

COMPOSITIONS AND METHODS FOR ALTERING A NUCLEOBASE IN A TRANSTHYRETIN POLYNUCLEOTIDE

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 The present application claims priority to U.S. Provisional Application No. 63/624,030, filed January 23, 2024, the entire contents of which are hereby incorporated by reference in its entirety.

SEQUENCE LISTING

- 10 This application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The Sequence Listing XML file, created on January 13, 2025, is named 180802-056202PCT_SL.xml, and is 1,550,753 bytes in size.

BACKGROUND

- 15 Amyloidosis is a condition characterized by the buildup of abnormal deposits of amyloid protein in the body's organs and tissues. These protein deposits can occur in the peripheral nervous system, which is made up of nerves connecting the brain and spinal cord to muscles and sensory cells that detect sensations such as touch, pain, heat, and sound.
- 20 Protein deposits in these nerves can result in a loss of sensation in the extremities (peripheral neuropathy). The autonomic nervous system, which controls involuntary body functions, such as blood pressure, heart rate, and digestion, can also be affected by amyloidosis. In some cases, the brain and spinal cord (central nervous system) are affected. Mutations in the transthyretin (TTR) gene are associated with transthyretin amyloidosis. Furthermore, patients
- 25 expressing wild-type TTR may also develop amyloidosis. Liver transplant remains the gold standard for treating transthyretin amyloidosis. However, there are a limited number of organ donors, and patients may wait years for an available organ. Accordingly, there is a need for compositions and methods for treating amyloidosis.

SUMMARY

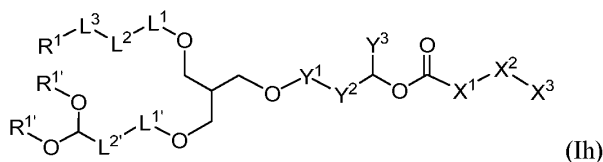
- 30 Provided herein are compositions for modifying or editing a polynucleotide (e.g., DNA, RNA, genomic DNA, gene) and methods of using the same to treat or prevent conditions associated with the extracellular deposition in various tissues of amyloid fibrils formed by the aggregation of misfolded transthyretin (TTR) proteins. Such conditions

include, but are not limited to, polyneuropathy due to hereditary transthyretin amyloidosis (hATTR-PN) and hereditary cardiomyopathy due to transthyretin amyloidosis (hATTR-CM), both of which are associated with autosomal dominant mutations of the *TTR* gene, and an age-related cardiomyopathy associated with wild-type TTR proteins (ATTRwt), also known as senile cardiac amyloidosis. Compositions and methods for editing TTR polynucleotides using base editor systems are disclosed herein.

In one aspect, the disclosure features a lipid nanoparticle (LNP) containing components A), B), and C). Component A) is a guide RNA, or a polynucleotide encoding the guide RNA, where the guide RNA contains a spacer containing a nucleotide sequence selected from GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472) and those listed in any of Tables 1B, 1C, 1D, 1E, 2A, 2B, 2D, or 2E. Component B) is an mRNA molecule, or a polynucleotide encoding the mRNA molecule, where the mRNA molecule encodes a base editor polypeptide containing a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain. The adenosine deaminase domain contains an amino acid sequence having at least 90% identity to the following TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1). The adenosine deaminase also contains a combination of amino acid alterations relative to the TadA*7.10 amino acid sequence selected from one or more of: a) I76Y, V82T, Y123H, Y147R, and Q154R; b) V82T, Y123H, D147R, and Q154R; c) V82T, Y123H, D147T, and Q154S; d) V82T and Q154R; e) V82T, Y147T, and Q154S; and f) I76Y, V82T, Y123H, Y147T, and Q154S. Component C) is an ionizable lipid according to any one of the following formulas, or a pharmaceutically acceptable salt thereof:

i) a compound of Formula (Ih):



where:

each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-OC(O)-$;

each L^2 and $L^{2'}$ is independently an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

L^3 is a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

R^1 is optionally substituted C_{1-20} aliphatic, $\text{---}L^{CyA}\text{---}Cy^A$, or $\text{---}L^{Ra}\text{---}\begin{array}{c} O-R^a \\ | \\ O-R^a \end{array}$;

- 5 L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

- 10 L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

each R^a and $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic;

Y^1 is $-C(O)-$ or $-C(O)O-$;

Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain;

- 15 Y^3 is optionally substituted C_{1-20} aliphatic;

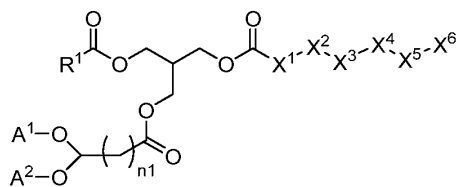
X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

- 20 X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

- 25 each R is independently hydrogen or optionally substituted C_{1-6} aliphatic;

ii) a compound of Formula (Ia):



Ia

where:

R¹ is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation;

X¹ and X² are each independently absent or selected from -O-, -NR²- and $\begin{array}{c} \text{-(CH)}_a\text{-} \\ | \\ \text{X}^7 \end{array}$, where each R² is independently hydrogen or C₁-C₆ alkyl; each a is independently an integer between 1 and 6;

- 5 X³ and X⁴ are each independently absent or selected from one or more of: 4- to 8-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered aryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 4- to 7-membered cycloalkyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, -O- and -NR³-, where each R³ is a independently
10 a hydrogen atom or C₁-C₆ alkyl and where X¹-X²-X³-X⁴ does not contain any oxygen-oxygen, oxygen-nitrogen or nitrogen-nitrogen bonds;

X⁵ is -(CH₂)_b-, where b is an integer between 0 and 6;

- X⁶ is hydrogen, C₁-C₆ alkyl, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, or -NR⁴R⁵, where R⁴ and R⁵ are each independently hydrogen or C₁-C₆
15 alkyl; or alternatively R⁴ and R⁵ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, where the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;

- each X⁷ is independently hydrogen, hydroxyl or -NR⁶R⁷, where R⁶ and R⁷ are each
20 independently hydrogen or C₁-C₆ alkyl; or alternatively R⁶ and R⁷ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, where the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;

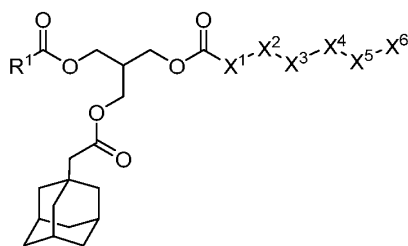
at least one of X¹, X², X³, X⁴, and X⁵ is present;

- 25 A¹ and A² are each independently selected from one or more of: C₅-C₁₂ haloalkyl, C₅-C₁₂ alkenyl, C₅-C₁₂ alkynyl, (C₅-C₁₂ alkoxy)-(CH₂)_{n2}-, (C₅-C₁₀ aryl)-(CH₂)_{n3}- optionally ring substituted with one or two halo, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ alkoxy groups, and (C₃-C₈ cycloalkyl)-(CH₂)_{n4}- optionally ring substituted with 1 or 2 C₁-C₆ alkyl groups; or
30 alternatively A¹ and A² join together with the atoms to which they are bound to form a 5- to 6-membered cyclic acetal substituted with 1 or 2 C₄-C₁₀ alkyl groups;

n₁, n₂ and n₃ are each individually an integer between 1 and 4; and

n₄ is an integer between zero and 4;

iii) A compound of Formula (Ib):



Ib

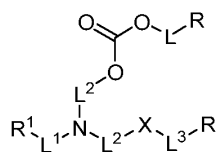
where:

R¹ is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation;

- 5 X¹ and X² are each independently absent or selected from -O-, NR², and $-(\text{CH})_a-$, where R² is C₁-C₆ alkyl, and where X¹ and X² are not both -O- or NR²; a is an integer between 1 and 6;
- X³ and X⁴ are each independently absent or selected from one or more of: 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, and -NR³-, where each R³ is a hydrogen atom or C₁-C₆ alkyl;
- 10 X⁵ is -(CH₂)_b-, where b is an integer between 0 and 6;
- X⁶ is hydrogen, C₁-C₆ alkyl, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, or -NR⁴R⁵, where R⁴ and R⁵ are each independently hydrogen or C₁-C₆ alkyl;
- 15 or alternatively R⁴ and R⁵ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, where the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;
- X⁷ is hydrogen or -NR⁶R⁷, where R⁶ and R⁷ are each independently hydrogen or C₁-C₆ alkyl;
- 20 or alternatively R⁶ and R⁷ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, where the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;
- at least one of X¹, X², X³, X⁴, and X⁵ is present; and

- 25 provided that when either X¹ or X² is -O-, neither X³ nor X⁴ is , and when either X¹ or X² is -O-, R⁴ and R⁵ are not both ethyl;

iv) A compound of Formula (Ic):



Ic

or its N-oxide, or a salt thereof, where

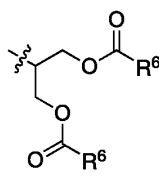
L1 is C1-6 alkylenyl, or C₂₋₆ heteroalkylenyl;

- 5 each L² is independently C₂₋₁₀ alkylenyl, or C₃₋₁₀ heteroalkylenyl;

L is absent, C₁₋₁₀ alkylenyl, or C₂₋₁₀ heteroalkylenyl;

L³ is absent, C₁₋₁₀ alkylenyl, or C₂₋₁₀ heteroalkylenyl;

X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;

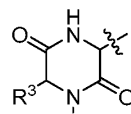
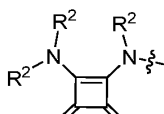


each R is independently hydrogen, $\text{O}=\text{R}^6$, or an optionally substituted group

- 10 selected from C₆₋₂₀ aliphatic, C₆₋₂₀ haloaliphatic, a 3- to 7-membered cycloaliphatic ring, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

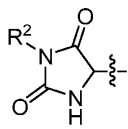
R¹ is hydrogen, a 3- to 7-membered cycloaliphatic ring, a 3- to 7-membered heterocyclic ring containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and

- sulfur, -OR², -C(O)OR², -C(O)SR², -OC(O)R², -OC(O)OR², -CN, -N(R²)₂, -C(O)N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR², -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -N(OR²)C(O)N(R²)₂, -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂, -C(NR²)R², -C(O)N(R²)OR², -


$$\text{C(R}^2\text{)N(R}^2\text{)}_2\text{C(O)OR}^2,$$
 $-, -\text{CR}^2(\text{OR}^2)\text{R}^3,$

$\triangle$, or

, or



- 20

each R² is independently hydrogen, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, a 3- to 7-membered

cycloaliphatic ring, and a 3- to 7-membered heterocyclic ring containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or

two occurrences of R^2 , taken together with the atom(s) to which they are attached, form an optionally substituted 4- to 7-membered heterocyclic ring containing 0-1 additional

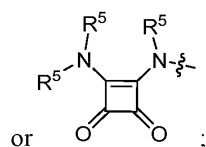
5 heteroatom selected from nitrogen, oxygen, and sulfur;

each R^3 is independently $-(CH_2)_n-R^4$, or

two occurrences of R^3 , taken together with the atoms to which they are attached, form an optionally substituted 5- to 6-membered heterocyclic ring containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

10 each R^4 is independently

hydrogen, $-OR^5$, $-N(R^5)_2$, $-OC(O)R^5$, $-OC(O)OR^5$, $-CN$, $-C(O)N(R^5)_2$, $-NR^5C(O)R^5$, $-OC(O)N(R^5)_2$, $-N(R^5)C(O)OR^5$, $-NR^5S(O)_2R^5$, $-NR^5C(O)N(R^5)_2$, $-NR^5C(S)N(R^5)_2$, $-NR^5C(NR^5)N(R^5)_2$,



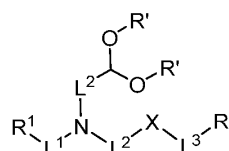
each R^5 is independently hydrogen, optionally substituted C_{1-6} aliphatic, or

15 two occurrences of R^5 , taken together with the atom(s) to which they are attached, form an optionally substituted 4- to 7-membered heterocyclic ring containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

each R^6 is independently C_{4-12} aliphatic; and

n is 0 to 4;

20 v) A compound of Formula (Id):



Id

or its N-oxide, or a pharmaceutically acceptable salt thereof, where

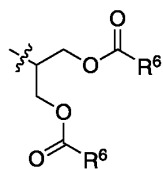
L_1 is absent, C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

25 each L_2 is independently optionally substituted C_{2-15} alkylenyl, or optionally substituted C_{3-15} heteroalkylenyl;

L^3 is absent, optionally substituted C_{1-10} alkylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

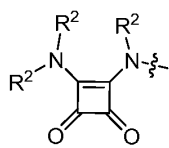
X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;

each R' is independently an optionally substituted group selected from C₄₋₁₂ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic containing 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;



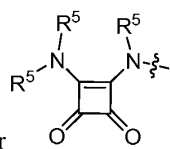
R is hydrogen, $\text{O}=\text{C}-\text{R}^6$, or an optionally substituted group selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic containing 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

- 10 R¹ is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl containing 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic
- 15 heteroaryl containing 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, -OR², -C(O)OR², -C(O)SR², -OC(O)R², -OC(O)OR², -CN, -N(R²)₂, -C(O)N(R²)₂, -S(O)₂N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR², -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -N(OR²)C(O)N(R²)₂, -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂, -C(NR²)R², -C(O)N(R²)OR², -C(R²)N(R²)₂C(O)OR², -CR²(R³)₂, -OP(O)(OR²)₂, or -
- 20



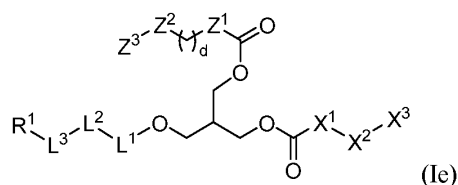
- P(O)(OR²)₂; or R¹ is $\text{O}=\text{C}-\text{R}^6$, or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, where the cycloaliphatic or heterocyclyl ring is
- 25 optionally substituted with 1-4 R² or R³ groups;
- each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl containing 1-4 heteroatoms

- independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or two occurrences of R^2 , taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl containing 0-1 additional heteroatom
- 5 selected from nitrogen, oxygen, and sulfur;
- each R^3 is independently $-(CH_2)_n-R^4$; or
- two occurrences of R^3 , taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- 10 each R^4 is independently hydrogen, $-OR^5$, $-N(R^5)_2$, $-OC(O)R^5$, $-OC(O)OR^5$, $-CN$, $-C(O)N(R^5)_2$, $-NR^5C(O)R^5$, $-OC(O)N(R^5)_2$, $-N(R^5)C(O)OR^5$, $-NR^5S(O)_2R^5$, $-NR^5C(O)N(R^5)_2$,



- $-NR^5C(S)N(R^5)_2$, $-NR^5C(NR^5)N(R^5)_2$, or
- each R^5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or
- two occurrences of R^5 , taken together with the atom(s) to which they are attached, form
- 15 optionally substituted 4- to 7-membered heterocyclyl containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- each R^6 is independently C_{4-12} aliphatic; and
- each n is independently 0 to 4;

(vi) A compound of Formula (Ie):



20

or a pharmaceutically acceptable salt thereof, where:

L^1 is a covalent bond, $-C(O)-$, or $-OC(O)-$;

L^2 is a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or

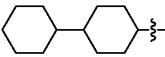
- 25 branched C_{1-12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{Cy}^A \left(\text{---} \right)_m \right]$;

Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

L^3 is a covalent bond, $-C(O)-$, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;

R^1 is $\text{---} \left(\text{---} \right)_p \text{---} \text{Cy}^B$, or an optionally substituted saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and phenyl; p is 0, 1, 2, or 3;

X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C_1 - C_{12} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$, $-NR-$, or $-\text{Cy}^C$;

Cy^C is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen or an optionally substituted C_1 - C_6 aliphatic group;

Z^1 is a covalent bond or $-O-$;

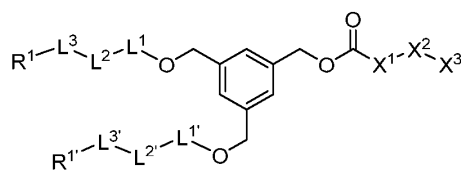
Z^2 is an optionally substituted group selected from 4- to 12-membered saturated or partially unsaturated carbocyclyl, phenyl, 1-adamantyl, and 2-adamantyl;

Z^3 is hydrogen, or an optionally substituted group selected from C_1 - C_{10} aliphatic, and 4- to 12-membered saturated or partially unsaturated carbocyclyl; and

d is 0, 1, 2, 3, 4, 5, or 6;

provided that when L^3 is a covalent bond, then R^1 must be $\text{---} \left(\text{---} \right)_p \text{---} \text{Cy}^B$;

vii) A compound of Formula (If):



(If)

or a pharmaceutically acceptable salt thereof, where:

each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-C(O)O-$;

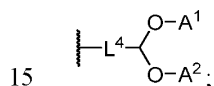
- 5 each L^2 and $L^{2'}$ is independently a covalent bond, an optionally substituted bivalent saturated

or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{Cy}^A \left(\text{---} \right)_m \right]$;

each Cy^A is independently an optionally substituted ring selected from phenylene or a 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

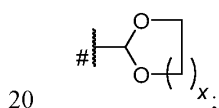
- 10 each L^3 and $L^{3'}$ is independently a covalent bond, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;
 each R^1 and $R^{1'}$ is independently an optionally substituted group selected from a saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$, a 3- to 12-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, sterolyl, phenyl, and



each L^4 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_6 hydrocarbon chain;

each A^1 and A^2 is independently an optionally substituted C_1 - C_{20} aliphatic or $-L^5-R^5$;

or A^1 and A^2 , together with their intervening atoms, may form an optionally substituted ring:



where

x is selected from 1 or 2; and

represents the point of attachment to L^4 ;

each L^5 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_{20}

- 25 hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

each R^5 is independently an optionally substituted group selected from a 5- to 10-membered aryl ring and a 3- to 8-membered carbocyclic ring ;

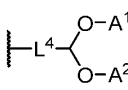
X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C_1 - C_{12} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

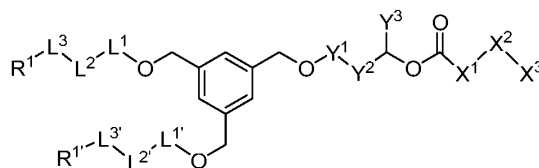
X^3 is hydrogen or $-Cy^B$;

Cy^B is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C_1 - C_6 aliphatic group;

provided that when X^3 is hydrogen, at least one of R^1 or $R^{1'}$ is ;

viii) a compound of Formula (Ig):

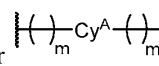


(Ig)

or a pharmaceutically acceptable salt thereof, where:

each of L^1 and $L^{1'}$ is independently a covalent bond, $-C(O)-$, or $-OC(O)-$;

each of L^2 and $L^{2'}$ is independently a covalent bond, an optionally substituted bivalent

saturated or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or ;

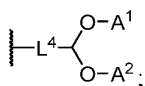
each Cy^A is independently an optionally substituted ring selected from phenylene or 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

each of L^3 and $L^{3'}$ is independently a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

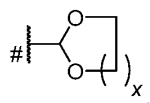
each of R^1 and $R^{1'}$ is independently an optionally substituted group selected from saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$, a 3- to 7-membered saturated or

partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, sterolyl, phenyl, or



each L⁴ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain;

- 5 each A¹ and A² is independently an optionally substituted C₁-C₂₀ aliphatic or -L⁵-R⁵,
or A¹ and A², together with their intervening atoms, may form an optionally substituted ring;



where

x is selected from 1 or 2; and

- 10 # represents the point of attachment to L^4 ;

each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

each R⁵ is independently an optionally substituted group selected from a 6- to 10-membered

- 15 aryl ring or a 3- to 8-membered carbocyclic ring;

Y¹ is a covalent bond, -C(O)-, or -C(O)O-;

Y² is a bivalent saturated or unsaturated, straight or branched C₁-C₆ hydrocarbon chain, where 1-2 methylene units are optionally and independently replaced with cyclopropylene, -O-, or -NR-;

- 20 Y³ is an optionally substituted group selected from saturated or unsaturated, straight or branched C₁-C₁₄ hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, or phenyl;

X¹ is a covalent bond, -O-, or -NR-;

- 25 X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁-C₁₂ hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with -O-, -NR^B-, or -Cv^B-;

each Cy^B is independently an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered heterocyclene

- 30 having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-

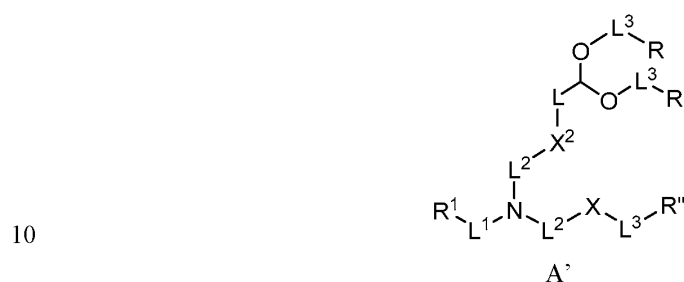
membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X³ is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3

- 5 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

ix) a compound of formula A':



or its N-oxide, or a pharmaceutically acceptable salt thereof, where

L¹ is absent, C₁₋₆ alkylenyl, or C₂₋₆ heteroalkylenyl;

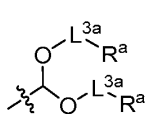
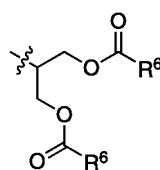
each L² is independently optionally substituted C₂₋₁₅ alkylenyl, or optionally substituted C₃₋₁₅

- 15 heteroalkylenyl;

L is C₁₋₁₀ alkylenyl, or C₂₋₁₀ heteroalkylenyl;

X² is -OC(O)-, -C(O)O-, or -OC(O)O-;

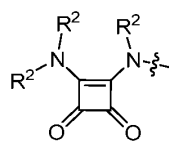
X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;

- 20 R'' is hydrogen, , , or an optionally substituted group selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic containing 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;
- 25 each of R and R^a is independently hydrogen, or an optionally substituted group selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic containing 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl

each of L^3 and L^{3a} is independently absent, optionally substituted C_{1-10} alkylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

R^1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl containing 1-3

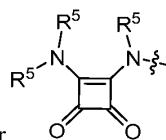
- 5 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl containing 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl containing 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-OR^2$, $-C(O)OR^2$, $-C(O)SR^2$, $-OC(O)R^2$, $-OC(O)OR^2$, $-CN$,
 10 $-N(R^2)_2$, $-C(O)N(R^2)_2$, $-S(O)_2N(R^2)_2$, $-NR^2C(O)R^2$, $-OC(O)N(R^2)_2$, $-N(R^2)C(O)OR^2$, $-NR^2S(O)_2R^2$, $-NR^2C(O)N(R^2)_2$, $-NR^2C(S)N(R^2)_2$, $-NR^2C(NR^2)N(R^2)_2$, $-NR^2C(CHR^2)N(R^2)_2$, $-N(OR^2)C(O)R^2$, $-N(OR^2)S(O)_2R^2$, $-N(OR^2)C(O)OR^2$, $-N(OR^2)C(O)N(R^2)_2$, $-N(OR^2)C(S)N(R^2)_2$, $-N(OR^2)C(NR^2)N(R^2)_2$, $-N(OR^2)C(CHR^2)N(R^2)_2$, $-C(NR^2)N(R^2)_2$, $-C(NR^2)R^2$, $-C(O)N(R^2)OR^2$, $-C(R^2)N(R^2)_2C(O)OR^2$, $-CR^2(R^3)_2$, $-OP(O)(OR^2)_2$, or $-P(O)(OR^2)_2$; or



R^1 is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, where the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R^2 or R^3 groups;

- 20 each R^2 is independently hydrogen, oxo, $-CN$, $-NO_2$, $-OR^4$, $-S(O)_2R^4$, $-S(O)_2N(R^4)_2$, $-(CH_2)_n-R^4$, or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl containing 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl containing 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or
 25 two occurrences of R^2 , taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
 each R^3 is independently $-(CH_2)_n-R^4$; or
 two occurrences of R^3 , taken together with the atom(s) to which they are attached, form
 30 optionally substituted 5- to 6-membered heterocyclyl containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

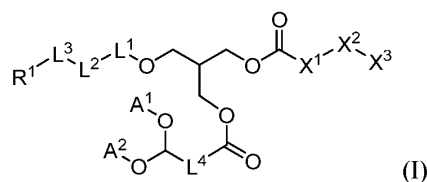
each R^4 is independently hydrogen, $-OR^5$, $-N(R^5)_2$, $-OC(O)R^5$, $-OC(O)OR^5$, $-CN$, $-C(O)N(R^5)_2$, $-NR^5C(O)R^5$, $-OC(O)N(R^5)_2$, $-N(R^5)C(O)OR^5$, $-NR^5S(O)_2R^5$, $-NR^5C(O)N(R^5)_2$,



$-NR^5C(S)N(R^5)_2$, $-NR^5C(NR^5)N(R^5)_2$, or

- each R^5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or
 5 two occurrences of R^5 , taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl containing 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
 each R^6 is independently C_{4-12} aliphatic; and
 each n is independently 0 to 4; or

- 10 x) a compound of Formula I:



or a pharmaceutically acceptable salt thereof, where:

L^1 is a covalent bond, $-C(O)-$, or $-OC(O)-$;

L^2 is a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or

- 15 branched C_{1-12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{---} \text{Cy}^A \text{---} \left(\text{---} \right)_m \right]$;

Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered saturated or partially unsaturated carbocyclene;

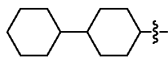
each m is independently 0, 1, or 2;

L^3 is a covalent bond, $-C(O)-$, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;

- 20 R^1 is $\left[\left(\text{---} \right)_p \text{---} \text{Cy}^B \right]$, an optionally substituted saturated or unsaturated, straight or branched C_{1-20} hydrocarbon chain where 1-3 methylene units are optionally and independently

replaced with $-O-$ or $-NR-$, or $\left[\text{---} \text{L}^4 \text{---} \begin{array}{c} \text{O}-A^1 \\ \text{O}-A^2 \end{array} \right]$;

Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or partially

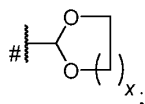
unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and phenyl;

p is 0, 1, 2, or 3;

each L^4 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_6 hydrocarbon chain;

each A^1 and A^2 is independently an optionally substituted C_1 - C_{20} aliphatic or $-L^5-R^5$;

- 5 or A^1 and A^2 , together with their intervening atoms, may form an optionally substituted ring:



where

x is selected from 1 or 2; and

represents the point of attachment to L^4 ;

- 10 each L^5 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

each R^5 is independently an optionally substituted group selected from a 5- to 10-membered aryl ring or a 3- to 8-membered carbocyclic ring ;

- 15 X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C_1 - C_{12} hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-O-$, $-NR-$, or $-Cy^C$;

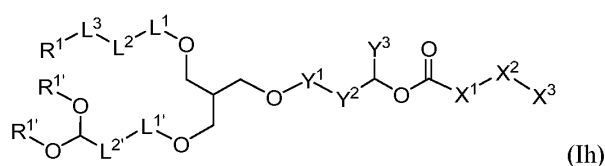
Cy^C is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

- 20 X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C_1 - C_6 aliphatic group;

- 30 provided that when L^3 is a covalent bond, then R^1 must be $\text{H}-(\text{---})_p\text{---}Cy^B$.

- In another aspect, the disclosure features a lipid nanoparticle (LNP) containing components A), B), and C). Component A) is a guide RNA, or a polynucleotide encoding the guide RNA, where the guide RNA is capable of directing a base editor polypeptide to alter a nucleotide in a transthyretin (TTR) polynucleotide. Component B) is an mRNA molecule, or a polynucleotide encoding the mRNA molecule, where the mRNA molecule encodes a base editor polypeptide containing a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain. The adenosine deaminase domain contains i) an amino acid sequence having at least 90% identity to the following TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:
- MSEVEFSHEYWMRHALLAKRARDEREVPVGAVLVLNLRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1). The adenosine deaminase domain also contains a combination of amino acid alterations relative to the TadA*7.10 amino acid sequence selected from one or more of: a) I76Y, V82T, Y123H, Y147R, and Q154R; b) V82T, Y123H, D147R, and Q154R; c) V82T, Y123H, D147T, and Q154S; d) V82T and Q154R; e) V82T, Y147T, and Q154S; f) I76Y, V82T, Y123H, Y147T, and Q154S; g) Y123H, Y147R, Q154R; h) I76Y, Y147R, and Q154R; i) Y147R, Q154R, and T166R; j) Y147T and Q154R; k) Y147T and Q154S; l) I76Y, Y123H, Y147R, and Q145R; m) I76Y and V82S; n) V82S and Y147R; o) V82S, Y123H, and Y147R; p) V82S and Q154R; q) V82S, Y123H, and Q154R; r) V82S, Y123H, Y147R, and Q154R; s) I76Y, V82S, Y123H, Y147R, and Q154R. Component C) is an ionizable lipid according to Formula (I), or a pharmaceutically acceptable salt thereof:



- where:
- each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-OC(O)-$;
- each L^2 and $L^{2'}$ is independently an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;
- L^3 is a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

R^1 is optionally substituted C_{1-20} aliphatic, $\text{---}L^{CyA}\text{---}Cy^A$, or $\text{---}L^{Ra}\text{---}\begin{matrix} O-R^a \\ O-R^a \end{matrix}$;

L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

5 L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

each R^a and $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic;

Y^1 is $-\text{C}(\text{O})-$ or $-\text{C}(\text{O})\text{O}-$;

10 Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain;

Y^3 is optionally substituted C_{1-20} aliphatic;

X^1 is a covalent bond, $-\text{O}-$, or $-\text{NR}-$;

X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12}

15 hydrocarbon chain, where 1-3 methylene units are optionally and independently replaced with $-\text{O}-$ or $-\text{NR}-$;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

In another aspect, the disclosure features a lipid nanoparticle (LNP) containing components A), B), and C). Component A) is a guide RNA having the following nucleotide sequence:

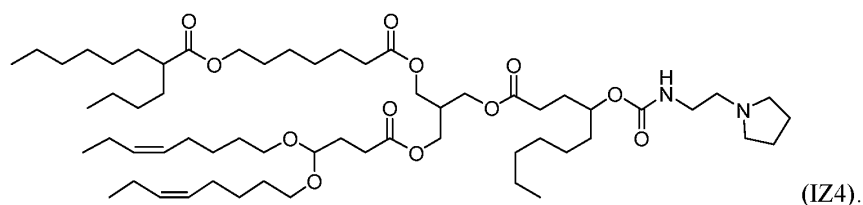
25 mGsmCsmCsAUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAmAGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUUmUmCAAmCmUmUGmAmAmAmAmAmGmUmGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUsmUsmUsmU (SEQ ID NO: 477), where A is adenosine; C is cytidine; G is guanosine; U is uridine; mA is 2'-O-methyladenosine; mC is 2'-O-methylcytidine; mG is 2'-O-methylguanosine; mU is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage. Component B) is a base editor polypeptide, or a polynucleotide encoding the base editor polypeptide, where the base editor polypeptide

contains a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain. The adenosine deaminase domain contains an amino acid sequence with at least 90% identity to the following amino TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:

5 MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1). The

adenosine deaminase domain also contains the following combination of amino acid alterations relative to the TadA*7.10 amino acid sequence: I76Y, V82T, Y123H, Y147R, and

10 Q154R. Component C) is an ionizable lipid having the following structure, or a pharmaceutically acceptable salt thereof:



In another aspect, the disclosure features a base editor system containing components
15 A), and B). Component A) is a guide RNA, or a polynucleotide encoding the guide RNA, where the guide RNA is capable of directing a base editor polypeptide to alter a nucleotide in a transthyretin (TTR) polynucleotide. Component B) is a base editor polypeptide, or one or more polynucleotide encoding the base editor polypeptide, where the base editor polypeptide contains a nucleic acid programmable DNA binding protein (napDNAbp) domain and an
20 adenosine deaminase domain. The adenosine deaminase domain contains an amino acid sequence with at least 90% identity to the following amino TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
25 GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1). The

adenosine deaminase domain also contains a combination of amino acid alterations relative to the TadA*7.10 amino acid sequence selected from one or more of: a) V82T, Y123H, D147R, and Q154R; b) V82T, Y123H, D147T, and Q154S; c) V82T and Q154R; d) V82T, Y147T, and Q154S; e) I76Y, V82T, Y123H, Y147R, and Q154R; and f) I76Y, V82T, Y123H,

30 Y147T, and Q154S.

In another aspect, the disclosure features a polynucleotide or set of polynucleotides encoding the base editor system of any aspect of the disclosure, or embodiments thereof.

In another aspect, the disclosure features a cell containing the base editor system or the polynucleotide or set of polynucleotides of any aspect of the disclosure, or embodiments thereof.

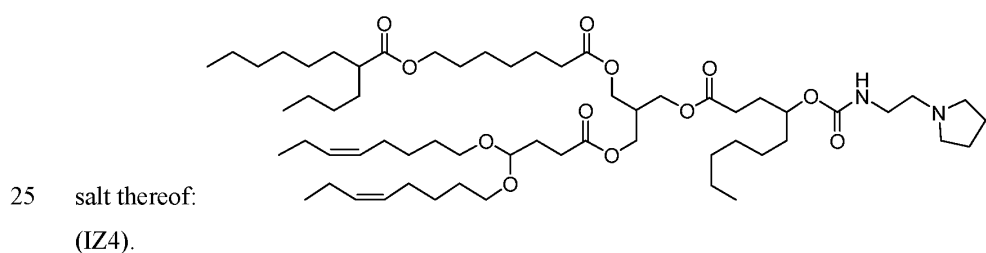
In another aspect, the disclosure features a pharmaceutical composition containing the lipid nanoparticle, the base editor system, the polynucleotide or set of polynucleotides, or the cell of any aspect of the disclosure, or embodiments thereof, and a pharmaceutically acceptable carrier or excipient.

In another aspect, the disclosure features a kit containing the lipid nanoparticle, the base editor system, the polynucleotide or set of polynucleotides, the cell, or the pharmaceutical composition of any aspect of the disclosure, or embodiments thereof, and a container.

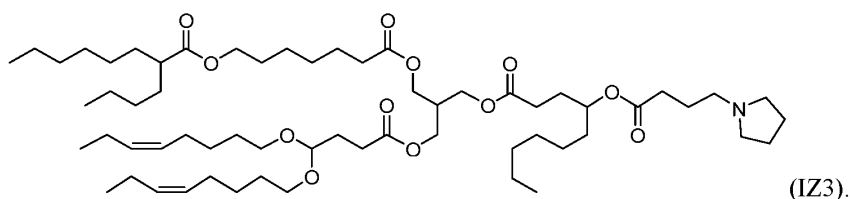
In another aspect, the disclosure features a method for modifying a target nucleobase in a transthyretin (*TTR*) polynucleotide in a cell. The method involves contacting the cell with the lipid nanoparticle or the base editor system of any aspect of the disclosure, or embodiments thereof, thereby modifying the target nucleobase in the *TTR* polynucleotide.

In another aspect, the disclosure features a method of treating a disease in a subject in need thereof. The disease is associated with a pathogenic mutation in a transthyretin (*TTR*) polynucleotide in the subject. The method involves administering to the subject the lipid nanoparticle or the base editor system of any aspect of the disclosure, or embodiments thereof, thereby altering a target nucleobase in the *TTR* polynucleotide.

In any aspect of the disclosure, or embodiments thereof, the lipid nanoparticle contains an ionizable lipid having the following structure, or a pharmaceutically acceptable



In any aspect of the disclosure, or embodiments thereof, the lipid nanoparticle contains an ionizable lipid having the following structure, or a pharmaceutically acceptable salt thereof:



In any aspect of the disclosure, or embodiments thereof, the LNP has a molar ratio of ionizable nitrogen atoms in an ionizable lipid to the total negative charge in the payload (N:P ratio) of between about 30:1 to about 1:1. In any aspect of the disclosure, or embodiments thereof, the LNP has an N:P ratio of about 6:1.

In any aspect of the disclosure, or embodiments thereof, the guide RNA contains a scaffold with the following nucleotide sequence:

gUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugG
 10 caccgagucggugcuusus (SEQ ID NO: 478), where A is adenosine; C is cytidine; G is guanosine; U is uridine; a is 2'-O-methyladenosine; c is 2'-O-methylcytidine; g is 2'-O-methylguanosine; u is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage. In any aspect of the disclosure, or embodiments thereof, the guide RNA contains a

spacer containing the following nucleotide sequence: GCCAUCCUGCCAAGAAUGAG (SEQ ID
 15 NO: 472). In any aspect of the disclosure, or embodiments thereof, the guide RNA contains the following nucleotide sequence:

mGsmCsmCsAUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAm
 AGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUUmUmCAAmCmUmUGmAmAmAmAmGmU
 mGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUsmUsmUsmU (SEQ ID NO: 477), where A
 20 is adenosine; C is cytidine; G is guanosine; U is uridine; mA is 2'-O-methyladenosine; mC is 2'-O-methylcytidine; mG is 2'-O-methylguanosine; mU is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage. In any aspect of the disclosure, or embodiments thereof, the guide RNA contains a spacer selected from those listed in any of **Tables 1B, 1C, 1D, 1E, 2A, 2B, 2D, or 2E**. In any aspect of the disclosure, or embodiments thereof, the
 25 guide RNA contains 2-5 contiguous 2'-O-methylated nucleobases at the 3' end and at the 5' end. In any aspect of the disclosure, or embodiments thereof, the guide RNA contains 2-5 contiguous nucleobases at the 3' end and at the 5' end that contain phosphorothioate internucleotide linkages.

In any aspect of the disclosure, or embodiments thereof, the napDNAbp domain contains a Cas9 polypeptide. In any aspect of the disclosure, or embodiments thereof, the napDNAbp is a nickase.

In any aspect of the disclosure, or embodiments thereof, the base editor polypeptide
5 contains an amino acid sequence with at least about 90% identity to the following amino acid sequence:

ABE9.51

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLYDATLYTTFEPVCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHP
10 GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSSGSSGGSSGSETPGTSES
ATPESSSGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRLLIYLALAHMIKFRGHFLIEGDL
NPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
15 LFGNLIALSLGLTPNFKSNFDLAEDAKQLSKDYYDDLDNLLAQIGDQYADFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYFPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEFVVDK
GASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
20 AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLTKI IKDKDFLD
NEENEDILEDIVLTTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDLVKVMGRHKPENIVIMARENQTTQKGQKNSRERMKRIEIEGKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
25 LTRSDKNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTTALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNI
MNFCKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLKSVKELLGI
30 TIMERSSEFKNPIDFLEAKGYKEVKDLIIKLPKYSLELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKV
LSAYNKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463).

In any aspect of the disclosure, or embodiments thereof, the cell is in a subject.

In any aspect of the disclosure, or embodiments thereof, an ionizable lipid of the lipid nanoparticle has a half-life in the liver of the subject that is less than 14 days.

In any aspect of the disclosure, or embodiments thereof, the target nucleobase is altered with an editing efficiency of at least about 50% or 60% in the cell and/or in the liver of the subject. In any aspect of the disclosure, or embodiments thereof, altering the target nucleobase results in a reduction in TTR polypeptide levels in the cell and/or in the subject. In any aspect of the disclosure, or embodiments thereof, TTR polypeptide levels are reduced by at least about 80% or 90%. In any aspect of the disclosure, or embodiments thereof, the method is associated with only a transient increase or no increase in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate aminotransferase (AST) levels in the subject. In any aspect of the disclosure, or embodiments thereof, the method is associated with no increase in ALP levels in the subject.

In any aspect of the disclosure, or embodiments thereof, the disease is selected from one or more of amyloidosis, cardiomyopathy, familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), familial transthyretin amyloidosis (FTA), senile systemic amyloidosis (SSA), and transthyretin amyloidosis.

In any aspect of the disclosure, or embodiments thereof, the LNP has a mean diameter of from about 20 nm to about 1000 nm, from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 50 nm to 90 nm, from about 55 nm to 85 nm, from about 55 nm to 75 nm, from about 50 nm to about 80 nm, from about 60 nm to about 80 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, or from about 70 nm to about 80 nm. In any aspect of the disclosure, or embodiments thereof, the LNP has a mean diameter of about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, 150 nm, or greater. In any aspect of the disclosure, or embodiments thereof, the LNP has a mean diameter of about 70 nm +/- 10 nm, 70 nm +/- 5 nm, 65 nm +/- 10 nm, 65 nm +/- 5 nm, 60 nm +/- 10 nm, or 60 nm +/- 5 nm.

In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at from about 30 mole percent to about 70 mole percent, based on total moles of components of the lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at from about 33 mole percent to about 60 mole percent, based on total moles of components of the

- lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at from about 34 mole percent to about 55 mole percent, based on total moles of components of the lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at from about 33 mole percent to about 51 mole percent, based on total moles of components of the lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at about 34.7 mole percent, based on total moles of components of the lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at about 50 mole percent, based on total moles of components of the lipid nanoparticle. In any aspect of the disclosure, or embodiments thereof, the ionizable lipid is present in the lipid nanoparticle (LNP) at about 40 mole percent to about 60 mole percent or about 45 mole percent to about 55 mole percent, based on total moles of components of the lipid nanoparticle.
- 15 In any aspect of the disclosure, or embodiments thereof, the LNP contains from about 0 mole percent to about 5 mole percent conjugate-linker lipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 1.5 mole percent conjugate-linker lipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 2.0 mole percent conjugate-linker lipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 2.5 mole percent conjugate-linker lipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 3 mole percent conjugate-linker lipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 3.5 mole percent conjugate-linker lipid.
- 25 In any aspect of the disclosure, or embodiments thereof, the LNP contains from about 5 mole percent to 25 mole percent of phospholipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 5 mole percent to 15 mole percent of phospholipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 9 mole percent to 11 mole percent of phospholipid. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 10 mole percent of phospholipid.
- 30 In any aspect of the disclosure, or embodiments thereof, the LNP contains from about 25 mole percent to about 45 mole percent sterol. In any aspect of the disclosure, or embodiments thereof, the LNP contains from about 30 mole percent to about 45 mole percent sterol. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 40 mole percent sterol. In any aspect of the disclosure, or embodiments thereof, the LNP

contains about 35.75 mole percent sterol. In any aspect of the disclosure, or embodiments thereof, the LNP contains about 32.5 mole percent cholesterol.

In any aspect of the disclosure, or embodiments thereof, the LNP about 30 mole percent to about 70 mole percent ionizable lipid, about 5 mole percent to about 25 mole percent phospholipid, about 25 mole percent to about 45 mole percent sterol, and about 0 mole percent to about 5 mole percent conjugate-linker lipid.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 47.5 mole percent ionizable lipid, about 10 mole percent phospholipid, about 40 mole percent sterol, and about 2.5 mole percent conjugate-linker lipid.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 51.25 mole percent ionizable lipid, about 10 mole percent phospholipid, about 35.75 mole percent sterol, and about 3 mole percent conjugate-linker lipid.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 55 mole percent ionizable lipid, about 10 mole percent phospholipid, about 32.5 mole percent sterol, and about 2.5 mole percent conjugate-linker lipid.

In any aspect of the disclosure, or embodiments thereof, the LNP about 45 mole percent to about 60 mole percent ionizable lipid of any provided compound, about 9 mole percent to about 11 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 1 mole percent to about 5 mole percent PEG₂₀₀₀-DMG, and about 30 mole percent to about 45 mole percent cholesterol, based on the total moles of these four ingredients.

In any aspect of the disclosure, or embodiments thereof, the LNP is the formulation LNP1, LNP2, LNP3, or LNP4 in **Table 10A**.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 47.5 mole percent ionizable lipid IZ1, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 40 mole percent cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload containing mRNA encoding an adenosine base editor (ABE) containing a Tada*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and a guide RNA containing a spacer containing the nucleotide sequence GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472). In some embodiments, the guide RNA is GA521 of **Table 1B**.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 55 mole percent ionizable lipid IZ2, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 32.5 mole

percent cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload containing mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and a guide RNA containing a spacer
 5 containing the nucleotide sequence GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472). In some embodiments, the guide RNA is GA521 of **Table 1B**.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 51.25 mole percent ionizable lipid IZ3, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 3 mole percent PEG₂₀₀₀-DMG, and about 35.75 mole percent
 10 cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload containing mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and a guide RNA containing a spacer containing the nucleotide sequence GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472). In
 15 some embodiments, the guide RNA is GA521 of **Table 1B**.

In any aspect of the disclosure, or embodiments thereof, the LNP contains about 47.5 mole percent ionizable lipid IZ4, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 40 mole percent
 20 cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload containing mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and a guide RNA containing a spacer containing the nucleotide sequence GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472). In
 some embodiments, the guide RNA is GA521 of **Table 1B**.

25 In any aspect of the disclosure, or embodiments thereof, the N/P ratio of the lipid nanoparticle is from about 1 to about 30. In any aspect of the disclosure, or embodiments thereof, the N/P ratio of the lipid nanoparticle is from about 2 to about 15, from about 2 to about 10, from about 2 to about 8, from about 2 to about 6, from about 3 to about 15, from
 30 about 3 to about 10, from about 3 to about 8, from about 3 to about 6, from about 4 to about 15, from about 4 to about 10, from about 4 to about 8, or from about 4 to about 6. In any aspect of the disclosure, or embodiments thereof, the N/P ratio of the lipid nanoparticle is about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about
 6.5, about 7, about 7.5, about 8, about 9, or about 10. In any aspect of the disclosure, or

embodiments thereof, the N/P ratio of the lipid nanoparticle is N/P ratio is from about 5 to about 7. In any aspect of the disclosure, or embodiments thereof, the N/P ratio of the lipid nanoparticle is about 6.

In any aspect provided herein, or embodiments thereof, the method is not a process
5 for modifying the germline genetic identity of human beings.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this disclosure belongs.

10 The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton *et al.*, Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following
15 terms have the meanings ascribed to them below, unless specified otherwise.

By “TadA*8.8 polypeptide” is meant an adenosine deaminase domain having deaminase activity, comprising the alterations Y123H, Y147R, and Q154R relative to the following reference sequence, and having at least about 85% amino acid sequence identity to the following reference sequence:

20 MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1;

TadA*7.10). In some embodiments, TadA*8.8 comprises further alterations (e.g., 1, 2, 3, 4,
5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or
25 more alterations) relative to the reference sequence.

