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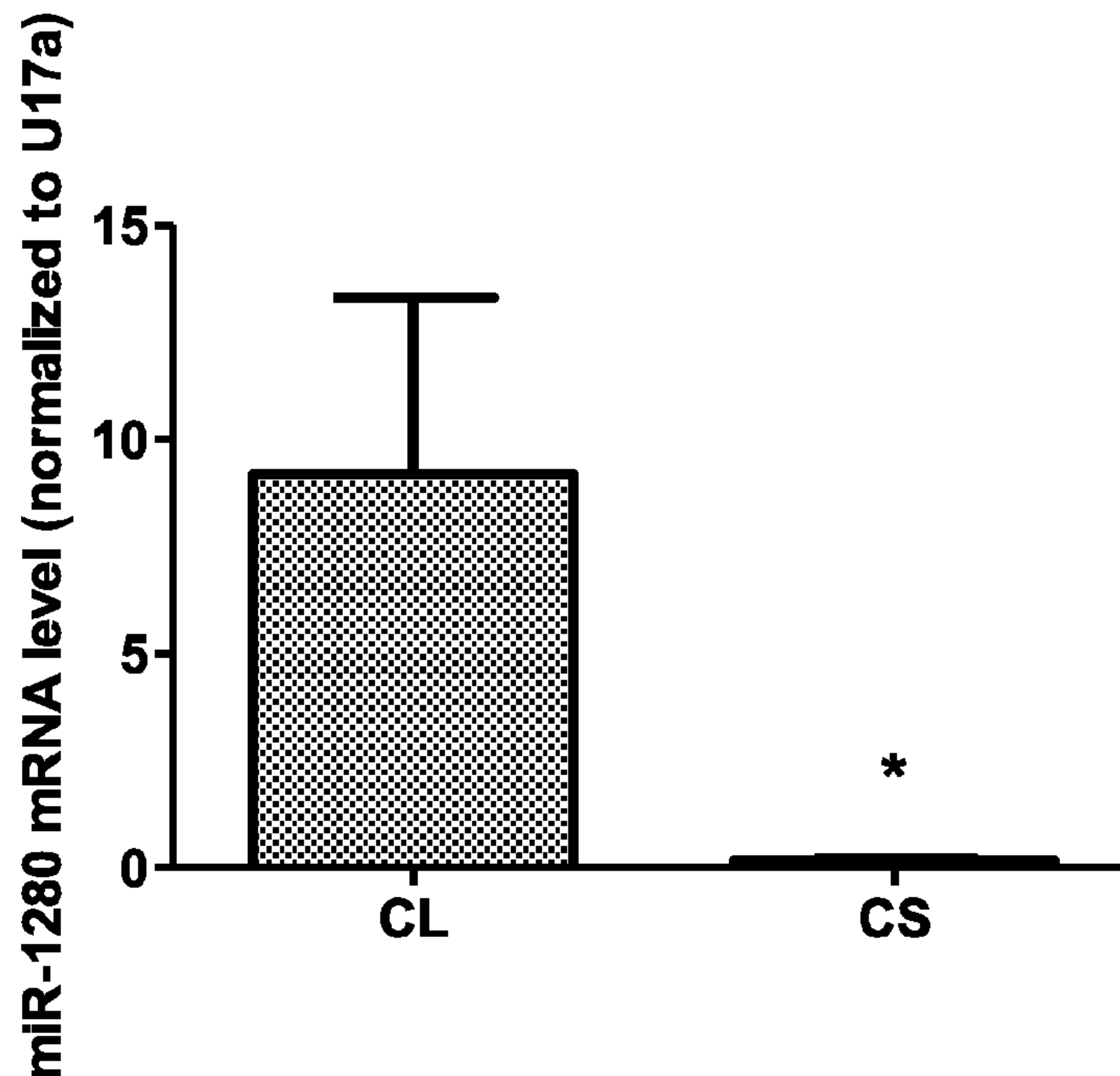
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(71) Demandeur/Applicant:
RHODE ISLAND HOSPITAL, US
(72) Inventeurs/Inventors:
TEREK, RICHARD, US;
CHEN, QIAN, US;
SUN, XIAOJUAN, US
(74) Agent: MBM INTELLECTUAL PROPERTY LAW LLP

(54) Titre : CIBLAGE DE MICRO-ARN POUR LE TRAITEMENT DU CANCER
(54) Title: TARGETING MICRORNA FOR CANCER TREATMENT

FIG. 1



(57) **Abrégé/Abstract:**

This invention is directed to, inter alia, compositions and methods for restoring normal microRNA (miR) expression in chondrosarcoma cells as well as methods for treating and diagnosing chondrosarcoma in individuals in need thereof.

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(71) Applicant: RHODE ISLAND HOSPITAL [US/US]; 593 Eddy Street, Providence, RI 02903 (US).

(72) Inventors: TEREK, Richard; 536 Wayland Avenue, Providence, RI 02906 (US). CHEN, Qian; 8 Tallwood Drive, Barrington, RI 02806 (US). SUN, Xiaojuan; 40 Wallis Avenue, Barrington, RI 02806 (US).

(74) Agents: BEATTIE, Ingrid, A. et al.; Mintz Levin Cohn Ferris Glovsky And Popeo, P.C, One Financial Center, Boston, MA 02111 (US).

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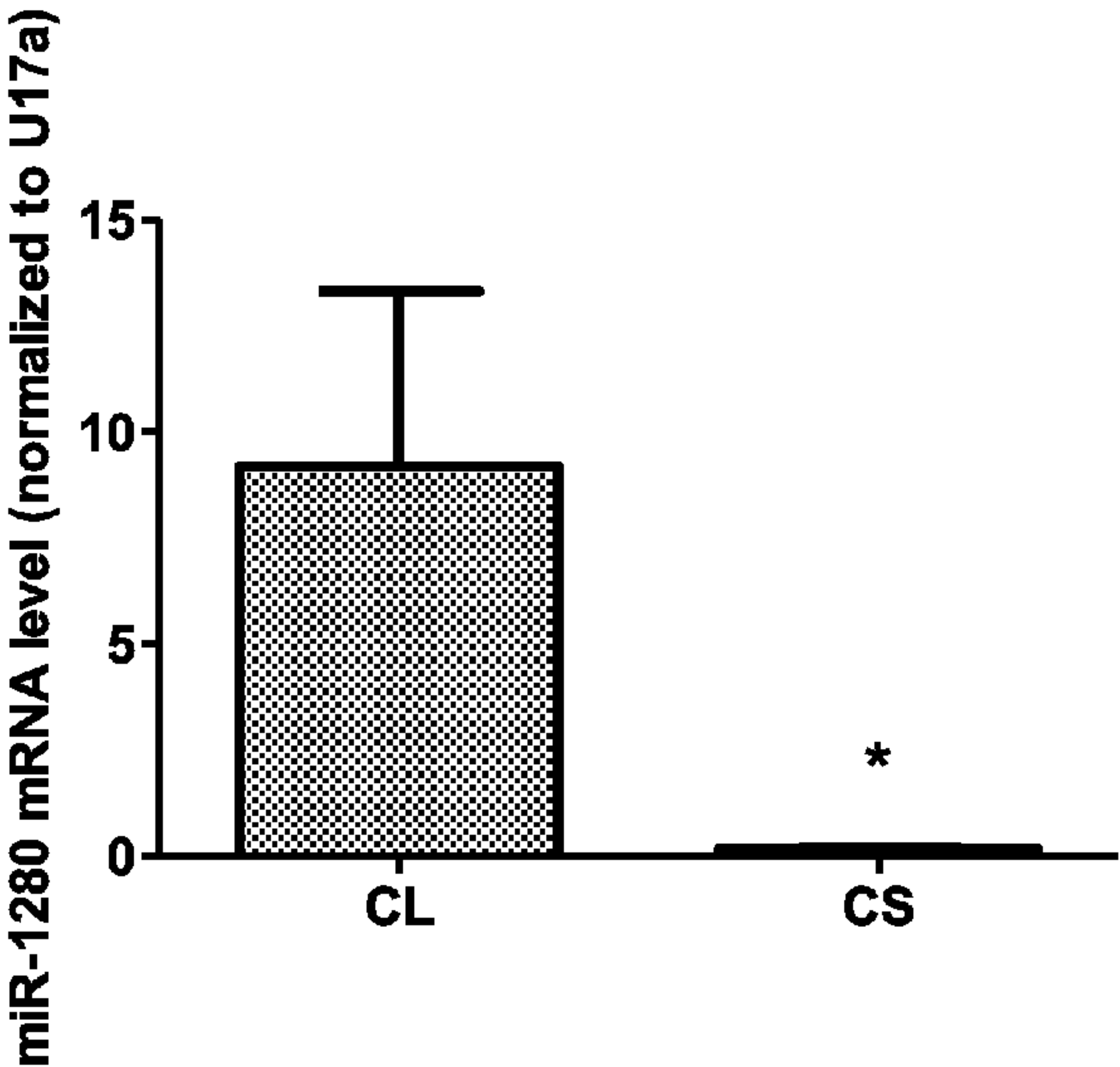
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(54) Title: TARGETING MICRORNA FOR CANCER TREATMENT

FIG. 1



(57) Abstract: This invention is directed to, *inter alia*, compositions and methods for restoring normal microRNA (miR) expression in chondrosarcoma cells as well as methods for treating and diagnosing chondrosarcoma in individuals in need thereof.

WO 2017/152182 A1

WO 2017/152182 A1



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TARGETING MICRORNA FOR CANCER TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/304,048, filed March 4, 2016, the disclosure of which is incorporated by reference herein in its entirety.

GOVERNMENT INTEREST

[0002] The invention was made with government support under grant numbers 1R01CA166089-01 and P20GM104937 awarded by The National Institutes of Health. The government has certain rights in the present invention.

FIELD OF INVENTION

[0003] This invention is directed to, *inter alia*, compositions and methods for the treatment and diagnosis of chondrosarcoma via manipulation of cellular levels of microRNAs.

BACKGROUND

[0004] Chondrosarcoma is the second most common malignancy in bone and is a highly metastatic cancer with no effective systemic treatments. Chondrosarcoma results from unregulated growth of mesenchymal stem cells and is a cancer of cartilage. It tends to be locally invasive and then metastatic. One of the biggest problems associated with chondrosarcoma is that it does not respond to either chemotherapy or radiation. In the past several decades, mesenchymal malignancies such as osteosarcoma and Ewing sarcoma have seen a dramatic increase in long term survival. However, other mesenchymal malignancies, such as human chondrosarcoma, have a poor prognosis due to the absence of an effective adjuvant therapy. The failure of currently available treatments to offer significant increases in long-term survival for individuals with chondrosarcoma indicates an urgent need for the development of new therapies for the treatment of this disease.

SUMMARY

[0005] The invention provides a solution to the clinical problem of treatment for chondrosarcoma, e.g., an adjuvant-based therapy. Adjuvant therapy is an additional cancer treatment given after the primary treatment to lower the risk that the cancer will come back. In the case of chondrosarcoma, primary treatment is typically surgical resection. Adjuvant therapy may include chemotherapy, radiation therapy, hormone therapy, targeted therapy, or biological therapy. Accordingly, in some aspects, a method for treating an individual with chondrosarcoma is provided by administering to the individual a therapeutically effective amount of an inhibitor of one or more microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-93b, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-let-7a-2*, miR-181a, and miR-30e. The methods described herein leads to inhibition of tumor progression (i.e. growth and metastasis) and can be used alone or in combination with other treatments for chondrosarcoma, such as surgical ablation.

[0006] Suitable compounds for inhibiting miR gene expression include, without limitation, antagomirs, double-stranded RNA (such as short- or small-interfering RNA or "siRNA"), antisense nucleic acids, enzymatic RNA molecules such as ribozymes, or molecules capable of forming a triple helix with the miR gene. In some embodiments, the inhibitor molecule causes post-transcriptional silencing of the miR. In some embodiments, the inhibitor molecule inhibits maturation of the miR (i.e. inhibits or prevents expression or function of the stem-loop-based precursor molecule). In some embodiments, the inhibitor molecule is administered as naked RNA, in conjunction with a delivery agent (such as, for example, an anionic lipid-based delivery agent).

[0007] Nucleotide sequences of miRs targeted for inhibition by the methods disclosed herein (and their corresponding mature forms) are listed below. Exemplary miRs range in size from 50-90 nucleotides in length (or any length within that range, with an average length of approximately 70 nucleotides) for miR stem-loop precursors and exemplary mature oligonucleotide compounds are 17 to 25 nucleotides in length, e.g., are 17, 18, 19,

20, 21, 22, 23, 24 or 25 nucleotides in length. For example, a stem-loop precursor is approximately 70 nucleotides and the mature nucleotide product is approximately 22 nucleotides (such as any of about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 nucleotides) in length. A stem-loop precursor comprises a stem-loop secondary structure.

miR-199a-3p (MIMAT0000232):

Mature: ACAGUAGUCUGCACAUUGGUUA (SEQ ID NO:1)

Stem loop:

GCCAACCCAGUGUUCAGACUACCUGUUCAGGAGGCUCUCA AUGUGUACAGUAG
UCUGCACAUUGGUUAGGC (SEQ ID NO:2)

miR-26a (MIMAT0000082):

Mature: UUCAAGUAAUCCAGGAUAGGCU (SEQ ID NO:3)

Stem loop:

GUGGCCUCGUUCAAGUAAUCCAGGAUAGGCUGUGCAGGUCCCAAUGGGCCUAU
UCUUGGUUACUUGCACGGGGACGC (SEQ ID NO:4)

miR-762 (MIMAT0010313):

Mature: GGGGCUGGGGCCGGGGCCGAGC (SEQ ID NO:5)

Stem loop:

GGCCCGGCUCCGGGUCUCGGCCCGUACAGUCCGGCCGGCCAUGCUGGCGGGGC
UGGGGCCGGGGCCGAGCCCGCGGCGGGGCC (SEQ ID NO:6)

miR-125a-5p (MIMAT0000443):

Mature: UCCCUGAGACCCUUUAACCUGUGA (SEQ ID NO:7)

Stem loop:

UGCCAGUCUCUAGGUCCCUGAGACCCUUUAACCUGUGAGGACA UCCAGGGUCA
CAGGUGAGGUUCUUGGGAGCCUGGCGUCUGGCC (SEQ ID NO:8)

miR-let-7G (MIMAT0000414):

Mature: UGAGGUAGUAGUUUGUACAGUU (SEQ ID NO:9)

Stem loop:

AGGCUGAGGUAGUAGUUUGUACAGUUUGAGGGUCUAUGAUACCACCCGGUAC

AGGAGUAACUGUACAGGCCACUGCCUUGCCA (SEQ ID NO:10)

miR-16 (MIMAT0000069):

Mature: UAGCAGCACGUAAAUAUUGGCG (SEQ ID NO:11)

Stem loop:

GUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUA
UCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGAC (SEQ ID NO:12)

let-7f (MIMAT0000067):

Mature: UGAGGUAGUAGAUUGUAUAGUU (SEQ ID NO:13)

Stem loop:

UCAGAGUGAGGUAGUAGAUUGUAUAGUUGUGGGGUAGUGAUUUUACCCUGUU
CAGGAGUAACUAUACAAUCUAUUGCCUUCCCUGA (SEQ ID NO:14)

miR-21 (MIMAT0000076):

Mature: UAGCUUAUCAGACUGAUGUUGA (SEQ ID NO:15)

Stem loop:

UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACC
AGUCGAUGGGCUGUCUGACA (SEQ ID NO:16)

miR-Let-7a (MIMAT0000062):

Mature: UGAGGUAGUAGGUUGUAUAGUU (SEQ ID NO:17)

Stem loop:

UGGGAUGAGGUAGUAGGUUGUAUAGUUUAGGGUCACACCCACCACUGGGAG
AUAACUAUACAAUCUACUGUCUUUCCUA (SEQ ID NO:18)

miR-638 (MIMAT0003308):

Mature: AGGGAUCGCGGGCGGGUGGCGGCCU (SEQ ID NO:19)

Stem loop:

GUGAGCGGGCGCGGCAGGGAUCGCGGGCGGGUGGCGGCCUAGGGCGCGGAGG
GCGGACCGGGAAUGGCGCGCCGUGCGCCGCCGGCGUAACUGCGGCGCU (SEQ ID
NO:102)

miR-23a (MIMAT0000078):

Mature: AUCACAUUGCCAGGGAUUUCC (SEQ ID NO:20)

Stem loop:

GGCCGGCUGGGGUUCCUGGGGAUGGGAUUUGCUUCCUGUCACAAAUCACAUU
GCCAGGGAUUUCCAACCGACC (SEQ ID NO:21)

miR-92a (MIMAT0000092):

Mature: UAUUGCACUUGUCCCCGGCCUGU (SEQ ID NO:22)

Stem loop:

CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUG
CACUUGUCCCCGGCCUGUUGAGUUUGG (SEQ ID NO:23)

miR-15b (MIMAT0000417):

Mature: UAGCAGCACAUCAUGGUUUACA (SEQ ID NO:24)

Stem loop:

UUGAGGCCUUAAGUACUGUAGCAGCACAUCAUGGUUUACAUGCUACAGUCA
AGAUGCGAAUCAUUAUUUGCUGCUCUAGAAAUUUAAGGAAAUUCAU (SEQ ID
NO:25)

miR-23b (MIMAT0000418):

Mature: AUCACAUUGCCAGGGAUUACC (SEQ ID NO:26)

Stem loop:

CUCAGGUGCUCUGGCUGCUUGGGUUCCUGGCAUGCUGAUUUGUGACUUAAGA
UUAAAUCACAUUGCCAGGGAUUACCACGCAACCACGACCUUGGC (SEQ ID
NO:27)

miR-451 (MIMAT0001631):

Mature: AAACCGUUACCAUACUGAGUU (SEQ ID NO:28)

Stem loop:

CUUGGGAAUGGCAAGGAAACCGUUACCAUACUGAGUUUAGUAAUGGUAUUG
GUUCUCUUGCUAUACCCAGA (SEQ ID NO:29)

miR-483-5P (MIMAT0004761):

Mature: AAGACGGGAGGAAAGAAGGGAG (SEQ ID NO:30)

Stem loop:

GAGGGGGAAGACGGGAGGAAAGAAGGGAGUGGUUCCAUCACGCCUCCUCACU
CCUCUCCUCCCGUCUUCUCCUCUC (SEQ ID NO:103)miR-15a (MIMAT0000068):

Mature: UAGCAGCACAUAAUGGUUUGUG (SEQ ID NO:31)

Stem loop:

CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGCA
GGCCAUAUUGUGCUGCCUCAAAAAUACAAGG (SEQ ID NO:32)miR-27a (MIMAT0000084):

Mature: UUCACAGUGGCUAAGUUCCGC (SEQ ID NO:33)

Stem loop:

CUGAGGAGCAGGGCUUAGCUGCUUGUGAGCAGGGUCCACACCAAGUCGUGUUC
ACAGUGGCUAAGUUCCGCCCCCAG (SEQ ID NO:34)miR-26b (MIMAT0000083):

Mature: UUCAAGUAAUUCAGGAUAGGU (SEQ ID NO:35)

Stem loop:

CCGGGACCCAGUUCAAGUAAUUCAGGAUAGGUUGUGUGCUGUCCAGCCUGUUC
UCCAUAUACUUGGCUCGGGGACCGG (SEQ ID NO:36)miR-let-7d (MIMAT0000065):

Mature: AGAGGUAGUAGGUUGCAUAGUU (SEQ ID NO:37)

Stem loop:

CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUUGCCCACA
AGGAGGUAAUAUACGACCUGCUGCCUUCUAGG (SEQ ID NO:38)miR-27b(MIMAT0000419):

Mature: UUCACAGUGGCUAAGUUCUGC (SEQ ID NO:39)

Stem loop:

miR-98 (MIMAT0000096):

Mature: UGAGGUAGUAAGUUGUAUUGUU (SEQ ID NO:40)

Stem loop:

AGGAUUCUGCUCAUGCCAGGGUGAGGUAGUAAGUUGUAUUGUUGUGGGGUAG
GGAUAUUAGGCCCCAAUUAGAAGAUAAUAUACAACUACUACUUUCCCUGG
UGUGUGGCAUAUUA (SEQ ID NO:41)

miR-145 (MIMAT0000437):

Mature: GUCCAGUUUUCCCAGGAAUCCCU (SEQ ID NO:42)

Stem loop:

CACCUUGUCCUCACGGUCCAGUUUUCCCAGGAAUCCCUUAGAUGCUAAGAUGG
GGAUUCCUGGAAAUACUGUUCUUGAGGUCAUGGUU (SEQ ID NO:43)

miR-143 (MIMAT0000435):

Mature: UGAGAUGAAGCACUGUAGCUC (SEQ ID NO:44)

Stem loop:

GCGCAGCGCCCUGUCUCCCAGCCUGAGGUGCAGUGCUGCAUCUCUGGUCAGUU
GGGAGUCUGAGAUGAAGCACUGUAGCUCAGGAAGAGAGAAGUUGUUCUGCAG
C (SEQ ID NO:45)

miR-1915 (MIMAT0007892):

Mature: CCCCAGGGCGACGCGGCGGG (SEQ ID NO:46)

Stem loop:

UGAGAGGCCGCACCUUGCCUUGCUGCCCGGGCCGUGCACCCGUGGGCCCCAGG
GCGACGCGGCGGGGGCGGCCCCUAGCGA (SEQ ID NO:47)

mir-149* (MIMAT0004609):

Mature: AGGGAGGGACGGGGGCUGUGC (SEQ ID NO:48)

Stem loop:

GCCGGCGCCCGAGCUCUGGCUCCGUGUCUUCACUCCCGUGCUUGUCCGAGGAG
GGAGGGAGGGACGGGGGCUGUGCUGGGGCAGCUGGA (SEQ ID NO:49)

miR-7i (MIMAT0000415):

Mature: UGAGGUAGUAGUUUGUGCUGUU (SEQ ID NO:50)

Stem loop:

CUGGCUGAGGUAGUAGUUUGUGCUGUUGGUCGGGUUGUGACAUUGCCCGCUG
UGGAGAUAAACUGCGCAAGCUACUGCCUUGCUA (SEQ ID NO:51)let-7c (MIMAT0000064):

Mature: UGAGGUAGUAGGUUGUAUGGUU (SEQ ID NO:52)

Stem loop:

GCAUCCGGGUUGAGGUAGUAGGUUGUAUGGUUUAGAGUUACACCCUGGGAGU
UAAACUGUACAACCUUCUAGCUUUCCUUGGAGC (SEQ ID NO:53)let-7e (MIMAT0000066):

Mature: UGAGGUAGGAGGUUGUAUAGUU (SEQ ID NO:54)

Stem loop:

CCCGGGCUGAGGUAGGAGGUUGUAUAGUUUGAGGAGGACACCCAAGGAGAUCA
CUAUACGGCCUCCUAGCUUUCCCCAGG (SEQ ID NO:55)miR-936 (MIMAT0004979):

Mature: ACAGUAGAGGGAGGAAUCGCAG (SEQ ID NO:56)

Stem loop:

UCAAGGCCACUGGGACAGUAGAGGGAGGAAUCGCAGAAAUCACUCCAGGAGC
AACUGAGAGACCUUGCUUCUACUUUACCAGGUCCUGCUGGCCCCAGA (SEQ ID
NO:57)miR-let-7b (MIMAT0000063):

Mature: UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO:58)

Stem loop:

CGGGGUGAGGUAGUAGGUUGUGUGGUUUCAGGGCAGUGAUGUUGCCCCUCGG
AAGAUAACUAUACAACCUACUGCCUUCCCUG (SEQ ID NO:59)miR-30c (MIMAT0000244):

Mature: UGUAAACAUCCUACACUCUCAGC (SEQ ID NO:60)

Stem loop:

AGAUACUGUAAACAUCCUACACUCUCAGCUGUGGAAAGUAAGAAAGCUGGGA
GAAGGCUGUUUACUCUUUCU (SEQ ID NO:61)

miR-181d (MIMAT0002821):

Mature: AACAUUCAUUGUUGUCGGUGGGU (SEQ ID NO:62)

Stem loop:

GUCCCCUCCCCUAGGCCACAGCCGAGGUCACAAUCAACAUUCAUUGUUGUCGG
UGGGUUGUGAGGACUGAGGCCAGACCCACCGGGGGGAUGAAUGUCACUGUGGC
UGGGCCAGACACGGCUUAAGGGGGAUGGGGAC (SEQ ID NO:63)

miR-148a (MIMAT0000243):

Mature: UCAGUGCACUACAGAACUUUGU (SEQ ID NO:64)

Stem loop:

GAGGCAAAGUUCUGAGACACUCCGACUCUGAGUAUGAUAGAAGUCAGUGCAC
UACAGAACUUUGUCUC (SEQ ID NO:65)

miR-181c (MIMAT0000258):

Mature: AACAUUCAACCUGUCGGUGAGU (SEQ ID NO:66)

Stem loop:

CGGAAA AUUUGCCAAGGGUUUGGGGGAACA UUCAACCUGUCGGUGAGUUUGG
GCAGCUCAGGCAAACCAUCGACCGUUGAGUGGACCCUGAGGCCUGGAAUUGCC
AUCU (SEQ ID NO:67)

miR-196a (MIMAT0000226):

Mature: UAGGUAGUUUCAUGUUGUUGGG (SEQ ID NO:68)

Stem loop:

GUGAAUUAGGUAGUUUCAUGUUGUUGGGCCUGGGUUUCUGAACACAACAACA
UUAACCACCCGAUUCAC (SEQ ID NO:69)

miR-30a (MIMAT0000087):

Mature: UGUAAACAUCCUCGACUGGAAG (SEQ ID NO:70)

Stem loop:

GCGACUGUAAACAUCCUCGACUGGAAGCUGUGAAGCCACAGAUGGGCUUUCAG
UCGGAUGUUUGCAGCUGC (SEQ ID NO:71)

miR-214 (MIMAT0000271):

Mature: ACAGCAGGCACAGACAGGCAGU (SEQ ID NO:72)

Stem loop:

GGCCUGGCUGGACAGAGUUGUCAUGUGUCUGCCUGUCUACACUUGCUGUGCAG
AACAUCCGCUCACCUGUACAGCAGGCACAGACAGGCAGUCACAUGACAACCCA
GCU (SEQ ID NO:73)

miR-187* (MIMAT0004561):

Mature: GGCUACAACACAGGACCCGGGC (SEQ ID NO:74)

Stem loop:

GGUCGGGCUCACCAUGACACAGUGUGAGACCUCGGGCUACAACACAGGACCCG
GGCGCUGCUCUGACCCCUCGUGUCUUGUGUUGCAGCCGGAGGGACGCAGGUCC
GCA (SEQ ID NO:75)

miR-663 (MIMAT0003326):

Mature: AGGCGGGGCGCCGCGGGACCGC (SEQ ID NO:76)

Stem loop:

CCUUCCGGCGUCCCAGGCGGGGCGCCGCGGGACCGCCCUCGUGUCUGUGGCGG
UGGGAUCCCGCGGCCGUGUUUUCUGGUGGCCCCGGCCAUG (SEQ ID NO:77)

miR-146a (MIMAT0000449):

Mature: UGAGAACUGAAUCCAUGGGUU (SEQ ID NO:78)

Stem loop:

CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUCCAUGGGUUGUGUCAGUGU
CAGACCUCUGAAAUUCAGUUCUUCAGCUGGGAUAUCUCUGUCAUCGU (SEQ ID
NO:79)

miR-30d (MIMAT0000245):

Mature: UGUAAACAUCCCCGACUGGAAG (SEQ ID NO:80)

Stem loop:

GUUGUUGUAAACAUCCCCGACUGGAAGCUGUAAGACACAGCUAAGCUUUCAG
UCAGAUGUUUGCUGCUAC (SEQ ID NO:81)

miR-365 (MIMAT0000710):

Mature: UAAUGCCCCUAAAAAUCCUUAU (SEQ ID NO:82)

Stem loop:

ACCGCAGGGAAAAUGAGGGACUUUUGGGGGCAGAUGUGUUUCCAUUCCACUA
UCAUAAUGCCCCUAAAAAUCCUUAUUGCUCUUGCA (SEQ ID NO:83)

miR-424 (MIMAT0001341):

Mature: CAGCAGCAAUUCAUGUUUUGAA (SEQ ID NO:84)

Stem loop:

CGAGGGGAUACAGCAGCAAUUCAUGUUUUGAAGUGUUCUAAAUGGUUCAAAA
CGUGAGGCGCUGCUAUACCCCCUCGUGGGGAAGGUAGAAGGUGGGG (SEQ ID
NO:85)

miR-1231 (MIMAT0005586):

Mature: GUGUCUGGGCGGACAGCUGC (SEQ ID NO:86)

Stem loop:

GUCAGUGUCUGGGCGGACAGCUGCAGGAAAGGGAAGACCAAGGCUUGCUGUC
UGUCCAGUCUGCCACCCUACCCUGUCUGUUCUUGCCACAG (SEQ ID NO:87)

miR-424* (MIMAT0004749):

Mature: CAAAACGUGAGGCGCUGCUAU (SEQ ID NO:88)

Stem loop:

CGAGGGGAUACAGCAGCAAUUCAUGUUUUGAAGUGUUCUAAAUGGUUCAAAA
CGUGAGGCGCUGCUAUACCCCCUCGUGGGGAAGGUAGAAGGUGGGG (SEQ ID
NO:89)

miR-454 (MIMAT0003885):

Mature: UAGUGCAAUAUUGCUUAUAGGGU (SEQ ID NO:90)

Stem loop:

UCUGUUUAUCACCAGAUCCUAGAACCCUAUCAUAUUGUCUCUGCUGUGUAAA
UAGUUCUGAGUAGUGCAAUAUUGCUUAUAGGGUUUUGGUGUUUGGAAAGAAC
AAUGGGCAGG (SEQ ID NO:91)

miR-455-5p (MIMAT0003150):

Mature: UAUGUGCCUUUGGACUACAUCG (SEQ ID NO:92)

Stem loop:

UCCCUGGCGUGAGGGUAUGUGCCUUUGGACUACAUCGUGGAAGCCAGCACCAU
GCAGUCCAUGGGCAUAUACACUUGCCUCAAGGCCUAUGUCAUC (SEQ ID NO:93)

miR-337-3p (MIMAT0000754):

Mature: CUCCUAUAUGAUGCCUUUCUUC (SEQ ID NO:94)

Stem loop:

GUAGUCAGUAGUUGGGGGGUGGGGAACGGCUUCAUACAGGAGUUGAUGCACAG
UUAUCCAGCUCCUAUAUGAUGCCUUUCUUCAUCCCCUCAA (SEQ ID NO:95)

miR-381 (MIMAT0000736):

Mature: UAUACAAGGGCAAGCUCUCUGU (SEQ ID NO:96)

Stem loop:

UACUUAAGCGAGGUUGCCCUUUGUAUAUUCGGUUUAUUGACAUGGAAUAUA
CAAGGGCAAGCUCUCUGUGAGUA (SEQ ID NO:97)

miR-30e (MIMAT0000692):

Mature: UGUAAACAUCUUGACUGGAAG (SEQ ID NO:98)

Stem loop:

GGGCAGUCUUUGCUACUGUAAACAUCUUGACUGGAAGCUGUAAGGUGUUCA
GAGGAGCUUUCAGUCGGAUGUUUACAGCGGCAGGCUGCCA (SEQ ID NO:99)

miR-181a (MI0000269)

Mature: ACAUUCAACGCUGUCGGUGAGU (SEQ ID NO:100)

Stem loop:

AGAAGGGCUAUCAGGCCAGCCUUCAGAGGACUCCAAGGAACAUUCAACGCUGU
CGGUGAGUUUGGGAUUUGAAAAAACCACUGACCGUUGACUGUACCUUGGGGU
CCUUA (SEQ ID NO:101)

[0008] In other aspects, a method for treating an individual with chondrosarcoma is provided by administering to the individual a therapeutically effective amount of a nucleic acid encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-1280, miR-126, and miR-324-3p. In some embodiments, the nucleic acid is administered on a vector, for example, a viral vector or nanoparticle.

