



(51) International Patent Classification:

C12N 15/11 (2006.01) A61P 21/00 (2006.01)

C12N 15/113 (2010.01)

(21) International Application Number:

PCT/US2021/048611

(22) International Filing Date:

01 September 2021 (01.09.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/073,304 01 September 2020 (01.09.2020) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

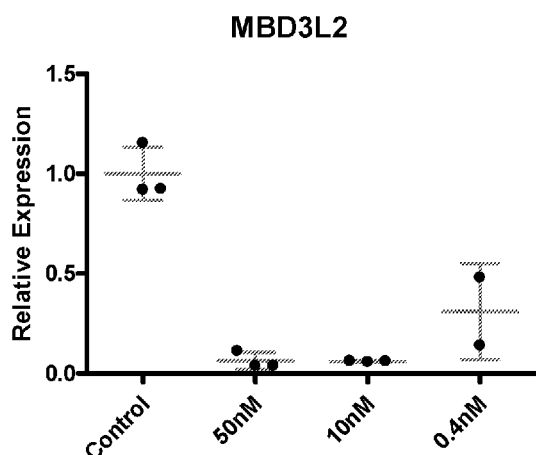
kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: DUX4 INHIBITORS AND METHODS OF USE THEREOF

Fig. 1A



(57) **Abstract:** This application relates to double-stranded small interfering RNAs that modulate DUX4 gene expression and describes methods of inhibiting DUX4 gene expression by contacting a cell with said double-stranded small interfering RNAs. The application further provides compositions comprising said double-stranded small interfering RNAs and their use in methods of preventing or treating a disease or disorder associated with aberrant expression of DUX4, such as facioscapulohumeral dystrophy (FSHD) or cancer, in a subject.

DUX4 INHIBITORS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/073,304, filed September 1, 2020, the contents of which are hereby incorporated by reference in their entirety.

REFERENCE TO A SEQUENCE LISTING

[0002] This application contains a Sequence Listing submitted electronically as a text file by EFS-Web. The text file, named "8957-5-PCT_Seq_Listing_ST25.txt", has a size in bytes of 194,000 bytes, and was recorded on 25 August 2021. The information contained in the text file is incorporated herein by reference in its entirety pursuant to 37 CFR § 1.52(e)(5).

TECHNICAL FIELD OF THE INVENTION

[0003] This invention relates to double-stranded small interfering RNAs (siRNAs) that modulate DUX4 gene expression, and their applications in research, diagnostics, and/or therapeutics. In some embodiments, it relates to compositions and methods comprising the said siRNAs in the prevention and/or treatment of facioscapulohumeral muscular dystrophy (FSHD).

BACKGROUND OF THE INVENTION

[0004] Facioscapulohumeral muscular dystrophy (FSHD) is a rare genetic disease affecting about one in 10,000 people worldwide. FSHD patients exhibit progressive, asymmetric muscle weakness and up to 20% of affected individuals become severely disabled. Non-muscular symptoms include subclinical sensorineural hearing loss telangiectasia.

[0005] Aberrant expression of the DUX4 protein in skeletal muscle due to inefficient epigenetic repression of the DUX4 gene is thought to cause FSHD. DUX4 is a retrogene encoded in each unit of the D4Z4 macrosatellite repeat array. D4Z4 repeats are bi-directionally transcribed in somatic tissues and generate long stretches of RNA and small RNA fragments that may have a role in epigenetic silencing. The more prevalent form of FSHD (FSHD1) is caused by the deletion of a subset of D4Z4 macrosatellite repeats in the subtelomeric region of chromosome 4q. Unaffected individuals have 11-100 of the 3.3 kb D4Z4 repeat units, whereas FSHD1 individuals have 10 or fewer repeats. FSHD2 is associated with decreased DNA methylation of the D4Z4 repeats on the same 4qA haplotype. Thus, administration of agents

that suppress expression of the DUX4 gene is a promising therapeutic approach for preventing or treating FSHD1 and FSHD2. Beyond their potential utility in the prevention or treatment of FSHD, DUX4-targeted treatments may also improve the success of cancer immunotherapies, as DUX4 expression been found to suppress MHC class I to promote cancer immune evasion and mediate resistance to anti-CTLA-4 therapy. *See Chew et al.*, 2019, *Dev Cell* 50(5): 525-6.

[0006] Double-stranded oligonucleotides have been used to modulate gene expression for use in research, diagnostics, and/or therapeutics. One method of modulation of gene expression is RNA interference (RNAi), which generally refers to gene silencing involving the introduction of double-stranded RNA (dsRNA) leading to the sequence-specific reduction of targeted endogenous mRNA levels. The reduction of target mRNA may occur by one of several different mechanisms, depending on the sequence or structure of the dsRNA. For example, it may lead to degradation of the target mRNA through formation of RNA induced silencing complex (RISC), or transcriptional silencing in which transcription of the mRNA is inhibited in a process called RNA-induced transcriptional silencing (RITS), or by modulation of microRNA (miRNA) function. MicroRNAs are small non-coding RNAs that regulate the expression of messenger RNAs. The binding of an RNAi compound to a microRNA prevents that microRNA from binding to its messenger RNA targets, and thus interferes with the function of the microRNA. The sequence-specificity of RNAi compounds makes them promising candidates as therapeutics to selectively modulate the expression of genes involved in the pathogenesis of diseases.

[0007] There continues to be a need in the art for methods and agents for treatment of FSHD. The present application addresses this need by providing oligonucleotides, and compositions and methods comprising them, that can suppress aberrant expression of DUX4 gene and thus can ameliorate, prevent or treat FSHD.

SUMMARY OF THE INVENTION

[0008] The present invention provides double-stranded small interfering RNA (siRNA) molecules for reducing or inhibiting the expression of the DUX4 gene. The present invention also provides a method of reducing or inhibiting expression of DUX4 in a cell comprising contacting the cell with a double-stranded siRNA molecule targeted to DUX4, thereby reducing or inhibiting expression of DUX4. In another aspect, the invention provides compositions and methods for the prevention or treatment of various disorders, including facioscapulohumeral

muscular dystrophy (FSHD) by administering the double-stranded siRNA molecules and compositions comprising the same to a subject.

[0009] Accordingly, in one aspect, the present invention includes double-stranded small interfering RNA (siRNA) molecules, each molecule comprising a sense strand and an antisense strand, that are useful for reducing or inhibiting the aberrant expression of the DUX4 gene in a cell. In some embodiments, the double-stranded small interfering RNA comprises at least one modified nucleoside.

[0010] In some embodiments, the DUX4 gene comprises a nucleobase sequence that is at least 85%, at least 90% identical, or at least 95% identical to SEQ ID NO: 593. In certain embodiments, DUX4 comprises a nucleobase sequence that is 100% identical to SEQ ID NO: 593.

[0011] In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is at least 85%, at least 90% or at least 95% complementary to an equal length portion of SEQ ID NO: 593. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is 100% complementary to an equal length portion of SEQ ID NO: 593.

[0012] In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is complementary to at least 8 contiguous nucleobases of an equal length portion of SEQ ID NO: 593. In various embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is complementary to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleobases of an equal length portion of SEQ ID NO: 593.

[0013] In one embodiment, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is complementary to at least 8 contiguous nucleobases of an equal length portion within nucleobases 4605 to 7485 of SEQ ID NO: 593. For example, in various embodiments, the antisense strand of the double-stranded small interfering RNA may comprise a nucleobase sequence that is complementary to at least 8 contiguous nucleobases of an equal length portion within nucleobases 4605-4638, 4693-4727, 4765-4783, 4933-4951, 4990-5011, 5075-5093, 5127-5148, 5161-5193, 5201-5228, 5243-5279, 5305-5327, 5353-5397, 5433-5461, 5464-5509, 5522-5540, 5651-5670, 5809-5830, 5842-5865,

5969-5990, 6066-6086, 6109-6135, 6183-6229, 6328-6369, 6403-6451, 7120-7138, 7162-7190, 7239-7285, 7404-7437, or 7452-7485 of SEQ ID NO: 593. In various embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is complementary to at least at least 8, at least 9, at least 10, at least 11, at least 12, 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleobases of an equal length portion of nucleobases 4605 to 7485 of SEQ ID NO: 593, for example, within nucleobases 4605-4638, 4693-4727, 4765-4783, 4933-4951, 4990-5011, 5075-5093, 5127-5148, 5161-5193, 5201-5228, 5243-5279, 5305-5327, 5353-5397, 5433-5461, 5464-5509, 5522-5540, 5651-5670, 5809-5830, 5842-5865, 5969-5990, 6066-6086, 6109-6135, 6183-6229, 6328-6369, 6403-6451, 7120-7138, 7162-7190, 7239-7285, 7404-7437, or 7452-7485 of SEQ ID NO: 593.

[0014] In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences listed in Table 1, *i.e.*, a sequence selected from the group consisting of SEQ ID NOs: 2, 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, 122, 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, 322, 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, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, and 592. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences selected from the group consisting of SEQ ID NOs: 216, 218,

220, 312, 324, 340, 342, 348, 350, 352, 354, 364, 372, 376, 400, 402, 404, 410, 434, 446, 448, 450, 462 and 564. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 216, 218, 220, 312, 324, 340, 342, 348, 350, 352, 354, 364, 372, 376, 400, 402, 404, 410, 434, 446, 448, 450, 462 and 564.

[0015] In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences listed in Table 5, *i.e.*, a sequence selected from the group consisting of SEQ ID NOs: 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, and 688. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences selected from the group consisting of SEQ ID NOs: 602, 604, 606, 616, 684, 686, and 688. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 602, 604, 606, 616, 684, 686, and 688.

[0016] In some embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence at least 85% complementary to the antisense strand of the double-stranded small interfering RNA. In various embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence at least 90%, at least 95%, or 100% complementary to the antisense strand of the double-stranded small interfering RNA.

[0017] In some embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is identical to at least 8 contiguous nucleobases of an equal length portion within nucleobases 4605 to 7485 of SEQ ID NO: 593. For example, in various embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is identical to at least 8 contiguous nucleobases of an equal length portion within nucleobases 4605-4638, 4693-4727, 4765-4783, 4933-4951, 4990-5011, 5075-5093, 5127-5148, 5161-5193, 5201-5228, 5243-5279, 5305-5327, 5353-5397, 5433-5461, 5464-5509, 5522-5540, 5651-5670, 5809-5830, 5842-5865, 5969-5990, 6066-6086, 6109-

6135, 6183-6229, 6328-6369, 6403-6451, 7120-7138, 7162-7190, 7239-7285, 7404-7437, or 7452-7485 of SEQ ID NO: 593. In various embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence that is identical to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleobases of an equal length portion of nucleobases 4605 to 7485 of SEQ ID NO: 593, for example, nucleobases 4605-4638, 4693-4727, 4765-4783, 4933-4951, 4990-5011, 5075-5093, 5127-5148, 5161-5193, 5201-5228, 5243-5279, 5305-5327, 5353-5397, 5433-5461, 5464-5509, 5522-5540, 5651-5670, 5809-5830, 5842-5865, 5969-5990, 6066-6086, 6109-6135, 6183-6229, 6328-6369, 6403-6451, 7120-7138, 7162-7190, 7239-7285, 7404-7437, or 7452-7485 of SEQ ID NO: 593.

[0018] In some embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any one of the nucleobase sequences listed in Table 1, *i.e.*, a sequence selected from the group consisting of SEQ ID NOs: 1, 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, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, and 591. In some embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences selected from the group consisting SEQ ID NOs: 215, 217, 219, 311, 323, 339, 341, 347, 349, 351, 353, 363, 371, 375, 399, 401, 403, 409, 433, 445, 447, 449, 461

and 563. In some embodiments, the sense strand of the double-stranded small interfering RNA comprises or consists of a nucleobase sequence of any one of the nucleobase sequences of SEQ ID NOs: 215, 217, 219, 311, 323, 339, 341, 347, 349, 351, 353, 363, 371, 375, 399, 401, 403, 409, 433, 445, 447, 449, 461 and 563.

[0019] In some embodiments, the sense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any one of the nucleobase sequences listed in Table 5, *i.e.*, a sequence selected from the group consisting of SEQ ID NOs: 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, and 687. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous nucleobases of any of the nucleobase sequences selected from the group consisting of SEQ ID NOs: 601, 603, 605, 615, 683, 685, and 687. In some embodiments, the antisense strand of the double-stranded small interfering RNA comprises or consists of a nucleic acid sequence of any one of SEQ ID NOs: 601, 603, 605, 615, 683, 685, and 687.

[0020] In some embodiments, the double-stranded small interfering RNA comprises at least one modified nucleoside. In some embodiments, the sense strand of the double-stranded small interfering RNA comprises at least one modified nucleoside. In some embodiments, each nucleoside of the sense strand of the double-stranded small interfering RNA comprises a modified nucleoside.

[0021] In some embodiments, at least one nucleoside of the sense strand of the double-stranded siRNA comprises a modified sugar. In some embodiments, each nucleoside of the sense strand of the double-stranded siRNA comprises a modified sugar. In some embodiments, the modified nucleoside comprises a 2'-F modified sugar and/or a 2'-OMe modified sugar. In some embodiments, the modified nucleoside comprises a 2'-OMe modified sugar. In some embodiments, the modified nucleoside comprises a 2'-F modified sugar modified sugar.

[0022] In some embodiments, the antisense and/or the sense strand of the double-stranded small interfering RNA comprises a TT overhang at the 3' end.

[0023] In some embodiments, the sense strand of the double-stranded small interfering RNA comprises at least one modified internucleoside linkage. In some embodiments, the sense strand of the double-stranded small interfering RNA comprises at least 2, 3, 4, or 5 modified internucleoside linkages. In some embodiments, the modified internucleoside linkage is a phosphorothioate linkage.

[0024] In some embodiments, the double-stranded small interfering RNA is conjugated to a lipophilic molecule, an antibody, an aptamer, a ligand, a peptide, or a polymer. In some embodiments, the lipophilic molecule may be a long chain fatty acid (LCFA). In some embodiments, the antibody is an anti-transferrin receptor antibody.

[0025] In another aspect, the present invention includes a pharmaceutical composition comprising a double-stranded small interfering RNA described herein or a salt thereof, and at least one pharmaceutically acceptable carrier. The pharmaceutical composition may be for use in medical therapy. The pharmaceutical composition may be for use in the treatment of a human or animal body. In another aspect, the present invention includes a use of the pharmaceutical composition for preparing or manufacturing a medicament for ameliorating, preventing, delaying onset, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof. In another aspect, the present invention includes a method for ameliorating, preventing, delaying onset, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof by administering the pharmaceutical composition to the subject. The disease or disorder may be facioscapulohumeral muscular dystrophy (FSHD), and may be FSHD1 or FSHD2. In another aspect, the present invention includes a method of ameliorating, preventing, delaying onset, or treating facioscapulohumeral muscular dystrophy (which includes FSHD1 and FSHD2) by administering the pharmaceutical composition to the subject.

[0026] In various embodiments, the administration may be intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, nasal, or via inhalation. In some embodiments, the administration may be once daily, weekly, every two weeks, monthly, every two months, quarterly, or yearly. In some embodiments, the administration may comprise an effective dose of from 0.01 to 100 mg/kg. In some embodiments, the administration inhibits the expression of DUX4 in the subject.

[0027] In another aspect, the invention comprises a kit comprising one or more double-stranded siRNA and a device for administering said double-stranded siRNA.

[0028] In another aspect, the present invention includes a method of ameliorating, preventing, delaying onset, or treating facioscapulohumeral muscular dystrophy (which includes FSHD1 and FSHD2) comprising administering a double-stranded small interfering RNA described herein. In another aspect, the present invention includes use of a double-stranded small interfering RNA described herein for the treatment of facioscapulohumeral muscular dystrophy.

[0029] In another aspect, the present invention includes a method of ameliorating, preventing, delaying onset, or treating cancer, comprising administering a double-stranded small interfering RNA described herein. In some embodiments, the method may further comprise the administration of a checkpoint inhibitor such as an anti-CTLA-4 agent.

[0030] In another aspect, the present invention includes use of a double-stranded small interfering RNA described herein for the preparation of a medicament for the treatment of facioscapulohumeral muscular dystrophy.

[0031] In another aspect, the present invention includes a method of inhibiting expression of DUX4 in a cell, comprising contacting a cell with a double-stranded small interfering RNA described here, and thereby inhibiting expression of DUX4. In some embodiments, the contacting is performed *in vitro*. In some embodiments, the contacting is performed *in vivo*. In some embodiments, the cell is in an animal. In some embodiments, the animal is a human. In some embodiments, the expression of DUX4 is inhibited by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%. In some embodiments, the expression of DUX4 is abolished.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] **FIG. 1A** shows *in vitro* MBD3L2 expression data obtained with unmodified siRNA molecules targeting DUX4.

[0033] **FIG. 1B** shows *in vitro* MBD3L2 expression data obtained with modified siRNA molecules targeting DUX4.

DETAILED DESCRIPTION OF THE INVENTION

[0034] Described herein are double-stranded small interfering RNA (siRNA) molecules that target sequences within the DUX4 gene. Also described are methods of reducing or inhibiting expression of DUX4 in a cell comprising contacting the cell with the said siRNA molecules.

Further described herein are methods for the prevention or treatment of facioscapulohumeral muscular dystrophy (FSHD) by administering to a subject the double-stranded siRNA molecules and compositions comprising the same.

Definitions

[0035] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

[0036] As used herein, the term “DUX4” may refer to a DUX4 protein or a DUX4 nucleic acid, *i.e.*, a nucleic acid sequence encoding a DUX4 protein. A DUX4 nucleic acid may refer to a DNA sequence encoding DUX4 protein, an RNA sequence transcribed from DNA encoding DUX4 (including genomic DNA comprising introns and exons) including a non-protein encoding (*i.e.*, non-coding) RNA sequence, and an mRNA sequence encoding DUX4. In some embodiments, DUX4 nucleic acid sequence comprises GENBANK Accession No. FJ439133.1 (SEQ ID NO: 593).

[0037] “Double-stranded small interfering RNA” means any duplex RNA structure comprising two anti-parallel and substantially complementary nucleic acid strands. In certain embodiments, double-stranded small interfering RNA comprise a sense strand and an antisense strand, wherein the antisense strand is complementary to a target nucleic acid.

[0038] “Complementarity” means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0039] “Contiguous nucleobases” means nucleobases immediately adjacent to each other.

[0040] “Deoxyribonucleotide” means a nucleotide having a hydrogen at the 2' position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

[0041] “Expression” includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

[0042] “Fully complementary” or “100% complementary” means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

[0043] “Inhibiting the expression or activity” refers to a reduction or blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

[0044] “Internucleoside linkage” refers to the chemical bond between nucleosides.

[0045] “Linked nucleosides” means adjacent nucleosides linked together by an internucleoside linkage.

[0046] “Modified internucleoside linkage” refers to a substitution or any change from a naturally occurring internucleoside bond (*i.e.*, a phosphodiester internucleoside bond).

[0047] “Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid. “Modified nucleobase” means any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An “unmodified nucleobase” means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

[0048] “Nucleoside” means a nucleobase linked to a sugar. “Modified nucleoside” means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

[0049] “Modified nucleotide” means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase.

[0050] “Modified sugar” means substitution and/or any change from a natural sugar moiety. In certain embodiments modified sugars include 2'-F modified sugars and 2'-OMe modified sugars.

[0051] “Nucleobase complementarity” refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a

certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

[0052] “Nucleobase sequence” means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

[0053] “Phosphorothioate linkage” means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

[0054] “Sites,” as used herein, are defined as unique nucleobase positions within a target nucleic acid.

[0055] “Subject” means a human or non-human animal selected for treatment or therapy.

[0056] “Target gene” refers to a gene encoding a target.

[0057] “Target nucleic acid” refers to a nucleic acid, the modulation of which is desired.

Double-stranded Small Interfering RNA (siRNA) Molecules

[0058] In one aspect, the present invention includes double-stranded oligonucleotides, such as small interfering RNA (siRNA) compounds, and compositions comprising the same. In some embodiments, siRNA compounds may comprise at least one modified RNA nucleoside (*i.e.*, independently, a modified sugar moiety and/or modified nucleobase), and/or modified internucleoside linkages. In certain embodiments, siRNA compounds may comprise modified RNA nucleosides, modified DNA nucleosides, and/or modified internucleoside linkages.

[0059] Some embodiments relate to double-stranded molecules wherein each strand comprises a motif defined by the location of one or more modified or unmodified nucleosides.

[0060] In some embodiments, compositions are provided comprising a first and a second oligomeric compound that are fully or at least partially hybridized to form a duplex region and further comprising a region that is complementary to and hybridizes to a nucleic acid target. Such a composition may comprise a first oligomeric compound that is an antisense strand having full or partial complementarity to a nucleic acid target and a second oligomeric compound that is a sense strand having one or more regions of complementarity to and forming at least one duplex region with the first oligomeric compound.

[0061] In some embodiments, the compositions of the present invention modulate gene expression by hybridizing to a nucleic acid target resulting in loss of its normal function. In some embodiments, the degradation of the target nucleic acid is facilitated by an activated RISC complex that is formed with compositions of the invention.

