Compositions and Methods for Inhibiting Gene Expression of Hif2alpha

BACKGROUND

EPAS1 is a member of the HIF (hypoxia inducible factor) gene family. Also known as Hif2alpha or Hif2α, EPAS1 encodes half of a transcription factor involved in the induction of genes regulated by oxygen, and which is induced as oxygen levels fall (a condition known as hypoxia).

Certain variants of this gene provide protection for people living at high altitude. However, at low altitude, over-expression of wild-type (WT) EPAS1 is associated with increased hypertension and stroke, and with symptoms similar to mountain sickness. Mutations in this gene are associated with erythrocytosis familial type 4 and pulmonary hypertension. EPAS1 can cause excessive production of red blood cells, leading to inhibited reproductive abilities or even death.

EPAS1 has been shown to be required for expression of, or enhance the expression of, various genes involved in an assortment of diseases, including tumor progression. For example, EPAS1 may play a role in the progression of uveal melanomas, possibly by promoting the autocrine loop VEGF-pVEGFR2/KDR, and by enhancing the expression of LDHA, thus conferring a growth advantage.

EPAS1 has also been shown to be involved in, or upregulates expression of, other factors, including: cMyc (which favors cell proliferation, transformation, neoplasia and tumorigenesis, and which is highly expressed in most cancers); Interleukin 8 (a pro-inflammatory mediator, e.g., in gingivitis and psoriasis); SP-1 (a transcription factor involved in IL-8 regulation and a coactivator of cMyc); LDH5 (which is linked with tumor necrosis and increased tumor size); and LANA (Latency Associated Nuclear Antigen, which is associated with Kaposi's sarcoma-associated Herpesvirus). In addition, HIF (hypoxia induced factor) activity may play a role in angiogenesis required for cancer tumor growth. EPAS 1 may also be involved in several other diseases, including inflammation, chronic inflammation, neovascular diseases, rheumatoid arthritis, renal cancer, clear cell renal cell carcinoma (and metastases of this and other cancers), melanoma, uveal melanoma, chondrosarcoma, and multiple myeloma.

Mutations in EPAS1 gene have been correlated to early onset of neuroendocrine tumors such

as paragangliomas, somatostatinomas and/or pheochromocytomas. The mutations are commonly somatic missense mutations located in the primary hydroxylation site of HIF-2α. These mutations are believed to disrupt the protein hydroxylation/degradation mechanism and lead to protein stabilization and pseudohypoxic signaling. In addition, neuroendocrine tumors release erythropoietin (EPO) into circulating blood, and lead to polycythemia.

SUMMARY

Described herein are Hif2α (also termed EPAS, or Hif2alpha) gene-specific RNA interference (RNAi) trigger molecules (also termed RNAi agent, RNAi trigger, or trigger) able to selectively and efficiently decrease expression of Hif2α. Each RNAi trigger includes at least a sense strand and an antisense strand. The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the RNAi trigger sense and antisense strands described herein each can be 16 to 30 nucleotides in length. In some embodiments, the sense and antisense strands are independently 17 to 26 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. The RNAi triggers described herein, upon delivery to a cell expressing the Hif2α gene, inhibit the expression of the Hif2α gene *in vitro* or *in vivo*. Examples of Hif2α RNAi trigger sense strands and antisense strands that can be used in a Hif2α RNAi trigger are provided in Tables 1-2 and 5.

A sense strand of an Hif2α RNAi trigger contains a nucleotide sequence having at least 90% identity over a core stretch of at least 16 consecutive nucleotides to a sequence in an Hif2α mRNA. In some embodiments, the nucleotide sequence having at least 90% identity to a sequence in the Hif2α mRNA is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. An antisense strand of an Hif2α RNAi trigger contains a nucleotide sequence having at least 90% complementary over a core stretch of at least 16 consecutive nucleotides to a sequence in the Hif2α mRNA and the corresponding sense strand. In some embodiments, the nucleotide sequence having at least 90% complementarity to a sequence in the Hif2α mRNA or the corresponding sense strand is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

In some embodiments, one or more Hif2α RNAi triggers are delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. Nucleic acid delivery methods include, but are not limited to, by encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, proteinaceous vectors or Dynamic

Polyconjugates™ (DPCs). In some embodiments, an Hif2α RNAi trigger is conjugated to a targeting group, such as a integrin-binding compound. In some embodiments, an Hif2α RNAi trigger is conjugated to a delivery polymer or vehicle. The delivery polymer can be a reversibly modified membrane active polyamine. The delivery polymer can also be an integrin-targeted reversibly modified membrane active polyamine.

An integrin-targeted reversibly modified membrane active polyamine comprises a membrane active polyamine conjugated to one or more integrin-binding compounds via reversible physiologically labile covalent linkages. In some embodiments, the integrin targeted reversibly modified membrane active polyamine further comprises the membrane active polyamine conjugated to one or more steric stabilizers via reversible physiologically labile covalent linkages. Integrin-binding compounds can be, but are not limited to, RGD peptides and RGD mimics. Reversible physiologically labile covalent linkages include, but are not limited to, dipeptide amidobenzyl carbamate linkages, tetrapeptide linkages, and disubstituted maleamate linkages.

The Hif2α RNAi triggers are optionally combined with one or more additional (i.e., second, third, etc.) therapeutics. A second therapeutic can be another Hif2α RNAi trigger (e.g., a Hif2α RNAi trigger which targets a different sequence within the Hif2α target). An additional therapeutic can also be a small molecule drug, antibody, antibody fragment, and/or vaccine. The Hif2α RNAi triggers, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

The present disclosure also encompasses methods of treating a human subject having a pathological state mediated at least in part by Hif2α expression, the methods comprising the step(s) of administering to the subject a therapeutically effective amount of an Hif2α RNAi trigger or Hif2α RNAi trigger-containing composition. The method of treating a subject with an Hif2α RNAi trigger or Hif2α RNAi trigger-containing composition can optionally be combined with one or more steps of administering one or more additional (i.e., second) therapeutics or treatments. The Hif2α RNAi trigger and additional therapeutics can be administered in a single composition or they made be administered separately. Non-limited examples of additional therapeutics include, but are not limited to, VEGFR inhibitors (such as SUTENT®, NEXAVAR®, VOTRIENT®, AVASTIN®, INLYTA®, CABOZANTINIB®), Cytokines (such as IL-2, IFN-α), mTor inhibitors (such as EVEROLIMUS®,

TEMSIROLIMUS®), anti-PD1 drugs (such as OPDIVO® and KEYTRUDA®), anti-CTLA4 (such as YERVOY®), drugs targeting signal transduction pathway components in cancer cells (such as VEGF, PI-3-kinase, MEK, JAK, Akt, MYC, Met, Src-family kinases, Abl, Axl, Mer), anti-PD-L1, anti-PD-L2, anti-TIM3, anti-LAG3, anti-CD28, anti-OX40, anti-OX-40L, anti-CD39. anti-CD40, anti-CD80, anti-CD86, anti-CD137, anti-41BBL, anti-TIGIT, anti-GITR, anti-GIRTL, anti-CD155, anti-Fas, anti-FasL, anti-TRAIL/TRAIL-L, IDO-1 inhibitor, and TDO-2 inhibitor.

The pharmaceutical compositions can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (e.g., by a transdermal patch), pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal), epidermal, transdermal, oral or parenteral. Parenteral administration includes, but is not limited to, intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal (e.g., via an implanted device), intracranial, intraparenchymal, intrathecal, and intraventricular, administration.

The described Hif2α RNAi triggers and/or compositions can be used in methods for therapeutic treatment of diseases, including but not limited to: cancer, renal cancer, clear cell renal cell carcinoma, non-small cell lung cancer, astrocytoma (brain cancer), bladder cancer, breast cancer, chondrosarcoma, colorectal carcinoma, gastric carcinoma, glioblastoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, lung adenocarcinoma, neuroblastoma, melanoma, multiple myeloma, ovarian cancer, rectal cancer, metastases, gingivitis, psoriasis, Kaposi's sarcoma-associated herpesvirus, preemclampsia, inflammation, chronic inflammation, neovascular diseases, and rheumatoid arthritis. Such methods comprise administration of an Hif2α RNAi trigger as described herein to a subject, e.g., a human or animal subject.

Unless otherwise defined, 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 belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification,

including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Chemical structures representing PEG_n-FCitFP-TFP modifying agents.

FIG. 2. Chemical structures representing RGD-PEG_n-FCitFP-TFP modifying agents.

FIG. 3. Chemical structures representing RGD-PEG_n-ACit-PABC-PNP modifying agents.

FIG. 4. Chemical structures representing aldehyde-PEG_n-FCit-PABC-PNP modifying agents.

FIG. 5. Chemical structures representing aldehyde-PEG_n-ACit-PABC-PNP modifying agents.

FIG. 6. Chemical structures representing SPDP-PEG_n-FCit-PABC-PNP modifying agents.

FIG. 7. Chemical structures representing PEG_n-ACit-PABC-PNP and PEG_n-FCit-PABC-PNP modifying agents.

FIG. 8. Graph illustrating serum SEAP levels during treatment in mice. Fold-changed in serum SEAP levels relative to day (−1) pre-dose levels. For G1 and G2, n = 4. For G3, n=3.

FIG. 9. Tumor gross morphology after 3 weekly treatments in mice . G1, vehicle; G2 400 μg Hif2α-ITG-DPC; G3 280 μg Hif2α-ITG-DPC. Both kidneys from each animal are shown. Tumor was implanted into the kidney shown on the right. Tumor from treatment groups were all significantly smaller and showed some discoloration when compared to controls.

FIG. 10. H&E staining of tumor formalin fixed paraffin sections. Thin arrows indicate apoptotic cells. Thick arrows indicate macrophage infiltration. Panel A, G1 vehicle treated. Typical tubular type RCC morphology with rare apoptotic cells (thin arrows). Panel B, G2 400 μg Hif2α-ITG-DPC treated. Massive necrotic center with numerous apoptotic cells in surrounding areas and overall loss of tumor structure. Panel C, G3 280 μg Hif2α-ITG-DPC treated. Destruction of typical tubular tumor structures with macrophage infiltration and numerous apoptotic cells.

DETAILED DESCRIPTION

Described herein are RNAi triggers for inhibiting expression of the Hif2α gene (referred to herein as Hif2α RNAi triggers). Each Hif2α RNAi trigger comprises a sense strand and an antisense strand. The sense strand and the antisense strand are partially, substantially, or fully

complementary to each other. In some embodiments, the length of the herein described RNAi trigger sense and antisense strands are independently 16 to 30 nucleotides in length. In some embodiments, the length of the herein described RNAi trigger sense and antisense strands are independently 17 to 26 nucleotides in length. In some embodiments, the herein described RNAi trigger sense and antisense strands are independently 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 nucleotides in length. The sense and antisense strands can be either the same length or they can be different lengths. In some embodiments, the sense strand is about 19 nucleotides in length while the antisense strand is about 21 nucleotides in length. In some embodiments, the sense strand is about 21 nucleotides in length while the antisense strand is about 23 nucleotides in length. In other embodiments, the sense and antisense strands are independently 17-21 nucleotides in length. In some embodiments, both the sense and antisense strands are each 21-26 nucleotides in length. Examples of nucleotide sequences used in forming Hif2α RNAi trigger molecules are provided in Tables 1-2 and 5.

RNAi triggers include, but are not limited to: short interfering RNAs (siRNAs), double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), and dicer substrates (US patent no. 8,084,599 8,349,809 and 8,513,207). The RNAi triggers described herein, upon delivery to a cell expressing the Hif2α gene, inhibit or knockdown expression of Hif2α gene *in vitro* or *in vivo* through the biological process of RNA interference (RNAi).

An Hif2α RNAi trigger comprises a sense strand and an antisense strand each containing a core sequence of 16-23 nucleobases in length. An antisense strand core sequence is 100% (perfectly) complementary or at least 90% (substantially) complementary to a nucleotide sequence (sometimes referred to, e.g. as a target sequence) present in the Hif2α mRNA. A sense strand core sequence is 100% (perfectly) complementary or at least 90% (substantially) complementary to a sequence in the antisense strand and thus the sense strand core sequence is perfectly identical or at least 90% identical to a nucleotide sequence (target sequence) present in the Hif2α mRNA. A sense strand core sequence can be the same length as a corresponding antisense core sequence or it can be a different length. In some embodiments, the antisense strand core sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, the sense strand core sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

The Hif2α RNAi trigger sense and antisense strands typically anneal to form a duplex. Within

the complementary duplex region, the sense strand core sequence is at least 90% complementary or 100% complementary to the antisense core sequence. In some embodiments, the sense strand core sequence contains a sequence of at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 nucleotides that is at least 90% or 100% complementary to a corresponding 16, 17, 18, 19, 20, or 21 nucleotide sequence of the antisense strand core sequence (i.e., the sense strand and antisense core sequences of an Hif2α RNAi trigger have a region of at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 nucleotides that is at least 90% base paired or 100% base paired.)

As used herein, the term "sequence" or "nucleotide sequence" refers to a succession or order of nucleobases, nucleotides, and/or nucleosides, described with a succession of letters using the standard nucleotide nomenclature and the key for modified nucleotides described herein.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence (e.g., RNAi trigger sense strand or Hif2α mRNA) in relation to a second nucleotide sequence (e.g., RNAi trigger antisense strand), refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize (form base pair hydrogen bonds) and form a duplex or double helical structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence. Complementary sequences include Watson-Crick base pairs or non-Watson-Crick base pairs and include natural or modified nucleotides or nucleotide mimics as long as the above requirements with respect to their ability to hybridize are fulfilled. "Perfectly complementary" or "fully complementary" means that all (100%) of the bases in a contiguous sequence of a first polynucleotide will hybridize with the same number of bases in a contiguous sequence of a second polynucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence. As used herein, "partial complementary" means that in a hybridized pair of nucleobase sequences, at least 70% of the bases in a contiguous sequence of a first polynucleotide will hybridize with the same number of bases in a contiguous sequence of a second polynucleotide. As used herein, "substantial complementary" means that in a hybridized pair of nucleobase sequences, at least 85% of the bases in a contiguous sequence of a first polynucleotide will hybridize with the same number of bases in a contiguous sequence of a second polynucleotide. The terms "complementary", "fully complementary" and "substantially complementary" as used herein may be used with respect to the base matching between the sense strand and the antisense strand of an RNAi trigger, or between the antisense

strand of an RNAi trigger and a sequence of an Hif2α mRNA. Sequence identity or complementarity is independent of modification. For the purposes of determining identity or complementarity, for example, a and Af are complementary to U (or T) and identical to A.

The sense strand and/or the antisense strand may optionally and independently contain an additional 1, 2, 3, 4, 5, or 6 nucleotides (extension) at the 3′ end, the 5′ end, or both the 3′ and 5′ ends of the core sequences. The antisense strand additional nucleotides, if present, may or may not be complementary to the corresponding sequence in the Hif2α mRNA. The sense strand additional nucleotides, if present, may or may not be identical to the corresponding sequence in the Hif2α mRNA. The antisense strand additional nucleotides, if present, may or may not be complementary to the corresponding sense strand's additional nucleotides, if present.

As used herein, an extension comprises 1, 2, 3, 4, 5, or 6 nucleotides at the 5' and/or 3' end of the sense strand core sequence and/or antisense strand core sequence. The extension nucleotides on a sense strand may or may not be complementary to nucleotides, either core sequence nucleotides or extension nucleotides, in the corresponding antisense strand. Conversely, the extension nucleotides on an antisense strand may or may not be complementary to nucleotides, either core sequence nucleotides or extension nucleotides, in the corresponding sense strand. In some embodiments, both the sense strand and the antisense strand of an RNAi trigger contain 3′ and 5′ extensions. In some embodiments, one or more of the 3′ extension nucleotides of one strand base pairs with one or more 5′ extension nucleotides of the other strand. In other embodiments, one or more of 3′ extension nucleotides of one strand do not base pair with one or more 5′ extension nucleotides of the other strand. In some embodiments, an Hif2α RNAi trigger has an antisense strand having a 3′ extension and a sense strand having a 5′ extension.

In some embodiments an Hif2α RNAi trigger molecule comprises an antisense strand having a 3′ extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In other embodiments, an Hif2α RNAi trigger molecule comprises an antisense strand having a 3′ extension of 1, 2, or 3 nucleotides in length. In some embodiments, one or more of the antisense strand extension nucleotides comprise uracil or thymidine nucleotides or nucleotides which are complementary to the corresponding Hif2α mRNA sequence. In some embodiments, the antisense strand extension can be, but is not limited to: uAu, uGu, udTsdT, usdTsdT, UfAu, Aua, Afsusa, UAU, uAfu,

uau, udAu, uscu, usgu, uscsu, cAu, aUa, aua, u(invdA)u, cag, agu, gcg, caa, usasu, uAMTM, or usTMsAM (each listed 5′ to 3′, notation is the same as for Table 2).

In some embodiments, an Hif2α RNAi trigger molecule comprises an antisense strand having a 5′ extension of 1, 2, 3, 4, or 5 nucleotides in length. In other embodiments, an Hif2α RNAi trigger molecule comprises an antisense strand having a 5′ extension of 1 or 2 nucleotides in length. In some embodiments, one or more of the antisense strand extension nucleotides comprises uracil or thymidine nucleotides or nucleotides which are complementary to the corresponding Hif2α mRNA sequence. In some embodiments, the antisense strand extension includes or consists of dA, dT, pdT, vpdT, or u, wherein dA and dT represent deoxyadenosine and deoxythimidine nucleotides respectively, pdT represents a deoxythimidine nucleotide having a 5′ phosphate, vpdT represents a vinylphosphonate deoxythimidine nucleotide, and u represents a 2′-OMe modified uracil nucleotide. An antisense strand may have any of the 3' extensions described above in combination with any of the 5′ antisense strand extensions described, if present.

In some embodiments, an Hif2α RNAi trigger molecule comprises a sense strand having a 3′ extension of 1, 2, 3, 4, or 5 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprises adenosine, uracil, or thymidine nucleotides, AT dinucleotide, or nucleotides which correspond to nucleotides in the Hif2α mRNA sequence. In some embodiments, the 3′ sense strand extension includes or consists of Af, invdA, invdT, A(invdT), Af(invdT), U(invdT), Uf(invdT), AfAbuAu, dTdT, or dTsdT, wherein Af and Uf represent 2′-fluoro adenosine and uracil nucleotides respectively, invdA and invdT represent 3′-3′ linked (inverted) deoxyadenosine and deoxythimidine nucleotides respectively, Ab represents an abasic ribose, u represents a 2′-OMe modified uracil nucleotide, dT represents a deoxythimidine nucleotide, sdT represents a deoxythimidine nucleotide having a 5′ phosphorothioate, and U and A represent uracil and adenosine ribonucleotides.

In some embodiments, an Hif2α RNAi trigger molecule comprises a sense strand having a 5′ extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprise uracil or adenosine nucleotides or nucleotides which correspond to nucleotides in the Hif2α mRNA sequence. In some embodiments, the sense strand 5′ extension can be, but is not limited to: uAuAus, uAuAu, UAUUAGfs, UfaUfaA, uauaA, AUAUU, AfuAfuU, auauU, uaUfau, uAuA(UUNA), uauau, udAudAu, uuAga, uuAuu,

uuGAu, uuaga, uAuga, aUaGas, uauaus, uAuaas, udAuau, adTaga, auaga, u(invdA)uau, gacau, ugaau, gcgau, uauga, uugga, or auaga (each listed 5′ to 3′, notation is the same as for Table 2). A sense strand may have a 3′ extension and/or a 5′ extension.

Unmodified Hif2α RNAi trigger sense strand and antisense strand sequences are provided in Tables 1 and Table 5. In forming Hif2α RNAi triggers, each of the nucleotides in each of the sequences listed in Tables 1 and 5 may be a modified nucleotide.

Table 1. Unmodified Hif2α RNAi trigger antisense strand and sense strand sequences.

SEQ ID NO.	Antisense Strand Sequence 5′ → 3′	SEQ ID NO.	Sense Strand Sequence 5′ → 3′
1	AGUAAAACAAUUGUGUACUUU	57	AGUACACAAUUGUUUUACUT
2	AGUAAAACAAUUGUGUACUUUAA	44	AAAGUACACAAUUGUUUUACT
3	AUUCAUGAAAUCGUUACGUTG	52	ACGUAACGAUUUCAUGAATT
4	AUUCAUGAAAUCGUUACGUUG	52	ACGUAACGAUUUCAUGAATT
4	AUUCAUGAAAUCGUUACGUUG	53	ACGUAACGAUUUCAUGAAU
4	AUUCAUGAAAUCGUUACGUUG	54	ACGUAACGAUUUCAUGAAUT
4	AUUCAUGAAAUCGUUACGUUG	73	UAUACGUAACGAUUUCAUGAAUT
4	AUUCAUGAAAUCGUUACGUUG	74	UAUACGUAACGAUUUCAUGAAUTT
5	AUUCAUGAAAUCGUUACGUUGAT	47	AACGUAACGAUUUCAUGAAUT
6	AUUCAUGAAAUCGUUACGUUGGC	77	UAUCAACGUAACGAUUUCAUGAAUTT
7	TAAAUCGUUACGUUGACAGTT	67	CUGUCAACGUAACGAUUUAT
8	TAACCACAUACGUUGGAGUTT	55	ACUCCAACGUAUGUGGUUAT
9	TAAGUUAAGCUCCCAUACATT	81	UGUAUGGGAGCUUAACUUAT
10	TAAUCGUUACGUUGACAGGTT	63	CCUGUCAACGUAACGAUUAT
11	TACGUUGACAGGUAGGGUUTT	45	AACCCUACCUGUCAACGUAT
12	TAGAGGAGCUUGUGUGUUCTT	68	GAACACACAAGCUCCUCUAT
13	TAGCUUGUGUGUUCGCAGGTT	62	CCUGCGAACACACAAGCUAT
14	TAGGAGCUUGUGUGUUCGCTT	69	GCGAACACACAAGCUCCUAT
15	TAUCGUUACGUUGACAGGUTT	49	ACCUGUCAACGUAACGUAAT
15	TAUCGUUACGUUGACAGGUTT	71	UAUACCUGUCAACGUAACGUAAT
16	TCAUGAAAUCGUUACGUUGTT	60	CAACGUAACGAUUUCAUGAT
17	TCGUUACGUUGACAGGUAGTT	65	CUACCUGUCAACGUAACGAT
18	TCUAGCAACAAAACCUUAATT	82	UUAAGGUUUUGUUGCUAGAT
19	TGAGCUUGUGUGUUCGCAGTT	66	CUGCGAACACACAAGCUCAT
19	TGAGCUUGUGUGUUCGCAGTT	68	GAACACACAAGCUCCUCUAT
20	TGAGGAGCUUGUGUGUUCGTT	64	CGAACACACAAGCUCCUCAT
21	TGGAGCUUGUGUGUUCGCATT	79	UGCGAACACACAAGCUCCAT
22	TGGUACUGGGUGGCGUAGCTT	70	GCUACGCCACCCAGUACCAT
23	TGUAAAACAAUUGUGUACUTT	56	AGUACACAAUUGUUUUACAT
24	TUACGUUGACAGGUAGGGUTT	48	ACCCUACCUGUCAAGGUAAT
25	TUCGUUACGUUGACAGGUATT	78	UCACUGUCAACGUAACGAAT

26	TUGAUAAACACUUAACCCATT	80	UGGGUUAAGUGUUUAUCAAT
27	TUGUCACGAUGCGGUGGUUTT	61	CAGUGCAACGCCACCCAGAT
28	TUUCAUGAAAUCGUUACGUCGGCUAU	76	UAUAUCGACGUAACGAUUUCAUGAAA
29	TUUCAUGAAAUCGUUACGUCGGCUGU	76	UAUAUCGACGUAACGAUUUCAUGAAA
30	TUUCAUGAAAUCGUUACGUTT	50	ACGUAACGAUUUCAUGAAA
30	TUUCAUGAAAUCGUUACGUTT	51	ACGUAACGAUUUCAUGAAAT
30	TUUCAUGAAAUCGUUACGUTT	72	UAUACGUAACGAUUUCAUGAAAT
31	TUUCAUGAAAUCGUUACGUUGGC	59	CAACGUAACGAUUUCAUGAAA
32	TUUCAUGAAAUCGUUACGUUGGCUAU	75	UAUAUCAACGUAACGAUUUCAUGAAA
33	TUUCAUGAAAUCGUUACGUUGGCUGU	75	UAUAUCAACGUAACGAUUUCAUGAAA
34	TUUCAUGAAAUCGUUACGUUGGCUTT	75	UAUAUCAACGUAACGAUUUCAUGAAA
35	UCAUGAAAUCGUUACGUUGTT	58	CAACGUAACGAUUUCAUGA
35	UCAUGAAAUCGUUACGUUGTT	58	CAACGUAACGAUUUCAUGA
36	UCUAGCAACAAAACCUUAATT	82	UUAAGGUUUUGUUGCUAGAT
37	UGUAAAACAAUUGUGUACUTT	56	AGUACACAAUUGUUUUACAT
38	UGUAAAACAAUUGUGUACUUU	56	AGUACACAAUUGUUUUACAT
39	UGUAAAACAAUUGUGUACUUUAA	43	AAAGUACACAAUUGUUUUACA
40	UUUCAUGAAAUCGUUACGUTT	51	ACGUAACGAUUUCAUGAAAT
40	UUUCAUGAAAUCGUUACGUTT	72	UAUACGUAACGAUUUCAUGAAAT
41	UUUCAUGAAAUCGUUACGUUG	50	ACGUAACGAUUUCAUGAAA
41	UUUCAUGAAAUCGUUACGUUG	51	ACGUAACGAUUUCAUGAAAT
41	UUUCAUGAAAUCGUUACGUUG	72	UAUACGUAACGAUUUCAUGAAAT
42	UUUCAUGAAAUCGUUACGUUGAT	46	AACGUAACGAUUUCAUGAAAT

The Hif2α RNAi triggers described herein are formed by annealing an antisense strand with a sense strand. In some embodiments, an Hif2α RNAi trigger antisense strand comprises a nucleotide sequence of any of the sequences in Tables 1 and 5. In some embodiments, an Hif2α RNAi trigger antisense strand comprises the sequence of nucleotides 1-17, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, 2-24, 1-25, 2-25, 1-26, or 2-26 of any of the sequences in Tables 1 and 5. In some embodiments, an Hif2α RNAi trigger sense strand comprises the nucleotide sequence of any of the sequences in Tables 1 and 5. In some embodiments, an Hif2α RNAi trigger sense strand comprises the sequence of nucleotides 1-17, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, 2-24, 1-25, 2-25, 1-26, or 2-26 of any of the sequences in Tables 1 and 5.

In some embodiments, the sense and antisense strands of the RNAi triggers described herein contain the same number of nucleotides. In some embodiments the sense and antisense strands of the RNAi triggers described herein contain different numbers of nucleotides. In some embodiments, the sense strand 5′ end and the antisense strand 3′ end of an RNAi trigger form a blunt end. In some embodiments, the sense strand 3′ end and the antisense strand 5′ end of an

RNAi trigger form a blunt end. In some embodiments, both ends of an RNAi trigger form a blunt end. In some embodiments, neither end of an RNAi trigger is blunt-ended. As used herein a blunt end refers to an end of a double stranded trigger molecule in which the terminal nucleotides of the two annealed strands are complementary (form a complementary base-pair). In some embodiments, the sense strand 5′ end and the antisense strand 3′ end of an RNAi trigger form a frayed end. In some embodiments, the sense strand 3′ end and the antisense strand 5′ end of an RNAi trigger form a frayed end. In some embodiments, both ends of an RNAi trigger form a frayed end. In some embodiments, neither end of an RNAi trigger is a frayed end. As used herein a frayed end refers to an end of a double stranded trigger molecule in which the terminal nucleotides of the two annealed strands from a pair (i.e. do not form an overhang) but are not complementary (i.e. form a non-complementary pair). As used herein, an overhang is a stretch of one or more unpaired nucleotides at the end of one strand of a double stranded RNAi trigger molecule. The unpaired nucleotides may be on the sense strand or the antisense strand, creating either 3' or 5' overhangs. In some embodiments the RNAi trigger molecule contains: a blunt end and a frayed end, a blunt end and 5′ overhang end, a blunt end and a 3′ overhang end, a frayed end and a 5′ overhand end, a frayed end and a 3′ overhang end, two 5′ overhang ends, two 3′ overhang ends, a 5′ overhang end and a 3′ overhand end, two frayed ends, or two blunt ends.

A nucleotide base (or nucleobase) is a heterocyclic pyrimidine or purine compound which is a constituent of all nucleic acids and includes adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). As used herein, "G", "g", "C", "c", "A", "a", "U", "u", and "T", each generally stand for a nucleobase, nucleoside, nucleotide or nucleotide mimic that contains guanine, cytosine, adenine, uracil and thymidine as a base. Also as used herein, the term "nucleotide" can include a modified nucleotide or nucleotide mimic, abasic site, or a surrogate replacement moiety.

As used herein, a "modified nucleotide" is a nucleotide other than a ribonucleotide (2′-hydroxyl nucleotide). In some embodiments, an Hif2α RNAi trigger contains one or more modified nucleotides. In some embodiments, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the nucleotides are modified. Modified nucleotides include, but are not limited to, deoxynucleotides, nucleotide mimics, abasic nucleotides (represented herein as X or Ab), 2′-modified nucleotides, 3′ to 3′ linkages (inverted) nucleotides (represented herein as invdN, invN, invn, invX), non-natural base-comprising nucleotides, bridged

nucleotides, peptide nucleic acids, 2′,3′-seco nucleotide mimics (unlocked nucleobase analogues, represented herein as N_{UNA} or NUNA), locked nucleotides (represented herein as N_{LNA} or NLNA), 3′-O-Methoxy (2′ internucleotide linked) nucleotides (represented herein as 3′-OMen), 2′-F-Arabino nucleotides (represented herein as NfANA or Nf_{ANA}), morpholino nucleotides, vinyl phosphonate deoxyribonucleotides (represented herein as vpdN), and vinyl phosphonate nucleotides. 2′-modified nucleotides (i.e. a nucleotide with a group other than a hydroxyl group at the 2′ position of the five-membered sugar ring) include, but are not limited to, 2′-O-methyl nucleotides (represented herein as a lower case letter 'n' in a nucleotide sequence), 2′-deoxy-2′-fluoro nucleotides (represented herein as Nf, also represented herein as 2′-fluoro nucleotide), 2′-deoxy nucleotides (represented herein as dN), 2′-methoxyethyl (2′-O-2-methoxylethyl) nucleotides (represented herein as NM or 2′-MOE), 2′-amino nucleotides, 2′-alkyl nucleotides. It is not necessary for all positions in a given compound to be uniformly modified. Conversely, more than one modification may be incorporated in a single Hif2α RNAi trigger or even in a single nucleotide thereof. The Hif2α RNAi trigger sense strands and antisense strands may be synthesized and/or modified by methods known in the art. Modification at one nucleotide is independent of modification of another nucleotide.

Modified nucleotides also include nucleotides having modified nucleobases. Modified nucleobases include, but are not limited to, synthetic and natural nucleobases, 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2- thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8- hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5- trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

In some embodiments 20% or fewer of the modified nucleotides are 2′-fluoro modified nucleotides. In some embodiments, an Hif2α RNAi trigger sense strand contains a 2′-F nucleotide at position 11 from the 3′ end. In some embodiments, an Hif2α RNAi trigger sense strand contains a 2′-F nucleotide at position 12 from the 3′ end. In some embodiments, an Hif2α

RNAi trigger sense strand contains a 2′-F nucleotide at position 13 from the 3′ end. In some embodiments, an Hif2α RNAi trigger sense strand contains at least two 2′-F nucleotides at positions 11, 12, and 13 from the 3′ end. In some embodiments, an Hif2α RNAi trigger sense strand contains 2′-F nucleotides at positions 11 and 12, positions 11 and 13, or positions 12 and 13 from the 3′ end. In some embodiments, an Hif2α RNAi trigger sense strand contains 2′-F nucleotides at positions 11, 12, and 13 from the 3′ end.

In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2′-F nucleotide at position 2 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2'-F nucleotide at position 14 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains 2′-F nucleotides at positions 2 and 14 from the 5′ end. In some embodiments, an Hif2α RNAi trigger contains at least two 2′-F nucleotides at positions 11, 12, and 13 from the 3′ end of the sense strand and at positions 2 and 14 from the 5′ end of the antisense strand.

In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2′-F nucleotide at position 4 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2'-F nucleotide at position 6 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2'-F nucleotide at position 8 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2′-F nucleotide at position 10 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2′-F nucleotide at position 12 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains at least two 2′-F nucleotides at positions 4, 6, 8, 10, and 12 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains 2′-F nucleotides at positions 4 and 6, positions 4 and 8, positions 4 and 10, positions 4 and 12, positions 6 and 8, positions 6 and 10, positions 6 and 12, positions 8 and 10, positions 8 and 12, or positions 10 and 12 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains at three 2′-F nucleotides at positions 4, 6, 8, 10, and 12 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains at least four 2′-F nucleotides at positions 4, 6, 8, 10, and 12 from the 5′ end. In some embodiments, an Hif2α RNAi trigger antisense strand contains 2′-F nucleotides at positions 4, 6, 8, and 10, positions 4, 6, 8, and 12, positions 4, 6, 10, and 12, positions 4, 8, 10, and 12 or positions 6, 8, 10, and 12 from the 5′ end.

In some embodiments, an Hif2α RNAi trigger antisense strand contains a 2′-F nucleotide at position 2 and/or position 14 and one, two, or three 2′-F nucleotides at positions 11, 12, and 13 from the 5′ end. In some embodiments, an Hif2α RNAi trigger contains a 2′-F nucleotide at position 2 and/or position 14 and one, two, or three 2′-F nucleotides at positions 11, 12, and 13 from the 5′ end of the antisense strand, and at least two 2′-F nucleotides at positions 11, 12, and 13 from the 3′ end of the sense strand.

In some embodiments, one or more nucleotides of an Hif2α RNAi trigger are linked by non-standard linkages or backbones (i.e. modified internucleoside linkages or modified backbones). In some embodiments, a modified internucleoside linkage is a non-phosphate-containing covalent internucleoside linkage. Modified internucleoside linkages or backbones include, but are not limited to, phosphorothioates, 5′-phosphorothioate group (represented herein as a lower case 's' before a nucleotide, as in sN, sn, sNf, or sdN), chiral phosphorothioates, thiophosphate, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, methyl and other alkyl phosphonates including 3′-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3′-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkyl-phosphonates, thionoalkylphosphotriesters, morpholino linkages, and boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′. In other embodiments, a modified internucleoside linkage or backbone lacks a phosphorus atom. Modified internucleoside linkages lacking a phosphorus atom include, but are not limited to, short chain alkyl or cycloalkyl inter-sugar linkages, mixed heteroatom and alkyl or cycloalkyl inter-sugar linkages, or one or more short chain heteroatomic or heterocyclic inter-sugar linkages. In some embodiments, modified internucleoside backbones include, but are not limited to, siloxane backbones, sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones, methylene formacetyl and thioformacetyl backbones, alkene containing backbones, sulfamate backbones, methyleneimino and methylenehydrazino backbones, sulfonate and sulfonamide backbones, amide backbones; and others having mixed N, O, S, and CH_2 component parts.