[0009] Nucleotide sequences of miR-encoding nucleic acids (such as miR-encoding nucleic acids which are part of a delivery construct, such as a viral vector) and combinations of the same (and their corresponding stem-loop forms) for use in the methods disclosed herein are listed below.

miR-320c (MIMAT0005793):

Mature: AAAAGCUGGGUUGAGAGGGGU (SEQ ID NO:104)

Stem loop:

UUUGCAUUA AAAAUGAGGCCUUCUCU UCCCAGUUCU UCCCAGAGUCAGGAAAA
GCUGGGUUGAGAGGGUAGAAAAAAAUGAUGUAGG

miR-320b (MIMAT0005792):

Mature: AAAAGCUGGGUUGAGAGGGCAA (SEQ ID NO:105)

Stem loop:

AAUUA AUCCCUCUCU UUCUAGUUCU UCCUAGAGUGAGGAAAAGCUGGGUUGA
GAGGGCAAACAAUUAACUAAUUA AUU (SEQ ID NO:106)

miR-320a (MIMAT0000510):

Mature: AAAAGCUGGGUUGAGAGGGCGA (SEQ ID NO:107)

Stem loop:

GCUUCGCUCCCCUCCGCCUUCUCUUCCCGGUUCUUCCCGGAGUCGGGAAAAGC
UGGGUUGAGAGGGGCGAAAAAGGAUGAGGU (SEQ ID NO:108)

miR-127-3p (MIMAT0000446):

Mature: UCGGAUCCGUCUGAGCUUGGCU (SEQ ID NO:109)

Stem loop:

UGUGAUCACUGUCUCCAGCCUGCUGAAGCUCAGAGGGCUCUGAUUCAGAAAGA
UCAUCGGAUCCGUCUGAGCUUGGCUGGUCGGAAGUCUCAUCAUC (SEQ ID
NO:110)

miR-1260 (MIMAT0005911):

Mature: AUCCCACCUCUGCCACCA (SEQ ID NO:111)

Stem loop:

ACCUUUCAGCUCAUCCCACCUCUGCCACCAAAACACUCAUCGCGGGGGUCAGA
GGGAGUGCCAAAAAAGGUAA (SEQ ID NO:112)

miR-140-3p (MIMAT0004597):

Mature: UACCACAGGGUAGAACCACGG (SEQ ID NO:113)

Stem loop:

UGUGUCUCUCUCUGUGUCCUGCCAGUGGUUUUACCCUAUGGUAGGUUACGUCA
UGCUGUUCUACCACAGGGUAGAACCACGGACAGGAUACCGGGGCACC (SEQ ID
NO:114)

miR-22 (MIMAT0000077):

Mature: AAGCUGCCAGUUGAAGAACUGU (SEQ ID NO:115)

Stem loop:

GGCUGAGCCGCAGUAGUUCUUCAGUGGCAAGCUUUAUGUCCUGACCCAGCUAA
AGCUGCCAGUUGAAGAACUGUUGCCCUCUGCC (SEQ ID NO:116)

miR-146b-5p (MIMAT0002809):

Mature: UGAGAACUGAAUCCAUAAGGCU (SEQ ID NO:117)

Stem loop:

CCUGGCACUGAGAACUGAAUCCAUAAGGCUGUGAGCUCUAGCAAUGCCCUGUG

GACUCAGUUCUGGUGCCCGG (SEQ ID NO:118)

miR-107 (MIMAT0000104):

Mature: AGCAGCAUUGUACAGGGCUAUA (SEQ ID NO:119)

Stem loop:

CUCUCUGCUUUCAGCUUCUUUACAGUGUUGCCUUGUGGCAUGGAGUUCAAGCA
GCAUUGUACAGGGCUAUCAAAGCACAGA (SEQ ID NO:120)

miR-320d (MIMAT0006764):

Mature: AAAAGCUGGGUUGAGAGGA (SEQ ID NO:121)

Stem loop:

UUCUCGUCCCAGUUCUUCCCAAAGUUGAGAAAAGCUGGGUUGAGAGGA (SEQ
ID NO:122)

miR-423-5p (MIMAT0004748):

Mature: UGAGGGGCAGAGAGCGAGACUUU (SEQ ID NO:123)

Stem loop:

AUAAAGGAAGUUAGGCUGAGGGGCAGAGAGCGAGACUUUUCUAUUUUCCAAA
AGCUCGGUCUGAGGCCCCUCAGUCUUGCUUCCUAACCCGCGC (SEQ ID NO:124)

miR-1974 (MIMAT0009449):

Mature: UGGUUGUAGUCCGUGCGAGAAUA (SEQ ID NO:125)

Stem loop:

UGUUCUUGUAGUUGAAAUACAACGAUGGUUUUUCUAUAUCAUUGGUCGUGGUU
GUAGUCCGUGCGAGAAUA (SEQ ID NO:126)

miR-455-3p (MIMAT0004784):

Mature: GCAGUCCAUGGGCAUAUACAC (SEQ ID NO:127)

Stem loop:

UCCUGGCGUGAGGGUAUGUGCCUUGGACUACAUCGUGGAAGCCAGCACCAU
GCAGUCCAUGGGCAUAUACACUUGCCUCAAGGCCUAUGUCAUC (SEQ ID
NO:128)

miR-193b* (MIMAT0002819):

Mature: AACUGGCCCUCAAAGUCCCGCU (SEQ ID NO:129)

Stem loop:

GUGGUCUCAGAAUCGGGGUUUUGAGGGGCGAGAUGAGUUUAUGUUUUAUCCAA
CUGGCCCUCAAAGUCCCGCUUUUGGGGUCAU (SEQ ID NO:130)

miR-103 (MIMAT0000101):

Mature: AGCAGCAUUGUACAGGGCUAUGA (SEQ ID NO:131)

Stem loop:

UUGUGCUUUCAGCUUCUUUACAGUGCUGCCUUGUAGCAUUCAGGUCAAGCAGC
UUGUACAGGGCUAUGAAAGAACCA (SEQ ID NO:132)

miR-432 (MIMAT0002814):

Mature: UCUUGGAGUAGGUCAUUGGGUGG (SEQ ID NO:133)

Stem loop:

UGACUCCUCCAGGUCUUGGAGUAGGUCAUUGGGUGGAUCCUCUAUUUCCUAC
GUGGGCCACUGGAUGGCUCCUCCAUGUCUUGGAGUAGAUA (SEQ ID NO:134)

miR-151-3p (MIMAT0000757):

Mature: CUAGACUGAAGCUCCUUGAGG (SEQ ID NO:135)

Stem loop:

UUUCCUGCCCUCGAGGAGCUCACAGUCUAGUAUGUCUCAUCCCCUACUAGACU
GAAGCUCCUUGAGGACAGGGAUGGUCAUACUCACCUC (SEQ ID NO:136)

miR-31 (MIMAT0000089):

Mature: AGGCAAGAUGCUGGCAUAGCU (SEQ ID NO:137)

Stem loop:

GGAGAGGAGGCAAGAUGCUGGCAUAGCUGUUGAACUGGGAACCUGCUAUGCC
AACAUAUUGCCAUCUUUCC (SEQ ID NO:138)

miR-664* (MIMAT0005948):

Mature: ACUGGCUAGGGAAAAUGAUUGGAU (SEQ ID NO:139)

Stem loop:

GAACAUUGAAACUGGCUAGGGAAAAUGAUUGGAUAGAAACUAUUAUUCUAUU
CAUUUAUCCCCAGCCUACAAAAUGAAAAAA (SEQ ID NO:140)

miR-486-5p (MIMAT0002177):

Mature: UCCUGUACUGAGCUGCCCCGAG (SEQ ID NO:141)

Stem loop:

GCAUCCUGUACUGAGCUGCCCCGAGGCCCUUCAUGCUGCCCAGCUCGGGGCAG
CUCAGUACAGGAUAC (SEQ ID NO:142)

miR-99a (MIMAT0000097):

Mature: AACCCGUAGAUCCGAUCUUGUG (SEQ ID NO:143)

Stem loop:

CCCAUUGGCAUAAACCCGUAGAUCCGAUCUUGUGGUGAAGUGGACCGCACAAG
CUCGCUUCUAUGGGUCUGUGUCAGUGUG (SEQ ID NO:144)

miR-24 (MIMAT0000080):

Mature: UGGCUCAGUUCAGCAGGAACAG (SEQ ID NO:145)

Stem loop:

CUCCGGUGCCUACUGAGCUGAUUAUCAGUUCUCAUUUUACACACUGGCUCAGUU
CAGCAGGAACAGGAG (SEQ ID NO:146)

miR-191 (MIMAT0000440):

Mature: CAACGGAAUCCCAAAGCAGCUG (SEQ ID NO:147)

Stem loop:

CGGCUGGACAGCGGGCAACGGAAUCCCAAAGCAGCUGUUGUCUCCAGAGCAU
UCCAGCUGCGCUUGGAUUUCGUCCCCUGCUCUCCUGCCU (SEQ ID NO:148)

miR-99b (MIMAT0000689):

Mature: CACCCGUAGAACCGACCUUGCG (SEQ ID NO:149)

Stem loop:

GGCACCCACCCGUAGAACCGACCUUGCGGGGCCUUCGCCGCACACAAGCUCGU
GU CUGUGGGUCCGUGUC (SEQ ID NO:150)

miR-574-5p (MIMAT0004795):

Mature: UGAGUGUGUGUGUGUGAGUGUGU (SEQ ID NO:151)

Stem loop:

GGGACCUGCGUGGGUGCGGGCGUGUGAGUGUGUGUGUGAGUGUGUGUCGC
UCCGGGUCCACGCUCAUGCACACACCCACACGCCCACACUCAGG (SEQ ID
NO:152)

miR-151-5p (MIMAT0004697):

Mature: UCGAGGAGCUCACAGUCUAGU (SEQ ID NO:153)

Stem loop:

UUUCCUGCCCUCGAGGAGCUCACAGUCUAGUAUGUCUCAUCCCCUACUAGACU
GAAGCUCCUUGAGGACAGGGAUGGUCAUACUCACCUC (SEQ ID NO:154)

miR-193a-5p (MIMAT0004614):

Mature: UGGGUCUUUGCGGGCGAGAUGA (SEQ ID NO:155)

Stem loop:

CGAGGAUGGGAGCUGAGGGCUGGGUCUUUGCGGGCGAGAUGAGGGUGUCGGA
UCAACUGGCCUACAAAGUCCCAGUUCUCGGCCCCCG (SEQ ID NO:156)

miR-1246 (MIMAT0005898):

Mature: AAUGGAUUUUUGGAGCAGG (SEQ ID NO:157)

Stem loop:

UGUAUCCUUGAAUGGAUUUUUGGAGCAGGAGUGGACACCUGACCCAAAGGAA
AUCAAUCCAUAAGGCUAGCAAU (SEQ ID NO:158)

miR-877 (MIMAT0004949):

Mature: GUAGAGGAGAUGGCGCAGGG (SEQ ID NO:159)

Stem loop:

GUAGAGGAGAUGGCGCAGGGGACACGGGCAAAGACUUGGGGGUUCCUGGGAC
CCUCAGACGUGUGUCCUCUUCUCCUCCUCCAG (SEQ ID NO:160)

miR-940 (MIMAT0004983):

Mature: AAGGCAGGGCCCCCGCUCCCC (SEQ ID NO:161)

Stem loop:

GUGAGGUGUGGGCCCCGGCCCCAGGAGCGGGGCCUGGGCAGCCCCGUGUGUUGA
GGAAGGAAGGCAGGGCCCCCGCUCCCCGGGGCCUGACCCAC (SEQ ID NO:162)

miR-1281 (MIMAT0005939):

Mature: UGCCCUCCUCCUCUCCC (SEQ ID NO:163)

Stem loop:

AGGGGGCACCGGGAGGAGGUGAGUGUCUCUUGUCGCCUCCUCCUCUCCCCCU
U (SEQ ID NO:164)

miR-494 (MIMAT0002816):

Mature: UGAAACAUACACGGGAAACCUC (SEQ ID NO:165)

Stem loop:

GAUACUCGAAGGAGAGGUUGUCCGUGUUGUCUUCUCUUUAUUUAUGAUGAAA
CAUACACGGGAAACCUCUUUUUUAGUAUC (SEQ ID NO:166)

miR-125b-2* (MIMAT0004603):

Mature: UCACAAGUCAGGCUCUUGGGAC (SEQ ID NO:167)

Stem loop:

ACCAGACUUUCCUAGUCCCUGAGACCCUAACUUGUGAGGUAUUUUAGUAACA
UCACAAGUCAGGCUCUUGGGACCUAGGCGGAGGGGA (SEQ ID NO:168)

miR-210 (MIMAT0000267):

Mature: CUGUGCGUGUGACAGCGGCUGA (SEQ ID NO:169)

Stem loop:

ACCCGGCAGUGCCUCCAGGCGCAGGGCAGCCCCUGCCCACCGCACACUGCGCU
GCCCCAGACCCACUGUGCGUGUGACAGCGGCUGAUCUGUGCCUGGGCAGCGCG
ACCC (SEQ ID NO:170)

miR-1249 (MIMAT0005901):

Mature: ACGCCCUUCCCCCCCCUUCUUCA (SEQ ID NO:171)

Stem loop:

GGGAGGAGGGAGGAGAUGGGCCAAGUUCCCUCUGGCUGGAACGCCCUUCCCCC
CCUUCUUCACCUG (SEQ ID NO:172)

miR-874 (MIMAT0004911):

Mature: CUGCCCUGGCCCCGAGGGACCGA (SEQ ID NO:173)

Stem loop:

UUAGCCCUGCGGCCCCACGCACCAGGGUAAGAGAGACUCUCGCUUCCUGCCCU
GGCCCGAGGGACCGACUGGCUGGGC (SEQ ID NO:174)

miR-23a* (MIMAT0004496):

Mature: GGGGUUCCUGGGGAUGGGAUUU (SEQ ID NO:175)

Stem loop:

GGCCGGCUGGGGUUCCUGGGGAUGGGAUUUUGCUUCCUGUCACAAAUCACAUU
GCCAGGGAUUUCCAACCGACC (SEQ ID NO:176)

miR-30b* (MIMAT0004589):

Mature: CUGGGAGGUGGAUGUUUACUUC (SEQ ID NO:177)

Stem loop:

ACCAAGUUUCAGUUCAUGUAAACAUCCUACACUCAGCUGUAAUACAUGGAUU
GGCUGGGAGGUGGAUGUUUACUUCAGCUGACUUGGA (SEQ ID NO:178)

miR-296-5p (MIMAT0000690):

Mature: AGGGCCCCCCCCUCAAUCCUGU (SEQ ID NO:179)

Stem loop:

AGGACCCUUCAGAGGGCCCCCCCCUCAAUCCUGUUGUGCCUAAUUCAGAGGGU
UGGGUGGAGGCUCUCCUGAAGGGCUCU (SEQ ID NO:180)

miR-744 (MIMAT0004945):

Mature: UGCGGGGCUAGGGCUAACAGCA (SEQ ID NO:181)

Stem loop:

UUGGGCAAGGUGCGGGGCUAGGGCUAACAGCAGUCUUACUGAAGGUUUCUG
GAAACCACGCACAUGCUGUUGCCACUAACCUCAACCUUACUCGGUC (SEQ ID
NO:)

miR-197 (MIMAT0000227):

Mature: UUCACCACCUUCUCCACCCAGC (SEQ ID NO:182)

Stem loop:

GGCUGUGCCGGGUAGAGAGGGCAGUGGGAGGUAAGAGCUCUUCACCCUUCACC
ACCUUCUCCACCCAGCAUGGCC (SEQ ID NO:183)miR-27b* (MIMAT0004588):

Mature: AGAGCUUAGCUGAUUGGUGAAC (SEQ ID NO:184)

Stem loop:

ACCUCUCUAACAAGGUGCAGAGCUUAGCUGAUUGGUGAACAGUGAUUGGUUU
CCGCUUUGUUCACAGUGGCCUAAGUUCUGCACCUGAAGAGAAGGUG (SEQ ID
NO:185)miR-324-3p *(MIMAT0000762):

Mature: ACUGCCCCAGGUGCUGCUGG (SEQ ID NO:186)

Stem loop:

CUGACUAUGCCUCCCCGCAUCCCCUAGGGCAUUGGUGUAAAGCUGGAGACCCA
CUGCCCCAGGUGCUGCUGGGGGUUGUAGUC (SEQ ID NO:187)miR-126 (MI0000471):

Mature: CAUUAUUACUUUUGGUACGCG (SEQ ID NO:188)

Stem loop:

CGCUGGCGACGGGACAUAUAUUACUUUUGGUACGCGCUGUGACACUCAAACUC
GUACCGUGAGUAAUAAUGCGCCGUCCACGGCA (SEQ ID NO:189)miR-34a (MI0000268)

Mature: AACAUUCAACGCUGUCGGUGAGU (SEQ ID NO:190)

Stem loop:

GGCCAGCUGUGAGUGUUUCUUUGGCAGUGUCUUAGCUGGUUGUUGUGAGCAA
UAGUAAGGAAGCAAUCAGCAAGUAUACUGCCCUAGAAGUGCUGCACGUUGUG
GGGCCC (SEQ ID NO:191)miR-34b (MI0000742)

Mature: UAGGCAGUGUCAUAGCUGAUU (SEQ ID NO:192)

Stem loop:

GUGCUCGGUUUGUAGGCAGUGUCAUAGCUGAUUGUACUGUGGUGGUUACAA
UCACUAAACUCCACUGCCAUCAAAACAAGGCAC (SEQ ID NO:193)

miR-34c (MI0000743)

Mature: AGGCAGUGUAGUUAGCUGAUUGC (SEQ ID NO:194)

Stem loop:

AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUGC UAAUAGUACCAAUCACU
AACCACACGGCCAGGUAAAAAGAUU (SEQ ID NO:195)

miR-1280 (MI0006437)

Mature: UCCCACCGCUGCCACCC (SEQ ID NO:196)

Stem loop:

UCUGUCCCACCGCUGCCACCCUCCCCUCUGCCUCAGUGUGCCAGGCAUCAG
CACUCACUCACAGAGGCAGGCUGGAUGGCGGGUGGGACAACAG (SEQ ID
NO:197)

[0010] MicroRNA inhibitors or nucleic acids encoding miRs (such as nucleic acid constructs, for example, vectors) can be administered in any number of ways including, without limitation, by nanopiece, via direct injection into a chondrosarcoma, or via intravenous administration.

[0011] The methods disclosed herein can be used to treat any form of chondrosarcoma, including, without limitation, conventional chondrosarcoma, periosteal chondrosarcoma, mesenchymal chondrosarcoma, dedifferentiated chondrosarcoma, clear-cell chondrosarcoma, or extraskeletal myxoid chondrosarcoma. In other embodiments, the methods for treating chondrosarcoma disclosed herein can further include administration of one or more additional anti-cancer therapies to the individual (for example, surgical ablation of the chondrosarcoma). The individual is preferably a mammal in need of such treatment, e.g., a subject that has been diagnosed with chondrosarcoma or a predisposition thereto. The mammal can be, e.g., any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a horse, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs,

chickens, and goats. In a preferred embodiment, the mammal is a human.

[0012] In other aspects, the invention provides a method for diagnosing an individual with chondrosarcoma by assessing the expression level of one or more miR(s) present in a biological sample obtained from the individual selected from miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-181a, and miR-30e. In one embodiment, the individual is diagnosed with chondrosarcoma if the expression levels of one or more of the miRs listed above are expressed at a higher level versus that of the corresponding miR(s) in a sample obtained from an individual without chondrosarcoma.

[0013] In yet other aspects, provided herein are methods for diagnosing an individual with chondrosarcoma by assessing the expression level of one or more miR(s) present in a biological sample obtained from the individual selected from miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p. In one embodiment, the individual is diagnosed with chondrosarcoma if the expression levels of one or more of the miRs listed above are expressed at a decreased level compared to that of the corresponding miR(s) in a sample obtained from an individual without chondrosarcoma or in normal tissue from an individual with chondrosarcoma.

[0014] Each of the aspects and embodiments described herein are capable of being used together, unless excluded either explicitly or clearly from the context of the embodiment or aspect.

[0015] Throughout this specification, various patents, patent applications and other types of publications (e.g., journal articles, electronic database entries, etc.) are referenced. The

disclosure of all patents, patent applications, and other publications cited herein are hereby incorporated by reference in their entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 depicts a graph showing miR-1280 RNA levels as measured by qRT-PCR in normal cartilage and chondrosarcoma tissue.

[0017] FIG. 2 depicts graphs showing the results of an ELISA measuring VEGF expression in chondrosarcoma cells. miR-126 was administered under both normoxic and hypoxic conditions in concentrations from 10-80nM (top). VEGF concentration was also assessed following administration of miR-126 or anti-miR-126 under both normoxic and hypoxic conditions (bottom). Upper panel concentrations in nM. Lower panel 20 nM for miR-126 and 80nM for anti-miR-126. Hypoxia is 2% oxygen concentration.

[0018] FIG. 3 depicts the results of an angiogenesis antibody array analysis in chondrosarcoma cells following treatment with a miR control (top) and miR-126 (bottom).

[0019] FIG. 4 depicts a graph showing miR-126 RNA expression as measured by qRT-PCR following overexpression of hif1a (Hif1a) in chondrosarcoma cells or treatment with an antisense inhibitor of hif1a expression (Hif1asi). EV is empty vector control.

[0020] FIG. 5 depicts a western blot showing Met protein level under control miRNA conditions and after overexpression of miR-126 in chondrosarcoma cells. Expression of actin protein is used as a loading control.

[0021] FIG. 6 depicts a graph showing proliferation of chondrosarcoma cells treated with both a control miR and miR-126a over time as measured by fluorescence.

[0022] FIG. 7A depicts a graph showing VEGF protein expression in chondrosarcoma cells transfected with control miR, miR-34a, an antisense control oligonucleotide, or an antisense-miR34a oligonucleotide as measured by ELISA. FIG. 7B depicts a graph showing VEGF protein expression in chondrosarcoma cells transfected with miR-34a relative to transfection with control miR in chondrosarcoma cells (* $p < 0.02$, $n=3$). FIG. 7C depicts a western blot showing VEGF protein expression in chondrosarcoma cells transfected with miR-34a compared to control cells and cells transfected with an anti-miR-34a construct. m-34a is miR-34a mimic, the same sequence as miR-34a. A-34a is the antagomir, (antisense

sequence).

[0023] FIG. 8 depicts a graph showing expression of VEGF protein secreted from chondrosarcoma cells transfected with control miR and miR-34a over time as measured by ELISA (* $p < 0.001$).

[0024] FIG. 9 depicts a graph showing chondrosarcoma cell proliferation over time in cells transfected with miR-34a compared to cells transfected with control miR (* $p < 0.001$).

[0025] FIG. 10A depicts a graph showing expression of SSX1 mRNA in chondrosarcoma cells transfected with control miR, miR-34a, anti-miR control sequence, and cells transfected with an antisense-miR-34a oligonucleotide as measured by qRT-PCR. FIG. 10B depicts a western blot showing SSX1 protein expression in chondrosarcoma cells after the same transfections in FIG. 10A.

[0026] FIG. 11A depicts a graph showing expression of VEGF mRNA in chondrosarcoma cells transfected with an SSX1 siRNA compared to control siRNA and cells transfected with an SSX4 siRNA as measured by qRT-PCR. FIG. 11B is a graph showing expression of VEGF protein in a monolayer culture of chondrosarcoma cells transfected with an SSX1 siRNA compared to control siRNA as measured by ELISA. FIG. 11C depicts a graph showing expression of VEGF protein in a 3D tumor cell spheroid growth culture of chondrosarcoma cells transfected with an SSX1 siRNA compared to control siRNA as measured by ELISA.

[0027] FIG. 12 depicts a graph showing VEGF expression in chondrosarcoma cells transfected with anti-miR-181a oligonucleotides, transfected with miR-34a, or combination treatment with an anti-miR-181a and with miR-34a compared to control cells as measured by ELISA.

[0028] FIG. 13A depicts fluorescent (left) and bright field (right) micrographs showing transfection of chondrosarcoma cells with a fluorescently labeled anti-miR-control carried by nanopieces (top, left), and anti-miR-control alone (bottom, left) indicating cells are transfected when nanopieces are used for delivery. FIG. 13B depicts a graph showing miR-181a RNA levels as measured by qRT-PCR in control miR/nanopiece versus anti-miR-181a/nanopiece-treated chondrosarcoma cells.

[0029] FIGS. 14A-C depict intracellular nanopiece delivery of nucleotide sequences. FIG. 14A shows a xenograft tumor in mouse injected with molecular beacon for GAPDH mRNA alone or in combination with nanopieces. FIG. 14B depicts miR-181a expression measured by qPCR in xenograft tumors treated with local injection of control miR or anti-miR-181a delivered by nanopieces. FIG. 14C shows the effect on MMP1 expression as measured by ELISA.

[0030] FIG. 15 depicts the *in vivo* effect of nanopiece plus anti-miR-181a delivered via tail vein injection on miR-181a expression 2 days after 1 injection compared to nanopiece plus control anti-miR.

[0031] FIG. 16 is a graph depicting the results of a qRT-PCR analysis of mir-181a RNA expression levels after seven injections over three weeks under both control conditions as well as following treatment with anti-mir-181a, indicating sustained suppression of miR-181a. mir-181a RNA levels are normalized to expression of U17a (#, $p < 0.01$).

[0032] FIG. 17 is a graph depicting tumor weight in a mouse xenograft cancer model of chondrosarcoma following seven injections over three weeks under both control conditions as well as following treatment with anti-mir-181a (*, $p < 0.037$).

[0033] FIG. 18 is a graph depicting MMP probe content as measured by Fluorescence Molecular Tomography (FMT) in an *in vivo* mouse tumor model of chondrosarcoma following seven injections over three weeks under both control conditions as well as following treatment with anti-mir-181a (#, $p < 0.043$).

[0034] FIG. 19 is a graph showing MMP1 expression in chondrosarcoma cells following treatment with AMD3100 (plerixafor) and/or anti-miR-181a under both hypoxic and normoxic conditions.

DETAILED DESCRIPTION

[0035] MicroRNAs (miRs) are small (about 22-nucleotide) RNAs that are derived from larger pre-miRs. MiRs act as repressors of target mRNAs by promoting their degradation, when their sequences are perfectly complementary or inhibiting translation when their sequences contain mismatches. MicroRNAs are emerging as important regulators of cellular differentiation, their importance underscored by the fact that they are often dysregulated

during carcinogenesis. Under a standardized nomenclature system, capitalized "miR-" refers to the mature form of the miRNA, while the uncapitalized "mir-" refers to the pre-miRNA, and "MIR" refers to the gene that encodes them.

[0036] Multiple species of miRs were found to be either underexpressed or overexpressed in chondrosarcoma cells in comparison to the expression of these miRs in normal chondrocytes. As such, the compositions and methods provided herein are directed to restoring normal miR expression in individuals with chondrosarcoma, thereby providing an alternative or an adjuvant-based treatment for this particularly radiation- and chemotherapy-resistant neoplasm. Thus, use of the methods and compositions disclosed herein can not only lead to earlier diagnosis of chondrosarcoma, but can also minimize or eliminate the need for disfiguring surgery for successful treatment of this disease.

I. Definitions

[0037] As used herein, "adjuvant-based therapy" or "adjuvant-based cancer treatment" refers to additional treatment (e.g., chemotherapy, radiotherapy), usually given after a primary treatment such as surgery (e.g., surgery for chondrosarcoma), where all detectable disease has been removed, but where there remains a statistical risk of relapse due to occult disease. Typically, statistical evidence is used to assess the risk of disease relapse before deciding on a specific adjuvant-based therapy. The aim of adjuvant treatment is to improve disease-specific and overall survival. Because the treatment is essentially for a risk, rather than for provable disease, it is accepted that a proportion of patients who receive adjuvant therapy will already have been cured by their primary surgery. The primary goal of adjuvant chemotherapy is to control systemic relapse of a disease to improve long-term survival. Adjuvant radiotherapy is given to control local and/or regional recurrence.

[0038] An "individual" can be a vertebrate, a mammal, or a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. In one aspect, an individual is a human. An "individual in need thereof" refers to an individual diagnosed with or thought to have chondrosarcoma. An individual can be diagnosed with chondrosarcoma using any means known in the art including, without limitation, radiographs ("x-rays"), computerized tomography (CT), technesium bone scan, PET scan, and magnetic resonance imaging (MRI) (*see* Leddy et al., *Cancer Treat Res.* 2014;162:117-30; Qasem et al. *Semin Diagn Pathol.* 2014 Jan;31(1):10-20).