By “TadA*8.8 polynucleotide” is meant a polynucleotide encoding a TadA*8.8 polypeptide.

By “TadA*8.13 polypeptide” is meant an adenosine deaminase domain having deaminase activity, comprising the alterations I76Y, Y123H, Y147R, and Q154R relative to
30 the following reference sequence and having at least about 85% amino acid sequence identity to the following reference sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1;

TadA*7.10). In some embodiments, TadA*8.13 comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more total alterations relative to the reference sequence.

By “TadA*8.13 polynucleotide” is meant a polynucleotide encoding a TadA*8.13 polypeptide.

By “TadA*8.17 polypeptide” is meant an adenosine deaminase domain having deaminase activity, comprising the alterations V82S and Q154R relative to the following reference sequence, and having at least about 85% amino acid sequence identity to the following reference sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1;

TadA*7.10). In some embodiments, TadA*8.17 comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more total alterations relative to the reference sequence.

By “TadA*8.17 polynucleotide” is meant a polynucleotide encoding a TadA*8.17 polypeptide.

By “TadA*8.20 polypeptide” is meant an adenosine deaminase domain having deaminase activity, comprising the alterations I76Y, V82S, Y123H, Y147R, and Q154R relative to the following reference sequence, and having at least about 85% amino acid sequence identity to the following reference sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1;

TadA*7.10). In some embodiments, TadA*8.20 comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more total alterations relative to the reference sequence.

By “TadA*8.20 polynucleotide” is meant a polynucleotide encoding a TadA*8.20 polypeptide.

In various embodiments, any adenosine deaminase domain provided herein (e.g., TadA*8.8, TadA*8.17, or TadA*8.20, and variants thereof) comprises the following alterations relative to the TadA*7.10 reference sequence: V82T; or V82T, Y147T, and Q154S.

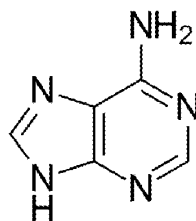
By “ABE9.51 polypeptide” is meant an adenosine deaminase base editor comprising a TadA*8.20 adenosine deaminase polypeptide variant having the amino acid alteration V82T, and a nucleic acid programmable DNA binding protein (napDNAbp) domain. In some embodiments, ABE9.51 comprises an amino acid sequence with at least about 85% sequence identity to the following polypeptide sequence, or a functional fragment thereof capable of deaminating an adenosine nucleotide:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
 LRQGGGLVMQNYRLYDATLYTTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHP
 GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSES
 10 ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVP SKKFKVLGNTDRHSIKKNLIGA
 LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKK
 HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRILIYALAHMIKFRGHFLIEGDL
 NPDNSDVKLFQILVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
 LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSD
 15 AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
 GYIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAI
 LRRQEDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEFVVDK
 GASAQSFIERMTNFDKNLPNEKVLPKHSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
 AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLD
 20 NEENEDILEDIVLTTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
 RDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
 IKKGILQTVKVVDLVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
 ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
 LTRSDKNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
 25 RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKREINN
 YHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNI
 MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGG
 FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLKSVKELLGI
 TIMERSSFENPIDFLEAKGYKEVKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
 30 PSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIEQISEFSKRVLADANLDKV
 LSAYNKHDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSI
 TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463). In some
 embodiments, an ABE9.51 napDNAbp comprises an spCas9 nickase.

In embodiments, ABE9.51 contains one or more uracil glycosylase inhibitor (UGI) domains. In some embodiments, ABE9.51 contains two UGI domains.

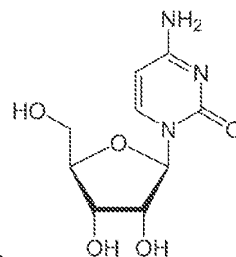
By “ABE9.51 polynucleotide” is meant a polynucleotide encoding an ABE9.51 polypeptide.

5 By “adenine” or “9*H*-Purin-6-amine” is meant a purine nucleobase with the molecular



formula $C_5H_5N_5$, having the structure , and corresponding to CAS No. 73-24-5.

By “adenosine” or “4-Amino-1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2(1*H*)-one” is meant an adenine molecule attached to



10 a ribose sugar via a glycosidic bond, having the structure , and corresponding to CAS No. 65-46-3. Its molecular formula is $C_{10}H_{13}N_5O_4$.

By “adenosine deaminase” or “adenine deaminase” is meant a polypeptide or fragment thereof capable of catalyzing the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase catalyzing the hydrolytic deamination of adenosine to inosine or deoxy adenosine to deoxyinosine. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (*e.g.*, engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism (*e.g.*, eukaryotic, prokaryotic), including but not limited to
 15 algae, bacteria, fungi, plants, invertebrates (*e.g.*, insects), and vertebrates (*e.g.*, amphibians, mammals). In some embodiments, the adenosine deaminase is an adenosine deaminase variant with one or more alterations and is capable of deaminating both adenine and cytosine in a target polynucleotide (*e.g.*, DNA, RNA) and may be referred to as a “dual deaminase”. Non-limiting examples of dual deaminases include those described in PCT/US22/22050. In
 20

some embodiments, the target polynucleotide is single or double stranded. In some
embodiments, the adenosine deaminase variant is capable of deaminating both adenine and
cytosine in DNA. In some embodiments, the adenosine deaminase variant is capable of
deaminating both adenine and cytosine in single-stranded DNA. In some embodiments, the
5 adenosine deaminase variant is capable of deaminating both adenine and cytosine in RNA. In
embodiments, the adenosine deaminase variant is selected from those described in
PCT/US2020/018192, PCT/US2020/049975, PCT/US2017/045381, and
PCT/US2020/028568, the full contents of which are each incorporated herein by reference in
their entireties for all purposes.

10 By “adenosine deaminase activity” is meant catalyzing the deamination of adenine or
adenosine to guanine in a polynucleotide.

By “Adenosine Base Editor (ABE)” is meant a base editor comprising an adenosine
deaminase.

15 By “Adenosine Base Editor (ABE) polynucleotide” is meant a polynucleotide
encoding an ABE.

By “Adenosine Base Editor 8 (ABE8) polypeptide” or “ABE8” is meant a base editor
as defined herein comprising an adenosine deaminase or adenosine deaminase variant
comprising one or more of the alterations listed in **Table 5B**, one of the combinations of
alterations listed in **Table 5B**, or an alteration at one or more of the amino acid positions
20 listed in **Table 5B**, such alterations are relative to the following reference sequence:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1), or a
corresponding position in another adenosine deaminase. In embodiments, ABE8 comprises
25 alterations at amino acids 82 and/or 166 of SEQ ID NO: 1. In some embodiments, ABE8
comprises further alterations, as described herein, relative to the reference sequence.

By “Adenosine Base Editor 8 (ABE8) polynucleotide” is meant a polynucleotide
encoding an ABE8 polypeptide.

30 “Administering” is referred to herein as providing one or more compositions
described herein to a patient or a subject. By way of example and without limitation,
composition administration (e.g., injection) can be performed by intravenous (i.v.) injection,
sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or
intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral
administration can be, for example, by bolus injection or by gradual perfusion over time. In

some embodiments, parenteral administration includes infusing or injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally. Alternatively, or concurrently,
5 administration can be by the oral route.

By “agent” is meant any small molecule chemical compound, antibody, nucleic acid molecule, lipid nanoparticle, polypeptide, polypeptide complex, or fragments thereof. In some embodiments, the agent is a lipid nanoparticle of the disclosure. In some embodiments, the lipid nanoparticle contains a base editor system of the disclosure. In some cases, the agent
10 is a base editor system of the disclosure, or a component thereof. The base editor system contains a base editor, or a polynucleotide (e.g., an mRNA molecule) encoding the base editor, and a guide RNA, or a polynucleotide encoding the guide RNA.

The term “aliphatic” or “aliphatic group”, as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely
15 saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as “carbocycle,” “carbocyclic,” “cycloaliphatic” or “cycloalkyl”), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-6 aliphatic
20 carbon atoms. In some embodiments, aliphatic groups contain 1-5 carbon atoms. In some embodiments, aliphatic groups contain 1-4 carbon atoms. In some embodiments, aliphatic groups contain 1-3 carbon atoms, and in some embodiments, aliphatic groups contain 1-2 carbon atoms. In some embodiments, “carbocyclic” (or “cycloaliphatic” or “carbocycle” or “cycloalkyl”) refers to an optionally substituted monocyclic C₃-C₈ hydrocarbon, or an
25 optionally substituted C₆-C₁₂ bicyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

30 As used herein, the term “alkenyl” refers to an alkyl group, as defined herein, having one or more double bonds. In some embodiments, the term “alkenyl”, used alone or as part of a larger moiety, refers to an optionally substituted straight or branched hydrocarbon chain having at least one double bond and having (unless otherwise specified) 2-20, 2-18, 2-16, 2-14, 2-12, 2-10, 2-8, 2-6, 2-4, or 2-3 carbon atoms (e.g., C₂₋₂₀, C₂₋₁₈, C₂₋₁₆, C₂₋₁₄, C₂₋₁₂, C₂₋₁₀,

C₂₋₈, C₂₋₆, C₂₋₄, or C₂₋₃). Exemplary alkenyl groups include ethenyl, propenyl, butenyl, pentenyl, hexenyl, and heptenyl.

The term “alkenylene” refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one double bond in which one or more
5 hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

As used herein, the term “alkyl” is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and
10 cycloalkyl substituted alkyl groups. In some embodiments, alkyl has 1-100 carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 carbon atoms in its backbone (e.g., C₁-C₂₀ for straight chain, C₂-C₂₀ for branched chain), and alternatively, about 1-10. In some embodiments, a cycloalkyl ring has from about 3-10 carbon atoms in their ring structure where such rings are monocyclic or bicyclic, and alternatively about 5, 6
15 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1-4 carbon atoms (e.g., C₁-C₄ for straight chain lower alkyls).

The term “alkylenyl” or “alkylene” refers to a bivalent alkyl group (i.e., a bivalent saturated hydrocarbon chain) that is a straight-chain (i.e., unbranched) or branched,
20 substituted or unsubstituted. Any of the above mentioned monovalent alkyl groups may be an alkylenyl by abstraction of a second hydrogen atom from the alkyl. In some embodiments, an “alkylenyl” is a polymethylene group, i.e., $-(CH_2)_n-$, wherein n is a positive integer, preferably from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, from 1 to 2, from 2 to 5, or from 4 to 8. A substituted alkylenyl is
25 a polymethylene group in which one or more methylene hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

As used herein, the term “alkynyl” refers to an alkyl group, as defined herein, having one or more triple bonds. In some embodiments, the term “alkynyl”, used alone or as part of
30 a larger moiety, refers to an optionally substituted straight or branched chain hydrocarbon group having at least one triple bond and having (unless otherwise specified) 2-20, 2-18, 2-16, 2-14, 2-12, 2-10, 2-8, 2-6, 2-4, or 2-3 carbon atoms (e.g., C₂₋₂₀, C₂₋₁₈, C₂₋₁₆, C₂₋₁₄, C₂₋₁₂, C₂₋₁₀, C₂₋₈, C₂₋₆, C₂₋₄, or C₂₋₃). Exemplary alkynyl groups include ethynyl, propynyl, butynyl, pentynyl, hexynyl, and heptynyl.

By “alteration” is meant a change in the level, structure, or activity of an analyte, gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a change (e.g., increase or reduction) in expression levels. In embodiments, the increase or reduction in expression levels is by 10%, 25%, 40%, 50% or
5 greater. In some embodiments, an alteration includes an insertion, deletion, or substitution of a nucleobase or amino acid (by, e.g., genetic engineering). In some embodiments, a base editor described herein alters the sequence of a TTR polynucleotide.

By “ameliorate” is meant reduce, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease. In embodiments, the disease is amyloidosis, and a
10 base editor system described herein reduces the accumulation of amyloid in a tissue of a subject.

By “analog” is meant a molecule that is not identical but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical
15 modifications that enhance the analog’s function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog’s protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

The term “aryl” refers to monocyclic and bicyclic ring systems having a total of six to
20 fourteen ring members (e.g., C₆₋₁₄), wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term “aryl” may be used interchangeably with the term “aryl ring”. In some embodiments, “aryl” refers to an aromatic ring system which includes, but is not limited to, phenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Unless otherwise specified, “aryl” groups are
25 hydrocarbons.

By “base editor (BE),” or “nucleobase editor polypeptide (NBE)” is meant an agent that binds a polynucleotide and has nucleobase modifying activity. In various embodiments, the base editor comprises a nucleobase modifying polypeptide (e.g., a deaminase) and a polynucleotide programmable nucleotide binding domain (e.g., Cas9 or Cpf1).
30 Representative nucleic acid and protein sequences of base editors include those sequences having about or at least about 85% sequence identity to any base editor sequence provided in the sequence listing, such as those corresponding to SEQ ID NOs: 2-11.

By “BE4 cytidine deaminase (BE4) polypeptide,” is meant a base editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain, a cytidine deaminase

domain, and two uracil glycosylase inhibitor domains (UGIs). In embodiments, the napDNAbp is a Cas9n (D10A) polypeptide. Non-limiting examples of cytidine deaminase domains include rAPOBEC, ppAPOBEC, RrA3F, AmAPOBEC1, and SsAPOBEC3B.

By “BE4 cytidine deaminase (BE4) polynucleotide,” is meant a polynucleotide
5 encoding a BE4 polypeptide.

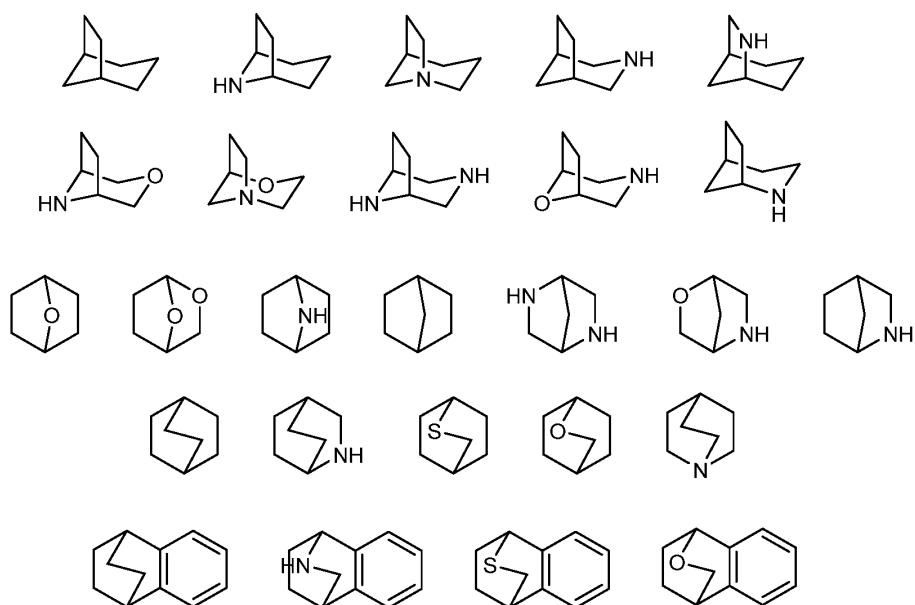
By “base editing activity” is meant acting to chemically alter a base within a polynucleotide. In one embodiment, a first base is converted to a second base. In one embodiment, the base editing activity is cytidine deaminase activity, *e.g.*, converting target C•G to T•A. In another embodiment, the base editing activity is adenosine or adenine
10 deaminase activity, *e.g.*, converting A•T to G•C.

The term “base editor system” or “base editing system” refers to an intermolecular complex for editing a nucleobase of a target nucleotide sequence. In various embodiments, the base editor (BE) system comprises (1) a polynucleotide programmable nucleotide binding domain, a deaminase domain (*e.g.*, cytidine deaminase or adenosine deaminase) for
15 deaminating nucleobases in the target nucleotide sequence; and (2) one or more guide polynucleotides (*e.g.*, guide RNA) in conjunction with the polynucleotide programmable nucleotide binding domain. In various embodiments, the base editor (BE) system comprises a nucleobase editor domain selected from an adenosine deaminase or a cytidine deaminase, and a domain having nucleic acid sequence specific binding activity. In some embodiments, the
20 base editor system comprises (1) a base editor (BE) comprising a polynucleotide programmable DNA binding domain and a deaminase domain for deaminating one or more nucleobases in a target nucleotide sequence; and (2) one or more guide RNAs in conjunction with the polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable
25 DNA binding domain. In some embodiments, the base editor is a cytidine base editor (CBE). In some embodiments, the base editor is an adenine or adenosine base editor (ABE). In some embodiments, the base editor is an adenine or adenosine base editor (ABE) or a cytidine or cytosine base editor (CBE). In some embodiments, the base editor system (*e.g.*, a base editor system comprising a cytidine deaminase) comprises a uracil glycosylase inhibitor or other
30 agent or peptide (*e.g.*, a uracil stabilizing protein such as provided in WO2022015969, the disclosure of which is incorporated herein by reference in its entirety for all purposes) that inhibits the inosine base excision repair system.

As used herein, the term “bivalent” refers to a chemical moiety with two points of attachment. For example, a “bivalent C₁₋₈ (or C₁₋₆) saturated or unsaturated, straight or

branched, hydrocarbon chain”, refers to bivalent alkylene, alkenylene, and alkynylene chains that are straight or branched as defined herein.

As used herein, the term “bridged bicyclic” refers to any bicyclic ring system, i.e. carbocyclic or heterocyclic, saturated or partially unsaturated, having at least one bridge. As defined by IUPAC, a “bridge” is an unbranched chain of atoms or an atom or a valence bond connecting two bridgeheads, where a “bridgehead” is any skeletal atom of the ring system which is bonded to three or more skeletal atoms (excluding hydrogen). In some embodiments, a bridged bicyclic group has 7-12 ring members and 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Such bridged bicyclic groups are well known in the art and include those groups set forth below where each group is attached to the rest of the molecule at any substitutable carbon or nitrogen atom. Unless otherwise specified, a bridged bicyclic group is optionally substituted with one or more substituents as set forth for aliphatic groups. Additionally or alternatively, any substitutable nitrogen of a bridged bicyclic group is optionally substituted. Exemplary bridged bicyclics include but are not limited to:



The terms “carbocyclyl,” “carbocycle,” and “carbocyclic ring” as used herein, refer to saturated or partially unsaturated cyclic aliphatic monocyclic, bicyclic, or polycyclic ring systems, as described herein, having from 3 to 14 members, wherein the aliphatic ring system is optionally substituted as described herein. Carbocyclic groups include, without limitation,

cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cycloheptenyl, cyclooctyl, cyclooctenyl, norbornyl, adamantyl, and cyclooctadienyl. In some embodiments, “carbocyclyl” (or “cycloaliphatic”) refers to an optionally substituted monocyclic C₃-C₈ hydrocarbon, or an optionally substituted C₆-C₁₂ bicyclic hydrocarbon that
 5 is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. The term “cycloalkyl” refers to an optionally substituted saturated ring system of about 3 to about 10 ring carbon atoms. In some embodiments, cycloalkyl groups have 3–6 carbons. Exemplary monocyclic cycloalkyl rings include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and
 10 cycloheptyl. The term “cycloalkenyl” refers to an optionally substituted non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and having about 3 to about 10 carbon atoms. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl, and cycloheptenyl.

The term “Cas9” or “Cas9 domain” refers to an RNA guided nuclease comprising a
 15 Cas9 protein, or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to sometimes as a casn1 nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease.

The term “conservative amino acid substitution” or “conservative mutation” refers to
 20 the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can
 25 be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., *supra*). Non-limiting examples of conservative mutations include amino acid substitutions of amino acids, for example, lysine for arginine and vice versa such that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such
 30 that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free –NH₂ can be maintained.

Amino acids generally can be grouped into classes according to the following common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

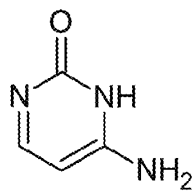
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gin;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- 5 (6) aromatic: Trp, Tyr, Phe.

In some embodiments, conservative substitutions can involve the exchange of a member of one of these classes for another member of the same class. In some embodiments, non-conservative amino acid substitutions can involve exchanging a member of one of these classes for another class.

10 The term “coding sequence” or “protein coding sequence” as used interchangeably herein refers to a segment of a polynucleotide that codes for a protein. Coding sequences can also be referred to as open reading frames. The region or sequence is bounded nearer the 5' end by a start codon and nearer the 3' end with a stop codon. Stop codons useful with the base editors described herein include the following: TAG, TAA, and TGA.

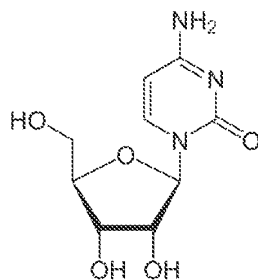
15 By “complex” is meant a combination of two or more molecules whose interaction relies on inter-molecular forces. Non-limiting examples of inter-molecular forces include covalent and non-covalent interactions. Non-limiting examples of non-covalent interactions include hydrogen bonding, ionic bonding, halogen bonding, hydrophobic bonding, van der Waals interactions (e.g., dipole-dipole interactions, dipole-induced dipole interactions, and
20 London dispersion forces), and π -effects. In an embodiment, a complex comprises polypeptides, polynucleotides, or a combination of one or more polypeptides and one or more polynucleotides. In one embodiment, a complex comprises one or more polypeptides that associate to form a base editor (e.g., base editor comprising a nucleic acid programmable DNA binding protein, such as Cas9, and a deaminase) and a polynucleotide (e.g., a guide
25 RNA). In an embodiment, the complex is held together by hydrogen bonds. It should be appreciated that one or more components of a base editor (e.g., a deaminase, or a nucleic acid programmable DNA binding protein) may associate covalently or non-covalently. As one example, a base editor may include a deaminase covalently linked to a nucleic acid programmable DNA binding protein (e.g., by a peptide bond). Alternatively, a base editor
30 may include a deaminase and a nucleic acid programmable DNA binding protein that associate noncovalently (e.g., where one or more components of the base editor are supplied in trans and associate directly or via another molecule such as a protein or nucleic acid). In an embodiment, one or more components of the complex are held together by hydrogen bonds.

By “cytosine” or “4-Aminopyrimidin-2(1*H*)-one” is meant a purine nucleobase with



the molecular formula $C_4H_5N_3O$, having the structure to CAS No. 71-30-7.

By “cytidine” is meant a cytosine molecule attached to a ribose sugar via a glycosidic



5 bond, having the structure , and corresponding to CAS No. 65-46-3. Its molecular formula is $C_9H_{13}N_3O_5$.

By “Cytidine Base Editor (CBE)” is meant a base editor comprising a cytidine deaminase.

By “Cytidine Base Editor (CBE) polynucleotide” is meant a polynucleotide encoding
10 a CBE.

By “cytidine deaminase” or “cytosine deaminase” is meant a polypeptide or fragment thereof capable of deaminating cytidine or cytosine. In embodiments, the cytidine or cytosine is present in a polynucleotide. In one embodiment, the cytidine deaminase converts cytosine to uracil or 5-methylcytosine to thymine. The terms “cytidine deaminase” and “cytosine
15 deaminase” are used interchangeably throughout the application. *Petromyzon marinus* cytosine deaminase 1 (PmCDA1) (SEQ ID NO: 13-14), Activation-induced cytidine deaminase (AICDA) (SEQ ID NOs: 15-21), and APOBEC (SEQ ID NOs: 12-61) are exemplary cytidine deaminases. Further exemplary cytidine deaminase (CDA) sequences are provided in the Sequence Listing as SEQ ID NOs: 62-66 and SEQ ID NOs: 67-189. Non-
20 limiting examples of cytidine deaminases include those described in PCT/US20/16288, PCT/US2018/021878, 180802-021804/PCT, PCT/US2018/048969, and PCT/US2016/058344.

By “cytosine deaminase activity” is meant catalyzing the deamination of cytosine or cytidine. In one embodiment, a polypeptide having cytosine deaminase activity converts an

amino group to a carbonyl group. In an embodiment, a cytosine deaminase converts cytosine to uracil (*i.e.*, C to U) or 5-methylcytosine to thymine (*i.e.*, 5mC to T). In some embodiments, a cytosine deaminase as provided herein has increased cytosine deaminase activity (*e.g.*, at least 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold or
5 more) relative to a reference cytosine deaminase.

The term “deaminase” or “deaminase domain,” as used herein, refers to a protein or fragment thereof that catalyzes a deamination reaction.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Exemplary diseases include diseases amenable to
10 treatment using the methods and/or compositions of the present disclosure include as non-limiting examples amyloidosis, cardiomyopathy, familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), familial transthyretin amyloidosis (FTA), senile systemic amyloidosis (SSA), transthyretin amyloidosis, and the like. The disease can be any disease associated with a mutation in a transthyretin (TTR) polynucleotide sequence.

By “dual editing activity” or “dual deaminase activity” is meant having adenosine deaminase and cytidine deaminase activity. In one embodiment, a base editor having dual editing activity has both A→G and C→T activity, wherein the two activities are approximately equal or are within about 10% or 20% of each other. In another embodiment, a dual editor has A→G activity that no more than about 10% or 20% greater than C→T
15 activity. In another embodiment, a dual editor has A→G activity that is no more than about 10% or 20% less than C→T activity. In some embodiments, the adenosine deaminase variant has predominantly cytosine deaminase activity, and little, if any, adenosine deaminase activity. In some embodiments, the adenosine deaminase variant has cytosine deaminase activity, and no significant or no detectable adenosine deaminase activity.
20

By “effective amount” is meant the amount of an agent (*e.g.*, a base editor, cell) as described herein, that is required to ameliorate the symptoms of a disease relative to an untreated patient or an individual without disease, *i.e.*, a healthy individual, or is the amount of the agent sufficient to elicit a desired biological response. The effective amount of active compound(s) used to practice embodiments of the present disclosure for therapeutic treatment
25 of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount. In one embodiment, an effective amount is the amount of a base editor of the disclosure sufficient to introduce an alteration in a gene of interest in a cell (*e.g.*, a cell *in*
30

vitro or *in vivo*). In one embodiment, an effective amount is the amount of a base editor required to achieve a therapeutic effect. Such therapeutic effect need not be sufficient to alter a pathogenic gene in all cells of a subject, tissue or organ, but only to alter the pathogenic gene in about 1%, 5%, 10%, 25%, 50%, 75% or more of the cells present in a subject, tissue
 5 or organ. In one embodiment, an effective amount is sufficient to ameliorate one or more symptoms of a disease. In one embodiment, an effective amount of a base editor is sufficient to alter the sequence of a TTR polynucleotide in 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more cells of a tissue.

The term “encapsulated” is used herein to refer to substances that are completely
 10 surrounded by another material. In an embodiment, an mRNA molecule encoding a base editor and/or a guide polynucleotide is encapsulated by a lipid nanoparticle of the disclosure.

By “fragment” is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain
 15 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids. In some embodiments, the fragment is a functional fragment.

By “guide polynucleotide” is meant a polynucleotide or polynucleotide complex which is specific for a target sequence and can form a complex with a polynucleotide programmable nucleotide binding domain protein (*e.g.*, Cas9 or Cpf1). In an embodiment, the
 20 guide polynucleotide is a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule.

The term “haloaliphatic” refers to an aliphatic group substituted by one or more halogen atoms (*e.g.*, one, two, three, four, five, six, or seven halo, such as fluoro, iodo, bromo, or chloro). In some embodiments, haloaliphatic groups contain 1-7 halogen atoms.
 25 In some embodiments, haloaliphatic groups contain 1-5 halogen atoms. In some embodiments, haloaliphatic groups contain 1-3 halogen atoms.

The term “haloalkyl” refers to an alkyl group substituted by one or more halogen atoms (*e.g.*, one, two, three, four, five, six, or seven halo, such as fluoro, iodo, bromo, or chloro). In some embodiments, haloalkyl groups contain 1-7 halogen atoms. In some
 30 embodiments, haloalkyl groups contain 1-5 halogen atoms. In some embodiments, haloalkyl groups contain 1-3 halogen atoms.

The term “heteroalkylenyl” or “heteroalkylene”, as used herein, denotes an optionally substituted straight-chain (*i.e.*, unbranched), or branched bivalent alkyl group (*i.e.*, bivalent saturated hydrocarbon chain) having, in addition to carbon atoms, from one to five

heteroatoms. The term “heteroatom” is described below. In some embodiments, heteroalkylenyl groups contain 2–10 carbon atoms wherein 1–3 carbon atoms are optionally and independently replaced with heteroatoms selected from oxygen, nitrogen, and sulfur. In some embodiments, heteroalkylenyl groups contain 2–8 carbon atoms wherein 1–3 carbon atoms are optionally and independently replaced with heteroatoms selected from oxygen, nitrogen, and sulfur. In some embodiments, heteroalkylenyl groups contain 4–8 carbon atoms, wherein 1–3 carbon atoms are optionally and independently replaced with heteroatoms selected from oxygen, nitrogen, and sulfur. In some embodiments, heteroalkylenyl groups contain 2–5 carbon atoms, wherein 1–2 carbon atoms are optionally and independently replaced with heteroatoms selected from oxygen, nitrogen, and sulfur. In yet other embodiments, heteroalkylenyl groups contain 1–3 carbon atoms, wherein 1 carbon atom is optionally and independently replaced with a heteroatom selected from oxygen, nitrogen, and sulfur. Suitable heteroalkylenyl groups include, but are not limited to $-\text{CH}_2\text{O}-$, $-(\text{CH}_2)_2\text{O}-$, $-\text{CH}_2\text{OCH}_2-$, $-\text{O}(\text{CH}_2)_2-$, $-(\text{CH}_2)_3\text{O}-$, $-(\text{CH}_2)_2\text{OCH}_2-$, $-\text{CH}_2\text{O}(\text{CH}_2)_2-$, $-\text{O}(\text{CH}_2)_3-$, $-(\text{CH}_2)_4\text{O}-$, $-(\text{CH}_2)_3\text{OCH}_2-$, $-\text{CH}_2\text{O}(\text{CH}_2)_3-$, $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_2-$, $-\text{O}(\text{CH}_2)_4-$. Unless otherwise specified, C_x heteroalkylenyl refers to heteroalkylenyl having x number of carbon atoms prior to replacement with heteroatoms.

The terms “heteroaryl” and “heteroar-”, used alone or as part of a larger moiety, e.g., “heteroaralkyl”, or “heteroaralkoxy”, refer to monocyclic or bicyclic ring groups having 5 to 10 ring atoms (e.g., 5- to 6-membered monocyclic heteroaryl or 9- to 10-membered bicyclic heteroaryl); having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. Exemplary heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridonyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizynyl, purinyl, naphthyridinyl, pteridinyl, imidazo[1,2-a]pyrimidinyl, imidazo[1,2-a]pyridinyl, thienopyrimidinyl, triazolopyridinyl, and benzoisoxazolyl. The terms “heteroaryl” and “heteroar-”, as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring (i.e., a bicyclic heteroaryl ring having 1 to 3 heteroatoms). Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzothiazolyl, benzothiadiazolyl, benzoxazolyl, quinolyl, isoquinolyl, cinnolynyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4*H*-quinolizynyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolynyl, tetrahydroisoquinolynyl, pyrido[2,3-*b*]-1,4-oxazin-

3(4H)-one, and benzoisoxazolyl. The term “heteroaryl” may be used interchangeably with the terms “heteroaryl ring”, “heteroaryl group”, or “heteroaromatic”, any of which terms include rings that are optionally substituted.

The term “heteroatom” means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR⁺ (as in N-substituted pyrrolidinyl)).

The terms “heterocycle”, “heterocyclyl”, “heterocyclic radical”, and “heterocyclic ring” are used interchangeably herein, and refer to a stable 3- to 8-membered monocyclic, a 7- to 12-membered bicyclic, or a 10- to 16-membered polycyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, such as one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term “nitrogen” includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl), or NR⁺ (as in N-substituted pyrrolidinyl). A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, azetidiny, oxetanyl, tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, piperidinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, tetrahydropyranyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl,



thiamorpholinyl, and . A heterocyclyl group may be mono-, bi-, tri-, or polycyclic, preferably mono-, bi-, or tricyclic, more preferably mono- or bicyclic. The term “heterocyclalkyl” refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted. A bicyclic heterocyclic ring also includes groups in which the heterocyclic ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings. Exemplary bicyclic heterocyclic groups include indolinyl, isoindolinyl, benzodioxolyl, 1,3-dihydroisobenzofuranyl, 2,3-dihydrobenzofuranyl, and tetrahydroquinolinyl. A bicyclic heterocyclic ring can also be a spirocyclic ring system (e.g., 7- to 11-membered spirocyclic fused heterocyclic ring having, in addition to carbon atoms, one or more heteroatoms as defined above (e.g., one, two, three or four heteroatoms)). A

bicyclic heterocyclic ring can also be a bridged ring system (e.g., 7- to 11-membered bridged heterocyclic ring having one, two, or three bridging atoms.

“Hybridization” means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By “increases” is meant a positive alteration of at least 10%, 25%, 50%, 75%, or 100%, or about 1.5 fold, about 2 fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, or about 100-fold.

The terms “inhibitor of base repair”, “base repair inhibitor”, “IBR” or their grammatical equivalents refer to a protein that is capable in inhibiting the activity of a nucleic acid repair enzyme, for example a base excision repair enzyme.

The terms “isolated,” “purified,” or “biologically pure” refer to material that is free to varying degrees from components which normally accompany it as found in its native state. “Isolate” denotes a degree of separation from original source or surroundings. “Purify” denotes a degree of separation that is higher than isolation. A “purified” or “biologically pure” protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this disclosure is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term “purified” can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By “isolated polynucleotide” is meant a nucleic acid molecule that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the disclosure is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that

exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide.

5 By an “isolated polypeptide” is meant a polypeptide of the disclosure that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. In embodiments, the preparation is at least 75%, at least 90%, or at least 99%, by weight, a polypeptide of the disclosure. An isolated
10 polypeptide of the disclosure may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

The term “linker”, as used herein, refers to a molecule that links two moieties. In one
15 embodiment, the term “linker” refers to a covalent linker (*e.g.*, covalent bond) or a non-covalent linker. For example, those of ordinary skill in the art appreciate that a polypeptide whose structure includes two or more functional or organizational domains often includes a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element “L” has an overall structure of the
20 general form S1-L'-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker. In some embodiments, a polypeptide linker is at least 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, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a
25 rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (*e.g.*, fusion polypeptides) known in the art (see *e.g.*, Holliger, P., et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R. J., et al. (1994) *Structure* 2: 1 121-1123).

As used herein, a “lipid nanoparticle (LNP) composition” is a nanoparticle
30 composition comprising one or more ionizable lipids. Exemplary LNP compositions include, but are not limited to, phospholipids, conjugate-linker lipids (*e.g.*, PEG-lipids), sterols, and ionizable lipids (*e.g.*, IZ1, IZ4).

The term “mutation,” as used herein, refers to a substitution of a residue within a sequence, *e.g.*, a nucleic acid or amino acid sequence, with another residue, or a deletion or

insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, 5 for example, Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)).

As used herein, the “N/P ratio” is the molar ratio of ionizable nitrogen atoms in a lipid(s) to phosphate groups in a nucleic acid molecular entity(ies). In some embodiments, the nitrogen atoms are ionizable within a physiological pH range. In some embodiments, the 10 molecular entities in a nanoparticle composition include a lipid component and an RNA. Ionizable nitrogen atoms can include, for example, nitrogen atoms that can be protonated at about pH 1, about pH 2, about pH 3, about pH 4, about pH 4.5, about pH 5, about pH 5.5, about pH 6, about pH 6.5, about pH 7, about pH 7.5, or about pH 8 or higher. The physiological pH range can include, for example, the pH range of different cellular 15 compartments (such as organs, tissues, and cells) and bodily fluids (such as blood, CSF, gastric juice, milk, bile, saliva, tears, and urine). In certain specific embodiments, the physiological pH range refers to the pH range of blood in a mammal, for example, from about 7.35 to about 7.45. Similarly, for phosphate charge neutralizers that have one or more ionizable nitrogen atoms, the N/P ratio can refer to a molar ratio of ionizable nitrogen atoms 20 in the phosphate charge neutralizer to the phosphate groups in a nucleic acid. In some embodiments, ionizable nitrogen atoms refer to those nitrogen atoms that are ionizable within a pH range between 5 and 14.

For a payload that does not contain a phosphate group, the N/P ratio can refer to a molar ratio of ionizable nitrogen atoms in a lipid to the total negative charge in the payload. 25 For example, the N/P ratio of an LNP composition can refer to a molar ratio of the total ionizable nitrogen atoms in the LNP composition to the total negative charge in the payload that is present in the composition.

As used herein, the term “nanoparticle” refers to a particle having a diameter of less than 1000 nanometers (nm). In some embodiments, a nanoparticle has a diameter of less than 30 300 nm, as defined by the National Science Foundation. In some embodiments, a nanoparticle has a diameter of less than 100 nm as defined by the National Institutes of Health. In some embodiments, nanoparticles are micelles in that they comprise an enclosed compartment, separated from the bulk solution by a micellar membrane, typically comprised of amphiphilic entities which surround and enclose a space or compartment (*e.g.*, to define a

lumen). In some embodiments, a micellar membrane is comprised of at least one polymer, such as for example a biocompatible and/or biodegradable polymer. In some embodiments, lipid nanoparticles described herein can have an average hydrodynamic diameter from about 30 to about 170 nm. In some embodiments, lipid nanoparticles described herein can have an average hydrodynamic diameter that is about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, 150 nm, 155 nm, 160 nm, 165 nm, 170 nm, 175 nm, 180 nm, 185 nm, 190 nm, 195 nm, 200 nm, 205 nm, 210 nm, 215 nm, 220 nm, or any range having endpoints defined by any two of the aforementioned values. For example, in some embodiments, lipid nanoparticles described herein have an average hydrodynamic diameter from between 50 nm to 100 nm. For example, in some embodiments, compositions, preparations, nanoparticles, and/or nanomaterials described herein have an average hydrodynamic diameter from between 50 nm to 90 nm. In some embodiments, lipid nanoparticles described herein have an average hydrodynamic diameter from about 60 to about 80 nm. For example, in some embodiments, lipid nanoparticles described herein have an average hydrodynamic diameter from between 50 nm to 70 nm.

As used herein, the term “nanoparticle composition” refers to a composition that contains at least one nanoparticle and at least one additional agent or ingredient. In some embodiments, a nanoparticle composition contains a substantially uniform collection of nanoparticles as described herein.

The terms “nucleic acid” and “nucleic acid molecule,” as used herein, refer to a compound comprising a nucleobase and an acidic moiety, *e.g.*, a nucleoside, a nucleotide, or a polymer of nucleotides. Typically, polymeric nucleic acids, *e.g.*, nucleic acid molecules comprising three or more nucleotides are linear molecules, in which adjacent nucleotides are linked to each other via a phosphodiester linkage. In some embodiments, “nucleic acid” refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some embodiments, “nucleic acid” refers to an oligonucleotide chain comprising three or more individual nucleotide residues. As used herein, the terms “oligonucleotide” and “polynucleotide” can be used interchangeably to refer to a polymer of nucleotides (*e.g.*, a string of at least three nucleotides). In some embodiments, “nucleic acid” encompasses RNA as well as single and/or double-stranded DNA. Nucleic acids may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a non-naturally occurring

molecule, *e.g.*, a recombinant DNA or RNA, an artificial chromosome, an engineered genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides. Furthermore, the terms “nucleic acid,” “DNA,” “RNA,” and/or similar terms include nucleic acid analogs, *e.g.*, analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, nucleic acids comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (*e.g.* adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5'-N-phosphoramidite linkages).

The term “nuclear localization sequence,” “nuclear localization signal,” or “NLS” refers to an amino acid sequence that promotes import of a protein into the cell nucleus. Nuclear localization sequences are known in the art and described, for example, in Plank *et al.*, International PCT application, PCT/EP2000/011690, filed November 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In other embodiments, the NLS is an optimized NLS described, for example, by Koblan *et al.*, Nature Biotech. 2018 doi:10.1038/nbt.4172. In some embodiments, an NLS comprises the amino acid sequence KRTADGSEFESPKKKRKV (SEQ ID NO: 190), KRPAATKKAGQAKKKK (SEQ ID NO: 191), KKTELQTTNAENKTKKL (SEQ ID NO: 192), KRGINDRNFWRGENGRKTR (SEQ ID NO: 193), RKSGKIAAIVVKRPRK (SEQ ID NO: 194), PKKKRKV (SEQ ID NO: 195), MDSLMLNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 196),

PKKKRKVEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 328), or
 RKSGKIAAIVVKRPRKPKKKRKV (SEQ ID NO: 329).

The term “nucleobase,” “nitrogenous base,” or “base,” used interchangeably herein, refers to a nitrogen-containing biological compound that forms a nucleoside, which in turn is a component of a nucleotide. The ability of nucleobases to form base pairs and to stack one upon another leads directly to long-chain helical structures such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Five nucleobases – adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U) – are called primary or canonical. Adenine and guanine are derived from purine, and cytosine, uracil, and thymine are derived from pyrimidine. DNA and RNA can also contain other (non-primary) bases that are modified. Non-limiting exemplary modified nucleobases can include hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine (m5C), and 5-hydromethylcytosine. Hypoxanthine and xanthine can be created through mutagen presence, both of them through deamination (replacement of the amine group with a carbonyl group). Hypoxanthine can be modified from adenine. Xanthine can be modified from guanine. Uracil can result from deamination of cytosine. A “nucleoside” consists of a nucleobase and a five carbon sugar (either ribose or deoxyribose). Examples of a nucleoside include adenosine, guanosine, uridine, cytidine, 5-methyluridine (m5U), deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine, and deoxycytidine. Examples of a nucleoside with a modified nucleobase includes inosine (I), xanthosine (X), 7-methylguanosine (m7G), dihydrouridine (D), 5-methylcytidine (m5C), and pseudouridine (Ψ). A “nucleotide” consists of a nucleobase, a five carbon sugar (either ribose or deoxyribose), and at least one phosphate group. Non-limiting examples of modified nucleobases and/or chemical modifications that a modified nucleobase may include are the following: pseudo-uridine, 5-Methyl-cytosine, 2'-O-methyl-3'-phosphonoacetate, 2'-O-methyl thioPACE (MSP), 2'-O-methyl-PACE (MP), 2'-fluoro RNA (2'-F-RNA), constrained ethyl (S-cEt), 2'-O-methyl (‘M’), 2'-O-methyl-3'-phosphorothioate (‘MS’), 2'-O-methyl-3'-thiophosphonoacetate (‘MSP’), 5-methoxyuridine, phosphorothioate, and N1-Methylpseudouridine.

The term “nucleic acid programmable DNA binding protein” or “napDNAbp” may be used interchangeably with “polynucleotide programmable nucleotide binding domain” to refer to a protein that associates with a nucleic acid (*e.g.*, DNA or RNA), such as a guide nucleic acid or guide polynucleotide (*e.g.*, gRNA), that guides the napDNAbp to a specific nucleic acid sequence. In some embodiments, the polynucleotide programmable nucleotide

binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 protein. A Cas9 protein

5 can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that is complementary to the guide RNA. In some embodiments, the napDNAbp is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpf1, Cas12b/C2cl, Cas12c/C2c3,

10 Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, and Cas12j/CasΦ (Cas12j/Casphi). Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpf1, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Cas12j/CasΦ, Cpf1, Csy1, Csy2, Csy3, Csy4, Cse1,

15 Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or

20 engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" *CRISPR J.* 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" *Science*. 2019 Jan

25 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference. Exemplary nucleic acid programmable DNA binding proteins and nucleic acid sequences encoding nucleic acid programmable DNA binding proteins are provided in the Sequence Listing as SEQ ID NOs: 197-245, 254-260, and 378. In some embodiments, the napDNAbp is a (CRISPR-associated system) Cas9 endonuclease, for

30 example, Cas9 (Csn1) from *Streptococcus pyogenes* (*e.g.*, SEQ ID NO: 197), Cas9 from *Neisseria meningitidis* (NmeCas9; SEQ ID NO: 208), Nme2Cas9 (SEQ ID NO: 209), *Streptococcus constellatus* (ScoCas9), or derivatives thereof (*e.g.*, a sequence with at least about 85% sequence identity to a Cas9, such as Nme2Cas9 or spCas9).

The terms “nucleobase editing domain” or “nucleobase editing protein,” as used herein, refers to a protein or enzyme that can catalyze a nucleobase modification in RNA or DNA, such as cytosine (or cytidine) to uracil (or uridine) or thymine (or thymidine), and adenine (or adenosine) to hypoxanthine (or inosine) deaminations, as well as non-templated
5 nucleotide additions and insertions. In some embodiments, the nucleobase editing domain is a deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase; or a cytidine deaminase or a cytosine deaminase).

As used herein, “obtaining” as in “obtaining an agent” includes synthesizing, purchasing, or otherwise acquiring the agent.
10 “Patient in need thereof” or “subject in need thereof” is referred to herein as a patient diagnosed with, at risk or having, predetermined to have, or suspected of having a disease or disorder.

The terms “pathogenic mutation”, “pathogenic variant”, “disease causing mutation”, “disease causing variant”, “deleterious mutation”, or “predisposing mutation” refers to a
15 genetic alteration or mutation that is associated with a disease or disorder or that increases an individual’s susceptibility or predisposition to a certain disease or disorder. In some embodiments, the pathogenic mutation comprises at least one wild-type amino acid substituted by at least one pathogenic amino acid in a protein encoded by a gene. In some embodiments, the pathogenic mutation is in a terminating region (*e.g.*, stop codon). In some
20 embodiments, the pathogenic mutation is in a non-coding region (*e.g.*, intron, promoter, etc.).

The term “pharmaceutically acceptable salt”, as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, *i.e.*, salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like,
25 and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977).

As used herein, a “phospholipid” can refer to a lipid that includes a phosphate moiety and one or more carbon chains, such as unsaturated fatty acid chains. A phospholipid may
30 include one or more multiple (*e.g.*, double or triple) bonds. In some embodiments, a phospholipid may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (*e.g.*, a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more

elements of an LNP to pass through the membrane, i.e., delivery of the one or more elements to a cell.

The terms “protein”, “peptide”, “polypeptide”, and their grammatical equivalents are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. A protein, peptide, or polypeptide can be naturally occurring,
5 recombinant, or synthetic, or any combination thereof.

