

TREM COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/009,669, filed on April 14, 2020, the entire contents of which is hereby incorporated by reference.

BACKGROUND

Transfer RNAs (tRNAs) are complex, naturally occurring RNA molecules that possess a number of functions including initiation and elongation of proteins.

SUMMARY

The present disclosure features modified tRNA-based effector molecules (TREMs, e.g., a TREM or TREM fragment), as well as related compositions and uses thereof. As provided herein, TREMs are complex molecules which can mediate a variety of cellular processes. The TREMs disclosed herein comprise at least one modification (e.g., a non-naturally occurring modification), e.g., on a component nucleotide (e.g., a nucleobase or sugar) or within an internucleotide region, e.g., the TREM backbone. In one aspect, the present disclosure provides a tRNA-based effector molecule (TREM) comprising a sequence of Formula A:

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH

Domain]-[L4]-[ASt Domain2], wherein:

independently, [L1] and [VL Domain], are optional;

[L1], [L2], [L3] and [L4] are linkers, [ASt Domain1] and [ASt Domain2] are acceptor stem domains, the [DH Domain] is a dihydrouridine domain, the [ACH Domain] is a domain that contains an anticodon that binds a respective codon in an mRNA, the [VL Domain] is a variable loop domain, and the [TH Domain] is a thymine hairpin domain;

one of [L1], [ASt Domain1], [L2]-[DH Domain], [L3], [ACH Domain], [VL Domain], [TH Domain], [L4], and [ASt Domain2] comprises a nucleotide having a non-naturally occurring modification,

the non-naturally occurring modification is present on a nucleotide within the [ASt Domain2] and

the TREM is capable of (i) mediating acceptance of an amino acid, (ii) transfer of the amino acid in the initiation or elongation of a polypeptide chain, and/or (iii) readthrough of a premature termination codon.

5 In one aspect, the present disclosure provides a pharmaceutical composition comprising a TREM of the disclosure.

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10 In one example, provided herein is a TREM comprising a sequence of Formula A: [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2], wherein independently, [L1] and [VL Domain], are optional; and one of [L1], [ASt Domain1], [L2]-[DH Domain], [L3], [ACH Domain], [VL Domain], [TH Domain], [L4], and [ASt Domain2] comprises a nucleotide comprising a non-naturally occurring modification.

15 In an example, the TREM: (a) has the ability to: (i) support protein synthesis, (ii) be charged by a synthetase, (iii) be bound by an elongation factor, (iv) introduce an amino acid into a peptide chain, (v) support elongation, or (vi) support initiation; (b) comprises at least X contiguous nucleotides without a non-naturally occurring modification, wherein X is greater than 3, 4, 5, 6, 7, 8, 9, or 10; (c) comprises at least 3, but less than all of the nucleotides of a type (e.g.,
20 A, T, C, G or U) comprise the same non-naturally occurring modification; (d) comprises at least X nucleotides of a type (e.g., A, T, C, G or U) that do not comprise a non-naturally occurring modification, wherein X=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48,

49 or 50; (e) comprises no more than 5, 10, or 15 nucleotides of a type (e.g., A, T, C, G or U) that comprise a non-naturally occurring modification; and/or (f) comprises no more than 5, 10, or 15 nucleotides of a type (e.g., A, T, C, G or U) that do not comprise a non-naturally occurring modification.

5 In an example, the TREM comprises feature (a)(i). In an example, the TREM comprises feature (a)(ii). In an example, the TREM comprises feature (a)(iii). In an example, the TREM comprises feature (a)(iv). In an example, the TREM comprises feature (a)(v). In an example, the TREM comprises feature (a)(vi). In an example, the TREM comprises feature (b). In an example, the TREM comprises feature (c). In an example, the TREM comprises feature (d). In an example, the TREM comprises feature (e). In an example, the TREM comprises feature (f). In an example, the TREM comprises all of features (a)-(f) or a combination thereof.

In an example, the TREM Domain comprising the non-naturally occurring modification has a function, e.g., a domain function described herein.

