

RIBOZYMES

TECHNICAL FIELD

5 The present disclosure relates broadly to a ribozyme engineered to comprise one or more target/trigger-binding domains.

BACKGROUND

10 The levels and profiles of coding and non-coding RNA in cells and in individuals present substantial information about biological and disease states. Accordingly, methods for RNA detection and quantification have been mainstays of molecular biology, and have continued to evolve with increasing technological sophistication. Traditional RT-qPCR and in situ hybridisation methods are still
15 routinely used, supplemented with state-of-the-art single-cell RNA-sequencing and spatial transcriptomics methods to answer ever-more complex biological questions. The RNA content of most cells can now be determined with precision, allowing the probing of molecular and cellular functions of the transcriptome in normal and disease states. In addition, cellular and systemic RNA biomarkers are important for
20 disease diagnosis and to guide clinical decision-making.

 While in situ RNA detection has many important applications, there is currently no generalisable method that can directly sense and convert a specific RNA signal into a second functional, non-coding RNA readout. Such a method could be genetically encoded and act as a compact gene switch to transduce RNA context to
25 functional outputs. RNA-sensing gene switches have been developed for some gene regulatory systems, most notably, CRISPR. For example, guide RNAs have been modified to respond to antisense blocking sequences at the guide spacer or other regions, so that they can be activated or deactivated in response to RNA triggers or ligands via toehold-mediated strand displacement, and CRISPR machinery have
30 been modified to be conditionally activated upon microRNA function, e.g. microRNA-directed Ago2 cleavage and release of sgRNA, and miRNA-regulated Cas9 mRNA. Many of these systems have sequence constraints as their designs involve strand displacement of critical regions of the gRNA or require multiple RNA or protein components. When the guide RNA itself is modified in this way, careful design of the
35 switching mechanism is required so that sgRNA function and specificity is not

affected. They are also CRISPR-specific and are not generalisable for transducing RNA signals to other functional non-coding RNA pathways, e.g. shRNA, anti-sense or splice-switching oligonucleotides and RNA aptamers.

Therefore, there is a need for a molecular system that can transduce binding of an RNA or other nucleic acid signal, into release of a functional RNA. The present disclosure provides a solution in the form of a ribozyme engineered to be capable of releasing functional RNA upon binding to a sequence-complementary trigger.

SUMMARY

In one aspect, the present disclosure relates to a ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently 1 nucleotide or, 2, 3 or 4 nucleotides in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain

motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

5 motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

10 In one example, there is provided a ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each
15 cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

20 wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

25 wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

30 motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain;

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motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

5 motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

10 In some examples, the linker between motifs [C] and [D] is selected from the group consisting of two-way junction, three-way junction, four-way junction, a stem, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges and combinations thereof.

In some examples, the linker between motifs [C] and [D] comprises a three-way junction and a stem.

15 In some examples, the stem sequence connecting the junction to motif [D] is 4 to 12 nucleotides in length.

In some examples, the stem sequence connecting motif [C] and [c] to the junction is 4 to 12 nucleotides in length.

20 In some examples, the trigger nucleic acid molecule comprises a region that is complementary to the trigger-binding domain, wherein said region is more than 10 nucleotides in length, optionally the one or more trigger -binding domains are for binding the same trigger nucleic acid molecule.

In some examples, the releasable RNA segment is 6 to 150 nucleotides in length.

25 In some examples, the releasable RNA segment comprises a sequence that is identical to at least one of the one or more trigger RNA molecules.

In some examples, the releasable RNA segment is a functional RNA selected from the group consisting of single-guide RNA (sgRNA), guide RNA (gRNA), short hairpin RNA (shRNA), and RNA aptamer.

30 In some examples, motifs [B] and [b] are independently 1 or more nucleotides in length, optionally 3 or more nucleotides in length.

In some examples, motifs [B] and [b] has a sequence selected from the group consisting of SEQ ID NO: 1 (5'-ACG/CGU-3'), SEQ ID NO: 2 (5'-ACG/CGA-3'), SEQ ID NO: 449 (5'- ACG/UGA -3'), SEQ ID NO: 450 (5'- AUG/CGA -3'), SEQ ID NO: 451 (5'- AUG/UGA -3'), SEQ ID NO: 452 (5'- CG/CG -3'), SEQ ID NO: 453 (5'-

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UUG/UGG -3'), SEQ ID NO: 454 (5'- UAU/AUA -3'), SEQ ID NO: 455 (5'- ACU/AGA -3'), SEQ ID NO: 456 (5'- AUG/CAA -3'), SEQ ID NO: 457 (5'- CU/AG -3'), and SEQ ID NO: 458 (5'- UG/CA -3').

In some examples, if motifs [e] and [E] are partially complementary to each other, the complementarity between motif [e] and [E] is characterised by alternating regions of complementarity and regions of non-complementarity.

In some examples, motif [D] comprises a mutation of nucleotide N₇ to pair with nucleotide N₊₃.

In some examples, the optional linker regions individually or collectively form one or more secondary structures, optionally the one or more secondary structures are selected from the group consisting of: single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges, stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots, symmetrical internal loops, asymmetrical internal loops, three stem junctions (3-way junctions), four stem junctions (4-way junctions), two-stem junctions (2-way junctions) or coaxial stacks or combinations thereof.

In some examples, the ribozyme complex comprises the sequences of any one or more of SEQ ID NOs: 3 to SEQ ID NOs: 448.

In some examples, the ribozyme further comprises one or more modification.

In some examples, the trigger nucleic acid comprises one or more modified nucleotide.

In another aspect, there is provided a method of detecting presence of a target/trigger nucleic acid molecule in a sample, wherein the method comprises:

incubating the sample with a ribozyme as disclosed herein at temperature T₁ which allows the binding of the target/trigger nucleic acid molecule with one or more target/trigger -binding domains comprised in the ribozyme;

incubating the sample at temperature T₂ which allows the nucleic acid molecule and the RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme.

In yet another aspect, there is provided a method of detecting presence of a sequence or mutation of interest on a nucleic acid of interest in a sample, wherein the method comprises:

incubating the sample with a ribozyme as disclosed herein, thereby allowing binding of the nucleic acid molecule of interest with one or more target/trigger-binding domains comprised in the ribozyme;

incubating the sample which allows the nucleic acid molecule and a releasable RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme;

wherein the releasable RNA segment is an sgRNA or shRNA; wherein
5 detection of the sequence or mutation of interest in the sample results in a signal being generated.

In some examples, the trigger nucleic acid molecule is a genome of a virus, or a fragment thereof.

In yet another aspect, there is provided a kit comprising the ribozyme as
10 disclosed herein.

DESCRIPTION OF EMBODIMENTS

Detection of specific RNA or other nucleic acid sequences is integral to many
15 applications in research, disease diagnosis, and therapeutics. Such applications will be further enabled if a detected nucleic acid signal can be directly functionally transduced via a second signal. This need is addressed by the present invention, which is a modular ribozyme whose self-cleavage is activated by binding of a specific complementary nucleic acid trigger sequence, leading to release of a second embedded RNA product
20 without alteration of the original trigger. This reaction is entirely encoded within one single strand of RNA, and does not require any protein or DNA cofactors.

The inventors of the present disclosure show that the ribozymes disclosed herein are specific and sensitive. The inventors demonstrate that the ribozymes can be modularly designed for cell-free and in-cell applications. Thus, it is a versatile platform
25 for which many potential applications can be envisioned.

The present disclosure describes a modular RNA signal transduction platform based on an altered self-cleaving ribozyme with one trigger-binding site and two cleavage sites, between which is embedded a releasable RNA cleavage product. The ribozyme's self-cleavage activity is dependent on complementary detection and binding
30 of a specific trigger nucleic acid. Upon trigger-binding, ribozyme self-cleavage is activated to release the embedded RNA cleavage product. Hence, the ribozyme acts simultaneously as a direct RNA signal detector and transducer.

Accordingly, in one aspect of the present disclosure, there is provided a ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain;

motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

In some examples, the one or more motifs [A], [B], [C], [D], [E], [D'], [e], [c], [b], to [a] is in the 5' to 3' directionality or the 3' to 5' directionality. In some

examples, the one or more motifs [A], [B], [D], [E], [D'], [e], [b], to [a] is in the 5' to 3' directionality or the 3' to 5' directionality.

In some examples, there is provided a ribozyme comprising:

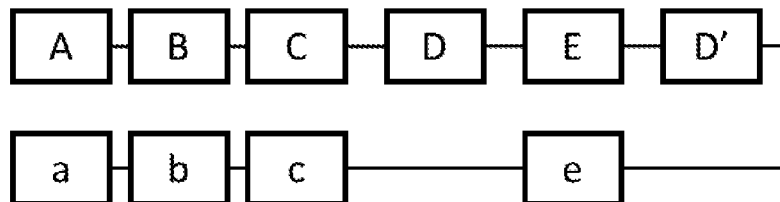
a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with the following structure:



(structure I),

wherein [A] to [a] is in the 5' to 3' directionality or the 3' to 5' directionality, and wherein:

motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

motifs [C] and [c] constitute the catalytic domain;

motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

5 motif [E] comprises the releasable RNA segment;

motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

each of the horizontal lines connecting the motifs represents an optional linker region.

10 Whereby a catalytic domain refers to a domain or domains that comprise nucleotides that are required for or participate in ribozyme catalysis, or motifs that comprise nucleotides that together are required for or participate in catalysis. In some examples, there is no single catalytic domain that switches between an active state and an inactive state. In some examples, trigger-binding is required for a ribozyme to fold into
15 a conformation that allows cleavage by nucleotides that participate in catalysis. In some examples, the cleavage at the cleavage site may be catalysed by the one or more catalytic nucleotides located within catalytic domains. As a result, the ribozyme is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the ribozyme is in an active
20 state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule

In some examples, the ribozyme may comprise:

25 a) one or more catalytic domains capable of switching between an active state and an inactive state,

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

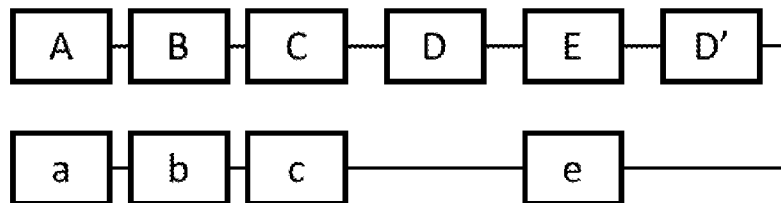
30 c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid
35 molecule, and wherein the catalytic domain is in an active state when the trigger-

binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved when catalysed by the one or more catalytic domains, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with the following structure:



(structure I),

wherein [A] to [a] is in the 5' to 3' directionality or the 3' to 5' directionality, and wherein:

motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

motifs [C] and [c] constitute the catalytic domain;

motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [E] comprises the releasable RNA segment;

motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

each of the horizontal lines connecting the motifs represents an optional linker region.

As used herein, the term “ribozyme” refers to an RNA molecule that is capable of catalysing specific biochemical reactions. Common examples of such reactions include the cleavage or ligation of RNA and DNA, and peptide bond formation. The term “ribozyme” as used herein includes both natural and artificial ribozymes. Artificial ribozymes include synthetic ribozymes and ribozymes modified or engineered from natural ribozymes. The term “ribozymes” also encompasses

ribozyme fusions and ribozyme complexes derived from natural or artificial ribozymes.

In some examples, the term “ribozyme” may include ribozymes comprising one or more modifications to their phosphate backbone, sugar, or nucleobase, or with conjugations. That is, the ribozymes as disclosed herein may contain alterations to their phosphate backbone (e.g. phosphorothioate instead of phosphate linkages). They may contain nucleotides with modified sugar moieties or sugar moiety analogs. Sugar moiety modifications include, but are not limited to, 2'-O-aminoethoxy, 2'-O-amonioethyl (2'-OAE), 2'-O-methoxy, 2-guanidoethyl (2'-OGE), 2'-O,4'-C-methylene (LNA), 2'-O—(N-(methyl)acetamido) (2'-OMA), 2'-O-methyl, 2'-fluoro, 2'-O-(methoxyethyl) (2'-OME), and the like. They can also contain nucleobase modifications, e.g. 5-methylcytosine or pseudouridine. Such modifications are introduced to improve stability and reduce immunogenicity of the ribozymes. Methods of introducing such modification to an RNA (such as ribozymes) are common general knowledge in the art.

As used herein, the term “nucleic acid” or “polynucleotide” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

As used herein, the term “catalytic domain” refers to a domain or domains within a ribozyme that comprise nucleotides that are required for or participate in catalyzing the biochemical reactions as mentioned above, or motifs that together are required for or participate in catalysis. The domain may not be one contiguous segment or structure of the ribozyme and may instead comprise nucleotides located in different parts of the ribozyme sequence or structure. In some examples, trigger-binding is required for a ribozyme to fold into a conformation that allows cleavage by nucleotides that participate in catalysis. In some examples, the cleavage at the cleavage site may be catalysed by the one or more catalytic nucleotides, which are generally referred to as a catalytic domain. As a result, the ribozyme is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the ribozyme is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule. In one example, in a ribozyme capable of cleaving RNA, the catalytic

domain or domains are participate in catalyzing the cleavage of the RNA backbone at a ribozyme cleavage site.

The term “ribozyme cleavage site” refers to the sequences recognized and cleaved by a ribozyme catalytic domain or domains. Unless specified otherwise, the term “cleavage site” as used herein refers a ribozyme cleavage site. A ribozyme is in an “active state” when it is capable of catalyzing the biochemical reaction; whereas a ribozyme is in an “inactive state” when it is incapable of catalyzing the biochemical reaction. In a further example, a ribozyme is in an “active state” when it is capable of cleaving a ribozyme cleavage site; whereas a ribozyme is in an “inactive state” when it is incapable of cleaving a ribozyme cleavage site.

The term “target-binding domain” refers to a domain that is capable of binding a target nucleic acid molecule. In some examples, the term “target-binding domain” may be used interchangeably with the term “trigger-binding domain”. In some examples, the binding between the target/trigger nucleic acid molecule and the target/trigger-binding domain occurs through the annealing of complementary sequences between the two. Thus, it is possible to design or modify the sequence of the one or more target/trigger-binding domains so that they can bind to different target nucleic acid molecules with specific sequences. In some examples, the target nucleic acid molecules may be a target RNA molecule and/or a target DNA molecule.

The term “complementary” as used herein describes a relationship between two nucleotides or two polynucleotides. When referring to nucleic acid complementarity, the nucleotide A is complementary to the nucleotide U, and vice versa, and the nucleotide C is complementary to the nucleotide G, and vice versa. Complementary nucleotides include those that undergo Watson and Crick base pairing and those that base pair in alternative modes, for example the G:U wobble base-pair. It should be understood that, unless explicitly specified (e.g. by assigning a percentage or the term “fully” or “partially”), the term “complementary” when used in relation to a nucleotide, includes varying degrees of complementarity. As used herein, the term “complementarity” refers to the degree and pattern by which one nucleic acid strand or segment is complementary to another nucleic acid strand or segment. When a percentage is assigned to a “complementarity” or a “degree of complementarity” between two polynucleotides (or segments thereof), the percentage refers to the percentage of nucleotides in one polynucleotide (or a segment thereof) that are complementary to the other polynucleotide (or a segment thereof). Therefore, a reference to two polynucleotide strands being

“complementary” should be understood to cover both full and partial complementarity.

The term “target nucleic acid molecule” and the term “trigger nucleic acid molecule” may be used interchangeably in the present disclosure. Both terms as used herein refer to nucleic acid molecules of interest that are to be sensed and bound by the target-binding domain or trigger-binding domain. In some examples, the trigger nucleic acid when bound to the trigger-binding domain switches the ribozyme from an inactive to an active state. Without wishing to be bound by theory, the present disclosure also includes the possibility that trigger binding stabilises the ribozyme to enable the ribozyme to fold into the conformation required to cleave the cleavage site. Therefore, in some examples, the trigger binding may serve to stabilise the ribozyme to allow cleavage by nucleotides that participate in the catalysis. In some examples, trigger binding may include both scenarios where trigger binding switches catalytic domain from inactive to active state as well as trigger binding stabilising ribozyme to allow structural conformation required for cleavage.

The term “linked” refers to the relationship between two domains, and can refer to physical linkage, functional linkage, or both. In one example of the present invention, a catalytic domain is linked to a target-binding domain when the capability of the catalytic nucleotides to carry out catalysis is determined by the state of the target-binding domain, specifically whether the target-binding domain is bound to its corresponding target RNA molecule.

The term “flanked” refers to a polynucleotide sequence that is adjacent to another sequence or that is in between an upstream polynucleotide sequence and/or a downstream polynucleotide sequence, i.e., 5' and/or 3', relative to the sequence. For example, “a releasable RNA segment that is “flanked” by two cleavage sites” indicates that one cleavage site is located 5' to the releasable RNA segment and the other cleavage site is located 3' to the releasable RNA segment; however, there may be intervening sequences therebetween.

As the cleavage sites are comprised on the ribozyme itself, the ribozyme of the present disclosure is considered a self-cleaving ribozyme, and the “releasable RNA segment” can be considered a cleavage product of the self-cleaving activity. As the release of the releasable RNA segment from the ribozyme is a result of the ribozyme binding with its one or more target nucleic acid molecules, the ribozyme can be used to detect the presence of target nucleic acid molecules. As the target

nucleic acid molecule(s) can be released from the ribozyme to activate more ribozymes and trigger the release of more “releasable RNA segments”, the presence of the target nucleic acid molecules can be amplified through the “releasable RNA segments” released in higher copies. The “releasable RNA segment” is variable and can be designed to comprise a large variety of sequences. In some examples where the “releasable RNA segment” comprises the same sequence as the target nucleic acid molecule, the target nucleic acid molecule (or the sequence thereof) is amplified using the ribozyme of the present disclosure. In some examples, the releasable RNA segment may comprise any one or more sequence(s) SEQ ID NO: 307 to 409.

In some examples, the binding of the target nucleic acid molecule to the target-binding domain enables the catalytic nucleotides to carry out catalysis, which results in the cleavage of both cleavage sites and the subsequent release of the releasable nucleic acid segment.

In view of the definition of “complementary” provided earlier in the present description, it would be understood by a person skilled in the art that the expression “optionally complementary” as used herein and the present description encompasses not only full complementarity (100% complementary) and partially complementary (between 0% and 100% complementarity), but also non-complementarity (0% complementarity). In some examples, the present disclosure includes about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% complementarity.

The term “directionality” as used herein refers to the end-to-end chemical orientation of a single strand of the RNA molecule. In a single strand of RNA, the chemical convention of naming carbon atoms in the nucleotide sugar-ring means that there will be a 5'-end, which contains a phosphate (or modified phosphate) group attached to the 5' carbon of the ribose ring, and a 3'-end, which in natural RNA contains -OH at the 2' position, but can also include various modifications, including, but is not limited to, 2'-fluoro, 2'-O-methyl, 2'-methoxyethyl, and the like. As an illustrative example, when [A] to [A'] of strand S1 is in the 5' to 3' direction, [a] to [a'] of strand S2 will be in the 3' to 5' direction.

As used herein, the term “motif” refers to a region on an RNA strand that has a specific structure or is involved with a specific function. The term “domain” as used herein refers to a region of the ribozyme that has a specific structure or is involved with a specific function. As used herein, the term “domain” is used when referring to a functional entity formed by more than one RNA strand or by more than one motif

of one RNA strand. As an illustrative example, the target-binding domain comprises both motifs [A] and [a], and the target-binding domain is considered “bound” to a target RNA molecule only when both motifs are bound to the RNA molecule.

In some examples, the ribozyme as disclosed herein further comprises one or more inhibitory domains; wherein each of the one or more catalytic domains is functionally linked to one of the one or more inhibitory domains, wherein the catalytic domain is in an inactive state due to inhibition from the inhibitory domain, said inhibitory domain being linked to one of the one or more target-binding domains; wherein when one of the one or more target-binding domains is bound to the target nucleic acid molecule, the inhibitory domain linked to said target-binding domain ceases to inhibit the catalytic domain linked to said inhibitory domain, which results in the catalytic domain switching to an active state. In this example, the linkage between a target-binding domain and a catalytic domain is achieved by an inhibitory domain, which is linked to both the target-binding domain and the catalytic domain.

As is commonly known in the art, secondary structures are commonly formed within a ribozyme. In the examples of the present disclosure, one or more secondary structures are either formed individually by any of the motifs or the optional linker regions, or formed collectively by motifs, linker regions, or combinations thereof. In some examples, the optional linker regions individually or collectively form one or more secondary structures.

As used herein, the term “secondary structure” refers to structures formed by the interactions between nucleotides in one or more polynucleotides. Examples for secondary structures include, but are not limited to, single-nucleotide bulges, three-nucleotide bulges, stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots, symmetrical internal loops, asymmetrical internal loops, three stem junctions (3-way junctions), four stem junctions (4-way junction), two-stem junctions (2-way junctions) or coaxial stacks or combinations thereof. Specific examples of secondary structures include stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots or combinations thereof. As used herein, the term “stem loop”, also known as a “hairpin loop”, refers to a secondary nucleic acid structure that forms when two regions of the same strand, usually complementary in nucleotide sequence when read in opposite directions, base-pair to form a double helix that ends with an unpaired loop.

Therefore, in some examples, the optional linker regions in the ribozymes as disclosed herein may individually or collectively form one or more secondary

structures. In some examples, the one or more secondary structures may include, but are not limited to, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges, stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots, symmetrical internal loops, asymmetrical internal loops, three stem junctions (3-way junctions), four stem junctions (4-way junctions), two-stem junctions (2-way junctions) or coaxial stacks or combinations thereof.

As exemplified in the Experimental Section and Drawings, the ribozyme complex as disclosed herein may comprise a linker having a motif such as, but is not limited to, two-way junction, three-way junction, four-way junction, a stem, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges and combinations thereof. In some examples, the linker(s) between motifs [C] and [D] (i.e. Helices 2 and 3) may be, but is not limited to, a two-way junction, a three-way junction, a four-way junction, a stem, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges and combinations thereof. In some examples, the linker between motifs [C] and [D] may comprise a three-way junction and a stem (or may be referred to as a 4-way junction).

In some examples, the stem sequence connecting the junction to motif [D] is 4 to 12 nucleotides in length.

In some examples, the stem sequence connecting the junction to motif [D] is 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more nucleotides in length. In some examples, the stem sequence connecting the junction to motif [D] is about 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides in length. In some examples, the stem sequence connecting to the junction to motif [D] is about 5, 6, 7, 8, 10, 11, or 12 nucleotides in length.

In some examples, the junction is selected from the group consisting of helix–helix (HHH), helix[minus 1 nucleotide]–strand[7 nucleotides long]–helix (H1S7H), helix–helix–strand[4 nucleotides long]–helix (HHS4H), and helix–helix–strand[2 nucleotides long]–helix (HHHS2H), where strand indicates unpaired nucleotides.

In some examples, the stem sequence connecting motif [C] and [c] to the junction to 4 to 15 nucleotides in length. In some examples, the stem sequence

connecting the junction to motif [C] and [c] is 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more nucleotides in length. In some examples, the stem sequence connecting the junction to motif [C] and [c] is about 4, 5, 6, 7, 8, 9, 10, 11, or 12
5 nucleotides in length. In some examples, the stem sequence connecting motif [C] and [c] to the junction is about 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 or 15 nucleotides in length.

In some examples, the junction is selected from the group consisting of helix–helix–helix (HHH), helix[minus 1 nucleotide]–strand[7 nucleotides long]–helix (H
10 1S7H), helix–helix–strand[4 nucleotides long]–helix (HHS4H), and helix–helix–strand[2 nucleotides long]–helix (HHHS2H), where strand indicates unpaired nucleotides

In some examples, the trigger nucleic acid molecule comprises a region that
15 is complementary to the trigger-binding domain, wherein said region is more than 10, more than 20, more than 30, more than 40, more than 50, more than 60, more than 70, more than 80, more than 90, more than 100, more than 110, more than 120, more than 130, more than 140, more than 150, more than 160, more than 170, more than 180, more than 190, or 200 nucleotides in length. In some examples, the region
20 is no more than 200, no more than 190, no more than 180, no more than 170, no more than 160, no more than 150, no more than 140, no more than 130, no more than 120, no more than 110, no more than 100, no more than 90, no more than 80, no more than 70, no more than 60, no more than 50, no more than 40, no more than 30, no more than 20, or no more than 10 nucleotides in length. In some examples,
25 the trigger nucleic acid molecule may be of any size from no more than 5 nucleotides in length up to many thousands or tens of thousands of nucleotides in length or more. Without wishing to be bound by theory, it would be understood by the person skilled in the art that the ribozymes as described herein is capable of binding to a trigger nucleic acid of any size provided the ribozymes can bind to a binding site of the
30 trigger nucleic acid.

In some examples, the nucleotides of motifs [A] and [a] may bind to any site of the target/trigger nucleic acid molecule that the motifs can bind to. For example, where the nucleotides of motifs [A] and [a] are 10 nucleotides each and the trigger nucleic acid is 2000 nucleotides in length, [A] and [a] can bind to any two 10
35 nucleotides regions that the motifs can bind to. In some examples, motifs [A] and [a]

may be able to bind to any two regions of the target/trigger nucleic acid that are not sequentially adjacent to one another.

In some examples, the nucleotides of motifs [A] and [a] may complementarily bind to the opposite ends of the target nucleic acid molecule respectively. For example, motif [A] may complementarily bind to the 5' end of the target nucleic acid molecule, while motif [a] may complementarily bind to the 3' end of the target nucleic acid molecule, and vice versa.

The lengths of motifs [A] and [a] are independent from each other, and can be the same or different. For example, each of motifs [A] and [a] can be between 3 to 5, or between 5 to 10, or between 10 to 15, or between 15 to 20, or between 20 to 30, or between 30 to 40, or between 40 to 50, or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 nucleotides in length. In some other examples, each of motifs [A] or [a] is between 1 to 5, or between 5 to 10, or between 10 to 15, or between 15 to 20, or between 20 to 30, or between 30 to 40, or between 40 to 50, between 50 to 60, between 60 to 70, between 70 to 80, between 80 to 90, between 90 to 100, between 100 to 110, between 110 to 120, between 130 to 140, between 140 to 150, between 150 to 160 nucleotides in length. In some examples, motif [a] is 11 nucleotides long. In some examples, the above descriptions for motifs [A] and [a] also apply to motifs [A'] and [a'].