The term “fusion protein” as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins.

The term “recombinant” as used herein in the context of proteins or nucleic acids
10 refers to proteins or nucleic acids that do not occur in nature but are the product of human engineering. For example, in some embodiments, a recombinant protein or nucleic acid molecule comprises an amino acid or nucleotide sequence that comprises at least one, at least two, at least three, at least four, at least five, at least six, or at least seven mutations as compared to any naturally occurring sequence.

15 By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or control condition. In one embodiment, the reference is a wild-type or healthy cell. In other embodiments and without limitation, a reference is an untreated cell that is not subjected to a test condition, or is subjected to
20 placebo or normal saline, medium, buffer, and/or a control vector that does not harbor a polynucleotide of interest. The reference can be a cell or subject with a pathogenic mutation in a transthyretin (TTR) polynucleotide sequence and/or a transthyretin (TTR) polypeptide sequence. A reference can be a subject or cell with an amyloidosis (e.g., a transthyretin amyloidosis) or a subject or cell without an amyloidosis. In one embodiment, the level of
25 amyloidosis in a cell or tissue treated with a base editor system described herein is compared to the level of amyloidosis in a corresponding untreated cell or tissue.

A “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or
30 gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, at least about 20 amino acids, at least about 25 amino acids, about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, at least about 60 nucleotides, at least about 75 nucleotides, about 100

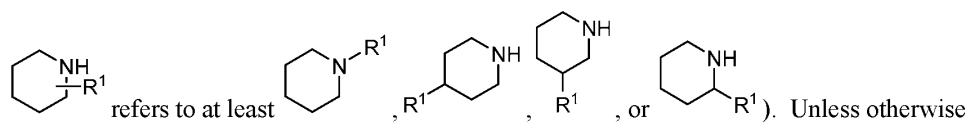
nucleotides or about 300 nucleotides or any integer thereabout or therebetween. In some embodiments, a reference sequence is a wild-type sequence of a protein of interest. In other embodiments, a reference sequence is a polynucleotide sequence encoding a wild-type protein.

5 The term “sterolyl,” as used herein, refers to a 17-membered fused polycyclic ring moiety that is either saturated or partially unsaturated and substituted with at least one hydroxyl group, and has a single point of attachment to the rest of the molecule at any substitutable carbon or oxygen atom. In some embodiments, a sterolyl group is a cholesterolyl group, or a variant or derivative thereof. In some embodiments, a cholesterolyl group is modified. In some embodiments, a cholesterolyl group is an oxidized cholesterolyl group (e.g., oxidized on the beta-ring structure or on the hydrocarbon tail structure). In some
10 embodiments, a cholesterolyl group is an esterified cholesterolyl group. In some embodiments, a sterolyl group is a phytosterolyl group. Exemplary sterolyl groups include but are not limited to 25-hydroxycholesterolyl (25-OH), 20 α -hydroxycholesterolyl (20 α -OH),
15 27-hydroxycholesterolyl, 6-keto-5 α -hydroxycholesterolyl, 7-ketocholesterolyl, 7 β -hydroxycholesterolyl, 7 α -hydroxycholesterolyl, 7 β -25-dihydroxycholesterolyl, beta-sitosterolyl, stigmasterolyl, brassicasterolyl, and campesterolyl.

 By “subject” or “patient” is meant a mammal, including, but not limited to, a human or non-human mammal. In embodiments, the mammal is a bovine, equine, canine, ovine,
20 rabbit, rodent, nonhuman primate, or feline. In an embodiment, “patient” refers to a mammalian subject with a higher than average likelihood of developing a disease or a disorder. Exemplary patients can be humans, non-human primates, cats, dogs, pigs, cattle, cats, horses, camels, llamas, goats, sheep, rodents (e.g., mice, rabbits, rats, or guinea pigs) and other mammals that can benefit from the therapies disclosed herein. Exemplary
25 human patients can be male and/or female.

 As described herein, compounds of this disclosure may be described as “substituted” or “optionally substituted”. That is, compounds may contain optionally substituted and/or substituted moieties. In general, the term “substituted,” whether preceded by the term
30 “optionally” or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. “Substituted” applies to one or more hydrogens that are either

explicit or implicit from the structure (e.g.,  refers to at least ; and



indicated, an “optionally substituted” group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the

5 substituent may be either the same or different at every position. Combinations of substituents envisioned by this disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term “stable,” as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for

10 one or more of the purposes disclosed herein. Groups described as being “substituted” preferably have between 1 and 4 substituents, more preferably 1 or 2 substituents. Groups described as being “optionally substituted” may be unsubstituted or be “substituted” as described above.

Suitable monovalent substituents include halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$; $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}Ph$, which may be substituted with R° ; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R° ; $-CH=CHPh$, which may be substituted with R° ; $-(CH_2)_{0-4}O(CH_2)_{0-1}pyridyl$ which may be substituted with R° ; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; $-SiR^\circ_3$; $-OSiR^\circ_3$; $-(C_{1-4} \text{ straight or branched alkylene})O-N(R^\circ)_2$; or $-(C_{1-4} \text{ straight or branched alkylene})C(O)O-N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$ -(5-6 membered heteroaryl ring), or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the

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definition above, two independent occurrences of R° , taken together with their intervening atom(s), form a 3–12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

- 5 Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, –
 (CH₂)_{0–2}R[•], –(haloR[•]), –(CH₂)_{0–2}OH, –(CH₂)_{0–2}OR[•], –(CH₂)_{0–2}CH(OR[•])₂, –O(haloR[•]), –CN,
 –N₃, –(CH₂)_{0–2}C(O)R[•], –(CH₂)_{0–2}C(O)OH, –(CH₂)_{0–2}C(O)OR[•], –(CH₂)_{0–2}C(O)NH₂, –(CH₂)_{0–}
 2C(O)NHR[•], –(CH₂)_{0–2}C(O)NR[•]₂, –(CH₂)_{0–2}SR[•], –(CH₂)_{0–2}SH, –(CH₂)_{0–2}NH₂, –(CH₂)_{0–}
 10 2NHR[•], –(CH₂)_{0–2}NR[•]₂, –NO₂, –SiR[•]₃, –OSiR[•]₃, –C(O)SR[•], –(C_{1–4} straight or branched
 alkylene)C(O)OR[•], or –SSR[•] wherein each R[•] is unsubstituted or where preceded by “halo”
 is substituted only with one or more halogens, and is independently selected from C_{1–}
 4 aliphatic, –CH₂Ph, –O(CH₂)_{0–1}Ph, or a 5–6-membered saturated, partially unsaturated, or
 aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.
- 15 Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

- Suitable divalent substituents include the following: =O, =S, =NNR^{*}₂, =NNHC(O)R^{*},
 =NNHC(O)OR^{*}, =NNHS(O)₂R^{*}, =NR^{*}, =NOR^{*}, –O(C(R^{*})₂)_{2–3}O–, or –S(C(R^{*})₂)_{2–3}S–,
 wherein each independent occurrence of R^{*} is selected from hydrogen, C_{1–6} aliphatic which
 may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially
 20 unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen,
 oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable
 carbons of an “optionally substituted” group include: –O(CR^{*})_{2–3}O–, wherein each
 independent occurrence of R^{*} is selected from hydrogen, C_{1–6} aliphatic which may be
 substituted as defined below, or an unsubstituted 5–6-membered saturated, partially
 25 unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen,
 oxygen, or sulfur.

- Suitable substituents on the aliphatic group of R^{*} include halogen, –R[•], –(haloR[•]), –
 OH, –OR[•], –O(haloR[•]), –CN, –C(O)OH, –C(O)OR[•], –NH₂, –NHR[•], –NR[•]₂, or –NO₂,
 wherein each R[•] is unsubstituted or where preceded by “halo” is substituted only with one or
 30 more halogens, and is independently C_{1–4} aliphatic, –CH₂Ph, –O(CH₂)_{0–1}Ph, or a 5–6–
 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently
 selected from nitrogen, oxygen, or sulfur.

In some embodiments, suitable substituents on a substitutable nitrogen include $-R^{\dagger}$, $-NR^{\dagger}_2$, $-C(O)R^{\dagger}$, $-C(O)OR^{\dagger}$, $-C(O)C(O)R^{\dagger}$, $-C(O)CH_2C(O)R^{\dagger}$, $-S(O)_2R^{\dagger}$, $-S(O)_2NR^{\dagger}_2$, $-C(S)NR^{\dagger}_2$, $-C(NH)NR^{\dagger}_2$, or $-N(R^{\dagger})S(O)_2R^{\dagger}$; wherein each $R^{\dagger}$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted $-OPh$, or an

5 unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3–12–membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen,

10 oxygen, or sulfur.

Suitable substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, $-(haloR^{\bullet})$, $-OH$, $-OR^{\bullet}$, $-O(haloR^{\bullet})$, $-CN$, $-C(O)OH$, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6–

15 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

The term “stable,” when applied to compositions herein, means that the compositions maintain one or more aspects of their physical structure (*e.g.*, size range and/or distribution of particles) over a period of time. In some embodiments, a stable nanoparticle composition is

20 one for which the average particle size, the maximum particle size, the range of particle sizes, and/or the distribution of particle sizes (*i.e.*, the percentage of particles above a designated size and/or outside a designated range of sizes) is maintained for a period of time under specified conditions. In some embodiments, a stable provided composition is one for which a biologically relevant activity is maintained for a period of time. In some embodiments, the

25 period of time is at least about one hour; in some embodiments the period of time is about 5 hours, about 10 hours, about one (1) day, about one (1) week, about two (2) weeks, about one (1) month, about two (2) months, about three (3) months, about four (4) months, about five (5) months, about six (6) months, about eight (8) months, about ten (10) months, about twelve (12) months, about twenty-four (24) months, about thirty-six (36) months, or longer.

30 In some embodiments, the period of time is within the range of about one (1) day to about twenty-four (24) months, about two (2) weeks to about twelve (12) months, about two (2) months to about five (5) months, etc. For example, if a population of nanoparticles is subjected to prolonged storage, temperature changes, and/or pH changes, and a majority of the nanoparticles in the composition maintain a diameter within a stated range, the

nanoparticle composition is stable. In some embodiments, a stable composition is stable at ambient conditions. In some embodiments, a stable composition is stable under biologic conditions (i.e. 37 °C in phosphate buffered saline).

By “substantially identical” is meant a polypeptide or nucleic acid molecule
5 exhibiting at least 50% identity to a reference amino acid sequence. In one embodiment, a reference sequence is a wild-type amino acid or nucleic acid sequence. In another embodiment, a reference sequence is any one of the amino acid or nucleic acid sequences described herein. In one embodiment, such a sequence is at least about 60%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or even 99.99%, identical at the amino acid level or
10 nucleic acid level to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches
15 identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

20 Nucleic acid molecules useful in the methods of the disclosure include any nucleic acid molecule that encodes a polypeptide of the disclosure or a functional fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one
25 strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the disclosure include any nucleic acid molecule that encodes a polypeptide of the disclosure or a functional fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are
30 typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (*e.g.*, a gene described herein), or portions thereof, under various conditions of stringency. (See, *e.g.*, Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

The term “target site” refers to a nucleotide sequence or nucleobase of interest within a nucleic acid molecule that is modified. In embodiments, the modification is deamination of a base. The deaminase can be a cytidine or an adenine deaminase. The fusion protein or base editing complex comprising a deaminase may comprise a dCas9-adenosine deaminase fusion
 5 protein, a Cas12b-adenosine deaminase fusion, or a base editor disclosed herein.

By “transthyretin (TTR) polypeptide” is meant a polypeptide or fragment thereof having at least about 95% amino acid sequence identity to an amino acid sequence provided at NCBI Reference Sequence No. NP_000362.1, or a fragment thereof, that binds an anti-TTR antibody. In some embodiments, a TTR polypeptide or fragment thereof has holo-
 10 retinol-binding protein (RBP) and/or thyroxine (T4) transport activity. In embodiments, TTR is capable of forming a tetramer. An exemplary TTR polypeptide sequence follows (the signal peptide sequence is in bold; therefore, the mature TTR polypeptide corresponds to amino acids 21 to 147 of the following sequence):

MASHRLLLCLAGLVFVSEAGPTGTGESKCPMLVKVLDAVRGSPAINVAVHVFRKAADDTWE
 15 PFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEEVFTANDSGPRR
 YTIAALLSPYSYSTTAVVTNPKE (SEQ ID NO: 464).

By “transthyretin (TTR) polynucleotide” is meant a nucleic acid molecule that encodes a TTR polypeptide, as well as the introns, exons, 3' untranslated regions, 5' untranslated regions, and regulatory sequences associated with its expression, or fragments
 20 thereof. In embodiments, the regulatory sequence is a promoter region. In embodiments, a TTR polynucleotide is the genomic sequence, cDNA, mRNA, or gene associated with and/or required for TTR expression. An exemplary *TTR* polynucleotide sequence (corresponding to Consensus Coding Sequence (CCDS) No. 11899.1) is provided below. Further exemplary *TTR* polynucleotide sequences include Gene Ensembl ID: ENSG00000118271 and Transcript
 25 Ensembl ID: ENST00000237014.8.

ATGGCTTCTCATCGTCTGCTCCTCCTCTGCCTTGCTGGACTGGTATTTGTGTCTGAGGCTGG
 CCCTACGGGCACCGGTGAATCCAAGTGTCTCTGATGGTCAAAGTTCTAGATGCTGTCCGAG
 GCAGTCTCGCCATCAATGTGGCCGTGCATGTGTTTCAGAAAGGCTGCTGATGACACCTGGGAG
 CCATTTGCCTCTGGGAAAACAGTGAGTCTGGAGAGCTGCATGGGCTCACAACCTGAGGAGGA
 30 ATTTGTAGAAGGGATATACAAAGTGGAAATAGACACCAAATCTTACTGGAAGGCACTTGGCA
 TCTCCCCATTCCATGAGCATGCAGAGGTGGTATTCACAGCCAACGACTCCGGCCCCCGCCGC
 TACACCATTGCCGCCCTGCTGAGCCCCTACTCCTATTCCACCACGGCTGTCGTCACCAATCC
 CAAGGAATGA (SEQ ID NO: 465).

A further exemplary TTR polynucleotide sequence is provided at NCBI Reference Sequence No. NG_009490.1 and follows (where exons encoding the TTR polypeptide are in bold, introns are in italics, and exemplary promoter regions are indicated by the combined underlined and bold-underlined text (promoter positions -1 to -177) and by the bold-

5 underlined text (promoter positions -106 to -176):

TTATGTGTTTATTCAACAATGGCGGAGGAGAGGCATGCCAGATAAGGCAGACACGGGCATTCC
 CAAACACAAGAAAGGTATGTGCTGCAGAGAAGTCAGATAAACTTTCTAGGCTCTCCTGCAGT
 CCGGATGAAATACTCTCAAAAAATTAGCCCGGGCCCTTTGCTCCAATTTTTTCGTTACCTAG
 CAACCATCTAACTATTAATTAAATTGGTATTATGGTTTAAACATGAATCTTTTATGATTTGC
 10 TTACCATTAATCAAACCCCCGAGGCTTATTACCTCAAGGGGAGCTGACAAAGTTGAATTAT
 TCAACCTGCAAAGATCCAGGGCCCCCAAATACTGTCATTTCCACTCTCCCCTAACCCCCACC
 ATGAGGCCCAGTCTCAGCACTCGGCCAGCCTATGCCCAACTCGGGGTAATCAGCTTAGACAT
 ATTAATATTAGTGGGCATTTTCAGTATCAACAGATCACTGTCTAGCAGCTGACAGGCACCCTC
 AGAAAAATAAACCAAGAAGAAAGGGTTTATCTATAATATCAAAATTTTTTCATAGATAAACCTG
 15 CCCATTATAAGGAAGAGGGCAGAAGAACCCTAACTAAGAGCCAGGCAACTTGTTTCATTAAT
 CACAGCATATTCCATAGAAGGAGGAGAAATGTTGTCATCAAATTCATCTTTTTTCACCTCAAT
 TAAACATCTATGCTACAGCTCCACAGTCAGATTGAGAGGAAAAACAGTACGTAGCTAAGAAA
 AGACATAGACTTGTAAGTAAATGCTTCACTGGTGCTCCTTTTGTTTAAAGGCATTGGATCT
 TCATAGCTACTGATCGTGCCCAAGCACACAGTATCTGCAGCAACCACTTAGGCCTCCAGGAA
 20 TGTGGTGACCATTGACCCTAATTCATTCCTTCATGGATCCTATGTAACCATCCTCCAAAA
 AGAGCTTTTCGCAAACCTCAAATAAACACAGGAAAGGAAGACCTTCTTATCTTTGAGAGTATAT
 GTTTAGCCCTATAACCCTCTCTTATCATAAATTGCTTCTTAGGCAAGAAACACTGGATTTTT
 CTTGTATTTGTCATTGCCATTGGTTCCATACAAGCATTCAATTAACAAATAATACATTCCAT
 CTCCGTTTTTTTGCTTTTTCTTTCATGCTTCGGCCTTGTTTCTCTCACCTAAAACACTACA
 25 AGCTTCTTCTCCCAGAGCTCTCACTTTGACTCCAGACTACCTACATTTAATCTTTAATTCTC
 TACCAAAATTTTCTCTTATCTTTATATCTTTTTTAATTTTAATTTTATTTTTGTGGATACAT
 AGTAGGTGTATATATTTATGGGTACATGCAATGTTTAAATACAGGCATGCAATGTGAAATA
 AGGACATCATGGAGAATGGGTATCCATCTCCTCAAGCATTTATCTTTTCAGTTACAAACAA
 TCCAATTACACTCTTTATTTTAAATATACAATTATTAATTATAGTCACTCTGTTGTGCTAT
 30 CAAATAGTAGATCTTATTCTTTCTATTTTTTGTACCCTCTCGTCTTTTATATTAATAAATA
 ATCTTTATCTCTGTAAGCTTCATCAGTGATTTTCCAATGAAATTTAGGATCTTCTCTATACC
 CTGAATTGCCTTACTTTCTCCCCACTTCCTTGTCTTATTCAAATGCAGATTTCAATTAATGAT
 TTCCAGATCAATGATAGTTCAGAAAGCAAGCAAGTCAAAGTGACCAAGGGCATGGCCTGAAA
 ACTGTTCTAAGAGAGGAATTTACAGAACAACCTATTAAATGATGTCAATAGGATTGTATTAGT

CCGTTTTTCATACGGCTATAAAGAACTGCCTGAGACTGGGTAAATTTATAAAGGAAAGAGGTTT
AATTGACTCACAGTTCAGCACAACTGGGCAGGCCTCAGGAACTTACAATCATGGTAGAAGG
TGAAGGGGAAGCAAAGCACCTTCCTCACAAGGCGTCAGGAAGAAGTGCCAAGCAAAGGGGGA
AAAGCCCCCTTGTA AAACTACCAGAACCTGTGAGAACTCAATCACTATCACAAGAACAGCATG
5 AGGGAACCGCCCCCTCGTGATTCAATTACCTCCACCTGGTCTCTCCCTTGACACATGGGGATT
ATGGGTGTTACAATTCAAGATGAGATTTGGGTGGGGACACAAAGCCTAACCATATCAAGGAT
CAAGTGGTGGGTGAAACTAACAGGATGAGATATATCAGATACAAACACAGGGTCCCATATT
TGGGTAAAATTCATAAATGATCAAAGCACAGGATGACAGATAATATAGGTCATTTTAGATT
ATTGTGGCCAACAGATCACAGTGGGTAGTGTTATGACGAAGGGAGGGTCACAGTTACTACAG
10 TTACAGATGGATTCTGGGTACAACATTTGCACTAAAGTGCCCTTGCCAAGGGAGGCAACAGT
CTCGACATCCTGTGGCCTGATCTACTTCAGGGACTGTGTCTTGTTCAGAGCATCACATTTGA
AGAGAACTTTGACCAAGGGGAATATGCCAGAAAAGGAAGTTCGGGATGCTGAGGATCTTAGG
AACTATGTCTAAACAAGATTCATTCACAGAAGTGGGAATGTCTATTTGGCAAAAAGAAAATA
CTACTTACATGGCTGTTGGAAGACCAGCAATCACAACTCAGTTTTTCAAAGGCTGGGCAG
15 AAACACAGATGAAAGAAACAGGCCATGTTTAAGAAAAGATAAAAGCTCACGCATGATATGCC
ACTAGAGAATCACCTAGCCTCAGTGTGGCGGGAGGCCTGGGGAGTCTTGATGTCTGAGAG
TGACATTCTGATGATCACTGTCTGTGTAATGTTGGCCTAAAGCTGCCAATATTTTTGATT
TAAGAGAAGCAAGAAATGCAAATTTTTATGCAGCATGTCTCAATTTTTAATTTTGGCAACTA
TTACAAAATGTTTAAAGAGACTCTGTGCAGCCCAAATATAACATATCTATGGGCTGATGGCA
20 GCCCAGCGTTGCCAGTTCACAGGGTCTACAAGAGATGATTCTTAGTTTCAACAGGGTGCAGT
GCTGAAACGCGTGACAGTAGATTTTGCTTCGGTTATGAAAGAACTTCCAAATATTTATGAT
TCATAGCCAGAGAAAAGGCTCTCTATCCAGGTTCTGAACAATAGGAAATCATCAAGAGGATA
TTGGATGACAATATATGAAAGATGTTATTTGAGAAAGGATTCTCTCCTGAGGCATAGATGTT
GAACCAAATTCTATTAGTTATGCTTTTACAGCAAGATAGTGGTTTACAGCTTACAAAAGGCT
25 TGTACATCCTCTCATATTAAAAGTTATTAGAACAGTCCTTTGAAGTAGAAAAGTAGGCATTT
CTATTTTACAAACGAGTTGGCCGAGTATCTGAGATAGTAGATAACTCATAGAAGGTCATCCG
GGAAACGGGGCAGCAGAACTGGGATCGAATGACTCTGGTCATCCAACCTCCAAATGCAAAAGT
CTTTCTGCTGCTGCTTCCTAGTTAACTCTAAGGGTCTAAGACTCCATTCTAGTTATGGTC
TCAACTACATTTGCTCATTGCTGTGAGGGGTCAACCCACCTCCCGGAGTCCTCTCCTGCACA
30 TTCTCATGTTCCCTGAAAGGCTTTTCTGTCCCTTCCACTACTCCCTGTAAGCTCCTGTGCTTC
ACAATTTCTTGTTGAATTTTTTCTAATCTGACTCTATCAGTTATGGGAATGTTCCCTCAATT
CTTAGTGCTCCAAACCGGACTTGCTCTGGCTTGTTATTTGTCCAAAATATTTGTCTTCTCTA
TGTTTTCTACATGTTTGTCTTATAAGGACAAAACCTGCCTTAGTTTATCCATGAACAAAGC
CACGCATGCTAGTGGACACACACACATGCGCGTGCGCGGCACACACACACACACACA

TACACACAGAGACTTTGTATGTGAGTAATGAATCATCAAATCATCATAATTTCTGGACTTGT
ATTAATAAGTCGGCCAGGAGGAAAAAGAATCTGCTGTCAATCATGGCTTCTGGTTCTCACAGT
CATCTCTACTTTTCTTCCAGCAAGTTTGGTTCTGTCAAAAACCAGCTGTCAGCCTTGTTCCTG
CATGCCCAATGCAGAAGAGTCAGTAAAGAAGATTTGGTTCTCTGTATTTTCAGGGGCATCAAT
5 GCCAGGTTGAAATATGCCATTCTGGCCCAGCTCAGTGGCTCACACGTGTAATCCCAGCACTT
TGGAAGGCCAAAGCGGGTGGATTGCTTGAGCTCAGGAGTTCGAGACCAGCCTGGGCAAGAGG
CTGAGGTGGGAGGATGACCTGAGCCCGGGAGGTCAAGGCTGCAGCGAGCTGTGATCGTGCCA
CTGCACTCGAGCCAGGGCGTTGGAGTGAGACCCTGTCAAAAAAAAAAAAAAAAAAGGAAGGAA
AAAAGGAAGGAAGGAAGGGAGGGAGGGAAGATGCCATTCTTAGATTGAAGTGGACTTTATCT
10 GGCAGAACACACACACATACACACATGCACACACACATTGTGGAGAAATTGCTGACTAA
GCAAAGCTTCCAATGACTTAGTTTGGCTAAAATGTAGGCTTTTAAAAATGTGAGCACTGCC
AAGGGTTTTTCTTGTGGACCCATGGATCCATCAAGTGCAAACATTTTCTAATGCATAAT
TTAAGCCTGTGCAGCTAGATGTCATTCAACATGAAATACATTATTACAACCTGCATCTGTCT
AAAATCTTGCATCTAAAATGAGAGACAAAAAATCTATAAAAAATGAAAAACATGCATAGAAAT
15 ATGTGAGGGAGGAAAAAATTACCCCCAAGAATGTTAGTGCACGCAGTCACACAGGGAGAAGA
CTATTTTTGTTTTGTTTTGATTGTTTTGTTTTGTTTTGGTTGTTTTGTTTTGGTGACCTAAC
TGGTCAAATGACCTATTAAGAATATTTCATAGAACGAATGTTCCGATGCTCTAATCTCTCTA
GACAAGGTTCATATTTGTATGGGTTACTTATTCTCTCTTTGTTGACTAAGTCAATAATCAGA
ATCAGCAGGTTTGCAGTCAGATTGGCAGGGATAAGCAGCCTAGCTCAGGAGAAAGTGAGTATA
20 AAAGCCCCAGGCTGGGAGCAGCCATCACAGAAGTCCACTCATTTCTGGCAGGATGGCTTCTC
ATCGTCTGCTCCTCCTCTGCCTTGCTGGACTGGTATTTGTGTCTGAGGCTGGCCCTACG*GTG*
AGTGTTTCTGTGACATCCCATTCCTACATTTAAGATTACGCTAAATGAAGTAGAAGTGACT
CCTTCCAGCTTTGCCAACCAGCTTTTATTACTAGGGCAAGGGTACCCAGCATCTATTTTTAA
TATAATTAAATCAAACCTTCAAAAAGAATGAAGTTCCACTGAGCTTACTGAGCTGGGACTTGA
25 *ACTCTGAGCATTCTACCTCATTTGCTTTGGTGCATTAGGTTTGTAATATCTGGTACCTCTGTT*
TCCTCAGATAGATGATAGAAATAAAGATATGATATTAAGGAAGCTGTTAATACTGAATTTTC
AGAAAAGTATCCCTCCATAAAATGTATTTGGGGGACAAACTGCAGGAGATTATATTCTGGCC
CTATAGTTATTCAAACGTATTTATTGATTAATCTTTAAAGGCTTAGTGAACAATATTCTA
GTCAGATATCTAATTCTTAAATCCTCTAGAAGAATTAATACTATAAAATGGGTCTGGA
30 *TGTAGTTCTGACATTATTTTATAACAACTGGTAAGAGGGAGTGACTATAGCAACAATAAAA*
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TCAAATGGGAGACTTTTAACAAAGCAACTGTTCTCAGGGGACCTATTTTCTCCCTTAAATTC
ATTATACACATCCCTGGTTGATAGCAGTGTGTCTGGAGGCAGAAACCATTCTTGCTTTGGAA
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GAATCCAAGTGTCTCTGATGGTCAAAGTTCTAGATGCTGTCCGAGGCAGTCTGCCATCAA
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5 AAGTTGCCAAAGAACCCTCCACAGGACTTGGTTTTATCTTCCCGTTTGCCCCCTCACTTGGT
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CTGTGGTTTGCAACCCTTTTGGGTACAGAACACTTTATGTAGGTGATGAAAAGTAAACATT
10 CTATGCCCAGAAAAAATGCACAGATACACACACATACAAAATCATATATGTGATTTTAGGAG
TTTCACAGATTCCCTGGTGTCCCTGGGTAACACCAAAGCTAAGTGTCTTGTCTTAGAATTT
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AACCAGGCATCTGTCTGTAGTTAATATGGATCACCCAAAACCAAGGCTTTTGCCTAATGAA
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15 ATAGAAGAGATGGATCCATGAGGCAAAGTACAGCCCCAGGCTAATCCCACGATCACCCGACT
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CCCCACGTAGAGTGTATGTAACAAGAGATGCACCATTTTATTTCTCGAAAACCCGTAACATT
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15 ACACCATGTGGCAGACACAATTATAAGTGTTTTCCATATTTAACCTACTTAATCCTCAGGAA
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CTTAGTCATGGATGAGACTTTCAATTGCTATAAAGTGGTTCCTTTATTAGACAATGTTACCA
GGGAAACAACAGGGGTTTGTTTGAAGTCTGGGGCCCAAGTCAACAAGAGAGCCCCATCTA
25 CCAAGGAGCATGTCCCTGACTACCCCTCAGCCAGCAGCAAGACATGGACCCAGTCAGGGCA
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CGAGTAATAGATCTGCCTACCTGAGACTGTTGTTTGCCCAAGAGCTGGGTCTCAGCCTGATG
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TGCAACCTCCGCCTCCCAGGTTCAAGCAATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGAC
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5 ACGTTGGCCAGGCTGGTCTGAACTCCAGACCTCAGGTGATTACCTGTCTCAGCCTCCCAA
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10 ACTCAGATCCTTTTTCTCCTATCCCTAAGTAATCCGGGTTTCTTTTTCCCATATTTAGAA
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15 AGCTCTAGTGCATGCATATATATTTCAAAATTCCACCATGATCTTCCACACTCTGTATTGTA
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AGGAGACTTGCCCTTCTACTATGGTTCCATCAGAATGTAGACTGGGACAATAACAATAATTCA
AGTCTGGTTTGCTCATCTGTAAATTGGGAAGAATGTTTCCAGCTCCAGAATGCTAAATCTCT
20 AAGTCTGTGGTTGGCAGCCACTATTGCAGCAGCTCTTCAATGACTCAATGCAGTTTTGCATT
CTCCCTACCTTTTTTTTTCTAAAACCAATAAAATAGATACAGCCTTTAGGCTTTCTGGGATTT
CCCTTAGTCAAGCTAGGGTCATCCTGACTTTTCGGCGTGAATTTGCAAAACAAGACCTGACTC
TGTA CTCTGCTCTAAGGACTGTGCATGGTTCCAAAGGCTTAGCTTGCCAGCATATTTGAGC
TTTTTCCTTCTGTTCAAACGTGTTCCAAAATATAAAAGAATAAAATTAATTAAGTTGGCACTG
25 GACTTCCGGTGGTCAGTCATGTGTGTCATCTGTACGTTTTTCGGGCTCTGGTGAAATGGA
TCTGTCTGTCTTCTCTCATAG**GTGGTATTCACAGCCAACGACTCCGGCCCCCGCCGCTACAC**
CATTGCCGCCCTGCTGAGCCCCCTACTCCTATTCCACCACGGCTGTCGTCACCAATCCCAAGG
AATGAGGGACTTCTCCTCCAGTGACCTGAAGGACGAGGGATGGGATTTTCATGTAACCAAGA
GTATTCCATTTTTACTAAAGCAGTGTTTTCACCTCATATGCTATGTTAGAAAGTCCAGGCAGA
30 GACAATAAAACATTCTGTGAAAGGCACTTTTTCATTCCACTTTAACTTGATTTTTTAAATTC
CCTTATTGTCCCTTCCAAAAAAGAGAATCAAAATTTTACAAAGAATCAAAGGAATTCTAG
AAAGTATCTGGGCAGAACGCTAGGAGAGATCCAAATTTCCATTGTCTTGCAAGCAAAGCACG
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 GGGGAAAACCAAAGAACCCACACACCCAATTTACTTAGGCTGATCCAAAATCCATGTATGG
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 TTGATAAGTTGAGAGAGCAGACGACGATTGAGAAATGTTGAAGGGCAAAGGTATTGAGGTGGG
 15 ATGGGTTATGCTGCTATCACAAATAACCCCAAATCTCGGGGGCTTAACAAAGTAAAAGTTTA
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 CCTTCTAATGGTGAAGTGTGAGACAGTTTGATTTAGTCTTGTGGCTTCAAGGTCACTCTGG
 TGATATTTAGCCAGCAGACTGAGGGAACATAGTATGGTATTAGACCCCTCTGTGCTGAAGTG
 TCACACATGAGTCCCATTGACTTCTCACTGGCCAGAGCTAGTTACATGCCCCATCTAGATG
 20 TGCTGAGAAATGTGGCCCTGGCTGGGAGCCATTTCCAGAACTAACTCTATGCTCTGG
 AAGAGGAGCACTAATCTGAGTTGGCCAACAACCATCTCTACCACAGTAGGGTTGGGACTGGT
 GGGGCATGAGGCTGGAGTGAAGGTTGGTTTTATCTGCCACGCGTTACAGCTGTGAATTTGTC
 TTGAAAGCAACATGGGTCCATTGAAGGGAACCTTGACATCAGTCATGTGGCTGGGACAAGAA
 TAGTTACCACTTGCCCGTAATCTCCAACCAGGATTCTCCAGGAGAACCTGAGTTAGACACAT
 25 GGCTTAGGCCTAAACCTACCTGAGTGGTCTTTCTATTTTCCTCCAAATTCAAATCTCAAATC
 TTGCTACCCCTCTAACTGGCTATGTTGAGAGAGGAAAAAACTTGAAGAGAATGCAGTGTAGCT
 TTGGAGTTTTTTCACATGCACTTTTCCCAAGATACATAGCAAAATCAATGTCTCCAATTCTAT
 TAATGTTGTTAGCAAGTCCTTGTTCATGCATATTGGTTAATCCATAGCAAAATTGCCATTTT
 TATAGACTAAATGGTCAAATATTGGCAATTTTATAAGGTTTCACTCTATTACCTACCATGATT
 30 GTATTGGTCACTAACCTGCCTATTTTTAGAAATGCTACATATTCATTTGGCTGTTGTTAAATA
 GCTATGGATTTTTATAATCAAAACAGGTTGAAAATATGAATCAGTTTAAAACCATACA
 (SEQ ID NO: 466).

In the above TTR polynucleotide sequence provided at NCBI Reference Sequence
 No. NG_009490.1, exons encoding the TTR polypeptide correspond to the union of

nucleotides 5137..5205, 6130..6260, 8354..8489, and 11802..11909, and the intervening sequences correspond to intron sequences. The union of nucleotides 5137..5205, 6130..6260, 8354..8489, and 11802..11909 corresponds to Consensus Coding Sequence (CCDS) No. 11899.1.

5 By “transthyretin amyloidosis” is meant a disease associated with an accumulation of amyloid in a tissue of a subject.

As used herein, the terms “treat,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith or obtaining a desired pharmacologic and/or physiologic effect. It will be appreciated that, although not precluded, 10 treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated. In some embodiments, the effect is therapeutic, *i.e.*, without limitation, the effect partially or completely reduces, diminishes, abrogates, abates, alleviates, reduces the intensity of, or cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the effect is preventative, *i.e.*, the 15 effect protects or prevents an occurrence or reoccurrence of a disease or condition. To this end, the presently disclosed methods comprise administering a therapeutically effective amount of a composition as described herein.

By “uracil glycosylase inhibitor” or “UGI” is meant an agent that inhibits the uracil-excision repair system. Base editors comprising a cytidine deaminase convert cytosine to 20 uracil, which is then converted to thymine through DNA replication or repair. In various embodiments, a uracil DNA glycosylase (UGI) prevent base excision repair which changes the U back to a C. In some instances, contacting a cell and/or polynucleotide with a UGI and a base editor prevents base excision repair which changes the U back to a C. An exemplary UGI comprises an amino acid sequence as follows:

25 >spIP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDA
PEYKPPWALVIQDSNGENKIKML (SEQ ID NO: 231).

In some embodiments, the agent inhibiting the uracil-excision repair system is a uracil stabilizing protein (USP). See, e.g., WO 2022015969 A1, incorporated herein by reference.

30 As used herein, the term “vector” refers to a means of introducing a nucleic acid molecule into a cell, resulting in a transformed cell. Vectors include plasmids, transposons, phages, viruses, liposomes, lipid nanoparticles, and episomes.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of

numbers, or sub-range from the group consisting 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.

The recitation of a listing of chemical groups in any definition of a variable herein
5 includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have
10 the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains

In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. In this application,
15 the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as
20 “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended. This wording indicates that specified elements, features, components, and/or method steps are present, but does not exclude the presence of other elements, features, components, and/or method steps. Any embodiments specified as “comprising” a particular
25 component(s) or element(s) are also contemplated as “consisting of” or “consisting essentially of” the particular component(s) or element(s) in some embodiments. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present
30 disclosure.

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system.

Reference in the specification to “some embodiments,” “an embodiment,” “one embodiment” or “other embodiments” means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A and 1B provide plots and charts showing that, under similar conditions, base editor systems containing a guide RNA targeting a human TTR polynucleotide sequence for base editing (i.e., human gRNA or “human”) and administered to primary human

10 hepatocytes were associated with higher levels of base editing than base editor systems containing a guide RNA targeting a non-human primate TTR polynucleotide sequence (i.e., cyno gRNA or “cyno”) and administered to primary cyno hepatocytes. The base editor systems contained mRNA encoding an adenosine deaminase base editor containing a Tada*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to

15 throughout the figures as “ABE9.51”). **FIG. 1A** provides a plot and chart showing base editing rates measured for base editing of a synthetic polynucleotide sequence (“AAVS1 Cassette”) in human embryonic kidney (HEK293) cells that contained a polynucleotide having the target sites for both the human gRNA and for the cyno gRNA. The base editor systems were delivered to the HEK293T cells using a commercially available lipid

20 transfection reagent, Lipofectamine® MessengerMAX mRNA Transfection Reagent (LipoMM). **FIG. 1B** provides plots and a chart showing base editing rates measured in primary human hepatocytes (PHH) and primary cyno hepatocytes (PCH) at the TTR gene (left panel) or at the ALAS1 gene control (right panel). The ALAS1 gene (right panel) was base edited using a base editor system containing the control guide RNA sg23 known to be

25 effective for use in targeting base editors to alter a nucleobase within the ALAS1 gene and an mRNA molecule encoding a base editor containing a Tada*8.8 adenosine deaminase domain. The base editor systems of **FIG. 1B** were delivered using lipid nanoparticles containing the ionizable lipid IZ1. In **FIGs. 1A and 1B**, the terms PHH-A, PHH-B, and PHH-C indicate three different human donors from which primary human hepatocytes (PHH)

30 were obtained, “cTTR” indicates a cyno TTR gene, and “Conserved” indicates the ALAS1 gene.

FIGs. 2A and 2B provide plots and charts showing that, under similar conditions, base editor systems containing a guide RNA targeting a human TTR polynucleotide sequence for base editing (i.e., human gRNA or “human”) and administered to primary human

hepatocytes were associated with higher levels of base editing than base editor systems containing a guide RNA targeting a non-human primate TTR polynucleotide sequence (i.e., cyno gRNA or “cyno”) and administered to primary cyno hepatocytes (PCH). The base editor systems were administered to the cells using lipid nanoparticles containing the ionizable lipid IZ4. **FIG. 2A** provides a plot and a chart showing base editing rates measured in primary human hepatocytes (PHH) and primary cyno hepatocytes (PCH) at the TTR gene. The base editor systems administered to the PHH contained the guide RNA gRNA2944, and the base editor systems administered to the PCH contained the guide RNA gRNA2945. The base editor systems contained mRNA encoding a base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”). **FIG. 2B** provides a plot and a chart showing base editing rates measured for base editing measured in primary human hepatocytes (PHH) and primary cyno hepatocytes (PCH) at the ALAS1 gene. The ALAS1 gene was base edited using a base editor system containing the control guide RNA sg23 known to be effective for use in targeting base editors to alter a nucleobase within the ALAS1 gene and an mRNA molecule encoding a base editor containing a TadA*8.8 adenosine deaminase domain. In **FIGs. 2A and 2B**, the terms PHH1, PHH2, and PHH3 indicate primary human hepatocytes (PHH) from three different human donors. Throughout the figures the term “surrogacy” refers to evaluating or using base editing measurements for a non-human primate polynucleotide sequence or cell to infer base editing rates achieved under similar or analogous conditions (e.g., using a base editor system containing a guide RNA targeting a human polynucleotide rather than a non-human primate polynucleotide for base editing) in a human polynucleotide sequence or cell.

FIGs. 3A to 3F provide bar graphs showing liver enzyme data for male (**FIGs. 3A-3C**) and female (**FIGs. 3D-3F**) non-human primates administered the indicated doses of lipid nanoparticles containing the lipid IZ1 and a base editor system containing a guide RNA targeting the TTR gene for base editing at the HM17 target site and an mRNA molecule encoding an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain variant with a V82T amino acid alteration. The groupings of bars from left-to-right in **FIGs. 3A to 3F** correspond to Control, 0.5 mg/kg, 1 mg/kg, and 2 mg/kg, respectively. In **FIGs. 3A to 3F**, the term “AST” represents “aspartate transaminase,” the term “ALT” represents “alanine transaminase,” and the term “ALP” represents “alkaline phosphatase.” The x-axes in **FIGs. 3A to 3F** represent the number of days following lipid nanoparticle administration (day 0). This experiment indicates that LNP administration of a base editor system had no sustained deleterious effect.

FIG. 4 provides bar graphs showing rates of on-target (TTR gene A>G nucleotide alterations) and off-target (EIF3E gene A>G nucleotide alteration) base editing measured in primary human hepatocytes from donors PHH Donor 1 (PHH1 of **FIGs. 2A and 2B**) and PHH Donor 2 (PHH2 of **FIGs. 2A and 2B**) administered lipid nanoparticles containing base editor systems containing a guide RNA targeting the TTR gene for base editing at the HM17 target site and mRNA encoding an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”) or a TadA*8.20 adenosine deaminase domain with the amino acid alterations V82T, Y147T, and Q154S (referred to throughout the figures as “ABE9.52”). For each pair of bars of **FIG. 4**, the left bar corresponds to “TTR, A>G” and the right bar represents “EIF3E, A>G.”

FIGs. 5A and 5B provide images of primary human hepatocyte cells. **FIG. 5A** provides an image of primary cyno hepatocyte cells that have not been administered any base editor system. **FIG. 5B** provides an image of primary cyno hepatocyte cells one day after having been administered lipid nanoparticles containing a base editor system containing a guide RNA targeting a non-human primate TTR gene for base editing and mRNA encoding an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”).

FIGs. 6A and 6B provide plots and charts showing that, under similar conditions, base editor systems containing a guide RNA targeting a human TTR polynucleotide sequence for base editing (i.e., human gRNA or “human”) and administered to primary human hepatocytes were associated with higher levels of base editing than base editor systems containing a guide RNA targeting a non-human primate TTR polynucleotide sequence (i.e., cyno gRNA or “cyno”) and administered to primary cyno hepatocytes (PCH). The base editor systems were administered to the cells using lipid nanoparticles containing the ionizable lipid IZ4. **FIG. 6A** provides a plot and a chart showing base editing rates measured in primary human hepatocytes (PHH) and primary cyno hepatocytes (PCH) at the TTR gene. The base editor systems administered to the PHH contained the guide RNA gRNA2944, and the base editor systems administered to the PCH contained the guide RNA gRNA2945. The base editor systems contained mRNA encoding a base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”). **FIG. 6B** provides a plot and a chart showing base editing rates measured for base editing measured in primary human hepatocytes (PHH) and primary cyno hepatocytes (PCH) at the ALAS1 gene. The ALAS1 gene was base edited using a base editor system

containing the control guide RNA sg23 known to be effective for use in targeting base editors to alter a nucleobase within the ALAS1 gene and an mRNA molecule encoding a base editor containing a TadA*8.8 adenosine deaminase domain. In **FIGs. 6A and 6B**, the terms PHH1, PHH2, and PHH3 indicate primary human hepatocytes (PHH) from three different human
5 donors, where the three donors were the same donors as those of **FIGs. 2A and 2B**.

DETAILED DESCRIPTION

Provided herein are compositions for polynucleotide (e.g., a gene) modification or editing and methods of using the same to treat or prevent conditions associated with the
10 extracellular deposition in various tissues of amyloid fibrils formed by the aggregation of misfolded transthyretin (TTR) proteins. Such conditions include, but are not limited to, polyneuropathy due to hereditary transthyretin amyloidosis (hATTR-PN) and hereditary cardiomyopathy due to transthyretin amyloidosis (hATTR-CM), both associated with autosomal dominant mutations of the *TTR* gene, and an age-related cardiomyopathy
15 associated with wild-type TTR proteins (ATTRwt), also known as senile cardiac amyloidosis. Compositions and methods directed to editing a TTR polynucleotide using an editing system, such as one comprising a base editor and guide RNAs are disclosed.

The invention is based, at least in part, on the discovery that base editor systems can be used to disrupt expression of a transthyretin polypeptide or to edit a pathogenic mutation
20 in a transthyretin polypeptide and that the base editor systems can be effectively delivered to cells using lipid nanoparticles of the disclosure. In one embodiment, the disclosure provides guide RNA sequences that are effective for use in conjunction with a base editing system for editing a transthyretin (TTR) gene sequence to disrupt splicing or correct a pathogenic mutation. In another embodiment, the invention provides guide RNA sequences that target a
25 Cas12b nuclease to edit a *TTR* gene sequence, thereby disrupting TTR polypeptide expression.

Accordingly, the disclosure provides guide RNA sequences suitable for use with an ABE and/or a BE4 for transthyretin (TTR) gene splice site disruption and guide RNA sequences suitable for use with bhCas12b nucleases for disruption of the transthyretin (TTR)
30 gene. In embodiments, the compositions and methods of the present invention can be used for editing a TTR gene in a hepatocyte. The methods provided herein can include reducing or eliminating expression of TTR in a hepatocyte cell to treat an amyloidosis.

TRANSTHYRETIN PROTEIN AND GENE

Transthyretin (TTR), originally known as prealbumin, is a 55-kDa transport protein for both thyroxine (T4) and retinol-binding protein, that circulates in soluble form in the serum and cerebrospinal fluid (CSF) of healthy humans. TTR is understood to be primarily synthesized in the liver. Under normal conditions, TTR circulates as a homotetramer with a central channel. An exemplary wild-type TTR monomer is 147 amino acids in length and has the amino acid sequence below:

MASHRLLLLCLAGLVFVSEAGPTGTGESKCPMLVKVLDAVRGSPAINVAVHVFRKAADDTWE
PFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDSGPRR
YTIAALLSPYSYSTTAVVTNPKE (SEQ ID NO: 464).

