUGCAGACUGCC UGCUUGGCGCAC UUCAGAUUUAAC GACGAUUGGACC UGCUAAGCUAUCG GGUUGAAAUUCG AAGUGGUCAG GCCGGGACCUU CCGCCGACUGAG GGGCACUCCAC CACGGGGCC [SEQ ID NO:1920]
miR-1-l_M	3302	-2.57066	0.05742	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CGCAATC AGCCCTTG TCGCAA [SEQ ID NO:1647]	CATGCAGACTGC CTGCTTGGCGC AAATCAGCCCTT GTCGAATATGG ACCTGCTAAGCT ATTGAACAAGG GCTGATTTGGGT CAGCCGGGACC TCTTCGCCGCAC TGAGGGGCACTC CACACCACGGG GCC [SEQ ID NO:1723]	UUGAACAA GGGCGUGA UUUGG [SEQ ID NO:1216]	CGCAAUUC AGCCCUU GUCGCAA [SEQ ID NO:1842]	CAUGCAGACUGCC UGCUUGGCGCAA AUCAGCCCUUGU CGCAAUUGGACC UGCUAAGCUAUU GAACAAGGGCU GAUUUGGCUCA GGCCGGGACCUU UCCGCCGACUGA GGGGCACUCCACA CCACGGGGGCC [SEQ ID NO:1924]
miR-1-l_M	3330	-2.47751	0.392579	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CGACATTAT CAGTCTCAG GAATA [SEQ ID NO:1659]	CATGCAGACTGC CTGCTTGGCGC CATTATCAGTCTC AGGAATATATGG ACCTGCTAAGCT ATATGCTGAGAC TGATAATGTGGT CAGCCGGGACC TCTTCGCCGCAC TGAGGGGCACTC [SEQ ID NO:1727]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CGCAUUA UCAGUCUC AGGAUA [SEQ ID NO:1854]	CAUGCAGACUGCC UGCUUGGCGACA UUAUCAGUCUCAG GAUAUUGGAC CUGCUAAGCUAUA UGCUAGACUGA UAAUGGUCUCA GGCCGGGACCUU UCCGCCGACUGA GGGGCACUCCACA CCACGGGGGCC

miR-1-l_M	2586	-2.46317	0.179187					CACACCACGGGG GCC [SEQ ID NO:1743]	UAGAUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CGAAGUUC UACUUCUG AACGCUA [SEQ ID NO:1857]	CAUGCAGACUGCC UGCUGGGCGAA GUUCUACUUCUGA ACGCUAUUGGAC CUGCUAAGCUAUA GAUUCAGAAUA GAACUUGGUCACAG GCCGGGACCUU CCGCCGACUCAG GGGACUCCACAC CACGGGGGCC [SEQ ID NO:1944]
miR-1-l_M	3272	-2.45517	0.050153					CATGCAGACTGC CTGCTTGGGGA ATATGTTGCTAC AGCGCTTATGG ACCTGCTAAGCT AAGGACTGTAGG CAACATATTCCT CAGCCGGGACC TCTTCGCCGCAC TGAGGGGCACTC CACACCACGGG GCC [SEQ ID NO:1747]	AGGACUGU AGCAACA UAUUGC [SEQ ID NO:1814]	GGAUAUG UUGCCUAC AGCGCCU [SEQ ID NO:1858]	CAUGCAGACUGCC UGCUGGGCGAA UAUUGGCUACA GCGCCUUAUGGAC CUGCUAAGCUAAG GACUGAAGGCAAC AUUUGGCUACAG CCGGGACCUUUC CGCCGACUCAGG GGCACUCCACAC ACGGGGGCC [SEQ ID NO:1945]
miR-1-l_M	2943	-2.45216	0.338577					TGTCAGCCA AAGCCTTCT CCCTA [SEQ ID NO:1650]	UAGUAGAA GGCUUGG CUGAGA [SEQ ID NO:1211]	UGUCAGCC AAAGCCUU CUCUUUA [SEQ ID NO:1845]	CAUGCAGACUGCC UGCUGGGUGUC AGCCAAAGCCUUC UCCCUAUUGGAC CUGCUAAGCUAUA GUAGAAGGCUU GGCUGAGACUCAG GCCGGGACCUU CCGCCGACUCAG GGGACUCCACAC CACGGGGGCC

miR-1-l_M	3301	-2.38494	0.327128	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TGCCAAATC AGCCCTTG CGTCA [SEQ ID NO:1673]	ACACCACGGGG CC [SEQ ID NO:1749]	UGAACAAAG GGGCUAGU UUGGGA [SEQ ID NO:1215]	UGCCAAAU CAGCCCU UGCGUCA [SEQ ID NO:1868]	CAUGCAGACUGCC UGCUUGGUGCCA AAUCAGCCCUUG CGUCAUUGGACC UGCUAAGCUAUG AACAAAGGGGUG AUUUGGACUCA GGCCGGGACCUCU UCCGCCGACUGA GGGGCACUCCACA CCACGGGGGCC [SEQ ID NO:1967]
miR-1-l_M	2602	-2.37919	0.040602	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ATTCTATGG ATCAACTAC CGAAA [SEQ ID NO:1681]	CATGCAGACTGC CTGCTTGGGATTC TATGGATCAACT ACCGAAATATGG ACCTGCTAAGCT ATTTAGTAGTTGA TCCATAGATTCTC AGCCGGGACCT CTTCCGCCGCACT GAGGGCACTCC ACACCACGGGG CC [SEQ ID NO:1772]	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202] (Same guide as XD-14837)	AUUCUAUG GAUCAACU ACCGAAA [SEQ ID NO:1876]	CAUGCAGACUGCC UGCUUGGGAUUC UAGGUAUAAU ACCGAAAUUUGG ACCUAGUAAGCUA UUUAGUAGUUGA UCCAUAGAUUCUC AGCCGGGACCCUC UUCGCCGCACTC AGGGGCACUCCAC ACCAACGGGGGCC [SEQ ID NO:1969]
miR-100	3302	-2.68521	-0.3818	TTGAACAA GGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAACC AGACCTTG CTCAT [SEQ ID NO:1638]	CCCAAAGAGAG AAGATATTGAGG CCTGTTGCCACAT TGAACAAGGGC TGATTGGGGTAT TAGTCCGCCCAA ACCAGACCTTG CTCATTTGTCTG TTAGGCAATCTCA CGGACCTGGGC [SEQ ID NO:1772]	UUGAACAA GGGCGUGA UUUGGG [SEQ ID NO:1216]	CCCAAACC AGACCCUU GCUCAU [SEQ ID NO:1833]	CCCAAAGAGAGA AGAUUUGAGGC CUGUUGCCACAUU GAACAAGGGCU GAUUUGGGUUAU UAGUCCGCCCAA CCAGACCCUUGCU CAUUGUGUCUGU UAGGCAUUCAC GGACCUUGGGCUU UGCUUAUAGCC

miR-100	3043	-2.67985	-0.0584	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCTGCTT GGTTTGCAA CAAT [SEQ ID NO:1639]	TTTGCTTATATGC C [SEQ ID NO:1716]	UUUGGUGC AAAACAAA CAGGCU [SEQ ID NO:1812]	AGCCUGCU UGGUUUGC AACAAU [SEQ ID NO:1834]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAUC UGGUGCAAAACA AACAGGCGUAU UAGUCCGAGCCUG CUUGGUUUGCAAC AAUUGUGUCUGU UAGGCAAUUCAC GGACCUGGGGCUU UGCUUAUAGCC [SEQ ID NO:1914]
miR-100	1755	-2.66915	-0.04161	TCGGGTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTCCA GCTTTCAAA CCGT [SEQ ID NO:1640]	CCCAAAAGAGAG AAGATATTGAGG CCTGTTGCCACAT CGGTTGAAATC TGAAGTGTGTA TTAGTCCGCACAC TCCAGCTTTCAAA CCGTTGTGTCTGT TAGGCAATCTCA CGGACCTGGGC TTTGCTTATATGC C [SEQ ID NO:1717]	UCGGGUUG AAAUUGA AGUGG [SEQ ID NO:1185]	CACACUCC AGCUUUC AACCGU [SEQ ID NO:1835]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAUC GGGUUGAAAUUCU GAAGUGUGUAU UAGUCCGCACACU CCAGCUUUCAAAC CGUUGUGUCUGU UAGGCAAUUCAC GGACCUGGGGCUU UGCUUAUAGCC [SEQ ID NO:1915]
miR-100	3272	-2.65898	-0.1606	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATACG TTTCTTACA ATCCA [SEQ ID NO:1641]	CCCAAAAGAGAG AAGATATTGAGG CCTGTTGCCACAA GGACTGTAGCA ACATATTGCTAT TAGTCCGCAAT ACGTTTCTACAA TCCATGTGTCTGT TAGGCAATCTCA CGGACCTGGGC C [SEQ ID NO:1718]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAAUACG UUUCCUAC AAUCCA [SEQ ID NO:1836]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAAG GACUAGGCAAC AUUUGCGUAU AGUCCGCAUAC GUUCCUACAUC CAUGUGUCUUC AGGCAAUUCACG GACCUGGGGCUU GCUUAUAGCC

miR-100	1578	-2.43871	-0.43493	TGGAATTTC TCTGAACTG CTGT [SEQ ID NO:1626]	ACAGCAAT TCCGAGAA ACTCCT [SEQ ID NO:1665]	TTTGCTTATATGC C [SEQ ID NO:1719]	UGGAAUUU CUCUGAAC UGCUGU [SEQ ID NO:1820]	ACAGCAAU UCCGAGAA ACUCCU [SEQ ID NO:1860]	CCCCAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAUG GAAUUUCUGA ACUGCUGUGAU UAGUCCGACAGCA AUCCGAGAAACU CCUUGUGUCUGUU AGGCAAUUCACG GACCUGGGCUUU GCUUAUAGCC [SEQ ID NO:1948]
miR-100	2945	-2.38747	-0.10088	TGTAGTAG AAGGCTTIG GCTGA [SEQ ID NO:685]	TCAGCCCA AGACTTCTA ATACT [SEQ ID NO:1678]	TTTGCTTATATGC C [SEQ ID NO:1751]	UGUAGUAG AAGGCUUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UCAGCCCA AGACUUCU AAUACU [SEQ ID NO:1873]	CCCCAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAUG UAGUAGAAAGGU UUGGCUAGAU UAGUCCGACAGCC CAAGACUUCUAAU ACUUGUCUGU UAGGCAAUUCAC GGACCUGGGCUU UGCUUAUAGCC [SEQ ID NO:1965]
miR-100	2586	-2.32164	-0.18294	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAATCCT AATTCTGAC TCTT [SEQ ID NO:1692]	TTTGCTTATATGC C [SEQ ID NO:1768]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAAGUCC UAAUUCUG ACUCUU [SEQ ID NO:1887]	CCCCAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAUA GAUUCAGAGUA GAACUUGGUU UAGUCCGCAAGU CCUAAUUCUGACU CUUUGUCUGU UAGGCAAUUCAC GGACCUGGGCUU UGCUUAUAGCC [SEQ ID NO:1982]

miR-100	3341	-2.20897	0.022563	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GTCTCAACA TACTCATGC CTAA [SEQ ID NO:2008]	[SEQ ID NO:1785] CCCAAAAGAGAG AAGATATTGAGG CCTGTTGCCACAA TAGACATGAGGA TGCTGAGACGTA TTAGTCCGGTCTC AACATACTCATG CCTAATGTGTCTG TTAGGCAATCTCA CGGACCTGGGC TTTGCTTATATGC C	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GUCUCAAC AUACUCAU GCCUAA [SEQ ID NO:2010]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAAU AGACAUAGAGAU GCUGAGACGUU UAGUCCGGUCUCA ACAUACUAGGCC UAAUGUGUCUGU UAGGCAAUUCAC GGACCUUGGGCUU UGCUUAUAGGCC [SEQ ID NO:2011]
miR-100	893	-2.15837	-0.34705	TTTGTTACT GTTTCGACC TCTG [SEQ ID NO:2012]	CAGAGGCC GACACAGT ACCAAT [SEQ ID NO:2013]	[SEQ ID NO:2009] CCCAAAAGAGAG AAGATATTGAGG CCTGTTGCCACAT TTGTTACTGTTTC GACCTCTGGTATT AGTCCGCAGAGG CCGACACAGTAC CAATTGTGTCTGT TAGGCAATCTCA CGGACCTGGGC TTTGCTTATATGC C	UUUGUUAC UGUUUCGA CCUCUG [SEQ ID NO:2015]	CAGAGGCC GACACAGU ACCAAU [SEQ ID NO:2016]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAAU UGUUACUGUUUC GACCUUGGUUUU AGUCCGCAGAGGC CGACACAGUACCA AUUGUGUCUGU AGGCAAUUCACG GACCUUGGGCUU GCUUAUAGGCC [SEQ ID NO:2017]
miR-100	3330	-2.15241	0.12385	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCACATCAT CCGTCTCAA CATT [SEQ ID NO:2018]	[SEQ ID NO:2014] CCCAAAAGAGAG AAGATATTGAGG CCTGTTGCCACAT ATGCTGAGACTG ATAATGTGGTA TTAGTCCGCCACA TCATCCGTCTCAA CATTGTGTCTGT TAGGCAATCTCA CGGACCTGGGC TTTGCTTATATGC C	UAUGCUGA GACUGAU AUGUGG [SEQ ID NO:1811]	CCACAUC UCCGUCUC AACAUU [SEQ ID NO:2020]	CCCAAAAGAGAGA AGAUUUUGAGGC CUGUUGCCACAAU UCCUGAGACUGA UAAUGUGGUU UAGUCCGCCACAU CAUCCGUCUAC AUUGUGUCUGU UAGGCAAUUCAC GGACCUUGGGCUU UGCUUAUAGGCC [SEQ ID NO:2021]

miR-100_M	3302	-2.62238	-0.19177	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAAACC AGACCCCTTG CTCAT [SEQ ID NO:1638]	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAT TGAACAAGGGC TGATTTGGGTAT TAGTCCGCCAA ACCAGACCCCTTG CTATTGTGTCTG TTAGGCTATTCCA CGGACCTGGGC TTTGCTTATATGC C [SEQ ID NO:1724]	UUGAACAA GGGGCUGA UUUGG [SEQ ID NO:1216]	CCCAAAACC AGACCCUU GCUCAU [SEQ ID NO:1833]	CCCAAAAGAGAGA AGAUUUUGAUGC CUGUUGCCACAUA GAACAAGGGCU GAUUUGGGUUAU UAGUCGCCCAAA CCAGACCCUUGCU CAUUGUGUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UGCUUAUUGCC [SEQ ID NO:1921]
miR-100_M	3272	-2.56927	-0.01914	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATACG TTTCTTACA ATCCA [SEQ ID NO:1641]	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAA GGACTGTAGGA ACATATTGCGTAT TAGTCCGGCAAT ACGTTTCTTACAA TCCATGTGTCTGT TAGGCTATTCCAC GGACCTGGGGCT TTGCTTATATGCC [SEQ ID NO:1729]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAAUACG UUUCCUAC AAUCCA [SEQ ID NO:1836]	CCCAAAAGAGAGA AGAUUUUGAUGC CUGUUGCCACAAG GACUGUAGGCAAC AUUUUGGUAU AGUCCGGCAUAC GUUCCUACAACU CAUGUGUCUGUU AGGCUAUUCCACG GACCUGGGGCUU GCUUAUUGCC [SEQ ID NO:1926]
miR-100_M	3133	-2.49525	0.238806	TATGTCTTG GCTTGATTG ACTG [SEQ ID NO:1624]	CAGTGACTC ACGCCAAG CCATT [SEQ ID NO:1656]	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAT ATGCTTTGGCTTG ATTCACCTGGTATT AGTCCGCAGTGA CTCAGGCCAAGC CATTTGTGTCTGT TAGGCTATTCCAC GGACCTGGGGCT TTGCTTATATGCC [SEQ ID NO:1740]	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO:1819]	CAGUGACU CACGCCAA GCCAUU [SEQ ID NO:1851]	CCCAAAAGAGAGA AGAUUUUGAUGC CUGUUGCCACAUA UGUCUUGGCUUG AUUCACUGGUUAU UAGUCCGCAGUGA CUCAGGCCAAGCC AUUUGUGUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UGCUUAUUGCC [SEQ ID NO:1937]
miR-100_M	1755	-2.4723	-0.07055	TCGGGTGA AATCTGAA GTGIG	CACACTCCA GCTTTCAA CCGT	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAT [SEQ ID NO:1740]	UCGGGUUG AAUUCUGA AGUGUG	CACACUCC AGCUUCA AACCGU	CCCAAAAGAGAGA AGAUUUUGAUGC CUGUUGCCACAUC

miR-100_M	1231	-2.37228	-0.26919	[SEQ ID NO:657] TTCACCTTA GCACTGAT AGCAG [SEQ ID NO:1627]	[SEQ ID NO:1640] CTGTACCA GGGCTAAA ATGAT [SEQ ID NO:1682]	CGGGTTGAAATC TGAAGTGTGTA TTAGTCGCACAC TCCAGTTTCAAA CCGTTGTGTCTGT TAGGCTATTCCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1745]	[SEQ ID NO:1185] UUCACUUU AGCACUGA UAGCAG [SEQ ID NO:1825]	[SEQ ID NO:1835] CUGCACU AGGGCUAA AAUGAU [SEQ ID NO:1877]	GGGUUGAAAUUCU GAAGUGGUUAU UAGUCGCACACU CCAGCUUCAAAC CGUUGUCUGU UAGCUAUUCCAC GGACUUGGGGCUU UGCUUAUAGCC [SEQ ID NO:1942]
miR-100_M	3043	-2.35911	0.045827	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCTGCTT GGTTTGCAA CAAT [SEQ ID NO:1639]	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAT TTGGTGCAAAAC AAACAGGCTGTA TTAGTCCGAGCCT GCTTGGTTGCAA CAATTGTGTCTGT TAGGCTATTCCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1773]	UUUGGUGC AAAACAAA CAGGCU [SEQ ID NO:1812]	AGCCUGCU UGGUUUGC AACAAU [SEQ ID NO:1834]	CCCAAAAGAGAG AGAUUUUGAUGC CUGUUGCCACAUU UGGUGCAAAACA AACAGGCUUAU UAGUCCGAGCCUG CUUGGUUUGCAAC AAUUGUGUCUGU UAGGCUAUUCCAC GGACUUGGGGCUU UGCUUAUAGCC [SEQ ID NO:1970]
miR-100_M	3301	-2.30882	-0.2279	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCCAACTC ATCCCCCTTA TTCT [SEQ ID NO:1695]	CCCAAAAGAGAG AAGATATTGATG CCTGTTGCCACAT GAACAAGGGCT GATTGGGAGTA TTAGTCCGTCCCA ACTCATCCCTTA	UGAACAAAG GGGCUGAU UUGGGA [SEQ ID NO:1215]	UCCCAACU CAUCCCCU UAUUCU [SEQ ID NO:1890]	CCCAAAAGAGAG AGAUUUUGAUGC CUGUUGCCACAUU AACAAAGGGGUG AUUUGGAGUAU UAGUCCGCUUUAU CUCAUCCCCCUUAU [SEQ ID NO:1973]

miR-100_M	2945	-2,26187	0.044364	TGTAGTAG AAGGCTTTG GCTGA [SEQ ID NO:685]	TCAGCCCA AGACTTCTA ATACT [SEQ ID NO:1678]	TTCTTGTTCTGT TAGGCTATCCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1788]	CCCAAAAGAGAG AAGTATTGATG CCTGTTGCCACAT GTAGTAGAAGGC TTTGGCTGAGTAT TAGTCCGTCAGCC CAAGACTTCTAAT ACTTGTTCTGT AGGCTATTCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1797]	UGUAGUAG AAGGCUUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UCAGCCCA AGCUUCU AAUACU [SEQ ID NO:1873]	CCCAAAAGAGAGA AGAUUTGAUGC CUGUUGCCACAUG UAGUAGAAGGCU UUGGUCGAGUAAU UAGUCCGUCAGCC CAAGACUUCUAAU ACUUGUCUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UGCUUAUAUGCC [SEQ ID NO:1994]	UUCUUGUCUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UGCUUAUAUGCC [SEQ ID NO:1985]
miR-100_M	1578	-2,24051	-0.33444	TGGAATTTC TCTGAACTG CTGT [SEQ ID NO:1626]	ACAGCAAT TCCGAGAA ACTCCT [SEQ ID NO:1665]	TTCTTGTTCTGT TAGGCTATCCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1808]	UGGAAUUU CUCUGAAC UGCUGU [SEQ ID NO:1820] UAGUCCGACAGCA ATTCCGAGAAAC TCCTTGTTCTGT TAGGCTATCCAC GGACCTGGGCT TTGCTTATATGCC [SEQ ID NO:1808]	ACUGGAAU UUCUCUGA ACUGCU [SEQ ID NO:1817]	ACGAGUCC AGCGAAU UACAGA [SEQ ID NO:2024]	CCCAAAAGAGAGA AGAUUTGAUGC CUGUUGCCACAAC UAGAAUUUCUCU GAACUCGUGUAAU UAGUCCGACGAGU CCAGCGAAUUAUAC AGAUUGUCUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UUGCUUAUAUGCC [SEQ ID NO:2005]	CCCAAAAGAGAGA AGAUUTGAUGC CUGUUGCCACAAC UAGAAUUUCUCU GAACUCGUGUAAU UAGUCCGACGAGU CCAGCGAAUUAUAC AGAUUGUCUCUGU UAGGCUAUUCCAC GGACCUGGGGCUU UUGCUUAUAUGCC [SEQ ID NO:2005]

miR-122	3332			-2.50104	-0.209	AGGATGCT GAGACTGA TAATGT [SEQ ID NO:1623]	ACATTATCA GTATCAGC ATAAT [SEQ ID NO:1655]	GGCTACAGAGTT TCCTTAGCAGAG CTGAGGATGCTG AGACTGATAATG TTGTCTAAACTAT ACATTATCAGTAT CAGCATAATTAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGCTTGG CATCGTTTGCTTT G	[SEQ ID NO:2023]	AGGAUGCU GAGACUGA UAAUGU [SEQ ID NO:1818]	ACAUUAUC AGUAUCAG CAUAAU [SEQ ID NO:1850]	[SEQ ID NO:2025]
miR-122	3341			-2.4857	0.187025	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GTCTCAGCA TCATCATGT CGCT [SEQ ID NO:1657]	GGCTACAGAGTT TCCTTAGCAGAG CTGATAGACATG AGGATGCTGAGA GTGTCTAAACTAT GTCTCAGCATCAT CATGTCGCTTAGC TACTGCTAGGCA ATCCTTCCCTCGA TAAATGCTTTGGC ATCGTTTGCTTTG [SEQ ID NO:1741]	[SEQ ID NO:1739]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GUCUCAGC AUCAUCAU GUCGCU [SEQ ID NO:1852]	[SEQ ID NO:1936]
miR-122	1755			-2.42198	0.034447	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTTCA GACTTCAAC CATA [SEQ ID NO:1646]	GGCTACAGAGTT TCCTTAGCAGAG CTGTCCGGTTGA AATCTGAAAGTGT GTGTCTAAACTAT CACACTTCAGACT TCAACCATATAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGCTTTG CATCGTTTGCTTT G	[SEQ ID NO:1760]	UCGGGUUG AAAUUCUGA AGUGUG [SEQ ID NO:1185]	CACACUUC AGACUUCA ACCAUA [SEQ ID NO:1841]	[SEQ ID NO:1938]