The complementary binding is either partially complementary or fully complementary. For example, motif [A] is between about 70 to about 80%, or between about 80% to about 90%, or between about 90% to about 100%, or between about 75% to about 85%, or between about 85% to about 95%, or between about 95% to about 100%, or between about 88% to about 98%, or about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% complementary to the a region of the target/trigger nucleic acid molecule; and motif [a] is between about 70 to about 80%, or between about 80% to about 90%, or between about 90% to about 100%, or between about 75% to about 85%, or between about 85% to about 95%, or between about 95% to about 100%, or between about 88% to about 98%, or about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% complementary to another region of the target/trigger RNA molecule, and vice versa.

In some examples, the one or more trigger-binding domains are for binding the same target/trigger nucleic acid molecule. Examples of target or trigger nucleic

acid molecules may include, but are not limited to, viral nucleic acid, bacterial nucleic acid, modified nucleic acid (such as mutation in a nucleic acid), messenger nucleic acid, coding nucleic acid, genomic nucleic acid, and the like.

5 Examples of target or trigger DNA molecules may include, but are not limited to viral DNA, cDNA, circulating DNA, cell free DNA (cfDNA), foetal DNA, modified DNA (e.g. mutation), single stranded DNA, double stranded DNA, mitochondrial DNA, and the like.

10 In some examples, the target/trigger nucleic acid may include, but is not limited to, viral RNA, a microRNA (miRNA), short interfering RNA (siRNA), small RNA (sRNA), messenger RNA (mRNA), non-coding RNA (ncRNA), short non-coding RNA, transfer RNA (tRNA), ribosomal RNA (rRNA), transfer-messenger RNA (tmRNA), clustered regularly interspaced short palindromic repeats RNA (CRISPR RNA), antisense RNA, pre-mRNA, circular RNA or pre-miRNA, or fragment thereof. In some examples, the trigger RNA molecule is a micro-RNA, or a precursor thereof, 15 or a fragment thereof. In some examples, the trigger RNA may be a Let-7 microRNA precursor. In some examples, the trigger nucleic acid may comprise any one or more sequence(s) SEQ ID NO: 221 to 306.

20 In some examples, the target/trigger nucleic acid molecule may include one or more modified nucleotide. In some examples, the target/trigger nucleic acid molecule may include nucleotides with modified phosphate backbone, modified sugar moieties or sugar moiety analogs, or modified nucleobases. Sugar moiety modifications include, but are not limited to, 2'-O-aminoethoxy, 2'-O-aminoethyl (2'-OAE), 2'-O-methoxy, 2-guanidoethyl (2'-OGE), 2'-O,4'-C-methylene (LNA), 2'-O—(N-(methyl)acetamido) (2'-OMA), 2'-O-methyl, 2'-fluoro, 2'-O-(methoxyethyl) (2'-OME), and the like. Modified phosphate linkages include phosphorothioate and phosphorothiolate linkages. Modified nucleobases can include hundreds of naturally occurring and synthetic modified examples, e.g. 5-methylcytosine, methyladenosine, and pseudouridine, to cite a few of the most common

30 The term “micro-RNA” (abbreviated miRNA) as used herein refers to a small non-coding RNA molecule. It generally functions in RNA silencing and post-transcription regulation of gene expression. While the majority of miRNAs are located within the cell, some miRNAs, commonly known as circulating miRNAs or extracellular miRNAs, have also been found in the extracellular environment, including various biological fluids and cell culture media.

In some examples, the releasable RNA segment is more than 5, more than 10, more than 15, more than 20, more than 30, more than 40, more than 50, more than 60, more than 70, more than 80, more than 90, more than 100, more than 110, more than 120, more than 130, more than 140, or 150 nucleotides in length. In some examples, the releasable RNA segment is no more than 150, no more than 140, no more than 130, no more than 120, no more than 110, no more than 100, no more than 90, no more than 80, no more than 70, no more than 60, no more than 50, no more than 40, no more than 30, no more than 20, or no more than 10 nucleotides in length. In some examples, the releasable RNA segment may be about 1 to about 200, 1 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, 91 to 100, 101 to 110, 111 to 120, 121 to 130, 131 to 140, or 141 to 150 nucleotides in length. In some examples, the releasable RNA segment is 6 to 150 nucleotides in length.

Some of the ribozymes as disclosed herein can function with full or partial complementarity between the releasable cleavage product and its complementary strand. In some examples, the releasable RNA segment comprises a sequence that is identical to at least one of the one or more target/trigger nucleic acid molecules.

In some examples, each of motifs [E] and [e] is between 6 to 160 nucleotides in length. In some examples, each of motifs [E] and [e] is between 5 to 10, or between 10 to 15, or between 15 to 20, or between 20 to 30, or between 30 to 40, or between 40 to 50, between 50 to 60, between 60 to 70, between 70 to 80, between 80 to 90, between 90 to 100, between 100 to 110, between 110 to 120, between 120 to 130, between 130 to 140, between 140 to 150, or 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 nucleotides in length.

In some examples, if motifs [e] and [E] are partially complementary to each other, the complementarity between motif [e] and [E] is characterised by alternating regions of complementarity and regions of non-complementarity.

In some examples, motifs [E] and [e] is less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, or less than 30%, or about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90% complementary to each other. In some examples, the total complementarity between motifs [E] and [e] is at least 20%.

In some examples, the complementarity between motifs [E] and [e] is characterized by alternating regions of complementarity and regions of non-

complementarity. The inventors envisage the complementarity of the nucleotides may include limitless patterns of complementarity. A non-exhaustive list of possible complementarity patterns include, but is not limited to, 5' – [1 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [1 nucleotide complementary – 2 nucleotide non-complementary] n – 3'; 5' – [1 nucleotide complementary – 3 nucleotide non-complementary] n – 3'; 5' – [1 nucleotide complementary – 4 nucleotide non-complementary] n – 3'; 5' – [2 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [2 nucleotide complementary – 2 nucleotide non-complementary] n – 3'; 5' – [2 nucleotide complementary – 3 nucleotide non-complementary] n – 3'; 5' – [2 nucleotide complementary – 4 nucleotide non-complementary] n – 3'; 5' – [3 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [3 nucleotide complementary – 2 nucleotide non-complementary] n – 3'; 5' – [3 nucleotide complementary – 3 nucleotide non-complementary] n – 3'; 5' – [3 nucleotide complementary – 4 nucleotide non-complementary] n – 3'; 5' – [4 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [4 nucleotide complementary – 2 nucleotide non-complementary] n – 3'; 5' – [4 nucleotide complementary – 3 nucleotide non-complementary] n – 3'; 5' – [4 nucleotide complementary – 4 nucleotide non-complementary] n – 3'; 5' – [2 nucleotide complementary – 2 nucleotide non-complementary – 1 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [3 nucleotide complementary – 3 nucleotide non-complementary – 1 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; 5' – [4 nucleotide complementary – 4 nucleotide non-complementary – 1 nucleotide complementary – 1 nucleotide non-complementary] n – 3'; and the like. In some examples, n may be an integer from 1 to 200.

Examples of 5' – [3 nucleotide complementary – 2 nucleotide non-complementary] n – 3' are SEQ ID NO: 66, SEQ ID NO: 106, and the like; Example of 5' – [3 nucleotide complementary – 3 nucleotide non-complementary] n – 3' is SEQ ID NO: 99; Example of 5' – [4 nucleotide complementary – 1 nucleotide non-complementary] n – 3' is SEQ ID NO: 108; Example of full complementarity is SEQ ID NO: 130.

In some examples, the alternating regions of complementarity and regions of non-complementarity may not have a pattern (or random in nature). The possible combinations can be determined as required when designing a ribozyme of interest.

In some examples, the complementarity pattern may be determined by the

target/trigger nucleic acid of interest. In some examples, the complementarity pattern may be determined by the cleaved product.

In some examples, each region of complementarity is not more than 3 consecutive nucleotides in length, and each region of non-complementarity is at least 3 consecutive nucleotides in length. In some examples, each region of complementarity between motifs [E] and [e] is not more than 3 consecutive nucleotides in length, wherein each region of non-complementarity between motifs [E] and [e] is at least 2 consecutive nucleotides long.

As used herein, a “region of complementarity” refers to a region of the ribozyme in which the first and second RNA strand are fully complementary to each other; and a “region of non-complementarity” refers to a region of the ribozyme in which the first and second RNA strand are not complementary to each other.

In some examples, the complementarity follows the pattern of 5′– 3 nucleotides complementary – 3 nucleotides non-complementary [2 nucleotides complementary – 3 nucleotides non-complementary – 2 nucleotides complementary]_n – 3 nucleotides non-complementary – 3 nucleotides complementary – 3′; wherein n is the number of repeats of the pattern required to cover the length of motifs [E] and [e]. In some examples, n can be a number between 1 to 50, or 1 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200.

In some examples, the releasable RNA segment may be a functional RNA, such as, but is not limited to, single-guide RNA (sgRNA), guide RNA (gRNA), short hairpin RNA (shRNA), RNA aptamer, and the like.

Without wishing to be bound by theory, it is observed that various forms of motifs [B] and [b] as described herein (i.e. the Helix 4 communication module) advantageously confers properties of very low background cleavage, low leakiness and high cleavage signal, which is ideal for various applications.

Without wishing to be bound by theory, it is observed that modified RNA (such as locked nucleic acid modification as disclosed herein) can bind more strongly to the ribozyme than unmodified RNA. In some examples, modified RNA can bind more strongly to modified ribozymes (such as ribozymes modified with locked nucleic acid nucleotides as disclosed herein). As such, in some examples, motif [B] and [b] may be as short as 1 nucleotide in length. In some examples, motifs [B] and [b] are

independently 1 or more nucleotides in length, optionally 2 or more nucleotides in length, optionally 3 or more nucleotides in length. In some examples, the motif [B] and [b] are independently 1 or more, 2 or more, 3 or more, or 4 or no more than 4 nucleotides in length. In some examples, the motif [B] and [b] are independently 1, 2, 3, or 4 nucleotides in length.

In some examples, motifs [B] and [b] has a sequence selected from the group consisting of SEQ ID NO: 1 (5'-ACG/CGU-3'), SEQ ID NO: 2 (5'-ACG/CGA-3'), SEQ ID NO: 449 (5'- ACG/UGA -3'), SEQ ID NO: 450 (5'- AUG/CGA -3'), SEQ ID NO: 451(5'- AUG/UGA -3'), SEQ ID NO: 452 (5'- CG/CG -3'), SEQ ID NO: 453 (5'- UUG/UGG -3'), SEQ ID NO: 454 (5'- UAU/AUA -3'), SEQ ID NO: 455 (5'- ACU/AGA -3'), SEQ ID NO: 456 (5'- AUG/CAA -3'), SEQ ID NO: 457 (5'- CU/AG -3'), and SEQ ID NO: 458 (5'- UG/CA -3').

In some examples, each of motifs [C], [c], [D] and [d] is independently between 1 to 100 nucleotides in length. In some examples, each of [C], [c], [D] and [d] is between 1 to 5, or between 5 to 10, or between 10 to 15, or between 15 to 20, or between 20 to 30, or between 30 to 40, or between 40 to 50, between 50 to 60, between 60 to 70, between 70 to 80 or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotides in length. In some specific examples, motif [C] has the same length as motif [c].

As illustrated in the Experimental Section, the inventors of the present disclosure found that a mutation in a specific site of a motif advantageously increase cleavage of the ribozymes. Thus, the ribozymes as disclosed herein may comprise a mutation at nucleotide N₇ to pair with nucleotide N₊₃, wherein the mutation increases cleavage when the sequence at the cleavage site deviates from the canonical sequences. In some examples, motif [D] comprises a mutation of nucleotide N7 to pair with nucleotide N+3.

Without wishing to be bound by theory, it is believed that one or more or all of the features mentioned above allows for the formation of a trigger-activated ribozyme (or dual ribozyme). That is, in some examples, the present disclosure provides a (dual) ribozyme comprising one or more features such as a circularly permuted (dual) ribozyme, reduced complementarity of the cleavage product, a 4-way junction, a shortened Helix 4, and an altered Helix 4 to encompass a branched nucleic acid-binding trigger region. In some examples, the present disclosure comprises a (dual) ribozyme comprising two or more features, or three or more, or all four features as disclosed herein. As disclosed

herein, each element of the ribozyme as disclosed herein may be combined with the others in different combinations.

Thus, as disclosed herein are examples of ribozymes with one or more of the motifs as disclosed herein:

5 1) Sequence motifs in the Helix 4 communication module of the ribozyme that result in ribozymes with very low background cleavage in absence of the target/trigger RNA, and high cleavage rates in presence of the target/trigger nucleic acid, applicable over a series of ribozymes.

10 2) Altered and lengthened Helix 2 domains, which reduce the background cleavage of the cleavage site proximal to the catalytic domain.

 3) Ribozymes with increased and full complementarity between the releasable cleavage product and its complementary strand, to reduce background cleavage product release.

15 4) A mutation of nucleotide N₇ to pair with nucleotide N₊₃, to increase cleavage when the sequence at or near the cleavage site deviates from canonical cleavage site sequences.

 5) Altered and lengthened Helix 3 domains, which reduce the background cleavage of the cleavage site proximal to the catalytic domain.

20 In some examples, the ribozymes as disclosed herein may comprise two or more, three or more, or all four of the motifs as disclosed herein.

 In some examples, the ribozymes may comprise a sequence as disclosed herein. In some examples, the ribozymes may comprise a sequence as disclosed in
25 Table 1. In some examples, the ribozyme may comprise a sequence of one or more of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID
30 NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID
35 NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID

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35 377, SEQ ID NO: 378, SEQ ID NO: 379, SEQ ID NO: 380, SEQ ID NO: 381, SEQ

ID NO: 382, SEQ ID NO: 383, SEQ ID NO: 384, SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, SEQ ID NO: 406, SEQ ID NO: 407, SEQ ID NO: 408, SEQ ID NO: 409, SEQ ID NO: 410, SEQ ID NO: 411, SEQ ID NO: 412, SEQ ID NO: 413, SEQ ID NO: 414, SEQ ID NO: 415, SEQ ID NO: 416, SEQ ID NO: 417, SEQ ID NO: 418, SEQ ID NO: 419, SEQ ID NO: 420, SEQ ID NO: 421, SEQ ID NO: 422, SEQ ID NO: 423, SEQ ID NO: 424, SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO: 435, SEQ ID NO: 436, SEQ ID NO: 437, SEQ ID NO: 438, SEQ ID NO: 439, SEQ ID NO: 440, SEQ ID NO: 441, SEQ ID NO: 442, SEQ ID NO: 443, SEQ ID NO: 444, SEQ ID NO: 445, SEQ ID NO: 446, SEQ ID NO: 447, and SEQ ID NO: 448. In some examples, the ribozyme complex comprises the sequences of any one or more of SEQ ID NOs: 3 to SEQ ID NOs: 448.

As would be appreciated by the person skilled in the art, the ribozyme as disclosed herein can be used for detecting and/or amplifying a trigger / target nucleic acid molecule in a sample. Therefore, in a second aspect of the present disclosure, there is provided a method of detecting presence of a trigger nucleic acid molecule in a sample, wherein the method comprises:

incubating the sample with a ribozyme as disclosed herein at temperature T1 which allows the binding of the trigger nucleic acid molecule with one or more trigger -binding domains comprised in the ribozyme;

incubating the sample at temperature T2 which allows the nucleic acid molecule and a RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme.

One exemplary method for using the ribozymes disclosed herein includes a method of detecting presence of a trigger nucleic acid molecule in a sample, wherein the method comprises:

a) incubating the sample with a ribozyme as disclosed herein at temperature T1 which allows the binding of the trigger RNA molecule with one or more trigger -binding domains comprised in the ribozyme;

- b) incubating the sample at temperature T2 which allows the nucleic acid molecule and a releasable RNA segment to be released from the ribozyme;
- c) detecting the release of the releasable RNA segment from the ribozyme.

In some examples, step c) is carried out by detecting the presence of the releasable RNA segment in the sample. Any RNA detection method or RNA detection systems known in the art can be used. Exemplary and non-exhaustive examples of RNA detection methods include: Reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR (RT-qPCR), probe-based RNA detection (such as northern blotting, microarrays and molecular beacons). Exemplary and non-exhaustive examples of RNA detection systems include: NanoString Technologies' nCounter® miRNA expression assay and Exiqon's Smart Flares), RNA-activated fluorescent sensors such as the Pandan fluorescent sensor (PCT patent PCT/SG2017/050086; Aw et. al., Nucleic Acids Research 2016), and CRISPR-Cas based nucleic acid detection systems such as DETECTR (Chen, J. S. et al. CRISPR-Cas12a target-binding unleashes indiscriminate single-stranded DNase activity. Science 360, 436-439, doi:10.1126/science.aar6245 (2018)) and SHERLOCK (Gootenberg, J. S. et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360, 439-444, doi:10.1126/science.aaq0179 (2018)).

In some examples, steps a) to b) are repeated for one or more times before step c) is carried out, wherein the RNA molecules released from step b) are for binding to another copy of the ribozyme. In this example, the sample can be incubated with an excess amount of the ribozyme when performing step a) for the first time, so that when performing a) for the second or subsequent time, additional ribozymes may not be supplemented to the sample. As the target nucleic acid molecules released from the ribozymes in step b) can further bind to new ribozymes, the copy number of released cleavage products (the "releasable RNA segment") can be many folds higher than the copy number of target/trigger molecules in the sample. Therefore, the presence or the amount of the released "releasable RNA segment" serve as an amplified signal for the presence or the amount of the target/trigger nucleic acid molecules in the sample. Thus, this method can improve the sensitivity of existing nucleic acid detection technologies when used in combination.

The releasable RNA segment can comprise a sequence identical to the target/trigger nucleic acid, in which case the released "releasable RNA segments" can further bind to new ribozymes as target nucleic acid molecules themselves. By repeating steps a) to b) as above, substantial amplification of the target/trigger nucleic acid

molecule (or the sequence the target/trigger nucleic acid molecule) can be achieved. Therefore, a method of amplifying a target/trigger nucleic acid molecule is also considered to be part of the present disclosure. Such methods may comprise the steps of a) incubating the target/trigger nucleic acid molecule with an ribozyme of the present disclosure at temperature T1 which allows the binding of the target/trigger nucleic acid molecule with one or more target-binding domains comprised in the ribozyme, and wherein the releasable RNA segment comprises a sequence identical to the target/trigger nucleic acid molecule; b) incubating the ribozyme bound to the target/trigger nucleic acid molecule at temperature T2 which allows the target/trigger nucleic acid molecule and the RNA segment to be released from the ribozyme. In some examples, steps a) to b) are repeated for one or more times, wherein the target/trigger nucleic acid molecules and the releasable RNA segment released from step b) are for binding to another copy of the ribozyme.

In some examples, the releasable RNA segment comprises a sequence that is identical to at least one of the one or or more trigger nucleic acid molecules. In some examples, the ribozyme comprises two trigger-binding domains for binding a specific trigger nucleic acid molecule, wherein the trigger nucleic acid molecule comprises a sequence that is identical to the trigger nucleic acid molecule. In some examples, the binding of trigger nucleic acid molecules leads to the release of an RNA molecule comprising the same sequence as the trigger nucleic acid molecule.

In some examples, the binding of the trigger nucleic acid molecule with the one or more trigger-binding domains of the ribozyme occurs at a temperature T1. In some examples, T1 is a temperature not more than 50°C. In some examples, T1 is a temperature between 0°C to 50°C, a temperature between 15°C to 45°C a temperature between 25°C to 45°C, a temperature between 30°C to 40°C, a temperature between 35°C to 38°C, a temperature between 36.5°C to 37.5°C. In a specific example, T1 is a temperature of about 37°C.

In some examples, the temperatures T1 and T2 are identical. In another example, temperature cycling does not occur and/or is not required. Instead, the method requires sample incubation at one specific temperature. In some examples, the ribozymes are incubated at 37°C for 4 hours. In one example, such a method is a cleavage assay.

In some examples, the trigger nucleic acid molecule bound to a trigger-binding domain of the ribozyme is released from said target-binding domain at a temperature T2. In some examples, wherein the two cleavage sites flanking the releasable RNA segment are cleaved, the releasable RNA segment is released at a preferred

temperature T2. In some examples, T2 is a temperature between 20°C to 100°C, a temperature between 25°C to 80°C, a temperature between 30°C to 80°C, a temperature between 35°C to 80°C, a temperature between 40°C to 80°C, a temperature between 45°C to 80°C, a temperature between 50°C to 80°C, a temperature between 55°C to 75°C, or a temperature between 57°C to 63°C. In a specific example, T2 is a temperature of about 60°C.

It should be understood that T1 and T2 as described above refer to temperatures which allow the binding (of the targeting RNA molecule) and the release (of the target nucleic acid molecules and the releasable RNA segment) respectively. T1 should not be taken to mean a temperature under which no trigger nucleic acid molecules or releasable RNA segments can be released; and T2 should not be taken to mean a temperature under which no target nucleic acid molecule can bind with the trigger-binding domain. It is generally understood in the art that the binding (also known as “annealing”) and release (also known as “melting”) of complementary RNA strands can occur simultaneously, albeit with differing kinetics, across a wide range of temperatures, therefore T1 and T2 can be the same or different. In some examples, the trigger nucleic acid molecules can bind to the trigger-binding domains of the ribozyme, triggering the cleavage and release of the releasable RNA segment, and release from the ribozyme, all at a temperature between 35°C to 38°C. However, when the ribozyme is used to detect and amplify trigger molecules, the implementation of annealing (under T1) and melting (under T2), wherein T2 is higher than T1, drives the reaction forward and can result in increased number of released releasable RNA products.

In yet another aspect, there is provided a method of detecting presence of a sequence or mutation of interest on an nucleic acid of interest in a sample, wherein the method comprises:

incubating the sample with a ribozyme as disclosed herein, thereby allowing binding of the trigger nucleic acid molecule with one or more trigger-binding domains comprised in the ribozyme;

incubating the sample which allows the nucleic acid molecule and a releasable RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme;

wherein the releasable RNA segment is an sgRNA or shRNA; wherein detection of the sequence or mutation of interest in the sample results in a signal being generated.

In some examples, the trigger nucleic acid molecule is an nucleic acid molecule obtained from animals, viruses, bacteria, yeast or plants. Therefore, in some examples, the target or trigger nucleic acid molecules may include, but are not limited to, viral nucleic acid, bacterial nucleic acid, modified nucleic acid (such as mutation in a nucleic acid), messenger nucleic acid, coding nucleic acid, genomic nucleic acid, and the like.

Examples of target or trigger DNA molecules may include, but are not limited to viral DNA, cDNA, circulating DNA, cell free DNA (cfDNA), foetal DNA, modified DNA (e.g. mutation), single stranded DNA, double stranded DNA, mitochondrial DNA, and the like.

In some examples, the target/trigger nucleic acid may include, but is not limited to, viral RNA, a microRNA (miRNA), short interfering RNA (siRNA), small RNA (sRNA), messenger RNA (mRNA), non-coding RNA (ncRNA), short non-coding RNA, transfer RNA (tRNA), ribosomal RNA (rRNA), transfer-messenger RNA (tmRNA), clustered regularly interspaced short palindromic repeats RNA (CRISPR RNA), antisense RNA, pre-mRNA, circular RNA or pre-miRNA, or fragment thereof. In some examples, the trigger RNA molecule is a micro-RNA, or a precursor thereof, or a fragment thereof. the trigger nucleic acid molecule is an RNA or DNA molecule, or modified RNA or DNA molecule.

In some examples, the trigger nucleic acid molecule is a genome of a virus, or a fragment thereof. In some examples, the virus may include, but is not limited to, a Retroviridae virus, a Lentiviridae virus, a Coronaviridae virus, a Picornaviridae virus, a Caliciviridae virus, a Flaviviridae virus, a Togaviridae virus, a Bornaviridae, a Filoviridae, a Paramyxoviridae, a Pneumoviridae, a Rhabdoviridae, an Arenaviridae, a Bunyaviridae, an Orthomyxoviridae, a Deltavirus.

In some examples, the virus may include, but is not limited to, Lymphocytic choriomeningitis virus, Coronavirus, human immunodeficiency virus (HIV), Severe acute respiratory syndrome virus (SARS), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Poliovirus, Rhinovirus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Norwalk virus, Yellow fever virus, West Nile virus, Dengue fever virus, Zika virus, Rubella virus, Ross River virus, Rhinovirus C, Sindbis virus, Chikungunya virus, Coxsackievirus virus, Borna disease virus, Ebola virus, Marburg virus, Measles virus, Mumps virus, Nipah virus, Hendra virus, Newcastle disease virus, Human respiratory syncytial virus, Rabies virus, Lassa virus, Hantavirus, Crimean-Congo hemorrhagic fever virus, Influenza virus (such as

influenza A virus), smallpox virus (variola virus), Japanese encephalitis virus, Seoul virus, adenovirus, and Herpes simplex virus.

The inventors have exemplified in the Experimental Section, potential applications for the ribozyme for cell-free RNA signal amplification and as an *in vitro* and *in vivo* gene reporter and signal transducer, in fish and human cells.

Therefore, in some examples, the methods or ribozymes as disclosed herein may be used in any one of mammalian cells, mammalian cell lines, human cell, human cell lines, zebrafish cells, and zebrafish embryos. In some examples, the methods or ribozymes as disclosed herein may be used either *in vitro* or *in vivo*.

In some examples, the ribozymes as disclosed herein may be used in any one of mammalian cells, mammalian cell lines, human cell, human cell lines, zebrafish cells, and zebrafish embryos.

In yet another aspect, there is provided a polynucleotide encoding the ribozymes as disclosed herein. In some examples where the ribozyme is comprised of more than one RNA strand, the RNA strands can be encoded together on one polynucleotide or separately on several polynucleotides.

The isolated nucleotide sequence as disclosed herein can be used to transduce the binding signal of a target trigger nucleic acid sequence into release of a second functional nucleic acid sequence. The isolated nucleic acid sequence as disclosed herein contains a trigger-binding domain comprised of two trigger-binding arms that are sequence-complementary to an nucleic acid trigger of interest and is capable of forming bimolecular interactions with the target trigger nucleic acid sequence. The isolated nucleic acid sequence as disclosed herein also forms a ternary complex allowing self-cleavage of itself when binding the target nucleic acid sequence, releasing an RNA cleavage product fragment that can take the form of a functional RNA with tertiary structure, e.g. CRISPR guide RNA (gRNA or sgRNA), short hairpin RNA (shRNA), RNA aptamer and the like. In some examples, the ribozymes as disclosed herein release CRISPR gRNA, shRNA or an RNA aptamer in presence of the trigger nucleic acid. Therefore, the ribozymes as disclosed herein has the ability to function *in vitro* and *in vivo* for gene regulation. For example, the Experimental Section has demonstrated functional gene regulation by the ribozyme *in vitro* and *in vivo*, in fish and mammalian cells.