In some embodiments, an Hif2α RNAi trigger contains one or more modified nucleotides and one or more modified internucleoside linkages. In some embodiments, a 2′-modified nucleotide is combined with modified internucleoside linkage. For example, in some embodiments, a sense strand of an Hif2α RNAi trigger can contain 1, 2, 3, 4 phosphorothioate linkages, an

antisense strand of a Hif2α RNAi trigger can contain 1, 2, 3, or 4 phosphorothioate linkages, or both the sense strand and the antisense strand independently can contain 1, 2, 3, or 4 phosphorothioate linkages.

In some embodiments, an Hif2α RNAi trigger sense strand contains two phosphorothioate internucleoside linkages. In some embodiments, the two phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 3' end of the sense strand. In some embodiments, the two phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3, 2-4, 3-5, 4-6, 4-5, or 6-8 from the 5' end of the sense strand. In some embodiments, an Hif2α RNAi trigger antisense strand contains four phosphorothioate internucleoside linkages. In some embodiments, the four phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 5' end of the sense strand and between the nucleotides at positions 19-21, 20-22, 21-23, 22-24, 23-25, or 24-26 from the 5' end. In some embodiments, an Hif2α RNAi trigger contains two phosphorothioate internucleoside linkages in the sense strand and four phosphorothioate internucleoside linkages in the antisense strand.

In some embodiments, an Hif2α RNAi trigger is prepared or provided as a salt, mixed salt, or a free-acid.

Examples of antisense strands containing modified nucleotides are provided in Table 2A and Table 5B. Examples of sense strands containing modified nucleotides are provided in Table 2B and Table 5B. In Tables 2A, 2B and 5B, the following notations are used to indicate modified nucleotides:

N	=	2′-OH (unmodified) ribonucleotide (capital letter without f or d indication)
n	=	2′-OMe modified nucleotide
Nf	=	2′-fluoro modified nucleotide
dN	=	2′-deoxy nucleotides
N_{UNA}	=	2′,3′-seco nucleotide mimics (unlocked nucleobase analogs)
NM	=	2′-methoxyethyl nucleotide
(invdN)	=	inverted deoxyribonucleotide (3′-3′ linked nucleotide)
(invAb)	=	inverted abasic nucleotide
s	=	phosphorothioate linked nucleotide
p	=	phosphate

vpdN = vinyl phosphonate deoxyribonucleotide

Table 2A. Hif2α RNAi trigger antisense strands having modified nucleotides.

Antisense Strand ID	Antisense Strand Sequence (5' → 3')	SEQ ID NO.	Unmod. SEQ ID NO.
AM00159-AS	dTGfgAfgCfuUfgUfgUfgUfuCfgCfadTsdT	112	21
AM00160-AS	dTGfgUfaCfuGfgGfuGfgCfgUfaGfcdTsdT	113	22
AM00161-AS	dTCfgUfuAfcGfuUfgAfcAfgGfuAfgdTsdT	106	17
AM00162-AS	dTAfgCfuUfgUfgUfgUfuCfgCfaGfgdTsdT	103	13
AM00163-AS	dTGfaGfcUfuGfuGfuGfuUfcGfcAfgdTsdT	110	19
AM00164-AS	dTAfgGfaGfcUfuGfuGfuGfuUfcGfcdTsdT	104	14
AM00165-AS	dTGfaGfgAfgCfuUfgUfgUfgUfuCfgdTsdT	111	20
AM00166-AS	dTAfgAfgGfaGfcUfuGfuGfuGfuUfcdTsdT	102	12
AM00167-AS	dTUfgUfcAfcGfaUfgCfgGfuGfgUfudTsdT	137	27
AM00168-AS	dTAfcGfuUfgAfcAfgGfuAfgGfgUfudTsdT	101	11
AM00169-AS	dTUfaCfgUfuGfaCfaGfgUfaGfgGfudTsdT	135	24
AM00170-AS	dTUfcGfuUfaCfgUfuGfaCfaGfgUfadTsdT	136	25
AM00171-AS	dTAfuCfgUfuAfcGfuUfgAfcAfgGfudTsdT	105	15
AM00172-AS	dTAfaUfcGfuUfaCfgUfuGfaCfaGfgdTsdT	100	10
AM01770-AS	dTGfuAfaAfaCfaAfuUfgUfgUfaCfudTsdT	114	23
AM01772-AS	dTGfuAfaAUNAaCfaAfuUfgUfgUfaCfudTsdT	116	23
AM01773-AS	dTGfuAfaAfAUNACfaAfuUfgUfgUfaCfudTsdT	115	23
AM01775-AS	dTCfuAfgCfaAfcAfaAfaCfcUfuAfadTsdT	107	18
AM01777-AS	dTCfuAfgCUNAaAfcAfaAfaCfcUfuAfadTsdT	109	18
AM01778-AS	dTCfuAfgCfAUNAAfcAfaAfaCfcUfuAfadTsdT	108	18
AM01780-AS	dTAfaAfuCfgUfuAfcGfuUfgAfcAfgdTsdT	94	7
AM01782-AS	dTAfaAfuCUNAgUfuAfcGfuUfgAfcAfgdTsdT	96	7
AM01783-AS	dTAfaAfuCfGUNAUfuAfcGfuUfgAfcAfgdTsdT	95	7
AM01784-AS	dTUfuCfaUfgAfaAfuCfgUfuAfcGfudTsdT	138	30
AM01786-AS	dTUfuCfaUUNAgAfaAfuCfgUfuAfcGfudTsdT	145	30
AM01787-AS	dTUfuCfaUfGUNAAfaAfuCfgUfuAfcGfudTsdT	144	30
AM01789-AS	dTAfaGfuUfaAfgCfuCfcCfaUfaCfadTsdT	97	9
AM01791-AS	dTAfaGfuUUNAaAfgCfuCfcCfaUfaCfadTsdT	99	9
AM01792-AS	dTAfaGfuUfAUNAAfgCfuCfcCfaUfaCfadTsdT	98	9
AM02090-AS	dTUfuCfaUfgAUNAaAfuCfgUfuAfcGfudTsdT	143	30
AM02091-AS	dTUfuCfaUfgAfAUNAAfuCfgUfuAfcGfudTsdT	142	30
AM02092-AS	dTUfuCfaUfgAfaAfuCfgUfUUNAAfcGfudTsdT	139	30
AM02133-AS	dTUfuCfaUfgAfaAfucgUfuAfcGfudTsdT	141	30
AM02140-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfuUfgsgsc	128	31
AM02145-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfuUfggscsuGu	127	33
AM02146-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfuCfggscsuGu	123	29
AM02147-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfuUfggscsuAu	126	32
AM02150-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfuUfggcusdTsdT	125	34

AM02327-AS	pdTUfuCfaUfgAfaAfuCfgUfuAfcGfudTsdT	147	30
AM02341-AS	dTsUfsuCfaUfgAUNAaAfuCfgUfuAfcGfuCfggscsuAu	130	28
AM02342-AS	dTsUfuCfaUfgAUNAaAfuCfgUfuAfcGfuCfggcsuAu	134	28
AM02345-AS	dTsUfsuCfaUfgAfAUNAAfuCfgUfuAfcGfuCfggscsuAu	129	28
AM02346-AS	dTsUfuCfaUfgAfAUNAAfuCfgUfuAfcGfuCfggcsuAu	133	28
AM02508-AS	dTsUfsuCfaUfgAfaAfuCfgUfuAfcGfudTsdT	120	30
AM02509-AS	dTUfuCfaUfgAfaAfucGfUfuAfcGfudTsdT	140	30
AM02523-AS	dTsAfsaCfcAfcAfuAfcGfuUfgGfaGfudTsdT	117	8
AM02525-AS	dTsUfsgAfuAfaAfcAfcUfuAfaCfcCfadTsdT	119	26
AM02527-AS	dTsCfsaUfgAfaAfuCfgUfuAfcGfuUfgdTsdT	118	16
AM02529-AS	usCfsaUfgAfaAfuCfgUfuAfcGfuUfgdTsdT	148	35
AM02604-AS	dTsUfsuCfaUfgAfaAfucGfUfuAfcGfudTsdT	122	30
AM02605-AS	dTsUfsuCfaUfgAfaAfucgUfuAfcGfudTsdT	124	30
AM02848-AS	dTsUfuCfaUfgAfaAfucGfUfuAfcGfudTsdT	132	30
AM02849-AS	dTsUfuCfaUfgAfaAfuCfgUfUfacGfudTsdT	131	30
AM02850-AS	dTsUfsuCfaUfgAfaAfuCfgUfUfacGfudTsdT	121	30
AM02998-AS	usUfsuCfaUfgAfaAfucgUfuAfcGfudTsdT	160	40
AM03000-AS	usCfsuAfgCfaAfcAfaaAfCfcUfuAfadTsdT	151	36
AM03001-AS	usCfsuAfgCfaaCfAfaaAfCfcUfuAfadTsdT	149	36
AM03002-AS	usCfsuAfgCfaAfcAfaAfaCfCfuuAfadTsdT	152	36
AM03003-AS	usCfsuAfgCfaaCfAfaAfaCfCfuuAfadTsdT	150	36
AM03008-AS	usGfsuAfaAfaCfaAfuuGfUfgUfaCfudTsdT	154	37
AM03009-AS	usGfsuAfaAfaCfaAfuUfgUfGfuaCfudTsdT	153	37
AM03059-AS	pdTsUfsuCfaUfgAfaAfucgUfuAfcGfudTsdT	146	30
AM03465-AS	usUfsuCfaUfgAfaAfucgUfuAfcGfususg	161	41
AM03513-AS	usUfsuCfaUfgAfaAfucguuacgususg	162	41
AM03514-AS	usUfsuCfaUfgAfaaucguuacgususg	165	41
AM03517-AS	asUfsuCfaUfgAfaAfucgUfuAfcGfususg	90	4
AM03685-AS	usUfsuCfaUfgAfaAfucgUfuacgususg	159	41
AM03688-AS	usUfsUfCfaugAfaAfucgUfuacgususg	167	41
AM03689-AS	usUfsuCfaUfgAfaaucgUfuacgususg	163	41
AM03690-AS	usUfsUfcaugAfaAfucgUfuacgususg	166	41
AM04001-AS	usGfsuAfaAfaCfaAfuugUfgUfaCfususu	155	38
AM04004-AS	asGfsuAfaAfaCfaAfuugUfgUfaCfususu	84	1
AM04007-AS	usGfsuAfaAfaCfaAfuugUfgUfaCfuuusasa	156	39
AM04010-AS	asGfsuAfaAfaCfaAfuugUfgUfaCfuuusasa	85	2
AM04015-AS	asGfsuAfaAfaCfaAfuugUfguaCfuuusasa	83	2
AM04018-AS	asGfsuAfaAfaCfaauugUfguacuuusasa	86	2
AM04040-AS	vpusUfsuCfaUfgAfaAfucgUfuAfcGfususg	168	41
AM04101-AS	asUfsuCfaUfgAfaaucgUfuacgususg	91	4
AM04102-AS	usUfsuCfaUfgAfaaucgUfuacguugsas(invdT)	164	42
AM04103-AS	asUfsuCfaUfgAfaaucgUfuacguugsas(invdT)	92	5
AM04104-AS	usUfsucaugAfaAfucgUfuacgususg	157	41
AM04105-AS	asUfsucaugAfaAfucgUfuacgususg	88	4

AM04106-AS	asUfsucaugAfaAfucgUfuacgusTMsGM	87	3
AM04244-AS	usUfsuCfaUfgaAfaucgUfuacgususg	158	41
AM04452-AS	asUfsuCfaUfgAfaaucgUfuAfcguugsgsc	93	6
AM04455-AS	asUfsuCfaUfgaAfaucgUfuAfcguugsgsc	89	6

Table 2B. Hif2α RNAi trigger sense strands having modified nucleotides.

Sense Strand ID	SS Sequence 5' → 3'	SEQ ID NO.	Unmod. SEQ ID NO.
AM00158-SS	CfuGfuCfaAfcGfuAfaCfgAfuUfuAf(invdT)	277	67
AM00188-SS	(NH2-C6)uAuUfgCfgAfaCfaCfaCfaAfgCfuCfcAf(invdT)	265	79
AM00189-SS	(NH2-C6)uAuGfcUfaCfgCfcAfcCfcAfgUfaCfcAf(invdT)	264	70
AM00190-SS	(NH2-C6)uAuCfuAfcCfuGfuCfaAfcGfuAfaCfgAf(invdT)	263	65
AM00191-SS	(NH2-C6)uAuCfcUfgCfgAfaCfaCfaCfaAfgCfuAf(invdT)	262	62
AM00207-SS	(NH2-C6)CfuGfcGfaAfcAfcAfcAfaGfcUfcAf(invdT)	258	66
AM00208-SS	(NH2-C6)GfcGfaAfcAfcAfcAfaGfcUfcCfuAf(invdT)	260	69
AM00209-SS	(NH2-C6)CfgAfaCfaCfaCfaAfgCfuCfcUfcAf(invdT)	256	64
AM00210-SS	(NH2-C6)GfaAfcAfcAfcAfaGfcUfcCfuCfuAf(invdT)	259	68
AM00211-SS	(NH2-C6)CfaGfuGfcAfaCfgCfcAfcCfcAfgAf(invdT)	253	61
AM00212-SS	(NH2-C6)AfaCfcCfuAfcCfuGfuCfaAfcGfuAf(invdT)	247	45
AM00213-SS	(NH2-C6)AfcCfcUfaCfcUfgUfcAfaGfgUfaAf(invdT)	248	48
AM00214-SS	(NH2-C6)UfcAfcUfgUfcAfaCfgUfaAfcGfaAf(invdT)	266	78
AM00219-SS	(NH2-C6)AfcCfuGfuCfaAfcGfuAfaCfgUfaAf(invdT)	249	49
AM00220-SS	(NH2-C6)CfcUfgUfcAfaCfgUfaAfcGfaUfuAf(invdT)	255	63
AM00221-SS	(NH2-C6)UfgCfgAfaCfaCfaCfaAfgCfuCfcAf(invdT)	267	79
AM00222-SS	(NH2-C6)GfcUfaCfgCfcAfcCfcAfgUfaCfcAf(invdT)	261	70
AM00223-SS	(NH2-C6)CfuAfcCfuGfuCfaAfcGfuAfaCfgAf(invdT)	257	65
AM00224-SS	(NH2-C6)CfcUfgCfgAfaCfaCfaCfaAfgCfuAf(invdT)	254	62
AM00366-SS	(Alk-SS-C6)CfuGfcGfaAfcAfcAfcAfaGfcUfcAf(invdT)	220	66
AM00367-SS	(Alk-SS-C6)GfcGfaAfcAfcAfcAfaGfcUfcCfuAf(invdT)	223	69
AM00369-SS	(Alk-SS-C6)GfaAfcAfcAfcAfaGfcUfcCfuCfuAf(invdT)	222	68
AM00530-SS	(Alk-SS-C6)AfcCfcUfaCfcUfgUfcAfaGfgUfaAf(invdT)	212	48
AM00531-SS	(Alk-SS-C6)UfcAfcUfgUfcAfaCfgUfaAfcGfaAf(invdT)	224	78
AM00543-SS	(Alk-SS-C6)AfaCfcCfuAfcCfuGfuCfaAfcGfuAf(invdT)	211	45
AM00544-SS	(Alk-SS-C6)AfcCfuGfuCfaAfcGfuAfaCfgUfaAf(invdT)	213	49
AM00545-SS	(Alk-SS-C6)CfuAfcCfuGfuCfaAfcGfuAfaCfgAf(invdT)	219	65
AM01771-SS	AfgUfaCfaCfaAfuUfgUfuUfuAfcAf(invdT)	271	56
AM01776-SS	UfuAfaGfgUfuUfuGfuUfgCfuAfgAf(invdT)	284	82
AM01785-SS	AfcGfuAfaCfgAfuUfuCfaUfgAfaAf(invdT)	269	51
AM01790-SS	UfgUfaUfgGfgAfgCfuUfaAfcUfuAf(invdT)	283	81
AM01859-SS	(Alk-SS-C6)AfgUfaCfaCfaAfuUfgUfuUfuAfcAf(invdT)	218	56
AM01860-SS	(Alk-SS-C6)UfuAfaGfgUfuUfuGfuUfgCfuAfgAf(invdT)	226	82
AM01861-SS	(Alk-SS-C6)CfuGfuCfaAfcGfuAfaCfgAfuUfuAf(invdT)	221	67
AM01862-SS	(Alk-SS-C6)AfcGfuAfaCfgAfuUfuCfaUfgAfaAf(invdT)	215	51

AM01863-SS	(Alk-SS-C6)UfgUfaUfgGfgAfgCfuUfaAfcUfuAf(invdT)	225	81
AM01994-SS	(Alk-C6)uAuAfcCfuGfuCfaAfcGfuAfaCfgUfaAf(invdT)	169	71
AM02043-SS	(Me-Alk-SS-C6)AfcGfuAfaCfgAfuUfuCfaUfgAfaAf(invdT)	231	51
AM02093-SS	(DBCO-TEG)uAuAfcGfuAfaCfgAfuUfuCfaUfgAfaAf(invdT)	228	72
AM02135-SS	(Alk-SS-C6)AfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(invdT)	216	51
AM02137-SS	(Alk-SS-C6)AfcGfuAfAfCfgAfuUfuCfaUfgAfaAf(invdT)	217	51
AM02139-SS	(Alk-SS-C6)AfcGfuaaCfgAfuUfuCfaUfgAfaAf(invdT)	214	51
AM02142-SS	CfsasAfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	276	59
AM02144-SS	CUNAsasAfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	278	59
AM02149-SS	uAuAusCfsaAfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	280	75
AM02163-SS	uAuAusCfsgAfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	282	76
AM02363-SS	(DBCO-TEG)uAuAusCfsgAfcGfuAfaCfgAfuUfuCfaUfgAfa(invdA)	230	76
AM02364-SS	uAuAusCfsgAfcGfuAfaCfgAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	281	76
AM02365-SS	uAuAusCfgAfcGfuAfaCfgAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	279	76
AM02456-SS	(Alk-SMPT-C6)AfcGfuAfaCfgAfuUfuCfaUfgAfaAf(invdT)	193	51
AM02510-SS	(Me-Alk-SS-C6)AfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(invdT)	232	51
AM02512-SS	(Me-Alk-SS-C6)AfcGfuAfacGfAfuUfuCfaUfgAfaAf(invdT)	233	51
AM02522-SS	(NH2-C6)AfcUfcCfaAfcGfuAfuGfuGfgUfuAf(invdT)	250	55
AM02524-SS	(NH2-C6)UfgGfgUfuAfaGfuGfuUfuAfuCfaAf(invdT)	268	80
AM02526-SS	(NH2-C6)CfaAfcGfuAfaCfgAfuUfuCfaUfgAf(invdT)	252	60
AM02528-SS	(NH2-C6)CfaAfcGfuAfaCfgAfuUfuCfaUfg(invdA)	251	58
AM02546-SS	(Me-Alk-SS-C6)AfcUfcCfaAfcGfuAfuGfuGfgUfuAf(invdT)	235	55
AM02547-SS	(Me-Alk-SS-C6)UfgGfgUfuAfaGfuGfuUfuAfuCfaAf(invdT)	242	80
AM02548-SS	(Me-Alk-SS-C6)CfaAfcGfuAfaCfgAfuUfuCfaUfgAf(invdT)	241	60
AM02549-SS	(Me-Alk-SS-C6)CfaAfcGfuAfaCfgAfuUfuCfaUfg(invdA)	240	58
AM02852-SS	AfscGfuAfacGfAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	272	50
AM02853-SS	AfscGfUfaaCfgAfuUfuCfaUfgAfaAf(C6-SS-Alk-Me)	273	50
AM02856-SS	(Me-Alk-SS-C6)AfcGfUfaaCfgAfuUfuCfaUfgAfaAf(invdT)	234	51
AM02999-SS	(DBCO-TEG)uAuAfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(invdT)	229	72
AM03004-SS	(Me-Alk-SS-C6)UfuAfaGfguUfUfuGfuUfgCfuAfgAf(invdT)	244	82
AM03005-SS	(Me-Alk-SS-C6)uuAfaGfguUfUfuguUfgCfuAfga(invdT)	246	82
AM03006-SS	(Me-Alk-SS-C6)UfuAfAfggUfuUfuGfuUfgCfuAfgAf(invdT)	243	82
AM03007-SS	(Me-Alk-SS-C6)uuAfAfggUfuUfuguUfgCfuAfga(invdT)	245	82
AM03010-SS	(Me-Alk-SS-C6)AfgUfaCfacAfAfuUfgUfuUfuAfcAf(invdT)	236	56
AM03011-SS	(Me-Alk-SS-C6)agUfaCfacAfAfuUfgUfuuuAfca(invdT)	238	56
AM03012-SS	(Me-Alk-SS-C6)AfgUfAfcaCfaAfuUfgUfuUfuAfcAf(invdT)	237	56
AM03013-SS	(Me-Alk-SS-C6)agUfAfcaCfaAfuUfgUfuuuAfca(invdT)	239	56
AM03058-SS	AfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(invdT)	270	51
AM03307-SS	agUfAfcaCfaAfuUfgUfuuuAfca(invdT)	274	56
AM03308-SS	(Alk-C6-C6)AfcGfuAfaCfGfAfuUfuCfaUfgAfaAf(invdT)	170	51
AM03467-SS	(Alk-SMPT-C6)AfscGfuAfaCfGfAfuUfuCfaUfgAfaa(invdT)	194	51
AM03469-SS	(Alk-SMPT-C6)ascGfuAfaCfGfAfuuuCfaUfgAfaa(invdT)	201	51
AM03471-SS	(Alk-SMPT-C6)ascguAfaCfgAfuuuCfaUfgAfaa(invdT)	208	51
AM03473-SS	(Alk-SMPT-C6)ascGfuaaCfgAfuuuCfaugAfaa(invdT)	200	51

AM03475-SS	(Alk-SMPT-C6)ascguaaCfgAfuuuCfaugaaa(invdT)	204	51
AM03516-SS	(Alk-SMPT-C6)ascguaaCfgAfuuucaugaaa(invdT)	203	51
AM03519-SS	(Alk-SMPT-C6)ascGfuAfaCfGfAfuuuCfaUfgAfau(invdT)	202	54
AM03687-SS	(Alk-SMPT-C6)ascguaaCfGfAfuuucaugaaa(invdT)	205	51
AM03692-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuucaugaaa(invdT)	185	72
AM03694-SS	(Alk-PEG5-C6)uAuascGfuAfaCfGfAfuuuCfaUfgAfau(invdT)	183	73
AM03708-SS	(Alk-PEG5-C6)uAuascGfuAfaCfGfAfuuuCfaUfgAfaa(invdT)	182	72
AM03710-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuuCfaugaaa(invdT)	189	72
AM03712-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuuCfaugaau(invdT)	190	73
AM03714-SS	(Alk-PEG5-C6)(Alk-PEG5-Ser)–uAuascguaaCfGfAfuuuCfaugaaa(invdT)	180	72
AM03774-SS	(Alk-C6-SMPT-C6)ascguaaCfgAfuuuCfaugaaa(invdT)	174	51
AM03829-SS	(Alk-PEG5-C6)uAuascguaaCfgAfuuuCfaugaaa(invdT)	184	72
AM03830-SS	(Chol-TEG)uAuascguaaCfgAfuuuCfaugaaa(invdT)	227	72
AM03831-SS	ascguaaCfgAfuuuCfaugaaa(NAG13)	275	50
AM04003-SS	(Alk-SMPT-C6)asgUfaCfaCfAfAfuUfgUfuuuAfca(invdT)	209	56
AM04006-SS	(Alk-SMPT-C6)asgUfaCfaCfAfAfuUfgUfuuuAfcu(invdT)	210	57
AM04009-SS	(Alk-SMPT-C6)asaAfgUfaCfaCfAfAfuUfgUfuuuAfc(invdA)	195	43
AM04012-SS	(Alk-SMPT-C6)asaAfgUfaCfaCfAfAfuUfgUfuuuAfc(invdT)	196	44
AM04014-SS	(Alk-SMPT-C6)asaaguaCfaCfAfAfuuguuuuac(invdT)	198	44
AM04017-SS	(Alk-SMPT-C6)asaagUfaCfaCfAfAfuUfgUfuuuac(invdT)	199	44
AM04020-SS	(Alk-SMPT-C6)asaaguacaCfAfAfuuguuuuac(invdT)	197	44
AM04107-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaaa(invdT)	175	51
AM04107-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaaa(invdT)	175	51
AM04109-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaAMTM(invdT)	176	52
AM04111-SS	(Alk-C6-SMPT-C6)AMsCMguaaCfGfAfuuucaugaAMTM(invdT)	171	52
AM04113-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaasus(invdT)	178	54
AM04115-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaasus(invAb)	177	53
AM04117-SS	(Alk-C6-SMPT-C6)ascguaaCfGfAfuuucaugaau(invdT)	179	54
AM04119-SS	(Alk-C6-SMPT-C6)asacguaaCfGfAfuuucaugaau(invdT)	173	47
AM04121-SS	(Alk-C6-SMPT-C6)asacguaaCfGfAfuuucaugaaa(invdT)	172	46
AM04122-SS	(Alk-PEG5-C6)ascguaaCfGfAfuuucaugaaa(invdT)	181	51
AM04241-SS	(Alk-SMPT-C6)ascguaaCfGfAfuuucaugaaAM(invdT)	206	51
AM04243-SS	(Alk-SMPT-C6)ascguaaCfGfAfuuucaugaaAMs(invdT)	207	51
AM04246-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuucaugaaAMs(invdT)	186	72
AM04248-SS	(Alk-PEG5-C6)uauascguaaCfGfAfuuucaugaaAMs(invdT)	187	72
AM04451-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuucaugaaudTs(invdT)	188	74
AM04454-SS	(Alk-PEG5-C6)uAucsasacguAfaCfGfAfuuucaugAfaudTs(invdT)	192	77
AM04457-SS	(Alk-PEG5-C6)uAucsaacguAfaCfGfAfuuucaugAfaudTs(invdT)	191	77
AM03710-SS	(Alk-PEG5-C6)uAuascguaaCfGfAfuuuCfaugaaa(invdT)	189	72

Table 3. Hif2α RNAi trigger duplexes with Duplex ID numbers.

Duplex ID	Antisense Strand ID	SEQ ID NO.	Unmod. SEQ ID NO.	Sense Strand ID	SEQ ID NO.	Unmod. SEQ ID NO.
AD00086	AM00159-AS	112	21	AM00188-SS	265	79
AD00087	AM00160-AS	113	22	AM00189-SS	264	70
AD00088	AM00161-AS	106	17	AM00190-SS	263	65
AD00089	AM00162-AS	103	13	AM00191-SS	262	62
AD00102	AM00163-AS	110	19	AM00207-SS	258	66
AD00103	AM00164-AS	104	14	AM00208-SS	260	69
AD00104	AM00165-AS	111	20	AM00209-SS	256	64
AD00105	AM00166-AS	102	12	AM00210-SS	259	68
AD00106	AM00167-AS	137	27	AM00211-SS	253	61
AD00107	AM00168-AS	101	11	AM00212-SS	247	45
AD00108	AM00169-AS	135	24	AM00213-SS	248	48
AD00109	AM00170-AS	136	25	AM00214-SS	266	78
AD00110	AM00171-AS	105	15	AM00219-SS	249	49
AD00111	AM00172-AS	100	10	AM00220-SS	255	63
AD00112	AM00159-AS	112	21	AM00221-SS	267	79
AD00113	AM00160-AS	113	22	AM00222-SS	261	70
AD00114	AM00161-AS	106	17	AM00223-SS	257	65
AD00115	AM00162-AS	103	13	AM00224-SS	254	62
AD00215	AM00163-AS	110	19	AM00369-SS	222	68
AD00268	AM00166-AS	102	12	AM00369-SS	222	68
AD00269	AM00169-AS	135	24	AM00530-SS	212	48
AD00270	AM00170-AS	136	25	AM00531-SS	224	78
AD00274	AM00168-AS	101	11	AM00543-SS	211	45
AD00275	AM00171-AS	105	15	AM00544-SS	213	49
AD00276	AM00161-AS	106	17	AM00545-SS	219	65
AD00285	AM00163-AS	110	19	AM00366-SS	220	66
AD00286	AM00164-AS	104	14	AM00367-SS	223	69
AD00373	AM00169-AS	135	24	AM00659-SS	212	48
AD00374	AM00170-AS	136	25	AM00660-SS	224	78
AD00375	AM00168-AS	101	11	AM00679-SS	211	45
AD00376	AM00171-AS	105	15	AM00661-SS	213	49
AD00377	AM00161-AS	106	17	AM00662-SS	219	65
AD00988	AM01772-AS	116	23	AM01771-SS	271	56
AD00989	AM01777-AS	109	18	AM01776-SS	284	82
AD00990	AM01782-AS	96	7	AM00158-SS	277	67
AD00991	AM01786-AS	145	30	AM01785-SS	269	51
AD00992	AM01791-AS	99	9	AM01790-SS	283	81
AD00993	AM01773-AS	115	23	AM01771-SS	271	56
AD00994	AM01778-AS	108	18	AM01776-SS	284	82
AD00995	AM01783-AS	95	7	AM00158-SS	277	67
AD00996	AM01787-AS	144	30	AM01785-SS	269	51
AD00997	AM01792-AS	98	9	AM01790-SS	283	81

AD01020	AM01772-AS	116	23	AM01859-SS	218	56
AD01021	AM01773-AS	115	23	AM01859-SS	218	56
AD01022	AM01770-AS	114	23	AM01859-SS	218	56
AD01023	AM01777-AS	109	18	AM01860-SS	226	82
AD01024	AM01778-AS	108	18	AM01860-SS	226	82
AD01025	AM01775-AS	107	18	AM01860-SS	226	82
AD01026	AM01782-AS	96	7	AM01861-SS	221	67
AD01027	AM01783-AS	95	7	AM01861-SS	221	67
AD01028	AM01780-AS	94	7	AM01861-SS	221	67
AD01029	AM01786-AS	145	30	AM01862-SS	215	51
AD01030	AM01787-AS	144	30	AM01862-SS	215	51
AD01031	AM01784-AS	138	30	AM01862-SS	215	51
AD01032	AM01791-AS	99	9	AM01863-SS	225	81
AD01033	AM01792-AS	98	9	AM01863-SS	225	81
AD01034	AM01789-AS	97	9	AM01863-SS	225	81
AD01180	AM00171-AS	105	15	AM01994-SS	169	71
AD01214	AM01784-AS	138	30	AM02043-SS	231	51
AD01255	AM01784-AS	138	30	AM02093-SS	228	72
AD01256	AM02090-AS	143	30	AM01862-SS	215	51
AD01257	AM02091-AS	142	30	AM01862-SS	215	51
AD01258	AM02092-AS	139	30	AM01862-SS	215	51
AD01288	AM02133-AS	141	30	AM02135-SS	216	51
AD01289	AM01784-AS	138	30	AM02137-SS	217	51
AD01290	AM01784-AS	138	30	AM02139-SS	214	51
AD01291	AM02140-AS	128	31	AM02142-SS	276	59
AD01292	AM02140-AS	128	31	AM02144-SS	278	59
AD01293	AM02145-AS	127	33	AM02149-SS	280	75
AD01294	AM02146-AS	123	29	AM02163-SS	282	76
AD01295	AM02147-AS	126	32	AM02149-SS	280	75
AD01296	AM02150-AS	125	34	AM02149-SS	280	75
AD01391	AM01784-AS	138	30	AM01785-SS	269	51
AD01392	AM02327-AS	147	30	AM01785-SS	269	51
AD01404	AM02341-AS	130	28	AM02364-SS	281	76
AD01405	AM02341-AS	130	28	AM02365-SS	279	76
AD01406	AM02342-AS	134	28	AM02364-SS	281	76
AD01407	AM02342-AS	134	28	AM02365-SS	279	76
AD01408	AM02345-AS	129	28	AM02364-SS	281	76
AD01409	AM02345-AS	129	28	AM02365-SS	279	76
AD01410	AM02346-AS	133	28	AM02364-SS	281	76
AD01411	AM02346-AS	133	28	AM02365-SS	279	76
AD01424	AM02345-AS	129	28	AM02363-SS	230	76
AD01476	AM01784-AS	138	30	AM02456-SS	193	51
AD01522	AM02133-AS	141	30	AM02510-SS	232	51
AD01523	AM02509-AS	140	30	AM02512-SS	233	51
AD01524	AM02508-AS	120	30	AM02043-SS	231	51
AD01525	AM02523-AS	117	8	AM02522-SS	250	55

AD01526	AM02525-AS	119	26	AM02524-SS	268	80
AD01527	AM02527-AS	118	16	AM02526-SS	252	60
AD01528	AM02529-AS	148	35	AM02528-SS	251	58
AD01546	AM02523-AS	117	8	AM02546-SS	235	55
AD01547	AM02525-AS	119	26	AM02547-SS	242	80
AD01548	AM02527-AS	118	16	AM02548-SS	241	60
AD01549	AM02529-AS	148	35	AM02549-SS	240	58
AD01554	AM02604-AS	122	30	AM02512-SS	233	51
AD01555	AM02605-AS	124	30	AM02510-SS	232	51
AD01654	AM02848-AS	132	30	AM02852-SS	272	50
AD01655	AM02848-AS	132	30	AM02512-SS	233	51
AD01656	AM02849-AS	131	30	AM02853-SS	273	50
AD01657	AM02849-AS	131	30	AM02856-SS	234	51
AD01658	AM02850-AS	121	30	AM02853-SS	273	50
AD01659	AM02850-AS	121	30	AM02856-SS	234	51
AD01884	AM02998-AS	160	40	AM02510-SS	232	51
AD01885	AM02998-AS	160	40	AM02999-SS	229	72
AD01886	AM03000-AS	151	36	AM03004-SS	244	82
AD01887	AM03001-AS	149	36	AM03005-SS	246	82
AD01888	AM03002-AS	152	36	AM03006-SS	243	82
AD01889	AM03003-AS	150	36	AM03007-SS	245	82
AD01890	AM03008-AS	154	37	AM03010-SS	236	56
AD01891	AM03008-AS	154	37	AM03011-SS	238	56
AD01892	AM03009-AS	153	37	AM03012-SS	237	56
AD01893	AM03009-AS	153	37	AM03013-SS	239	56
AD01910	AM02605-AS	124	30	AM03058-SS	270	51
AD01911	AM03059-AS	146	30	AM03058-SS	270	51
AD02073	AM03009-AS	153	37	AM03307-SS	274	56
AD02074	AM02605-AS	124	30	AM03308-SS	170	51
AD02691	AM03465-AS	161	41	AM03467-SS	194	51
AD02692	AM03465-AS	161	41	AM03469-SS	201	51
AD02693	AM03465-AS	161	41	AM03471-SS	208	51
AD02694	AM03465-AS	161	41	AM03473-SS	200	51
AD02695	AM03465-AS	161	41	AM03475-SS	204	51
AD02733	AM03513-AS	162	41	AM03516-SS	203	51
AD02734	AM03514-AS	165	41	AM03516-SS	203	51
AD02735	AM03517-AS	90	4	AM03519-SS	202	54
AD02857	AM03685-AS	159	41	AM03687-SS	205	51
AD02858	AM03688-AS	167	41	AM03687-SS	205	51
AD02859	AM03689-AS	163	41	AM03687-SS	205	51
AD02860	AM03690-AS	166	41	AM03687-SS	205	51
AD02861	AM03685-AS	159	41	AM03692-SS	185	72
AD02862	AM03517-AS	90	4	AM03694-SS	183	73
AD02873	AM03465-AS	161	41	AM03708-SS	182	72
AD02874	AM03465-AS	161	41	AM03710-SS	189	72
AD02875	AM03517-AS	90	4	AM03712-SS	190	73