The *TTR* gene, composed of four exons, is located on chromosome 18 at 18q12.1. The full sequence of the human *TTR* gene is available at UniProtKB - P02766 (TTHY_HUMAN). Over 120 TTR variants have so far been identified, the great majority of which are pathogenic. The most common pathogenic variant consists of a point mutation leading to replacement of valine by methionine at position 30 of the mature protein. This Val30Met mutation is responsible for hATTR amyloidosis and is the most frequent amyloidogenic mutation worldwide, accounting for about 50% of TTR variants.

Hereditary transthyretin amyloidosis (hATTR) is a disease caused by mutations in the *TTR* gene. Autosomal dominant mutations destabilize the TTR tetramer and enhance dissociation into monomers, resulting in misfolding, aggregation, and the subsequent extracellular deposition of TTR amyloid fibrils in different tissue sites. This multisystem extracellular deposition of amyloid (amyloidosis) results in dysfunction of different organs and tissues. In particular, polyneuropathy due to transthyretin amyloidosis (ATTR-PN) and cardiomyopathy due to transthyretin amyloidosis (ATTR-CM) are severe disorders associated with significant morbidity and mortality.

When there is clinical suspicion for hATTR-PN, diagnosis is typically done by tissue biopsy with staining for amyloid, amyloid typing (using immunohistochemistry or mass spectrometry), and/or TTR gene sequencing. When there is clinical suspicion for ATTR-CM, the key diagnostic tools are either endomyocardial biopsy (with tissue staining and amyloid typing by immunohistochemistry or mass spectrometry) or 99m technetium-pyrophosphate scan. Both of these approaches can provide a diagnosis of ATTR-CM. TTR gene sequencing can be used to differentiate between the hATTR-CM (mutation positive) and ATTRwt-CM (mutation negative).

In some embodiments, the compositions described herein include guide polynucleotides containing a spacer having a nucleotide sequence that functions as a guide to direct a gene editing protein (e.g., a base editor) to alter a *TTR* gene, for example by introducing one or more nucleobase alterations in the *TTR* gene. These point mutations may be used to disrupt gene function, by the introduction of a missense mutation(s) that results in production of a less functional, or non-functional protein, thus silencing the *TTR* gene. Alternatively, it is contemplated herein that corrections to one or more point mutation(s) may be made using a gene editing protein to alter a mutated gene to correct the underlying mutation causing the dysfunction in the *TTR* gene or otherwise mitigate against dysfunction of the gene.

AMYLOIDOSIS

Amyloidosis is a disorder that involves extracellular deposition of amyloid in an organ or tissue (e.g., the liver). Amyloidosis may occur when mutant transthyretin polypeptides aggregate (e.g., as fibrils). An amyloidosis caused by a mutation to the transthyretin gene can be referred to as a “transthyretin amyloidosis”. Some forms of transthyretin amyloidosis are not associated with a mutation to the transthyretin gene. Non-limiting examples of mutations to the mature transthyretin (TTR) protein that can lead to amyloidosis include the alterations T60A, V30M, V30A, V30G, V30L, V122I, V122A, and V122(-). One method for treatment of transthyretin amyloidosis includes disrupting expression or activity of transthyretin in a cell of a subject, optionally a hepatocyte cell. Accordingly, provided herein are methods for reducing or eliminating expression of transthyretin in a cell. The transthyretin in the cell can be a pathogenic variant. Expression of transthyretin in a cell can be disrupted by disrupting splicing of a transthyretin transcript.

Transthyretin amyloidosis is a progressive condition characterized by the buildup of protein deposits in organs and/or tissues. These protein deposits can occur in the peripheral nervous system, which is made up of nerves connecting the brain and spinal cord to muscles and sensory cells that detect sensations such as touch, pain, heat, and sound. Protein deposits in these nerves result in a loss of sensation in the extremities (peripheral neuropathy). The autonomic nervous system, which controls involuntary body functions such as blood pressure, heart rate, and digestion, may also be affected by amyloidosis. In some cases, the brain and spinal cord (i.e., central nervous system) are affected. Other areas of amyloidosis include the heart, kidneys, eyes, liver, and gastrointestinal tract. The age at which symptoms begin to develop can be between the ages of 20 and 70.

There are three major forms of transthyretin amyloidosis, which are distinguished by their symptoms and the body systems they effect: neuropathic, leptomeningeal, and cardiac.

The neuropathic form of transthyretin amyloidosis primarily affects the peripheral and autonomic nervous systems, resulting in peripheral neuropathy and difficulty controlling
5 bodily functions. Impairments in bodily functions can include sexual impotence, diarrhea, constipation, problems with urination, and a sharp drop in blood pressure upon standing (orthostatic hypotension). Some people experience heart and kidney problems as well. Various eye problems may occur, such as cloudiness of the clear gel that fills the eyeball (vitreous opacity), dry eyes, increased pressure in the eyes (glaucoma), or pupils with an
10 irregular or "scalloped" appearance. Some people with this form of transthyretin amyloidosis develop carpal tunnel syndrome, which can involve numbness, tingling, and weakness in the hands and fingers.

The leptomeningeal form of transthyretin amyloidosis primarily affects the central nervous system. In people with this form, amyloidosis occurs in the leptomeninges, which are
15 two thin layers of tissue that cover the brain and spinal cord. A buildup of protein in this tissue can cause stroke and bleeding in the brain, an accumulation of fluid in the brain (hydrocephalus), difficulty coordinating movements (ataxia), muscle stiffness and weakness (spastic paralysis), seizures, and loss of intellectual function (dementia). Eye problems similar to those in the neuropathic form may also occur. When people with leptomeningeal
20 transthyretin amyloidosis have associated eye problems, they are said to have the oculoleptomeningeal form.

The cardiac form of transthyretin amyloidosis affects the heart. People with cardiac amyloidosis may have an abnormal heartbeat (arrhythmia), an enlarged heart (cardiomegaly), or orthostatic hypertension. These abnormalities can lead to progressive heart failure and
25 death. Occasionally, people with the cardiac form of transthyretin amyloidosis have mild peripheral neuropathy.

Mutations in the transthyretin (TTR) gene cause transthyretin amyloidosis. Transthyretin transports vitamin A (retinol) and a hormone called thyroxine throughout the body. Not being bound by theory, to transport retinol and thyroxine, transthyretin must form
30 a tetramer. Transthyretin is produced primarily in the liver (i.e., in hepatic cells). A small amount of transthyretin (TTR) is produced in an area of the brain called the choroid plexus and in the retina.

TTR gene mutations can alter the structure of transthyretin, impairing its ability to bind to other transthyretin proteins. The TTR gene mutation can be autosomal dominant.

SPLICE SITES

Gene splice sites and splice site motifs are well known in the art and it is within the skill of a practitioner to identify splice sites in sequence (see, e.g., Sheth, *et al.*, “Comprehensive splice-site analysis using comparative genomics”, *Nucleic Acids Research*, 34:3955-3967 (2006); Dogan, *et al.*, “AplicePort – an interactive splice-site analysis tool”, *Nucleic Acids Research*, 35:W285-W291 (2007); and Zuallaert, *et al.*, “SpliceRover: interpretable convolutional neural networks for improved splice site prediction”, *Bioinformatics*, 34:4180-4188 (2018)).

- 10 Canonical splice donors contain the DNA sequence GT on the sense strand, whereas canonical splice acceptors comprise the DNA sequence AG. Alteration of the sequence disrupts normal splicing. Splice donors can be disrupted by adenine base editing of the complementary base in the second position in the antisense strand (GT→GC), and splice acceptors can be disrupted by adenine base editing of the first position in the sense strand
- 15 (AG→GG).

EDITING OF TARGET GENES

- To edit the transthyretin (TTR) gene, a cell (e.g., a hepatocyte) is contacted with a guide RNA and a nucleobase editor polypeptide comprising a nucleic acid programmable
- 20 DNA binding protein (napDNAbp) and a cytidine deaminase or adenosine deaminase to edit a base of a gene sequence. Editing of the base can result in disruption of a splice site (e.g., through alteration of a splice-site motif nucleobase). Editing of the base can result in replacement of a pathogenic variant amino acid with a non-pathogenic variant amino acid. As a non-limiting example, editing of the base can result in replacing a T60A, V30M, V30A,
- 25 V30G, V30L, V122I, V122A, or a V122(-) alteration in the mature transthyretin (TTR) polypeptide with a non-pathogenic variant or the wild-type valine residue. The cytidine deaminase can be BE4 (e.g., saBE4). The adenosine deaminase can be ABE (e.g., saABE.8.8). In some embodiments, multiple target sites are edited simultaneously. In some embodiments, the TTR gene is edited by contacting a cell with a nuclease and a guide RNA
- 30 to introduce an indel into a gene sequence. The indel can be associated with a reduction or elimination of expression of the gene. The nuclease can be Cas12b (e.g., bhCas12b). The cells can be edited *in vivo* or *ex vivo*. The guide RNA can be a single guide or a dual guide. In some embodiments, cells to be edited are contacted with at least one nucleic acid, wherein at least one nucleic acid encodes a guide RNA, or two or more guide RNAs, and a nucleobase

editor polypeptide comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase, e.g., an adenosine or a cytidine deaminase. In some embodiments, the gRNA comprises nucleotide analogs. These nucleotide analogs can inhibit degradation of the gRNA by cellular processes. Exemplary single guide RNA (sgRNA) sequences are provided in **Tables 1A to 1E** and exemplary spacer sequences and target sequences (e.g., protospacer sequences) are provided in **Tables 1B, 1C, and 2A to 2E**.

With the present disclosure, guide polynucleotides and spacer sequences suitable for use therein for targeting a base editor to alter a nucleobase within the human *TTR* gene to either disrupt the start codon, or disrupt splice sites, whether donors or acceptors, via A→G editing within an editing window (roughly positions 4 to 7 in the 20-nt protospacer region corresponding to the spacer of the guide polynucleotide).

Protospacer, corresponding to guide RNA GA457, has the sequence 5'-GCCATCCTGCCAAGAATGAG-3' (SEQ ID NO: 467) and is located at 34,879 to 34,898 bp of the human *TTR* gene.

Protospacer, corresponding to guide RNA GA459, has the sequence 5'-GCAACTTACCCAGAGGCAAA-3' (SEQ ID NO: 468) and is located at 36,007 to 36,026 bp of the human *TTR* gene.

Protospacer, corresponding to guide RNA GA460, has the sequence 5'-TATAGGAAAACCAAGTGAGTC-3' (SEQ ID NO: 469) and is located at 38,106-38,125 bp of the human *TTR* gene.

Protospacer, corresponding to guide RNA GA461, has the sequence 5'-TACTCACCTCTGCATGCTCA-3' (SEQ ID NO: 470) and is located at 38,234-38,253 of the human *TTR* gene.

Protospacer, corresponding to guide RNA GA458, has the sequence 5'-GCCATCCTGCCAAGAACGAG-3' (SEQ ID NO: 471) represents the sequence within the cynomolgus macaque *TTR* gene corresponding to the human protospacer sequence corresponding to guide RNA GA459.

The present disclosure includes a guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-GCCAUCCUGCCAAGAAUGAG-3' (SEQ ID NO: 472) (GA457).

The present disclosure includes a guide polynucleotide having the sequence 5'-GCCAUCCUGCCAAGAAUGAG-3' (SEQ ID NO: 472) (GA457).

The present disclosure includes a modified guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-**GCC**AUCCUGCCAAGAAUGAG-3' (SEQ ID NO: 472), wherein the nucleotides **GCC** shown in **bold** are modified by methylation (GA521) (C is modified to 2'-O-methylcytidine, G is modified to 2'-O-methylguanosine). The present disclosure includes a modified guide polynucleotide having the sequence 5'-mGsmCsmCAUCCUGCCAAGAAUGAG-3' (SEQ ID NO: 472) (GA521), wherein mC: 2'-O-methylcytidine, mG: 2'-O-methylguanosine and s: phosphorothioate (PS) backbone linkage.

The present disclosure includes a guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-GCCAUCCUGCCAAGAACGAG-3' (SEQ ID NO: 473) (GA458). The present disclosure includes a guide polynucleotide having the sequence 5'-GCCAUCCUGCCAAGAACGAG-3' (SEQ ID NO: 473) (GA458).

The present disclosure includes a guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-GCAACUUACCCAGAGGCAAA-3' (SEQ ID NO: 474) (GA459). The present disclosure includes a guide polynucleotide having the sequence 5'-GCAACUUACCCAGAGGCAAA-3' (SEQ ID NO: 474) (GA459).

The present disclosure includes a guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-UAUAGGAAAACCAGUGAGUC-3' (SEQ ID NO: 475) (GA460). The present disclosure includes a guide polynucleotide having the sequence 5'-UAUAGGAAAACCAGUGAGUC-3' (SEQ ID NO: 475) (GA460).

The present disclosure includes a guide polynucleotide having a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to the sequence 5'-UACUCACCUCUGCAUGCUC-3' (SEQ ID NO: 476) (GA461). The present disclosure includes a guide polynucleotide having the sequence 5'-UACUCACCUCUGCAUGCUC-3' (SEQ ID NO: 476) (GA461).

In some aspects, provided herein is a guide RNA, comprising a sequence defined by mG*mC*mC*AUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAmAGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUUmUmCAAmCmUmUGmAmAmAmAmAmGmUmGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (GA521, (SEQ ID NO: 477), wherein A is adenosine, C is cytidine, G is guanosine, U is uridine, mA* is 2'-O-

methyladenosine, mC* is 2'-O-methylcytidine, mG* is 2'-O-methylguanosine, mU* is 2'-O-methyluridine, and wherein nucleotides represented in bold are linked by a phosphorothioate (PS) backbone linkage.

Alternatively, GA521 is represented as

5 mG*smC*smC*AUCCUGCCAAGAAUGAGmGsUsUsUsUsAsGsmAsmGsmCsmUsmA
sGsmAsmAsmAsmUsmAsmGsmCsmAsmAsGsUsUsmAsAsmAsAsmUsAsmAsmGsmGsm
CsmUsmAsGsUsmCsmCsGsUsUsAsmUsmCsAsAsmCsmUsmUsGsmAsmAsmAsmAs
mGsmUsmGsGsmCsmAsmCsmCsmGsmAsmGsmUsmCsmGsmGsmUsmGsmCsmU*smU*sm
U*smU (GA521, SEQ ID NO: 477), wherein A is adenosine, C is cytidine, G is guanosine, U
10 is uridine, mA* is 2'-O-methyladenosine, mC* is 2'-O-methylcytidine, mG* is 2'-O-
methylguanosine, mU* is 2'-O-methyluridine, and wherein nucleotides are linked by a
phosphorothioate (PS) backbone linkage represented by the letter 's'.

In some embodiments,

mG*mc*mc*AUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAm
15 AGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUUmUmCAAmCmUmUGmAmAmAmAmAmGmU
mGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU (GA521, SEQ ID NO: 477),
wherein A is adenosine, C is cytidine, G is guanosine, U is uridine, mA* is 2'-O-
methyladenosine, mC* is 2'-O-methylcytidine, mG* is 2'-O-methylguanosine, mU* is 2'-O-
methyluridine, and wherein nucleotides represented in bold are linked by a phosphorothioate
20 (PS) backbone linkage.

Alternatively, GA521 is represented as

mG*mc*mc*AUCCUGCCAAGAAUGAGmGsUsUsUsUsAsGsmAsmsGsmCsmUsmAs
GsmAsmAsmAsmUsmAsmGsmCsmAsmAsGsUsUsmAsAsmAsAsmUsAsmAsmGsmGsmC
smUsmAsGsUsmCsmCsGsUsUsAsmUsmCsAsAsmCsmUsmUsGsmAsmAsmAsmAsm
25 GsmUsmGsGsmCsmAsmCsmCsmGsmAsmGsmUsmCsmGsmGsmUsmGsmCsmU*mU*mU*mU
U (GA521, SEQ ID NO: 477), wherein A is adenosine, C is cytidine, G is guanosine, U is
uridine, mA* is 2'-O-methyladenosine, mC* is 2'-O-methylcytidine, mG* is 2'-O-
methylguanosine, mU* is 2'-O-methyluridine, and wherein nucleotides represented in bold
are linked by a phosphorothioate (PS) backbone linkage represented by the letter 's'.

Exemplary guide RNAs, spacer sequences, and target sequences are provided in
Tables 1A to 1E and Tables 2A to 2E.

30 In various instances, it is advantageous for a spacer sequence to include a 5' and/or a
3' "G" nucleotide. In some cases, for example, any spacer sequence or guide polynucleotide
provided herein comprises or further comprises a 5' "G", where, in some embodiments, the 5'

“G” is or is not complementary to a target sequence. In some embodiments, the 5′ “G” is added to a spacer sequence that does not already contain a 5′ “G.” For example, it can be advantageous for a guide RNA to include a 5′ terminal “G” when the guide RNA is expressed under the control of a U6 promoter or the like because the U6 promoter prefers a “G” at the transcription start site (*see* Cong, L. *et al.* “Multiplex genome engineering using CRISPR/Cas systems. *Science* 339:819-823 (2013) doi: 10.1126/science.1231143). In some cases, a 5′ terminal “G” is added to a guide polynucleotide that is to be expressed under the control of a promoter, but is optionally not added to the guide polynucleotide if or when the guide polynucleotide is not expressed under the control of a promoter.

10 Variants of the spacer sequences provided herein comprising 1, 2, 3, 4, or 5 nucleobase alterations are contemplated. For example, variation of a target polynucleotide sequence within a population (e.g., single nucleotide polymorphisms) may require said alterations to a spacer sequence to allow the spacer to better bind a variant of a target sequence in a subject.

In embodiments, a guide RNA comprises a sequence complementary to a promoter region of a TTR polynucleotide sequence. In embodiments, the promoter region spans from positions +10, +5, +1, -1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -15, -20, -25, -30, -35, -40, -45, -50, -55, -60, -65, -70, -75, -80, -85, -90, -95, -100, -105, -110, -115, -120, -125, -130, -135, -140, -145, -150, -155, -160, -165, -170, -175, -180, -185, -190, -195, -200, -250, or -300 to position +5, +1, -1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -15, -20, -25, -30, -35, -40, -45, -50, -55, -60, -65, -70, -75, -80, -85, -90, -95, -100, -105, -110, -115, -120, -125, -130, -135, -140, -145, -150, -155, -160, -165, -170, -175, -180, -185, -190, -195, -200, -250, -300, or -400, where position +1 corresponds to the first A of the start codon (ATG) of the TTR polynucleotide sequence.

15 In various embodiments of the disclosure, a guide RNA contains the following polynucleotide sequence, where the sequence may be modified by adding or removing one or more of the “Ns” (i.e., the spacer sequence, shown in bold, can be extended or truncated by, e.g., 1, 2, 3, 4, or 5 nucleotides):

nsnsns**NNNNNNNNNNNNNNNNNNNN**gUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGU
20 ccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususus (SEQ ID NO: 1235).

Where, “n” is a, c, g, or u; “N” is A, C, G, or U; A is adenosine; C is cytidine; G is guanosine; U is uridine; a is 2′-O-methyladenosine; c is 2′-O-methylcytidine; g is 2′-O-methylguanosine; u is 2′-O-methyluridine and s is a phosphorothioate (PS) backbone linkage and wherein bold type represents the spacer sequence.

Table 1A. Exemplary guide RNAs for editing transthyretin (TTR) splice sites and/or introducing indels into the TTR gene (e.g., using bhCas12b)

sgRNA ID	Sequence	SEQ ID NO
sgRNA_361	mUsmAsmUsAGGAAAACCACTGAGTCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCmUsmUsmUsU	484
sgRNA_362	mUsmAsmCsUCACCUCUGCAUGCUCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCmUsmUsmUsU	485
sgRNA_363	mAsmCsmUsCACCUCUGCAUGCUCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCmUsmUsmUsU	486
sgRNA_364	mUsmAsmCsCACCUAUGAGAGAAGACGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCmUsmUsmUsU	487
sgRNA_365	mAsmUsmAsCUCACCUCUGCAUGCUCAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUAACUUGUUGGCGAGAmUsmUsmUsU	488
sgRNA_366	mAsmCsmUsGGUUUCCUAUAAGGUGUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUAACUUGUUGGCGAGAmUsmUsmUsU	489
sgRNA_367	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUUGGCGAGAUUGGCUUCUCmAsmUsmCsG	490
sgRNA_368	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUCCUAUAAGGUGUGAAAGmUsmCsmUsG	491
sgRNA_369	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUGAGCCCAUGCAGCUCUCmCsmAsmGsA	492
sgRNA_370	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUCCUCAGUUGUGAGCCmAsmUsmGsC	493
sgRNA_371	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACGUAGAAGGGAUAUACAAmGsmUsmGsG	494
sgRNA_372	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACCCACUUGUAUAUCCCUUmCsmUsmAsC	495
sgRNA_373	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACGGUGUCUAUUUCCACUUUmGsmUsmAsU	496
sgRNA_374	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACCAUGAGCAUGCAGAGGUGmAsmGsmUsA	497

sgRNA ID	Sequence	SEQ ID NO
gRNA1594	mCsmAsmAsCUUACCCAGAGGCAAUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	498
gRNA1595	mAsmAsmUsGGCUCCCAGGUGUCAUCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	499
gRNA1596	mGsmGsmCsUCCCAGGUGUCAUCAGCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	500
gRNA1597	mCsmUsmCsUCAUAGGUGGUAUUCACGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	501
gRNA1598	mUsmAsmUsAGGAAAACCAGUGAGUCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	628
gRNA1599	mUsmAsmCsUCACCUCUGCAUGCUCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	629
gRNA1600	mGsmCsmAsACUUAACCCAGAGGCAAAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	502
gRNA1601	mUsmCsmUsGUUAUCUCACCUCUGCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	503
gRNA1602	mGsmAsmAsACACUCACCGUAGGGCCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	504
gRNA1603	mCsmUsmCsUACACCCAGGGCACCGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	505
gRNA1604	mAsmCsmAsCCUUAUAGGAAAACCAGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	506
gRNA1605	mAsmUsmAsGGAAAACCAGUGAGUCUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	507
gRNA1606	mAsmCsmUsCACCUCUGCAUGCUCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	508
gRNA1607	mCsmUsmCsACCGUAGGGCCAGCCUCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	509
gRNA1746	mAsmAsmCsCUGCUGAUUCUGAUUAUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	510
gRNA1747	mAsmAsmGsAGAGAAUAAGUAACCCAUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	511

sgRNA ID	Sequence	SEQ ID NO
gRNA1748	mAsmAsmGsCAGCCUAGCUCAGGAGAAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	512
gRNA1749	mAsmAsmGsUCCACUCAUUCUUGGCAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	513
gRNA1750	mAsmCsmGsAUGAGAAGCCAUCCUGCCGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	514
gRNA1751	mAsmGsmAsCAAGGUUCAUUAUUUGUAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	515
gRNA1752	mAsmGsmGsCUGGGAGCAGCCAUCAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	516
gRNA1753	mAsmUsmAsAGUAACCAUAACAAUAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	517
gRNA1754	mAsmUsmAsCUCACUUCUCCUGAGCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	518
gRNA1755	mAsmUsmUsAUUGACUAGUCAACAAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	519
gRNA1756	mCsmAsmAsAUAUGAACCUUGUCUAGGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	520
gRNA1757	mCsmAsmGsAAGUCCACUCAUUCUUGGGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	521
gRNA1758	mCsmAsmGsGCUGGGAGCAGCCAUCACGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	522
gRNA1759	mCsmCsmAsUCCUGCCAAGAAUGAGUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	523
gRNA1760	mCsmCsmUsGCUGAUUCUGAUUAUUGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	524
gRNA1761	mCsmGsmAsUGCUCUAAUCUCUCUAGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	525
gRNA1762	mCsmUsmAsAGUCAAAUAUCAGAAUCAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	526
gRNA1763	mCsmUsmAsGACAAGGUUCAUUAUUUGUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	527

sgRNA ID	Sequence	SEQ ID NO
gRNA1764	mGsmAsmAsCCUUGUCUAGAGAGAUUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	528
gRNA1765	mGsmAsmAsGUCCACUCAUUCUUGGCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	529
gRNA1766	mGsmAsmAsUCAGCAGGUUUGCAGUCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	530
gRNA1767	mGsmAsmAsUGAGUGGACUUCUGUGAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	531
gRNA1768	mGsmAsmCsUGCAAACCGUCUGAUUCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	532
gRNA1769	mGsmAsmCsUUAGUCAACAAAGAGAGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	533
gRNA1770	mGsmAsmUsAAGCAGCCUAGCUCAGGGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	534
gRNA1771	mGsmAsmUsGAGAAGCCAUCUGCCAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	535
gRNA1772	mGsmCsmCsAUCCUGCCAAGAAUGAGGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	630
gRNA1773	mGsmCsmUsUUUAUACUCACUUCUCCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	536
gRNA1774	mGsmGsmAsUAAGCAGCCUAGCUCAGGGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	537
gRNA1775	mGsmUsmCsUAGAGAGAUUAGAGCAUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	538
gRNA1776	mGsmUsmGsAUGGCUGCCCCAGCCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	539
gRNA1777	mUsmAsmCsUUAUUCUCUCUUUGUUGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	540
gRNA1778	mUsmAsmUsUCUCUCUUUGUUGACUAAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	541
gRNA1779	mUsmAsmUsUGACUUAGUCAACAAAGGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	542

sgRNA ID	Sequence	SEQ ID NO
gRNA1780	mUsmAsmUsUGACUUAGUCAACAAAGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	543
gRNA1781	mUsmCsmAsGAAUCAGCAGGUUUGCAGGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	544
gRNA1782	mUsmCsmCsACUCAUUCUUGGCAGGAGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	545
gRNA1783	mUsmCsmUsCUCUUUGUAGCUAAGUCGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	546
gRNA1784	mUsmGsmAsGAAGCCAUCUGCCAAGAGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	547
gRNA1785	mUsmGsmAsGCUAGGCGUCUUAUCCUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	548
gRNA1786	mUsmGsmAsGUUAAAAAGCCCCAGGCGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	549
gRNA1787	mUsmGsmAsUGGCGUCUCCAGCCUGGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	550
gRNA1788	mUsmGsmCsCAAGAAUGAGUGGACUUCGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	551
gRNA1789	mUsmGsmCsCAAUCUGACUGCAAACCUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	552
gRNA1790	mUsmGsmUsUGACUAGUCAAUAAUCGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	553
gRNA1791	mUsmUsmGsACUUAGUCAACAAAGAGGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	554
gRNA1792	mUsmUsmUsGUUGACUAGUCAAUAAUGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	555
gRNA-#1	mAsmAsmAsAGCCCCAGGCUGGGAGCGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	556
gRNA-#2	mAsmAsmGsUGAGUAUAAAAGCCCCAGUUUUAGAGCUAGAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	557
gRNA-#3	mAsmAsmUsAAUCAGAAUCAGCAGGUUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	558

sgRNA ID	Sequence	SEQ ID NO
gRNA-#4	mAsmAsmUsAUGAACCUUGUCUAGAGGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	559
gRNA-#5	mAsmAsmUsGAGUGGACUUCUGUGAUGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	560
gRNA-#6	mAsmCsmAsAAUAUGAACCUUGUCUAGGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	561
gRNA-#7	mAsmCsmAsGAAGUCCACUCAUUCUUGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	562
gRNA-#8	mAsmCsmCsUUGUCUAGAGAGAUUAGGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	563
gRNA-#9	mAsmGsmAsAGCCAUCCUGCCAAGAAGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	564
gRNA-#10	mAsmGsmCsAGGUUUGCAGUCAGAUUGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	565
gRNA-#11	mAsmGsmGsGAUAAGCAGCCUAGCUCGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	566
gRNA-#12	mAsmGsmGsUUUGCAGUCAGAUUGGCGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	567
gRNA-#13	mAsmGsmUsAUAAAAGCCCCAGGCUGGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	568
gRNA-#14	mAsmGsmUsCAAUAUCAGAAUCAGCGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	569
gRNA-#15	mAsmUsmAsAUCAGAAUCAGCAGGUUGUUUUAGAGCUAGAAAUA GCAAGUUAUUUUUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	570
gRNA-#16	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUUGACUUAAGUCAACAAAGAsmGsmAsmG	571
gRNA-#17	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUCUCUUUGUUGACUAAGUCsmAsmAsmU	572
gRNA-#18	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUGAUUAUUGACUUAAGUCAAsmCsmAsmA	573
gRNA-#19	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUUGGACGAUGGCUUCUCAsmUsmCsmG	574

sgRNA ID	Sequence	SEQ ID NO
gRNA-#20	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAGCACACUUAGUCAACAAAGAGAGsmAsmAsmU	575
gRNA-#21	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAGCACGCAGGGAUAAGCAGCCUAGsmCsmUsmC	576
gRNA-#22	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAGCACGUAUGGUAUACUUAUUCUCsmUsmCsmU	577
gRNA-#23	mCsmAsmAsGAAUGAGUGGACUUCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	578
gRNA-#24	mCsmAsmAsUCUGACUGCAAACCGUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	579
gRNA-#25	mCsmAsmCsAGAAGUCCACUCAUUCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	580
gRNA-#26	mCsmAsmGsACGAUGAGAAGCCAUCGUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	581
gRNA-#27	mCsmAsmGsCAGGUUUGCAGUCAGAUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	582
gRNA-#28	mCsmAsmGsGAUGGCUUCUCAUCGUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	583
gRNA-#29	mCsmAsmGsGUUUGCAGUCAGAUUGGCGUUUUAGUACUCUGUAA UGAAAAUACAGAAUCUACUAAAACAAGGCAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	584
gRNA-#30	mCsmAsmGsUCAGAUUGGCAGGGAUAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	585
gRNA-#31	mCsmCsmAsCUCAUUCUUGGCAGGAUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	586
gRNA-#32	mCsmUsmAsAGUCAUAUAUCAGAAUCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	587
gRNA-#33	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAGCACGUCACAAAGAGAGAAUAAsmGsmUsmA	588
gRNA-#34	mCsmUsmUsAUCCCUGCCAAUCUGACGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	589
gRNA-#35	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAGCACUCCCUGCCAAUCUGACUGCsmAsmAsmA	590

sgRNA ID	Sequence	SEQ ID NO
gRNA-#36	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUUCUCUCUUUGUUGACUAAmGsmUsmC	591
gRNA-#37	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUCCUGAGCUAGGCUGCUUAAmUsmCsmC	592
gRNA-#38	mCsmUsmUsCUGUGAUGGCUGCUCGCCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	593
gRNA-#39	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUGUGAUGGCUGCUCGCCAGCsmCsmUsmG	594
gRNA-#40	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACGAGGAUGGCUUCUCAUCGsmUsmCsmU	595
gRNA-#41	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUCUAGAGAGAUUAGAGCAUsmCsmGsmG	596
gRNA-#42	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACGUUGACUAAGUCAUAAUCsmAAsmGsmA	597
gRNA-#43	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACUAUACUCACUUCUCCUGAGsmCsmUsmA	598
gRNA-#44	mGsmAsmAsGUGAGUAUAAAAGCCCCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	599
gRNA-#45	mGsmAsmCsAAGGUUCAUAAUUGUAUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	600
gRNA-#46	mGsmAsmGsUAUAAAAGCCCCAGGCUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	601
gRNA-#47	mGsmAsmGsUGGACUUCUGUGAUGGCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	602
gRNA-#48	mGsmAsmUsGGCUGCUCGCCAGCCUGGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	603
gRNA-#49	mGsmCsmAsGCCUAGCUCAGGAGAAGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	604
gRNA-#50	mGsmCsmUsGCUUAUCCUGCCAAUCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	605
gRNA-#51	mGsmGsmGsAUAAGCAGCCUAGCUCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	606

sgRNA ID	Sequence	SEQ ID NO
gRNA-#52	mGsmGsmUsUUGCAGUCAGAUUGGCAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	607
gRNA-#53	mGsmUsmUsACUUAUUCUCUCUUUGUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	608
gRNA-#54	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACCUUAUUCUCUCUUUGUUGAsmCsmUsmA	609
gRNA-#55	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACAUUUUGUAUGGGUUACUUsmAsmUsmU	610
gRNA-#56	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACACUAAGUCAUAUAUCAGAAAsmUsmCsmA	611
gRNA-#57	mGsmUsmUsUGCAGUCAGAUUGGCAGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	612
gRNA-#58	mGsmUsmUsCUGUCUUUUGGUCAGGACAACCGUCUAGCUAUAAG UGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUU ACGAGGCAUUAAGCACGAGUCAGAUUGGCAGGGAsmUsmAsmA	613
gRNA-#59	mUsmAsmCsAAAAUUGAACCUUGUCUGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	614
gRNA-#60	mUsmAsmCsUCACUUCUCCUGAGCUAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	615
gRNA-#61	mUsmAsmUsAAAAGCCCCAGGCUGGGGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	616
gRNA-#62	mUsmCsmAsCUUCUCCUGAGCUAGGCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	617
gRNA-#63	mUsmCsmAsGAUUGGCAGGGAUAAGCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	618
gRNA-#64	mUsmCsmAsGGAGAAGUGAGUAUAAGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	619
gRNA-#65	mUsmCsmUsGACUGCAAACCGUGCUGAUGUUUUAGUACUCUGUAA UGAAAAUUAACAGAAUCUACUAAAACAAGGCAAAUAGCCGUGUUU AUCUCGUCAACUUGUUGGCGAGAUsmUsmUsmU	620
gRNA-#66	mUsmGsmAsGCUAGGCGUCUUAUCCCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	621
gRNA-#67	mUsmGsmCsCAAUCUGACUGCAAACCGUUUUAGAGCUAGAAAUA GCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	622

sgRNA ID	Sequence	SEQ ID NO
gRNA-#68	mUsmGsmCsUCUAAUCUCUCUAGACAGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	623
gRNA-#69	mUsmGsmUsGAUGGCUGCUCUCCAGCCGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	624
gRNA-#70	mUsmUsmGsGCAGGGAUAAGCAGCCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	625
gRNA-#71	mUsmUsmUsUAUACUCACUUCUCCUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUsmUsmUsmU	626

Lowercase m indicates 2'-O-methylated nucleobases (e.g., mA, mC, mG, mU), and "s" indicates phosphorothioates.

5 Table 1B. Exemplary guide RNAs for editing a TTR polynucleotide (spacer sequences within a gRNA sequence are in bold)

gRNA ID	Species	Spacer Sequence (5'-3')	SEQ ID NO	gRNA sequence (5'-3')	SEQ ID NO
GA457	Human	GCCAUCCUG CCAAGAAUG AG	472	gscscsAUCCUGCCAAGAAUGAG GUU UUAGAGCUAGAAAUAAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUsusu su	627
GA519 (gRNA2945)	Cyno	GCCAUCCUG CCAAGAACG AG	473	gscscsAUCCUGCCAAGAACGAG gUU UUAGagcuaGaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuusus us	631
GA458	Cyno	GCCAUCCUG CCAAGAACG AG	473	gscscsAUCCUGCCAAGAACGAG GUU UUAGAGCUAGAAAUAAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUsusu su	632
GA459	Human	GCAACUUAC CCAGAGGCA AA	474	gscsasACUUACCCAGAGGCAAA GUU UUAGAGCUAGAAAUAAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUsusu su	633
GA460	Human/ Cyno	UAUAGGAAA ACCAGUGAG UC	475	usasusAGGAAAACCAGUGAGUC GUU UUAGAGCUAGAAAUAAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUsusu su	634

GA520	Human/ Cyno	UAUAGGAAA ACCAGUGAG UC	475	usasusAGGAAAACCAGUGAGUC gUU UUAGagcuaGaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuusus us	635
GA461	Human/ Cyno	UACUCACCU CUGCAUGCU CA	476	usascsUCACCUCUGCAUGCUCAGUU UUAGAGCUAGAAAUAAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCU susu	636
GA521¹ (gRNA A2944)	Human	GCCAUCCUG CCAAGAAUG AG	472	mG*mC*mC*ATCCTGCCAAGAATGAG mGUUUUAGmAmGmCmUmAGmAmAm UmAmGmCmAmAGUUmAaAmAmUmAm GmGmCmUmAGUmCmCGUUAmUmCAAm CmUmUGmAmAmAmAmAmGmUmGGmCm AmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU	637

Letters in the sequences:- A: adenosine; C: cytidine; G: guanosine; U: uridine; a or mA: 2'-O-methyladenosine; c or mC: 2'-O-methylcytidine; g or mG: 2'-O-methylguanosine; u or mU: 2'-O-methyluridine; and s or *: phosphorothioate (PS) backbone linkage. Bold type in gRNA sequence denotes spacer sequence corresponding to Protospacer. GA460 and GA520 have the same protospacer sequence but have different chemical modifications in the gRNA sequence.

Table 1C. Exemplary guide RNAs for editing a TTR polynucleotide (spacer sequences within gRNA sequences are shown in bold)

LN P	gRNA ID	Species	Spacer Sequence (5'-3')	Equivalent Human gRNA	gRNA Sequence (5'-3')
1	GA519	Cyno	GCCAUCCUGC CAAGAACGAG (SEQ ID NO: 473)	GA457	gscscsAUCCUGCCAAGAAC GAG gUUUUAGagcuaGaaau agcaaGUUaAaAuAaggcua GUccGUUAucAAcuuGaaaa agugGcaccgagucggugcu ususus (SEQ ID NO: 631)
2	GA520	Cyno	UAUAGGAAAA CCAGUGAGUC (SEQ ID NO: 475)	GA460	usasusAGGAAAACCAGUGA GUC gUUUUAGagcuaGaaau agcaaGUUaAaAuAaggcua GUccGUUAucAAcuuGaaaa agugGcaccgagucggugcu ususus (SEQ ID NO: 635)

¹ The region of the *TTR* gene targeted by this base editor, either in humans or non-human primates, is referred to as "HM17."

Letters in the sequences: A = adenosine; C = cytidine; G = guanosine; U = uridine; a = 2'-O-methyladenosine; c = 2'-O-methylcytidine; g = 2'-O-methylguanosine; u = 2'-O-methyluridine; s = phosphorothioate (PS) backbone linkage. C = nucleotide that differs in NHP from human TTR sequence. Bold type in gRNA sequence denotes spacer sequence

5 corresponding to Protospacer.

Table 1D. Exemplary guide RNA sequences (spacer sequences are in bold)

Guide RNA sequence (5'-3')	SEQ ID NO
gscscsAUCCUGCCAAGAAUGAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	627
AUCCUGCCAAGAACGAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUAG UCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	638
gscsasACUUACCCAGAGGCAAAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	633
usasusAGGAAAACAGUGAGUC GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	634
usascsUCACCUCUGCAUGCUCAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	636
gscscsAUCCUGCCAAGAACGAG gUUUUAGagcuaGaaauagcaaGUUaAaAuAa ggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususus	631
usasusAGGAAAACAGUGAGUC gUUUUAGagcuaGaaauagcaaGUUaAaAuAa ggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususus	635

A is adenosine; C is cytidine; G is guanosine; U is uridine; a is 2'-O-methyladenosine; c is 2'-O-methylcytidine; g is 2'-O-methylguanosine; u is 2'-O-methyluridine and s is phosphorothioate (PS) backbone linkage and wherein bold type represents the spacer sequence.

10

Table 1E. Exemplary guide RNA sequences (spacer sequences are in bold)

gRNA sequence (5'-3')	SEQ ID NO
GCCAUCCUGCCAAGAAUGAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	639
GCCAUCCUGCCAAGAACGAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	640
GCAACUUACCCAGAGGCAAAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	641
UAUAGGAAAACAGUGAGUC GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	642
UACUCACCUCUGCAUGCUCAG GUUUUAGAGCUAGAAAUAGCAAGUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	643

GCCAUCUGCCAAGAACGAGGUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU	640
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Table 2A. Exemplary Spacer and Target Site Sequences.²

sgRNA	Spacer sequence	SEQ ID NO	Target site ID	Target site sequence (target bases for base editing are in bold and underlined)	SEQ ID NO	Target Base (s)
sgRNA_361	UAUAGGAAAACCA GUGAGUC	475	TSBTx2 602	TAT <u>AGG</u> AAAACCAGTG AGTC	469	4A
sgRNA_362	UACUCACCUCUGC AUGCUC	476	TSBTx2 603	TACTC <u>AC</u> CTCTGCATG CTCA	470	6A, 7C
sgRNA_363	ACUCACCUCUGCA UGCUC	644	TSBTx2 604	ACTC <u>AC</u> CTCTGCATGC TCAT	658	5A, 6C
sgRNA_364	UACCACCUAUGAG AGAAGAC	645	TSBTx2 605	TACCAC <u>C</u> TATGAGAGA AGAC	659	7C
sgRNA_365	AUACUCACCUCUG CAUGCUC	646	TSBTx2 606	ATACTC <u>AC</u> CTCTGCAT GCTCA	660	7A, 8C
sgRNA_366	ACUGGUUUUCCUA UAAGGUGU	647	TSBTx2 607	ACTGGTTTTTC <u>C</u> TATAA GGTGT	661	11C
sgRNA_367	UUGGCAGGAUGGC UUCUCAUCG	648	TSBTx2 608	TTGGC <u>AGG</u> <u>A</u> TGGCTTC TCATCG	662	6A, 9A
sgRNA_368	UCCUAUAAGGUGU GAAAGUCUG	649	TSBTx2 609	GTTTTCTATAAGGTG TGAAAGTCTG	663	
sgRNA_369	UGAGCCCAUGCAG CUCUCCAGA	650	TSBTx2 610	GTTGTGAGCCCATGCA GCTCTCCAGA	664	
sgRNA_370	CUCCUCAGUUGUG AGCCCAUGC	651	TSBTx2 611	ATTCTCTCTCAGTTGT GAGCCCATGC	665	
sgRNA_371	GUAGAAGGGUAU ACAAAGUGG	652	TSBTx2 612	ATTTGTAGAAGGGATA TACAAAGTGG	666	
sgRNA_372	CCACUUUGUAU CCCUUCUAC	653	TSBTx2 613	ATTTCCACTTTGTATA TCCCTTCTAC	667	
sgRNA_373	GGUGUCUAUUUCC ACUUUGUAU	654	TSBTx2 614	ATTTGGTGTCTATTTT CACTTTGTAT	668	
sgRNA_374	CAUGAGCAUGCAG AGGUGAGUA	655	TSBTx2 615	ATTCCATGAGCATGCA GAGGTGAGTA	669	
sgRNA_375	GGCUAUCGUCACC AAUCCCA	656		GGCT <u>A</u> TCGTCACCAAT CCCA	670	5A

² One of skill in the art will understand that some of the target site sequences correspond to a reverse-complement to the above-provided transthyretin polynucleotide sequence; i.e., the target sequences may correspond to either strand of a dsDNA molecule encoding a transthyretin polynucleotide. Further, it is to be understood that a C base can be targeted by a cytidine deaminase and that an A base can be targeted by an adenine deaminase.

sgRNA	Spacer sequence	SEQ ID NO	Target site ID	Target site sequence (target bases for base editing are in bold and underlined)	SEQ ID NO	Target Base (s)
sgRNA_376	GCUAUCGUCACCA AUCCCAA	657		GCT <u>A</u> TCGTCACCAATC CCAA	671	4A
sgRNA_377	GGCUAUCGUCACC AAUCCCA	656		GGCT <u>A</u> TCGTCACCAAT CCCA	670	5A

Table 2B. Exemplary Spacer and Target Site Sequences.