This isolated nucleotide sequence also comprises one or both distinct features of: A) A catalytic domain that is stabilised by binding of the trigger nucleic acid, and B) A communication module between the catalytic domain and the trigger arms. While a range

of sequence motifs can be envisioned to work, and indeed the best module for any particular ribozyme may need to be empirically optimised, 5-10 sequence motifs were identified in Helix 4 that allow for optimised low background/high-cleavage activity in absence and presence of the trigger nucleic acid respectively. It was also found that
5 lengthening of Helix 2 or Helix 3 decreases background cleavage of the cleavage site proximal to the catalytic domain in absence of the trigger.

In yet another aspect, there is provided a kit comprising the ribozymes as disclosed herein.

In some examples, the kit further comprises a nucleic acid detection system.

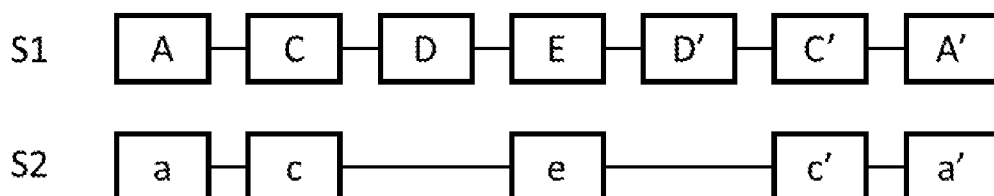
10 In some examples, the nucleic acid detection system comprises an RNA-activated fluorescent sensor. In some examples, the sensor is a Pandan fluorescent sensor or detection system (PCT patent PCT/SG2017/050086; Aw et. al., Nucleic Acids Research 2016). In some examples the nucleic acid detection system is a CRISPR-Cas based nucleic acid detection system. CRISPR Cas-based nucleic acid detection
15 methods and systems are known in the art, and are disclosed for example in Chen, J. S. et al. CRISPR-Cas12a target-binding unleashes indiscriminate single-stranded DNase activity. Science 360, 436-439, doi:10.1126/science.aar6245 (2018), and Gootenberg, J. S. et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360, 439-444, doi:10.1126/science.aaq0179
20 (2018).

In some examples, the ribozymes as disclosed herein may also act as an nucleic acid signal transducer and amplifier.

In some examples, the ribozyme is modified from a naturally existing
25 ribozyme. In some examples, the ribozyme is modified from an artificial ribozyme, fusion ribozyme, fragments and derivatives thereof. In some examples, the ribozyme is characteristic of a hairpin ribozyme or a hammerhead ribozyme, or fragments and fusions thereof. In some examples, the ribozyme is a twin ribozyme or duplex ribozyme. In some examples, the ribozyme comprises a twin-hairpin ribozyme
30 structure. Design of ribozymes as described herein is illustrated in an earlier application, PCT/SG2020/050226, the content of which is incorporated herein and described briefly below.

As is commonly known in the art, a ribozyme can comprise one or more RNA strands. In a specific example, the ribozyme comprises a first RNA strand and a
35 second RNA strand. When a ribozyme is comprised of two RNA strands, the two

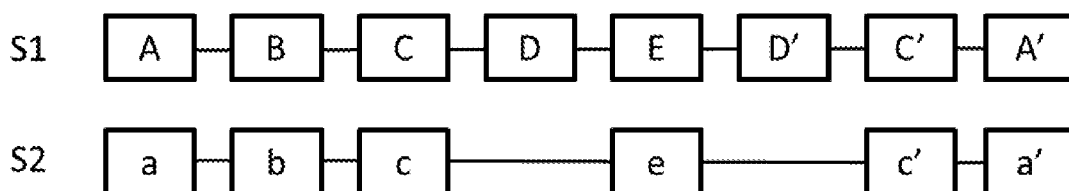
RNA strands have sufficient complementarity so that they are bound to each other. Typically, the two RNA strands are not fully complementary across their entire lengths. Each RNA strand can form secondary structures independently, as is generally known in the art. In one example, the ribozyme comprises the following structure:



(structure III),

wherein: S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; and motifs [A] and [a] constitute a first trigger-binding domain for binding a first trigger nucleic acid molecule, motifs [C] and [c] constitute a first catalytic domain, motif [D] comprises a first cleavage site capable of being cleaved, motifs [A'] and [a'] constitute a second trigger-binding domain for binding a second trigger nucleic acid molecule, motifs [C'] and [c'] constitute a second catalytic domain, motif [D'] comprises a second cleavage site capable of being cleaved by the second catalytic domain, motif [E] comprises a releasable RNA segment, motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E], each of the horizontal lines connecting the motifs represents an optional linker region; and wherein the first catalytic domain is in an active state when the first target-binding domain is bound to first target RNA molecule; and wherein the second catalytic domain is in an active state when the second target-binding domain is bound to the second target nucleic acid molecule.

In another example, the ribozyme comprises the following structure:



(structure IV),

wherein: S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; wherein motifs [A] and [a] constitute a first trigger-binding domain for binding a first trigger nucleic acid molecule, motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

or, wherein motifs [B] and [b] constitute a first inhibitory domain, motifs [C] and [c] constitute a first catalytic domain, motif [D] comprises a first cleavage site capable of being cleaved by the first catalytic domain, motifs [A'] and [a'] constitute a second trigger-binding domain for binding a second trigger nucleic acid molecule; motifs [C'] and [c'] constitute a second catalytic domain, motif [D'] comprises a second cleavage site capable of being cleaved by the second catalytic domain, motif [E] comprises a releasable RNA segment, motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E], each of the horizontal lines connecting the motifs represents an optional linker region, wherein the first inhibitory domain is characterized by i) or ii) below:

i) motif [b] anneals with [C] when the first target-binding domain is not bound by the first target nucleic acid molecule, but anneals with [B] when the first target-binding domain is bound by the first target nucleic acid molecule,

ii) motif [B] anneals with motif [c] when the first target-binding domain is not bound by the first target nucleic acid molecule, but anneals with [b] when the first target-binding domain is bound by the first target nucleic acid molecule,

wherein the first catalytic domain is in an active state when motif [b] is annealed with motif [B].

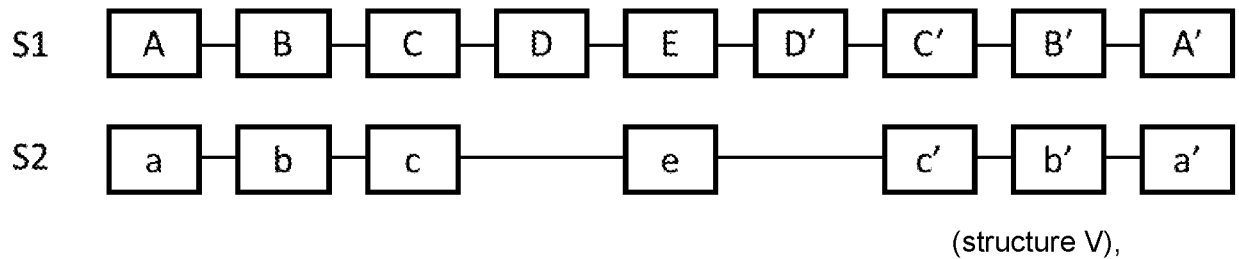
In a further example, the first inhibitory domain is further characterized by i) or ii) below: i) motif [b] is at least 50% complementary to motif [C], and at least 20% complementary to motif [B], and ii) motif [B] is at least 50% complementary to motif [c], and at least 20% complementary to motif [b].

In a further example, the first inhibitory domain is further characterized by i) or ii) below:

i) motif [b] is at least 60%, at least 70%, at least 80% or at least 90% complementary to motif [C], and at least 20% complementary to motif [B], or

ii) motif [B] is at least 60%, at least 70%, at least 80% or at least 90% complementary to motif [c], and at least 20% complementary to motif [b].

In another example, the ribozyme comprises the following structure:



wherein:

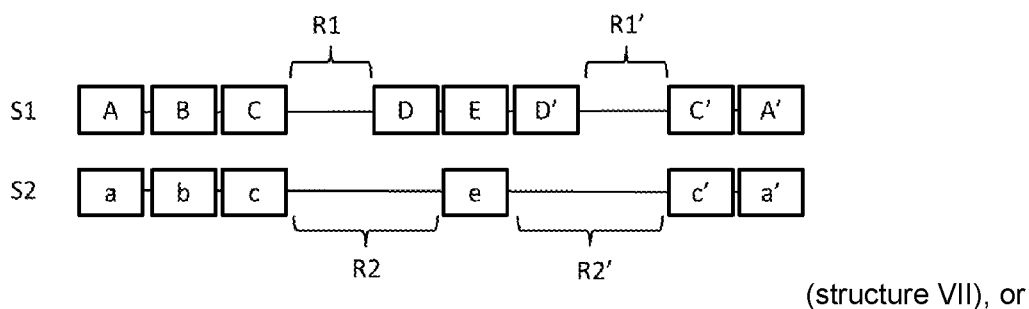
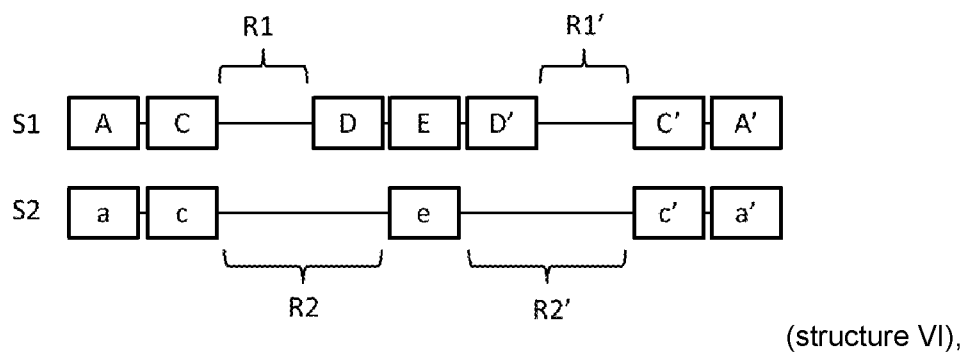
- 5 S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; wherein motifs [A] and [a] constitute a first trigger-binding domain for binding a first trigger nucleic acid molecule, motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are
- 10 independently at least 1 nucleotide in length;
or, wherein motifs [B] and [b] constitute a first inhibitory domain, motifs [C] and [c] constitute a first catalytic domain, motif [D] comprises a first cleavage site capable of being cleaved by the first catalytic domain, motifs [A'] and [a'] constitute a second trigger-binding domain for binding a second target RNA molecule; motifs [B'] and [b'] constitute
- 15 a second linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B'] and [b'] are independently at least 1 nucleotide in length;
or, wherein motifs [B'] and [b'] constitute a second inhibitory domain, motifs [C'] and [c'] constitute a second catalytic domain, motif [D'] comprises a second cleavage site
- 20 capable of being cleaved by the second catalytic domain, motif [E] comprises a releasable RNA segment, motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E], each of the horizontal lines connecting the motifs represents an optional linker region; wherein the first inhibitory domain is characterized by i) or ii) below:
- 25 i) motif [b] anneals with [C] when the first target-binding domain is not bound by the first target nucleic acid molecule, but anneals with [B] when the first target-binding domain is bound by the first target nucleic acid molecule,
- ii) motif [B] anneals with motif [c] when the first target-binding domain is not bound by the first target nucleic acid molecule, but anneals with [b] when the first target-binding
- 30 domain is bound by the first target nucleic acid molecule,
- wherein the second inhibitory domain is characterized by i) or ii) below:

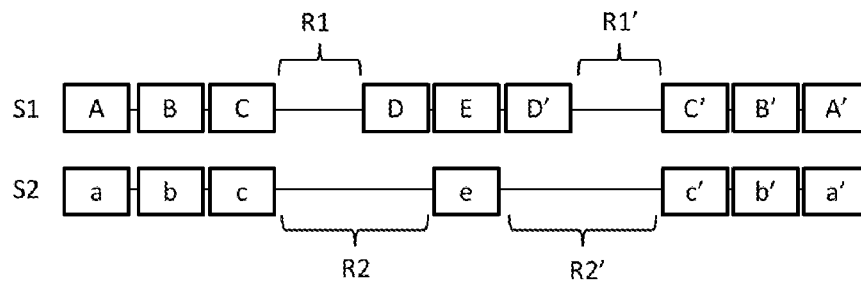
i) motif [b'] anneals with motif [C'] when the second target-binding domain is not bound by the first target nucleic acid molecule, but anneals with [B'] when the second target-binding domain is bound by the second target nucleic acid molecule,

5 ii) motif [B'] anneals with motif [c'] when the second target-binding domain is not bound by the second target RNA molecule, but anneals with [b'] when the second target-binding domain is bound by the second target RNA molecule,

wherein the first catalytic domain is in an active state when motif [b] is annealed with motif [B], and the second catalytic domain is in an active state when motif [b'] is
10 annealed with motif [B'].

One or more secondary structures can be formed either individually by any of the motifs or optional linker regions, or formed collectively by motifs, linker regions, or combinations thereof. In an example, the optional linker regions individually or collectively form one or more secondary structures. In some examples of the ribozyme
15 where the ribozymes comprise two target-binding domains and two catalytic domains, the ribozyme comprises one of the following structures:





(structure VIII),

wherein linker regions R1 and R2 individually or collectively form one or more secondary structures, and linker regions R1' and R2' individually or collectively form one or more secondary structures.

Table 1. Sequence listing table

Ribozyme Description	SEQ ID NO :	Trigger Sequence	Cleavage Product Name	SEQ ID NO :	Cleavage Product Sequence	SEQ ID NO :	Full Sequence (S1)		Full Sequence (S2)
Chong Hui Circularly permuted ribozyme 8-nt Helix 4		-	29nt-clvRNA	307	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUUUACCU GGUCCC	410	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA GUAAAACUU UCGACUAAG AGAAGUCAA CCAGAGAAA CACACUCC C
Chong Hui Circularly permuted ribozyme 6-nt Helix 4		-	29nt-clvRNA	308	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUUUACCU GGUU	411	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA GUAAAACUU UCGACUAAG AGAAGUCAA CCAGAGAAA CACACUUC
Chong Hui Circularly permuted ribozyme 4-nt Helix 4		-	29nt-clvRNA	309	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUUUACCU GG	412	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA GUAAAACUU UCGACUAAG AGAAGUCAA CCAGAGAAA CACACU
Chong Hui Circularly permuted		-	29nt-clvRNA	310	GUCCU UAGUC GAAAGU UUUACU	6	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC	413	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA

ed ribozyme 3-nt Helix 4					AGAGU CA		GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUAUUACCU G		GUAAAACUU UCGACUAAG AGAAGUCA CCAGAGAAA CACAC
Chong Hui Circularly permutat ed ribozyme 2-nt Helix 4		-	29nt- clvR NA	3 1 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	7	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUAUUACCU	4 1 4	GGGAACCAG AGAAACACA CGAAUCAGA GAAGACUCU AGUAAAACU UUCGACUAA GAGAAGUCA ACCAGAGAA ACACA
Chong Hui Circularly permutat ed ribozyme 1-nt Helix 4		-	29nt- clvR NA	3 1 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	8	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUAUUACC	4 1 5	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA GUAAAACUU UCGACUAAG AGAAGUCA CCAGAGAAA CAC
Chong Hui Circularly permutat ed ribozyme 0-nt Helix 4		-	29nt- clvR NA	3 1 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	9	GGGAAGUGGU AUUUUACCU GUACGCGUUC ACGGUUCGCC GUGAACGCGU UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGAGGUCG UGAGUCCACG ACCUCCGUGG UAUAUUAC	4 1 6	GGGACCAGA GAAACACAC GAAUCAGAG AAGACUCUA GUAAAACUU UCGACUAAG AGAAGUCA CCAGAGAAA CA
Chong Hui IN- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 4-nt Helix 4	2 2 1	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 0	GGGAAGUCA ACCAAAGUGG UAUAUUACCU GGUUUUCGAU CGAAAGAUCG ACGAGGUGAA AACCUCGUGA CAGUCCUUAG UCGAAAGUUU	4 1 7	GGGACCGG UUUUCGAUU UGGUUUGAC UUUCGAGUC AAACCAACC AGAGAAACA CACGAAUCA GAGAAGACG AGCGUCCCA

							UACUAGAGUC AGUCCUGAUU GUGCUCGAAA GAGCACAGAC CUGAAAAGGU CUUUCGUGGU AUUUUACCUG GAUCGAAAAC CGG		CGGGCGCA GAAGAGAAG UCAACCAGA GAAACACAC UAUCGAAAA CCGGUUCGC CGGUUUUCG AUUUGGUUU GACU
Chong Hui IN- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 3-nt Helix 4	2 2 2	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 1	GGGAAGUCA ACCAAAGUGU AUUUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUGAC AGUCCUUAGU CGAAAGUUUU ACUAGAGUCA GUCCUGAUUG UGCUCGAAAG AGCACAGACC UGAAAAGGUC UUUCGUGGUA UAUUACCUGA UCGAAAACCG G	4 1 8	GGGACCGG UUUUCGAUU UGGUUUUGAC UUUCGAGUC AAACCAACA GAGAAACAC ACGAAUCAG AGAAGACGA GCGUCCAC GGGCGCAGA AGAGAAGUC AACCAGAGA AACAACUUA CGAAAACCG GUUCGCCG GUUUUCGAU UUGGUUUGA CU
Chong Hui IN- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 2-nt Helix 4	2 2 3	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 2	GGGAAGUCA ACCAAAGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCCUUAGUC GAAAGUUUUA CUAGAGUCAG UCCUGAUUGU GCUCGAAAGA GCACAGACCU GAAAAGGUCU UUCGUGGUUA AUUACCUAUC GAAAACCGG	4 1 9	GGGACCGG UUUUCGAUU UGGUUUUGAC UUUCGAGUC AAACCAAAG AGAAACACA CGAAUCAGA GAAGACGAG CGUCCACG GGCGCAGAA GAGAAGUCA ACCAGAGAA ACACUAUCG AAAACCGGU UCGCCGGUU UUCGAUUUG GUUUGACU
Chong Hui IN- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 1-nt Helix 4	2 2 4	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 3	GGGAAGUCA ACCAAAGUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUGUG CUCGAAAGAG CACAGACCUG AAAAGGUCUU	4 2 0	GGGACCGG UUUUCGAUU UGGUUUUGAC UUUCGAGUC AAACCAAGA GAAACACAC GAAUCAGAG AAGACGAGC GUCCCACGG GCGCAGAAG AGAAGUCA CCAGAGAAA CAUAUCGAA AACCGGUUC

						UCGUGGUAUA UUACCAUCGA AAACCGG		GCCGGUUUU CGAUUUGGU UUGACU
Chong Hui OUT- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 8-nt Helix 4	2 2 5	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 4	4 2 1	GGGAAGUCA AACCAAGGG ACCAGAGAA ACACACGAA UCAGAGAAG ACGAGCGUC CCACGGGCG CAGAAAGAG AGUCAACCA GAGAAACAC ACUUCCCAU CGAAAACCG G
Chong Hui OUT- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 4-nt Helix 4	2 2 6	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 1 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 5	4 2 2	GGGAAGUCA AACCAACCA GAGAAACAC ACGAAUCAG AGAAGACGA GCGUCCCAC GGGCGCAGA AGAGAAGUC AACCAGAGA AACACACUA UCGAAAACC GG
Chong Hui OUT- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 3-nt Helix 4	2 2 7	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 2 0	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 6	4 2 3	GGGAAGUCA AACCAACAG AGAAACACA CGAAUCAGA GAAGACGAG CGUCCCACG GGCGCAGAA GAGAAGUCA ACCAGAGAA ACACACAU GAAAACCGG

							UUUCGUGGUA UAUUACCUGA UCGAAAACCG G		
Chong Hui OUT- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 2-nt Helix 4	2 2 8	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 2 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 7	GGGAAGUCA ACCAAUGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGUGACA GUCCUUAGUC GAAAGUUUUA CUAGAGUCAG UCCUGAUUGU GCUCGAAAGA GCACAGACCU GAAAAGGUCU UUCGUGGUAU AUUACCUAUC GAAAACCGG	4 2 4	GGGAAGUCA AACCAAAGA GAAACACAC GAAUCAGAG AAGACGAGC GUCCCACGG GCGCAGAAG AGAAGUCA CCAGAGAAA CACAAUCGA AAACCGG
Chong Hui OUT- form ribozyme 4WJ (HHHS2 H) 2 x 3/4 1-nt Helix 4	2 2 9	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 2 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 8	GGGAAGUCA ACCAAGGUAU AUUACCUGGU UUUCGAUCGA AAGAUUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUGUG CUCGAAAGAG CACAGACCUG AAAAGGUCUU UCGUGGUAUA UUACCAUCGA AAACCGG	4 2 5	GGGAAGUCA AACCAAGAG AAACACACG AAUCAGAGA AGACGAGCG UCCCACGGG CGCAGAAGA GAAGUCAAC CAGAGAAAC ACAUCGAAA ACCGG
Dual ribozyme 2WJ Paired		-	29nt- clvR NA	3 2 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	1 9	gggaagtGTAT ATTACctggtTCT TTCTgacAGTCc TTAGTCGAAA GTTTTACTAGA GTcAGTCctgatT CTTTCTcgtgGT ATATTACctggtc cc	4 2 6	gggaccagAGA AACAcacgatc agAGAAgACT CTAGTAAAA CTTTCGACT AAgAGAAgtca ccagAGAAAC Acacttccc
Dual ribozyme 3WJ (HHH) Paired		-	29nt- clvR NA	3 2 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 0	gggaagtGTAT ATTACctggtacg cggtcacggTTCG ccgtgaacgcgtga cAGTCcTTAGT CGAAAGTTTTA CTAGAGTcAGT Cctgattgaggtcgt qAGTCcacgacct	4 2 7	gggaccagAGA AACAcacgaat cagAGAAgAC TCTAGTAAAA CTTTCGACT AAgAGAAgtca accagAGAAA CAcacttccc

						ccgtgGTATATT ACctggtccc			
Dual ribozyme 3WJ (HHH) Unpaired		-	29nt- clvR NA	3 2 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 1	gggaagtgGTAT ATTACctggtacg cgttcacggTTCG ccgtgaacgcggtga cAGTCcTTAGT CGAAAGTTTTA CTAGAGTcAGT Cctgattgagggtcgt gAGTCcacgacct ccgtgGTATATT ACctggtccc	4 2 8	gggaccagAGA AACAcacgaat cagAGAAgAC AGATCATTTT GAAAGCTGA AAgAGAAgtca accagAGAAA CAcacttccc
Dual ribozyme 3WJ (HHS4H) Paired		-	29nt- clvR NA	3 2 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 2	gggaagtgGTAT ATTACctggtCTT TacgcgttcacggT TCGccgtgaacgc gttgacAGTCcTT AGTCGAAAGT TTTACTAGAGT cAGTCcctgattgag gtcgtgAGTCcac gacctcCTTTcgtg GTATATTACctg gtccc	4 2 9	gggaccagAGA AACAcacgaat cagAGAAgAC TCTAGTAAAA CTTTCGACT AAgAGAAgtca accagAGAAA CAcacttccc
Dual ribozyme 3WJ (HHS4H) Unpaired		-	29nt- clvR NA	3 2 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 3	gggaagtgGTAT ATTACctggtCTT TacgcgttcacggT TCGccgtgaacgc gttgacAGTCcTT AGTCGAAAGT TTTACTAGAGT cAGTCcctgattgag gtcgtgAGTCcac gacctcCTTTcgtg GTATATTACctg gtccc	4 3 0	gggaccagAGA AACAcacgaat cagAGAAgAC AGATCATTTT GAAAGCTGA AAgAGAAgtca accagAGAAA CAcacttccc
Dual ribozyme 4WJ (HHHS2 H) Paired		-	29nt- clvR NA	3 2 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 4	gggaagtgGTAT ATTACctggtTTtc gatacGAAAgatcg acgaggtGAAAac ctcgtgacAGTCc TTAGTCGAAA GTTTTACTAGA GTcAGTCcctgatt gtgctcGAAAgag cacagacctGAAA aggtctTTcgtgGT ATATTACctggtc cc	4 3 1	gggaccagAGA AACAcacgaat cagAGAAgAC TCTAGTAAAA CTTTCGACT AAgAGAAgtca accagAGAAA CAcacttccc
Dual ribozyme 4WJ (HHHS2 H) Unpaired		-	29nt- clvR NA	3 2 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 5	gggaagtgGTAT ATTACctggtTTtc gatacGAAAgatcg acgaggtGAAAac ctcgtgacAGTCc TTAGTCGAAA	4 3 2	gggaccagAGA AACAcacgaat cagAGAAgAC AGATCATTTT GAAAGCTGA AAgAGAAgtca

							GTTTTACTAGA GTcAGTCctgatt gtgctcGAAAgag cacagacctGAAA aggtctTTcgtgGT ATATTACctggtc cc		accagAGAAA CAcacttccc
Dual ribozyme 4WJ (HHHS2 H) Unpaired #3		-	29nt- clvR NA	3 3 0	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 6	gggaagtgGTAT ATTACctggtTTtc gatacGAAAgatcg acgaggtGAAAac ctcgtgacAGTCc TTAGTCGAAA GTTTTACTAGA GTcAGTCctgatt gtgctcGAAAgag cacagacctGAAA aggtctTTcgtgGT ATATTACctggtc cc	4 3 3	gggaccagAGA AACAcacgaat cagAGAAgAC GAGCTGCCC CAGGGATCA GAAgAGAAgt caaccagAGAA ACAcacttccc
Dual ribozyme 4WJ (HHHS2 H) 3 x 2 #4		-	29nt- clvR NA	3 3 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 7	gggaagtgGTAT ATTACctggtTTtc gatacGAAAgatcg acgaggtGAAAac ctcgtgacAGTCc TTAGTCGAAA GTTTTACTAGA GTcAGTCctgatt gtgctcGAAAgag cacagacctGAAA aggtctTTcgtgGT ATATTACctggtc cc	4 3 4	gggaccagAGA AACAcacgaat cagAGAAgAC GAGAGTCCA ACGGTCGCA GAAgAGAAgt caaccagAGAA ACAcacttccc
Dual ribozyme 4WJ (HHHS2 H) 2 x 3/4 #5		-	29nt- clvR NA	3 3 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 8	gggaagtgGTAT ATTACctggtTTtc gatacGAAAgatcg acgaggtGAAAac ctcgtgacAGTCc TTAGTCGAAA GTTTTACTAGA GTcAGTCctgatt gtgctcGAAAgag cacagacctGAAA aggtctTTcgtgGT ATATTACctggtc cc	4 3 5	gggaccagAGA AACAcacgaat cagAGAAgAC GAGCGTCCC ACGGGCGCA GAAgAGAAgt caaccagAGAA ACAcacttccc
Dual ribozyme T-let- 7f_C1- 29nt- clvRNA	2 3 0	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 3 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	2 9	gggaAACUAUA CAAUGUAUAU UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGUGCUCG	4 3 6	gggaAACUAU ACAAUGUAA UAUAAGAAA CACACGAU CAGAGAAGA CGAGCGUCC CACGGGCGC AGAAGAGAA GUCAACCAG AGAAACUA AUAUACCUA CUACCUCA