miR-122	2586	-2.35707	0.153236	TAGATTTCAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAGTTCT ACCTCTGAA TAGA [SEQ ID NO:1685]	GGCTACAGAGTT TCCTTAGCAGAG CTGTAGATTTCAG AAGTAGAACTTG GTGTCTAAACTAT CCAAAGTTCTACCT CTGAATAGATAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGTTTGG CATCGTTTGCTTT G	UAGAUCA GAAGUAGA ACUUG [SEQ ID NO:1816]	CCAAGUUC UACCUCUG AAUAGA [SEQ ID NO:1880]	GGCUACAGAGUU UCCUAGCAGAGC UGUAGAUUCAGA AGUAGAAUUGG UGUCUAAACUAC CAAGUUCUACCUC UGAAUAGAUAGC UACUGCUAGGCAA UCCUCCCUUGAU AAUGUCUUGGC AUCGUUUGCUUU G
miR-122	3273	-2.30907	-0.11515	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATATGTT GCATACAG TCAGA [SEQ ID NO:1694]	GGCTACAGAGTT TCCTTAGCAGAG CTGTAGGACTGT AGGCAACATATT GTGTCTAAACTAT CAATATGTTGCAT ACAGTCAGATAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGTTTGG CATCGTTTGCTTT G	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAUAUUGU UGCAUACA GUCAGA [SEQ ID NO:1889]	GGCUACAGAGUU UCCUAGCAGAGC UGUAGGACUGUA GGCAACAUAUUG UGUCUAAACUAC AAUUGUUGCAU ACAGUCAGAUAGC UACUGCUAGGCAA UCCUCCCUUGAU AAUGUCUUGGC AUCGUUUGCUUU G
miR-122	1162	-2.2647	-0.33024	AACTGTACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTG TTATGGTAC ATGT [SEQ ID NO:1701]	GGCTACAGAGTT TCCTTAGCAGAG CTGAACCTGTACC ACAACAAAGTCT GTGTCTAAACTAT CAGACTTTGTTAT GGTACATGTTAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGTTTGG CATCGTTTGCTTT G	AACUGUAC CACAAACA AGUCUG [SEQ ID NO:1180]	CAGACUUU GUUAUGGU ACAUGU [SEQ ID NO:1896]	GGCUACAGAGUU UCCUAGCAGAGC UGAACUGUACCAC AACAAAGUCUGU GUCUAAACUACA GACUUUGUUAUG GUACAUGUAGC UACUGCUAGGCAA UCCUCCCUUGAU AAUGUCUUGGC AUCGUUUGCUUU G

miR-122	3301	-2.1436	0.009402	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCAAATC AGACCCTTG TGAA [SEQ ID NO:1702]	GGTACAGAGTT TCCTTAGCAGAG CTGTGAACAAGG GGTGATTGGG ATGTCTAAACTAT TCCAAATCAGA CCCTTGTAATAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGCTTGG CATCGTTTGCTTT G [SEQ ID NO:2026]	UGAACAAAG GGGUGAU UUGGGA [SEQ ID NO:1215]	UCCCAAU CAGACCCU UGUGAA [SEQ ID NO:1897]	GGCUACAGAGUU UCCUAGCAGAGC UGUGAACAAAGG GCUGAUUUGGA UGUCUAAACUUA UCCAAAUACAGAC CCUUGUGAAUAGC UACUGCUAGGCAA UCCUCCCUUGAU AAUGUCUUGGC AUCGUUUGCUUU G [SEQ ID NO:2027]
miR-122	3330	-2.06194	0.224544	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCACATTAT CAATCTCAG CCGA [SEQ ID NO:2028]	GGCTACAGAGTT TCCTTAGCAGAG CTGTATGCTGAG ACTGATAATGTG GTGTCTAAACTAT CCACATTATCAAT CTCAGCCGATAG CTACTGCTAGGC AATCCTTCCCTCG ATAAATGCTTGG CATCGTTTGCTTT G [SEQ ID NO:2029]	UAUGCUGA GACUGAU AUGUGG [SEQ ID NO:1811]	CCACAUUA UCAAUUC AGCCGA [SEQ ID NO:2030]	GGCUACAGAGUU UCCUAGCAGAGC UGUAGCUGAGA CUGAUAAUGUGG UGUCUAAACUUC CACAUUAUCAUC UCAGCCGAUAGCU ACUGCUAGGCAU CCUCCCUUGCA AAUGUCUUGGCA UCGUUUGCUUG G [SEQ ID NO:2031]
miR-122	3272	-2.04615	-0.54779	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATATGT TGACTACA GTAAT [SEQ ID NO:2032]	GGCTACAGAGTT TCCTTAGCAGAG CTGAGGACTGTA GGCAACATATTG CTGTCTAAACTAT GCAATATGTTGA CTACAGTAATTA GCTACTGCTAGG CAATCCTTCCCTC GATAAATGCTTGG GCATCGTTTGCTT TG [SEQ ID NO:2033]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAUAUG UUGACUAC AGUAAU [SEQ ID NO:2034]	GGCUACAGAGUU UCCUAGCAGAGC UGAGGACUGUAG GCAACAUUUGCU GUCUAAACUAGC AAUAGUUGACU ACAGUAAUAGC UACUGCUAGGCAA UCCUCCCUUGAU AAUGUCUUGGC AUCGUUUGCUUU G [SEQ ID NO:2261]

miR-122	3133	-2.03169	0.05703	TATGTCCTTG GCTTGATTC ACTG [SEQ ID NO:1624]	CAGTGAAT CAAACCAA GACCGA [SEQ ID NO:1666]	GGCTACAGAGTT TCCTTAGCAGAG CTGTATGCTTGG CTTGATTCAGTGT GTCTAAACTATCA GTGAATCAAAC AAGACCGATAGC TACTGCTAGGCA ATCCTTCCCTCGA TAAATGCTTGGC ATCGTTTGCTTGG [SEQ ID NO:2262]	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO:1819]	CAGUGAAU CAAACCAA GACCGA [SEQ ID NO:1861]	GGCUACAGAGUU UCCUAGCAGAGC UGUAUGUCUUGG CUUGAUUCACUGU GUCUAAACUAUCA GUGAAUCAAAACCA AGACCGAUAGCUA CUGCUAGGCAUUC CUUCCUCGUAUA AUGUCUUGGCAUC GUUUGCUUUG [SEQ ID NO:2263]
miR-122_M	1755	-2.60043	-0.03541	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTTCA GACTTCAAC CATA [SEQ ID NO:1646]	GGCTACAGAGTT TGCTTAGCAGAG CTGTGCGGTTGA AATCTGAAGTGT GTGTCTAACTAT CACACTTCAGACT TCAACCATATAG CTACTGCTAGGCC ATCCTTCCCTCGA TAAATGCTTGGC ATCGTTTGCTTGG [SEQ ID NO:1725]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CACACUUC AGACUUA ACCAUA [SEQ ID NO:1841]	GGCUACAGAGUU UGCUAGCAGAGC UGUCGGGUUGAA AUCUGAAGUGUG UGUCUAAACUAUC ACACUUCAGACUU CAACCAUAUAGCU ACUGCUAGGCCAU CCUCCUCGUAUA AAUGUCUUGGCA UCGUUUGCUUUG [SEQ ID NO:1922]
miR-122_M	3133	-2.43848	0.197681	TATGTCCTTG GCTTGATTC ACTG [SEQ ID NO:1624]	CAGTGAAT CAAACCAA GACCGA [SEQ ID NO:1666]	GGCTACAGAGTT TGCTTAGCAGAG CTGTATGCTTGG CTTGATTCAGTGT GTCTAAACTATCA GTGAATCAAAC AAGACCGATAGC TACTGCTAGGCC ATCCTTCCCTCGA TAAATGCTTGGC ATCGTTTGCTTGG [SEQ ID NO:1725]	UAUGUCUU GGCUUGAU UCACUG [SEQ ID NO:1819]	CAGUGAAU CAAACCAA GACCGA [SEQ ID NO:1861]	GGCUACAGAGUU UGCUAGCAGAGC UGUAUGUCUUGG CUUGAUUCACUGU GUCUAAACUAUCA GUGAAUCAAAACCA AGACCGAUAGCUA CUGCUAGGCAUUC CUUCCUCGUAUA AUGUCUUGGCAUC GUUUGCUUUG [SEQ ID NO:1949]
miR-122_M	3302	-2.40308	-0.14782	TTGAACAA GGGGCTGA TTTGGG	CCCAATC AGCACCTTG TTACA	GGCTACAGAGTT TGCTTAGCAGAG CTGTGGAACAAG GGGCTGATTGG	UUGAACAA GGGGCUGA UUUGGG	CCCAAAUC AGCACCUU GUUACA	GGCUACAGAGUU UGCUAGCAGAGC UGUUGAACAAAG GGCUGAUUUUGG

miR-122_M	3301	-2.26423	0.053866	[SEQ ID NO:688]	[SEQ ID NO:1675]	GTGTCTAAACTAT CCCAATCAGCA CCTTGTACATAG CTACTGCTAGGCC ATCCTTCCCTCGA TAAATGTCTTGGC ATCGTTTGCTTG [SEQ ID NO:1765]	[SEQ ID NO:1216]	[SEQ ID NO:1870]	UGUCUAAACUAUC CCAAAU/CAGCACC UUGUACAUAGC UACUGCUAGGCCA UCCUCCUCGAU AAAGUCUUGGC AUCGUUUGCUUU G [SEQ ID NO:1962]
				TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCCAAATC AGACCCCTTG TGAA [SEQ ID NO:1702]	GGCTACAGAGTT TGCTTAGCAGAG CTGTGAACAAGG GGCTGATTGGG ATGTCTAAACTAT TCCCAAATCAGA CCCTTGTAATAG CTACTGCTAGGCC ATCCTTCCCTCGA TAAATGTCTTGGC ATCGTTTGCTTG [SEQ ID NO:1795]	UGAACAAAG GGGUGAU UUGGGA [SEQ ID NO:1215]	UCCCAAU CAGACCU UGUGAA [SEQ ID NO:1897]	GGCUACAGAGUU UGCUUAGCAGAGC UGUGAACAAAGG GCUGAUUUGGA UGUCUAAACUAU UCCCAAUCAGAC CCUUGUGAAUAGC UACUGCUAGGCCA UCCUCCUCGAU AAAGUCUUGGC AUCGUUUGCUUU G [SEQ ID NO:1992]
miR-122_M	3132	-2.24518	0.049515	ATGTCTTGG CTTGATCA CTGG [SEQ ID NO:1631]	CCAGTGAA TCACGCCA AGAACT [SEQ ID NO:1707]	GGCTACAGAGTT TGCTTAGCAGAG CTGATGTCTTGGC TTGATTCACTGGT GTCTAAACTATCC AGTGAATCACGC CAAGAAGCTTAGC TACTGCTAGGCC ATCCTTCCCTCGA TAAATGTCTTGGC ATCGTTTGCTTG [SEQ ID NO:1804]	AUGUCUUG GCUUGAU CACUGG [SEQ ID NO:1826]	CCAGUGAA UCACGCCA AGAACT [SEQ ID NO:1904]	GGCUACAGAGUU UGCUUAGCAGAGC UGUGUUCUUGGC UUGAUUCACUGG UGUCUAAACUAUC CAGUGAAUCACGC CAAGAACUUAAGCU ACUGCUAGGCCAU CCUCCUCGAUA AAUGUCUUGGCA UCCGUUUGCUUUG [SEQ ID NO:2001]
				ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GTCTCAGCA TCATCATGT CGCT [SEQ ID NO:1657]	GGCTACAGAGTT TGCTTAGCAGAG CTGATAGACATG AGGATGCTGAGA CTGTCTAAACTAT GTCTCAGCATCAT	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GUCUCAGC AUCAUCAU GUCGCU [SEQ ID NO:1852]	GGCUACAGAGUU UGCUUAGCAGAGC UGAUAGACAUGA GGAUGCUGAGAC UGUCUAAACUAU GUCUCAGCAUCAU

						CATGTCGCTTAGC TACTGTAGGCC ATCCTTCCCTCGA TAAATGCTTGGC ATCGTTTGCTTG [SEQ ID NO:2264]					CAUGUCGCUUAGC UACUGCUAGGCCA UCCUCCCUCCGAU AAUGUCUUGGC AUCGUUUUGCUU G [SEQ ID NO:2265]
miR-122_M	3332	-2.06545	-0.17324	AGGATGCT GAGACTGA TAATGT [SEQ ID NO:1623]	ACATTATCA GTATCAGC ATAAT [SEQ ID NO:1655]	GGCTACAGAGTT TGCTTAGCAGAG CTGAGGATGCTG AGACTGATAATG TTGCTAAACTAT ACATTATCAGTAT CAGCATAATTAG CTACTGCTAGGCC ATCCTTCCCTCGA TAAATGCTTGGC ATCGTTTGCTTG [SEQ ID NO:2266]	AGGACUGU GAGACUGA UAAUGU [SEQ ID NO:1818]	ACAUUAUC AGUAUCAG CAUAAU [SEQ ID NO:1850]			GGCUACAGAGUU UGCUUAGCAGAGC UGAGGAUGCUGA GACUGAAUUGU UGUCUAAACUAAU ACAUUAUCAGUA UCAGCAUAAUUA GCUACUGCAGGC CAUCCUCCUCUG AUAAUUGUCUUG GCAUCGUUUUGCUU UG [SEQ ID NO:2267]
miR-122_M	3272	-2.0434	-0.02715	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATATGT TGACTACA GTAAT [SEQ ID NO:2032]	GGCTACAGAGTT TGCTTAGCAGAG CTGAGGACTGTA GGCAACATATTG CTGCTAAACTAT GCAATATGTTGA CTACAGTAAATTA GCTACTGCTAGG CCATCCTTCCCTC GATAAATGCTTG GCATCGTTTGCTT TG [SEQ ID NO:2268]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAAUAUG UUGACUAC AGUAAU [SEQ ID NO:2034]		GGCUACAGAGUU UGCUUAGCAGAGC UGAGGACUGUAG GCAACAUUUGCU GUCUAAACUAGC AAUUGUUGACU ACAGUAAUAGC UACUGCUAGGCCA UCCUCCCUCCGAU AAUUGUCUUGGC AUCGUUUUGCUU G [SEQ ID NO:2035]	
miR-122_M	3338	-1.99732	-0.07815	TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TCAGTCTCA GCCTCCTCA TTGA [SEQ ID NO:2036]	GGCTACAGAGTT TGCTTAGCAGAG CTGTACATGAGG ATGCTGAGACTG ATGTCTAAACTAT TCAGTCTCAGCCT CCTCATTCATAGC [SEQ ID NO:2269]	UACAUGAG GAUGCUGA GACUGA [SEQ ID NO:314]	UCAGUCUC AGCCUCCU CAUUGA [SEQ ID NO:2038]		GGCUACAGAGUU UGCUUAGCAGAGC UGUACAUAGAGG UGCUGAGACUGA UGUCUAAACUAAU UCAGUCUAGCCU CCTCATTCATAGC [SEQ ID NO:2039]	

									(Same guide as XD-14893)			
miR-122_M	1162	-1.99443	-0.12785	AACTGTACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTG TTATGGTAC ATGT [SEQ ID NO:1701]	TACTGCTAGGCC ATCCTTCCTCGA TAAATGCTTGGC ATCGTTTGCTTTG [SEQ ID NO:2037]	GGCTACAGAGTT TGCTTAGCAGAG CTGAACCTGTACC CTAAACAAAGTCT GTGCTAAACTAT CAGACTTTGTTAT GGTACATGTTAG CTACTGCTAGGCC ATCCTTCCTCGA TAAATGCTTGGC ATCGTTTGCTTTG [SEQ ID NO:2040]	AACUGUAC CACAAACA AGUCUG [SEQ ID NO:1180]	CAGACUUU GUUAUGGU ACAUGU [SEQ ID NO:1896]	GGCUACAGAGUU UGCUUAGCAGAGC UGAACUGUACCAC AACAAAGUCUGU GUCUAAACUACA GACUUUGUUAUG GUACAUGUUAGC UACUGCUAGGCCA UCCUCCUCUGAU AAUGUCUUGGC AUCGUUUGUUU G	[SEQ ID NO:2039]	UACUGCUAGGCCA UCCUCCUCUGAU AAUGUCUUGGC AUCGUUUGUUU G
miR-124	2928	-1.82454	0.049772	TCTGAGAG AAGGAACG TGGGTT [SEQ ID NO:668]	ACGCCACG TTCATTCTGA TCAGA [SEQ ID NO:2042]	TTCTTCTCTCAGG AGAAAGGCCTCT CTCAGCCACGTT CATTCGATCAGA ATTTAAATGTCCA TACAATTCTGAG AGAAGGAACGTG GGTTGAATGGGG CTGGCTGAGCAC CGTGGTGGCGG AGGGCCCCCAA GGA [SEQ ID NO:2043]	UCUGAGAG AAGGAACG UGGGUU [SEQ ID NO:1196]	ACGCCACG UUCAUUCG AUCAGA [SEQ ID NO:2044]	UUCUCCUCACAGG AGAAAGGCUCUC UACGCCACGUUC AUUCGAUCAGAA UUUAAAUGUCCA UACAAUUCUGAG AGAAGGAACGUG GGUUGAAUGGGG CUGGCUGAGCAC GUGGUUCGGCGA GGGCCCGCCAAGG A	[SEQ ID NO:2041]	[SEQ ID NO:2045]	
miR-124	3043	-1.7881	0.031105	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AAGCTGTTT GTGTTGACC CAAA [SEQ ID NO:2046]	TTCTTCTCTCAGG AGAAAGGCCTCT CTCAAGCTGTTTG TGTTGACCCAAA ATTTAAATGTCCA TACAATTTGGTG CAAAACAAACAG CCTGAATCGGCG	UUUGGUGC AAAAACAA CAGGCU [SEQ ID NO:1812]	AAGCUGUU UGUGUUGA CCCAAA [SEQ ID NO:2048]	UUCUCCUCACAGG AGAAAGGCUCUC UCAAGCUGUUUG UGUUGACCCAAA UUUAAAUGUCCA UACAAUUCUUGU GCAAAACAAACAG CCTGAATCGGCG	[SEQ ID NO:2045]	[SEQ ID NO:2048]	

						TGGCTGAGCACCT GTGGTTCGGCGA GGCCCCGCCAAG GA [SEQ ID NO:2047]	UUAUUGCGU GGAGUAAG CUGGUG [SEQ ID NO:308] (Same as XD- 14890)	CCGCAGCU UACGCCAA ACAAUA [SEQ ID NO:2052]	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCAGCUUAC GCCAAACAUAU UUAUUGUCCAU ACAAUAUUGCG UGGAGUAAGCUG GUGGAUUGGGCG UGGUGAGCACCG UGGUUCGGCGAG GGCCCCCAAGGA [SEQ ID NO:2053]	UGGUGAGCACCG UGGUUCGGCGAG GGCCCCCAAGGA [SEQ ID NO:2049]
miR-124	3256	-1.65786	0.035613	TATTGCGTG GAGTAAGC TGGTG [SEQ ID NO:618]	CCGCAGCTT ACGCCAAA CAATA [SEQ ID NO:2050]	TTCCTTCTCAGG AGAAAGGCCTCT CTCCCGCAGCTTA CGCCAAACAATA ATTAAATGTCCA TACAATTATTGCG TGGAGTAAGCTG GTGGAATGGGGC TGGCTGAGCACCT GTGGTTCGGCGA GGCCCCGCCAAG GA [SEQ ID NO:2051]	UUCGAUGC AGGACUAG CAGGCG [SEQ ID NO:166] (Same as XD- 14819)	CAGCUGCU AGUACUGA CUCGAA [SEQ ID NO:2056]	UUCUUCUCUACAGG AGAAAGCCUCUC UCCAGCUCUAGU ACUGACUCGAAU UUAUUGUCCAU ACAAUUCGAGUC AGGACUAGCAGGC GGAAUGGGGCGUG GCUAGCACCCUG GGUCGGCGAGGGC CCGCCAAGGA [SEQ ID NO:2057]	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCUAUCAGU UUCACCGUGAAAU UUAUUGUCCAU ACAAUUCACUUU AGCACUGAUAGCA AGGAAUGGGGCGU GCUAGCACCCUG
miR-124	2353	-1.64493	0.114403	TTCGATGCA GGACTAGC AGGCG [SEQ ID NO:612]	CAGCTGCTA GTACTGACT CGAA [SEQ ID NO:2054]	TTCCTTCTCAGG AGAAAGGCCTCT CTCCAGCTGTAG TACTGACTGAA ATTAAATGTCCA TACAATTTCGATG CAGGACTAGCAG CGGGAATGGGGC TGGTGAGCACCT GTGGTTCGGCGA GGCCCCGCCAAG GA [SEQ ID NO:2055]	UUCGAUUU AGCACUGA UAGCAG [SEQ ID NO:1825]	CCCCUAC AGUUCUAC CGUGAA [SEQ ID NO:2060]	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCUAUCAGU UUCACCGUGAAAU UUAUUGUCCAU ACAAUUCACUUU AGCACUGAUAGCA AGGAAUGGGGCGU GCUAGCACCCUG	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCUAUCAGU UUCACCGUGAAAU UUAUUGUCCAU ACAAUUCACUUU AGCACUGAUAGCA AGGAAUGGGGCGU GCUAGCACCCUG
miR-124	1231	-1.5099	-0.27848	TTCACCTTA GCACTGAT AGCAG [SEQ ID NO:1627]	CCCTATCA GTTCTACCG TGAA [SEQ ID NO:2058]	TTCCTTCTCAGG AGAAAGGCCTCT CTCCCTATCAG TTCTACCTGAAA TTTAAATGTCCAT ACAATTTCACCTT AGCACTGATAGC AGGAATGGGCT GGCTGAGCACCG	UUCACUUU AGCACUGA UAGCAG [SEQ ID NO:1825]	CCCCUAC AGUUCUAC CGUGAA [SEQ ID NO:2060]	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCUAUCAGU UUCACCGUGAAAU UUAUUGUCCAU ACAAUUCACUUU AGCACUGAUAGCA AGGAAUGGGGCGU GCUAGCACCCUG	UUCUUCUCUACAGG AGAAAGCCUCUC UCCCGCUAUCAGU UUCACCGUGAAAU UUAUUGUCCAU ACAAUUCACUUU AGCACUGAUAGCA AGGAAUGGGGCGU GCUAGCACCCUG