[0039] By the phrases "therapeutically effective amount," "in an amount effective" and "effective dosage" is meant an amount sufficient to produce a therapeutically (e.g., clinically) desirable result; the exact nature of the result will vary depending on the nature of the disorder being treated. For example, where the disorder to be treated is chondrosarcoma, the result can be inhibition of growth of chondrosarcoma cells and shrinkage of chondrosarcoma tumors. Therapeutically effective amount can also refer to the amount sufficient to decrease invasion or metastasis of chondrosarcoma (such as a decrease in invasion or metastasis by any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, inclusive of values falling in between these percentages). The compositions described herein can be administered from one or more times per day to one or more times per week. A person having ordinary skill in the art will appreciate that certain factors can influence the dosage and timing required to effectively treat an individual, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the individual, and other diseases present. Moreover, treatment of an individual with a therapeutically effective amount of the compositions of the invention can include a single treatment or a series of treatments. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art.

[0040] "Purified," as used herein, refers to molecules, either nucleic acid or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

[0041] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

[0042] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

[0043] As used herein, the term "about" in the context of a numerical value or range means

$\pm 10\%$ of the numerical value or range recited or claimed.

[0044] As used herein, the singular terms “a,” “an,” and “the” include the plural reference unless the context clearly indicates otherwise.

II. Methods of the Invention

[0045] Chondrosarcoma accounts for about 20% of primary malignant bone tumors, and shows the second highest occurrence frequency following that of osteosarcoma. It is histologically classified into conventional chondrosarcoma, periosteal chondrosarcoma, mesenchymal chondrosarcoma, dedifferentiated chondrosarcoma, clear-cell chondrosarcoma, extraskeletal myxoid chondrosarcoma, and the like. Typical chondrosarcoma frequently occurs in individuals aged 30 to 50, and slightly more frequently occurs in men. It tends to appear in the pelvic bone, but also regularly occurs in rib, proximal femur, proximal humerus, and distal femur. Mesenchymal chondrosarcoma usually occurs in persons from between the age of 10 and 19 but is also observed in people in their 30's. Mesenchymal chondrosarcoma frequently occurs in jaw, spine, iliac bone, rib, and the distal part of the femur. Dedifferentiated chondrosarcoma is a combination of a spindle cell sarcoma and benign or low-grade cartilage tumor. which can develop from conventional chondrosarcoma or benign cartilaginous tumors such as enchondroma. It occurs in individuals in their 50's or 60's, and most frequently occurs in femur but is also observed in pelvis and humerus. Clear-cell chondrosarcoma frequently occurs in persons in their 20's to 50's, and in about 2/3 of the patients, it occurs in the humeral head or femoral head. It also occurs in cranial bone, spine, and the bones of hand and foot. Extraskeletal myxoid chondrosarcoma frequently occurs in people in their 40's and 50's, and may occur in soft tissues of extremities such as thigh, as well as the distal portions of extremities, the mediastinum and the retroperitoneum.

[0046] Nearly all chondrosarcoma patients appear to be in good health. Often, patients are not aware of a growing sarcoma until there is a noticeable lump or pain. Earlier diagnosis is generally accidental, when a patient undergoes testing for another problem and physicians discover the cancer. Prognosis depends on how early the cancer is discovered and treated. For the least aggressive grade, about 90% of patients survive more than five years after diagnosis. People usually have a good survival rate at the low grade volume of cancer. However, for the most aggressive grade, only 10% of patients will survive one year.

[0047] Surgery is currently the main form of treatment for chondrosarcoma.

Musculoskeletal tumor specialists or orthopedic oncologists are usually chosen to treat chondrosarcoma, unless it is located in the skull, spine, or chest cavity, in which case, a neurosurgeon or thoracic surgeon experienced with sarcomas is chosen. Often, a limb-sparing operation can be performed; however in some cases amputation is unavoidable. Amputation of the arm, leg, jaw, or half of the pelvis (called a hemipelvectomy) may be necessary in some cases.

A. Methods for treating chondrosarcoma

[0048] The present invention is directed, *inter alia*, to methods for inhibiting the symptoms or conditions (disabilities, impairments) associated chondrosarcoma as described in detail below. As such, it is not required that all effects of the condition be entirely prevented or reversed, although the effects of the presently disclosed methods likely extend to a significant therapeutic benefit for the patient. As such, a therapeutic benefit is not necessarily a complete prevention or cure for the condition, but rather, can encompass a result which includes reducing or preventing the symptoms that result from chondrosarcoma, reducing or preventing the occurrence of such symptoms (either quantitatively or qualitatively), reducing the severity of such symptoms or physiological effects thereof, and/or enhancing the recovery of the individual after experiencing chondrosarcoma symptoms.

[0049] Specifically, the therapies of the present invention, when administered to an individual, can treat or prevent one or more of the symptoms or conditions associated with chondrosarcoma and/or reduce or alleviate symptoms of or conditions associated with this disorder. As such, protecting an individual from the effects or symptoms resulting from chondrosarcoma includes both preventing or reducing the occurrence and/or severity of the effects of the disorder and treating a patient in which the effects of the disorder are already occurring or beginning to occur. A beneficial effect can easily be assessed by one of ordinary skill in the art and/or by a trained clinician who is treating the patient. Preferably, there is a positive or beneficial difference in the severity or occurrence of at least one clinical or biological score, value, or measure used to evaluate such patients in those who have been treated with the methods of the present invention as compared to those that have not. In some embodiments, a positive or beneficial difference is a reduction in tumor size following treatment, such as a decrease of any of about 5%, 10%, 15%, 20%, 25%, 30%,

35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in tumor size or weight, inclusive of values falling in between these percentages. In some embodiments, a positive or beneficial difference is prevention or a delay in the occurrence of metastatic disease, which is most commonly to the lungs, but can also be to other organs, including the skeleton. In some embodiments, a positive or beneficial difference is reduction in size of metastases or a decrease in rate of growth, such as a decrease of any of about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in tumor size, or weight, inclusive of values falling in between these percentages.

[0050] The methods of the invention may be practiced in an adjuvant setting. "Adjuvant setting" refers to a clinical setting in which an individual has had a history of a proliferative disease, particularly a chondrosarcoma and generally (but not necessarily) has been responsive to therapy, which includes, but is not limited to, surgery, radiotherapy, and chemotherapy. However, because of a history of the proliferative disease (such as a chondrosarcoma), these individuals are considered at risk of developing a recurrence of that disease. In some embodiments, treatment or administration in the "adjuvant setting" refers to a subsequent mode of treatment.

[0051] The methods provided herein may also be practiced in a "neoadjuvant setting," that is, the method may be carried out before the primary/definitive therapy. In some aspects, the individual has previously been treated. In other aspects, the individual has not previously been treated. In some aspects, the treatment is a first line therapy. The individual may be a human or may be a non-human mammal.

1. Inhibition of miR expression

[0052] Provided herein are methods for treating an individual with chondrosarcoma by administering to the individual a therapeutically effective amount of an inhibitor of one or more microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-93b, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-

424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-let-7a-2*, miR-126, miR-181a, and miR-30e.

[0053] Suitable compounds for inhibiting miR gene expression include double-stranded RNA (such as short- or small-interfering RNA or "siRNA"), antagomirs, antisense nucleic acids, enzymatic RNA molecules such as ribozymes, or molecules capable of forming a triple helix with the miR gene. Another class of inhibitor compound can cause hyper-methylation of the miR gene product promoter, resulting in reduced expression of the miR gene. Each of these compounds can be targeted to a given miR gene product to inhibit (e.g., destroy, induce the destruction of, or otherwise reduce the level of) the target miR.

[0054] For example, expression of a given miR can be inhibited by inducing RNA interference of the miR with an isolated double-stranded RNA ("dsRNA") molecule which has at least 90%, for example at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with at least a portion of the miR. In a particular embodiment, the dsRNA molecule is a "short or small interfering RNA" or "siRNA." siRNA useful in the present methods comprise short double-stranded RNA from about 17 nucleotides to about 29 nucleotides in length, or from about 19 to about 25 nucleotides in length. siRNAs have a sense RNA strand and a complementary antisense RNA strand annealed together by standard Watson-Crick base-pairing interactions (hereinafter "base-paired"). The sense strand comprises a nucleic acid sequence which is substantially identical to a nucleic acid sequence contained within the target miR gene product. As used herein, a nucleic acid sequence in an siRNA which is "substantially identical" to a target sequence contained within the target mRNA is a nucleic acid sequence that is identical to the target sequence, or that differs from the target sequence by one or two nucleotides. The sense and antisense strands of the siRNA can comprise two complementary, single-stranded RNA molecules, or can comprise a single molecule in which two complementary portions are base-paired and are covalently linked by a single-stranded "hairpin" area.

[0055] The siRNA can also be altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or to one or more internal nucleotides of the siRNA, or modifications that make the siRNA resistant to nuclease digestion, or the substitution of one or more nucleotides in the siRNA with deoxyribo-nucleotides. Further, the siRNA can also be engineered to contain

certain "drug like" properties. Such modifications include chemical modifications for stability and cholesterol conjugation for delivery. Such modifications impart better pharmacological properties to the siRNA and using such modifications, pharmacologically active siRNAs can achieve broad biodistribution and efficient silencing of miRNAs in most tissues *in vivo*.

[0056] One or both strands of the siRNA can also comprise a 3' overhang. As used herein, a "3' overhang" refers to at least one unpaired nucleotide extending from the 3'-end of a duplexed RNA strand. Thus, in certain embodiments, the siRNA comprises at least one 3' overhang of from 1 to about 6 nucleotides (which includes ribonucleotides or deoxyribonucleotides) in length, from 1 to about 5 nucleotides in length, from 1 to about 4 nucleotides in length, or from about 2 to about 4 nucleotides in length. In one embodiment, the 3' overhang is present on both strands of the siRNA, and is 2 nucleotides in length. For example, each strand of the siRNA can comprise 3' overhangs of dithymidylic acid ("TT") or diuridylic acid ("uu").

[0057] siRNA can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing dsRNA or siRNA molecules are described in U.S. Patent Application Publication Nos. 2002/0173478 and 2004/0018176, the disclosures of which are incorporated herein by reference.

[0058] In another aspect, one or more antagomirs can be used to inhibit a microRNA in the methods of the disclosed invention. Antagomirs are single stranded, double stranded, partially double stranded and hairpin-structured chemically-modified oligonucleotides that specifically target a microRNA. Antagomirs have at least 12 or more contiguous nucleotides substantially complementary to an endogenous miRNA or pre-miRNA (stem-loop) nucleotide sequence. As used herein, "partially double stranded" refers to double stranded structures that contain less nucleotides than the complementary strand. An antagomir typically includes a nucleotide sequence sufficiently complementary to hybridize to a miRNA target sequence of about 12 to 25 nucleotides, (such as about 15 to 23 nucleotides, or any of about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides). Ideally, the target sequence differs by no more than 1, 2, or 3 nucleotides from the complementary antagomir sequence. Delivery of antagomirs is often facilitated by the attachment of a moiety that promotes cellular diffusion and transport. For example, the

antagomir can include a non-nucleotide moiety, e.g., a cholesterol moiety. The non-nucleotide moiety can be attached, e.g., to the 3' or 5' end of the antagomir.

[0059] Antagomirs can be further stabilized against nucleolytic degradation such as by the incorporation of a modification, e.g., a nucleotide modification. For example, the antagomir can include a phosphorothioate moiety at the first, second, or third internucleotide linkage at the 5' or 3' end of the nucleotide sequence. In another non-limiting embodiment, the antagomir includes a 2'-modified nucleotide, e.g., a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA). In other embodiments, the antagomir includes at least one 2'-O-methyl-modified nucleotide, and in some embodiments, all of the nucleotides of the antagomir include a 2'-O-methyl modification. Methods for synthesizing and validating a therapeutically effective antagomir engineered to silence miRNAs *in vivo* is described in Krutzfeldt J, et al. (2005), Silencing of microRNAs in vivo with 'antagomirs,' *Nature* 438(7068):685-9, the entire content of which is incorporated herein by reference.

[0060] Expression of a given miR gene can also be inhibited by an antisense nucleic acid. As used herein, an "antisense nucleic acid" refers to a nucleic acid molecule that stably binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-peptide nucleic acid interactions, which alters the activity of the target RNA. Antisense nucleic acids suitable for use in the present methods are single-stranded nucleic acids (e.g., RNA, DNA, RNA-DNA chimeras, PNA) that generally comprise a nucleic acid sequence complementary to a contiguous nucleic acid sequence in a miR gene product. The antisense nucleic acid can comprise a nucleic acid sequence that is 50-100% complementary, 75-100% complementary, or 95-100% complementary, such as any of about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 100% complementary to a contiguous nucleic acid sequence in a miR. Without wishing to be bound by any theory, it is believed that the antisense nucleic acids activate RNase H or another cellular nuclease that digests the miR gene product/antisense nucleic acid duplex.

[0061] For example, in eukaryotes, RNA polymerase catalyzes the transcription of a structural gene to produce mRNA. A DNA molecule can be designed to contain an RNA polymerase template in which the RNA transcript has a sequence that is complementary to

that of a preferred mRNA. The RNA transcript is termed an "antisense RNA". Antisense RNA molecules can inhibit mRNA expression (for example, Rylova et al., *Cancer Res.*, 62(3):801-8, 2002; Shim et al., *Int. J. Cancer*, 94(1):6-15, 2001). Antisense nucleic acids can also contain modifications to the nucleic acid backbone or to the sugar and base moieties (or their equivalent) to enhance target specificity, nuclease resistance, delivery or other properties related to efficacy of the molecule. Such modifications include cholesterol moieties, duplex intercalators, such as acridine, or one or more nuclease-resistant groups. Antisense nucleic acids can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing are within the skill in the art; see, e.g., Stein and Cheng (1993), *Science* 261:1004 and U.S. Pat. No. 5,849,902 to Woolf et al., the entire disclosures of which are incorporated herein by reference.

[0062] Expression of a given miR can also be inhibited by an enzymatic nucleic acid. As used herein, an "enzymatic nucleic acid" refers to a nucleic acid comprising a substrate binding region that has complementarity to a contiguous nucleic acid sequence of a miR, and which is able to specifically cleave the miR. The enzymatic nucleic acid substrate binding region can be, for example, 50-100% complementary, 75- 100% complementary, or 95-100%, such as any of about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 100% complementary to a contiguous nucleic acid sequence in an miR. The enzymatic nucleic acids can also comprise modifications at the base, sugar, and/or phosphate groups. An exemplary enzymatic nucleic acid for use in the present methods is a ribozyme.

[0063] Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. A review is provided in Rossi, *Current Biology*, 4:469-471 (1994). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. A composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include a well-known catalytic sequence responsible for mRNA cleavage (U.S. Pat. No. 5,093,246, incorporated by reference herein). Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the molecule of interest for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the miR gene containing the cleavage site can

be evaluated for predicted structural features, for example, secondary structure, that can render an oligonucleotide sequence unsuitable. The suitability of candidate sequences also can be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

[0064] Enzymatic nucleic acids can be produced chemically or biologically, or can be expressed from a recombinant plasmid or viral vector, as described above for the isolated miR gene products. Exemplary methods for producing and testing dsRNA or siRNA molecules are described in Werner and Uhlenbeck (1995), Nucl. Acids Res. 23:2092-96; Hammann et al. (1999), Antisense and Nucleic Acid Drug Dev. 9:25-31; and U.S. Pat. No. 4,987,071 to Cech et al, the entire disclosures of which are incorporated herein by reference.

[0065] Triple helix forming molecules can be used in reducing the level of a target miR. Nucleic acid molecules that can associate together in a triple-stranded conformation (triple helix) and that thereby can be used to inhibit translation of a target gene, should be single helices composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines on one strand of a duplex. Nucleotide sequences can be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide bases complementary to a purine -rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules can be chosen that are purine-rich, for example, those that contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines on one strand of a duplex.

[0066] In some embodiments, inhibition of one or more microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451,

miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-93b, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-let-7a-2*, miR-181a, and miR-30e according to any of the methods disclosed herein results in decreased chondrosarcoma progression (tumor weight or size compared to tumors that are not treated with inhibitors of the one or more miRs, or delay in onset or progression of metastatic disease). The reduction in progression can be any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, inclusive of values falling in between these percentages. In some embodiments, the miR inhibitor is an inhibitory nucleic acid such as, but not limited to, an antisense oligonucleotide or an siRNA. In other embodiments, the inhibitor is delivered to the chondrosarcoma cell by a nanopiece. In one embodiment, the miR inhibitor is an inhibitor of miR-181a. In a further embodiment, the miR inhibitor (such as miR-181a) is co-administered with a chemotherapeutic (such as, but not limited to AMD3100). In some embodiments, the treatment occurs under normoxic conditions. In other embodiments, the treatment occurs under hypoxic conditions.

[0067] In further embodiments, inhibition of one or more microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-93b, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-let-7a-2*, miR-181a, and miR-30e according to any of the methods disclosed herein results in decreased expression of matrix metalloproteinase (MMP) (such as, but not limited to, MMP1), vascular endothelial growth factor (VEGF), or other molecules related to tumor growth and or metastasis, by chondrosarcoma cells compared to expression of molecules in cells that are not treated with inhibitors of the one or more miRs. The reduction in expression of MMP and other molecules can be any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, inclusive of values falling in between these percentages. In some embodiments, the miR inhibitor is an inhibitory nucleic acid such as, but not limited to, an antisense oligonucleotide or an siRNA. In other embodiments, the inhibitor is delivered to the chondrosarcoma cell by a nanopiece. In one

embodiment, the miR inhibitor is an inhibitor of miR-181a.

2. Restoration of miR expression

[0068] In other aspects, provided herein are methods for treating chondrosarcoma in an individual in need thereof. The method is performed by administering to the individual a therapeutically effective amount of a nucleic acid encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p.

[0069] The administered microRNA-encoding nucleic acids lead to transient or permanent overexpression of the desired microRNA(s) in the target cell or tissue (such as chondrosarcoma cells). Thus, the nucleic acids increase the level of an endogenous microRNA sequence expressed in a cell or tissue. Similarly, administration of microRNA delivery constructs such as lentiviruses lead to permanent expression of microRNAs (stem-loop sequence or mature sequence) in the cells.

[0070] In one embodiment, the administered nucleic acid is a microRNA mimic. As used herein, the term “microRNA mimic” refers to synthetic non-coding RNAs that are capable of entering the RNAi pathway and regulating gene expression. MicroRNA mimics imitate the function of endogenous microRNAs and can be designed as mature, double-stranded molecules or mimic precursors (e.g., pri- or pre-microRNAs). MicroRNA mimics can be comprised of modified or unmodified RNA, DNA, RNA-DNA hybrids or alternative nucleic acid chemistries. Accordingly, the invention provides microRNA mimics corresponding any of the miRs disclosed above which comprise a consensus sequence, wherein the microRNA mimics are capable of mimicking the endogenous activity of any naturally-expressed miR. Therefore, restoration of microRNA expression is achieved through the use of these microRNA mimics.

[0071] To improve efficiency, the methods of the present invention can employ a

microRNA mimic comprising a structurally and chemically modified double-stranded RNA. In exemplary embodiments, non-toxic chemical modifications to the mimic sequence can be introduced to improve stability, reduce off-target effects and increase activity.

[0072] In particular embodiments, the microRNA mimics of the invention contemplate the use of nucleotides that are modified to enhance their activities. Such nucleotides include those that are at the 5' or 3' terminus of the RNA as well as those that are internal within the molecule.

[0073] In other aspects, modifications may be made to the sequence of a microRNA or a pre-microRNA without disrupting microRNA activity. As used herein, the term "functional variant" of a microRNA sequence refers to an oligonucleotide sequence that varies from the natural microRNA sequence, but retains one or more functional characteristics of the microRNA (e.g. enhancement of cancer cell susceptibility to chemotherapeutic agents, cancer cell proliferation inhibition, induction of cancer cell apoptosis, specific microRNA target inhibition). In some embodiments, a functional variant of a microRNA sequence retains all of the functional characteristics of the microRNA. In certain embodiments, a functional variant of a microRNA has a nucleobase sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the microRNA or precursor thereof over a region of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleobases, or that the functional variant hybridizes to the complement of the microRNA or precursor thereof under stringent hybridization conditions. Accordingly, in certain embodiments the nucleotide base sequence of a functional variant may be capable of hybridizing to one or more target sequences of the microRNA. Other modifications contemplated in the practice of the invention can be found in U.S. Patent Application Publication No. 2012/0259001, which is incorporated herein by reference in its entirety.

[0074] In some embodiments, administering to an individual (such as, for example, intravenous, intratumoral, or parenteral administration) a therapeutically effective amount (e.g., from about 1 ng/kg to about 100 ng/kg of body weight) of a nucleic acid encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-

5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p results in decreased expression of one or more angiogenesis or metastasis-promoting molecules by chondrosarcoma cells. These angiogenesis or metastasis-promoting molecules can include, without limitation, matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or Met. In some embodiments, administration of one or more of the miRs above results in decreased expression of the one or more angiogenesis-promoting molecules by any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, inclusive of values falling in between these percentages compared to chondrosarcoma cells that are not administered the one or more miRs. In some embodiments, the one or more miRs are delivered to the chondrosarcoma cell by a nanopiece. In another embodiment, the miR is one or more of miR-126, miR-34a, miR-34b, miR-34c, and/or miR1280.

[0075] In other embodiments, administering to an individual (such as, for example, intravenous, intratumoral, or parenteral administration) a therapeutically effective amount (e.g., from about 1 ng/kg to about 100 ng/kg of body weight) of a nucleic acid encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p results in decreased proliferation by chondrosarcoma cells. In some embodiments, administration of one or more of the miRs above results in decreased proliferation of chondrosarcoma cells by any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, inclusive of values falling in between these percentages compared to chondrosarcoma cells that are not administered the one or more miRs. In some embodiments, the one or more miRs are delivered to the chondrosarcoma cell by a nanopiece. In another embodiment, the miR is one or more of miR-126, miR-34a, miR-34b, miR-34c, and/or miR1280.

3. Delivery of miR inhibitors and/or nucleic acid constructs

[0076] The inhibitors and miR nucleic acid for use in the methods disclosed herein can be introduced into a cell (such as a chondrosarcoma cell) by any method known, e.g., transfection or transduction. Transfection is the process of introducing nucleic acids into cells by non-viral methods, and transduction is the process whereby foreign DNA is introduced into another cell via a viral vector (such as a lentiviral vector or an adenoviral vector). The invention also includes use of nanopieces either *de novo* or linked with polyethylene glycol (PEG), aptamers, and or peptides to deliver microRNAs and miR inhibitors alone or in combination.

[0077] Other methods of administering nucleic acids are well known in the art. In particular, the routes of administration already in use for nucleic acid therapeutics, along with formulations in current use, provide preferred routes of administration and formulation for the nucleic acids. Nucleic acid compositions can be administered by a number of routes including, but not limited to: intratumoral, oral, intravenous, intrapleural, intraperitoneal, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means.

[0078] In some embodiments, miR inhibitors or miR nucleic acid constructs are administered to an organism using one or more reagents that facilitate or enhance delivery, e.g., a compound which enhances transit through the cell membrane. Such reagents can include, without limitation, a lipophilic moiety; a transfection agent (e.g., an ion or other substance which substantially alters cell permeability to an oligonucleotide agent); or (iii) a commercial transfecting agent such as Lipofectamine™ (Invitrogen, Carlsbad, Calif.), Lipofectamine 2000™, TransIT-TKO™ (Mirus, Madison, Wis.), FuGENE 6 (Roche, Indianapolis, Ind.), polyethylenimine, X-tremeGENE Q2 (Roche, Indianapolis, Ind.), DOTAP, DOSPER, Metafectene™ (Biontex, Munich, Germany), and the like.

[0079] In other embodiments, the miR inhibitors or miR nucleic acid constructs are administered using a Nanopiece delivery vehicle. Nanopieces are co-assembled rosette nanotubes (RNTs) and nucleic acids (such as an inhibitory nucleic acid, for example, an siRNA). The RNT and the nucleic acid cargo are joined by completely non-covalent bindings. Once Nanopieces deliver their nucleic acid cargo, their degradation products are highly biocompatible due to the biomimetic G^AC base motif of the RNT. The ability of Nanopiece to deliver cargo effectively and degrade safely allows minimal levels of cytotoxicity, a prerequisite for *in vivo* therapeutic applications. Furthermore, Nanopieces have a nano-rod-like shape, 20-30 nm in diameter. This is more than 2000 times smaller in

volume than Lipofectamine™ spherical particles, allowing the Nanopiece to transfect cells that are shielded by dense extracellular matrix. Information related to constructing and using nanostructures, e.g., nanopieces, for delivering nucleic acids and other therapeutics to cells can be found in PCT/US2015/020801 (International Patent Application Publication No. WO 2015/139051) and International Patent Application No.: PCT/US2015/061193, the disclosures of which are incorporated by reference herein in its entirety. Exemplary NPs useful in the therapeutic methods described herein include those with a length of 1 nm to 200 nm, e.g. a length of about 100 nm, and a width or diameter of 1 nm to 60 nm, e.g., 20 nm. For example, the length is in the range of 50-150 nm and the width/diameter is in the range of 20-40 nm. Typically, the nanopieces are characterized by a length of about 100 nm and width/diameter of about 20 nm.

[0080] In one embodiment, a recombinant vector can be used for delivering one or more miR inhibitor or miR nucleic acid constructs (such as any of those disclosed herein) to the individual. This can include both systemic delivery and delivery localized to a particular region of the body (such as, the location of a chondrosarcoma). Any vector capable of enabling recombinant production of one or more miR inhibitor or miR nucleic acid and/or which can deliver one or more oligo miR inhibitors or miR nucleic acid into a host cell (such as a chondrosarcoma cell). The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. The vector can be part of a DNA vaccine or used as part of any other method for delivering a heterologous gene for expression in a host cell that is known to one having skill in the art. Recombinant vectors are capable of replicating when transformed into a suitable host cell. Viral vectors infect a wide range of non-dividing human cells and have been used extensively in live vaccines without adverse side effects. A viral vector (such as, but not limited to, an adenoviral vector or an adeno-associated viral (AAV) vector (e.g. AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, etc. or hybrid AAV vectors comprising the same) is an example of a vector for use in the present methods for delivering one or more miR inhibitor or miR nucleic acid constructs to chondrosarcoma cancer cells (see, e.g. U.S. Patent Application Publication No. 2004/0224389, the disclosure of which is incorporated by reference herein).

4. Other anti-cancer therapies

[0081] In some aspects, any of the methods of treatment described herein can comprise administering one or more additional anti-cancer therapies to the individual. Various classes

of anti-cancer agents can be used. Non-limiting examples include: alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, podophyllotoxin, antibodies (e.g., monoclonal or polyclonal), tyrosine kinase inhibitors (e.g., imatinib mesylate (Gleevec® or Glivec®)), hormone treatments, soluble receptors and other antineoplastics.

[0082] Topoisomerase inhibitors are also another class of anti-cancer agents that can be used. Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibition of type I or type II topoisomerases interferes with both transcription and replication of DNA by upsetting proper DNA supercoiling. Some type I topoisomerase inhibitors include camptothecins: irinotecan and topotecan. Examples of type II inhibitors include amsacrine, etoposide, etoposide phosphate, and teniposide. These are semisynthetic derivatives of epipodophyllotoxins, alkaloids naturally occurring in the root of American Mayapple (*Podophyllum peltatum*).

[0083] Antineoplastics include the immunosuppressant dactinomycin, doxorubicin, epirubicin, bleomycin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. The antineoplastic compounds generally work by chemically modifying a cell's DNA.

[0084] Alkylating agents can alkylate many nucleophilic functional groups under conditions present in cells. Cisplatin and carboplatin, and oxaliplatin are alkylating-like agents. They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.

[0085] Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). The vinca alkaloids include: vincristine, vinblastine, vinorelbine, and vindesine.

[0086] Anti-metabolites resemble purines (azathioprine, mercaptopurine) or pyrimidine and prevent these substances from becoming incorporated into DNA during the "S" phase of the cell cycle, stopping normal development and division. Anti-metabolites also affect RNA synthesis.

[0087] Plant alkaloids and terpenoids are derived from plants and block cell division by preventing microtubule function. Since microtubules are vital for cell division, without them, cell division cannot occur. The main examples are vinca alkaloids and taxanes.

[0088] Podophyllotoxin is a plant-derived compound which has been reported to help with digestion as well as used to produce two other cytostatic drugs, etoposide and teniposide. They prevent the cell from entering the G1 phase (the start of DNA replication) and the replication of DNA (the S phase).

[0089] Taxanes as a group includes paclitaxel and docetaxel. Paclitaxel is a natural product, originally known as Taxol and first derived from the bark of the Pacific Yew tree. Docetaxel is a semi- synthetic analogue of paclitaxel. Taxanes enhance stability of microtubules, preventing the separation of chromosomes during anaphase.

[0090] In some aspects, the anti-cancer therapeutics can be selected from remicade, docetaxel, celecoxib, melphalan, dexamethasone (Decadron®), steroids, gemcitabine, cisplatin, temozolomide, etoposide, cyclophosphamide, temodar, carboplatin, procarbazine, gliadel, tamoxifen, topotecan, methotrexate, Arisa®, taxol, taxotere, fluorouracil, leucovorin, irinotecan, xeloda, CPT-11, interferon alpha, pegylated interferon alpha (e.g., PEG INTRON- A), capecitabine, cisplatin, thiotepa, fludarabine, carboplatin, liposomal daunorubicin, cytarabine, doxetaxol, paclitaxel, vinblastine, IL-2, GM-CSF, dacarbazine, vinorelbine, zoledronic acid, palmitronate, biaxin, busulphan, prednisone, bortezomib (Velcade®), bisphosphonate, arsenic trioxide, vincristine, doxorubicin (Doxil®), paclitaxel, ganciclovir, adriamycin, estrainustine sodium phosphate (Emcyt®), sulindac, or etoposide.