							AAAGAGCACA GACCUGAAAA GGUCUUUCGU GGUAUAUUAC UCUCUGAACU ACUACCUCA		
Dual ribozyme T-ban- 5p_Cl- 29nt- clvRNA	2 3 1	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 3 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 0	GGGAAGUCA ACCAAGUAUA UUAGUAUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUUAUAUA CUCUCUGAAA UCGAAAACCG G	4 3 7	GGGAAGUCA AACCAAGUA AUUAUAGAA ACACACGAA UCAGAGAAG ACGAGCGUC CCACGGGCG CAGAAGAGA AGUCAACCA GAGAAACAU AAUAUACAU CGAAAACCG G
ban-5p double catalytic mutant	2 3 2	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 3 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 1	GGGAAGUCA ACCAAGUAUA UUAGUuUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUuUAUA CUCUCUGAAA UCGAAAACCG G	4 3 8	GGGAAGUCA AACCAAGUA AUUAUAGAA AgACACGAA UCAGAGAAG ACGAGCGUC CCACGGGCG CAGAAGAGA AGUCAACCA GAGAAAgAU AAUAUACAU CGAAAACCG G
ban-5p distal catalytic mutant	2 3 3	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 3 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 2	GGGAAGUCA ACCAAGUAUA UUAGUAUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA	4 3 9	GGGAAGUCA AACCAAGUA AUUAUAGAA AgACACGAA UCAGAGAAG ACGAGCGUC CCACGGGCG CAGAAGAGA AGUCAACCA GAGAAACAU AAUAUACAU CGAAAACCG G

							AGGUCUUUCG UGGUuUAUUA CUCUCUGAAA UCGAAAACCG G		
ban-5p proximal catalytic mutant	2 3 4	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 3 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 3	GGGAAGUCA ACCAAGUA UUAGUuUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUUAUUA CUCUCUGAAA UCGAAAACCG G	4 4 0	GGGAAGUCA AACCAAGUA AUUAAGAA ACACACGAA UCAGAGAAG ACGAGCGUC CCACGGGCG CAGAAGAGA AGUCAACCA GAGAAgAU AAUAUACAU CGAAAACCG G
Dual ribozyme T-mir- 451a_Cl- 29nt- clvRNA	2 3 5	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 3 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 4	GGGAUUUGGC AAUGGGUAUA UUAGUAUUAU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUUAUUA CUCUCUGAAU AAUGACUCAA	4 4 1	GGGAUUUG GCAAUGGGU AAUAUAAGA AACACACGA AUCAGAGAA GACGAGCGU CCCACGGGC GCAGAAGAG AAGUCAACC AGAGAAACA UAAUAUACU AAUGACUCA A
mir-451a double catalytic mutant	2 3 6	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 3 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 5	GGGAUUUGGC AAUGGGUAUA UUAGUuUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG	4 4 2	GGGAUUUG GCAAUGGGU AAUAUAAGA AAgACACGA AUCAGAGAA GACGAGCGU CCCACGGGC GCAGAAGAG AAGUCAACC AGAGAAAgA UAAUAUACU AAUGACUCA A

							UGGUuUAUUA CUCUCUGAAU AAUGACUCAA		
mir-451a distal catalytic mutant	2 3 7	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 4 0	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 6	GGGAUUUGGC AAUGGGUAUA UUAGUAUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUuUAUUA CUCUCUGAAU AAUGACUCAA	4 4 3	GGGAUUUG GCAAUGGGU AAUAUAAGA AAgACACGA AUCAGAGAA GACGAGCGU CCCACGGGC GCAGAAGAG AAGUCAACC AGAGAAACA UAAUAUACU AAUGACUCA A
mir-451a proximal catalytic mutant	2 3 8	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 4 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 7	GGGAUUUGGC AAUGGGUAUA UUAGUuUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU GAUUGUGCUC GAAAGAGCAC AGACCUGAAA AGGUCUUUCG UGGUAUAUUA CUCUCUGAAU AAUGACUCAA	4 4 4	GGGAUUUG GCAAUGGGU AAUAUAAGA AACACACGA AUCAGAGAA GACGAGCGU CCCACGGGC GCAGAAGAG AAGUCAACC AGAGAAAgA UAAUAUACU AAUGACUCA A
Dual ribozyme T-let- 7f_CI- 29nt- clvRNA	2 3 9	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 4 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 8	gggaAACUAUA CAAUGUAUAU UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGUGCUCG AAAGAGCACA GACCUGAAA GGUCUUUCGU GGUAUAUUAC	4 4 5	gggaAACUAU ACAAUGUAA UAUAAGAAA CACACGAU CAGAGAAGA CGAGCGUCC CACGGGCGC AGAAGAGAA GUCAACCAG AGAAACUA AUAUACCUA CUACCUCA

							UCUCUGAACU ACUACCUC		
let-7f double catalytic mutant	2 4 0	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 4 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	3 9	gggaAACUAUA CAAUGUAUAU UAGUuUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGUGCUCG AAAGAGCACA GACCUGAAAA GGUCUUUCGU GGUuUAUUAC UCUCUGAACU ACUACCUC	4 4 6	gggaAACUAU ACAAUGUAA UAUAAGAAA gACACGAU CAGAGAAGA CGAGCGUCC CACGGGCGC AGAAGAGAA GUCAACCAG AGAAAgUA AUAUACCUC CUACCUC
let-7f distal catalytic mutant	2 4 1	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 4 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 0	gggaAACUAUA CAAUGUAUAU UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGUGCUCG AAAGAGCACA GACCUGAAAA GGUCUUUCGU GGUuUAUUAC UCUCUGAACU ACUACCUC	4 4 7	gggaAACUAU ACAAUGUAA UAUAAGAAA gACACGAU CAGAGAAGA CGAGCGUCC CACGGGCGC AGAAGAGAA GUCAACCAG AGAAACUA AUAUACCUC CUACCUC
let-7f proximal catalytic mutant	2 4 2	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 4 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 1	gggaAACUAUA CAAUGUAUAU UAGUuUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUGUGCUCG AAAGAGCACA GACCUGAAAA GGUCUUUCGU GGUuUAUUAC	4 4 8	gggaAACUAU ACAAUGUAA UAUAAGAAA CACACGAU CAGAGAAGA CGAGCGUCC CACGGGCGC AGAAGAGAA GUCAACCAG AGAAAgUA AUAUACCUC CUACCUC

							UCUCUGAACU ACUACCUCA		
One- sided T- ban5p_CI -29nt- clvRNA	2 4 3	CCGG UUUU CGAU UUGG UUUG ACU	29nt- clvR NA	3 4 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 2	gggaAGUCAAA CCAAGUAUUA UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC AUCGAAAACC GG		
One- sided T- mir- 451a_CI- 29nt- clvRNA	2 4 4	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 4 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 3	gggaAACUCAG UAAUGUAUUA UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC GGUAACGGUU U		
One- sided T- E- gene_CI- 29nt- clvRNA	2 4 5	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 4 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 4	gggaACGUACC UGUGUAUAUU AGUAUAUUAC CUGGUUUUCG AUCGAAAGAU CGACGAGGUG AAAACCUCGU GACAGUCCUU AGUCGAAAGU UUUACUAGAG UCAGUCCUGA UUUUCGAAUC		

						AGAGAAGACG AGCGUCCCAC GGGCGCAGAA GAGAAGUCAA CCAGAGAAAC AUAAUAUACC UCUUCCGAA			
One- sided T- mir- 184_CI- 29nt- clvRNA	2 4 6	UGGA CGGA GAAC UGAU AAGG GC	29nt- clvR NA	3 4 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 5	gggaGCCCUUA UCAGGUUAU UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC UUCUCCGUCC A		
One- sided T- mir- 252_CI- 29nt- clvRNA	2 4 7	CUAA GUAC UAGU GCCG CAGG AG	29nt- clvR NA	3 5 0	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 6	gggaCUCCUGC GGCAGUAUUA UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC CUAGUACUUA G		
One- sided T- mir- 263a_CI- 29nt- clvRNA	2 4 8	AAUG GCAC UGGA AGAA UUCA CGGG	29nt- clvR NA	3 5 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 7	gggaCCCGUGA AUUCUGUAUA UUAGUAUAUU ACCUGGUUUU CGAUCGAAAG AUCGACGAGG UGAAAACCUC GUGACAGUCC UUAGUCGAAA GUUUUACUAG AGUCAGUCCU		

							GAUUUUCGAA UCAGAGAAGA CGAGCGUCCC ACGGGCGCAG AAGAGAAGUC AACCAGAGAA ACAUAAUAUA CUCCAGUGCC AUU		
One- sided T- Orf1ab- 20nt_CI- 29nt- clvRNA	2 4 9	GGGA CAGA TCTAA TGGC TGC	29nt- clvR NA	3 5 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 8	gggaGCAGCCA UUAGUAUAUU AGUAUAUUAC CUGGUUUUCG AUCGAAAGAU CGACGAGGUG AAAACCUCGU GACAGUCCUU AGUCGAAAGU UUUACUAGAG UCAGUCCUGA UUUUCGAAUC AGAGAAGACG AGCGUCCAC GGGCGCAGAA GAGAAGUCAA CCAGAGAAAC AUAUAUACG AUCUGUCCC		
One- sided T- Orf1ab- 40nt_CI- 29nt- clvRNA	2 5 0	GGGA GTAG ACAAT TCTAG TCTTA CTATT AAGA AACCT AAT	29nt- clvR NA	3 5 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	4 9	gggaAUUAGGU UUCUUAUAG UAAGUAUAUU AGUAUAUUAC CUGGUUUUCG AUCGAAAGAU CGACGAGGUG AAAACCUCGU GACAGUCCUU AGUCGAAAGU UUUACUAGAG UCAGUCCUGA UUUUCGAAUC AGAGAAGACG AGCGUCCAC GGGCGCAGAA GAGAAGUCAA CCAGAGAAAC AUAUAUACG ACUAGAAUUG UCUACUCCC		
One- sided T- let-7f_CI- 29nt- clvRNA	2 5 1	UGAG GUAG UAGA UUGU AUAG UU	29nt- clvR NA	3 5 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 0	gggaAACUAUA CAAUGUAUAU UAGUAUAUUA CCUGGUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG		

							UUUUACUAGA GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC CUACUACCUC A		
One- sided T- Egene_C I-29nt- clvRNA 8-nt Helix 4	2 5 2	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 5 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 1	gggaACGUACC UGUGUAUAAU AGUAUAUUAC CUGGUUUUCG AUCGAAAGAU CGACGAGGUG AAAACCUCGU GACAGUCCUU AGUCGAAAGU UUUACUAGAG UCAGUCCUGA UUUUCGAAUC AGAGAAGACG AGCGUCCAC GGGCGCAGAA GAGAAGUCAA CCAGAGAAAC AUAAUAUACC UCUUCCGAA		
One- sided T- Egene_C I-29nt- clvRNA 5-nt Helix 4	2 5 3	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 5 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 2	gggaACGUACC UGUUAAUUAGU AUUUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUGAC AGUCCUUAGU CGAAAGUUUU ACUAGAGUCA GUCCUGAUUU UCGAAUCAGA GAAGACGAGC GUCCCACGGG CGCAGAAGAG AAGUCAACCA GAGAAACAU AUACUCUCC GAA		
One- sided T- Egene_C I-29nt- clvRNA 3-nt Helix 4 v1	2 5 4	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 5 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 3	gggaACGUACC UGUACGGUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC		

							UAGAGUCAGU CCUGAUUUUUC GAAUCAGAGA AGACGAGCGU CCCACGGGCG CAGAAGAGAA GUCAACCAGA GAAACACGUC UCUUCCGAA		
One- sided T- Egene_C l-29nt- clvRNA 3-nt Helix 4 v2	2 5 5	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 5 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 4	gggaACGUACC UGUACGGUUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUUUUC GAAUCAGAGA AGACGAGCGU CCCACGGGCG CAGAAGAGAA GUCAACCAGA GAAACACGAC UCUUCCGAA		
One- sided T- Egene_C l-29nt- clvRNA 2-nt Helix 4	2 5 6	UUCG GAAG AGAC AGGU ACGU	29nt- clvR NA	3 5 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 5	gggaACGUACC UGUACGUUAU UUACCUGGUU UUCGAUCGAA AGAUCGACGA GGUGAAAACC UCGUGACAGU CCUUAGUCGA AAGUUUUACU AGAGUCAGUC CUGAUUUUUCG AAUCAGAGAA GACGAGCGUC CCACGGGCGC AGAAGAGAAG UCAACCAGAG AAACAGUCUC UUCCGAA		
One- sided T- mir451a_ Cl-29nt- clvRNA 8-nt Helix 4	2 5 7	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 6 0	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 6	gggaAACUCAG UAAUGUAUUA UAGUAUAUUA CCUGGUUUUUC GAUCGAAAGA UCGACGAGGU GAAAACCUCG UGACAGUCCU UAGUCGAAAG UUUUACUAGA GUCAGUCCUG AUUUUCGAU CAGAGAAGAC		

							GAGCGUCCCA CGGGCGCAGA AGAGAAGUCA ACCAGAGAAA CAUAAUAUAC GGUAACGGUU U		
One- sided T- mir451a_ CI-29nt- clvRNA 5-nt Helix 4	2 5 8	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 6 1	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 7	gggaAACUCAG UAAUUUUUAG UAUAUUACCU GGUUUUUCGAU CGAAAGAUCG ACGAGGUGAA AACCUCGUGA CAGUCCUUAG UCGAAAGUUU UACUAGAGUC AGUCCUGAUU UUCGAAUCAG AGAAGACGAG CGUCCCACGG GCGCAGAAGA GAAGUCAACC AGAGAAACAU AAUAGGUAAC GGUUU		
One- sided T- mir451a_ CI-29nt- clvRNA 3-nt Helix 4 v1	2 5 9	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 6 2	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 8	gggaAACUCAG UAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCCUUAGUC GAAAGUUUUA CUAGAGUCAG UCCUGAUUUU CGAAUCAGAG AAGACGAGCG UCCCACGGGC GCAGAAGAGA AGUCAACCAG AGAAACACGU GGUAACGGUU U		
One- sided T- mir451a_ CI-29nt- clvRNA 3-nt Helix 4 v2	2 6 0	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 6 3	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	5 9	gggaAACUCAG UAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCCUUAGUC GAAAGUUUUA CUAGAGUCAG UCCUGAUUUU CGAAUCAGAG AAGACGAGCG		

							UCCCACGGGC GCAGAAGAGA AGUCAACCAG AGAAACACGA GGUAACGGUU U		
One- sided T- mir451a_ Cl-29nt- clvRNA 2-nt Helix 4	2 6 1	AAAC CGUU ACCA UUAC UGAG UU	29nt- clvR NA	3 6 4	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 0	gggaAACUCAG UAAUACGUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUUUC GAAUCAGAGA AGACGAGCGU CCCACGGGCG CAGAAGAGAA GUCAACCAGA GAAACAGUGG UAACGGUUU		
One- sided T- Sgene_C l-29nt- clvRNA 8-nt Helix 4	2 6 2	CUUC UUCA GGUU GGAC AGCU	29nt- clvR NA	3 6 5	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 1	gggaAGCUGUC CAAGUAUAAU AGUAUAUUAC CUGGUUUUCG AUCGAAAGAU CGACGAGGUG AAAACCUCGU GACAGUCCUU AGUCGAAAGU UUUACUAGAG UCAGUCCUGA UUUUCGAAUC AGAGAAGACG AGCGUCCCAC GGGCGCAGAA GAGAAGUCAA CCAGAGAAAC AUAAUAUACC CUGAAGAAG		
One- sided T- Sgene_C l-29nt- clvRNA 5-nt Helix 4	2 6 3	CUUC UUCA GGUU GGAC AGCU	29nt- clvR NA	3 6 6	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 2	gggaAGCUGUC CAAUAUUAGU AUUUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUGAC AGUCCUUAGU CGAAAGUUUU ACUAGAGUCA GUCCUGAUUU UCGAAUCAGA GAAGACGAGC GUCCCACGGG CGCAGAAGAG		

							AAGUCAACCA GAGAAACUA AUACCUGAAG AAG		
One- sided T- Sgene_C I-29nt- clvRNA 3-nt Helix 4 v1	2 6 4	CUUC UUCA GGUU GGAC AGCU	29nt- clvR NA	3 6 7	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 3	gggaAGCUGUC CAAACGGUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUUUC GAAUCAGAGA AGACGAGCGU CCCACGGGCG CAGAAGAGAA GUCAACCAGA GAAACACGUC CUGAAGAAG		
One- sided T- Sgene_C I-29nt- clvRNA 3-nt Helix 4 v2	2 6 5	CUUC UUCA GGUU GGAC AGCU	29nt- clvR NA	3 6 8	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 4	gggaAGCUGUC CAAACGGUAU AUUACCUGGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCCUUAGUCG AAAGUUUUAC UAGAGUCAGU CCUGAUUUUC GAAUCAGAGA AGACGAGCGU CCCACGGGCG CAGAAGAGAA GUCAACCAGA GAAACACGAC CUGAAGAAG		
One- sided T- Sgene_C I-29nt- clvRNA 2-nt Helix 4	2 6 6	CUUC UUCA GGUU GGAC AGCU	29nt- clvR NA	3 6 9	GUCCU UAGUC GAAAGU UUUACU AGAGU CA	6 5	gggaAGCUGUC CAAACGUAUA UUACCUGGUU UUCGAUCGAA AGAUCGACGA GGUGAAAACC UCGUGACAGU CCUUAGUCGA AAGUUUUACU AGAGUCAGUC CUGAUUUUCG AAUCAGAGAA GACGAGCGUC CCACGGGCGC AGAAGAGAAG UCAACCAGAG		

							AAACAGUCCU GAAGAAG		
T-let- 7f_CI- sgRNA- GFP	2 6 7	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 0	GUCCU UGGGC ACGGG CAGCU UGCCG GGUUU UAGAG CUAGAA AUAGCA AGUUAA AAUAAG GCUAG UCCGU UAUCAA CUUGAA AAAGUG GCACC GAGUC GGUGC UUUUU UUGUC A	6 6	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCCUUGGGC ACGGGCAGCU UGCCGGGUUU UAGAGCUAGA AAUAGCAAGU UAAAAUAAGG CUAGUCCGUU AUCAACUUGA AAAAGUGGCA CCGAGUCGGU GCUUUUUUUG UCAGUCCUGA UUUUCGAAUC AGAGAAGACC CCAACCUAUC CCCUUAAAUA GGCAAUUGAA AAAGAGAAGU CAACCAGAGA AACACGACUA CUACCUCA		
T-let- 7f_CI- sgRNAG FP_A7	2 6 8	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 1	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	6 7	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GGGCACGGG CAGCUUGCCG GGUUUUAGAG CUAGAAAUAG CAAGUUAAAA UAAGGCUAGU CCGUUAUCAA CUUGAAAAAG UGGCACCGAG UCGGUGCGUC AGUCCUGAUU UUCGAAUCAG AGAAGACUAU CCACCUUAAA UAGGCAAGUG AGAAGUCAAC CAGAGAAACA		

							CGACUACUAC CUCA		
T-let- 7f_CI- sgRNAG FP_C7	2 6 9	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 2	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	6 8	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGUGACA GGGCACGGG CAGCUUGCCG GGUUUUAGAG CUAGAAAUAG CAAGUUAAAA UAAGGCUAGU CCGUUAUCAA CUUGAAAAAG UGGCACCGAG UCGGUGCGUC AGUCCUGAUU UUCGAAUCAG AGAAGACUAU CCACCUUAAA UAGGCAAGUG AGAAGUCAAC CAGAGAAACA CGACUACUAC CUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC1_8R H2_A7R _A7L	2 7 0	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 3	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	6 9	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGAGAAGAC CCAUGAUAA UUAUUUUAAC UUUCUAAAAC CCGGCAAGCU		

							GCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGACUACUA CCUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC2_8R H2_A7R _A7L	2 7 1	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 4	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 0	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC CCAUGAUAAC UUUUUUUAAC UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCGUGA GAAGUCAUAC GACCAGAGAA ACACGACUAC UACCUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC3_8R H2_A7R _A7L	2 7 2	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 5	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 1	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU		

							CAGAGAAGAC CCAUGAUAAAC GGACUAGCCU UAUUUUAAACU UGCUAUUUCU AGCUCUAAAA CCCGGCAAGC UGCCCGUGAG AAGUCAUACG ACCAGAGAAA CACGACUACU ACCUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC4_8R H2_A7R _A7L	2 7 3	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 7 6	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 2	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GCACCGACUC GGUGCCACUU UUUCAAGUUG AUAACGGACU AGCCUUUUUU UAACUUGCUA UUUCUAGCUC UAAAACCCGG CAAGCUGCCC GUGAGAAGUC AUACGACCAG AGAAACACGA CUACUACCUC A		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC1_8R H2_A7R _A7L	2 7 4	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 7 7	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU	7 3	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA		

					AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA		AAUAAGGCU AGUCCGUUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGAGAAGAC CCAUGAUUAC UUUUUUUAAAC UUUCUAAAAC CCGGCAAGCU GCCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGAUCGGAG UACAAA		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC2_8R H2_A7R _A7L	2 7 5	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 7 8	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 4	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAUAAGGCU AGUCCGUUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGAGAAGAC CCAUGAUUAC UUUUUUUAAAC UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCCGUGA GAAGUCAUAC GACCAGAGAA ACACGAUCGG AGUACAAA		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC3_8R H2_A7R _A7L	2 7 6	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 7 9	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU	7 5	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA		

					CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA		UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC CCAUGAUAAAC GGACUAGCCU UAUUUUAACU UGCUAUUUCU AGCUCUAAAA CCCGGCAAGC UGCCCGUGAG AAGUCAUACG ACCAGAGAAA CACGAUCGGA GUACAAA		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC4_8R H2_A7R _A7L	2 7 7	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 8 0	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 6	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU CAGAGAAGAC GCACCGACUC GGUGCCACUU UUUCAAGUUG AUAACGGACU AGCCUUUUUU UAACUUGCUA UUUCUAGCUC UAAAACCCGG CAAGCUGCCC GUGAGAAGUC AUACGACCAG AGAAACACGA UCGGAGUACA AA		

T-let-7f_CI-sgRNA-GFP_mo dC1_8R H2_A7R_C7L	2 7 8	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 8 1	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 7	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAUAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGCGAAGAC CCAUGAUAA UUUUUUUAA UUUCUAAAAC CCGGCAAGCU GCCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGACUACUA CCUCA	
T-let-7f_CI-sgRNA-GFP_mo dC2_8R H2_A7R_C7L	2 7 9	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 8 2	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 8	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAUAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGCGAAGAC CCAUGAUAA UUUUUUUAA UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCGUGA GAAGUCAUAC GACCAGAGAA	

							ACACGACUAC UACCUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC1_8R H2_A7R _G7L	2 8 0	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 8 3	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	7 9	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAAU CAGGGAAGAC CCAUGAUAAC UUUUUUUAAC UUUCUAAAAC CCGGCAAGCU GCCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGACUACUA CCUCA		
T-let- 7f_CI- sgRNA- GFP_mo dC2_8R H2_A7R _G7L	2 8 1	UGAG GUAG UAGA UUGU AUAG UU	sgRN A- GFP	3 8 4	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC	8 0	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG		

					GGUGC GUCA		AUUUUCGAU CAGGGAAGAC CCAUGAUAA UUUUUUUAA UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCGUGA GAAGUCAUAC GACCAGAGAA ACACGACUAC UACCUCA		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC1_8R H2_A7R _C7L	2 8 2	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 8 5	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	8 1	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGCGAAGAC CCAUGAUAA UUUUUUUAA UUUCUAAAAC CCGGCAAGCU GCCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGAUCGGAG UACAAA		
T-cel-mir- 238_CI- sgRNA- GFP_mo dC2_8R H2_A7R _C7L	2 8 3	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 8 6	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC	8 2	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC		

					GGUGC GUCA		GUCAGUCCUG AUUUUCGAU CAGCGAAGAC CCAUGAUAA UUUUUUUAAC UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCGUGA GAAGUCAUAC GACCAGAGAA ACACGAUCGG AGUACAAA		
T-cel-mir- 238_C1- sgRNA- GFP_mo dC1_8R H2_A7R _G7L	2 8 4	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 8 7	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC GAGUC GGUGC GUCA	8 3	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGGGAAGAC CCAUGAUAA UUUUUUUAAC UUUCUAAAAC CCGGCAAGCU GCCCCGUGAGA AGUCAUACGA CCAGAGAAAC ACGAUCGGAG UACAAA		
T-cel-mir- 238_C1- sgRNA- GFP_mo dC2_8R H2_A7R _G7L	2 8 5	UUUG UACU CCGA UGCC AUUC AGA	sgRN A- GFP	3 8 8	GGGCA CGGGC AGCUU GCCGG GUUUU AGAGC UAGAAA UAGCAA GUUAAA AUAAGG CUAGU CCGUU AUCAAC UUGAAA AAGUG GCACC	8 4	gggaUCUGAAU GGCAACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGGGCAC GGGCAGCUUG CCGGGUUUUA GAGCUAGAAA UAGCAAGUUA AAAUAAAGGCU AGUCCGUUAU CAACUUGAAA AAGUGGCACC		

					GAGUC GGUGC GUCA		GAGUCGGUGC GUCAGUCCUG AUUUUCGAU CAGGGAAGAC CCAUGAUAA UUUUUUUAA UUGCUAUUUC UAGCUCUAAA ACCCGGCAAG CUGCCCGUGA GAAGUCAUAC GACCAGAGAA ACACGAUCGG AGUACAAA		
T-let- 7f_CI- shRNA- GFP	2 8 6	UGAG GUAG UAGA UUGU AUAG UU	shRN A- GFP	3 8 9	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCACC ACCUGA ACUUCA GGGUC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	8 5	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAGCA GUGCCUGCAA GCUGACCCUG AAGUUCACCA CCUGAACUUC AGGGUCAGCU UGCAAGUAAG GUUGACCAUA CUCUACAGUC CUGAUUUUCG AAUCAGAGAA GUAUCUGAUG AUUUAGCUCA AGAAGUCAAC CAGAGAAACA CGACUACUAC CUCA		
T-let- 7f_CI- shRNA- GFP_8R H2	2 8 7	UGAG GUAG UAGA UUGU AUAG UU	shRN A- GFP	3 9 0	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCACC ACCUGA ACUUCA GGGUC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	8 6	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGCGUAU GACAGUCUGA UAGCAAUGUC AGCAGUGCCU GCAAGCUGAC CCUGAAGUUC ACCACCUGAA CUUCAGGGUC AGCUUGCAAG UAAGGUUGAC CAUACUCUAC AGUCCUGAUU UUCGAAUCAG		