						TGGGTGGCGAG GGCCCGCCAAGG A [SEQ ID NO:2059]	UAGCUUAGC UCAUCAGG CUGCGA [SEQ ID NO:2066]		GGUGCGAGGGC CCGCCAAGGA [SEQ ID NO:2061]
miR-124	3546	-1.46171	-0.28183	TCGCATACT GCTGAGCA AGGGA [SEQ ID NO:2062]	TAGCTTGCT CATCAGGCT GCGA [SEQ ID NO:2063]	TTCTTCTCTCAGG AGAAAGGCTCT CTCTAGCTTGCTC ATCAGGCTGCGA ATTAAATGTCCA TACAATTCGCATA CTGCTGAGCAAG GGAGAAATGGGCG TGGCTGAGCAC GTGGGTGCGCGA GGGCCCGCCAAG GA [SEQ ID NO:2064]	UAGCUUAGC UCAUCAGG CUGCGA [SEQ ID NO:2066]	UUCUUCUUCAGG AGAAAGGCUUC UCUAGCUUGCUA UCAGGUCGCAAU UUAAAUGUCCAU ACAAUUCGCAUAC UGCUGAGCAAGG GAGAAUGGGGCU GGCUGAGCACCGU GGGUCGGCGAGG GCCGCGCAAGGA [SEQ ID NO:2067]	
miR-124	893	-1.42389	-0.31114	TTTGTTACT GTTTCGACC TCTG [SEQ ID NO:2012]	CCCAGGTC GAACCAAG CACAAA [SEQ ID NO:2068]	TTCTTCTCTCAGG AGAAAGGCTCT CTCCCCAGGTCG AACCAAGGCACAA AATTTAAATGTCC ATACAATTTGTT ACTGTTTCGACCT CTGGAATGGGC TGGCTGAGCAC GTGGGTGCGCGA GGGCCCGCCAAG GA [SEQ ID NO:2069]	CCCAGGUC GAACCAAG CACAAA [SEQ ID NO:2070]	UUCUUCUUCAGG AGAAAGGCUUC UCCCCAGGUCGAA CCAGGCACAAAAU UUAAAUGUCCAU ACAAUUCGCAUAC CUGUUCGACCUUC UGGAAUGGGGCU GGUGAGCACCGU GGGUCGGCGAGG GCCCGCCAAGGA [SEQ ID NO:2071]	
miR-124	2602	-1.34373	0.049733	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ACACTATG GATAAAG CCTAAA [SEQ ID NO:2072]	TTCTTCTCTCAGG AGAAAGGCTCT CTCACACTATGG ATAAAGCCTTAA AATTTAAATGTCC ATACAATTTAGT AGTTGATCCATA GATTGAATGGGG CTGGCTGAGCAC CTGCGCTGCGCG [SEQ ID NO:2073]	ACACUAG GAUAAACG CCUAAA [SEQ ID NO:2074]	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202]	UUCUUCUUCAGG AGAAAGGCUUC UCACACUAGGGAU AAACGCUAAAAU UUAAAUGUCCAU ACAAUUCGCAUAC GUUGAUCCAUAG AUUGAAUGGGGC UGGCUAGACACCG

miR-124	2944	-1.33679	0.075742	TTAGTAGA AGGCTTTGG CTGAG [SEQ ID NO:684]	CCGAGCCA AAGACTTA CACTAA [SEQ ID NO:2076]	AGGCCCGCCAA GGA [SEQ ID NO:2073]	UAGUAGA AGGCUUUG GCUGAG [SEQ ID NO:246] (Same guide as XD-14859)	CCGAGCCA AAGACUUA CACUAA [SEQ ID NO:2078]	UGGUCGGCGAG GGCCGCAAGGA [SEQ ID NO:2075] UUCUUCCUCAGG AGAAAGCCUCUC UCCGAGCCAAAG ACUACACUAAU UUAAUUGUCCAU ACAAUUAGUAG AAGCUUUGGU GAGGAUUGGGC UGGUGAGCACCG UGGUCGGCGAG GGCCGCAAGGA [SEQ ID NO:2079]
miR-124	3270	-1.2645	0.050943	TACTGTAGG CAACATATT GCGT [SEQ ID NO:2080]	AACCAATA TGTGGCCGC CAGTA [SEQ ID NO:2081]	TTCTTCTCAGG AGAAAGCCCTCT CTCAACCAATAT GTGGCCGCCAGT AATTAAATGTCC ATACAATTACTGT AGGCAACATATT GCGTGAATGGG CTGGCTGAGCAC CGTGGTGGCG AGGCCCGCCAA GGA [SEQ ID NO:2077]	UACUGUAG GCAACAUA UUGCGU [SEQ ID NO:2083]	AACCAUA UGUGGCCG CCAGUA [SEQ ID NO:2084]	UUCUUCCUCAGG AGAAAGCCUCUC UCAACCAAUUGU GGCCGCCAGUAAU UUAAUUGUCCAU ACAAUACUGUA GGCAACAUUUGC GUGAAUGGGCU GGCUGAGCACCGU GGGUCGGCGAGG GGCCGCAAGGA [SEQ ID NO:2085]
miR-130a	3302	-2.62809	0.136636	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CGCAATC AGACCTTG TTCAC [SEQ ID NO:1644]	GCAGGCCGGCA TGCTCTGCTGCT GGCCACGCAAT CAGACCTTGTTC ACCTGTCTGCACC TGTCAGTAGTTGA ACAAGGGGTGA TTTGGGTGGCCGT GTAGTGTACCC AGCGTGGCTGC CTCCTCAGCATG	UUGAACAA GGGCGUGA UUUGGG [SEQ ID NO:1216]	CGCAAUUC AGACCCUU GUUCAC [SEQ ID NO:1839]	GCAGGCCGGCAU GCCUCUGCUG GCCACGCAAUCA GACCUUGUUCAC CUGUCGACCCUG UCACUAGUAGAC AAGGGCUGAU UGGGUGGCCGUG UAGUCUACCCAG CGCUGGCGCCUC CUCAGCAUUG

miR-130a	1755	-2.56989	0.011195	TCGGGTGGA AATCTGAA GTGTG [SEQ ID NO:657]	CTCACTTCA GCTTTCAAT TCGC [SEQ ID NO:1648]	GCAGGGCCGGCA TGCTCTGCTGCT GGCCACTCACTTC AGCTTTCAATTGC CCTGTCTGCACCT GTCACTAGTCGG GTTGAAATCTGA AGTGTGTGGCCG TGTAAGTGTACCC AGCGTGGCTGC CTCTCAGCATTG [SEQ ID NO:1722]	UCGGGUUG AAAUUCUGA AGUGUG [SEQ ID NO:1185]	CUCACUUC AGCUUUC AUUCGC [SEQ ID NO:1843]	[SEQ ID NO:1919] GCAGGGCCGGCAU GCCUCUGUCUG GCCACUCACUUA GCUUCAAUUCG CUGUCUACCCUG UCACUAGUCGGU UGAAUUCUGAAG UGUGUGGCCGUG UAGUGCUACCCAG CGCUGGUGCCUC CUCAGCAUUG [SEQ ID NO:1925]
miR-130a	3301	-2.55451	-0.07529	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TGCCAAATC ATCCCCCTTG TTCC [SEQ ID NO:1649]	GCAGGGCCGGCA TGCTCTGCTGCT GGCCATGCCAAA TCATCCCTTGT CCCTGTCTGCACC TGTCAGTAGTA ACAAAGGGCTGA TTTGGGATGGCC GTGTAAGTGTACC CAGCGTGGCTG CCTCCTCAGCATT G [SEQ ID NO:1728]	UGAACAAAG GGGCUGAU UUGGGA [SEQ ID NO:1215]	UGCCAAAU CAUCCCCU UGUUC [SEQ ID NO:1844]	GCAGGGCCGGCAU GCCUCUGUCUG GCCAUGCCAAUUC AUCUUUGUUC CUGUCUGACCCUG UCACUAGUGAACA AGGGGCUGAUU GGGAUGGCCGUG UAGUGCUACCCAG CGCUGGUGCCUC CUCAGCAUUG [SEQ ID NO:1927]
miR-130a	3341	-2.43802	0.12379	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GACTCAGC ATACTCATG TTTAC [SEQ ID NO:1667]	GCAGGGCCGGCA TGCTCTGCTGCT GGCCAGACTCAG CATACTCATGTTT ACCTGTCTGCACC TGTCAGTAGATA GACATGAGGATG CTGAGACTGGCC GTGTAAGTGTACC CAGCGTGGCTG CCTCCTCAGCATT G [SEQ ID NO:1730]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GACUCAGC AUACUCAU GUUUAC [SEQ ID NO:1862]	GCAGGGCCGGCAU GCCUCUGUCUG GCCAGACUCAGCA UACUCAUGUUAC CUGUCUGACCCUG UCACUAGUAGAC AUGAGGAUGCUG AGACUGGCCGUGU AGUGCUACCCAGC GCUGGUGCCUCC UCAGCAUUG [SEQ ID NO:1950]

miR-130a	3330	-2.43606	0.221874	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CGACATTAT CCGTCTCAG TATC [SEQ ID NO:1668]	GCAGGGCCGGCA TGCCTCTGCTGCT GGCCACGACATT ATCCGTCTCAGTA TCCTGTCTGCACC TGTCAGTAGTATG CTGAGACTGATA ATGTGGTGGCCG TGAGTGTACCC AGCGTGGCTGC CTCCTCAGCATTG [SEQ ID NO:1754]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CGACAUUA UCCGUCUC AGUAUC [SEQ ID NO:1863]	GCAGGGCCGGCAU GCCUCUGUCUG GCCACGACAUUAU CCGUCACUAUUC CUGUCUGACCCUG UCACUAGUAUGCU GAGACUGAUAAU GUGGUGGCCGUG UAGUGCUACCCAG CGCUGGCGCCUC CUCAGCAUUG [SEQ ID NO:1951]
miR-130a	3255	-2.43262	0.01754	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:1617]	CGACCAGC TTCCTCCAC GTAAC [SEQ ID NO:1669]	GCAGGGCCGGCA TGCTCTGCTGCT GGCCACGACCCAG CTTCCTCACGTA ACCTGTCTGCACC TGTCAGTAGATTG CGTGGAGTAAGC TGGTGGTGGCCG TGAGTGTACCC AGCGTGGCTGC CTCCTCAGCATTG [SEQ ID NO:1755]	AUUGCUG GAGUAAGC UGGUGG [SEQ ID NO:306] (Same guide as XD-14889)	CGACCAGC UUCUCCA CGUAAC [SEQ ID NO:1864]	GCAGGGCCGGCAU GCCUCUGUCUG GCCACGACCCAGCU UCCUCCACGUAAC CUGUCUGACCCUG UCACUAGAUUGCG UGGAGUAAGCUG GUGGUGGCCGUG UAGUGCUACCCAG CGCUGGCGCCUC CUCAGCAUUG [SEQ ID NO:1952]
miR-130a	3133	-2.42664	0.412413	TATGTCTTG GCTTGATTC ACTG [SEQ ID NO:1624]	CTGTGAATC ACGCCAAG ATATC [SEQ ID NO:1671]	GCAGGGCCGGCA TGCTCTGCTGCT GGCCACTGTGAA TCACGCCAAGAT ATCCTGTCTGCAC CTGTCACTAGTAT GTCTTGGCTTGAT TCACTGTGGCCGT GTAGTGTACCC AGCGTGGCTGC CTCCTCAGCATTG [SEQ ID NO:1757]	UAUGCUG GGCUUGAU UCACUG [SEQ ID NO:1819]	CUGUGAAU CACGCCAA GAUAUC [SEQ ID NO:1866]	GCAGGGCCGGCAU GCCUCUGUCUG GCCACUGUGAAUC ACGCCAAGUAUC CUGUCUGACCCUG UCACUAGUAUGUC UUGGUUGAUUC ACUGUGGCCGUGU AGUGCUACCCAGC GCUGGCGCCUCC UCAGCAUUG [SEQ ID NO:1954]
miR-130a	3842	-2.39495	-0.55009	AACGTGAG AAGGATGG ATCGTA	TTCGATCCA TACTTCTCA TGTC	GCAGGGCCGGCA TGCTCTGCTGCT GGCCATTGATCC ATACTTCTCATGT	AACGUGAG AAGGAUGG AUCGUA	UUCGAUCC AUACUUCU CAUGUC	GCAGGGCCGGCAU GCCUCUGUCUG GCCAUUCGAUCCA UACUUCUCAUGUC

					[SEQ ID NO:1625]	[SEQ ID NO:1677]	CCTGTCTGCACCT GTCACTAGAAGC TGAGAAGGATGG ATCGTATGGCCGT GTAGTGTACCC AGCGTGGCTGC CTCCTCAGCATTG [SEQ ID NO:1767]	[SEQ ID NO:1824]	[SEQ ID NO:1872]	CUGUCUGCACCUG UCACUAGAACGUG AAGAAGUGGAU CGUAUGGCCGUGU AGUGCUACCCAGC GUGGUGGCCUCC UCAGCAUUG [SEQ ID NO:1964]
miR-130a	2586	-2.38521	0.042149	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CGAAGTTCT AATTCTGAA TCTC [SEQ ID NO:1679]	GCAGGGCCGGCA TGCCCTCTGCTGCT GGCCACGAAGTT CTAATTCTGAATC TCCTGTCTGCACC TGTCACCTAGTAG ATTCAGAAAGTAG AACTTGGTGGCC GTGTAGTGCTACC CAGCGTGGCTG CCTCTCAGCATTT G [SEQ ID NO:1769]	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CGAAGUUUC UAAUUCUG AAUCUC [SEQ ID NO:1874]	GCAGGGCCGGCAU GCCUCUGCUGCUG GCCACGAAGUUCU AAUUCUGAAUCUC CUGUCUGCACCUG UCACUAGUAGAU UCAGAAGUAGAA CUUGGUGGCCGUG UAGUGCUACCCAG CUGUGGUGCCUC CUCAGCAUUG [SEQ ID NO:1966]	
miR-130a	2602	-2.30701	-0.14983	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ATTCTATGG AGCAACTA TTAAC [SEQ ID NO:1696]	GCAGGGCCGGCA TGCCCTCTGCTGCT GGCCAATTCTATG GAGCAACTATTA ACCTGTCTGCACC TGTCACCTAGTTA GTAGTTGATCCAT AGATTTGGCCGT GTAGTGTACCC AGCGTGGCTGC CTCCTCAGCATTG [SEQ ID NO:1789]	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202] (Same guide as XD-14837)	AUUCUAUG GAGCAACU AUUAAC [SEQ ID NO:1891]	GCAGGGCCGGCAU GCCUCUGCUGCUG GCCAAUUCUAUGG AGCAAUAUUAAC CUGUCUGCACCUG UCACUAGUUUAG UAGUUGAUCCAU AGAUUUGGCCGU GUAGUGCUACCCA GUGUGGUGCCU CCUCAGCAUUG [SEQ ID NO:1986]	
miR-132	3341	-2.48196	0.14613	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GCCTCAGC ATCATAATG TCTAT [SEQ ID NO:1658]	GCCGTCGCGCG CCCCGCCCGCG GTCTCCAGGGGC CTCAGCATCAT ATGTCTATCTGTG GGAAGTGGAGGA TAGACATGAGGA	AUAGACAU GAGGAUC UGAGAC [SEQ ID NO:1813]	GCUCAGC AUCUAUU GUCUUA [SEQ ID NO:1853]	GCCGUCGCGCGC CCCCGCCCGCG CUCGAGGGCGUC AGCAUCAUAAUG UCUAUCUGGGG AACUGGAGGAU GACAUGAGGAUG	

miR-132	3302	-2.43028	0.018802				TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CACAAATC AGCACATT GTTCAA [SEQ ID NO:1670]	GCCGTCGCGCG CCCCGCCCCG GTCTCCAGGCA CAAATCAGACA TTGTTCAACTGT GGAACCTGGAGT TGAACAAGGGC TGATTTGGGCCC GCAGCAGCCCA CGCGCGCGCCA CGCCGCGCCCG AGCC [SEQ ID NO:1756]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CACAAAU AGCACAU GUUCAA [SEQ ID NO:1865]	GCCGUCCGCGCG CCCCGCCCCG CUCCAGGCA AUCAGCACAUUG UCAACUGUGGA ACUGGAGGUAGA ACAAGGGGCUA UUUGGGCCCCGA GCAGCCCCACGG CCGCGCCACGCG CGCCCCGAGCC [SEQ ID NO:1953]
miR-132	1755	-2.41844	0.196175				TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CCCACTTCA GAGTGCAA CCCGA [SEQ ID NO:1672]	GCCGTGCGCGCG CCCCGCCCCG GTCTCCAGGCG CACTTCAGAGTG CAACCCGACTGT GGGAACTGGAGG TCGGGTTGAAAT CTGAAGTGTGCC CCGCAGCACGCC CACGCGCGCGC CACGCGCGCCC CGAGCC [SEQ ID NO:1762]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CCCAUUC AGAGUGA ACCCGA [SEQ ID NO:1867]	GCCGUCCGCGCG CCCCGCCCCG CUCCAGGCGCAC UUCAGAGUGCAAC CCGACUGUGGAA CUGGAGGUCGG UUGAAAUUCGAA GUGUGCCCCGAG CACGCCACGCGC CGCGCCACGCGC GCCCGGAGCC [SEQ ID NO:1959]
miR-132	2602	-2.40987	-0.18915				TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	ACTCTATGG ATAACCTAC TAAA [SEQ ID NO:1674]	GCCGTGCGCGCG CCCCGCCCCG GTCTCCAGGGAC TCTATGGATAACC TACTAAACTGTG GGAAGTGGAGGT TTAGTAGTTGATC CATAGATTCCCCG (Same guide as XD-14837)	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202]	ACUCUAUG GAUAAACU ACUAAA [SEQ ID NO:1869]	GCCGUCCGCGCG CCCCGCCCCG CUCCAGGACUCU AUGGAUAACCUAC UAAACUGUGGA ACUGGAGGUUA GUAGUUGAUCCA UAGAUUCCCCGA

miR-132	1784	-2.34143	-0.26117				ATTAATAC TCTTTGGTC TGAA [SEQ ID NO:608]	TCCAGACC AAATATTA GTTAAT [SEQ ID NO:1686]	CAGACGCCAC GGCGCGCCAC GCC [SEQ ID NO:1764]	AUUAACUA CUCUUUGG UCUGAA [SEQ ID NO:112] (Same guide as XD-14792)	UCCAGACC AAUAUUA GUUAAU [SEQ ID NO:1881]	GACGCCACGCG CCGCCACGCG CGCCCGAGCC [SEQ ID NO:1961]
miR-132	3338	-2.2984	-0.03531				TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TAAGTCTCA GCCTACTCA TGTA [SEQ ID NO:1698]	GCGTCCGCGG CCCGCCCCCG GTCTCCAGGTA AGTCTCAGCTAC TCATGTACTGTG GAACTGGAGTA CATGAGGATGCT GAGACTGACCC GCAGCACGCCA CGCGCCGCCA CGCCGCCCGG AGCC [SEQ ID NO:1791]	UACAUGAG GAUGCUGA GACUGA [SEQ ID NO:314] (Same guide as XD-14893)	UAAGUCUC AGCCUACU CAUGUA [SEQ ID NO:1893]	GCCGUCCGCGG CCCGCCCCCGG CUCAGGUAAGU CUCAGCCUACUA UGUACUGGGA ACUGGAGGUA UGAGGAUGCUA GACUGACCCGCA GCAGGCCACGCG CCGCCACGCGG CGCCCGAGCC [SEQ ID NO:1988]
miR-132	2945	-2.18195	0.054975				TGTAGTAG AAGCTTTG GCTGA [SEQ ID NO:685]	TAAGCCAA AGCATGCT ACTACA [SEQ ID NO:2086]	GCGTCCGCGG CCCGCCCCCG GTCTCCAGGTA AGCCAAAGCATG CTACTACACTGTG GGAAGTGGAGT GTAGTAGAGGC TTTGGCTGACCC GCAGCACGCCA [SEQ ID NO:1791]	UGUAGUAG AAGGCUU GGCUGA [SEQ ID NO:1213] (Same guide as XD-14860)	UAAGCCAA AGCAUGCU ACUACA [SEQ ID NO: 2088]	GCCGUCCGCGG CCCGCCCCCGG CUCAGGUAAGC CAAAGCAUGCUAC UACACUGGGA ACUGGAGGUA GUAGAAAGCUU GGCUGACCCGCA GCAGGCCACGCG

miR-132	3256	-2.15429	0.256242	TATTGCGTG GAGTAAGC TGGTG [SEQ ID NO:618]	CCCAGCTT ACGCAACG CAATA [SEQ ID NO:2090]	CGCGCCGCGCCA CGCCGCGCCCG AGCC [SEQ ID NO:2087]	UUAUUGCU GAGUAAG CUGGUG [SEQ ID NO:308] (Same as XD- 14890)	CCCCAGCU UACGCAAC GCAUA [SEQ ID NO:2092]	CGCGCCACGCGC CGCCCGAGCC [SEQ ID NO:2089]
miR-132	3255	-2.09429	0.188946	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CAACCAGC TTAATACAC GCAAT [SEQ ID NO:2094]	GCGGTCCGCGCG CCCCGCGCCCG GTCTCCAGGCA ACGAGCTTAATA CAGCAATCTGT GGGAATGGAGG ATTGCGTGGAGT AAGCTGTGGCC CCGAGCACGCC CAGCGCGCGCG CAGCGCGCGCC CGAGCC [SEQ ID NO:2091]	AUUGCGUG GAGUAAGC UGGUG [SEQ ID NO:306] (Same guide as XD-14889)	CAACCAGC UUAUAACA CGCAAU [SEQ ID NO:2096]	GCCGUCGCGCGC CCCCGCGCCCGU CUCCAGGCAACC AGCUAAUACACG CAAUCUGGGA ACUGGAGAUUG CGUGGAGUAAGC UGGUGGCGCCGCA GCACGCGCACGCC CGCGCCACGCGC CGCCCGAGCC [SEQ ID NO:2097]
miR-132	2928	-2.08992	0.223976	TCTGAGAG AAGGAACG TGGGTT [SEQ ID NO:668]	ACCCACGT TCATGCTCT CAGA [SEQ ID NO:2098]	GCCGTCCGCGCG CCCCGCGCCCG GTCTCCAGGAC CCCACGTTTCATGC TCTCAGACTGTGG GAACTGGAGGTC TGAGAGAAGGAA CGTGGGTTCCCGC CAGCACGCCAC GCGCGCGGCCAC [SEQ ID NO:2095]	UCUGAGAG AAGGAACG UGGUGU [SEQ ID NO:1196]	ACCCACG UUAUGCU CUCAGA [SEQ ID NO:2100]	GCCGUCGCGCGC CCCCGCGCCCGU CUCCAGGACCCC ACGUCAUGUCU CAGACUGUGGA ACUGGAGUCUG AGAGAAGGAACG UGGUUCCCGCA GCACGCGCACGCC

miR-138-2	3338	-2.01915	-0.127			TACATGAG GATGCTGA GACTGA [SEQ ID NO:1620]	TCAGAAACA GCATCCTCA TGA [SEQ ID NO:2102]	GCCGGGCCCGA GCC [SEQ ID NO:2099]	UACAUGAG GAUGCUGA GACUGA [SEQ ID NO:314] (Same guide as XD-14893)	UCAGAACA GCAUCCUC AUGA [SEQ ID NO:2104]	CCGGCCACGCCG CGCCCCGAGCC [SEQ ID NO:2101]
miR-138-2	3330	-1.83218	0.060443			TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCACTGATC AGTCTCAGC AA [SEQ ID NO:2106]	GCCGGGGAGTT CTGGTATCGTTGC TGCTATGCTGAG ACTGATAATGTC GGACGAGCAGCG CATCCTCTTACCC CCACTGATCAGTC TCAGCAAGTTGC ATCATACCCATCC TCTCCAGGCGAG CCTCGTGGGACC GG [SEQ ID NO:2103]	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	CCACUGAU CAGUCUCA GCAA [SEQ ID NO:2108]	GCCGGGGAGUUC UGGUAUCGUUGC UGCUAUGCUGAG ACUGAAUUGUG GGACGAGCAGCG AUCCUUUACCCC CACUGAUCAGUCU CAGCAAGUUGCAU CAUACCCAUCCUC UCCAGGCGAGCCU CGUGGACCCG [SEQ ID NO:2109]
miR-138-2	3043	-1.6949	0.083253			TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCCATTG TTTGCACC AT [SEQ ID NO:2110]	GCCGGGGAGTT CTGGTATCGTTGC TGCTTTGGTGCAA AACAAACAGGCT GACGAGCAGCGC ATCCTCTTACCCA GCCATTGTTTG CACCATGTTGCAT CATACCCATCCTC TCCAGGCGAGCC TCGTGGGACCGG [SEQ ID NO:2107]	UUUGGUGC AAAACAA CAGGCU [SEQ ID NO:1812]	AGCCAUUU GUUUUGCA CCAU [SEQ ID NO:2112]	GCCGGGGAGUUC UGGUAUCGUUGC UGCUUUGGUGCA AAACAAACAGGCU GACGAGCAGCGA UCCUUUACCCAG CCAUUUUUUGC ACCAUGUUGCAUC AUACCAUCCUCU CCAGGCGAGCCUC GUGGACCCG