AD02876	AM03465-AS	161	41	AM03714-SS	180	72
AD02949	AM03465-AS	161	41	AM03774-SS	174	51
AD03011	AM03465-AS	161	41	AM03829-SS	184	72
AD03012	AM03465-AS	161	41	AM03830-SS	227	72
AD03013	AM03465-AS	161	41	AM03831-SS	275	50
AD03187	AM04001-AS	155	38	AM04003-SS	209	56
AD03188	AM04004-AS	84	1	AM04006-SS	210	57
AD03189	AM04007-AS	156	39	AM04009-SS	195	43
AD03190	AM04010-AS	85	2	AM04012-SS	196	44
AD03191	AM04010-AS	85	2	AM04014-SS	198	44
AD03192	AM04015-AS	83	2	AM04017-SS	199	44
AD03193	AM04018-AS	86	2	AM04020-SS	197	44
AD03215	AM04040-AS	168	41	AM03469-SS	201	51
AD03216	AM04040-AS	168	41	AM03475-SS	204	51
AD03253	AM03689-AS	163	41	AM04107-SS	175	51
AD03254	AM04101-AS	91	4	AM04117-SS	179	54
AD03255	AM04102-AS	164	42	AM04121-SS	172	46
AD03256	AM04103-AS	92	5	AM04119-SS	173	47
AD03257	AM04104-AS	157	41	AM04107-SS	175	51
AD03258	AM04105-AS	88	4	AM04117-SS	179	54
AD03259	AM04106-AS	87	3	AM04109-SS	176	52
AD03260	AM04105-AS	88	4	AM04109-SS	176	52
AD03261	AM04105-AS	88	4	AM04111-SS	171	52
AD03262	AM04105-AS	88	4	AM04113-SS	178	54
AD03263	AM04105-AS	88	4	AM04115-SS	177	53
AD03264	AM03690-AS	166	41	AM04107-SS	175	51
AD03265	AM03689-AS	163	41	AM03692-SS	185	72
AD03266	AM03689-AS	163	41	AM04122-SS	181	51
AD03345	AM03689-AS	163	41	AM04241-SS	206	51
AD03346	AM03689-AS	163	41	AM04243-SS	207	51
AD03347	AM04244-AS	158	41	AM04243-SS	207	51
AD03348	AM03689-AS	163	41	AM04246-SS	186	72
AD03349	AM03689-AS	163	41	AM04248-SS	187	72
AD03505	AM04101-AS	91	4	AM04451-SS	188	74
AD03506	AM04452-AS	93	6	AM04454-SS	192	77
AD03507	AM04455-AS	89	6	AM04454-SS	192	77
AD03508	AM04452-AS	93	6	AM04457-SS	191	77

A sense strand containing a sequence listed in Table 2B can be hybridized to any antisense strand containing a sequence listed in Table 2A provided the two sequences have a region of at least 90% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence. Representative Hif2α RNA triggers are represented by the Duplex ID Nos. shown in Table 3. In some embodiments an Hif2α RNAi trigger consists of any of the Duplex ID Nos. presented herein. In some embodiments an Hif2α RNAi trigger comprises of any of the Duplex ID Nos.

presented herein. In some embodiments, an Hif2α RNAi trigger comprises the sense strand and antisense strand nucleotide sequences of any of the Duplex ID Nos. presented herein. In some embodiments, an Hif2α RNAi trigger comprises the sense strand and antisense strand nucleotide sequences of any of the Duplex ID Nos. presented herein and a targeting group and/or linking group wherein the targeting group and/or linking group is covalently linked to the sense strand or the antisense strand. In some embodiments, an Hif2α RNAi trigger comprises the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein. In some embodiments, an Hif2α RNAi trigger comprises the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein and a targeting group and/or linking group wherein the targeting group and/or linking group is covalently linked to the sense strand or the antisense strand.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of nucleotides 2-21 of SEQ ID NO. 4. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of nucleotides 2-21 of SEQ ID NO. 4 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 53.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 88, SEQ ID NO. 157, SEQ ID NO. 159, or SEQ ID NO. 163. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 88 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 179. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 88 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 177. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 157 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 175. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 159 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 185. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 163 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 185.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ

ID NO. 88, SEQ ID NO. 157, SEQ ID NO. 159, or SEQ ID NO. 163. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 88 and a sense strand comprising SEQ ID NO. 179. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 88 and a sense strand comprising SEQ ID NO. 177. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 157 and a sense strand comprising SEQ ID NO. 175. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 159 and a sense strand comprising SEQ ID NO. 185. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 163 and a sense strand comprising SEQ ID NO. 185.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of nucleotides 2-21 of SEQ ID NO. 38. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of nucleotides 2-21 of SEQ ID NO. 38 and a sense strand comprising a nucleotide base sequence of nucleotides 1-19 of SEQ ID NO. 56.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 86, SEQ ID NO. 155, SEQ ID NO. 156. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 156 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 195. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 86 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 197. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising a nucleotide base sequence of SEQ ID NO. 155 and a sense strand comprising a nucleotide base sequence of SEQ ID NO. 209.

In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 86, SEQ ID NO. 155, SEQ ID NO. 156. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 156 and a sense strand comprising SEQ ID NO. 195. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 86 and a sense strand comprising SEQ ID NO. 197. In some embodiments, a Hif2α RNAi trigger comprises an antisense strand comprising SEQ ID NO. 155 and a sense strand comprising SEQ ID NO. 209.

In some embodiments, an Hif2α RNAi trigger contains or is conjugated to a targeting group, linking group, delivery polymer, delivery vehicle, and/or other non-nucleotide group. The targeting group, linking group, delivery polymer, delivery vehicle, and/or other non-nucleotide group can be covalently linked to the 3′ and/or 5′ end of either the sense strand and/or the antisense strand. In some embodiments, an Hif2α RNAi trigger can contains a targeting group, linking group, delivery polymer, delivery vehicle, or other non-nucleotide group linked to the 3′ and/or 5′ end of the sense strand. In some embodiments a targeting group, linking group, delivery polymer, delivery vehicle, or other non-nucleotide group is linked to the 5′ end of an Hif2α RNAi trigger sense strand. In some embodiments, the targeting group, linking group, delivery polymer, delivery vehicle, and/or other non-nucleotide group is linked directly or indirectly to the trigger via a linker/linking group. In some embodiments, targeting group, linking group, delivery polymer, delivery vehicle, and/or other non-nucleotide group is linked to the trigger via a labile, cleavable, or reversible bond or linker.

A targeting group can enhance the pharmacokinetic or biodistribution properties of an RNAi trigger or conjugate to which it is attached to improve cell- or tissue-specific distribution and cell-specific uptake of the conjugate. In some instances, binding of a targeting group to a cell or cell receptor may initiate endocytosis. A targeting group can be monovalent, divalent, trivalent, tetravalent, or have higher valency. Representative targeting groups include, without limitation, compounds with affinity to cell surface molecule, cell receptor ligands, hapten, antibodies, monoclonal antibodies, antibody fragments, and antibody mimics with affinity to cell surface molecules.

The RNAi trigger molecules described herein may be synthesized having a reactive group, such as an amine group, at the 5′-terminus. The reactive group may be used to subsequently attach a targeting moiety using methods typical in the art.

In some embodiments, an Hif2α RNAi trigger includes a linking group conjugated to the trigger. The linking group facilitates covalent linkage of the trigger to a targeting group or delivery polymer or delivery vehicle. The linking group can be linked to the 3′ or the 5′ end of the RNAi trigger sense strand or antisense strand. In some embodiments, the linking group is linked to the RNAi trigger sense strand. In some embodiments, the linking group is conjugated to the 5′ or 3′ end of an RNAi trigger sense strand. In some embodiments a linking group is

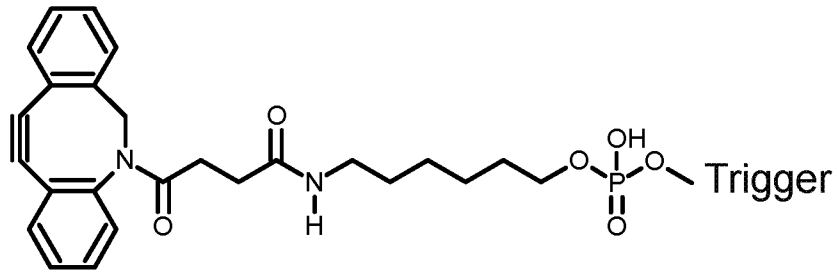
conjugated to the 5′ end of an RNAi trigger sense strand. Examples of linking groups, include, but are not limited to: Alk-SMPT-C6, Alk-SS-C6, DBCO-TEG, Me-Alk-SS-C6, and C6-SS-Alk-Me, reactive groups such a primary amines and alkynes, alkyl groups, abasic ribose, ribitol, and/or PEG groups.

A linker or linking group is a connection between two atoms that links one chemical group (such as an RNAi trigger) or segment of interest to another chemical group (such as a targeting group or delivery polymer) or segment of interest via one or more covalent bonds. A labile linkage contains a labile bond. A linkage may optionally include a spacer that increases the distance between the two joined atoms. A spacer may further add flexibility and/or length to the linkage. Spacers may include, but are not be limited to, alkyl groups, alkenyl groups, alkynyl groups, aryl groups, aralkyl groups, aralkenyl groups, and aralkynyl groups; each of which can contain one or more heteroatoms, heterocycles, amino acids, nucleotides, and saccharides. Spacer groups are well known in the art and the preceding list is not meant to limit the scope of the description.

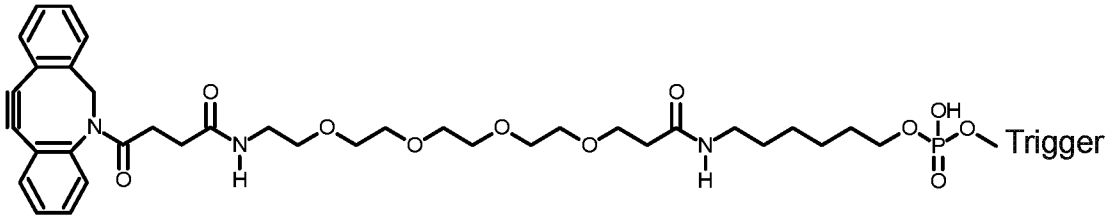
Targeting groups and linking groups include, but are not limited to, (Alk-C6), (Alk-C6-C6), (Alk-C6-SMPT-C6), (Alk-PEG5-C6), (Alk-PEG5-C6)(Alk-PEG5-Ser), (Alk-SMPT-C6), (Alk-SS-C6), (C6-SS-Alk-Me), (Chol-TEG), (DBCO-TEG), (Me-Alk-SS-C6), (NAG13), (NH2-C6). In some embodiments, any of the Hif2α RNAi trigger sense strands listed in Table 2B which contains a 3′ or 5′ targeting group or linking group, may alternatively contain no 3′ or 5′ targeting group or linking group, or may contain a different 3′ or 5′ targeting group or linking group including, but not limited to, those depicted in Table 4.

In some of the targeting group and linking group structures shown in Table 4, the RNAi trigger is shown and denoted by Trigger, RNA, R, or R1 or R2 (i.e. Trigger, RNA or R1 or R2 each comprises the RNAi trigger). For example, with respect to (Alk-C6-Ser), (Alk-PEG5-Ser), and (Alk-PEG13-Ser), one of R1 and R2 comprises the RNAi trigger and the other can be a hydrogen.

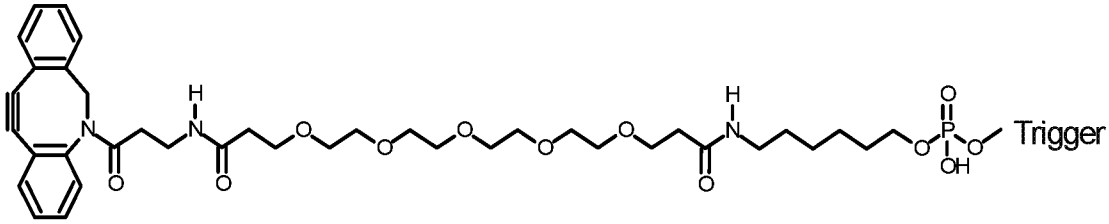
Table 4. Structures representing targeting groups and linking groups.



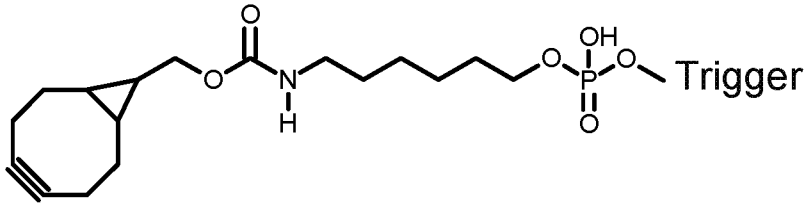
(Alk-C6)−Trigger



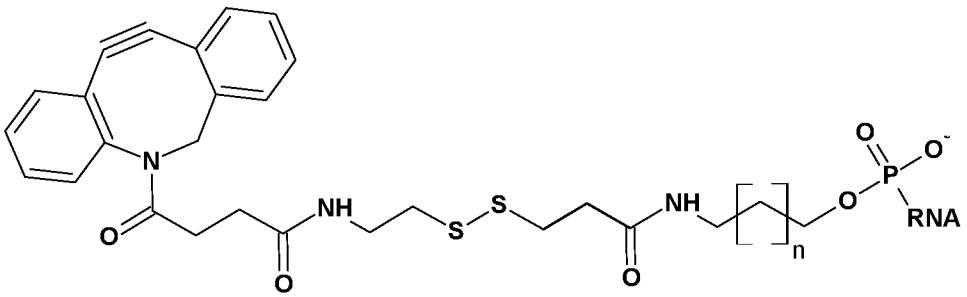
(Alk-PEG4-C6)−Trigger



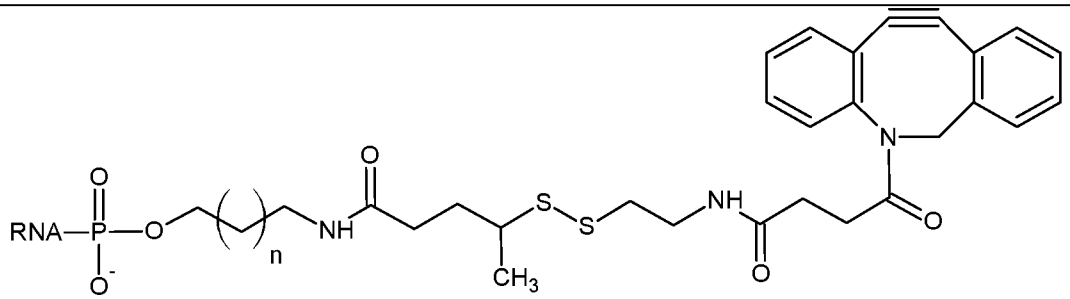
(Alk-PEG5-C6)−Trigger or Trigger−(C6-PEG5-Alk)



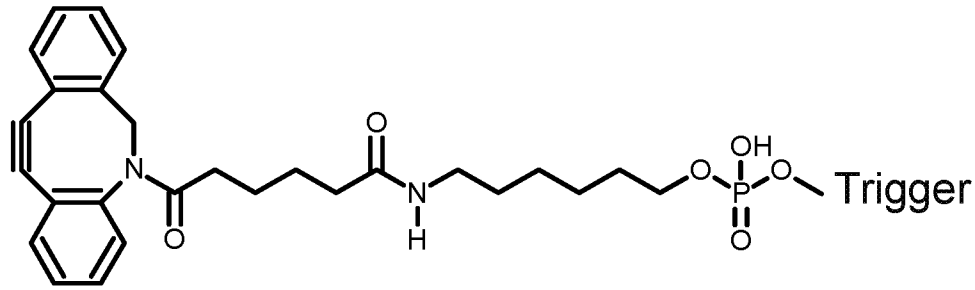
(Alk-BC9-C6)−Trigger



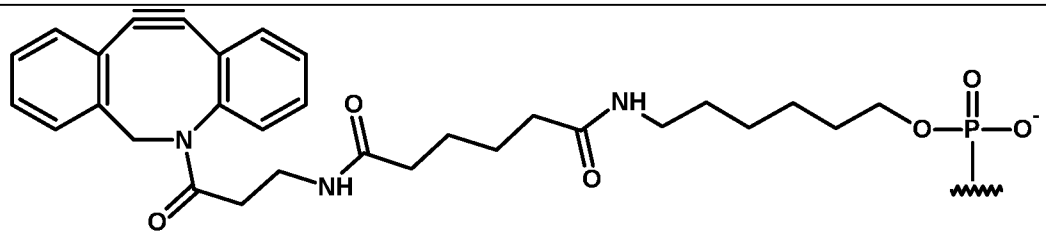
(Alk-SS-C6)−RNA or RNA−(C6-SS-Alk); (n = 1-10), In some embodiments, n = 4



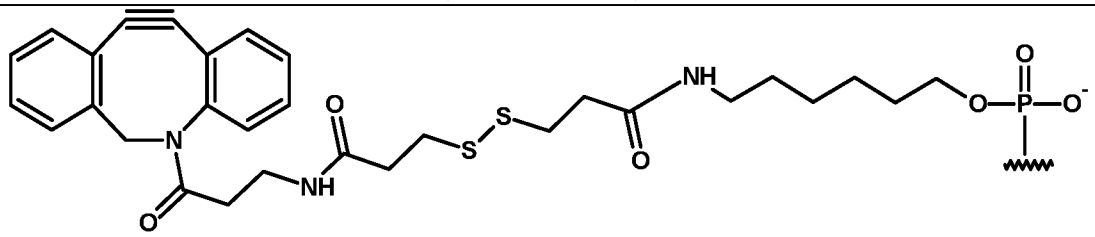
RNA−(C6-SS-Alk-Me) or ((Me-Alk-SS-C6)−RNA; (n = 1-10), In some embodiments, n = 4.



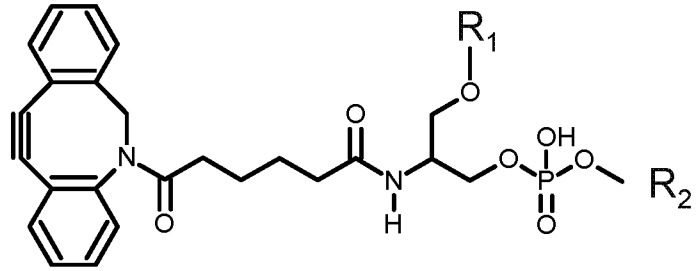
(Alk-C6-C6)−Trigger or Trigger−(C6-C6-Alk)



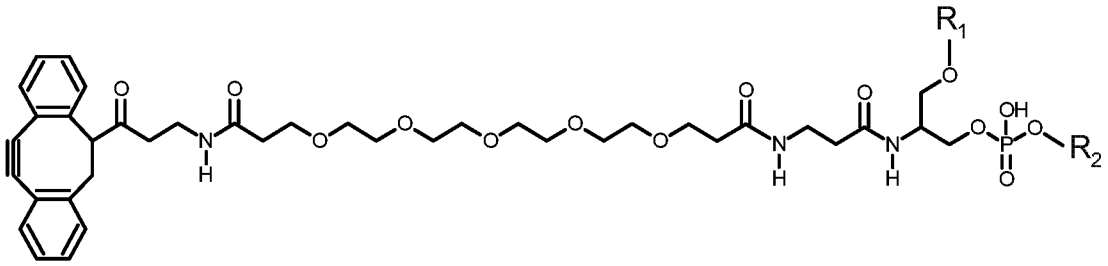
(Alk-NHCO-C6)



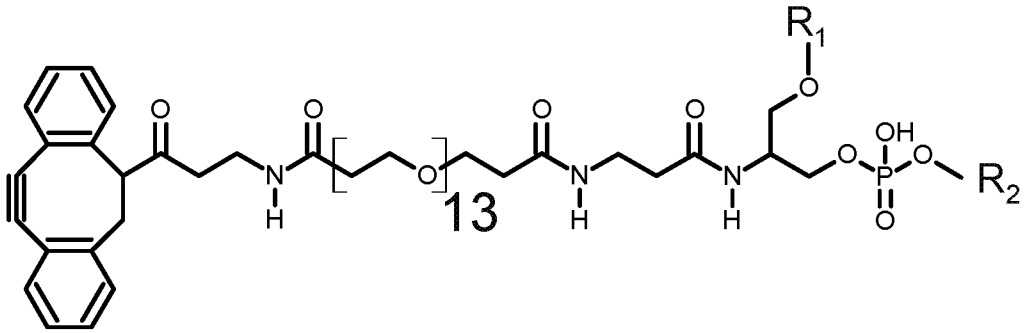
(Alk-NHCO-SS-C6)



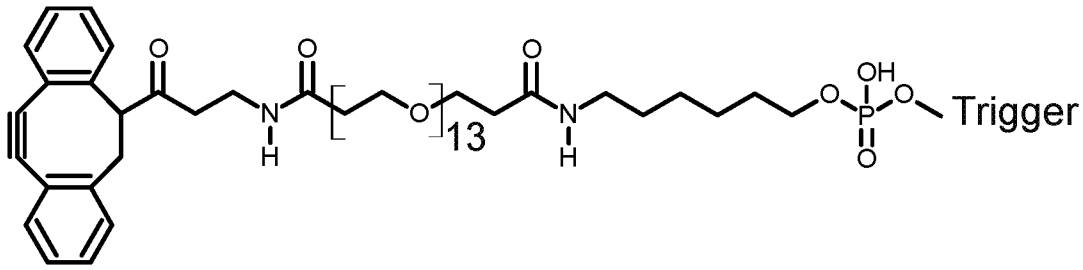
(Alk-C6-Ser)−RNA or RNA−(Ser-C6-Alk), RNA is R1 or R2



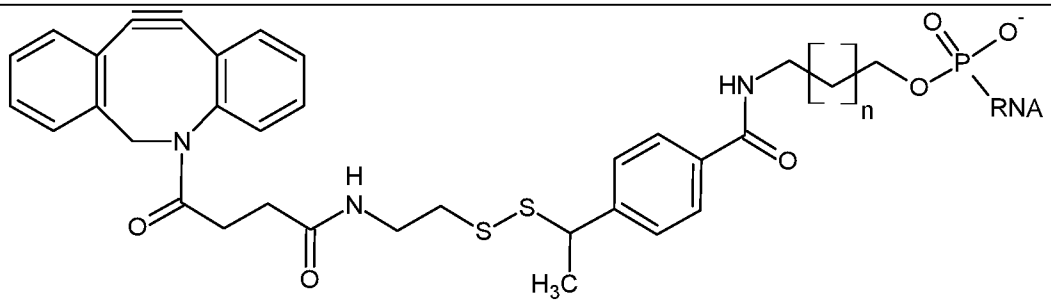
(Alk-PEG5-Ser)−RNA, RNA is R1 or R2



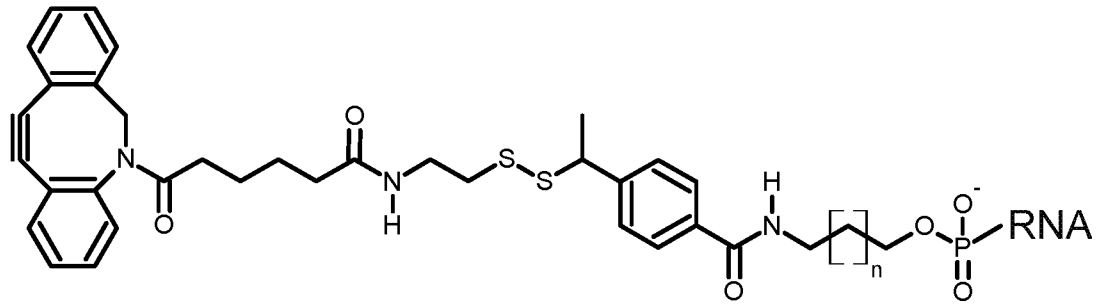
(Alk-PEG13-Ser)−RNA, RNA is R1 or R2



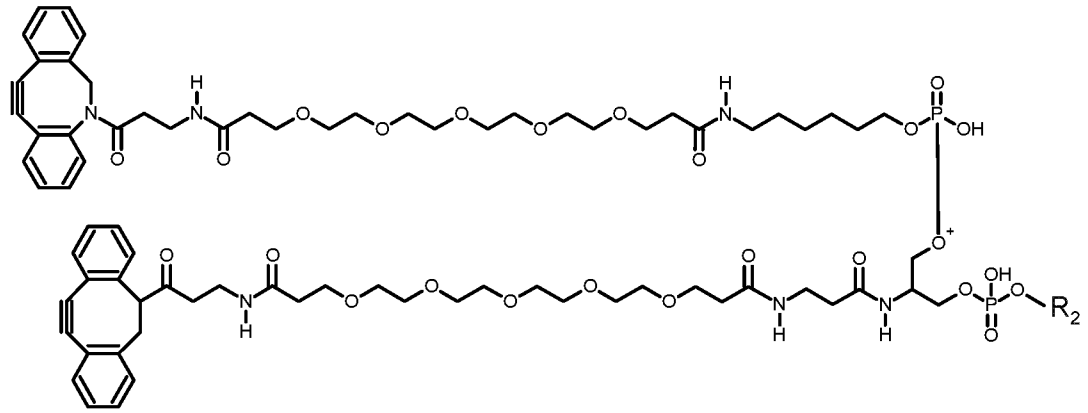
(Alk-PEG13-C6)−Trigger



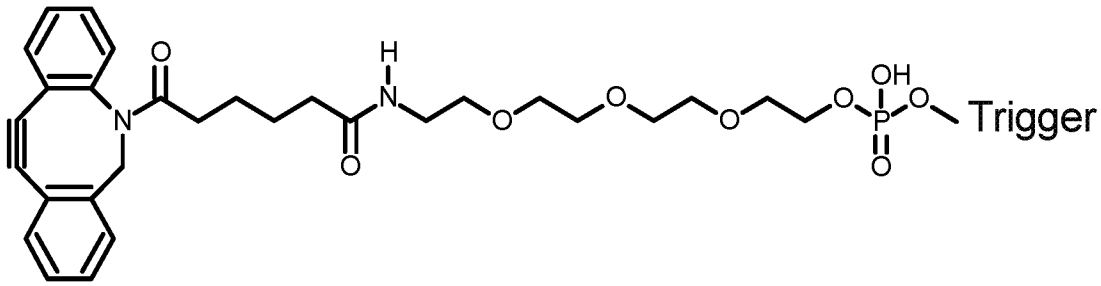
RNA−(C6-SMPT-Alk) or (Alk-SMPT-C6)−RNA, n = 1-10, In some embodiments, n = 4



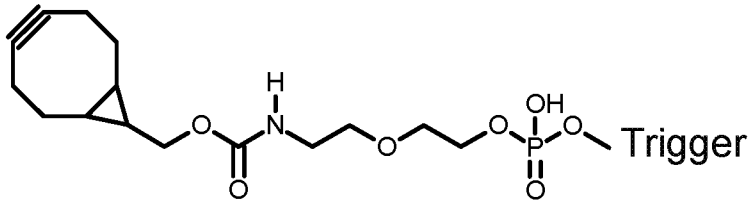
(Alk-C6-SMPT-C6)−RNA or RNA-(C6-SMPT-C6-Alk),
n=1-10, In some embodiments, n = 4



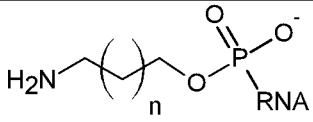
(Alk-PEG5-C6)(Alk-PEG5-Ser)−RNA



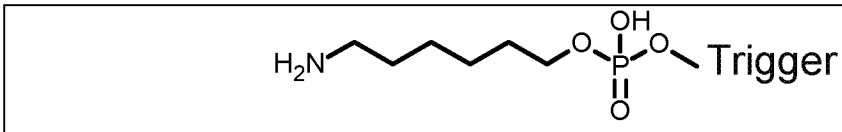
(DBCO-TEG)−Trigger



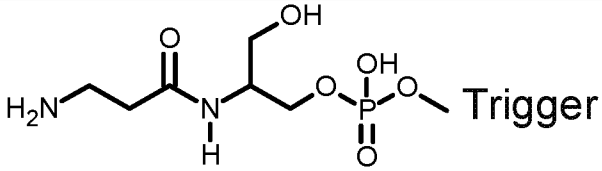
(BCN)−Trigger



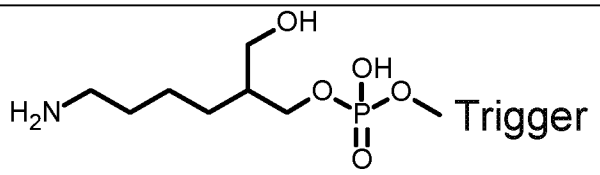
(NH_2-C_n)-RNA, n = 1-10



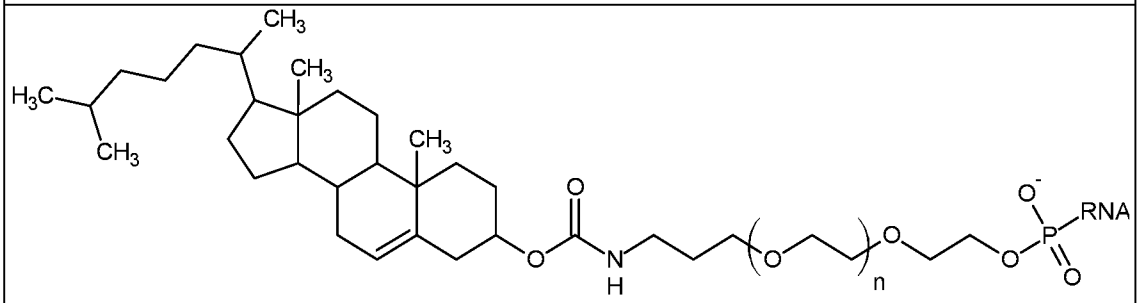
(NH2-C6)−Trigger or Trigger−(C6-NH2)



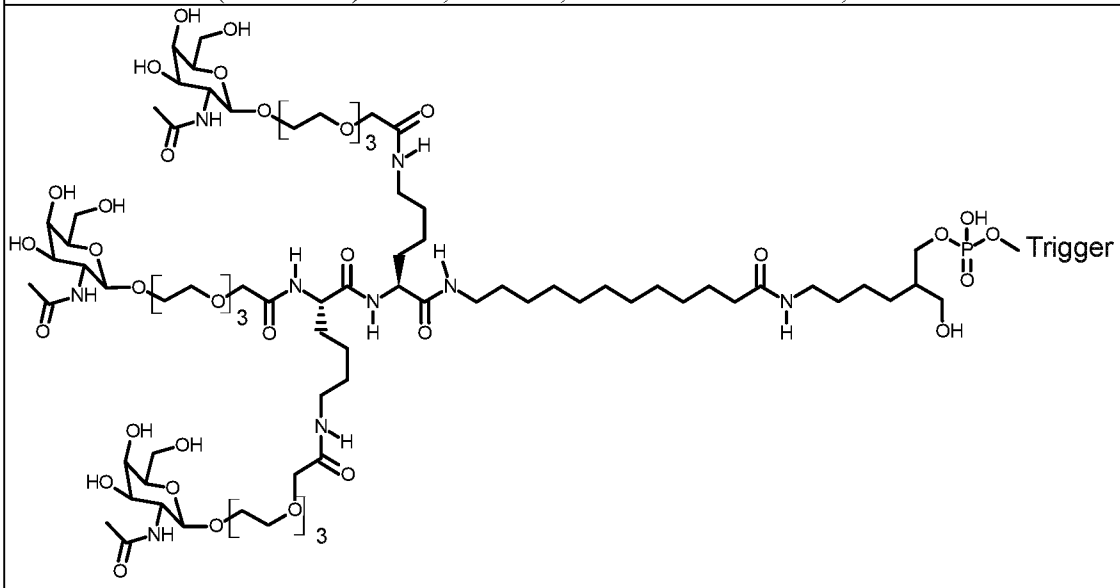
(NH2-Ser)−Trigger or Trigger−(Ser-NH2)



(NH2-C7)−Trigger



(Chol-TEG)−RNA, n = 1-10, In some embodiments, n = 2.



(NAG13)−Trigger

In some embodiments, a delivery vehicle may be used to deliver an RNAi trigger to a cell or tissue. A delivery vehicle is a compound that improves delivery of the RNAi trigger to a cell

or tissue. A delivery vehicle can include, or consist of, but is not limited to: a polymer, such as an amphipathic polymer, a membrane active polymer, a peptide, a melittin peptide, a melittin-like peptide, a lipid, a reversibly modified polymer or peptide, or a reversibly modified membrane active polyamine.

In some embodiments, the RNAi triggers can be combined with lipids, nanoparticles, polymers, liposomes, micelles, DPCs or other delivery systems available in the art. The RNAi triggers can also be chemically conjugated to targeting groups, lipids (including, but not limited to cholesterol and cholesteryl derivatives), nanoparticles, polymers, liposomes, micelles, DPCs (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, WO 2013/032829, WO 2013/158141, each of which is incorporated herein by reference), or other delivery systems available in the art.

In some embodiments, pharmaceutical compositions for delivering an Hif2α RNAi trigger to a tumor cell *in vivo* are described. Such pharmaceutical compositions can include, but are not limited to, an Hif2α RNAi trigger conjugated to delivery polymer to form an RNAi trigger-delivery polymer conjugate. In some embodiments, the delivery polymer is a membrane active polyamine. In some embodiments, the delivery polymer is a reversibly modified membrane active polyamine.

Hif2α RNAi Trigger-Delivery Polymer Conjugates

In some embodiments, we describe compositions represented by the formula:

$$(\text{RNAi trigger})_n\text{–}\mathbf{P}\begin{matrix}\diagup (L^1\text{–}M^1)_x \\ \diagdown (L^2\text{–}M^2)_y,\end{matrix} \qquad \text{(formula 1)}$$

wherein RNAi trigger is an Hif2α RNAi trigger as described herein, P is a membrane active polyamine, M^1 comprises a targeting group linked to P via reversible physiologically labile linkage L^1, and M^2 comprises a steric stabilizer linked to P via reversible physiologically labile linkage L^2, x is greater than 1, y is greater than or equal to 0. $(M^2\text{–}L^2)_y\text{–}P\text{–}(L^1\text{–}M^1)_x$, is not membrane active. As used herein, $(M^2\text{–}L^2)_y\text{–}P\text{–}(L^1\text{–}M^1)_x$ refers to a delivery polymer. Cleavage of $(L^1\text{–}M^1)$ and $(M^2\text{–}L^2)$ restores P to a membrane active state. In some embodiments, the value of x+y is greater than 80%, greater than 90%, or greater than 95% of the number of primary amines of P. In some embodiments, the value of x+y is greater than 80%, greater than 90%, or greater than 95% of the number of primary amines on a population of P. The value of

n can be from 0.25 to 5 (one (1) RNAi trigger per every 4 polymers to 5 RNAi triggers per polymer). In some embodiments, the value of n is 0.5 to 5. In some embodiments, n is 0.5-2. In some embodiments, n is 0.8-1.6. In some embodiments, x is 1-20, 2-20, 3-20, 4-20, 5-20, 6-20, 7-20, 8-20, 9-20, 10-20, 11-20, 12-20, 13-20, 14-20, or 15-20.