							AGAAGUAUCU GAUGAUUUAG CUCAAGAAGU CAUACGACCA GAGAAACACG ACUACUACCU CA		
T-let- 7f_CI- shRNA- GFP_A3 8mutant	2 8 8	UGAG GUAG UAGA UUGU AUAG UU	shRN A- GFP	3 9 1	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCACC ACCUGA ACUUCA GGGUC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	8 7	GGGAAACUAU ACAAUACGGU UUAUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAGC AGUGCCUGCA AGCUGACCCU GAAGUUCACC ACCUGAACUU CAGGGUCAGC UUGCAAGUAA GGUUGACCAU ACUCUACAGU CCUGAUUUUUC GAAUCAGAGA AGUAUCUGAU GAUUUAGCUC AAGAAGUCAA CCAGAGAAAC ACGACUACUA CCUCA		
T-let- 7f_CI- shRNA- GFP_tar getingmu tant	2 8 9	UGAG GUAG UAGA UUGU AUAG UU	shRN A- GFP- targe tingmu tant	3 9 2	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GCAACU GAAGU UCACCA CCUGAA CUUCA GUUGC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	8 8	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAGC AGUGCCUGCA AGCUGCAACU GAAGUUCACC ACCUGAACUU CAGUUGCAGC UUGCAAGUAA GGUUGACCAU ACUCUACAGU CCUGAUUUUUC GAAUCAGAGA AGUAUCUGAU GAUUUAGCUC AAGAAGUCAA CCAGAGAAAC ACGACUACUA CCUCA		

T-cel-mir-238_CI-shRNA-GFP	290	UUUG UACU CCGA UGCC AUUC AGA	shRN A- GFP	393	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCACC ACCUGA ACUUCA GGGUC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	89	gggaUCUGAAU GGCAACGGUA UAAUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAGCA GUGCCUGCAA GCUGACCCUG AAGUUCACCA CCUGAACUUC AGGGUCAGCU UGCAAGUAAG GUUGACCAUA CUCUACAGUC CUGAUUUUCG AAUCAGAGAA GUAUCUGAUG AUUUAGCUCA AGAAGUCAAC CAGAGAAACA CGAUCGGAGU ACAAA		
T-cel-mir-238_CI-shRNA-GFP_A38mutant	291	UUUG UACU CCGA UGCC AUUC AGA	shRN A- GFP	394	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCACC ACCUGA ACUUCA GGGUC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA	90	gggaUCUGAAU GGCAACGGUU UAAUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAGCA GUGCCUGCAA GCUGACCCUG AAGUUCACCA CCUGAACUUC AGGGUCAGCU UGCAAGUAAG GUUGACCAUA CUCUACAGUC CUGAUUUUCG AAUCAGAGAA GUAUCUGAUG AUUUAGCUCA AGAAGUCAAC CAGAGAAACA CGAUCGGAGU ACAAA		
T-cel-mir-238_CI-shRNA-GFP_targetingmutant	292	UUUG UACU CCGA UGCC AUUC AGA	shRN A- GFP- targeting mutant	395	<i>GUCUG</i> <i>AUAGCA</i> <i>AUGUCA</i> GCAGU GCCUG CAAGCU GCAACU GAAGU	91	gggaUCUGAAU GGCAACGGUA UAAUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC		

					UCACCA CCUGAA CUUCA GUUGC AGCUU GCAAG UAAGG UUGAC CAUACU CUACA		AAUGUCAGCA GUGCCUGCAA GCUGCAACUG AAGUUCACCA CCUGAACUUC AGUUGCAGCU UGCAAGUAAG GUUGACCAUA CUCUACAGUC CUGAUUUUCG AAUCAGAGAA GUAUCUGAUG AUUUAGCUCA AGAAGUCAAC CAGAGAAACA CGAUCGGAGU ACAAA		
T-let- 7f_CI- RedBro coli_ML	2 9 3	UGAG GUAG UAGA UUGU AUAG UU	Bro coli RNA apta mer	3 9 6	GUCCG AGACG GUCGG GUCCA GUCCC AACGAU GUUGG CUGUU GAGUA GUGUG UGGGC UCCA	9 2	gggaAACUAUA CAAUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCCGAGACG GUCGGGUCCA GUCCCAACGA UGUUGGCUGU UGAGUAGUGU GUGGGCUCCA GUCCUGAUUU UCGAAUCAGA GAAGACCCCA ACCUAUCCCC UUAAAUAGGC AAUUGAAAAA GAGAAGUCAA CCAGAGAAAC ACGACUACUA CCUCA		
tSJ057_sh GFP1Rz	2 9 4	UGAG GUAG UAGA UUGU AUAG UU	shGF P1	3 9 7	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCU GACCC UGAAG UUCAUC UGCAG AUGAAC UUCAG GGUCA GCUUG CAAGUA AGGUU GACCAU	9 3	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUUGUGAC AGUCUGAUAG CAAUGUCAGC AGUGCCUGCA AGCUGACCCU GAAGUUCAUC UGCAGAUCAA CUUCAGGGUC AGCUUGCAAG UAAGGUUGAC CAUACUCUAC AGUCCUGAUU		

					ACUCUA CA		UUCGAAUCAG AGAAGUAUCU GAUGUAAUUU AGCUCAAGAA GUCAACCAGA GAAACACGAC UACUACCUCA		
tSJ058_sh GFP2Rz	2 9 5	UGAG GUAG UAGA UUGU AUAG UU	shGF P2	3 9 8	GUCUG AUAGCA AUGUCA GCAGU GCCUG CAAGCT GACCCT GAAGTT CAccacc TGAAGT TCAGG GTCAGC TTGCaa GUAAG GUUGA CCAUAC UCUACA	9 4	GGGAAACUAU ACAAUACGGU AUUUUACCU GUUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCCUGAC AGUCUGAUAG CAAUGUCAGC AGUGCCUGCA AGCUGACCCU GAAGUUCACC ACCUGAACUU CAGGGUCAGC UUGCAAGUAA GGUUGACCAU ACUCUACAGU CCUGAUUUUC GAAUCAGAGA AGUAUCUGAU GUAAUUUAGC UCAAGAAGUC AACCAGAGAA ACACGACUAC UACCUCA		
tSJ059_sh GFP3	2 9 6	UGAG GUAG UAGA UUGU AUAG UU	shGF P3	3 9 9	GUCUG AUAGCA AUGUCA ACCUC GCCUG CAAGCT GACCCT GAAGTT CAccacc TGAAGT TCAGG GTCAGC TTGCag GCGCG GUUGA CCAUAC CGUCC A	9 5	GGGAAACUAU ACAAUACGGU AUUUUACCU GUUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCCUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUGCA AGCUGACCCU GAAGUUCACC ACCUGAACUU CAGGGUCAGC UUGCAGGCGC GGUUGACCAU ACCGUCCAGU CCUGAUUUUC GAAUCAGAGA AGGAAUUGAU GUAAUUUAGC UCAAGAAGUC AACCAGAGAA ACACGACUAC UACCUCA		

tSJ060_sh GFP3mut	2 9 7	UGAG GUAG UAGA UUGU AUAG UU	shGF P3mut	4 0 0	GUCUG AUAGCA AUGUCA ACCUC GCCUG CAAGCT GCAACT GAAGTT CAccacc TGAAGT TCAGG GTCAGC TTGCag GCGCG GUUGA CCAUAC CGUCC A	9 6	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUGCA AGCUGCAACU GAAGUUCACC ACCUGAACUU CAGGGUCAGC UUGCAGGCGC GGUUGACCAU ACCGUCCAGU CCUGAUUUUC GAAUCAGAGA AGGAAUUGAU GUAAUUUAGC UCAAGAAGUC AACCAGAGAA ACACGACUAC UACCUCA	
tSJ061_sh GFP4Rz	2 9 8	UGAG GUAG UAGA UUGU AUAG UU	shGF P4	4 0 1	GUCUG CUAGCA AUGUCA ACCUC GCCUG CAAGCT GACCCT GAAGTT CATgtgc caccucua ATGAAC TTCAGG GTCAGC TTGCag GCGCG GUUGA CCAUAC CGUCC A	9 7	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGCUAG CAAUGUCAAC CUCGCCUGCA AGCUGACCCU GAAGUUCAUG UGCCACCUCU AAUGAACUUC AGGGUCAGCU UGCAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAUUUGAUG UAAUUUAGCG CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA	

tSJ062_sh GFP5Rz	2 9 9	UGAG GUAG UAGA UUGU AUAG UU	shGF P5	4 0 2	GUCUG CUAGCA AUGUCA ACCUC GCCUG CAAGCT GACCCT GAAGTT CATgtgtt attcttgAT GAACTT CAGGG TCAGCT TGCagG CGCGG UUGAC CAUACC GUCCA	9 8	GGGAAACUAU ACAAUACGGU AUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGCUAG CAAUGUCAAC CUCGCCUGCA AGCUGACCCU GAAGUUCAUG UGUUUUUCUU GAUGAACUUC AGGGUCAGCU UGCAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCG CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
tSJ063_sh GFP6Rz	3 0 0	UGAG GUAG UAGA UUGU AUAG UU	shGF P6	4 0 3	GUCUG AUAGCA AUGUCA ACCUC GCCUAT GAACTT CAGGG TCAGCT TGCgtgtt attcttgGC AAGCTG ACCCTG AAGTTC ATagGC GCGGU UGACCA UACCG UCCA	9 9	GGGAAACUAU ACAAUACGGU AUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

tSJ064_sh GFP7Rz	3 0 1	UGAG GUAG UAGA UUGU AUAG UU	shGF P7	4 0 4	GUCUG AUAGCA AUGUCA ACCUCC UTGCAG ATGAAC TTCAGG GTCAGC Tgtgttattc ttgAGCT GACCCT GAAGTT CATCTG CAagGC GGUUG ACCAUA CCGUC CA	1 0 0	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCCUUGCAG AUGAACUUCA GGGUCAGCUG UGUUUAUUCUU GAGCUGACCC UGAAGUUCAU CUGCAAGGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA	
tSJ065_sh GFP8Rz	3 0 2	UGAG GUAG UAGA UUGU AUAG UU	shGF P8	4 0 5	GUCcag ccagcuua uUGcgaa uCUCga TGCAGA TGAAC TCAGG GTCAGC Tgtgttattc ttgAGCT GACCCT GAAGTT CATCTG CAtcGC GauucgC UtattCG UCuuug CA	1 0 1	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCCAGCCA GCUUAUUGCG AAUCUCGAUG CAGAUGAACU UCAGGGUCAG CUGUGUUUU CUUGAGCUGA CCCUGAAGUU CAUCUGCAUC GCGAUUCGCU UAUUCGUCUU UGCAGUCCUG AUUUUCGAU CAGAGAAGCA CCUACGCCUA AUAAAUCCGC UUUCUGAGAA GUCAACCAGA GAAACACGAC UACUACCUCA	

tSJ066_sh GFP9Rz	3 0 3	UGAG GUAG UAGA UUGU AUAG UU	shGF P9	4 0 6	GUCugu aucauu cuUGugu auCUCg cTGCAG ATGAAC TTCAGG GTCAGC Tgtgttattc ttgAGCT GACCCT GAAGTT CATCTG CAgcGC Gguacac CUtattC GUCcaa uCA	1 0 2	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGUAUC AAUUCUUGUG UAUCUCGCUG CAGAUGAACU UCAGGGUACG CUGUGUUUU CUUGAGCUGA CCCUGAAGUU CAUCUGCAGC GCGGUACACC UUUUUCGUCC AAUCAGUCCU GAUUUUCGAA UCAGAGAAGA UGUUACGCCU AAUAAAGCCU UGCGACAAGA AGUCAACCAG AGAAACACGA CUACUACCUC A	
tSJ067_sh GFP10Rz	3 0 4	UGAG GUAG UAGA UUGU AUAG UU	shGF P10	4 0 7	GUCUG AUAGCA AUGUCA ACCUC GCCUT GGTGC AGATGA ACTTCA GGGTgt gttattcttg ACCCTG AAGTTC ATCTGC ACCAag GCGCG GUUGA CCAUAC CGUCC A	1 0 3	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUUGG UGCAGAUCAA CUUCAGGGUG UGUUUUUCUU GACCCUGAAG UUCUUCUGCA CCAAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA	

tSJ068_sh GFP11Rz	3 0 5	UGAG GUAG UAGA UUGU AUAG UU	shGF P11	4 0 8	GUCcag ccagcuua uUGcgaa uCUCga TGGTGC AGATGA ACTTCA GGGTgt gttattcttg ACCCTG AAGTTC ATCTGC ACCAtc GCGauu cgCUtatt CGUCuu ugCA	1 0 4	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCCAGCCA GCUUAUUGCG AAUCUCGAUG GUGCAGAUGA ACUUCAGGGU GUGUUUUUCU UGACCCUGAA GUUCAUCUGC ACCAUCGCGA UUCGCUUAUU CGUCUUUGCA GUCCUGAUUU UCGAAUCAGA GAAGCACCUA CGCCUAAUAA AUCCGCUUUC UGAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA	
tSJ069_sh GFP12Rz	3 0 6	UGAG GUAG UAGA UUGU AUAG UU	shGF P12	4 0 9	GUCugu aucaauu cuUGugu auCUCg ccgTGG TGCAGA TGA ACT TCAGG GTgtgttat tcttgACC CTGAAG TTCATC TGCACC AuggcG CGguaca cCUtattC GUCcaa uCA	1 0 5	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGUAUC AAUUCUUGUG UAUCUCGCCG UGGUGCAGAU GAACUUCAGG GUGUGUUUU CUUGACCCUG AAGUUCAUCU GCACCAUGGC GCGGUACACC UUUUUCGUCC AAUCAGUCCU GAUUUUUCGAA UCAGAGAAGA UGUUACGCCU AAUAAAGCCU UGCGACAAGA AGUCAACCAG AGAAACACGA CUACUACCUC A	

1sided_T let7f_Csg RNA- GFP_ML _modA						1 0 6	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCCGUAAG AGGCCAUUGC AAAAGAGAAG UCAACCAGAG AAACACGACU ACUACCUC A		
1sided_T let7f_Csg RNA- GFP_ML _modA_ C25_A38 _mut						1 0 7	GGGAAACUAU ACAAUACGGU UUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCCGUAAG AGGCCAUUGC AAAAGAGAAG UCAACCAGAG AAAGACGACU ACUACCUC A		

1sided_T let7f_Csg RNA- GFP_ML _modB						1 0 8	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCCGGAAG CGGCCCUUGC CAAAGAGAAG UCAACCAGAG AAACACGACU ACUACCUCA		
1sided_T let7f_Csg RNA- GFP_ML _modB_ C25_A38 _mut						1 0 9	GGGAAACUAU ACAAUACGGU UUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCCGGAAG CGGCCCUUGC CAAAGAGAAG UCAACCAGAG AAAGACGACU ACUACCUCA		

1sided_T let7f_Csg RNA- GFP_ML _modC_ C25_A38 _mut						1 1 0	GGGAAACUAU ACAAUACGGU UUAUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCCGGCAAG CUGCCCGUGC CCAAGAGAAG UCAACCAGAG AAAGACGACU ACUACCUCA		
1sided_T let7f_Csg RNA- GFP_ML _modD						1 1 1	GGGAAACUAU ACAAUACGGU AUAUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC UUUUUUU</u> <u>GUCAGUCCUG</u> AUUUUCGAU CAGAGAAGAC AAAAAAUAU CCCCGGCAAG CUGCCCGUGC CCAAGAGAAG UCAACCAGAG AAACACGACU ACUACCUCA		

1sided_T let7f_Csg RNA- GFP_ML _modD_ C25_A38 _mut						1 1 2	<u>GGGAAACUAU</u> <u>ACAAUACGGU</u> <u>UUUUUACCU</u> <u>GUUUUCGAUC</u> <u>GAAAGAU</u> <u>CGAGGUGAAA</u> <u>ACCUCGUGAC</u> <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC</u> <u>GUCAGUCCUG</u> <u>AUUUUCGAU</u> <u>CAGAGAAGAC</u> <u>AAAAAAUAU</u> <u>CCCCGGCAAG</u> <u>CUGCCCGUGC</u> <u>CCAAGAGAAG</u> <u>UCAACCAGAG</u> <u>AAAGACGACU</u> <u>ACUACCUCA</u>		
1sided_T let7f_Csg RNA- GFP_ML _modC						1 1 3	<u>GGGAAACUAU</u> <u>ACAAUACGGU</u> <u>AUAUUACCU</u> <u>GUUUUCGAUC</u> <u>GAAAGAU</u> <u>CGAGGUGAAA</u> <u>ACCUCGUGAC</u> <u>AGUCCUUGGG</u> <u>CACGGGCAGC</u> <u>UUGCCGGGUU</u> <u>UUAGAGCUAG</u> <u>AAAUAGCAAG</u> <u>UUAAAAUAAG</u> <u>GCUAGUCCGU</u> <u>UAUCAACUUG</u> <u>AAAAAGUGGC</u> <u>ACCGAGUCGG</u> <u>UGC</u> <u>GUCAGUCCUG</u> <u>AUUUUCGAU</u> <u>CAGAGAAGAC</u> <u>CCCAACCUAU</u> <u>CCCCGGCAAG</u> <u>CUGCCCGUGC</u> <u>CCAAGAGAAG</u> <u>UCAACCAGAG</u> <u>AAACACGACU</u> <u>ACUACCUCA</u>		

1sided_Tlet7f _CsgRNA- GFP_ML_mo dC1_8H2_ext ra_A7_addU C					1 1 4	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCGUA UGACAGGGCA CGGGCAGCUU GCCGGGUUUU AGAGCUAGAA AUAGCAAGUU AAAAUAAGGC UAGUCCGUUA UCAACUUGAA AAAGUGGCAC CGAGUCGGUG CGUCAGUCCU GAUUUUCGAA UCAGAGAAGA CCCAUGAUAA CUUAUUUUAA CUUUCUAAAA CCCGGCAAGC UGCCCGUGAG AAGUCAUACG ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csg RNA- GFP_ML _modC_ A38mut					1 1 5	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCCUUGGG CACGGGCAGC UUGCCGGGUU UUAGAGCUAG AAAUAGCAAG UUAAAAUAAG GCUAGUCCGU UAUCAACUUG AAAAAGUGGC ACCGAGUCGG UGCUUUUUUU GUCAGUCCUG AUUUUCGAU CAGAGAAGAC CCCAACCUAU CCCGGCAAG CUGCCCGUGC CCAAGAGAAG UCAACCAGAG AAACACGACU ACUACCUCA		

1sided_Tlet7f _CsgRNA- GFP_ML_mo dC1_8H2_ext ra_A7_addU C_A38mut					1 1 6	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCGUA UGACAGGGCA CGGGCAGCUU GCCGGGUUUU AGAGCUAGAA AUAGCAAGUU AAAAUAAGGC UAGUCCGUUA UCAACUUGAA AAAGUGGCAC CGAGUCGGUG CGUCAGUCCU GAUUUUCGAA UCAGAGAAGA CCCAUGAUAA CUUAUUUUAA CUUUCUAAAA CCCGGCAAGC UGCCCGUGAG AAGUCAUACG ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA6- GFP_ML					1 1 7	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6mut _ML						1 1 8	GGGAAACUAU ACAAUACGGU AUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUACUGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCAGUAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_M L_A38mut						1 1 9	GGGAAACUAU ACAAUACGGU UUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T Egene20 _CshRN A- GFP6_M L						1 2 0	<u>GGGAACGUAC</u> CUGUACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GAAUUGAUGU AAUUUAGCUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6mut _ML						1 2 1	<u>GGGAACGUAC</u> CUGUACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUACUGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CAGUAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GAAUUGAUGU AAUUUAGCUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		

1sided_T Egene20 _CshRN A- GFP6_M L_A38mu t					1 2 2	GGGAACGUAC CUGUACGGUU UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GAAUUGAUGU AAUUUAGCUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		
1sided_T let7f_Csh RNA- GFP6_D 1_ML_A3 8mut					1 2 3	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUAUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG AUUUAGCUCA AGAAGUCAAC CAGAGAAACA CGACUACUAC CUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_A3 8mut						1 2 4	GGGAAACUAU ACAAUACGGU UUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 3_ML_A3 8mut						1 2 5	GGGAAACUAU ACAAUACGGU UUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGUGCGU UAAUUUCUCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 4_ML_A3 8mut						1 2 6	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA UGACGUGAUG UAAUUUAUUA CCAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 5_ML_A3 8mut						1 2 7	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA UGACGUGAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 6_ML_A3 8mut						1 2 8	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 1_ML						1 2 9	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGAAUUGAUG AUUUUAGCUA AGAAGUCAAC CAGAGAAACA CGACUACUAC CUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML						1 3 0	GGGAAACUAU ACAAUACGGU AUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6mut _D2_ML						1 3 1	GGGAAACUAU ACAAUACGGU AUUUUACCG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUacUGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCagUAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 3_ML						1 3 2	<u>GGGAAACUAU</u> ACAAUACGGU AUUUUACCU GUUUUCGAUC GAAAGAU CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUUCG AAUCAGAGAA GGACGUGCGU UAAUUUCUCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 4_ML						1 3 3	<u>GGGAAACUAU</u> ACAAUACGGU AUUUUACCU GUUUUCGAUC GAAAGAU CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUUCG AAUCAGAGAA UGACGUGAUG UAAUUUAU CCAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 5_ML						1 3 4	GGGAAACUAU ACAAUACGGU AUUUUACCU GUUUUCGAUC GAAAGAUCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA UGACGUGAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 6_ML						1 3 5	GGGAAACUAU ACAAUACGGU AUUUUACCU GUUUUCGAUC GAAAGAUCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUUAGCU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T Egene20 _CshRN A- GFP6_D 2_ML						1 3 6	GGGAACGUAC CUGUACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6_D 2_ML_A3 8mut						1 3 7	GGGAACGUAC CUGUACGGUU UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		

1sided_T mir222_ CshRNA- GFP6_D 2_ML						1 3 8	<u>GGGAACCCAG</u> UAGCACGGUA UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACAGAUG UAGCU		
1sided_T mir222_ CshRNA- GFP6_D 2_ML_A3 8mut						1 3 9	<u>GGGAACCCAG</u> UAGCACGGUU UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACAGAUG UAGCU		

1sided_T mir222_ CshRNA- GFP6mut _D2_ML						1 4 0	GGGAACCCAG UAGCACGGUA UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUacUGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CagUAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACAGAUG UAGCU		
1sided_T let7f_Csh RNA- GFP6_D 2_CM1						1 4 1	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUAUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM2						1 4 2	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ A38mut						1 4 3	GGGAAACUAU ACAAUAUGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM3						1 4 4	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUAUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM4						1 4 5	GGGAAACUAU ACAAUCGGUA UAUUUACCUGG UUUUCGAUCG AAAGAUCCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGCUACUAC CUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM5						1 4 6	GGGAAACUAU ACAAUUUGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGGCUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM6						1 4 7	GGGAAACUAU ACAAUUAUGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAAUACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM7						1 4 8	GGGAAACUAU ACAAUACUGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAAGACUACU ACCUCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM8						1 4 9	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACAACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM9						1 5 0	<u>GGGAAACUAU</u> ACAAUCUGUA UAAUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAGCUACUAC CUCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM10						1 5 1	<u>GGGAAACUAU</u> ACAAUUGGUA UAAUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACACUACUAC CUCA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM1						1 5 2	<u>GGGAACGUAC</u> CUGUACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6_D 2_CM2						1 5 3	<u>GGGAACGUAC</u> CUGUAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM2_ A38mut						1 5 4	GGGAACGUAC CUGUAUGGUU UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6_D 2_CM3						1 5 5	GGGAACGUAC CUGUAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGACUCUUC CGAA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM4						1 5 6	GGGAACGUAC CUGUCGGUUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUUUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA CGCUCUUCGG AA		
1sided_T Egene20 _CshRN A- GFP6_D 2_CM5						1 5 7	GGGAACGUAC CUGUUUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGGCUCUUC CGAA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM6						1 5 8	GGGAACGUAC CUGUUAUGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAUACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6_D 2_CM7						1 5 9	GGGAACGUAC CUGUACUGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAGACUCUUC CGAA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM8						1 6 0	GGGAACGUAC CUGUAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACAACUCUUC CGAA		
1sided_T Egene20 _CshRN A- GFP6_D 2_CM9						1 6 1	GGGAACGUAC CUGUCUGUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUAUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA AGCUCUCCG AA		

1sided_T Egene20 _CshRN A- GFP6_D 2_CM10						1 6 2	GGGAACGUAC CUGUUGGUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUUUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA CACUCUUCGG AA		
1sided_T mir222_ CshRNA- GFP6_D 2_CM1						1 6 3	GGGAACCCAG UAGCACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGACAGAUG UAGCU		

1sided_T mir222_ CshRNA- GFP6_D 2_CM2						1 6 4	<u>GGGAACCCAG</u> UAGCAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGACAGAUG UAGCU		
1sided_T mir222_ CshRNA- GFP6_D 2_CM3						1 6 5	<u>GGGAACCCAG</u> UAGCAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGACAGAUG UAGCU		

1sided_T mir222_ CshRNA- GFP6_D 2_CM4						1 6 6	GGGAACCCAG UAGCCGGUUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUUUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA CGCAGAUGUA GCU		
1sided_T mir222_ CshRNA- GFP6_D 2_CM5						1 6 7	GGGAACCCAG UAGCUUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AUGGCAGAUG UAGCU		

1sided_T mir222_ CshRNA- GFP6_D 2_CM6						1 6 8	<u>GGGAACCCAG</u> UAGCUAUGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAUACAGAUG UAGCU		
1sided_T mir222_ CshRNA- GFP6_D 2_CM7						1 6 9	<u>GGGAACCCAG</u> UAGCACUGUA UAUUACCUGG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAGACAGAUG UAGCU		

1sided_T mir222_ CshRNA- GFP6_D 2_CM8						1 7 0	GGGAACCCAG UAGCAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACAACAGAUG UAGCU		
1sided_T mir222_ CshRNA- GFP6_D 2_CM9						1 7 1	GGGAACCCAG UAGCCUGUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUAUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA AGCAGAUGUA GCU		