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA1747	AAGAGAGAAUAAGU AACCCAU	672	AAGAGAGAATAAGTAA CCCAT	689
gRNA1748	AAGCAGCCUAGCUC AGGAGAA	673	AAGCAGCCTAGCTCAG GAGAA	690
gRNA1749	AAGUCCACUCAUUC UUGGCA	674	AAGTCCACTCATTCTT GGCA	691
gRNA1750	ACGAUGAGAAGCCA UCCUGCC	675	ACGATGAGAAGCCATC CTGCC	692
gRNA1751	AGACAAGGUUCAUA UUUGUA	676	AGACAAGGTTCATATT TGTA	693
gRNA1752	AGGCUGGGAGCAGC CAUCAC	677	AGGCTGGGAGCAGCCA TCAC	694
gRNA1753	AUAAGUAACCCAU CAAAUA	678	ATAAGTAACCCATACA AATA	695
gRNA1754	AUACUCACUUCUCC UGAGCU	679	ATACTCACTTCTCCTG AGCT	696
gRNA1755	AUUUUUGACUUAGU CAACAA	680	ATTATTGACTTAGTCA ACAA	697
gRNA1756	CAAAUAUGAACCUU GUCUAG	681	CAAATATGAACCTTGT CTAG	698
gRNA1757	CAGAAGUCCACUCA UUCUUGG	682	CAGAAGTCCACTCATT CTTGG	699
gRNA1758	CAGGCUGGGAGCAG CCAUCAC	683	CAGGCTGGGAGCAGCC ATCAC	700
gRNA1759	CCAUCCUGCCAAGA AUGAGU	684	CCATCCTGCCAAGAAT GAGT	701
gRNA1760	CCUGCUGAUUCUGA UUAUUGA	685	CCTGCTGATTCTGATT ATTGA	702
gRNA1761	CGAUGCUCUAAUCU CUCUAGA	686	CGATGCTCTAATCTCT CTAGA	703
gRNA1762	CUAAGUCAUAAUC AGAAUCA	687	CTAAGTCAATAATCAG AATCA	704
gRNA1763	CUAGACAAGGUUCA UAUUUGU	688	CTAGACAAGGTTCATA TTTGT	705

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA1764	GAACCUUGUCUAGA GAGAUU	706	GAACCTTGTCTAGAGA GATT	1218
gRNA1765	GAAGUCCACUCAUU CUUGGC	707	GAAGTCCACTCATTCT TGGC	1219
gRNA1766	GAAUCAGCAGGUUU GCAGUC	708	GAATCAGCAGGTTTGC AGTC	1220
gRNA1767	GAAUGAGUGGACUU CUGUGA	709	GAATGAGTGGACTTCT GTGA	1221
gRNA1768	GACUGCAAACCUGC UGAUUC	710	GACTGCAAACCTGCTG ATTC	1222
gRNA1769	GACUUAGUCAACAA AGAGAGA	711	GACTTAGTCAACAAAG AGAGA	1223
gRNA1770	GAUAAGCAGCCUAG CUCAGG	712	GATAAGCAGCCTAGCT CAGG	1224
gRNA1771	GAUGAGAAGCCAUC CUGCCA	713	GATGAGAAGCCATCCT GCCA	1225
gRNA1772	GCCAUCCUGCCAAG AAUGAG	472	GCCATCCTGCCAAGAA TGAG	467
gRNA1773	GCUUUUAUACUCAC UUCUCC	714	GCTTTTATACTCACTT CTCC	1226
gRNA1774	GGAUAAAGCAGCCUA GCUCAGG	715	GGATAAGCAGCCTAGC TCAGG	1227
gRNA1775	GUCUAGAGAGAUUA GAGCAU	716	GTCTAGAGAGATTAGA GCAT	1228
gRNA1776	GUGAUGGCUGCUCC CAGCCU	717	GTGATGGCTGCTCCCA GCCT	1229
gRNA1777	UACUUAUUCUCUCU UUGUUGA	718	TACTTATTCTCTCTTT GTTGA	1230
gRNA1778	UAUUCUCUCUUUGU UGACUAA	719	TATTCTCTCTTTGTTG ACTAA	1231
gRNA1779	UAUUGACUUAGUCA ACAAAG	720	TATTGACTTAGTCAAC AAAG	1232
gRNA1780	UAUUGACUUAGUCA ACAAAGA	721	TATTGACTTAGTCAAC AAAGA	1233
gRNA1781	UCAGAAUCAGCAGG UUUGCAG	722	TCAGAATCAGCAGGTT TGCAG	1234
gRNA1782	UCCACUCAUUCUUG GCAGGA	723	TCCACTCATTCTTGGC AGGA	741
gRNA1783	UCUCUCUUUGUUGA CUAAGUC	724	TCTCTCTTTGTTGACT AAGTC	742
gRNA1784	UGAGAAGCCAUCCU GCCAAGA	725	TGAGAAGCCATCCTGC CAAGA	743
gRNA1785	UGAGCUAGGCUGCU UAUCCCU	726	TGAGCTAGGCTGCTTA TCCCT	744
gRNA1786	UGAGUAUAAAAGCC CCAGGC	727	TGAGTATAAAAGCCCC AGGC	745

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA1787	UGAUGGCUGCUCCC AGCCUG	728	TGATGGCTGCTCCCAG CCTG	746
gRNA1788	UGCCAAGAAUGAGU GGACUUC	729	TGCCAAGAATGAGTGG ACTTC	747
gRNA1789	UGCCAUCUGACUG CAAACCU	730	TGCCAATCTGACTGCA AACCT	748
gRNA1790	UGUUGACUAAGUCA AUAUUC	731	TGTTGACTAAGTCAAT AATC	749
gRNA1791	UUGACUUAGUCAAC AAAGAG	732	TTGACTTAGTCAACAA AGAG	750
gRNA1792	UUUGUUGACUAAGU CAUAUAAU	733	TTTGTGACTAAGTCA ATAAT	751
gRNA1746	AACCUGCUGAUUCU GAUUAU	734	AACCTGCTGATTCTGA TTAT	752
gRNA1594	CAACUUACCCAGAG GCAAAU	735	CAACTTACCCAGAGGC AAAT	753
gRNA1595	AAUGGCUCCCAGGU GUCAUC	736	AATGGCTCCCAGGTGT CATC	754
gRNA1596	GGCUCCCAGGUGUC AUCAGC	737	GGCTCCCAGGTGTCAT CAGC	755
gRNA1597	CUCUCAUAGGUGGU AUUCAC	738	CTCTCATAGGTGGTAT TCAC	756
gRNA1598	UAUAGGAAAACCAG UGAGUC	475	TATAGGAAAACCAGTG AGTC	469
gRNA1599	UACUCACCUCUGCA UGCUC	476	TACTCACCTCTGCATG CTCA	470
gRNA1600	GCAACUUACCCAGA GGCAAA	474	GCAACTTACCCAGAGG CAAA	468
gRNA1601	UCUGUAUACUCACC UCUGCA	739	TCTGTATACTCACCTC TGCA	757
gRNA1602	GAAACACUCACCGU AGGGCC	740	GAAACACTCACCGTAG GGCC	758
gRNA1603	CUCUACACCCAGGG CACCGG	759	CTCTACACCCAGGGCA CCGG	779
gRNA1604	ACACCUUAUAGGAA AACCAG	760	ACACCTTATAGGAAAA CCAG	780
gRNA1605	AUAGGAAAACCAGU GAGUCU	761	ATAGGAAAACCAGTGA GTCT	781
gRNA1606	ACUCACCUCUGCAU GCUCAU	644	ACTCACCTCTGCATGC TCAT	658
gRNA1607	CUCACCGUAGGGCC AGCCUC	762	CTCACCGTAGGGCCAG CCTC	782
gRNA-#1	AAAAGCCCCAGGCU GGGAGC	763	AAAAGCCCCAGGCTGG GAGC	783
gRNA-#2	AAGUGAGUAUAAAA GCCCCA	764	AAGTGAGTATAAAAGC CCCA	784

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA-#3	AAUAAUCAGAAUCA GCAGGUU	765	AATAATCAGAATCAGC AGGTT	785
gRNA-#4	AAUAUGAACCUUGU CUAGAG	766	AATATGAACCTTGTCT AGAG	786
gRNA-#5	AAUGAGUGGACUUC UGUGAU	767	AATGAGTGGACTTCTG TGAT	787
gRNA-#6	ACAAUAUGAACCU UGUCUAG	768	ACAAATATGAACCTTG TCTAG	788
gRNA-#7	ACAGAAGUCCACUC AUUCUU	769	ACAGAAGTCCACTCAT TCTT	789
gRNA-#8	ACCUUGUCUAGAGA GAUUAG	770	ACCTTGTCTAGAGAGA TTAG	790
gRNA-#9	AGAAGCCAUCCUGC CAAGAA	771	AGAAGCCATCCTGCCA AGAA	791
gRNA-#10	AGCAGGUUUGCAGU CAGAUU	772	AGCAGGTTTGCAGTCA GATT	792
gRNA-#11	AGGGAUAAAGCAGCC UAGCUC	773	AGGGATAAGCAGCCTA GCTC	793
gRNA-#12	AGGUUUGCAGUCAG AUUGGC	774	AGGTTTGCAGTCAGAT TGGC	794
gRNA-#13	AGUAUAAAAGCCCC AGGCUG	775	AGTATAAAAAGCCCCAG GCTG	795
gRNA-#14	AGUCAUAAUCAGA AUCAGC	776	AGTCAATAATCAGAAT CAGC	796
gRNA-#15	AUAAUCAGAAUCAG CAGGUU	777	ATAATCAGAATCAGCA GGTT	797
gRNA-#16	UUGACUUAGUCAAC AAAGAGAG	778	TTGACTTAGTCAACAA AGAGAG	798
gRNA-#17	UCUCUUUGUUGACU AAGUCAAU	799	TCTCTTTGTTGACTAA GTCAAT	820
gRNA-#18	UGAUUAUUGACUUA GUCAACAA	800	TGATTATTGACTTAGT CAACAA	821
gRNA-#19 (sgRNA 367)	UUGGCAGGAUGGCU UCUCAUCG	648	TTGGCAGGATGGCTTC TCATCG	662
gRNA-#20	ACUUAGUCAACAAA GAGAGAAU	801	ACTTAGTCAACAAAGA GAGAAT	822
gRNA-#21	GCAGGGAUAAAGCAG CCUAGCUC	802	GCAGGGATAAGCAGCC TAGCTC	823
gRNA-#22	GUAUGGGUUACUUA UUCUCUCU	803	GTATGGGTTACTTATT CTCTCT	824
gRNA-#23	CAAGAAUGAGUGGA CUUCUG	804	CAAGAATGAGTGGACT TCTG	825
gRNA-#24	CAAUCUGACUGCAA ACCUGC	805	CAATCTGACTGCAAAC CTGC	826
gRNA-#25	CACAGAAGUCCACU CAUUCU	806	CACAGAAGTCCACTCA TTCT	827

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA-#26	CAGACGAUGAGAAG CCAUCC	807	CAGACGATGAGAAGCC ATCC	828
gRNA-#27	CAGCAGGUUUGCAG UCAGAU	808	CAGCAGGTTTGCAGTC AGAT	829
gRNA-#28	CAGGAUGGCUUCUC AUCGUC	809	CAGGATGGCTTCTCAT CGTC	830
gRNA-#29	CAGGUUUGCAGUCA GAUUGGC	810	CAGGTTTGCAGTCAGA TTGGC	831
gRNA-#30	CAGUCAGAUUGGCA GGGAUA	811	CAGTCAGATTGGCAGG GATA	832
gRNA-#31	CCACUCAUUCUUGG CAGGAU	812	CCACTCATTCTTGGCA GGAT	833
gRNA-#32	CUAAGUCAUAUAUC AGAAUC	813	CTAAGTCAATAATCAG AATC	834
gRNA-#33	GUCAACAAAGAGAG AAUAAGUA	814	GTCAACAAAGAGAGAA TAAGTA	835
gRNA-#34	CUUAUCCCUGCCAA UCUGAC	815	CTTATCCCTGCCAATC TGAC	836
gRNA-#35	UCCCUGCCAAUCUG ACUGCAAA	816	TCCCTGCCAATCTGAC TGCAAA	837
gRNA-#36	UUCUCUCUUUGUUG ACUAAGUC	817	TTCTCTCTTTGTTGAC TAAGTC	838
gRNA-#37	UCCUGAGCUAGGCU GCUUAUCC	818	TCCTGAGCTAGGCTGC TTATCC	839
gRNA-#38	CUUCUGUGAUGGCU GCUCCC	819	CTTCTGTGATGGCTGC TCCC	860
gRNA-#39	UGUGAUGGCUGCUC CCAGCCUG	840	TGTGATGGCTGCTCCC AGCCTG	861
gRNA-#40	GCAGGAUGGCUUCU CAUCGUCU	841	GCAGGATGGCTTCTCA TCGTCT	862
gRNA-#41	UCUAGAGAGAUUAG AGCAUCGG	842	TCTAGAGAGATTAGAG CATCGG	863
gRNA-#42	GUUGACUAAGUCAA UAAUCAGA	843	GTTGACTAAGTCAATA ATCAGA	864
gRNA-#43	UAUACUCACUUCUC CUGAGCUA	844	TATACTCACTTCTCCT GAGCTA	865
gRNA-#44	GAAGUGAGUAUAAA AGCCCC	845	GAAGTGAGTATAAAAAG CCCC	866
gRNA-#45	GACAAGGUUCAUUAU UUGUAU	846	GACAAGGTTTCATATTT GTAT	867
gRNA-#46	GAGUAUAAAAGCCC CAGGCU	847	GAGTATAAAAAGCCCCA GGCT	868
gRNA-#47	GAGUGGACUUCUGU GAUGGC	848	GAGTGGACTTCTGTGA TGGC	869
gRNA-#48	GAUGGCUGCUCCCA GCCUGG	849	GATGGCTGCTCCAGC CTGG	870

gRNA_Name	Spacer Sequence	SEQ ID NO:	Target Site/Protospacer Sequence	SEQ ID NO:
gRNA-#49	GCAGCCUAGCUCAG GAGAAG	850	GCAGCCTAGCTCAGGA GAAG	871
gRNA-#50	GCUGCUUAUCCUG CCAAUC	851	GCTGCTTATCCCTGCC AATC	872
gRNA-#51	GGGAUAAGCAGCCU AGCUCA	852	GGGATAAGCAGCCTAG CTCA	873
gRNA-#52	GGUUUGCAGUCAGA UUGGCA	853	GGTTTGCAGTCAGATT GGCA	874
gRNA-#53	GUUACUUAUUCUCU CUUUGU	854	GTTACTTATTCTCTCT TTGT	875
gRNA-#54	CUUAUUCUCUCUUU GUUGACUA	855	CTTATTCTCTCTTTGT TGACTA	876
gRNA-#55	AUAUUUGUAUGGGU UACUUAUU	856	ATATTTGTATGGGTTA CTTATT	877
gRNA-#56	ACUAAGUCAUAAU CAGAAUCA	857	ACTAAGTCAATAATCA GAATCA	878
gRNA-#57	GUUUGCAGUCAGAU UGGCAG	858	GTTTGCAGTCAGATTG GCAG	879
gRNA-#58	GCAGUCAGAUUGGC AGGGAUAA	859	GCAGTCAGATTGGCAG GGATAA	880
gRNA-#59	UACAAUAUGAACC UUGUCU	881	TACAAATATGAACCTT GTCT	894
gRNA-#60	UACUCACUUCUCCU GAGCUA	882	TACTCACTTCTCCTGA GCTA	895
gRNA-#61	UAUAAAAGCCCCAG GCUGGG	883	TATAAAAAGCCCCAGGC TGGG	896
gRNA-#62	UCACUUCUCCUGAG CUAGGC	884	TCACTTCTCCTGAGCT AGGC	897
gRNA-#63	UCAGAUUGGCAGGG AUAAGC	885	TCAGATTGGCAGGGAT AAGC	898
gRNA-#64	UCAGGAGAAGUGAG UAUAAA	886	TCAGGAGAAGTGAGTA TAAA	899
gRNA-#65	UCUGACUGCAAACC UGCUGAU	887	TCTGACTGCAAACCTG CTGAT	900
gRNA-#66	UGAGCUAGGCUGCU UAUCCC	888	TGAGCTAGGCTGCTTA TCCC	901
gRNA-#67	UGCCAAUCUGACUG CAAACC	889	TGCCAATCTGACTGCA AACC	902
gRNA-#68	UGCUCUAAUCUCUC UAGACA	890	TGCTCTAATCTCTCTA GACA	903
gRNA-#69	UGUGAUGGCUGCUC CCAGCC	891	TGTGATGGCTGCTCCC AGCC	904
gRNA-#70	UUGGCAGGGAUAAG CAGCCU	892	TTGGCAGGGATAAGCA GCCT	905
gRNA-#71	UUUUUAUCUCACUU CUCCUG	893	TTTATACTCACTTCT CCTG	906

Table 2C. Exemplary human TTR target site sequences and base editor + guide RNA combinations.

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1594	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	Splice Site	CAACTTACCCAG AGGCAAATGGC	907
gRNA1594	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	Splice Site	CAACTTACCCAG AGGCAAATGGC	908
gRNA1594	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	Splice Site	CAACTTACCCAG AGGCAAATGGC	909
gRNA1595	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	Stop Codon	AATGGCTCCAG GTGTCATCAGC	910
gRNA1596	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	Stop Codon	GGCTCCCAGGTG TCATCAGCAGC	911
gRNA1597	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	Splice Site	CTCTCATAGGTG GTATTCACAGC	912
gRNA1597	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	Splice Site	CTCTCATAGGTG GTATTCACAGC	913
gRNA1598	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	Splice Site	TATAGGAAAACC AGTGAGTCTGG	914
gRNA1599	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	Splice Site	TACTCACCTCTG CATGCTCATGG	915
gRNA1600	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	Splice Site	GCAACTTACCCA GAGGCAAATGG	916
gRNA1601	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	Splice Site	TCTGTATACTCA CCTCTGCATGC	917
gRNA1602	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	Splice Site	GAAACACTCACC GTAGGGCCAGC	918
gRNA1603	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	Splice Site	CTCTACACCCAG GGCACCGGTGA	919
gRNA1604	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	Splice Site	ACACCTTATAGG AAAACCAAGTGA	920
gRNA1605	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	Splice Site	ATAGGAAAACCA GTGAGTCTGGA	921
gRNA1606	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	Splice Site	ACTCACCTCTGC ATGCTCATGGA	922

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1607	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	Splice Site	CTCACCGTAGGG CCAGCCTCAGA	923
gRNA1746	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AACCTGCTGATT CTGATTATTGA	924
gRNA1746	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AACCTGCTGATT CTGATTATTGA	925
gRNA1746	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AACCTGCTGATT CTGATTATTGA	926
gRNA1747	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	AAGAGAGAATAA GTAACCCATACA AAT	927
gRNA1747	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	AAGAGAGAATAA GTAACCCATACA AAT	928
gRNA1748	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	AAGCAGCCTAGC TCAGGAGAAGTG AGT	929
gRNA1748	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	AAGCAGCCTAGC TCAGGAGAAGTG AGT	930
gRNA1749	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AAGTCCACTCAT TCTTGGCAGGA	931
gRNA1749	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AAGTCCACTCAT TCTTGGCAGGA	932
gRNA1749	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AAGTCCACTCAT TCTTGGCAGGA	933
gRNA1750	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	ACGATGAGAAGC CATCCTGCCAAG AAT	934
gRNA1750	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	ACGATGAGAAGC CATCCTGCCAAG AAT	935
gRNA1751	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	AGACAAGGTTCA TATTTGTATGG	936
gRNA1751	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	AGACAAGGTTCA TATTTGTATGG	937
gRNA1751	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	AGACAAGGTTCA TATTTGTATGG	938

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1752	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AGGCTGGGAGCA GCCATCACAGA	939
gRNA1752	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AGGCTGGGAGCA GCCATCACAGA	940
gRNA1752	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AGGCTGGGAGCA GCCATCACAGA	941
gRNA1753	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	ATAAGTAACCCA TACAAATATGA	942
gRNA1753	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	ATAAGTAACCCA TACAAATATGA	943
gRNA1753	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	ATAAGTAACCCA TACAAATATGA	944
gRNA1754	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	ATACTCACTTCT CCTGAGCTAGG	945
gRNA1754	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	ATACTCACTTCT CCTGAGCTAGG	946
gRNA1754	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	ATACTCACTTCT CCTGAGCTAGG	947
gRNA1755	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	ATTATTGACTTA GTCAACAAAGA	948
gRNA1755	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	ATTATTGACTTA GTCAACAAAGA	949
gRNA1755	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	ATTATTGACTTA GTCAACAAAGA	950
gRNA1756	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	CAAATATGAACC TTGTCTAGAGA	951
gRNA1756	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	CAAATATGAACC TTGTCTAGAGA	952
gRNA1756	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	CAAATATGAACC TTGTCTAGAGA	953
gRNA1757	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	CAGAAGTCCACT CATTCTTGGCAG GAT	954

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1757	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	CAGAAGTCCACT CATTCTTGGCAG GAT	955
gRNA1758	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	CAGGCTGGGAGC AGCCATCACAGA AGT	956
gRNA1758	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	CAGGCTGGGAGC AGCCATCACAGA AGT	957
gRNA1759	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	CCATCCTGCCAA GAATGAGTGGA	958
gRNA1759	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	CCATCCTGCCAA GAATGAGTGGA	959
gRNA1759	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	CCATCCTGCCAA GAATGAGTGGA	960
gRNA1760	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	CCTGCTGATTCT GATTATTGACTT AGT	961
gRNA1760	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	CCTGCTGATTCT GATTATTGACTT AGT	962
gRNA1761	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	CGATGCTCTAAT CTCTCTAGACAA GGT	963
gRNA1761	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	CGATGCTCTAAT CTCTCTAGACAA GGT	964
gRNA1762	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	CTAAGTCAATAA TCAGAATCAGCA GGT	965
gRNA1762	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	CTAAGTCAATAA TCAGAATCAGCA GGT	966
gRNA1763	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	CTAGACAAGGTT CATATTTGTATG GGT	967
gRNA1763	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	CTAGACAAGGTT CATATTTGTATG GGT	968
gRNA1764	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GAACCTTGTCTA GAGAGATTAGA	969

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1764	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GAACCTTGTCTA GAGAGATTAGA	970
gRNA1764	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GAACCTTGTCTA GAGAGATTAGA	971
gRNA1765	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GAAGTCCACTCA TTCTTGGCAGG	972
gRNA1765	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GAAGTCCACTCA TTCTTGGCAGG	973
gRNA1765	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GAAGTCCACTCA TTCTTGGCAGG	974
gRNA1766	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GAATCAGCAGGT TTGCAGTCAGA	975
gRNA1766	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GAATCAGCAGGT TTGCAGTCAGA	976
gRNA1766	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GAATCAGCAGGT TTGCAGTCAGA	977
gRNA1767	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GAATGAGTGGAC TTCTGTGATGG	978
gRNA1767	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GAATGAGTGGAC TTCTGTGATGG	979
gRNA1767	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GAATGAGTGGAC TTCTGTGATGG	980
gRNA1768	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GACTGCAAACCT GCTGATTCTGA	981
gRNA1768	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GACTGCAAACCT GCTGATTCTGA	982
gRNA1768	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GACTGCAAACCT GCTGATTCTGA	983
gRNA1769	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	GACTTAGTCAAC AAAGAGAGAATA AGT	984
gRNA1769	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	GACTTAGTCAAC AAAGAGAGAATA AGT	985
gRNA1770	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GATAAGCAGCCT AGCTCAGGAGA	986

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1770	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GATAAGCAGCCT AGCTCAGGAGA	987
gRNA1770	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GATAAGCAGCCT AGCTCAGGAGA	988
gRNA1771	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GATGAGAAGCCA TCCTGCCAAGA	989
gRNA1771	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GATGAGAAGCCA TCCTGCCAAGA	990
gRNA1771	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GATGAGAAGCCA TCCTGCCAAGA	991
gRNA1772	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GCCATCCTGCCA AGAATGAGTGG	992
gRNA1772	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GCCATCCTGCCA AGAATGAGTGG	993
gRNA1772	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GCCATCCTGCCA AGAATGAGTGG	994
gRNA1773	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GCTTTTATACTC ACTTCTCCTGA	995
gRNA1773	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GCTTTTATACTC ACTTCTCCTGA	996
gRNA1773	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GCTTTTATACTC ACTTCTCCTGA	997
gRNA1774	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	GGATAAGCAGCC TAGCTCAGGAGA AGT	998
gRNA1774	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	GGATAAGCAGCC TAGCTCAGGAGA AGT	999
gRNA1775	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GTCTAGAGAGAT TAGAGCATCGG	1000
gRNA1775	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GTCTAGAGAGAT TAGAGCATCGG	1001
gRNA1775	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GTCTAGAGAGAT TAGAGCATCGG	1002
gRNA1776	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GTGATGGCTGCT CCCAGCCTGGG	1003

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1776	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GTGATGGCTGCT CCCAGCCTGGG	1004
gRNA1776	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GTGATGGCTGCT CCCAGCCTGGG	1005
gRNA1777	ABE_NNNRRT_2Int_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TACTTATTCTCT CTTTGTTGACTA AGT	1006
gRNA1777	CBE_NNNRRT_2Int_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TACTTATTCTCT CTTTGTTGACTA AGT	1007
gRNA1778	ABE_NNNRRT_2Int_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TATTCTCTCTTT GTTGACTAAGTC AAT	1008
gRNA1778	CBE_NNNRRT_2Int_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TATTCTCTCTTT GTTGACTAAGTC AAT	1009
gRNA1779	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	TATTGACTTAGT CAACAAAGAGA	1010
gRNA1779	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	TATTGACTTAGT CAACAAAGAGA	1011
gRNA1779	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	TATTGACTTAGT CAACAAAGAGA	1012
gRNA1780	ABE_NNGRRT_2Int_5-14_011	saCas9	saCas9 ABE	TTR Promoter	TATTGACTTAGT CAACAAAGAGAG AAT	1013
gRNA1780	CBE_NNGRRT_2Int_3-12_012	saCas9	saCas9 CBE	TTR Promoter	TATTGACTTAGT CAACAAAGAGAG AAT	1014
gRNA1781	ABE_NNNRRT_2Int_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TCAGAATCAGCA GGTTTGCAGTCA GAT	1015
gRNA1781	CBE_NNNRRT_2Int_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TCAGAATCAGCA GGTTTGCAGTCA GAT	1016
gRNA1782	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	TCCACTCATTCT TGGCAGGATGG	1017
gRNA1782	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	TCCACTCATTCT TGGCAGGATGG	1018
gRNA1782	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	TCCACTCATTCT TGGCAGGATGG	1019

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1783	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TCTCTCTTTGTT GACTAAGTCAAT AAT	1020
gRNA1783	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TCTCTCTTTGTT GACTAAGTCAAT AAT	1021
gRNA1784	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	TGAGAAGCCATC CTGCCAAGAATG AGT	1022
gRNA1784	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	TGAGAAGCCATC CTGCCAAGAATG AGT	1023
gRNA1785	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TGAGCTAGGCTG CTTATCCCTGCC AAT	1024
gRNA1785	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TGAGCTAGGCTG CTTATCCCTGCC AAT	1025
gRNA1786	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	TGAGTATAAAAAG CCCCAGGCTGG	1026
gRNA1786	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	TGAGTATAAAAAG CCCCAGGCTGG	1027
gRNA1786	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	TGAGTATAAAAAG CCCCAGGCTGG	1028
gRNA1787	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	TGATGGCTGCTC CCAGCCTGGGG	1029
gRNA1787	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	TGATGGCTGCTC CCAGCCTGGGG	1030
gRNA1787	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	TGATGGCTGCTC CCAGCCTGGGG	1031
gRNA1788	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TGCCAAGAATGA GTGGACTTCTGT GAT	1032
gRNA1788	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TGCCAAGAATGA GTGGACTTCTGT GAT	1033
gRNA1789	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TGCCAATCTGAC TGCAAACCTGCT GAT	1034
gRNA1789	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TGCCAATCTGAC TGCAAACCTGCT GAT	1035
gRNA1790	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	TGTTGACTAAGT CAATAATCAGA	1036

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA1790	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	TGTTGACTAAGT CAATAATCAGA	1037
gRNA1790	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	TGTTGACTAAGT CAATAATCAGA	1038
gRNA1791	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	TTGACTTAGTCA ACAAAGAGAGA	1039
gRNA1791	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	TTGACTTAGTCA ACAAAGAGAGA	1040
gRNA1791	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	TTGACTTAGTCA ACAAAGAGAGA	1041
gRNA1792	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	TTTGTGACTAA GTCAATAATCAG AAT	1042
gRNA1792	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	TTTGTGACTAA GTCAATAATCAG AAT	104
gRNA-#1	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	AAAAGCCCCAGG CTGGGAGCAGC	1044
gRNA-#1	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	AAAAGCCCCAGG CTGGGAGCAGC	1045
gRNA-#1	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	AAAAGCCCCAGG CTGGGAGCAGC	1046
gRNA-#2	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	AAGTGAGTATAA AAGCCCCAGGC	1047
gRNA-#2	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	AAGTGAGTATAA AAGCCCCAGGC	1048
gRNA-#2	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	AAGTGAGTATAA AAGCCCCAGGC	1049
gRNA-#3	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	AATAATCAGAAT CAGCAGGTTTGC AGT	1050
gRNA-#3	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	AATAATCAGAAT CAGCAGGTTTGC AGT	1051
gRNA-#4	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AATATGAACCTT GTCTAGAGAGA	1052

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#4	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AATATGAACCTTGTCTAGAGAGA	1053
gRNA-#4	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AATATGAACCTTGTCTAGAGAGA	1054
gRNA-#5	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	AATGAGTGGACTTCTGTGATGGC	1055
gRNA-#5	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	AATGAGTGGACTTCTGTGATGGC	1056
gRNA-#5	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	AATGAGTGGACTTCTGTGATGGC	1057
gRNA-#6	ABE_NNNRRT_21nt_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	ACAAATATGAACCTTGTCTAGAGAGAT	1058
gRNA-#6	CBE_NNNRRT_21nt_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	ACAAATATGAACCTTGTCTAGAGAGAT	1059
gRNA-#7	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	ACAGAAGTCCACTCATTCTTGGC	1060
gRNA-#7	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	ACAGAAGTCCACTCATTCTTGGC	1061
gRNA-#7	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	ACAGAAGTCCACTCATTCTTGGC	1062
gRNA-#8	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	ACCTTGTCTAGAGAGATTAGAGC	1063
gRNA-#8	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	ACCTTGTCTAGAGAGATTAGAGC	1064
gRNA-#8	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	ACCTTGTCTAGAGAGATTAGAGC	1065
gRNA-#9	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AGAAGCCATCCTGCCAAGAATGA	1066
gRNA-#9	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AGAAGCCATCCTGCCAAGAATGA	1067
gRNA-#9	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AGAAGCCATCCTGCCAAGAATGA	1068

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#10	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	AGCAGGTTTGCA GTCAGATTGGC	1069
gRNA-#10	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	AGCAGGTTTGCA GTCAGATTGGC	1070
gRNA-#10	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	AGCAGGTTTGCA GTCAGATTGGC	1071
gRNA-#11	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	AGGGATAAGCAG CCTAGCTCAGG	1072
gRNA-#11	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	AGGGATAAGCAG CCTAGCTCAGG	1073
gRNA-#11	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	AGGGATAAGCAG CCTAGCTCAGG	1074
gRNA-#12	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	AGGTTTGCAGTC AGATTGGCAGG	1075
gRNA-#12	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	AGGTTTGCAGTC AGATTGGCAGG	1076
gRNA-#12	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	AGGTTTGCAGTC AGATTGGCAGG	1077
gRNA-#13	CBE_NGA_20n t_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	AGTATAAAAGCC CCAGGCTGGGA	1078
gRNA-#13	ABE_NGA_20n t_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	AGTATAAAAGCC CCAGGCTGGGA	1079
gRNA-#13	ABE_NGA_20n t_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	AGTATAAAAGCC CCAGGCTGGGA	1080
gRNA-#14	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	AGTCAATAATCA GAATCAGCAGG	1081
gRNA-#14	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	AGTCAATAATCA GAATCAGCAGG	1082
gRNA-#14	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	AGTCAATAATCA GAATCAGCAGG	1083
gRNA-#15	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	ATAATCAGAATC AGCAGGTTTGC	1084
gRNA-#15	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	ATAATCAGAATC AGCAGGTTTGC	1085
gRNA-#15	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	ATAATCAGAATC AGCAGGTTTGC	1086

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#16	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTATTGACTTA GTCAACAAAGAG AG	1087
gRNA-#17	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTCTCTCTTTG TTGACTAAGTCA AT	1088
gRNA-#18	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTCTGATTATT GACTTAGTCAAC AA	1089
gRNA-#19 (sgRNA_36 7)	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTCTTGGCAGG ATGGCTTCTCAT CG	1090
gRNA-#20	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTGACTTAGTC AACAAAGAGAGA AT	1091
gRNA-#21	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTGGCAGGGAT AAGCAGCCTAGC TC	1092
gRNA-#22	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	ATTTGTATGGGT TACTTATTCTCT CT	1093
gRNA-#23	CBE_NGA_20n t_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	CAAGAATGAGTG GACTTCTGTGA	1094
gRNA-#23	ABE_NGA_20n t_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	CAAGAATGAGTG GACTTCTGTGA	1095
gRNA-#23	ABE_NGA_20n t_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	CAAGAATGAGTG GACTTCTGTGA	1096
gRNA-#24	CBE_NGA_20n t_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	CAATCTGACTGC AAACCTGCTGA	1097
gRNA-#24	ABE_NGA_20n t_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	CAATCTGACTGC AAACCTGCTGA	1098
gRNA-#24	ABE_NGA_20n t_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	CAATCTGACTGC AAACCTGCTGA	1099
gRNA-#25	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	CACAGAAGTCCA CTCATTCTTGG	1100
gRNA-#25	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	CACAGAAGTCCA CTCATTCTTGG	1101
gRNA-#25	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	CACAGAAGTCCA CTCATTCTTGG	1102

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#26	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CAGACGATGAGA AGCCATCCTGC	1103
gRNA-#26	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CAGACGATGAGA AGCCATCCTGC	1104
gRNA-#26	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CAGACGATGAGA AGCCATCCTGC	1105
gRNA-#27	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	CAGCAGGTTTGC AGTCAGATTGG	1106
gRNA-#27	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	CAGCAGGTTTGC AGTCAGATTGG	1107
gRNA-#27	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	CAGCAGGTTTGC AGTCAGATTGG	1108
gRNA-#28	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CAGGATGGCTTC TCATCGTCTGC	1109
gRNA-#28	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CAGGATGGCTTC TCATCGTCTGC	1110
gRNA-#28	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CAGGATGGCTTC TCATCGTCTGC	1111
gRNA-#29	ABE_NNGRRT_21nt_5-14_011	saCas9	saCas9 ABE	TTR Promoter	CAGGTTTGCAGT CAGATTGGCAGG GAT	1112
gRNA-#29	CBE_NNGRRT_21nt_3-12_012	saCas9	saCas9 CBE	TTR Promoter	CAGGTTTGCAGT CAGATTGGCAGG GAT	1113
gRNA-#30	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CAGTCAGATTGG CAGGGATAAGC	1114
gRNA-#30	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CAGTCAGATTGG CAGGGATAAGC	1115
gRNA-#30	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CAGTCAGATTGG CAGGGATAAGC	1116
gRNA-#31	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CCACTCATTCTT GGCAGGATGGC	1117
gRNA-#31	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CCACTCATTCTT GGCAGGATGGC	1118
gRNA-#31	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CCACTCATTCTT GGCAGGATGGC	1119

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#32	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CTAAGTCAATAA TCAGAATCAGC	1120
gRNA-#32	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CTAAGTCAATAA TCAGAATCAGC	1121
gRNA-#32	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CTAAGTCAATAA TCAGAATCAGC	1122
gRNA-#33	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTAGTCAACAA AGAGAGAATAAG TA	1123
gRNA-#34	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CTTATCCCTGCC AATCTGACTGC	1124
gRNA-#34	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CTTATCCCTGCC AATCTGACTGC	1125
gRNA-#34	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CTTATCCCTGCC AATCTGACTGC	1126
gRNA-#35	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTATCCCTGCC AATCTGACTGCA AA	1127
gRNA-#36	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTATTCTCTCT TTGTTGACTAAG TC	1128
gRNA-#37	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTCTCCTGAGC TAGGCTGCTTAT CC	1129
gRNA-#38	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	CTTCTGTGATGG CTGCTCCCAGC	1130
gRNA-#38	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	CTTCTGTGATGG CTGCTCCCAGC	1131
gRNA-#38	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	CTTCTGTGATGG CTGCTCCCAGC	1132
gRNA-#39	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTCTGTGATGG CTGCTCCCAGCC TG	1133
gRNA-#40	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTGGCAGGATG GCTTCTCATCGT CT	1134
gRNA-#41	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTGTCTAGAGA GATTAGAGCATC GG	1135

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#42	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTTGTTGACTA AGTCAATAATCA GA	1136
gRNA-#43	ABE_VTTN_22 nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	CTTTTATACTCA CTTCTCCTGAGC TA	1137
gRNA-#44	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GAAGTGAGTATA AAAGCCCCAGG	1138
gRNA-#44	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GAAGTGAGTATA AAAGCCCCAGG	1139
gRNA-#44	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GAAGTGAGTATA AAAGCCCCAGG	1140
gRNA-#45	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GACAAGGTTTCAT ATTTGTATGGG	1141
gRNA-#45	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GACAAGGTTTCAT ATTTGTATGGG	1142
gRNA-#45	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GACAAGGTTTCAT ATTTGTATGGG	1143
gRNA-#46	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GAGTATAAAAAGC CCCAGGCTGGG	1144
gRNA-#46	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GAGTATAAAAAGC CCCAGGCTGGG	1145
gRNA-#46	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GAGTATAAAAAGC CCCAGGCTGGG	1146
gRNA-#47	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	GAGTGGACTTCT GTGATGGCTGC	1147
gRNA-#47	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	GAGTGGACTTCT GTGATGGCTGC	1148
gRNA-#47	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	GAGTGGACTTCT GTGATGGCTGC	1149
gRNA-#48	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	GATGGCTGCTCC CAGCCTGGGGC	1150
gRNA-#48	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	GATGGCTGCTCC CAGCCTGGGGC	1151
gRNA-#48	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	GATGGCTGCTCC CAGCCTGGGGC	1152
gRNA-#48	CBE_NGA_20n t_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GCAGCCTAGCTC AGGAGAAGTGA	1153

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#48	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GCAGCCTAGCTC AGGAGAAGTGA	1154
gRNA-#48	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GCAGCCTAGCTC AGGAGAAGTGA	1155
gRNA-#50	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GCTGCTTATCCC TGCCAATCTGA	1156
gRNA-#50	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GCTGCTTATCCC TGCCAATCTGA	1157
gRNA-#50	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GCTGCTTATCCC TGCCAATCTGA	1158
gRNA-#51	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GGGATAAGCAGC CTAGCTCAGGA	1159
gRNA-#51	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GGGATAAGCAGC CTAGCTCAGGA	1160
gRNA-#51	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GGGATAAGCAGC CTAGCTCAGGA	1161
gRNA-#52	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	GGTTTGCAGTCA GATTGGCAGGG	1162
gRNA-#52	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	GGTTTGCAGTCA GATTGGCAGGG	1163
gRNA-#52	ABE_NGG_20nt_3-12_018	spCas9	spCas9 IBE	TTR Promoter	GGTTTGCAGTCA GATTGGCAGGG	1164
gRNA-#53	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GTTACTTATTCT CTCTTTGTTGA	1165
gRNA-#53	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GTTACTTATTCT CTCTTTGTTGA	1166
gRNA-#53	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GTTACTTATTCT CTCTTTGTTGA	1167
gRNA-#54	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	GTTACTTATTCT CTCTTTGTTGAC TA	1168
gRNA-#55	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	GTTTCATATTGT ATGGGTTACTTA TT	1169

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#56	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	GTTGACTAAGTC AATAATCAGAATCA	1170
gRNA-#57	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	GTTTGCAGTCAG ATTGGCAGGGA	1171
gRNA-#57	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	GTTTGCAGTCAG ATTGGCAGGGA	1172
gRNA-#57	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	GTTTGCAGTCAG ATTGGCAGGGA	1173
gRNA-#58	ABE_VTTN_22nt_5-9_017	cas12b	cas12b ABE	TTR Promoter	GTTTGCAGTCAG ATTGGCAGGGATAA	1174
gRNA-#59	CBE_NGA_20nt_4-9_006	spCas9	spCas9 VRQR CBE	TTR Promoter	TACAAATATGAA CCTTGTCTAGA	1175
gRNA-#59	ABE_NGA_20nt_3-9_005	spCas9	spCas9 VRQR ABE	TTR Promoter	TACAAATATGAA CCTTGTCTAGA	1176
gRNA-#59	ABE_NGA_20nt_3-12_019	spCas9	spCas9 VRQR IBE	TTR Promoter	TACAAATATGAA CCTTGTCTAGA	1177
gRNA-#60	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TACTCACTTCTC CTGAGCTAGGC	1178
gRNA-#60	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TACTCACTTCTC CTGAGCTAGGC	1179
gRNA-#60	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TACTCACTTCTC CTGAGCTAGGC	1180
gRNA-#61	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TATAAAAGCCCC AGGCTGGGAGC	1181
gRNA-#61	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TATAAAAGCCCC AGGCTGGGAGC	1182
gRNA-#61	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TATAAAAGCCCC AGGCTGGGAGC	1183
gRNA-#62	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TCACTTCTCCTG AGCTAGGCTGC	1184
gRNA-#62	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TCACTTCTCCTG AGCTAGGCTGC	1185

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#62	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TCACTTCTCCTG AGCTAGGCTGC	1186
gRNA-#63	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TCAGATTGGCAG GGATAAGCAGC	1187
gRNA-#63	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TCAGATTGGCAG GGATAAGCAGC	1188
gRNA-#63	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TCAGATTGGCAG GGATAAGCAGC	1189
gRNA-#64	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TCAGGAGAAGTG AGTATAAAAGC	1190
gRNA-#64	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TCAGGAGAAGTG AGTATAAAAGC	1191
gRNA-#64	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TCAGGAGAAGTG AGTATAAAAGC	1192
gRNA-#65	ABE_NNNRRT_2Int_5-14_014	saCas9	saCas9 KKH ABE	TTR Promoter	TCTGACTGCAAA CCTGCTGATTCT GAT	1193
gRNA-#65	CBE_NNNRRT_2Int_3-12_015	saCas9	saCas9 KKH CBE	TTR Promoter	TCTGACTGCAAA CCTGCTGATTCT GAT	1194
gRNA-#66	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TGAGCTAGGCTG CTTATCCCTGC	1195
gRNA-#66	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TGAGCTAGGCTG CTTATCCCTGC	1196
gRNA-#66	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TGAGCTAGGCTG CTTATCCCTGC	1197
gRNA-#67	ABE_NGC_20nt_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TGCCAATCTGAC TGCAAACCTGC	1198
gRNA-#67	CBE_NGC_20nt_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TGCCAATCTGAC TGCAAACCTGC	1199
gRNA-#67	ABE_NGC_20nt_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TGCCAATCTGAC TGCAAACCTGC	1200
gRNA-#68	ABE_NGG_20nt_3-9_002	spCas9	spCas9 ABE	TTR Promoter	TGCTCTAATCTC TCTAGACAAGG	1201
gRNA-#68	CBE_NGG_20nt_4-9_003	spCas9	spCas9 CBE	TTR Promoter	TGCTCTAATCTC TCTAGACAAGG	1202

gRNA Name	Editor Name	Cas Name	Editor Alias	Editing Strategy	Target Site/Protospacer + PAM Sequence	SEQ ID NO
gRNA-#68	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	TGCTCTAATCTC TCTAGACAAGG	1203
gRNA-#69	ABE_NGG_20n t_3-9_002	spCas9	spCas9 ABE	TTR Promoter	TGTGATGGCTGC TCCCAGCCTGG	1204
gRNA-#69	CBE_NGG_20n t_4-9_003	spCas9	spCas9 CBE	TTR Promoter	TGTGATGGCTGC TCCCAGCCTGG	1205
gRNA-#69	ABE_NGG_20n t_3-12_018	spCas9	spCas9 IBE	TTR Promoter	TGTGATGGCTGC TCCCAGCCTGG	1206
gRNA-#70	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TTGGCAGGGATA AGCAGCCTAGC	1207
gRNA-#70	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TTGGCAGGGATA AGCAGCCTAGC	1208
gRNA-#70	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TTGGCAGGGATA AGCAGCCTAGC	1209
gRNA-#71	ABE_NGC_20n t_3-9_008	spCas9	spCas9 NGC ABE	TTR Promoter	TTTATACTCAC TTCTCCTGAGC	1210
gRNA-#71	CBE_NGC_20n t_4-9_009	spCas9	spCas9 NGC CBE	TTR Promoter	TTTATACTCAC TTCTCCTGAGC	1211
gRNA-#71	ABE_NGC_20n t_3-12_020	spCas9	spCas9 NGC IBE	TTR Promoter	TTTATACTCAC TTCTCCTGAGC	1212

Table 2C (CONTINUED)

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA1594	GGC	chr18	31593011	31593034	-	8
gRNA1594	GGC	chr18	31593011	31593034	-	7
gRNA1594	GGC	chr18	31593011	31593034	-	7
gRNA1595	AGC	chr18	31592994	31593017	-	9
gRNA1596	AGC	chr18	31592991	31593014	-	7,6
gRNA1597	AGC	chr18	31598558	31598581	+	8
gRNA1597	AGC	chr18	31598558	31598581	+	8
gRNA1598	TGG	chr18	31595114	31595137	+	4
gRNA1599	TGG	chr18	31595239	31595262	-	6
gRNA1600	TGG	chr18	31593012	31593035	-	8
gRNA1601	TGC	chr18	31595245	31595268	-	12
gRNA1602	AGC	chr18	31591959	31591982	-	10