miR-138-2	1689		-1.57706	-0.53719	TACGCGGT GAATTCGT CTCCC [SEQ ID NO:605]	GGGACCAG AATTCACCG CGA [SEQ ID NO:2114]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTACGCGGTG AAATCTGCTCCC GACGAGCAGCG ATCTCTTACCG GGACCAGAAATTC ACCGGAGTTGC ATCATACCATCC TCTCAGGCGAG CCTGTGGGACC GG	[SEQ ID NO:2111]	UACGCGGU GAAUUCUG UCUCCC [SEQ ID NO:100] (Same guide as XD-14786)	GGGACCAG AAUUCACC GCGA [SEQ ID NO:2116]	[SEQ ID NO:2113]
miR-138-2	3270		-1.41498	0.087791	TACTGTAGG CAACATATT GCGT [SEQ ID NO:2080]	ACGCTCATG TTGCCTACA GA [SEQ ID NO:1901]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTACTGTAGG AACATATTGCGT GACGAGCAGCG ATCTCTTACCG CGTCTATGTTGC TACAGAGTTGCA TCATACCATCCT CTCAGGCGAGC CTCGTGGGACCG G	[SEQ ID NO:2115]	UACUGUAG GCAACAU UUGCGU [SEQ ID NO:2083]	ACGCUCAU GUUGCCUA CAGA [SEQ ID NO:2119]	[SEQ ID NO:2117]
miR-138-2	2928		-1.36141	0.122452	TCTGAGAG AAGGAACG TGGGTT [SEQ ID NO:668]	AACGCGGT CCTTCTCTC AC [SEQ ID NO:2121]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTCTGAGAGA AGGAACGTGGT TGACGAGCAGCG CATCTCTTACCG AACGCGTTCCTT CTCTACGTTGCA TCATACCATCCT CTCAGGCGAGC CTCGTGGGACCG G	[SEQ ID NO:2118]	UCUGAGAG AAGGAACG UGGGUU [SEQ ID NO:1196]	AACGCGU UCCUUCUC UCAC [SEQ ID NO:2123]	[SEQ ID NO:2120]
miR-138-2	2928		-1.36141	0.122452	TCTGAGAG AAGGAACG TGGGTT [SEQ ID NO:668]	AACGCGGT CCTTCTCTC AC [SEQ ID NO:2121]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTCTGAGAGA AGGAACGTGGT TGACGAGCAGCG CATCTCTTACCG AACGCGTTCCTT CTCTACGTTGCA TCATACCATCCT CTCAGGCGAGC CTCGTGGGACCG G	[SEQ ID NO:2118]	UCUGAGAG AAGGAACG UGGGUU [SEQ ID NO:1196]	AACGCGU UCCUUCUC UCAC [SEQ ID NO:2123]	[SEQ ID NO:2124]

miR-138-2	3273	-1.32764	0.033491	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATTGTTG CCTACAGTC CA [SEQ ID NO:2125]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTAGGACTGT AGGCAACATATT GGACGAGCAGCG CATCTCTTACCC CAATTGTTGCCTA CAGTCCAGTTGC ATCATACCCATCC TCTCCAGGCGAG CCTCGTGGGACC GG [SEQ ID NO:2126]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAAUUGUU GCCUACAG UCCA [SEQ ID NO:2127]	GCCGGCGGAGUUC UGGUUUCGUUGC UGCUAGGACUGU AGGCAACAUUU GGACGAGCAGCGC AUCUUCUACCCC AAUUGUUGCUAC AGUCCAGUUGCAU CAUACCCAUCCUC UCCAGGCGAGCCU CGUGGGACCGG [SEQ ID NO:2128]
miR-138-2	3255	-1.19435	0.020971	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CCACGCCTT ACTCCACGC AT [SEQ ID NO:2129]	GCCGGCGGAGTT CTGGTATCGTTGC TGCAATTGCGTGG AGTAAGCTGGTG GGACGAGCAGCG CATCTCTTACCC CCACGCCTTACTC CAGCATGTTGC ATCATACCCATCC TCTCCAGGCGAG CCTCGTGGGACC GG [SEQ ID NO:2130]	AUUGCGUG GAGUAAGC UGGUG [SEQ ID NO:306] (Same guide as XD-14889)	CCACGCCU UACUCCAC GCAU [SEQ ID NO:2131]	GCCGGCGGAGUUC UGGUUUCGUUGC UGCAUUGCGUGG AGUAAGCUUGUG GGACGAGCAGCGC AUCUUCUACCCC CACGCCUUAUCC ACGCAUUGUUGCAU CAUACCCAUCCUC UCCAGGCGAGCCU CGUGGGACCGG [SEQ ID NO:2132]
miR-138-2	3302	-1.02575	-0.2011	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCATCCAG CCTCTTGT CT [SEQ ID NO:2133]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTTGAAACAAG GGGCTGATTGG GGACGAGCAGCG CATCTCTTACCC CCCATCCAGCCTC TTGTTCTGTGCA TCATACCCATCCT CTCCAGGCGAGC CTCGTGGGACCG G [SEQ ID NO:2134]	UUGAACAA GGGGCUGA UUUGGG [SEQ ID NO:1216]	CCCAUCCA GCCUCUUG UUUCU [SEQ ID NO:2135]	GCCGGCGGAGUUC UGGUUUCGUUGC UGCUUGAACAAAG GGGCUGAUUUGG GGACGAGCAGCGC AUCUUCUACCCC CCAUCCAGCCUCU UGUUCUUGUCA UCAUACCCAUCCU CUCCAGGCGAGCC UCGUGGGACCGG [SEQ ID NO:2136]

miR-138-2	1436	-0.97653	0.111912	TGAGTTATC TCTTTCTAA GGGC [SEQ ID NO:1632]	GCCAGGA AAGAGATA ACTG [SEQ ID NO:2137]	GCCGGCGGAGTT CTGGTATCGTTGC TGCTGAGTTATCT CTTCTAAGGGCG ACGAGCAGCGCA TCCTCTTACCCGC CCAGGAAAGAGA TAACCTGGTTGCAT CATACCATCTCTC TCCAGGCGAGCC TCGTGGGACCGG [SEQ ID NO:2138]	UGAGUUAU CUCUUUCU AAGGGC [SEQ ID NO:1827]	GCCCAGGA AAGAGUA ACUG [SEQ ID NO:2139]	GCCGGCGGAGUUC UGGUUUCGUUC UCUGAGUUAUC UCUUUAAGGGC GACGAGCAGCGCA UCCUUACCCGC CCAGGAAAGAGA UACUGGUUGCA UCAUACCCAUCCU CUCAGGCGAGCC UCGUGGGACCGG [SEQ ID NO:2140]
miR-144	3043	-2.80802	0.325474	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	CCGTTTGT TTATGCACC AAA [SEQ ID NO:1635]	TCAAGCCATGCTT CCTGTGCCCCAG TGGGGCCCTGGC TCCGGTTTGTTA TGCACCAAAAGT TTGCGATGAGAC ACTTTGGTGCAA AACAAACAGGAG TCCGGGCACCCC CAGCTCTGAGC CTGACAAGGAGG ACA [SEQ ID NO:1713]	UUUGGUGC AAAAACAA CAGGCU [SEQ ID NO:1812]	CCGGUUG UUUAUGCA CCAAA [SEQ ID NO:1830]	UCAAGCCAUUCU CCUGGCCCCAG UGGGCCCUUGGU CCGGUUUGUUA UGCACCAAAAGU UCCGAUGAGACAC UUUGUGCAAAA CAACACAGGAGCC GGGCACCCCCAGC UCUGGAGCCUGAC AAGGAGACA [SEQ ID NO:1910]
miR-144	2602	-2.76068	-0.25422	TTTAGTAGT TGATCCATA GATT [SEQ ID NO:1616]	TCGATGGAT CATACTACT AAA [SEQ ID NO:1636]	TCAAGCCATGCTT CCTGTGCCCCAG TGGGGCCCTGGC TTCGATGGATCAT ACTACTAAAAGT TTGCGATGAGAC ACTTTAGTAGTTG ATCCATAGAAAT CCGGGCACCCCC AGCTCTGGAGCC TGACAAGGAGGA CA [SEQ ID NO:1714]	UUUAGUAG UUGAUCCA UAGAUU [SEQ ID NO:202]	UCGAUGGA UCAUACUA CUAAA [SEQ ID NO:1831]	UCAAGCCAUUCU CCUGGCCCCAG UGGGCCCUUGGU UCGAUGGAUCAU ACUACUAAAAGU UUGCGAUGAGAC ACUUUAGUAGUU GAUCCAUAGAAG UCCGGGCACCCCC AGCUCUGGAGCCU GACAAGGAGGAC A [SEQ ID NO:1911]

miR-144	3255	-2.47679	-0.08595	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	ACAAGCTT ACTGCCAC GCAAT [SEQ ID NO:1660]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TACAAGCTTACTG CCACGCAATAGT TTGCGATGAGAC ACATTGCGTGGA GTAAGCTGCTAG TCCGGGCACCCC CAGCTCTGGAGC CTGACAAGGAGG ACA [SEQ ID NO:1744]	AUUGCUG GAGUAAGC UGGUGG [SEQ ID NO:306] (Same guide as XD-14889)	ACAAGCUU ACUGCCAC GCAAU [SEQ ID NO:1855]	UCAAGCCAUAGCUU CCUGGCCCCCAG UGGGCCCUUGGU ACAAGCUUACUGC CACGCAUAAGUU GCGAUGAGACACA UUGGUGGAGUA AGCUGGUAUCCG GGCACCCCAGCU CUGGAGCCUGACA AGGAGGACA [SEQ ID NO:1941]
miR-144	1231	-2.16776	-0.36035	TTCACTTTA GCACTGAT AGCAG [SEQ ID NO:1627]	GCGATCAG TGCATAAA GTGAA [SEQ ID NO:2141]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TGCATCAGTGC ATAAAGTGAAAG TTTGGATGAGA CACTTCACTTTAG CACTGATAGCAG TCCGGGCACCCC CAGCTCTGGAGC CTGACAAGGAGG ACA [SEQ ID NO:2142]	UUCACUUU AGCACUGA UAGCAG [SEQ ID NO:1825]	GCGAUCAG UGCAUAAA GUGAA [SEQ ID NO:2143]	UCAAGCCAUAGCUU CCUGGCCCCCAG UGGGCCCUUGGU GCGAUCAGUGCAU AAAGUGAAAGUU UGCGAUGAGACAC UUCACUUUAGCAC UGAUGCAGUCCG GGCACCCCAGCU CUGGAGCCUGACA AGGAGGACA [SEQ ID NO:2144]
miR-144	3845	-2.11363	-0.36405	TTGAACGTG AGAAGGAT GGATC [SEQ ID NO:696]	TCAATCCTT CTGCACGTT CAA [SEQ ID NO:2145]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TTCAATCCTTCTG CACGTTCAAAGTT TGCATGAGACA CTTGAACGTGAG AAGGATGGAAGT CCGGGCACCCC AGCTCTGGAGCC TGACAAGGAGGA CA [SEQ ID NO:2146]	UUGAACGU GAGAAGGA UGGAUC [SEQ ID NO:1224]	UCAAUCCU UCUGCACG UUCAA [SEQ ID NO:2147]	UCAAGCCAUAGCUU CCUGGCCCCCAG UGGGCCCUUGGU UCAAUCCUUCUGC ACGUCAAAGUU UGCGAUGAGACAC UUGAACGUGAGA AGGAUGGAAGUC CGGGACCCCAG CUCUGGAGCCUGA CAAGGAGGACA [SEQ ID NO:2148]

miR-144	915	-2.04132	-0.09574	AAATCGTA GACTGAGG CAGTCC [SEQ ID NO:2149]	ACGGCCTC AGTGCTAC GATT [SEQ ID NO:2150]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TACGGCCTCAGT GCTACGATTAGT TTGCGATGAGAC ACAAATCGTAGA CTGAGGCAGTAG TCCGGGCACCCC CAGCTCTGGAGC CTGACAAGGAGG ACA [SEQ ID NO:2151]	AAATCGUA GACUGAGG CAGUCC [SEQ ID NO:2152]	ACGGCCUC AGUCUAC GAUU [SEQ ID NO:2153]	UCAAGCCAUUCUU CCUGUGCCCCCAG UGGGCCCUUGGU ACGGCCUCAGUGC UACGAUUUAGUU UGCGAUGAGACAC AAUUCGUAGACU GAGGCAGUAGUCC GGGCACCCCCAGC UCUGGAGCCUGAC AAGGAGGACA [SEQ ID NO:2154]
miR-144	3301	-1.88293	0.071952	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	CCCAATCA GCCGCTTG TTCA [SEQ ID NO:2155]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TCCCAATCAGCC GCCTTGTTCAAGT TTGCGATGAGAC ACTGAACAAGGG GCTGATTTGGAGT CCGGGCACCCCC AGCTCTGGAGCC TGACAAGGAGGA CA [SEQ ID NO:2156]	UGAACAAAG GGGCUAGU UUGGGA [SEQ ID NO:1215]	CCCAAUCA GCCGCCUU GUUCA [SEQ ID NO:2157]	UCAAGCCAUUCUU CCUGUGCCCCCAG UGGGCCCUUGGU CCCAUUCAGCCGC CUUGUCAAAGUU UGCGAUGAGACAC UGAACAAAGGGC UGAUUUGGAGUC CGGGCACCCCCAG CUCUGGAGCCUGA CAAGGAGGACA [SEQ ID NO:2158]
miR-144	2932	-1.63	0.064183	TTTGGCTGA GAGAAAGGA ACGTG [SEQ ID NO:672]	CGGTCCTTC TCATCAGCC AAA [SEQ ID NO:2159]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TCGGTCTTCTCA TCAGCCAAAAGT TTGCGATGAGAC ACTTTGGCTGAG AGAAAGAACGAG TCCGGGCACCCC CAGCTCTGGAGC CTGACAAGGAGG ACA [SEQ ID NO:2160]	UUUGGCUG AGAGAAAG AACGUG [SEQ ID NO:1200]	CGGUCCUU CUCAUCAG CCAAA [SEQ ID NO:2161]	UCAAGCCAUUCUU CCUGUGCCCCCAG UGGGCCCUUGGU CGGUCCUUCUCAU CAGCCAAAAGUU GCGAUGAGACACU UUGGCUAGAGAG AGGAACGAGUCCG GGCACCCCCAGCU CUGGAGCCUGACA AGGAGGACA [SEQ ID NO:2162]

miR-144	3842	-1.57752	-0.42898	AACGTGAG AAGGATGG ATCGTA [SEQ ID NO:1625]	CGTCCATC CTATCTCAC GTT [SEQ ID NO:2163]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TCGCTCCATCCTA TCTACGTTAGTT TGCATGAGACA CAACGTGAGAAG GATGGATCGAGT CCGGGCACCCCC AGCTCTGGAGCC TGACAAGGAGGA CA	AACGUGAG AAGGAUGG AUCGUA [SEQ ID NO:1824]	CGCUCCAU CCUAUCUC ACGUU [SEQ ID NO:2165]	UCAAGCCAUAGCUU CCUGGCCCCCAG UGGGCCCUAGCU CGCUCCAUCCAU CUCACGUUAGUU GCCAUGAGACACA ACGUAGAGAAGGA UGGAUCGAGUCCG GGCACCCCAGCU CUGGAGCCUGACA AGGAGGACA [SEQ ID NO:2166]
miR-144	2353	-1.4808	0.451244	TTCGATGCA GGACTAGC AGGCG [SEQ ID NO:612]	CCGGCTAGT CCATGCATC GAA [SEQ ID NO:2167]	TCAAGCCATGCTT CCTGTGCCCCCAG TGGGGCCCTGGC TCCGGCTAGTCCA TGCATCGAAAGT TTGCGATGAGAC ACTTCGATGCAG GACTAGCAGGAG TCCGGGCACCCC CAGCTCTGGAGC CTGACAAGGAGG ACA [SEQ ID NO:2168]	UUCGAUGC AGGACUAG CAGGCG [SEQ ID NO:166] (Same as XD- 14819)	CCGGCUAG UCCAUGCA UCGAA [SEQ ID NO:2169]	UCAAGCCAUAGCUU CCUGGCCCCCAG UGGGCCCUAGCU CCGGCUAGUCCAU GCAUCGAAAGUU UCCGAUGAGACAC UUCGAUGCAGGAC UAGCAGGAGUCCG GGCACCCCAGCU CUGGAGCCUGACA AGGAGGACA [SEQ ID NO:2170]
miR-155E	3302	-2.52291	0.018122	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAAAATC GCCCTTGTT CAA [SEQ ID NO:1652]	CTGGAGGCTTGCT TTGGGCTGTATGC TGTTGAACAAGG GGCTGATTGGGT TTTGCCCTCTGAC TGACCCAAATCG CCCTTGTTCAACA GGACAAGGCCCT TTATCAGCACTCA CATGGAACAAAT GGCCACCGTGGG [SEQ ID NO:1734]	UUGAACAA GGGCGUGA UUUGGG [SEQ ID NO:1216]	CCCAAAUC GCCCUUGU UCAA [SEQ ID NO:1847]	CUGGAGCCUAGCU UUGGGCUUAUG CUGUGAACAAAG GGGCUGAUUUGG GUUUUGGCCUCUG ACUGACCCAAAU GCCCUUGUCAAAC AGGACAAGGCCCU UUAUACAGCACUCA CAUGGAACAAAU GGCCACCGUGGG [SEQ ID NO:1931]

miR-155E	2586	-2.5179	-0.10172	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAGTTCA CTCTGAATC TA [SEQ ID NO:1653]	CTGAGGCTTGCT TTGGGCTGTATGC TGATAGATTGAG AGTAGAACTTGG TTTGGCTCTGA CTGACCAAGTTC ACTCTGAATCTAC AGGACAAGGCC TTTATCAGCACTC ACATGGAACAAA TGGCCACCGTGG G [SEQ ID NO:1736]	UAGAUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAAGUUC ACUCUGAA UCUA [SEQ ID NO:1848]	CUGAGGCUUGCU UUGGGCUUAUG CUGUAGAUUCAG AAGUAGAAUUG GUUUUGGCUUG ACUGACCAAGUUC ACUCUGAAUUCAC AGGACAAGGCCU UUUACAGCACUA CAUGGAACAAU GGCCACCGUGGG [SEQ ID NO:1933]
miR-155E	1755	-2.39604	-0.03915	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTTCG ATTCAACCC GA [SEQ ID NO:1676]	CTGGAGGCTTGCT TTGGGCTGTATGC TGTCGGTTGAA ATCTGAAGTGTG TTTGGCTCTGAC TGACACACTTCG ATTCAACCGAC AGGACAAGGCC TTTATCAGCACTC ACATGGAACAAA TGGCCACCGTGG G [SEQ ID NO:1766]	UCGGGUUG AAAUUGA AGUGG [SEQ ID NO:1185]	CACACUUC GAUUC AAC CCGA [SEQ ID NO:1871]	CUGAGGCUUGCU UUGGGCUUAUG CUGUAGGCUUGA AAUCUGAAGUG GUUUUGGCUUG ACUGACACUUC GAUUC AACCGAC AGGACAAGGCCU UUUACAGCACUA CAUGGAACAAU GGCCACCGUGGG [SEQ ID NO:1963]
miR-155E	3273	-2.36984	-0.0801	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATATGTG CCACAGTCC TA [SEQ ID NO:1683]	CTGGAGGCTTGCT TTGGGCTGTATGC TGATGAGCTGTA GGCAACATATTG TTTGGCTCTGA CTGACAATATGT GCCACAGTCTTA CAGGACAAGGCC CTTATCAGCACT CACATGGAACAA ATGGCCACCGTG GG [SEQ ID NO:1774]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAUAUUGU GCCACAGU CCUA [SEQ ID NO:1878]	CUGAGGCUUGCU UUGGGCUUAUG CUGUAGGACUGU AGGCAACAUU GUUUUGGCUUG ACUGACAAUUG UGCCACAGUCCUA CAGGACAAGGCC UUUACAGCACUC ACAUGGAACAAA UGGCCACCGUGGG [SEQ ID NO:1971]

miR-155E	3272	-2.34055	-0.19485	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATATGT GCTACAGTC CT [SEQ ID NO:1687]	CTGAGGGCTTGCT TTGGGCTGTATGC TGAGGACTGTAG GCAACATATTGCT TTTGGCTCTGAC TGAGCAATATGT GCTACAGTCTCA GGACAAGGCCCT TTATCAGCACTCA CATGGAACAAAT GGCCACCGTGG [SEQ ID NO:1780]	AGGACUGU AGGCAACA UAUUGC [SEQ ID NO:1814]	GCAUAUUG UGCACAG UCCU [SEQ ID NO:1882]	CUGAGGCUUGCU UUGGCUUAUG CUGAGGACUGA GGCAACAUAUUGC UUUUGGCUUGA CUGAGCAUAUUG UGCUACAGUCCUC AGGACAAGGCCCU UUUACAGCACUCA CAUGGAACAAU GGCCACCGUGGG [SEQ ID NO:1977]
miR-155E	3330	-2.26554	0.033188	TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCACATTAC AGCTCAGC ATA [SEQ ID NO:1700]	CTGAGGGCTTGCT TTGGGCTGTATGC TGTATGCTGAGA CTGATAATGTGGT TTTGGCTCTGAC TGACCACATTAC AGCTCAGCATAC AGGACAAGGCC TTTATCAGCACTC ACATGGAACAAA TGCCACCGTGG G [SEQ ID NO:1793]	UAUGCUGA GACUGAU AUGUGG [SEQ ID NO:1811]	CCACAUUA CAGCUCAG CAUA [SEQ ID NO:1895]	CUGAGGCUUGCU UUGGCUUAUG CUGUAUGCUGAG ACUGAUAAUGUG GUUUGGCUUG ACUGAGCACAUUA CAGCUCAGCAUAC AGGACAAGGCCCU UUUACAGCACUCA CAUGGAACAAU GGCCACCGUGGG [SEQ ID NO:1990]
miR-155E	1162	-2.26036	-0.20831	AACGTGACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTT TGGGTACA GTT [SEQ ID NO:1706]	CTGAGGGCTTGCT TTGGGCTGTATGC TGAACGTACCA CAACAAAGTCTG TTTGGCTCTGA CTGACAGACTTTT TGGGTACAGTTC AGGACAAGGCC TTTATCAGCACTC ACATGGAACAAA TGCCACCGTGG G [SEQ ID NO:1801]	AACUGUAC CACAAACA AGUCUG [SEQ ID NO:1180]	CAGACUUU UUGGCUAC AGUU [SEQ ID NO:1905]	CUGAGGCUUGCU UUGGCUUAUG CUGAACUGUACCA CAACAAAGUCUGU UUUGGCUUGAC UGACAGACUUU UGGUAACAGUUC AGGACAAGGCCCU UUUACAGCACUCA CAUGGAACAAU GGCCACCGUGGG [SEQ ID NO:1998]

miR-155E	1580	-2.18059	-0.26086	ACTGGAATT TCTCTGAAC TGCT [SEQ ID NO:1622]	AGCAGTTC GAGAAATC CAGT [SEQ ID NO:2171]	CTGAGGGCTTGCT TTGGGCTGTATGC TGACTGGAATTC TCTGAACTGCTTT TTGGCTCTGACT GAAGCAGTTTGA GAATTCAGTCA GGACAAGGCCCT TTATCAGCACTCA CATGGAACAAAT GGCCACCGTGG [SEQ ID NO:2172]	ACUGGAU UUCUCUGA ACUGCU [SEQ ID NO:1817]	AGCAGUUC GAGAAUUC CAGU [SEQ ID NO:2173]	CUGAGGCUUGCU UUGGCUUAUG CUGACUGAAU UCUCUGAACUGCU UUUGGCUUGA CUGAAGCAGUUCG AGAAUUCAGUCA GGACAAGGCCCUU UAUCAGCACUCAC AUGGAACAAUG GCCACCGUGG [SEQ ID NO:2174]
miR-155E	1436	-2.08545	0.133624	TGAGTTATC TCTTCTAA GGGC [SEQ ID NO:1632]	GCCCTTAGA AGGATAAC TCA [SEQ ID NO:1710]	CTGAGGGCTTGCT TTGGGCTGTATGC TGTGAGTTATCTC TTTCTAAGGGCTT TTGGCTCTGACT GAGCCCTTAGAA GGATAACTCACA GGACAAGGCCCT TTATCAGCACTCA CATGGAACAAAT GGCCACCGTGG [SEQ ID NO:2175]	UGAGUUAU CUCUUUCU AAGGC [SEQ ID NO:1827]	GCCCUUAG AAGGAUAA CUCA [SEQ ID NO:1900]	CUGAGGCUUGCU UUGGCUUAUG CUGUGAGUUAUC UCUUUUAAGGGC UUUGGCUUGA CUGAGCCCUAGA AGGAUAAUCACA GGACAAGGCCCUU UAUCAGCACUCAC AUGGAACAAUG GCCACCGUGG [SEQ ID NO:2176]
miR-155E	3270	-2.03222	0.002948	TACTGTAGG CAACATATT GCGT [SEQ ID NO:2080]	ACGCAATA GTTCTTACA GTA [SEQ ID NO:2177]	CTGAGGGCTTGCT TTGGGCTGTATGC TGACTGTAGGC AACATATTGCGT TTTGCCCTCTGAC TGAACGCAATAG TTCCTACAGTACA GGACAAGGCCCT TTATCAGCACTCA CATGGAACAAAT GGCCACCGTGG [SEQ ID NO:2178]	UACUGUAG GCAACAU UUGCGU [SEQ ID NO:2083]	ACGCAUA GUUCCUAC AGUA [SEQ ID NO:2179]	CUGAGGCUUGCU UUGGCUUAUG CUGUACUGAAGGC AACAUUUGCGU UUUGGCUUGA CUGAACGCAUAG UUCUACAGUACA GGACAAGGCCCUU UAUCAGCACUCAC AUGGAACAAUG GCCACCGUGG [SEQ ID NO:2180]
miR-190a	3330	-2.86183	0.46905	TATGCTGAG ACTGATAAT GTGG	CCATTATCA GTTCTCAGCA CC	GAGCTCAGTCAA ACCTGGATGCTT TTCTGAGGCTC TGTGTATGCTGAG	UAUGCUGA GACUGAUA AUGGG	CCAUUAC AGUCUCAG CACC	GAGCUCAGCAAA CCUGAUGCCUU UCUGCAGGCCU GUGUAUGCUGAG