[0091] In other embodiments, the anti-cancer therapeutics can be selected from bortezomib, cyclophosphamide, dexamethasone, doxorubicin, interferon-alpha, lenalidomide, melphalan, pegylated interferon-alpha, prednisone, thalidomide, or vincristine.

[0092] In other embodiments, the anti-cancer therapeutic is AMD3100 (plerixafor).

B. Methods for diagnosing chondrosarcoma

[0093] Also provided herein are methods for diagnosing chondrosarcoma in an individual via detecting the expression level of one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50) microRNAs (miRs) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-

143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-181a, and miR-30e in a biological sample obtained from the individual; wherein the individual is diagnosed with chondrosarcoma if expression of one or more of the miRs is increased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma. In some embodiments, the individual is diagnosed with chondrosarcoma if expression of one or more of the miRs listed above is any of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% increased in the biological sample from the individual relative to the expression level of the corresponding miR(s) from a sample derived from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma. In other embodiments, the individual is diagnosed with chondrosarcoma if expression of one or more of the miRs listed above is any of about 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold more highly expressed in the biological sample from the individual relative to the expression level of the corresponding miR(s) from a sample derived from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma.

[0094] Further, also provided herein is a method for diagnosing chondrosarcoma in an individual by detecting the expression level of one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50) miR(s) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p in the biological sample obtained from the individual, wherein the individual is diagnosed with chondrosarcoma if expression of one or more of the miR(s) of is decreased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma. In some embodiments, the individual is diagnosed with chondrosarcoma if expression of one or more of the miRs listed above is any of about 10%,

20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% decreased in the biological sample from the individual relative to the expression level of the corresponding miR(s) from a sample derived from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma. In other embodiments, the individual is diagnosed with chondrosarcoma if expression of one or more of the miRs listed above is any of about 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold less highly expressed in the biological sample from the individual relative to the expression level of the corresponding miR(s) from a sample derived from an individual without chondrosarcoma or relative to normal tissue from the individual with chondrosarcoma.

[0095] The assessment of miR expression is at the level of the transcribed RNA. Assessment of RNA expression levels of gene transcripts is routine and well known in the art. For example, one flexible and sensitive quantitative method for assessing RNA expression levels derived from a biological sample (such as a biopsy) is by quantitative RT-PCR (qRT-PCR) or by any other comparable quantitative PCR-based method. Additional methods for assessing RNA expression include, but are not limited to, Northern blotting, microarrays, *in situ* hybridization, serial analysis of gene expression (SAGE), dot blot, oligonucleotide arrays for chimeric RNA and antisense chimeric RNAs, amplification of the RNA by *in vitro* transcription mediated amplification (TMA), or ribonuclease protection assays.

[0096] In other embodiments, chondrosarcoma is diagnosed using additional methods. Imaging studies - including radiographs ("x-rays"), technetium bone scan, PET scan, computerized tomography (CT), and magnetic resonance imaging (MRI) – can also be used to make a presumptive diagnosis of chondrosarcoma. Further, a definitive diagnosis may also be made based on the identification of malignant cancer cells which produce cartilage in a biopsy specimen.

[0097] In yet other embodiments, the methods for diagnosing chondrosarcoma described above can further include treatment of the individual (using any of the methods for treating chondrosarcoma described above) if the diagnostic method indicates that the individual has chondrosarcoma

III. Compositions of the Invention

[0098] Also provided herein are compositions containing one or more inhibitors of a

microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-181a, miR-381, and miR-30e in an amount effective to inhibit growth of human chondrosarcoma cell. Any inhibitor or combination of inhibitors of the miRs described above is suitable for use in the pharmaceutical compositions of the present invention, including those inhibitors disclosed herein. Furthermore, the miR inhibitors disclosed herein can be suitably formulated for delivery according to any of the delivery methods described herein.

[0099] Additionally, provided herein are compositions containing one or more nucleic acids encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p; and a pharmaceutically acceptable carrier or diluent. Any nucleic acid or combination of nucleic acids encoding one or more of the miRs described above is suitable for use in the pharmaceutical compositions of the present invention, including those disclosed herein. Furthermore, nucleic acids encoding the miRs disclosed herein can be suitably formulated for delivery according to any of the delivery methods described herein.

A. Pharmaceutical compositions

[0100] Any of the therapies for chondrosarcoma (such as nucleic-acid-based therapies, for example, use of antagomir miR-inhibitors) disclosed herein can be administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including intratumoral, oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the

pharmaceutical art and comprise at least one active compound. When employed as oral compositions, the oligonucleotides and another disclosed herein are protected from acid digestion in the stomach by a pharmaceutically acceptable protectant.

[0101] Specifically, this invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the miR inhibitor, miR inhibitor expressing constructs, miR-encoding nucleic acid, or miR-expressing nucleic acid constructs disclosed herein associated with one or more pharmaceutically acceptable excipients or carriers. In making the compositions of this invention, the active ingredient (e.g., miR inhibitor or miR-expressing nucleic acid construct) is usually mixed with an excipient or carrier, diluted by an excipient or carrier or enclosed within such an excipient or carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient or carrier serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

[0102] In preparing a formulation, it may be necessary to mill the active lyophilized compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

[0103] Some examples of suitable excipients or carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

[0104] The miR inhibitor or miR nucleic acid compositions can be formulated in a unit dosage form, each dosage containing from about 1 ng to about 100 mg or more, such as any of about 1 ng to about 25 ng, about 1 ng to about 50 ng, about 1 ng to about 100 ng, about 1 ng to about 500 ng, about 1 ng to about 1000 ng, about 1 ng to about 1500 ng, about 1 ng to about 5000 ng, about 1 ng to about 7500 ng, about 1 to about 1 μ g, about 100 ng to about 500 ng, about 500 ng to about 2000 ng, about 1 mg to about 5 mg, 1 mg to about 10 mg, about 1 mg to about 20 mg, about 1 mg to about 30 mg, about 1 mg to about 40 mg, about 1 mg to about 50 mg, about 1 mg to about 60 mg, about 1 mg to about 70 mg, about 1 mg to about 80 mg, or about 1 mg to about 90 mg, inclusive, including any range in between these values, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for individuals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient or carrier. It will be understood, however, that the amount of the anti-cancer therapies actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[0105] In general, dosage is from about 1 ng/kg to about 100 ng/kg of body weight (such as any of about 1 ng/kg, 2 ng/kg, 3 ng/kg, 4 ng/kg, 5 ng/kg, 6 ng/kg, 7 ng/kg, 8 ng/kg, 9 ng/kg, 10 ng/kg, 11 ng/kg, 12 ng/kg, 13 ng/kg, 14 ng/kg, 15 ng/kg, 16 ng/kg, 17 ng/kg, 18 ng/kg, 19 ng/kg, 20 ng/kg, 21 ng/kg, 22 ng/kg, 23 ng/kg, 24 ng/kg, 25 ng/kg, 26 ng/kg, 27 ng/kg, 28 ng/kg, 29 ng/kg, 30 ng/kg, 31 ng/kg, 32 ng/kg, 33 ng/kg, 34 ng/kg, 35 ng/kg, 36 ng/kg, 37 ng/kg, 38 ng/kg, 39 ng/kg, 40 ng/kg, 41 ng/kg, 42 ng/kg, 43 ng/kg, 44 ng/kg, 45 ng/kg, 46 ng/kg, 47 ng/kg, 48 ng/kg, 49 ng/kg, 50 ng/kg, 51 ng/kg, 52 ng/kg, 53 ng/kg, 54 ng/kg, 55 ng/kg, 56 ng/kg, 57 ng/kg, 58 ng/kg, 59 ng/kg, 60 ng/kg, 61 ng/kg, 62 ng/kg, 63 ng/kg, 64 ng/kg, 65 ng/kg, 66 ng/kg, 67 ng/kg, 68 ng/kg, 69 ng/kg, 70 ng/kg, 71 ng/kg, 72 ng/kg, 73 ng/kg, 74 ng/kg, 75 ng/kg, 76 ng/kg, 77 ng/kg, 78 ng/kg, 79 ng/kg, 80 ng/kg, 81 ng/kg, 82 ng/kg, 83 ng/kg, 84 ng/kg, 85 ng/kg, 86 ng/kg, 87 ng/kg, 88 ng/kg, 89 ng/kg, 90 ng/kg, 91 ng/kg, 92 ng/kg, 93 ng/kg, 94 ng/kg, 95 ng/kg, 96 ng/kg, 97 ng/kg, 98 ng/kg, 99 ng/kg, or 100 ng/kg of body weight), from about 100 ng/kg to 500 ng/kg, from about 250 ng/kg to 750 ng/kg, from about 500 ng/kg to 1000 ng/kg, from about 750 ng/kg to 1250 ng/kg, from about 1000 ng/kg to 1500 ng/kg, from about 1250 ng/kg to 1750 ng/kg, from about 1500 ng/kg to 2000 ng/kg, from about 1750 ng/kg to 2250 ng/kg, from about 2000 ng/kg to 2500 ng/kg,

from about 2250 ng/kg to 2750 ng/kg, from about 2500 ng/kg to 3000 ng/kg, from about 2750 ng/kg to 3250 ng/kg, from about 3000 ng/kg to 3500 ng/kg, from about 3250 ng/kg to 3750 ng/kg, from about 3500 ng/kg to 4000 ng/kg, from about 3750 ng/kg to 4250 ng/kg, from about 4000 ng/kg to 4500 ng/kg, from about 4250 ng/kg to 4750 ng/kg, from about 4500 ng/kg to 5000 ng/kg, from about 4750 ng/kg to 5250 ng/kg, from about 5000 ng/kg to 5500 ng/kg, from about 5250 ng/kg to 5750 ng/kg, from about 5500 ng/kg to 6000 ng/kg, from about 5750 ng/kg to 6250 ng/kg, from about 6000 ng/kg to 6500 ng/kg, from about 6250 ng/kg to 6750 ng/kg, from about 6500 ng/kg to from about 7000 ng/kg, from about 6750 ng/kg to 7250 ng/kg, from about 7000 ng/kg to 7500 ng/kg, from about 7250 ng/kg to 7750 ng/kg, from about 7500 ng/kg to 8000 ng/kg, from about 7750 ng/kg to 8250 ng/kg, from about 8000 ng/kg to 8500 ng/kg, from about 8250 ng/kg to 8750 ng/kg, from about 8500 ng/kg to 9000 ng/kg, from about 8750 ng/kg to 9250 ng/kg, from about 9000 ng/kg to 9500 ng/kg, from about 9250 ng/kg to 9750 ng/kg, from about 9500 ng/kg to 10 µg/kg, from about 0.01 µg to 100 g per kg of body weight, from 0.1 µg to 10 g per kg of body weight, from 1.0 µg to 1 g per kg of body weight, from 10.0 µg to 100 mg per kg of body weight, from 100 µg to 10 mg per kg of body weight, or from 1 mg to 5 mg per kg of body weight, and may be given once or more daily, weekly, monthly or yearly.

[0106] Pharmaceutical compositions which contain, as the active ingredient, one or more of the miR inhibitor, miR inhibitor expressing constructs, miR-encoding nucleic acid, or miR-expressing nucleic acid constructs disclosed herein are effective over a wide dosage range and are generally administered in a therapeutically effective amount. It will be understood, however, that the amount of the composition actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[0107] For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient or carrier to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

[0108] Tablets or pills of the present invention can be coated or otherwise compounded to

provide a dosage form affording the advantage of prolonged action and to protect the one or more of the miR inhibitor, miR inhibitor expressing constructs, miR-encoding nucleic acid, or miR-expressing nucleic acid constructs disclosed herein compounds from acid hydrolysis in the stomach. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0109] The liquid forms in which the pharmaceutical compositions of the present invention can be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles. Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions can contain suitable pharmaceutically acceptable excipients as described above. The compositions can be administered by the oral or nasal respiratory route for local or systemic effect. Compositions in pharmaceutically acceptable solvents can be nebulized by use of inert gases. Nebulized solutions can be inhaled directly from the nebulizing device or the nebulizing device can be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can also be administered, orally or nasally, from devices which deliver the formulation in an appropriate manner.

B. Oligonucleotide modifications

[0110] The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. The oligonucleotides (for example, an antisense oligonucleotide or an siRNA oligonucleotide or an synthetic oligonucleotide used to compensate or restore expression of a naturally-occurring counterpart) used for treating chondrosarcoma according to any of the compositions or methods disclosed herein can have one or more modified, i.e. non-naturally occurring, internucleoside linkages. With respect to therapeutics, modified internucleoside linkages are often selected over oligonucleotides having naturally occurring

internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

[0111] Oligonucleotides (such as an antisense oligonucleotide an siRNA oligonucleotide) having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiester, phosphotriester, methylphosphonate, phosphoramidate, and phosphorothioate. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

[0112] In one embodiment, oligonucleotides (such as antisense oligonucleotides) targeted to the miR molecules disclosed herein comprise one or more modified internucleoside linkages. In some embodiments, the modified internucleoside linkages are phosphorothioate linkages. In other embodiments, each internucleoside linkage of an oligonucleotide compound is a phosphorothioate internucleoside linkage.

[0113] As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[0114] Specific though nonlimiting examples of oligonucleotides (such as antisense oligonucleotides or siRNA oligonucleotides) useful in the methods of the present invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones

include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[0115] In some embodiments, modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotri-esters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thiono-phosphoramidates, thionoalkylphosphonates, thionoalkylphospho-triesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof) can also be employed. Various salts, mixed salts and free acid forms are also included. Oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

[0116] In other embodiments, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases

are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

[0117] Representative United States patents that teach the preparation of the above phosphorus-containing and non-phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, each of which is herein incorporated by reference.

[0118] Modified oligonucleotides (such as antisense oligonucleotides) used as anticancer therapies in conjunction with any of the methods or compositions disclosed herein may also contain one or more substituted sugar moieties. For example, the furanosyl sugar ring can be modified in a number of ways including substitution with a substituent group, bridging to form a bicyclic nucleic acid "BNA" and substitution of the 4'-O with a heteroatom such as S or N(R) as described in U.S. Pat. No. 7,399,845, hereby incorporated by reference herein in its entirety. Other examples of BNAs are described in published International Patent Application No. WO 2007/146511, hereby incorporated by reference herein in its entirety.

[0119] In other embodiments, the modified oligonucleotide comprises a bicyclic sugar moiety having a bridge group between the 2' and the 4'-carbon atoms. In certain such embodiments, the bridge group comprises from 1 to linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises from 1 to 4 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 or 3 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 linked biradical groups. In certain embodiments, a linked biradical group is selected from ---O--- , ---S--- , ---N(R1)- , ---C(R1)(R2)- , ---C(R1)=C(R1)- , ---C(R1)=N--- , ---C(=NR1)- , ---Si(R1)(R2)- , $\text{---S(=O)}_2\text{---}$, ---S(O)--- , ---C(=O)--- and ---C(=S)--- ; where each R1 and R2 is, independently, H, hydroxyl, C1-C12 alkyl, substituted C1-C12 alkyl, C2-C12 alkenyl, substituted C2-C12 alkenyl, C2-C12 alkynyl, substituted C2-C12 alkynyl, C5-C20 aryl, substituted C5-C20 aryl, a heterocycle radical, a substituted hetero-cycle radical, heteroaryl, substituted heteroaryl, C5-C7 alicyclic

radical, substituted C₅-C₇ alicyclic radical, halogen, substituted oxy (—O—), amino, substituted amino, azido, carboxyl, substituted carboxyl, acyl, substituted acyl, CN, thiol, substituted thiol, sulfonyl (S(=O)₂-H), substituted sulfonyl, sulfoxyl (S(=O)—H) or substituted sulfoxyl; and each substituent group is, independently, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, amino, substituted amino, acyl, substituted acyl, C₁-C₁₂ amino alkyl, C₁-C₁₂ aminoalkoxy, substituted C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkoxy or a protecting group.

[0120] Oligonucleotides (such as antisense oligonucleotides) for use in any of the methods disclosed herein may also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. Nucleobase modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to oligonucleotide compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an oligonucleotide compound (such as an antisense oligonucleotide compound) for a target nucleic acid (such as a miR). Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, U.S. Pat. Nos. 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, each of which is herein incorporated by reference.

[0121] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0122] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

EXAMPLES

Example 1: Identification of miRNAs over and underexpressed in human chondrosarcoma

[0123] As a first step in identifying miRNAs that are aberrantly expressed in human chondrosarcoma, miRNA array analysis was performed on two human chondrosarcomas (Grade II and III) with their normal articular cartilage used as a control.

Materials and Methods

[0124] RNA isolation and MicroRNA Microarray: Total RNA including miRNA was isolated from two human chondrosarcoma (Grades II and III), the same patients' normal articular cartilage, which were then pooled, primary chondrocytes, chondrocyte cell line CS-1, using a miRNeasy Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The concentration and purity of total RNA were determined by a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Human microarray assay containing 894 human miRNA sequences was performed (LC Sciences, Houston, TX, USA) using miRNA from the individual tumors and the pooled normal cartilage samples. The specific miRNA that were over- and underexpressed compared with the pooled normal articular cartilage (with $p < 0.01$, Student's t-test) were identified for further analysis. Institutional review board approval was obtained for the study.

Results

[0125] Using the criteria of statistically significant differences in expression between tumor and normal cartilage in both tumors, the overexpressed and underexpressed miRs shown in Table 1 were identified.

Table 1: Over and underexpressed miRs identified in microarray experiments. CS1 = chondrosarcoma human tumor 1; CL1 = cartilage control 1; CS2 = human tumor 2; CL2 = cartilage control 2.

miR	p-value CS1/CL1	CS1 mean relative expression	CL1 mean relative expression	p-value CS2/CL2	CS2 mean relative expression	CL2 mean relative expression

miR	p-value CS1/CL1	CS1 mean relative expression	CL1 mean relative expression	p-value CS2/CL2	CS2 mean relative expression	CL2 mean relative expression
hsa-miR-199a-3p	1.34E-10	15,413	5,662	4.69E-06	18,800	5,662
hsa-miR-26a	3.29E-08	29,197	10,912	3.46E-02	95	6
hsa-miR-762	1.17E-07	9,177	3,943	1.44E-07	9,568	3,943
hsa-miR-125a-5p	9.74E-07	5,933	3,735	6.84E-06	6,606	3,735
hsa-let-7g	1.20E-06	14,801	7,116	4.90E-07	13,929	7,116
hsa-miR-16	1.43E-06	5,894	1,950	3.78E-07	6,968	1,950
hsa-let-7f	3.47E-06	39,289	20,457	2.99E-05	29,699	20,457
hsa-miR-21	4.18E-06	14,652	232	4.45E-06	13,347	232
hsa-let-7a	4.77E-06	44,857	22,244	2.93E-05	35,011	22,244
hsa-miR-638	8.95E-06	14,477	8,444	1.45E-05	14,065	8,444
hsa-miR-23a	8.97E-06	20,252	9,221	1.79E-05	17,645	9,221
hsa-miR-92a	9.63E-06	3,834	2,025	1.68E-04	2,843	2,025
hsa-miR-15b	1.10E-05	3,995	1,917	7.55E-04	2,658	1,917
hsa-miR-23b	2.69E-05	21,344	10,678	1.56E-05	18,412	10,678
hsa-miR-451	3.48E-05	7,053	816	6.57E-06	4,866	816
hsa-miR-483-5p	4.58E-05	8,749	4,836	1.16E-06	15,561	4,836
hsa-miR-15a	6.75E-05	1,548	97	1.30E-05	1,175	97
hsa-miR-27a	9.29E-05	8,140	4,592	1.05E-05	7,069	4,592
hsa-miR-26b	9.30E-05	13,046	2,605	4.07E-05	21,074	2,605
hsa-let-7d	9.63E-05	37,833	21,780	5.70E-04	29,131	21,780
hsa-miR-27b	9.90E-05	9,054	6,307	5.79E-04	7,387	6,307
hsa-miR-98	1.08E-04	16,759	10,737	8.14E-04	13,406	10,737
hsa-miR-145	1.12E-04	2,555	154	5.01E-04	903	154
hsa-miR-143	1.45E-04	1,203	63	2.99E-04	649	63
hsa-miR-1915	1.79E-04	1,857	1,287	2.28E-06	3,557	1,287
hsa-miR-149*	2.02E-04	4,407	2,436	2.05E-06	6,594	2,436
hsa-let-7i	2.50E-04	14,711	10,284	7.88E-06	15,770	10,284
hsa-let-7c	2.53E-04	39,082	23,088	1.79E-03	31,121	23,088
hsa-let-7e	2.58E-04	29,233	16,914	7.47E-04	24,514	16,914
hsa-let-7b	3.21E-04	33,841	23,114	7.92E-03	27,705	23,114
hsa-miR-30c	6.85E-04	2,836	1,191	1.82E-03	1,875	1,191
hsa-miR-181d	7.96E-04	1,304	68	8.76E-03	203	68
hsa-miR-148a	9.69E-04	1,477	704	7.23E-05	4,071	704
hsa-miR-181c	1.00E-03	710	41	1.14E-03	291	41
hsa-miR-196a	1.11E-03	702	274	1.08E-04	928	274
hsa-miR-30a	1.67E-03	946	191	1.39E-03	1,025	191
hsa-miR-214	2.59E-03	14,210	12,212	2.32E-03	14,481	12,212
hsa-miR-187*	3.60E-03	648	144	2.12E-03	612	144
hsa-miR-663	4.00E-03	2,205	1,340	9.08E-04	2,252	1,340
hsa-miR-146a	4.19E-03	510	75	4.95E-03	463	75
hsa-miR-30d	5.42E-03	1,335	939	2.25E-03	1,448	939
hsa-miR-365	5.56E-03	804	183	2.47E-03	844	183
hsa-miR-424	6.27E-03	1,374	45	8.85E-03	875	45
hsa-miR-1231	8.56E-03	492	246	3.17E-04	795	246
hsa-miR-424*	8.21E-04	110	23	2.87E-03	52	23
hsa-miR-454	1.20E-03	215	24	4.29E-04	181	24
hsa-miR-455-5p	1.66E-03	181	95	3.60E-03	153	95
hsa-miR-337-3p	4.22E-03	57	12	8.16E-04	91	12

miR	p-value CS1/CL1	CS1 mean relative expression	CL1 mean relative expression	p-value CS2/CL2	CS2 mean relative expression	CL2 mean relative expression
hsa-miR-381	5.48E-03	26	10	2.53E-03	47	10
hsa-miR-30e	8.50E-03	311	43	8.91E-03	292	43
hsa-miR-320c	1.93E-09	3,908	22,585	7.03E-05	7,531	22,585
hsa-miR-320b	4.15E-09	3,597	22,088	7.61E-05	7,422	22,088
hsa-miR-720	2.27E-08	1,193	29,654	1.32E-05	172	29,654
hsa-miR-320a	4.64E-08	4,104	23,550	3.16E-05	6,754	23,550
hsa-miR-127-3p	4.71E-08	177	1,445	3.47E-08	200	1,445
hsa-miR-1260	7.52E-08	12	6,444	1.03E-07	5	6,444
hsa-miR-140-3p	1.21E-07	8,171	19,485	3.59E-05	9,375	19,485
hsa-miR-22	2.06E-07	655	2,138	5.72E-05	257	2,138
hsa-miR-146b-5p	2.69E-07	247	719	6.50E-05	317	719
hsa-miR-107	8.81E-07	1,159	2,340	5.60E-07	871	2,340
hsa-miR-320d	1.73E-06	3,067	22,085	8.91E-03	292	43
hsa-miR-1280	4.67E-06	915	24,004	3.75E-06	200	24,004
hsa-miR-423-5p	6.83E-06	2,133	6,213	1.57E-06	1,693	6,213
hsa-miR-455-3p	1.76E-05	2,877	11,567	7.27E-08	1,015	11,567
hsa-miR-193b*	2.02E-05	60	1,233	1.89E-05	131	1,233
hsa-miR-103	2.04E-05	1,162	2,289	1.08E-04	882	2,289
hsa-miR-432	2.23E-05	341	1,497	1.37E-05	234	1,497
hsa-miR-151-3p	2.54E-05	296	645	7.96E-05	296	645
hsa-miR-31	2.66E-05	50	911	8.60E-06	15	911
hsa-miR-664*	4.61E-05	58	695	1.04E-04	41	695
hsa-miR-1978	4.98E-05	632	2,360	1.42E-07	235	2,360
hsa-miR-486-5p	8.60E-05	379	662	3.29E-06	151	662
hsa-miR-99a	3.31E-04	3,683	6,554	5.85E-04	4,133	6,554
hsa-miR-24	3.43E-04	5,623	7,317	2.31E-03	5,276	7,317
hsa-miR-191	3.65E-04	2,992	4,694	3.05E-05	2,052	4,694
hsa-miR-99b	5.10E-04	672	1,888	8.62E-05	555	1,888
hsa-miR-574-5p	7.12E-04	1,897	5,214	9.83E-04	1,896	5,214
hsa-miR-151-5p	7.18E-04	2,207	2,684	4.94E-05	1,900	2,684
hsa-miR-193a-5p	7.91E-04	369	1,319	1.02E-04	303	1,319
hsa-miR-1246	1.51E-03	19,632	21,591	8.58E-04	13,676	21,591
hsa-miR-877	2.85E-03	87	364	3.88E-04	58	364
hsa-miR-940	3.43E-03	475	1,254	2.91E-04	70	1,254
hsa-miR-1281	3.61E-03	176	713	5.85E-05	50	713
hsa-miR-494	3.85E-03	88	526	4.66E-03	98	526
hsa-miR-125b-2*	4.93E-06	32	169	1.42E-04	30	169
hsa-miR-210	5.71E-06	70	468	2.04E-03	241	468
hsa-miR-1249	6.02E-05	29	124	3.00E-03	14	124
hsa-miR-874	1.24E-03	33	137	1.01E-03	39	137
hsa-miR-23a*	1.62E-03	28	166	1.79E-05	17,645	9,221
hsa-miR-30b*	2.26E-03	33	75	4.14E-03	40	75
hsa-miR-296-5p	3.35E-03	29	101	2.69E-04	18	101
hsa-miR-744	4.34E-03	76	170	3.26E-04	53	170
hsa-miR-197	7.61E-03	110	300	2.42E-03	63	300
hsa-miR-27b*	8.00E-03	56	172	7.66E-04	32	172
hsa-miR-324-3p	9.98E-03	33	115	1.62E-03	21	115

[0126] With respect to miR-1280, a qRT-PCR analysis confirms that expression of this miR is significantly decreased and/or absent in chondrosarcoma tissue (CS1, CS2) relative to its expression in normal cartilage (CL1, CL2; FIG. 1).

Example 2: Expression or restoration of miR-126 expression inhibits growth of chondrosarcoma cells

[0127] This Example demonstrates that enhancing expression of miR-126 in chondrosarcoma cells is associated with decreased expression of the angiogenesis-promoting proteins vascular endothelial growth factor (VEGF), placental growth factor (PGF), and thrombospondin-1 (TSP-1), decreased cellular proliferation, and decreased Met protein expression.

Materials and Methods

[0128] Cell lines: Human primary chondrocytes and chondrosarcoma cell lines were cultured as previously described (Kulshreshtha et al., Cell Cycle. 2007;6:1426–1431). CS-1 (a gift from Dr. Francis Hornicek, Harvard Medical School, Boston, MA) was cultured in Gibco RPMI 1640 Medium (Life Technologies, Grand Island, NY), with 10% FBS in a humidified incubator (NuAire Inc, Plymouth, MN) under 5% CO₂ and either normoxia (ambient oxygen) or hypoxia (2% O₂)(11). CS-1 was derived from human grade III chondrosarcoma and metastasizes in a xenograft mouse model(Susa M, Morii T, Yabe H, Horiuchi K, Toyama Y, Weissbach L, et al. Alendronate inhibits growth of high-grade chondrosarcoma cells. *Anticancer Res.* 2009 Jun;29(6):1879–88.). All cells were cultured in a humidified incubator (NuAire Inc, Plymouth, MN, USA) under 5% CO₂ and either normoxia or hypoxia (2% O₂) (Lin et al., *J Orthop Res.* 2004;22:1175–1181).

[0129] Transfection with microRNA, anti-microRNA: Transient miR knockdown or overexpression was achieved with syn-hsa-miR-181a, 34a, 126 or others miScript miRNA mimic, control miR, anti-hsa-miR-181a, 34a, 126 or others miScript miRNA inhibitor, and miScript inhibitor negative control (Applied Biosystems). Transfections were performed with GenMute transfection reagent (SignaGen Laboratories, Gaithersburg, MD). pmiRZIP lentivector expressing anti-miR-181a or control sequence (SBI, Mountain View, CA) was used for permanent miR-181a knockdown experiments. Transduction-ready FIV-based pseudoviral particles were generated using pPACK-H1 Lentivector Packaging System

together with 293TN cell line (SBI), at a titer of 1.06×10^9 IFU/ml. Control was Lenti-scramble Hairpin control pseudoviral particles at a titer of 1×10^9 IFU/ml. Cells were cultured in 12-well plates at a density of 1×10^5 /well for 1 day, infected by pseudoviral particles (using a multiplicity of infection of 100 viruses per cell) and cultured for 72 hrs, then selected for puromycin (5 μ g/ml) resistance for stable cell lines. Stably transduced cells were used for vitro and in vivo experiments.

[0130] Angiogenesis antibody array: Human Angiogenesis Array Kit is a membrane-based sandwich immunoassay. Samples are mixed with a cocktail of biotinylated detection antibodies and then incubated with the array membrane which is spotted in duplicate with capture antibodies to specific target proteins. Captured proteins are visualized using chemiluminescent detection reagents. The signal produced is proportional to the amount of bound analyte. It is sensitive and economical tool to simultaneously detect the relative levels of 55 angiogenesis-related proteins in a single sample.

[0131] ELISA: Conditioned medium was obtained 72 hours after transfection. Soluble VEGF-165 and MMP1 were detected using VEGF and MMP1 Immunoassay kits (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. VEGF-165 and MMP1 levels were measured two times for each condition and with normalization to the number of cells at the end of the culture period. Each experiment was repeated three times. Twenty mg of xenograft tumor tissue in RIPA buffer containing proteinase inhibitors (Roche) was homogenized on ice using TissueRuptor (Qiagen). Tissue lysates were centrifuge at 14000 rpm for 30 min, supernatant saved at -80°C for later use. VEGF-165 levels in xenograft tumor lysates were normalized to total protein.