15 In an example, provided herein is a TREM core fragment comprising a sequence of Formula B:

$[L1]_y-[ASt\ Domain1]_x-[L2]_y-[DH\ Domain]_y-[L3]_y-[ACH\ Domain]_x-[VL\ Domain]_y-[TH\ Domain]_y-[L4]_y-[ASt\ Domain2]_x,$

wherein $x=1$ and $y=0$ or 1 ; and one of [ASt Domain1], [ACH Domain], and [ASt Domain2] comprises a nucleotide having a non-naturally occurring modification.

20 In an example, the TREM has the ability to support protein synthesis. In an example, the TREM has the ability to be able to be charged by a synthetase. In an example, the TREM has the ability to be bound by an elongation factor. In an example, the TREM has the ability to introduce an amino acid into a peptide chain. In an example, the TREM has the ability to support elongation. In an example, the TREM has the ability to support initiation.

25 In an example, the [ASt Domain 1] and/or [ASt Domain 2] comprising the non-naturally occurring modification has the ability to initiate or elongate a polypeptide chain.

In an example, the [ACH Domain] comprising the non-naturally occurring modification has the ability to mediate pairing with a codon.

In an example, y=1 for any one, two, three, four, five, six, all or a combination of [L1], [L2], [DH Domain], [L3], [VL Domain], [TH Domain], [L4].

5 In an example, y=0 for any one, two, three, four, five, six, all or a combination of [L1], [L2], [DH Domain], [L3], [VL Domain], [TH Domain], [L4].

In an example, y=1 for linker [L1], and L1 comprises a nucleotide having a non-naturally occurring modification.

In an example, y=1 for linker [L2], and L2 comprises a nucleotide having a non-naturally occurring modification.

10 In an example, y=1 for [DH Domain (DHD)], and DHD comprises a nucleotide having a non-naturally occurring modification. In an example, the DHD comprising the non-naturally occurring modification has the ability to mediate recognition of aminoacyl-tRNA synthetase.

In an example, y=1 for linker [L3], and L3 comprises a nucleotide having a non-naturally occurring modification.

15 In an example, y=1 for [VL Domain (VLD)], and VLD comprises a nucleotide having a non-naturally occurring modification.

In an example, y=1 for [TH Domain (THD)], and THD comprises a nucleotide having a non-naturally occurring modification. In an example, the THD comprising the non-naturally occurring modification has the ability to mediate recognition of the ribosome.

20 In an example, y=1 for linker [L4], and L4 comprises a nucleotide having a non-naturally occurring modification.

In another example, the disclosure provides a TREM fragment comprising a portion of a TREM, wherein the TREM comprises a sequence of Formula A:

25 [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2], and wherein the TREM fragment comprises a non-naturally occurring modification.

30 In an example, the TREM fragment comprises one, two, three or all or any combination of the following: (a) a TREM half (*e.g.*, from a cleavage in the ACH Domain, *e.g.*, in the anticodon sequence, *e.g.*, a 5' half or a 3' half); (b) a 5' fragment (*e.g.*, a fragment comprising the 5' end, *e.g.*, from a cleavage in a DH Domain or the ACH Domain); (c) a 3' fragment (*e.g.*, a fragment comprising the 3' end, *e.g.*, from a cleavage in the TH Domain); or (d)

an internal fragment (*e.g.*, from a cleavage in any one of the ACH Domain, DH Domain or TH Domain).

In an example, the TREM fragment comprise (a) a TREM half which comprises a nucleotide having a non-naturally occurring modification.

5 In an example, the TREM fragment comprise (b) a 5' fragment which comprises a nucleotide having a non-naturally occurring modification.

In an example, the TREM fragment comprise (c) a 3' fragment which comprises a nucleotide having a non-naturally occurring modification.

10 In an example, the TREM fragment comprise (d) an internal fragment which comprises a nucleotide having a non-naturally occurring modification.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM Domain comprises a plurality of nucleotides each having a non-naturally occurring modification. In an example, the non-naturally occurring modification comprises a nucleobase modification, a sugar (*e.g.*, ribose) modification, or a backbone
15 modification. In an example, the non-naturally occurring modification is a sugar (*e.g.*, ribose) modification. In an example, the non-naturally occurring modification is 2'-ribose modification, *e.g.*, a 2'-OMe, 2'-halo (*e.g.*, 2'-F), 2'-MOE, or 2'-deoxy modification. In an example, the non-naturally occurring modification is a backbone modification, *e.g.*, a phosphorothioate modification.