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miR-190a	3301	-2.36785	0.195332	TGAACAAG GGGCTGATT TGGGA [SEQ ID NO:687]	TCCAATCAG CCCCTTGTT AC [SEQ ID NO:1684]	TCAACCCACCTAC AGTGTCTTGCCCT GTCTCCGGGGTT CCTAATAAAG [SEQ ID NO:1771]	UGAACAAAG GGGUGAU UUGGGA [SEQ ID NO:1215]	UCCAAUCA GCCCCUUG UUAC [SEQ ID NO:1879]	UUAACCCACCUA CAGUCUUGCCC UGUCGGGGGU UCCUAAUAAAG [SEQ ID NO:1968]
miR-190a	3043	-2.26041	0.277711	TTTGGTGCA AAACAAAC AGGCT [SEQ ID NO:1615]	AGCGTTTGT TTTGACCA CC [SEQ ID NO:1664]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCTC TGTGTTTGGTGCA AAACAAACAGGC TTGTTATTAAATC CAAGCGTTTGT TGCACCACTTAC AGTGTCTTGCCCT GTCTCCGGGGTT CCTAATAAAG [SEQ ID NO:1800]	UUUGGUGC AAAACAAA CAGGCU [SEQ ID NO:1812]	AGCGUUUG UUUUGCAC CACC [SEQ ID NO:1859]	GAGUCAGUCAAA CCUGGAUGCCUUU UCUGCAGGCCUU GUGUUUGGUGCA AAACAAACAGGCU UGUUAUUUAUC CAAGCGUUUGU UUGCACCACCUA CAGUGUCUUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:1997]
miR-190a	2586	-2.24846	0.172174	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCATTCTAC TTCTGAATC CC [SEQ ID NO:1661]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCTC TGTGTAGATTGAG AAGTAGAACTTG GTGTTATTAAATC CACCATTCTACTT CTGAATCCCTAC AGTGTCTTGCCCT	UAGAUUCA GAAGUAGA ACUUGG [SEQ ID NO:1816]	CCAUUCUA CUUCUGAA UCCC [SEQ ID NO:1856]	GAGUCAGUCAAA CCUGGAUGCCUUU UCUGCAGGCCUU GUGUAGAUCAG AAGUAGAUCUUG GUGUUAUUUAU CCACCAUUCUACU UCUGAAUCCCUA CAGUGUCUUGCCC

miR-190a	967	-2.23671	-0.08895	ACTGATGTA AGTATATG AACCA [SEQ ID NO:1619]	TGGCATATA CTTACATCA AG [SEQ ID NO:1642]	GTCTCCGGGGTT CCTAATAAAG [SEQ ID NO:1803]	ACUGAUGU AAGUAU GAACCA [SEQ ID NO:1815]	UGGCAU ACUACAU CAAG [SEQ ID NO:1837]	UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:2000]
miR-190a	3255	-2.21465	0.46382	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CCAAGCTTA CTCCACGCA CG [SEQ ID NO:1697]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCTC TGTGATTGCTGG AGTAAGCTGGT GTGTTATTAAATC CACCAAGCTTACT CCACGCACGCTA CAGTGTCTTGCC TGCTCCGGGGT TCCTAATAAAG [SEQ ID NO:1809]	AUUGCUG GAGUAAGC UGGUG [SEQ ID NO:306] (Same guide as XD-14889)	CCAAGCU ACUCCACG CACG [SEQ ID NO:1892]	GAGCUCAGUCAA CCUGGAUGCCUU UCUGCAGGCCUCU GUGAUUGCGUG AGUAAGCUGGUG GUGUAUUUAAU CCACCAAGCUAC UCCACGCACGCUA CAGUGUCUUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:2006]
miR-190a	3341	-2.17489	0.322497	ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GTCAGCATC CTCATGTCT CG [SEQ ID NO:1703]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCTC TGTGATAGACAT GAGGATGCTGAG ACTGTTATTAAAT CCAGTCAGCATC CTCATGTCTGCT ACAGTGCTTGCC CTGTCTCGGGG GTTCTTAATAAA G [SEQ ID NO:2183]	AUAGACAU GAGGAUGC UGAGAC [SEQ ID NO:1813]	GUCAGCAU CCUCAUGU CUCG [SEQ ID NO:1898]	GAGCUCAGUCAA CCUGGAUGCCUU UCUGCAGGCCUCU GUGAUAGACAU AGGAUGCUGAGA CUGUAUUUAAU CCAGUCAGCAUCC UCAUGUCGCUA CAGUGUCUUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:2184]

miR-190a_M	967	-2.64307	0.113083	ACTGATGTA AGTATATG AACCA [SEQ ID NO:1619]	TGGCATATA CTTACATCA AG [SEQ ID NO:1642]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGCGTC TGTGATGATGTA AGTATATGAAC ATGTTATTAAATC CATGGCATATACT TACATCAAGCTA CAGTCTCTTGCCC TGCTCCGGGGGT TCCTAATAAAG [SEQ ID NO:1720]	ACUGAUGU AAGUAU GAACCA [SEQ ID NO:1815]	UGGCAU ACUUAU CAAG [SEQ ID NO:1837]	GAGCUCAGUCAA CCUGGAUCCUUU UCUGCAGCGUCU GUGACUGAUGUA AGUAUUAUAAU AUGUAUUAUAAU CCAUGCAUUAUAC UUACAUCAAGCUA CAGUCUUCUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:1917]
miR-190a_M	3302	-2.52395	0.249656	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCATCAGC CCCTTGTTT CC [SEQ ID NO:1643]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGCGTC TGTGTTGAACAA GGGCTGATTG GGTGTATTAAAT CCACCCATCAGC CCCTTGTTCCCT ACAGTCTCTTGCC CTGTCTCGGGG GTTCTTAATAAA G [SEQ ID NO:1733]	UUGAACAA GGGGCUGA UUUGG [SEQ ID NO:1216]	CCCAUCAG CCCCUUGU UCCC [SEQ ID NO:1838]	GAGCUCAGUCAA CCUGGAUCCUUU UCUGCAGCGUCU GUGUAGAUUCAG AAGUAGAAUUG GUGUAUUAUAAU CCACCAUACGCG CCUUGUCCCUA CAGUCUUCUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:1930]
miR-190a_M	2586	-2.46486	0.014777	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCATTCTAC TTCTGAATC CC [SEQ ID NO:1661]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGCGTC TGTGATGATTCAG AAGTAGAACTTG GTGTTATTAAATC CACCATTCTACTT CTGAATCCCTAC AGTCTCTTGCCCT GTCTCCGGGGGT CCTAATAAAG [SEQ ID NO:1746]	UAGAUCA GAAGUAGA ACUUG [SEQ ID NO:1816]	CCAUCUA CUUCUGAA UCCC [SEQ ID NO:1856]	GAGCUCAGUCAA CCUGGAUCCUUU UCUGCAGCGUCU GUGUAGAUUCAG AAGUAGAAUUG GUGUAUUAUAAU CCACCAUUCUACU UCUGAAUCCCUA CAGUCUUCUGCCC UGUCUCCGGGGU UCCUAAUAAAG [SEQ ID NO:1943]
miR-190a_M	3043	-2.44458	0.289334	TTTGGTGCA AAACAAAC AGGCT	AGCGTTTGT TTTGCACCA CC	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGCGTC	UUUGGUC AAACAA CAGGCU	AGCGUUUG UUUUGCAC CACC	GAGCUCAGUCAA CCUGGAUCCUUU UCUGCAGCGUCU

					[SEQ ID NO:1615]	[SEQ ID NO:1664]	TGTGTTTGGTGCA AAACAAACAGGC TTGTTATTTAATC CAAGCGTTTGT TGACACACCTAC AGTCTTTGCCCT GTCTCGGGGTT CCTAATAAAG [SEQ ID NO:1750]	[SEQ ID NO:1812]	[SEQ ID NO:1859]	GUGUUUGGUGCA AAACAAACAGGCU UGUUUUUAAUC CAAGCGUUUGUU UUGCACCAACCUA CAGUCUUGGCC UGUCUGGGGGU UCCUAAUAAAG [SEQ ID NO:1947]
miR-190a_M	1755	-2.34216	0.272299	TCGGGTTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACCTCAGA TTTCAACCC AC [SEQ ID NO:1680]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCGTC TGTGTCGGGTGA AATCTGAAGTGT GTGTTATTTAATC CACACTTCAGATT TCAACCCACCTAC AGTCTTTGCCCT GTCTCGGGGGTT CCTAATAAAG [SEQ ID NO:1778]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CACUUCAG AUUCAAAC CCAC [SEQ ID NO:1875]	GAGCUCAGUCAA CCUGGAUGCCUU UCUGCAGGCGUCU GUGUCGGGUUA AAUCUGAAGUGU GUGUUUUUAAU CCACACUUCAGAU UUCACCCACCUA CAGUCUCUUGCCC UGUCUGGGGGU UCCUAAUAAAG [SEQ ID NO:1975]	
miR-190a_M	3269	-2.3297	0.037293	ACTGTAGG CAACATATT GCGTG [SEQ ID NO:1629]	CACAATAT GTTGCCTAC AAG [SEQ ID NO:1689]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCGTC TGTGACTGTAGG CAACATATTGCGT GTGTTATTTAATC CACACAATATGTT GCCTACAAGCTA CAGTCTTTGCC TGTCTCGGGGGT TCCTAATAAAG [SEQ ID NO:1782]	ACUGUAGG CAACAUAU UGCGUG [SEQ ID NO:1822]	CACAAUUA GUUGCCUA CAAG [SEQ ID NO:1884]	GAGCUCAGUCAA CCUGGAUGCCUU UCUGCAGGCGUCU GUGACUGUAGGC AACAUUUGCGU GUGUUUUUAAU CCACACAUAUGU UGCCUACAAGCUA CAGUCUUGGCC UGUCUGGGGGU UCCUAAUAAAG [SEQ ID NO:1979]	
miR-190a_M	3255	-2.30348	0.418205	ATTGCGTGG AGTAAGCT GGTGG [SEQ ID NO:617]	CCAAGCTTA CTCCACGCA CG [SEQ ID NO:1697]	GAGCTCAGTCAA ACCTGGATGCTT TTCTGCAGGCGTC TGTGATTGCGTGG AGTAAGCTGGT GTGTTATTTAATC CACCAAGCTTACT	AUUGCGUG GAGUAAGC UGGUGG [SEQ ID NO:306]	CCAAGCUU ACUCCACG CACG [SEQ ID NO:1892]	GAGCUCAGUCAA CCUGGAUGCCUU UCUGCAGGCGUCU GUGAUUGCGUGG AGUAAGCUGGUG GUGUUUUUAAU CCACCAAGCTUAC	

									(Same guide as XD-14889)				
miR-190a_M	3341	-2.26383	0.394316						ATAGACAT GAGGATGC TGAGAC [SEQ ID NO:1617]	GTCAGCATC CTCATGTCT CG [SEQ ID NO:1703]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGGCGTC TGTGATGACAT GAGGATGCTGAG ACTGTTATTTAAT CCAGTCAGCATC CTCATGTCTCGCT ACAGTCTCTTGCC CTGTCTCCGGGG GTTCTTAATAAA G	CCACGCACGCTA CAGTCTCTTGCCC TGTCCTCGGGGT TCCTAATAAG [SEQ ID NO:1790]	UCCACGCACGCUA CAGUCUCUUGCCC UGUCUCGGGGGU UCCUAAUAAAG [SEQ ID NO:1987]
miR-190a_M	1436	-2.18684	0.218825						TGAGTTATC TCTTTCTAA GGGC [SEQ ID NO:1632]	GCCTAGAA AGAGATAA CTAC [SEQ ID NO:2185]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGGCGTC TGTGTGAGTTATC TCTTTCTAAGGGC TGTTATTATATCC AGCTAGAAAGA GATAACTACCTA CAGTCTCTTGCCC TGTCCTCGGGGT TCCTAATAAAG [SEQ ID NO:2186]	UGAGUUUU CUCUUUCU AAGGGC [SEQ ID NO:1827]	GAGCUCAGUCUAAA CCUGGAUGCCUUU UCUGCAGGCGUCU GUGUGAGUUUUC UCUUUUCUAAAGGC UGUUUUUUUUUC CAGCCUAGAAAGA GAUAAUACCUAC AGUCUCUUGCCCU GUCUCGGGGGUU CCUAAUAAAG [SEQ ID NO:2187]
miR-190a_M	3330	-2.17382	0.344749						TATGCTGAG ACTGATAAT GTGG [SEQ ID NO:1614]	CCATTATCA GTCTCAGCA CC [SEQ ID NO:1634]	GAGCTCAGTCAA ACCTGGATGCCTT TTCTGCAGGCGTC TGTGTATGCTGAG ACTGATAATGTG GTGTTATTTAATC CACCATTTATCAGT CTCAGCACCCCTAC AGTCTCTGCCCC	UAUGCUGA GACUGAUA AUGUGG [SEQ ID NO:1811]	GAGCUCAGUCUAAA CCUGGAUGCCUUU UCUGCAGGCGUCU GUGUAUGCUGAG ACUGAAUUAUGUG GUGUUUUUUUU CCACCAUUUAUCAG UCUCAGCACCCUA CAGUCUCUUGCCC

miR155-M	3302	-2.42428	0.285147	TTGAACAA GGGGCTGA TTTGGG [SEQ ID NO:688]	CCCAATC GCCCTTGT CAA [SEQ ID NO:1652]	GTCTCGGGGTT CCTAATAAG [SEQ ID NO:2188]	UUGAACAA GGGCUGA UUUGG [SEQ ID NO:1216]	CCCAAUUC GCCCCUGU UCAA [SEQ ID NO:1847]	UGUCUCGGGGU UCCUAAUAAAG [SEQ ID NO:2189]
miR155-M	2586	-2.4228	-0.13209	TAGATTGAG AAGTAGAA CTTGG [SEQ ID NO:1621]	CCAAATTCA CTCTGAATC TA [SEQ ID NO:1653]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTAGATTCA GAAGTAGAACTT GGTTTGGCCACT GACTGACCAAGT TCACTCTGAATCT ACAGGACACAAG GCCTGTACTAGC ACTCACATGGAA CAAATGGCCACC GG [SEQ ID NO:1758]	UAGAUCA GAAUGA ACUUGG [SEQ ID NO:1816]	CCAAGUUC ACUCUGAA UCUA [SEQ ID NO:1848]	CCUGAGGCUUGC UGAAGGCUUAU GCUGAGAUUA GAAGUAGAACTU GGUUUGGCCACU GACUGACCAAGU CACUCUGAAUCUA CAGGACACAAGGC CUGUACUAGCAC UCACAUUGAACAA AUGGCCACCGG [SEQ ID NO:1955]
miR155-M	1436	-2.22884	0.141256	TGAGTTATC TCTTCTAA GGGC [SEQ ID NO:1632]	GCCCTTAGA AGGATAAC TCA [SEQ ID NO:1710]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTGAGTTAT CTCTTCTAAGGG CTTTGGCCACTG ACTGAGCCCTTA GAAGGATACTC ACAGGACACAAG GCCTGTACTAGC ACTCACATGGAA [SEQ ID NO:1759]	UGAGUUAU CUCUUUCU AAGGC [SEQ ID NO:1827]	GCCUUAG AAGGAUAA CUCA [SEQ ID NO:1900]	CCUGAGGCUUGC UGAAGGCUUAU GCUGAGAUUAU CUCUUUAAGGG CUUUGGCCACUG ACUGAGCCCUAG AAGGAUACUCAC AGGACACAAGGCC UGUACUAGCACU CACAUUGAACAA UGGCCACCGG

miR155-M	3272	-2.02279	-0.09047	AGGACTGT AGGCAACA TATTGC [SEQ ID NO:1618]	GCAATATGT GCTACAGTC CT [SEQ ID NO:1687]	CAAATGGCCACC GG [SEQ ID NO:1810]	AGGACUGU AGCAACA UAUUGC [SEQ ID NO:1814]	GCAUAUG UGCACAG UCCU [SEQ ID NO:1882]	[SEQ ID NO:2007]
miR155-M	1755	-1.98201	-0.05599	TCGGGTGA AATCTGAA GTGTG [SEQ ID NO:657]	CACACTTCG ATTCAACCC GA [SEQ ID NO:1676]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTCGGGTG AAATCTGAAGTG TGTTTTGGCCACT GACTGACACACT TCGATTCAACCCG ACAGGACACAAG GCCTGTTACTAGC ACTCACATGGAA CAAATGGCCACC GG [SEQ ID NO:2190]	UCGGGUUG AAAUUGA AGUGUG [SEQ ID NO:1185]	CACACUUC GAUUC AAC CCGA [SEQ ID NO:1871]	CCUGGAGGCUUGC UGAAGGCUUUAU GCUGCGGGUUG AAUUCUGAAGUG UGUUUGGCCACU GACUGACACACU CGAUUCAACCCGA CAGGACACAAGG CUGUACUAGCAC UCACAUUGGAACA AUGGCCACCGG [SEQ ID NO:2193]
miR155-M	1162	-1.86763	-0.12823	AACTGTACC ACAACAAA GTCTG [SEQ ID NO:652]	CAGACTTTT TGGGTACA GTT [SEQ ID NO:1706]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGAACGTGAC CACACAAAGTC TGTTTTGGCCACT GACTGACAGACT TTTTGGGTACAGT TCAGGACACAAG GCCTGTTACTAGC ACTCACATGGAA	AACUGUAC CACAAACA AGUCUG [SEQ ID NO:1180]	CAGACUUU UUGGGUAC AGUU [SEQ ID NO:1905]	CCUGGAGGCUUGC UGAAGGCUUUAU GCUGAACUGUACC ACAACAAAGUCUG UUUUGGCCACUGA CUGACAGACUUU UGGGUACAGUUC AGGACACAAGGCC UGUUACUAGCACU CACAUUGGAACAA UGGCCACCGG

miR155-M	1580	-1.73791	-0.21175	ACTGGAATT TCTCTGAAC TGCT [SEQ ID NO:1622]	AGCAGTTC GAGAAATTC CAGT [SEQ ID NO:2171]	CAAATGGCCACC GG [SEQ ID NO:2194]	ACUGGAUU UUCUCUGA ACUGCU [SEQ ID NO:1817]	AGCAGUUC GAGAAUUC CAGU [SEQ ID NO:2173]	[SEQ ID NO:2195]
miR155-M	3273	-1.30321	-0.03402	TAGGACTGT AGGCAACA TATTG [SEQ ID NO:1628]	CAATATGTG CCACAGTCC TA [SEQ ID NO:1683]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTAGGACT GTAGGCAACATA TTGTTTGGCCAC TGACTGACAATA TGTGCCACAGTCC TACAGGACACAA GGCCTGTTACTAG CACTCACATGGA ACAAATGGCCAC CGG [SEQ ID NO:2196]	UAGGACUG UAGGCAAC AUUUG [SEQ ID NO:1821]	CAUAUGU GCCACAGU CCUA [SEQ ID NO:1878]	CCUGAGGCUUGC UGAAGGCUUUAU GCUGUAGGACUG UAGGCAACAUU UGUUUGGCCACU GACUGACAUAU GUGCCACAGUCCU ACAGGACACAAGG CCUGUACUAGCA CUCACUUGGAACA AAUGGCCACCCG [SEQ ID NO:2199]
miR155-M	3291	-1.28366	0.074799	TCTGATTG GGAAGTGC TGAGG [SEQ ID NO:2200]	CCTCAGCAT TCCAAATCA GA [SEQ ID NO:2201]	CCTGGAGGCTTG CTGAAGGCTGTA TGCTGTCTGATTT GGGAACCTGCTGA GGTTTGGCCACT GACTGACCTCAG CATTCCAAATCA GACAGGACACAA GGCCTGTTACTAG CACTCACATGGA	UCUGAUUU GGGAACUG CUGAGG [SEQ ID NO:2203]	CCUCAGCA UUCCAAU CAGA [SEQ ID NO:2204]	CCUGAGGCUUGC UGAAGGCUUUAU GCUGUUGAUUU GGGAACUGCUA GGUUUUGGCCACU GACUGACCUACG AUUCCAAU/CAGA CAGGACACAAGG CUGUACUAGCAC UCACAUUGGAACA AUGGCCACCCG

[illegible]

Methods

Oligo pool design and synthesis:

A total of 7500 elements of 210bp length were designed for synthesis, split approximately evenly across 20 miRNA backbones. There were more elements in the miR-1-1, miR-155, and miR-16-2 backbones as elements that had been tested in arrayed experiments were also included in this screen. ATXN2 targeting sequences accounted for about 60% of the library.

Each element included the 138nt pri-miRNA, flanked by dual 18nt adapter pairs. The outer adapter pair was miR-specific and the inside adapter pair was universal.

Full DS2 library cloning strategy

Oligonucleotide pools were synthesized (Twist Bioscience) and were reconstituted in nuclease free water. For cloning the EF1A oligo pool into pLVX_EF1A-MCS-WPRE-CMV-Puro, the vector was first linearized by XbaI and EcoRI restriction digest and gel purified. The primers DS2_EF1A_fw and DS2_EF1A_rv were used to amplify the oligo pool through 10 cycles of PCR and purified. The purified pooled insert and purified linearized vector were assembled with NEB HiFi assembly, precipitated, concentrated, and electroporated into Lucigen Endura electrocompetent cells, recovered and maxiprep. Oligo pools were PCR amplified with the following conditions.