[0132] qRT-PCR: miR expression evaluated with qRT-PCR using Hs_miR-181a primer 5'-AACAUUCAACGCUGU-CGGUGAGU-3' (SEQ ID NO:198) and 1280: 5'UCCCACCGCUGCCACCC-3' (SEQ ID NO:199) (Qiagen). The comparative threshold cycle (Ct) method, i.e., $2^{-\Delta\Delta\text{Ct}}$ method is used to calculate fold amplification. For quantification of mRNA, total RNA was isolated from chondrocytes, CS-1 cells, and xenograft mouse tumors using the RNAqueous® Kit (Ambion, Austin, TX, USA). SYBR real-time PCR was carried out using two-step real-time qRT-PCR (Qiagen) with normalization to 18S rRNA (18S). The comparative threshold cycle (Ct) method, i.e., the $2^{-\Delta\Delta\text{Ct}}$ method, was used for the calculation of fold amplification. Each experiment was evaluated with three PCR reactions and each experiment was repeated three times, the

sample size necessary to maintain power at 0.80 to detect a 50% decrease with an alpha of 0.025 (one-tailed *t*-test).

[0133] Western blot: Cell or tumor lysates containing forty µg of protein were separated via SDS-PAGE (Bio-Rad, Hercules, CA) and probed with antibodies for VEGF (VEGFA), MMP-1, SSX, and actin (Santa Cruz Biotechnology, Santa Cruz, CA). The fluorescent signals were detected using a fluorescently-labeled secondary goat anti-rabbit antibody (Alexa Fluor 680) (Molecular Probes, Eugene, OR) and analyzed on Licor Odyssey Scanner (LI-COR Biosciences, Lincoln, NE). Western Blot analyses were performed as previously described with specific antibodies (IMGNEX, San Diego, CA) and actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Protein concentrations were determined using the Quick Start Bradford protein assay (Bio-Rad).

[0134] Cell proliferation assay: CyQUANT® Cell Proliferation Assay Kit according to manufacturer's protocol

Results

[0135] VEGF is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can also cause vascular disease in the retina of the eye and other parts of the body.

[0136] As shown in FIG. 2 (top), miR-126 inhibited VEGF expression in a dose-dependent manner (10-80nM of miR-126) in chondrosarcoma cells under both normoxia and hypoxia as measured by ELISA. FIG. 2 (bottom) also shows that transient transfection of chondrosarcoma cells with miR-126 resulted in reduced VEGF expression under both normoxic and hypoxic conditions. However, this effect was obviated when cells were administered an anti-miR-126 antagomir.

[0137] Similarly, as shown in FIG. 3, an angiogenesis antibody array showed miR-126 reduces the expression of not only VEGF, but also placental growth factor (PLGF) and thrombospondin-1 (TSP-1). PLGF is a member of the VEGF sub-family and is considered to be a key molecule in angiogenesis and vasculogenesis, in particular during embryogenesis and neovascularization (Moons et al., *Circulation*, 2005, 111(21):2828–2836). TSP-1 is an

adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Various domains of and receptors for TSP1 have been shown to have pro-adhesive and chemotactic activities for cancer cells, suggesting that this molecule may have a direct effect on cancer cell biology (Taraboletti et al., *J. Cell Biol.*, 1987, 105(5):2409–15).

[0138] Based on the observation that VEGF expression goes up under hypoxic conditions (FIG. 2), expression of miR-126 was examined in response to expression of hypoxia-inducible factor 1-alpha (hif1a). Hif1a is a subunit of a heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1) and is considered as the “master” transcriptional regulator of cellular and developmental response to hypoxia (Wang et al., *PNAS*, 1995, 92(12):5510–5514). As shown in FIG. 4, hif1a regulated miR-126 expression as measured by qRT-PCR. Specifically, reduction of hif1a expression by a specific hif1a siRNA (20nM) increased miR-126 expression while overexpression of hif1a by transfection with an hif1a-expressing construct decreased miR-126 expression in under normoxic conditions. These results show that tumor hypoxia effects are partially mediated by expression of microRNA 126

[0139] In addition, by restoring expression of miR-126, expression of Met protein decreased in chondrosarcoma cells compared to cells treated with an miRNA control, as shown in FIG.5. Met has been shown to play important roles in the development of cancer through activation of key oncogenic pathways (*e.g.*, RAS, PI3K, STAT3, beta-catenin); induction of angiogenesis; and cellular dissociation due to metalloprotease production, which often leads to metastasis.

[0140] Finally, chondrosarcoma cells transfected with 20 nM miR-126 grew more slowly over time compared to cells treated with a control miR as measured by as measured by CyQUANT® Cell Proliferation Assay Kit (FIG. 6).

[0141] In summary, expression of miR-126 in chondrosarcoma cells is associated with downregulation of multiple pro-angiogenic and metastasis-promoting factors, including VEGF, PLGF, TSP-1, and Met. Moreover, hif1a appears to negatively regulate the expression of miR-126 under hypoxic conditions. Following transfection with miR-126, chondrosarcoma cells were observed to grow more slowly over time as compared to control-treated cells.

Example 3: Expression or restoration of miR-34a expression inhibits expression of VEGF and growth of chondrosarcoma cells

[0142] This Example shows that increased or restored miR-34a expression in chondrosarcoma cells results in decreased VEGF and SSX-1 expression as well as decreased cellular proliferation.

Materials and Methods

[0143] ELISA, western blot, qRT-PCR, and cell proliferation assays were performed as described above.

Results

[0144] Transfection of chondrosarcoma cells with miR-34 (20 nM) significantly decreased expression of VEGF protein compared to control cells (cells treated with control miR or control anti-miR sequences) or cells treated with an anti-miR-34a construct (FIG. 7A-7C). Transfection with miR-34a was also associated with decreased VEGF expression over time as measured by ELISA, as shown in FIG. 8. Additionally, as shown in FIG. 9, chondrosarcoma cells transfected with miR-34a exhibited significantly decreased growth rates over time compared to control cells.

[0145] Synovial sarcoma X-1 (SSX1) is a tumor antigen involved in the t(X;18) translocation characteristically found in all synovial sarcomas. This translocation results in the fusion of the synovial sarcoma translocation gene on chromosome 18 to one of the SSX genes on chromosome X. The encoded hybrid proteins are thought to be responsible for transforming activity (Sun et al., *Oncogene*, 25(7):1042–52). As shown in FIG. 10A, transfection of chondrosarcoma cells with miR-34a (20 nM) resulted in decreased SSX1 mRNA expression as measured by qRT-PCR compared to control cells or cells treated with an anti-miR-34a oligonucleotide construct. Similar results were observed with respect to SSX1 protein expression following transfection of chondrosarcoma cells with miR-34a (FIG. 10B). However, an siRNA directed against SSX4 did not result in decreased VEGF mRNA expression (FIG. 10A). Further, as shown in FIG. 11A-C, transfection with an SSX1 siRNA (20nM) resulted in decreased VEGF mRNA and protein expression in chondrosarcoma cells both in monolayer and a 3D tumor cell spheroid growth culture conditions (Matrigel suspension).

[0146] As shown in FIG. 12, co-administration of miR-34a with an anti-miR181a oligonucleotide (20 nM) construct resulted in decreased VEGF protein expression in chondrosarcoma cells.

[0147] In summary, this example shows that expression of miR-34a results in decreased expression of VEGF and SSX1 as well as significantly decreased proliferation in chondrosarcoma cells. As shown in FIG. 10-11, miR-34a may exert some of its effects on VEGF expression by decreasing the expression of SSX1.

Example 4: Nanopiece delivery of a miR-181a inhibitor to chondrosarcoma cells

[0148] This example shows that nanopieces can be used to enhance transfection of chondrosarcoma cells with nucleotide sequences (oligos and molecular beacons) both *in vitro* and *in vivo*.

Materials and Methods

[0149] Preparation of nanopieces (NP) for IV injection into nude mice: the antagomir amount needed is calculated as below: each IV injection for one mouse is 6 μ L (50 μ M). siRNA and JAK (AAT or RNT) were thawed at room temperature. [“JAK”: Janus base with Amine or lysine (K), and “AAT”: fused Amino Adenine with Thymine]. The following were mixed in an Eppendorf tube: siRNA: 6 μ L, water: 60 μ L, AAT/RNT: 90 μ L. The tube was sonicated in Qsonica Sonicator for 2.5 minutes. The Eppendorf tubes were then centrifuged to spin down aqueous droplets in the tube. Next, 3.9 μ L sterile PEG (polyethylene glycol) 400 and 2.16 μ L sterile 45% glucose were added into the Eppendorf tube containing assembled NP (total volume is 162 μ L). The NP was stored on ice before animal injection. Exemplary NPs useful in the therapeutic methods described herein include those with a length of 1 nm to 200 nm, e.g. a length of about 100 nm, and a width or diameter of 1 nm to 60 nm, e.g., 20 nm. For example, the length is in the range of 50-150 nm and the width/diameter is in the range of 20-40 nm. Typically, the nanopieces are characterized by a length of about 100 nm and width/diameter of about 20 nm.

[0150] Cell line and xenograft tumor model: CS-1 cells (100 μ L of 1×10^6 cells) were mixed with 300 μ L Matrigel™ (BD Biosciences, San Jose, CA) and injected subcutaneously in the back of nude mice (nu/nu 6–8 week old female, Charles River Laboratory, Wilmington, MA).

[0151] FMT assays: *in vivo* bioimaging with fluorescence-based tomography (FMT, PerkinElmer, Waltham, MA) was performed at three weeks after injection of tumor cells. Twenty-four hours before imaging, mice were injected via tail vein with 2 nmol MMPsense 680 and Angiosense 750 (PerkinElmer, Waltham, MA). Mice were anesthetized with ketamine (ip) during FMT imaging. FMT is acquired with a continuous wave-type scanner capable of acquiring transillumination, reflectance and absorption data at 680 nm excitation and 700 nm emission or 750 nm excitation and 780 nm emission (PerkinElmer). AngioSense and MMPsense content in xenograft tumors was determined by region of interest analysis. Fluorochrome concentration in the target was calculated from reconstructed images and expressed as femtomoles of fluorochrome per defined target volume (the primary tumor).

Results

[0152] Oligonucleotide sequences cannot gain entry into a cell unless the cell membrane is permeabilized as is done in cell culture experiments with lipophilic agents, or by utilizing a vector, such as a virus, or utilizing nanopieces. Chondrosarcoma cells treated with fluorescent labeled control oligonucleotide for 24 hours followed by washing do not fluoresce, indicating lack of entry to the cell (FIG. 13A (bottom)). In contrast, if the oligos are delivered with nanopieces for 24 hours and then washed, the cells do fluoresce, indicating that the nanopieces have transfected the cells with the oligonucleotides (FIG. 13A (top)). Bright field views (FIG. 13A) demonstrate the presence of cells in both conditions. When the experiment is repeated with control antagomiR and one directed against miR-181a, expression of miR-181a is decreased when measured by qPCR (FIG. 13B).

[0153] To provide further proof that nanopieces can deliver either antagomirs or replacement miR oligonucleotides to tumors, nanopieces with a molecular beacon or molecular beacon alone were injected into xenograft chondrosarcoma tumors. Molecular beacon is complementary to the mRNA sequence for the house keeping gene GAPDH and only fluoresces if the beacon hybridizes to GAPDH mRNA. GAPDH mRNA only exists inside the cell, so that fluorescence indicates the beacon is intracellular. As shown in FIG. 14A, fluorescence is produced only if the beacon is injected into the tumor with nanopieces (RNT). When the experiment was repeated using an antagomir to miR-181a instead of the molecular beacon, expression of miR-181 was decreased in the tumor compared to control sequence (FIG. 14B) and MMP1 protein in the tumor decreased when measured by ELISA (FIG. 14C).

[0154] To further demonstrate the feasibility of using a systemic treatment approach to altering microRNA expression using nanopieces for delivery, anti-miR181a in combination with nanopieces was delivered via tail vein injection into mice harboring xenograft tumors. After a single injection, this resulted in decreased miR-181 expression in the tumors (FIG. 15). After seven injections over a three week time span after establishment of xenograft tumors, miR-181a expression was again decreased (FIG. 16) tumor weight was decreased (FIG. 17), as was MMP activity as measured by FMT bioimaging (FIG. 18).

[0155] These results indicate that anti-miR-181a therapy is effective for reducing tumor burden in a mouse model of chondrosarcoma.

[0156] Taken together, the Examples demonstrate that analysis of specific chondrosarcoma tumors with microRNA array can be used to identify over and underexpressed microRNAs that are relevant biologic targets whose expression can be inhibited in the case of those overexpressed and restored in the case of those underexpressed via delivery of nucleotide sequences with nucleotide based nanopieces, resulting in inhibition of tumor progression.

Example 5: Treatment with anti-miR181a enhances the sensitivity of chemotherapy in chondrosarcoma cells

[0157] This Example shows the ability of anti-miR-181a treatment to enhance the sensitivity of chemotherapy in chondrosarcoma cells under both hypoxic and normoxic conditions.

Materials and Methods

[0158] Cell culture, transfection, and FMT assays were performed as described above.

Results

[0159] AMD3100 (plerixafor) is an immunostimulant used to mobilize hematopoietic stem cells in cancer patients into the bloodstream and which also has been shown to reduce metastasis in mice (Sun et al., Mol Cancer Ther. 2013 Jul;12(7):1163-70; Smith et al., Cancer Research, 2004, 64(23):8604–8612). AMD3100 was administered alone and in combination with anti-miR-181a (80nM under both normoxia and hypoxia (2% oxygen)). As shown in FIG. 19, co-administration of anti-miR-181a and AMD3100 synergistically decreased MMP1 expression under both normoxic and hypoxic conditions. Expression of metalloproteases, such as MMP1, can lead to cellular dissociation of cancer cells from

underlying basal lamina and subsequent metastasis.

[0160] These results indicate that anti-miR-181a therapy can synergistically enhance the anti-tumor effects of AMD3100 when co-administered to chondrosarcoma cells under both hypoxic and normoxic conditions.

CLAIMS

We claim:

1. A method for treating chondrosarcoma in an individual in need thereof, the method comprising administering to the individual a therapeutically effective amount of one or more chondrosarcoma-inhibiting microRNA (miR), wherein said method results in one or more of a) in decreased expression of one or more of matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or tyrosine-protein kinase Met (Met); or b) decreased chondrosarcoma cellular proliferation, invasion, or metastasis.
2. The method of claim 1, wherein said chondrosarcoma-inhibiting miR comprises miR-34a.
3. The method of claim 1 or 2, wherein said one or more chondrosarcoma-inhibiting miR is selected from the group consisting of miR-34a, miR-34b, miR-34c, miR-126, miR-1280, miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, and miR-324-3p.
4. The method of any one of claims 1-3, wherein the nucleic acid is administered in a vector.
5. The method of claim 4, wherein the vector is a viral vector.
6. The method of any one of claim 1-5, wherein the nucleic acid encoding one or more miRs is a microRNA mimic.
7. The method of any one of claims 1-6, wherein said administration results in decreased expression of one or more of vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or Met.

8. The method of any one of claims 1-7, wherein said administration results in decreased chondrosarcoma cellular proliferation.
9. The method of any one of claims 1-8, further comprising administering a chemotherapeutic to the individual.
10. The method of claim 9, wherein said chemotherapeutic is AMD3100.
11. The method of any one of claim 1-10, wherein said miR is one or more of miR-34a, miR-34b, miR-34c, miR-126, miR-1280.
12. The method of any one of claim 1-11, further comprising administering to the individual a therapeutically effective amount of an inhibitor of miR-181a.
13. A method for treating chondrosarcoma in an individual in need thereof, the method comprising administering to the individual a therapeutically effective amount of an inhibitor of one or more chondrosarcoma-promoting microRNA (miR), wherein said method results in one or more of a) in decreased expression of one or more of matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or tyrosine-protein kinase Met (Met); or b) decreased chondrosarcoma cellular proliferation, invasion, or metastasis.
14. The method of claim 13, wherein said chondrosarcoma- promoting miR comprises miR-181a.
15. The method of claim 13 or claim 14, wherein said one or more chondrosarcoma-promoting miR is selected from the group consisting of miR-181a, miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, and miR-30e.
16. The method of any one of claims 13-15, wherein the inhibitor is an antagomir specific for said miR.

17. The method of any one of claims 13-15, wherein the inhibitor molecule causes post-transcriptional silencing of the miR.
18. The method of any one of claims 13-15, wherein the inhibitor molecule inhibits maturation of the miR.
19. The method of any one of claims 13-15, wherein the inhibitor molecule is an antisense oligonucleotide of the miR.
20. The method of any one of claims 13-15, wherein the inhibitor molecule is a small interfering RNA (siRNA).
21. The method of any one of claims 13-15, wherein the inhibitor molecule is a molecule capable of forming a triple helix with a gene coding for the miR.
22. The method of any one of claims 13-15, wherein the inhibitor molecule is a ribozyme.
23. The method of any one of claims 13-15, wherein the inhibitor molecule is administered as naked RNA, in conjunction with a delivery agent.
24. The method of any one of claims 13-15, wherein the inhibitor is administered on a vector.
25. The method of claim 24, wherein the vector is a viral vector.
26. The method of any one of claims 13-25, wherein said administration results in decreased expression of one or more of matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or Met.
27. The method of any one of claims 13-26, wherein said administration results in decreased chondrosarcoma cellular proliferation.
28. The method of any one of claims 13-27, further comprising administering a chemotherapeutic to the individual.
29. The method of claim 28, wherein said chemotherapeutic is AMD3100.

- 30.** The method of any one of claims 1-29, wherein said inhibitor or said nucleic acid is administered by a nanopiece.
- 31.** The method of any one of claims 1-30, wherein said inhibitor or said nucleic acid is administered intravenously.
- 32.** The method of any one of claims 1-30, wherein said inhibitor or said nucleic acid is administered via direct injection into the chondrosarcoma.
- 33.** The method of any one of claims 1-32, wherein the individual is diagnosed with conventional chondrosarcoma, periosteal chondrosarcoma, mesenchymal chondrosarcoma, dedifferentiated chondrosarcoma, clear-cell chondrosarcoma, or extraskeletal myxoid chondrosarcoma.
- 34.** The method of any one of claims 1-33, wherein the method further comprises administration of one or more additional anti-cancer therapies to the individual.
- 35.** The method of claim 34, wherein said additional anti-cancer therapy is surgical ablation of the chondrosarcoma.
- 36.** The method of any one of claims 1-35, wherein the individual is a human.
- 37.** A pharmaceutical composition comprising (a) one or more inhibitors of a microRNA (miR) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-181a, and miR-30e in an amount effective to inhibit growth of human chondrosarcoma cells; and (b) a pharmaceutically acceptable carrier or diluent.
- 38.** A pharmaceutical composition comprising (a) a nucleic acid encoding one or more microRNA (miR) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31,

miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-1280, miR-126, and miR-324-3p; and (b) a pharmaceutically acceptable carrier or diluent.

39. A method for diagnosing chondrosarcoma in an individual, the method comprising

(a) detecting the expression level of one or more microRNAs (miRs) selected from the group consisting of miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, miR-181a, and miR-30e in a biological sample obtained from the individual; or

(b) detecting the expression level of one or more microRNAs (miRs) selected from the group consisting of miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, miR-34a, miR-34b, miR-34c, miR-126, miR-1280, and miR-324-3p in the biological sample obtained from the individual,

wherein the individual is diagnosed with chondrosarcoma if (i) expression of one or more of the miRs of (a) is increased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or in normal tissue from the individual with chondrosarcoma or (ii) expression of one or more of the miRs of (b) is decreased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or in normal tissue from the individual with chondrosarcoma.

40. The method of claim 39, further comprising (c) treating the individual with the method of any one of claims 13-36 if expression of one or more of the miRs of (a) is increased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or in normal tissue from the individual with chondrosarcoma; or (d) treating the individual with the method of any one of claims 1-12 or 28-36 if expression of one or more of the miRs of (b) is decreased relative to the expression level of said one or more miRs in a biological sample obtained from an individual without chondrosarcoma or in normal tissue from the individual with chondrosarcoma.

41. A composition for use in the treatment of chondrosarcoma in an comprising a therapeutically effective amount of one or more chondrosarcoma-inhibiting microRNA (miR), wherein said composition results in one or more of a) in decreased expression of one or more of matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or Met; or b) decreased chondrosarcoma cellular proliferation, invasion, or metastasis.

42. The composition for use of claim 41, wherein said chondrosarcoma-inhibiting miR is miR-34a.

43. The composition for use of claim 41 or 42, wherein said one or more chondrosarcoma-inhibiting miR is selected from the group consisting of miR-34a, miR-34b, miR-34c, miR-126, miR-1280, miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, and miR-324-3p.

44. A composition for use in the treatment of chondrosarcoma in an individual comprising a therapeutically effective amount of one or more of a chondrosarcoma-promoting microRNA (miR), wherein said method results in one or more of a) in decreased expression of one or more of matrix metalloproteinases (MMP), vascular endothelial growth factor (VEGF), placental growth factor (PGF), thrombospondin-1 (TSP-1) and/or Met; or b) decreased chondrosarcoma cellular proliferation, invasion, or metastasis.

45. The composition for use of claim 44, wherein said chondrosarcoma-promoting miR comprises miR-181a.

46. The composition for use of claim 44 or claim 45, wherein said one or more chondrosarcoma- promoting miR is selected from the group consisting of miR-181a, miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, and miR-30e.

47. A method for treating chondrosarcoma in an individual in need thereof, the method comprising administering to the individual a therapeutically effective amount of (a) one or more chondrosarcoma-inhibiting microRNA (miR); or (b) an inhibitor of one or more chondrosarcoma-promoting miR using a nanopiece delivery vehicle.

48. The method of claim 47, wherein said one or more chondrosarcoma-inhibiting miR is selected from the group consisting of miR-34a, miR-34b, miR-34c, miR-126, miR-1280, miR-320c, miR-320b, miR-320a, miR-127-3p, miR-1260, miR-140-3p, miR-22, miR-146b-5p, miR-107, miR-320d, miR-423-5p, miR-1974, miR-455-3p, miR-193b*, miR-103, miR-432, miR-151-3p, miR-31, miR-664*, miR-486-5p, miR-99a, miR-24, miR-191, miR-99b, miR-574-5p, miR-151-5p, miR-193a-5p, miR-1246, miR-877, miR-940, miR-1281, miR-494, miR-125-b-2*, miR-210, miR-1249, miR-874, miR-23a*, miR-30b*, miR-296-5p, miR-744, miR-197, miR-27b*, and miR-324-3p.

49. The method of claim 47, wherein said one or more chondrosarcoma- promoting miR is selected from the group consisting of miR-181a, miR-199a-3p, miR-26a, miR-762, miR-125a-5p, miR-let-7g, miR-16, miR-let-7f, miR-21, miR-let-7a, miR-638, miR-23a, miR-92a, miR-15b, miR-23b, miR-451, miR-483-5p, miR-15a, miR-27a, miR-26b, miR-let-7d, miR-27-b, miR-98, miR-145, miR-143, miR-1915, miR-149*, miR-7i, miR-7c, miR-7e, miR-936, miR-let-7b, miR-30c, miR-181d, miR-148a, miR-181c, miR-196a, miR-30a, miR-214, miR-187*, miR-663, miR-146a, miR-30d, miR-365, miR-424, miR-1231, miR-424*, miR-454, miR-455-5p, miR-337-3p, miR-381, and miR-30e.

50. The method of any one of claims 47-49, wherein the nanopiece comprises a length of about 100 nm and width/diameter of about 20 nm.

51. The method of any one of claims 1-36 or 47-50, wherein the chondrosarcoma-inhibiting microRNA (miR); or the inhibitor of one or more chondrosarcoma-promoting miR is administered to a tumor bed in the individual following surgical resection of a tumor.

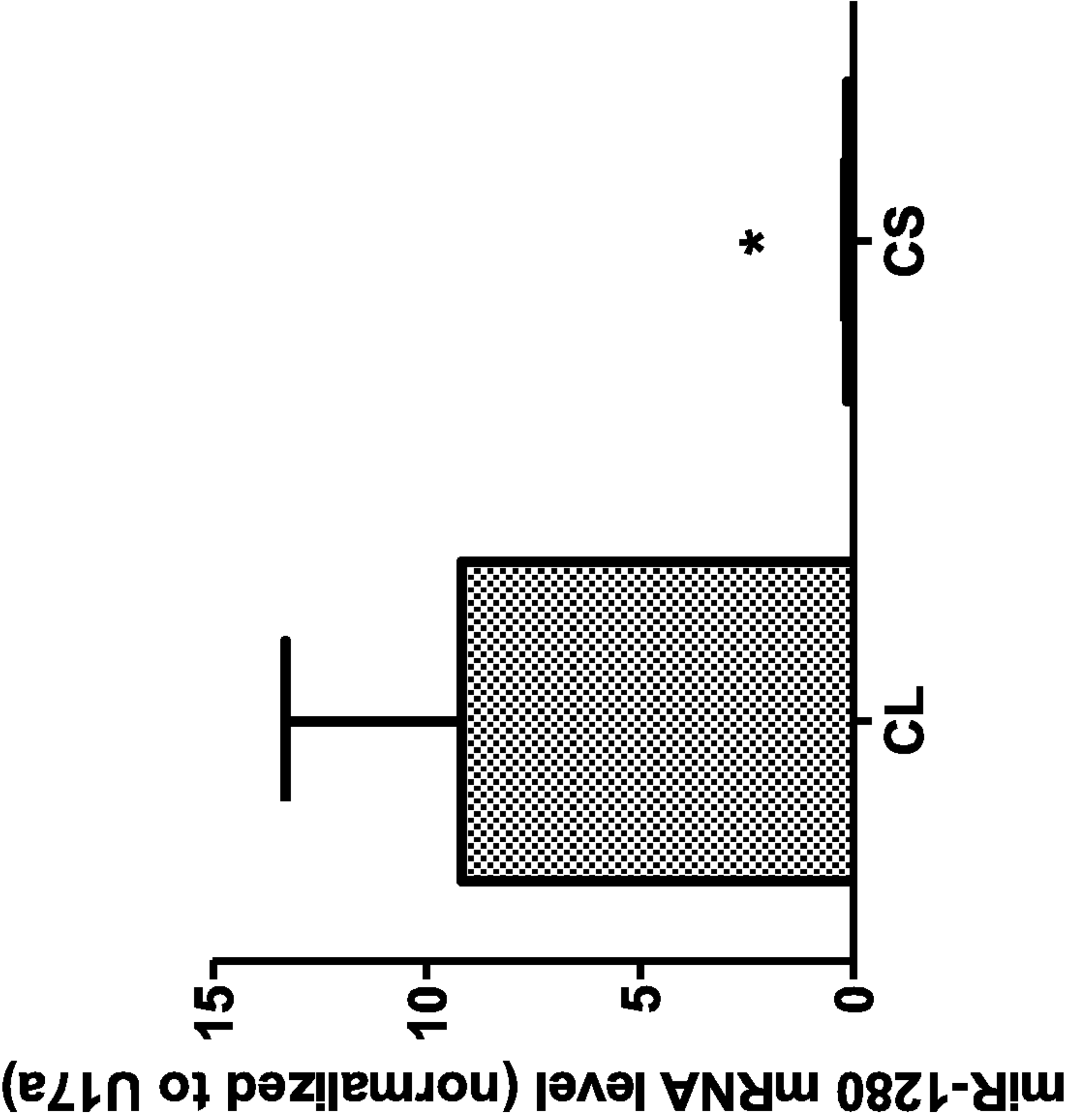
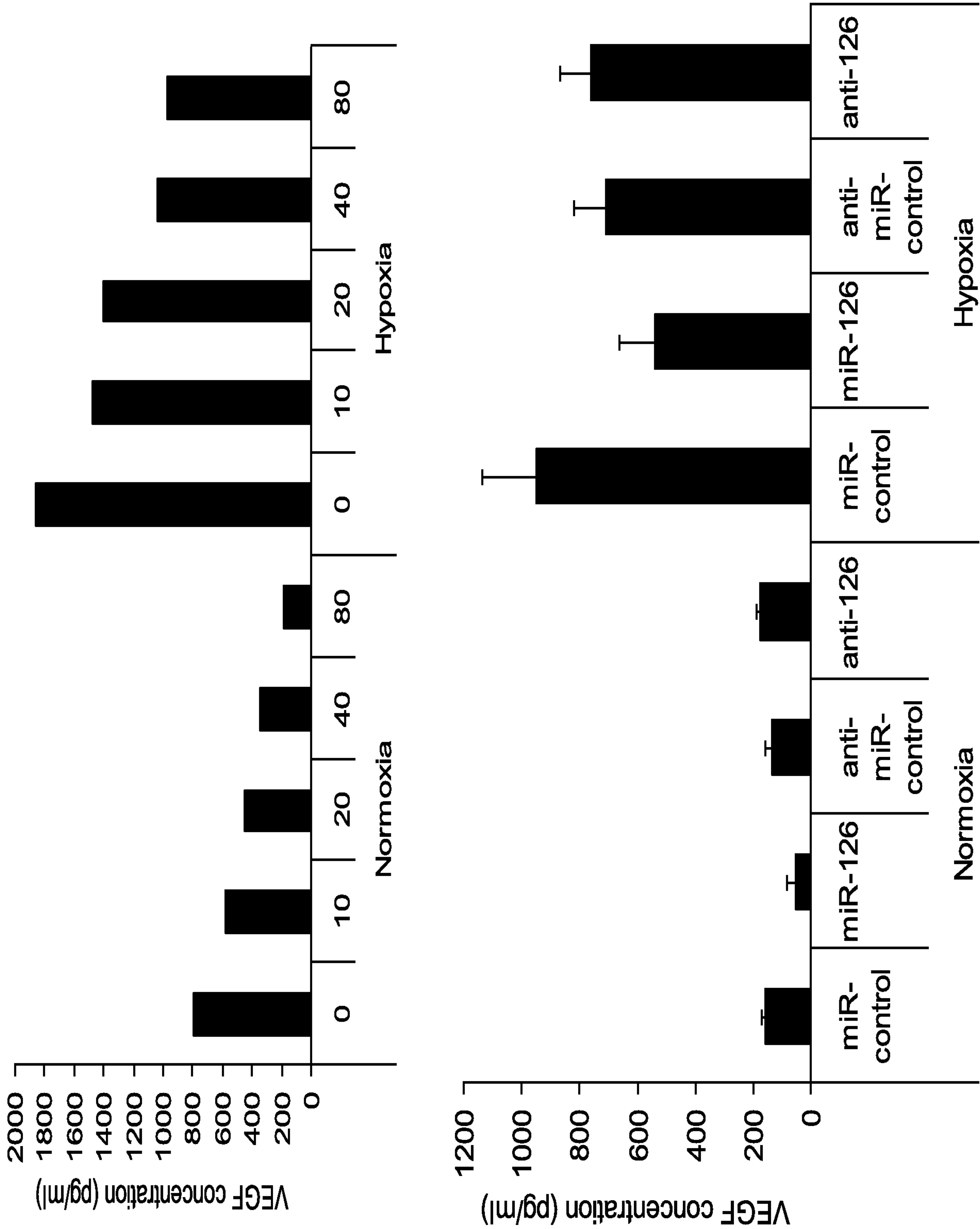