[0062] In some embodiments, one of the strands is useful in, for example, influencing the preferential loading of the opposite strand into the RISC (or cleavage) complex. In some embodiments, the compositions of the present invention hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

[0063] Some embodiments are drawn to double-stranded compositions wherein both the strands comprises a hemimer motif, a fully modified motif, a positionally modified motif or an alternating motif. Each strand of the compositions of the present invention may be modified to fulfill a particular role in for example the siRNA pathway. Using a different motif in each strand or the same motif with different chemical modifications in each strand permits targeting the antisense strand for the RISC complex while inhibiting the incorporation of the sense strand. Within this model, each strand may be independently modified such that it is enhanced for its particular role. The antisense strand may be modified at the 5'-end to enhance its role in one region of the RISC while the 3'-end may be modified differentially to enhance its role in a different region of the RISC.

[0064] The double-stranded oligonucleotide molecules may comprise self-complementary sense and antisense regions, wherein the antisense region comprises a nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide molecules can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (*i.e.*, each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double-stranded structure, for example wherein the double-stranded region is about 12 to about 30, *e.g.*, about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (*e.g.*,

about 12 to about 30 or more nucleotides of the double-stranded oligonucleotide molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the double-stranded oligonucleotide may be assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siRNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

[0065] The double-stranded oligonucleotide may have a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siRNA molecule capable of mediating RNAi.

[0066] In some embodiments, the double-stranded oligonucleotide comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linker molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der Waals interactions, hydrophobic interactions, and/or stacking interactions. In some embodiments, the double-stranded oligonucleotide comprises a nucleotide sequence that is complementary to a nucleotide sequence of a target gene. In another embodiment, the double-stranded oligonucleotide interacts with a nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene.

[0067] As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term

RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, double-stranded oligonucleotides can be used to epigenetically silence genes at both the post-transcriptional level and the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siRNA molecules of the invention can result from siRNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel et al., 2004, Science, 303, 672-676; Pal-Bhadra et al., 2004, Science, 303, 669-672; Allshire, 2002, Science, 297, 1818-1819; Volpe et al., 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall et al., 2002, Science, 297, 2232-2237).

[0068] It is contemplated that compounds and compositions of some embodiments provided herein can target by a dsRNA-mediated gene silencing or RNAi mechanism, including, *e.g.*, “hairpin” or stem-loop double-stranded RNA effector molecules in which a single RNA strand with self-complementary sequences is capable of assuming a double-stranded conformation, or duplex dsRNA effector molecules comprising two separate strands of RNA. In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as the RNA/DNA hybrids disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.

[0069] As used herein, double-stranded oligonucleotides need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules may lack ribonucleotides or 2'-hydroxy (2'-OH) containing nucleotides. Such double-stranded oligonucleotides that do not require the presence of ribonucleotides within the molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. In some embodiments, the double-stranded oligonucleotides can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions.

[0070] The dsRNA or dsRNA effector molecule may be a single molecule with a region of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In various embodiments, a dsRNA that consists of a single molecule consists entirely of ribonucleotides or includes a region of ribonucleotides

that is complementary to a region of deoxyribonucleotides. Alternatively, the dsRNA may include two different strands that have a region of complementarity to each other.

[0071] In various embodiments, both strands consist entirely of ribonucleotides, one strand consists entirely of ribonucleotides and one strand consists entirely of deoxyribonucleotides, or one or both strands contain a mixture of ribonucleotides and deoxyribonucleotides.

[0072] In certain embodiments, the regions of complementarity are at least 70, 80, 90, 95, 98, or 100% complementary to each other and to a target nucleic acid sequence. In certain embodiments, the region of the dsRNA that is present in a double-stranded conformation includes at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75, 100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in a cDNA or other target nucleic acid sequence being represented in the dsRNA. In some embodiments, the dsRNA does not contain any single stranded regions, such as single stranded ends, or the dsRNA is a hairpin. In other embodiments, the dsRNA has one or more single stranded regions or overhangs. In certain embodiments, RNA/DNA hybrids include a DNA strand or region that is an antisense strand or region (*e.g.*, has at least 70, 80, 90, 95, 98, or 100% complementarity to a target nucleic acid) and an RNA strand or region that is a sense strand or region (*e.g.*, has at least 70, 80, 90, 95, 98, or 100% identity to a target nucleic acid), and vice versa.

[0073] In various embodiments, the RNA/DNA hybrid is made *in vitro* using enzymatic or chemical synthetic methods such as those described herein or those described in WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. In other embodiments, a DNA strand synthesized *in vitro* is complexed with an RNA strand made *in vivo* or *in vitro* before, after, or concurrent with the transformation of the DNA strand into the cell.

[0074] In yet other embodiments, the dsRNA is a single circular nucleic acid containing a sense and an antisense region, or the dsRNA includes a circular nucleic acid and either a second circular nucleic acid or a linear nucleic acid (see, for example, WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999). Exemplary circular nucleic acids include lariat structures in which the free 5' phosphoryl group of a nucleotide becomes linked to the 2' hydroxyl group of another nucleotide in a loop back fashion. Chemically synthesized RNA duplexes in the 25-30 base length range have been shown to exhibit increased potency compared with shorter 21-mer siRNAs and the increase in potency is attributed to the action of Dicer endonuclease enzyme which uses the longer dsRNA as substrate, cleaves it and facilitates

the loading of the cleaved dsRNA into the RISC. Thus, in some embodiments, the dsRNA may be a dicer substrate RNA. For example, in some embodiments, the dsRNA is a 25/27 mer.

Modified Nucleotides and Chemically Modified siRNAs

[0075] In various embodiments described herein, a double-stranded siRNA of the invention may comprise one or more (*e.g.*, two, three, four, five, or more) modified nucleic acid monomers (*i.e.*, nucleotides). Various examples of modified nucleotides are disclosed in US Patent Nos. 9,035,039, 9,951,338, 10,036,024, 10,538,763, and International Patent Publication No. WO/2018/222926, each of which is herein incorporated by reference in its entirety.

[0076] Examples of modified nucleotides suitable for use in the present invention include, but are not limited to, ribonucleotides or arabinonucleotides having a 2'-O-methyl (2'-OMe), 2'-deoxy-2'-fluoro (2'-F), 2'-deoxy, 5-C-methyl, 2'-O-(2-methoxyethyl) (MOE), 4'-thio, 2'-amino, or a 2'-C-allyl group. In some embodiments, these may include 2'-deoxy-2'-fluoro arabinoguanosine nucleotides.

[0077] Modified nucleotides having a conformation such as those described in the art, for example in Saenger, Principles of Nucleic Acid Structure, Springer-Verlag Ed. (1984), are also suitable for use in siRNA molecules. Other modified nucleotides include, without limitation, locked nucleic acid (LNA) nucleotides, unlocked nucleic acid (UNA) nucleotides, G-clamp nucleotides, or nucleotide base analogs. LNA nucleotides include but need not be limited to 2'-O, 4'-C-methylene-(D-ribofuranosyl)nucleotides, 2'-O-(2-methoxyethyl) (MOE) nucleotides, 2'-methyl-thio-ethyl nucleotides, 2'-deoxy-2'-fluoro (2'-F) nucleotides, 2'-deoxy-2'-chloro (2'-Cl) nucleotides, 2'-azido nucleotides, and (S)-constrained ethyl (cEt) nucleotides.

[0078] In some embodiments, a double-stranded siRNA molecule may comprise one or more chemical modifications such as terminal cap moieties, phosphate backbone modifications, and the like. Examples of classes of terminal cap moieties include, without limitation, inverted deoxy abasic residues, glyceryl modifications, 4',5'-methylene nucleotides, 1-(beta-D-erythrofuransyl) nucleotides, 4'-thio nucleotides, carbocyclic nucleotides, 1,5-anhydrohexitol nucleotides, L-nucleotides, alpha-nucleotides, modified base nucleotides, threo pentofuranosyl nucleotides, acyclic 3',4'-seco nucleotides, acyclic 3,4-dihydroxybutyl nucleotides, acyclic 3,5-dihydroxypentyl nucleotides, 3'-3'-inverted nucleotide moieties, 3'-3'-inverted abasic moieties, 3'-2'-inverted nucleotide moieties, 3'-2'-inverted abasic moieties, 5'-5'-inverted nucleotide

moieties, 5'-5'-inverted abasic moieties, 3'-5'-inverted deoxy abasic moieties, 5'-amino-alkyl phosphate, 1,3-diamino-2-propyl phosphate, 3 aminopropyl phosphate, 6-aminoethyl phosphate, 1,2-aminododecyl phosphate, hydroxypropyl phosphate, 1,4-butanediol phosphate, 3'-phosphoramidate, 5' phosphoramidate, hexylphosphate, aminoethyl phosphate, 3'-phosphate, 5'-amino, 3'-phosphorothioate, 5'-phosphorothioate, phosphorodithioate, and bridging or non-bridging methylphosphonate or 5'-mercapto moieties. Non-limiting examples of phosphate backbone modifications (*i.e.*, resulting in modified internucleoside linkages) include phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate, carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and alkylsilyl substitutions. Such chemical modifications can occur at the 5'-end and/or 3'-end of the sense strand, antisense strand, or both strands of the siRNA.

[0079] In some embodiments, a double-stranded siRNA of the invention may comprise at least one modified internucleoside linkage. Examples of modified internucleoside linkage include, without limitation, peptide linkage, phosphorothioate (PS) linkage, and phosphorodiamidate morpholino (PMO) linkage. In some embodiments, a double-stranded siRNA of the invention comprises 2, 3, 4, 5, or more modified internucleoside linkages. In some embodiments, a double-stranded siRNA of the invention comprises a sense strand, an antisense strand, or both, where all internucleoside linkages are modified. In some embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

[0080] In some embodiments, the sense and/or antisense strand may comprise a 5'-terminal or 3'-terminal overhang having 1, 2, 3, 4, or more 2'-deoxyribonucleotides (*e.g.*, A, G, C, or T) and/or any combination of modified and unmodified nucleotides. In some embodiments, a double-stranded siRNA may comprise a TT overhang at the 3' end of the sense strand. In some embodiments, a double-stranded siRNA may comprise a TT overhang at the 3' end of the antisense strand. In some exemplary embodiments, a double-stranded siRNA may comprise a TT overhang at the 3' end of sense strand and at the 3' end of the antisense strand.

Conjugated siRNAs

[0081] In some embodiments, a double-stranded siRNA of the invention may be conjugated to at least one other molecule. Conjugation of the double-stranded siRNA with an appropriate molecule provides a means for improving delivery of the siRNA into target cells. Such conjugate molecules may interact with the lipid components of the cell membrane, bind to

specific cell surface proteins or receptors, and/or penetrate the cell through endogenous transport mechanisms carrying the siRNA with them.

[0082] The conjugate can be attached at the 5'- and/or the 3'-end of the sense and/or the antisense strand of the siRNA via a covalent attachment such as a nucleic acid or non-nucleic acid linker. The conjugate can be attached to the siRNA through a carbamate group or other linking group (*see, e.g.*, U.S. Patent Publication Nos. 20050074771, 20050043219, and 20050158727). A conjugate may be added to siRNA for any of a number of purposes. For example, the conjugate may be a molecular entity that facilitates the delivery of siRNA into a cell or may be a molecule that comprises a drug or label. Examples of conjugate molecules suitable for attachment to siRNA of the present invention include, without limitation, lipophilic molecules (*e.g.*, fatty acids), cholesterol, glycols such as polyethylene glycol (PEG), human serum albumin (HSA), carotenoids, terpenes, bile acids, folates (*e.g.*, folic acid, folate analogs and derivatives thereof), sugars (*e.g.*, galactose, galactosamine, N-acetyl galactosamine, glucose, mannose, fructose, fucose, etc.), phospholipids, peptides, ligands for cellular receptors capable of mediating cellular uptake, antibodies, aptamers, and combinations thereof (*see, e.g.*, U.S. Patent Publication Nos. 20030130186, 20040110296, and 20040249178; U.S. Pat. No. 6,753,423).

[0083] The type of conjugate used and the extent of conjugation to the siRNA can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of the siRNA while retaining activity. As such, one skilled in the art can screen siRNA molecules having various conjugates attached thereto to identify siRNA conjugates having improved properties using any of a variety of well-known *in vitro* cell culture or *in vivo* animal models including the negative-controlled expression studies described above. Examples of siRNA bioconjugates are described in, *e.g.*, Chernikov *et al.*, 2019, *Front. Pharmacol.* 10: 1-25 and Osborn *et al.*, 2018, *Nuc. Acid Ther.* 28(3): 128-136.

[0084] In some embodiments, a double-stranded siRNA of the invention may be conjugated to a lipophilic molecule (for example, a long chain fatty acid or LCFA), an antibody (for example, anti-transferrin receptor antibody), an aptamer, a ligand, a peptide, or a polymer.

[0085] In one embodiment, the double-stranded siRNA may be conjugated to a lipophilic molecule, *e.g.*, a long chain fatty acid. In some exemplary embodiments, a double-stranded siRNA of the invention is conjugated to a long chain fatty acid described in International Patent Publication No. WO/2019/232255.

[0086] In some embodiments, a double-stranded siRNA of the invention may be conjugated to an antibody. In some embodiments, the antibody is a muscle-targeting antibody. In some embodiments, the muscle-targeting antibody is an anti-transferrin receptor antibody. In some exemplary embodiments, a double-stranded siRNA of the invention is conjugated to an anti-transferrin receptor antibody described in International Patent Publication No. WO/2020/028864.

siRNA Delivery with Liposomes, Lipid Nanoparticles (LNPs), and Other Carriers

[0087] In some embodiments, a double-stranded siRNA of the invention may be delivered via liposomes, nanoparticles, lipid nanoparticles (LNPs), polymers, microparticles, microcapsules, micelles, or extracellular vesicles.

[0088] In some embodiments, a double-stranded siRNA of the invention is delivered via a lipid nanoparticle (LNP). Examples of LNPs capable of delivering a double-stranded siRNA of the invention are described in International Patent Publication Nos. WO/2015/074085, WO/2016/081029, WO/2017/117530, WO/2018/118102, WO/2018/119163, WO/2018/222926, WO/2019/191780, and WO/2020/154746. In some embodiments, an LNP may be decorated with targeting moiety, *e.g.*, an antibody, a receptor, or a fragment thereof capable of binding to a target ligand.

[0089] In one embodiment, a lipid nanoparticle for use in the instant invention comprises (a) a nucleic acid (*e.g.*, a double-stranded siRNA), (b) a cationic lipid, (c) an aggregation reducing agent (such as a PEG-lipid), (d) optionally a non-cationic lipid (such as a neutral lipid), and (e) optionally a sterol. In one embodiment, the lipid nanoparticle comprises (i) at least one cationic lipid; (ii) a neutral lipid, *e.g.*, DSPC; (iii) a sterol, *e.g.*, cholesterol; and (iv) a PEG-lipid, in a molar ratio of about 20-65% cationic lipid: 5-25% neutral lipid: 25-55% sterol; 0.5-15% PEG-lipid. In some embodiments, the cationic lipid is selected from ATX-002, ATX-081, ATX-095, or ATX-126, as described in WO/2018/222926.

[0090] In some embodiments, a double-stranded siRNA of the invention is delivered via a nanocarrier comprising a molecule enabling specific receptor-mediated endosomal uptake. In one embodiment, said molecule can enable receptor binding, endosomal uptake, controlled breakdown of the endosomal membrane, and release of siRNA into a target cell. Examples of nanocarriers capable of delivering a double-stranded siRNA of the invention are described in WO/2009/141257. In some embodiments, the nanocarrier is a lipid-based nanocarrier, *e.g.*, a lipid nanoparticle (LNP).

DUX4

[0091] In another aspect, the present invention includes a method of reducing expression of DUX4 in a cell comprising contacting the cell with a double-stranded small interfering RNA compound targeted to DUX4. In certain embodiments, DUX4 comprises a nucleic acid sequence at least 85% identical to SEQ ID NO: 593. In certain embodiments, DUX4 comprises a nucleic acid sequence at least 85% complementary to SEQ ID NO: 593.

[0092] The inefficient epigenetic repression of DUX4 in skeletal muscle leads to aberrant expression of the DUX4 protein and facioscapulohumeral muscular dystrophy (FSHD) 1 and 2. FSHD1 and 2 patients exhibit progressive, asymmetric muscle weakness. Therefore, in certain embodiments it is desirable to inhibit expression of DUX4. In certain embodiments it is desirable to inhibit expression of DUX4 in a subject having 10 or fewer D4Z4 repeats.

[0093] In certain embodiments, DUX4 expression is inhibited by contacting a cell with a double-stranded small interfering RNA compound. In certain embodiments, DUX4 expression is inhibited by contacting a cell with a double-stranded small interfering RNA compound disclosed herein.

Pharmaceutical Compositions

[0094] In certain embodiments, the present invention provides pharmaceutical compositions comprising one or more the double-stranded small interfering RNA compounds. In certain embodiments, such pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more antisense compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more antisense compound. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one or more antisense compound and sterile water. In certain embodiments, the sterile saline is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one or more antisense compound and sterile phosphate-buffered saline (PBS). In certain embodiments, the sterile saline is pharmaceutical grade PBS.

[0095] In certain embodiments, the double-stranded small interfering RNA compounds may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0096] Pharmaceutical compositions comprising the double-stranded small interfering RNA compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters. In certain embodiments, pharmaceutical compositions comprising the double-stranded small interfering RNA compounds comprise one or more oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of the double-stranded small interfering RNA compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0097] In certain embodiments, one or more the double-stranded small interfering RNA compounds provided herein is formulated as a prodrug. A prodrug can include the incorporation of additional nucleosides at one or both ends of a double-stranded small interfering RNA compound which are cleaved by endogenous nucleases within the body, to form the active antisense oligomeric compound. In certain embodiments, upon *in vivo* administration, a prodrug is chemically or enzymatically converted to the biologically, pharmaceutically or therapeutically more active form of an oligonucleotide. In certain embodiments, prodrugs are useful because they are easier to administer than the corresponding active form or to be processed by RISC. For example, in certain instances, a prodrug may be more bioavailable (*e.g.*, through oral administration) than is the corresponding active form. In certain instances, a prodrug may have improved solubility compared to the corresponding active form. In certain embodiments, prodrugs are less water soluble than the corresponding active form. In certain instances, such prodrugs possess superior transmittal across cell membranes, where water solubility is detrimental to mobility.

[0098] In certain embodiments, pharmaceutical compositions provided herein comprise one or more the double-stranded small interfering RNA compounds and one or more excipients. In certain such embodiments, excipients are selected from water, salt solutions, alcohol,

polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

Administration and Dosages

[0099] The siRNA molecules and the compositions comprising them may be administered to a subject by any suitable route. For example, the administration may be intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, nasal, or *via* inhalation.

[00100] For example, in certain embodiments, a pharmaceutical composition provided herein is prepared for oral administration. In certain embodiments, a pharmaceutical composition is prepared for administration by injection (*e.g.*, intravenous, subcutaneous, intramuscular, dermal, intraperitoneal etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In certain embodiments, other ingredients are included (*e.g.*, ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

[00101] In certain embodiments, a pharmaceutical composition is prepared for transmucosal administration. In some embodiments, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. In certain embodiments, a pharmaceutical composition is prepared for pulmonary delivery, *e.g.* intratracheal, intranasal or *via* inhalation. Compositions suitable for intranasal preparation can be administered into the nasal cavity as nasal suspensions. Compositions suitable for inhalation may be provided as pharmaceutical aerosols, for example, solution aerosols or powder aerosols,

and may be administered using devices such as inhalers, *e.g.* metered dose inhalers (MDIs) or dry powder inhalers (DPIs), and nebulizers. By controlling the particle characteristics and deposition mechanics, or by specifically targeting certain cell types in the lung, for example by coupling ligands that bind to receptors expressed on the surface of target cells, compositions can be delivered to specific regions in the pulmonary tract. In certain embodiments, a pharmaceutical composition is prepared for intranasal administration. In certain embodiments it is administered via inhalation.

[00102] In certain embodiments, a pharmaceutical composition provided herein comprises the double-stranded small interfering RNA in a therapeutically effective amount. In certain embodiments, the therapeutically effective amount is sufficient to prevent, alleviate or ameliorate symptoms of a disease or to prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. In some embodiments, the administration comprises an effective dose of from 0.01 to 100 mg/kg. The administration may be once daily, weekly, every two weeks, monthly, every two months, or quarterly.

[00103] In certain embodiments, the present invention provides compositions and methods for reducing the amount or activity of a target nucleic acid in a cell. In certain embodiments, the cell is in an animal. In certain embodiments, the animal is a mammal. In certain embodiments, the animal is a rodent. In certain embodiments, the animal is a primate. In certain embodiments, the animal is a non-human primate. In certain embodiments, the animal is a human.