The PCR mix consisted of:

Component	Volume (ul)
NEBNext 2x mix (M0541L)	50
DMSO (D9170-5VL)	2
Betaine (Sigma, B0300-1VL)	10
100uM FW primer (DS2_EF1A_fw)	0.5
100uM RV primer (DS2_EF1A_rv)	0.5

1ng EF1A oligo pool in nuclease free water	37
Total	100

The PCR cycling parameters were:

STEP	TEMP	TIME
Initial Denaturation	98°C	30s
10 cycles	98°C	10s
	64°C	30s
	72°C	15s
Final Extension	72°C	2min
Hold	4–10°C	

5 PCR products of 210bp length were purified by agarose gel extraction (Zymoclean gel DNA recovery kit, D4002). Agilent Tapestation High Sensitivity D1000 was used to quantify the molarity of the 210bp peak and to confirm removal of contaminating bands.

HiFi assembly of the pooled library was performed by assembling at 5 to 1 insert to backbone molar ratio. 15ul of 2x HiFi assembly master mix (NEB, E2621L) and 15ul of insert and backbone (about 0.375 pmol purified miR library insert to 0.075 pmol purified backbone) and incubating for 1hr at 50°C.

Assembled DNA was precipitated by adding 1ul of 20 mg/mL glycogen, one-tenth volume of 3M sodium acetate pH 5.5, and 2.2x volume of ethanol, mixed and stored overnight at -80°C.

15 Samples were spun at 16,000 xg for 15min at 4°C. Supernatant was removed and discarded. Pellets were washed twice with 1ml of 70% ice cold ethanol and let to dry, then dissolved in 4ul nuclease free water.

Purified DNA and 0.1 cm Gene Pulser Cuvettes (Bio-rad, 165-2089) were placed on ice for 10 min. 50ul of Lucigen Endura electrocompetent cells were thawed briefly on ice. 4ul of precipitated HiFi reaction was added to 25ul of

electrocompetent cells, mixed, and transferred to the cuvette. DNA and cell mixes were electroporated with the following parameters: 1800 Volts, 10 uF, 600 Ohms, 0.1cm cuvette. Cuvettes were immediately flushed twice with 1 mL Lucigen recovery media. Cells were recovered at 37°C for 1 hour at 230 rpm.

5 To titer the transformed bacteria, 2 uL of each culture was diluted into 200 uL of LB and 100 ul or 10ul of this plated at a 1:100 dilution onto LB agar plates plus appropriate antibiotic. The number of colonies were counted the next day to determine total number of transformants.

10 Liquid cultures were inoculated into the appropriate amount of LB with antibiotic for maxi prep. Pooled plasmid libraries were prepared with a Qiagen Plasmid Maxi Kit following the manufacturer's instructions.

Preparation and titering of pooled EF1A library

15 Lentivirus was produced with the Takara packaging plasmid system in Lenti-X 293T cells. Functional titers were determined by Cell Titer Glo following infection and puromycin selection for 3 days to identify conditions to achieve MOI = 0.1.

Execution of full library screens for Atxn2 levels and dropout

20 Concurrent ATXN2 levels and dropout screens were conducted similarly to DS1. U2OS cells were infected at day 0 with the lentiviral pooled EF1A library at 2000x coverage and MOI = 0.1.

25 For the dropout screen, a T0 baseline sample was collected at day 1. Puromycin was added on day 2 and MOI was confirmed by plating cells for Cell Titer Glo titer assessment at day 5. After day 7, puromycin was removed and cells were passaged at a minimum of 20 million cells to day 18, upon which the T1 final cell population was collected.

30 For the ATXN2 protein levels screen, on day 7 cells were harvested and fixed in 6% sucrose/8% PFA for 10-15min at room temperature, centrifuged 600 xg for 3 minutes, washed thrice using the permeabilization buffer (eBioscience, 00-5523-00), mixed with wash buffer and incubated for 15-20min at room temperature. Anti-ATXN2 primary antibody (1:200, BD, 611378) was incubated for 30-60min at RT.

Cells were washed thrice and AF647 secondary (1:200, Biolegend, 405322) was added and incubated for 45min. After three washes, cells were resuspended in FACS buffer and sorted on a BD FACS Aria Fusion. After gating for singlets, 25% high and low Atxn2 gates were drawn, adjusting for cell size by sorting on an APC/SSC ratiometric gate. Once 3-3.5 million cells were collected for the 25% high and low sort gates, remaining cells were sorted on a 10% low gate (1 million cells collected) to further enrich for high performing guides. The reference population was collected by sorting for singlets.

Fixed populations of sorted cells were decrosslinked with 1% SDS/1% sodium bicarbonate and incubated overnight at 65°C. Genomic DNA was extracted with Machery Nagel NucleoSpin L kit and proceeded to nested PCR to prepare sequencing libraries.

Sequencing library preparation

Nested PCR was performed to produce Illumina adapted sequencing amplicons. The first PCR reaction was performed on all genomic DNA extracted from each cell pellet. A maximum of 5µg genomic DNA was used per 100µl PCR reaction using the conditions listed below.

Component	Volume (ul)
NEBNext 2x mix (M0541L)	50
DMSO (D9170-5VL)	2
Betaine (Sigma, B0300-1VL)	10
100uM FW primer (EF1A_F_intron)	0.5
100uM RV primer (WPRE_R_CG)	0.5
5ug genomic DNA and nuclease free water	37
Total	100

STEP	TEMP	TIME
Initial Denaturation	98°C	30s

20 cycles	98°C	10s
	64°C	30s
	72°C	20s
Final Extension	72°C	2 minutes
Hold	4–10°C	

Following PCR, all reactions from a given sample were consolidated into a single tube.

Bead purification of the first PCR product of 564bp expected size was performed with 0.5x and 0.9x double sided SPRI bead ratios. Specifically, 25ul of
5 SPRIselect (Beckman, B23318) was added to 50ul first PCR product, mixed well by pipetting, and incubated at room temperature for 10min. Samples were placed on a magnetic stand for 5min. The supernatant was transferred to a new tube. 45ul SPRIselect was added to the transferred supernatant, mixed well by pipetting, and incubated at room temperature for 10min. Samples were placed on a magnetic stand for
10 5min. Supernatant was then removed. Beads were washed twice with 1ml fresh 80% ethanol over 2min incubations. Beads with bound DNA were air dried for 5-10min and eluted with 20ul elution buffer from the Machery Nagel kit.

A second PCR was performed to add sample barcodes and Illumina adapters with the following conditions:

Component	Volume (ul)
NEBNext 2x mix (M0541L)	50
DMSO (D9170-5VL)	2
Betaine (Sigma, B0300-1VL)	10
100uM FW primer (P5-DS2-FW)	0.5
100uM RV primer (RV primer P7-DS2-RV-1 to 12 for multiplexing onto MiSeq run)	0.5
1 st PCR bead purification product	5
Nuclease free water	32

Total	100
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STEP	TEMP	TIME
Initial Denaturation	98°C	30s
10 cycles	98°C	10s
	64°C	30s
	72°C	20s
Final Extension	72°C	2 minutes
Hold	4–10°C	

Bead purification of the second PCR product with 300bp expected size was performed with 0.7x and 1.2x double sided SPRI bead ratios. Specifically, 35ul of SPRIselect (Beckman, B23318) was added to 50ul first PCR product, mixed well by pipetting, and incubated at room temperature for 10min. Samples were placed on a magnetic stand for 5min. The supernatant was transferred to a new tube. 60ul SPRIselect was added to the transferred supernatant, mixed well by pipetting, and incubated at room temperature for 10min. Samples were placed on a magnetic stand for 5min. Supernatant was then removed. Beads were washed twice with 1ml fresh 80% ethanol over 2min incubations. Beads with bound DNA were air dried for 5-10min and eluted with 20ul elution buffer from the Machery Nagel kit.

Final bead purified 2nd PCR product was quantified by TapeStation High Sensitivity D1000 (Agilent) and multiplexed at equimolar ratio for sequencing on a MiSeq (Illumina). Using manufacturer's protocols, 15pM libraries were denatured and mixed with 2% PhiX control. DS2-EF1A-READ1 primer was spiked into position 12 of the MiSeq v3 cartridge (Illumina). Read 1 was set to 139 cycles and index reads was set to 6 cycles.

Data were demultiplexed using the fastq generation module and analyzed.

20 Primers

Name	Sequence
WPRE_R_CG	Catagcgtaaaaggagcaaca (SEQ ID NO:628)

EF1A_F_intron	Ccaggcacctcgattagttc (SEQ ID NO:2212)
DS2-EF1A-READ1	AAGTAAGcctgcaggAATTgCCTAGGgt (SEQ ID NO:2213)
P5-DS2-FW	aatgatacggcgaccaccgagatctacacAAGTAAGcctgcaggAATTgCCTAGGgt (SEQ ID NO:2214)
P7-DS2-RV_1	CAAGCAGAAGACGGCATAACGAGATCTTGTAGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2215)
P7-DS2-RV_2	CAAGCAGAAGACGGCATAACGAGATGCCAATGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2216)
P7-DS2-RV_3	CAAGCAGAAGACGGCATAACGAGATAGTTCCGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2217)
P7-DS2-RV_4	CAAGCAGAAGACGGCATAACGAGATTAGCTTGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2218)
P7-DS2-RV_5	CAAGCAGAAGACGGCATAACGAGATTTAGGCGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2219)
P7-DS2-RV_6	CAAGCAGAAGACGGCATAACGAGATATCACGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2220)
P7-DS2-RV_7	CAAGCAGAAGACGGCATAACGAGATGAGTGGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2221)
P7-DS2-RV_8	CAAGCAGAAGACGGCATAACGAGATAGTCAAGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2222)
P7-DS2-RV_9	CAAGCAGAAGACGGCATAACGAGATACAGTGGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2223)
P7-DS2-RV_10	CAAGCAGAAGACGGCATAACGAGATTGACCAAGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2224)
P7-DS2-RV_11	CAAGCAGAAGACGGCATAACGAGATCAGATCGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2225)
P7-DS2-RV_12	CAAGCAGAAGACGGCATAACGAGATGGCTACGTGAC TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGtctc gacctggcttactagtG (SEQ ID NO:2226)

EXAMPLE 6: EVALUATION OF miR BACKBONES IN AAV PLASMIDS

A subset of these miR backbones were subsequently evaluated in *cis* plasmids for AAV production. As described in Example 4 for AAV packaging of miR-16-2 backbone containing amiRNA vectors, *cis* plasmids containing an H1 promoter

(nucleotides 113-203 of SEQ ID NO:1522) and a stuffer sequence (“AMELY_ITR_Stuffer_V1” - nucleotides 348-2228 of SEQ ID NO:1522) and various miR backbones were used to package AAV, and then the uniformity of vector genomes produced was assessed by agarose gel electrophoresis. SEQ ID NO:1522 provides an example of such a sequence from 5’ ITR to 3’ ITR, where for each library element the plasmid would be as shown but with the bases denoted with ‘n’ in the miR backbone insert (nucleotides 204-341 of SEQ ID NO:1522) replaced by the appropriate 138-bp artificial miRNA sequence (backbone, guide, and passenger insert. **FIG. 35** shows the indicated set of AAVs, with indicated ATXN2 guide sequence (targeting position 4402 in *ATXN2* transcript, – SEQ ID NO:1279 (RNA)), and overall miR cassette sequences constructed from the rules in Table 8. Among the miR backbones assessed, miR-100 and miR-128 backbone-embedded miRs had more uniform gel patterns. To more generally assess the vector integrity of AAV containing different miR backbones, libraries of *cis* plasmids, each containing the complete set of ATXN2 targeting amiRNA guide sequences as in Deep Screen 2, were used to package AAV as before. The oligonucleotide amplification strategy used in this experiment does not distinguish between parent and “_M” forms of the miR backbones where both were originally present in the Deep Screen 2 library, so the libraries include mixtures of, for example, miR-100 and miR-100_M backbone containing elements; miR-1-1 and mir-1-1_M backbones. **FIG. 36** shows that, as with AAVs containing a miR-100 backbone and the specific guide sequence 4402 (SEQ ID NO:751 (DNA); SEQ ID NO:1279 (RNA)), AAVs derived from a library of miRs embedded in the miR-100 and miR-100_M backbones exhibit a more uniform gel electrophoresis pattern than AAVs with other miR backbones. Although the specific composition of the *cis* plasmid libraries was not assessed after packaging and confirmed to be consistent across libraries with different miR backbones, the simplest interpretation of this data is that on average, across a range of specific miRs, AAV vector genomes with a miR-100 backbone exhibit more uniform, full-length size, than other backbones.

Based on the combined properties of good knockdown performance and good AAV vector genome uniformity, miR-100 and the slightly modified miR-100_M

were prioritized as backbones for advancement. ‘Micropool’ plasmid libraries comprising amiRNAs inserted into unpackaged AAV cis plasmid scAAV_AMELY_V1_H1 (SEQ ID NO:1522; amiRNA insert located at nucleotides 204-341) were tested by transfecting plasmid library into HEK293T cells and harvesting small RNA. As above the oligonucleotide amplification strategy to construct the plasmid library did not distinguish between the miR100 and miR100_M backbones, and so the library represents a mix of both; however, given the similar performance overall of miRs from parent and _M form backbones, the mix of backbones in the library is unlikely to degrade the overall ability to detect precisely processed miRNAs. This small RNAseq data was integrated to evaluate processing precision of individual amiRNAs within the library, as in the below examples.

Methods

AAV micropool cloning

To clone micropools into the scAAV_AMELY_V1_H1 backbone (to yield plasmids as set forth in SEQ ID NO:1522), the backbone was first linearized by AarI digestion of a cloning site region and agarose gel purified.

Micropools were amplified using the following conditions, using miR-1-1 as an example. All miRNA backbone specific primer pairs are listed in the table below.

Component	Volume (ul)
NEBNext 2x mix (M0541L)	50
DMSO (D9170-5VL)	2
Betaine (Sigma, B0300-1VL)	10
100uM FW primer (miR-1-1-AAV-H1-AMELY-V1-AarI-FW)	0.5
100uM RV primer (miR-1-1-AAV-H1-AMELY-V1-AarI-RV)	0.5
1ng EF1A oligo pool from Twist and nuclease free water	37
Total	100

STEP	TEMP	TIME
Initial Denaturation	98°C	30s
20 cycles	98°C	10s
	64°C	30s
	72°C	15s
Final Extension	72°C	2 minutes
Hold	4–10°C	

Double sided bead purification with 0.7x SPRI beads and 1.2x SPRI beads ratios was used on the PCR product, which was in turn used as the insert in the HiFi assembly.

5 HiFi assembly of the pooled library was performed by assembling at 5 to 1 insert to backbone molar ratio. 15ul of 2x HiFi assembly master mix (NEB, E2621L) and 15ul of insert and backbone (about 0.375 pmol purified miR library insert to 0.075 pmol purified backbone) and incubating for 1hr at 50°C.

10 Assembled DNA was precipitated by adding 1ul of 20 mg/mL glycogen, one-tenth volume of 3M sodium acetate pH 5.5, and 2.2x volume of ethanol, mixed and stored overnight at -80°C.

Samples were spun at 16,000 xg for 15min at 4°C. Supernatant was removed and discarded. Pellets were washed twice with 1ml of 70% ice cold ethanol and let to dry, then dissolved in 4ul nuclease free water.

15 Purified DNA and 0.1 cm Gene Pulser Cuvettes (Bio-rad, 165-2089) were placed on ice for 10 min. 50ul of Lucigen Endura electrocompetent cells were thawed briefly on ice. 4ul of precipitated HiFi reaction was added to 25ul of electrocompetent cells, mixed, and transferred to the cuvette. DNA and cell mixes were electroporated with the following parameters: 1800 Volts, 10 uF, 600 Ohms, 0.1cm
20 cuvette. Cuvettes were immediately flushed 2X with 1 mL Lucigen recovery media. Cells were recovered at 37°C for 1 hour at 230rpm.

To titer the transformed bacteria, 2 uL of each culture was diluted into 200 uL of LB and plated 100 ul and 10ul of this 1:100 dilution onto LB agar plates plus appropriate antibiotic. The number of colonies were counted the next day to determine
25 total number of transformants.

Liquid cultures were inoculated into the appropriate amount of LB with antibiotic for maxi prep. Pooled plasmid libraries were prepared with a Qiagen Plasmid Maxi Kit following the manufacturer's instructions.

Primers

Name	Sequence
miR-1-1-AAV-H1-AMELY-V1-AarI-FW	TaagttctgtatgagaccacatgcagactgcctgctTGG (SEQ ID NO:2227)
miR-100-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacCCCAAAAGAGAGAAGATATT (SEQ ID NO:2228)
miR-124-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacTTCCTTCCTCAGGAGAAAGG (SEQ ID NO:2229)
miR-128-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacATTTtgcaataattggcctt (SEQ ID NO:2230)
miR-122-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacggctacagagtttCCTTAGC (SEQ ID NO:2231)
miR-130a-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacgcaggcgccggcatgcctcTG (SEQ ID NO:2232)
miR-132-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacGCCGTCCGCGCGCCCCGCC (SEQ ID NO:2233)
miR-138-2-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacggcgccggagttctggatC (SEQ ID NO:2234)
miR-144-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacTCAAGCCATGCTTCCTGTGC (SEQ ID NO:2235)
miR-155-M-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacCCTGGAGGCTTGCTGAAGGC (SEQ ID NO:2236)
miR-155E-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacCTGGAGGCTTGCTTTGGGCT (SEQ ID NO:2237)
miR-16-2-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacTTATGTTTGGATGAAGTAC (SEQ ID NO:2238)
miR-190a-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacGAGCTCAGTCAAACCTGGAT (SEQ ID NO:2239)
miR-223-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacTCCCCACAGAAGCTCTTGGC (SEQ ID NO:2240)
miR-451a-AAV-H1-AMELY-V1-AarI-FW	taagttctgtatgagaccacGCTCTCTGCTCAGCCTGTCA (SEQ ID NO:2241)
miR-1-1-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaggccccgtgtgtggagtg (SEQ ID NO:2242)
miR-100-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaGGCATATAAGCAAAGCCCCA (SEQ ID NO:2243)
miR-124-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaatcctTGCGGGGCCCTCGCCG (SEQ ID NO:2244)
miR-128-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaagcagtggaaacctgagtaa (SEQ ID NO:2245)
miR-122-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaacaagcaaacgatccaaga (SEQ ID NO:2246)
miR-130a-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaacaatgctgaggaggcagcca (SEQ ID NO:2247)
miR-132-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaGGCTCGGGGCGCGCGCTGGC (SEQ ID NO:2248)
miR-138-2-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaCCggtccacgaggctcgcc (SEQ ID NO:2249)

miR-144-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaatgtccTCCTTGTCAGGCTCC (SEQ ID NO:2250)
miR-155-M-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaCCGGTGGCCATTGTTCCAT (SEQ ID NO:2251)
miR-155E-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaCCCACGGTGGCCATTGTTC (SEQ ID NO:2252)
miR-16-2-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaAAACAATTGATAAAATAGTT (SEQ ID NO:2253)
miR-190a-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaCTTTATTAGGAACCCCGGA (SEQ ID NO:2254)
miR-223-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaGGCCTAGAGCTGGTAAGCAT (SEQ ID NO:2255)
miR-451a-AAV-H1-AMELY-V1-AarI-RV	TATGTGATATGCATAATAaaaaaaCTGAGTTCTCTTCC TGGCAC (SEQ ID NO:2256)

Pooled AAV production

AAV micropools served as cis-plasmids to package with Ad helper and AAV9 RepCap using standard three plasmid AAV packaging methods at Vector

5 BioLabs.

Crude lysate processing and gel visualization

To extract vector genomes, crude lysates underwent 4 freeze thaw cycles (37°C and dry ice-ethanol bath) and were passed through a 0.45µm filter (Chemglass, CLS-2005-017). Each 100µl of passthrough was treated with 2µl DNase 1 (NEB, M0303L) and 0.2µl RNase A (ThermoScientific, EN0531) for 30min at 37°C. Vector genomes were extracted with the Quick Viral DNA kit (Zymo, D3015). 1.5% agarose gels with either SYBRsafe or SYBRgold to stain DNA were used for visualization.

10

Pooled expression of micropools for small RNAseq

Micropools of miR100 and miR100_M backbone miRs, embedded in the plasmid scAAV_AMELY_V1_H1, were transfected into HEK293 cells using a lipid based method (Lipofectamine LTX, ThermoFisher) in cells grown in 6 well plates. 600,000 cells were seeded per well and were transfected the following day in duplicate, with 2.5 micrograms of micropool library transfected per well. Media was changed at day 2 and collected in Trizol at day 3. Total RNA was extracted by chloroform phase separation and purification by Zymo Direct-zol column elution using manufacturer's protocols.

15

20

Small RNAseq

Small RNAseq libraries were prepared using the Nextflex v3 small RNA seq kit (Bioo Scientific Corp, NOVA-5132-05). Briefly, library preparation was initiated with 0.5-2ug of RNA input. 14-18 cycles of PCR were performed for each sample. Two rounds of double-sided bead cleanup were performed prior to pooling samples based on Tapestation High Sensitivity D1000 quantitation of the 150bp band. Illumina adapted libraries were multiplexed and loaded onto a MiSeq (Illumina), loading the library at 9pM with 10% phiX on a MiSeq v3 kit and with read 1 set to 75 cycles and index set to 6 cycles.

10 **EXAMPLE 7: RANKING OF TOP ARTIFICIAL MIRNAS EMBEDDED IN miR-100 AND miR-100_M BACKBONES**

Top amiRNAs embedded in miR-100 and miR-100_M backbones were ranked by knockdown performance in Deep Screen 2; by guide to passenger ratio; and by minimal depletion at late (T₁, 18 day) versus early (T₀) timepoints (dropout). (Noting, as above, that the guide:passenger ratios are from a small RNAseq library including a mix of miR100 and miR100_M backbones). Additionally, the set of potential off-target transcripts with 1 or 2 bp mismatches was assessed for each ranked candidate. After eliminating candidates with low guide:passenger ratios, low T₁/T₀ ratios, and candidates with CNS expressed transcripts with near-complementarity of only 1 bp mismatch, a set of 9 active miRNAs, and 2 911 control miRNAs, were cloned into *cis* plasmids downstream of an H1 promoter, and packaged with a Rep/Cap helper plasmid encoding for AAV-DJ capsid components. Data from Deep Screen 2 (**FIG. 37**) and small RNAseq profiling for these candidates are listed in **Table 25**. For these selected hits, the mean of replicate 1 and replicate 2 T₁/T₀ log₂ ratios were all within 1 standard deviation (0.22) of the median (-0.07) log₂ ratio of miR100 and miR100_M amiRNAs targeting ATXN2.