20 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM sequence comprises a CCA sequence on a terminus, *e.g.*, the 3' terminus. In an example, the TREM sequence does not comprise a CCA sequence on a terminus, *e.g.*, the 3' terminus.

25 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a modification in a base or a backbone of a nucleotide, *e.g.*, a modification chosen from any one of Tables 5, 6, 7, 8 or or 9.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 5.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 6.

5 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 7.

10 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a backbone modification chosen from a modification listed in Table 8.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a backbone modification chosen from a modification listed in Table 9.

15 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 1, e.g., any one of SEQ ID NOs 1-451.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a consensus sequence chosen from any one of SEQ ID NOs: 562-621.

20 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in any one of Tables 15-22, e.g., any one of SEQ ID NOs: 622-1187. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 15, e.g., any one of SEQ ID NOs: 622-698. In an example, the TREM, TREM core
25 fragment, or TREM fragment is encoded by a sequence provided in Table 16, e.g., any one of SEQ ID NOs: 699-774. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 17, e.g., any one of SEQ ID NOs: 775-841. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 18, e.g., any one of SEQ ID NOs: 842-917. In an example, the TREM, TREM
30 core fragment, or TREM fragment is encoded by a sequence provided in Table 19, e.g., any one of SEQ ID NOs: 918-992. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 20, e.g., any one of

SEQ ID NOs: 993-1078. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 21, e.g., any one of SEQ ID NOs: 1079-1154. In an example, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 22, e.g., any one of SEQ ID NOs: 1155-1187.

5 In another example, the disclosure provides a pharmaceutical composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein.

In another example, the disclosure provides a method of making a TREM, a TREM core fragment, or a TREM fragment disclosed herein, comprising linking a first nucleotide to a second nucleotide to form the TREM.

10 In an example, the TREM, TREM core fragment or TREM fragment is non-naturally occurring (e.g., synthetic).

In an example, the TREM, TREM core fragment or TREM fragment is made by cell-free solid phase synthesis.

15 In another example, the disclosure provides a method of modulating a tRNA pool in a cell comprising: providing a TREM, a TREM core fragment, or a TREM fragment disclosed herein, and contacting the cell with the TREM, TREM core fragment or TREM fragment, thereby modulating the tRNA pool in the cell.

20 In an example, the disclosure provides a method of contacting a cell, tissue, or subject with a TREM, a TREM core fragment, or a TREM fragment disclosed herein, comprising: contacting the cell, tissue or subject with the TREM, TREM core fragment or TREM fragment, thereby contacting the cell, tissue, or subject with the TREM, TREM core fragment or TREM fragment.

25 In another example, the disclosure provides a method of delivering a TREM, TREM core fragment or TREM fragment to a cell, tissue, or subject, comprising: providing a cell, tissue, or subject, and contacting the cell, tissue, or subject, a TREM, a TREM core fragment, or a TREM fragment disclosed herein.

In an example, the disclosure provides a method of modulating a tRNA pool in a cell comprising an endogenous open reading frame (ORF), which ORF comprises a codon having a first sequence, comprising:

30 optionally, acquiring knowledge of the abundance of one or both of (i) and (ii), e.g., acquiring knowledge of the relative amounts of: (i) and (ii) in the cell, wherein (i) is a tRNA moiety having an anticodon that pairs with the codon of the ORF having a first sequence (the

first tRNA moiety) and (ii) is an isoacceptor tRNA moiety having an anticodon that pairs with a codon other than the codon having the first sequence (the second tRNA moiety) in the cell;

contacting the cell with a TREM, a TREM core fragment, or a TREM fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with: the codon having the first sequence; or the codon other than the codon having the first sequence, in an amount and/or for a time sufficient to modulate the relative amounts of the first tRNA moiety and the second tRNA moiety in the cell, thereby modulating the tRNA pool in the cell.

In another example, the disclosure provides a method of modulating a tRNA pool in a subject having an ORF, which ORF comprises a codon having a first sequence, comprising:

optionally, acquiring knowledge of the abundance of one or both of (i) and (ii), e.g., acquiring knowledge of the relative amounts of: (i) and (ii) in the subject, wherein (i) is a tRNA moiety having an anticodon that pairs with the codon of the ORF having a first sequence (the first tRNA moiety) and (ii) is an isoacceptor tRNA moiety having an anticodon that pairs with a codon other than the codon having the first sequence (the second tRNA moiety) in the subject;

contacting the subject with a TREM, a TREM core fragment, or a TREM fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with: the codon having the first sequence; or the codon other than the codon having the first sequence, in an amount and/or for a time sufficient to modulate the relative amounts of the first tRNA moiety and the second tRNA moiety in the subject, thereby modulating the tRNA pool in the subject.