[00104] In certain embodiments, the present invention provides methods of administering a pharmaceutical composition comprising a double-stranded small interfering RNA compound of the present disclosure to an animal. Suitable administration routes include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, through inhalation, intrathecal, intracerebroventricular, intraperitoneal, intranasal, intratumoral, and parenteral (*e.g.*, intravenous, intramuscular, intramedullary, and subcutaneous). In certain embodiments, pharmaceutical intrathecal are administered to achieve local rather than systemic exposures. For example, pharmaceutical compositions may be injected directly in the area of desired effect (*e.g.*, into the ears).

[00105] While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples

serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

[00106] Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

[00107] Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence “ATCGATCG” (SEQ ID NO:689) encompasses any oligomeric compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” (SEQ ID NO: 690) and those having some DNA bases and some RNA bases such as “AUCGATCG” (SEQ ID NO: 691) and oligomeric compounds having other modified or naturally occurring bases, such as “ATmeCGAUCG,” (SEQ ID NO: 692) wherein meC indicates a cytosine base comprising a methyl group at the 5-position.

[00108] Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

[00109] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

[00110] It is understood that the sequence set forth in each SEQ ID NO described herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase.

EXAMPLES

Example 1: Design and Sequences of siRNA Targeting the DUX4 Coding and Upstream D4Z4 Repeat Region

[00111] Double-stranded small interfering RNA (siRNA) targeting the human DUX4 promoter region and coding region were designed *in silico*. The sequences are listed below in Table 1. Each siRNA listed in Table 1 is targeted against the human genomic DUX4 sequence (GENBANK Accession No. FJ439133.1, SEQ ID NO: 593). “Start” indicates the 5'-most nucleoside to which the siRNA is targeted in the genomic DUX4 sequence. “Stop” indicates the 3'-most nucleoside to which the siRNA is targeted in the genomic DUX4 sequence.

Table 1: Double-Stranded siRNA Targeting Human DUX4

Oligo name	SEQ ID NO.	sense	SEQ ID NO:	antisense	Start site	Stop site
UGNX394	1	GGCAGAGAUGGAGAGAGGA	2	UCCUCUCUCCAUCUCUGCC	4605	4623
UGNX482	3	AGGGAGGAACGGAGGGGAAA	4	UUUCCCUCCGUUCCUCCCU	4693	4711
UGNX554	5	GAGGAAGGCAGGGAGGAAA	6	UUUCCUCCUGCCUUCUC	4765	4783
UGNX722	7	CGGUUCCUCCGGGACAAA	8	UUUGUCCCGGAGGAAACCG	4933	4951
UGNX779	9	CGGUUCACAGACCGCACAU	10	AUGUGCGGUCUGUGAACCG	4990	5008
UGNX864	11	GACGACGGAGGCGUGAUUU	12	AAAUCACGCCUCCGUCGUC	5075	5093
UGNX916	13	GCCUGUUGCUCACGUCUCU	14	AGAGACGUGAGCAACAGGC	5127	5145
UGNX950	15	CUGGCCAUGCCGACUGUUU	16	AAACAGUCGGCAUGGCCAG	5161	5179
UGNX991	17	CCGAAACAUGCAGGGAAG	18	CUUCCUGCAUGUUUCCGG	5202	5220
UGNX1032	19	UUCGCUCUCCUUGCCAGGU	20	ACCUGGCAAGGAGAGCGAA	5243	5261
UGNX1094	21	GGAAUCCAUCGUCAGGCCA	22	UGGCCUGACGAUGGAUUC	5305	5323
UGNX1142	23	GUCUCGCUCUGGUCUUCUA	24	UAGAAGACCAGAGCGAGAC	5353	5371
UGNX1223	25	CACAGGCAUUGCCUCCUUC	26	GAAGGAGGCAUUGCCUGUG	5434	5452
UGNX1253	27	GCCUGGCACACUCAAGACU	28	AGUCUUGAGUGUGCCAGGC	5464	5482
UGNX1311	29	AGGCUGGUUUCUCCUGCU	30	AGCAGGGAGAAACCAGCCU	5522	5540
UGNX1440	31	CUUCCUCUUCGUCUCUCCG	32	CGGAGAGACGAAGAGGAAG	5651	5669
UGNX1599	33	CUCACCGCCAUUCAUGAAG	34	CUUCAUGAAUGGCGGUGAG	5810	5828
UGNX1631	35	CUGCCUGUGGGCCUUUACA	36	UGUAAAGGCCACAGGCAG	5842	5860

UGNX1758	37	GCGUCCGUCCGUGAAAUUC	38	GAAUUUCACGGACGGACGC	5969	5987
UGNX1855	39	CGACCGAGACUCGUUUGGA	40	UCCAAACGAGUCUCCGUCG	6066	6084
UGNX1898	41	GAGCCUGCUUUGAGCGGAA	42	UUCCGCUCAAAAGCAGGCUC	6109	6127
UGNX1972	43	GAGCCCAGGGUCCAGAUUU	44	AAAUCUGGACCCUGGGCUC	6183	6201
UGNX2119	45	CUCCUCCGAGCCUUUGAGA	46	UCUCAAAAGGCUCGGAGGAG	6330	6348
UGNX2192	47	UCCCGGAGUCCAGGAUUA	48	UGAAUCCUGGACUCCGGGA	6403	6421
UGNX2909	49	UGCUGCUGGAUGAGCUCCU	50	AGGAGCUCAUCCAGCAGCA	7120	7138
UGNX2952	51	GGCGCAACCUUCCUAGAA	52	UUCUAGGAGAGGUUGCGCC	7163	7181
UGNX3028	53	CUCAGCGAGGAAGAAUACC	54	GGUAUUUUCUCCUCGCUGAG	7239	7257
UGNX3194	55	CUGGGAUUCUGCCUUCUA	56	UAGAAGGCAGGAAUCCAG	7405	7423
UGNX3242	57	GCGGAGAACUGCCAUUCUU	58	AAGAAUGGCAGUUCUCCGC	7453	7471
UGNX395	59	GCAGAGAUGGAGAGAGGAA	60	UUCCUCUCUCCAUCUCUG	4606	4624
UGNX396	61	CAGAGAUGGAGAGAGGAAC	62	GUUCCUCUCUCCAUCUCUG	4607	4625
UGNX397	63	AGAGAUGGAGAGAGGAACG	64	CGUUCUUCUCUCCAUCUCU	4608	4626
UGNX398	65	GAGAUGGAGAGAGGAACGG	66	CCGUUCCUCUCUCCAUCUC	4609	4627
UGNX399	67	GAUGGAGAGAGGAACGGG	68	CCCGUCCUCUCUCCAUCU	4610	4628
UGNX400	69	GAUGGAGAGAGGAACGGGA	70	UCCCGUCCUCUCUCCAUC	4611	4629
UGNX401	71	AUGGAGAGAGGAACGGGAG	72	CUCCCGUCCUCUCUCCAUC	4612	4630
UGNX402	73	UGGAGAGAGGAACGGGAGA	74	UCUCCCGUCCUCUCUCCA	4613	4631
UGNX405	75	AGAGAGGAACGGGAGACCU	76	AGGUCUCCCGUCCUCUCU	4616	4634
UGNX406	77	GAGAGGAACGGGAGACCUA	78	UAGGUCUCCCGUCCUCUC	4617	4635
UGNX407	79	AGAGGAACGGGAGACCUAG	80	CUAGGUCUCCCGUCCUCU	4618	4636
UGNX408	81	GAGGAACGGGAGACCUAGA	82	UCUAGGUCUCCCGUCCUC	4619	4637
UGNX409	83	AGGAACGGGAGACCUAGAG	84	CUCUAGGUCUCCCGUCCUC	4620	4638
UGNX484	85	GGAGGAACGGAGGGAAAGA	86	UCUUUCCCUCCGUUCCUC	4695	4713
UGNX485	87	GAGGAACGGAGGGAAAGAC	88	GUCUUUCCCUCCGUUCCUC	4696	4714
UGNX486	89	AGGAACGGAGGGAAAGACA	90	UGUCUUUCCCUCCGUUCCUC	4697	4715
UGNX487	91	GGAACGGAGGGAAAGACAG	92	CUGUCUUUCCCUCCGUUCC	4698	4716
UGNX488	93	GAACGGAGGGAAAGACAGA	94	UCUGUCUUUCCCUCCGUUCC	4699	4717
UGNX489	95	AACGGAGGGAAAGACAGAG	96	CUCUGUCUUUCCCUCCGUU	4700	4718
UGNX490	97	ACGGAGGGAAAGACAGAGC	98	GCUCUGUCUUUCCCUCCGU	4701	4719
UGNX492	99	GGAGGGAAAGACAGAGCGA	100	UCGCUCUGUCUUUCCCUCC	4703	4721
UGNX493	101	GAGGGAAAGACAGAGCGAC	102	GUCGUCUGUCUUUCCCUCC	4704	4722
UGNX494	103	AGGGAAAGACAGAGCGACG	104	CGUCGUCUCUGUCUUUCCCU	4705	4723
UGNX496	105	GGAAAGACAGAGCGACGCA	106	UGCGUCGUCUCUGUCUUUCC	4707	4725
UGNX497	107	GAAAGACAGAGCGACGCA	108	CUGCGUCGUCUCUGUCUUUCC	4708	4726
UGNX498	109	AAAGACAGAGCGACGCA	110	CCUGCGUCGUCUCUGUCUUU	4709	4727
UGNX780	111	GGUUCACAGACCGCACAUCC	112	GAUGUGCGGUCUGUGAAC	4991	5009
UGNX781	113	GUUCACAGACCGCACAUCC	114	GGAUGUGCGGUCUGUGAAC	4992	5010
UGNX782	115	UUCACAGACCGCACAUCCC	116	GGGAUGUGCGGUCUGUGAA	4993	5011
UGNX917	117	CCUGUUGCUCACGUCUCUC	118	GAGAGACGUGAGCAACAGG	5128	5146
UGNX918	119	CUGUUGCUCACGUCUCUCC	120	GGAGAGACGUGAGCAACAG	5129	5147
UGNX919	121	UGUUGCUCACGUCUCUCCG	122	CGGAGAGACGUGAGCAACA	5130	5148
UGNX954	123	CCAUGCCGACUGUUUGCUC	124	GAGCAAACAGUCGGCAUGG	5165	5183
UGNX955	125	CAUGCCGACUGUUUGCUC	126	GGAGCAAACAGUCGGCAUG	5166	5184
UGNX956	127	AUGCCGACUGUUUGCUC	128	GGGAGCAAACAGUCGGCAU	5167	5185
UGNX964	129	UGUUUGCUCUCCGGAGCUCU	130	AGAGCUCCGGAGCAAACA	5175	5193
UGNX990	131	CCCGGAAACAUGCAGGGAA	132	UUCCUGCAUGUUUCCGGG	5201	5219
UGNX992	133	CGGAAACAUGCAGGGAAAGG	134	CCUCCUGCAUGUUUCCG	5203	5221
UGNX993	135	GGAAACAUGCAGGGAAAGGG	136	CCCUCCUGCAUGUUUCC	5204	5222
UGNX994	137	GAAACAUGCAGGGAAAGGGU	138	ACCCUCCUGCAUGUUUCC	5205	5223
UGNX995	139	AAACAUGCAGGGAAAGGGUG	140	CACCCUCCUGCAUGUUU	5206	5224
UGNX996	141	AACAUGCAGGGAAAGGGUGC	142	GCACCCUCCUGCAUGUU	5207	5225
UGNX998	143	CAUGCAGGGAAAGGGUGCAA	144	UUGCACCCUCCUGCAUG	5209	5227
UGNX999	145	AUGCAGGGAAAGGGUGCAAG	146	CUUGCACCCUCCUGCAU	5210	5228
UGNX1033	147	UCGCUCUCCUUGCCAGGUU	148	AACCUGGCAAGGAGAGCGA	5244	5262
UGNX1036	149	CUCUCCUUGCCAGGUCCA	150	UGGAACCUGGCAAGGAGAG	5247	5265

UGNX1037	151	UCUCCUUGCCAGGUUCCAA	152	UUGGAACCUUGGCAAGGAGA	5248	5266
UGNX1038	153	CUCCUUGCCAGGUUCCAAA	154	UUUGGAACCUUGGCAAGGAG	5249	5267
UGNX1039	155	UCCUUGCCAGGUUCCAAAC	156	GUUUGGAACCUUGGCAAGGA	5250	5268
UGNX1040	157	CCUUGCCAGGUUCCAAACC	158	GGUUUGGAACCUUGGCAAGG	5251	5269
UGNX1041	159	CUUGCCAGGUUCCAAACCG	160	CGGUUUGGAACCUUGGCAAG	5252	5270
UGNX1042	161	UUGCCAGGUUCCAAACCGG	162	CCGGUUUGGAACCUUGGCAA	5253	5271
UGNX1049	163	GUUCCAAACCGGCCACACU	164	AGUGUGGCCCGUUUGGAAC	5260	5278
UGNX1050	165	UUCCAAACCGGCCACACUG	166	CAGUGUGGCCCGUUUGGAA	5261	5279
UGNX1095	167	GAAUCCAUCGUCAGGCCAU	168	AUGGCCUGACGAUGGAUUC	5306	5324
UGNX1096	169	AAUCCAUCGUCAGGCCAUC	170	GAUGGCCUGACGAUGGAUU	5307	5325
UGNX1097	171	AUCCAUCGUCAGGCCAUCA	172	UGAUGGCCUGACGAUGGAU	5308	5326
UGNX1098	173	UCCAUCGUCAGGCCAUAC	174	GUGAUGGCCUGACGAUGGA	5309	5327
UGNX1143	175	UCUCGUCUGGUCUUCUAC	176	GUAGAAGACCAGAGCGAGA	5354	5372
UGNX1144	177	CUCGUCUGGUCUUCUACG	178	CGUAGAAGACCAGAGCGAG	5355	5373
UGNX1145	179	UCGUCUGGUCUUCUACGU	180	ACGUAGAAGACCAGAGCGA	5356	5374
UGNX1146	181	CGCUCUGGUCUUCUACGUG	182	CACGUAGAAGACCAGAGCG	5357	5375
UGNX1147	183	GCUCUGGUCUUCUACGUGG	184	CCACGUAGAAGACCAGAGC	5358	5376
UGNX1148	185	CUCUGGUCUUCUACGUGGA	186	UCCACGUAGAAGACCAGAG	5359	5377
UGNX1149	187	UCUGGUCUUCUACGUGGAA	188	UUCCACGUAGAAGACCAGA	5360	5378
UGNX1150	189	CUGGUCUUCUACGUGGAAA	190	UUUCCACGUAGAAGACCAG	5361	5379
UGNX1151	191	UGGUCUUCUACGUGGAAAU	192	AUUUCCACGUAGAAGACCA	5362	5380
UGNX1152	193	GGUCUUCUACGUGGAAAU	194	CAUUUCCACGUAGAAGACC	5363	5381
UGNX1153	195	GUCUUCUACGUGGAAAU	196	UCAUUUCCACGUAGAAGAC	5364	5382
UGNX1154	197	UCUUCUACGUGGAAAU	198	UUCAUUUCCACGUAGAAGA	5365	5383
UGNX1155	199	CUUCUACGUGGAAAU	200	GUUCAUUUCCACGUAGAAG	5366	5384
UGNX1156	201	UUCUACGUGGAAAU	202	CGUUCAUUUCCACGUAGAA	5367	5385
UGNX1157	203	UCUACGUGGAAAU	204	UCGUUCAUUUCCACGUAGA	5368	5386
UGNX1158	205	CUACGUGGAAAU	206	CUCGUUCAUUUCCACGUAG	5369	5387
UGNX1159	207	UACGUGGAAAU	208	UCUCGUUCAUUUCCACGUA	5370	5388
UGNX1160	209	ACGUGGAAAU	210	CUCUCGUUCAUUUCCACGU	5371	5389
UGNX1161	211	CGUGGAAAU	212	GCUCUCGUUCAUUUCCACG	5372	5390
UGNX1162	213	GUGGAAAU	214	GGCUCUCGUUCAUUUCCAC	5373	5391
UGNX1163	215	UGGAAAU	216	UGGCUCUCGUUCAUUUCCA	5374	5392
UGNX1164	217	GGAAAU	218	GUGGCUCUCGUUCAUUUCC	5375	5393
UGNX1165	219	GAAAU	220	UGUGGCUCUCGUUCAUUUC	5376	5394
UGNX1166	221	AAAU	222	GUGUGGCUCUCGUUCAUUU	5377	5395
UGNX1167	223	AAU	224	CGUGUGGCUCUCGUUCAUU	5378	5396
UGNX1168	225	AUA	226	GCGUGUGGCUCUCGUUCAU	5379	5397
UGNX1222	227	CCACAGGCAUUGCCUCCU	228	AAGGAGGCAAUGCCUGUGG	5433	5451
UGNX1224	229	ACAGGCAUUGCCUCCU	230	UGAAGGAGGCAAUGCCUGU	5435	5453
UGNX1225	231	CAGGCAUUGCCUCCU	232	GUGAAGGAGGCAAUGCCUG	5436	5454
UGNX1226	233	AGGCAUUGCCUCCU	234	CGUGAAGGAGGCAAUGCCU	5437	5455
UGNX1228	235	GCAUUGCCUCCU	236	UCCGUGAAGGAGGCAAUGC	5439	5457
UGNX1229	237	CAUUGCCUCCU	238	CUCCGUGAAGGAGGCAAUG	5440	5458
UGNX1230	239	AUUGCCUCCU	240	UCUCCGUGAAGGAGGCAAU	5441	5459
UGNX1231	241	UUGCCUCCU	242	CUCUCCGUGAAGGAGGCAA	5442	5460
UGNX1232	243	UGCCUCCU	244	UCUCUCCGUGAAGGAGGCA	5443	5461
UGNX1254	245	CCUGGCACACUCAAGACUC	246	GAGUCUUGAGUGUGCCAGG	5465	5483
UGNX1255	247	CUGGCACACUCAAGACUCC	248	GGAGUCUUGAGUGUGCCAG	5466	5484
UGNX1256	249	UGGCACACUCAAGACUCCC	250	GGGAGUCUUGAGUGUGCCA	5467	5485
UGNX1257	251	GGCACACUCAAGACUCCCA	252	UGGGAGUCUUGAGUGUGCC	5468	5486
UGNX1258	253	GCACACUCAAGACUCCCA	254	GUGGGAGUCUUGAGUGUGC	5469	5487
UGNX1259	255	CACACUCAAGACUCCCA	256	CGUGGGAGUCUUGAGUGUG	5470	5488
UGNX1260	257	ACACUCAAGACUCCCA	258	CCGUGGGAGUCUUGAGUGU	5471	5489
UGNX1261	259	CACUCAAGACUCCCA	260	UCCGUGGGAGUCUUGAGUG	5472	5490
UGNX1262	261	ACUCAAGACUCCCA	262	CUCCGUGGGAGUCUUGAGU	5473	5491
UGNX1264	263	UCAAGACUCCCA	264	ACCUCGUGGGAGUCUUGA	5475	5493