In some embodiments, M^1 comprises an integrin-binding compound. In some embodiments, the integrin-binding compound comprises an $\alpha_v\beta_3$-binding compound. In some embodiments, the integrin-binding compound comprises an RGD ligand. In some embodiments, the $\alpha_v\beta_3$-binding compound comprises an RGD ligand. In some embodiments the RGD ligand comprises an RGD mimic. In some embodiments, the steric stabilizer comprises a polyethylene glycol (PEG). In some embodiments, cleavage of L^1 and/or L^2 restores an unmodified amine on P. In some embodiments, (L^1–M^1) and (L^2–M^2) are independently tetrapeptide modifying agents and/or dipeptide modifying agents. In some embodiments, L^1 and L^2 are independently tetrapeptide linkages or dipeptide-PABC (*p*-amidobenzyl-carbamate) linkages. In some embodiments, L^1 and L^2 are tetrapeptide linkages. In other embodiments, L^1 and L^2 are dipeptide-PABC linkages. In some embodiments, L^1 is a dipeptide-PABC linkage and L^2 is a tetrapeptide linkage. In other embodiments, L^1 is a tetrapeptide linkage and L^2 is a dipeptide-PABC linkage. In some embodiments, a tetrapeptide linkage is an FCitFP (Phenylalanine-Citrulline-Phenylalanine-Proline) tetrapeptide linkage. In some embodiments, a dipeptide-PABC linkage is an ACit-PABC linkage. For x = 2 or more, L^1 can be all tetrapeptide linkages, all dipeptide-PABC linkages, or a combination tetrapeptide linkages and dipeptide-PABC linkages. For y = 2 or more, L^2 and be all tetrapeptide linkages, all dipeptide-PABC linkages, or a combination tetrapeptide linkages and dipeptide-PABC linkages.

In some embodiments, a described Hif2α RNAi trigger is conjugated to a reversibly modified membrane active polyamine to form an RNAi trigger-delivery polymer conjugate. In some embodiments, the RNAi trigger-delivery polymer conjugate comprises the formula represented by:

$$(\text{RNAi trigger})_n\text{–poly}(\mathbf{A}_a\text{–}\textit{co}\text{–}(\mathbf{B}_b\text{–}\textit{graft}\text{–}(\mathbf{C}_c; \mathbf{D}_d))) \quad \text{(formula 2)}$$

wherein

A is a hydrophobic group-containing monomeric unit,

B is a primary amine-containing monomeric unit,

C comprises an integrin-binding ligand linked (i.e., *grafted*) to a primary amine-containing monomeric unit via a reversible physiologically labile linkage,

D comprises a steric stabilizer linked (i.e., *grafted*) to a primary amine-containing monomeric unit via a reversible physiologically labile linkage,

a is an integer greater than zero,

b is an integer greater than or equal to two,

c is an integer greater than or equal to one,

d is an integer greater than or equal to one,

the value of **c**+ **d** is greater than 80%, greater than 85%, greater than 90%, or greater than 95% of the value of **b**,

poly($\mathbf{A_a}$–*co*–$\mathbf{B_b}$) is a membrane active polyamine copolymer having **A** and **B** monomeric units

RNAi trigger comprises a Hif2α RNAi trigger described herein, and

n has a value from 0.25 (i.e., conjugated to only one out of every four delivery polymers) to 5.0.

Poly($\mathbf{A_a}$–*co*–($\mathbf{B_b}$–*graft*–($\mathbf{C_c}$; $\mathbf{D_d}$))) is not membrane active. In some embodiments, the integrin-binding compound comprises an $\alpha_v\beta_3$-binding compound. In some embodiments, the integrin-binding compound comprises an RGD ligand, such as an RGD mimic. In some embodiments, the $\alpha_v\beta_3$-binding compound comprises an RGD ligand, such as an RGD mimic. In some embodiments, the steric stabilizer comprises a polyethylene glycol (PEG). In some embodiments, the PEG contains 2 to 25 ethylene glycol units. In some embodiments, c is any integer from 1-75, 1-50, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 10-35, 10-30, 10-25, 10-20, or 15-20. In some embodiments, n has a value from 0.5 to 2. In some embodiments, the ratio **A**:**B** (i.e., **a**:**b**) is 30:70 to 60:40. In some embodiments, the ratio **A**:**B** is 60:40 to 40:60. In some embodiments, the ratio **A**:**B** is about 45±5:55±5. In some embodiments, the ratio **A**:**B** is about 44:56. In some embodiments, the ratio **A**:**B** is about 46:54. In some embodiments, the molecular weight (Mw) of the polymer is 30 kDa - 70 kDa. In other embodiments, the Mw of the polymer is 40 kDa - 60 kDa. In other embodiments, the Mw of the polymer is 40 kDa - 50 kDa. In yet other embodiments, the Mw of the polymer about 43 kDa to about 48 kDa. In some embodiments, the polymer has a polydispersity index (PDI) less than 1.4, less than 1.3, 1.25, less than 1.2, less than 1.15, or less than 1.1. In some embodiments, the polymer contains a terminal azide group for attachment of an RNAi trigger. In some embodiments, n is 0.8-1.6. In some embodiments, n is 1±0.5. In some embodiments, **c** is 1-20, 2-20, 3-20, 4-20, 5-20, 6-20, 7-20, 8-20, 9-20, 10-20, 11-20, 12-20, 13-20, 14-20, or 15-20. In some embodiments, the value of **c**+**d** is greater than 80%, greater than 90%, or greater than 95% of the value of **b**. In some

embodiments, **C** is RGD-PEG$_x$-FcitFPro and **D** is PEG$_y$–ACit–PABC, wherein x is 1-50, y is 4-30. In some embodiments, x is greater than y.

In some embodiments, polyamine poly(**A**$_a$–*co*–**B**$_b$) is a poly(acrylate) random copolymer wherein **A** is a hydrophobic group-containing acrylate monomer and **B** is a primary amine-containing acrylate monomer. In some embodiments **A** is a propyl acrylate monomer and **B** is an ethoxy-ethylamine acrylate monomer.

Membrane active polyamines are membrane active and therefore capable of disrupting plasma membranes or lysosomal/endocytic membranes. As used herein, membrane active polyamines are surface active, amphipathic polymers that are able to induce one or more of the following effects upon a biological membrane: an alteration or disruption of the membrane that allows non-membrane permeable molecules to enter a cell or cross the membrane, pore formation in the membrane, fission of membranes, or disruption or dissolving of the membrane. As used herein, a membrane, or cell membrane, comprises a lipid bilayer. The alteration or disruption of the membrane can be functionally defined by the peptide's activity in at least one the following assays: red blood cell lysis (hemolysis), liposome leakage, liposome fusion, cell fusion, cell lysis, and endosomal release. Peptides, or modified peptides that preferentially cause disruption of endosomes or lysosomes over plasma membranes are considered endosomolytic. A reversibly modified membrane active polyamine is an example of an endosomolytic peptide. The effect of membrane active polymers on a cell membrane may be transient. Membrane active polymers possess affinity for the membrane and cause a denaturation or deformation of bilayer structures. Delivery of a RNAi trigger to a cell is mediated by the membrane active polyamine disrupting or destabilizing the plasma membrane or an internal vesicle membrane (such as an endosome or lysosome), including forming a pore in the membrane, or disrupting endosomal or lysosomal vesicles thereby permitting release of the contents of the vesicle into the cell cytoplasm. A preferred polymer is an amphipathic poly(acrylate) random copolymer.

Integrin-Binding Compound

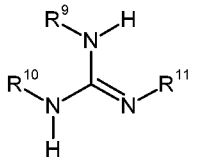
An integrin-binding compound has affinity for one or more integrins expressed on a cell surface. A non-limiting example of an integrin includes an $\alpha_v\beta_3$ integrin. Examples of integrin-binding compounds include, but are not limited to: $\alpha_v\beta_3$-binding compounds, RGD ligand. RGD ligands include RGD peptide-containing compounds and RGD mimic-containing

compounds. As used herein, an RGD peptide comprises an arginine-glycine-aspartate tripeptide. An RGD peptide may be conformationally constrained. An RGD peptide may have non-peptide components linked to the RGD amino acid sequence.

As used herein, an RGD ligand comprises an RGD peptide or RGD mimic <1500 kDa in size that binds to (has affinity for) an integrin, such as an alpha v/beta 3 (αvβ3 or $\alpha_v\beta_3$) integrin.

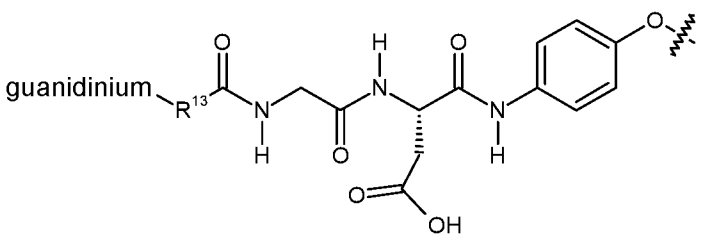
As used herein, an RGD mimic is a non-peptide synthetic molecule other than an RDG peptide that biologically mimics the active determinants of an RGD peptide, an integrin-binding RGD portion of an integrin-binding protein, or an $\alpha_v\beta_3$ integrin-binding RGD motif. An RGD mimic may contain one or two naturally occurring amino acids linked via amide bonds. An RGD mimetic may be a modified peptide, contain non-standard amino acids or non-standard amino acid side chains.

In one embodiment, an RGD ligand comprises a guanidinium group linked to a glycine-aspartate dipeptide via an amide bond. Guanidinium groups of the invention have the structure represented by:

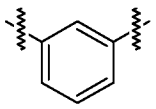
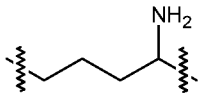


wherein R^9 and R^{10} are independently hydrogen or alkyl and may by connected to form a ring, and R^{11} is a linker connecting the guanidinium group to the glycine-aspartate dipeptide. The guanidinium group includes both the structure represented above and its resonance structures. A preferred linker is: −(CRR′)−(CRR′)−(CRR′)− or −(CRR′)−(CRR′)−(CRR′)−(CRR')−, wherein: a) each R is independently optional and if present is independently hydrogen, alkyl, or aryl, b) R' is independently hydrogen, alkyl, aryl, or NH_2, and c) each carbon (C) may be linked by single bonds, a single bond and a double bond, or aromatic bonds.

In some embodiments, an RGD mimic contains a phenoxy group attached to the aspartate amino acid. In some embodiments, an RGD mimic comprises a quanidinium-glycine-aspartate-4-aminophenoxy compound. In some embodiments, a quanidinium-glycine-aspartate-4-aminophenoxy compound comprises the structure represented by:

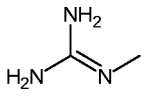


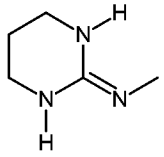
wherein R^{13} is:


or


.

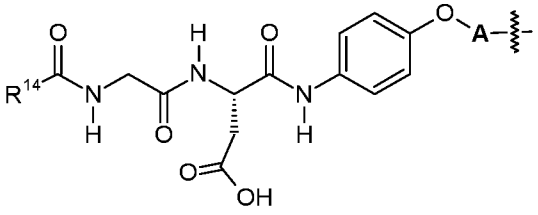
In some embodiments, a guanidinium is



or


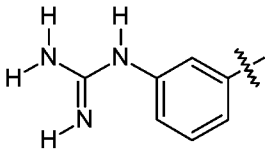
and their resonance structures.

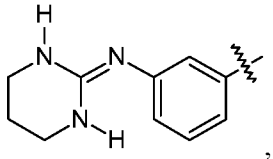
In some embodiments, an RGD mimic comprises the structure represented by:



wherein:

R^{14} is



or


, and

A comprises a linker. The linker connects the RGD mimic to another molecule such as a dipeptide amidobenzyl-carbonate or tetrapeptide, provides for increased solubility, or provides a means for covalent linkage to another molecule.

Steric stabilizer

As used herein, a steric stabilizer is a non-ionic hydrophilic polymer (either natural, synthetic, or non-natural) that prevents or inhibits intramolecular or intermolecular interactions of a polymer to which it is attached relative to the polymer containing no steric stabilizer. A steric

stabilizer hinders a polymer to which it is attached from engaging in electrostatic interactions. Electrostatic interaction is the non-covalent association of two or more substances due to attractive forces between positive and negative charges. Steric stabilizers can inhibit interaction with blood components and therefore opsonization, phagocytosis, and uptake by the reticuloendothelial system. Steric stabilizers can thus increase circulation time of molecules to which they are attached. Steric stabilizers can also inhibit aggregation of a polymer. In some embodiments, a steric stabilizer is a polyethylene glycol (PEG) or PEG derivative. In some embodiments, a PEG can have about 1-500 ethylene monomers or units. In some embodiments, the PEG contains 2-25 ethylene units. In some embodiments, the PEG contains 4-30 ethylene units. In some embodiments, PEG contains 5-24 ethylene units. In some embodiments, a PEG has a molecular weight average of about 85-20,000 Daltons (Da). In some embodiments a PEG has a molecular weight of about 85-1000 Da. As used herein, steric stabilizers prevent or inhibit intramolecular or intermolecular interactions of a polymer to which it is attached relative to the polymer containing no steric stabilizer in aqueous solution.

Reversible Physiologically Labile Linkages/Modifying Agents

A membrane active polyamine may be reversibly modified. Reversible modification can be accomplished through reversible attachment of modifying agents to primary amines of the membrane active polyamine.

In some embodiments, a reversible physiologically labile linkage comprises a tetrapeptide linkage. In some embodiments, $P–(L^1–M^1)_x$ and/or $P–(L^2–M^2)_y$ (of formula 1) comprises:

$$R^5–A^4–A^3–A^2–A^1–R^6$$

wherein

R^5 comprises a targeting group (M^1) or a steric stabilizer (M^2),

A^4 is a natural, non-natural isomeric, or synthetic hydrophobic L amino acid wherein the hydrophobicity index (Monera et al, J. Protein Sci. 1995, 1, 319) at pH 7 is 41 or greater, normalized to glycine, as it relates to the composition of the amino acid side chain (R-group),

A^3 is an uncharged hydrophilic L amino acid wherein the hydrophobicity index (Monera et al, J. Protein Sci. 1995, 1, 319) at pH 7 is −28 or less, normalized to glycine, as it relates to the composition of amino acid side chain (R-group),

A^2 is a natural, non-natural isomeric, or synthetic hydrophobic L amino acid wherein the hydrophobicity index (Monera et al, J. Protein Sci. 1995, 1, 319) at pH 7 is 41 or greater,

normalized to glycine, as it relates to the composition of the amino acid side chain (R-group),

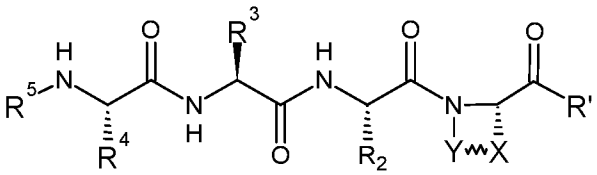
A^1 is L-proline, L-leucine, or L-N-methyl alanine, and

R^6 is P, wherein P is a membrane active polyamine of formula 1.

In some embodiments, A^1 is proline, A^2 and A^4 are independently alanine, valine, leucine, isoleucine or phenylalanine (side chains of $-CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$, or $-CH_2C_6H_6$, respectively), and A^3 is citrulline or asparagine (side chains or $-(CH_2)_3NHCONH_2$ or $-CH_2CONH_2$, respectively).

In some embodiments, A^1 is proline, A^2 and A^4 are phenylalanine, and A^3 is citrulline (FCitFPro). In some embodiments, A^1 is proline, A^2 is phenylalanine, and A^3 is citrulline, and A^4 is alanine (ACitFPro).

In some embodiments, a tetrapeptide modifying agent has the structure represented by:



wherein,

R^5 comprises a targeting group (M^1) or a steric stabilizer (M^2),

R^4 is a side chain of a natural, non-natural isomeric, or synthetic hydrophobic amino acid,

R^3 is a side chain of an uncharged hydrophilic amino acid, preferably citrulline,

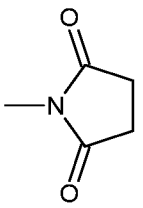
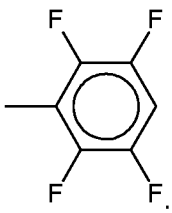
R^2 is a side chain of a natural, non-natural isomeric, or synthetic hydrophobic amino acid, preferably phenylalanine,

X and Y are:

a) $(CH_2)_2(CH_3)_2$ and H, respectively (tetrapeptide A^1 is Leucine),

b) CH_3- and CH_3-, respectively (tetrapeptide A^1 is N-methyl alanine), or

c) CH_2- and CH_2-CH_2-, respectively (tetrapeptide A^1 is proline); and

R' is  or .

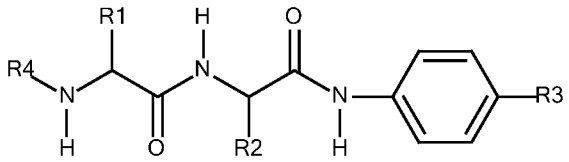
Reaction of the tetrapeptide modifying agent with a polyamine yields P–(L–M).

In some embodiments, R^4 is a side chain of phenylalanine or alanine. In some embodiments, R^3 is a side chain of citrulline. In some embodiments, R^2 is a side chain of phenylalanine.

In some embodiments, the membrane active polyamine is modified with dipeptide modifying agents (dipeptide-PABC-PNP modifying agent) having the general form:

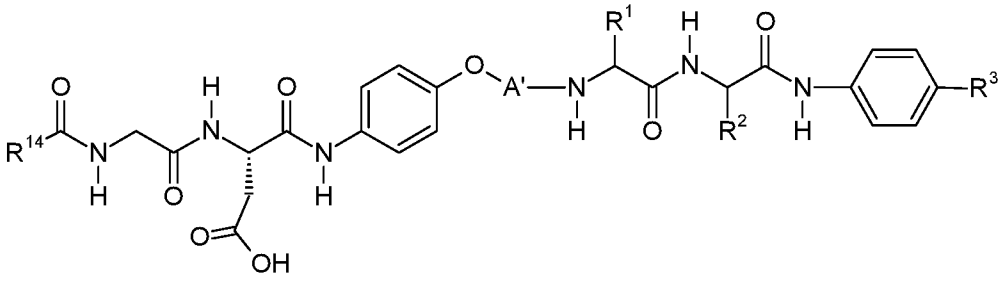
R–A^1A^2-amidobenzyl-carbonate.

wherein R comprises a steric stabilizer or targeting group, A^1 is a hydrophobic amino acid, and A^2 is a hydrophilic uncharged amino acid. Reaction of the modifying agent carbonate with a polymer amine yields a carbamate linkage. In some embodiments, the amidobenzyl group is a p-amidobenzyl group. In some embodiments, the carbonate is an activated amine reactive carbonate. In some embodiments, dipeptide-PABC cleavable linkers have the general structure:



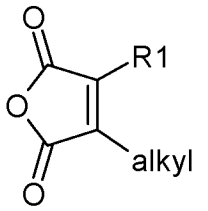
wherein R4 comprises a targeting group or steric stabilizer, R3 comprises an amine reactive carbonate moiety, such as a para-nitrophenyl group, R1 is the side chain of a hydrophobic amino acid, such as Phenylalanine or Alanine and R2 is the side chain of a hydrophilic uncharged are amino acid, such as citrulline (Cit). In some embodiments, R1 is the side chain of Phenylalanine or Alanine. In some embodiments, R2 is the side chain of citrulline (Cit).

In some embodiments, an RGD modifying agent comprises the structure represented by:



wherein R^{14} is a guanidinium-containing group as defined above, A' comprises a PEG-containing linker, R^1 is a side chain of a Phenylalanine or Alanine, R^2 is a side chain of citrulline, and R^3 is an amine-reactive carbonate.

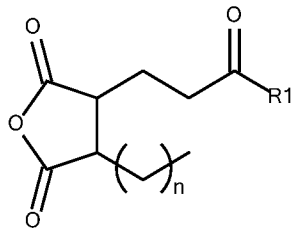
A delivery polymer can include a polyamine reversibly modified by reaction of primary amines on the polymer with a disubstituted alkylmaleic anhydride:



,

wherein R1 comprises a targeting group or a steric stabilizer.

In some embodiments, the disubstituted alkylmaleic anhydride has the structure represented by:



wherein R1 comprises an targeting group or a steric stabilizer.

In some embodiments, a targeting group (e.g., RGD ligand) is linked to a modifying agent via a linker, such as a PEG linker. The PEG linker can have 1-50 ethylene units.

RGD and PEG modifying agents are shown in FIGs. 1-7.

In some embodiments, we describe compositions represented by the formula:

$$(\text{RNAi trigger})_n\text{–}\mathbf{P}\begin{matrix}\diagup (L^1\text{–}M^1)_x \\ \diagdown (L^2\text{–}M^2)_y\end{matrix},$$

wherein: RNAi trigger is an Hif2α RNAi trigger, n is 0.5-5, **P** is a membrane active polyamine, L^1–M^1 comprises RGD-PEG$_a$-FCitFPro–, a is 1-50, x is 1-20, L^2–M^2 comprises PEG$_b$–ACit–PABC–, b is 4-30, and y is greater than or equal to 0, and $(M^2\text{–}L^2)_y$–P–$(L^1\text{–}M^1)_x$ is not membrane active. In some embodiments, the value of x+y is greater than 80%, greater than 90%, or greater than 95% of the number of primary amines of **P**. In some embodiments, n is 0.5-2. In some embodiments, n is 0.8-1.6. In some embodiments, x is 2-20, 3-20, 4-20, 5-

20, 6-20, 7-20, 8-20, 9-20, 10-20, 11-20, 12-20, 13-20, 14-20, or 15-20. In some embodiments, the value of *a* is greater than the value of *b*.

Pharmaceutical compositions

In some embodiments, at least one of the described Hif2α RNAi triggers is used in the preparation of a pharmaceutical composition (i.e., medicament) for treatment of a subject that would benefit from reduction or inhibition in Hif2α expression. These pharmaceutical compositions are useful in the inhibition of the expression of the Hif2α gene in a cell, a tissue, or an organism. In some embodiments, the described pharmaceutical compositions are used to treat a subject having a disease or disorder that would benefit from reduction or inhibition in Hif2α expression.

As used herein, a pharmaceutical composition or medicament comprises a pharmacologically effective amount of at least one of the described Hif2α RNAi triggers or Hif2α RNAi trigger-containing conjugates and one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients (excipients) are substances other than the Active Pharmaceutical ingredient (API, therapeutic product, e.g., RNAi trigger) that have been appropriately evaluated for safety and are intentionally included in the drug delivery system. Excipients do not exert or are not intended to exert a therapeutic effect at the intended dosage. Excipients may act to a) aid in processing of the drug delivery system during manufacture, b) protect, support or enhance stability, bioavailability or patient acceptability of the API, c) assist in product identification, and/or d) enhance any other attribute of the overall safety, effectiveness, of delivery of the API during storage or use. A pharmaceutically acceptable excipient may or may not be an inert substance.

Excipients include, but are not limited to: absorption enhancers, anti-adherents, anti-foaming agents, anti-oxidants, binders, binders, buffering agents, carriers, coating agents, colors, delivery enhancers, dextran, dextrose, diluents, disintegrants, emulsifiers, extenders, fillers, flavors, glidants, humectants, lubricants, oils, polymers, preservatives, saline, salts, solvents, sugars, suspending agents, sustained release matrices, sweeteners, thickening agents, tonicity agents, vehicles, water-repelling agents, and wetting agents.

A pharmaceutical composition can contain other additional components commonly found in pharmaceutical compositions. Such additional components include, but are not limited to: anti-

pruritics, astringents, local anesthetics, or anti-inflammatory agents (e.g., antihistamine, diphenhydramine, etc.). It is also envisioned that cells, tissues or isolated organs that express or comprise the herein defined RNAi triggers may be used as "pharmaceutical compositions". As used herein, "pharmacologically effective amount," "therapeutically effective amount," or simply "effective amount" refers to that amount of an RNAi trigger to produce the intended pharmacological, therapeutic or preventive result.

In some embodiments, a described Hif2α RNAi trigger is combined one or more additional therapeutics or treatments including, but not limited to: a second Hif2α RNAi trigger or other RNAi agent, a small molecule drug, an antibody, an antibody fragment, and/or a vaccine.

The described RNAi triggers and pharmaceutical compositions comprising Hif2α RNAi triggers disclosed herein may be packaged or included in a kit, container, pack, or dispenser. The Hif2α RNAi triggers and pharmaceutical compositions comprising said Hif2α RNAi triggers may be packaged in pre-filled syringes or vials.

Cells, tissues, and non-human organisms that include at least one of the Hif2α RNAi triggers described herein is contemplated. The cell, tissue, or non-human organism is made by delivering the RNAi trigger to the cell, tissue, or non-human organism by any means available in the art. In some embodiments, the cell is a mammalian cell, including, but no limited to, a human cell. The cell, tissue, or non-human organisms are useful for research or as research tools (e.g., drug testing or diagnoses).

Method of Treatment

In some embodiments, the Hif2α RNAi triggers described herein are used to treat a subject having a disease or disorder that would benefit from reduction or inhibition in Hif2α expression. In some embodiments, the described Hif2α RNAi triggers are used to treat or prevent at least one symptom in a subject having a disease or disorder that would benefit from reduction or inhibition in Hif2α expression. The subject is administered a therapeutically effective amount of any one or more of the described RNAi triggers thereby treating the symptom.

In some embodiments, the Hif2α RNAi triggers are used to treat or manage a clinical presentation wherein a subject in need of such treatment, prevention or management is

administered a therapeutically or prophylactically effective amount of one or more of the Hif2α RNAi triggers or Hif2α RNAi trigger-containing compositions described herein. In some embodiments, the method comprises administering a composition comprising an Hif2α RNAi trigger molecule described herein to a mammal to be treated.

Representative diseases that would benefit from a reduction and/or inhibition of Hif2α gene expression include, but are not limited to, cancer, renal cancer, clear cell renal cell carcinoma, non-small cell lung cancer, astrocytoma (brain cancer), bladder cancer, breast cancer, chondrosarcoma, colorectal carcinoma, gastric carcinoma, glioblastoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, lung adenocarcinoma, neuroblastoma, melanoma, multiple myeloma, ovarian cancer, rectal cancer, metastases, gingivitis, psoriasis, Kaposi's sarcoma-associated herpesvirus, preeclampsia, inflammation, chronic inflammation, neovascular diseases, and rheumatoid arthritis.

In some embodiments, an Hif2α RNAi trigger can be used to inhibit expression of Hif2α in a cell, group of cells, or a tissue, e.g., in a subject. In some embodiments, an Hif2α RNAi trigger can be used to formulate a composition for inhibiting expression of Hif2α in a cell, group of cells, or a tissue, e.g., in a subject. In some embodiments, a therapeutically effective amount of one type (or several different types) of Hif2α RNAi triggers as described herein is administered to a subject, thereby inhibiting expression of Hif2α in the subject (e.g., an amount effective to inhibit expression of Hif2α in the subject).

As used herein, the terms "silence," "reduce," "inhibit," "down-regulate," or "knockdown gene expression," when referring to an Hif2α gene, mean that the expression of the gene, as measured by the level of RNA transcribed from the gene or the level of polypeptide, protein, or protein subunit translated from the mRNA in a cell, group of cells, or tissue, in which the Hif2α gene is transcribed, is reduced when the cell, group of cells, or tissue, is treated with the described Hif2α RNAi triggers as compared to a second cell, group of cells, or tissue that has or has not been so treated or compared to the same cell, group of cells, or tissue, prior to administration of the Hif2α RNAi trigger.

In some embodiments, the gene expression level and/or mRNA level of Hif2α in a subject to whom a described Hif2α RNAi trigger is administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,

95%, or 98% relative to the subject prior to being administered the Hif2α RNAi trigger or to a subject not receiving the Hif2α RNAi trigger. The gene expression level and/or mRNA level in the subject may be reduced in a cell, group of cells, and/or tissue of the subject. In some embodiments, the protein level of Hif2α in a subject to whom a described Hif2α RNAi trigger has been administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98% relative to the subject prior to being administered the Hif2α RNAi trigger or to a subject not receiving the Hif2α RNAi trigger. The protein level in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject. A reduction in gene expression, mRNA, or protein levels can be assessed by any methods known in the art. Reduction or decrease in Hif2α mRNA level and/or protein level are collectively referred to herein as a reduction or decrease in Hif2α or inhibiting or reducing the expression of Hif2α.

"Introducing into a cell", when referring to an RNAi trigger, means functionally delivering the RNAi trigger into a cell. By functional delivery, it is meant that the RNAi trigger is delivered to the cell and has the expected biological activity, (e.g., sequence-specific inhibition of gene expression).

The route of administration is the path by which an RNAi trigger is brought into contact with the body. In general, methods of administering drugs and nucleic acids for treatment of a subject are well known in the art and can be applied to administration of the compositions described herein. The compounds described herein can be administered via any suitable route in a preparation appropriately tailored to the particular route. Thus, the compounds described herein can be administered by injection, for example, intravenously, intramuscularly, intracutaneously, subcutaneously, or intraperitoneally.

In some embodiments, the Hif2α RNAi trigger molecules or compositions described herein can be delivered to a cell, group of cells, tissue, or subject using oligonucleotide delivery technologies known in the art. In general, any suitable method recognized in the art for delivering a nucleic acid molecule (in vitro or in vivo) can be adapted for use with an Hif2α RNAi trigger described herein. For example, delivery can be by local administration, (e.g., direct injection, implantation, or topical administering), systemic administration, or subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway

(aerosol), nasal, rectal, or topical (including buccal and sublingual) administration, In certain embodiments, the compositions are administered by subcutaneous or intravenous infusion or injection.

The above provided embodiments and items are now illustrated with the following, non-limiting examples.

EXAMPLES

Example 1. *RNAi trigger synthesis.*

A) Synthesis. RNAi trigger molecules were synthesized according to phosphoramidite technology on solid phase used in oligonucleotide synthesis. Depending on the scale either a MerMade96E (Bioautomation) or a MerMade12 (Bioautomation) was used. Syntheses were performed on a solid support made of controlled pore glass (CPG, 500 Å or 600Å, obtained from Prime Synthesis, Aston, PA, USA). All DNA, 2′-modified RNA, and UNA phosphoramidites were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA). Specifically, the following 2′-O-Methyl phosphoramidites were used: (5′-O-dimethoxytrityl-N^6-(benzoyl)-2′-O-methyl-adenosine-3′-O-(2-cyanoethyl-N,N-diisopropy-lamino) phosphoramidite, 5′-O-dimethoxy-trityl-N^4-(acetyl)-2′-O-methyl-cytidine-3′-O-(2-cyanoethyl-N,N-diisopropyl-amino) phosphoramidite, (5′-O-dimethoxytrityl-N^2-(isobutyryl)-2′-O-methyl-guanosine-3′-O-(2-cyano-ethyl-N,N-diisopropylamino)phosphoramidite, and 5′-O-dimethoxy-trityl-2′-O-methyl-uridine-3′-O-(2-cyanoethyl-N,N-diisopropylamino)phosphoramidite. The 2′-Deoxy-2′-fluoro-phosphor-amidites carried the same protecting groups as the 2′-O-methyl RNA amidites. The following UNA phosphoramidites were used: 5′-(4,4'-Dimethoxytrityl)-N-benzoyl-2′,3′-seco-adenosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphor-amidite, 5′-(4,4'-Dimethoxytrityl)-N-acetyl-2′,3′-seco-cytosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5′-(4,4'-Dimethoxytrityl)-N-isobutyryl-2′,3′-seco-guanosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, and 5′-(4,4'-Dimethoxy-trityl)-2′,3′-seco-uridine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N- diisopropyl)]-phosphoramidite. All amidites were dissolved in anhydrous acetonitrile (50 mM) and molecular sieves (3Å) were added. In order to introduce the TEG-Cholesterol at the 5′-end of the oligomers, the 1-Dimethoxytrityloxy-3-O-(N-cholesteryl-3-aminopropyl)-triethyleneglycol-glyceryl-2-O-(2-cyanoethyl)-(N,N,-diisopropyl)-phosphoramidite from Glen Research (Sterling, VA, USA) was employed. The 5′-modifications were introduced without any modification of the synthesis cycle. 5-Benzylthio-1H-tetrazole (BTT, 250 mM in acetonitrile) was used as activator solution.

Coupling times were 10 min (RNA), 180 sec (Cholesterol), 90 sec (2′OMe and UNA), and 60 sec (2′F and DNA). In order to introduce phosphorothioate linkages, a 100 mM solution of 3-phenyl 1,2,4-dithiazoline-5-one (POS, obtained from PolyOrg, Inc., Leominster, MA, USA) in anhydrous Acetonitrile was employed. See Tables 1-2 and 5 for specific sequences.

B. Cleavage and deprotection of support bound oligomer. After finalization of the solid phase synthesis, the dried solid support was treated with a 1:1 volume solution of 40 wt. % methylamine in water and 28% ammonium hydroxide solution (Aldrich) for two hours at 30°C. The solution was evaporated and the solid residue was reconstituted in water (see below).

C. Purification. Crude Cholesterol containing oligomers were purified by reverse phase HPLC using a Waters XBridge BEH300 C4 5u Prep column and a Shimadzu LC-8 system. Buffer A was 100 mM TEAA, pH 7.5 and contained 5% Acetonitrile and buffer B was 100 mM TEAA and contained 95% Acetonitrile. UV traces at 260 nm were recorded. Appropriate fractions were then run on size exclusion HPLC using a GE Healthcare XK 16/40 column packed with Sephadex G-25 medium with a running buffer of 100mM ammonium bicarbonate, pH 6.7 and 20% Acetonitrile. Other crude oligomers were purified by anionic exchange HPLC using a TKSgel SuperQ-5PW 13u column and Shimadzu LC-8 system. Buffer A was 20 mM Tris, 5 mM EDTA, pH 9.0 and contained 20% Acetonitrile and buffer B was the same as buffer A with the addition of 1.5 M sodium chloride. UV traces at 260 nm were recorded. Appropriate fractions were pooled then run on size exclusion HPLC as described for cholesterol containing oligomers.