FIG. 1



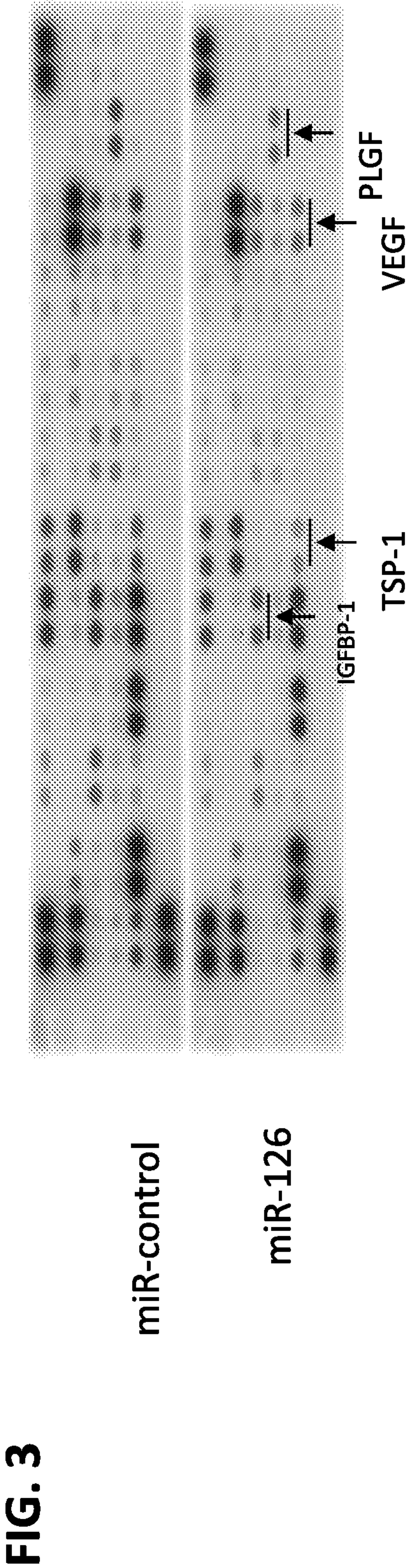


FIG. 4

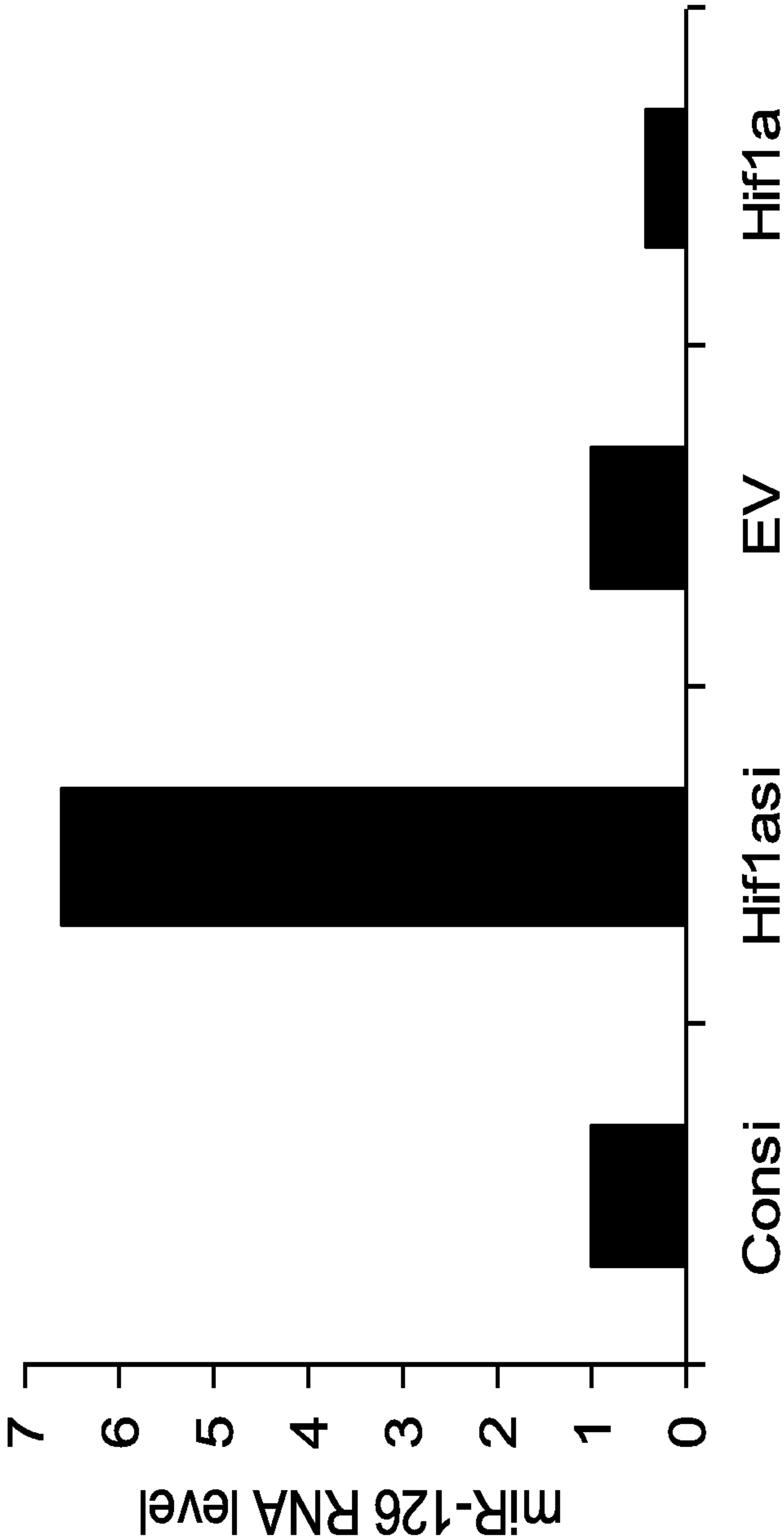


FIG. 5

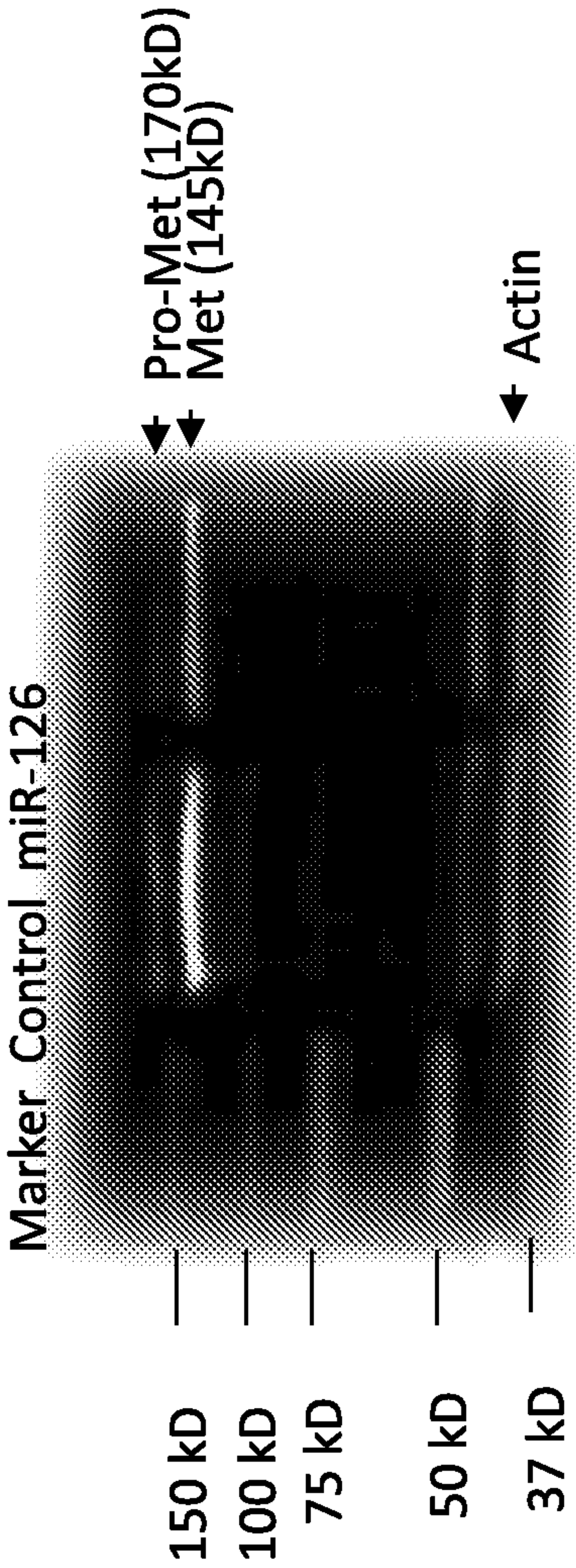


FIG. 6

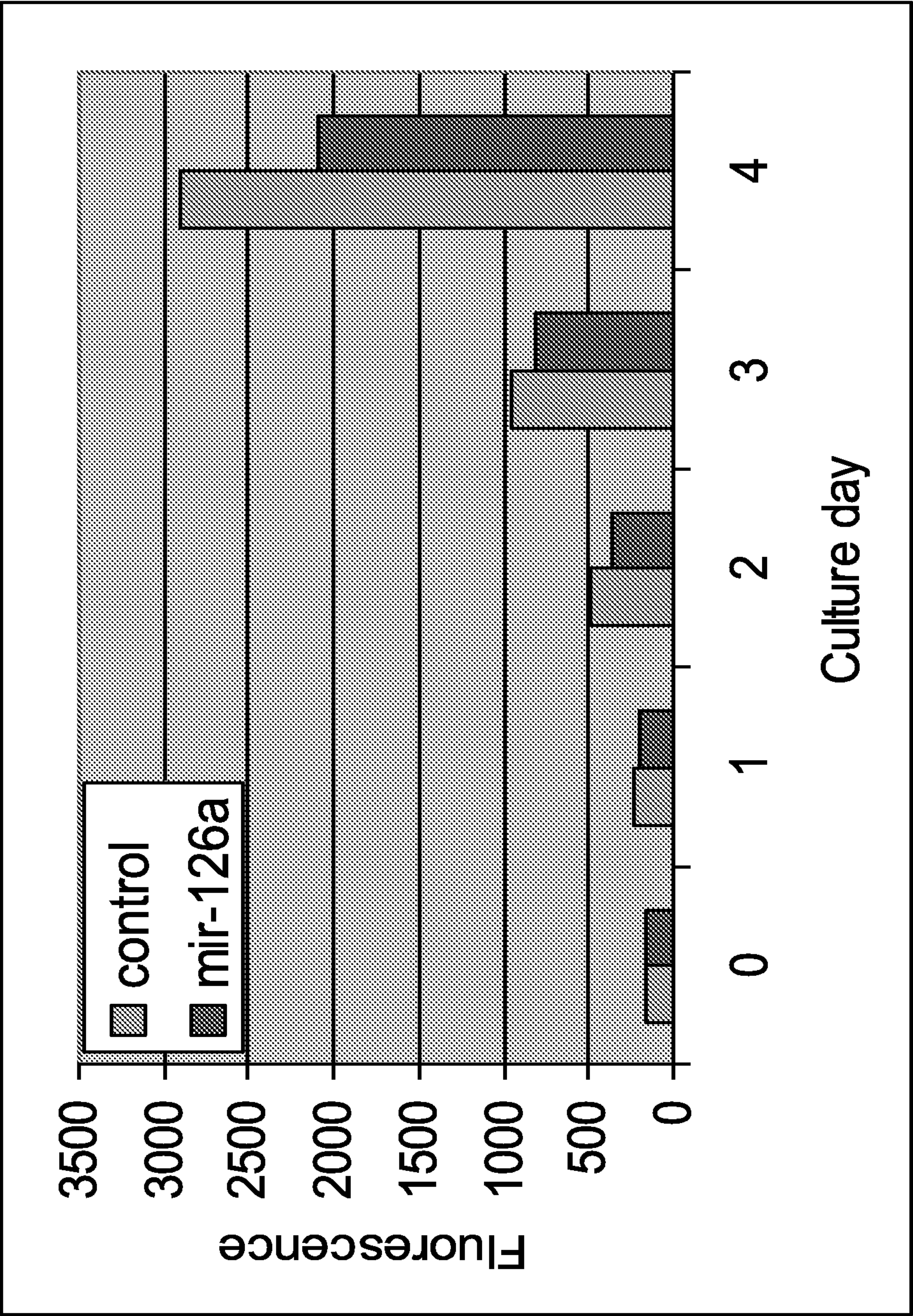


FIG. 7A

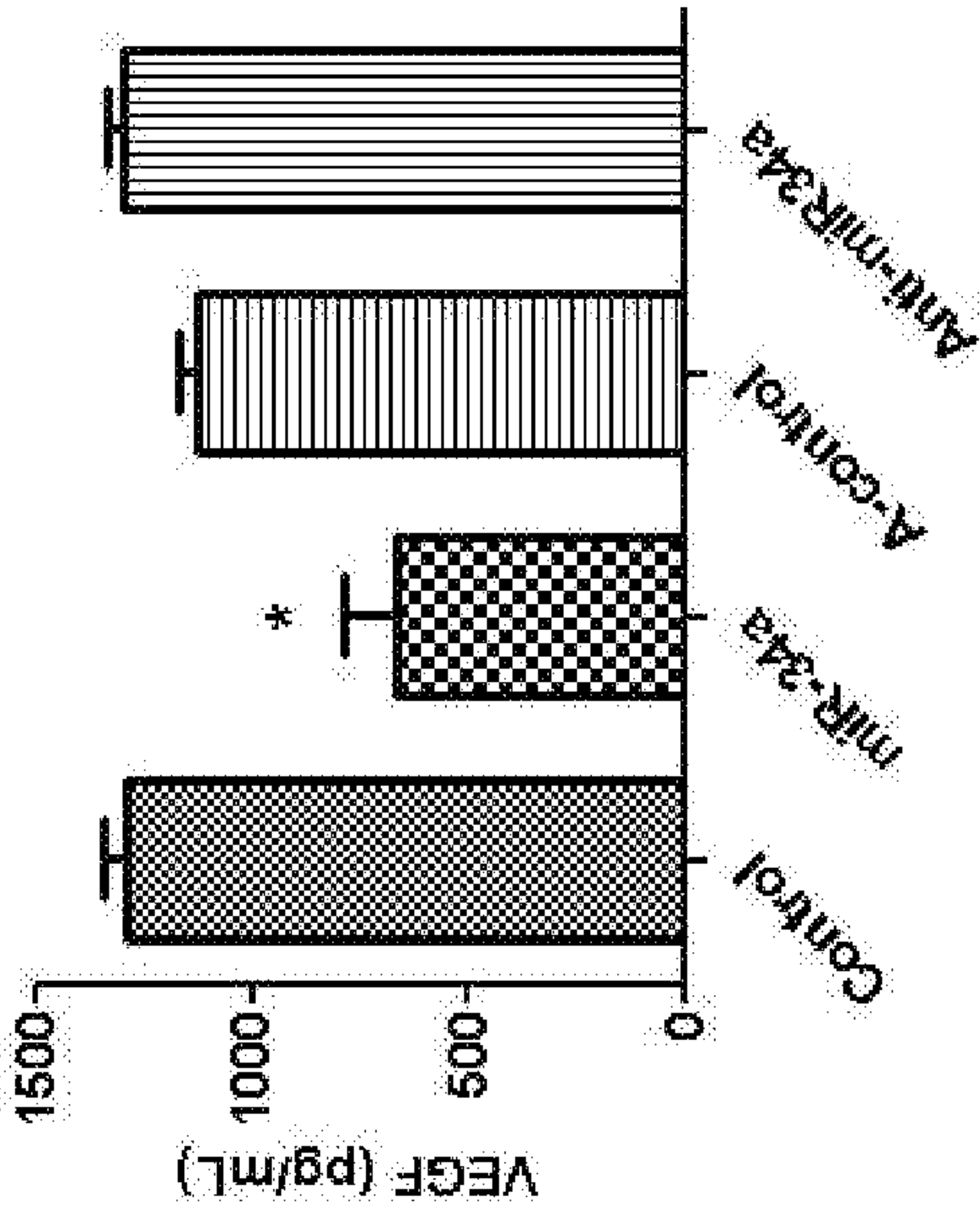


FIG. 7B

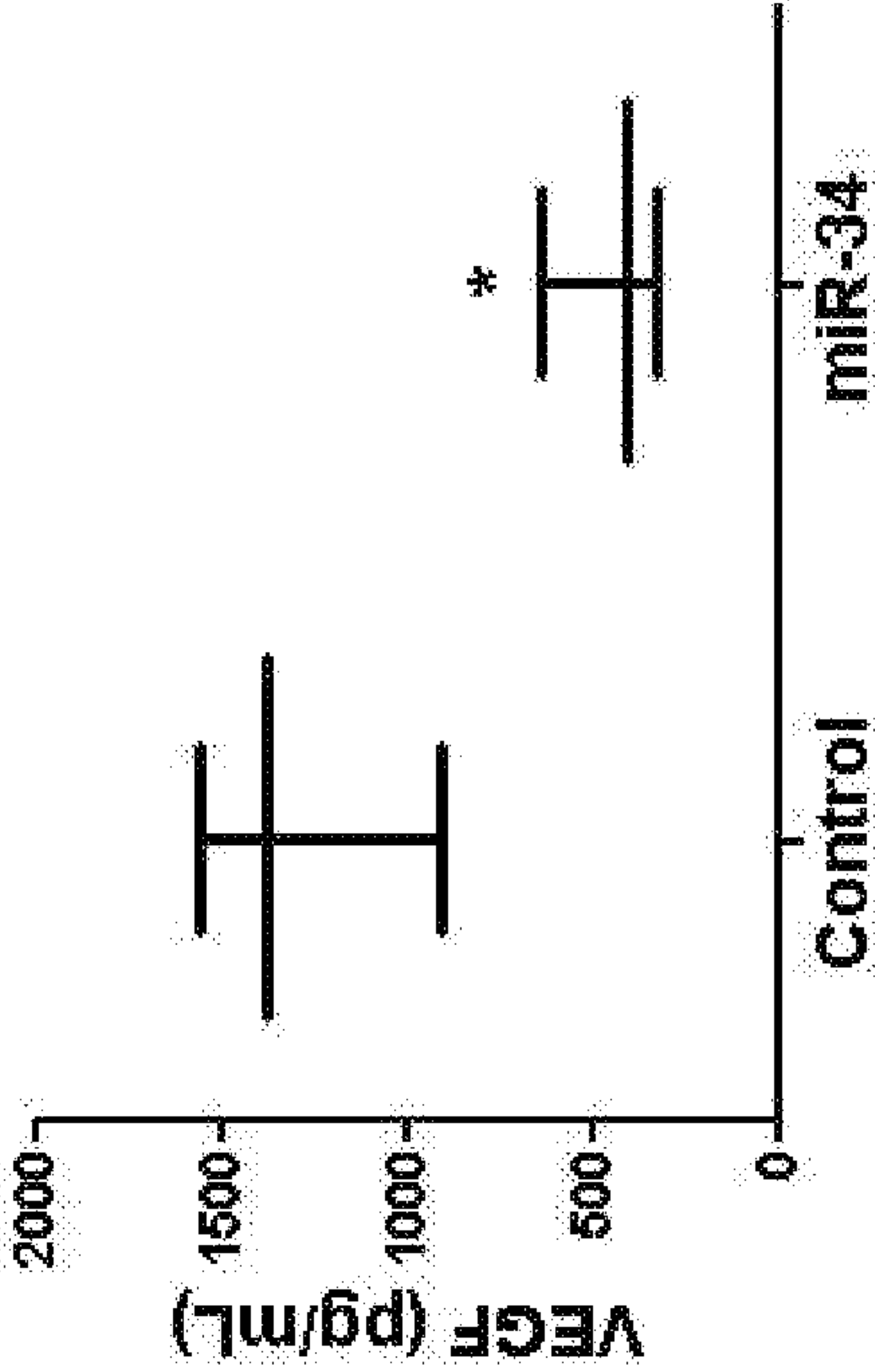


FIG. 7C

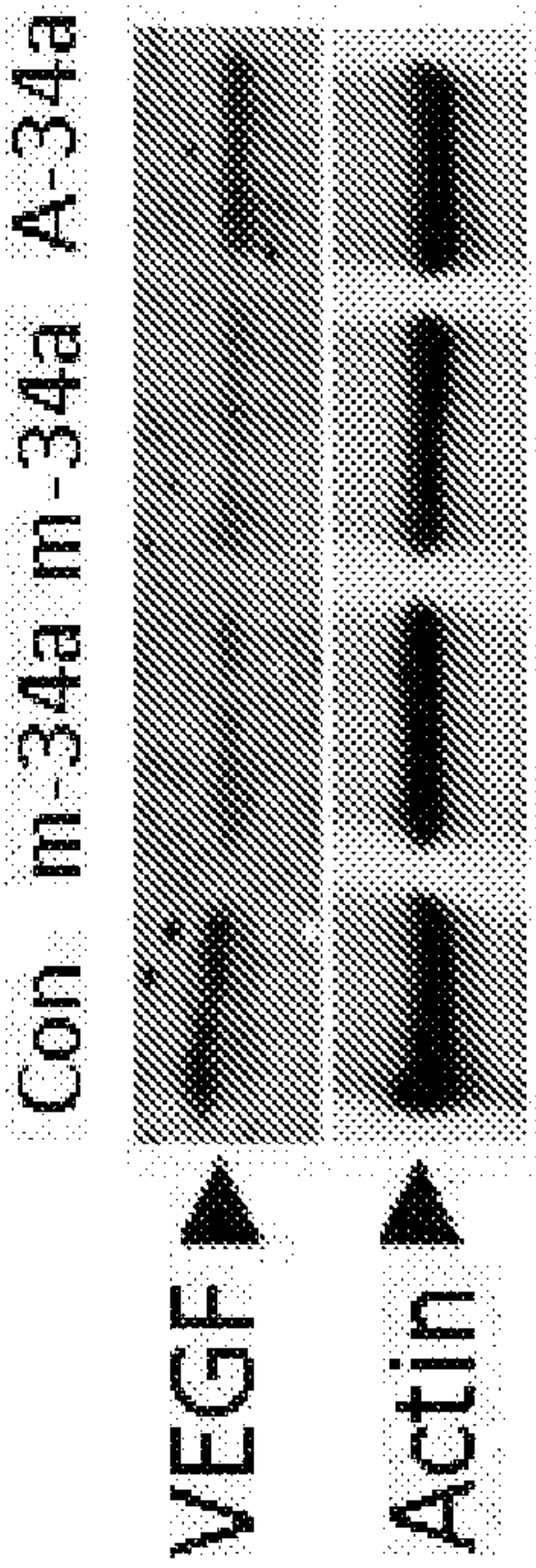


FIG. 8