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA1603	TGA	chr18	31592883	31592906	+	11
gRNA1604	TGA	chr18	31595108	31595131	+	10
gRNA1605	GGA	chr18	31595115	31595138	+	3
gRNA1606	GGA	chr18	31595238	31595261	-	5
gRNA1607	AGA	chr18	31591953	31591976	-	4
gRNA1746	TGA	chr18	31591776	31591799	-	
gRNA1746	TGA	chr18	31591776	31591799	-	
gRNA1746	TGA	chr18	31591776	31591799	-	
gRNA1747	ACAAAT	chr18	31591738	31591765	-	
gRNA1747	ACAAAT	chr18	31591738	31591765	-	
gRNA1748	GTGAGT	chr18	31591820	31591847	+	
gRNA1748	GTGAGT	chr18	31591820	31591847	+	
gRNA1749	GGA	chr18	31591880	31591903	+	
gRNA1749	GGA	chr18	31591880	31591903	+	
gRNA1749	GGA	chr18	31591880	31591903	+	
gRNA1750	AAGAAT	chr18	31591890	31591917	-	
gRNA1750	AAGAAT	chr18	31591890	31591917	-	
gRNA1751	TGG	chr18	31591725	31591748	+	
gRNA1751	TGG	chr18	31591725	31591748	+	
gRNA1751	TGG	chr18	31591725	31591748	+	
gRNA1752	AGA	chr18	31591858	31591881	+	
gRNA1752	AGA	chr18	31591858	31591881	+	
gRNA1752	AGA	chr18	31591858	31591881	+	
gRNA1753	TGA	chr18	31591734	31591757	-	
gRNA1753	TGA	chr18	31591734	31591757	-	
gRNA1753	TGA	chr18	31591734	31591757	-	
gRNA1754	AGG	chr18	31591826	31591849	-	
gRNA1754	AGG	chr18	31591826	31591849	-	
gRNA1754	AGG	chr18	31591826	31591849	-	
gRNA1755	AGA	chr18	31591761	31591784	-	
gRNA1755	AGA	chr18	31591761	31591784	-	
gRNA1755	AGA	chr18	31591761	31591784	-	
gRNA1756	AGA	chr18	31591720	31591743	-	
gRNA1756	AGA	chr18	31591720	31591743	-	
gRNA1756	AGA	chr18	31591720	31591743	-	
gRNA1757	CAGGAT	chr18	31591877	31591904	+	
gRNA1757	CAGGAT	chr18	31591877	31591904	+	
gRNA1758	AGAAGT	chr18	31591857	31591884	+	
gRNA1758	AGAAGT	chr18	31591857	31591884	+	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA1759	GGA	chr18	31591883	31591906	-	
gRNA1759	GGA	chr18	31591883	31591906	-	
gRNA1759	GGA	chr18	31591883	31591906	-	
gRNA1760	CTAGT	chr18	31591770	31591797	-	
gRNA1760	CTAGT	chr18	31591770	31591797	-	
gRNA1761	CAAGGT	chr18	31591707	31591734	+	
gRNA1761	CAAGGT	chr18	31591707	31591734	+	
gRNA1762	GCAGGT	chr18	31591771	31591798	+	
gRNA1762	GCAGGT	chr18	31591771	31591798	+	
gRNA1763	ATGGGT	chr18	31591723	31591750	+	
gRNA1763	ATGGGT	chr18	31591723	31591750	+	
gRNA1764	AGA	chr18	31591713	31591736	-	
gRNA1764	AGA	chr18	31591713	31591736	-	
gRNA1764	AGA	chr18	31591713	31591736	-	
gRNA1765	AGG	chr18	31591879	31591902	+	
gRNA1765	AGG	chr18	31591879	31591902	+	
gRNA1765	AGG	chr18	31591879	31591902	+	
gRNA1766	AGA	chr18	31591786	31591809	+	
gRNA1766	AGA	chr18	31591786	31591809	+	
gRNA1766	AGA	chr18	31591786	31591809	+	
gRNA1767	TGG	chr18	31591871	31591894	-	
gRNA1767	TGG	chr18	31591871	31591894	-	
gRNA1767	TGG	chr18	31591871	31591894	-	
gRNA1768	TGA	chr18	31591783	31591806	-	
gRNA1768	TGA	chr18	31591783	31591806	-	
gRNA1768	TGA	chr18	31591783	31591806	-	
gRNA1769	ATAAGT	chr18	31591751	31591778	-	
gRNA1769	ATAAGT	chr18	31591751	31591778	-	
gRNA1770	AGA	chr18	31591817	31591840	+	
gRNA1770	AGA	chr18	31591817	31591840	+	
gRNA1770	AGA	chr18	31591817	31591840	+	
gRNA1771	AGA	chr18	31591892	31591915	-	
gRNA1771	AGA	chr18	31591892	31591915	-	
gRNA1771	AGA	chr18	31591892	31591915	-	
gRNA1772	TGG	chr18	31591884	31591907	-	
gRNA1772	TGG	chr18	31591884	31591907	-	
gRNA1772	TGG	chr18	31591884	31591907	-	
gRNA1773	TGA	chr18	31591832	31591855	-	
gRNA1773	TGA	chr18	31591832	31591855	-	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA1773	TGA	chr18	31591832	31591855	-	
gRNA1774	AGAAAGT	chr18	31591816	31591843	+	
gRNA1774	AGAAAGT	chr18	31591816	31591843	+	
gRNA1775	CGG	chr18	31591706	31591729	-	
gRNA1775	CGG	chr18	31591706	31591729	-	
gRNA1775	CGG	chr18	31591706	31591729	-	
gRNA1776	GGG	chr18	31591855	31591878	-	
gRNA1776	GGG	chr18	31591855	31591878	-	
gRNA1776	GGG	chr18	31591855	31591878	-	
gRNA1777	CTAAGT	chr18	31591750	31591777	+	
gRNA1777	CTAAGT	chr18	31591750	31591777	+	
gRNA1778	GTCAAT	chr18	31591754	31591781	+	
gRNA1778	GTCAAT	chr18	31591754	31591781	+	
gRNA1779	AGA	chr18	31591759	31591782	-	
gRNA1779	AGA	chr18	31591759	31591782	-	
gRNA1779	AGA	chr18	31591759	31591782	-	
gRNA1780	GAGAAT	chr18	31591755	31591782	-	
gRNA1780	GAGAAT	chr18	31591755	31591782	-	
gRNA1781	TCAGAT	chr18	31591783	31591810	+	
gRNA1781	TCAGAT	chr18	31591783	31591810	+	
gRNA1782	TGG	chr18	31591883	31591906	+	
gRNA1782	TGG	chr18	31591883	31591906	+	
gRNA1782	TGG	chr18	31591883	31591906	+	
gRNA1783	AATAAT	chr18	31591757	31591784	+	
gRNA1783	AATAAT	chr18	31591757	31591784	+	
gRNA1784	ATGAGT	chr18	31591886	31591913	-	
gRNA1784	ATGAGT	chr18	31591886	31591913	-	
gRNA1785	GCCAAT	chr18	31591808	31591835	-	
gRNA1785	GCCAAT	chr18	31591808	31591835	-	
gRNA1786	TGG	chr18	31591842	31591865	+	
gRNA1786	TGG	chr18	31591842	31591865	+	
gRNA1786	TGG	chr18	31591842	31591865	+	
gRNA1787	GGG	chr18	31591854	31591877	-	
gRNA1787	GGG	chr18	31591854	31591877	-	
gRNA1787	GGG	chr18	31591854	31591877	-	
gRNA1788	TGTGAT	chr18	31591873	31591900	-	
gRNA1788	TGTGAT	chr18	31591873	31591900	-	
gRNA1789	GCTGAT	chr18	31591788	31591815	-	
gRNA1789	GCTGAT	chr18	31591788	31591815	-	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA1790	AGA	chr18	31591765	31591788	+	
gRNA1790	AGA	chr18	31591765	31591788	+	
gRNA1790	AGA	chr18	31591765	31591788	+	
gRNA1791	AGA	chr18	31591757	31591780	-	
gRNA1791	AGA	chr18	31591757	31591780	-	
gRNA1791	AGA	chr18	31591757	31591780	-	
gRNA1792	CAGAAT	chr18	31591763	31591790	+	
gRNA1792	CAGAAT	chr18	31591763	31591790	+	
gRNA-#1	AGC	chr18	31591849	31591872	+	
gRNA-#1	AGC	chr18	31591849	31591872	+	
gRNA-#1	AGC	chr18	31591849	31591872	+	
gRNA-#2	GGC	chr18	31591839	31591862	+	
gRNA-#2	GGC	chr18	31591839	31591862	+	
gRNA-#2	GGC	chr18	31591839	31591862	+	
gRNA-#3	TGCAGT	chr18	31591778	31591805	+	
gRNA-#3	TGCAGT	chr18	31591778	31591805	+	
gRNA-#4	AGA	chr18	31591718	31591741	-	
gRNA-#4	AGA	chr18	31591718	31591741	-	
gRNA-#4	AGA	chr18	31591718	31591741	-	
gRNA-#5	GGC	chr18	31591870	31591893	-	
gRNA-#5	GGC	chr18	31591870	31591893	-	
gRNA-#5	GGC	chr18	31591870	31591893	-	
gRNA-#6	AGAGAT	chr18	31591717	31591744	-	
gRNA-#6	AGAGAT	chr18	31591717	31591744	-	
gRNA-#7	GGC	chr18	31591876	31591899	+	
gRNA-#7	GGC	chr18	31591876	31591899	+	
gRNA-#7	GGC	chr18	31591876	31591899	+	
gRNA-#8	AGC	chr18	31591711	31591734	-	
gRNA-#8	AGC	chr18	31591711	31591734	-	
gRNA-#8	AGC	chr18	31591711	31591734	-	
gRNA-#9	TGA	chr18	31591888	31591911	-	
gRNA-#9	TGA	chr18	31591888	31591911	-	
gRNA-#9	TGA	chr18	31591888	31591911	-	
gRNA-#10	GGC	chr18	31591791	31591814	+	
gRNA-#10	GGC	chr18	31591791	31591814	+	
gRNA-#10	GGC	chr18	31591791	31591814	+	
gRNA-#11	AGG	chr18	31591814	31591837	+	
gRNA-#11	AGG	chr18	31591814	31591837	+	
gRNA-#11	AGG	chr18	31591814	31591837	+	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA-#12	AGG	chr18	31591794	31591817	+	
gRNA-#12	AGG	chr18	31591794	31591817	+	
gRNA-#12	AGG	chr18	31591794	31591817	+	
gRNA-#13	GGA	chr18	31591844	31591867	+	
gRNA-#13	GGA	chr18	31591844	31591867	+	
gRNA-#13	GGA	chr18	31591844	31591867	+	
gRNA-#14	AGG	chr18	31591774	31591797	+	
gRNA-#14	AGG	chr18	31591774	31591797	+	
gRNA-#14	AGG	chr18	31591774	31591797	+	
gRNA-#15	TGC	chr18	31591779	31591802	+	
gRNA-#15	TGC	chr18	31591779	31591802	+	
gRNA-#15	TGC	chr18	31591779	31591802	+	
gRNA-#16	ATTA	chr18	31591758	31591784	-	
gRNA-#17	ATTC	chr18	31591755	31591781	+	
gRNA-#18	ATTC	chr18	31591764	31591790	-	
gRNA-#19	ATTC	chr18	31591890	31591916	+	
gRNA-#20	ATTG	chr18	31591755	31591781	-	
gRNA-#21	ATTG	chr18	31591808	31591834	+	
gRNA-#22	ATTT	chr18	31591738	31591764	+	
gRNA-#23	TGA	chr18	31591874	31591897	-	
gRNA-#23	TGA	chr18	31591874	31591897	-	
gRNA-#23	TGA	chr18	31591874	31591897	-	
gRNA-#24	TGA	chr18	31591789	31591812	-	
gRNA-#24	TGA	chr18	31591789	31591812	-	
gRNA-#24	TGA	chr18	31591789	31591812	-	
gRNA-#25	TGG	chr18	31591875	31591898	+	
gRNA-#25	TGG	chr18	31591875	31591898	+	
gRNA-#25	TGG	chr18	31591875	31591898	+	
gRNA-#26	TGC	chr18	31591897	31591920	-	
gRNA-#26	TGC	chr18	31591897	31591920	-	
gRNA-#26	TGC	chr18	31591897	31591920	-	
gRNA-#27	TGG	chr18	31591790	31591813	+	
gRNA-#27	TGG	chr18	31591790	31591813	+	
gRNA-#27	TGG	chr18	31591790	31591813	+	
gRNA-#28	TGC	chr18	31591898	31591921	+	
gRNA-#28	TGC	chr18	31591898	31591921	+	
gRNA-#28	TGC	chr18	31591898	31591921	+	
gRNA-#29	AGGGAT	chr18	31591793	31591820	+	
gRNA-#29	AGGGAT	chr18	31591793	31591820	+	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA-#30	AGC	chr18	31591801	31591824	+	
gRNA-#30	AGC	chr18	31591801	31591824	+	
gRNA-#30	AGC	chr18	31591801	31591824	+	
gRNA-#31	GGC	chr18	31591884	31591907	+	
gRNA-#31	GGC	chr18	31591884	31591907	+	
gRNA-#31	GGC	chr18	31591884	31591907	+	
gRNA-#32	AGC	chr18	31591771	31591794	+	
gRNA-#32	AGC	chr18	31591771	31591794	+	
gRNA-#32	AGC	chr18	31591771	31591794	+	
gRNA-#33	CTTA	chr18	31591750	31591776	-	
gRNA-#34	TGC	chr18	31591800	31591823	-	
gRNA-#34	TGC	chr18	31591800	31591823	-	
gRNA-#34	TGC	chr18	31591800	31591823	-	
gRNA-#35	CTTA	chr18	31591797	31591823	-	
gRNA-#36	CTTA	chr18	31591752	31591778	+	
gRNA-#37	CTTC	chr18	31591816	31591842	-	
gRNA-#38	AGC	chr18	31591860	31591883	-	
gRNA-#38	AGC	chr18	31591860	31591883	-	
gRNA-#38	AGC	chr18	31591860	31591883	-	
gRNA-#39	CTTC	chr18	31591857	31591883	-	
gRNA-#40	CTTG	chr18	31591893	31591919	+	
gRNA-#41	CTTG	chr18	31591706	31591732	-	
gRNA-#42	CTTT	chr18	31591762	31591788	+	
gRNA-#43	CTTT	chr18	31591828	31591854	-	
gRNA-#44	AGG	chr18	31591838	31591861	+	
gRNA-#44	AGG	chr18	31591838	31591861	+	
gRNA-#44	AGG	chr18	31591838	31591861	+	
gRNA-#45	GGG	chr18	31591726	31591749	+	
gRNA-#45	GGG	chr18	31591726	31591749	+	
gRNA-#45	GGG	chr18	31591726	31591749	+	
gRNA-#46	GGG	chr18	31591843	31591866	+	
gRNA-#46	GGG	chr18	31591843	31591866	+	
gRNA-#46	GGG	chr18	31591843	31591866	+	
gRNA-#47	TGC	chr18	31591867	31591890	-	
gRNA-#47	TGC	chr18	31591867	31591890	-	
gRNA-#47	TGC	chr18	31591867	31591890	-	
gRNA-#48	GGC	chr18	31591853	31591876	-	
gRNA-#48	GGC	chr18	31591853	31591876	-	
gRNA-#48	GGC	chr18	31591853	31591876	-	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA-#48	TGA	chr18	31591822	31591845	+	
gRNA-#48	TGA	chr18	31591822	31591845	+	
gRNA-#48	TGA	chr18	31591822	31591845	+	
gRNA-#50	TGA	chr18	31591804	31591827	-	
gRNA-#50	TGA	chr18	31591804	31591827	-	
gRNA-#50	TGA	chr18	31591804	31591827	-	
gRNA-#51	GGA	chr18	31591815	31591838	+	
gRNA-#51	GGA	chr18	31591815	31591838	+	
gRNA-#51	GGA	chr18	31591815	31591838	+	
gRNA-#52	GGG	chr18	31591795	31591818	+	
gRNA-#52	GGG	chr18	31591795	31591818	+	
gRNA-#52	GGG	chr18	31591795	31591818	+	
gRNA-#53	TGA	chr18	31591748	31591771	+	
gRNA-#53	TGA	chr18	31591748	31591771	+	
gRNA-#53	TGA	chr18	31591748	31591771	+	
gRNA-#54	GTTA	chr18	31591748	31591774	+	
gRNA-#55	G TTC	chr18	31591732	31591758	+	
gRNA-#56	G TTG	chr18	31591766	31591792	+	
gRNA-#57	GGA	chr18	31591796	31591819	+	
gRNA-#57	GGA	chr18	31591796	31591819	+	
gRNA-#57	GGA	chr18	31591796	31591819	+	
gRNA-#58	G T T T	chr18	31591796	31591822	+	
gRNA-#59	AGA	chr18	31591722	31591745	-	
gRNA-#59	AGA	chr18	31591722	31591745	-	
gRNA-#59	AGA	chr18	31591722	31591745	-	
gRNA-#60	GGC	chr18	31591825	31591848	-	
gRNA-#60	GGC	chr18	31591825	31591848	-	
gRNA-#60	GGC	chr18	31591825	31591848	-	
gRNA-#61	AGC	chr18	31591846	31591869	+	
gRNA-#61	AGC	chr18	31591846	31591869	+	
gRNA-#61	AGC	chr18	31591846	31591869	+	
gRNA-#62	TGC	chr18	31591822	31591845	-	
gRNA-#62	TGC	chr18	31591822	31591845	-	
gRNA-#62	TGC	chr18	31591822	31591845	-	
gRNA-#63	AGC	chr18	31591804	31591827	+	
gRNA-#63	AGC	chr18	31591804	31591827	+	
gRNA-#63	AGC	chr18	31591804	31591827	+	
gRNA-#64	AGC	chr18	31591832	31591855	+	
gRNA-#64	AGC	chr18	31591832	31591855	+	

gRNA Name	PAM	Human Chromosome Location	Start Site	End Site	Strand	Target Base Position(s)
gRNA-#64	AGC	chr18	31591832	31591855	+	
gRNA-#65	TCTGAT	chr18	31591782	31591809	-	
gRNA-#65	TCTGAT	chr18	31591782	31591809	-	
gRNA-#66	TGC	chr18	31591812	31591835	-	
gRNA-#66	TGC	chr18	31591812	31591835	-	
gRNA-#66	TGC	chr18	31591812	31591835	-	
gRNA-#67	TGC	chr18	31591792	31591815	-	
gRNA-#67	TGC	chr18	31591792	31591815	-	
gRNA-#67	TGC	chr18	31591792	31591815	-	
gRNA-#68	AGG	chr18	31591710	31591733	+	
gRNA-#68	AGG	chr18	31591710	31591733	+	
gRNA-#68	AGG	chr18	31591710	31591733	+	
gRNA-#69	TGG	chr18	31591856	31591879	-	
gRNA-#69	TGG	chr18	31591856	31591879	-	
gRNA-#69	TGG	chr18	31591856	31591879	-	
gRNA-#70	AGC	chr18	31591809	31591832	+	
gRNA-#70	AGC	chr18	31591809	31591832	+	
gRNA-#70	AGC	chr18	31591809	31591832	+	
gRNA-#71	AGC	chr18	31591830	31591853	-	
gRNA-#71	AGC	chr18	31591830	31591853	-	
gRNA-#71	AGC	chr18	31591830	31591853	-	

Table 2D. Exemplary gRNA spacer sequences with PS linkage at 5' end

gRNA spacer sequence (5'-3')	SEQ ID NO
gscscsAUCCUGCCAAGAAUGAG	1213
gscscsAUCCUGCCAAGAACGAG	1214
gscsasACUUACCCAGAGGCAAA	1215
usasusAGGAAAACCAGUGAGUC	1216
usascsUCACCUCUGCAUGCUCU	1217
gscscsAUCCUGCCAAGAACGAG	1214

A is adenosine; C is cytidine; G is guanosine; U is uridine; a is 2'-O-methyladenosine; c is 2'-O-methylcytidine; g is 2'-O-methylguanosine; u is 2'-O-methyluridine and s is phosphorothioate (PS) backbone linkage.

Table 2E. Exemplary gRNA spacer sequence without PS linkage

gRNA spacer sequence (5'-3')	SEQ ID NO
GCCAUCCUGCCAAGAAUGAG	472
GCCAUCCUGCCAAGAACGAG	473

gRNA spacer sequence (5'-3')	SEQ ID NO
GCAACUUACCCAGAGGCAAA	474
UAUAGGAAAACCAGUGAGUC	475
UACUCACCUCUGCAUGCUCA	476
GCCAUCCUGCCAAGAACGAG	473

A is a modified or unmodified adenosine; C is a modified or unmodified cytidine; G is modified or unmodified guanosine; and U is a modified or unmodified uridine.

- The guide polynucleotide sg23 was used throughout the disclosure as a control guide RNA known to be effective in targeting base editor systems to alter a nucleobase in an ALAS1 gene. The sg23 guide RNA contained the following spacer sequence: CAGGAUCCGCACAGACUCCA (SEQ ID NO: 480). The sg23 guide RNA targeted the following sequence for base editing, where the PAM sequence is shown in **bold**: CAGGATCCGCACAGACTCCA**GGG** (SEQ ID NO: 481).
- The spacer sequences in **Table 2A** corresponding to sgRNAs sgRNA_361, sgRNA_362, sgRNA_363, sgRNA_364, sgRNA_365, sgRNA_366, and sgRNA_367 may be used for targeting a base editor to alter a nucleobase of a splice site of the transthyretin polynucleotide. The spacer sequences in **Table 2A** corresponding to sgRNAs sgRNA_368, sgRNA_369, sgRNA_370, sgRNA_371, sgRNA_372, sgRNA_373, and sgRNA_374 may be used for targeting an endonuclease to a transthyretin (TTR) polynucleotide sequence. The three spacer sequences in **Table 2A** corresponding to sgRNA_375, sgRNA_376, and sgRNA_377 may be used to alter a nucleobase of a transthyretin (TTR) polynucleotide. The alteration of the nucleobase may result in an alteration of an isoleucine (I) to a valine (V) (e.g., to correct a V122I mutation in a transthyretin polypeptide encoded by the transthyretin polynucleotide). In embodiments, a transthyretin polynucleotide may be edited using the following combinations of base editors and sgRNA sequences (see **Tables 1A and 2A**): ABE8.8 and sgRNA_361; ABE8.8 and sgRNA_362; ABE8.8-VRQR and sgRNA_363; BE4-VRQR and sgRNA_363; BE4-VRQR and sgRNA_364; saABE8.8 and sgRNA_365; saBE4 and sgRNA_365; saBE4-KKH and sgRNA_366, ABE-bhCas12b and sgRNA_367; spCas9-ABE and sgRNA_375; spCas9-VRQR-ABE and sgRNA_376; or saCas9-ABE and sgRNA_377. The PAM sequence of spCas9-ABE can be AGG. The PAM sequence of spCas9-VRQR-ABE can be GGA. The PAM sequence of saCas9-ABE can be AGGAAT.

- In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity of the fusion proteins. For example, any of the fusion proteins provided herein may comprise a Cas9 domain that has reduced nuclease

activity. In some embodiments, any of the fusion proteins provided herein may have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9). Without wishing to be bound by any particular theory, the presence of the catalytic residue (e.g., H840) maintains the activity of the Cas9 to cleave the non-edited (e.g., non-methylated) strand opposite the targeted nucleobase. Mutation of the catalytic residue (e.g., D10 to A10) prevents cleavage of the edited strand containing the targeted A residue. Such Cas9 variants can generate a single-strand DNA break (nick) at a specific location based on the gRNA-defined target sequence, leading to repair of the non-edited strand, ultimately resulting in a nucleobase change on the non-edited strand.

NUCLEOBASE EDITORS

Useful in the methods and compositions described herein are nucleobase editors that edit, modify or alter a target nucleotide sequence of a polynucleotide. Nucleobase editors described herein typically include a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (e.g., adenosine deaminase, cytidine deaminase, or a dual deaminase). A polynucleotide programmable nucleotide binding domain, when in conjunction with a bound guide polynucleotide (e.g., gRNA), can specifically bind to a target polynucleotide sequence and thereby localize the base editor to the target nucleic acid sequence desired to be edited.

Polynucleotide Programmable Nucleotide Binding Domain

Polynucleotide programmable nucleotide binding domains bind polynucleotides (e.g., RNA, DNA). A polynucleotide programmable nucleotide binding domain of a base editor can itself comprise one or more domains (e.g., one or more nuclease domains). In some embodiments, the nuclease domain of a polynucleotide programmable nucleotide binding domain comprises an endonuclease or an exonuclease.

Disclosed herein are base editors comprising a polynucleotide programmable nucleotide binding domain comprising all or a portion (e.g., a functional portion) of a CRISPR protein (i.e., a base editor comprising as a domain all or a portion (e.g., a functional portion) of a CRISPR protein (e.g., a Cas protein), also referred to as a “CRISPR protein-derived domain” of the base editor). A CRISPR protein-derived domain incorporated into a base editor can be modified compared to a wild-type or natural version of the CRISPR protein. A CRISPR protein-derived domain can comprise one or more mutations, insertions,

deletions, rearrangements and/or recombinations relative to a wild-type or natural version of the CRISPR protein.

Cas proteins that can be used herein include class 1 and class 2. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 or Csx12), Cas10, Csy1, Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Cas12a/Cpf1, Cas12b/C2c1 (e.g., SEQ ID NO: 232), Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, and Cas12j/CasΦ, CARF, DinG, homologues thereof, or modified versions thereof. A CRISPR enzyme can direct cleavage of one or both strands at a target sequence, such as within a target sequence and/or within a complement of a target sequence. For example, a CRISPR enzyme can direct cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence.

A vector that encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. A Cas protein (e.g., Cas9, Cas12) or a Cas domain (e.g., Cas9, Cas12) can refer to a polypeptide or domain with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild-type exemplary Cas polypeptide or Cas domain. Cas (e.g., Cas9, Cas12) can refer to the wild-type or a modified form of the Cas protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

In some embodiments, a CRISPR protein-derived domain of a base editor can include all or a portion (e.g., a functional portion) of Cas9 from *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquis* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1); *Listeria innocua* (NCBI Ref: NP_472073.1); *Campylobacter jejuni* (NCBI

Ref: YP_002344900.1); *Neisseria meningitidis* (NCBI Ref: YP_002342100.1), *Streptococcus pyogenes*, or *Staphylococcus aureus*.

Some aspects of the disclosure provide high fidelity Cas9 domains. High fidelity Cas9 domains are known in the art and described, for example, in Kleinstiver, B.P., *et al.* “High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects.” *Nature* 529, 490-495 (2016); and Slaymaker, I.M., *et al.* “Rationally engineered Cas9 nucleases with improved specificity.” *Science* 351, 84-88 (2015); the entire contents of each of which are incorporated herein by reference. An Exemplary high fidelity Cas9 domain is provided in the Sequence Listing as SEQ ID NO: 233.

10 In some embodiments, any of the Cas9 fusion proteins or complexes provided herein comprise one or more of a D10A, N497X, a R661X, a Q695X, and/or a Q926X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. .

Typically, Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a
15 “protospacer adjacent motif (PAM)” or PAM-like motif, which is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial adaptive immune system. The presence of an NGG PAM sequence is required to bind a particular nucleic acid region, where the “N” in “NGG” is adenosine (A), thymidine (T), or cytosine (C), and the G is guanosine. In some embodiments, any of the
20 fusion proteins or complexes provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (*e.g.*, NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, “Engineered CRISPR-Cas9
25 nucleases with altered PAM specificities” *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, “Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition” *Nature Biotechnology* 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

30 In some embodiments, the napDNAbp is a circular permutant (*e.g.*, SEQ ID NO: 238).

In some embodiments, the polynucleotide programmable nucleotide binding domain comprises a nickase domain. Herein the term “nickase” refers to a polynucleotide programmable nucleotide binding domain comprising a nuclease domain that is capable of cleaving only one strand of the two strands in a duplexed nucleic acid molecule (*e.g.*, DNA).

For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can include a D10A mutation and a histidine at position 840. In another example, a Cas9-derived nickase domain comprises an H840A mutation, while the amino acid residue at position 10 remains a D.

5 In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase, referred to as an “nCas9” protein (for “nickase” Cas9; SEQ ID NO: 201). The Cas9 nickase may be a Cas9 protein that is capable of cleaving only one strand of a duplexed nucleic acid molecule (*e.g.*, a duplexed DNA molecule). In some embodiments the Cas9 nickase comprises an amino acid sequence that is
10 at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the Cas9 nickases provided herein. Additional suitable Cas9 nickases will be apparent to those of skill in the art based on this disclosure and knowledge in the field and are within the scope of this disclosure.

15 Also provided herein are base editors comprising a polynucleotide programmable nucleotide binding domain which is catalytically dead (*i.e.*, incapable of cleaving a target polynucleotide sequence). For example, in the case of a base editor comprising a Cas9 domain, the Cas9 can comprise both a D10A mutation and an H840A mutation. In further embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain
20 comprises a point mutation (*e.g.*, D10A or H840A) as well as a deletion of all or a portion (*e.g.*, a functional portion) of a nuclease domain. dCas9 domains are known in the art and described, for example, in Qi *et al.*, “Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression.” *Cell*. 2013; 152(5):1173-83, the entire contents of which are incorporated herein by reference.

25 The term “protospacer adjacent motif (PAM)” or PAM-like motif refers to a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by a nucleic acid programmable DNA binding protein. In some embodiments, the PAM can be a 5' PAM (*i.e.*, located upstream of the 5' end of the protospacer). In other embodiments, the PAM can be a 3' PAM (*i.e.*, located downstream of the 5' end of the protospacer). The PAM sequence can
30 be any PAM sequence known in the art. Suitable PAM sequences include, but are not limited to, NGG, NGA, NGC, NGN, NGT, NGTT, NGCG, NGAG, NGAN, NGNG, NGCN, NGCG, NGTN, NNGRRT, NNNRRT, NNGRR(N), TTTV, TYCV, TYCV, TATV, NNNNGATT, NNAGAAW, or NAAAAC. Y is a pyrimidine; N is any nucleotide base; W is A or T.

A base editor provided herein can comprise a CRISPR protein-derived domain that is capable of binding a nucleotide sequence that contains a canonical or non-canonical protospacer adjacent motif (PAM) sequence.

5 In some embodiments, the PAM is an “NRN” PAM where the “N” in “NRN” is adenine (A), thymine (T), guanine (G), or cytosine (C), and the R is adenine (A) or guanine (G); or the PAM is an “NYN” PAM, wherein the “N” in NYN is adenine (A), thymine (T), guanine (G), or cytosine (C), and the Y is cytidine (C) or thymine (T), for example, as described in R.T. Walton *et al.*, 2020, *Science*, 10.1126/science.aba8853 (2020), the entire contents of which are incorporated herein by reference.

10 Several PAM variants are described in **Table 3** below.

Table 3. Cas9 proteins and corresponding PAM sequences. N is A, C, T, or G; and V is A, C, or G.

Variant	PAM
spCas9	NGG
spCas9-VRQR	NGA
spCas9-VRER	NGCG
xCas9 (sp)	NGN
saCas9	NNGRRT
saCas9-KKH	NNNRRT
spCas9-MQKSER	NGCG
spCas9-MQKSER	NGCN
spCas9-LRKIQK	NGTN
spCas9-LRVSQK	NGTN
spCas9-LRVSQL	NGTN
spCas9-MQKFRAER	NGC
Cpf1	5 ' (TTTV)
SpyMac	5 ' -NAA-3 '

15 In some embodiments, the PAM is NGC. In some embodiments, the NGC PAM is recognized by a Cas9 variant. In some embodiments, the NGC PAM Cas9 variant includes one or more amino acid substitutions selected from D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (collectively termed “MQKFRAER”) of spCas9

(SEQ ID No: 197), or a corresponding mutation in another Cas9. In some embodiments, the Cas9 variant contains one or more amino acid substitutions selected from D1135V, G1218R, R1335Q, and T1337R (collectively termed VRQR) of spCas9 (SEQ ID No: 197), or a corresponding mutation in another Cas9. In some embodiments, the Cas9 variant contains

5 one or more amino acid substitutions selected from D1135V, G1218R, R1335E, and T1337R (collectively termed VRER) of spCas9 (SEQ ID No: 197), or a corresponding mutation in another Cas9. In some embodiments, the Cas9 variant contains one or more amino acid substitutions selected from E782K, N968K, and R1015H (collectively termed KHH) of saCas9 (SEQ ID NO: 218). In some embodiments, the Cas9 variant includes one or more

10 amino acid substitutions selected from D1135M, S1136Q, G1218K, E1219S, R1335E, and T1337R (collectively termed "MQKSER") of spCas9 (SEQ ID No: 197), or a corresponding mutation in another Cas9. In some embodiments, the Cas9 variant includes one or more amino acid substitutions selected from D1135M, S1136Q, G1218K, E1219S, R1335E, and T1337R (collectively termed "MQKSER") of spCas9 (SEQ ID No: 197), or a corresponding

15 mutation in another Cas9.

In some embodiments, a CRISPR protein-derived domain of a base editor comprises all or a portion (e.g., a functional portion) of a Cas9 protein with a canonical PAM sequence (NGG). In other embodiments, a Cas9-derived domain of a base editor can employ a non-canonical PAM sequence. Such sequences have been described in the art and would be

20 apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition" *Nature Biotechnology* 33, 1293-1298 (2015); R.T. Walton *et al.*

25 "Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants" *Science* 10.1126/science.aba8853 (2020); Hu *et al.* "Evolved Cas9 variants with broad PAM compatibility and high DNA specificity," *Nature*, 2018 Apr. 5, 556(7699), 57-63; Miller *et al.*, "Continuous evolution of SpCas9 variants compatible with non-G PAMs" *Nat. Biotechnol.*, 2020 Apr;38(4):471-481; the entire contents of each are hereby incorporated by

30 reference.

Fusion Proteins or Complexes Comprising a NapDNAbp and a Cytidine Deaminase and/or Adenosine Deaminase

Some aspects of the disclosure provide fusion proteins or complexes comprising a Cas9 domain or other nucleic acid programmable DNA binding protein (*e.g.*, Cas12) and one or more cytidine deaminase, adenosine deaminase, or cytidine adenosine deaminase domains. It should be appreciated that the Cas9 domain may be any of the Cas9 domains or Cas9 proteins (*e.g.*, dCas9 or nCas9) provided herein. In some embodiments, any of the Cas9 domains or Cas9 proteins (*e.g.*, dCas9 or nCas9) provided herein may be fused with any of the cytidine deaminases and/or adenosine deaminases provided herein. The domains of the base editors disclosed herein can be arranged in any order.

In some embodiments, the fusion proteins or complexes comprising a cytidine deaminase or adenosine deaminase and a napDNAbp (*e.g.*, Cas9 or Cas12 domain) do not include a linker sequence. In some embodiments, a linker is present between the cytidine or adenosine deaminase and the napDNAbp. In some embodiments, cytidine or adenosine deaminase and the napDNAbp are fused via any of the linkers provided herein. For example, in some embodiments the cytidine or adenosine deaminase and the napDNAbp are fused via any of the linkers provided herein.

It should be appreciated that the fusion proteins or complexes of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein or complex may comprise inhibitors, cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins or complexes. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (*e.g.*, Softag 1, Softag 3), strep-tags, biotin ligase tags, FLAsH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein or complex comprises one or more His tags.

Exemplary, yet nonlimiting, fusion proteins are described in International PCT Application Nos. PCT/US2017/045381, PCT/US2019/044935, and PCT/US2020/016288, each of which is incorporated herein by reference for its entirety.

Fusion Proteins or Complexes with Internal Insertions

Provided herein are fusion proteins or complexes comprising a heterologous polypeptide fused to a nucleic acid programmable nucleic acid binding protein, for example, a napDNAbp. The heterologous polypeptide can be fused to the napDNAbp at a C-terminal
 5 end of the napDNAbp, an N-terminal end of the napDNAbp, or inserted at an internal location of the napDNAbp. In some embodiments, the heterologous polypeptide is a deaminase (*e.g.*, cytidine or adenosine deaminase) or a functional fragment thereof. For example, a fusion protein can comprise a deaminase flanked by an N-terminal fragment and a C-terminal fragment of a Cas9 or Cas12 (*e.g.*, Cas12b/C2c1), polypeptide.

10 The deaminase can be a circular permutant deaminase. In some embodiments, the deaminase is a circular permutant TadA, circularly permuted at amino acid residue 116, 136, or 65 as numbered in a TadA reference sequence.

The fusion protein or complexes can comprise more than one deaminase. The fusion protein or complex can comprise, for example, 1, 2, 3, 4, 5 or more deaminases. The
 15 deaminases in a fusion protein or complex can be adenosine deaminases, cytidine deaminases, or a combination thereof.

In some embodiments, the napDNAbp in the fusion protein or complex contains a Cas9 polypeptide or a fragment thereof. The Cas9 polypeptide can be a variant Cas9 polypeptide. The Cas9 polypeptide can be a circularly permuted Cas9 protein.

20 The heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp (*e.g.*, Cas9 or Cas12 (*e.g.*, Cas12b/C2c1)) at a suitable location, for example, such that the napDNAbp retains its ability to bind the target polynucleotide and a guide nucleic acid. A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase (dual deaminase)) can be inserted into a napDNAbp without
 25 compromising function of the deaminase (*e.g.*, base editing activity) or the napDNAbp (*e.g.*, ability to bind to target nucleic acid and guide nucleic acid).

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted in regions of the Cas9 polypeptide comprising higher than average B-factors (*e.g.*, higher B factors compared to the
 30 total protein or the protein domain comprising the disordered region). Cas9 polypeptide positions comprising a higher than average B-factor can include, for example, residues 768, 792, 1052, 1015, 1022, 1026, 1029, 1067, 1040, 1054, 1068, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence. Cas9 polypeptide regions comprising a

higher than average B-factor can include, for example, residues 792-872, 792-906, and 2-791 as numbered in the above Cas9 reference sequence.

In some embodiments, a heterologous polypeptide (*e.g.*, deaminase) is inserted in a flexible loop of a Cas9 polypeptide. The flexible loop portions can be selected from the group consisting of 530-537, 569-570, 686-691, 943-947, 1002-1025, 1052-1077, 1232-1247, or 1298-1300 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The flexible loop portions can be selected from the group consisting of: 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted into a Cas9 polypeptide region corresponding to amino acid residues: 1017-1069, 1242-1247, 1052-1056, 1060-1077, 1002 – 1003, 943-947, 530-537, 568-579, 686-691, 1242-1247, 1298 – 1300, 1066-1077, 1052-1056, or 1060-1077 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted in place of a deleted region of a Cas9 polypeptide. The deleted region can correspond to an N-terminal or C-terminal portion of the Cas9 polypeptide. Exemplary internal fusions base editors are provided in **Table 4A** below:

Table 4A: Insertion loci in Cas9 proteins

BE ID	Modification	Other ID
IBE001	Cas9 TadA ins 1015	ISLAY01
IBE002	Cas9 TadA ins 1022	ISLAY02
IBE003	Cas9 TadA ins 1029	ISLAY03
IBE004	Cas9 TadA ins 1040	ISLAY04
IBE005	Cas9 TadA ins 1068	ISLAY05
IBE006	Cas9 TadA ins 1247	ISLAY06
IBE007	Cas9 TadA ins 1054	ISLAY07
IBE008	Cas9 TadA ins 1026	ISLAY08
IBE009	Cas9 TadA ins 768	ISLAY09
IBE020	delta HNH TadA 792	ISLAY20
IBE021	N-term fusion single TadA helix truncated 165-end	ISLAY21
IBE029	TadA-Circular Permutant 116 ins1067	ISLAY29
IBE031	TadA- Circular Permutant 136 ins1248	ISLAY31
IBE032	TadA- Circular Permutant 136ins 1052	ISLAY32
IBE035	delta 792-872 TadA ins	ISLAY35
IBE036	delta 792-906 TadA ins	ISLAY36
IBE043	TadA-Circular Permutant 65 ins1246	ISLAY43

BE ID	Modification	Other ID
IBE044	TadA ins C-term truncate2 791	ISLAY44

A heterologous polypeptide (e.g., deaminase) can be inserted within a structural or functional domain of a Cas9 polypeptide. A heterologous polypeptide (e.g., deaminase) can be inserted between two structural or functional domains of a Cas9 polypeptide. A

5 heterologous polypeptide (e.g., deaminase) can be inserted in place of a structural or functional domain of a Cas9 polypeptide, for example, after deleting the domain from the Cas9 polypeptide. The structural or functional domains of a Cas9 polypeptide can include, for example, RuvC I, RuvC II, RuvC III, Rec1, Rec2, PI, or HNH.

A fusion protein can comprise a linker between the deaminase and the napDNAbp

10 polypeptide. The linker can be a peptide or a non-peptide linker. For example, the linker can be an XTEN, (GGGS)_n (SEQ ID NO: 246), SGGSSGGGS (SEQ ID NO: 330), (GGGGS)_n (SEQ ID NO: 247), (G)_n, (EAAAK)_n (SEQ ID NO: 248), (GGS)_n, SGSETPGTSESATPES (SEQ ID NO: 249). In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein

15 comprises a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the N-terminal and C-terminal fragments of napDNAbp are connected to the deaminase with a linker. In some embodiments, the N-terminal and C-terminal fragments are joined to the deaminase domain without a linker. In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase but does not

20 comprise a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein comprises a linker between the C-terminal Cas9 fragment and the deaminase but does not comprise a linker between the N-terminal Cas9 fragment and the deaminase.

In some embodiments, the napDNAbp in the fusion protein or complex is a Cas12

25 polypeptide, e.g., Cas12b/C2c1, or a functional fragment thereof capable of associating with a nucleic acid (e.g., a gRNA) that guides the Cas12 to a specific nucleic acid sequence. The Cas12 polypeptide can be a variant Cas12 polypeptide. In other embodiments, the N- or C-terminal fragments of the Cas12 polypeptide comprise a nucleic acid programmable DNA binding domain or a RuvC domain. In other embodiments, the fusion protein contains a linker

30 between the Cas12 polypeptide and the catalytic domain. In other embodiments, the amino acid sequence of the linker is GGSGGS (SEQ ID NO: 250) or GSSGSETPGTSESATPESG (SEQ ID NO: 251). In other embodiments, the linker is a rigid linker. In other embodiments

of the above aspects, the linker is encoded by GGAGGCTCTGGAGGAAGC (SEQ ID NO: 252) or GGCTCTTCTGGATCTGAAACACCTGGCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGC (SEQ ID NO: 253).

In other embodiments, the fusion protein or complex contains a nuclear localization signal (*e.g.*, a bipartite nuclear localization signal). In other embodiments, the amino acid sequence of the nuclear localization signal is MAPKKKRKVGIIHGVPA (SEQ ID NO: 261). In other embodiments of the above aspects, the nuclear localization signal is encoded by the following sequence:

ATGGCCCCAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCC (SEQ ID NO: 262). In other embodiments, the Cas12b polypeptide contains a mutation that silences the catalytic activity of a RuvC domain. In other embodiments, the Cas12b polypeptide contains D574A, D829A and/or D952A mutations.

In some embodiments, the fusion protein or complex comprises a napDNAbp domain (*e.g.*, Cas12-derived domain) with an internally fused nucleobase editing domain (*e.g.*, all or a portion (*e.g.*, a functional portion) of a deaminase domain, *e.g.*, an adenosine deaminase domain). In some embodiments, the napDNAbp is a Cas12b. In some embodiments, the base editor comprises a BhCas12b domain with an internally fused TadA*8 domain inserted at the loci provided in **Table 4B** below.

Table 4B: Insertion loci in Cas12b proteins

BhCas12b	Insertion site	Inserted between aa
position 1	153	PS
position 2	255	KE
position 3	306	DE
position 4	980	DG
position 5	1019	KL
position 6	534	FP
position 7	604	KG
position 8	344	HF
BvCas12b	Insertion site	Inserted between aa
position 1	147	PD
position 2	248	GG
position 3	299	PE
position 4	991	GE
position 5	1031	KM
AaCas12b	Insertion site	Inserted between aa
position 1	157	PG
position 2	258	VG
position 3	310	DP

position 4	1008	GE
position 5	1044	GK

In some embodiments, the base editing system described herein is an ABE with TadA inserted into a Cas9. Polypeptide sequences of relevant ABEs with TadA inserted into a Cas9 are provided in the attached Sequence Listing as SEQ ID NOs: 263-308.

- 5 Exemplary, yet nonlimiting, fusion proteins are described in International PCT Application Nos. PCT/US2020/016285 and U.S. Provisional Application Nos. 62/852,228 and 62/852,224, the contents of which are incorporated by reference herein in their entireties.

A to G Editing

- 10 In some embodiments, a base editor described herein comprises an adenosine deaminase domain. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. In some embodiments, an A-to-G base editor further comprises an inhibitor of inosine base excision repair, for example, a
- 15 uracil glycosylase inhibitor (UGI) domain or a catalytically inactive inosine specific nuclease. Without wishing to be bound by any particular theory, the UGI domain or catalytically inactive inosine specific nuclease can inhibit or prevent base excision repair of a deaminated adenosine residue (*e.g.*, inosine), which can improve the activity or efficiency of the base editor.
- 20 A base editor comprising an adenosine deaminase can act on any polynucleotide, including DNA, RNA and DNA-RNA hybrids. In an embodiment an adenosine deaminase domain of a base editor comprises all or a portion (*e.g.*, a functional portion) of an ADAT comprising one or more mutations which permit the ADAT to deaminate a target A in DNA. For example, the base editor can comprise all or a portion (*e.g.*, a functional portion) of an
- 25 ADAT from *Escherichia coli* (EcTadA) comprising one or more of the following mutations: D108N, A106V, D147Y, E155V, L84F, H123Y, I156F, or a corresponding mutation in another adenosine deaminase. Exemplary ADAT homolog polypeptide sequences are provided in the Sequence Listing as SEQ ID NOs: 1 and 309-315.

- 30 The adenosine deaminase can be derived from any suitable organism (*e.g.*, *E. coli*). In some embodiments, the adenosine deaminase is from *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shewanella putrefaciens*, *Haemophilus influenzae*, *Caulobacter crescentus*, or *Bacillus subtilis*. In some embodiments, the adenine deaminase is a naturally-

occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). The corresponding residue in any homologous protein can be identified by *e.g.*, sequence alignment and determination of homologous residues. The mutations in any naturally-occurring adenosine deaminase (*e.g.*,
5 having homology to ecTadA) that correspond to any of the mutations described herein (*e.g.*, any of the mutations identified in ecTadA) can be generated accordingly.

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least
10 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine
15 deaminase comprises an amino acid sequence that has 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, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein.

It should be appreciated that any of the mutations provided herein (*e.g.*, based on a
20 TadA reference sequence, such as TadA*7.10 (SEQ ID NO: 1)) can be introduced into other adenosine deaminases, such as *E. coli* TadA (ecTadA), *S. aureus* TadA (saTadA), or other adenosine deaminases (*e.g.*, bacterial adenosine deaminases). In some embodiments, the TadA reference sequence is TadA*7.10 (SEQ ID NO: 1). It would be apparent to the skilled artisan that additional deaminases may similarly be aligned to identify homologous amino
25 acid residues that can be mutated as provided herein. Thus, any of the mutations identified in a TadA reference sequence can be made in other adenosine deaminases (*e.g.*, ecTadA) that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein can be made individually or in any combination in a TadA reference sequence or another adenosine deaminase.

30 In some embodiments, the adenosine deaminase comprises an alteration or set of alterations selected from those listed in **Tables 5A-5E** below:

Table 5A. Adenosine Deaminase Variants. Residue positions in the *E. coli* TadA variant (TadA*) are indicated.