D. Annealing. Complementary strands were mixed by combining equimolar solutions (sense and antisense) in 0.2× PBS (Phosphate-Buffered Saline, 1×, Corning, Cellgro) to form the RNAi triggers. This solution was placed into a thermomixer at 70°C, heated to 95°C, held at 95°C for 5 min, and cooled to room temperature slowly. Some RNAi triggers were lyophilized and stored at −15 to −25°C. Duplex concentration was determined by measuring the solution absorbance on a UV-Vis spectrometer in 0.2× PBS. The solution absorbance at 260 nm was then multiplied by a conversion factor and the dilution factor to determine the duplex concentration. Unless otherwise stated, all conversion factor was 0.037 mg/(mL·cm). For some experiments, a conversion factor was calculated from an experimentally determined extinction coefficient.

Example 2. *Synthesis of APN 1170-100A (100A), APN 1203-006 (006), APN 1203-064 (064) amphipathic membrane active polyamines.*

Polymer	MW (protected)	Theoretical MW (deprotected)	PDI	% Amine Incorp.	% Alkyl Incorp.	% End Group Removal	Azides/ Polymer
APN 1170-100A	64,430	45,765	1.22	56	44	0	1.25
APN 1203-006	60,330	43,578	1.05	56	44	99	1.14
APN 1203-062	65,170	46,736	1.05	54	46	99	0.96

A. *Materials.* 2,2'-Azobis(2,4-dimethyl valeronitrile) (V-65, radical initiator) was purchased from Wako Pure Chemical Industries. Propyl acrylate was purchased from Polysciences Inc. N-Boc-ethoxy-ethylamine acrylate was obtained from WuXi Inc. 2-(Dodecylthio-carbonothioylthio)-2-methylpropionic acid (DDMAT, RAFT Agent), 1,1'-Azobis-(cyclo-hexanecarbonitrile) (ACHN), 1-Ethylpiperidine hypophosphite (EPHP), Pentafluorophenol, N,N'-Dicyclohexylcarbodiimide and N,N-diisopropyl-ethylamine were purchased from Sigma Aldrich. O-(2-Aminoethyl)-O'-(2-azidoethyl)triethylene Glycol (azido-PEG_4-amine) was purchased from Biomatrik Inc.

B. *RAFT copolymer of N-Boc-ethoxyethylamine acrylate and propyl acrylate (EAP).* Solutions of V-65 (2 mg/mL) and RAFT agent DDMAT (10 mg/mL) in butyl acetate were prepared. Monomer molar feed was 52% N-Boc-ethoxyethylamine acrylate, 48% propyl acrylate. Theoretical Mw was 75,000. RAFT agent (DDMAT) to initiator (V-65) molar ratio was 6.67:1.

N-Boc-ethoxyethylamine acrylate (1.778 g, 6.86 mmol), propyl acrylate (0.794 mL, 0.722 g, 6.33 mmol), DDMAT solution (1.215 mL, 0.0333 mmol), V-65 solution (0.621 mL, 0.005 mmol), and butyl acetate (10.2 mL) were added to a 20 mL glass vial with a stir bar. The vial was sealed with a septa cap and the solution bubbled with nitrogen using a long needle with a second needle as the outlet for 1 h. The needles were removed and the vial was heated to 50°C for 24h with stirring. The solution was allowed to cool to room temperature and transferred equally between two 50 mL centrifuge tube before hexane (35 mL) was added to both tubes. The solution was centrifuged for 2 min at 4400 rpm. The supernatant layer was carefully decanted and the bottom layer rinsed with hexane. The bottom layer of each tube was then re-dissolved in dichloromethane (7 mL), precipitated in hexane (40 mL) and centrifuged once more. The supernatant was decanted and the bottom layer rinsed with hexane before the layers were combined to one 50 mL centrifuge tube and the polymer was dried under reduced pressure for several hours. The yield of crude EAP copolymer was 2.1 g. Samples of the copolymer were taken for multi-angle light scattering (MALS), and ^{1}H-NMR.

Polymer 006: The composition determined by ^{1}H-NMR was 55% N-Boc-ethoxyethylamine acrylate and 45% propyl acrylate. The Mw for 006 determined by MALS was 58,600 g/mol with a polydispersity index (PDI) of 1.04.

Polymer 100A: Composition by 1H-NMR: 56% N-Boc-ethoxyethylamine acrylate and 44% propyl acrylate. MW by MALS: 65,150, PDI of 1.122.

Polymer 064: Composition by 1H-NMR: 54% N-Boc-ethoxyethylamine acrylate and 46% propyl acrylate. The Mw for 064 determined by MALS was 57,957 g/mol with a polydispersity index (PDI) of 1.07.

C. *Radical induced ω-end group removal (polymers 006 and 064).* Solutions of 1,1'-Azobis-(cyclohexanecarbonitrile) (ACHN, 20 mg/mL) and 1-Ethylpiperidine hypophosphite (EPHP, 100 mg/mL) were prepared in toluene. EAP (2 g, 0.035 mmol), ACHN (0.213 mL, 0.5 eq, 0.0174 mmol), EPHP (1.25 mL, 20 eq, 0.697 mmol), and toluene (25.2 mL) were added to a 40 mL glass vial with a stir bar. The vial was sealed with a septa cap and the solution bubbled with nitrogen using a long needle with a second needle as the outlet for 1 h. The needles were removed and the vial was heated to 100°C for 2 h. The solution was allowed to cool to room temperature and ~20 mL toluene was removed by rotary evaporation. The remaining solution was transferred to a 50 mL centrifuge vial, and hexane (35 mL) was added. The solution was centrifuged for 2 min at 4400 rpm. The supernatant layer was carefully decanted and the bottom layer rinsed with hexane. The bottom layer was then re-dissolved in dichloromethane (7 mL), precipitated in hexane (40 mL) and centrifuged once more. The supernatant was decanted and the bottom layer rinsed with hexane before the polymer was dried under reduced pressure for ~ 1 h. The polymer was dissolved in methyl tert-butyl ether (80 mL) and transferred to a separatory funnel. The solution was then washed with 3 × 30 mL volumes of H_2O followed by 3 × 30 mL volumes of saturated NaCl. The polymer solution was then dried over sodium sulfate, and vacuum filtered through 0.45 μm GHP filters. MTBE was removed via rotary evaporation and high vacuum. A sample was taken for monitoring of end group removal using a UV spectrophotometer. End group removal was calculated to be 99%. Samples were taken for MALS, GC-FID, and ^{1}H-NMR. The composition of 006 by ^{1}H-NMR was 55% N-Boc-eth oxyethylamine acrylate and 45% propyl acrylate. The conversion of 006 determined by GC-FID was 81.4% for the N-Boc-ethoxyethylamine acrylate and 77.3% for the propyl acrylate. The conversion of 100A determined by GC-FID conversion was 87% for N-Boc-ethoxyethylamine acrylate and 83% for propyl acrylate. The Mw for polymer 006 determined by MALS was 57,700 g/mol with a polydispersity index (PDI) of 1.06.

D. *Pentafluorophenol activation of α-end group.* EAP polymer (2 g, 0.0347 mmol), pentafluorophenol (63.8 mg, 0.3466 mmol), N,N'-Dicyclohexylcarbodiimide (71.5 mg, 0.3466 mmol), and dichloromethane (40mL) were added to a 100 mL round bottom flask with a stir bar. The flask was stoppered with a rubber septum and the system was purged with nitrogen for 15 min. The solution was stirred for 16 h at room temperature. Additional Pentafluorophenol (63.8 mg, 0.3466 mmol) and N,N'-Dicyclohexylcarbodiimide (71.5 mg, 0.3466 mmol) were added, the flask stoppered with a rubber septum, and the system was purged with nitrogen for 15 min. The solution was stirred for 3 h at room temperature. The polymer was precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal dichloromethane, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal ethyl acetate, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer precipitate was dried under high vacuum until the solid reached a constant weight.

E. *Azide functionalization of α-end group.* In a 100 ml round bottom flask equipped with a rubber septum and stir bar, polymer from the previous step (1.9 g, 0.0329 mmol) was dissolved in dichloromethane (38 mL). Azido-PEG_4-Amine (86.4 mg, 0.3293 mmol) and N,N-Diisopropylethylamine (46.8 mg, 63.1 µL, 0.3622 mmol) were added to the flask with stirring. The system was purged with nitrogen for 15min, and the reaction was left to stir at room temperature overnight. Additional Azido PEG_4 Amine (86.4 mg, 0.3293 mmol) and N,N-Diisopropylethylamine (46.8 mg, 63.1 µL, 0.3622 mmol) were added to the flask, the system was purged with N_2 gas, and the reaction was stirred for 3h at room temperature. The polymer was precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal dichloromethane, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer precipitate was dried under high vacuum until the solid reached a constant weight. The yield of Azide functionalized EAP was 1.77g. Samples of the copolymer were taken for multi-angle light scattering (MALS), and ^{1}H-NMR.

Polymer 006: The composition determined by ^{1}H-NMR was 56% N-Boc-ethoxyethylamine acrylate and 44% propyl acrylate. The Mw determined by MALS was 60,330 g/mol with a polydispersity index (PDI) of 1.05.

Polymer 100A: The composition by ^{1}H-NMR was 56% N-Boc-ethoxyethylamine acrylate and 44% propyl acrylate. The Mw determined by MALS: 64,430 with PDI of 1.217.

Polymer 064: The composition by 1H-NMR was 54% N-Boc-ethoxyethylamine acrylate and 46% propyl acrylate. The Mw determined by MALS: 65,170 with PDI of 1.05

Mono-Azide: The term “mono-azide” or "mono-azide polymer" indicates that steps D and E of the procedures above were done and an azide group was coupled to the α-end group of the polymer.

F. *Boc Deprotection and Tangential Flow Filtration.* In a 100 mL round bottom flask, 2M HCl in acetic acid (28mL) was added to Azide functionalized EAP copolymer (1.67g, 0.0277 mmol). The reaction was stirred at room temperature for 1 h. De-ionized H_2O (56 mL) was added, and stirred for 10 min. The solution was then immediately exchanged with 10 equivalent volumes of 5 mM Phosphate-Citrate buffer (pH 5) using a mPES 30kD 115cm^2 filter module equipped with a tangential flow filtration system (KrosFlo Research). The solution was then concentrated using the apparatus to 55 mL final volume. A pH value of 5.1 was recorded. Samples were taken for concentration determination by headspace gas chromatography. An aliquot was lyophilized and then reconstituted in 33.3% Acetonitrile-d in Deuterium Oxide at a concentration of 10 mg/mL for ^{1}H-NMR analysis. Theoretical MW was calculated to be 43,026 g/mol 45,765 g/mol for 006 and 100A respectively.

G. Using similar techniques, similar amphipathic membrane active polyamines can be readily formed. Particularly, amphipathic membrane active polyamines with molecular weight (Mw) 40-120k protected (25k to 85k deprotected), PDI ranges of 1.03 to 1.2, and monomer ratios of 35% amine monomer/65% hydrophobic group monomer to 70% amine monomer/30% hydrophobic group monomer.

Example 3. Synthesis of APN 1095-126 (126).

MW (protected)	Theoretical MW (deprotected)	PDI	% Amine Incorporation	% Alkyl Incorporation	% End Group Removal	Azides Per Polymer
66,670	47,606	1.11	56	44	0	4.1

Synthesis of APN 1095-126 used dithiobenzoate moiety RAFT agent and AIBN RAFT initiator, compared to the trithiocarbonate moiety RAFT agent and V-65 RAFT initiator used for synthesis of 100A and 006. The conditions for this polymerization required different heating temperatures and times. In addition, this polymer required fractional precipitation. The

polymer was not end capped, but the method of azide addition was the same as 100A and 006.

A. *Materials.* Propyl acrylate was purchased from Polysciences Inc. N-Boc-ethoxyethylamine acrylate was obtained from WuXi Inc. 4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPCPA, RAFT Agent), 2,2'-Azobis(2-methylpropionitrile) (AIBN, radical initiator), Pentafluorophenol, N,N'-Dicyclohexylcarbodiimide and N,N-diisopropylethylamine were purchased from Sigma Aldrich. O-(2-Aminoethyl)-O'-(2-azidoethyl)triethylene Glycol (azido-PEG_4-amine) was purchased from Biomatrik Inc.

B. *RAFT copolymer of N-Boc-ethoxyethylamine acrylate and propyl acrylate (EAP).* The following procedure was repeated 8 times to yield a total of 4.5513 g fractionated EAP copolymer. Solutions of AIBN (1.035 mg/mL) and RAFT agent CPCPA (50.54 mg/mL) in butyl acetate were prepared. Monomer molar feed was 52% N-Boc-ethoxyethylamine acrylate, 48% propyl acrylate. Theoretical Mw was 75,000. RAFT agent (CPCPA) to initiator (AIBN) molar ratio was 6.67:1.

N-Boc-ethoxyethylamine acrylate (1.7879 g, 6.9 mmol), propyl acrylate (0.774 mL, 0.7121 g, 6.24 mmol), CPCPA solution (0.184 mL, 0.0333 mmol), AIBN solution (0.793 mL, 0.005 mmol), and butyl acetate (11.02 mL) were added to a 20 mL glass vial with a stir bar. The vial was sealed with a septa cap and the solution bubbled with nitrogen using a long needle with a second needle as the outlet for 1 h. The needles were removed and the vial was heated to 50°C for 24 h with stirring. The solution was allowed to cool to room temperature and transferred to a 50 mL centrifuge tube before hexane (35 mL) was added. The solution was centrifuged for 2 min at 4400 rpm. The supernatant layer was carefully decanted and the bottom layer rinsed with hexane. The bottom layer of each tube was then re-dissolved in dichloromethane (7 mL), precipitated in hexane (40 mL) and centrifuged once more. The supernatant was decanted and the bottom layer rinsed with Hexane before the polymer was dried under reduced pressure for several hours. The yield of crude EAP copolymer was 1.734 g. Samples of the crude copolymer were taken for multi-angle light scattering (MALS), and ^{1}H-NMR. The dried, crude copolymer was dissolved in DCM (100 mg/mL). Hexane was added until just after the cloud point was reached. The resulting milky solution was centrifuged. The bottom layer was extracted and fully precipitated into hexane. The fraction was centrifuged, after which the copolymer was isolated and dried under vacuum. The yield of isolated fraction of EAP copolymer was 0.602 g. Samples of the fractionated copolymer were taken for ^{1}H-NMR and MALS. The composition determined by ^{1}H-NMR was 56% N-Boc-

ethoxyethylamine acrylate and 44% propyl acrylate. The Mw determined by MALS was 62,010 g/mol with a polydispersity index (PDI) of 1.14.

C. *Pentafluorophenol activation of α-end group.* EAP polymer (2 g, 0.0347 mmol), pentafluorophenol (63.8 mg, 0.3466 mmol), N,N'-Dicyclohexylcarbodiimide (71.5 mg, 0.3466 mmol), and dichloromethane (40mL) were added to a 100 mL round bottom flask with a stir bar. The flask was stoppered with a rubber septum and the system was purged with nitrogen for 15 min. The solution was stirred for 16h at room temperature. Additional Pentafluorophenol (63.8 mg, 0.3466 mmol) and N,N'-Dicyclohexylcarbodiimide (71.5 mg, 0.3466 mmol) were added, the flask stoppered with a rubber septum, and the system was purged with nitrogen for 15min. The solution was stirred for 3h at room temperature. The polymer was precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal dichloromethane, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal ethyl acetate, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer precipitate was dried under high vacuum until the solid reached a constant weight.

D. *Azide functionalization of α-end group.* In a 100 ml round bottom flask equipped with a rubber septum and stir bar, polymer from the previous step (1.9g, 0.0329 mmol) was dissolved in dichloromethane (38 mL). Azido-PEG_4-Amine (86.4 mg, 0.3293 mmol) and N,N-Diisopropyl-ethylamine (46.8 mg, 63.1 µL, 0.3622 mmol) were added to the flask with stirring. The system was purged with nitrogen for 15min, and the reaction was left to stir at room temperature overnight. Additional Azido PEG_4 Amine (86.4 mg, 0.3293 mmol) and N,N-Diisopropyl-ethylamine (46.8 mg, 63.1 µL, 0.3622 mmol) were added to the flask, the system was purged with N_2 gas, and the reaction was stirred for 3h at room temperature. The polymer was precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer was dissolved in minimal dichloromethane, precipitated with hexane (~10× volume), centrifuged, and the solvent was decanted. The polymer precipitate was dried under high vacuum until the solid reached a constant weight. The yield of Azide functionalized EAP was 1.77g. Samples of the copolymer were taken for multi-angle light scattering (MALS), and ^{1}H-NMR. The composition determined by ^{1}H-NMR was 56% N-Boc-ethoxyethylamine acrylate and 44% propyl acrylate. The Mw determined by MALS was 66,670 g/mol with a polydispersity index (PDI) of 1.11.

E. *Boc Deprotection and Tangential Flow Filtration.* In a 100 mL round bottom flask, 2M HCl in acetic acid (28mL) was added to Azide functionalized EAP copolymer (1.67 g, 0.0277 mmol). The reaction was stirred at room temperature for 1 hour. De-ionized H_2O (56 mL) was added, and stirred for 10 min. The solution was then immediately exchanged with 10 equivalent volumes of 5 mM Phosphate-Citrate buffer (pH 5) using a mPES 30kD 115cm^2 filter module equipped with a tangential flow filtration system (KrosFlo Research). The solution was then concentrated using the apparatus to 55 mL final volume. A pH value of 5.1 was recorded. Samples were taken for concentration determination by headspace gas chromatography. An aliquot was lyophilized and then reconstituted in 33.3% Acetonitrile-d in Deuterium Oxide at a concentration of 10 mg/mL for ^{1}H-NMR analysis. Theoretical MW was calculated to be 43,026 g/mol.

Example 4. *Polymer analytics.*

(i) *MALS analysis.* Approximately 10 mg of the copolymer was dissolved in 0.5 mL 75% dichloromethane, 20% tetrahydrofuran, and 5% acetonitrile. The molecular weight and polydispersity (PDI) were measured using a Wyatt Heleos II multiangle light scattering detector attached to a Shimadzu Prominence HPLC using a Jordi 5 µm 7.8 x 300 Mixed Bed LS DVB column. Molecular weight (polymer 006) before de-protection: 60,330 (PDI 1.05).

(ii) *Monomer conversion by Gas Chromatography.* Approximately 40 µL of copolymer solution (section B) was taken after mixing (pre-N_2 bubbling), after N_2 bubbling, and after reaction completion. Samples were diluted 100 fold into ethyl acetate. The samples were analyzed with a Shimadzu GC-2010 plus equipped with a flame ionization detector using a Phenomenex Zebron capillary column (ZB-5, 30 m, 0.25 mm ID, 0.5 µm film thickness). Using the pre-N_2 bubbled sample as a single point calibration, monomer conversion was measured by comparing post reaction monomer concentrations with pre reaction/post N_2 bubbling monomer concentrations.

(iii) *Polymer concentration by propanol content using headspace gas chromatography (HS-GC).* Deprotected polymer solution (~20 mg/mL) was diluted 50 fold into 3M NaOH using 1-Butanol as an internal standard. The reaction tube was sealed and shaken for 1 h. The reaction was then incubated for at least 6 h at room temperature. In a 10 mL headspace vial, hydrolyzed test article (250 µL) was added to saturated NaCl (500 µL) and HCl (4M, 250 µL) and the system was sealed. Test articles were analyzed using a Shimadzu GC-2010 plus with HS-20 headspace sampler using a Phenomenex ZB-WAX plus gc column (30.0 m, 0.25 mm ID, 0.25 µm film thickness). Propanol concentration was then quantitated using an external standard

curve or propanol containing the same NaCl/HCl/NaOH matrix. Polymer concentration was then calculated by dividing propanol concentration by the amount of propanol per polymer as determined by monomer incorporation.

(iv) *Azide quantitation using UV Spectroscopy.* Deprotected polymer solution (~20 mg/mL) was diluted to ~5 mg/mL in 60 mM MES, pH 6. The polymer was then reacted with DBCO-amine (2.5 molar eq.) at room temperature for at least 6 h. The difference in absorbance at 310 nm was calculated and azide content per polymer was determined.

Example 5. *Tangential Flow Filtration and Analysis of Conjugate.* Following conjugate formation, i.e., modification of polymer by addition of RGD and PEG modifying agents and attachment of RNAi trigger (see example 9 below) the conjugate solution (2 mg/mL, 10 mL) was exchanged with 10 equivalent volumes of 10 mM Phosphate-Citrate buffer (pH 5) using a mPES 30kD 20 cm^2 filter module equipped with a tangential flow filtration system (KrosFlo Research). A pH value of 5.1 was recorded.

A. *Conjugate characterization and analysis.*

(i) *Polymer concentration throughout conjugation.* The same method as section G(iii) was used throughout the assembly of the conjugate to monitor polymer concentration.

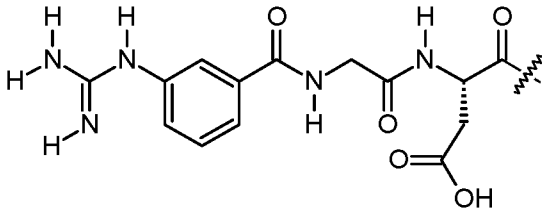
(ii) *Impurity quantitation by HPLC-reverse-phase chromatography.* Polymer conjugate (after TFF purification) was diluted to 1 mg/mL with H_2O and injected onto a Shimadzu Prominence HPLC with a SPD-20A UV detector and a Waters Xbridge C18 5 µm 4.6 x 250 mm column. The method used a binary gradient consisting of H_2O/Acetonitrile/0.1% formic acid with detection set to 247 nm. Concentrations of PEG_n-ACit-PABOH, RGD-PEG_n-FCFP-COOH, and PNP were calculated using external standard quantitation.

(iii) *RGD-PEG_n-FCitFP-TFP and PEG_n-ACit-PABC-PNP modification through amino acid analysis.* Polymer conjugate (after TFF purification) with NorValine as an internal standard was hydrolyzed for 16 h in HCl (6 M) at 110°C in a sealed hydrolysis tube. The hydrolysis solution was then neutralized with NaOH, diluted with borate buffer (pH 10.1), and derivatized with phthaldialdehyde/ 3-mercaptopropionic acid. The sample was then injected onto a Shimadzu Nexera HPLC system with SIL-30A autosampler, SPD-20A photo diode array detector, and a Waters Xbridge C18 5 µm 4.6 x 250 mm column. Sample was eluted using a 10 mM Sodium tetraborate decahydrate/10 mM dibasic sodium phosphate/5mM Sodium azide and 45% Methanol/45% Acetonitrile/10% H_2O binary gradient. UV detection was set to 338 nm. Alanine and Phenylalanine concentrations were calculated using external standard curves.

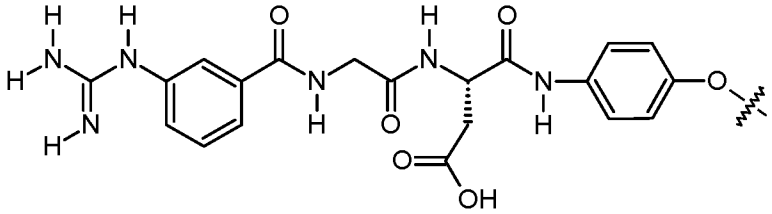
Alanine and Phenylalanine concentrations along with polymer concentration and monomer incorporations were used to calculate total amine group modification, as well as the ratio between both ligands.

(iv) *RNA trigger quantitation and conjugate purity by size exclusion chromatography.* A Shimadzu Prominence HPLC equipped with SPD-20A UV detector and Acclaim SEC-300 4.6mm x 300mm, 5µm, 300Å size exclusion column (1st in series) connected to Acclaim SEC-1000 4.6mm x 300mm, 7µm, 1000Å (2nd in series) size exclusion column was assembled. The method used was isocratic, with 200 mM NaBr, 10 mM Tris pH 8, 1mM EDTA, and 20% Acetonitrile as mobile phase and detection at 260 nm. A sample of polymer conjugate (after RNA trigger addition) was diluted into mobile phase and injected onto the system. Another sample of conjugate followed the same dilution scheme but was treated with 200 mM dithiothreitol for 2h before injection onto the system. RNA trigger concentration for both samples was calculated using an external standard curve. Amount of conjugated RNA trigger was calculated by subtracting DTT treated RNA levels from untreated RNA levels. Post-TFF purity of the conjugate was also determined using this method.

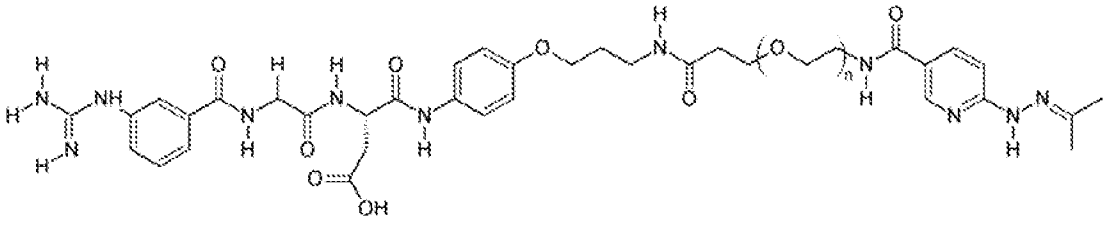
Example 6. *RGD ligands (RGD mimic).*



, or

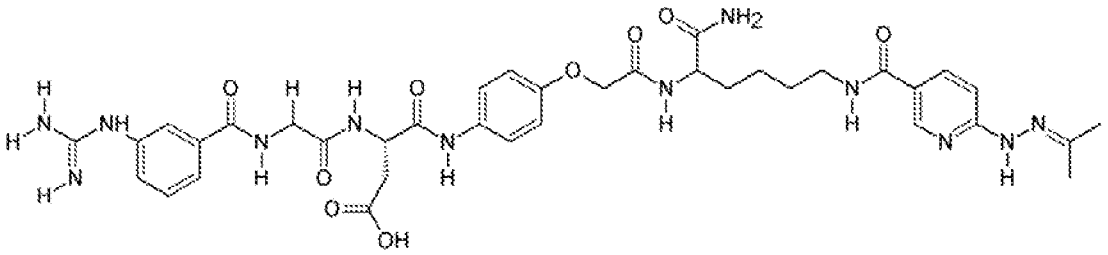


A. RGD mimic #1-PEG$_n$-HyNic, MW 1272.

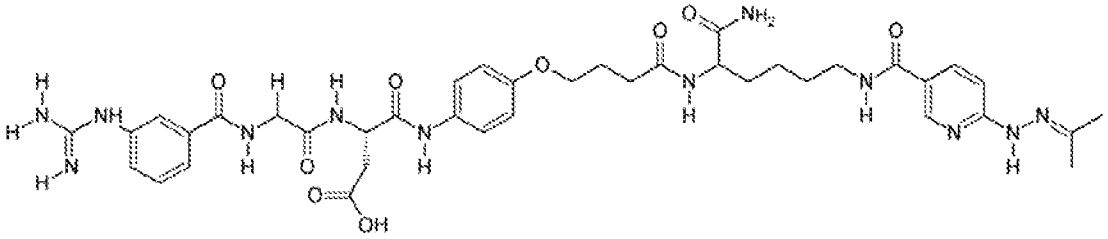


n = 4-24 (preferably 8-12)

B. RGD mimic #1a-HyNic, MW 802.8.



C. RGD mimic #1b-HyNic, MW 830.9 (RGD).



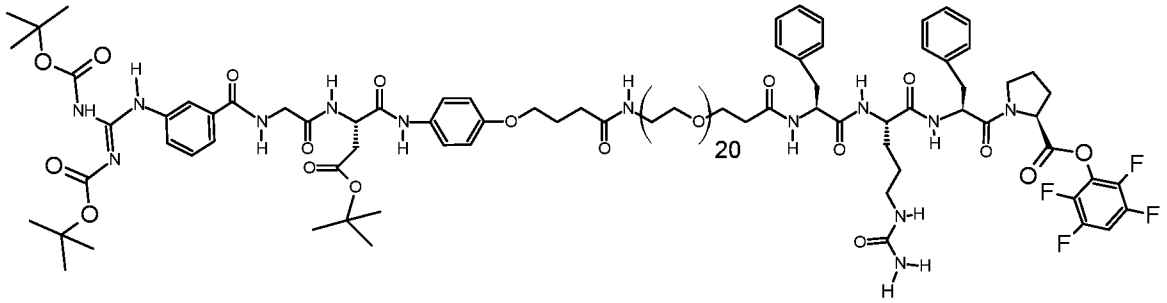
Example 7. *RGD and PEG modifying agents*

A. Dipeptide RGD-dipeptide and PEG-dipeptide modifying agents were made as described in US-2012-0172412-A1 (WO 2012/092373) and US 2015-0045573 A1 (WO 2015/021092) (both of which are incorporated herein by reference). FIG. 3-7.

B. *RGD-PEG$_n$-FCitFP-TFP and PEG$_n$-FCitFP-TFP modifying agent synthesis.* The modifying agent precursor (di-Boc)RGD(OtBu)-APBA-PEG$_n$-FCitFPro-COOH was prepared using general Fmoc chemistry solid phase synthesis using 2-Cl-Trt resin preloaded with Fmoc-Proline-OH. To Resin-Pro-Fmoc was added sequentially (following Fmoc deprotection at each step): FMoc-Phe-OH, Fmoc-Cit-OH, Fmoc-Phe-OH, Fmoc-NH-PEG$_n$-COOH, 4-(N-Fmoc-p-aminophenoxy)-butyric acid (APBA), Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, and diboc-m-guanidino benzoic acid.

(diboc)RGD(OtBu)-APBA-PEG$_n$-FCitFPro-COOH (458 mg, 0.200 mmols) and TFP (66.5 mg, 0.400 mmols) were dissolved in anhydrous DCM (5.0 mL) and cooled to 0°C in an ice/water bath while stirring under Argon. EDC (77 mg, 0.400 mmols) was added and the reaction mixture stirred in an ice/water bath at 0°C for 30 min. Reaction progress was monitored by TLC (8.5:1.5 $CHCl_3$:MeOH) and was complete after 90 min with no starting material observed by TLC. The reaction mixture was diluted to 100 mL total volume with DCM, washed 3 × 40 mL with DI H_2O (pH=5), and washed 1 × 40 mL aqueous saturated NaCl solution. The organics were then dried over Na_2SO_4, and concentrated on a rotovap to yield 448 mg (92 % yield) of a tan/orange foam. The structure was confirmed by ^1H NMR, and ESI

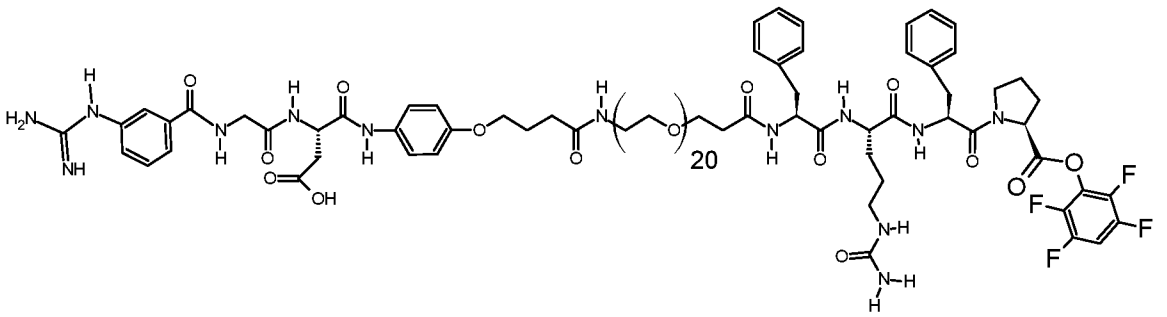
MS (Reaction shown above for PEG_{20} (n = 20)).



(diboc)RGD(OtBu)-APBA-PEG_n-FCitFPro-TFP

(shown for n = 20)

(diboc)RGD(OtBu)-PEGn-FCitFPro-TFP (497 mg, 0.204 mmols) was dissolved in [9.25:0.75:0.50] TFA:H_2O:Thioanisole (5.0 mL) and stirred at room temperature in a closed flask for 45 min. Reaction completion was confirmed by MS (ESI, scan neg, 300-3000) with no masses related to starting material or partially deprotected intermediates observed. The reaction mixture was then precipitated into 45 mL diethyl ether, spun down, the supernatant poured off, and washed 2×10 mL diethyl ether and dried on high vacuum overnight. The final product was purified on prep HPLC using a Thermo Aquasil C18 5 um semi prep column, with mobile phases 0.1% TFA in H_2O and ACN. Each injection was 50 mg of crude material dissolved in 3.0 mL of 0.1% TFA in [61:39] H_2O:ACN run on a gradient of (indicated in %B) 39-(5)-39-(35)-43-(5)-95-(10)-95-(2)-39-(5)-39. Each sample for injection was prepared (dissolved) within 15 minutes of being injected and positive fractions were pooled in one flask and kept cold in the freezer until the last injection of the day had finished. The positive fractions were then concentrated on the rotovap with a bath temperature of 32°C to dryness, then chased 2× with ACN/Toluene, then 3× with ACN and then dried on high vacuum overnight. Out of 257 mg injected crude, 180 mg (70%) was isolated as pure material (Reaction shown above for PEG_{20} (n = 20)).



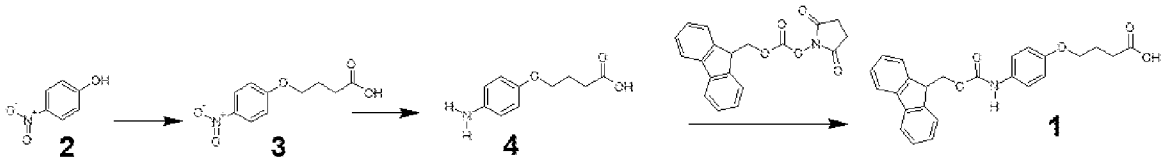
RGD-PEG$_n$-FCitFPro-TFP

(shown for n = 20)

*4-(N-Fmoc-p-aminophenoxy)-butyric acid **1** synthesis.* p-Nitro-phenol (**2**) (7.5 g, 53.9 mmole) was combined with ethyl 4-bromobutyrate (8.45 ml, 59 mmol) and K_2CO_3 (7.5 g, 54 mmole) in DMF (75 mL). The mixture was stirred for 2 h at 100°C. DMF was removed and the crude product was diluted in a mixture of 3:1 mixture of 2 N NaOH and methanol and stirred 4 h at RT. The reaction mixture was acidified with 6 M HCl. The white precipitate was collected to yield 4-(p-Nitrophenyloxy)-butyric acid **3**: (10.9 g, 90% yield).

4-(p-Nitrophenyloxy)-butyric acid **3** (37.1 g, 165 mmole) was dissolved in MeOH (1 L) with ammonium formate (35 g, 555 mmole) and 10% Pd/C (Degussa Type) (3.5 g) was added. The mixture was refluxed at 65°C overnight. The reaction was filtered with celite to yield a reddish brown solid of product 4-(p-Aminophenyloxy)-butyric acid **4** (30.5 g, 95 % yield).

4-(p-Aminophenyloxy)-butyric acid **4** (5.1 g, 26 mmole) was dissolved in 6:4 a mixture of an aqueous saturated $NaHCO_3$ (3.36 g, 40 mmol) in H_2O (450 mL) and THF (300 ml) to make a white slurry. Fmoc-OSu (8.82 g, 26.1 mmole) was added and the reaction was stirred for 4 h. The acetone was removed, the reaction was acidified (HCl), and the off-white precipitate was collected and triturated in 1N HCl to yield 9.6 g of product 4-(N-Fmoc-*p*-aminophenoxy)-butyric acid **1** (88% yield).



diBoc-m-guanidino-benzoic acid **5** was synthesized according to Riches AG et al. Tetrahedron (2012) 68, p. 9448-9455.