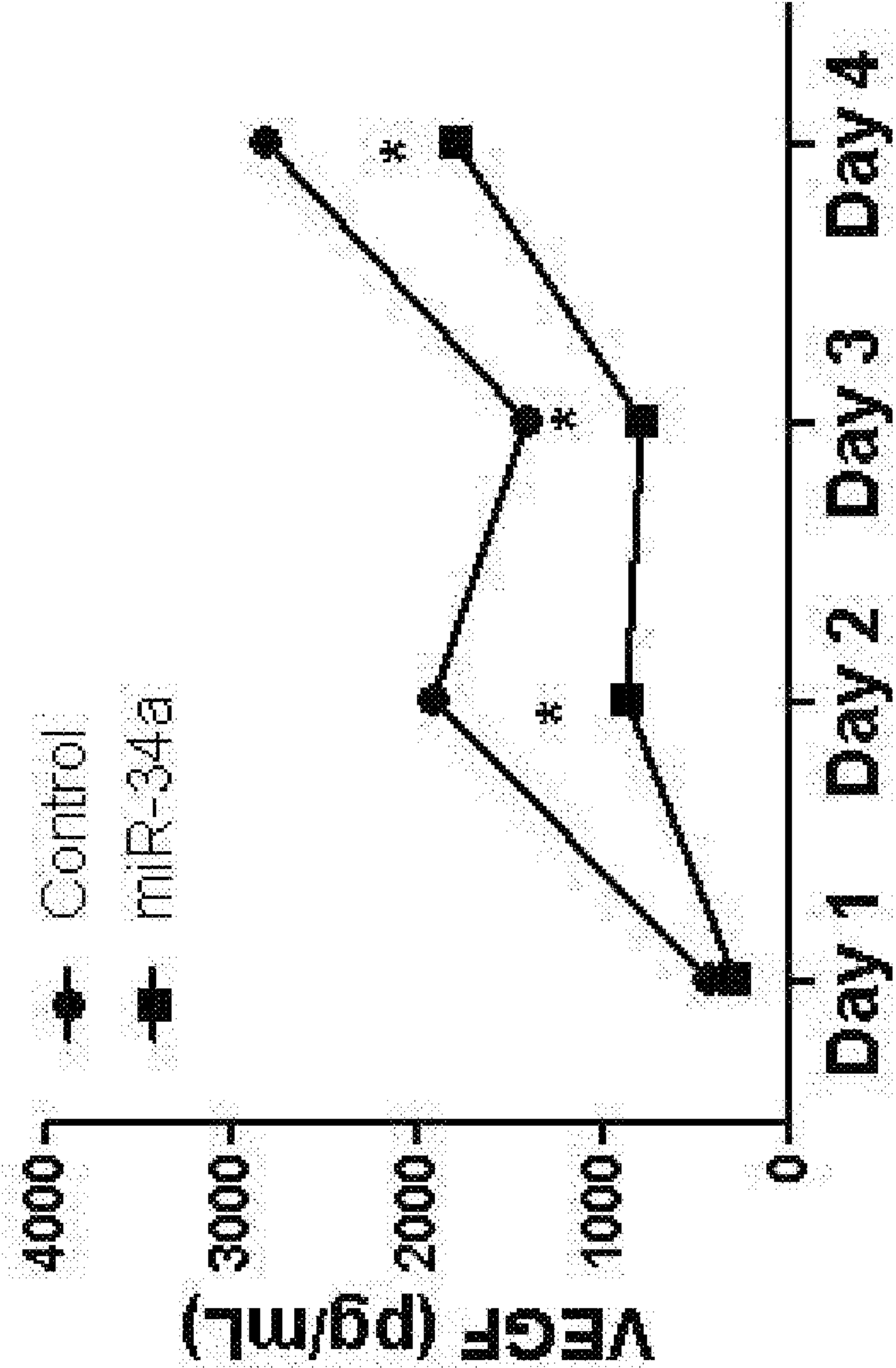


FIG. 9

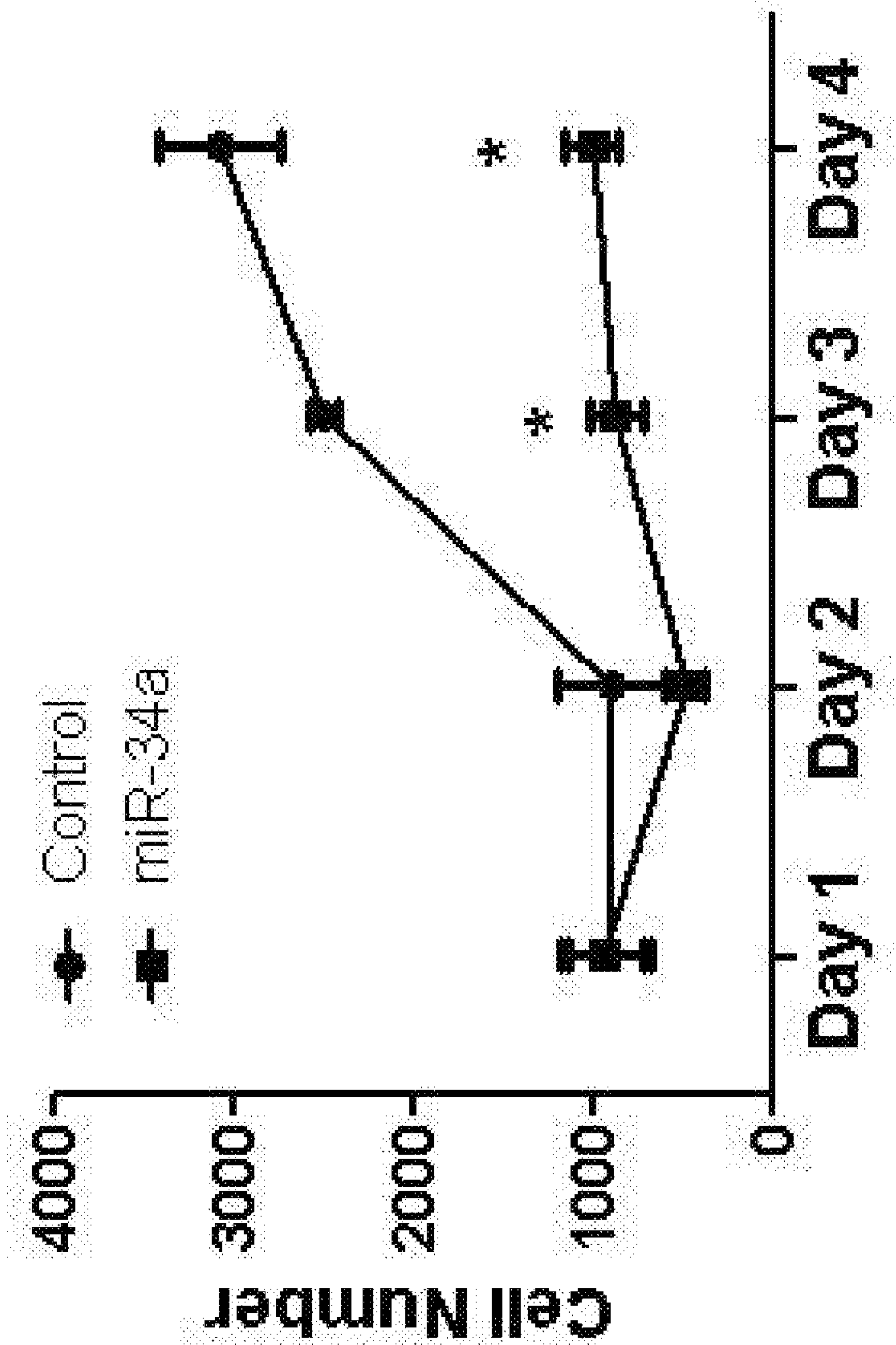


FIG. 10A

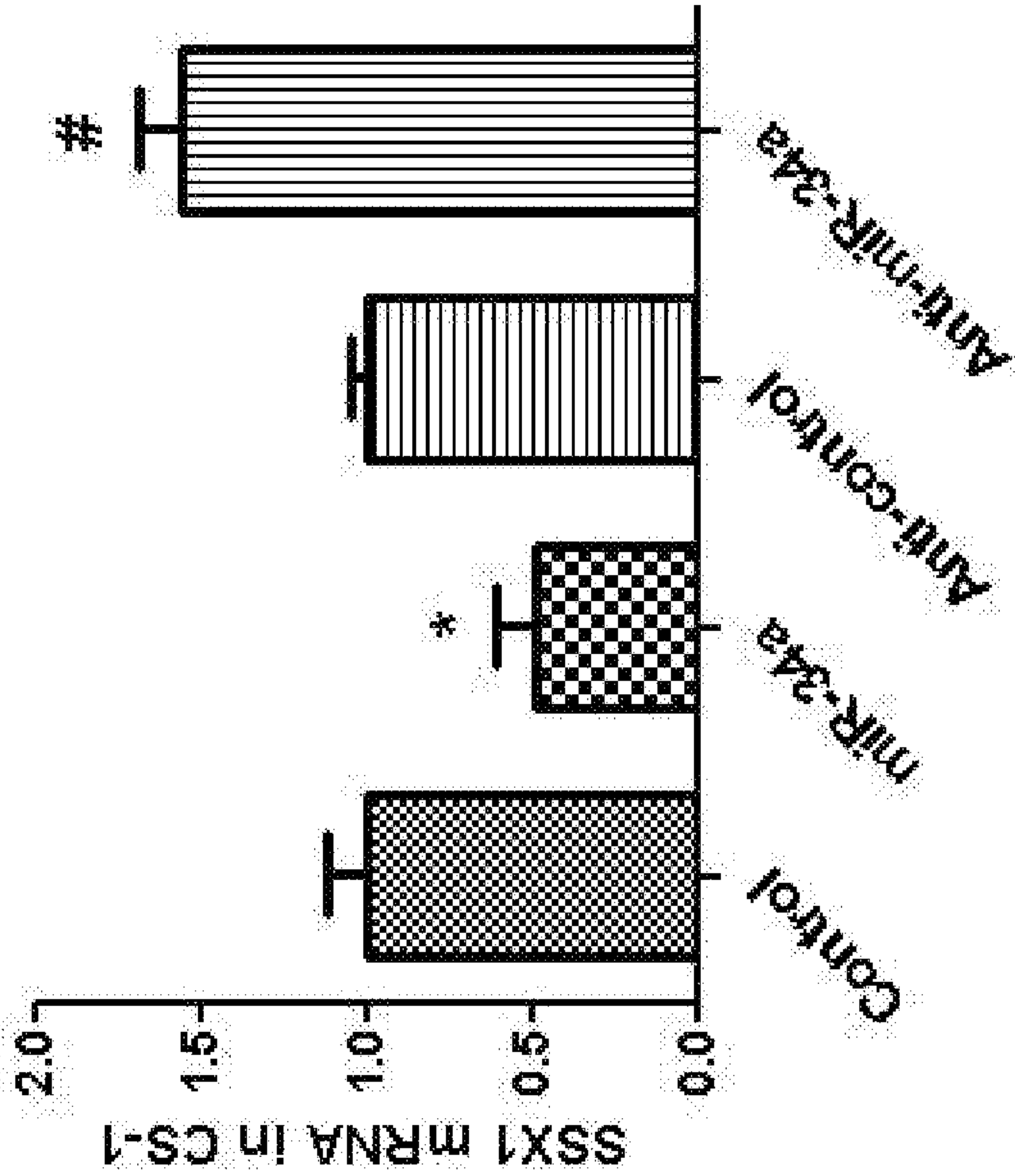


FIG. 10B

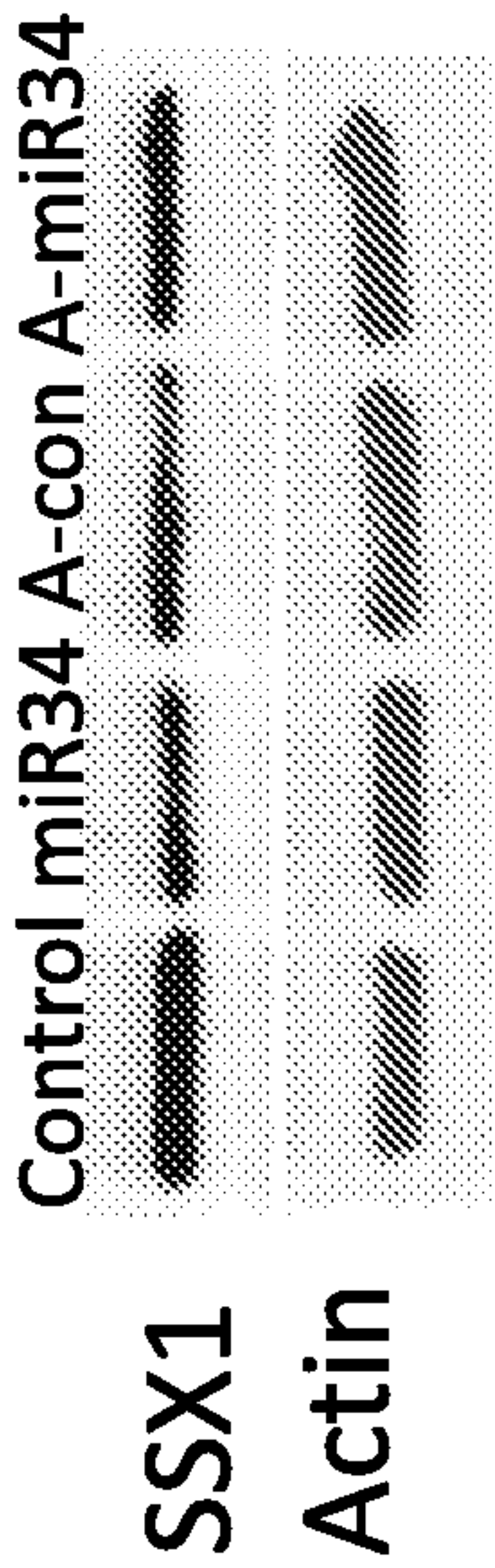


FIG. 11B

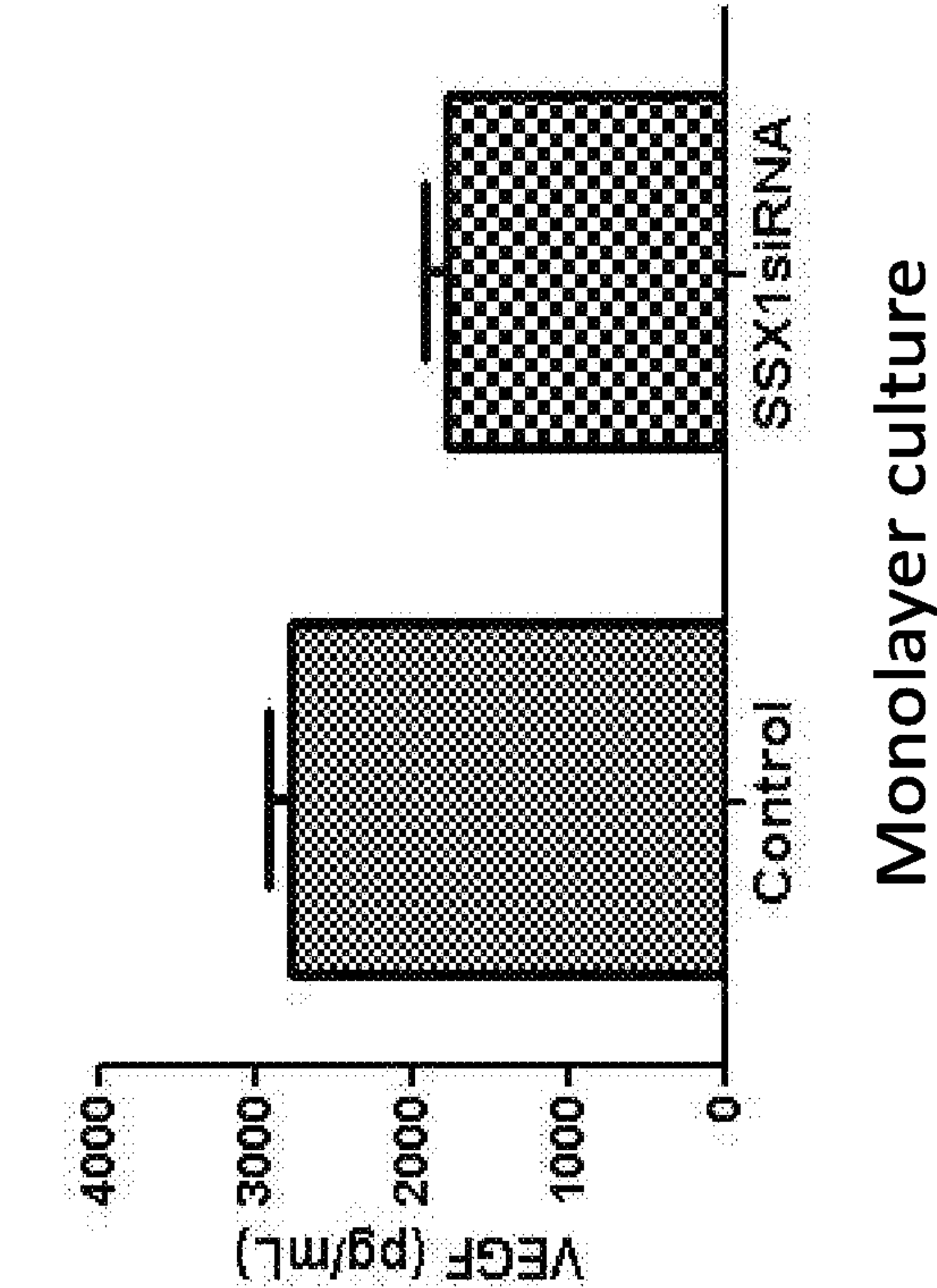


FIG. 11A

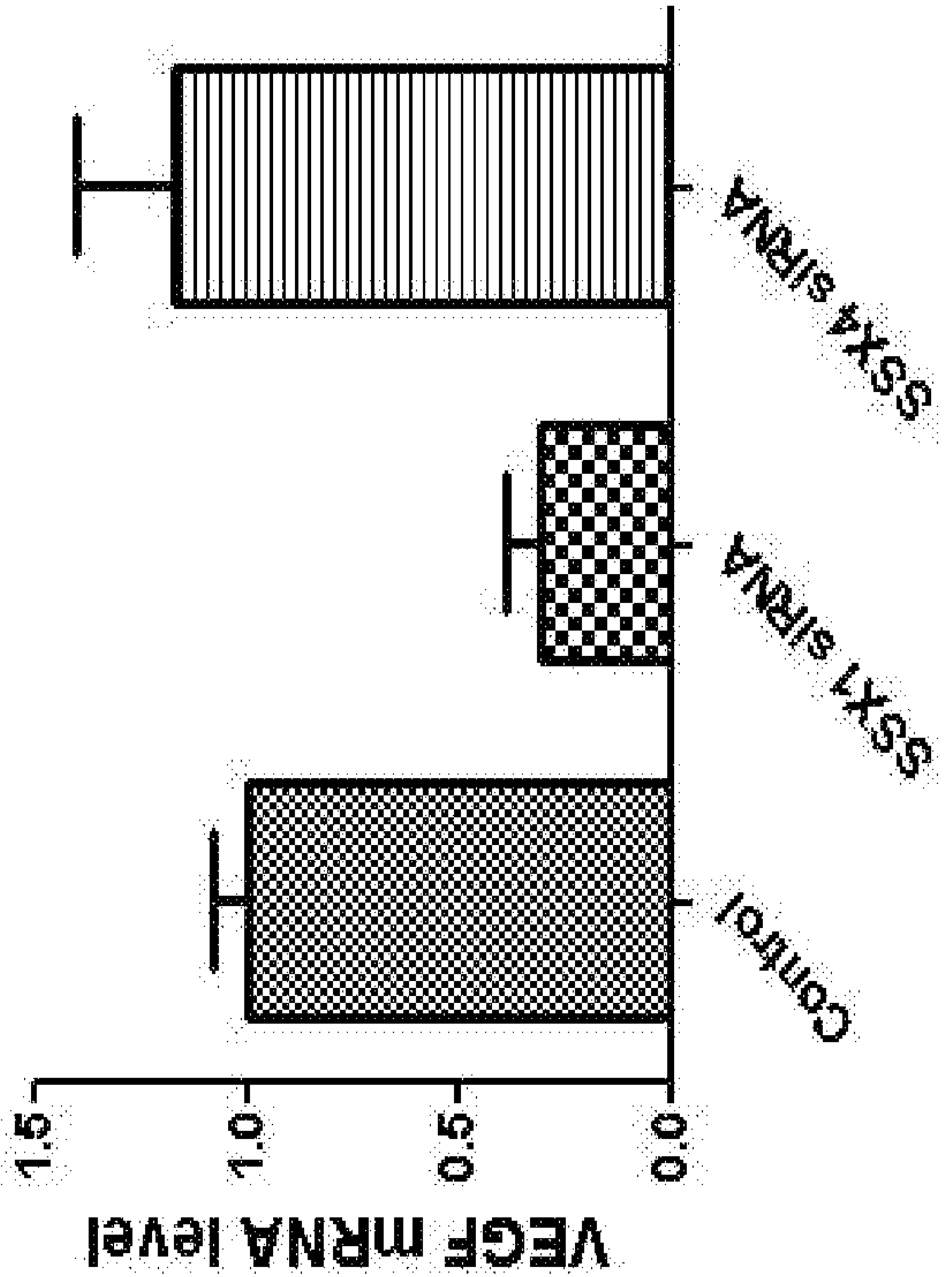


FIG. 11C

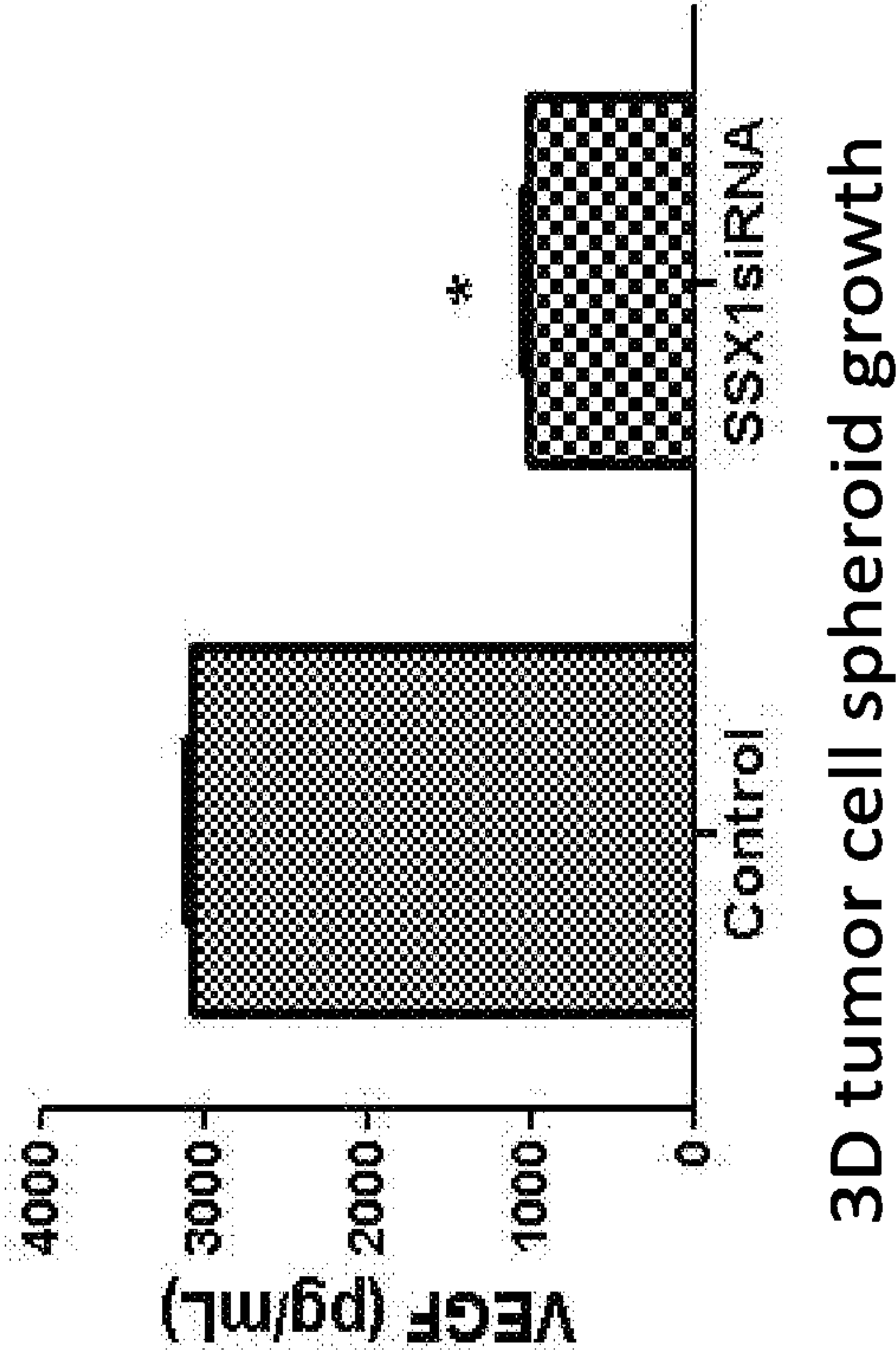
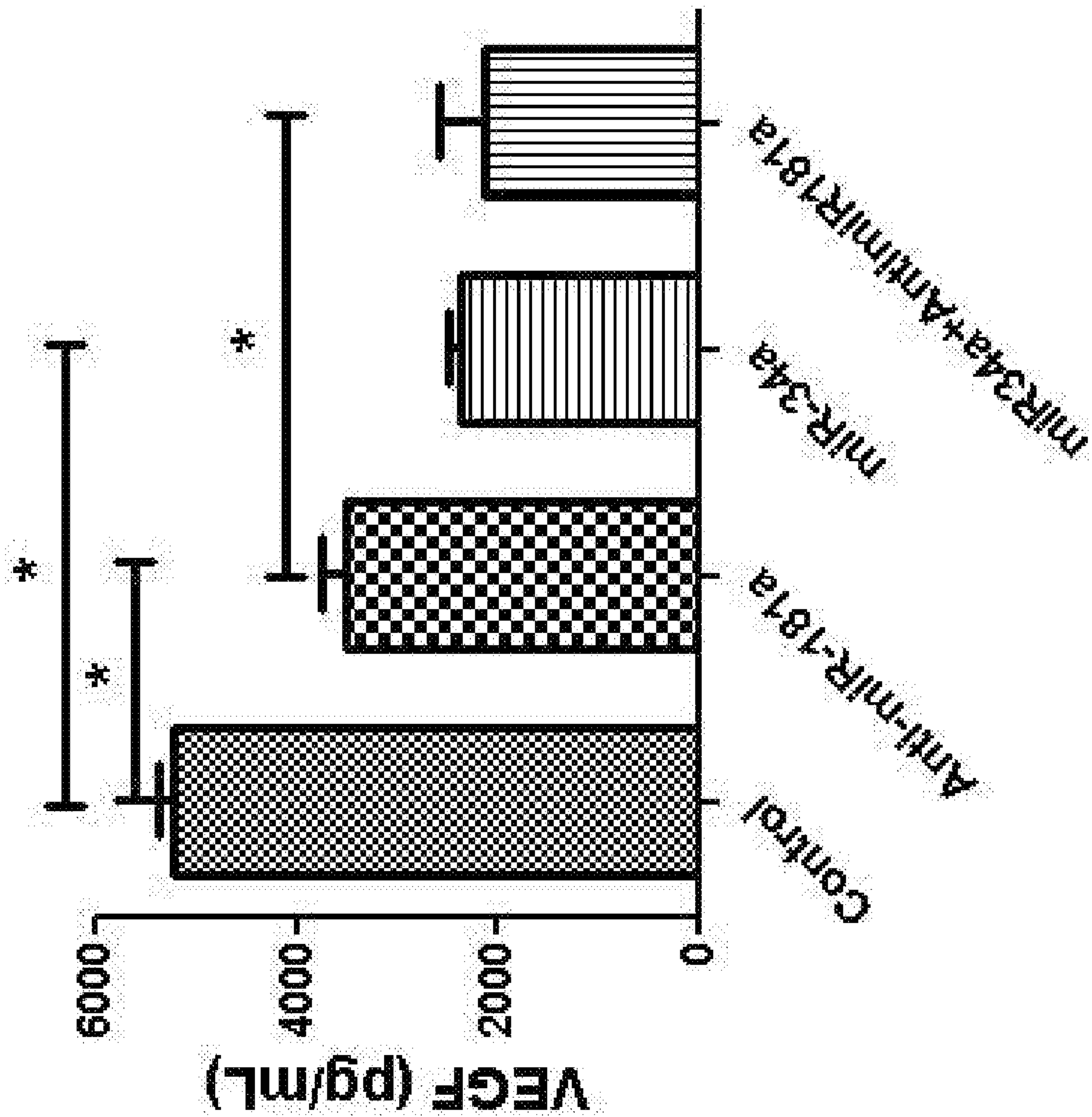