UGNX1265	265	CAAGACUCCACGGAGGUU	266	AACCUCGUGGGAGUCUUG	5476	5494
UGNX1266	267	AAGACUCCACGGAGGUUC	268	GAACCUCCGUGGGAGUCUU	5477	5495
UGNX1267	269	AGACUCCACGGAGGUUCA	270	UGAACCUCCGUGGGAGUCU	5478	5496
UGNX1269	271	ACUCCACGGAGGUUCAGU	272	ACUGAACCUCCGUGGGAGU	5480	5498
UGNX1270	273	CUCCACGGAGGUUCAGUU	274	AACUGAACCUCCGUGGGAG	5481	5499
UGNX1271	275	UCCACGGAGGUUCAGUUC	276	GAACUGAACCUCCGUGGGA	5482	5500
UGNX1273	277	CCACGGAGGUUCAGUCCA	278	UGGAACUGAACCUCCGUGG	5484	5502
UGNX1274	279	CACGGAGGUUCAGUCCAC	280	GUGGAACUGAACCUCCGUG	5485	5503
UGNX1275	281	ACGGAGGUUCAGUCCACA	282	UGUGGAACUGAACCUCCGU	5486	5504
UGNX1276	283	CGGAGGUUCAGUCCACAC	284	GUGUGGAACUGAACCUCCG	5487	5505
UGNX1277	285	GGAGGUUCAGUCCACACU	286	AGUGUGGAACUGAACCUCC	5488	5506
UGNX1278	287	GAGGUUCAGUCCACACUC	288	GAGUGUGGAACUGAACCUCC	5489	5507
UGNX1279	289	AGGUUCAGUCCACACUCC	290	GGAGUGUGGAACUGAACCU	5490	5508
UGNX1280	291	GGUUCAGUCCACACUCCC	292	GGGAGUGUGGAACUGAACCC	5491	5509
UGNX1441	293	UUCUCUUCGUCUCUCCGG	294	CCGGAGAGACGAAGAGGAA	5652	5670
UGNX1598	295	GCUCACCGCCAUUCAUGAA	296	UUCAUGAAUGGCGUGAGC	5809	5827
UGNX1600	297	UCACCGCCAUUCAUGAAGG	298	CCUUCAGAAUGGCGGUGA	5811	5829
UGNX1601	299	CACCGCCAUUCAUGAAGGG	300	CCCUUCAGAAUGGCGGUG	5812	5830
UGNX1632	301	UGCCUGUGGGCCUUUACAA	302	UUGUAAAGGCCACAGGCA	5843	5861
UGNX1633	303	GCCUGUGGGCCUUUACAAG	304	CUUGUAAAGGCCACAGGC	5844	5862
UGNX1634	305	CCUGUGGGCCUUUACAAGG	306	CCUUGUAAAGGCCACAGG	5845	5863
UGNX1635	307	CUGUGGGCCUUUACAAGGG	308	CCCUUGUAAAGGCCACAG	5846	5864
UGNX1636	309	UGUGGGCCUUUACAAGGGC	310	GCCCUUGUAAAGGCCACA	5847	5865
UGNX1759	311	CGUCCGUCCGUGAAAUUCC	312	GGAAUUUCACGGACGGACG	5970	5988
UGNX1760	313	GUCCGUCCGUGAAAUUCCG	314	CGGAUUUCACGGACGGAC	5971	5989
UGNX1761	315	UCCGUCCGUGAAAUUCCGG	316	CCGGAUUUCACGGACGGA	5972	5990
UGNX1856	317	GACGGAGACUCGUUUGGAC	318	GUCCAAACGAGUCUCCGUC	6067	6085
UGNX1857	319	ACGGAGACUCGUUUGGACC	320	GGUCCAAACGAGUCUCCGU	6068	6086
UGNX1899	321	AGCCUGCUUUGAGCGGAAC	322	GUUCCGCUCAAAGCAGGCU	6110	6128
UGNX1904	323	GCUUUGAGCGGAACCCGUA	324	UACGGGUUCCGCUCAAAGC	6115	6133
UGNX1905	325	CUUUGAGCGGAACCCGUAC	326	GUACGGGUUCCGCUCAAAG	6116	6134
UGNX1906	327	UUUGAGCGGAACCCGUACC	328	GGUACGGGUUCCGCUCAA	6117	6135
UGNX1975	329	CCCAGGGUCCAGAUUUGGU	330	ACCAAUCUGGACCCUGGG	6186	6204
UGNX1976	331	CCAGGGUCCAGAUUUGGUU	332	AACCAAUCUGGACCCUGG	6187	6205
UGNX1977	333	CAGGGUCCAGAUUUGGUUU	334	AAACCAAUCUGGACCCUG	6188	6206
UGNX1978	335	AGGGUCCAGAUUUGGUUUC	336	GAAACCAAUCUGGACCCU	6189	6207
UGNX1979	337	GGGUCCAGAUUUGGUUUC	338	UGAAACCAAUCUGGACCC	6190	6208
UGNX1980	339	GGUCCAGAUUUGGUUUCAG	340	CUGAAACCAAUCUGGACC	6191	6209
UGNX1981	341	GUCCAGAUUUGGUUUCAGA	342	UCUGAAACCAAUCUGGAC	6192	6210
UGNX1982	343	UCCAGAUUUGGUUUCAGAA	344	UUCUGAAACCAAUCUGGA	6193	6211
UGNX1983	345	CCAGAUUUGGUUUCAGAAU	346	AUUCUGAAACCAAUCUGG	6194	6212
UGNX1984	347	CAGAUUUGGUUUCAGAAUG	348	CAUUCUGAAACCAAUCUG	6195	6213
UGNX1985	349	AGAUUUGGUUUCAGAAUGA	350	UCAUUCUGAAACCAAUCU	6196	6214
UGNX1986	351	GAUUGGUUUCAGAAUGAG	352	CUCAUUCUGAAACCAAUC	6197	6215
UGNX1987	353	AUUUGGUUUCAGAAUGAGA	354	UCUCAUUCUGAAACCAAU	6198	6216
UGNX1988	355	UUUGGUUUCAGAAUGAGAG	356	CUCUCAUUCUGAAACCAA	6199	6217
UGNX1989	357	UUGGUUUCAGAAUGAGAGG	358	CCUCUCAUUCUGAAACCAA	6200	6218
UGNX1990	359	UGGUUUCAGAAUGAGAGGU	360	ACCUCUCAUUCUGAAACCA	6201	6219
UGNX1991	361	GGUUUCAGAAUGAGAGGUC	362	GACCUCUCAUUCUGAAACC	6202	6220
UGNX1992	363	GUUUCAGAAUGAGAGGUCA	364	UGACCUUCAUUCUGAAAC	6203	6221
UGNX1993	365	UUUCAGAAUGAGAGGUCAC	366	GUGACCUUCAUUCUGAAA	6204	6222
UGNX1994	367	UUCAGAAUGAGAGGUCACG	368	CGUGACCUUCAUUCUGAA	6205	6223
UGNX1995	369	UCAGAAUGAGAGGUCACGC	370	GCGUGACCUUCAUUCUGA	6206	6224
UGNX1996	371	CAGAAUGAGAGGUCACGCC	372	GGCGUGACCUUCAUUCUG	6207	6225
UGNX1997	373	AGAAUGAGAGGUCACGCCA	374	UGGCGUGACCUUCAUUCU	6208	6226
UGNX1998	375	GAAUGAGAGGUCACGCCAG	376	CUGGCGUGACCUUCAUUC	6209	6227
UGNX1999	377	AAUGAGAGGUCACGCCAGC	378	GCUGGCGUGACCUUCAUU	6210	6228

UGNX2000	379	AUGAGAGGUCACGCCAGCU	380	AGCUGGCGUGACCUCUCAU	6211	6229
UGNX2117	381	UGCUCUCCGAGCCUUUGA	382	UCAAAGGCUCGGAGGAGCA	6328	6346
UGNX2120	383	UCCUCCGAGCCUUUGAGAA	384	UUCUCAAAAGGCUCGGAGGA	6331	6349
UGNX2121	385	CCUCCGAGCCUUUGAGAAG	386	CUUCUCAAAAGGCUCGGAGG	6332	6350
UGNX2122	387	CUCCGAGCCUUUGAGAAGG	388	CCUUCUCAAAAGGCUCGGAG	6333	6351
UGNX2123	389	UCCGAGCCUUUGAGAAGGA	390	UCCUUCUCAAAAGGCUCGGA	6334	6352
UGNX2124	391	CCGAGCCUUUGAGAAGGAU	392	AUCCUUCUCAAAAGGCUCGG	6335	6353
UGNX2125	393	CGAGCCUUUGAGAAGGAUC	394	GAUCCUUCUCAAAAGGCUCG	6336	6354
UGNX2126	395	GAGCCUUUGAGAAGGAUCG	396	CGAUCCUUCUCAAAAGGCUC	6337	6355
UGNX2127	397	AGCCUUUGAGAAGGAUCGC	398	GCGAUCCUUCUCAAAAGGCUC	6338	6356
UGNX2128	399	GCCUUUGAGAAGGAUCGCU	400	AGCGAUCCUUCUCAAAAGGC	6339	6357
UGNX2129	401	CCUUUGAGAAGGAUCGCUU	402	AAGCGAUCCUUCUCAAAAGG	6340	6358
UGNX2130	403	CUUUGAGAAGGAUCGCUUU	404	AAAGCGAUCCUUCUCAAAAG	6341	6359
UGNX2131	405	UUUGAGAAGGAUCGCUUUC	406	GAAAGCGAUCCUUCUCAAA	6342	6360
UGNX2132	407	UUGAGAAGGAUCGCUUUC	408	GGAAAGCGAUCCUUCUCA	6343	6361
UGNX2133	409	UGAGAAGGAUCGCUUCCA	410	UGGAAAGCGAUCCUUCUCA	6344	6362
UGNX2134	411	GAGAAGGAUCGCUUCCAG	412	CUGGAAAGCGAUCCUUCUC	6345	6363
UGNX2135	413	AGAAGGAUCGCUUCCAGG	414	CCUGGAAAGCGAUCCUUCU	6346	6364
UGNX2136	415	GAAGGAUCGCUUCCAGGC	416	GCCUGGAAAGCGAUCCUUC	6347	6365
UGNX2137	417	AAGGAUCGCUUCCAGGCA	418	UGCCUGGAAAGCGAUCCU	6348	6366
UGNX2138	419	AGGAUCGCUUCCAGGCAU	420	AUGCCUGGAAAGCGAUCCU	6349	6367
UGNX2139	421	GGAUCGCUUCCAGGCAUC	422	GAUGCCUGGAAAGCGAUCC	6350	6368
UGNX2140	423	GAUCGCUUCCAGGCAUCG	424	CGAUGCCUGGAAAGCGAUC	6351	6369
UGNX2194	425	CCGGAGUCCAGGAUUCAGA	426	UCUGAAUCCUGGACUCCGG	6405	6423
UGNX2195	427	CGGAGUCCAGGAUUCAGAU	428	AUCUGAAUCCUGGACUCCG	6406	6424
UGNX2196	429	GGAGUCCAGGAUUCAGAU	430	GAUCUGAAUCCUGGACUCC	6407	6425
UGNX2197	431	GAGUCCAGGAUUCAGAU	432	AGAUCUGAAUCCUGGACUC	6408	6426
UGNX2198	433	AGUCCAGGAUUCAGAU	434	CAGAUUCUGAAUCCUGGACU	6409	6427
UGNX2199	435	GUCCAGGAUUCAGAU	436	CCAGAUUCUGAAUCCUGGAC	6410	6428
UGNX2200	437	UCCAGGAUUCAGAU	438	ACCAGAUUCUGAAUCCUGGA	6411	6429
UGNX2201	439	CCAGGAUUCAGAU	440	AACCAGAUUCUGAAUCCUGG	6412	6430
UGNX2202	441	CAGGAUUCAGAU	442	AAACCAGAUUCUGAAUCCUG	6413	6431
UGNX2203	443	AGGAUUCAGAU	444	GAAACCAGAUUCUGAAUCCU	6414	6432
UGNX2204	445	GGAUUCAGAU	446	UGAAACCAGAUUCUGAAUCC	6415	6433
UGNX2205	447	GAUUCAGAU	448	CUGAAACCAGAUUCUGAAUC	6416	6434
UGNX2206	449	AUUCAGAU	450	UCUGAAACCAGAUUCUGAAU	6417	6435
UGNX2207	451	UUCAGAU	452	UUCUGAAACCAGAUUCUGAA	6418	6436
UGNX2208	453	UCAGAU	454	AUUCUGAAACCAGAUUCUGA	6419	6437
UGNX2209	455	CAGAU	456	GAUUCUGAAACCAGAUUCUG	6420	6438
UGNX2210	457	AGAUC	458	CGAUUCUGAAACCAGAUUCU	6421	6439
UGNX2211	459	GAUC	460	UCGAUUCUGAAACCAGAU	6422	6440
UGNX2212	461	AUC	462	UUCGAUUCUGAAACCAGAU	6423	6441
UGNX2213	463	UCUG	464	CUUCGAUUCUGAAACCAGA	6424	6442
UGNX2214	465	CUGG	466	CCUUCGAUUCUGAAACCAG	6425	6443
UGNX2215	467	UGGU	468	CCCUUCGAUUCUGAAACCA	6426	6444
UGNX2216	469	GGUU	470	GCCCUUCGAUUCUGAAACC	6427	6445
UGNX2217	471	GUUUC	472	GGCCCUUCGAUUCUGAAAC	6428	6446
UGNX2218	473	UUUC	474	UGGCCCUUCGAUUCUGAAA	6429	6447
UGNX2219	475	UUC	476	CUGGCCCUUCGAUUCUGAA	6430	6448
UGNX2220	477	UC	478	CCUGGCCCUUCGAUUCUGA	6431	6449
UGNX2222	479	AG	480	UGCCUGGCCCUUCGAUUCU	6433	6451
UGNX2951	481	AGGCGCAACCUCUCCUAGA	482	UCUAGGAGAGGUUGCGCCU	7162	7180
UGNX2953	483	GCGCAACCUCUCCUAGAAA	484	UUUCUAGGAGAGGUUGCGC	7164	7182
UGNX2954	485	CGCAACCUCUCCUAGAAAC	486	GUUUCUAGGAGAGGUUGCG	7165	7183
UGNX2955	487	GCAACCUCUCCUAGAAACG	488	CGUUUCUAGGAGAGGUUGC	7166	7184
UGNX2956	489	CAACCUCUCCUAGAAACGG	490	CCGUUUCUAGGAGAGGUUG	7167	7185
UGNX2957	491	AACCUCUCCUAGAAACGGA	492	UCCGUUUCUAGGAGAGGUU	7168	7186

UGNX2958	493	ACCUCUCCUAGAAACGGAG	494	CUCCGUUUUCUAGGAGAGGU	7169	7187
UGNX2959	495	CCUCUCCUAGAAACGGAGG	496	CCUCCGUUUUCUAGGAGAGG	7170	7188
UGNX2960	497	CUCUCCUAGAAACGGAGGC	498	GCCUCCGUUUUCUAGGAGAG	7171	7189
UGNX2961	499	UCUCCUAGAAACGGAGGCC	500	GGCCUCCGUUUUCUAGGAGA	7172	7190
UGNX3029	501	UCAGCGAGGAAGAAUACCG	502	CGGUUUUCUCCUCGCU	7240	7258
UGNX3030	503	CAGCGAGGAAGAAUACCGG	504	CCGGUAAUUCUCCUCGCU	7241	7259
UGNX3031	505	AGCGAGGAAGAAUACCGGG	506	CCCGGUAAUUCUCCUCGCU	7242	7260
UGNX3033	507	CGAGGAAGAAUACCGGGCU	508	AGCCCGGUAAUUCUCCUCG	7244	7262
UGNX3034	509	GAGGAAGAAUACCGGGCUC	510	GAGCCCGGUAAUUCUCCUC	7245	7263
UGNX3035	511	AGGAAGAAUACCGGGCUCU	512	AGAGCCCGGUAAUUCUCCU	7246	7264
UGNX3036	513	GGAAGAAUACCGGGCUCUG	514	CAGAGCCCGGUAAUUCUCC	7247	7265
UGNX3037	515	GAAGAAUACCGGGCUCUGC	516	GCAGAGCCCGGUAAUUCUUC	7248	7266
UGNX3038	517	AAGAAUACCGGGCUCUGCU	518	AGCAGAGCCCGGUAAUUCU	7249	7267
UGNX3039	519	AGAAUACCGGGCUCUGCUG	520	CAGCAGAGCCCGGUAAUUCU	7250	7268
UGNX3041	521	AAUACCGGGCUCUGCUGGA	522	UCCAGCAGAGCCCGGUAAU	7252	7270
UGNX3049	523	GCUCUGCUGGAGGAGCUUU	524	AAAGCUCCUCCAGCAGAGC	7260	7278
UGNX3050	525	CUCUGCUGGAGGAGCUUUA	526	UAAAGCUCCUCCAGCAGAG	7261	7279
UGNX3051	527	UCUGCUGGAGGAGCUUUAG	528	CUAAAGCUCCUCCAGCAGA	7262	7280
UGNX3052	529	CUGCUGGAGGAGCUUUAGG	530	CCUAAAGCUCCUCCAGCAG	7263	7281
UGNX3053	531	UGCUGGAGGAGCUUUAGGA	532	UCCUAAAGCUCCUCCAGCA	7264	7282
UGNX3054	533	GCUGGAGGAGCUUUAGGAC	534	GUCCUAAAGCUCCUCCAGC	7265	7283
UGNX3055	535	CUGGAGGAGCUUUAGGACG	536	CGUCCUAAAGCUCCUCCAG	7266	7284
UGNX3056	537	UGGAGGAGCUUUAGGACGC	538	GCGUCCUAAAGCUCCUCCA	7267	7285
UGNX3193	539	CCUGGGAUUCUGCCUUCU	540	AGAAGGCAGGAAUCCAGG	7404	7422
UGNX3195	541	UGGGAUUCUGCCUUCUAG	542	CUAGAAGGCAGGAAUCCCA	7406	7424
UGNX3196	543	GGGAUUCUGCCUUCUAGG	544	CCUAGAAGGCAGGAAUCCC	7407	7425
UGNX3197	545	GGAUUCUGCCUUCUAGGU	546	ACCUAGAAGGCAGGAAUCC	7408	7426
UGNX3198	547	GAUUCUGCCUUCUAGGUC	548	GACCUAGAAGGCAGGAAUC	7409	7427
UGNX3199	549	AUUCUGCCUUCUAGGUCU	550	AGACCUAGAAGGCAGGAAU	7410	7428
UGNX3200	551	UUCUGCCUUCUAGGUCUA	552	UAGACCUAGAAGGCAGGAA	7411	7429
UGNX3201	553	UCCUGCCUUCUAGGUCUAG	554	CUAGACCUAGAAGGCAGGA	7412	7430
UGNX3202	555	CCUGCCUUCUAGGUCUAGG	556	CCUAGACCUAGAAGGCAGG	7413	7431
UGNX3203	557	CUGCCUUCUAGGUCUAGGC	558	GCCUAGACCUAGAAGGCAG	7414	7432
UGNX3204	559	UGCCUUCUAGGUCUAGGCC	560	GGCCUAGACCUAGAAGGCA	7415	7433
UGNX3208	561	UUCUAGGUCUAGGCCCGGU	562	ACCGGGCCUAGACCUAGAA	7419	7437
UGNX3241	563	CGCGGAGAACUGCCAUCU	564	AGAAUGGCAGUUCUCCGCG	7452	7470
UGNX3243	565	CGGAGAACUGCCAUCUUCU	566	AAAGAAUGGCAGUUCUCCG	7454	7472
UGNX3244	567	GGAGAACUGCCAUCUUCU	568	GAAAGAAUGGCAGUUCUCC	7455	7473
UGNX3245	569	GAGAACUGCCAUCUUCUCC	570	GGAAAGAAUGGCAGUUCUC	7456	7474
UGNX3246	571	AGAACUGCCAUCUUCUCCU	572	AGGAAAGAAUGGCAGUUCU	7457	7475
UGNX3247	573	GAACUGCCAUCUUCUCCUG	574	CAGGAAAGAAUGGCAGUUC	7458	7476
UGNX3248	575	AACUGCCAUCUUCUCCUGG	576	CCAGGAAAGAAUGGCAGUU	7459	7477
UGNX3249	577	ACUGCCAUCUUCUCCUGGG	578	CCCAGGAAAGAAUGGCAGU	7460	7478
UGNX3250	579	CUGCCAUCUUCUCCUGGGC	580	GCCCAGGAAAGAAUGGCAG	7461	7479
UGNX3251	581	UGCCAUCUUCUCCUGGGCA	582	UGCCCAGGAAAGAAUGGCA	7462	7480
UGNX3252	583	GCCAUCUUCUCCUGGGCAU	584	AUGCCCAGGAAAGAAUGGC	7463	7481
UGNX3253	585	CCAUCUUCUCCUGGGCAUC	586	GAUGCCCAGGAAAGAAUGG	7464	7482
UGNX3254	587	CAUCUUCUCCUGGGCAUCC	588	GGAUGCCCAGGAAAGAAUG	7465	7483
UGNX3255	589	AUUCUUCUCCUGGGCAUCC	590	GGGAUGCCCAGGAAAGAAU	7466	7484
UGNX3256	591	UUCUUCUCCUGGGCAUCCCG	592	CGGAUGCCCAGGAAAGAA	7467	7485

Example 2: Silencing of DUX4 Gene by Double Stranded siRNA

[00112] The double-stranded small interfering RNA (siRNA) listed in Table 1 were tested for inhibition of DUX4 gene expression *in vitro* as described below.

[00113] Cells and Cell Culture. Immortalized human FSHD1 (54-2) (Krom *et al.*, 2012) and FSHD2 (MB200) (Stadler *et al.*, 2011) cell lines were used. Immortalized myoblasts were grown in Ham's F-10 Nutrient Mix (Gibco, Waltham, MA, USA) supplemented with 20% Corning USDA Approved Source Fetal Bovine Serum (Corning, Corning, NY, USA), 100 U/100 µg penicillin/streptomycin (Gibco), 10 ng/ml recombinant human fibroblast growth factor (Promega Corporation, Madison, WI, USA) and 1 µM dexamethasone (Sigma-Aldrich). Differentiation of myoblasts into myotubes was achieved by switching confluent myoblast monolayers into DMEM:F-12 Nutrient Mixture (1:1, Gibco) supplemented with 2% KnockOut Serum Replacement (Gibco), 100 U/100 µg penicillin/streptomycin, 10 µg/ml insulin and 10 µg/ml transferrin (KSR media) for 40 hours.

[00114] Small interfering RNA (siRNA) transfections. Unmodified duplex 21-mer siRNAs listed in Table 1 containing 19 base-pair complementary sequences and dTdT 3 prime overhangs were synthesized and obtained from Thermo Fisher Scientific or Integrated DNA Technologies. Transfections of siRNAs into FSHD1 and FSHD2 myoblasts were carried out using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. Briefly, cells were seeded at 1×10^5 cells/well in 12-well plates and transfected approximately 20 hours later with 2 µl Lipofectamine RNAiMAX and 10 pmol or less of either gene-specific siRNAs or a scrambled non-silencing control siRNA diluted in 100 µl Opti-MEM Reduced Serum Medium. 24, 48 or 72 hours following transfection, cells were incubated in differentiation medium for 40 hours to induce differentiation into myotubes and harvested for total RNA analysis.