	23	26	36	37	48	49	51	72	84	87	106	108	123	125	142	146	147	152	155	156	157	161	
TadA*0.1	W	R	H	N	P			R	N	L	S	A	D	H	G	A	S	D	R	E	I	K	K
TadA*0.2	W	R	H	N	P			R	N	L	S	A	D	H	G	A	S	D	R	E	I	K	K
TadA*1.1	W	R	H	N	P			R	N	L	S	A	N	H	G	A	S	D	R	E	I	K	K
TadA*1.2	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	D	R	E	I	K	K
TadA*2.1	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.2	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.3	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.4	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.5	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.6	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.7	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.8	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.9	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.10	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.11	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*2.12	W	R	H	N	P			R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
TadA*3.1	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.2	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.3	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.4	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.5	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.6	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.7	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*3.8	W	R	H	N	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*4.1	W	R	H	N	P			R	N	L	S	V	N	H	G	N	S	Y	R	V	I	K	K
TadA*4.2	W	G	H	N	P			R	N	L	S	V	N	H	G	N	S	Y	R	V	I	K	K
TadA*4.3	W	R	H	N	P			R	N	F	S	V	N	Y	G	N	S	Y	R	V	F	K	K
TadA*5.1	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.2	W	R	H	S	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	T
TadA*5.3	W	R	L	N	P			L	N	I	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.4	W	R	H	S	P			R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	T
TadA*5.5	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.6	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.7	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.8	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.9	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.10	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.11	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.12	W	R	L	N	P			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*5.13	W	R	H	N	P			L	D	F	S	V	N	Y	A	A	S	Y	R	V	F	K	K
TadA*5.14	W	R	H	N	S			L	N	F	C	V	N	Y	G	A	S	Y	R	V	F	K	K
TadA*6.1	W	R	H	N	S			L	N	F	S	V	N	Y	G	N	S	Y	R	V	F	K	K
TadA*6.2	W	R	H	N	T	V		L	N	F	S	V	N	Y	G	N	S	Y	R	V	F	N	K
TadA*6.3	W	R	L	N	S			L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*6.4	W	R	L	N	S			L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K

	23	26	36	37	48	49	51	72	84	87	106	108	123	125	142	146	147	152	155	156	157	161
TadA*6.5	W	R	L	N	T	V	L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*6.6	W	R	L	N	T	V	L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
TadA*7.1	W	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*7.2	W	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
TadA*7.3	L	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*7.4	R	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
TadA*7.5	W	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	H	V	F	N	K
TadA*7.6	W	R	L	N	A		L	N	I	S	V	N	Y	G	A	C	Y	P	V	F	N	K
TadA*7.7	L	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	P	V	F	N	K
TadA*7.8	L	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
TadA*7.9	L	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	P	V	F	N	K
TadA*7.10	R	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	P	V	F	N	K

Table 5B. TadA*8 Adenosine Deaminase Variants. Residue positions in the *E. coli* TadA variant (TadA*) are indicated. Alterations are referenced to TadA*7.10 (first row).

	2 3	3 6	4 8	5 1	7 6	8 2	8 4	10 6	10 8	12 3	14 6	14 7	15 2	15 4	15 5	15 6	15 7	166
TadA*7.10	R	L	A	L	I	V	F	V	N	Y	C	Y	P	Q	V	F	N	T
TadA*8.1												T						
TadA*8.2												R						
TadA*8.3														S				
TadA*8.4										H								
TadA*8.5						S												
TadA*8.6																		R
TadA*8.7														R				
TadA*8.8										H		R		R				
TadA*8.9					Y							R		R				
TadA*8.10												R		R				R
TadA*8.11												T		R				
TadA*8.12												T		S				
TadA*8.13					Y					H		R		R				
TadA*8.14					Y	S												
TadA*8.15						S						R						
TadA*8.16						S				H		R						
TadA*8.17						S								R				
TadA*8.18						S				H				R				
TadA*8.19						S				H		R		R				
TadA*8.20					Y	S				H		R		R				
TadA*8.21												R		S				
TadA*8.22						S								S				
TadA*8.23						S				H								
TadA*8.24						S				H		T						

Table 5C. TadA*9 Adenosine Deaminase Variants. Alterations are referenced to TadA*7.10. Additional details of TadA*9 adenosine deaminases are described in International PCT Application No. PCT/US2020/049975, which is incorporated herein by reference in its entirety for all purposes.

TadA*9 Description	Alterations
TadA*9.1	E25F, V82S, Y123H, T133K, Y147R, Q154R
TadA*9.2	E25F, V82S, Y123H, Y147R, Q154R
TadA*9.3	V82S, Y123H, P124W, Y147R, Q154R
TadA*9.4	L51W, V82S, Y123H, C146R, Y147R, Q154R
TadA*9.5	P54C, V82S, Y123H, Y147R, Q154R
TadA*9.6	Y73S, V82S, Y123H, Y147R, Q154R
TadA*9.7	N38G, V82T, Y123H, Y147R, Q154R
TadA*9.8	R23H, V82S, Y123H, Y147R, Q154R
TadA*9.9	R21N, V82S, Y123H, Y147R, Q154R
TadA*9.10	V82S, Y123H, Y147R, Q154R, A158K
TadA*9.11	N72K, V82S, Y123H, D139L, Y147R, Q154R,
TadA*9.12	E25F, V82S, Y123H, D139M, Y147R, Q154R
TadA*9.13	M70V, V82S, M94V, Y123H, Y147R, Q154R
TadA*9.14	Q71M, V82S, Y123H, Y147R, Q154R
TadA*9.15	E25F, V82S, Y123H, T133K, Y147R, Q154R
TadA*9.16	E25F, V82S, Y123H, Y147R, Q154R
TadA*9.17	V82S, Y123H, P124W, Y147R, Q154R
TadA*9.18	L51W, V82S, Y123H, C146R, Y147R, Q154R
TadA*9.19	P54C, V82S, Y123H, Y147R, Q154R
TadA*9.2	Y73S, V82S, Y123H, Y147R, Q154R
TadA*9.21	N38G, V82T, Y123H, Y147R, Q154R
TadA*9.22	R23H, V82S, Y123H, Y147R, Q154R
TadA*9.23	R21N, V82S, Y123H, Y147R, Q154R
TadA*9.24	V82S, Y123H, Y147R, Q154R, A158K
TadA*9.25	N72K, V82S, Y123H, D139L, Y147R, Q154R,
TadA*9.26	E25F, V82S, Y123H, D139M, Y147R, Q154R
TadA*9.27	M70V, V82S, M94V, Y123H, Y147R, Q154R
TadA*9.28	Q71M, V82S, Y123H, Y147R, Q154R
TadA*9.29	E25F I76Y V82S Y123H Y147R Q154R
TadA*9.30	I76Y V82T Y123H Y147R Q154R
TadA*9.31	N38G I76Y V82S Y123H Y147R Q154R
TadA*9.32	N38G I76Y V82T Y123H Y147R Q154R
TadA*9.33	R23H I76Y V82S Y123H Y147R Q154R
TadA*9.34	P54C I76Y V82S Y123H Y147R Q154R
TadA*9.35	R21N I76Y V82S Y123H Y147R Q154R
TadA*9.36	I76Y V82S Y123H D138M Y147R Q154R
TadA*9.37	Y72S I76Y V82S Y123H Y147R Q154R
TadA*9.38	E25F I76Y V82S Y123H Y147R Q154R
TadA*9.39	I76Y V82T Y123H Y147R Q154R
TadA*9.40	N38G I76Y V82S Y123H Y147R Q154R
TadA*9.41	N38G I76Y V82T Y123H Y147R Q154R
TadA*9.42	R23H I76Y V82S Y123H Y147R Q154R
TadA*9.43	P54C I76Y V82S Y123H Y147R Q154R

TadA*9 Description	Alterations
TadA*9.44	R21N I76Y V82S Y123H Y147R Q154R
TadA*9.45	I76Y V82S Y123H D138M Y147R Q154R
TadA*9.46	Y72S I76Y V82S Y123H Y147R Q154R
TadA*9.47	N72K V82S, Y123H, Y147R, Q154R
TadA*9.48	Q71M V82S, Y123H, Y147R, Q154R
TadA*9.49	M70V, V82S, M94V, Y123H, Y147R, Q154R
TadA*9.50	V82S, Y123H, T133K, Y147R, Q154R
TadA*9.51a	V82S, Y123H, T133K, Y147R, Q154R, A158K
TadA*9.52a	M70V, Q71M, N72K, V82S, Y123H, Y147R, Q154R
TadA*9.53	N72K V82S, Y123H, Y147R, Q154R
TadA*9.54	Q71M V82S, Y123H, Y147R, Q154R
TadA*9.55	M70V, V82S, M94V, Y123H, Y147R, Q154R
TadA*9.56	V82S, Y123H, T133K, Y147R, Q154R
TadA*9.57	V82S, Y123H, T133K, Y147R, Q154R, A158K
TadA*9.58	M70V, Q71M, N72K, V82S, Y123H, Y147R, Q154R

In some embodiments, the adenosine deaminase comprises a TadA*8.20 adenosine deaminase variant further comprising an F149Y amino acid alteration. In some embodiments, the adenosine deaminase comprises a TadA*8.20 adenosine deaminase variant further comprising the amino acid alterations R147D, F149Y, T166I, and D167N (TadA*8.10+). In some embodiments, the adenosine deaminase comprises a TadA*8.20 adenosine deaminase variant further comprising the amino acid alterations S82T and F149Y (TadA*9v1). In some embodiments, the adenosine deaminase comprises a TadA*8.20 adenosine deaminase variant further comprising the amino acid alterations Y147D, F149Y, T166I, D167N and S82T (TadA*9v2).

In some embodiments, the adenosine deaminase comprises one or more of M1I, M1S, S2A, S2E, S2H, S2R, S2L, E3L, V4D, V4E, V4M, V4K, V4S, V4T, V4A, E5K, F6S, F6G, F6H, F6Y, F6I, F6E, S7K, H8E, H8Y, H8H, H8Q, H8E, H8G, H8S, E9Y, E9K, E9V, E9E, Y10F, Y10W, Y10Y, M12S, M12L, M12R, M12W, R13H, R13I, R13Y, R13R, R13G, R13S, H14N, A15D, A15V, A15L, A15H, T17T, T17A, T17W, T17L, T17F, T17R, T17S, L18A, L18E, L18N, L18L, L18S, A19N, A19H, A19K, A19A, A19D, A19G, A19M, R21N, K20K, K20A, K20R, K20E, K20G, K20C, K20Q R21A, R21R, R21N, R21Y, R21C G22P, A22W, A22R, W23D, R23H, W23G, W23Q, W23L, W23R, W23H W23D W23M, W23W, W23I, D24E, D24G, D24W, D24D, D24R, E25F, E25M, E25D, E25A, E25G, E25R, E25E, E25H E25V, E25S, E25Y, R26D, R26E, R26G, R26N, R26Q, R26C, R26L, R26K, R26W, R26C, R26P, R26R, R26A, R26H, E27E, E27Q, E27H, E27C, E27G, E27K, E27S, E27P, E27R, E27L, E27V, E27D, V28V, V28A, V28C, V28G, V28P, V28S, V28T, P29V, P29P,

P29A, P29G, P29K, P29L, V30V, V30I, V30L, V30F, V30G, V30A, V30M, L34S, L34V,
L34L, L34M, L34W, L34G, H36E, H36V, L36H, H36L, H36N, N37N, N37H, N37R, N37T,
N37S, N38G, N38R, N38N, N38E, V40I, W45A, W45W, W45R, W45L, W45N, N46N,
N46M, N46P, N46G, N46L, N46R, N46V, R46W, R46F, R46Q, R46M, R47A, R47Q, R47F,
5 R47K, R47P, R47W, R47M, R47R, R47G, R47S, R47V, R47H, P48T, P48L, P48A, P48I,
P48S, P48R, P48K, P48D, P48E, P48H, P48G, P48P, P48N, I49G, I49H, I49V, I49F, I49H,
I49I, I49M, I49N, I49K, I49Q, I49T, G50L, G50S, G50R, G50G, R51H, R51L, R51N,
L51W, R51Y, R51G, R51V, R51R, H52D, H52Y, H52I, H52H, D53D, D53E, D53G, D53P,
P54C, P54T, P54P, P54E, A55H, T55A, T55I, T55V, T55G, T55T, A56A, A56H, A56W,
10 A56E, A56S, H57P, H57A, H57H, H57N, A58G, A58E, A58A, A58R, E59A, E59G, E59I,
E59Q, E59W, E59E, E59T, E59H, E59P, M61A, M61I, M61L, M61V, M61P, M61G, M61I,
L63S, L63V, L63T, L63R, L63H, L63A, R64A, R64Q, R64R, R64D, Q65V, Q65H, Q65G,
Q65P, Q65F, Q65Q, Q65R, G66V, G66E, G66T, G66G, G66C, G67G, G67W, G67I, G67A,
G67D, G67L, G67V, L68Q, L68M, L68V, L68H, L68L, L68G, V69A, V69M, V69V,
15 M70V, M70L, E70A, M70A, M70M, M70E, M70T, M70v, Q71M, Q71N, Q71L, Q71R,
Q71Q, Q71I, N72A, N72K, N72S, N72D, N72Y, N72N, N72H, N72G, N72M, Y73G, Y73I,
Y73K, Y73R, Y73S, Y73Y, Y73H, Y73A, R74A, R74Q, R74G, R74K, R74L, R74N, R74G,
R74K, R74R, I76H, I76R, I76W, I76Y, I76V, I76Q, I76L, I76D, I76F, I76I, I76N, I76T,
I76Y, D77G, D77D, D77A, D77Q, A78Y, A78T, A78G, A78A, A78I, T79M, T79R, T79L,
20 T79T, L80M, L80Y, L80I, L80V, L80L, Y81D, Y81V, Y81Y, Y81M, V82A, V82S, V82G,
V82T, V82V, V82Q, V82Y, T83L, T83F, T83T, T83N, L84E, L84F, L84Y, L84I, L84L,
L84M, L84A, L84T, L84S, E85K, E85G, E85P, E85S, E85E, E85F, E85V, E85R, P86T,
P86C, P86P, P86L, P86N, P86K, P86H, C87M, C87I, C87S, C87N, C87P, S87C, S87L,
S87V, V88A, V88M, V88V, V88T, V88E, V88D, V88S, C90S, C90P, C90A, C90T,
25 C90M, A91A, A91G, A91S, A91V, A91T, A91C, A91L, G92T, G92M, G92A, G92Y,
G92G, A93I, A93C, A93M, A93V, A93A, M94M, M94T, M94A, M94V, M94L, M94I,
M94H, I95S, I95G, I95L, I95H, I95V, H96A, H96L, H96R, H96S, H96H, H96N, H96E,
S97C, S97G, S97I, S97M, S97R, S97S, S97P, R98K, R98I, R98N, R98Q, R98G, R98H,
R98C, R98L, R98R, G100R, G100V, G100K, G100A, G100S, G100M, G100I, R101V,
30 R101R, R101S, R101C, V102A, V102F, V102I, V102V, D103A, V103A, V103G, V103F,
V103V, F104G, D104N, F104V, F104I, F104L, F104A, F104F, F104R, G105V, G105W,
G105G, G105M, G105A, A106T, V106Q, V106F, V106W, V106M, A106A, A106Q,
A106F, A106G, A106W, A106M, A106V, A106R, A106L, A106S, A106B, A106I, R107C,
R107G, R107P, R107K, R107A, R107N, R107W, R107H, R107S, R107R, R107F, D108N,

D108F, D108G, D108V, D108A, D108Y, D108H, D108I, D108K, D108L, D108M, D108Q,
N108Q, N108F, N108W, N108M, N108K, D108K, D108F, D108M, D108Q, D108R,
D108W, D108S, D108E, D108T, D108R, D108D, A109H, A109K, A109R, A109S, A109T,
A109V, A109A, A109D, K110G, K110H, K110I, K110R, K110T, K110K, K110A, K110I,
5 T111A, T111G, T111H, T111R, T111T, T111K, G112A, G112G, G112H, G112T, G112R,
A113N, A114G, A114H, A114V, A114C, A114S, A114A, G115S, G115G, G115M, G115L,
G115A, G115F, L117M, L117L, L117W, L117A, L117S, L117N, L117V, M118D, M118G,
M118K, M118N, M118V, M118M, M118L, M118R, D119L, D119N, D119S, D119V,
D119D, V120H, V120L, V120V, V120T, V120A, V120E, V120G, V120D, L121D, L121M,
10 L121N, L121K, L121L, H122H, H122N, H122P, H122R, H122S, H122Y, H122G, H122T,
H122L, H123C, H123G, H123P, H123V, H123Y, Y123H, H123Y, H123H, P124P, P124H,
P124A, P124Y, P124D, P124G, P124I, P124L, P124W, G125H, G125I, G125A, G125M,
G125K, G125G, G125P, M126D, M126H, M126K, M126I, M126N, M126O, M126S,
M126Y, M126M, M126G, N127H, N127S, N127D, N127K, N127R, N127N, N127I, N127P,
15 N127M, H128R, H128N, H128L, H128H, R129H, R129Q, R129V, R129I, R129E, R129V,
R129R, R129M, R129P, V130R, V130V, V130E, V130D, E131E, E131I, E131V, E131K,
I132I, I132F, I132T, I132L, I132V, I132E, T133V, T133E, T133G, T133K, T133T, T133A,
T133H, T133F, T133I, E134A, E134E, E134G, E134I, E134H, E134K, E134T, G135G,
G135V, G135I, G135P, G135E, I136G, I136L, I136T, I136I, I137A, I137D, I137E, L137M,
20 I137S, L137L, L137I, A138D, A138E, A138G, S138A, A138N, A138S, A138T, A138V,
A138Y, A138A, A138M, A138L, D139E, D139I, D139C, D139L, D139M, D139D, D139G,
D139H, D139A, E140A, E140C, E140L, E140R, E140K, E140E, E140D, C141S, C141A,
C141C, C141V, C141E, A142N, A142D, A142G, A142A, A142L, A142S, A142T, A142N,
A142S, A142V, A142E, A142C, A143D, A143E, A143G, , A143D, A143G, A143E, A143L,
25 A143W, A143M, A143S, A143Q, A143R, A143A, A143I, L144S, L144L, L144T, L144A,
L145A, L145F, L145G, L145D, L145L, L145C, L145E, L145s, C146R, S146A, S146C,
S146D, S146F, S146R, S146T, S146D, S146G, S146S, S146L, D147D, D147L, D147F,
D147G, D147Y, Y147T, Y147R, Y147D, D147R, D147Y, D147A, D147T, D147H, D147F,
D147U, D147V, D147I, D147C, F148L, F148F, F148R, F148Y, F148A, F148T, F149C,
30 F149M, F149R, F149Y, F149N, F149F, F149A, F149T, F149V R150R, R150M, R150D,
R150F, M151F, M151P, M151R, M151V, M151M, M151E, R152C, R152F, R152H, R152P,
R152R, R152P, R152Q, R152M, R152O, R153C, R153Q, R153R, R153V, R153E, R153A,
R153P, Q154E, Q154H, Q154M, Q154R, Q154L, Q154S, Q154V, Q154Q, Q154F, Q154I,
Q154A, Q154K, E155F, E155G, E155I, E155K, E155P, E155V, E155D, E155E, E155L,

E155Q, I156V, I156A, I156I, I156L, I156F, I156D, I156K, I156N, I156R, I156Y, E157A, E157F, E157I, E157P, E157T, E157V, N157K, K157N, K157V, K157P, K157I, K157F, K157F, K157T, K157A, K157S, K157R, A158Q, A158K, A158V, A158A, A158D, A158S, A158T, A158N, Q159S, Q159Q, Q159A, Q159F, Q159K, Q159L, Q159N, K160A, K160S, 5 K160E, K160K, K160N, K160F, K160Q, K161T, K161K, K161R, K161I, K161A, K161N, K161Q, K161S, K161T, A162D, A162Q, R162H, R162P, A162S, A162A, A162N, A162M, A162K, Q163G, Q163S, Q163Q, Q163A, Q163H, Q163N, Q163R, S164F, S164S, S164Q, S164I, S164R, S164Y, S165S, S165P, S165Q, S165A, S165D, S165I, S165T, S165Y, T166T, T166Q, T166E, T166S, T166D, T166K, T166I, T166N, T166P, T166R, D167S 10 D167D, D167I, D167G, D167T, D167A and/or D167N mutation in a TadA reference sequence (e.g., TadA*7.10, ecTadA, or TadA8e), and any alternative mutation at the corresponding position, or one or more corresponding mutations in another adenosine deaminase. Additional mutations are described in U.S. Patent Application Publication No. 2022/0307003 A1 U.S. Patent No. 11,155,803, and International Patent Application 15 Publications No. WO 2023/288304 A2, PCT/CN2022/143408, WO 2018/027078 A1, WO 2021/158921 A1 and WO 2023/034959 A2, the disclosures of which are incorporated herein by reference in their entirety for all purposes.

In some embodiments, the disclosure provides TadA variants comprising a V82T, Y147T, and/or a Q154S mutation. In some embodiments, the disclosure provides TadA 20 variants comprising a V82T, Y147T, and/or a Q154S mutation. In some embodiments, the disclosure provides TadA*8.8 further comprising a V82T mutation. In some embodiments, the disclosure provides TadA*8.8 further comprising a V82T, a Y147T, and a Q154S mutation. In some embodiments, the disclosure provides TadA*8.17 further comprising a V82T mutation. In some embodiments, the disclosure provides TadA*8.17 further 25 comprising a V82T, a Y147T, and a Q154S mutation. In some embodiments, the disclosure provides TadA*8.20 further comprising a V82T mutation. In some embodiments, the disclosure provides TadA*8.20 further comprising a V82T, a Y147T, and a Q154S mutation.

In embodiments, a variant of TadA*7.10 comprises one or more alterations selected from any of those alterations provided herein.

30 In particular embodiments, an adenosine deaminase heterodimer comprises a TadA*8 domain and an adenosine deaminase domain selected from *Staphylococcus aureus* (*S. aureus*) TadA, *Bacillus subtilis* (*B. subtilis*) TadA, *Salmonella typhimurium* (*S. typhimurium*) TadA, *Shewanella putrefaciens* (*S. putrefaciens*) TadA, *Haemophilus influenzae* F3031 (*H.*

influenzae) TadA, *Caulobacter crescentus* (*C. crescentus*) TadA, *Geobacter sulfurreducens* (*G. sulfurreducens*) TadA, or TadA*7.10.

In some embodiments, the TadA*8 is a variant as shown in **Table 5D**. **Table 5D** shows certain amino acid position numbers in the TadA amino acid sequence and the amino acids present in those positions in the TadA-7.10 adenosine deaminase. **Table 5D** also shows amino acid changes in TadA variants relative to TadA-7.10 following phage-assisted non-continuous evolution (PANCE) and phage-assisted continuous evolution (PACE), as described in M. Richter *et al.*, 2020, Nature Biotechnology, doi.org/10.1038/s41587-020-0453-z, the entire contents of which are incorporated by reference herein. In some embodiments, the TadA*8 is TadA*8a, TadA*8b, TadA*8c, TadA*8d, or TadA*8e. In some embodiments, the TadA*8 is TadA*8e. In one embodiment, an adenosine deaminase is a TadA*8 that comprises or consists essentially of SEQ ID NO: 316 or a fragment thereof having adenosine deaminase activity.

Table 5D. Select TadA*8 Variants

	TadA	TadA amino acid number									
		26	88	109	111	119	122	147	149	166	167
	TadA-7.10	R	V	A	T	D	H	Y	F	T	D
PANCE 1					R						
PANCE 2				S/T	R						
PACE	TadA-8a	C		S	R	N	N	D	Y	I	N
	TadA-8b		A	S	R	N	N		Y	I	N
	TadA-8c	C		S	R	N	N		Y	I	N
	TadA-8d		A		R	N			Y		
	TadA-8e			S	R	N	N	D	Y	I	N

In some embodiments, the TadA variant is a variant as shown in **Table 5E**. **Table 5E** shows certain amino acid position numbers in the TadA amino acid sequence and the amino acids present in those positions in the TadA*7.10 adenosine deaminase. In some embodiments, the TadA variant is MSP605, MSP680, MSP823, MSP824, MSP825, MSP827, MSP828, or MSP829. In some embodiments, the TadA variant is MSP828. In some embodiments, the TadA variant is MSP829.

Table 5E. TadA Variants

Variant	TadA Amino Acid Number								
	36	76	82	147	149	154	157	167	

TadA-7.10	L	I	V	Y	F	Q	N	D
MSP605			G	T		S		
MSP680		Y	G	T		S		
MSP823	H		G	T		S	K	
MSP824			G	D	Y	S		N
MSP825	H		G	D	Y	S	K	N
MSP827	H	Y	G	T		S	K	
MSP828		Y	G	D	Y	S		N
MSP829	H	Y	G	D	Y	S	K	N

In particular embodiments, the fusion proteins or complexes comprise a single (*e.g.*, provided as a monomer) TadA* (*e.g.*, TadA*8 or TadA*9). Throughout the present disclosure, an adenosine deaminase base editor that comprises a single TadA* domain is

5 indicates using the terminology ABEm or ABE#m, where “#” is an identifying number (*e.g.*, ABE8.20m), where “m” indicates “monomer.” In some embodiments, the TadA* is linked to a Cas9 nickase. In some embodiments, the fusion proteins or complexes of the disclosure comprise as a heterodimer of a wild-type TadA (TadA(wt)) linked to a TadA*. Throughout the present disclosure, an adenosine deaminase base editor that comprises a single TadA*

10 domain and a TadA(wt) domain is indicates using the terminology ABEd or ABE#d, where “#” is an identifying number (*e.g.*, ABE8.20d), where “d” indicates “dimer.” In other embodiments, the fusion proteins or complexes of the disclosure comprise as a heterodimer of a TadA*7.10 linked to a TadA*. In some embodiments, the base editor is ABE8 comprising a TadA* variant monomer. In some embodiments, the base editor is ABE

15 comprising a heterodimer of a TadA* and a TadA(wt). In some embodiments, the base editor is ABE comprising a heterodimer of a TadA* and TadA*7.10. In some embodiments, the base editor is ABE comprising a heterodimer of a TadA*. In some embodiments, the TadA* is selected from **Tables 5A-5E**.

In some embodiments, the adenosine deaminase is expressed as a monomer. In other

20 embodiments, the adenosine deaminase is expressed as a heterodimer. In some embodiments, the deaminase or other polypeptide sequence lacks a methionine, for example when included as a component of a fusion protein. This can alter the numbering of positions. However, the skilled person will understand that such corresponding mutations refer to the same mutation.

Any of the mutations provided herein and any additional mutations (*e.g.*, based on the

25 ecTadA amino acid sequence) can be introduced into any other adenosine deaminases. Any of the mutations provided herein can be made individually or in any combination in a TadA reference sequence or another adenosine deaminase (*e.g.*, ecTadA).

Details of A to G nucleobase editing proteins are described in International PCT Application No. PCT/US2017/045381 (WO2018/027078) and Gaudelli, N.M., *et al.*, “Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage” Nature, 551, 464-471 (2017), the entire contents of which are hereby incorporated by
5 reference.

C to T Editing

In some embodiments, a base editor disclosed herein comprises a fusion protein or complex comprising cytidine deaminase capable of deaminating a target cytidine (C) base of
10 a polynucleotide to produce uridine (U), which has the base pairing properties of thymine. In some embodiments, for example where the polynucleotide is double-stranded (*e.g.*, DNA), the uridine base can then be substituted with a thymidine base (*e.g.*, by cellular repair machinery) to give rise to a C:G to a T:A transition. In other embodiments, deamination of a C to U in a nucleic acid by a base editor cannot be accompanied by substitution of the U to a
15 T.

The deamination of a target C in a polynucleotide to give rise to a U is a non-limiting example of a type of base editing that can be executed by a base editor described herein. In another example, a base editor comprising a cytidine deaminase domain can mediate conversion of a cytosine (C) base to a guanine (G) base. For example, a U of a polynucleotide
20 produced by deamination of a cytidine by a cytidine deaminase domain of a base editor can be excised from the polynucleotide by a base excision repair mechanism (*e.g.*, by a uracil DNA glycosylase (UDG) domain), producing an abasic site. The nucleobase opposite the abasic site can then be substituted (*e.g.*, by base repair machinery) with another base, such as a C, by for example a translesion polymerase. Although it is typical for a nucleobase opposite
25 an abasic site to be replaced with a C, other substitutions (*e.g.*, A, G or T) can also occur.

Accordingly, in some embodiments a base editor described herein comprises a deamination domain (*e.g.*, cytidine deaminase domain) capable of deaminating a target C to a U in a polynucleotide. Further, as described below, the base editor can comprise additional domains which facilitate conversion of the U resulting from deamination to, in some
30 embodiments, a T or a G. For example, a base editor comprising a cytidine deaminase domain can further comprise a uracil glycosylase inhibitor (UGI) domain to mediate substitution of a U by a T, completing a C-to-T base editing event. In another example, the base editor can comprise a uracil stabilizing protein as described herein. In another example, a base editor can incorporate a translesion polymerase to improve the efficiency of C-to-G

base editing, since a translesion polymerase can facilitate incorporation of a C opposite an abasic site (*i.e.*, resulting in incorporation of a G at the abasic site, completing the C-to-G base editing event).

5 A base editor comprising a cytidine deaminase as a domain can deaminate a target C in any polynucleotide, including DNA, RNA and DNA-RNA hybrids.

In some embodiments, a cytidine deaminase of a base editor comprises all or a portion (e.g., a functional portion) of an apolipoprotein B mRNA editing complex (APOBEC) family deaminase. APOBEC is a family of evolutionarily conserved cytidine deaminases. Members of this family are C-to-U editing enzymes. The N-terminal domain of APOBEC like proteins 10 is the catalytic domain, while the C-terminal domain is a pseudocatalytic domain. More specifically, the catalytic domain is a zinc dependent cytidine deaminase domain and is important for cytidine deamination. APOBEC family members include APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D (“APOBEC3E” now refers to this), APOBEC3F, APOBEC3G, APOBEC3H, APOBEC4, and Activation-induced 15 (cytidine) deaminase.

Other exemplary deaminases that can be fused to Cas9 according to aspects of this disclosure are provided below. In embodiments, the deaminases are activation-induced deaminases (AID). It should be understood that, in some embodiments, the active domain of the respective sequence can be used, *e.g.*, the domain without a localizing signal (nuclear 20 localization sequence, without nuclear export signal, cytoplasmic localizing signal).

Some aspects of the present disclosure are based on the recognition that modulating the deaminase domain catalytic activity of any of the fusion proteins or complexes described herein, for example by making point mutations in the deaminase domain, affect the processivity of the fusion proteins (*e.g.*, base editors) or complexes. For example, mutations 25 that reduce, but do not eliminate, the catalytic activity of a deaminase domain within a base editing fusion protein or complexes can make it less likely that the deaminase domain will catalyze the deamination of a residue adjacent to a target residue, thereby narrowing the deamination window. The ability to narrow the deamination window can prevent unwanted deamination of residues adjacent to specific target residues, which can reduce or prevent off- 30 target effects.

In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise one or more mutations selected from the group consisting of H121R, H122R, R126A, R126E, R118A, W90A, W90Y, and R132E of rAPOBEC1; D316R, D317R, R320A, R320E, R313A, W285A, W285Y, and R326E of hAPOBEC3G; and any alternative mutation

at the corresponding position, or one or more corresponding mutations in another APOBEC deaminase.

A number of modified cytidine deaminases are commercially available, including, but not limited to, SaBE3, SaKKH-BE3, VQR-BE3, EQR-BE3, VRER-BE3, YE1-BE3, EE-BE3, 5 YE2-BE3, and YEE-BE3, which are available from Addgene (plasmids 85169, 85170, 85171, 85172, 85173, 85174, 85175, 85176, 85177). In some embodiments, a deaminase incorporated into a base editor comprises all or a portion (e.g., a functional portion) of an APOBEC1 deaminase.

In some embodiments, the fusion proteins or complexes of the disclosure comprise 10 one or more cytidine deaminase domains. In some embodiments, the cytidine deaminases provided herein are capable of deaminating cytosine or 5-methylcytosine to uracil or thymine. In some embodiments, the cytidine deaminases provided herein are capable of deaminating cytosine in DNA. The cytidine deaminase may be derived from any suitable organism. In some embodiments, the cytidine deaminase is a naturally-occurring cytidine 15 deaminase that includes one or more mutations corresponding to any of the mutations provided herein. One of skill in the art will be able to identify the corresponding residue in any homologous protein, e.g., by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring cytidine deaminase that corresponds to any of the mutations described 20 herein. In some embodiments, the cytidine deaminase is from a prokaryote. In some embodiments, the cytidine deaminase is from a bacterium. In some embodiments, the cytidine deaminase is from a mammal (e.g., human).

In some embodiments, the cytidine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 25 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the cytidine deaminase amino acid sequences set forth herein. It should be appreciated that cytidine deaminases provided herein may include one or more mutations (e.g., any of the mutations provided herein). Some embodiments provide a polynucleotide molecule encoding the cytidine deaminase nucleobase editor polypeptide of 30 any previous aspect or as delineated herein. In some embodiments, the polynucleotide is codon optimized.

In embodiments, a fusion protein of the disclosure comprises two or more nucleic acid editing domains.

Details of C to T nucleobase editing proteins are described in International PCT Application No. PCT/US2016/058344 (WO2017/070632) and Komor, A.C., *et al.*, “Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage” Nature 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference.

Cytidine Adenosine Base Editors (CABEs)

In some embodiments, a base editor described herein comprises an adenosine deaminase variant that has increased cytidine deaminase activity. Such base editors may be referred to as “cytidine adenosine base editors (CABEs)” or “cytosine base editors derived from TadA* (CBE-Ts),” and their corresponding deaminase domains may be referred to as “TadA* acting on DNA cytosine (T_{AD}C)” domains. In some instances, an adenosine deaminase variant has both adenine and cytosine deaminase activity (i.e., is a dual deaminase). In some embodiments, the adenosine deaminase variants deaminate adenine and cytosine in DNA. In some embodiments, the adenosine deaminase variants deaminate adenine and cytosine in single-stranded DNA. In some embodiments, the adenosine deaminase variants deaminate adenine and cytosine in RNA. In some embodiments, the adenosine deaminase variant predominantly deaminates cytosine in DNA and/or RNA (e.g., greater than 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of all deaminations catalyzed by the adenosine deaminase variant, or the number of cytosine deaminations catalyzed by the variant is about or at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 25-fold, 50-fold, 75-fold, 100-fold, 500-fold, or 1,000-fold greater than the number adenine deaminations catalyzed by the variant). In some embodiments, the adenosine deaminase variant has approximately equal cytosine and adenosine deaminase activity (e.g., the two activities are within about 10% or 20% of each other). In some embodiments, the adenosine deaminase variant has predominantly cytosine deaminase activity, and little, if any, adenosine deaminase activity. In some embodiments, the adenosine deaminase variant has cytosine deaminase activity, and no significant or no detectable adenosine deaminase activity. In some embodiments, the target polynucleotide is present in a cell *in vitro* or *in vivo*. In some embodiments, the cell is a bacteria, yeast, fungi, insect, plant, or mammalian cell.

In some embodiments, the CABE comprises a bacterial TadA deaminase variant (e.g., ecTadA). In some embodiments, the CABE comprises a truncated TadA deaminase variant.

In some embodiments, the CAGE comprises a fragment of a TadA deaminase variant. In some embodiments, the CAGE comprises a TadA*8.20 variant.

In some embodiments, an adenosine deaminase variant of the disclosure is a TadA adenosine deaminase comprising one or more alterations that increase cytosine deaminase activity (*e.g.*, at least about 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold or more increase) while maintaining adenosine deaminase activity (*e.g.*, at least about 30%, 40%, 50% or more of the activity of a reference adenosine deaminase (*e.g.*, TadA*8.20 or TadA*8.19)). In some instances, the adenosine deaminase variant comprises one or more alterations that increase cytosine deaminase activity (*e.g.*, at least about 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold or more increase) relative to the activity of a reference adenosine deaminase and comprise undetectable adenosine deaminase activity or adenosine deaminase activity that is less than 30%, 20%, 10%, or 5% of that of a reference adenosine deaminase. In some embodiments, the reference adenosine deaminase is TadA*8.20 or TadA*8.19.

In some embodiments, the adenosine deaminase variant is an adenosine deaminase comprising two or more alterations at an amino acid position selected from the group consisting of 2, 4, 6, 8, 13, 17, 23, 27, 29, 30, 47, 48, 49, 67, 76, 77, 82, 84, 96, 100, 107, 112, 114, 115, 118, 119, 122, 127, 142, 143, 147, 149, 158, 159, 162, 165, 166, and 167, of an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or greater identity to SEQ ID NO: 1, or a corresponding alteration in another deaminase. I

In some embodiments, the adenosine deaminase variant is an adenosine deaminase comprising one or more alterations selected from the group consisting of S2H, V4K, V4S, V4T, V4Y, F6G, F6H, F6Y, H8Q, R13G, T17A, T17W, R23Q, E27C, E27G, E27H, E27K, E27Q, E27S, E27G, P29A, P29G, P29K, V30F, V30I, R47G, R47S, A48G, I49K, I49M, I49N, I49Q, I49T, G67W, I76H, I76R, I76W, Y76H, Y76R, Y76W, F84A, F84M, H96N, G100A, G100K, T111H, G112H, A114C, G115M, M118L, H122G, H122R, H122T, N127I, N127K, N127P, A142E, R147H, A158V, Q159S, A162C, A162N, A162Q, and S165P of an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or greater identity to SEQ ID NO: 1, or a corresponding alteration in another deaminase.

In some embodiments, the adenosine deaminase variant is an adenosine deaminase comprising an amino acid alteration or combination of amino acid alterations selected from those listed in any of **Tables 6A-6F**.

The residue identity of exemplary adenosine deaminase variants that are capable of deaminating adenine and/or cytidine in a target polynucleotide (*e.g.*, DNA) is provided in

Tables 6A-6F below. Further examples of adenosine deaminase variants include the following variants of 1.17 (see Table 6A): 1.17+E27H; 1.17+E27K; 1.17+E27S; 1.17+E27S+I49K; 1.17+E27G; 1.17+I49N; 1.17+E27G+I49N; and 1.17+E27Q. In some embodiments, any of the amino acid alterations provided herein are substituted with a conservative amino acid. Additional mutations known in the art can be further added to any of the adenosine deaminase variants provided herein.

In some embodiments, the base editor systems comprising a CABE provided herein have at least about a 30%, 40%, 50%, 60%, 70% or more C to T editing activity in a target polynucleotide (*e.g.*, DNA). In some embodiments, a base editor system comprising a CABE as provided herein has an increased C to T base editing activity (*e.g.*, increased at least about 30-fold, 40-fold, 50-fold, 60-fold, 70-fold or more) relative to a reference base editor system comprising a reference adenosine deaminase (*e.g.*, TadA*8.20 or TadA*8.19).

Table 6A. Adenosine Deaminase Variants. Mutations are indicated with reference to TadA*8.20. “S” indicates “Surface,” and “NAS” indicates “Near Active Site.”

location in structure	N/A	S _{h1}	S _{h1}	S _{h1}	NAS	NAS	NAS	NAS		S	
Amino Acid No. (*START Met is AA#1)	2	8	13	17	27	47	48	49	67	76	77
TadA*8.20	S	H	R	T	E	R	A	I	G	Y	D
TadA*8.19										I	
1.1					H					I	
1.2					H			K		I	
1.3					S			K		I	
1.4					S			K		I	
1.5					K						
1.6					K						
1.7					H					I	
1.8					S			K	W		
1.9								T	W		
1.10					C					I	
1.11			G		Q						
1.12				A	H			M		I	
1.13								Q		I	
1.14	H							K		I	
1.15						S					
1.16		Q						Q		I	
1.17				A			G				
1.18					G						
1.19					G			N			
1.20					G						G

Table 6A (continued). Adenosine Deaminase Variants. Mutations are indicated with reference to TadA*8.20. “I” indicates “Internal,” “S” indicates “Surface,” and “NAS” indicates “Near Active Site.”

location in structure	I	NAS		NAS	S		S	S	S		S	
Amino Acid No. (*START Met is AA#1)	82	84	96	107	112	115	118	119	127	142	162	165
TadA*8.20	S	F	H	R	G	G	M	D	N	A	A	S
TadA*8.19												
1.1		M										
1.2												
1.3												
1.4											N	
1.5												
1.6								N				
1.7												
1.8												
1.9			N									
1.10								N				
1.11									K			
1.12							L					
1.13						M						
1.14					H							
1.15				C								
1.16												
1.17	T									E		
1.18												
1.19												
1.20												P

5

Table 6B. Adenosine deaminase variants. Mutations are indicated with reference to TadA*8.20.

Position No.	27	29	30	49	82	84	107	112	115	142
TadA*8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.1	S			K	T					
S1.2	S			K	T		C			
S1.3	S			K	T			H		
S1.4	S			K	T				M	
S1.5	S			K	T					E
S1.6	S			K	T		C	H		
S1.7	S			K	T		C		M	

Position No.	27	29	30	49	82	84	107	112	115	142
TadA *8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.8	S			K	T		C			E
S1.9	S			K	T			H		E
S1.10	S			K	T				M	E
S1.11	S			K	T		C	H	M	E
S1.12	S		I	K	T					
S1.13	S		I	K	T		C			
S1.14	S		I	K	T			H		
S1.15	S		I	K	T				M	
S1.16	S		I	K	T					E
S1.17	S		I	K	T		C	H		
S1.18	S		I	K	T		C		M	
S1.19	S		I	K	T		C			E
S1.20	S		I	K	T			H		E
S1.21	S		I	K	T				M	E
S1.22	S		I	K	T		C	H	M	E
S1.23	S		L	K	T					
S1.24	S		L	K	T		C			
S1.25	S		L	K	T			H		
S1.26	S		L	K	T				M	
S1.27	S		L	K	T					E
S1.28	S		L	K	T		C	H		
S1.29	S		L	K	T		C		M	
S1.30	S		L	K	T		C			E
S1.31	S		L	K	T			H		E
S1.32	S		L	K	T				M	E
S1.33	S		L	K	T		C	H	M	E
S1.34	S		F	K	T	A				
S1.35	S		F	K	T	A	C			
S1.36	S		F	K	T	A		H		
S1.37	S		F	K	T	A			M	
S1.38	S		F	K	T	A				E
S1.39	S		F	K	T	A	C	H		
S1.40	S		F	K	T	A	C		M	
S1.41	S		F	K	T	A	C			E
S1.42	S		F	K	T	A		H		E
S1.43	S		F	K	T	A			M	E
S1.44	S		F	K	T	A	C	H	M	E
S1.45	S			K	T	L				
S1.46	S			K	T	L	C			
S1.47	S			K	T	L		H		
S1.48	S			K	T	L			M	
S1.49	S			K	T	L				E

Position No.	27	29	30	49	82	84	107	112	115	142
TadA *8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.50	S			K	T	L	C	H		
S1.51	S			K	T	L	C		M	
S1.52	S			K	T	L	C			E
S1.53	S			K	T	L		H		E
S1.54	S			K	T	L			M	E
S1.55	S			K	T	L	C	H	M	E
S1.56	S		I	K	T	L				
S1.57	S		I	K	T	L	C			
S1.58	S		I	K	T	L		H		
S1.59	S		I	K	T	L			M	
S1.60	S		I	K	T	L				E
S1.61	S		I	K	T	L	C	H		
S1.62	S		I	K	T	L	C		M	
S1.63	S		I	K	T	L	C			E
S1.64	S		I	K	T	L		H		E
S1.65	S		I	K	T	L			M	E
S1.66	S		I	K	T	L	C	H	M	E
S1.67	S	G		K	T					
S1.68	S	G		K	T		C			
S1.69	S	G		K	T			H		
S1.70	S	G		K	T				M	
S1.71	S	G		K	T					E
S1.72	S	G		K	T		C	H		
S1.73	S	G		K	T		C		M	
S1.74	S	G		K	T		C			E
S1.75	S	G		K	T			H		E
S1.76	S	G		K	T				M	E
S1.77	S	G		K	T		C	H	M	E
S1.78		G		K	T					
S1.79		G		K	T		C			
S1.80		G		K	T			H		
S1.81		G		K	T				M	
S1.82		G		K	T					E
S1.83		G		K	T		C	H		
S1.84		G		K	T		C		M	
S1.85		G		K	T		C			E
S1.86		G		K	T			H		E
S1.87		G		K	T				M	E
S1.88		G		K	T		C	H	M	E
S1.89		K		K	T					
S1.90		K		K	T		C			
S1.91		K		K	T			H		

Position No.	27	29	30	49	82	84	107	112	115	142
TadA*8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.92		K		K	T				M	
S1.93		K		K	T					E
S1.94		K		K	T		C	H		
S1.95		K		K	T		C		M	
S1.96		K		K	T		C			E
S1.97		K		K	T			H		E
S1.98		K		K	T				M	E
S1.99		K		K	T		C	H	M	E
S1.100		K	I	K	T					
S1.101		K	I	K	T		C			
S1.102		K	I	K	T			H		
S1.103		K	I	K	T				M	
S1.104		K	I	K	T					E
S1.105		K	I	K	T		C	H		
S1.106		K	I	K	T		C		M	
S1.107		K	I	K	T		C			E
S1.108		K	I	K	T			H		E
S1.109		K	I	K	T				M	E
S1.110		K	I	K	T		C	H	M	E
S1.111		K		K	T	L				
S1.112		K		K	T	L	C			
S1.113		K		K	T	L		H		
S1.114		K		K	T	L			M	
S1.115		K		K	T	L				E
S1.116		K		K	T	L	C	H		
S1.117		K		K	T	L	C		M	
S1.118		K		K	T	L	C			E
S1.119		K		K	T	L		H		E
S1.120		K		K	T	L			M	E
S1.121		K		K	T	L	C	H	M	E
S1.122		K	I	K	T	L				
S1.123		K	I	K	T	L	C			
S1.124		K	I	K	T	L		H		
S1.125		K	I	K	T	L			M	
S1.126		K	I	K	T	L				E
S1.127		K	I	K	T	L	C	H		
S1.128		K	I	K	T	L	C		M	
S1.129		K	I	K	T	L	C			E
S1.130		K	I	K	T	L		H		E
S1.131		K	I	K	T	L			M	E
S1.132		K	I	K	T	L	C	H	M	E
S1.133	G			K	T					

Position No.	27	29	30	49	82	84	107	112	115	142
TadA*8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.134	G			K	T		C			
S1.135	G			K	T			H		
S1.136	G			K	T				M	
S1.137	G			K	T					E
S1.138	G			K	T		C	H		
S1.139	G			K	T		C		M	
S1.140	G			K	T		C			E
S1.141	G			K	T			H		E
S1.142	G			K	T				M	E
S1.143	G			K	T		C	H	M	E
S1.144	H			K	T					
S1.145	H			K	T		C			
S1.146	H			K	T			H		
S1.147	H			K	T				M	
S1.148	H			K	T					E
S1.149	H			K	T		C	H		
S1.150	H			K	T		C		M	
S1.151	H			K	T		C			E
S1.152	H			K	T			H		E
S1.153	H			K	T				M	E
S1.154	H			K	T		C	H	M	E
S1.155	S				T					
S1.156	S				T		C			
S1.157	S				T			H		
S1.158	S				T				M	
S1.159	S				T					E
S1.160	S				T		C	H		
S1.161	S				T		C		M	
S1.162	S				T		C			E
S1.163	S				T			H		E
S1.164	S				T				M	E
S1.165	S				T		C	H	M	E
S1.166		A			T					
S1.167		A			T		C			
S1.168		A			T			H		
S1.169		A			T				M	
S1.170		A			T					E
S1.171		A			T		C	H		
S1.172		A			T		C		M	
S1.173		A			T		C			E
S1.174		A			T			H		E
S1.175		A			T				M	E

Position No.	27	29	30	49	82	84	107	112	115	142
TadA*8.20	E	P	V	I	S	F	R	G	G	A
Alterations Evaluated	G/S/H	G/A/K	I/L/F	K	T	L/A	C	H	M	E
S1.176		A			T		C	H	M	E
S1.177	S		I		T					
S1.178	S		I		T		C			
S1.179	S		I		T			H		
S1.180	S		I		T				M	
S1.181	S		I		T					E
S1.182	S		I		T		C	H		
S1.183	S		I		T		C		M	
S1.184	S		I		T		C			E
S1.185	S		I		T			H		E
S1.186	S		I		T				M	E
S1.187	S		I		T		C	H	M	E
S1.188		A	I		T	L				
S1.189		A	I		T	L	C			
S1.190		A	I		T	L		H		
S1.191		A	I		T	L			M	
S1.192		A	I		T	L				E
S1.193		A	I		T	L	C	H		
S1.194		A	I		T	L	C		M	
S1.195		A	I		T	L	C			E
S1.196		A	I		T	L		H		E
S1.197		A	I		T	L			M	E
S1.198		A	I		T	L	C	H	M	E
S1.199	S	A	L	K	T	L	C	H	M	E

Table 6C. Adenosine deaminase variants. Mutations are indicated with reference to variant 1.2 (Table 6A) .