Table 25: Data from Deep Screen 2 and small RNAseq profiling

Position	Rep. 1 lo10/ unsort log2FC	Rep. 2 lo10/ unsort log2FC	Rep. 1 T ₁ /T ₀ log2FC	Rep. 2 T ₁ /T ₀ log2FC	miR_with_ suffix	Guide Sequences (DNA)	Guide: Passenger ratio
1755	-2.70	-2.64	0.04	-0.12	miR-100	TCGGGTTGAAATCTGAAGTGTG [SEQ ID NO:657]	161
2586	-2.40	-2.24	-0.28	-0.08	miR-100	TAGATTTCAGAAAGTAGAACTTGG [SEQ ID NO:1621]	390
2945	-2.61	-2.17	0.10	-0.31	miR-100	TGTAGTAGAAGGCTTTGGCTGA [SEQ ID NO:685]	>778:0
3133	-2.66	-2.33	0.21	0.27	miR-100_M	TATGTCTTGGCTTGATTCACTG [SEQ ID NO:1624]	78
3270	-2.09	-1.46	0.05	0.03	miR-100	TACTGTAGGCAACATATTGCGT [SEQ ID NO:2080]	>334:0
3301	-2.52	-2.09	-0.19	-0.27	miR-100_M	TGAACAAAGGGGCTGATTGGGA [SEQ ID NO:687]	92
3302	-2.89	-2.36	-0.09	-0.30	miR-100_M	TTGAACAAGGGGCTGATTGGG [SEQ ID NO:688]	41
3330	-2.18	-2.12	0.30	-0.06	miR-100	TATGCTGAGACTGATAATGTGG [SEQ ID NO:1614]	39
3338	-1.91	-2.25	0.03	-0.15	miR-100	TACATGAGGATGCTGAGACTGA [SEQ ID NO:1620]	212

The above miRNAs as well as 911 controls for 1755 (guide sequence SEQ ID NO:1185) and 2945 (guide sequence SEQ ID NO:1213) were tested for knockdown of *ATXN2* in stem-cell derived motor neurons. amiRNAs were packaged in cis plasmids to generate self-complementary AAV-DJ vectors containing a long H1 promoter (nucleotides 113-343 of SEQ ID NO:2257), and a stuffer sequence “PSG11_V5” (nucleotides 489-2185 of SEQ ID NO:2257). Sequences for vectors encoding amiRNAs miR100_1755 (SEQ ID NO:1915), miR100_2586 (SEQ ID NO:1982), miR100_2945 (SEQ ID NO:1965), and miR100_3330 (SEQ ID NO:2021) from 5' ITR to 3' ITR are provided in SEQ ID NO:2257, SEQ ID NO:2258, SEQ ID NO:2259, and SEQ ID NO:2260, respectively. After titering each vector, and based on hemacytometer based quantification of number of cells plated, vectors were added at intended doses of 3.16E3 and 3.16E4 vector genomes per cell. 7 days after addition of vectors, neurons were harvested and RNA isolated with miRNeasy Tissue/Cells Advanced Mini Kit (Qiagen, P/N 217604) *ATXN2* knockdown was assessed by digital droplet RT-PCR, measuring the ratio of *ATXN2* expression to housekeeping controls *GUSB* and *B2M*.

FIG. 38 shows individual data points, and **Table 26** shows mean and standard deviation of knockdown across these constructs, at the two doses of 3.16E3 vg/cell and 3.16E4 vg/cell, normalized to *ATXN2* expression values from untransduced cells, which were treated with an equivalent volume of AAV diluent.

Table 26: *ATXN2* Knockdown by amiRNAs in stem-cell derived motor neurons at two different doses

miR	mean <i>ATXN2</i> 3160	SD	N	mean <i>ATXN2</i> 31600	SD	N
miR100_1755	26.8	1.7	6	14.0	2.1	6
miR100_2586	41.8	2.9	6	29.3	1.0	6
miR100_2945	36.7	1.7	6	26.8	2.6	6
miR100_3270	72.1	5.1	6	47.9	2.2	6
miR100_3330	44.1	6.3	6	32.2	2.7	6
miR100_3338	36.5	3.6	6	23.6	5.5	6
miR100_M_3133	30.6	1.0	5	23.8	1.5	6
miR100_M_3301	83.7	9.1	6	49.1	3.7	6
miR100_M_3302	38.0	3.0	6	32.3	3.3	6

Untransduced	100	9.5	12			
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Dose response studies

The candidates AAVs were also tested at a more extensive range of doses in motor neurons. As before, RNA was isolated from the cultures after 7 days of culture, and *ATXN2* knockdown assessed. **FIG. 39** shows plots of knockdown across different concentrations of each vector added. Concentration of *ATXN2* mRNA, normalized for each data point by B2M expression, and collectively to the *ATXN2* expression level in neurons treated with vehicle (PBS + .001% PF-68) was measured by digital droplet RT-ddPCR. By examination, differences in potencies of amiRNAs can be observed; for example miR100_1755 exhibits knockdown at lower vector genome exposures than other amiRs; mir100_3301 and miR100_3270 appear to exhibit reduced potency relative to other vectors.

Neurons dosed at 3.16E3 vector genomes per cell were additionally subject to small RNA sequencing. **Table 27** shows the abundance of the amiRNA, as a fraction of total miRNA. There was a surprising range of expression levels, and several amiRs (1755, 2586, 2945, and 3270) had considerably less amiRNA detected than other amiRNAs.

For these small RNA experiments, reads were not ‘deduplicated’ (by eliminating reads with identical flanking 5’ and 3’ 4-mer random adapters) as in small RNA analysis for deep screen 1 libraries, because the number of reads of the artificial miRNAs in some cases approached the number of unique combinations of nucleotides in the adapters.

Table 27: Abundance of amiRNA, as a fraction of total miRNA

Guide	Rep1 amiRNA/total miRNA (%)	Rep2 amiRNA/total miRNA (%)
mir100_1755	1.80	2.77
mir100_2586	2.53	2.73
mir100_2945	3.20	3.30
mir100_3270	1.21	1.43
mir100_3330	16.83	17.75
mir100_3338	23.84	25.00

mir100M_3133	22.82	22.57
mir100M_3301	22.07	17.53
mir100M_3302	38.04	36.78

To assess whether AAV amiRNA treatment had any obvious impact on neuronal morphology or cell counts, neurons grown in 96-well format were treated with AAV or vehicle at a dose of 1E4 vector genomes/cell, and 7 days later fixed and stained with Hoechst, anti-Isl1, and anti-Beta3 tubulin antibodies to visualize nuclei, a motor neuron marker, and neuronal processes respectively. **FIG. 40** shows representative images from cultures treated with indicated amiRNA AAVs and controls, demonstrating that no AAV miRNA exhibited obvious impacts on neuronal morphology. **FIG. 41A** shows zoomed in images comparing miR100_1755 and miR100_1755_911 (a 911 control, rendered inactive for slicing Atxn2 by complementing bases 9, 10 and 11 of the 1755 amiRNA). No obvious differences can be seen, suggesting that Atxn2 knockdown does not cause dramatic changes in neuronal process or nuclear morphology. Panels on right quantify the total number of Hoechst+ nuclei (**FIG. 41B**) and the % of total nuclei that are Isl1+ (**FIG. 41C**). Compared to vehicle-treated (PBS + .001% PF-68) wells, significant differences ($p < .05$) were observed for a few of the AAV-amiRNA treatments, with a reduction in total number of nuclei per field. However, one of these treatments (miR100_1755) were also showed with a significant increase in the fraction of cells that were Isl1+, and an apparent trend toward increasing Isl+ neurons was apparent for other AAV-DJ amiRNAs, arguing against any alteration in total motor neuron numbers. There were no significant differences between neurons transduced with any of the active AAV amiRNAs and the inactive 911 control AAV amiRNAs.

RNAseq studies

RNA was collected from motor neurons 7 days after dosing with 1E4 vector genomes/cell of the above AAVs. There were 6 replicates per condition, except miR100_1755_911, which had 5. To determine if ATXN2 knockdown from AAV expression impacts the transcriptome in neurons, RNA expression was compared

between neurons transduced with active amiRNA-expressing vectors and vectors expressing a cognate 911 control. **FIG. 42** shows ‘volcano plots’ of differential expression for miR100_1755 vs. miR100_1755_911 and miR100_2945 and miR100_2945_911. A large separation can be seen in nominal p-values for the differential expression calculated for *ATXN2* versus all other genes. Remarkably, after adjustment of nominal p values for multiple comparisons using the Benjamini-Hochberg procedure, only *ATXN2* or one other gene exceeded a 10% false discovery rate threshold for 1755 and 2945, respectively.

To further investigate whether there was any impact on any of the predicted off-target genes (the set of transcripts with 2 or fewer mismatches to bases 2 – 18 of each amiRNA), each amiRNA was compared to data from all other active amiRNAs (**FIG. 43**). For this set of selected amiRNAs, few of the predicted off-targets exceed the 10% false discovery rate threshold. This suggests that these amiRNAs yield specific knockdown of *ATXN2*.

Methods

ddPCR AAV titering

To titer AAVs, each vector was serially diluted in Salmon Sperm DNA solution (20 ng/ul Salmon Sperm DNA, 0.001% PF-68, 10 mM Tris-HCl pH 7.5, 50 mM KCl, 1.5 mM MgCl₂) and subsequently heated at 95°C for 10 minutes to release the vector genome from the AAV9 capsid. After an incubation with SmaI to reduce secondary structure, known to inhibit the rAAV PCR reactions, (NEB, R0141L), droplets were generated using DG32 Automated Droplet Generator (Bio-Rad), followed by a PCR amplification with vector-specific primer/probe sets. Once complete, droplets were analyzed using QX200 Droplet Digital PCR System (Bio-Rad), and positive and negative populations were defined, and the dilution factor applied to determine the concentration of the undiluted vector stock.

Motor neuron immunocytochemistry

Motor neuron cultures were fixed in 4% Paraformaldehyde for 10 minutes at room temperature. Fixed cultures were permeabilized and blocked in PBS

- containing 0.2% Triton-X-100 and 10% donkey serum solution for 45 minutes at room temperature. Cells were then incubated in blocking solution (10% donkey serum in 0.1% Tween-PBS) containing primary antibody overnight at 4C. Cells were washed 3 times with PBS-0.1% Tween and then incubated in blocking solution containing
- 5 secondary antibodies for 1-2 hours at room temperature followed by 3 washes with PBS-0.1% Tween and a rinse with a PBS solution containing Dapi. Stained cultures were imaged on the Perkin Elmer Operetta high content imager with 20x water objective. 40-60 fields were imaged for every well. Cell quantifications were done using the Perkin Elmer Harmony software. Statistical analysis was done using
- 10 GraphPad Prism software. Primary antibodies used: TUJ1 (1:500 dilution) ISL1 (1:200 dilution) secondary antibodies: AlexaFluor 488 and AlexFluor 647 (1:500 dilutions).

Reagent	Vendor	Cat. No.
32% Paraformaldehyde	Fisher Scientific	50-980-495
Triton X-100	Sigma	T8787-100ML
Tween 20	Sigma	P1379-100ML
Donkey Serum	Jackson Immuno Research	017-000-121
Donkey anti-chicken secondary antibody-Alexa fluor 647	Jackson Immuno Research	703-606-155
ISL1 antibody	Abcam	ab109517
TUJ1 antibody	Abcam	ab41489
Donkey anti-rabbit secondary antibody-Alexa fluor 488	ThermoFisher	A32790
DAPI solution	ThermoFisher	62248
Phosphate-Buffered Saline (PBS) pH 7.4	ThermoFisher	10010023

Off-target prediction

- 15 To generate a set of predicted off-targets, bases 2 – 18 of amiRNAs were aligned to the human transcriptome using bowtie commands:
- bowtie -n 2 -l 17 -e 81 -seed [pseudorandom number to enforce reproducibility] –nomaqround –tryhard –chunkmbs 256 –all –time (and additional commands for input/output handling). To ensure that only 2 or fewer mismatches occurred, fastq file
- 20 inputs to the bowtie alignment containing amiRNAs to be tested were constructed in which each amiRNA was given a phred score ‘mask’ of IHHHHHHHHHHHH, such that alignments of the amiRNA with transcripts where more than 2 mismatches occurred

would exceed the weight threshold. The amiRNAs were aligned to the build
Homo_sapiens.GRCh38.cdna.all,
Macaca_fascicularis.Macaca_fascicularis_5.0.cdna.all, or
Mus_musculus.GRCm38.cdna.all.

5

RNAseq

Stem-cell derived motor neuron cultures were plated at a density of 200,000
cells per well of 6-well plates. 6 days after plating, cells were transduced with AAV
vectors at a dose of 10,000 vector genomes (calculated by titering method described
10 above) per cell. 7 days later, cells were harvested for RNA. Lysis of transduced
samples was conducted by addition of 300ul of Buffer RLT Plus, followed by overnight
freeze at -80. Samples were thawed on ice and processed according to the remainder of
the RNeasy Plus standard protocol. (Qiagen RNeasy Plus Micro Kit (Catalog 74034)),
according to manufacturer's instructions. All purified RNA samples were quantified by
15 Qubit (using RNA HS standard). A selection of samples with low, mid, and high RNA
concentrations (16 in total) were further characterized by Tapestation (High Sensitivity
RNA) to check purity (RINe score) and verify Qubit quantification. All RINe scores
were in the 9.9-10 range, near maximal.

Purified RNAs were then used as input into QuantSeq [Lexogen catalog #015
20 (QuantSeq 3' mRNA-Seq Library Prep Kit for Illumina (FWD))]. Target RNA input
was 100ng per reaction (for lower concentration samples, the maximum input volume
of 5ul was used). The standard Quantseq protocol was followed with the following
modifications: (1) UMI addition at step 7 using the "UMI Second Strand Synthesis
Module" (Lexogen Cat. No. 081). (2) 20 cycles for library amplification. Resulting
25 libraries were quantified by Qubit (DNA HS) and QC spot-checked on Tapestation (HS
D5000). Libraries were pooled based on Qubit quantifications and sequenced on an
Illumin NovaSeq (Seqmatic). Sequencing parameters were as follows: NovaSeq S1
run, single-read 100bp, single index 6bp.

30

RNAseq analysis

To analyze RNAseq data, SeqTK was used to split each of the single-end reads obtained from each sample into fastq files containing the UMI and read sequence, respectively:

```
5          seqtk trimfq -b 10 raw.fastq > sequence.fastq
          seqtk trimfq -e [readsize - 6] raw.fastq > umi.fastq
```

The resulting sequences were then pseudoaligned with kallisto version 0.46.0 (Bray et al., Nature Biotechnology 2016 34: 525-527). in batch mode to a transcriptome assembly derived from the the trailing 600 bp of all cdnas present in Ensembl release 96 (kmer
10 length = 19) using the following command:

```
          kallisto pseudo --umi --quant --single -t 8 -i [kallisto_index] -o
[output_file] -          b [batch_file.txt]
```

Aligning reads were summed across all transcripts annotated to each gene to generate gene-level count matrices. Genes with five or more counts observed in all replicates of at
15 least one experimental condition were considered in downstream analyses. Sample read counts were converted to base-2-log(CPM) and normalized via TMM (edgeR::calcNormFactors) prior to probability weight estimation via limma::voom. (Law et al., Genome Biology (2014) 15:R29). Evidence for differential expression was quantified by fitting a genewise linear model on the normalized expression values, with
20 fold changes extracted from the model coefficients and associated *P*-values estimated using a Wald test. Genewise *P*-values were corrected for multiple testing using the FDR approach.

EXAMPLE 8: *In Vivo* TESTING OF CANDIDATE AMIRNAS IN WILD-TYPE MOUSE.

Two additional studies of *in vivo* performance of amiRNAs embedded in
25 self-complementary AAV9 vectors were conducted. In a first study, amiRNA 1784 and 3330, in the miR1-1 or miR100 backbone, respectively, were tested in a variety of vector genomes containing different promoters and stuffers. The specific miR cassettes used for *in vivo* testing are provided in **Table 28**.

Table 28: Specific miR cassettes used *in vivo*

Cassette	Sequence
miR1-1.1784	CATGCAGACTGCCTGCTTGGGTACAGACCAAAGAGTA GTCGAATTATGGACCTGCTAAGCTAATTAATACTACTCTT TGGTCTGAACTCAGGCCGGGACCTCTCTCGCCGCACTG AGGGGCACTCCACACCACGGGGGCC (SEQ ID NO:538)
miR100.3330	CCCAAAGAGAGAAGATATTGAGGCCTGTTGCCACAT ATGCTGAGACTGATAATGTGGGTATTAGTCCGCCACAT CATCCGTCTCAACATTTGTGTCTGTTAGGCAATCTCAC GGACCTGGGGCTTTGCTTATATGCC (SEQ ID NO:2019)

The vector designs, including specific promoter and stuffer, are described separately.

- 5 Here the performance of the amiRNAs is compared in several overall vector formats and promoters. AAV was dosed to wild-type mice either intravenously (dose: 3.21E9 vg/gram mouse) or by intrastriatal injection (dose: 7.5E9 vg total).

Table 29 shows mean *ATXN2* knockdown as assessed in liver 3 weeks after intravenous dosing, relative to animals dosed with vehicle (PBS with 0.001% PF-68). *Atxn2* expression was assessed by digital droplet RT-PCR, and knockdown was taken as the mean of *Atxn2/Hprt* and *Atxn2/Gusb* ratios, as measured by ddPCR.

Table 29: ATXN2 Knock-down in Liver After I.V. amiRNA Dosing

miR	mean <i>Atxn2</i> (% Vehicle injected)	SD	N
miR1-1.1784	10.8	6.5	12
miR100.3330	14.7	4.7	12

- 15 For striatal samples, vector biodistribution after collection of punch biopsies was more variable from sample to sample. Vector distribution was assessed by digital droplet PCR, measuring the relevant number of droplets amplifying for primer/probesets recognizing the AAV vector genome versus primer/probesets recognizing the Tert gene in the mouse genome. Because there are a fixed number of
- 20 copies of the Tert gene per cell (2), the number of vector genomes per cell (diploid genome) can be measured in this way. By assessing AAV vector distribution in the

same biopsies as *ATXN2* mRNA was quantified, a clear dose response trend can be seen (FIGS. 44, 45A-45B). It should be noted that the amount of nuclear vector genomes versus cytoplasmic or extracellular vector was not assessed, such as by histological methods; it is possible that vector introduced by intraparenchymal injections may accumulate extracellularly. Nonetheless, the clear dose response shows that even if not all of the vector genomes measured are in the nucleus, available to express the amiRNA, there is a clear correlation between any such total vector genome exposure and functionally active vector genomes.

To determine the relationship between amiRNA expressed and knockdown, amiRNA was quantified in two ways. First, libraries using TaqMan Advanced miRNA cDNA Synthesis Kit (Thermo, P/N A28007) were generated for all striatal punch biopsy samples, using RNA isolated with a kit which enriches for small RNAs (Qiagen, P/N 217604). To generate a cDNA library for TaqMan qPCR, 3' poly-A tailing is first complete, then 5' ligation to add on an adaptor. After reverse transcription, the cDNA is PCR amplified for 14 cycles, then a dilution of the final amplification product is subject to qPCR with primer probe sets specific to exogenous and endogenous miRNAs. Primer/probesets designed to target exogenous amiRNAs were used (Thermo), as well as primer/probesets targeting endogenous miRNAs miR-21a-5p (Thermo, P/N mmu482709_mir) and miR-124-3p (Thermo, P/N mmu480901_mir) as controls. The abundance of miRNA is assessed by the qPCR cycle number at which target amplification occurs. Comparing the qPCR cycle where amplification occurs (CT) between primer/probesets targeting different miRNAs allows assessing the relative abundance of miRNAs.

FIGS. 45A-45B plots the difference in CT value between amiRNA and endogenous control, as well as the difference between two endogenous miRNAs (miR-21 and miR-124), against the vector biodistribution in the same sample. As can be seen, there is no obvious change in the difference in CT thresholds between endogenous miRNAs with increasing detection of AAV vector genome. By contrast, there is what appears to be a log-linear relationship between the expected increase in the CT

separation between the amiRNA and endogenous miRNA and vector exposure, consistent with greater amiRNA expression with increased exposure to AAV.

For a subset of samples, small RNAseq was additionally conducted. As above, amiRNA expression normalized by total miRNA expression was quantified for each sample. Since for these samples amiRNA expression was quantified both by small RNAseq and qPCR, a model could be fit to establish how qPCR predicts amiRNA expression as a function of total miRNA. Therefore a linear model was fit (**FIG. 46**), with good explanation of the variance ($R^2 > .89$) for both amiRNAs.

Using this model, the qPCR-assessed amiRNA expression values for miR100_3330 and miR1.1.1784 in all samples could be converted to an absolute scale, of amiRNA/total miRNA. Plotting *ATXN2* mRNA in striatal biopsies versus this metric of predicted amiRNA expression, there was considerably greater knockdown per miRNA expressed in samples expressing the miR100-3330 amiRNA versus samples expressing the miR1.1.1784 amiRNA (**FIG. 47**). Therefore, although as a function of vector dosed, more knockdown was induced by vectors expressing miR1.1.1784, as a function of amiRNA expressed, more knockdown was induced by miR100.3330. This suggests that *in vivo* the potency of the approximately 22 nucleotide final product of pri-miRNA processing is higher for miR100.3330 than for miR1.1.1784.

In a second study, self-complementary vectors expressing amiRNAs miR100_1755 (SEQ ID NO:1915), miR100_2945 (SEQ ID NO:1965), miR100_3330 (SEQ ID NO:2021), and miR100_2586 (SEQ ID NO:1982) were packaged in AAV9 with a cis plasmid as described above containing a stuffer sequence "PSG11_V5" (nucleotides 489-2185 of SEQ ID NO:2257), a long H1 promoter (nucleotides 113-343 of SEQ ID NO:2257) and dosed intravenously or intrastrially in adult wild-type mice. 5' ITR to 3' ITR sequences for these vectors, as described in Example 7, are provided in SEQ ID NO:2257 (scAAV_H1_long_miR100_1755_PSG11_V5_ITR_to_ITR.gb), SEQ ID NO:2258 (scAAV_H1_long_miR100_2586_PSG11_V5_ITR_to_ITR.gb), SEQ ID NO:2259 (scAAV_H1_long_miR100_2945_PSG11_V5_ITR_to_ITR.gb), and SEQ ID NO:2260 (scAAV_H1_long_miR100_3330_PSG11_V5_ITR_to_ITR.gb). Because the mouse *Atxn2* transcript has several mismatches to 2586, no knockdown of mouse *Atxn2* transcript is expected.

During the intravenous study, 4 animals were dosed per group for a 3-week study. There were no clinical observations noted during weekly observation. For ALT and AST analysis, blood was collected via submandibular vein into serum tubes and allowed to clot for 30 minutes. Samples were centrifuged at 12,000 rpm for 5 minutes at 4°C. Serum was collected into clean Eppendorf tubes and stored at -80°C until further analysis at IDEXX. Results were reported as AST (U/L) and ALT (U/L). **FIG. 48** shows liver enzyme data at 2 and 3 weeks post-dosing. All ALT and AST values were within normal ranges at these timepoints.

During the intrastriatal study, 6 animals were dosed 4 microliters per striatum per group for a 3-week study. There were no group wide clinical observations noted for 7 days following injection and during weekly observation and there were no unscheduled deaths. For one cage dosed with miR100-2586, fighting was observed but the bully was separated, and all animals completed the study.

Knockdown performance of vectors was tested in liver. **Table 30** quantifies remaining *Atxn2*, normalizing *Atxn2* to two different control genes (*Hprt* and *Gusb*) and further normalized to *Atxn2* expression levels in naïve animals. From the same liver samples, as above biodistribution was measured. Samples treated with different vectors had highly similar exposures in liver.

Table 30: Atxn2 knockdown of amiRNA containing vectors in liver

Treatment	Mean % Control <i>Atxn2</i> mRNA	mRNA SD	Mean Biodistribution (VG/DG)	SD (VG/DG)	N
miR100_1755	22.1	3.3	5.93	1.70	4
miR100_2945	29.3	5.0	5.77	0.50	4
miR100_3330	27.9	0.8	5.71	0.36	4
None	100.0	10.5	0.01	0.01	4

Knockdown performance of these vectors was further assessed in brain after intrastriatal injections. As in the study described above DNA, mRNA and small RNA were isolated from punch biopsies in order to simultaneously monitor vector biodistribution, *Atxn2* knockdown, and amiRNA expression. Although in this *in vivo*

study exposure levels were lower than in the above study with miR1.1.1784 and miR100_3330, for unknown reasons, a clear dose response is visible (**FIG. 49**).

amiRNA expression versus total miRNA expression was assessed in a subset of samples in both liver and striatal punch biopsies. **FIG. 50** shows, for each tissue, vector biodistribution-normalized miRNA expression. In both tissues, miR100_1755 has the lowest miRNA expression, followed by miR100_2945, miR100_3330, and lastly miR100_2586.