[00115] Measurement of DUX4 target gene expression in FSHD1 and FSHD2 myotubes. Total RNA was extracted from whole cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Isolated RNA was treated with DNase I (Thermo Fisher Scientific), heat inactivated and reverse transcribed into cDNA using Superscript III (Thermo Fisher Scientific) and oligo(dT) primers (Invitrogen) following the manufacturer's protocol. qPCR was performed on cDNA to measure expression of *DUX4*. Expression of *DUX4* can be measured by measuring expression of genes that are upregulated by *DUX4*, e.g., *MBD3L2*, *ZSCAN4*, *LEUTX*, *MYOG*, and *MYH2* using TaqMan Gene

Expression Assay ID numbers: MBD3L2, Hs00544743_m1; MYH2, Hs00430042_m1; MYOG, Hs01072232_m1; RPL30, Hs00265497_m1; LEUTX, Hs01028718_m1; ZSCAN4, Hs00537549_m1; or DUX4 with primers GCCGCCCAGGTACCA (SEQ ID NO: 594) and CAGCGAGCTCCCTTGCA (SEQ ID NO: 595) with probe CAGTGCGCACCCCG (SEQ ID NO: 596) having a florescent dye (6FAM) attached at the 5' end of the probe and a minor groove binder (MGB) and a non-florescent quencher (NFQ) attached at the 3' end of the probe.

[00116] The results are summarized in Tables 2 and 3 below. MB200 or 54-2 myoblasts were transfected as described above with an siRNA listed in Table 1. RNA was isolated and analyzed by qRT-PCR. The results for *MBD3L2* expression are shown in Tables 2 and 3 below as percent expression relative to the expression in cells that were transfected with the control. The results show that some antisense siRNAs inhibited DUX4 expression.

Table 2: % Inhibition in MB200 Cells

Oligo name	SEQ ID NO.	sense	SEQ ID NO.	antisense	% inhibition
UGNX394	1	GGCAGAGAUGGAGAGAGGA	2	UCCUCUCUCCAUCUCUGCC	36
UGNX482	3	AGGGAGGAACGGAGGGAAA	4	UUUCCCUCCGUUCCUCCCU	41
UGNX554	5	GAGGAAGGCAGGGAGGAAA	6	UUUCCCUCCGUUCCUCCCU	39
UGNX722	7	CGGUUUUCCUCCGGGACAAA	8	UUUGUCCCGGAGGAAACCG	49
UGNX779	9	CGGUUCACAGACCGCACAU	10	AUGUGCGGUCUGUGAACCG	-4
UGNX864	11	GACGACGGAGGCGUGAUUU	12	AAAUCACGCCUCCGUCGUC	45
UGNX916	13	GCCUGUUGCUCACGUCUCU	14	AGAGACGUGAGCAACAGGC	50
UGNX950	15	CUGGCAUGCCGACUGUUU	16	AAACAGUCGGCAUGGCCAG	50
UGNX991	17	CCGAAACAUGCAGGGAAAG	18	CUUCCUGCAUGUUUCCCG	67
UGNX1032	19	UUCGCUUCCUUGCCAGGU	20	ACCUGGCAAGGAGAGCGAA	44
UGNX1094	21	GGAAUCCAUCGUCAGGCCA	22	UGGCCUGACGAUGGAUUC	55
UGNX1142	23	GUCUCGCUUGGUCUUCUA	24	UAGAAGACCAGAGCGAGAC	55
UGNX1223	25	CACAGGCAUUGCCUCCUUC	26	GAAGGAGGCAAUGCCUGUG	27
UGNX1253	27	GCCUGGCACACUCAAGACU	28	AGUCUUGAGUGUGCCAGGC	58
UGNX1311	29	AGGCUGGUUUCUCCUUGCU	30	AGCAGGGAGAAACCAGCCU	66
UGNX1440	31	CUUCCUCUUCGUCUCUCCG	32	CGGAGAGACGAAGAGGAAG	51
UGNX1599	33	CUCACCGCCAUUCAUGAAG	34	CUUCAUGAAUGGCGGUGAG	68
UGNX1631	35	CUGCCUGUGGGCCUUUACA	36	UGUAAAGGCCACAGGCAG	75
UGNX1758	37	GCGUCCGUCCGUGAAAUUC	38	GAAUUUCACGGACGGACGC	92
UGNX1855	39	CGACGGAGACUCGUUUGGA	40	UCCAAACGAGUCUCCGUCG	85
UGNX1898	41	GAGCCUGCUUUGAGCGGAA	42	UUCGCGCUCAAAGCAGGCUC	86
UGNX1972	43	GAGCCAGGGUCCAGAUUU	44	AAAUCUGGACCCUGGGCUC	56
UGNX2119	45	CUCCUCCGAGCCUUUGAGA	46	UCUCAAGGCUCGGAGGAG	61
UGNX2192	47	UCCCGGAGUCCAGGAUUA	48	UGAAUCCUGGACUCCGGGA	66
UGNX2909	49	UGCUGCUGGAUGAGCUCCU	50	GGAGCUCAUCCAGCAGCA	61
UGNX2952	51	GGCGCAACCUCUCCUAGAA	52	UUCUAGGAGAGGUUGCGCC	89
UGNX3028	53	CUCAGCGAGGAAGAAUACC	54	GGUAUUCUCCUUCGUGAG	35
UGNX3194	55	CUGGGAUUCUCCUUCUA	56	UAGAAGGCAGGAUCCAG	69
UGNX3242	57	GCGGAGAUCGCCAUUCUU	58	AAGAAUGGCAGUUCUCCGC	84
UGNX395	59	GCAGAGAUGGAGAGAGGAA	60	UUCUCUCUCCAUCUCUG	40
UGNX396	61	CAGAGAUGGAGAGAGGAAC	62	GUUCCUCUCUCCAUCUCUG	74
UGNX397	63	AGAGAUGGAGAGAGGAACG	64	CGUCCUCUCUCCAUCUCU	21

UGNX398	65	GAGAUGGAGAGAGGAACGG	66	CCGUUCCUCUCUCCAUCUC	80
UGNX399	67	AGAUGGAGAGAGGAACGGG	68	CCCGUUCCUCUCUCCAUCU	65
UGNX400	69	GAUGGAGAGAGGAACGGGA	70	UCCCGUUCCUCUCUCCAUC	73
UGNX401	71	AUGGAGAGAGGAACGGGAG	72	CUCCCGUUCCUCUCUCCAUC	81
UGNX402	73	UGGAGAGAGGAACGGGAGA	74	UCUCCCGUUCCUCUCCAUC	91
UGNX405	75	AGAGAGGAACGGGAGACCU	76	AGGUCUCCCGUUCCUCUCU	77
UGNX406	77	GAGAGGAACGGGAGACCUA	78	UAGGUCUCCCGUUCCUCUC	90
UGNX407	79	AGAGGAACGGGAGACCUAG	80	CUAGGUCUCCCGUUCCUCU	74
UGNX408	81	GAGGAACGGGAGACCUAGA	82	UCUAGGUCUCCCGUUCCUC	56
UGNX409	83	AGGAACGGGAGACCUAGAG	84	CUCUAGGUCUCCCGUUCCU	57
UGNX484	85	GGAGGAACGGAGGGAAAGA	86	UCUUUCCCGUCCGUUCCUC	64
UGNX485	87	GAGGAACGGAGGGAAAGAC	88	GUCUUUCCCGUCCGUUCCUC	72
UGNX486	89	AGGAACGGAGGGAAAGACA	90	UGUCUUUCCCGUCCGUUCCU	42
UGNX487	91	GGAACGGAGGGAAAGACAG	92	CUGUCUUUCCCGUCCGUUCC	80
UGNX488	93	GAACGGAGGGAAAGACAGA	94	UCUGUCUUUCCCGUCCGUUCC	96
UGNX489	95	AACGGAGGGAAAGACAGAG	96	CUCUGUCUUUCCCGUCCGUU	79
UGNX490	97	ACGGAGGGAAAGACAGAGC	98	GCUCUGUCUUUCCCGUCCGU	81
UGNX492	99	GGAGGGAAAGACAGAGCGA	100	UCGUCUGUCUUUCCCGUCC	25
UGNX493	101	GAGGGAAAGACAGAGCGAC	102	GUCGUCUGUCUUUCCCGUC	80
UGNX494	103	AGGGAAAGACAGAGCGACG	104	CGUCGUCUGUCUUUCCCGU	38
UGNX496	105	GGAAAGACAGAGCGACGCA	106	UGCGUCGUCUGUCUUUCC	47
UGNX497	107	GAAAGACAGAGCGACGACG	108	CUGCGUCGUCUGUCUUUCC	41
UGNX498	109	AAAGACAGAGCGACGACGG	110	CCUGCGUCGUCUGUCUUU	63
UGNX780	111	GGUUCACAGACCGCACAUCC	112	GAUGUGCGGUCUGUGAAC	47
UGNX781	113	GUUCACAGACCGCACAUCC	114	GGAUGUGCGGUCUGUGAAC	93
UGNX782	115	UUCACAGACCGCACAUCCC	116	GGGAUGUGCGGUCUGUGAA	97
UGNX917	117	CCUGUUGCUCACGUCUCUC	118	GAGAGACGUGAGCAACAGG	-5
UGNX918	119	CUGUUGCUCACGUCUCUCC	120	GGAGAGACGUGAGCAACAG	31
UGNX919	121	UGUUGCUCACGUCUCUCCG	122	CGGAGAGACGUGAGCAACA	18
UGNX954	123	CCAUGCCGACUGUUUGCUC	124	GAGCAAACAGUCGGCAUGG	80
UGNX955	125	CAUGCCGACUGUUUGCUC	126	GGAGCAAACAGUCGGCAUG	48
UGNX956	127	AUGCCGACUGUUUGCUC	128	GGGAGCAAACAGUCGGCAU	65
UGNX964	129	UGUUUGCUCUCCCGAGCUCU	130	AGAGCUCCGGGAGCAAACA	94
UGNX990	131	CCCGGAAACAUGCAGGGAA	132	UUCCCUUGCAUGUUUCCGGG	93
UGNX992	133	CGGAAACAUGCAGGGAAAGG	134	CCUUCCCUUGCAUGUUUCCG	83
UGNX993	135	GGAAACAUGCAGGGAAAGGG	136	CCCUUCCCUUGCAUGUUUCC	76
UGNX994	137	GAAACAUGCAGGGAAAGGGU	138	ACCCUUCCCUUGCAUGUUUCC	21
UGNX995	139	AAACAUGCAGGGAAAGGGUG	140	CACCCUUCCCUUGCAUGUUU	43
UGNX996	141	AACAUGCAGGGAAAGGGUGC	142	GCACCCUUCCCUUGCAUGUU	5
UGNX998	143	CAUGCAGGGAAAGGGUGCAA	144	UUGCACCCUUCCCUUGCAUG	83
UGNX999	145	AUGCAGGGAAAGGGUGCAAG	146	CUUGCACCCUUCCCUUGCAU	13
UGNX1033	147	UCGUCUCCUUGCCAGGUUU	148	AACCUGGCAAGGAGAGCGA	57
UGNX1036	149	CUCUCCUUGCCAGGUUCCA	150	UGGAACCUGGCAAGGAGAG	77
UGNX1037	151	UCUCCUUGCCAGGUUCCAA	152	UUGGAACCUGGCAAGGAGA	42
UGNX1038	153	CUCUUGCCAGGUUCCAAA	154	UUUGGAACCUGGCAAGGAG	79
UGNX1039	155	UCCUUGCCAGGUUCCAAAC	156	GUUUGGAACCUGGCAAGGA	51
UGNX1040	157	CCUUGCCAGGUUCCAAACC	158	GGUUUGGAACCUGGCAAGG	36
UGNX1041	159	CUUGCCAGGUUCCAAACCG	160	CGGUUUGGAACCUGGCAAG	42
UGNX1042	161	UUGCCAGGUUCCAAACCGG	162	CCGGUUUGGAACCUGGCAA	89
UGNX1049	163	GUUCCAAACCGGCCACACU	164	AGUGUGGCCGGUUUGGAAC	77
UGNX1050	165	UUCCAAACCGGCCACACUG	166	CAGUGUGGCCGGUUUGGA	54
UGNX1095	167	GAAUCCAUCGUCAGGCCAU	168	AUGGCCUGACGAUGGAUUC	33
UGNX1096	169	AAUCCAUCGUCAGGCCAUC	170	GAUGGCCUGACGAUGGAUU	89
UGNX1097	171	AUCCAUCGUCAGGCCAUCA	172	UGAUGGCCUGACGAUGGAU	97
UGNX1098	173	UCCAUCGUCAGGCCAUAC	174	GUGAUGGCCUGACGAUGGA	55
UGNX1143	175	UCUCGCUCUGGUCUUCUAC	176	GUAGAAGACCAGAGCGAGA	18
UGNX1144	177	CUCGCUCUGGUCUUCUACG	178	CGUAGAAGACCAGAGCGAG	14

UGNX1145	179	UCGCUCUGGUCUUCUACGU	180	ACGUAGAAGACCAGAGCGA	73
UGNX1146	181	CGCUCUGGUCUUCUACGUG	182	CACGUAGAAGACCAGAGCG	93
UGNX1147	183	GCUCUGGUCUUCUACGUGG	184	CCACGUAGAAGACCAGAGC	-36
UGNX1148	185	CUCUGGUCUUCUACGUGGA	186	UCCACGUAGAAGACCAGAG	65
UGNX1149	187	UCUGGUCUUCUACGUGGAA	188	UCCACGUAGAAGACCAGA	92
UGNX1150	189	CUGGUCUUCUACGUGGAAA	190	UUUCCACGUAGAAGACCAG	37
UGNX1151	191	UGGUCUUCUACGUGGAAAU	192	AUUUCCACGUAGAAGACCA	33
UGNX1152	193	GGUCUUCUACGUGGAAAU	194	CAUUUCCACGUAGAAGACC	66
UGNX1153	195	GUCUUCUACGUGGAAAU	196	UCAUUUCCACGUAGAAGAC	34
UGNX1154	197	UCUUCUACGUGGAAAU	198	UUCAUUUCCACGUAGAAGA	80
UGNX1155	199	CUUCUACGUGGAAAU	200	GUUCAUUUCCACGUAGAAG	65
UGNX1156	201	UUCUACGUGGAAAU	202	CGUUCAUUUCCACGUAGAA	9
UGNX1157	203	UCUACGUGGAAAU	204	UCGUUCAUUUCCACGUAGA	66
UGNX1158	205	CUACGUGGAAAU	206	CUCGUUCAUUUCCACGUAG	75
UGNX1159	207	UACGUGGAAAU	208	UCUCGUUCAUUUCCACGUA	69
UGNX1160	209	ACGUGGAAAU	210	CUCUCGUUCAUUUCCACGU	70
UGNX1161	211	CGUGGAAAU	212	GCUCUCGUUCAUUUCCACG	83
UGNX1162	213	GUGGAAAU	214	GGCUCUCGUUCAUUUCCAC	50
UGNX1163	215	UGGAAAU	216	UGGCUCUCGUUCAUUUCCA	95
UGNX1164	217	GGAAAU	218	GUGGCUCUCGUUCAUUUCC	95
UGNX1165	219	GAAAU	220	UGUGGCUCUCGUUCAUUUC	99
UGNX1166	221	AAAU	222	GUGUGGCUCUCGUUCAUUU	77
UGNX1167	223	AAU	224	CGUGUGGCUCUCGUUCAUU	57
UGNX1168	225	AU	226	GCGUGUGGCUCUCGUUCAU	27
UGNX1222	227	CCACAGGCAUUGCCUCCU	228	AAGGAGGCAAUGCCUGUGG	86
UGNX1224	229	ACAGGCAUUGCCUCCU	230	UGAAGGAGGCAAUGCCUGU	85
UGNX1225	231	CAGGCAUUGCCUCCU	232	GUGAAGGAGGCAAUGCCUG	81
UGNX1226	233	AGGCAUUGCCUCCU	234	CGUGAAGGAGGCAAUGCCU	67
UGNX1228	235	GCAUUGCCUCCU	236	UCCGUGAAGGAGGCAAUGC	97
UGNX1229	237	CAUUGCCUCCU	238	CUCCGUGAAGGAGGCAAUG	54
UGNX1230	239	AUUGCCUCCU	240	UCUCCGUGAAGGAGGCAAU	11
UGNX1231	241	UUGCCUCCU	242	CUCUCCGUGAAGGAGGCAA	42
UGNX1232	243	UGCCUCCU	244	UCUCUCCGUGAAGGAGGCA	27
UGNX1254	245	CCUGGCACACUCAAGACUC	246	GAGUCUUGAGUGUGCCAGG	35
UGNX1255	247	CUGGCACACUCAAGACUCC	248	GGAGUCUUGAGUGUGCCAG	69
UGNX1256	249	UGGCACACUCAAGACUCCC	250	GGGAGUCUUGAGUGUGCCA	30
UGNX1257	251	GGCACACUCAAGACUCCCA	252	UGGGAGUCUUGAGUGUGCC	41
UGNX1258	253	GCACACUCAAGACUCCCA	254	GUGGGAGUCUUGAGUGUGC	39
UGNX1259	255	CACACUCAAGACUCCCA	256	CGUGGGAGUCUUGAGUGUG	-71
UGNX1260	257	ACACUCAAGACUCCCA	258	CCGUGGGAGUCUUGAGUGU	16
UGNX1261	259	CACUCAAGACUCCCA	260	UCCGUGGGAGUCUUGAGUG	30
UGNX1262	261	ACUCAAGACUCCCA	262	CUCCGUGGGAGUCUUGAGU	3
UGNX1264	263	UCAAGACUCCCA	264	ACCUCCGUGGGAGUCUUGA	80
UGNX1265	265	CAAGACUCCCA	266	AACCUCCGUGGGAGUCUUG	-20
UGNX1266	267	AAGACUCCCA	268	GAACCUCCGUGGGAGUCUU	44
UGNX1267	269	AGACUCCCA	270	UGAACCUCCGUGGGAGUCU	63
UGNX1269	271	ACUCCCA	272	ACUGAACCUCCGUGGGAGU	-8
UGNX1270	273	CUCCCA	274	AACUGAACCUCCGUGGGAG	67
UGNX1271	275	UCCCA	276	GAACUGAACCUCCGUGGGA	13
UGNX1273	277	CCACGGAGGUUCAGU	278	UGGAACUGAACCUCCGUGG	95
UGNX1274	279	CACGGAGGUUCAGU	280	GUGGAACUGAACCUCCGUG	69
UGNX1275	281	ACGGAGGUUCAGU	282	UGUGGAACUGAACCUCCGU	68
UGNX1276	283	CGGAGGUUCAGU	284	GUGUGGAACUGAACCUCCG	89
UGNX1277	285	GGAGGUUCAGU	286	AGUGUGGAACUGAACCUCC	72
UGNX1278	287	GAGGUUCAGU	288	GAGUGUGGAACUGAACCUCC	81
UGNX1279	289	AGGUUCAGU	290	GGAGUGUGGAACUGAACCU	83
UGNX1280	291	GGUUCAGU	292	GGGAGUGUGGAACUGAACCU	53