1sided_T mir222_ CshRNA- GFP6_D 2_CM10						1 7 2	GGGAACCCAG UAGCUGGUAU AUUACCUUGU UUUCGAUCGA AAGAUCGACG AGGUGAAAAC CUCGUGACAG UCUGAUAGCA AUGUCAACCU CGCCUAUGAA CUUCAGGGUC AGCUUGCGUG UUAUUCUUGG CAAGCUGACC CUGAAGUUCA UAGGCGCGGU UGACCAUACC GUCCAGUCCU GAUUUUCGAA UCAGAGAAGG ACGGUAUGUA AUUGCUAUCA AGAAGUCAAC CAGAGAAACA CACAGAUGUA GCU		
1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM1						1 7 3	GGGAUCUGAA UGGCAACGGU AUUUUACCU GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUUGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGAUCGGA GUACAAA		

1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM2						1 7 4	GGGAUCUGAA UGGCAAUGGU AUUUUACCU GUUUUCGAUC GAAAGAUCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACGAUCGGA GUACAAA		
1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM3						1 7 5	GGGAUCUGAA UGGCAAUGGU AUUUUACCU GUUUUCGAUC GAAAGAUCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGAUCGGA GUACAAA		

1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM4						1 7 6	GGGAUCUGAA UGGCACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGUCGGAGU ACAAA		
1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM5						1 7 7	GGGAUCUGAA UGGCAUUGGU AUUUUACCUUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUUGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUAUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAUGGUCGGA GUACAAA		

1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM6						1 7 8	GGGAUCUGAA UGGCAUAUGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAAUAUCGGA GUACAAA		
1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM7						1 7 9	GGGAUCUGAA UGGCAACUGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CAAGAUCGGA GUACAAA		

1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM8						1 8 0	GGGAUCUGAA UGGCAAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGUGAC AGUCUGAUAG CAAUGUCAAC CUCGCCUAUG AACUUCAGGG UCAGCUUGCG UGUUUUUCUU GGCAAGCUGA CCCUGAAGUU CAUAGGCGCG GUUGACCAUA CCGUCCAGUC CUGAUUUUCG AAUCAGAGAA GGACGGUAUG UAAUUGCUAU CAAGAAGUCA ACCAGAGAAA CACAAUCGGA GUACAAA		
1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM9						1 8 1	GGGAUCUGAA UGGCACUGUA UAUUUACCUGG UUUUUCGAUCG AAAGAUCCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUUUUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC AAGUCGGAGU ACAAA		

1sided_T cel-mir- 238_Csh RNA- GFP6_D 2_CM10						1 8 2	GGGAUCUGAA UGGCAUGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACAUCGGAGU ACAAA		
1sided_T Egene20 _alt1_Cs hRNA- GFP6_D 2_ML						1 8 3	GGGAGUUAAC AAUAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAUUGCAG CAGU		

1sided_T Egene20 _alt2_Cs hRNA- GFP6_D 2_ML						1 8 4	GGGAGAAGAA UUCAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAGAUUUU UAAC		
1sided_T Egene20 _alt3_Cs hRNA- GFP6_D 2_ML						1 8 5	GGGAUCAGAU UUUAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAUUGCAG CAGU		

1sided_T Egene20 _alt4_Cs hRNA- GFP6_D 2_ML						1 8 6	<u>GGGAAGUAAG</u> GAUGACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAGCUAGU GUAA		
1sided_T Egene20 _alt5_Cs hRNA- GFP6_D 2_ML						1 8 7	<u>GGGACCGAAA</u> CGAAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAUGAGUA CAUG		

1sided_T Egene20 _alt6_Cs hRNA- GFP6_D 2_ML						1 8 8	<u>GGGAAAGUAC</u> GCUAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAUUAACU AUUA		
1sided_T Egene20 _alt7_Cs hRNA- GFP6_D 2_ML						1 8 9	<u>GGGAAGUGUA</u> ACU AACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAGCAAGA AUAC		

1sided_T Egene20 _alt8_Cs hRNA- GFP6_D 2_ML					1 9 0	GGGAUACAAG ACUCACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAACGUUA ACAA		
1sided_T Egene20 _alt9_Cs hRNA- GFP6_D 2_ML					1 9 1	GGGAAGUAAA CGUAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAAAAAGA AGGU		

1sided_T Egene20 _alt10_C shRNA- GFP6_D 2_ML						1 9 2	<u>GGGAGAACUC</u> UAGAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAAGAAUU CAGA		
1sided_T Egene20 _alt11_C shRNA- GFP6_D 2_ML						1 9 3	<u>GGGAGACCAG</u> AAGAACGGUA UAUUACCUUG UUUUCGAUCG AAAGAUCGAC GAGGUGAAAA CCUCGUGACA GUCUGAUAGC AAUGUCAACC UCGCCUAUGA ACUUCAGGGU CAGCUUGCGU GUUAUUCUUG GCAAGCUGAC CCUGAAGUUC AUAGGCGCGG UUGACCAUAC CGUCCAGUCC UGAUUUUCGA AUCAGAGAAG GACGGUAUGU AAUUGCUAUC AAGAAGUCAA CCAGAGAAAC ACGAUCAGGA ACUC		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_5 RH2						1 9 4	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGAUGA CAGUCUGAUA GCAAUGUCAA CCUCGCCUAU GAACUUCAGG GUCAGCUUGC GUGUUUUUCU UGGCAAGCUG ACCCUGAAGU UCAUAGGCGC GGUUGACCAU ACCGUCCAGU CCUGAUUUUC GAAUCAGAGA AGGACGGUAU GUAAUUGCUA UCAAGAAGUC AUACCAGAGA AACACGACUA CUACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_5RH2_left_ cleav_site_m ut_v1						1 9 5	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGAUGA CAGUCUGAUA GCAAUGUCAA CCUCGCCUAU GAACUUCAGG GUCAGCUUGC GUGUUUUUCU UGGCAAGCUG ACCCUGAAGU UCAUAGGCGC GGUUGACCAU ACCGUCCAcU CCUGAUUUUC GAAUCAGAcA AGGACGGUAU GUAAUUGCUA UCAAGAAGUC AUACCAGAGA AACACGACUA CUACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_7 RH2					1 9 6	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGGUAU GACAGUCUGA UAGCAAUGUC AACCUCGCCU AUGAACUUCA GGGUCAGCUU GCGUGUUUU CUUGGCAAGC UGACCCUGAA GUUCAUAGGC GCGGUUGACC AUACCGUCCA GUCCUGAUUU UCGAAUCAGA GAAGGACGGU AUGUAAUUGC UAUCAAGAAG UCAUACACCA GAGAAACACG ACUACUACCU CA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_7RH2_left_ cleav_site_m ut_v1					1 9 7	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGGUAU GACAGUCUGA UAGCAAUGUC AACCUCGCCU AUGAACUUCA GGGUCAGCUU GCGUGUUUU CUUGGCAAGC UGACCCUGAA GUUCAUAGGC GCGGUUGACC AUACCGUCCA cUCCUGAUUU UCGAAUCAGA cAAGGACGGU AUGUAAUUGC UAUCAAGAAG UCAUACACCA GAGAAACACG ACUACUACCU CA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_8 RH2						1 9 8	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCGUA UGACAGUCUG AUAGCAAUGU CAACCUCGCC UAUGAACUUC AGGGUCAGCU UGCGUGUUAU UCUUGGCAAG CUGACCCUGA AGUUCAUAGG CGCGGUUGAC CAUACCGUCC AGUCCUGAUU UUCGAAUCAG AGAAGGACGG UAUGUAAUUG CUAUC AAGAA GUCAUACGAC CAGAGAAACA CGACUACUAC CUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_8RH2_left_ cleav_site_m ut_v1						1 9 9	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCGUA UGACAGUCUG AUAGCAAUGU CAACCUCGCC UAUGAACUUC AGGGUCAGCU UGCGUGUUAU UCUUGGCAAG CUGACCCUGA AGUUCAUAGG CGCGGUUGAC CAUACCGUCC AcUCCUGAUU UUCGAAUCAG AcAAGGACGG UAUGUAAUUG CUAUC AAGAA GUCAUACGAC CAGAGAAACA CGACUACUAC CUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_9 RH2					2 0 0	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGACGU AUGACAGUCU GAUAGCAAUG UCAACCUCGC CUAUGAACUU CAGGGUCAGC UUGCGUGUUA UUCUUGGCAA GCUGACCCUG AAGUUCAUAG GCGCGGUUGA CCAUACCGUC CAGUCCUGAU UUUCGAAUCA GAGAAGGACG GUAUGUAAUU GCUAUCAGA AGUCAUACGU ACCAGAGAAA CACGACUACU ACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_9RH2_left_ cleav_site_m ut_v1					2 0 1	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUCGACGU AUGACAGUCU GAUAGCAAUG UCAACCUCGC CUAUGAACUU CAGGGUCAGC UUGCGUGUUA UUCUUGGCAA GCUGACCCUG AAGUUCAUAG GCGCGGUUGA CCAUACCGUC CAcUCCUGAU UUUCGAAUCA GAcAAGGACG GUAUGUAAUU GCUAUCAGA AGUCAUACGU ACCAGAGAAA CACGACUACU ACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_10 RH2						2 0 2	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCACG UAUGACAGUC UGAUAGCAAU GUCAACCUCG CCUAUGAACU UCAGGGUCAG CUUGCGUGUU AUUCUUGGCA AGCUGACCCU GAAGUUCAUA GGCGCGGUU GACCAUACCG UCCAGUCCUG AUUUUCGAU CAGAGAAGGA CGGUAUGUAA UUGCUAUCAA GAAGUCAUAC GUGACCAGAG AAACACGACU ACUACCUCU		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_10RH2_left _cleav_site_ mut_v1						2 0 3	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGCACG UAUGACAGUC UGAUAGCAAU GUCAACCUCG CCUAUGAACU UCAGGGUCAG CUUGCGUGUU AUUCUUGGCA AGCUGACCCU GAAGUUCAUA GGCGCGGUU GACCAUACCG UCCAcUCCUG AUUUUCGAU CAGAcAAGGA CGGUAUGUAA UUGCUAUCAA GAAGUCAUAC GUGACCAGAG AAACACGACU ACUACCUCU		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_6 RH2					2 0 4	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_6RH2_left_ cleav_site_m ut_v1					2 0 5	GGGAAACUAU ACAAUACGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAc UCCUGAUUUU CGAAUCAGAc AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		

1sided_Tlet7f _CshRNA- GFP6_D2_M L_6RH2_righ t_cleav_site_ mut_v1					2 0 6	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAA cAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_M L_6RH2_bot h_cleav_site_ mut_v1					2 0 7	GGGAAACUAU ACAAUACGGU AUUUUACCGU GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAc UCCUGAUUUU CGAAUCAGAc AAGGACGGUA UGUAAUUGCU AUCAA cAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_ML_6 RH2_A3 8mut						2 0 8	GGGAAACUAU ACAAUACGGU UUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		
1sided_T Egene20 _CshRN A- GFP6_D 2_ML_6 RH2						2 0 9	GGGAACGUAC CUGUACGGUA UAUUACCUGG UUUUCGAUCG AAAGAU CGAC GAGGUGAAAA CCUCGUAUGA CAGUCUGAUA GCAAUGUCAA CCUCGCCUAU GAACUUCAGG GUCAGCUUGC GUGUUUUUCU UGGCAAGCUG ACCCUGAAGU UCAUAGGCGC GGUUGACCAU ACCGUCCAGU CCUGAUUUUC GAAUCAGAGA AGGACGGUAU GUAAUUGCUA UCAAGAAGUC AUAACCAGAG AAACACGACU CUUCCGAA		

1sided_T Egene20 _CshRN A- GFP6_D 2_ML_6 RH2_A3 8mut						2 1 0	GGGAACGUAC CUGUACGGUU UAUUACCUUG UUUUCGAUCG AAAGAUCCGAC GAGGUGAAAA CCUCGUAUGA CAGUCUGAUA GCAAUGUCAA CCUCGCCUAU GAACUUCAGG GUCAGCUUGC GUGUUUUUCU UGGCAAGCUG ACCCUGAAGU UCAUAGGCGC GGUUGACCAU ACCGUCCAGU CCUGAUUUUC GAAUCAGAGA AGGACGGUAU GUAAUUGCUA UCAAGAAGUC AUAACCAGAG AAACACGACU CUUCCGAA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 6RH2						2 1 1	GGGAAACUAU ACAAUAUGGU AUUUUACCUUG GUUUUCGAUC GAAAGAUCCA CGAGGUGAAA ACCUUGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCCGCUA UGAACUUCAG GGUCAGCUUG CGUGUUUUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 6RH2_A 38mut					2 1 2	GGGAAACUAU ACAAUAUGGU UUAUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUAUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_C M2_6RH2_lef t_cleav_site_ mut_v1					2 1 3	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAUCGA CGAGGUGAAA ACCUCGUAUG ACAGUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUAUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAc UCCUGAUUUU CGAAUCAGAc AAGGACGGUA UGUAAUUGCU AUCAAGAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		

1sided_Tlet7f _CshRNA- GFP6_D2_C M2_6RH2_ri ght_cleav_sit e_mut_v1					2 1 4	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUAUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAG UCCUGAUUUU CGAAUCAGAG AAGGACGGUA UGUAAUUGCU AUCAA cAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		
1sided_Tlet7f _CshRNA- GFP6_D2_C M2_6RH2_b oth_cleav_sit e_mut_v1					2 1 5	GGGAAACUAU ACAAUAUGGU AUUUUACCUG GUUUUCGAUC GAAAGAU CGA CGAGGUGAAA ACCUCGUAUG ACAUCUGAU AGCAAUGUCA ACCUCGCCUA UGAACUUCAG GGUCAGCUUG CGUGUUAUUC UUGGCAAGCU GACCCUGAAG UUCAUAGGCG CGGUUGACCA UACCGUCCAc UCCUGAUUUU CGAAUCAGAc AAGGACGGUA UGUAAUUGCU AUCAA cAAGU CAUAACCAGA GAAACACGAC UACUACCUCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 6RH3						2 1 6	GGGAAACTAT ACAATATGGTA TATTACACTGG TTTTCGATCGA AAGATCGACG AGGTGAAAAC CTCGTGACAG TCTGATAGCA ATGTCAACCT CGCCTATGAA CTTCAGGGTC AGCTTGCGTG TTATTCTTGGC AAGCTGACCC TGAAGTTCATA GGCGCGGTTG ACCATACCGT CCAGTCCTGA TTTTCGAATCA GAGAAGGACG GTATGTAATTG CTATCAAGAA GTCAACCAGT AGAAACACGA CTACTACCTCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 7RH3						2 1 7	GGGAAACTAT ACAATATGGTA TATTACGACTG GTTTTCGATC GAAAGATCGA CGAGGTGAAA ACCTCGTGAC AGTCTGATAG CAATGTCAAC CTCGCCTATG AACTTCAGGG TCAGCTTGCG TGTTATTCTTG GCAAGCTGAC CCTGAAGTTC ATAGGCGCGG TTGACCATAC CGTCCAGTCC TGATTTTCGAA TCAGAGAAGG ACGGTATGTA ATTGCTATCAA GAAGTCAACC AGTCAGAAAC ACGACTACTA CCTCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 8RH3						2 1 8	<u>GGGAAACTAT</u> ACAATATGGTA TATTACTGACT GGTTTTTCGAT CGAAAGATCG ACGAGGTGAA AACCTCGTGA CAGTCTGATA GCAATGTCAA CCTCGCCTAT GAACCTCAGG GTCAGCTTGC GTGTTATTCTT GGCAAGCTGA CCCTGAAGTT CATAGGCGCG GTTGACCATA CCGTCCAGTC CTGATTTTCGA ATCAGAGAAG GACGGTATGT AATTGCTATCA AGAAGTCAAC CAGTCAAGAA ACACGACTAC TACCTCA		
1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 9RH3						2 1 9	<u>GGGAAACTAT</u> ACAATATGGTA TATTACCTGAC TGGTTTTTCGAT CGAAAGATCG ACGAGGTGAA AACCTCGTGA CAGTCTGATA GCAATGTCAA CCTCGCCTAT GAACCTCAGG GTCAGCTTGC GTGTTATTCTT GGCAAGCTGA CCCTGAAGTT CATAGGCGCG GTTGACCATA CCGTCCAGTC CTGATTTTCGA ATCAGAGAAG GACGGTATGT AATTGCTATCA AGAAGTCAAC CAGTCAGAGA AACACGACTA CTACCTCA		

1sided_T let7f_Csh RNA- GFP6_D 2_CM2_ 10RH3						2 2 0	GGGAAACTAT ACAATATGGTA TATTACACTGA CTGGTTTTTCG ATCGAAAGAT CGACGAGGTG AAAACCTCGT GACAGTCTGA TAGCAATGTC AACCTCGCCT ATGAACTTCA GGGTCAGCTT GCGTGTTATT CTTGGCAAGC TGACCCTGAA GTTTCATAGGC GCGGTTGACC ATACCGTCCA GTCCTGATTTT CGAATCAGAG AAGGACGGTA TGTAATTGCTA TCAAGAAAGTC AACCAGTCAG TAGAAACACG ACTACTACCTC A		
1sided_T Egene20 _CsgRN A- Stoplight _modC_ 137						4 5 9	GGGAACGTAC CTGTACGGTA TATTACCTGGT TTTCGATCGAA AGATCGACGA GGTGAAAACC TCGTGACAGT CCTTGGACAG TACTCCGCTC GAGTGTTTTA GAGCTAGAAA TAGCAAGTTAA AATAAGGCTA GTCCGTTATC AACTTGAAAAA GTGGCACC GA GTCGGTGCTT TTTTTGTCAGT CCTGATTTTCG AATCAGAGAA GACCCCAACC TATCCACTCG AGCGGAGTAC TGTC CAAGAG AAGTCAACCA GAGAAACACG ACTCTTCCGA A		

1sided_T Egene20 _CsgRN A- Stoplight _modC_ 143							<u>GGGAACGTAC</u> CTGTACGGTA TATTACCTGGT TTTCGATCGAA AGATCGACGA GGTGAAAACC TCGCGTATGA CAGTCGGACA GTACTCCGCT CGAGTGTTTTA GAGCTAGAAA TAGCAAGTTAA 4 AATAAGGCTA 6 GTCCGTTATC 0 AACTTGAAAAA GTGGCACC GA GTCGGTGCGT CAGTCCTGAT TTTCGAATCAG AGAAGACCCA TGATAACTTAC TCGAGCGGAG TACTGTCCAG AAGTCATACG ACCAGAGAAA CACGACTCTT CCGAA		
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Note that a GGGA is introduced by T7 polymerase at the very 5' end of each ribozyme sequence; in some examples above this is underlined or presented in lowercase.

The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

The term "associated with", used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

Further, in the description herein, the word “substantially” whenever used is understood to include, but not restricted to, “entirely” or “completely” and the like. In addition, terms such as “comprising”, “comprise”, and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when “comprising” is used, reference to a “one” feature is also intended to be a reference to “at least one” of that feature. Terms such as “consisting”, “consist”, and the like, may in the appropriate context, be considered as a subset of terms such as “comprising”, “comprise”, and the like. Therefore, in embodiments disclosed herein using the terms such as “comprising”, “comprise”, and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as “consisting”, “consist”, and the like. Further, terms such as “about”, “approximately” and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the individual numerical values within the range also include integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a description of a range of 1% to 5% is intended to have specifically disclosed the ranges 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%, 1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.,) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless

otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DETAILED DESCRIPTION OF FIGURES

Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications related to the ribozymes may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments. The example embodiments should not be construed as limiting the scope of the disclosure.

Fig 1. Development of an RNA trigger-activated dual self-cleaving ribozyme with two trigger-binding catalytic domains.

Fig. 1A. Schematic of a representative hairpin ribozyme, which consists of two Loops A and B, each flanked by two helices. Arrow marks the cleavage site between the N-1 and guanine (G+1) nucleotides in Loop A. Key catalytic nucleotides in catalytic Loop B, A38 and C25, are labelled. 5' - *G*- 3' spans the cleavage site, and the most highly tolerated sequences with cleavage activity of at least 20% of the wildtype ribozyme are shown in the box.

Fig. 1Bi. Design strategy of an RNA trigger-activated self-cleaving dual ribozyme, which releases an embedded RNA product upon trigger-induced cleavage.

Fig. 1Bii. A dual tandem ribozyme.

Fig. 1Biii. Circularly permuted dual ribozyme.

Fig. 1Biv. IN-form of self-cleaving sensor ribozyme, where the RNA-trigger is built into the ribozyme structure.

Fig. 1Bv. OUT-form of the self-cleaving sensor ribozyme, where the trigger RNA is separated from the ribozyme structure.

Fig. 1C. An IN-form of the sensor ribozyme retains self-cleavage activity when Helix 4 is at least 2-3 bp long. Helix 4 configurations tested in Figs 1C, D and 9A are shown at top. Asterisks label bands corresponding to predicted cleavage products for the ribozyme with a 4bp Helix 4. Panel at right shows blot for the same gel probed for the 29-nt cleavage product.

Fig. 1D. Separation of trigger from the ribozyme allows for an RNA trigger-activated dual ribozyme. Asterisks mark bands corresponding to predicted cleavage products for the ribozyme with a 8bp Helix 4. Panel at bottom shows blot for the same gel probed for the 29-nt cleavage product. Hashtags indicate non-specific products produced in original in vitro transcription that are probe-negative.

Fig. 2A. Ribozymes with a single catalytic domain exhibit RNA-triggered dual self-cleavage. T-ban5p_CI-29nt-clvRNA RNA-triggered dual ribozyme with mutations at A38 of either vs both catalytic domains demonstrate that at least one catalytic domain is necessary and sufficient for dual cleavage.

Fig. 2B. Sequence and structure of exemplary dual cleavage site T-ban5p_CI-29nt-clvRNA ribozymes with either a single “right” wildtype catalytic domain or a single “left” reverse-joined catalytic domain.

Fig. 2C. Single ribozymes with dual cleavage sites and either a single “right” wildtype catalytic domain or a single “left” reverse-joined catalytic domain (structures similar to Fig. 2B) with sensor regions that are triggered by dme-ban-5p, hsa-mir-451 or SARS-CoV-2 E-gene fragment can cleave at two cleavage sites to release an embedded 29-nt RNA cleavage product.

Fig. 2D. Optimisation of the Helix 4 communication module in the T-SARS-CoV-2-E-gene_CI-29nt-clvRNA ribozyme and identification of 3-nt motifs that improve the signal-to-noise ratio of the ribozyme (Additional motifs have also been identified).

Fig. 2E. Cleavage product release from the T-SARS-CoV-2-E-gene_CI-29nt-clvRNA ribozyme increases with increasing concentration of E-gene trigger RNA. 600 nM ribozyme was used. Asterisks mark bands corresponding to predicted cleavage products.

Fig. 2F. Testing sequence variants of the E-gene test RNA against the T-SARS-CoV-2-E-gene_CI-29nt-clvRNA ribozyme shows that the ribozymes can distinguish between closely related trigger RNAs with 1-3 nt differences, while unrelated sequences do not trigger the ribozyme.

Fig. 2G. Ribozyme can detect its trigger from within a complex mixture of RNA, up to at least 1000 fold more non-specific RNA than trigger RNA.

Fig. 3A. Functional RNA can be embedded as cleavage products in ribozymes. Schematic of a ribozyme that comprises an embedded single guide RNA (sgRNA).

5 Fig. 3B. Schematic of a ribozyme that comprises an embedded short hairpin RNA (shRNA).

Fig. 3C. Schematic of a ribozyme that comprises an embedded RNA aptamer, Broccoli.

10 Fig. 3D. Cleavage assay for ribozyme T-let-7f_CI-sgRNAGFP. Asterisks mark bands corresponding to predicted cleavage products. Right panel shows blot for the same gel probed for the sgRNA cleavage product.

Fig. 3Ei. Cleavage assays for ribozyme T-let-7f_CI-shRNAGFP and 3Eii. Ribozyme T-let-7f_CI-shRNAGFP6. Asterisks mark bands corresponding to predicted cleavage products. Right panels show blots for the same gel probed for the shRNA
15 cleavage product.

Fig. 3F. Cleavage assay for ribozyme T-let-7f_CI-Broccoli aptamer. Top panel shows blot for the same gel probed for the aptamer cleavage product.

20 Fig. 3G. Members of the human let-7 microRNA family (Top) and cleavage assay for ribozyme T-let-7f_CI-sgRNAGFP when triggered by each member of the let-7 family (Bottom). Bottom-most panel shows blot for the same gel probed for the sgRNA cleavage product. Asterisks mark bands corresponding to predicted cleavage products.

Fig. 3H. Lengthening of Helix 2 on T-let-7f_CI-sgRNAGFP from 4 bp to 8- or 12 bp reduced trigger-independent cleavage at the proximal cleavage site (dashed boxes). Asterisks mark bands corresponding to predicted cleavage products for the ribozyme
25 with a 8-nt Helix 2. Right panel shows blot for the same gel probed for the sgRNA cleavage product.

Fig. 4A. Rate of editing in uninjected, positive control gRNA + Cas9-injected, ribozyme (triggered by let-7f to release a gRNA against GFP) + Cas9-injected, and ribozyme + Cas9 + let-7f morpholino-injected zebrafish embryos. Mann Whitney non-
30 parametric test was used.

Fig. 4B. Ribozyme (1sided_Tlet7f_CshRNA-GFP6 modified with 50% 2'-fluorinated C and U) detects modified let-7f trigger to increase down-regulation of GFP expression.

35 Fig. 4C. Ribozyme (1sided_TEgene20_CsgRNA-Stoplight_modC modified with 50% 2'-fluorinated C) detects modified Egene trigger to increase editing of GFP locus.

Fig. 5A. Original ribozyme without modifying A7 at the lower strand of the proximal cleavage loop.

Fig. 5B. Mutation of A7 to C7, to be able to pair with opposite strand (bottom C at the lower strand of the proximal cleavage loop).

5 Fig. 5C: Mutation of A7 to U7, to be able to pair with opposite strand (bottom U at the lower strand of the proximal cleavage loop).

Fig. 5D: Mutation of N7 to pair with N+3 improves cleavage activity of ribozymes with a non-canonical sequence in the cleavage site (cleavage product in outlined box).

10 Fig. 6A. 1sided_TCel-mir-238_CsgRNA-GFP_ML_modC1_8H2-A7 ribozyme with full complementary across most of the cleavage product (not alternating complementarity).

Fig. 6B: Cleavage assay for 1sided_TCel-mir-238_CsgRNA-GFP_ML_modC1_8H2-A7 ribozyme.

15 Fig. 6Ci-iv. 1sided_TCel-mir-238_CsgRNA-GFP_ML_modC1 (i) / or C2 (ii) / or C3 (iii) / or C4 (iv)_8H2-A7 ribozymes with full complementarity across most of the cleavage product (not alternating complementarity).

Fig. 6Di-iv. 1sided_Tlet7f_CsgRNA-GFP_ML_modC1 (i) / or C2 (ii) / or C3 (iii) / or C4 (iv)_8H2-A7 ribozymes with full complementarity across most of the cleavage product (not alternating complementarity).