Guide processing precision was also assessed *in vivo*, by counting reads that initiated at each position of the guide and predicted passenger sequences. **FIGS. 51, and 52A-51D** shows the count of reads aligning to the miR at the start position, where +0 is the expected typical cut position. **Table 31** quantifies the proportion out of all reads (including all guide and passenger strand reads) initiating at the +0 and +1 positions for each amiRNA. **FIGS. 52A-52D** show the read counts for the top 20 most common sequences for miR100_1755, miR100_2586, miR100_2945 and miR100_3330. Interestingly, by comparing the observed reads versus the pri-miRNA sequence, post-translational modifications such as 3' monoadenylation or monouridylation can be noted. Since 3' mismatches to the target transcript have in some cases been reported to increase the knockdown performance of miRNAs (Becker et al., Molecular Cell (2019) 75:741-755), it may be the case that this 3' modification of these amiRs may contribute to the high knockdown performance of Atxn2.

Table 31: Proportion of amiRNAs Initiating at Position +0 or +1

amiRNA	Proportion cut at +0	Proportion cut at +1
mir100_1755	97.67	1.07
mir100_2586	98.51	0.99
mir100_2945	98.34	0.94
mir100_3330	97.08	2.07

Table 32 quantifies the ratio of guide to passenger strand reads. The ratio of reads detected from guide versus passenger strand for all of these miR100 backbone amiRs

exceeded 300:1 *in vivo*. This high processing ratio may reduce the likelihood of off-target effects.

Table 32: Ratio of guide to passenger strand reads

amiRNA	Ratio guide:passenger
mir100_1755	399.8
mir100_2586	1061.7
mir100_2945	651.3
mir100_3330	508.3

5

Methods

For intravenous injection, vector was diluted in PBS with 0.001% PF-68 at 3.21E9vg/10 microliters, and mice were injected via tail vein based on weight (average total dose of 8.5E10 VG). Mice are placed in a restrainer and the tail is

10 swabbed with a sterile alcohol wipe to increase vein visibility. Once a lateral tail vein is located, a 32-gauge insulin syringe is used to administer the solution. 3-weeks post-injection, mice were fasted for 4 hours, blood collected via vena cava and serum processed for AST and ALT analysis. Following PBS perfusion, liver was cut into

15 snap frozen in liquid nitrogen. For tissue homogenization, Buffer RLT supplemented with beta-Mercaptoethanol was added to the sample and a Precellys Cryolys Evolution (Bertin Instruments) with program setting 3 x 45s at 5000 rpm with 15s pauses was performed. Samples tubes were centrifuged at 18,000 x g for 3 minutes and a fraction of the homogenate was used for DNA, RNA, and protein purification using the AllPrep

20 DNA/RNA/Protein Mini Kit (Qiagen, P/N 80004) and the other fraction of the homogenate was used for small RNA purification using the miRNeasy Tissue/Cells Advanced Mini Kit (Qiagen, P/N 217604).

For intrastriatal injections, vector was diluted in PBS with 0.001% PF-68 at 7.5E9vg/4 microliters, and mice were injected at coordinates (relative to Bregma) 1.5

25 mm anterior, +/- 1.6 mm lateral, and -4.0 mm ventral with 4 uL per hemisphere (Hamilton P/N 7635-01) over 5 minutes. After 3-weeks post-injection, mice were perfused transcardially with cold PBS and the brain placed in a matrix (CellPoint

Scientific, Alto Acrylic 1mm Mouse Brain Coronal 40-75gm), and a 2 mm coronal section containing the injection site was excised. Within the coronal section, a 2 mm punch biopsy of both the left and right striatum was collected and placed into separate homogenizing tubes (Precellys, P/N P000933-LYSK0-A) then snap frozen in liquid nitrogen. For tissue homogenization, Buffer RLT supplemented with beta-Mercaptoethanol was added to the sample and homogenization with a Precellys Cryolys Evolution (Bertin Instruments) with program setting 3 x 45 second at 5000 rpm with 15 second pauses was performed. Samples tubes were centrifuged at 18,000 x g for 3 minutes and a fraction of the homogenate was used for DNA, RNA, and protein purification using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, P/N 80004) and the other fraction of the homogenate was used for small RNA purification using the miRNeasy Tissue/Cells Advanced Mini Kit (Qiagen, P/N 217604).

EXAMPLE 9: PHARMACOLOGY STUDY OF AAV VECTOR EXPRESSED AMIRNA
TARGETING ATXN2 IN NON-HUMAN PRIMATES

Testing in non-human primates is conducted to establish knockdown of ATXN2 by ATXN2-targeting amiRNA rAAV vectors in tissues relevant to neurodegenerative disease, via clinically relevant routes of administration. Tissues to be assessed include spinal cord ventral horn, motor cortex, and cerebellum, which are relevant to neurodegenerative diseases such as ALS or Spinocerebellar ataxia-2.

In non-human primates, test articles (1×10^{12} - 1×10^{14} vg of amiRNA expressed with a H1 promoter and packaged in AAV9) or vehicle are administered into the cisterna magna by intrathecal cervical (IT-C) catheter. Male and female cynomolgus monkeys (*Macaca fascicularis*) of approximately 2.5-4 kg body weight, are implanted with an intrathecal cervical catheter for dose administration and sample collection. Test articles are administered (4 animals per test article) comprising a single 2.5 mL dose of vehicle or test article via the implanted intrathecal catheter at a rate of 0.3 mL/minute, followed by 0.1 mL of vehicle to flush the dose from the catheter. At 5 to 26 weeks following the administration, animals are sacrificed, and selected tissues harvested for bioanalytical and histological evaluation. ATXN2 protein and mRNA levels are

assessed for suppression after treatment with ATXN2 amiRNA packaged in AAV9 with a H1 promoter, relative to the vehicle group.

Vector Assessment

5 Test articles for dosing in non-human primates are assessed by multiple assays. One assessment is analytical ultracentrifugation (AUC) for empty and full capsids and quantification of aggregates. Absorbance scans are collected as the material sediments under the force of a gravitational field. Sample sedimentation profile is monitored in real time during centrifugation, which gives an absolute measurement of
10 molecule size and shape. The distribution movement over time is used to calculate the sedimentation coefficient. Fitting the raw data to the Lamm equation results in a continuous distribution, and area under each peak is proportional to the amount present in solution. Empty capsids are expected to sediment at 65S, partial capsids between 65 and 95S, full capsids at 95S, and aggregates at >110S. Measurements indicating
15 majority full capsids are desirable.

AAV9 capsid ELISA is used to assess intact AAV9 capsids. The capture-antibody detects a conformational epitope that is not present on unassembled capsid proteins. The ADK9 antibody is used as capture and detection antibody in the AAV9 titration ELISA. Assay results are expected to corroborate AUC assessment, by
20 comparing AAV9 capsid ELISA with vector genome titers.

Endotoxin is assessed by Limulus amoebocyte lysate (LAL). Detection and quantification of bacterial endotoxins less than 10EU / mL is desired.

Bioburden is assessed by direct inoculation, and less than 10 CFU / 100mL is desired.

25 For lot release and stability, an *in vitro* potency assay for gene therapy product potency is performed. *In vitro* potency is assessed by amiRNA expression by RT-qPCR, *ATXN2* mRNA levels by RT-ddPCR, and *ATXN2* protein levels by *ATXN2* protein FACS in 2v6.11 or Lec2 cells. Cells may be pre-treated with 1 ug/mL ponasterone A (Invitrogen, H10101), 50 mU/mL neuraminidase (Sigma, N7885), and
30 2mM hydroxyurea (Sigma, H8627) prior to transduction. Serial dilutions of vector are used to treat cells in a 96 well format, incubating at 4°C for 30min, and then 90min

following application of vector. Plates are then transferred to 37°C. After 2-3 days
amiRNA, *ATXN2* mRNA, and ATXN2 protein are assessed at each dose.

In vivo potency in some experiments is tested prior to dosing non-human
primates and is assessed by single dose (such as 8.5E10 vg / gram) administration of
5 test article intravenously into wild-type C57Bl/6 mice. Liver biopsy is collected,
homogenized, and DNA and RNA are extracted by Allprep DNA/RNA/Protein mini kit
(Qiagen, 80004) for assessment of vector distribution and *ATXN2* mRNA knockdown in
liver.

Median Tissue Culture Infectious Dose (TCID₅₀) to assess vector
10 infectivity is performed in HelaRC32 cells. HelaRC32 stably express AAV2 *rep* and
cap genes, and the assay involves serial dilutions of vector in a 96 well plate and co-
infection with Adenovirus 5 helper virus, lysing cells, extracting DNA, performing
qPCR or ddPCR on the vector genome to assess number of infected cells per well
across the dilution range.

15

Biodistribution and pharmacodynamic activity

Non-human primate brain and spinal cord tissue from rAAV vector and
control treated animals are collected by punch biopsy or as slabs at necropsy and snap
frozen. Samples are homogenized by addition of Buffer RLT (Qiagen) supplemented
20 with beta-mercaptoethanol. Ceramic bead-based homogenization (Precellys, CK14
2mL) is performed using 3 cycles of 15s at 6500rpm and 10s break. DNA, RNA and
protein are extracted with Allprep DNA/RNA/Protein Mini kit (Qiagen, 80004) and
small RNA are extracted with miRNEasy Tissue/Cells Advanced Mini kit (Qiagen,
217604).

25 For isolation of motor neurons from rAAV dosed non-human primates,
spinal cord tissues are frozen in liquid nitrogen at necropsy. Cryosections are generated
and stained with ARCTURUS HistoGene Quick H&E Stain for LCM, and motor
neurons are dissected from each section with the ARCTURUS XT LCM System.
DNA and RNA from LCM samples are extracted with PicoPure kits (Thermo Fisher).

30 For histological evaluations, non-human primate brain and spinal cord
tissue are collected at necropsy and fixed with 10% neutral buffered formalin for 24hr,
transferred to 70% ethanol for 3-10 days and embedded into paraffin blocks. Five-

micron sections are cut, mounted onto glass slides, and stained for hematoxylin and eosin for histology, or stained in separate protocols for immunohistochemistry or in-situ hybridization.

Vector biodistribution in tissues from animals dosed with rAAV is
5 assessed by ddPCR. Specifically, primer probes that amplify promoter and/or stuffer regions of the vector are used and compared to primer probes specific to host genome and results are expressed as vector genomes per diploid genome.

To isolate biodistribution in tissue material enriched for motor neurons, vector biodistribution is assessed by ddPCR on DNA isolated from spinal cord neurons
10 captured by laser capture microdissection (LCM). Specifically, primer probes that amplify promoter and/or stuffer regions of the vector are used and compared to host diploid genome and results are expressed as vector genomes per diploid genome. Biodistribution in tissue material enriched for other disease-relevant cell types such as motor cortex, containing motor neurons, and cerebellum, containing Purkinje cells, can
15 be assessed by the same ddPCR method in tissue punches from those brain regions.

To measure ATXN2 knockdown in spinal cord motor neurons, *ATXN2* mRNA is assessed by RT-ddPCR in spinal cord neurons captured by laser capture microdissection. Knockdown of *ATXN2* mRNA is assessed by comparison of spinal cord neurons in amiRNA treated subjects relative to the vehicle treated group, using the
20 ratio of *ATXN2* positive droplets to housekeeping genes (GUSB, B2M, TBP, or others). Significant knockdown of *ATXN2* in spinal cord neurons in animals dosed with *ATXN2* targeting amiRNAs relative to vehicle dosed animals is desirable.

ATXN2 mRNA in spinal cord neurons, cortical motor neurons, cerebellar purkinje cells and other relevant tissues is also assessed by *in situ* hybridization (ISH) in
25 tissue sections, and by RT-ddPCR in tissue punches. By *in situ* hybridization, knockdown of *ATXN2* mRNA is semi-quantitatively assessed by comparison of amiRNA treated subjects relative to the vehicle group. Significant knockdown in these tissues is desirable, with reductions in *ATXN2* mRNA in spinal and cortical motor neurons particularly relevant for ALS and knockdown in Purkinje cells particularly
30 relevant for SCA2. By RT-ddPCR, knockdown is assessed as described above.

ATXN2 protein in spinal cord neurons, cortical motor neurons, cerebellum, and other brain tissues is assessed by immunohistochemistry. Fixed slides

are stained with monoclonal ATXN2 antibody (BD, 611378) or polyclonal ATXN2 antibody (Sigma, HPA018295-100UL) using standard protocols.

Immunohistochemistry is used to semi-quantitatively assess knockdown of ATXN2 protein, and significant reduction in ATXN2 levels relative to vehicle treated animals is
5 desirable.

Other assays for the pharmacology of ATXN2 amiRNA vectors dosed via administration into the cerebrospinal fluid in non-human primates that may be conducted include ATXN2 assays using alphaLISA® or Simoa® bead technology; or amiRNA detection assays from tissue or body fluids using miRNA-ISH or miRNA RT-
10 qPCR.

ATXN2 protein in bulk tissue is assessed by alphaLISA. The capture antibody is monoclonal ATXN2 antibody (BD, 611378) and detection antibody is polyclonal ATXN2 antibody (ProteinTech, 21776-1-AP). ATXN2 protein in CSF is assessed by custom ATXN2 Simoa assay (Quanterix).

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The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet,
15 including but not limited to U.S. Provisional Application No. 62/971,873 filed on February 7, 2020, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the
20 above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. An isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising the nucleic acid sequence set forth in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

2. The isolated nucleic acid molecule of claim 1, wherein the guide strand sequence comprises:

(a) the nucleic acid sequence set forth in any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362;

(b) the nucleic acid sequence set forth in any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362;

(c) the nucleic acid sequence set forth in any one of SEQ ID NOS: 1908-2007; or

(d) the nucleic acid sequence set forth in any one of SEQ ID NOS: 100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209;

(e) the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314; or

(f) the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811.

3. The isolated nucleic acid molecule of claim 1 or 2, wherein the inhibitory nucleic acid is a siRNA duplex, shRNA, miRNA, or dsRNA.

4. The isolated nucleic acid molecule of any one of claims 1-3, wherein the inhibitory nucleic acid further comprises a passenger strand sequence, optionally wherein the passenger strand sequence is selected from Tables 1, 19, 23, and 24, or a passenger strand sequence selected from Tables 1, 19, 23, and 24 and having 1-10 insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof.

5. The isolated nucleic acid molecule of any one of claims 1-4, wherein the inhibitory nucleic acid is an artificial miRNA, wherein the guide strand sequence is contained within a miRNA backbone sequence.

6. The isolated nucleic acid molecule of claim 5, wherein the guide strand sequence and passenger strand sequence of the artificial miRNA are contained within a miRNA backbone sequence.

7. The isolated nucleic acid molecule of claim 5 or 6, wherein the miRNA backbone sequence is a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-

16-2 backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

8. The isolated nucleic acid molecule of claim 6 or 7, wherein the miRNA backbone sequence is a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-16-2 backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

9. The isolated nucleic acid molecule of any one of claims 6-8, wherein the miRNA backbone sequence is a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-124 backbone sequence, a miR-130a backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-144 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR-190a_M backbone sequence, or a miR-190a_M backbone sequence.

10. The isolated nucleic acid molecule of any one of claims 6-9, wherein the miRNA backbone sequence is a miR-100 backbone sequence or miR-100_M backbone sequence.

11. The isolated nucleic acid molecule of any one of claims 1-10, wherein the inhibitory nucleic acid is a miRNA comprising the nucleic acid sequence set forth in any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057,

2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

12. The isolated nucleic acid molecule of claim 11, wherein the inhibitory nucleic acid is a miRNA comprising:

- (a) the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007;
- (b) the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-1934, 1936-1977, 1979-1982, 1984-1994, 1997, 1998, 2000, 2001, 2005-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267;
- (c) the nucleic acid sequence set forth in any one of SEQ ID NOS:1915, 1982, 1965, 1937, 1985, 1921, and 2021; or
- (d) the nucleic acid sequence set forth in any one of SEQ ID NOS:1915, 1965, 1982, and 2021.

13. The isolated nucleic acid molecule of any one of claims 1-12, wherein the nucleic acid sequence encoding the inhibitory nucleic acid is located in an untranslated region of the expression construct.

14. The isolated nucleic acid molecule of claim 13, wherein the untranslated region is an intron, a 5' untranslated region (5' UTR), or a 3' untranslated region (3'UTR).

15. The isolated nucleic acid molecule of any one of claims 1-14, further comprising a promoter operably linked to the nucleic acid sequence encoding the inhibitory nucleic acid.

16. The isolated nucleic acid molecule of claim 15, wherein the promoter is a RNA pol III promoter (e.g., U6, H1, etc.), a chicken-beta actin (CBA) promoter, a CAG promoter, a H1 promoter, a CD68 promoter, a human synapsin promoter, or a JeT promoter.

17. The isolated nucleic acid molecule of claim 15 or 16, wherein the promoter is an H1 promoter comprising nucleotides 113-203 of SEQ ID NO:1522, nucleotides 1798-1888 of SEQ ID NO:1521, nucleotides 244-343 of SEQ ID NO:2257, or nucleotides 113-343 of SEQ ID NO:2257.

18. The isolated nucleic acid molecule of any one of claims 1-17, wherein the expression construct is flanked by a 5' adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence, or variants thereof.

19. The isolated nucleic acid molecule of claim 18, wherein one of the ITR sequences lacks a functional terminal resolution site.

20. The isolated nucleic acid molecule of claim 13 or 14, wherein the 5' and 3' ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVRh10, AAV11, and variants thereof.

21. The isolated nucleic acid molecule of any one of claims 18-20, wherein the 5' ITR comprises nucleotides 1-106 of SEQ ID NO:2257 and the 3' ITR comprises nucleotides 2192-2358 of SEQ ID NO:2257.

22. A vector comprising the isolated nucleic acid molecule of any one of claims 1-21.
23. The vector of claim 16, wherein the vector is a plasmid or viral vector.
24. The vector of claim 23, wherein the viral vector is a recombinant adeno-associated virus (rAAV) vector or a Baculovirus vector.
25. The vector of claim 24, wherein the vector is a self-complementary rAAV vector.
26. The vector of any one of claims 22-25, further comprising a stuffer sequence.
27. The vector of claim 26, wherein the stuffer sequence comprises nucleotides 348-2228 of SEQ ID NO:1522 or nucleotides 489-2185 of SEQ ID NO:2257.
28. The vector of any one of claims 22-27, comprising the nucleotide sequence of any one of SEQ ID NOS:2257-2260.
29. A recombinant adeno-associated (rAAV) particle comprising the isolated nucleic acid molecule of any one of claims 1-21 or the vector of claims 22-28.
30. The rAAV particle of claim 29, wherein the rAAV particle comprises a capsid protein.
31. The rAAV particle of claim 30, wherein the capsid protein is capable of crossing the blood-brain barrier.

32. The rAAV particle of claim 30 or 31, wherein the capsid protein is an AAV9 capsid protein or AAVrh.10 capsid protein.

33. The rAAV particle of any one of claims 29-32, wherein the rAAV particle transduces neuronal cells and/or non-neuronal cells of the central nervous system (CNS).

34. A pharmaceutical composition comprising the isolated nucleic acid molecule of any one of claims 1-21, the vector of any one of claims 22-28, or the rAAV particle of any one of claims 29-33, and optionally a pharmaceutically acceptable carrier.

35. A host cell comprising the isolated nucleic acid molecule of any one of claims 1-21, the vector of any one of claims 22-28, or the rAAV particle of any one of claims 29-33.

36. A method for treating a subject having or suspected of having a neurodegenerative disease, the method comprising administering to the subject the isolated nucleic acid molecule of any one of claims 1-21, the vector of any one of claims 22-28, the rAAV particle of any one of claims 29-33, or the pharmaceutical composition of claim 34.

37. The method of claim 36, wherein the administration comprises direct injection to the CNS of the subject.

38. The method of claim 37, wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, or any combination thereof.

39. The method of claim 37, wherein the direct injection is direct injection to the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or intralumbar injection.

40. The method of any one of claims 36-39, wherein the subject is characterized as having an *ATXN2* allele having at least 22 CAG trinucleotide repeats, optionally wherein the *ATXN2* allele has at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

41. The method of any one of claims 36-40, wherein the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

42. A method of inhibiting *ATXN2* expression in a cell, the method comprising delivering to the cell the isolated nucleic acid of any one of claims 1-21, the vector of any one of claims 22-28, the rAAV particle of any one of claims 29-33, or the pharmaceutical composition of claim 34.

43. The method of claim 42, wherein the cell has an *ATXN2* allele having at least 22 CAG trinucleotide repeats, optionally wherein the *ATXN2* allele has at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

44. The method of claim 42 or 43, wherein the cell is a cell in the CNS, optionally a neuron, glial cell, astrocyte, or microglial cell.

45. The method of any one of claims 42-44, wherein the cell is in vitro.

46. The method of any one of claims 42-45, wherein the cell is from a subject having one or more symptoms of a neurodegenerative disease.

47. The method of any one of claims 42-46, wherein the cell is from a subject having or suspected of having a neurodegenerative disease.

48. The method of claim 46 or 47, wherein the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

49. A method of inhibiting ATXN2 expression in the central nervous system of a subject, the method comprising administering to the subject the isolated nucleic acid of any one of claims 1-21, the vector of any one of claims 22-28, the rAAV particle of any one of claims 29-33, or the pharmaceutical composition of claim 34.

50. The method of claim 49, wherein the administration comprises direct injection to the CNS of the subject.

51. The method of claim 50, wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intraatrial injection, subpial injection, or any combination thereof.

52. The method of claim 50, wherein the direct injection is injection to the cerebrospinal fluid (CSF) of the subject, optionally wherein the direct injection is intracisternal injection, intraventricular injection, and/or intralumbar injection.

53. The method of any one of claims 49-52, wherein the subject has an *ATXN2* allele having at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

54. An artificial miRNA comprising a guide strand sequence and a passenger strand sequence, wherein the guide strand sequence comprises any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

55. The artificial miRNA of claim 54, wherein the guide strand sequence comprises:

(a) any one of SEQ ID NOS: 12, 14, 40, 60, 100, 104, 108, 112, 124, 126, 128, 166, 198, 220, 242, 302, 306, 308, 330, 336, and 362;

(b) any one of SEQ ID NOS: 14, 40, 100, 108, 112, 128, 166, 198, 242, 308, 336, and 362;

- (c) the nucleic acid sequence set forth in any one of SEQ ID NOS:1908-2007; or
- (d) the nucleic acid sequence set forth in any one of SEQ ID NOS:100, 112, 166, 202, 246, 306, 308, 314, 1180, 1185, 1196, 1200, 1211, 1213, 1215, 1216, 1224, 1811-1822, 1824-1827, 2015, 2065, 2083, 2152, 2203, and 2209;
- (e) the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, 1819, 2083, 1215, 1216, 1811, and 314; or
- (f) the nucleic acid sequence set forth in any one of SEQ ID NOS:1185, 1816, 1213, and 1811.

56. The artificial miRNA of claim 54 or 55, wherein the guide strand sequence and passenger strand sequence are contained within a miR backbone sequence.

57. The artificial miRNA of claim 56, wherein the miR backbone sequence is a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-16-2 backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, or a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

58. The artificial miRNA of claim 56 or 57, wherein the miRNA backbone sequence is a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence,

a miR-16-2 backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

59. The artificial miRNA of any one of claims 56-58, wherein the miRNA backbone sequence is a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100 backbone sequence, a miR-100_M backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-124 backbone sequence, a miR-130a backbone sequence, a miR-132 backbone sequence, a miR-138-2 backbone sequence, a miR-144 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR-190a_M backbone sequence, or a miR-190a_M backbone sequence.

60. The artificial miRNA of any one of claims 56-59, wherein the miRNA backbone sequence is a miR-100 backbone sequence or miR-100_M backbone sequence

61. The artificial miRNA of any one of claims 54-60, wherein the artificial miRNA comprises the sequence as set forth in any one of SEQ ID NOS: 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

62. An isolated RNA duplex comprising a guide strand sequence and a passenger strand sequence, wherein the guide strand sequence comprises the nucleic acid sequence set forth in any one of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146,

148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, and 1176-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, optionally wherein the guide strand sequence and passenger strand sequence are linked by a loop region to form a hairpin structure comprising a duplex structure and a loop region.

63. The isolated RNA duplex of claim 62, wherein the loop structure comprises from 6 to 25 nucleotides.