UGNX1441	293	UUCCUCUUCGUCUCUCCGG	294	CCGGAGAGACGAAGAGGAA	26
UGNX1598	295	GCUCACCGCCAUUCAUGAA	296	UUCAUGAAUGGCGGUGAGC	63
UGNX1600	297	UCACCGCCAUUCAUGAAGG	298	CCUUCAUGAAUGGCGGUGA	82
UGNX1601	299	CACCGCCAUUCAUGAAGGG	300	CCCUUCAUGAAUGGCGGUG	71
UGNX1632	301	UGCCUGUGGGCCUUUACAA	302	UUGUAAAAGGCCACAGGCA	62
UGNX1633	303	GCCUGUGGGCCUUUACAAG	304	CUUGUAAAAGGCCACAGGC	67
UGNX1634	305	CCUGUGGGCCUUUACAAGG	306	CCUUGUAAAAGGCCACAGG	49
UGNX1635	307	CUGUGGGCCUUUACAAGGG	308	CCCUUGUAAAAGGCCACAG	59
UGNX1636	309	UGUGGGCCUUUACAAGGGC	310	GCCCUUGUAAAAGGCCACA	30
UGNX1759	311	CGUCCGUCCGUGAAAUUC	312	GGAAUUUCACGGACGGACG	85
UGNX1760	313	GUCCGUCCGUGAAAUUCG	314	CGGAAUUUCACGGACGGAC	92
UGNX1761	315	UCCGUCCGUGAAAUUCGG	316	CCGGAUUUCACGGACGGA	35
UGNX1856	317	GACGGAGACUCGUUUGGAC	318	GUCCAAACGAGUCUCCGUC	81
UGNX1857	319	ACGGAGACUCGUUUGGACC	320	GGUCCAAACGAGUCUCCGU	63
UGNX1899	321	AGCCUGCUUUGAGCGGAAC	322	GUUCCGCUCAAAGCAGGCU	29
UGNX1904	323	GCUUUGAGCGGAACCCGUA	324	UACGGGUUCCGCUCAAAGC	96
UGNX1905	325	CUUUGAGCGGAACCCGUAC	326	GUACGGGUUCCGCUCAAAG	-5
UGNX1906	327	UUUGAGCGGAACCCGUACC	328	GGUACGGGUUCCGCUCAA	-15
UGNX1975	329	CCCAGGGUCCAGAUUUGGU	330	ACCAAAUCUGGACCCUGGG	11
UGNX1976	331	CCAGGGUCCAGAUUUGGUU	332	AACCAAAUCUGGACCCUGG	99
UGNX1977	333	CAGGGUCCAGAUUUGGUUU	334	AAACCAAAUCUGGACCCUG	22
UGNX1978	335	AGGGUCCAGAUUUGGUUUC	336	GAAACCAAAUCUGGACCCU	-21
UGNX1979	337	GGGUCCAGAUUUGGUUUC	338	UGAAACCAAAUCUGGACCC	80
UGNX1980	339	GGUCCAGAUUUGGUUUCAG	340	CUGAAACCAAAUCUGGACC	99
UGNX1981	341	GUCCAGAUUUGGUUUCAGA	342	UCUGAAACCAAAUCUGGAC	99
UGNX1982	343	UCCAGAUUUGGUUUCAGAA	344	UUCUGAAACCAAAUCUGGA	99
UGNX1983	345	CCAGAUUUGGUUUCAGAAU	346	AUUCUGAAACCAAAUCUGG	79
UGNX1984	347	CAGAUUUGGUUUCAGAAUG	348	CAUUCUGAAACCAAAUCUG	98
UGNX1985	349	AGAUUUGGUUUCAGAAUGA	350	UCAUUCUGAAACCAAAUCU	99
UGNX1986	351	GAUUGGUUUCAGAAUGAG	352	CUCAUUCUGAAACCAAAUC	96
UGNX1987	353	AUUUGGUUUCAGAAUGAGA	354	UCUCAUUCUGAAACCAAAU	96
UGNX1988	355	UUUGGUUUCAGAAUGAGAG	356	CUCUCAUUCUGAAACCAAA	64
UGNX1989	357	UUGGUUUCAGAAUGAGAGG	358	CCUCUCAUUCUGAAACCAA	62
UGNX1990	359	UGGUUUCAGAAUGAGAGGU	360	ACCUCUCAUUCUGAAACCA	74
UGNX1991	361	GGUUCAGAAUGAGAGGUC	362	GACCUCUCAUUCUGAAACC	99
UGNX1992	363	GUUUCAGAAUGAGAGGUCA	364	UGACCUCUCAUUCUGAAAC	97
UGNX1993	365	UUCAGAAUGAGAGGUCAC	366	GUGACCUCUCAUUCUGAAA	60
UGNX1994	367	UUCAGAAUGAGAGGUCACG	368	CGUGACCUCUCAUUCUGAA	44
UGNX1995	369	UCAGAAUGAGAGGUCACGC	370	GCGUGACCUCUCAUUCUGA	-48
UGNX1996	371	CAGAAUGAGAGGUCACGCC	372	GGCGUGACCUCUCAUUCUG	84
UGNX1997	373	AGAAUGAGAGGUCACGCCA	374	UGGCGUGACCUCUCAUUCU	15
UGNX1998	375	GAAUGAGAGGUCACGCCAG	376	CUGGCGUGACCUCUCAUUC	70
UGNX1999	377	AAUGAGAGGUCACGCCAGC	378	GCUGGCGUGACCUCUCAU	95
UGNX2000	379	AUGAGAGGUCACGCCAGCU	380	AGCUGGCGUGACCUCUCAU	33
UGNX2117	381	UGCUCUCCGAGCCUUUGA	382	UCAAGGCUCGGAGGAGCA	80
UGNX2120	383	UCCUCCGAGCCUUUGAGAA	384	UUCUCAAGGCUCGGAGGA	18
UGNX2121	385	CCUCCGAGCCUUUGAGAAG	386	CUUCUCAAGGCUCGGAGG	96
UGNX2122	387	CUCCGAGCCUUUGAGAAGG	388	CCUUCUCAAGGCUCGGAG	87
UGNX2123	389	UCCGAGCCUUUGAGAAGGA	390	UCCUUCUCAAGGCUCGGA	48
UGNX2124	391	CCGAGCCUUUGAGAAGGAU	392	AUCCUUCUCAAGGCUCGG	76
UGNX2125	393	CGAGCCUUUGAGAAGGAUC	394	GAUCCUUCUCAAGGCUCG	92
UGNX2126	395	GAGCCUUUGAGAAGGAUCG	396	CGAUCCUUCUCAAGGCUC	46
UGNX2127	397	AGCCUUUGAGAAGGAUCGC	398	GCGAUCCUUCUCAAGGCU	8
UGNX2128	399	GCCUUUGAGAAGGAUCGCU	400	AGCGAUCCUUCUCAAGGC	97
UGNX2129	401	CCUUUGAGAAGGAUCGCUU	402	AAGCGAUCCUUCUCAAGG	98
UGNX2130	403	CUUUGAGAAGGAUCGCUUU	404	AAAGCGAUCCUUCUCAAG	98
UGNX2131	405	UUUGAGAAGGAUCGCUUUC	406	GAAAGCGAUCCUUCUCAAA	10

UGNX2132	407	UUGAGAAGGAUCGCUUUC	408	GGAAAGCGAUCCUUCUCAA	35
UGNX2133	409	UGAGAAGGAUCGCUUUC	410	UGGAAAGCGAUCCUUCUCA	93
UGNX2134	411	GAGAAGGAUCGCUUUC	412	CUGGAAAGCGAUCCUUCUC	-150
UGNX2135	413	AGAAGGAUCGCUUUC	414	CCUGGAAAGCGAUCCUUCU	-180
UGNX2136	415	GAAGGAUCGCUUUC	416	GCCUGGAAAGCGAUCCUUC	31
UGNX2137	417	AAGGAUCGCUUUC	418	UGCCUGGAAAGCGAUCCU	30
UGNX2138	419	AGGAUCGCUUUC	420	AUGCCUGGAAAGCGAUCCU	-16
UGNX2139	421	GGAUCGCUUUC	422	GAUGCCUGGAAAGCGAUCC	98
UGNX2140	423	GAUCGCUUUC	424	CGAUGCCUGGAAAGCGAUC	35
UGNX2194	425	CCGAGUCCAGGAUUCAGA	426	UCUGAAUCCUGGACUCCG	99
UGNX2195	427	CGGAGUCCAGGAUUCAGA	428	AUCUGAAUCCUGGACUCCG	17
UGNX2196	429	GGAGUCCAGGAUUCAGAUC	430	GAUCUGAAUCCUGGACUCC	-41
UGNX2197	431	GAGUCCAGGAUUCAGAUCU	432	AGAUCUGAAUCCUGGACUC	4
UGNX2198	433	AGUCCAGGAUUCAGAUCUG	434	CAGAUUCUGAAUCCUGGACU	89
UGNX2199	435	GUCCAGGAUUCAGAUCUGG	436	CCAGAUUCUGAAUCCUGGAC	45
UGNX2200	437	UCCAGGAUUCAGAUCUGGU	438	ACCAGAUUCUGAAUCCUGGA	26
UGNX2201	439	CCAGGAUUCAGAUCUGGUU	440	AACCAGAUUCUGAAUCCUGG	-44
UGNX2202	441	CAGGAUUCAGAUCUGGUUU	442	AAACCAGAUUCUGAAUCCUG	-27
UGNX2203	443	AGGAUUCAGAUCUGGUUUC	444	GAAACCAGAUUCUGAAUCCU	-43
UGNX2204	445	GGAUUCAGAUCUGGUUUC	446	UGAAACCAGAUUCUGAAUCC	96
UGNX2205	447	GAUUCAGAUCUGGUUUCAG	448	CUGAAACCAGAUUCUGAAUC	98
UGNX2206	449	AUUCAGAUCUGGUUUCAGA	450	UCUGAAACCAGAUUCUGAAU	97
UGNX2207	451	UUCAGAUCUGGUUUCAGAA	452	UUCUGAAACCAGAUUCUGAA	92
UGNX2208	453	UCAGAUCUGGUUUCAGAAU	454	AUUCUGAAACCAGAUUCUGA	56
UGNX2209	455	CAGAUCUGGUUUCAGAAUC	456	GAUUCUGAAACCAGAUUCUG	98
UGNX2210	457	AGAUCUGGUUUCAGAAUCG	458	CGAUUCUGAAACCAGAUUCU	-5
UGNX2211	459	GAUCUGGUUUCAGAAUCGA	460	UCGAUUCUGAAACCAGAUUC	96
UGNX2212	461	AUCUGGUUUCAGAAUCGAA	462	UUCGAUUCUGAAACCAGAU	99
UGNX2213	463	UCUGGUUUCAGAAUCGAAG	464	CUUCGAUUCUGAAACCAGAU	-11
UGNX2214	465	CUGGUUUCAGAAUCGAAGG	466	CCUUCGAUUCUGAAACCAG	85
UGNX2215	467	UGGUUUCAGAAUCGAAGGG	468	CCCUUCGAUUCUGAAACCA	9
UGNX2216	469	GGUUUCAGAAUCGAAGGGC	470	GCCCUUCGAUUCUGAAACC	6
UGNX2217	471	GUUUCAGAAUCGAAGGGCC	472	GGCCCUUCGAUUCUGAAAC	52
UGNX2218	473	UUUCAGAAUCGAAGGGCCA	474	UGGCCCUUCGAUUCUGAAA	17
UGNX2219	475	UUCAGAAUCGAAGGGCCAG	476	CUGGCCCUUCGAUUCUGAA	-47
UGNX2220	477	UCAGAAUCGAAGGGCCAGG	478	CCUGGCCCUUCGAUUCUGA	-40
UGNX2222	479	AGAAUCGAAGGGCCAGGA	480	UGCCUGGCCCUUCGAUUCU	17
UGNX2951	481	AGGCGCAACCUCUCCUAGA	482	UCUAGGAGAGGUUGCGCCU	98
UGNX2953	483	GCGCAACCUCUCCUAGAAA	484	UUUCUAGGAGAGGUUGCGC	-11
UGNX2954	485	CGCAACCUCUCCUAGAAAC	486	GUUUCUAGGAGAGGUUGCG	-28
UGNX2955	487	GCAACCUCUCCUAGAAACG	488	CGUUUCUAGGAGAGGUUGC	10
UGNX2956	489	CAACCUCUCCUAGAAACGG	490	CCGUUUCUAGGAGAGGUUG	16
UGNX2957	491	AACCUCUCCUAGAAACGGA	492	UCCGUUUCUAGGAGAGGUU	52
UGNX2958	493	ACCUCUCCUAGAAACGGAG	494	CUCCGUUUCUAGGAGAGGU	4
UGNX2959	495	CCUCUCCUAGAAACGGAGG	496	CCUCCGUUUCUAGGAGAGG	-53
UGNX2960	497	CUCUCCUAGAAACGGAGGC	498	GCCUCCGUUUCUAGGAGAG	-17
UGNX2961	499	UCUCCUAGAAACGGAGGCC	500	GGCCUCCGUUUCUAGGAGA	-554
UGNX3029	501	UCAGCGAGGAAGAAUACCG	502	CGGUUUUCUCCUCGCUA	-40
UGNX3030	503	CAGCGAGGAAGAAUACCGG	504	CCGGUAAUUCUCCUCGCU	86
UGNX3031	505	AGCGAGGAAGAAUACCGGG	506	CCCGGUAAUUCUCCUCGCU	80
UGNX3033	507	CGAGGAAGAAUACCGGGCU	508	AGCCCGGUAAUUCUCCUCG	90
UGNX3034	509	GAGGAAGAAUACCGGGCUC	510	GAGCCCGGUAAUUCUCCUC	80
UGNX3035	511	AGGAAGAAUACCGGGCUCU	512	AGAGCCCGGUAAUUCUCCU	91
UGNX3036	513	GGAAGAAUACCGGGCUCUG	514	CAGAGCCCGGUAAUUCUCC	-78
UGNX3037	515	GAAGAAUACCGGGCUCUGC	516	GCAGAGCCCGGUAAUUCU	-101
UGNX3038	517	AAGAAUACCGGGCUCUGCU	518	AGCAGAGCCCGGUAAUUCU	-280
UGNX3039	519	AGAAUACCGGGCUCUGCUG	520	CAGCAGAGCCCGGUAAUUCU	-50

UGNX3041	521	AAUACCGGGCUCUGCUGGA	522	UCCAGCAGAGCCCGGUAAU	-27
UGNX3049	523	GCUCUGCUGGAGGAGCUUU	524	AAAGCUCCUCCAGCAGAGC	-66
UGNX3050	525	CUCUGCUGGAGGAGCUUUA	526	UAAAGCUCCUCCAGCAGAG	87
UGNX3051	527	UCUGCUGGAGGAGCUUUAG	528	CUAAAAGCUCCUCCAGCAGA	-52
UGNX3052	529	CUGCUGGAGGAGCUUUAGG	530	CCUAAAAGCUCCUCCAGCAG	-57
UGNX3053	531	UGCUGGAGGAGCUUUAGGA	532	UCCUAAAAGCUCCUCCAGCA	-18
UGNX3054	533	GCUGGAGGAGCUUUAGGAC	534	GUCCUAAAAGCUCCUCCAGC	-60
UGNX3055	535	CUGGAGGAGCUUUAGGACG	536	CGUCCUAAAAGCUCCUCCAG	28
UGNX3056	537	UGGAGGAGCUUUAGGACGC	538	GCGUCCUAAAAGCUCCUCCA	31
UGNX3193	539	CCUGGGAUUCUGCCUUCU	540	AGAAGGCAGGAAUCCAGG	-84
UGNX3195	541	UGGGAUUCUGCCUUCUAG	542	CUAGAAGGCAGGAAUCCCA	-36
UGNX3196	543	GGGAUUCUGCCUUCUAGG	544	CCUAGAAGGCAGGAAUCCC	0
UGNX3197	545	GGAUUCUGCCUUCUAGGU	546	ACCUAGAAGGCAGGAAUCC	2
UGNX3198	547	GAUUCUGCCUUCUAGGUC	548	GACCUAGAAGGCAGGAAUC	-6
UGNX3199	549	AUUCUGCCUUCUAGGUCU	550	AGACCUAGAAGGCAGGAAU	20
UGNX3200	551	UUCUGCCUUCUAGGUCUA	552	UAGACCUAGAAGGCAGGAA	-39
UGNX3201	553	UCCUGCCUUCUAGGUCUAG	554	CUAGACCUAGAAGGCAGGA	-39
UGNX3202	555	CCUGCCUUCUAGGUCUAGG	556	CCUAGACCUAGAAGGCAGG	-8
UGNX3203	557	CUGCCUUCUAGGUCUAGGC	558	GCCUAGACCUAGAAGGCAG	-48
UGNX3204	559	UGCCUUCUAGGUCUAGGCC	560	GGCCUAGACCUAGAAGGCA	-31
UGNX3208	561	UUCUAGGUCUAGGCCCGGU	562	ACCGGGCCUAGACCUAGAA	-71
UGNX3241	563	CGCGGAGAACUGCCAUCU	564	AGAAUGGCAGUUCUCCGCG	92
UGNX3243	565	CGGAGAACUGCCAUCUUCU	566	AAAGAAUGGCAGUUCUCCG	-121
UGNX3244	567	GGAGAACUGCCAUCUUCU	568	GAAAGAAUGGCAGUUCUCC	-43
UGNX3245	569	GAGAACUGCCAUCUUCUCC	570	GGAAAGAAUGGCAGUUCUC	59
UGNX3246	571	AGAACUGCCAUCUUCUCCU	572	AGGAAAGAAUGGCAGUUCU	10
UGNX3247	573	GAACUGCCAUCUUCUCCUG	574	CAGGAAAGAAUGGCAGUUC	-92
UGNX3248	575	AACUGCCAUCUUCUCCUGG	576	CCAGGAAAGAAUGGCAGUU	-70
UGNX3249	577	ACUGCCAUCUUCUCCUGGG	578	CCCAGGAAAGAAUGGCAGU	-25
UGNX3250	579	CUGCCAUCUUCUCCUGGGC	580	GCCCAGGAAAGAAUGGCAG	-22
UGNX3251	581	UGCCAUCUUCUCCUGGGCA	582	UGCCCAGGAAAGAAUGGCA	-65
UGNX3252	583	GCCAUCUUCUCCUGGGCAU	584	AUGCCCAGGAAAGAAUGGC	82
UGNX3253	585	CCAUCUUCUCCUGGGCAUC	586	GAUGCCCAGGAAAGAAUGG	-2
UGNX3254	587	CAUCUUCUCCUGGGCAUCC	588	GGAUGCCCAGGAAAGAAUG	-390
UGNX3255	589	AUUCUUCUCCUGGGCAUCCC	590	GGAUGCCCAGGAAAGAAU	-110
UGNX3256	591	UUCUUCUCCUGGGCAUCCG	592	CGGGAUGCCCAGGAAAGAA	-40

Table 3: % Inhibition in 54-2 Cells

siRNA name	SEQ ID NO.	sense	SEQ ID NO:	antisense	% inhibition in 54-2
UGNX394	1	GGCAGAGAUGGAGAGAGGA	2	UCCUCUCUCCAUCUCUGCC	29
UGNX482	3	AGGGAGGAACGGAGGGGAAA	4	UUUCCCUCCGUUCCUCCCU	30
UGNX554	5	GAGGAAGGCAGGGAGGAAA	6	UUUCCCUCCUGCCUCCUC	-11
UGNX722	7	CGGUUUCUCCGGGACAAA	8	UUUGUCCCGGAGGAAACCG	19
UGNX779	9	CGGUUCACAGACCGCACAU	10	AUGUGCGGUCUGUGAACCG	38
UGNX864	11	GACGACGGAGGCGUGAUUU	12	AAAUCACGCCUCCGUCGUC	19
UGNX916	13	GCCUGUUGCUCACGUCUCU	14	AGAGACGUGAGCAACAGGC	41
UGNX950	15	CUGGCCAUGCCGACUGUUU	16	AAACAGUCGGCAUGGCCAG	-57
UGNX991	17	CCGGAACAAUGCAGGGAAG	18	CUUCCUGCAUGUUUCCGG	29
UGNX1032	19	UUCGUCUCCUUGCCAGGU	20	ACCUGGCAAGGAGAGCGAA	31
UGNX1094	21	GGAAUCCAUCGUCAGGCCA	22	UGGCCUGACGAUGGAUUC	50
UGNX1142	23	GUCUCGUCUGGUCUUCUA	24	UAGAAGACCAGAGCGAGAC	5
UGNX1223	25	CACAGGCAUUGCCUCCUUC	26	GAAGGAGGCAAUGCCUGUG	-92