PEG$_n$-FCitFP modifying agents were made using similar chemistry. FIG. 1-2.

Example 8. *Tetrapeptide peptide linkages.*

A. *Tetrapeptide Syntheses.* All tetrapeptides were synthesized in the same manner using standard solid phase Fmoc procedures. Some peptides were synthesized from commercially

available 2-Cl-Trt resin (EMD Millipore, Billerica, MA) containing either proline, leucine, or alanine. For other peptides, 2-Cl-Trt resin was loaded with either FMOC-PEG_n-CO_2H or FMOC-N-methyl-Ala-CO_2H by adding a solution of DMF containing the amino acid or PEG (1eq) and DIEA (2eq) to 2-Cl-Trt resin for 16 h. Upon completion, resins were capped with MeOH. Stepwise addition was performed using PYBOP (4eq), amino acid (4eq), and DIEA (8eq) for coupling and 20% piperdine in DMF for Fmoc de-protection.

After peptide syntheses, the tetrapeptides were reacted with 2 eq of N-Hydroxysuccinimide (NHS) activated esters of either protected N-Acetyl-galactosamine, $NAG(OAc)_3$ (Rozema DB et al. "Protease-triggered siRNA Delivery Vehicles." J Control Release. 2015 Vol. 209:57-66 and US patent 8802773) or PEG_n in DMF containing 4 eq DIEA. Following attachment of $NAG(OAc)_3$ or PEG, the peptides were removed from resin using HFIP (30%) in DCM for 0.5 h. After solvent removal the residue was triturated with Et_2O.

Tetrapeptides were either purified and conjugated to activated esters to form modifying agents or conjugated to chromophore N-(p-Nitrophenyl)ethylenediamine (pNA) without purification to form substrates for physiological lability testing. Prior to purification, $NAG(OAc)_3$-containing substrates were de-acetylated by treatment with TEA (35%) in water (45%) and MeOH (20%) and stirred at room temp. For purification, tetrapeptide substrates were separated by HPLC using a Thermo Scientific Aquasil C18 reverse-phase column (250 x 21.2, Waltham, MA), eluting a gradient of acetonitrile and water buffered with 0.1% formic acid. Following purification, the substrates were lyophilized.

Attachment of amine-reactive groups to tetrapeptides. 1 eq HPLC purified peptide with N-terminal NAG (R_5=$NAG(OH)_3$ or PEG (R_5=PEG_n) in DMF or DCM was added to a flame dried flask at 0°C to give a 0.2 M concentration of peptide. NHS (3eq) and N,N'-Dicyclohexylcarbodiimide (DCC) (3eq) were added and allowed to stir at room temp. under argon overnight to yield the modifying agents. The mixture was partially concentrated, chilled to −20°C, and filtered. All solvents were then removed in vacuo. The residue was dissolved in a minimum of DCM and MeOH, precipitated into cold Et_2O and collected by decantation of the solvent after centrifugation. Precipitation into Et_2O was repeated until no residual DCU(dicyclohexylurea) was detectable. All prepared compounds were subsequently used without further purification.

Example 9. *Polymer modification.* Formation of siRNA delivery conjugate using RGD-PEG-HyNic, RGD-PEG-ACit-PNP, or RDG-PEG-FCitFP-TFP and PEG-dipeptide modifying agents.

1) *Protocol 1*. The indicated polymer was reacted with SMPT at a weight ratio of 1:0.015 (polymer:SMPT) in 5 mM HEPES, pH 8.0 buffer for 1 h at RT. The SMPT-modified polymer was then reacted with aldehyde-PEG-dipeptide modifying agent (aldehyde-PEG_{12}-FCit or aldehyde-PEG_{24}-ACit) at desired ratios for 1 h at RT. The modified polymer was then reacted with PEG_{12}-dipeptide modifying agent (PEG_{12}-FCit, PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:2 (polymer:PEG) in 100 mM HEPES, pH 9.0 buffer for 1 h at RT. The modified polymer was then reacted overnight with SATA-RNAi trigger at a weight ratio of 1:0.2 (polymer:SATA-RNAi trigger) in 100 mM HEPES, pH 9.0 buffer at RT to attach the RNAi trigger. Next, the modified polymer was reacted with protease cleavable PEG (PEG_{12}-FCit or PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:6 (polymer:PEG) in 100 mM HEPES, pH 9.0 buffer for 1 h at RT. The resultant conjugate was purified using a sephadex G-50 spin column.

RGD-HyNic (Example 6B) was attached to the modified polymer to form the full delivery conjugate by reaction with the modified polymer at a weight ratio of 1:0.7 (polymer:RGD-HyNic mimic) in 50 mM MES, pH 5.0 buffer for a minimum of 4 h at RT. The conjugate was purified using a sephadex G-50 spin column. RGD ligand attachment efficiency was determined as described above.

2) *Protocol 2*. The indicated polymer was reacted with SMPT at a weight ratio of 1:0.015 (polymer:SMPT) in 5 mM HEPES, pH 8.0 buffer for 1 h at RT. The SMPT-modified polymer was then reacted with aldehyde-PEG-dipeptide modifying agent (aldehyde-PEG_{24}-ACit) at a weight ratio of 1:0.5 (polymer:PEG) and with PEG-dipeptide modifying agent (PEG_{12}-FCit, PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:2 (polymer:PEG) in 100 mM HEPES, pH 9.0 buffer for 1 h at RT. The modified polymer was then reacted overnight with SATA-RNAi trigger at a weight ratio of 1:0.2 (polymer:SATA-RNAi trigger) in 100 mM HEPES, pH 9.0 buffer at RT to attach the RNAi trigger. Next, the modified polymer was reacted with protease cleavable-PEG (PEG_{12}-FCit or PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:6 (polymer:PEG) in 100 mM HEPES, pH 9.0 buffer for 1 h at RT. RGD-HyNic (Example 6) was attached to the modified polymer to form the full conjugate by reaction with the modified polymer at a weight ratio of 1:0.7 (polymer:RGD-HyNic) in 69 mM hydrogen chloride solution (HCl) overnight at RT. RGD ligand attachment efficiency was determined as described above.

3) *Protocol 3*. The indicated polymer was reacted with SMPT at a weight ratio of 1:0.015 (polymer:SMPT) in 5 mM HEPES, pH 8.0 buffer for 1 h at RT. The SMPT-modified polymer was then reacted with aldehyde-PEG-dipeptide modifying agent (aldehyde-PEG_{24}-ACit) at a weight ratio of 1:0.5 (polymer:PEG) and with PEG-dipeptide modifying agent (PEG_{12}-FCit, PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:2 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. The modified polymer was then reacted overnight with SATA-RNAi trigger at a weight ratio of 1:0.2 (polymer:SATA-RNAi trigger) in 50 mM HEPES, pH 9.0 buffer at RT to attach the RNAi trigger. Next, the modified polymer was reacted with protease cleavable-PEG (PEG_{12}-FCit or PEG_{12}-ACit or PEG_{24}-ACit) at a weight ratio of 1:6 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. RGD-HyNic (Example 6) was attached to the modified polymer to form the full delivery conjugate by reaction with the modified polymer at a weight ratio of 1:0.7 (polymer:RGD-HyNic mimic) in 100 mM MES free acid solution overnight at RT. RGD targeting ligand conjugation efficiency was determined as described above.

4) *Protocol 4*. The indicated polymer was reacted with Azido-PEG4-NHS at a weight ratio of 1:0.015 (polymer:Azido) in 5 mM HEPES, pH 8.0 buffer for 1 h at RT. The Azido-modified polymer was then reacted with aldehyde-PEG-dipeptide modifying agent (aldehyde-PEG_{24}-ACit) at a weight ratio of 1:0.5 (polymer:PEG) and with PEG-dipeptide modifying agent (PEG_{12}-ACit) at a weight ratio of 1:2 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. The modified polymer was then reacted overnight with Alkyne-RNAi trigger at a weight ratio of 1:0.2 (polymer:Alkyne-RNAi trigger) in 50 mM HEPES, pH 9.0 buffer at RT to attach the RNAi trigger. Next, the modified polymer was reacted with protease cleavable-PEG (PEG_{12}-ACit) at a weight ratio of 1:6 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. RGD-HyNic (Example 6) was attached to the modified polymer to form the full delivery conjugate by reaction with the modified polymer at a weight ratio of 1:0.7 (polymer:RGD-HyNic mimic) in 100 mM sodium acetate-acetic acid buffer solution, pH 5.0 overnight at RT. RGD targeting ligand conjugation efficiency was determined as described above.

5) *Protocol 5*. The mono azide-polymer was reacted with aldehyde-PEG-dipeptide modifying agent (aldehyde-PEG_{24}-ACit) at a weight ratio of 1:0.5 (polymer:PEG) and with PEG-dipeptide modifying agent (PEG_{12}-ACit) at a weight ratio of 1:2 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. The modified polymer was then reacted overnight with Alkyne-RNAi trigger at a weight ratio of 1:0.2 (polymer:Alkyne-RNAi trigger) in 50 mM HEPES, pH 9.0

buffer at RT to attach the RNAi trigger. Next, the modified polymer was reacted with protease cleavable-PEG (PEG_{12}-ACit) at a weight ratio of 1:6 (polymer:PEG) in 50 mM HEPES, pH 9.0 buffer for 1 h at RT. RGD-HyNic (Example 6) was attached to the modified polymer to form the full delivery conjugate by reaction with the modified polymer at a weight ratio of 1:0.7 (polymer:RGD-HyNic mimic) in 100 mM sodium acetate-acetic acid buffer solution, pH 5.0 overnight at RT. RGD targeting ligand conjugation efficiency was determined as described above.

6) *Protocol 6.* The mono azide-polymer was reacted with protease cleavable-RGD agent (RGD-PEG_8-ACit-PNP, RDG-PEG_8-FCitFP-TFP, RGD-PEG_{15}-FCitFP-TFP, RGD-PEG_{19}-FCitFP-TFP, or RGD-PEG_{20}-FCitFP-TFP) at weight ratios of 1:0.125, 1:0.25, 1:0.5, 1:1, 1:1.5, 1:2 (polymer:RGD) in 50 mM HEPES, pH 8.5 buffer for 4 h at RT. The modified polymer was then reacted with protease cleavable-PEG agent (PEG_6-ACit-PABC-PNP, PEG_{12}-ACit-PABC-PNP, PEG_{12}-FCit-PABC-PNP, PEG_{12}-FCitFP-TFP) at a weight ratio of 1:8 (polymer:PEG) in 50 mM HEPES, pH 8.5 buffer for 2 h at RT. Alkyne-RNAi trigger at a weight ratio of 1:0.3 (polymer:Alkyne-RNAi trigger) was added to the modified polymer in 100 mM sodium acetate-acetic acid buffer solution, pH 5.0 for 5 days at RT. The completed conjugate was TFF purified and conjugation efficiency determined.

7) Protocol 7. The mono azide-polymer was reacted with protease cleavable-RGD agent (RGD-PEG_{20}-FCitFP-TFP) at weight ratio of 1:1 (polymer:RGD) in 50 mM HEPES, pH 8.5 buffer for 2 h at RT. The modified polymer was then reacted with protease cleavable-PEG agent (PEG_{12}-ACit-PABC-PNP) at a weight ratio of 1:8 (polymer:PEG) in 50 mM HEPES, pH 8.5 buffer for 2 h at RT. The modified polymer was then TFF purified. Alkyne-RNAi trigger at a weight ratio of 1:0.4 (polymer:Alkyne-RNAi trigger) was added to the TFF purified polymer for 3 days at 37°C.

Example 10. *In vitro analysis of Hif2α RNAi triggers.* Candidate sequences were identified by in silico analysis and screened as chemically modified canonical siRNAs in vitro. For screening purposes, the human EPAS1 (Hif2α) cDNA sequence (accession #NM_001430) was synthesized and cloned (DNA 2.0, Menlo Park, CA) into a commercially-available reporter-based screening plasmid, psiCHECK2 (Promega, Madison, WI) which generated a Renilla luciferase/EPAS1 fusion mRNA. For RNAi trigger efficacy evaluation, Hep3B cells, a human

hepatocellular carcinoma line, were plated at ~10,000 cells per well in 96-well format. Each of the 187 EPAS1 RNAi triggers, in two subsets, was co-transfected at two concentrations, 1 nM and 0.1 nM, with 25 ng EPAS1-psiCHECK2 plasmid DNA per well and 0.2 μL LipoFectamine 2000 (Life Technologies) per well. Gene knockdown was determined by measuring Renilla luciferase levels normalized to the levels of constitutively-expressed firefly luciferase, also present on the psiCHECK-2 plasmid, using the Dual Luciferase Reporter Assay (Promega, Madison, WI) Table 5.

Table 5A. Unmodified Hif2α RNAi trigger antisense strand and sense strand sequences.

SEQ ID NO.	Sense Strand Sequence (5' → 3')	SEQ ID NO.	Antisense Strand Sequence (5' → 3')
285	GAGACUGUAUGGUCAGCUC	478	GAGCUGACCAUACAGUCUC
286	CUCCGACUCCUUCCGACUC	479	GAGUCGGAAGGAGUCGGAG
287	UCCGACUCCCAGCAUUCGA	480	UCGAAUGCUGGGAGUCGGA
288	CGACUCCCAGCAUUCGAGC	481	GCUCGAAUGCUGGGAGUCG
289	GACUCCCAGCAUUCGAGCC	482	GGCUCGAAUGCUGGGAGUC
290	CAGGUGCUCGGCGUCUGAA	483	UUCAGACGCCGAGCACCUG
291	GUGCUCGGCGUCUGAACGU	484	ACGUUCAGACGCCGAGCAC
292	UCGGCGUCUGAACGUCUCA	485	UGAGACGUUCAGACGCCGA
293	GGCGUCUGAACGUCUCAAA	486	UUUGAGACGUUCAGACGCC
294	CGUCUGAACGUCUCAAAGG	487	CCUUUGAGACGUUCAGACG
295	AAAAGGAGUAGCUCGGAGA	488	UCUCCGAGCUACUCCUUUU
296	GGGUUUCAUUGCCGUGGUG	489	CACCACGGCAAUGAAACCC
297	UUCAUGGGACUUACACAGG	490	CCUGUGUAAGUCCCAUGAA
298	GGGACUUACACAGGUGGAG	491	CUCCACCUGUGUAAGUCCC
299	ACACAGGUGGAGCUAACAG	492	CUGUUAGCUCCACCUGUGU
300	GAGCUAACAGGACAUAGUA	493	UACUAUGUCCUGUUAGCUC
301	GCUAACAGGACAUAGUAUC	494	GAUACUAUGUCCUGUUAGC
302	CUAACAGGACAUAGUAUCU	495	AGAUACUAUGUCCUGUUAG
303	GGACAUAGUAUCUUUGACU	496	AGUCAAAGAUACUAUGUCC
304	UCUUUGACUUCACUCAUCC	497	GGAUGAGUGAAGUCAAAGA
305	UCACUCAUCCCUGCGACCA	498	UGGUCGCAGGGAUGAGUGA
306	GAGAUUCGUGAGAACCUGA	499	UCAGGUUCUCACGAAUCUC
307	UUCGUGAGAACCUGAGUCU	500	AGACUCAGGUUCUCACGAA
308	UCGUGAGAACCUGAGUCUC	501	GAGACUCAGGUUCUCACGA
309	GACAUGUCCACAGAGCGGG	502	CCCGCUCUGUGGACAUGUC
310	GCGGGACUUCUUCAUGAGG	503	CCUCAUGAAGAAGUCCCGC
311	GGAUGAAGUGCACGGUCAC	504	GUGACCGUGCACUUCAUCC
312	CACGGUCACCAACAGAGGC	505	GCCUCUGUUGGUGACCGUG
313	UCACCAACAGAGGCCGUAC	506	GUACGGCCUCUGUUGGUGA
314	CACCAACAGAGGCCGUACU	507	AGUACGGCCUCUGUUGGUG

315	AGGCCGUACUGUCAACCUC	508	GAGGUUGACAGUACGGCCU
316	UCCUCACAAUAGUCUGUGU	509	ACACAGACUAUUGUGAGGA
317	AAUAGUCUGUGUGGCUACA	510	UGUAGCCACACAGACUAUU
318	CAGAACUGAUUGGUUACCA	511	UGGUAACCAAUCAGUUCUG
319	AGAACUGAUUGGUUACCAC	512	GUGGUAACCAAUCAGUUCU
320	CUGAUUGGUUACCACCCUG	513	CAGGGUGGUAACCAAUCAG
321	UUGGCCGCUCAGCCUAUGA	514	UCAUAGGCUGAGCGGCCAA
322	UAUGAAUUCUACCAUGCGC	515	GCGCAUGGUAGAAUUCAUA
323	AUGAAUUCUACCAUGCGCU	516	AGCGCAUGGUAGAAUUCAU
324	UGAAUUCUACCAUGCGCUA	517	UAGCGCAUGGUAGAAUUCA
325	GAAUUCUACCAUGCGCUAG	518	CUAGCGCAUGGUAGAAUUC
326	AAUUCUACCAUGCGCUAGA	519	UCUAGCGCAUGGUAGAAUU
327	UCUACCAUGCGCUAGACUC	520	GAGUCUAGCGCAUGGUAGA
328	AUGCGCUAGACUCCGAGAA	521	UUCUCGGAGUCUAGCGCAU
329	UGCGCUAGACUCCGAGAAC	522	GUUCUCGGAGUCUAGCGCA
330	GUAAGUGGCCAGUACCGGA	523	UCCGGUACUGGCCACUUAC
331	UAAGUGGCCAGUACCGGAU	524	AUCCGGUACUGGCCACUUA
332	CCAGUACCGGAUGCUCGCA	525	UGCGAGCAUCCGGUACUGG
333	AGUACCGGAUGCUCGCAAA	526	UUUGCGAGCAUCCGGUACU
334	UACCGGAUGCUCGCAAAGC	527	GCUUUGCGAGCAUCCGGUA
335	UGCUCGCAAAGCAUGGGGG	528	CCCCCAUGCUUUGCGAGCA
336	CGCAAAGCAUGGGGGCUAC	529	GUAGCCCCCAUGCUUUGCG
337	AGCAUGGGGGCUACGUGUG	530	CACACGUAGCCCCCAUGCU
338	GCAUGGGGGCUACGUGUGG	531	CCACACGUAGCCCCCAUGC
339	CAUCUACAACCCUCGCAAC	532	GUUGCGAGGGUUGUAGAUG
340	AUCUACAACCCUCGCAACC	533	GGUUGCGAGGGUUGUAGAU
341	CUACAACCCUCGCAACCUG	534	CAGGUUGCGAGGGUUGUAG
342	UACAACCCUCGCAACCUGC	535	GCAGGUUGCGAGGGUUGUA
343	UUUGAUAGCAGUGGCAAGG	536	CCUUGCCACUGCUAUCAAA
344	AGUAACUUCCUAUUCACCA	537	UGGUGAAUAGGAAGUUACU
345	UCGGGAAUCAGAACUUCGA	538	UCGAAGUUCUGAUUCCCGA
346	CUGCUCCACGCCCAAUAGC	539	GCUAUUGGGCGUGGAGCAG
347	UGCUCCACGCCCAAUAGCC	540	GGCUAUUGGGCGUGGAGCA
348	GCUCCACGCCCAAUAGCCC	541	GGGCUAUUGGGCGUGGAGC
349	ACGCCCAAUAGCCCUGAAG	542	CUUCAGGGCUAUUGGGCGU
350	CAUCUUUGGAUAACGACCU	543	AGGUCGUUAUCCAAAGAUG
351	CAAUGCAGUACCCAGACGG	544	CCGUCUGGGUACUGCAUUG
352	AUGCAGUACCCAGACGGAU	545	AUCCGUCUGGGUACUGCAU
353	AGUACCCAGACGGAUUUCA	546	UGAAAUCCGUCUGGGUACU
354	CUGUAGCCCCGCACAGUCC	547	GGACUGUGCGGGGCUACAG
355	AUCUUCUUUGAUGCCGGAA	548	UUCCGGCAUCAAAGAAGAU
356	CUUUGAUGCCGGAAGCAAA	549	UUUGCUUCCGGCAUCAAAG
357	GAUGCCGGAAGCAAAGCAU	550	AUGCUUUGCUUCCGGCAUC
358	AUGCCGGAAGCAAAGCAUC	551	GAUGCUUUGCUUCCGGCAU

359	GCCGGAAGCAAAGCAUCCC	552	GGGAUGCUUUGCUUCCGGC
360	CCCCCAGAUCCACCAUUAC	553	GUAAUGGUGGAUCUGGGGG
361	AGAUCCACCAUUACAUUUU	554	AAAAUGUAAUGGUGGAUCU
362	AUUUUGGGCCCACAAAGUG	555	CACUUUGUGGGCCCAAAAU
363	UUUUGGGCCCACAAAGUGG	556	CCACUUUGUGGGCCCAAAA
364	UUUGGGCCCACAAAGUGGG	557	CCCACUUUGUGGGCCCAAA
365	CCACAAAGUGGGCCGUCGG	558	CCGACGGCCCACUUUGUGG
366	CACAAAGUGGGCCGUCGGG	559	CCCGACGGCCCACUUUGUG
367	AGUGGGCCGUCGGGGAUCA	560	UGAUCCCCGACGGCCCACU
368	AAAGGGUUUUGGGGCUCGA	561	UCGAGCCCCAAAACCCUUU
369	GGCUCGAGGCCCAGACGUG	562	CACGUCUGGGCCUCGAGCC
370	GCUCGAGGCCCAGACGUGC	563	GCACGUCUGGGCCUCGAGC
371	CUCGAGGCCCAGACGUGCU	564	AGCACGUCUGGGCCUCGAG
372	GGUAGCCCUCUCCAACAAG	565	CUUGUUGGAGAGGGCUACC
373	CUUUGAUGCCGGACAAGCC	566	GGCUUGUCCGGCAUCAAAG
374	UUUGAUGCCGGACAAGCCA	567	UGGCUUGUCCGGCAUCAAA
375	UUGAUGCCGGACAAGCCAC	568	GUGGCUUGUCCGGCAUCAA
376	GGACAAGCCACUGAGCGCA	569	UGCGCUCAGUGGCUUGUCC
377	ACAAGCCACUGAGCGCAAA	570	UUUGCGCUCAGUGGCUUGU
378	GGACUACAGCCUGUCGUCA	571	UGACGACAGGCUGUAGUCC
379	GACUACAGCCUGUCGUCAG	572	CUGACGACAGGCUGUAGUC
380	CUACAGCCUGUCGUCAGCC	573	GGCUGACGACAGGCUGUAG
381	CCUGUCGUCAGCCCACAAG	574	CUUGUGGGCUGACGACAGG
382	GCAUGGCAAGCCGGCUGCU	575	AGCAGCCGGCUUGCCAUGC
383	CUGACCAGAUAUGACUGUG	576	CACAGUCAUAUCUGGUCAG
384	GAUAUGACUGUGAGGUGAA	577	UUCACCUCACAGUCAUAUC
385	GGUGAACGUGCCCGUGCUG	578	CAGCACGGGCACGUUCACC
386	UACAAGAUGGACUUACCUG	579	CAGGUAAGUCCAUCUUGUA
387	GGACUUACCUGGCAGACUU	580	AAGUCUGCCAGGUAAGUCC
388	UUUUUCUGAGAUGCUCACU	581	AGUGAGCAUCUCAGAAAAA
389	AGUACACAAUUGUUUUACC	582	GGUAAAACAAUUGUGUACU
390	ACAAGUUUGGUGCAUGUCU	583	AGACAUGCACCAAACUUGU
391	ACUAAAAAGAUUCCUCGUU	584	AACGAGGAAUCUUUUUAGU
392	AGGGUCAACUCCAACGUAU	585	AUACGUUGGAGUUGACCCU
393	GGGUCAACUCCAACGUAUG	586	CAUACGUUGGAGUUGACCC
394	GUCAACUCCAACGUAUGUG	587	CACAUACGUUGGAGUUGAC
395	UCAACUCCAACGUAUGUGG	588	CCACAUACGUUGGAGUUGA
396	CAACUCCAACGUAUGUGGU	589	ACCACAUACGUUGGAGUUG
397	CUCCAACGUAUGUGGUUAU	590	AUAACCACAUACGUUGGAG
398	UCCAACGUAUGUGGUUAUC	591	GAUAACCACAUACGUUGGA
399	CCAACGUAUGUGGUUAUCU	592	AGAUAACCACAUACGUUGG
400	AACGUAUGUGGUUAUCUGU	593	ACAGAUAACCACAUACGUU
401	UUAUAUCUGGGUUAAGUGU	594	ACACUUAACCCAGAUAUAA
402	CCACGGCCUGUACGGACAC	595	GUGUCCGUACAGGCCGUGG

403	ACGGCCUGUACGGACACUG	596	CAGUGUCCGUACAGGCCGU
404	UGUCGGCUUUUUGCCAUCU	597	AGAUGGCAAAAAGCCGACA
405	GUCGGCUUUUUGCCAUCUG	598	CAGAUGGCAAAAAGCCGAC
406	AUCUGUGAUAUGCCAUAGG	599	CCUAUGGCAUAUCACAGAU
407	UGCCAUAGGUGUGACAAUC	600	GAUUGUCACACCUAUGGCA
408	CCAUAGGUGUGACAAUCCG	601	CGGAUUGUCACACCUAUGG
409	CAUAGGUGUGACAAUCCGA	602	UCGGAUUGUCACACCUAUG
410	AUAGGUGUGACAAUCCGAG	603	CUCGGAUUGUCACACCUAU
411	GGUGUGACAAUCCGAGCAG	604	CUGCUCGGAUUGUCACACC
412	ACAAUCCGAGCAGUGGAGU	605	ACUCCACUGCUCGGAUUGU
413	CCGAGCAGUGGAGUCAUUC	606	GAAUGACUCCACUGCUCGG
414	GGGAGCACUGCGCGCUAUC	607	GAUAGCGCGCAGUGCUCCC
415	GGAGCACUGCGCGCUAUCC	608	GGAUAGCGCGCAGUGCUCC
416	AGCACUGCGCGCUAUCCCC	609	GGGGAUAGCGCGCAGUGCU
417	UAUUGCUGCCAAGAGGGUC	610	GACCCUCUUGGCAGCAAUA
418	GGUCUGAUGGCACGUUGUG	611	CACAACGUGCCAUCAGACC
419	CUGAUGGCACGUUGUGGGG	612	CCCCACAACGUGCCAUCAG
420	GGCACGUUGUGGGGUCGGG	613	CCCGACCCCACAACGUGCC
421	GCACGUUGUGGGGUCGGGG	614	CCCCGACCCCACAACGUGC
422	CACGUUGUGGGGUCGGGGG	615	CCCCCGACCCCACAACGUG
423	GCGGGGAAGUGCUCUAACU	616	AGUUAGAGCACUUCCCCGC
424	CGGGGAAGUGCUCUAACUU	617	AAGUUAGAGCACUUCCCCG
425	UUAAGGUUUUGUUGCUAGC	618	GCUAGCAACAAAACCUUAA
426	GUUGCUAGCCCUUCAAGUG	619	CACUUGAAGGGCUAGCAAC
427	GAGCUAUGUGACUCGGAUG	620	CAUCCGAGUCACAUAGCUC
428	GCUAUGUGACUCGGAUGGU	621	ACCAUCCGAGUCACAUAGC
429	CGGAUGGUCUUUCACACGG	622	CCGUGUGAAAGACCAUCCG
430	GAUGGUCUUUCACACGGCA	623	UGCCGUGUGAAAGACCAUC
431	UGGUCUUUCACACGGCACA	624	UGUGCCGUGUGAAAGACCA
432	AACUACCAUGAGAUGGUUU	625	AAACCAUCUCAUGGUAGUU
433	UACCAUGAGAUGGUUUAGA	626	UCUAAACCAUCUCAUGGUA
434	CCAAGCUCACGACCUUGGA	627	UCCAAGGUCGUGAGCUUGG
435	ACGACCUUGGAGCCCCGUG	628	CACGGGGCUCCAAGGUCGU
436	GGGUAAGAGGGACGACACC	629	GGUGUCGUCCCUCUUACCC
437	GGUAAGAGGGACGACACCU	630	AGGUGUCGUCCCUCUUACC
438	GUAAGAGGGACGACACCUC	631	GAGGUGUCGUCCCUCUUAC
439	UGGUUUUUCAAUACCAAUU	632	AAUUGGUAUUGAAAAACCA
440	UUCAAUACCAAUUACAUGG	633	CCAUGUAAUUGGUAUUGAA
441	AUACCAAUUACAUGGAACU	634	AGUUCCAUGUAAUUGGUAU
442	CCAACUAUUUAGUAAGCCC	635	GGGCUUACUAAAUAGUUGG
443	AACUAUUUAGUAAGCCCGG	636	CCGGGCUUACUAAAUAGUU
444	ACUAUUUAGUAAGCCCGGA	637	UCCGGGCUUACUAAAUAGU
445	AGAAAUUCCUUAGUCAUGG	638	CCAUGACUAAGGAAUUUCU
446	CAUUAAGGGCAUUUUACCC	639	GGGUAAAAUGCCCUUAAUG

447	UAAGGGCAUUUUACCCUUG	640	CAAGGGUAAAAUGCCCUUA
448	AGCUUCAUAUUAACCCUAC	641	GUAGGGUUAAUAUGAAGCU
449	UAUUAACCCUACCUGUCAA	642	UUGACAGGUAGGGUUAAUA
450	UUAACCCUACCUGUCAACG	643	CGUUGACAGGUAGGGUUAA
451	ACCCUACCUGUCAACGUAA	644	UUACGUUGACAGGUAGGGU
452	CCCUACCUGUCAACGUAAC	645	GUUACGUUGACAGGUAGGG
453	CCUACCUGUCAACGUAACG	646	CGUUACGUUGACAGGUAGG
454	CUACCUGUCAACGUAACGA	647	UCGUUACGUUGACAGGUAG
455	UACCUGUCAACGUAACGAU	648	AUCGUUACGUUGACAGGUA
456	ACCUGUCAACGUAACGAUU	649	AAUCGUUACGUUGACAGGU
457	CCUGUCAACGUAACGAUUU	650	AAAUCGUUACGUUGACAGG
458	CUGUCAACGUAACGAUUUC	651	GAAAUCGUUACGUUGACAG
459	UGUCAACGUAACGAUUUCA	652	UGAAAUCGUUACGUUGACA
460	UCAACGUAACGAUUUCAUG	653	CAUGAAAUCGUUACGUUGA
461	ACGUAACGAUUUCAUGAAC	654	GUUCAUGAAAUCGUUACGU
462	UAUUAUAUUGUCGAAUUCC	655	GGAAUUCGACAAUAUAAUA
463	UUAUAUUGUCGAAUUCCUA	656	UAGGAAUUCGACAAUAUAA
464	UAUUGUCGAAUUCCUACUG	657	CAGUAGGAAUUCGACAAUA
465	GAAUUCCUACUGACAACAU	658	AUGUUGUCAGUAGGAAUUC
466	UCCUACUGACAACAUUAUA	659	UAUAAUGUUGUCAGUAGGA
467	UAUAACUGUAUGGGAGCUU	660	AAGCUCCCAUACAGUUAUA
468	UAACUGUAUGGGAGCUUAA	661	UUAAGCUCCCAUACAGUUA
469	UGUAUGGGAGCUUAACUUU	662	AAAGUUAAGCUCCCAUACA
470	UUGACACUGGUAUCUUAUU	663	AAUAAGAUACCAGUGUCAA
471	AAGUAUUCUGAUCCUACCA	664	UGGUAGGAUCAGAAUACUU
472	CAACGUAACGAUUUCAUGAAA	665	UUCAUGAAAUCGUUACGUUGGC
473	UAUAUCAACGUAACGAUUUCAUGAAA	666	UUCAUGAAAUCGUUACGUUGGCU
474	UAUAUCAACGUAACGAUUUCAUGAAA	667	UUCAUGAAAUCGUUACGUUGGCUAU
475	UAUAUCAACGUAACGAUUUCAUGAAA	668	UUCAUGAAAUCGUUACGUUGGCUGU
476	UAUAUCGACGUAACGAUUUCAUGAAA	669	UUCAUGAAAUCGUUACGUCGGCUAU
477	UAUACGUAACGAUUUCAUGAAA	670	UUCAUGAAAUCGUUACGU

Table 5B. Hif2α RNAi trigger sequences having modified nucleotides.