FIG. 12



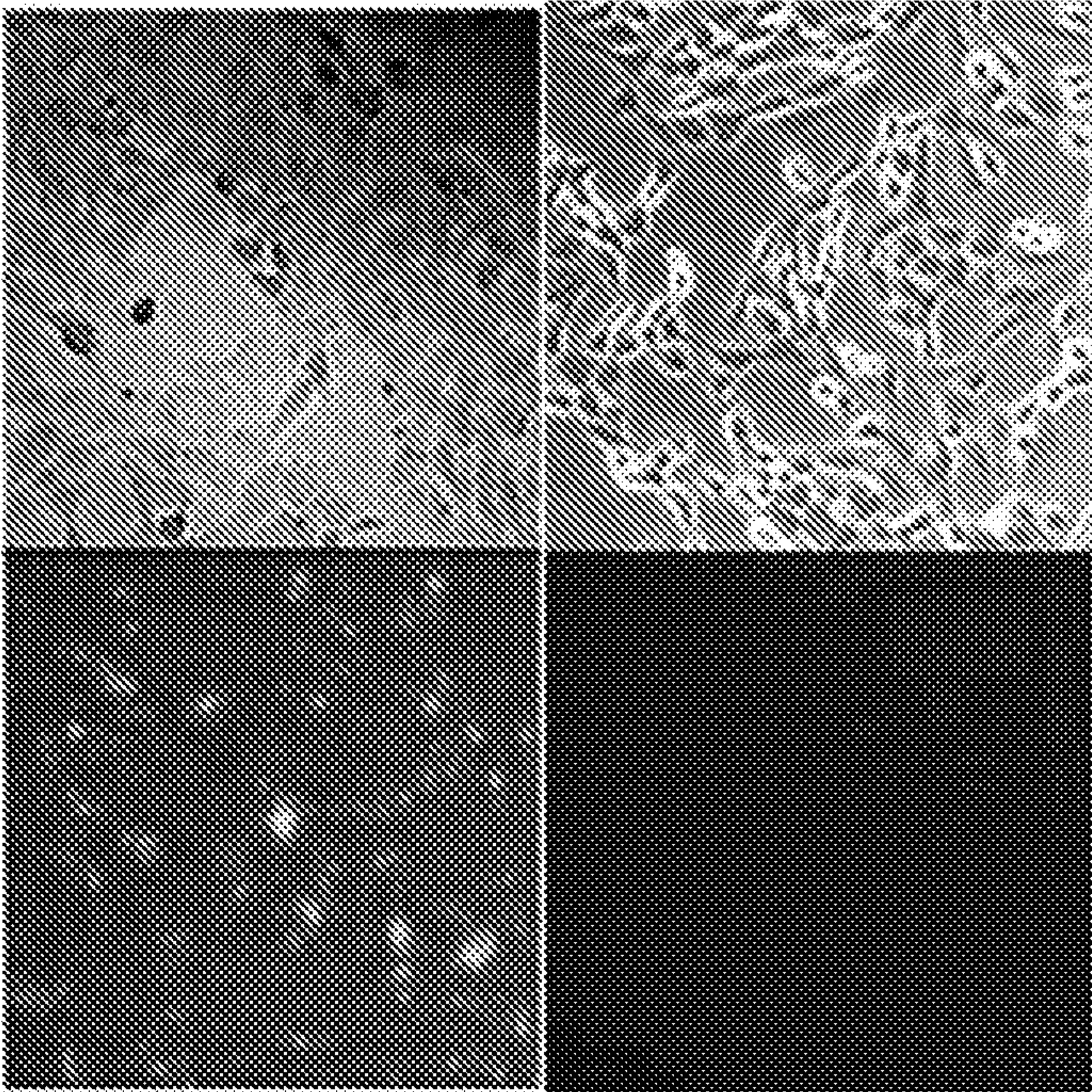


FIG. 13A

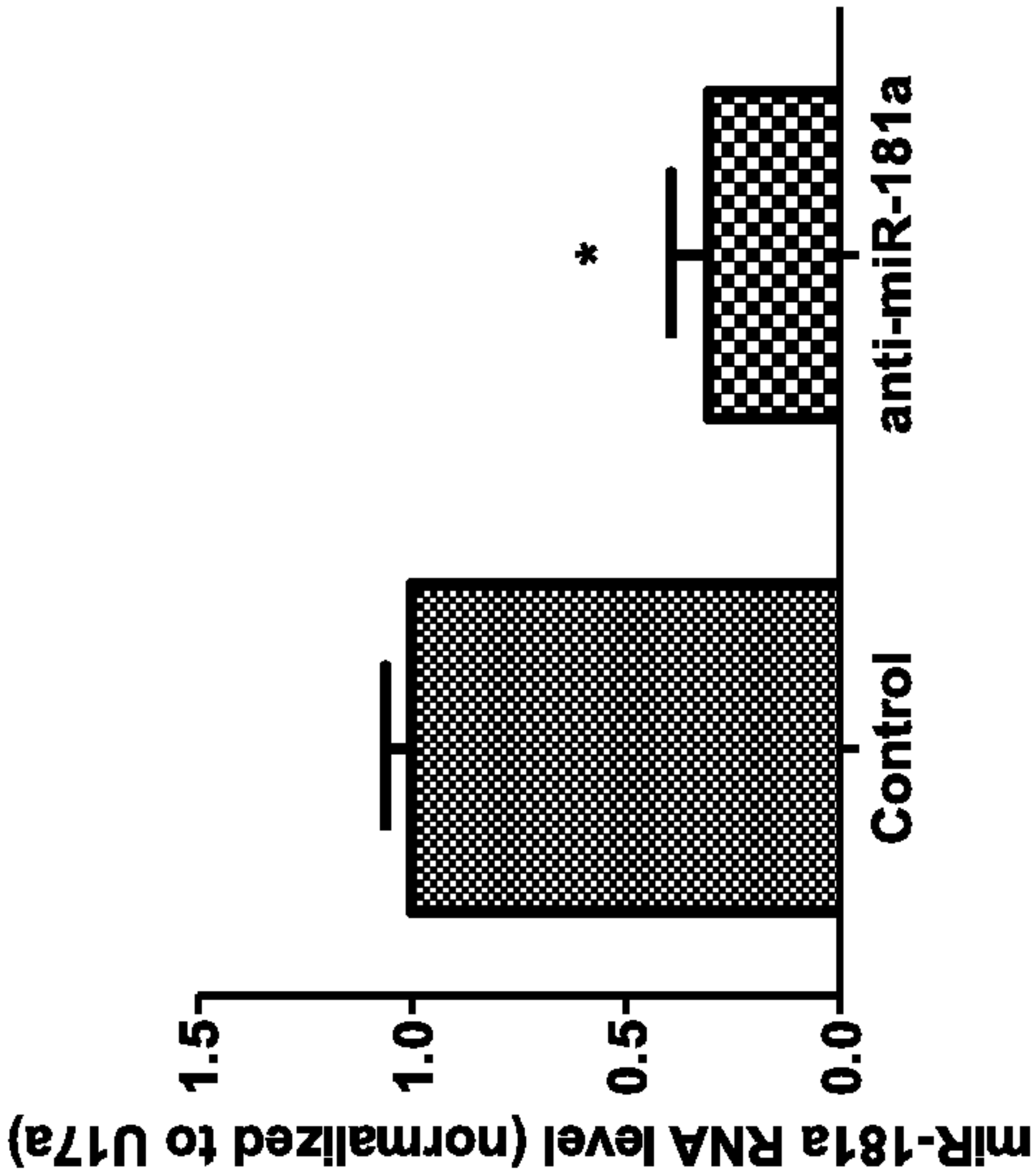


FIG. 13B

FIG. 14B

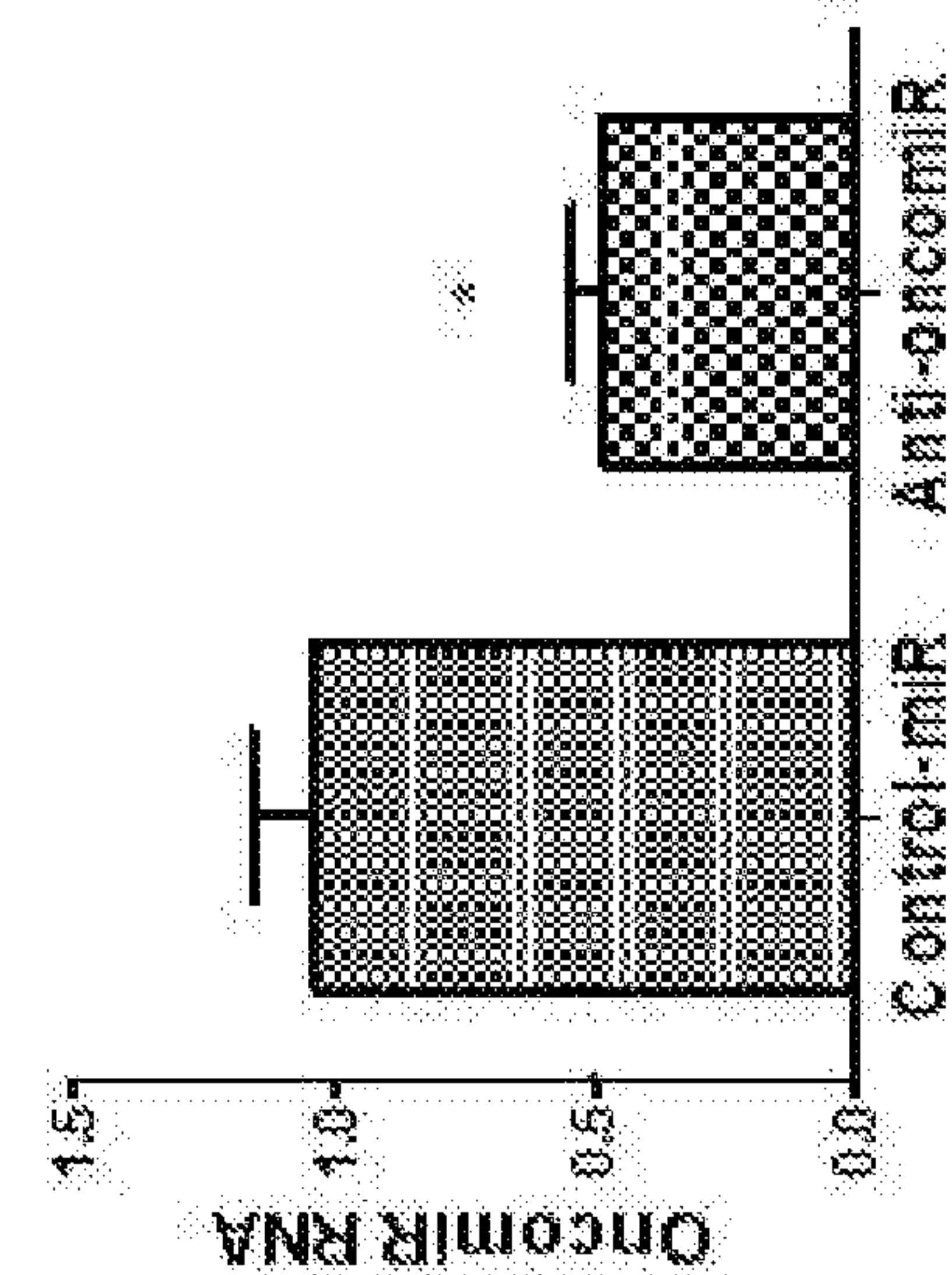


FIG. 14A

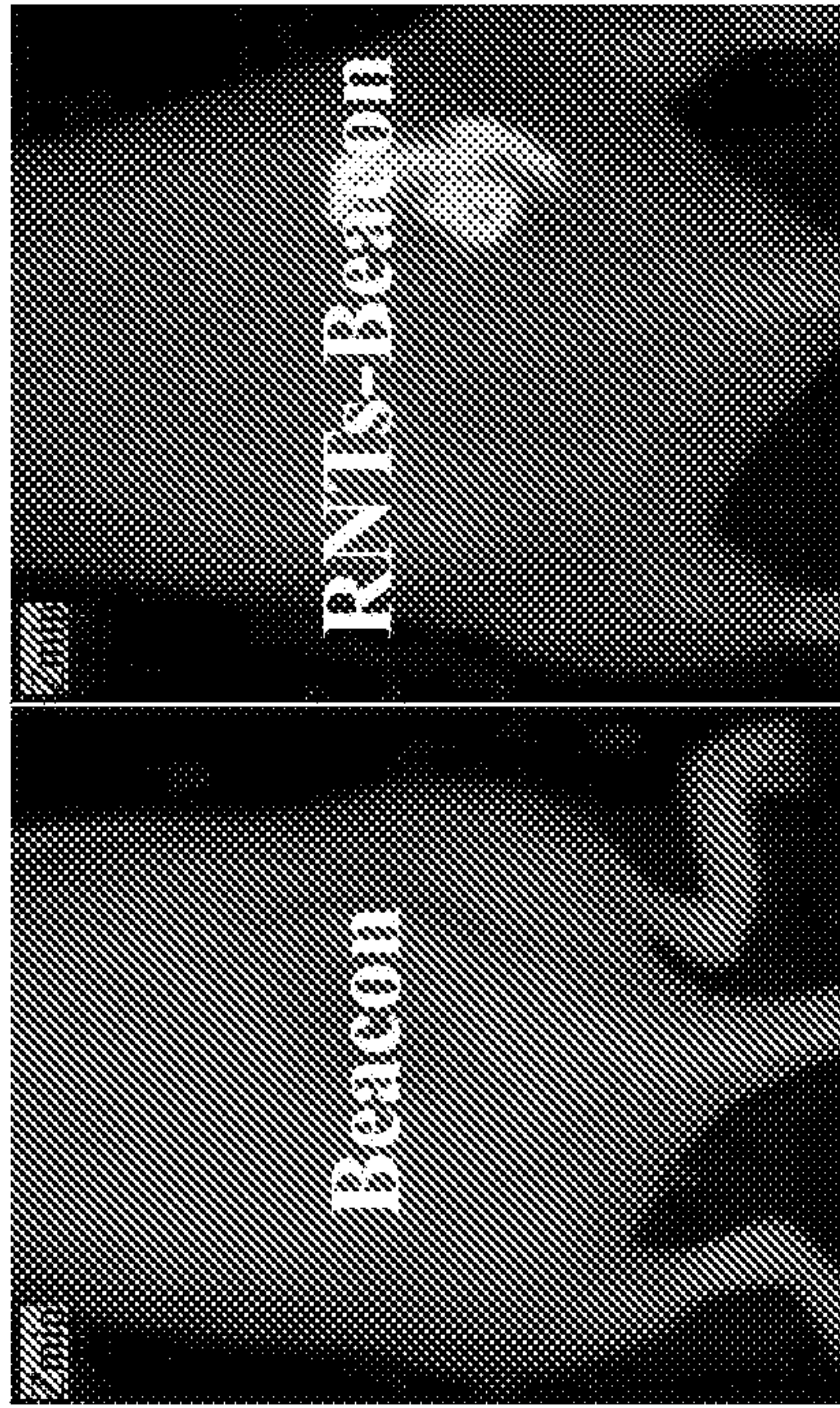


FIG. 14C

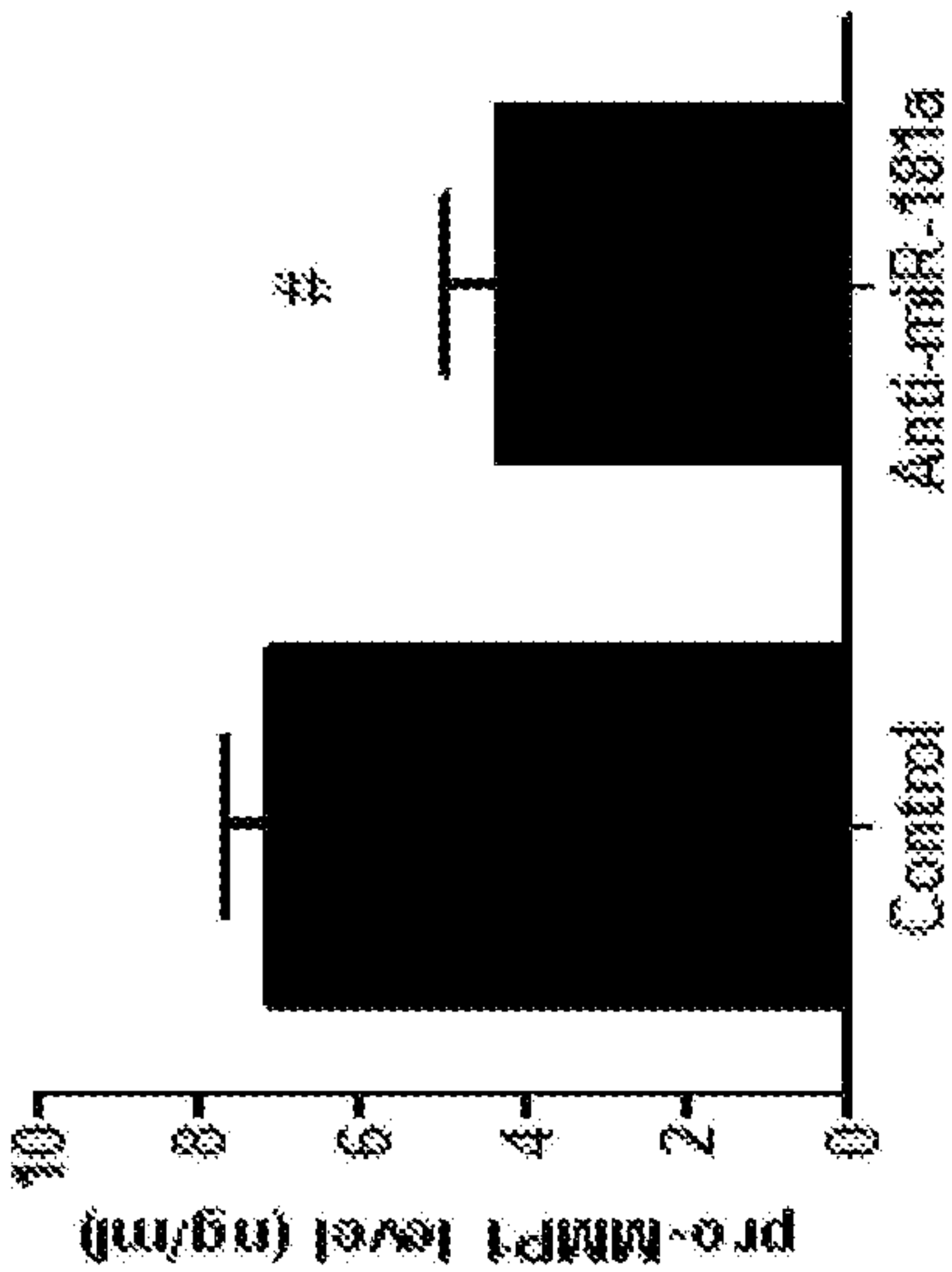


FIG. 15

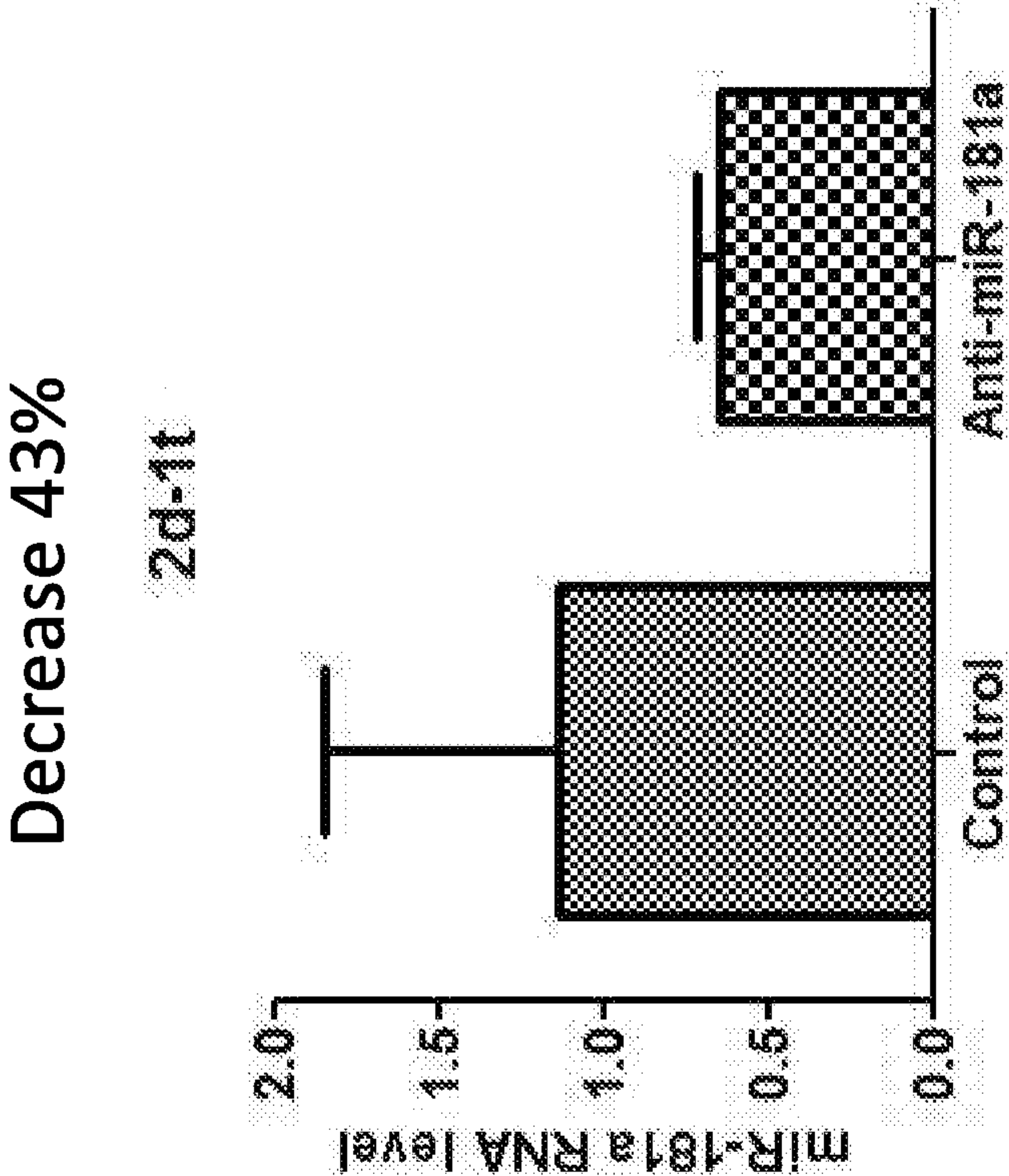


FIG. 16

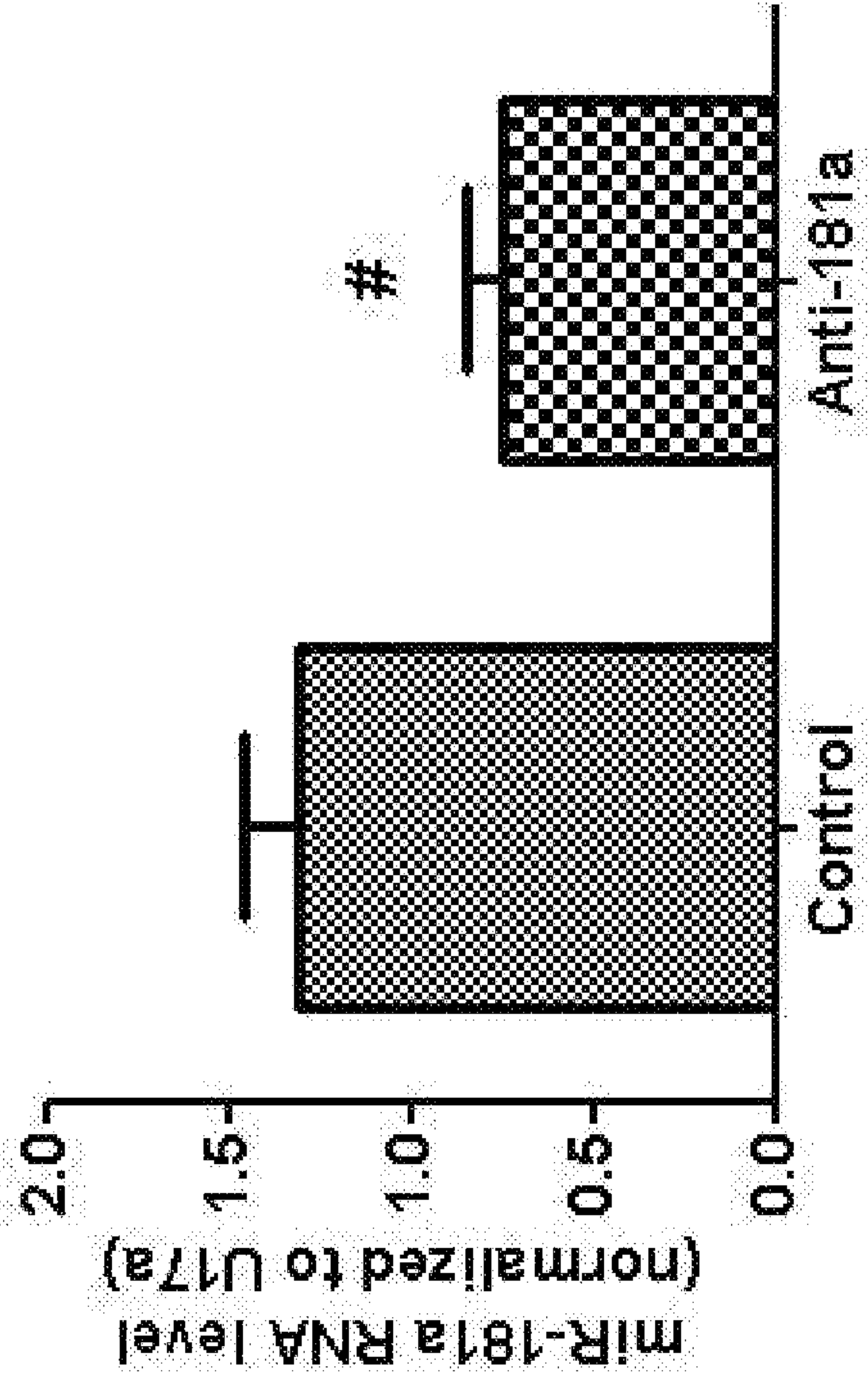


FIG. 17

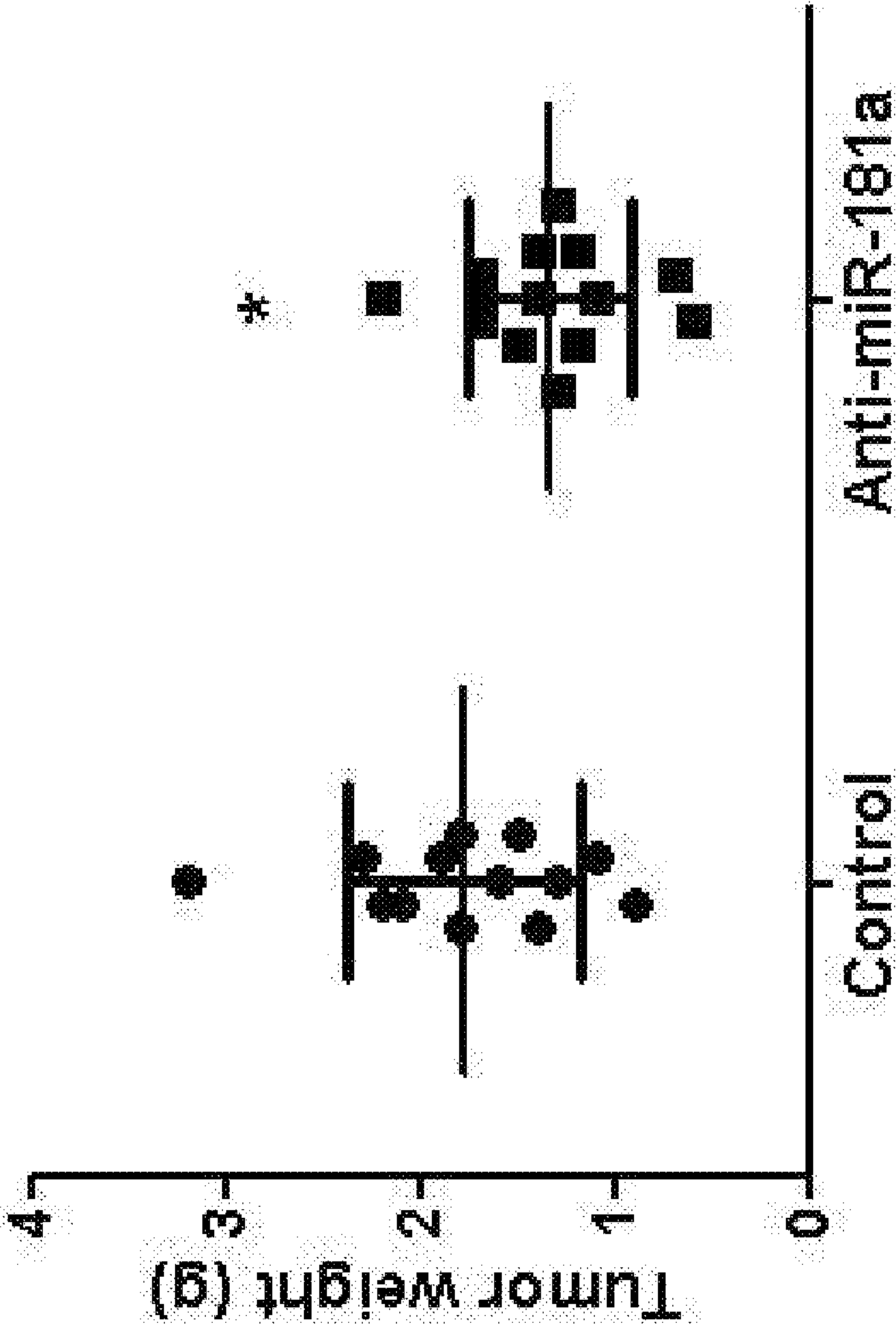


FIG. 18

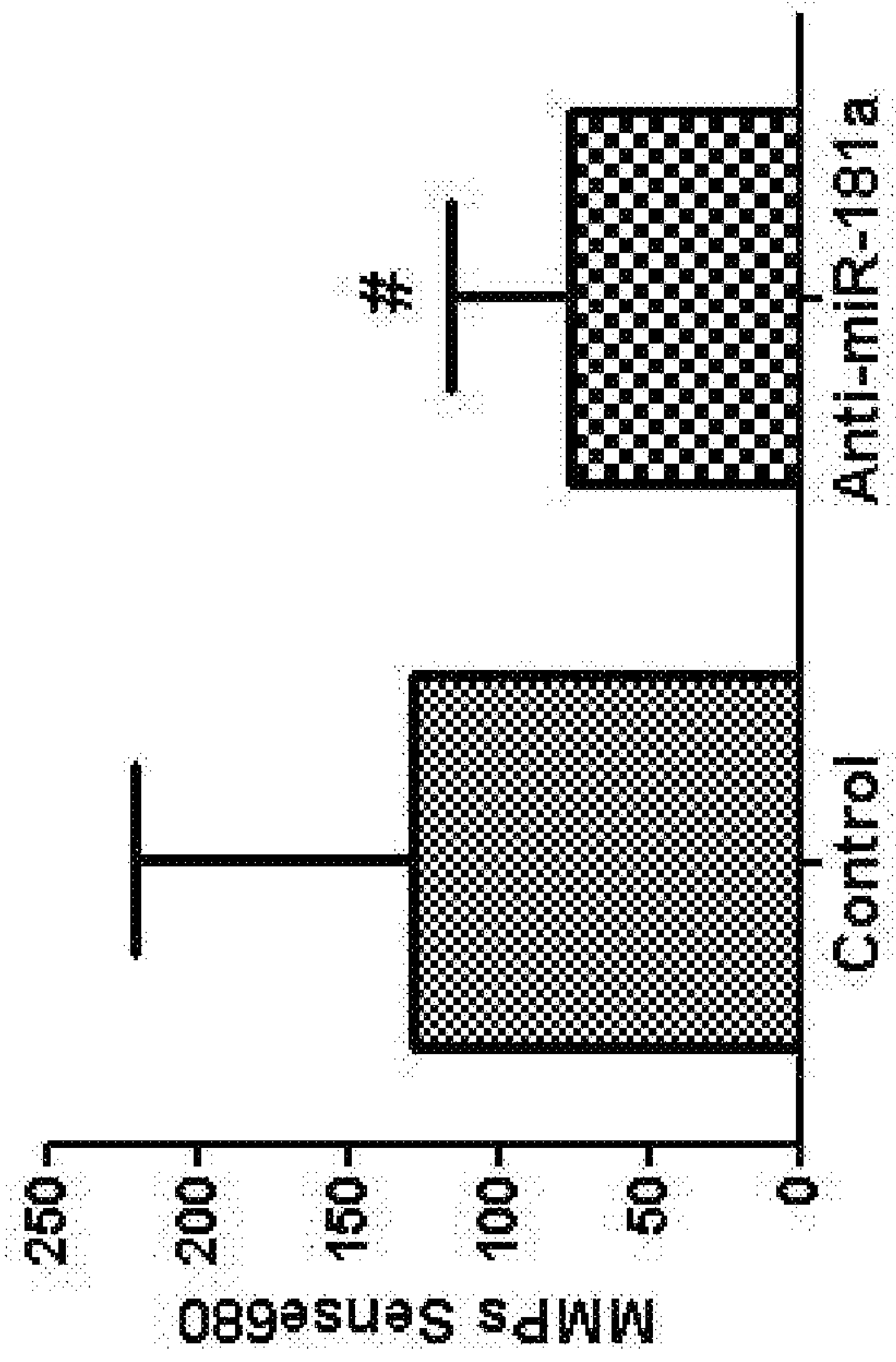


FIG. 19

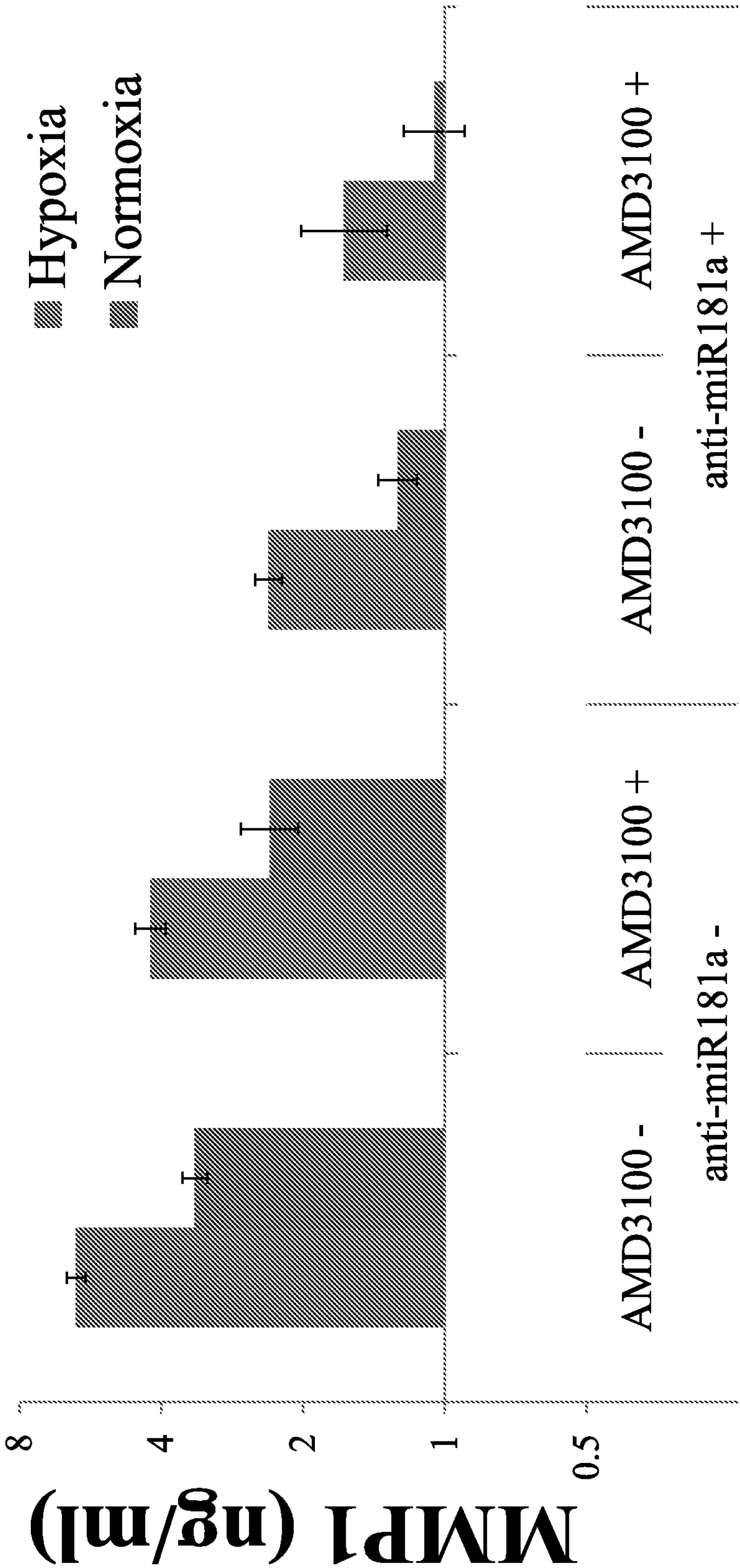


FIG. 1