20 Fig. 6E: Cleavage assay for 1sided_Tlet-7f or Cel-mir-238_CsgRNA-GFP_ML_modC1_8H2-A7 ribozymes.

Fig. 7: shRNA ribozymes work in human cells. Structure of 1sided_Tlet7f_shRNA-GFP6 ribozyme.

25 Fig. 8A. Ribozymes can cleave out RNA aptamers. Structure and sequence of ribozyme that cleaves out Red Broccoli fluorescent RNA aptamer.

Fig. 8B. Ribozymes can cleave out RNA aptamers. Trigger-induced cleavage and release of Red Broccoli fluorescent RNA aptamer.

30 Fig. 9A. A circularly permuted ribozyme with an 8-nt Helix 4 retains self-cleavage activity, while shortening of Helix 4 gradually reduces self-cleavage. Asterisks mark bands corresponding to predicted cleavage products for the 8-bp H2 ribozyme.

Fig. 9B. Schematic of optimal configurations of the hairpin ribozyme junction (i-iv) tested (v). Asterisks mark bands corresponding to predicted cleavage products for the 4WJ paired ribozyme. Hashtags mark unpredicted cleavage products that mostly appear in ribozymes with strong non-complementarity between cleavage product and ribozyme (unpaired).

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Fig. 9C. Pairing configurations of Helix 1 tested in 4WJ (HHHS2H) 8-nt Helix 4 ribozymes. Top: Cleavage product sequence in 3' to 5' direction. Bottom: Five configurations of pairing on the ribozyme with the cleavage product were tested; sequences are in the 5' to 3' direction and vertical lines indicate complementary pairing with the cleavage product at top.

Fig. 9D. Graph showing amount (normalised to the S2 strand) of cleavage product released by the IN-Form of the ribozyme when length of Helix 4 is varied from 4 bp to 1 bp (Refers to Fig. 1D).

Fig. 9E. Graph showing fold change in cleavage product released (Trigger Lane/Water Lane) by the OUT-Form of the ribozyme when length of Helix 4 is varied from 8 bp to 1 bp (Refers to Fig. 1E).

Fig. 9F. T-let-7f_CI-29nt-clvRNA dual ribozyme exhibits let-7f-induced cleavage release of the embedded 29-nt cleavage product.

Fig. 10A. Ribozymes with a single catalytic domain and triggered by dme-mir-184, dme-mir-252, dme-mir-263a, has-let-7f or SARS-CoV-2 Orf1ab gene RNA fragments can self-cleave at dual sites.

Fig. 10B. Mutation of either or both catalytic domains in the ban-5p, mir-451a- or E-gene-triggered dual ribozyme shows that one catalytic domain is sufficient for dual cleavage.

Fig. 10C. Optimisation of the Helix 4 communication module in the T-mir-451a_CI-29nt-clvRNA and T-SARS-CoV-2-S-gene_CI-29nt-clvRNA ribozyme and identification of a 3-nt v1 and v2 motifs that improve the signal-to-noise ratio of the ribozyme.

Fig. 10D. Testing mutant variants of the E-gene test RNA against the T-SARS-CoV-2-E-gene_CI-29nt-clvRNA ribozyme shows that the ribozymes can distinguish between closely related trigger RNAs with 1-3 nt differences. Bottom panel shows quantification of the ratio of the intensity of the variant over WT band, each normalised to its 40-nt spike-in control.

Fig. 11A. Editing efficiency for a range of GFP single guide RNAs tested. ^indicates the sgRNA selected for encoding within the ribozyme for zebrafish studies.

Fig. 11B. An sgRNA starting with GUC can be cleaved out from a ribozyme in a trigger-dependent manner (227R in previous panel, Fig. 11A).

Fig. 11Ci. Structure of ribozyme where A7 has been changed to C7 or U7 Cii. Mutation of A7 to C7 or U7 restores cleavage to ribozyme comprising the GFP-149R

sgRNA, which begins with GGGC. ii) Cleavage assay for ribozyme in Ci. Right panel shows blot for the same gel probed for the sgRNA cleavage product.

Fig. 11D. Lengthening of Helix 2 on T-let-7f_Ci-shRNAGFP from 4 bp to 8- or 12 bp reduced trigger-independent cleavage at the proximal cleavage site (dashed boxes).

5 Asterisks mark bands corresponding to predicted cleavage products for the ribozyme with the 8-nt Helix 2. Right panel shows blot for the same gel probed for the shRNA cleavage product.

Fig. 12A. Various Helix 4 motifs tested.

Fig. 12B. Cleavage assay showing that (CM2: 5'- AUG/CGA -3'), (CM7: 5'-
10 ACU/AGA -3') and (CM8: 5'- AUG/CAA -3') are additional Helix 4 motifs that perform well.

Fig. 13A. Sequence of shRNA-embedded ribozyme with original right Helix 2 and additional variations in Helix 2 tested.

Fig. 13B. Cleavage assay of ribozymes with varied Helix 2 as shown in Fig. 13A.
15 Thus, Fig. 13B shows extension of Helix 2 beyond 5-nt decreases background cleavage (lower asterisk).

Fig. 14A. Sequence of shRNA-embedded ribozyme with original pairing.

Fig. 14B. Top: Variations in pairing of shRNA ribozyme from Fig. 14A tested.
Bottom: Flow cytometry results of shRNA knockdown of GFP fluorescence by ribozymes
20 with varying degree of pairing between embedded shRNA and ribozyme. Thus, Fig. 19B shows that an increase in complementarity between the shRNA cleavage product and ribozyme increases the cleavage dependency of embedded shRNA function.

Fig. 15A. Design of original sgRNA-embedded ribozymes with partial complementarity between sgRNA spacer and ribozyme backbone, and changes in
25 spacer complementarity that were tested (mods A, B and C in boxes at right).

Fig. 15B. Cleavage assays of modA, modB and modC sgRNA-embedded ribozymes from (A).

Fig. 15C. Design of sgRNA-embedded ribozymes where part of the first stem loop of the sgRNA is "flattened" to pair with the ribozyme backbone, resulting in
30 increased complementarity between sgRNA and ribozyme backbone (also known as versions modC and modC1).

Fig. 15D. Cleavage assays of modC1 sgRNA-embedded ribozymes from (C).

Fig. 16. A. Structures of ribozymes with lengthened right Helix 3 (RH3). B. Cleavage assay results of ribozymes with lengthened right Helix 3 (RH3).

35 Fig. 17A: Detection of modified RNA triggers in cells.

Fig. 17Bi: Ribozyme can be triggered by 2' MOE modified synthetic RNA (E gene). Two E-gene-triggered shRNA ribozymes, with ML and CM2 communication modules, can be triggered by modified E-gene trigger to cleave out embedded RNA cleavage product (boxed in gel).

5 2'MOE let-7f sequence (/52MOErT//i2MOErG//i2MOErA/ rGrGrU rArGrU rArGrA rUrUrG rUrArU rA/i2MOErG//i2MOErT/ /32MOErT/) (SEQ ID NO: 506)

 2'MOE E gene sequence (/52MOErT//i2MOErT//i2MOErC/ rGrGrA rArGrA rGrArC rArGrG rUrA/i2MOErC/ /i2MOErG//32MOErT/) (SEQ ID NO: 507)

Fig. 17Bii: Ribozyme can be triggered by 2' MOE modified synthetic RNA (let7f).
10 Two let-7f-triggered shRNA ribozymes, with ML and CM2 communication modules, can be triggered by modified let-7f trigger to cleave out embedded RNA cleavage product (boxed in gel).

Fig. 17C. Ribozyme can be triggered by DNA. 3 ribozymes, triggered by S-gene fragment, E-gene fragment and mir-451a, respectively, can detect both RNA and DNA
15 triggers to cleave out embedded RNA cleavage product (boxed in gel). E-gene ribozyme potentially shows preference for RNA trigger over DNA trigger.

Fig. 17D. Ribozymes partially modified with 2'-fluoro nucleotides (using Durascribe) can cleave. Fully modified ribozymes (100% 2'F C & U) may not be able to
20 cleave.

EXPERIMENTAL DATA

Methods

25 **PCR amplification of template and *in vitro* transcription of RNA**

DNA templates and primers were ordered (Integrated DNA Technologies, USA), and PCR amplification was performed using Phusion high fidelity PCR mastermix (#F531L, Thermo Fisher Scientific, USA) according to manufacturer's instructions. PCR products were purified using QIAquick PCR purification kit (#28106, Qiagen, Germany),
30 and used as templates for *in vitro* transcription using AmpliScribe T7-flash transcription kit (#LGLC-ASF3507, Lucigen, USA) according to manufacturer's instructions. For some templates, DNA gene fragments (Twist Bioscience, USA) were used directly for *in vitro* transcription. For the experiments using modified ribozymes, Durascribe (#DS010925, Epicentre, USA) was used according to manufacturer's instructions. After the reaction,
35 280 µL of RNase-free water (#SH30538.02, Hyclone, USA) was added to 20 µL of the *in*

vitro transcription reaction. The RNA was purified by adding 300 µL of acid-phenol:chloroform, pH 4.5 (with IAA, 125:24:1) (#AM9722, Invitrogen, USA), mixed, and centrifuged at 14,000 rpm for 3 min at room temperature. The aqueous phase was transferred to a new tube, and 300 µL of chloroform (#07278-00, Kanto Chemical, Japan) was added. The mixture was centrifuged at 14,000 rpm for 3 min at room temperature. The aqueous phase was transferred to a new tube and the RNA was precipitated with 1/10 volume (25 µL) of 3 M sodium acetate (pH 5.2) and 2.5X volume (625 µL) of absolute ethanol. After overnight precipitation at -20°C, the samples were centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was discarded, the RNA pellet was washed with cold 75% ethanol, and centrifuged at 13,000 rpm for 20 min at 4°C. After centrifugation, the supernatant was removed completely, and the pellet allowed to air-dry. RNA was re-suspended in 50 µL of RNase-free water (#SH30538.02, Hyclone, USA) and its concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Cell-free cleavage assay

Assays were performed with 200 nM ribozyme RNA (*in vitro* transcribed) and 50 nM trigger RNA (Integrated DNA Technologies, USA) in 1X cleavage buffer (10 mM Tris, 7 mM magnesium chloride, 5 mM spermine, 2 mM sodium chloride, pH 6.4). 200 nM of an inert 40-nt RNA sequence (5'-GGGACAUGGAAGUCACACCUUCGGGAACGUGGUUGACCUA-3') (SEQ ID NO: 461) was spiked into the reaction as a loading control. Reactions were incubated at 37°C for 4 hours.

Detection and visualization of cleavage product

RNA was heat-denatured for 10 min at 70°C, loaded with 2x RNA Loading Dye (#B0363S, New England Biolabs, USA), and separated on a 10% denaturing polyacrylamide gel (#EC-833, National Diagnostics, USA) in 1X TBE (Tris/Boric Acid/EDTA) buffer (#1610770, Bio-Rad, USA) at 200 V for 1 hour, or until the dye front migrated to the bottom of the gel. Low range ssRNA ladder (#N0364S, New England Biolabs, USA) was loaded as a size marker, and 25 ng of a 29 nt oligo (5'-GUCCUUAGUCGAAAGUUUUACUAGAGUCA-3') (SEQ ID NO: 462) (Integrated DNA Technologies, USA) or an *in vitro* transcribed RNA sequence, corresponding to the size of the expected cleavage product, was spiked in to the ladder as an additional size marker. Where appropriate, 25 ng of the trigger and spike-in sequence was also added

into the ladder as size markers. Gels were stained with SYBR Gold at 1:10,000 dilution (#S11494, Invitrogen, USA), and visualized using ChemiDoc Imaging System (Bio-Rad, USA). Images were analyzed using Image Lab software (Bio-Rad, USA).

Using the Transblot SD semi-dry transfer cell (Bio-Rad, USA), RNA was transferred onto Hybond-N+ membrane (#RPN303B, GE Healthcare, USA) in 1X TBE (#1610770, Bio-Rad, USA) at 10 V and 300 mA for 1 hour in the cold room. The membrane was cross-linked using a UV crosslinker (Analytik Jena, USA), and pre-hybridized in PerfectHyb Plus Hybridization Buffer (#H7033-1L, Merck, USA) at 45°C for 5 min with rotation. 5' Alexa Fluor 647 or Cy5 labelled DNA probe against the cleavage product (Integrated DNA Technologies, USA) was added to the pre-hybridization solution and incubated in a hybridization oven at 45°C for 3 hours with rotation. Following hybridization, membranes were washed progressively with increasing stringency of wash buffer (low stringency wash buffer: 2X SSC, 0.1% SDS; high stringency wash buffer: 0.5X SSC, 0.1% SDS; ultra-high stringency wash buffer: 0.1X SSC, 0.1% SDS). All washing steps were performed at 45°C in a hybridization oven with rotation (except for low stringency wash, which was performed at room temperature with rotation). Membranes were visualized using ChemiDoc MP Imaging System (Bio-Rad, USA), and analyzed using Image Lab software (Bio-Rad, USA).

Table 2.

Probe name	Sequence	SEQ ID NO:
CsgRNA_probe_Alexa647	/5Alex647N/GGCAAGCTGCCCCGTGCCCAA	463
Cban3pR_probe_Cy5	/5Cy5/ACTCTAGTAAACTTTTCGAC	464
Exact_sgRNA_probe_647	/5Alex647N/CCGGCAAGCTGCCCCGTGCCC	465
CshRNA-GFP6_60nt_AF647	/5Alex647N/TGGACGGTATGGTCAACCGCGCCTATGA ACTTCAGGGTCAGCTTGCCAAGAATAACACGC	466

Maintenance of cell cultures

HEK293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Gibco #10270106), 1% penicillin/streptomycin (Gibco #15140122). HEK293-GFP cells (#SC001, Amsbio, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Gibco #10270106), 1% penicillin/streptomycin (Gibco #15140122), 2 mM L-glutamine, 0.1 mM MEM non-essential amino acids (Gibco #111140050), and 10 µg/ml blasticidin (Gibco #A1113903). All cultures were maintained at 37 °C and 5% CO₂.

Electroporation

Electroporation of RNA into HEK293-GFP cells was performed using the Neon transfection system (#MPK1096, Invitrogen, USA) according to manufacturer's instructions, with the parameters set as 1500 V, 30 ms pulse width, 1 pulse. The Alt-R CRISPR-Cas9 system (Integrated DNA Technologies, USA) was used for delivery of ribonucleoprotein complexes by electroporation using the Neon kit. After electroporation, cells were seeded on 96-well plates and incubated for 48 hours until analysis.

miRNA inhibitors

miRNA and control inhibitors were purchased from Integrated DNA Technologies, USA.

hsa-let-7f_inhibitor	mA/ZEN/mA mCmUmA mUmAmC mAmAmU mCmUmA mCmUmA mCmCmU mC/3ZEN/ (SEQ ID NO: 467)
NC1_neg_ctrl_inhibitor	mG/ZEN/mC mGmUmA mUmUmA mUmAmG mCmCmG mAmUmU mAmAmC mG/3ZEN/ (SEQ ID NO: 468)

Flow cytometry

Cells were washed with 1X PBS (#10010023, Gibco, USA), and dislodged from the cell culture plate with Trypsin-EDTA (0.05%), phenol red (#25300054, Gibco, USA). The trypsin was neutralized by adding complete media and the entire volume from each well was transferred to a U-bottom 96-well plate. The plate was spun down at 300 g for 4 min, and the supernatant discarded. Cells were stained with Live/Dead fixable near-IR dead cell stain (#L34975, Invitrogen, USA) diluted 1:1000 in 1X PBS (#10010023, Gibco, USA) for 15 min at room temperature. After staining, the plate was spun down at 300 g for 4 min, and the supernatant discarded. Cells were re-suspended in 1X PBS (#10010023,

Gibco, USA) and analysed on BD FACSymphony High Throughput Sampler (HTS). Data analysis was carried out using FlowJo (BD Biosciences, USA).

Cloning of plasmids

- 5 pSpCas9(BB)-2A-Puro V2.0 vector (#62988, Addgene, USA) was used as the base vector for cloning in gRNA or ribozyme sequences. The vector was cut with BbsI (#R0539S, New England Biolabs, USA) and ligated with the desired insert using T4 DNA ligase (#10716359001, Roche, USA). The gRNA insert was made by oligo annealing of sense and antisense strands of the gRNA sequence with BbsI overhangs. For cloning of
10 ribozymes into the same vector, a gblock (Integrated DNA technologies, USA) with the removal of the gRNA scaffold sequence and inclusion of a new BamHI restriction site was inserted between the AflIII and XbaI sites. This modified plasmid was then cut with BbsI and ligated with the desired ribozyme insert. The ribozyme insert was made with PCR amplification using Phusion high fidelity PCR mastermix ((#F531L, Thermo Fisher
15 Scientific, USA) to include BbsI overhangs by creating PaqCI sites, and then cut with PaqCI (#R0745S, New England Biolands, USA), as the ribozyme sequence contains an internal BbsI cut site.

Fish micro-injection

- 20 Ribonucleoprotein mixture (RNP) containing 150 ng of in vitro transcribed ribozyme and 0.5 µg of Cas9 protein (Integrated DNA Technologies, USA) was incubated at 37°C for 10 mins and allowed to cool to room temperature. Each embryo was injected with 0.75 nL of RNP mixture.

- Genomic DNA was extracted from single embryos 24 hours after micro-injection.
25 Each embryo was first rinsed with 1X PBS (#10010023, Gibco, USA), followed by addition of 20 µL alkaline lysis solution (25 mM sodium hydroxide, 0.2 mM EDTA) and incubation at 95°C for 30 min. The tubes were vortexed to check for complete lysis of embryos, and then incubated at 95°C for another 10 min. 20 µL of neutralization buffer (40 mM Tris-HCl, pH 8.0) was added to each tube, and 2 µL of the extracted genomic
30 DNA was used as template for PCR amplification of GFP using the following primers: SAW907_GFPPF2 and SAW908_GFPR2.

SAW907_GFPPF2	GTGGTGCCCATCCTGGTC (SEQ ID NO: 469)
SAW908_GFPR2	CTTGTACAGCTCGTCCATGC (SEQ ID NO: 470)

Genome editing and detection

sgRNA design was carried out using <http://crispor.tefor.net/>. Genomic DNA extracted from cells using QuickExtract DNA extraction solution (#QE09050, Lucigen, USA), and used as a template for PCR amplification of GFP. The desired band was gel extracted using ZR-96 Zymoclean gel DNA recovery kit (#D4022, Zymo Research, USA). DNA was eluted in RNase-free water (#SH30538.02, Hyclone, USA), and the concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

50 ng of DNA was used as template for a second PCR using fluorescent labelled primers. Capillary electrophoresis was run on ABI 3730xl DNA Analyzer (Applied Biosystems, USA). The fluorescently labelled DNA fragments were sized by comparison to a size standard (#4322682, Applied Biosystems, USA). Data analysis was performed using GeneMapper (Applied Biosystems, USA).

FW+M13 adaptor	TGTAAAACGACGGCCAGTACGTAAACGGCCACAAGT (SEQ ID NO: 471)
RV	TGAAGAAGATGGTGCCTC (SEQ ID NO: 472)

Results

An RNA trigger-activated self-cleaving dual ribozyme

Pandan, a miRNA sensor whose fluorescence is activated upon binding of a specific target miRNA, was previously developed by altering the structure of the fluorescent RNA Spinach2, so that hybridization of a target miRNA to its cognate RNA sensor backbone is required for stable binding of the fluorophore DFHBI (3,5-difluoro-4-hydroxybenzylidene imidazolinone). Pandan was designed by removing part of the structure of Spinach2, replacing it with sequences complementary to a target miRNA, so that stabilisation of the fluorophore DFHBI to the G-quadruplex structure of Spinach2 could only occur when the target RNA was bound. While Pandan sensors exhibited a substantial 50-fold increase in fluorescence in presence of 1 μ M of its target miRNA, the Pandan sensor system, unlike other methods for RNA detection, did not comprise an amplification step that could enable more sensitive applications.

It was reasoned that the principle used for designing Pandan could be applied to modify a ribozyme able to amplify and transduce RNA signals: A molecule with ribozyme activity could be assembled from two or more different RNA molecules if the structural

elements required for stable RNA folding into the catalytic conformation is only reconstituted upon base-pairing between a ribozyme sensor and its trigger RNA. This can be achieved if part of the ribozyme structure was removed and replaced by sequences complementary to a trigger RNA of interest. In addition, the inventors of the disclosure hypothesised that linkage of two such RNA-activated self-cleaving ribozymes could enable RNA signal amplification and transduction via release of a second RNA molecule from the dual-ribozyme.

After considering the structural characteristics of various ribozymes, the inventors selected as a proof-of-concept the self-cleaving hairpin ribozyme (HpRz), as mutagenesis and crystallography studies suggested that it exhibits the structural elements required. The HpRz consists of two independently folding domains (Domains A and B; Fig. 1A), each consisting of a loop region containing key catalytic nucleotides, flanked by two helices. The phosphodiester cleavage reaction occurs between the N₁ and guanine (G₊₁) nucleotides in Loop A (black arrow in Fig. 1A). The sequence requirements across the cleavage site has been systematically studied by many groups; the most highly tolerated sequences with activity of at least 20% of the wildtype ribozyme are shown in Figure 1A.

Figure 1Bi schematises our original design approach. Two HpRz, each with a modified Helix 4 containing complementary sequence to a trigger miRNA, would be arranged in tandem, one in a wild-type configuration ("right" side), and the other in a reverse-joined configuration ("left" side). Without the trigger RNA, catalytic Loop B is conformationally unstable, and ribozyme self-cleavage does not occur. Binding of the RNA trigger stabilises Loop B, activating cleavage to release an RNA cleavage product (Fig. 1Bi). This strategy requires three modifications to the original wildtype dual ribozyme (Fig 1Bii). First, the inventors would circularly permute it so that Helix 4 will no longer be a closed stem loop; instead, the positions of the 5' and 3' ends will be altered to reside within each Helix 4 (Fig. 1Biii; different junctional configurations are possible, and the 3-way junction is shown). This dual ribozyme would consist of two strands, the first comprising the cleavage sites and cleavage product (Strand 1; S1), and the second a non-cleaved strand (Strand 2; S2). Next, the inventors would introduce a second sequence-variable stem loop that branches off Helix 4 (IN-form; Fig. 1Biv); this mimics the structure that would be formed by binding of a trigger RNA. Finally, the inventors would assemble a functional self-cleaving ribozyme from three RNAs (S1, S2 and Trigger RNA) that are sequence-complementary at the new branched Helix 4 (OUT-form; Fig.

1Bv). The inventors systematically tested the feasibility of this strategy and optimised each feature of the ribozyme.

The inventors first selected an example trigger miRNA and an example cleavage product with which to start development. Since there was a wide range of performance efficacy for Pandan sensors ranging from 4 to 118 fluorescence fold-change, the inventors decided to first select as a trigger RNA the *Drosophila* microRNA *bantam-5p* (*ban-5p*), which was the trigger for the best-performing Pandan sensor. It was reasoned that a trigger RNA that binds well to its cognate Pandan sensor may also bind other similar branched sensor structures effectively. The inventors chose a 29-nt RNA fragment (29nt-clvRNA) as an example cleavage product, long enough to differentiate from the 23-nt *ban-5p* trigger miRNA on a gel.

The first step was to circularly permute the ribozyme (Fig. 1Biii). The inventors observed that a circularly permuted ribozyme retained self-cleavage activity to release the embedded 29-nt cleavage product (Fig. 9A). In order for the strategy of trigger-activated cleavage to work, the inventors believed that they ought to be able to destabilise Helix 4 by shortening its length; indeed, while ribozymes with an 8bp Helix 4 showed clear release of cleavage product, shortening of Helix 4 to fewer than 3-4 nucleotides impeded cleavage product release (Fig. 9A). These initial experiments were carried out with the ribozyme in the 3-way junction configuration (Fig. 1Biii), and the inventors observed that the rate of cleavage product formation was low, even when Helix 4 was 8-nt long, and a substantial fraction of S1 strands remained intact (Fig. 9A). Hence, the inventors took two approaches to boost cleavage. First, since re-ligation rates increase when the cleaved product is highly complementary within Helix 1, the inventors decreased base-pairing between the cleavage product and S2 to favour cleavage product release. In addition, it had been shown that HpRzs with a 3-way junction (HHH) configuration (Fig. 1Biii, 9A, 9Bi) cleaved poorly, while those with modified 2-way (H_1S_7H)(Fig. 9Bii), modified 3-way (HHS_4H)(Fig. 9Biii) or modified 4-way ($HHHS_2H$)(Fig. 9Biv) configurations cleaved well. Hence, the inventors tested the cleavage activity of ribozymes with these modified junctions, where the cleavage product was either paired or unpaired with the ribozyme (Fig. 9C). These modifications substantially improved cleavage product release, with the unpaired 3-way (HHS_4H) and 4-way ($HHHS_2H$) junctions showing the highest rate of cleavage (Fig. 9C). However, the fully unpaired ribozymes exhibited unexpected cleavage bands (Fig. 9C, hashtags), and the inventors hypothesised that non-complementarity of the cleavage product disrupted ribozyme folding to cause off-target cleavage. Hence, the inventors partially restored

complementarity between the cleavage product and the ribozyme (Fig. 9C). A configuration of 3/4-unpaired, 2-paired nucleotides, repeated along the cleavage product-ribozyme region, was very effective for cleavage product release (Fig. 9C, configuration #5).

5 Next, to determine whether the ribozyme could accommodate an RNA-binding trigger region, the inventors introduced branched stems into Helix 4 to mimic such a structure (Trigger-IN form, where the trigger RNA is introduced as part of the ribozyme, Fig. 1Biv). The optimised 4-way junction ribozyme with alternate base-pairing configuration was used (Fig. 9C, configuration #5). As Helix 4 stems longer than 4bp
10 released cleavage product in absence of trigger (Fig. 9A), the inventors tested Helix 4 stems of 1 to 4bp. Trigger-IN ribozymes could self-cleave to release substantial 29-nt cleavage product when Helix 4 was at least 3 bp long (Fig. 1C), with increased Helix 4 lengths associated with greater cleavage product release (Fig. 9D). Therefore, circularly permuting the dual ribozyme, reducing complementarity of the cleavage product,
15 introducing a 4-way junction, shortening Helix 4 and altering it to encompass a branched RNA-binding trigger region allowed for the possibility of a trigger-activated dual ribozyme.