Duplex ID No.	SEQ ID No.	Sense Strand Sequence (5' → 3')	SEQ ID No.	Antisense Strand Sequence (5' → 3')
2231	671	GfaGfaCfuGfuAfuGfgUfcAfgCfuAfdT	858	dTAfgCfuGfaCfcAfuAfcAfgUfcUfcdTsdT
2232	672	CfuCfcGfaCfuCfcUfuCfcGfaCfuAfdT	859	dTAfgUfcGfgAfaGfgAfgUfcGfgAfgdTsdT
2233	673	UfcCfgAfcUfcCfcAfgCfaUfuCfgAfdT	860	dTCfgAfaUfgCfuGfgGfaGfuCfgGfadTsdT
2234	674	CfgAfcUfcCfcAfgCfaUfuCfgAfgAfdT	861	dTCfuCfgAfaUfgCfuGfgGfaGfuCfgdTsdT
2235	675	GfaCfuCfcCfaGfcAfuUfcGfaGfcAfdT	862	dTGfcUfcGfaAfuGfcUfgGfgAfgUfcdTsdT
2236	676	CfaGfgUfgCfuCfgGfcGfuCfuGfaAfdT	863	dTUfcAfgAfcGfcCfgAfgCfaCfcUfgdTsdT
2237	677	GfuGfcUfcGfgCfgUfcUfgAfaCfgAfdT	864	dTCfgUfuCfaGfaCfgCfcGfaGfcAfcdTsdT
2238	678	UfcGfgCfgUfcUfgAfaCfgUfcUfcAfdT	865	dTGfaGfaCfgUfuCfaGfaCfgCfcGfadTsdT
2239	679	GfgCfgUfcUfgAfaCfgUfcUfcAfaAfdT	866	dTUfuGfaGfaCfgUfuCfaGfaCfgCfcdTsdT

2240	680	CfgUfcUfgAfaCfgUfcUfcAfaAfgAfdT	867	dTCfuUfuGfaGfaCfgUfuCfaGfaCfgdTsdT
2241	681	AfaAfaGfgAfgUfaGfcUfcGfgAfgAfdT	868	dTCfuCfcGfaGfcUfaCfuCfcUfuUfudTsdT
2242	682	GfgGfuUfuCfaUfuGfcCfgUfgGfuAfdT	869	dTAfcCfaCfgGfcAfaUfgAfaAfcCfcdTsdT
2243	683	UfuCfaUfgGfgAfcUfuAfcAfcAfgAfdT	870	dTCfuGfuGfuAfaGfuCfcCfaUfgAfadTsdT
2244	684	GfgGfaCfuUfaCfaCfaGfgUfgGfaAfdT	871	dTUfcCfaCfcUfgUfgUfaAfgUfcCfcdTsdT
2245	685	AfcAfcAfgGfuGfgAfgCfuAfaCfaAfdT	872	dTUfgUfuAfgCfuCfcAfcCfuGfuGfudTsdT
2246	686	GfaGfcUfaAfcAfgGfaCfaUfaGfuAfdT	873	dTAfcUfaUfgUfcCfuGfuUfaGfcUfcdTsdT
2247	687	GfcUfaAfcAfgGfaCfaUfaGfuAfuAfdT	874	dTAfuAfcUfaUfgUfcCfuGfuUfaGfcdTsdT
2248	688	CfuAfaCfaGfgAfcAfuAfgUfaUfcAfdT	875	dTGfaUfaCfuAfuGfuCfcUfgUfuAfgdTsdT
2249	689	GfgAfcAfuAfgUfaUfcUfuUfgAfcAfdT	876	dTGfuCfaAfaGfaUfaCfuAfuGfuCfcdTsdT
2250	690	UfcUfuUfgAfcUfuCfaCfuCfaUfcAfdT	877	dTGfaUfgAfgUfgAfaGfuCfaAfaGfadTsdT
2251	691	UfcAfcUfcAfuCfcCfuGfcGfaCfcAfdT	878	dTGfgUfcGfcAfgGfgAfuGfaGfuGfadTsdT
2252	692	GfaGfaUfuCfgUfgAfgAfaCfcUfgAfdT	879	dTCfaGfgUfuCfuCfaCfgAfaUfcUfcdTsdT
2253	693	UfuCfgUfgAfgAfaCfcUfgAfgUfcAfdT	880	dTGfaCfuCfaGfgUfuCfuCfaCfgAfadTsdT
2254	694	UfcGfuGfaGfaAfcCfuGfaGfuCfuAfdT	881	dTAfgAfcUfcAfgGfuUfcUfcAfcGfadTsdT
2255	695	GfaCfaUfgUfcCfaCfaGfaGfcGfgAfdT	882	dTCfcGfcUfcUfgUfgGfaCfaUfgUfcdTsdT
2256	696	GfcGfgGfaCfuUfcUfuCfaUfgAfgAfdT	883	dTCfuCfaUfgAfaGfaAfgUfcCfcGfcdTsdT
2257	697	GfgAfuGfaAfgUfgCfaCfgGfuCfaAfdT	884	dTUfgAfcCfgUfgCfaCfuUfcAfuCfcdTsdT
2258	698	CfaCfgGfuCfaCfcAfaCfaGfaGfgAfdT	885	dTCfcUfcUfgUfuGfgUfgAfcCfgUfgdTsdT
2259	699	UfcAfcCfaAfcAfgAfgGfcCfgUfaAfdT	886	dTUfaCfgGfcCfuCfuGfuUfgGfuGfadTsdT
2260	700	CfaCfcAfaCfaGfaGfgCfcGfuAfcAfdT	887	dTGfuAfcGfgCfcUfcUfgUfuGfgUfgdTsdT
2261	701	AfgGfcCfgUfaCfuGfuCfaAfcCfuAfdT	888	dTAfgGfuUfgAfcAfgUfaCfgGfcCfudTsdT
2262	702	UfcCfuCfaCfaAfuAfgUfcUfgUfgAfdT	889	dTCfaCfaGfaCfuAfuUfgUfgAfgGfadTsdT
2263	703	AfaUfaGfuCfuGfuGfuGfgCfuAfcAfdT	890	dTGfuAfgCfcAfcAfcAfgAfcUfaUfudTsdT
2264	704	CfaGfaAfcUfgAfuUfgGfuUfaCfcAfdT	891	dTGfgUfaAfcCfaAfuCfaGfuUfcUfgdTsdT
2265	705	AfgAfaCfuGfaUfuGfgUfuAfcCfaAfdT	892	dTUfgGfuAfaCfcAfaUfcAfgUfuCfudTsdT
2266	706	CfuGfaUfuGfgUfuAfcCfaCfcCfuAfdT	893	dTAfgGfgUfgGfuAfaCfcAfaUfcAfgdTsdT
2267	707	UfuGfgCfcGfcUfcAfgCfcUfaUfgAfdT	894	dTCfaUfaGfgCfuGfaGfcGfgCfcAfadTsdT
2268	708	UfaUfgAfaUfuCfuAfcCfaUfgCfgAfdT	895	dTCfgCfaUfgGfuAfgAfaUfuCfaUfadTsdT
2269	709	AfuGfaAfuUfcUfaCfcAfuGfcGfcAfdT	896	dTGfcGfcAfuGfgUfaGfaAfuUfcAfudTsdT
2270	710	UfgAfaUfuCfuAfcCfaUfgCfgCfuAfdT	897	dTAfgCfgCfaUfgGfuAfgAfaUfuCfadTsdT
2271	711	GfaAfuUfcUfaCfcAfuGfcGfcUfaAfdT	898	dTUfaGfcGfcAfuGfgUfaGfaAfuUfcdTsdT
2272	712	AfaUfuCfuAfcCfaUfgCfgCfuAfgAfdT	899	dTCfuAfgCfgCfaUfgGfuAfgAfaUfudTsdT
2273	713	UfcUfaCfcAfuGfcGfcUfaGfaCfuAfdT	900	dTAfgUfcUfaGfcGfcAfuGfgUfaGfadTsdT
2274	714	AfuGfcGfcUfaGfaCfuCfcGfaGfaAfdT	901	dTUfcUfcGfgAfgUfcUfaGfcGfcAfudTsdT
2275	715	UfgCfgCfuAfgAfcUfcCfgAfgAfaAfdT	902	dTUfuCfuCfgGfaGfuCfuAfgCfgCfadTsdT
2276	716	GfuAfaGfuGfgCfcAfgUfaCfcGfgAfdT	903	dTCfcGfgUfaCfuGfgCfcAfcUfuAfcdTsdT
2277	717	UfaAfgUfgGfcCfaGfuAfcCfgGfaAfdT	904	dTUfcCfgGfuAfcUfgGfcCfaCfuUfadTsdT
2278	718	CfcAfgUfaCfcGfgAfuGfcUfcGfcAfdT	905	dTGfcGfaGfcAfuCfcGfgUfaCfuGfgdTsdT
2279	719	AfgUfaCfcGfgAfuGfcUfcGfcAfaAfdT	906	dTUfuGfcGfaGfcAfuCfcGfgUfaCfudTsdT
2280	720	UfaCfcGfgAfuGfcUfcGfcAfaAfgAfdT	907	dTCfuUfuGfcGfaGfcAfuCfcGfgUfadTsdT
2281	721	UfgCfuCfgCfaAfaGfcAfuGfgGfgAfdT	908	dTCfcCfcAfuGfcUfuUfgCfgAfgCfadTsdT
2282	722	CfgCfaAfaGfcAfuGfgGfgGfcUfaAfdT	909	dTUfaGfcCfcCfcAfuGfcUfuUfgCfgdTsdT
2283	723	AfgCfaUfgGfgGfgCfuAfcGfuGfuAfdT	910	dTAfcAfcGfuAfgCfcCfcCfaUfgCfudTsdT

2284	724	GfcAfuGfgGfgGfcUfaCfgUfgUfgAfdT	911	dTCfaCfaCfgUfaGfcCfcCfcAfuGfcdTsdT
2285	725	CfaUfcUfaCfaAfcCfcUfcGfcAfaAfdT	912	dTUfuGfcGfaGfgGfuUfgUfaGfaUfgdTsdT
2286	726	AfuCfuAfcAfaCfcCfuCfgCfaAfcAfdT	913	dTGfuUfgCfgAfgGfgUfuGfuAfgAfudTsdT
2287	727	CfuAfcAfaCfcCfuCfgCfaAfcCfuAfdT	914	dTAfgGfuUfgCfgAfgGfgUfuGfuAfgdTsdT
2288	728	UfaCfaAfcCfcUfcGfcAfaCfcUfgAfdT	915	dTCfaGfgUfuGfcGfaGfgGfuUfgUfadTsdT
2289	729	UfuUfgAfuAfgCfaGfuGfgCfaAfgAfdT	916	dTCfuUfgCfcAfcUfgCfuAfuCfaAfadTsdT
2290	730	AfgUfaAfcUfuCfcUfaUfuCfaCfcAfdT	917	dTGfgUfgAfaUfaGfgAfaGfuUfaCfudTsdT
2291	731	UfcGfgGfaAfuCfaGfaAfcUfuCfgAfdT	918	dTCfgAfaGfuUfcUfgAfuUfcCfcGfadTsdT
2292	732	CfuGfcUfcCfaCfgCfcCfaAfuAfgAfdT	919	dTCfuAfuUfgGfgCfgUfgGfaGfcAfgdTsdT
2293	733	UfgCfuCfcAfcGfcCfcAfaUfaGfcAfdT	920	dTGfcUfaUfuGfgGfcGfuGfgAfgCfadTsdT
2294	734	GfcUfcCfaCfgCfcCfaAfuAfgCfcAfdT	921	dTGfgCfuAfuUfgGfgCfgUfgGfaGfcdTsdT
2295	735	AfcGfcCfcAfaUfaGfcCfcUfgAfaAfdT	922	dTUfuCfaGfgGfcUfaUfuGfgGfcGfudTsdT
2296	736	CfaUfcUfuUfgGfaUfaAfcGfaCfcAfdT	923	dTGfgUfcGfuUfaUfcCfaAfaGfaUfgdTsdT
2297	737	CfaAfuGfcAfgUfaCfcCfaGfaCfgAfdT	924	dTCfgUfcUfgGfgUfaCfuGfcAfuUfgdTsdT
2298	738	AfuGfcAfgUfaCfcCfaGfaCfgGfaAfdT	925	dTUfcCfgUfcUfgGfgUfaCfuGfcAfudTsdT
2299	739	AfgUfaCfcCfaGfaCfgGfaUfuUfcAfdT	926	dTGfaAfaUfcCfgUfcUfgGfgUfaCfudTsdT
2300	740	CfuGfuAfgCfcCfcGfcAfcAfgUfcAfdT	927	dTGfaCfuGfuGfcGfgGfgCfuAfcAfgdTsdT
2301	741	AfuCfuUfcUfuUfgAfuGfcCfgGfaAfdT	928	dTUfcCfgGfcAfuCfaAfaGfaAfgAfudTsdT
2302	742	CfuUfuGfaUfgCfcGfgAfaGfcAfaAfdT	929	dTUfuGfcUfuCfcGfgCfaUfcAfaAfgdTsdT
2303	743	GfaUfgCfcGfgAfaGfcAfaAfgCfaAfdT	930	dTUfgCfuUfuGfcUfuCfcGfgCfaUfcdTsdT
2304	744	AfuGfcCfgGfaAfgCfaAfaGfcAfuAfdT	931	dTAfuGfcUfuUfgCfuUfcCfgGfcAfudTsdT
2305	745	GfcCfgGfaAfgCfaAfaGfcAfuCfcAfdT	932	dTGfgAfuGfcUfuUfgCfuUfcCfgGfcdTsdT
2306	746	CfcCfcCfaGfaUfcCfaCfcAfuUfaAfdT	933	dTUfaAfuGfgUfgGfaUfcUfgGfgGfgdTsdT
2307	747	AfgAfuCfcAfcCfaUfuAfcAfuUfuAfdT	934	dTAfaAfuGfuAfaUfgGfuGfgAfuCfudTsdT
2308	748	AfuUfuUfgGfgCfcCfaCfaAfaGfuAfdT	935	dTAfcUfuUfgUfgGfgCfcCfaAfaAfudTsdT
2309	749	UfuUfuGfgGfcCfcAfcAfaAfgUfgAfdT	936	dTCfaCfuUfuGfuGfgGfcCfcAfaAfadTsdT
2310	750	UfuUfgGfgCfcCfaCfaAfaGfuGfgAfdT	937	dTCfcAfcUfuUfgUfgGfgCfcCfaAfadTsdT
2311	751	CfcAfcAfaAfgUfgGfgCfcGfuCfgAfdT	938	dTCfgAfcGfgCfcCfaCfuUfuGfuGfgdTsdT
2312	752	CfaCfaAfaGfuGfgGfcCfgUfcGfgAfdT	939	dTCfcGfaCfgGfcCfcAfcUfuUfgUfgdTsdT
2313	753	AfgUfgGfgCfcGfuCfgGfgGfaUfcAfdT	940	dTGfaUfcCfcCfgAfcGfgCfcCfaCfudTsdT
2314	754	AfaAfgGfgUfuUfuGfgGfgCfuCfgAfdT	941	dTCfgAfgCfcCfcAfaAfaCfcCfuUfudTsdT
2315	755	GfgCfuCfgAfgGfcCfcAfgAfcGfuAfdT	942	dTAfcGfuCfuGfgGfcCfuCfgAfgCfcdTsdT
2316	756	GfcUfcGfaGfgCfcCfaGfaCfgUfgAfdT	943	dTCfaCfgUfcUfgGfgCfcUfcGfaGfcdTsdT
2317	757	CfuCfgAfgGfcCfcAfgAfcGfuGfcAfdT	944	dTGfcAfcGfuCfuGfgGfcCfuCfgAfgdTsdT
2318	758	GfgUfaGfcCfcUfcUfcCfaAfcAfaAfdT	945	dTUfuGfuUfgGfaGfaGfgGfcUfaCfcdTsdT
2319	759	CfuUfuGfaUfgCfcGfgAfcAfaGfcAfdT	946	dTGfcUfuGfuCfcGfgCfaUfcAfaAfgdTsdT
2320	760	UfuUfgAfuGfcCfgGfaCfaAfgCfcAfdT	947	dTGfgCfuUfgUfcCfgGfcAfuCfaAfadTsdT
2321	761	UfuGfaUfgCfcGfgAfcAfaGfcCfaAfdT	948	dTUfgGfcUfuGfuCfcGfgCfaUfcAfadTsdT
2322	762	GfgAfcAfaGfcCfaCfuGfaGfcGfcAfdT	949	dTGfcGfcUfcAfgUfgGfcUfuGfuCfcdTsdT
2323	763	AfcAfaGfcCfaCfuGfaGfcGfcAfaAfdT	950	dTUfuGfcGfcUfcAfgUfgGfcUfuGfudTsdT
2324	764	GfgAfcUfaCfaGfcCfuGfuCfgUfcAfdT	951	dTGfaCfgAfcAfgGfcUfgUfaGfuCfcdTsdT
2325	765	GfaCfuAfcAfgCfcUfgUfcGfuCfaAfdT	952	dTUfgAfcGfaCfaGfgCfuGfuAfgUfcdTsdT
2326	766	CfuAfcAfgCfcUfgUfcGfuCfaGfcAfdT	953	dTGfcUfgAfcGfaCfaGfgCfuGfuAfgdTsdT
2327	767	CfcUfgUfcGfuCfaGfcCfcAfcAfaAfdT	954	dTUfuGfuGfgGfcUfgAfcGfaCfaGfgdTsdT

2328	768	GfcAfuGfgCfaAfgCfcGfgCfuGfcAfdT	955	dTGfcAfgCfcGfgCfuUfgCfcAfuGfcdTsdT
2329	769	CfuGfaCfcAfgAfuAfuGfaCfuGfuAfdT	956	dTAfcAfgUfcAfuAfuCfuGfgUfcAfgdTsdT
2330	770	GfaUfaUfgAfcUfgUfgAfgGfuGfaAfdT	957	dTUfcAfcCfuCfaCfaGfuCfaUfaUfcdTsdT
2331	771	GfgUfgAfaCfgUfgCfcCfgUfgCfuAfdT	958	dTAfgCfaCfgGfgCfaCfgUfuCfaCfcdTsdT
2332	772	UfaCfaAfgAfuGfgAfcUfuAfcCfuAfdT	959	dTAfgGfuAfaGfuCfcAfuCfuUfgUfadTsdT
2333	773	GfgAfcUfuAfcCfuGfgCfaGfaCfuAfdT	960	dTAfgUfcUfgCfcAfgGfuAfaGfuCfcdTsdT
2334	774	UfuUfuUfcUfgAfgAfuGfcUfcAfcAfdT	961	dTGfuGfaGfcAfuCfuCfaGfaAfaAfadTsdT
2335	775	AfgUfaCfaCfaAfuUfgUfuUfuAfcAfdT	962	dTGfuAfaAfaCfaAfuUfgUfgUfaCfudTsdT
2336	776	AfcAfaGfuUfuGfgUfgCfaUfgUfcAfdT	963	dTGfaCfaUfgCfaCfcAfaAfcUfuGfudTsdT
2337	777	AfcUfaAfaAfaGfaUfuCfcUfcGfuAfdT	964	dTAfcGfaGfgAfaUfcUfuUfuUfaGfudTsdT
2338	778	AfgGfgUfcAfaCfuCfcAfaCfgUfaAfdT	965	dTUfaCfgUfuGfgAfgUfuGfaCfcCfudTsdT
2339	779	GfgGfuCfaAfcUfcCfaAfcGfuAfuAfdT	966	dTAfuAfcGfuUfgGfaGfuUfgAfcCfcdTsdT
2340	780	GfuCfaAfcUfcCfaAfcGfuAfuGfuAfdT	967	dTAfcAfuAfcGfuUfgGfaGfuUfgAfcdTsdT
2341	781	UfcAfaCfuCfcAfaCfgUfaUfgUfgAfdT	968	dTCfaCfaUfaCfgUfuGfgAfgUfuGfadTsdT
2342	782	CfaAfcUfcCfaAfcGfuAfuGfuGfgAfdT	969	dTCfcAfcAfuAfcGfuUfgGfaGfuUfgdTsdT
2343	783	CfuCfcAfaCfgUfaUfgUfgGfuUfaAfdT	970	dTUfaAfcCfaCfaUfaCfgUfuGfgAfgdTsdT
2344	784	UfcCfaAfcGfuAfuGfuGfgUfuAfuAfdT	971	dTAfuAfaCfcAfcAfuAfcGfuUfgGfadTsdT
2345	785	CfcAfaCfgUfaUfgUfgGfuUfaUfcAfdT	972	dTGfaUfaAfcCfaCfaUfaCfgUfuGfgdTsdT
2346	786	AfaCfgUfaUfgUfgGfuUfaUfcUfgAfdT	973	dTCfaGfaUfaAfcCfaCfaUfaCfgUfudTsdT
2347	787	UfuAfuAfuCfuGfgGfuUfaAfgUfgAfdT	974	dTCfaCfuUfaAfcCfcAfgAfuAfuAfadTsdT
2348	788	CfcAfcGfgCfcUfgUfaCfgGfaCfaAfdT	975	dTUfgUfcCfgUfaCfaGfgCfcGfuGfgdTsdT
2349	789	AfcGfgCfcUfgUfaCfgGfaCfaCfuAfdT	976	dTAfgUfgUfcCfgUfaCfaGfgCfcGfudTsdT
2350	790	UfgUfcGfgCfuUfuUfuGfcCfaUfcAfdT	977	dTGfaUfgGfcAfaAfaAfgCfcGfaCfadTsdT
2351	791	GfuCfgGfcUfuUfuUfgCfcAfuCfuAfdT	978	dTAfgAfuGfgCfaAfaAfaGfcCfgAfcdTsdT
2352	792	AfuCfuGfuGfaUfaUfgCfcAfuAfgAfdT	979	dTCfuAfuGfgCfaUfaUfcAfcAfgAfudTsdT
2353	793	UfgCfcAfuAfgGfuGfuGfaCfaAfuAfdT	980	dTAfuUfgUfcAfcAfcCfuAfuGfgCfadTsdT
2354	794	CfcAfuAfgGfuGfuGfaCfaAfuCfcAfdT	981	dTGfgAfuUfgUfcAfcAfcCfuAfuGfgdTsdT
2355	795	CfaUfaGfgUfgUfgAfcAfaUfcCfgAfdT	982	dTCfgGfaUfuGfuCfaCfaCfcUfaUfgdTsdT
2356	796	AfuAfgGfuGfuGfaCfaAfuCfcGfaAfdT	983	dTUfcGfgAfuUfgUfcAfcAfcCfuAfudTsdT
2357	797	GfgUfgUfgAfcAfaUfcCfgAfgCfaAfdT	984	dTUfgCfuCfgGfaUfuGfuCfaCfaCfcdTsdT
2358	798	AfcAfaUfcCfgAfgCfaGfuGfgAfgAfdT	985	dTCfuCfcAfcUfgCfuCfgGfaUfuGfudTsdT
2359	799	CfcGfaGfcAfgUfgGfaGfuCfaUfuAfdT	986	dTAfaUfgAfcUfcCfaCfuGfcUfcGfgdTsdT
2360	800	GfgGfaGfcAfcUfgCfgCfgCfuAfuAfdT	987	dTAfuAfgCfgCfgCfaGfuGfcUfcCfcdTsdT
2361	801	GfgAfgCfaCfuGfcGfcGfcUfaUfcAfdT	988	dTGfaUfaGfcGfcGfcAfgUfgCfuCfcdTsdT
2362	802	AfgCfaCfuGfcGfcGfcUfaUfcCfcAfdT	989	dTGfgGfaUfaGfcGfcGfcAfgUfgCfudTsdT
2363	803	UfaUfuGfcUfgCfcAfaGfaGfgGfuAfdT	990	dTAfcCfcUfcUfuGfgCfaGfcAfaUfadTsdT
2364	804	GfgUfcUfgAfuGfgCfaCfgUfuGfuAfdT	991	dTAfcAfaCfgUfgCfcAfuCfaGfaCfcdTsdT
2365	805	CfuGfaUfgGfcAfcGfuUfgUfgGfgAfdT	992	dTCfcCfaCfaAfcGfuGfcCfaUfcAfgdTsdT
2366	806	GfgCfaCfgUfuGfuGfgGfgUfcGfgAfdT	993	dTCfcGfaCfcCfcAfcAfaCfgUfgCfcdTsdT
2367	807	GfcAfcGfuUfgUfgGfgGfuCfgGfgAfdT	994	dTCfcCfgAfcCfcCfaCfaAfcGfuGfcdTsdT
2368	808	CfaCfgUfuGfuGfgGfgUfcGfgGfgAfdT	995	dTCfcCfcGfaCfcCfcAfcAfaCfgUfgdTsdT
2369	809	GfcGfgGfgAfaGfuGfcUfcUfaAfcAfdT	996	dTGfuUfaGfaGfcAfcUfuCfcCfcGfcdTsdT
2370	810	CfgGfgGfaAfgUfgCfuCfuAfaCfuAfdT	997	dTAfgUfuAfgAfgCfaCfuUfcCfcCfgdTsdT
2371	811	UfuAfaGfgUfuUfuGfuUfgCfuAfgAfdT	998	dTCfuAfgCfaAfcAfaAfaCfcUfuAfadTsdT

2372	812	GfuUfgCfuAfgCfcCfuUfcAfaGfuAfdT	999	dTAfcUfuGfaAfgGfgCfuAfgCfaAfcdTsdT
2373	813	GfaGfcUfaUfgUfgAfcUfcGfgAfuAfdT	1000	dTAfuCfcGfaGfuCfaCfaUfaGfcUfcdTsdT
2374	814	GfcUfaUfgUfgAfcUfcGfgAfuGfgAfdT	1001	dTCfcAfuCfcGfaGfuCfaCfaUfaGfcdTsdT
2375	815	CfgGfaUfgGfuCfuUfuCfaCfaCfgAfdT	1002	dTCfgUfgUfgAfaAfgAfcCfaUfcCfgdTsdT
2376	816	GfaUfgGfuCfuUfuCfaCfaCfgGfcAfdT	1003	dTGfcCfgUfgUfgAfaAfgAfcCfaUfcdTsdT
2377	817	UfgGfuCfuUfuCfaCfaCfgGfcAfcAfdT	1004	dTGfuGfcCfgUfgUfgAfaAfgAfcCfadTsdT
2378	818	AfaCfuAfcCfaUfgAfgAfuGfgUfuAfdT	1005	dTAfaCfcAfuCfuCfaUfgGfuAfgUfudTsdT
2379	819	UfaCfcAfuGfaGfaUfgGfuUfuAfgAfdT	1006	dTCfuAfaAfcCfaUfcUfcAfuGfgUfadTsdT
2380	820	CfcAfaGfcUfcAfcGfaCfcUfuGfgAfdT	1007	dTCfcAfaGfgUfcGfuGfaGfcUfuGfgdTsdT
2381	821	AfcGfaCfcUfuGfgAfgCfcCfcGfuAfdT	1008	dTAfcGfgGfgCfuCfcAfaGfgUfcGfudTsdT
2382	822	GfgGfuAfaGfaGfgGfaCfgAfcAfcAfdT	1009	dTGfuGfuCfgUfcCfcUfcUfuAfcCfcdTsdT
2383	823	GfgUfaAfgAfgGfgAfcGfaCfaCfcAfdT	1010	dTGfgUfgUfcGfuCfcCfuCfuUfaCfcdTsdT
2384	824	GfuAfaGfaGfgGfaCfgAfcAfcCfuAfdT	1011	dTAfgGfuGfuCfgUfcCfcUfcUfuAfcdTsdT
2385	825	UfgGfuUfuUfuCfaAfuAfcCfaAfuAfdT	1012	dTAfuUfgGfuAfuUfgAfaAfaAfcCfadTsdT
2386	826	UfuCfaAfuAfcCfaAfuUfaCfaUfgAfdT	1013	dTCfaUfgUfaAfuUfgGfuAfuUfgAfadTsdT
2387	827	AfuAfcCfaAfuUfaCfaUfgGfaAfcAfdT	1014	dTGfuUfcCfaUfgUfaAfuUfgGfuAfudTsdT
2388	828	CfcAfaCfuAfuUfuAfgUfaAfgCfcAfdT	1015	dTGfgCfuUfaCfuAfaAfuAfgUfuGfgdTsdT
2389	829	AfaCfuAfuUfuAfgUfaAfgCfcCfgAfdT	1016	dTCfgGfgCfuUfaCfuAfaAfuAfgUfudTsdT
2390	830	AfcUfaUfuUfaGfuAfaGfcCfcGfgAfdT	1017	dTCfcGfgGfcUfuAfcUfaAfaUfaGfudTsdT
2391	831	AfgAfaAfuUfcCfuUfaGfuCfaUfgAfdT	1018	dTCfaUfgAfcUfaAfgGfaAfuUfuCfudTsdT
2392	832	CfaUfuAfaGfgGfcAfuUfuUfaCfcAfdT	1019	dTGfgUfaAfaAfuGfcCfcUfuAfaUfgdTsdT
2393	833	UfaAfgGfgCfaUfuUfuAfcCfcUfuAfdT	1020	dTAfaGfgGfuAfaAfaUfgCfcCfuUfadTsdT
2394	834	AfgCfuUfcAfuAfuUfaAfcCfcUfaAfdT	1021	dTUfaGfgGfuUfaAfuAfuGfaAfgCfudTsdT
2395	835	UfaUfuAfaCfcCfuAfcCfuGfuCfaAfdT	1022	dTUfgAfcAfgGfuAfgGfgUfuAfaUfadTsdT
2396	836	UfuAfaCfcCfuAfcCfuGfuCfaAfcAfdT	1023	dTGfuUfgAfcAfgGfuAfgGfgUfuAfadTsdT
2397	837	AfcCfcUfaCfcUfgUfcAfaCfgUfaAfdT	1024	dTUfaCfgUfuGfaCfaGfgUfaGfgGfudTsdT
2398	838	CfcCfuAfcCfuGfuCfaAfcGfuAfaAfdT	1025	dTUfuAfcGfuUfgAfcAfgGfuAfgGfgdTsdT
2399	839	CfcUfaCfcUfgUfcAfaCfgUfaAfcAfdT	1026	dTGfuUfaCfgUfuGfaCfaGfgUfaGfgdTsdT
2400	840	CfuAfcCfuGfuCfaAfcGfuAfaCfgAfdT	1027	dTCfgUfuAfcGfuUfgAfcAfgGfuAfgdTsdT
2401	841	UfaCfcUfgUfcAfaCfgUfaAfcGfaAfdT	1028	dTUfcGfuUfaCfgUfuGfaCfaGfgUfadTsdT
2402	842	AfcCfuGfuCfaAfcGfuAfaCfgAfuAfdT	1029	dTAfuCfgUfuAfcGfuUfgAfcAfgGfudTsdT
2403	843	CfcUfgUfcAfaCfgUfaAfcGfaUfuAfdT	1030	dTAfaUfcGfuUfaCfgUfuGfaCfaGfgdTsdT
2404	844	CfuGfuCfaAfcGfuAfaCfgAfuUfuAfdT	1031	dTAfaAfuCfgUfuAfcGfuUfgAfcAfgdTsdT
2405	845	UfgUfcAfaCfgUfaAfcGfaUfuUfcAfdT	1032	dTGfaAfaUfcGfuUfaCfgUfuGfaCfadTsdT
2406	846	UfcAfaCfgUfaAfcGfaUfuUfcAfuAfdT	1033	dTAfuGfaAfaUfcGfuUfaCfgUfuGfadTsdT
2407	847	AfcGfuAfaCfgAfuUfuCfaUfgAfaAfdT	1034	dTUfuCfaUfgAfaAfuCfgUfuAfcGfudTsdT
2408	848	UfaUfuAfuAfuUfgUfcGfaAfuUfcAfdT	1035	dTGfaAfuUfcGfaCfaAfuAfuAfaUfadTsdT
2409	849	UfuAfuAfuUfgUfcGfaAfuUfcCfuAfdT	1036	dTAfgGfaAfuUfcGfaCfaAfuAfuAfadTsdT
2410	850	UfaUfuGfuCfgAfaUfuCfcUfaCfuAfdT	1037	dTAfgUfaGfgAfaUfuCfgAfcAfaUfadTsdT
2411	851	GfaAfuUfcCfuAfcUfgAfcAfaCfaAfdT	1038	dTUfgUfuGfuCfaGfuAfgGfaAfuUfcdTsdT
2412	852	UfcCfuAfcUfgAfcAfaCfaUfuAfuAfdT	1039	dTAfuAfaUfgUfuGfuCfaGfuAfgGfadTsdT
2413	853	UfaUfaAfcUfgUfaUfgGfgAfgCfuAfdT	1040	dTAfgCfuCfcCfaUfaCfaGfuUfaUfadTsdT
2414	854	UfaAfcUfgUfaUfgGfgAfgCfuUfaAfdT	1041	dTUfaAfgCfuCfcCfaUfaCfaGfuUfadTsdT
2415	855	UfgUfaUfgGfgAfgCfuUfaAfcUfuAfdT	1042	dTAfaGfuUfaAfgCfuCfcCfaUfaCfadTsdT

2416	856	UfuGfaCfaCfuGfgUfaUfcUfuAfuAfdT	1043	dTAfuAfaGfaUfaCfcAfgUfgUfcAfadTsdT
2417	857	AfaGfuAfuUfcUfgAfuCfcUfaCfcAfdT	1044	dTGfgUfaGfgAfuCfaGfaAfuAfcUfudTsdT

Table 5C. Efficacy screen results of Hif2α RNAi triggers *in vitro*, as determined by dual-luciferase reporter assay.

duplex number	Relative R_{luc}-Hif2α		AD number	Relative R_{luc}-Hif2α	
	1 nM	0.1 nM		1nM	0.1 nM
2231	0.491 ± 0.198	0.544 ± 0.368	2325	1.379 ± 0.275	1.304 ± 0.134
2232	0.468 ± 0.032	0.684 ± 0.061	2326	0.546 ± 0.083	0.660 ± 0.114
2233	0.862 ± 0.125	0.913 ± 0.019	2327	1.073 ± 0.089	1.339 ± 0.179
2234	0.388 ± 0.046	0.508 ± 0.148	2328	1.192 ± 0.144	1.291 ± 0.109
2235	0.857 ± 0.090	0.743 ± 0.129	2329	0.456 ± 0.064	0.807 ± 0.117
2236	0.761 ± 0.042	0.693 ± 0.071	2330	0.464 ± 0.035	1.097 ± 0.416
2237	0.978 ± 0.083	0.746 ± 0.029	2331	0.783 ± 0.080	1.002 ± 0.321
2238	0.148 ± 0.024	0.208 ± 0.023	2332	0.871 ± 0.235	1.174 ± 0.027
2239	0.157 ± 0.017	0.225 ± 0.022	2333	0.291 ± 0.019	0.684 ± 0.147
2240	0.845 ± 0.052	0.841 ± 0.048	2334	0.389 ± 0.044	0.672 ± 0.082
2241	0.270 ± 0.068	0.385 ± 0.028	2335	0.104 ± 0.013	0.311 ± 0.032
2242	0.222 ± 0.064	0.411 ± 0.019	2336	0.299 ± 0.034	0.783 ± 0.037
2243	0.694 ± 0.107	0.688 ± 0.059	2337	0.131 ± 0.033	0.334 ± 0.066
2244	0.915 ± 0.115	0.760 ± 0.050	2338	0.334 ± 0.091	0.743 ± 0.070
2245	0.727 ± 0.066	0.761 ± 0.039	2339	0.189 ± 0.028	0.523 ± 0.134
2246	0.327 ± 0.042	0.509 ± 0.044	2340	0.444 ± 0.039	0.920 ± 0.114
2247	0.231 ± 0.048	0.439 ± 0.082	2341	0.202 ± 0.018	0.378 ± 0.116
2248	0.148 ± 0.036	0.215 ± 0.007	2342	0.549 ± 0.041	1.058 ± 0.064
2249	0.190 ± 0.028	0.303 ± 0.042	2343	0.254 ± 0.009	0.620 ± 0.208
2250	0.139 ± 0.046	0.255 ± 0.020	2344	0.276 ± 0.033	0.570 ± 0.071
2251	0.872 ± 0.121	0.857 ± 0.087	2345	0.129 ± 0.026	0.296 ± 0.096
2252	0.592 ± 0.061	0.696 ± 0.096	2346	0.273 ± 0.012	0.523 ± 0.133
2253	0.564 ± 0.043	0.646 ± 0.144	2347	0.530 ± 0.040	0.753 ± 0.092
2254	0.641 ± 0.054	0.756 ± 0.080	2348	0.454 ± 0.096	0.840 ± 0.270
2255	0.721 ± 0.055	0.653 ± 0.130	2349	0.504 ± 0.062	0.674 ± 0.172
2256	0.765 ± 0.124	0.796 ± 0.028	2350	0.678 ± 0.105	0.755 ± 0.085
2257	0.802 ± 0.044	0.647 ± 0.127	2351	1.306 ± 0.216	1.216 ± 0.415
2258	0.972 ± 0.168	0.882 ± 0.098	2352	0.749 ± 0.049	1.012 ± 0.048
2259	0.836 ± 0.110	0.743 ± 0.086	2353	0.324 ± 0.075	0.635 ± 0.156
2260	1.030 ± 0.187	0.861 ± 0.031	2354	0.741 ± 0.189	1.043 ± 0.239
2261	0.657 ± 0.067	0.568 ± 0.065	2355	0.248 ± 0.063	0.457 ± 0.066
2262	0.277 ± 0.071	0.755 ± 0.031	2356	0.210 ± 0.023	0.574 ± 0.006
2263	0.875 ± 0.059	1.126 ± 0.083	2357	0.435 ± 0.062	0.737 ± 0.082
2264	0.183 ± 0.019	0.381 ± 0.023	2358	0.731 ± 0.123	0.647 ± 0.113
2265	0.186 ± 0.045	0.448 ± 0.042	2359	0.354 ± 0.022	0.576 ± 0.208
2266	0.368 ± 0.046	0.808 ± 0.062	2360	0.962 ± 0.167	1.102 ± 0.119
2267	0.651 ± 0.049	1.029 ± 0.104	2361	0.603 ± 0.133	0.800 ± 0.198
2268	0.210 ± 0.037	0.409 ± 0.023	2362	0.454 ± 0.069	0.673 ± 0.089
2269	0.302 ± 0.080	0.530 ± 0.097	2363	0.452 ± 0.091	0.653 ± 0.199
2270	0.613 ± 0.147	0.658 ± 0.149	2364	0.855 ± 0.282	1.070 ± 0.042