UGNX1253	27	GCCUGGCACACUCAAGACU	28	AGUCUUGAGUGUGCCAGGC	-19
UGNX1311	29	AGGCUGGUUUCUCCUGCU	30	AGCAGGGAGAAACCAGCCU	41
UGNX1440	31	CUUCCUCUUCGUCUCUCCG	32	CGGAGAGACGAAGAGGAAG	44
UGNX1599	33	CUCACCGCCAUUCAUGAAG	34	CUUCAUGAAUGGCGGUGAG	68
UGNX1631	35	CUGCCUGUGGGCCUUUACA	36	UGUAAAGGCCACAGGCAG	68
UGNX1758	37	GCGUCCGUCCGUGAAAUUC	38	GAAUUUCACGGACGGACGC	90
UGNX1855	39	CGACGGAGACUCGUUUGGA	40	UCCAAACGAGUCUCCGUCG	82
UGNX1898	41	GAGCCUGCUUUGAGCGGAA	42	UUCCGCUCAAAGCAGGCUC	72
UGNX1972	43	GAGCCCAGGGUCCAGAUUU	44	AAAUCUGGACCCUGGGCUC	50
UGNX2119	45	CUCCUCCGAGCCUUUGAGA	46	UCUCAAGGCUCGGAGGAG	64
UGNX2192	47	UCCCGAGUCCAGGAUUCA	48	UGAAUCCUGGACUCCGGGA	69
UGNX2909	49	UGCUGCUGGAUGAGCUCCU	50	AGGAGCUCAUCCAGCAGCA	43
UGNX2952	51	GGCGCAACCUCUCCUAGAA	52	UUCUAGGAGAGGUUGCGCC	81
UGNX3028	53	CUCAGCGAGGAAGAAUACC	54	GGUAUUCUCCUCGUGAG	41
UGNX3194	55	CUGGGAUCCUGCCUUCUA	56	UAGAAGGCAGGAUCCAG	67
UGNX3242	57	GCGGAGAACUGCCAUCUU	58	AAGAAUGGCAGUUCUCCGC	87
UGNX1759	311	CGUCCGUCCGUGAAAUUCC	312	GGAAUUUCACGGACGGACG	85
UGNX1856	317	GACGGAGACUCGUUUGGAC	318	GUCCAAACGAGUCUCCGUC	-5
UGNX1857	319	ACGGAGACUCGUUUGGACC	320	GGUCCAAACGAGUCUCCGU	-9
UGNX1899	321	AGCCUGCUUUGAGCGGAAC	322	GUUCCGCUCAAAGCAGGCU	10
UGNX1905	325	CUUUGAGCGGAACCCGUAC	326	GUACGGGUUCCGCUCAAA	-79
UGNX1906	327	UUUGAGCGGAACCCGUACC	328	GGUACGGGUUCCGCUCAAA	-291
UGNX1975	329	CCCAGGGUCCAGAUUUGGU	330	ACCAAAUCUGGACCCUGGG	4
UGNX1977	333	CAGGGUCCAGAUUUGGUUU	334	AAACCAAAUCUGGACCCUG	-23
UGNX1978	335	AGGGUCCAGAUUUGGUUU	336	GAAACCAAAUCUGGACCCU	5
UGNX1979	337	GGGUCCAGAUUUGGUUUCA	338	UGAAACCAAAUCUGGACCC	66
UGNX1983	345	CCAGAUUUGGUUUUCAGAAU	346	AUUCUGAAACCAAAUCUGG	-9
UGNX1986	351	GAUUUGGUUUUCAGAAUGAG	352	CUCAUUCUGAAACCAAAUC	72
UGNX1988	355	UUUGGUUUUCAGAAUGAGAG	356	CUCUCAUUCUGAAACCAAA	51
UGNX1989	357	UUUGGUUUUCAGAAUGAGAGG	358	CCUCUCAUUCUGAAACCAA	42
UGNX1990	359	UGGUUUUCAGAAUGAGAGGU	360	ACCUCUCAUUCUGAAACCA	19
UGNX1991	361	GGUUUCAGAAUGAGAGGUC	362	GACCUCUCAUUCUGAAACC	10
UGNX1993	365	UUUCAGAAUGAGAGGUCAC	366	GUGACCUCUCAUUCUGAAA	37
UGNX1994	367	UUCAGAAUGAGAGGUCACG	368	CGUGACCUCUCAUUCUGAA	65
UGNX1995	369	UCAGAAUGAGAGGUCACGC	370	GCGUGACCUCUCAUUCUGA	-233
UGNX1996	371	CAGAAUGAGAGGUCACGCC	372	GGCGUGACCUCUCAUUCUG	95
UGNX1997	373	AGAAUGAGAGGUCACGCCA	374	UGGCGUGACCUCUCAUUCU	1
UGNX1998	375	GAAUGAGAGGUCACGCCAG	376	CUGGCGUGACCUCUCAUUC	70
UGNX1999	377	AAUGAGAGGUCACGCCAGC	378	GCUGGCGUGACCUCUCAU	23
UGNX2000	379	AUGAGAGGUCACGCCAGCU	380	AGCUGGCGUGACCUCUCAU	40
UGNX2117	381	UGCUCUCCGAGCCUUUGA	382	UCAAGGCUCGGAGGAGCA	28
UGNX2120	383	UCCUCCGAGCCUUUGAGAA	384	UUCUCAAGGCUCGGAGGA	5
UGNX2123	389	UCCGAGCCUUUGAGAAGGA	390	UCCUUCUCAAGGCUCGGA	-5
UGNX2124	391	CCGAGCCUUUGAGAAGGAU	392	AUCCUUCUCAAGGCUCGG	64
UGNX2125	393	CGAGCCUUUGAGAAGGAUC	394	GAUCCUUCUCAAGGCUCG	33
UGNX2126	395	GAGCCUUUGAGAAGGAUCG	396	CGAUCCUUCUCAAGGCUC	-8
UGNX2127	397	AGCCUUUGAGAAGGAUCGC	398	GCGAUCCUUCUCAAGGCUC	15
UGNX2131	405	UUUGAGAAGGAUCGCUUUC	406	GAAAGCGAUCCUUCUCAAA	3
UGNX2132	407	UUGAGAAGGAUCGCUUUC	408	GGAAAGCGAUCCUUCUCA	47

Example 3: Inhibition of DUX4 with Antisense siRNA

[00117] Further dose response studies were conducted using the concentrations of 10 nM, 2 nM and 0.4 nM. Transfections of siRNAs into FSHD1 and FSHD2 myoblasts were carried out

using Lipofectamine RNAiMAX (Invitrogen) as above. Briefly, cells were seeded at 1×10^5 cells/well in 1 ml regular growth medium in 12-well culture plates. Approximately 20 hours later, cells were transfected by first diluting 2 μ l Lipofectamine RNAiMAX and 10, 2 or 0.4 pmol of either gene-specific siRNAs or a scrambled non-silencing control siRNA in 100 μ l Opti-MEM Reduced Serum Medium to create lipofectamine/siRNA complexes. The lipofectamine/siRNA complexes were then added dropwise to one well of the culture plate containing myoblasts. 24 hours following transfection, cells were incubated in differentiation medium for 40 hours to induce differentiation into myotubes and harvested for total RNA analysis.

[00118] For determination of the 50% inhibitory concentration (IC₅₀) for each siRNA, seven-point concentration response curves were generated by first creating 3-fold serial dilutions of siRNAs from concentrated stocks in water in 96-well plates. Transfection then proceeded as above. IC₅₀s were determined by nonlinear regression using a four-parameter logistic equation (GraphPad Prism Software Inc., San Diego, CA; <http://www.graphpad.com>). Data is presented as IC₅₀s with two significant digits. Data from the dose response and IC₅₀ studies are summarized in Table 4.

Table 4: Dose Response and IC₅₀ Studies

siRNA name	SEQ ID NO.	sense	SEQ ID NO.	antisense	MB200			54-2			MB200	54-2
					10nM	2nM	0.4nM	10nM	2nM	0.4nM	IC50	IC50
UGNX 1758	37	GCGUCCG UCCGUGA AAUUC	38	GAAUU UCACGG ACGGAC GC	95	52	6	95	32	15		
UGNX 1855	39	CGACGGA GACUCGU UUGGA	40	UCCAAA CGAGUC UCCGUC G	92	38	29	87	35	22		
UGNX 1898	41	GAGCCUG CUUUGAG CGGAA	42	UUCCGC UCAAAG CAGGCU C	91	63	29	91	48	23		
UGNX 2952	51	GGCGCAA CCUCUCC UAGAA	52	UUCUAG GAGAG GUUGCG CC	94	65	15	93	62	32		
UGNX 3194	55	CUGGGAU UCCUGCC UUCUA	56	UAGAA GGCAGG AAUCCC AG	76	21	10	77	38	24		
UGNX 3242	57	GCGGAGA ACUGCCA UUCUU	58	AAGAA UGGCAG	96	89	27	95	77	25		

				UUCUCC GC								
UGNX 402	73	UGGAGAG AGGAACG GGAGA	74	UCUCCC GUUCCU CUCUCC A	87	9	31	77	48	38		
UGNX 406	77	GAGAGGA ACGGGAG ACCUA	78	UAGGUC UCCCGU UCCUCU C	92	79	52	80	59	43		
UGNX 488	93	GAACGGA GGGAAAG ACAGA	94	UCUGUC UUUCCC UCCGUU C	95	47	33	90	69	38		
UGNX 781	113	GUUCACA GACCGCA CAUCC	114	GGAUG UGCGGU CUGUGA AC	95	50	35	60	26	39		
UGNX 782	115	UUCACAG ACCGCAC AUCCC	116	GGGAU GUGCGG UCUGUG AA	87	33	31	55	34	34		
UGNX 964	129	UGUUUGC UCCCGGA GCUCU	130	AGAGCU CCGGGA GCAAAC A	90	42	40	39	47	36		
UGNX 990	131	CCCGGAA ACAUGCA GGGAA	132	UUCCCU GCAUGU UUCCGG G	72	51	38	80	46	38		
UGNX 1097	171	AUCCAUC GUCAGGC CAUCA	172	UGAUG GCCUGA CGAUGG AU	98	68	-2	70	51	35		
UGNX 1146	181	CGCUCUG GUCUUCU ACGUG	182	CACGUA GAAGAC CAGAGC G	77	34	34	78	60	32		
UGNX 1149	187	UCUGGUC UUCUACG UGGAA	188	UUCCAC GUAGA AGACCA GA	78	73	15	37	19	-8		
UGNX 1163	215	UGGAAAU GAACGAG AGCCA	216	UGGCUC UCGUUC AUUUC A	98	68	17	86	70	48		
UGNX 1164	217	GGAAAU AACGAGA GCCAC	218	GUGGCU CUCGUU CAUUUC C	96	57	2	91	64	47		
UGNX 1165	219	GAAAUGA ACGAGAG CCACA	220	UGUGGC UCUCGU UCAUUU C	99	88	71	93	67	52		
UGNX 1228	235	GCAUUGC CUCCUUC ACGGA	236	UCCGUG AAGGA GGCAAU GC	86	60	48	84	61	60		

UGNX 1273	277	CCACGGA GGUUCAG UUCCA	278	UGGAAC UGAACC UCCGUG G	89	69	35	84	61	63		
UGNX 1276	283	CGGAGGU UCAGUUC CACAC	284	GUGUG GAACUG AACCUC CG	90	64	37	77	55	48		
UGNX 1759	311	CGUCCGU CCGUGAA AUUCC	312	GGAAU UUCACG GACGGA CG	98	87	70				0.240	2.000
UGNX 1760	313	GUCCGUC CGUGAAA UUCCG	314	CGGAAU UUCACG GACGGA C	86	42	38					
UGNX 1904	323	GCUUUGA GCGGAAC CCGUA	324	UACGGG UUCCGC UCAAG C	90	49	50				0.870	1.600
UGNX 1976	331	CCAGGGU CCAGAUU UGGUU	332	AACCAA AUCUGG ACCCUG G	93	22	-6					
UGNX 1980	339	GGUCCAG AUUUGGU UUCAG	340	CUGAAA CCAAAU CUGGAC C	92	89	65				0.060	0.220
UGNX 1981	341	GUCCAGA UUUGGUU UCAGA	342	UCUGAA ACCAAA UCUGGA C	96	80	56				0.360	0.073
UGNX 1982	343	UCCAGAU UUGGUUU CAGAA	344	UUCUGA AACCAA AUCUGG A	87	50	-18					
UGNX 1984	347	CAGAUUU GGUUUCA GAAUG	348	CAUUCU GAAACC AAAUCU G	98	88	58				0.280	0.230
UGNX 1985	349	AGAUUUG GUUUCAG AAUGA	350	UCAUUC UGAAAC CAAUC U	99	95	90				0.028	0.041
UGNX 1986	351	GAUUUGG UUUCAGA AUGAG	352	CUCAUU CUGAAA CCAAAU C	85	45	4					
UGNX 1987	353	AUUUGGU UUCAGAA UGAGA	354	UCUCAU UCUGAA ACCAAA U	90	83	57				0.500	0.154
UGNX 1991	361	GGUUUCA GAAUGAG AGGUC	362	GACCUC UCAUUC UGAAAC C	93	60	13					

UGNX 1992	363	GUUUCAG AAUGAGA GGUCA	364	UGACCU CUCAUU CUGAAA C	96	91	89				0.035	0.100
UGNX 1996	371	CAGAAUG AGAGGUC ACGCC	372	GGCGUG ACCUCU CAUUCU G	88	63	51				0.610	3.8
UFNX 1998	375	GAAUGAG AGGUCAC GCCAG	376	CUGGCG UGACCU CUCAUU C	92	70	32					
UGNX 1999	377	AAUGAGA GGUCACG CCAGC	378	GCUGGC GUGACC UCUCAU U	46	12	30					
UGNX 2121	385	CCUCCGA GCCUUUG AGAAG	386	CUUCUC AAAGGC UCGGAG G	84	52	28					
UGNX 2125	393	CGAGCCU UUGAGAA GGAUC	394	GAUCCU UCUCAA AGGCUC G	66	-8	15					
UGNX 2128	399	GCCUUUG AGAAGGA UCGCU	400	AGCGAU CCUUCU CAAAGG C	94	80	59				0.061	0.084
UGNX 2129	401	CCUUUGA GAAGGAU CGCUU	402	AAGCGA UCCUUC UCAAAG G	94	84	67				0.170	0.110
UGNX 2130	403	CUUUGAG AAGGAUC GCUUU	404	AAAGCG AUCCUU CUCAAA G	98	96	94				0.023	0.230
UGNX 2133	409	UGAGAAG GAUCGCU UUCCA	410	UGGAA AGCGAU CCUUCU CA	98	92	37				0.370	0.840
UGNX 2139	421	GGAUCGC UUUCCAG GCAUC	422	GAUGCC UGGAA AGCGAU CC	96	89	45					
UGNX 2194	425	CCGGAGU CCAGGAU UCAGA	426	UCUGAA UCCUGG ACUCCG G	83	49	32					
UGNX 2198	433	AGUCCAG GAUUCAG AUCUG	434	CAGAUC UGAAUC CUGGAC U	98	90	60				0.700	0.540
UGNX 2204	445	GGAUUCA GAUCUGG UUUCA	446	UGAAAC CAGAUC UGAAUC C	91	82	60				0.210	0.370

UGNX 2205	447	GAUUCAG AUCUGGU UUCAG	448	CUGAAA CCAGAU CUGAAU C	95	85	61				0.220	0.047
UGNX 2206	449	AUUCAGA UCUGGUU UCAGA	450	UCUGAA ACCAGA UCUGAA U	91	80	74				0.100	0.032
UGNX 2207	451	UUCAGAU CUGGUUU CAGAA	452	UUCUGA AACCAG AUCUGA A	67	-23	2					
UGNX 2209	455	CAGAUCU GGUUUCA GAAUC	456	GAUUCU GAAACC AGAUCU G	91	62	39					
UGNX 2211	459	GAUCUGG UUUCAGA AUCGA	460	UCGAUU CUGAAA CCAGAU C	95	83	36					
UGNX 2212	461	AUCUGGU UUCAGAA UCGAA	462	UUCGAU UCUGAA ACCAGA U	71	86	69				0.180	0.040
UGNX 2951	481	AGGCGCA ACCUCUC CUAGA	482	UCUAGG AGAGG UUGCGC CU	85	66	31					
UGNX 3033	507	CGAGGAA GAAUACC GGGCU	508	AGCCCG GUAUUC UCCUC G	92	75	35					
UGNX 3035	511	AGGAAGA AUACCGG GCUCU	512	AGAGCC CGGUAU UCUCC U	88	35	-14					

Example 4: siRNA Modifications

[00119] siRNA UGNX-1898 (comprising sense strand sequence SEQ ID NO:41 and antisense strand sequence SEQ ID NO:42) was modified by addition of TT overhang at the 3' end of each of the sense and antisense strands, and by incorporation of 2'O-methyl modified ribose sugars in the sense strand as shown below (bold – TT overhangs, underlined – 2'O-methyl).

Sense strand (5'-3') GAGCCUGCUUUGAGCGGAATT (SEQ ID NO:41)
 Antisense strand (3'-5') TTCUCGGACGAAACUCGCCUU (SEQ ID NO:42)

[00120] Inhibition of *DUX4* by unmodified and modified siRNA was determined as explained in Example 2 by measuring the expression of *MBD3L2*. The data is summarized in

FIG. 1A (unmodified) and **FIG. 1B** (modified), and demonstrates that the modified siRNA showed minimal potency loss *in vitro*.

Example 5: siRNA Modifications

[00121] In this example, various chemical modifications were made to generate chemically modified siRNA compounds targeting DUX4. The chemically modified siRNAs are shown in Table 5.

Table 5: Chemically Modified siRNAs

siRNA name	SEQ ID NO.	sense	SEQ ID NO.	antisense
UGNX1165a	597	+GrArArAmUrGrArArCmGrAmGrAmGrCrCrArCrA+T+T	598	rUmGrUmGrGrCmUrCmUrCmGrUmUmCrAmUrUmUrCTT
UGNX1980a	599	+GrGmUrCrCrArGrAmUmUmUrGrGmUmUmUrCrArG+T+T	600	rCmUrGrArArArCrCrArArAmUrCmUrGmGrArCrCTT
UGNX1981a	601	+GmUrCrCrArGrAmUrUmUrGrGmUrUmUrCrAmGrA+T+T	602	rUrCmUrGrArArArCrCrArArAmUrCmUrGmGrArCTT
UGNX1985a	603	+AmGrAmUrUmUrGmGrUmUrUrCrAmGrArAmUrGrA+T+T	604	rUrCrArUmUrCmUrGrArArArCrCrArArAmUrCmUTT
UGNX1987a	605	+AmUrUmUrGrGmUrUmUrCrAmGrArAmUmGrAmGrA+T+T	606	rUrCmUrCrAmUrUrCmUrGrArArArCrCrArArAmUTT
UGNX1992a	607	+GmUrUmUrCrAmGrArAmUrGrAmGrArMrGmUrCrA+T+T	608	rUmGrArCrCmUrCmUrCrArUmUrCrUmGrArArArCTT
UGNX2128a	609	+GrCrCmUrUmUmGrAmGrArAmGrGrAmUrCmGrCmU+T+T	610	rAmGrCmGrAmUrCrCmUrUrCmUrCrArArAmGrGrCTT
UGNX2130a	611	+CmUrUmUmGrAmGrArAmGmGrAmUrCmGrCmUrUmU+T+T	612	rArArAmGrCmGrAmUrCrCmUmUrCmUrCrArArAmGTT
UGNX2205a	613	+GrAmUmUrCrAmGrAmUrCmUrGrGmUrUmUrCrAmG+T+T	614	rCmUrGrArArArCmCrArGrAmUrCmUrGrArAmUrCTT
UGNX2206a	615	+AmUmUrCrAmGrAmUrCmUrGrGmUmUmUrCrAmGrA+T+T	616	rUrCmUrGrArArArCrCrAmGrAmUrCmUrGrArAmUTT
UGNX1165b	617	+GrArArAmUrGrArArCmGrAmGrAmGrCrCrArCrA+T+T	618	mUmGmUmGrGrCmUrCmUrCmGmUmUmCrAmUmUmUrC+T+T
UGNX1980b	619	+GrGmUrCrCrArGrAmUmUmUrGrGmUmUmUrCrArG+T+T	620	rCmUrGrArArArCrCrArArAmUrCmUrGmGrArCrC+T+T
UGNX1981b	621	+GmUrCrCrArGrAmUmUmUrGrGmUmUmUrCrAmGrA+T+T	622	mUrCmUrGrArArArCrCrArArAmUrCmUrGmGrArC+T+T
UGNX1985b	623	+AmGrAmUmUmUrGmGmUmUmUrCrAmGrArAmUrGrA+T+T	624	mUrCrAmUmUrCmUrGrArArArCrCrArArAmUrCmU+T+T
UGNX1987b	625	+AmUmUmUrGrGmUmUmUrCrAmGrArAmUmGrAmGrA+T+T	626	mUrCmUrCrAmUmUrCmUmGrArArArCrCrArArAmU+T+T
UGNX1992b	627	+GmUmUmUrCrAmGrArAmUrGrAmGrArMrGmUrCrA+T+T	628	mUmGrArCrCmUrCmUrCrAmUmUrCmUmGrArArArC+T+T
UGNX2128b	629	+GrCrCmUmUmUmGrAmGrArAmGrGrAmUrCmGrCmU+T+T	630	rAmGrCmGrAmUrCrCmUmUrCmUrCrArArAmGrGrC+T+T
UGNX2130b	631	+CmUmUmUmGrAmGrArAmGmGrAmUrCmGrCmUmUmU+T+T	632	rArArAmGrCmGrAmUrCrCmUmUrCmUrCrArArAmG+T+T
UGNX2205b	633	+GrAmUmUrCrAmGrAmUrCmUrGrGmUmUmUrCrAmG+T+T	634	rCmUrGrArArArCrCrAmGrAmUrCmUrGrArAmUrC+T+T
UGNX2206b	635	+AmUmUrCrAmGrAmUrCmUrGrGmUmUmUrCrAmGrA+T+T	636	mUrCmUrGrArArArCrCrAmGrAmUrCmUrGrArAmU+T+T