In an example, the disclosure provides a method of modulating a tRNA pool in a subject having an endogenous ORF comprising a codon comprising a synonymous mutation (a synonymous mutation codon or SMC), comprising:

providing a composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment comprises an isoacceptor tRNA moiety comprising an anticodon sequence that pairs with the SMC (the TREM);

contacting the subject with the composition in an amount and/or for a time sufficient to modulate the tRNA pool in the subject, thereby modulating the tRNA pool in the subject.

In another example, the disclosure provides a method of modulating a tRNA pool in a cell comprising an endogenous ORF comprising a codon comprising a SMC, comprising:
providing a composition comprising a TREM, a TREM core fragment, or a TREM
5 fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment comprises an isoacceptor tRNA moiety comprising an anticodon sequence that pairs with the SMC (the TREM);

contacting the cell with the composition comprising a TREM in an amount and/or for a time sufficient to modulate the tRNA pool in the cell,
10 thereby modulating the tRNA pool in the cell.

In an example, the disclosure provides a method of modulating expression of a protein in a cell, wherein the protein is encoded by a nucleic acid comprising an ORF, which ORF comprises a codon having a mutation, comprising:

contacting the cell with a composition comprising a TREM, a TREM core fragment, or a
15 TREM fragment disclosed herein in an amount and/or for a time sufficient to modulate expression of the encoded protein,

wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the codon having the mutation,

thereby modulating expression of the protein in the cell.

20 In another example, the disclosure provides a method of modulating expression of a protein in a subject, wherein the protein is encoded by a nucleic acid comprising an endogenous ORF, which ORF comprises a codon having a mutation, comprising:

contacting the subject with a composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein, in an amount and/or for a time sufficient to modulate
25 expression of the encoded protein,

wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the codon having the mutation,

thereby modulating expression of the protein in the subject.

30 In an example of any of the methods disclosed herein, the mutation in the ORF is a nonsense mutation, e.g., resulting in a premature stop codon chosen from UAA, UGA or UAG.

In an example, the stop codon is UAA. In an example, the stop codon is UGA. In an example, the stop codon is UAG.

In an example of any of the methods disclosed herein, the TREM comprises an anticodon that pairs with a stop codon.

5 TREMs of the disclosure include TREMs, TREM core fragments and TREM fragments. TREMs, TREM core fragments or TREM fragments can be modified with non-naturally occurring modifications to, e.g., increase the level and/or activity (e.g., stability) of the TREM. Pharmaceutical TREM compositions, e.g., comprising TREMs having a non-naturally occurring modification, can be administered to cells, tissues or subjects to modulate these functions, *e.g., in*
10 *vitro* or *in vivo*. Disclosed herein are TREMs, TREM core fragments or TREM fragments comprising non-naturally occurring modifications, TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using the same.

Additional features of any of the aforesaid TREMs, TREM core fragments, TREM fragments, TREM compositions, preparations, methods of making TREM compositions and
15 preparations, and methods of using TREM compositions and preparations include one or more of the features in the Enumerated Embodiments, Figures, Description, Examples, or Claims.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following Enumerated
20 Embodiments, Figures, Description, Examples, or Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustrating the activity (log2 fold change) of modified TREMs containing a 2'-OMe, 2'-F, 2'-OME, 2'-deoxy, and PS modification at each position along an exemplary TREM sequence (TREM-Arg-TGA) over an unmodified TREM, as outlined in
25 Example 11.