RNA-triggered cleavage in cell-free assays

20 The sequences of both helices branching off Helix 4 could be varied. This allowed the inventors to encode sequences complementary to target RNAs into the ribozyme backbone. Hence, the possibility of reconstituting ribozyme activity from three separate RNAs was looked into by: A dual ribozyme with *ban-5p* trigger-binding domains (OUT-form, Fig. 1Bv), and a short RNA (*ban-5p*) complementary to the ribozyme trigger domain (Fig. 1D). This ribozyme was named Trigger RNA-ban-5p_Cleaved product-29nt-clvRNA
25 (T-ban-5p_CI-29nt-clvRNA). In the OUT-form, when Helix 4 was shorter than 2 bp, the ribozyme released very little cleavage product even in presence of the trigger (most S1 strands remained uncleaved; Fig. 1D). A Helix 4 of 2-3 bp length showed trigger-activated self-cleavage, releasing more cleavage product in presence of the trigger compared to in its absence. Increasing Helix 4 length to 8 bp led to high background
30 cleavage in absence of trigger, and no appreciable trigger-activated cleavage (Figs. 1D, 9E). A second ribozyme was tested, triggered by a different miRNA let-7f to release the same 29-nt cleavage product (T-let-7f_CI-29nt-clvRNA). This ribozyme also released the cleavage product in a trigger-dependent manner (Fig. 9F).

Therefore, the inventors have developed a ribozyme with two cleavage sites and two trigger-binding regions, which is activated by a trigger RNA of interest to release an embedded RNA cleavage product.

5 A single catalytic domain is sufficient for RNA-triggered dual self-cleavage

The observed cleavage patterns hinted at differential cleavage rates between the two sites (Figs. 1C, D). Hence, each catalytic domain was individually mutated to determine if one was more active. A₃₈ is a critical nucleobase involved in stabilising the transition state during catalysis, and mutation of this nucleotide abolishes cleavage. To
10 the inventors' surprise, mutation of A₃₈→U₃₈, in either the "right" wildtype catalytic or the "left" reverse-joined catalytic domain in the T-ban-5p_CI-29nt-clvRNA dual ribozyme (Fig. 1Bv, 1D with 8-nt H4), did not abolish cleavage at either cleavage site, nor substantially reduce release of the cleavage product, while mutation of both catalytic domains completely abolished cleavage (Fig. 2A). This suggested that either single
15 catalytic domain can cleave at both cleavage sites.

To test this, dual-cleavage site ribozymes were designed with either a single "right" wildtype catalytic domain or a single "left" reverse-joined catalytic domain (Fig. 2B). These ribozymes can now be encoded by a single strand of RNA. Surprisingly, both
20 single ribozymes were able to cleave at both sites to release the cleavage product (Fig. 2C). Addition of the trigger RNA increased cleavage product release for the *ban-5p* single "right-sided" ribozyme (Fig. 2C). The inventors chose to proceed with the single "right" wildtype catalytic domain, reasoning that it could be rationally optimised based on the abundance of functional studies that have been carried out on this canonical structure.
25 "Right-sided" single ribozymes, triggered by microRNAs *dme-mir-184*, *dme-mir-252*, *dme-mir-263a*, *hsa-let-7f*, as well as for fragments of the E-gene and Orf1ab transcripts of the SARS-CoV-2 genome, all could self-cleave at both sites to release the cleavage product (Figs. 2C, 10A), confirming that one catalytic domain was sufficient for dual cleavage. Mutation of A₃₈→U₃₈ in the single catalytic domain abolished cleavage (Fig.
30 2C). Several ribozymes showed additional cleavage product release when trigger RNA was added; however, most ribozymes showed cleavage product release even in absence of the trigger (Figs. 2C, 10A).

To optimise the signal-to-noise ratio in presence vs absence of the trigger, Helix 4 was varied, which acts as a trigger-responsive communication module, to identify an
35 optimal stem length and sequence. Ribozymes with Helix 4 longer than 5-nt cleaved in

a trigger-independent manner, while those with lengths of 2-nt were cleavage-resistant even in presence of trigger (Fig. 2D). Two 3-nt motifs, 5'-ACG/CGU-3' (3-nt v1) and 5'-ACG/CGA-3' (3-nt v2), greatly improved the signal-to-noise ratio of several ribozymes (Figs. 2D, 10B). Cleavage product increased with increasing trigger RNA concentration (Fig. 2E). The inventors named this trigger-activated, single-stranded dual ribozyme platform, able to self-cleave and release an embedded RNA product upon trigger RNA-binding, Unlocked By Activating RNA (UNBAR).

The inventors next investigated the ability of the ribozymes to discriminate between unrelated and closely related sequences by introducing sequence changes into the test RNA (Fig. 2F). An unrelated RNA (S-gene fragment) was unable to trigger cleavage. The effect of single nucleotide mismatches depended on the location of the mismatch, with position 4 reducing cleavage product formation by more than 80%. Dual and triple mutants exhibited strong reduction in cleavage activity (Figs. 2F, 10D). These results suggest that these ribozymes can be optimised to distinguish between similar target sequences.

For these ribozymes to be useful in biological samples, they must identify their triggers in complex mixtures. total RNA from HEK293T cells was prepared and assayed for the ability of the ribozyme to detect trigger RNA spiked into the RNA mixture at a range of concentrations. The ribozyme could detect its trigger in presence of at least 1000-fold excess competing RNA (Fig. 2G).

Functional RNA as cleavage products: CRISPR sgRNA and shRNA

To explore whether these ribozymes can be used for RNA context-specific gene regulation *in vivo*, the inventors engineered ribozymes encoding either a CRISPR single-guide RNA against GFP (sgRNA^{GFP}; Fig. 3A), a short hairpin RNA against GFP (shRNA^{GFP}; Fig. 3B), or an RNA aptamer (Broccoli, Fig. 3C) as the cleavage product. The inventors first carried out a cell-based screen to identify efficient sgRNAs; and found that three sgRNAs beginning with GUC, the canonical sequence at the hairpin ribozyme cleavage site (Fig. 1A), were inefficient at inducing editing (Fig. 11A), although they could be encoded within the ribozyme and released in a trigger-dependent manner (Fig. 11B). Hence, the inventors selected sgRNA GFP-149R, which is efficient (Fig. 11A) and previously characterised. The inventors first retained the conserved hairpin ribozyme sequences at the cleavage sites to test cleavage of these more complex products; hence, at the 5' and 3' ends of the sgRNA and shRNA, there were one to four nucleotides "leftover" that would be included in the cleavage product. Extra nucleotides at the 3' end

of the gRNA should not alter Cas9 targeting and DNA cleavage. While unpaired nucleotides at the 5' end of the sgRNA 20-nt protospacer sequence can inhibit DNA cleavage, these effects are context-dependent, and the sgRNA we chose can tolerate unpaired nucleotides at its 5' end. The inventors later found that this sgRNA can be made to be efficiently cleaved exactly without "leftover" nucleotides at the 5' end, despite beginning with GGG, by mutating the A7 in the WT cleavage loop to either U₇ or C₇ (Fig. 11C), to pair with G₊₃.

The inventors designed the shRNA ribozyme to resemble a primary microRNA, with single-stranded RNA (ssRNA) basal segments and double stranded (dsRNA) segments predicted to be a substrate for Drosha cleavage, continuous with a GFP^{shRNA}. This was achieved by designing and screening transcripts optimised with features important for microRNA processing⁴⁹, which also included 5' and 3' ribozyme scar sequences to enable subsequent embedding within the ribozyme. The inventors identified potential cleavage products that strongly decreased GFP expression. They then designed ribozymes that comprised the most potent cleavage product, shGFP6 (T-let-7f_CI-shRNA^{GFP6} (Fig. 7). Before cleavage, the ssRNA basal segments are base-paired with the ribozyme (Fig. 3B), and since single-stranded basal strands are essential for Drosha processing, uncleaved shRNA embedded in the ribozyme should not be processed for RNAi. After trigger-induced ribozyme self-cleavage, the cleavage product is released to become a potential substrate for Drosha, and to enter the miRNA maturation pathway. Since Drosha cleavage does not require specific conserved sequences, but rather "counts" from the junction of the ssRNA and dsRNA, the inventors hypothesised that incorporation of 5' and 3' scar nucleotides would not affect processing of the shRNA cleavage product. Finally, the inventors also designed and tested ribozymes that cleave out the fluorescent aptamer Broccoli (Fig. 3C).

The inventors chose as the trigger miRNA *let-7f*, as the *let-7* family is highly conserved, with let-7f one of its most abundant members (www.mirbase.org), allowing the inventors to test these ribozymes in both zebrafish embryos and human cell lines. T-let-7f_CI-sgRNA^{GFP}, T-let-7f_CI-shRNA^{GFP} (two different shRNA for GFP were tested for cleavage) and T-let-7f_CI-Broccoli were assayed for trigger-activated cleavage in cell-free assays. For all three ribozymes, substantially more cleavage product was released in presence of trigger than without (Figs. 3D-F). Therefore, these ribozymes can be triggered to release longer functional RNA with secondary structure, including sgRNA, shRNA and aptamers.

The let-7 miRNA family consists of 12 closely related members (Fig. 3G). We asked whether T-let-7f_CI-sgRNA^{GFP} could distinguish between them. T-let-7f_CI-sgRNA^{GFP} could distinguish between most let-7 family members that differed from let-7f by at least 3-nt (i.e., let-7b, let-7i and mir-98). Let-7d was not distinguishable despite differing by 3-nt, perhaps because the nucleotide at position 1 may have a weaker effect (Fig. 3G).

While cleavage product release from the ribozyme was dependent on presence of the trigger RNA, a substantial fraction of ribozymes cleaved at the site proximal to the catalytic domain in absence of trigger (Figs. 2, D-F), which could lead to leaky activity of the sgRNA or shRNA cleavage product *in vivo*. However, the distal cleavage site was not cleaved without trigger, and the inventors hypothesised that this stemmed from its increased distance from catalytic Loop B. To test this, the inventors lengthened Helix 2 of T-let-7f_CI-sgRNA^{GFP} from 4-bp to 8-bp or 12-bp (Fig. 3H). This substantially decreased trigger-independent cleavage at the proximal site (Fig. 3H). To ask if this was generalisable, the inventors similarly altered the T-let-7f_CI-shRNA^{GFP} ribozyme. This also reduced trigger-independent cleavage at the proximal site (Fig. 11D) Therefore, the length of Helix 2 can be tuned to refine cleavage regulation by the trigger RNA.

***In vivo* RNA signal transduction**

The inventors next asked whether these ribozymes can enable cell RNA context-dependent gene regulation *in vivo*. CRISPR-Cas9 editing can be carried out in zebrafish embryos by microinjection of RNA-Cas9 complexes. The T-let-7f_CI-sgRNA^{GFP} ribozyme can detect let-7a, c, d, e, f, g (Fig. 3E); let-7a, -c and -f account for 72% of these miRNAs in the early fish embryo (www.mirbase.org). One-cell injection of Cas9 protein with the positive control sgRNA^{GFP} led to high rates of genomic deletions, ~88% per embryo (Fig. 4A). Co-injection of T-let-7f_CI-sgRNA^{GFP} with Cas9 also led to robust editing, although lower than the gRNA alone, at ~29% per embryo ($p < 0.001$) (Fig. 4A). This provides evidence that the sgRNA-encoding ribozyme is stable *in vivo* and able to cause gene editing when delivered as RNA. To determine whether editing was let-7-dependent, the inventors co-expressed with the ribozyme a morpholino antisense inhibitor against let-7f. Co-injection of the inhibitor with the ribozyme reduced ribozyme-induced editing at the GFP locus from ~29% to ~12% ($p < 0.05$), providing support for gRNA function being let-7f-dependent (Fig. 4A).

The inventors next asked whether these ribozymes can function in human cells.

Expression of Durascribe-modified (50% of Cs and Us modified) T-let-7f_CI-shRNA^{GFP6}

ribozymes in HEK293-GFP cells with 2'MOE-modified let-7f led to a ~9% increase in GFP knockdown, while expression of 2'MOE-modified E-gen had no effect (Fig. 4B).

Similarly, expression of Durascribe-modified (50% of Cs modified) T-Egene20_CI-Stoplight_modC ribozymes in Stoplight cells (de Jong et al Nature Communications 2020) with 2'MOE-modified E-gene increased gene editing, while expression of 2'MOE-modified let-7f had no effect (Fig. 4C). Therefore regulation by the ribozyme is trigger-dependent in mammalian cells.

10 Sequence with A₇: (Note: This refers to Fig. 11, described above)

AACUAUACAAUACGGUAUAUUACCUGGUUUUCGAUCGAAAGAUCGACGA
GGUGAAAACCUCGUGACAGGGCACGGGCAGCUUGCCGGGUUUUAGAGCUAGAA
AUAGCAAGUUAUUAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG
UCGGUGCGUCAGUCCUGAUUUUCGAAUCAGAGAAGACUAUCCACCUUAAAUAG
15 GCAAGUGAGAAGUCAACCAGAGAAACACGACUACUACCUCA (SEQ ID NO: 473)

Sequence with C₇:

AACUAUACAAUACGGUAUAUUACCUGGUUUUCGAUCGAAAGAUCGACGA
GGUGAAAACCUCGUGACAGGGCACGGGCAGCUUGCCGGGUUUUAGAGCUAGAA
AUAGCAAGUUAUUAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG
20 UCGGUGCGUCAGUCCUGAUUUUCGAAUCAGAGAAGACUAUCCACCUUAAAUAG
GCAAGUGCGAAGUCAACCAGAGAAACACGACUACUACCUCA (SEQ ID NO: 474)

For clarity, the specific nucleotide sequences are known in the art by numerical positions based on the original hairpin ribozyme. Hence, in one example, A₇ and C₇ as mentioned herein are named based on that original hairpin ribozyme annotation, i.e. it (the location mentioned) does not depend on its specific position in a specific ribozyme, but rather this position refers to a nucleotide located in the bulge opposite the cleavage site in the canonical hairpin ribozyme. Shown in Fig. 1 is a sketch of the canonical hairpin ribozyme with an exemplary N₇ position indicated. One amendment disclosed herein which had an effect on cleavage efficacy is the change of the nucleotide identity of N₇ so that it will pair with nucleotide N₊₃ in the cleavage product. This is useful because it allows variance in the cleavage product sequence. So, exemplarily speaking, if nucleotide N₊₃ is a G in the cleavage product, then N₇ can be changed to a C (to pair with this G). But if N₊₃ is changed to an A, for example, then N₇ can be changed to a U (which pairs with A) to increase cleavage activity.

35

Disclosed herein are features of the ribozyme, whereby these features are shown to improve its signal to noise ratio, i.e. to decrease its background cleavage in absence of the target/trigger RNA, and increase its cleavage rate in presence of the target/trigger RNA. Preferably, any such modification should be applicable over a wide series of ribozymes. Towards this, the inventors tested several motifs for the communication module of the ribozyme, i.e. the region between the catalytic domain and the trigger arms (Fig. 1A). The inventors found that a 2-nt communication module was too short (Figs. 12B), while a 3-nt motif was generally preferred over the other tested lengths. Specifically, several 3-nt communication module dsRNA motifs, 5'-ACG/CGU-3' (SEQ ID NO: 1) and 5'-ACG/CGA-3' (SEQ ID NO: 2) were highly effective (Figs. 2D, 10C), as well as several other variants shown in Figs. 12A and 12B, especially (CM2: 5'-AUG/CGA -3') (SEQ ID NO: 450), (CM7: 5'- ACU/AGA -3') (SEQ ID NO: 455) and (CM8: 5'- AUG/CAA -3') (SEQ ID NO: 456), 5'- UAU/AUA -3' (SEQ ID NO: 454), and 5'- CU/AG-3' (SEQ ID NO: 457) (Figs. 12A, B). Therefore, changing the communication module to these variants could greatly improve the signal to noise ratio of the ribozyme.

Next, using these optimised communication modules, the inventors developed ribozymes that were triggered by a short RNA to release either an shRNA or a sgRNA against Green Fluorescent Protein (GFP), or a fluorescent aptamer (Figs. A-C). All of these ribozymes released the embedded shRNA , sgRNA or aptamer when triggered by RNA let-7f, at a high signal-to-noise ratio (Figs. 3D-F).

However, the inventors noticed that there was substantial background cleavage at the cleavage site that was proximal to the catalytic domain, with little to no background cleavage at the distal cleavage site. The inventors wondered whether the lower background cleavage at the distal site was due to its greater distance from the catalytic domain. Hence, the inventors asked whether extending the length of Helix 2 could decrease the level of background cleavage at the proximal site. Hence, the inventors extended Helix 2 from its normal 4-bp configuration to either 8-bp or 12-bp. This significantly decreased the background cleavage at the proximal site in absence of the trigger (Fig. 3H). Lengthening of Helix 2 to lengths 5-10 bp also had similar benefits (Fig. 13A). Therefore, lengthening of Helix 2 can decrease background cleavage in absence of the trigger.

The inventors also asked whether extension of Helix 3 could affect cleavage. They found that alteration of Helix 3 also dramatically decreased background cleavage in absence of trigger (Fig. 16).

Optimisations and other experimental data

1) *A new sequence motif in Helix 4 of the ribozyme that results in Rz with very high signal-to-noise ratio in presence of the trigger RNA, generalizable over many Rz*

Fig. 12A exemplified 10 additional motifs in Helix 4 of the ribozymes that has very high signal-to-noise ratio in the presence of the trigger RNA. These additional motifs include, but are not limited to (5'- ACG/UGA -3') (SEQ ID NO: 449), (5'- AUG/CGA -3') (SEQ ID NO: 450), (5'- AUG/UGA -3') (SEQ ID NO: 451), (5'- CG/CG -3') (SEQ ID NO: 452), (5'- UUG/UGG -3') (SEQ ID NO: 453), (5'- UAU/AUA -3') (SEQ ID NO: 454), (5'- ACU/AGA -3') (SEQ ID NO: 455), (5'- AUG/CAA -3') (SEQ ID NO: 456), (5'- CU/AG -3') (SEQ ID NO: 457), and (5'- UG/CA -3') (SEQ ID NO: 458).

Cleavage assays showed that at least 3 of these work as well as or better than SEQ ID1 and SEQ ID2 motifs (Fig. 12B) (CM2: 5'- AUG/CGA -3') (SEQ ID NO: 450), (CM7: 5'- ACU/AGA -3') (SEQ ID NO: 455) and (CM8: 5'- AUG/CAA -3') (SEQ ID NO: 456). Also, marginally, 5'- UAU/AUA -3' (SEQ ID NO: 454), and 5'- CU/AG-3' (SEQ ID NO: 457), which shows that a 2-nt Helix 4 can also work in some ribozyme contexts.

2) *A new, lengthened Helix 2 (we previously tested 8-nt and 12-nt), which reduces the background cleavage of the cleavage site proximal to the catalytic domain in absence of the trigger*

Fig. 13 shows the testing of additional modified Helix 2 lengths, from 5-10-nt, some of which also exhibit reduced background cleavage, e.g. 6-nt to 10-nt.

3) *Ribozymes with full complementarity exhibit increased cleavage-dependency of some functional ribozymes*

The inventors of the present disclosure made shRNA-releasing ribozymes that have increased complementarity between the cleavage product and ribozyme, including full complementarity (Fig. 14A,B, D2 design). These greatly improve the mild cleavage dependency of the original design (Fig. 14B). sgRNA-releasing ribozymes with increased complementarity between the cleavage product and ribozyme were also made and found to function (Fig. 15).

4) *Rz embedded with CRISPR sgRNA, shRNA and aptamers, that release them in presence of trigger RNA*

The inventors present examples of ribozymes embedded with shRNA and sgRNA. For shRNA, see Figs. 3, 12, 13, and 14. New sgRNA examples are in Figs. 3 and 15.

5 5) *Use of sgRNA and shRNA-releasing ribozymes in vivo and in vitro*, in zebrafish and mammalian cells, respectively.

Additional examples of shRNA and sgRNA-releasing ribozymes are listed in the Table 1 above. The functional efficacy of shRNA and sgRNA ribozymes in cells and in vivo are demonstrated in Figures 4A-C.

10 APPLICATIONS

Embodiments of the methods disclosed herein provide a sensitive, low to no background, specific, and functional ribozyme.

15 Advantageously, ribozymes as disclosed herein includes one or more surprising improvements including: 1) new sequence motifs in the Helix 4 communication module of the ribozyme that result in ribozymes with very low background cleavage in absence of the target/trigger RNA, and high cleavage rates in presence of the target/trigger RNA, applicable over a series of ribozymes, 2) new lengthened Helix 2 domains, which reduce
20 the background cleavage of the cleavage site proximal to the catalytic domain, 3) ribozymes with full complementarity between the releasable cleavage product and its complementary strand, to reduce background cleavage product release, 4) a mutation of nucleotide N₇ to pair with nucleotide N₊₃, to increase cleavage when the sequence at the cleavage site deviates from canonical cleavage site sequences, and 5) new
25 lengthened Helix 3 domains, which reduce the background cleavage of the cleavage site proximal to the catalytic domain.

Even more advantageously, the ribozymes as disclosed herein have very low background cleavage and leakiness that enable them to be useful for various cell-free, *in vitro* and *in vivo* applications.

30 The ribozymes as disclosed herein may be advantageously designed to comprise functional RNA such as, but not limited to, single guide RNA (sgRNA), short hairpin RNA (shRNA), or an RNA aptamer, and the like. Therefore, the ribozymes as disclosed herein are capable of releasing functional RNA in the presence of trigger RNA.

The ribozymes as disclosed herein are also advantageously capable of releasing functional RNA with secondary structure, such as, but not limited to, single guide RNA (sgRNA), short hairpin RNA (shRNA), an RNA aptamer, and the like.

The ribozymes as disclosed herein may be used *in vitro* and *in vivo* (such as
5 as exemplified in zebrafish and mammalian cells) to elicit gene knockdown (when in the case of shRNA ribozyme) or gene editing (in the case of sgRNA ribozyme) when in the presence of trigger RNA. Therefore, the ribozymes as disclosed herein may also be used for gene regulation, with the output dependent on the type of functional RNA encoded as the releasable cleavage product.

10 Disclosed herein are features of the ribozyme, whereby these features are shown to improve its signal-to-noise ratio, i.e. to decrease its background cleavage in absence of the target/trigger RNA, and increase its cleavage rate in presence of the target/trigger RNA. Preferably, any such modification should be applicable over a wide series of ribozymes.

15 It will be appreciated by a person skilled in the art that other variations and/or modifications may be made to the embodiments disclosed herein without departing from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like
20 across different exemplary embodiments. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

CLAIMS

1. A ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently 1 nucleotide or, 2, 3 or 4 nucleotides in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain; motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

2. The ribozyme of claim 1, wherein the linker between motifs [C] and [D] is selected from the group consisting of two-way junction, three-way junction, four-way junction, a stem, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges and combinations thereof.

3. The ribozyme of claim 1 or 2, wherein the linker between motifs [C] and [D] comprises a three-way junction and a stem.

4. The ribozyme of claim 3, wherein the stem sequence connecting the junction to motif [D] is 4 to 12 nucleotides in length.

5. The ribozyme of claim 3, wherein the stem sequence connecting motif [C] and [c] to the junction is 4 to 12 nucleotides in length.

6. The ribozyme of any one of the preceding claims, wherein the trigger nucleic acid molecule comprises a region that is complementary to the trigger-binding domain, wherein said region is more than 10 nucleotides in length, optionally the one or more trigger -binding domains are for binding the same trigger nucleic acid molecule.

7. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment is 6 to 150 nucleotides in length.

8. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment comprises a sequence that is identical to at least one of the one or more trigger RNA molecules.

9. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment is a functional RNA selected from the group consisting of single-guide RNA (sgRNA), guide RNA (gRNA), short hairpin RNA (shRNA), and RNA aptamer.

10. The ribozyme of any one of the preceding claims, wherein motifs [B] and [b] are independently 3 nucleotides in length.

11. The ribozyme of any one of the preceding claims, wherein motifs [B] and [b] has a sequence selected from the group consisting of SEQ ID NO: 1 (5'-ACG/CGU-3'), SEQ ID NO: 2 (5'-ACG/CGA-3'), SEQ ID NO: 449 (5'- ACG/UGA -3'), SEQ ID NO: 450 (5'- AUG/CGA -3'), SEQ ID NO: 451 (5'- AUG/UGA -3'), SEQ ID NO: 452 (5'- CG/CG -3'), SEQ ID NO: 453 (5'- UUG/UGG -3'), SEQ ID NO: 454 (5'- UAU/AUA -3'), SEQ ID NO: 455 (5'- ACU/AGA -3'), SEQ ID NO: 456 (5'- AUG/CAA -3'), SEQ ID NO: 457 (5'- CU/AG -3'), and SEQ ID NO: 458 (5'- UG/CA -3').
12. The ribozyme of any one of the preceding claims, wherein if motifs [e] and [E] are partially complementary to each other, the complementarity between motif [e] and [E] is characterised by alternating regions of complementarity and regions of non-complementarity.
13. The ribozyme of any one of the preceding claims, wherein motif [D] comprises a mutation of nucleotide N₇ to pair with nucleotide N₊₃.
14. The ribozyme of any one of the preceding claims, wherein the optional linker regions individually or collectively form one or more secondary structures, optionally the one or more secondary structures are selected from the group consisting of: single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges, stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots, symmetrical internal loops, asymmetrical internal loops, three stem junctions (3-way junctions), four stem junctions (4-way junctions), two-stem junctions (2-way junctions) or coaxial stacks or combinations thereof.
15. The ribozyme of any one of the preceding claims, wherein the ribozyme complex comprises the sequences of any one or more of SEQ ID NOs: 3 to SEQ ID NOs: 448.
16. The ribozyme of any one of the preceding claims, wherein the ribozyme further comprises one or more modification.
17. The ribozyme of any one of the preceding claims, wherein the trigger nucleic acid comprises one or more modified nucleotide.

18. A method of detecting presence of a target/trigger nucleic acid molecule in a sample, wherein the method comprises:

incubating the sample with a ribozyme according to any of the claims 1 to 17 at temperature T1 which allows the binding of the target/trigger nucleic acid molecule with one or more target/trigger -binding domains comprised in the ribozyme;

incubating the sample at temperature T2 which allows the nucleic acid molecule and the RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme.

19. A method of detecting presence of a sequence or mutation of interest on a nucleic acid of interest in a sample, wherein the method comprises:

incubating the sample with a ribozyme according to any of the claims 1 to 17, thereby allowing binding of the nucleic acid molecule of interest with one or more target/trigger-binding domains comprised in the ribozyme;

incubating the sample which allows the nucleic acid molecule and a releasable RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme;

wherein the releasable RNA segment is an sgRNA or shRNA; wherein detection of the sequence or mutation of interest in the sample results in a signal being generated.

20. The ribozyme of any one of claims 1 to 17 or the method of any one of claims 18 to 19, wherein the trigger nucleic acid molecule is a genome of a virus, or a fragment thereof.

21. A kit comprising the ribozyme of any one of claims 1 to 17 or 20.