UGNX1981c	637	+GmU*rCrCrArGrAmUrUmUrGrGmUrUmUrCrAmGrA+T+T	638	rU*rC*mUrGrArArArCrCrArArAmUrCmUrGmGrArC*T*T
UGNX1985c	639	+AmGrAmUrUmUrGmGrUmUrUrCrAmGrArAmUrGrA+T+T	640	rU*rC*rArUmUrCmUrGrArArArCrCrArArAmUrCmU*T*T
UGNX1987c	641	+AmUrUmUrGrGmUrUmUrCrAmGrArAmUmGrAmGrA+T+T	642	rU*rC*mUrCrAmUrUrCmUrGrArArArCrCrArArAmU*T*T
UGNX2128c	643	+GrCrCmUrUmUmGrAmGrArArGrGrAmUrCmGrCmU+T+T	644	rA*mG*rCrGrArUrCrCmUrUrCmUrCrArArAmGrGrC*T*T
UGNX2130c	645	+CmUrUmUmGrArGrArAmGmGmAmUmCmGrCrUmUmU+T+T	646	rA*rA*rAmGrCrGrArUrCrCrUmUrCmUrCrArArAmG*T*T
UGNX2130d	647	+CmUrUmUmGrArGrArAmGmGmAmUmCmGrCrUmUmU+T+T	648	rA*rA*rAmGrCrGrArUrCrCrUmUrCrUrCrArArAmG*T*T
UGNX1165c	649	+GrArArAmUrGrArArCmGrAmGrAmGrCrCrArCrA+T+T	650	rU*mG*rUmGrGrCmUrCmUrCmGrUmUmCrAmUrUmUrC*T*T
UGNX1165d	651	rG*rA*rArAmUrGrArArCmGrAmGrAmGrCrCrArCrA*T*T	652	+TmGrUmGrGrCmUrCmUrCmGrUmUmCrAmUrUmUrC+T+T
UGNX488c	653	+GrArArCmGrGrAmGmGmGrArArAmGrArCrAmGrA+T+T	654	mU*rC*mUrGmUrCrUmUmUrCrCrCmUrCrCmGrUmUrC*T*T
UGNX488d	655	rG*rA*rArCmGrGrAmGmGmGrArArAmGrArCrAmGrA*T*T	656	+TrCmUrGmUrCrUmUmUrCrCrCmUrCrCmGrUmUrC+T+T
UGNX1097c	657	+AmUrCrCrAmUrCmGmUrCrArGrGmCrCrAmUrCrA+T+T	658	mU*rG*rAmUrGrGmCrCmUrGrArCmGrAmUrGrGrAmU*T*T
UGNX1097d	659	rA*mU*rCrCrAmUrCmGmUrCrArGrGmCrCrAmUrCrA*T*T	660	+TrG*rAmUrGrGmCrCmUrGrArCmGrAmUrGrGrAmU+T+T
UGNX1981d	661	+GmU*rCrCrAmGrAmUrUmUmGrGmUrUmUrCrAmGrA+T+T	662	/5Phos/rU*rC*mUrGrAmArArCrCrAmArAmUrCmUrGmGrArC*T*T
UGNX1981e	663	+GmU*rCrCrAmGrAmUrUmUmGrGmUrUmUrCrAmGrA+T+T	664	/5Phos/rU*rC*mUrGrAmArArCrCrAmArAmUrCmUrGmGrArC+T+T
UGNX1981f	665	+GmU*rCrCrAmGrAmUrUmUmGrGmUrUmUrCrAmGrA+T+T	666	/5Phos/rU*rC*mU*rGrAmArArCrCrAmArAmUrCmUrGmGrArC+T+T
UGNX1985d	667	+AmGrAmUrUmUrGmGrUmUrUrCrAmGrArAmUrGrA+T+T	668	/5Phos/rU*rC*rArUmUrCmUrGmArAmArCrCrAmArAmUrCmU*T*T
UGNX1985e	669	+AmGrAmUrUmUrGmGrUmUrUrCrAmGrArAmUrGrA+T+T	670	/5Phos/rU*rC*rArUmUrCmUrGmArAmArCrCrAmArAmUrCmU+T+T
UGNX1987d	671	+AmUrUmUrGrGmUrUmUmCrAmGmArAmUmGrAmGrA+T+T	672	/5Phos/rU*rC*mUrCrAmUrUrCmUrGrAmArArCmCrAmArAmU*T*T
UGNX1987e	673	+AmUrUmUrGrGmUrUmUmCrAmGmArAmUmGrAmGrA+T+T	674	/5Phos/rU*rC*mUrCrAmUrUrCmUrGrAmArArCmCrAmArAmU+T+T
UGNX2128d	675	+GrCrCmUrUmUmGrAmGrArArGrGrAmUrCmGrCmU+T+T	676	/5Phos/rA*mG*rCrGrAmUrCrCmUrUrCmUrCrAmArAmGrGrC*T*T
UGNX2128e	677	+GrCrCmUrUmUmGrAmGrArArGrGrAmUrCmGrCmU+T+T	678	/5Phos/rA*mG*rCrGrAmUrCrCmUrUrCmUrCrAmArAmGrGrC+T+T
UGNX2130e	679	+CmUrUmUmGrAmGrArAmGmGrAmUrCmGrCmUrUmU+T+T	680	/5Phos/rA*mA*rAmGrCmGrAmUrCrCrUmUrCmUrCrAmArAmG*T*T
UGNX2130f	681	+CmUrUmUmGrAmGrArAmGmGrAmUrCmGrCmUrUmU+T+T	682	/5Phos/rA*mA*rAmGrCmGrAmUrCrCrUmUrCmUrCrAmArAmG+T+T
UGNX1985-Full	683	52FA*mG*I2FmUI2FmUI2FGmGI2FmUI2FmUI2FmGI2FmAI2FmUI2FmGI2FA*+T*+T	684	mU*I2FC*mAI2FmUI2FCmUI2FGmAI2FmAI2FCmUI2FmAI2FmUI2FCmU*T*T
UGNX2130-Full	685	52FC*mU*I2FmUI2FGmAI2FGmAI2FmGI2FGmAI2FmUI2FGmCI2FmUI2FU+T+T	686	mA*I2FA*mAI2FGmCI2FGmAI2FmUI2FCmUI2FmUI2FmUI2FmAI2FmG*T*T
UGNX2206-Full	687	52FA*mU*I2FmUI2FmGI2FmUI2FmUI2FCmUI2FGmGI2FmUI2FmUI2FCmUI2FmGI2FA*+T*+T	688	mU*I2FC*mUI2FGmAI2FmAI2FCmUI2FmGI2FmUI2FCmUI2FGmAI2FmU*T*T

Modifications in Table 5:

52FA = 5' 2'-Fluoro A (a 2-Fluoro A modification at the 5' end)

52FC = 5' 2'-Fluoro C (a 2-Fluoro C modification at the 5' end)

I2FA = Int 2'-Fluoro A (an internal 2-Fluoro A modification)

I2FC = Int 2'-Fluoro C (an internal 2-Fluoro C modification)

I2FG = Int 2'-Fluoro G (an internal 2-Fluoro G modification)

I2FU = Int 2'-Fluoro U (an internal 2-Fluoro U modification)

m = 2' O-Methyl RNA base

* = Phosphorothioated DNA (phosphorothioate bonds in these positions)

r = RNA base

+ = Locked Nucleic Acid (LNA)

[00122] Following synthesis, the chemically modified compounds shown in Table 5 were evaluated for potency and stability. Results are shown in Table 6.

[00123] MB200 and 54-2 potency assays were performed as described in Examples 2-3, while potency in a cultured human hepatocyte carcinoma cell line (HepG2) was measured as follows. Briefly, a human hepatocyte carcinoma cell line HepG2 was cryo-recovered and plated. Cells were seeded at 1×10^5 cells/well in 90 μ l EMEM + 10% FBS in 96-well culture plates. Chemically modified siRNA sequences were then transfected using Lipofectamine RNAiMAX (Thermo-Fisher Scientific) at varying amounts. After 24 hours post-transfection, the cells were lysed in lysis buffer and harvested for subsequent Quantigene Singleplex Gene Expression analysis (Thermo-Fisher Scientific). HepG2 potency values were measured as relative DUX4 gene expression normalized to negative control siRNA, with + = 0.5 and ++ = < 0.5 as illustrated in Table 6.

[00124] Meanwhile, the stability of the chemically modified siRNAs was measured in human serum as follows. First, 1 μ M of each siRNA sequence was incubated with 10% human serum for 2 hours in a heat block at 37°C. Additionally, 1 μ M of each siRNA sequence was incubated without serum at the same conditions. After incubation, the siRNA/serum and siRNA alone mixtures were snap frozen on dry ice. Next, to determine stability, the Agilent Small RNA Assay was run on the samples following manufacturer's protocol for preparation of the chip. Samples were run on Agilent 2100 Bioanalyzer instrument. Stability was determined by peak presence in electropherograms and band presence in translated electrophoresis gel. Quantitation was measured by size distribution of the peak to represent percent recovery, with 50% = +, 50-75% = ++, and greater than 75% = +++ as illustrated in Table 6.

Table 6: Potency and Stability of Chemically Modified siRNAs

siRNA name	Stability	Potency		
		MB200*	54-2*	HepG2
UGNX1165a	Not tested	Not active	Not active	+
UGNX1980a	Not tested	Not active	Not active	+
UGNX1981a	-	0.02	0.072	+
UGNX1985a	+	0.025	0.018	++
UGNX1987a	++	0.024	0.038	++
UGNX1992a	Not tested	1.1	0.79	++
UGNX2128a	Not tested	Not active	Not active	+
UGNX2130a	++	3.1	0.83	+
UGNX2205a	Not tested	Not active	Not active	-
UGNX2206a	-	0.018	0.022	+
UGNX1165b	Not tested	-	-	-
UGNX1980b	Not tested	27%	-	-
UGNX1981b	++	66%	-	-
UGNX1985b		0.27	0.69	-
UGNX1987b	Not tested	89%	7.2	-
UGNX1992b	Not tested	43%	-	-
UGNX2128b	Not tested	46%	-	-
UGNX2130b		52%	> 10	-
UGNX2205b	Not tested		-	-
UGNX2206b	Not tested	0.47	0.43	-
UGNX1165c	++	-	29%	-
UGNX1981c	-	0.92	1.5	+
UGNX1985c	+	0.35	0.3	+
UGNX1987c	++	0.58	0.57	+
UGNX2128c	++	-	-	-
UGNX2130c	++	-	-	-
UGNX488c		-	43%	-
UGNX1097c		-	33%	-
UGNX1165d	+	-	26%	-
UGNX1981d	-	66%	37%	+
UGNX1985d	+	0.75	0.72	+
UGNX1987d	++	3	5	+
UGNX2128d	++	2.5	-	+
UGNX2130d	++	1.2	0.38	-
UGNX488d	-	-	28%	-
UGNX1097d	-	-	25%	-
UGNX1981e	-	0.94	1.5	
UGNX1985e	+	0.28	0.86	+
UGNX1987e	++	1.1	0.99	+
UGNX2128e	+	-	-	+
UGNX2130e	++	-	-	+
UGNX1981f	++	2.9	3.3	+
UGNX2130f	++	-	-	+
UGNX1985-Full	+++	0.5	0.37	Not tested
UGNX2130-Full		0.81	0.64	Not tested
UGNX2206-Full	+++	1.2	1.6	Not tested

Nomenclature in Table 6:

* - Where a % value is provided, the % value is the percent inhibition at 10 nM of chemically modified siRNA; where a numerical value is provided, the numerical value represents the IC₅₀ of the chemically modified siRNA.
 (-) = Not active when used in reference to potency

WHAT IS CLAIMED IS:

1. A double-stranded small interfering RNA (siRNA) comprising a sense strand and an antisense strand,

wherein the antisense strand of the double-stranded siRNA comprises a nucleobase sequence of at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2, 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, 122, 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, 322, 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, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, and 592,

and wherein the double-stranded siRNA comprises at least one modified nucleoside.

2. The double-stranded siRNA of claim 1, wherein the antisense strand of the double-stranded siRNA comprises a nucleobase sequence of at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 216, 218, 220, 312, 324, 340, 342, 348, 350, 352, 354, 364, 372, 376, 400, 402, 404, 410, 434, 446, 448, 450, 462 and 564.

3. A double-stranded siRNA comprising a sense strand and an antisense strand,

wherein the sense strand of the double-stranded siRNA comprises a nucleobase sequence of at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 1, 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, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, and 591,

and wherein the double-stranded siRNA comprises at least one modified nucleoside.

4. A double-stranded siRNA comprising a sense strand and an antisense strand,

wherein the antisense strand of the double-stranded siRNA comprises a nucleobase sequence complementary to at least 8 contiguous nucleobases of an equal length portion of nucleobases 4605 to 7485 of SEQ ID NO: 593; and wherein said sense strand is at least 85% complementary to said antisense strand; and

wherein said double-stranded siRNA comprises at least one modified nucleoside.

5. The double-stranded siRNA of claim 4, wherein the antisense strand of the double-stranded siRNA comprises a nucleobase sequence complementary to at least 8 contiguous nucleobases of an equal length portion of nucleobases 4605-4638, 4693-4727, 4765-4783, 4933-4951, 4990-5011, 5075-5093, 5127-5148, 5161-5193, 5201-5228, 5243-5279, 5305-5327, 5353-5397, 5433-5461, 5464-5509, 5522-5540, 5651-5670, 5809-5830, 5842-5865, 5969-5990, 6066-6086, 6109-6135, 6183-6229, 6328-6369, 6403-6451, 7120-7138, 7162-7190, 7239-7285, 7404-7437, or 7452-7485 of SEQ ID NO: 593.
6. The double-stranded siRNA of any one of the preceding claims, wherein at least one nucleoside of the sense strand of the double-stranded siRNA comprises a modified sugar.
7. The double-stranded siRNA of any one of the preceding claims, wherein each nucleoside of the sense strand of the double-stranded siRNA comprises a modified sugar.
8. The double-stranded small interfering RNA of claim 6, wherein the modified sugar is selected from a 2'-OMe modified sugar and a 2'-F modified sugar.
9. The double-stranded siRNA of any one of the preceding claims, wherein the antisense strand comprises a TT overhang at the 3' end.
10. The double-stranded siRNA of any one of the preceding claims, wherein the sense strand comprises a TT overhang at the 3' end.
11. The double-stranded small interfering RNA of any one of the preceding claims, wherein the sense strand of the double-stranded siRNA comprises at least one modified internucleoside linkage.
12. The double-stranded siRNA of claim 11, wherein the sense strand of the double-stranded siRNA comprises at least five modified internucleoside linkages.

13. The double-stranded siRNA of claim 11, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.
14. The double-stranded siRNA of any one of the preceding claims, wherein the siRNA is conjugated to a lipophilic molecule, an antibody, an aptamer, a ligand, a peptide, or a polymer.
15. The double-stranded siRNA of claim 14, wherein the lipophilic molecule is a long chain fatty acid (LCFA).
16. The double-stranded siRNA of claim 14, wherein the antibody is an anti-transferrin receptor antibody.
17. A pharmaceutical composition comprising the double-stranded siRNA of any one of the preceding claims and a pharmaceutically acceptable carrier.
18. The pharmaceutical composition of claim 17 for use in medical therapy.
19. The pharmaceutical composition of claim 17 for use in the treatment of a human or animal body.
20. A use of the pharmaceutical composition of claim 17 for preparing or manufacturing a medicament for ameliorating, preventing, delaying onset, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof.
21. The use of claim 20, wherein the disease or disorder is facioscapulohumeral muscular dystrophy (FSHD).
22. The use of claim 21, wherein the FSHD is selected from the group consisting of FSHD1 and FSHD2.
23. A method for ameliorating, preventing, delaying onset of, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof, the

method comprising administering to the subject the pharmaceutical composition of claim 17.

24. The method of claim 23, wherein the disease or disorder is FSHD.

25. The method of claim 24, wherein the FSHD is selected from the group consisting of FSHD1 and FSHD2.

26. A method for ameliorating, preventing, delaying onset of, or treating FSHD in a subject need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 17.

27. The method of claim 26, wherein the FSHD is FSHD1.

28. The method of claim 26, wherein the FSHD is FSHD2.

29. A method of ameliorating, preventing, delaying onset, or treating cancer in a subject need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 17.

30. The method of any of claims 23-29, wherein the administration is intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, nasal, or via inhalation.

31. The method of any of claims 23-30, wherein the administration is once daily, weekly, every two weeks, monthly, every two months, quarterly, or yearly.

32. The method of any of claims 23-31, wherein the administration comprises an effective dose of from 0.01 to 100 mg/kg.

33. The method of any of claims 23-32, wherein the administration inhibits expression of DUX4 in the subject.

34. A kit comprising one or more double-stranded siRNA of any one of claims 1-16 and a device for administering said double-stranded siRNA.
35. A method of inhibiting expression of DUX4 in a cell, comprising contacting a cell with the double-stranded siRNA of any one of claims 1-16.
36. A double-stranded siRNA comprising a sense strand and an antisense strand, wherein the sense strand comprises at least 8 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, and 687.
37. A double-stranded siRNA comprising a sense strand and an antisense strand, wherein the antisense strand comprises at least 8 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, and 688.
38. A double-stranded siRNA comprising a sense strand and an antisense strand, wherein the sense strand comprises at least 8 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, and 687, and wherein the antisense strand comprises at least 8 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, and 688.

39. A pharmaceutical composition comprising the double-stranded siRNA of any one of claims 36-38 and a pharmaceutically acceptable carrier.
40. The pharmaceutical composition of claim 39 for use in medical therapy.
41. The pharmaceutical composition of claim 39 for use in the treatment of a human or animal body.
42. A use of the pharmaceutical composition of claim 39 for preparing or manufacturing a medicament for ameliorating, preventing, delaying onset, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof.
43. The use of claim 42, wherein the disease or disorder is FSHD.
44. The use of claim 43, wherein the FSHD is selected from the group consisting of FSHD1 and FSHD2.
45. A method for ameliorating, preventing, delaying onset of, or treating a disease or disorder associated with aberrant expression of DUX4 in a subject need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 17.
46. The method of claim 45, wherein the disease or disorder is FSHD.
47. The method of claim 46, wherein the FSHD is selected from the group consisting of FSHD1 and FSHD2.
48. A method for ameliorating, preventing, delaying onset of, or treating FSHD in a subject need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 39.
49. The method of claim 48, wherein the FSHD is FSHD1.

50. The method of claim 48, wherein the FSHD is FSHD2.
51. A method of ameliorating, preventing, delaying onset, or treating cancer in a subject need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 39.
52. The method of any of claims 45-51, wherein the administration is intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, nasal, or via inhalation.
53. The method of any of claims 45-52, wherein the administration is once daily, weekly, every two weeks, monthly, every two months, quarterly, or yearly.
54. The method of any of claims 45-53, wherein the administration comprises an effective dose of from 0.01 to 100 mg/kg.
55. The method of any of claims 45-54, wherein the administration inhibits expression of DUX4 in the subject.
56. A kit comprising one or more double-stranded siRNA of any one of claims 36-38 and a device for administering said double-stranded siRNA.
57. A method of inhibiting expression of DUX4 in a cell, comprising contacting a cell with the double-stranded siRNA of any one of claims 36-38.

Fig. 1A

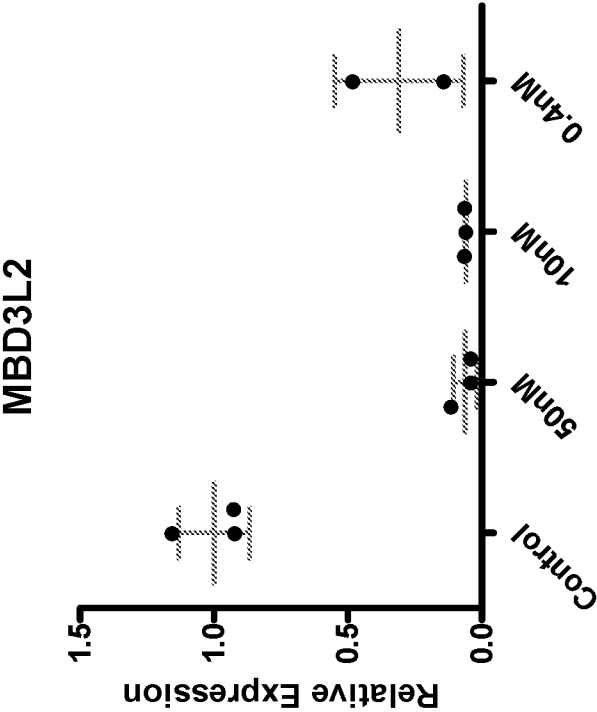
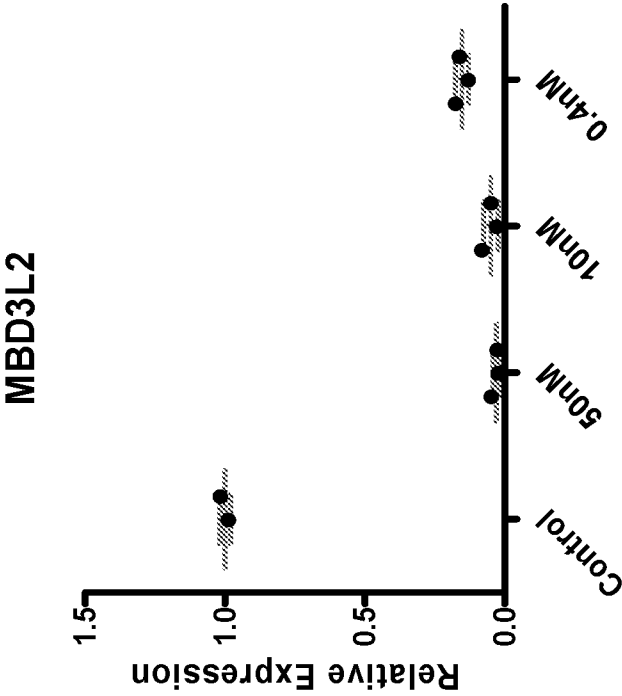


Fig. 1B



SEQUENCE LISTING

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Saint Louis University
Daugherty, Sean Christopher
Chen, Lishan
Sverdrup, Francis Michael

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