2271	0.930 ± 0.094	1.056 ± 0.291	2365	1.259 ± 0.247	1.132 ± 0.107
2272	0.864 ± 0.197	1.028 ± 0.281	2366	0.695 ± 0.115	0.879 ± 0.223
2273	0.588 ± 0.057	0.574 ± 0.033	2367	0.376 ± 0.039	0.665 ± 0.064
2274	0.506 ± 0.074	0.870 ± 0.108	2368	1.140 ± 0.221	1.036 ± 0.063
2275	0.464 ± 0.048	0.515 ± 0.264	2369	0.338 ± 0.055	0.464 ± 0.032
2276	0.330 ± 0.080	0.543 ± 0.029	2370	0.294 ± 0.026	0.498 ± 0.128
2277	0.702 ± 0.091	0.625 ± 0.384	2371	0.166 ± 0.037	0.193 ± 0.030
2278	0.564 ± 0.070	0.947 ± 0.044	2372	0.514 ± 0.044	0.831 ± 0.103
2279	0.280 ± 0.042	0.506 ± 0.086	2373	0.162 ± 0.032	0.369 ± 0.082
2280	0.911 ± 0.086	0.871 ± 0.147	2374	0.192 ± 0.014	0.355 ± 0.020
2281	0.651 ± 0.041	0.914 ± 0.177	2375	0.305 ± 0.040	0.481 ± 0.062
2282	0.824 ± 0.155	1.209 ± 0.132	2376	0.228 ± 0.029	0.546 ± 0.115
2283	0.882 ± 0.026	1.175 ± 0.194	2377	0.958 ± 0.189	0.900 ± 0.189
2284	0.787 ± 0.062	1.227 ± 0.266	2378	0.734 ± 0.170	0.994 ± 0.246
2285	0.723 ± 0.088	1.109 ± 0.247	2379	0.939 ± 0.204	0.858 ± 0.098
2286	0.878 ± 0.078	1.291 ± 0.367	2380	1.722 ± 0.101	1.128 ± 0.285
2287	0.751 ± 0.011	0.871 ± 0.102	2381	1.063 ± 0.191	0.756 ± 0.109
2288	0.932 ± 0.118	0.943 ± 0.029	2382	1.151 ± 0.365	1.070 ± 0.064
2289	1.039 ± 0.146	0.992 ± 0.153	2383	1.060 ± 0.292	0.833 ± 0.082
2290	0.353 ± 0.036	0.698 ± 0.155	2384	0.439 ± 0.101	0.533 ± 0.067
2291	0.434 ± 0.046	0.911 ± 0.092	2385	0.515 ± 0.089	0.701 ± 0.178
2292	0.832 ± 0.000	1.135 ± 0.048	2386	0.274 ± 0.087	0.603 ± 0.032
2293	0.925 ± 0.126	1.010 ± 0.045	2387	0.201 ± 0.063	0.436 ± 0.028
2294	0.546 ± 0.033	0.743 ± 0.100	2388	0.436 ± 0.021	0.901 ± 0.318
2295	0.863 ± 0.122	1.047 ± 0.184	2389	0.820 ± 0.184	1.085 ± 0.307
2296	0.213 ± 0.028	0.411 ± 0.079	2390	0.568 ± 0.108	0.772 ± 0.175
2297	0.643 ± 0.028	0.704 ± 0.083	2391	0.129 ± 0.015	0.273 ± 0.006
2298	0.695 ± 0.037	0.729 ± 0.092	2392	0.283 ± 0.052	0.636 ± 0.039
2299	0.827 ± 0.067	0.907 ± 0.171	2393	1.049 ± 0.089	0.862 ± 0.388
2300	0.786 ± 0.181	1.090 ± 0.106	2394	0.254 ± 0.026	0.533 ± 0.076
2301	0.423 ± 0.102	0.558 ± 0.034	2395	0.218 ± 0.029	0.494 ± 0.104
2302	0.147 ± 0.028	0.414 ± 0.019	2396	0.939 ± 0.299	1.321 ± 0.070
2303	0.294 ± 0.034	0.414 ± 0.002	2397	0.119 ± 0.024	0.254 ± 0.048
2304	0.302 ± 0.025	0.528 ± 0.116	2398	0.534 ± 0.043	1.047 ± 0.047
2305	0.992 ± 0.217	0.961 ± 0.120	2399	0.418 ± 0.081	0.784 ± 0.071
2306	0.613 ± 0.031	0.596 ± 0.038	2400	0.165 ± 0.020	0.478 ± 0.028
2307	0.702 ± 0.142	0.800 ± 0.248	2401	0.174 ± 0.003	0.375 ± 0.062
2308	0.998 ± 0.059	0.799 ± 0.084	2402	0.128 ± 0.015	0.389 ± 0.060
2309	1.081 ± 0.135	0.823 ± 0.096	2403	0.568 ± 0.106	0.930 ± 0.132
2310	0.923 ± 0.101	0.785 ± 0.122	2404	0.104 ± 0.019	0.234 ± 0.045
2311	0.841 ± 0.130	0.888 ± 0.051	2405	0.138 ± 0.035	0.261 ± 0.044
2312	0.725 ± 0.173	0.899 ± 0.124	2406	0.168 ± 0.044	0.241 ± 0.022
2313	0.886 ± 0.243	0.853 ± 0.133	2407	0.124 ± 0.021	0.222 ± 0.027
2314	0.920 ± 0.150	0.692 ± 0.141	2408	0.282 ± 0.004	0.730 ± 0.045
2315	0.876 ± 0.099	0.781 ± 0.206	2409	0.104 ± 0.020	0.301 ± 0.085
2316	0.579 ± 0.055	0.797 ± 0.211	2410	0.154 ± 0.022	0.228 ± 0.033
2317	0.843 ± 0.157	0.842 ± 0.239	2411	0.410 ± 0.083	0.796 ± 0.110
2318	0.780 ± 0.103	0.885 ± 0.370	2412	0.291 ± 0.014	0.515 ± 0.037
2319	0.234 ± 0.055	0.454 ± 0.101	2413	0.317 ± 0.055	0.675 ± 0.104
2320	0.486 ± 0.059	0.770 ± 0.312	2414	0.167 ± 0.038	0.422 ± 0.070

2321	0.665 ± 0.033	0.561 ± 0.043	2415	0.119 ± 0.015	0.180 ± 0.097
2322	0.306 ± 0.059	0.468 ± 0.012	2416	0.166 ± 0.031	0.247 ± 0.031
2323	0.664 ± 0.166	0.631 ± 0.085	2417	0.253 ± 0.037	0.295 ± 0.057
2324	1.046 ± 0.208	1.081 ± 0.293			

Example 11. *Hif2α RNAi trigger EC_{50} determination.* The eight best canonical sequences were further evaluated by determining the EC_{50} concentration. Each trigger was assessed for knockdown under the same conditions and assays as above, but at 10 different concentrations ranging from 0.00051 nM to 10 nM. EC_{50} were determined using GraphPad Prism software. Each of the top five canonical sequences were modified to contain UNA at sites 6 and 7. These triggers, along with their parent canonical sequences, were evaluated side-by-side for EC_{50} concentration determination using the same conditions and assays as above, Table 6.

Table 6. EC_{50} values (nM) determined *in vitro* for the indicated RNAi triggers.

EC50		EC50 UNAs		
ID number	EC50 (nM)	ID number	Mod.	EC50
XD-02335	0.0593	AD00988	UNA6	0.8406
		AD00993	UNA7	0.5504
XD-02337	0.1010			
XD-02371	0.0592	AD00989	UNA6	0.3754
		AD00994	UNA7	0.08068
XD-02391	0.1554			
XD-02397	0.0858			
XD-02404	0.0570	AD00990	UNA6	0.1534
		AD00995	UNA7	0.1689
XD-02407	0.0287	AD00991	UNA6	0.3503
		AD00996	UNA7	0.1176
XD-02415	0.0892	AD00992	UNA6	0.1419
		AD00997	UNA7	0.1827

Example 12. *Creation of SEAP-expressing clear cell renal cell carcinoma (ccRCC) A498 cells.* A pCR3.1 expression vector expressing the reporter gene secreted alkaline phosphatase (SEAP) under the CMV promoter was prepared by directional cloning of the SEAP coding sequence PCR amplified from Clontech's pSEAP2-basic vector. Convenient restriction sites were added onto primers used to amplify the SEAP coding sequence for cloning into the pCR3.1 vector (Invitrogen). The resultant construct pCR3-SEAP was used to create a SEAP-expressing A498 ccRCC cell line. Briefly, pCR3-SEAP plasmid was transfected into A498 ccRCC cells by electroporation following manufacturer's recommendation. Stable transfectants were selected by G418 resistance. Selected A498-SEAP clones were evaluated

for SEAP expression and integration stability.

Example 13. *Orthotopic RCC tumor bearing mice with A498 cell stably expressing SEAP.* Female athymic nude mice were anesthetized with ~3% isoflourane and placed in the right lateral decubitus position. A small, 0.5-1cm, longitudinally abdominal incision in the left flank was made. Using a moist cotton swab, the left kidney was lifted out of the peritoneum and gently stabilized. Just before injection, a 1.0 ml syringe was filled with the cell/Matrigel mixture and a 27 gauge needle catheter was attached to the syringe tip. The filled syringe was then attached to a syringe pump (Harvard Apparatus, model PHD2000) and primed to remove air. The tip of a 27-gauge needle catheter attached to a syringe was inserted just below the renal capsule near the caudal pole and the tip of the needle was then carefully advanced cranially along the capsule 3-4 mm. A 10 µl aliquot of 2:1 (vol:vol) cell/matrigel mixture containing about 300,000 cells was slowly injected into the kidney parenchyma using a syringe pump. The needle was left in the kidney for 15-20 seconds to ensure the injection was complete. The needle was then removed from the kidney and a cotton swab was placed over the injection site for 30 seconds to prevent leakage of the cells or bleeding. The kidney was then gently placed back into the abdomen and the abdominal wall was closed. Serum was collected every 7-14 days after implantation to monitor tumor growth using a commercial SEAP assay kit. For most studies, tumor mice were used 5-6 weeks after implantation, when tumor measurements were typically around 4-8 mm.

Example 14. *Evaluation of HiF2α-RNAi triggers in orthotopic RCC tumor bearing mice.* RGD targeted HiF2α-RNAi trigger delivery conjugates. Delivery polymers were modified using RGD-PEG-HyNic, RGD-PEG-ACit-PNP, or RDG-PEG-FCitFP-TFP and PEG-dipeptide modifying agents. The indicated amount of polymer 126 or 100A polymer was modified with 8× PEG_{12}-ACit-PABC-PNP/ 0.5× aldehyde-PEG_{24}-FCit-PABC-PNP (with RGD mimic #1-PEG-HyNic using protocol #1) and the indicated amount of the indicated Hif2α RNAi trigger. Polymer 064 was modified according to protocol 7. Kidney RCC tumor-bearing mice were generated as described and treated with a single tail vein injection of isotonic glucose (G1) or the indicated Hif2α RNAi trigger-delivery polymer conjugate. Mice were euthanized at the indicated time after injection and total RNA was prepared from kidney tumor using Trizol reagent following manufacturer's recommendation. Relative HiF2α mRNA levels were determined by RT-qPCR as described below and compared to mice treated with delivery buffer (isotonic glucose) only.

Table 7. Hif2α knockdown in mice following Hif2α RNAi trigger delivery. RNAi triggers were conjugated to the indicated reversibly modified delivery polymer.

RNAi trigger		Polymer		Relative Expression	
duplex number	μg	number	μg	day 4	low error/ high error
isotonic glucose	0		0	1.00	0.06/0.06
AD01031	80	126	400	0.20	0.04/0.05
AD01214	80	126	400	0.29	0.08/0.12
AD01214	112.5	006	300	0.36	0.07/0.09
AD01255	80	126	400	0.28	0.05/0.05
AD01476	115	100A	375	0.32	0.04/0.04
AD01291	80	126	400	0.19	0.03/0.03
AD01292	80	126	400	0.27	0.06/0.08
AD01293	80	126	400	0.20	0.01/0.01
AD01294	80	126	400	0.17	0.01/0.02
AD01295	80	126	400	0.22	0.02/0.02
AD01296	80	126	400	0.21	0.04/0.06
AD01029	80	126	400	0.94	0.08/0.09
AD01030	80	126	400	0.47	0.08/0.10
AD01256	80	126	400	0.22	0.05/0.07
AD01257	80	126	400	0.24	0.04/0.05
AD01258	80	126	400	0.38	0.03/0.03
AD01424	150	100A	300	0.54	0.06/0.07
AD01404	150	100A	300	0.58	0.11/0.13
AD01405	150	100A	300	0.51	0.10/0.12
AD01406	150	100A	300	0.45	0.06/0.07
AD01407	150	100A	300	0.47	0.06/0.07
AD01408	150	100A	300	0.50	0.07/0.09
AD01409	150	100A	300	0.55	0.03/0.03
AD01410	150	100A	300	0.41	0.10/0.12
AD01411	150	100A	300	0.36	0.01/0.01
AD01288	115	100A	375	0.32	0.02/0.02
AD01522	115	100A	375	0.44	0.07/0.09
AD01289	115	100A	375	0.28	0.06/0.08
AD01290	115	100A	375	0.39	0.02/0.02
AD01523	115	100A	375	0.49	0.04/0.05
AD01524	115	100A	375	0.26	0.05/0.07
AD01554	115	100A	375	0.29	0.04/0.04
AD01555	115	100A	375	0.20	0.03/0.03
AD01025	80	126	400	0.34	0.02/0.02
AD01023	80	126	400	0.93	0.10/0.12
AD01024	80	126	400	0.51	0.04/0.05

AD01028	80	126	400	0.39	0.04/0.04
AD01026	80	126	400	0.97	0.12/0.13
AD01027	80	126	400	0.92	0.08/0.09
AD01034	80	126	400	0.35	0.07/0.09
AD01032	80	126	400	1.00	0.09/0.10
AD01033	80	126	400	1.08	0.08/0.09
AD01022	80	126	400	0.53	0.07/0.09
AD01020	80	126	400	0.94	0.06/0.06
AD01021	80	126	400	0.90	0.05/0.05
AD01654	100	100A	250	0.185	0.01/0.01
AD01655	100	100A	250	0.234	0.02/0.02
AD01656	100	100A	250	0.184	0.01/0.01
AD01657	100	100A	250	0.256	0.03/0.03
AD01658	100	100A	250	0.138	0.01/0.01
AD01659	100	100A	250	0.249	0.04/0.05
AD01884	100	064	250	0.091	0.01/0.01
AD01885	100	064	250	0.146	0.02/0.02
AD01886	100	064	250	0.292	0.04/0.05
AD01887	100	064	250	0.329	0.05/0.06
AD01888	100	064	250	0.209	0.04/0.08
AD01889	100	064	250	0.282	0.04/0.05
AD01890	100	064	250	0.256	0.02/0.03
AD01891	100	064	250	0.189	0.02/0.03
AD01892	100	064	250	0.146	0.03/0.03
AD01893	100	064	250	0.115	0.02/0.03
AD02691	75	064	187.5	0.124	0.04/0.07
AD02692	75	064	187.5	0.089	0.03/0.04
AD02693	75	064	187.5	0.122	0.01/0.01
AD02694	75	064	187.5	0.099	0.01/0.01
AD02695	75	064	187.5	0.101	0.02/0.02
AD02733	75	064	187.5	0.283	0.02/0.03
AD02734	75	064	187.5	0.262	0.03/0.03
AD02735	75	064	187.5	0.080	0.01/0.02
AD01884	75	064	187.5	0.227	0.02/0.02
AD01884	50	064	125	0.203	0.03/0.03
AD02692	75	064	187.5	0.121	0.02/0.02
AD02692	50	064	125	0.092	0.01/0.01
AD02695	75	064	187.5	0.155	0.02/0.02
AD02695	50	064	125	0.123	0.03/0.03
AD02735	75	064	187.5	0.222	0.07/0.10
AD02735	50	064	125	0.144	0.03/0.03
AD02857	50	064	125	0.192	0.03/0.03
AD02858	50	064	125	0.192	0.04/0.05

AD02859	50	064	125	0.214	0.03/0.03
AD02860	50	064	125	0.165	0.02/0.02
AD02949	50	064	125	0.176	0.05/0.07
AD02074	50	064	125	0.566	0.01/0.13
AD02861	50	064	125	0.103	0.03/0.05
AD02862	50	064	125	0.093	0.05/0.11
AD02873	50	064	125	0.079	0.04/0.06
AD02875	50	064	125	0.101	0.01/0.02
AD03011	50	064	125	0.183	0.01/0.02
AD02874	50	064	125	0.138	0.05/0.07
AD03187	50	064	125	1.000	0.06/0.07
AD03188	50	064	125	0.308	0.08/0.07
AD03189	50	064	125	0.245	0.02/0.02
AD03190	50	064	125	0.269	0.08/0.12
AD03191	50	064	125	0.307	0.13/0.24
AD03192	50	064	125	0.286	0.01/0.01
AD03193	50	064	125	0.275	0.07/0.09
AD03125	50	064	125	0.205	0.04/0.05
AD03126	50	064	125	0.172	0.04/0.04
AD03253	50	064	125	0.188	0.04/0.05
AD03264	50	064	125	0.264	0.09/0.13
AD03265	50	064	125	0.294	0.07/0.08
AD03266	50	064	125	0.408	0.07/0.08
AD03254	50	064	125	0.295	0.06/0.07
AD03255	50	064	125	0.264	0.07/0.10
AD03256	50	064	125	0.333	0.06/0.08
AD03257	50	064	125	0.203	0.04/0.05
AD03258	50	064	125	0.137	0.02/0.02
AD03259	50	064	125	0.179	0.03/0.04
AD03260	50	064	125	0.175	0.03/0.04
AD03261	50	064	125	0.177	0.02/0.02
AD03262	50	064	125	0.197	0.01/0.01
AD03263	50	064	125	0.134	0.04/0.06

Quantitative Real-Time PCR assay. In preparation for quantitative PCR, total RNA was isolated from tissue samples homogenized in TriReagent (Molecular Research Center, Cincinnati, OH) following the manufacturer's protocol. Approximately 500 ng RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). For human (tumor) Hif2α (EPAS1) expression, pre-manufactured TaqMan

gene expression assays for human Hif2α (Catalog # 4331182) and CycA (PPIA) Catalog #: 4326316E) were used in biplex reactions in triplicate using TaqMan Gene Expression Master Mix (Life Technologies) or VeriQuest Probe Master Mix (Affymetrix). For human (tumor) VegFa (VEGFA) expression, pre-manufactured TaqMan gene expression assays for human VegFa (Catalog # 4331182, Assay ID: Hs00900055) and CycA (Part#: 4326316E) were used in biplex reactions in triplicate using TaqMan Gene Expression Master Mix (Life Technologies) or VeriQuest Probe Master Mix (Affymetrix). Quantitative PCR was performed by using a 7500 Fast or StepOnePlus Real-Time PCR system (Life Technologies). The $\Delta\Delta C_T$ method was used to calculate relative gene expression.

Example 15. *Multi-dose Hif2α RNAi trigger-delivery polymer conjugate inhibits tumor growth in orthotopic RCC tumor bearing mice.* Hif2α RNAi trigger-delivery polymer conjugate was prepared using protocol #1 with RNAi trigger duplex ID AD01031 and polymer Ant 126. The conjugate was then TFF purified and polymer concentration, RNAi trigger, RGD and modifying conjugation efficiency was determined as described above. Weekly doses of Hif2α RNAi trigger-delivery polymer conjugate containing either 400 μg (polymer weight) or 280 μg (polymer weight) were administered intravenously to 2 different groups of tumor bearing mice. Tumor bearing mice receiving isotonic glucose (IG) were used as treatment control. A total of 3 weekly doses were administered during the course of study. Tumor growth rates were evaluated by serum SEAP collected at 5-7 days interval during treatment. Tumor weight and volume was determined at necropsy. Gross tumor morphology and H&E histopathology were evaluated.

Table 8. Hif2α RNAi trigger-delivery polymer conjugate knockdown HiF2α and VEGFa

Treatment	Hif2α expression		VEGFa expression	
	Relative Expression	low/high error	Relative Expression	low/high error
G1 - IG	1.00	0.07/0.07	1.00	0.07/0.08
G2 - 400 μg	0.18	0.03/0.04	0.45	0.07/0.09
G3 - 280 μg	0.19	0.04/0.05	0.39	0.10/0.13

Expression of Hif2α in the 400 μg or 280 μg Hif2α RNAi trigger-delivery polymer conjugate group was 82% and 81% decreased, respectively, compared to control treatment (Table 8). Expression of VEGFa, a well characterized down-stream Hif2α regulated gene, was also decreased by 55% and 61%, respectively (Table 8).

Collectively, after 3 weekly Hif2α RNAi trigger-delivery polymer conjugate injections, tumor growth was dramatically inhibited in both dosages evaluated. This is supported by the overall tumor sizes and serum SEAP levels (FIGs. 8-9 and 12, Table 9). A downward trend of the SEAP levels after the third injections suggest beginning of tumor regression. In addition, tumor histopathology examination from H&E stained formalin fixed paraffin sections showed destruction of typical RCC tubular structure. The number of apoptotic cells was increased in treatment groups. Some tumor sample contained large areas of tumor necrosis (FIG. 10).

Table 9. Serum SEAP levels during treatment, G1 and G2, n = 4; G3, n=3

Treatment	SEAP (fold-changed relative to day −1)			
	Day −1	Day 7	Day 14	Day 21
G1 - IG	1.00 ±0.19	1.65 ±0.22	3.44 ±0.23	7.48 ±0.27
G2- 400 μg	1.00 ±0.45	0.79 ±0.43	1.36 ±0.61	1.25 ±0.68
G3- 280 μg	1.00 ±0.14	1.46 ±0.09	2.55 ±0.29	1.82 ±0.35

Example 16. *Evaluation of HiF2α-RNAi triggers in orthotopic RCC tumor bearing mice.* RGD targeted HiF2α-RNAi trigger delivery polymer conjugates were formed using polymer 126, 100A, or 006. The RNAi trigger, μg indicates the quantity of trigger reacted with polymer. The polymer was modified with the indicated RGD mimic and PEG modifying agents as described above. Kidney RCC tumor-bearing mice were generated as described and treated with a single tail vein injection of isotonic glucose (G1) or the indicated Hif2α RNAi trigger-delivery polymer conjugate. Mice were euthanized 72 h (day 4) after injection and total RNA was prepared from kidney tumor using Trizol reagent following manufacturer's recommendation. Relative HiF2α mRNA levels were determined by RT-qPCR as described and compared to mice treated with delivery buffer (isotonic glucose) only (Table 11).

Example 17. *HiF2α RNAi trigger / second therapeutic combination study.* HiF2α RNAi trigger-delivery polymer conjugate (125 μg polymer) was prepared using protocol 7 Duplex ID No. AD1884 and polymer 064. HiF2α RNAi trigger-delivery polymer conjugate was dosed every 4 weeks by iv injection, 4 doses total. Sunitinib (Malate salt) obtained from LC laboratories was suspended in Ora-plus/Ora sweet (50:50, vol:vol). Sunitinib treatment started 2 weeks after the first HiF2α RNAi trigger dose was administered. Mice were dosed by oral gavage 5 days/week for 2 weeks, then off 2 weeks, 3 cycles total.

Tumor growth rates were evaluated by serum SEAP collected at 5-7 days interval during treatment. Tumor weight and volume was determined at necropsy. Gross tumor morphology and H&E histopathology were evaluated. Relative HiF2α expression levels were of were 11.4%, 73.8%, and 77.6% decreased in the sunitinib alone, DPC + sunitinib and DPC alone treated groups, respectively (Table 10A). Combined HiF2α RNAi trigger and sunitinib treatments resulted in increased tumor growth inhibition response. Overall smaller tumor sizes were smaller and lower overall growth (as measured by overall-fold increase in SEAP) was observed (Table 10B).

Table 10A. HiF2α expression in RCC tumors in animal models treated with Sunitinib, HiF2α RNAi trigger, or HiF2α RNAi trigger + Sunitinib.

Treatment	HiF2α expression		
	Relative expression	Low error	High error
isotonic glucose	1.000	0.153	0.180
Sunitinib	0.886	0.070	0.076
HiF2α RNAi trigger	0.262	0.071	0.097
HiF2α RNAi trigger + Sunitinib	0.224	0.081	0.126

Table 10B. Tumor size and SEAP expression in RCC tumors in animal models treated with Sunitinib, HiF2α RNAi trigger, or HiF2α RNAi trigger + Sunitinib.

Treatment	Tumor weight (mg)	Tumor volume (mm3)	Fold increase in SEAP
isotonic glucose	4158.5 ±865	3576.3 ±279	21.8 ±11
Sunitinib	2385.7 ±845	2113.5 ±368	18.1 ±9.8
HiF2α RNAi trigger	2130.9 ±1066	1537.4 ±999	20.54 ±20
HiF2α RNAi trigger + Sunitinib	1075.8 ±600	1008.8 ±650	5.7 ±3.3

Other Embodiments: It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Table 11. Hif2α expression in RCC cells in RCC tumor bearing mice following Hif2α RNAi trigger delivery.

HiF2α RNAi trigger		polymer			RGD			PEG	Relative Expression		
										error	
duplex ID	μg	ID	μg	purification	modifying agent	amt.	no.	modifying agent	day 4	low	high
isotonic glucose									1.000	0.060	0.064
AD01031	80	126	280		RGD-PEG_8-HyNic	0.5×		PEG_{12}-ACit-PABC-PNP	0.300	0.074	0.098
AD01031	80	126	280		RGD-PEG_8-ACit-PABC-PNP	0.4×		PEG_6-ACit-PABC-PNP	0.311	0.055	0.067
AD01214	80	126	280	TFF	RGD-PEG_8-HyNic	0.5×		PEG_{12}-FCit-PABC-PNP	0.489	0.022	0.023
AD01214	80	126	280		RGD-PEG_8-HyNic	0.5×		PEG_{12}-FCitFP-TFP	0.210	0.032	0.038
AD01214	80	126	280		RGD-PEG_8-ACit-PABC-PNP	0.4×		PEG_6-ACit-PABC-PNP	0.360	0.019	0.021
AD01214	80	126	280		RGD-PEG_8-ACitFP-NHS	1×		PEG_6-ACit-PABC-PNP	0.288	0.005	0.005
AD01214	115	100A	375		RGD-PEG_8-HyNic	0.5×		PEG_{12}-FCitFP-TFP	0.258	0.033	0.038
AD01214	112.5	100A	375	TFF	RGD-PEG_{15}-FCitFP-TFP	0.5×	10.8	PEG_{12}-ACit-PABC-PNP	0.193	0.046	0.061
AD01214	112.5	100A	375	TFF	RGD-PEG_{15}-FCitFP-TFP	1×	16.1	PEG_{12}-ACit-PABC-PNP	0.182	0.007	0.008
AD01214	112.5	100A	375	TFF	RGD-PEG_{15}-FCitFP-TFP	2×	29.0	PEG_{12}-ACit-PABC-PNP	0.182	0.031	0.038
AD01214	112.5	100A	375	TFF	RGD-PEG_{19}-FCitFP-TFP	0.5×	10.7	PEG_{12}-ACit-PABC-PNP	0.163	0.023	0.027
AD01214	112.5	100A	375	TFF	RGD-PEG_{19}-FCitFP-TFP	1×	18.5	PEG_{12}-ACit-PABC-PNP	0.114	0.011	0.012
AD01214	112.5	100A	375	TFF	RGD-PEG_{19}-FCitFP-TFP	2×	31.1	PEG_{12}-ACit-PABC-PNP	0.182	0.047	0.063
AD01214	112.5	100A	375		RGD-PEG_{19}-FCitFP-TFP	1×		PEG_{12}-FCitFP-TFP	0.148	0.079	0.169
AD01214	112.5	100A	375		RGD-PEG_{19}-FCitFP-TFP	1×		PEG_{12}-FCitFP-TFP	0.188	0.026	0.03
AD01214	112.5	100A	375		RGD-PEG_{19}-FCitFP-TFP	1×		PEG_{12}-ACit-PABC-PNP	0.195	0.043	0.055
AD01214	112.5	006	300		RGD-PEG_8-HyNic			PEG_{12}-ACit-PABC-PNP	0.357	0.069	0.086
AD01214	112.5	100A	375	TFF	RGD-PEG_{20}-FCitFP-TFP	0.125×	1.9	PEG_{12}-ACit-PABC-PNP	0.169	0.052	0.075
AD01214	112.5	100A	375	TFF	RGD-PEG_{20}-FCitFP-TFP	0.25×	3.4	PEG_{12}-ACit-PABC-PNP	0.168	0.029	0.035
AD01214	112.5	100A	375	TFF	RGD-PEG_{20}-FCitFP-TFP	0.5×	6.6	PEG_{12}-ACit-PABC-PNP	0.130	0.004	0.005
AD01214	112.5	100A	375	TFF	RGD-PEG_{20}-FCitFP-TFP	1×	12.7	PEG_{12}-ACit-PABC-PNP	0.121	0.016	0.018
AD01214	112.5	100A	375	TFF	RGD-PEG_{20}-FCitFP-TFP	1.5×	20.3	PEG_{12}-ACit-PABC-PNP	0.135	0.018	0.020

Claims

1. A composition comprising an RNA interference (RNAi) trigger for inhibiting the expression of an Hif2α gene, wherein the RNAi trigger comprises a sense strand and an antisense strand, wherein the antisense strand comprises the base sequence (5′ → 3′) of UUUCAUGAAAUCGUUACGUUG (SEQ ID NO. 41), wherein the sense strand comprises a core sequence of 16 or more consecutive nucleobases that is at least substantially complementary to the antisense strand sequence, and wherein the sense strand and/or antisense strand independently comprises one or more modified nucleotides.

2. The composition of claim 1, wherein both the sense strand and the antisense strand each independently comprises one or more modified nucleotides or nucleotide mimics.

3. The composition of claim 1, wherein the modified nucleotide is selected from the group consisting of: 2′-O-methyl modified nucleotide, nucleotide comprising a 5′-phosphorothioate group, 2′-deoxy-2′-fluoro modified nucleotide, 2′-deoxy-modified nucleotide, locked nucleotide, abasic nucleotide, deoxythymidine, inverted deoxythymidine, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholino nucleotide, and non-natural base comprising nucleotide.

4. The composition of claim 3, wherein the sense strand contains one, two, or three 2′-deoxy-2′-fluoro modified nucleotides at positions 11, 12, and/or 13 from the 3′ end.

5. The composition of claim 3, wherein the antisense strand contains a 2′-deoxy-2′-fluoro modified nucleotide at position 2 from the 5′ end.

6. The composition of claim 3, wherein the antisense strand contains a 2′-deoxy-2′-fluoro modified nucleotide at position 14 from the 5′ end.

7. The composition of claim 3, wherein the antisense strand contains one, two, three, or four 2′-deoxy-2′-fluoro modified nucleotides at positions 4, 6, 8, 10, and 12 from the 5′ end.

8. The composition of claim 1, wherein the RNAi trigger comprises one or more phosphorothioate internucleoside linkages.

9. The composition of claim 8, wherein the sense strand comprises one or two phosphorothioate internucleoside linkages.

10. The composition of claim 8, wherein the antisense strand comprises one, two, three, or four phosphorothioate internucleoside linkages.

11. The composition of claim 1, further comprising one or more additional therapeutics or treatments.

12. The composition of claim 1, further comprising a pharmaceutically acceptable excipient.

13. The composition of claim 1, wherein the composition is packaged in a kit, container, pack, dispenser, pre-filled syringes, or vials.

14. The composition of claim 1, wherein the antisense strand further comprises a 3′ extension of 1-6 nucleotides in length.

15. The composition of claim 14, wherein the 3′ extension of the antisense strand comprises uAu, uGu, udTsdT, usdTsdT, UfAu, Aua, Afsusa, UAU, uAfu, uau, udAu, uscu, usgu, uscsu, cAu, aUa, aua, u(invdA)u, cag, agu, gcg, caa, usasu, uAMTM, or usTMsAM.

16. The composition of claim 1, wherein a targeting group is conjugated to the RNAi trigger.

17. The composition of claim 16, wherein the targeting group comprises a compound selected from the group consisting of: integrin-binding compound, $\alpha_v\beta_3$ integrin-binding, RGD ligand, RGD peptide, and RGD mimic.

18. The composition of claim 1, wherein a delivery polymer is conjugated to the RNAi trigger.

19. The composition of claim 1, wherein a linking group is conjugated to the RNAi trigger.

20. The composition of claim 1, wherein the antisense strand comprises the base sequence (5′ → 3′) of UUUCAUGAAAUCGUUACGUUG (SEQ ID NO. 41) and said sequence is located at positions 1-21 from the 5′ end of the antisense strand.

21. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3′) of ACGUAACGAUUUCAUGAAA (SEQ ID NO: 50).

22. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3′) of ACGUAACGAUUUCAUGAAAT (SEQ ID NO: 51).

23. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3′) of ACGUAACGAUUUCAUGAAU (SEQ ID NO: 53).

24. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3′) of CAACGUAACGAUUUCAUGA (SEQ ID NO: 58).

25. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3') of CAACGUAACGAUUUCAUGAAA (SEQ ID NO: 59).

26. The composition of claim 1, wherein the sense strand of the RNAi agent comprises the base sequence (5′ → 3') of UAUACGUAACGAUUUCAUGAAAT (SEQ ID NO: 72).

27. The composition of claim 1, wherein the sense strand of the RNAi agent is 16 to 30 nucleotides in length.

28. The composition of claim 27, wherein the antisense strand of the RNAi agent is 21 to 30 nucleotides in length.

29. A method for inhibiting Hif2α expression in an isolated cell or isolated tissue, the method comprising administering to the isolated cell or isolated tissue, an effective amount of the composition of claim 1.

30. The method of claim 29, wherein the cell or tissue is a renal cell carcinoma.

31. An RNAi trigger for inhibiting the expression of an Hif2α gene, wherein the RNAi trigger comprises a sense strand and an antisense strand, wherein the antisense strand comprises the base sequence (5′ → 3') of UUUCAUGAAAUCGUUACGUUG (SEQ ID NO. 41), wherein said sequence is located at positions 1-21 from the 5' end of the antisense strand, wherein the sense strand comprises a core sequence of 16 or more consecutive nucleobases that is at least substantially complementary to the antisense strand sequence, and wherein the sense strand and/or antisense strand independently comprises one or more modified nucleotides.