ENUMERATED EMBODIMENTS

Enumerated Embodiments I

30 1. A TREM comprising a sequence of Formula A:

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2],
wherein:

What is claimed is:

1. A tRNA-based effector molecule (TREM) comprising a sequence of Formula A:

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2], wherein:

independently, [L1] and [VL Domain], are optional;

[L1], [L2], [L3] and [L4] are linkers, [ASt Domain1] and [ASt Domain2] are acceptor stem domains, the [DH Domain] is a dihydrouridine domain, the [ACH Domain] is a domain that contains an anticodon that binds a respective codon in an mRNA, the [VL Domain] is a variable loop domain, and the [TH Domain] is a thymine hairpin domain;

one of [L1], [ASt Domain1], [L2]-[DH Domain], [L3], [ACH Domain], [VL Domain], [TH Domain], [L4], and [ASt Domain2] comprises a nucleotide having a non-naturally occurring modification,

the non-naturally occurring modification is present on a nucleotide within the [ASt Domain2] and

the TREM is capable of (i) mediating acceptance of an amino acid, (ii) transfer of the amino acid in the initiation or elongation of a polypeptide chain, and/or (iii) readthrough of a premature termination codon.

2. The TREM of claim 1, wherein the non-naturally occurring modification is present on the 2'-position of a nucleotide sugar or within the internucleotide region.

3. The TREM of claim 2, wherein the non-naturally occurring modification is selected from a 2-O-methyl (2-OMe), 2'-halo, 2'-O-methoxyethyl (2'MOE), or 2'deoxy modification.

4. The TREM of claim 3, wherein the non-naturally occurring modification is a 2'OMe modification.

5. The TREM of claim 3, wherein the non-naturally occurring modification is a 2'halo modification.

6. The TREM of claim 3, wherein the non-naturally occurring modification is a 2'MOE modification.
7. The TREM of claim 3, wherein the non-naturally occurring modification is a 2'-deoxy modification.
8. The TREM of claim 2, wherein the non-naturally occurring modification is present in the internucleotide region.
9. The TREM of claim 8, wherein the non-naturally occurring modification is a phosphorothioate modification.
10. The TREM of claim 2, wherein the TREM further comprises a non-naturally occurring modification on or within any one of nucleotides 1-76 of SEQ ID NO: 622, nucleotides 1-85 of SEQ ID NO: 993, or nucleotides 1-75 of SEQ ID NO: 1079.
11. The TREM of claim 2, wherein the TREM further comprises a non-naturally occurring modification on or within any one of nucleotides 1-76 of SEQ ID NO: 622.
12. The TREM of claim 10, wherein the TREM further comprises a non-naturally occurring modification on or within a nucleotide in the [ASt Domain1].
13. The TREM of claim 10, wherein the TREM further comprises a non-naturally occurring modification on or within a nucleotide in the [DH Domain], [ACH Domain], [VL Domain], or [TH Domain].
14. The TREM of claim 10, wherein the TREM further comprises a non-naturally occurring modification within a linker domain.

15. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 1, 2, 4, 6, 10, 12, 13, 17, 18, 20, 22, 29, 30, 42, 43, 45, 50, 52, 56, 59, 61, 65, 66, 68, 69, 71, 72, and 73 of SEQ ID NO: 622.

16. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 20, 29, 33, 40, 41, 44, 45, 48, 49, 50, 52, 53, 54, 56, 59, 61, 62, 63, 65, 67, 68, 69, 71, 72, 75, and 76 of SEQ ID NO: 622.

17. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 1, 4, 14, 15, 16, 17, 20, 29, 44, 45, 47, 49, 50, 52, 54, 56, 57, 59, 65, 72, and 73 of SEQ ID NO: 622.

18. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 3, 4, 5, 6, 14, 15, 16, 20, 22, 23, 33, 54, 59, 62, 63, 72, and 76 of SEQ ID NO: 622.

19. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 1, 2, 3, 9, 14, 15, 16, 17, 18, 19, 20, 21, 35, 37, 38, 44, 45, 46, 52, 54, 55, 56, 57, 58, 73, and 74 of SEQ ID NO: 622.

20. The TREM of claim 1, wherein the TREM comprises a non-naturally occurring modification on or within any one of nucleotides 1, 17, 18, 20, 29, 30, 50, 52, and 73 of SEQ ID NO: 622.

21. A pharmaceutical composition comprising a TREM of claim 1.

22. The pharmaceutical composition of claim 21, further comprising a pharmaceutically acceptable component.

23. A lipid nanoparticle formulation comprising a TREM of claim 1.

24. A lipid nanoparticle formulation comprising a pharmaceutical composition of claim 21.