Variant Name	Alternative Variant Names	Residue identity (START Met is amino acid #1)								
		4	6	17	23	76	77	100	111	114
Reference	1.2 (see Table 6A)	V	F	T	R	I	D	G	T	A
TadAC2.1	pDKL-135; 2.1	K								C
TadAC2.2	pDKL-136; 2.2	K					G			
TadAC2.3	pDKL-137; 2.3		Y					A		
TadAC2.4	pDKL-138; 2.4	T				R				
TadAC2.5	pDKL-139; 2.5		Y			W				
TadAC2.6	pDKL-140; 2.6		Y							
TadAC2.7	pDKL-141; 2.7		Y							C
TadAC2.8	pDKL-142; 2.8		Y							
TadAC2.9	pDKL-143; 2.9	K				W				

Variant Name	Alternative Variant Names	Residue identity (START Met is amino acid #1)								
		4	6	17	23	76	77	100	111	114
Reference	1.2 (see Table 6A)	V	F	T	R	I	D	G	T	A
TadAC2.10	pDKL-144; 2.10		G			R		K		
TadAC2.11	pDKL-145; 2.11		H							
TadAC2.12	pDKL-146; 2.12									C
TadAC2.13	pDKL-147; 2.13		Y			H				
TadAC2.14	pDKL-148; 2.14									
TadAC2.15	pDKL-149; 2.15				Q	R				
TadAC2.16	pDKL-150; 2.16					H				
TadAC2.17	pDKL-151; 2.17		Y						H	
TadAC2.18	pDKL-152; 2.18					W				
TadAC2.19	pDKL-153; 2.19								H	
TadAC2.20	pDKL-154; 2.20									
TadAC2.21	pDKL-155; 2.21		Y			R				
TadAC2.22	pDKL-156; 2.22			W		H				
TadAC2.23	pDKL-157; 2.23	S				Y				
TadAC2.24	pDKL-158; 2.24									

Table 6C. (CONTINUED)

Variant Name	Alternative Variant Names	Residue identity (START Met is amino acid #1)								
		119	122	127	143	144	145	148	156	166
Reference	1.2 (see Table 6A)	D	H	N	A	R	A	Q	A	T
TadAC2.1	pDKL-135; 2.1									
TadAC2.2	pDKL-136; 2.2									
TadAC2.3	pDKL-137; 2.3		R							
TadAC2.4	pDKL-138; 2.4		G							
TadAC2.5	pDKL-139; 2.5									
TadAC2.6	pDKL-140; 2.6	N								
TadAC2.7	pDKL-141; 2.7									
TadAC2.8	pDKL-142; 2.8									
TadAC2.9	pDKL-143; 2.9		T							
TadAC2.10	pDKL-144; 2.10									
TadAC2.11	pDKL-145; 2.11		N							
TadAC2.12	pDKL-146; 2.12									
TadAC2.13	pDKL-147; 2.13		R							I
TadAC2.14	pDKL-148; 2.14			P						
TadAC2.15	pDKL-149; 2.15									

Variant Name	Alternative Variant Names	Residue identity (START Met is amino acid #1)								
		1 1 9	1 2 2	1 2 7	1 4 3	1 4 7	1 5 8	1 5 9	1 6 2	16 6
Reference	1.2 (see Table 6A)	D	H	N	A	R	A	Q	A	T
TadAC2.16	pDKL-150; 2.16		R				V			
TadAC2.17	pDKL-151; 2.17									
TadAC2.18	pDKL-152; 2.18									
TadAC2.19	pDKL-153; 2.19		G						C	
TadAC2.20	pDKL-154; 2.20				E					
TadAC2.21	pDKL-155; 2.21									
TadAC2.22	pDKL-156; 2.22		G				V			
TadAC2.23	pDKL-157; 2.23				E			S		
TadAC2.24	pDKL-158; 2.24			I					Q	

Table 6D. Adenosine deaminase variants. Mutations are indicated with reference to TadA*8.20.

AA Positions	6	27	49	76	77	82	107	112	114	115	119	122	127	142	143
TadA*8.20	F	E	I	Y	D	S	R	G	A	G	D	H	N	A	A
S1.154	F	H	K	Y	D	T	C	H		M				E	
Alterations from Table 6C	Y			W	G				C		N	G	P		E
S2.1	Y	H	K	W		T	C	H		M				E	
S2.2	Y	H	K		G	T	C	H		M				E	
S2.3	Y	H	K			T	C	H	C	M				E	
S2.4	Y	H	K			T	C	H		M	N			E	
S2.5	Y	H	K			T	C	H		M		G		E	
S2.6	Y	H	K			T	C	H		M			P	E	
S2.7	Y	H	K			T	C	H		M				E	E
S2.8	Y	H	K			T	C	H		M				A	E
S2.9	Y	H	K	W	G	T	C	H		M				E	
S2.10	Y	H	K	W		T	C	H	C	M				E	
S2.11	Y	H	K	W		T	C	H		M	N			E	
S2.12	Y	H	K	W		T	C	H		M		G		E	
S2.13	Y	H	K	W		T	C	H		M			P	E	
S2.14	Y	H	K	W		T	C	H		M				E	E
S2.15	Y	H	K	W		T	C	H		M				A	E
S2.16	Y	H	K		G	T	C	H	C	M				E	

AA Positions	6	27	49	76	77	82	107	112	114	115	119	122	127	142	143
TadA*8.20	F	E	I	Y	D	S	R	G	A	G	D	H	N	A	A
S2.17	Y	H	K		G	T	C	H		M	N			E	
S2.18	Y	H	K		G	T	C	H		M		G		E	
S2.19	Y	H	K		G	T	C	H		M			P	E	
S2.20	Y	H	K		G	T	C	H		M				E	E
S2.21	Y	H	K		G	T	C	H		M				A	E
S2.22	Y	H	K			T	C	H	C	M	N			E	
S2.23	Y	H	K			T	C	H	C	M		G		E	
S2.24	Y	H	K			T	C	H	C	M			P	E	
S2.25	Y	H	K			T	C	H		M	N	G		E	
S2.26	Y	H	K			T	C	H		M	N		P	E	
S2.27	Y	H	K			T	C	H		M		G	P	E	
S2.28	Y	H	K	W	G	T	C	H	C	M				E	
S2.29	Y	H	K	W	G	T	C	H		M	N			E	
S2.30	Y	H	K	W	G	T	C	H		M		G		E	
S2.31	Y	H	K	W	G	T	C	H		M			P	E	
S2.32	Y	H	K	W	G	T	C	H		M				E	E
S2.33	Y	H	K	W	G	T	C	H		M				A	E
S2.34	Y	H	K	W		T	C	H	C	M	N			E	
S2.35	Y	H	K	W		T	C	H	C	M		G		E	
S2.36	Y	H	K	W		T	C	H	C	M			P	E	
S2.37	Y	H	K	W		T	C	H	C	M				E	E
S2.38	Y	H	K	W		T	C	H	C	M				A	E
S2.39	Y	H	K	W		T	C	H		M	N	G		E	
S2.40	Y	H	K	W		T	C	H		M	N		P	E	
S2.41	Y	H	K	W		T	C	H		M		G	P	E	
S2.42	Y	H	K	W		T	C	H	C	M	N	G		E	
S2.43	Y	H	K	W		T	C	H	C	M	N		P	E	
S2.44	Y	H	K	W		T	C	H	C	M		G	P	E	
S2.45	Y	H	K	W	G	T	C	H	C	M	N			E	
S2.46	Y	H	K	W	G	T	C	H	C	M		G		E	
S2.47	Y	H	K	W	G	T	C	H	C	M			P	E	
S2.48	Y	H	K	W	G	T	C	H	C	M				E	E
S2.49	Y	H	K	W	G	T	C	H	C	M				A	E
S2.50	Y	H	K	W	G	T	C	H	C	M	N	G		E	
S2.51	Y	H	K	W	G	T	C	H	C	M	N		P	E	
S2.52	Y	H	K	W	G	T	C	H	C	M		G	P	E	

AA Positions	6	27	49	76	77	82	107	112	114	115	119	122	127	142	143
TadA*8.20	F	E	I	Y	D	S	R	G	A	G	D	H	N	A	A
S2.53	Y	H	K	W		T	C	H	C	M	N	G	P	E	E
S2.54	Y	H	K	W		T	C	H	C	M	N	G	P	A	E
S2.55	Y	H	K	W	G	T	C	H	C	M	N	G	P	E	E
S2.56	Y	H	K	W	G	T	C	H	C	M	N	G	P	A	E

Table 6E. Hybrid constructs. Mutations are indicated with reference to TadA*7.10.

	TadA amino acid substitutions											
	76	82	109	111	119	122	123	147	149	154	166	167
TadA*7.10	I	V	A	T	D	H	Y	Y	F	Q	T	D
TadA*8c			S	R	N	N		D	Y		I	N
TadA*8.20	Y	S					H	R		R		
TadA*8.17		S								R		
pNMG-B878	Y	S					H	D		R		
pNMG-B879	Y	S					H	R	Y	R		
pNMG-B880	Y	S					H	R		R	I	
pNMG-B881	Y	S					H	R		R		N
pNMG-B882	Y	S					H	D	Y	R	I	N
pNMG-B883	Y	S		R	N		H	R		R		
pNMG-B884	Y	S	S	R	N	N	H	R		R		
pNMG-B885	Y	S	S				H	R		R		
pNMG-B886	Y	S		R			H	R		R		
pNMG-B887	Y	S			N		H	R		R		
pNMG-B888	Y	S				N	H	R		R		
pNMG-B889	Y	S	S	R			H	R		R		
pNMG-B890	Y	S			N	N	H	R		R		
pNMG-B891	Y	S	S	R	N	N	H	D	Y	R	I	N

5 Table 6F. Base editor variants. Mutations are indicated with reference to TadA*8.19/8.20.

AA positions:	17	27	48	49	76	82	84	118	142	147	149	166	167
ABE8.19m/8.20m	T	E	A	I	Y/I	S	F	M	A	Y	F	T	D
1.1 + 8c(B879)		H			I		M				Y		
1.2 + 8c(B879)		H		K	I						Y		
1.12 + 8c(B879)	A	H		M	I			L			Y		
1.17 + 8c(B879)	A		G			T			E		Y		
1.18 + 8c(B879)		G									Y		
1.19 + 8c(B879)		G		N							Y		
1.1 + 8c(B882)		H			I		M			D	Y	I	N
1.2 + 8c(B882)		H		K	I					D	Y	I	N

AA positions:	17	27	48	49	76	82	84	118	142	147	149	166	167
ABE8.19m/8.20m	T	E	A	I	Y/I	S	F	M	A	Y	F	T	D
1.12 + 8e(B882)	A	H		M	I			L		D	Y	I	N
1.17 + 8e(B882)	A		G			T			E	D	Y	I	N
1.18 + 8e(B882)		G								D	Y	I	N
1.19 + 8e(B882)		G		N						D	Y	I	N

Guide Polynucleotides

A polynucleotide programmable nucleotide binding domain, when in conjunction with a bound guide polynucleotide (*e.g.*, gRNA), can specifically bind to a target polynucleotide sequence (*i.e.*, via complementary base pairing between bases of the bound guide nucleic acid and bases of the target polynucleotide sequence) and thereby localize the base editor to the target nucleic acid sequence desired to be edited. In some embodiments, the target polynucleotide sequence comprises single-stranded DNA or double-stranded DNA. In some embodiments, the target polynucleotide sequence comprises RNA. In some embodiments, the target polynucleotide sequence comprises a DNA-RNA hybrid.

In an embodiment, a guide polynucleotide described herein can be RNA or DNA. In one embodiment, the guide polynucleotide is a gRNA.

In some embodiments, the guide polynucleotide is at least one single guide RNA (“sgRNA” or “gRNA”). In some embodiments, a guide polynucleotide comprises two or more individual polynucleotides, which can interact with one another via for example complementary base pairing (*e.g.*, a dual guide polynucleotide, dual gRNA). For example, a guide polynucleotide can comprise a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA) or can comprise one or more trans-activating CRISPR RNA (tracrRNA).

A guide polynucleotide may include natural or non-natural (or unnatural) nucleotides (*e.g.*, peptide nucleic acid or nucleotide analogs). In some cases, the targeting region of a guide nucleic acid sequence (*e.g.*, a spacer) can be at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some embodiments, the methods described herein can utilize an engineered Cas protein. A guide RNA (gRNA) is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic target to be modified. Exemplary gRNA scaffold sequences are provided in the sequence listing as SEQ ID NOs: 317-327 and 425. Thus, a skilled artisan can change the genomic target of the Cas protein specificity is partially determined by how specific the gRNA targeting sequence is for the genomic target compared to the rest of the genome. In embodiments, the spacer is about

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more nucleotides in length. The spacer of a gRNA can be or can be about 19, 20, or 21 nucleotides in length.

A gRNA or a guide polynucleotide can target any exon or intron of a gene target. In some embodiments, a composition comprises multiple gRNAs that all target the same exon or multiple gRNAs that target different exons. An exon and/or an intron of a gene can be targeted. A gRNA or a guide polynucleotide can target a nucleic acid sequence of about 20 nucleotides or less than about 20 nucleotides (*e.g.*, at least about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 nucleotides), or anywhere between about 1-100 nucleotides (*e.g.*, 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100). A target nucleic acid sequence can be or can be about 20 bases immediately 5' of the first nucleotide of the PAM. A gRNA can target a nucleic acid sequence. A target nucleic acid can be at least or at least about 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, or 1-100 nucleotides.

The guide polynucleotides can comprise standard ribonucleotides, modified ribonucleotides (*e.g.*, pseudouridine), ribonucleotide isomers, and/or ribonucleotide analogs.

In some embodiments, a base editor system may comprise multiple guide polynucleotides, *e.g.*, gRNAs. For example, the gRNAs may target to one or more target loci (*e.g.*, at least 1 gRNA, at least 2 gRNA, at least 5 gRNA, at least 10 gRNA, at least 20 gRNA, at least 30 gRNA, at least 50 gRNA) comprised in a base editor system. The multiple gRNA sequences can be tandemly arranged and may be separated by a direct repeat.

Modified Polynucleotides

To enhance expression, stability, and/or genomic/base editing efficiency, and/or reduce possible toxicity, the base editor-coding sequence (*e.g.*, mRNA) and/or the guide polynucleotide (*e.g.*, gRNA) can be modified to include one or more modified nucleotides and/or chemical modifications, *e.g.* pseudo-uridine, 5-Methyl-cytosine, 2'-*O*-methyl-3'-phosphonoacetate, 2'-*O*-methyl thioPACE (MSP), 2'-*O*-methyl-PACE (MP), 2'-fluoro RNA (2'-F-RNA), =constrained ethyl (S-cEt), 2'-*O*-methyl ('M'), 2'-*O*-methyl-3'-phosphorothioate ('MS'), 2'-*O*-methyl-3'-thiophosphonoacetate ('MSP'), 5-methoxyuridine, phosphorothioate, and N1-Methylpseudouridine. Chemically protected gRNAs can enhance stability and editing efficiency *in vivo* and *ex vivo*. Methods for using chemically modified mRNAs and guide RNAs are known in the art and described, for example, by Jiang et al., Chemical modifications of adenine base editor mRNA and guide RNA expand its application scope. Nat Commun 11, 1979 (2020). doi.org/10.1038/s41467-020-15892-8, Callum et al., N1-Methylpseudouridine substitution enhances the performance of synthetic mRNA switches in

cells, *Nucleic Acids Research*, Volume 48, Issue 6, 06 April 2020, Page e35, and Andries et al., *Journal of Controlled Release*, Volume 217, 10 November 2015, Pages 337-344, each of which is incorporated herein by reference in its entirety.

In some embodiments, the guide polynucleotide comprises one or more modified
5 nucleotides at the 5' end and/or the 3' end of the guide. In some embodiments, the guide polynucleotide comprises two, three, four or more modified nucleosides at the 5' end and/or the 3' end of the guide. In some embodiments, the guide polynucleotide comprises two, three, four or more modified nucleosides at the 5' end and/or the 3' end of the guide.

In some embodiments, the guide comprises at least about 50%-75% modified
10 nucleotides. In some embodiments, the guide comprises at least about 85% or more modified nucleotides. In some embodiments, at least about 1-5 nucleotides at the 5' end of the gRNA are modified and at least about 1-5 nucleotides at the 3' end of the gRNA are modified. In some embodiments, at least about 3-5 contiguous nucleotides at each of the 5' and 3' termini of the gRNA are modified. In some embodiments, at least about 20% of the nucleotides
15 present in a direct repeat or anti-direct repeat are modified. In some embodiments, at least about 50% of the nucleotides present in a direct repeat or anti-direct repeat are modified. In some embodiments, at least about 50-75% of the nucleotides present in a direct repeat or anti-direct repeat are modified. In some embodiments, at least about 100 of the nucleotides present in a direct repeat or anti-direct repeat are modified. In some embodiments, at least
20 about 20% or more of the nucleotides present in a hairpin present in the gRNA scaffold are modified. In some embodiments, at least about 50% or more of the nucleotides present in a hairpin present in the gRNA scaffold are modified. In some embodiments, the guide comprises a variable length spacer. In some embodiments, the guide comprises a 20-40 nucleotide spacer. In some embodiments, the guide comprises a spacer comprising at least
25 about 20-25 nucleotides or at least about 30-35 nucleotides. In some embodiments, the spacer comprises modified nucleotides. In some embodiments, the guide comprises two or more of the following:

- at least about 1-5 nucleotides at the 5' end of the gRNA are modified and at least about 1-5 nucleotides at the 3' end of the gRNA are modified;
- 30 • at least about 20% of the nucleotides present in a direct repeat or anti-direct repeat are modified;
- at least about 50-75% of the nucleotides present in a direct repeat or anti-direct repeat are modified;

- at least about 20% or more of the nucleotides present in a hairpin present in the gRNA scaffold are modified;
- a variable length spacer; and
- a spacer comprising modified nucleotides.

5 In embodiments, the gRNA contains numerous modified nucleotides and/or chemical modifications. Such modifications can increase base editing ~2 fold *in vivo* or *in vitro*. In embodiments, the gRNA comprises 2'-O-methyl or phosphorothioate modifications. In an embodiment, the gRNA comprises 2'-O-methyl and phosphorothioate modifications. In an embodiment, the modifications increase base editing by at least about 2 fold.

10 A guide polynucleotide can comprise one or more modifications to provide a nucleic acid with a new or enhanced feature. A guide polynucleotide can comprise a nucleic acid affinity tag. A guide polynucleotide can comprise synthetic nucleotide, synthetic nucleotide analog, nucleotide derivatives, and/or modified nucleotides.

A gRNA or a guide polynucleotide can also be modified by 5' adenylate, 5'
 15 guanosine-triphosphate cap, 5' N7-Methylguanosine-triphosphate cap, 5' triphosphate cap, 3' phosphate, 3' thiophosphate, 5' phosphate, 5' thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9, 3'-3' modifications, 2'-O-methyl thioPACE (MSP), 2'-O-methyl-PACE (MP), and constrained ethyl (S-cEt), 5'-5' modifications, abasic, acridine, azobenzene, biotin, biotin BB,
 20 biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3' DABCYL, black hole quencher 1, black hole quencher 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2'-deoxyribonucleoside analog purine, 2'-deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2'-O-methyl ribonucleoside
 25 analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2'-fluoro RNA, 2'-O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5'-triphosphate, 5'-methylcytidine-5'-triphosphate, or any combination thereof.

In some cases, a phosphorothioate enhanced RNA gRNA can inhibit RNase A, RNase
 30 T1, calf serum nucleases, or any combinations thereof. These properties can allow the use of PS-RNA gRNAs to be used in applications where exposure to nucleases is of high probability *in vivo* or *in vitro*. For example, phosphorothioate (PS) bonds can be introduced between the last 3-5 nucleotides at the 5'- or 3'-end of a gRNA which can inhibit exonuclease degradation.

In some cases, phosphorothioate bonds can be added throughout an entire gRNA to reduce attack by endonucleases.

Fusion Proteins or Complexes Comprising a Nuclear Localization Sequence (NLS)

5 In some embodiments, the fusion proteins or complexes provided herein further comprise one or more (*e.g.*, 2, 3, 4, 5) nuclear targeting sequences, for example a nuclear localization sequence (NLS). In one embodiment, a bipartite NLS is used. In some
10 embodiments, a NLS comprises an amino acid sequence that facilitates the importation of a protein, that comprises an NLS, into the cell nucleus (*e.g.*, by nuclear transport). In some
15 embodiments, the NLS is fused to the N-terminus or the C-terminus of the fusion protein. In some embodiments, the NLS is fused to the C-terminus or N-terminus of an nCas9 domain or a dCas9 domain. In some embodiments, the NLS is fused to the N-terminus or C-terminus of the Cas12 domain. In some embodiments, the NLS is fused to the N-terminus or C-terminus of the cytidine or adenosine deaminase. In some embodiments, the NLS is fused to the fusion
20 protein via one or more linkers. In some embodiments, the NLS is fused to the fusion protein without a linker. In some embodiments, the NLS comprises an amino acid sequence of any one of the NLS sequences provided or referenced herein. Additional nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank *et al.*, PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences.

In some embodiments, the NLS is present in a linker or the NLS is flanked by linkers, for example described herein. A bipartite NLS comprises two basic amino acid clusters, which are separated by a relatively short spacer sequence (hence bipartite - 2 parts, while
25 monopartite NLSs are not). The NLS of nucleoplasmin, KR [PAATKKAGQA] KKKK (SEQ ID NO: 191), is the prototype of the ubiquitous bipartite signal: two clusters of basic amino acids, separated by a spacer of about 10 amino acids. The sequence of an exemplary bipartite NLS follows:

PKKKRKVEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 328).

30 In some embodiments, any of the fusion proteins or complexes provided herein comprise an NLS comprising the amino acid sequence EGADKRTADGSEFESPKKKRKV (amino acids 8 to 29 of SEQ ID NO 328). In some embodiments, any of the adenosine base editors provided herein comprise the amino acid sequence EGADKRTADGSEFESPKKKRKV

(amino acids 8 to 29 of SEQ ID NO: 328). In some embodiments, the NLS is at a C-terminal portion of the adenosine base editor. In some embodiments, the NLS is at the C-terminus of the adenosine base editor.

5 *Additional Domains*

A base editor described herein can include any domain which helps to facilitate the nucleobase editing, modification or altering of a nucleobase of a polynucleotide. In some embodiments, a base editor comprises a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9), a nucleobase editing domain (*e.g.*, deaminase domain), and one or more
 10 additional domains. In some embodiments, the additional domain can facilitate enzymatic or catalytic functions of the base editor, binding functions of the base editor, or be inhibitors of cellular machinery (*e.g.*, enzymes) that could interfere with the desired base editing result. In some embodiments, a base editor comprises a nuclease, a nickase, a recombinase, a deaminase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a
 15 transcriptional activator, or a transcriptional repressor domain.

In some embodiments, a base editor comprises an uracil glycosylase inhibitor (UGI) domain. In some cases, a base editor is expressed in a cell in trans with a UGI polypeptide. In some embodiments, cellular DNA repair response to the presence of U: G heteroduplex DNA can be responsible for a reduction in nucleobase editing efficiency in cells. In such
 20 embodiments, uracil DNA glycosylase (UDG) can catalyze removal of U from DNA in cells, which can initiate base excision repair (BER), mostly resulting in reversion of the U:G pair to a C:G pair. In such embodiments, BER can be inhibited in base editors comprising one or more domains that bind the single strand, block the edited base, inhibit UGI, inhibit BER, protect the edited base, and /or promote repairing of the non-edited strand. Thus, this
 25 disclosure contemplates a base editor fusion protein or complex comprising a UGI domain and/or a uracil stabilizing protein (USP) domain.

BASE EDITOR SYSTEM

Provided herein are systems, compositions, and methods for editing a nucleobase
 30 using a base editor system. In some embodiments, the base editor system comprises (1) a base editor (BE) comprising a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, a deaminase domain) for editing the nucleobase; and (2) a guide polynucleotide (*e.g.*, guide RNA) in conjunction with the polynucleotide programmable nucleotide binding domain. In some embodiments, the base editor system is a

cytidine base editor (CBE) or an adenosine base editor (ABE). In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA or RNA binding domain. In some embodiments, the nucleobase editing domain is a deaminase domain. In some embodiments, a deaminase domain can be a cytidine deaminase or an cytosine deaminase. In some embodiments, a deaminase domain can be an adenine deaminase or an adenosine deaminase. In some embodiments, the adenosine base editor can deaminate adenine in DNA. In some embodiments, the base editor is capable of deaminating a cytidine in DNA.

Use of the base editor system provided herein comprises the steps of: (a) contacting a target nucleotide sequence of a polynucleotide (*e.g.*, double- or single stranded DNA or RNA) of a subject with a base editor system comprising a nucleobase editor (*e.g.*, an adenosine base editor or a cytidine base editor) and a guide polynucleotide (*e.g.*, gRNA), wherein the target nucleotide sequence comprises a targeted nucleobase pair; (b) inducing strand separation of said target region; (c) converting a first nucleobase of said target nucleobase pair in a single strand of the target region to a second nucleobase; and (d) cutting no more than one strand of said target region, where a third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase. It should be appreciated that in some embodiments, step (b) is omitted. In some embodiments, said targeted nucleobase pair is a plurality of nucleobase pairs in one or more genes. In some embodiments, the base editor system provided herein is capable of multiplex editing of a plurality of nucleobase pairs in one or more genes. In some embodiments, the plurality of nucleobase pairs is located in the same gene. In some embodiments, the plurality of nucleobase pairs is located in one or more genes, wherein at least one gene is located in a different locus.

The components of a base editor system (*e.g.*, a deaminase domain, a guide RNA, and/or a polynucleotide programmable nucleotide binding domain) may be associated with each other covalently or non-covalently. For example, in some embodiments, the deaminase domain can be targeted to a target nucleotide sequence by a polynucleotide programmable nucleotide binding domain, optionally where the polynucleotide programmable nucleotide binding domain is complexed with a polynucleotide (*e.g.*, a guide RNA). In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to a deaminase domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can target a deaminase domain to a target nucleotide sequence by non-covalently interacting with or associating with the deaminase domain. For example, in

some embodiments, the nucleobase editing component (*e.g.*, the deaminase component) comprises an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with a corresponding heterologous portion, antigen, or domain that is part of a polynucleotide programmable nucleotide binding domain and/or a guide polynucleotide (*e.g.*, a guide RNA) complexed therewith. In some

5 embodiments, the polynucleotide programmable nucleotide binding domain, and/or a guide polynucleotide (*e.g.*, a guide RNA) complexed therewith, comprises an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with a corresponding heterologous portion, antigen, or domain

10 that is part of a nucleobase editing domain (*e.g.*, the deaminase component). In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polypeptide. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional

15 heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion is capable of binding to a polynucleotide linker. An additional heterologous portion may be a protein domain. In some embodiments, an additional heterologous portion comprises a polypeptide, such as a 22

20 amino acid RNA-binding domain of the lambda bacteriophage antiterminator protein N (N22p), a 2G12 IgG homodimer domain, an ABI, an antibody (*e.g.* an antibody that binds a component of the base editor system or a heterologous portion thereof) or fragment thereof (*e.g.* heavy chain domain 2 (CH2) of IgM (MHD2) or IgE (EHD2), an immunoglobulin Fc region, a heavy chain domain 3 (CH3) of IgG or IgA, a heavy chain domain 4 (CH4) of IgM

25 or IgE, an Fab, an Fab2, miniantibodies, and/or ZIP antibodies), a barnase-barstar dimer domain, a Bel-xL domain, a Calcineurin A (CAN) domain, a Cardiac phospholamban transmembrane pentamer domain, a collagen domain, a Com RNA binding protein domain (*e.g.* SfMu Com coat protein domain, and SfMu Com binding protein domain), a Cyclophilin-Fas fusion protein (CyP-Fas) domain, a Fab domain, an Fe domain, a

30 fibrin foldon domain, an FK506 binding protein (FKBP) domain, an FKBP binding domain (FRB) domain of mTOR, a foldon domain, a fragment X domain, a GAI domain, a GID1 domain, a Glycophorin A transmembrane domain, a GyrB domain, a Halo tag, an HIV Gp41 trimerisation domain, an HPV45 oncoprotein E7 C-terminal dimer domain, a hydrophobic polypeptide, a K Homology (KH) domain, a Ku protein domain (*e.g.*, a Ku heterodimer), a

leucine zipper, a LOV domain, a mitochondrial antiviral-signaling protein CARD filament domain, an MS2 coat protein domain (MCP), a non-natural RNA aptamer ligand that binds a corresponding RNA motif/aptamer, a parathyroid hormone dimerization domain, a PP7 coat protein (PCP) domain, a PSD95-Dlg1-zo-1 (PDZ) domain, a PYL domain, a SNAP tag, a
 5 SpyCatcher moiety, a SpyTag moiety, a streptavidin domain, a streptavidin-binding protein domain, a streptavidin binding protein (SBP) domain, a telomerase Sm7 protein domain (*e.g.* Sm7 homoheptamer or a monomeric Sm-like protein), and/or fragments thereof. In embodiments, an additional heterologous portion comprises a polynucleotide (*e.g.*, an RNA motif), such as an MS2 phage operator stem-loop (*e.g.*, an MS2, an MS2 C-5 mutant, or an
 10 MS2 F-5 mutant), a non-natural RNA motif, a PP7 operator stem-loop, an SfMu phage Com stem-loop, a sterile alpha motif, a telomerase Ku binding motif, a telomerase Sm7 binding motif, and/or fragments thereof. Non-limiting examples of additional heterologous portions include polypeptides with at least about 85% sequence identity to any one or more of SEQ ID
 15 NOs: 380, 382, 384, 386-388, or fragments thereof. Non-limiting examples of additional heterologous portions include polynucleotides with at least about 85% sequence identity to any one or more of SEQ ID NOs: 379, 381, 383, 385, or fragments thereof.

In some instances, components of the base editing system are associated with one another through the interaction of leucine zipper domains (*e.g.*, SEQ ID NOs: 387 and 388). In some cases, components of the base editing system are associated with one another
 20 through polypeptide domains (*e.g.*, FokI domains) that associate to form protein complexes containing about, at least about, or no more than about 1, 2 (*i.e.*, dimerize), 3, 4, 5, 6, 7, 8, 9, 10 polypeptide domain units, optionally the polypeptide domains may include alterations that reduce or eliminate an activity thereof.

In some instances, components of the base editing system are associated with one another through the interaction of multimeric antibodies or fragments thereof (*e.g.*, IgG, IgD,
 25 IgA, IgM, IgE, a heavy chain domain 2 (CH2) of IgM (MHD2) or IgE (EHD2), an immunoglobulin Fc region, a heavy chain domain 3 (CH3) of IgG or IgA, a heavy chain domain 4 (CH4) of IgM or IgE, an Fab, and an Fab2). In some instances, the antibodies are dimeric, trimeric, or tetrameric. In embodiments, the dimeric antibodies bind a polypeptide or
 30 polynucleotide component of the base editing system.

In some cases, components of the base editing system are associated with one another through the interaction of a polynucleotide-binding protein domain(s) with a polynucleotide(s). In some instances, components of the base editing system are associated with one another through the interaction of one or more polynucleotide-binding protein

domains with polynucleotides that are self-complementary and/or complementary to one another so that complementary binding of the polynucleotides to one another brings into association their respective bound polynucleotide-binding protein domain(s).

In some instances, components of the base editing system are associated with one another through the interaction of a polypeptide domain(s) with a small molecule(s) (e.g., chemical inducers of dimerization (CIDs), also known as “dimerizers”). Non-limiting examples of CIDs include those disclosed in Amara, *et al.*, “A versatile synthetic dimerizer for the regulation of protein-protein interactions,” *PNAS*, 94:10618-10623 (1997); and Voß, *et al.*, “Chemically induced dimerization: reversible and spatiotemporal control of protein function in cells,” *Current Opinion in Chemical Biology*, 28:194-201 (2015), the disclosures of each of which are incorporated herein by reference in their entireties for all purposes. In some embodiments, the base editor inhibits base excision repair (BER) of the edited strand. In some embodiments, the base editor protects or binds the non-edited strand. In some embodiments, the base editor comprises UGI activity or USP activity. In some embodiments, the base editor comprises a catalytically inactive inosine-specific nuclease.

The base editors of the present disclosure can comprise any domain, feature or amino acid sequence which facilitates the editing of a target polynucleotide sequence. For example, in some embodiments, the base editor comprises a nuclear localization sequence (NLS). In some embodiments, an NLS of the base editor is localized between a deaminase domain and a polynucleotide programmable nucleotide binding domain. In some embodiments, an NLS of the base editor is localized C-terminal to a polynucleotide programmable nucleotide binding domain.

Protein domains included in the fusion protein can be a heterologous functional domain. Non-limiting examples of protein domains which can be included in the fusion protein include a deaminase domain (e.g., cytidine deaminase and/or adenosine deaminase), a uracil glycosylase inhibitor (UGI) domain, epitope tags, and reporter gene sequences. In some embodiments, the adenosine base editor (ABE) can deaminate adenine in DNA. In some embodiments, ABE is generated by replacing APOBEC1 component of BE3 with natural or engineered *E. coli* TadA, human ADAR2, mouse ADA, or human ADAT2. In some embodiments, ABE comprises an evolved TadA variant. In some embodiments, the base editor is ABE8.1, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity: SEQ ID NO: 331. Other ABE8 sequences are provided in the attached sequence listing (SEQ ID NOs: 332-354).

In some embodiments, the base editor includes an adenosine deaminase variant comprising an amino acid sequence, which contains alterations relative to an ABE 7*10 reference sequence, as described herein. The term “monomer” as used in **Table 7** refers to a monomeric form of TadA*7.10 comprising the alterations described. The term “heterodimer” as used in **Table 7** refers to the specified wild-type *E. coli* TadA adenosine deaminase fused to a TadA*7.10 comprising the alterations as described.

Table 7. Adenosine Deaminase Base Editor Variants

ABE	Adenosine Deaminase	Adenosine Deaminase Description
ABE-605m	MSP605	monomer_TadA*7.10 + V82G + Y147T + Q154S
ABE-680m	MSP680	monomer_TadA*7.10 + I76Y + V82G + Y147T + Q154S
ABE-823m	MSP823	monomer_TadA*7.10 + L36H + V82G + Y147T + Q154S + N157K
ABE-824m	MSP824	monomer_TadA*7.10 + V82G + Y147D + F149Y + Q154S + D167N
ABE-825m	MSP825	monomer_TadA*7.10 + L36H + V82G + Y147D + F149Y + Q154S + N157K + D167N
ABE-827m	MSP827	monomer_TadA*7.10 + L36H + I76Y + V82G + Y147T + Q154S + N157K
ABE-828m	MSP828	monomer_TadA*7.10 + I76Y + V82G + Y147D + F149Y + Q154S + D167N
ABE-829m	MSP829	monomer_TadA*7.10 + L36H + I76Y + V82G + Y147D + F149Y + Q154S + N157K + D167N
ABE-605d	MSP605	heterodimer_(WT)+(TadA*7.10 + V82G + Y147T + Q154S)
ABE-680d	MSP680	heterodimer_(WT)+(TadA*7.10 + I76Y + V82G + Y147T + Q154S)
ABE-823d	MSP823	heterodimer_(WT)+(TadA*7.10 + L36H + V82G + Y147T + Q154S + N157K)
ABE-824d	MSP824	heterodimer_(WT)+(TadA*7.10 + V82G + Y147D + F149Y + Q154S + D167N)
ABE-825d	MSP825	heterodimer_(WT)+(TadA*7.10 + L36H + V82G + Y147D + F149Y + Q154S + N157K + D167N)
ABE-827d	MSP827	heterodimer_(WT)+(TadA*7.10 + L36H + I76Y + V82G + Y147T + Q154S + N157K)
ABE-828d	MSP828	heterodimer_(WT)+(TadA*7.10 + I76Y + V82G + Y147D + F149Y + Q154S + D167N)
ABE-829d	MSP829	heterodimer_(WT)+(TadA*7.10 + L36H + I76Y + V82G + Y147D + F149Y + Q154S + N157K + D167N)

In some embodiments, the base editor comprises a domain comprising all or a portion (e.g., a functional portion) of a uracil glycosylase inhibitor (UGI) or a uracil stabilizing protein (USP) domain.

Linkers

In certain embodiments, linkers may be used to link any of the peptides or peptide domains of the disclosure. The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (*e.g.*, a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, *etc.*).

In some embodiments, any of the fusion proteins provided herein, comprise a cytidine or adenosine deaminase and a Cas9 domain that are fused to each other via a linker. Various linker lengths and flexibilities between the cytidine or adenosine deaminase and the Cas9 domain can be employed (*e.g.*, ranging from very flexible linkers of the form (GGGS)_n (SEQ ID NO: 246), (GGGGS)_n (SEQ ID NO: 247), and (G)_n to more rigid linkers of the form (EAAAK)_n (SEQ ID NO: 248), (SGGS)_n (SEQ ID NO: 355), SGSETPGTSESATPES (SEQ ID NO: 249) (see, *e.g.*, Guilinger JP, *et al.* Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32(6): 577-82; the entire contents are incorporated herein by reference) and (XP)_n in order to achieve the optimal length for activity for the cytidine or adenosine deaminase nucleobase editor. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises a (GGS)_n motif, wherein n is 1, 3, or 7. In some embodiments, cytidine deaminase or adenosine deaminase and the Cas9 domain of any of the fusion proteins provided herein are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 249), which can also be referred to as the XTEN linker.

In some embodiments, the domains of the base editor are fused via a linker that comprises the amino acid sequence of:

SGGSSGSETPGTSESATPESSGGS (SEQ ID NO: 356),
 SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 357), or
 GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPS
 EGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS (SEQ ID NO: 358).

In some embodiments, domains of the base editor are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 249), which may also be referred to as the XTEN linker. In some embodiments, a linker comprises the amino acid

sequence SGGG (SEQ ID NO: 355). In some embodiments, the linker is 24 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPES (SEQ ID NO: 359). In some embodiments, the linker is 40 amino acids in length. In some embodiments, the linker comprises the amino acid sequence: SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 360). In some embodiments, the linker is 64 amino acids in length. In some embodiments, the linker comprises the amino acid sequence: SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 361). In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence: PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPAT (SEQ ID NO: 362). In some embodiments, a linker comprises a plurality of proline residues and is 5-21, 5-14, 5-9, 5-7 amino acids in length, *e.g.*, PAPAP (SEQ ID NO: 363), PAPAPA (SEQ ID NO: 364), PAPAPAP (SEQ ID NO: 365), PAPAPAPA (SEQ ID NO: 366), P(AP)4 (SEQ ID NO: 367), P(AP)7 (SEQ ID NO: 368), P(AP)10 (SEQ ID NO: 369) (see, *e.g.*, Tan J, Zhang F, Karcher D, Bock R. Engineering of high-precision base editors for site-specific single nucleotide replacement. Nat Commun. 2019 Jan 25;10(1):439; the entire contents are incorporated herein by reference). Such proline-rich linkers are also termed “rigid” linkers.

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Nucleic Acid Programmable DNA Binding Proteins with Guide RNAs

Provided herein are compositions and methods for base editing in cells. Further provided herein are compositions comprising a guide polynucleotide sequence, *e.g.*, a guide RNA sequence, or a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more guide RNAs as provided herein. In some embodiments, a composition for base editing as provided herein further comprises a polynucleotide that encodes a base editor, *e.g.*, a C-base editor or an A-base editor. For example, a composition for base editing may comprise a mRNA sequence encoding a BE, a BE4, an ABE, and a combination of one or more guide RNAs as provided. A composition for base editing may comprise a base editor polypeptide and a combination of one or more of any guide RNAs provided herein. Such a composition may be used to effect base editing in a cell through different delivery approaches, for example, electroporation, nucleofection, viral transduction or transfection. In some embodiments, the composition for base editing comprises an mRNA sequence that

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encodes a base editor and a combination of one or more guide RNA sequences provided herein for electroporation.

Some aspects of this disclosure provide systems comprising any of the fusion proteins or complexes provided herein, and a guide RNA bound to a nucleic acid programmable DNA binding protein (napDNAbp) domain (*e.g.*, a Cas9 (*e.g.*, a dCas9, a nuclease active Cas9, or a Cas9 nickase) or Cas12) of the fusion protein or complex. These complexes are also termed ribonucleoproteins (RNPs). In some embodiments, the guide nucleic acid (*e.g.*, guide RNA) is from 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the target sequence is a DNA sequence. In some embodiments, the target sequence is an RNA sequence. In some embodiments, the target sequence is a sequence in the genome of a bacteria, yeast, fungi, insect, plant, or animal. In some embodiments, the target sequence is a sequence in the genome of a human. In some embodiments, the 3' end of the target sequence is immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is immediately adjacent to a non-canonical PAM sequence (*e.g.*, a sequence listed in **Table 3** or 5'-NAA-3'). In some embodiments, the guide nucleic acid (*e.g.*, guide RNA) is complementary to a sequence in a gene of interest (*e.g.*, a gene associated with a disease or disorder).

Some aspects of this disclosure provide methods of using the fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule with any of the fusion proteins or complexes provided herein, and with at least one guide RNA, wherein the guide RNA is about 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence.

The domains of the base editor disclosed herein can be arranged in any order.

A defined target region can be a deamination window. A deamination window can be the defined region in which a base editor acts upon and deaminates a target nucleotide. In some embodiments, the deamination window is within a 2, 3, 4, 5, 6, 7, 8, 9, or 10 base regions. In some embodiments, the deamination window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM.

The base editors of the present disclosure can comprise any domain, feature or amino acid sequence which facilitates the editing of a target polynucleotide sequence.

Methods of Using Fusion Proteins or Complexes Comprising a Cytidine or Adenosine Deaminase and a Cas9 Domain

Some aspects of this disclosure provide methods of using the fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule with any of the fusion proteins or complexes provided herein, and with at least one guide RNA described herein.

In some embodiments, a fusion protein or complex of the disclosure is used for editing a target gene of interest. In particular, a cytidine deaminase or adenosine deaminase nucleobase editor described herein is capable of making multiple mutations within a target sequence. These mutations may affect the function of the target. For example, when a cytidine deaminase or adenosine deaminase nucleobase editor is used to target a regulatory region the function of the regulatory region is altered and the expression of the downstream protein is reduced or eliminated.

Base Editor Efficiency

In some embodiments, the purpose of the methods provided herein is to alter a gene and/or gene product via gene editing. The nucleobase editing proteins provided herein can be used for gene editing-based human therapeutics in vitro or in vivo. It will be understood by the skilled artisan that the nucleobase editing proteins provided herein, *e.g.*, the fusion proteins or complexes comprising a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9) and a nucleobase editing domain (*e.g.*, an adenosine deaminase domain or a cytidine deaminase domain) can be used to edit a nucleotide from A to G or C to T.

Advantageously, base editing systems as provided herein provide genome editing without generating double-strand DNA breaks, without requiring a donor DNA template, and without inducing an excess of stochastic insertions and deletions as CRISPR may do. In some embodiments, the present disclosure provides base editors that efficiently generate an intended mutation, such as a STOP codon, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations.

The base editors of the disclosure advantageously modify a specific nucleotide base encoding a protein without generating a significant proportion of indels (*i.e.*, insertions or deletions). Such indels can lead to frame shift mutations within a coding region of a gene.

In some embodiments, the base editors provided herein are capable of generating a ratio of intended mutations to indels (*i.e.*, intended point mutations:unintended point

mutations) that is greater than 1:1. In some embodiments, the base editors provided herein are capable of generating a ratio of intended mutations to indels that is at least 1.5:1, at least 2:1, at least 2.5:1, at least 3:1, at least 3.5:1, at least 4:1, at least 4.5:1, at least 5:1, at least 5.5:1, at least 6:1, at least 6.5:1, at least 7:1, at least 7.5:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1, at least 100:1, at least 200:1, at least 300:1, at least 400:1, at least 500:1, at least 600:1, at least 700:1, at least 800:1, at least 900:1, or at least 1000:1, or more. The number of intended mutations and indels may be determined using any suitable method.

In some embodiments, the base editors provided herein can limit formation of indels in a region of a nucleic acid. In some embodiments, the region is at a nucleotide targeted by a base editor or a region within 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of a nucleotide targeted by a base editor. In some embodiments, any of the base editors provided herein can limit the formation of indels at a region of a nucleic acid to less than 1%, less than 1.5%, less than 2%, less than 2.5%, less than 3%, less than 3.5%, less than 4%, less than 4.5%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, less than 10%, less than 12%, less than 15%, or less than 20%.

Base editing is often referred to as a “modification”, such as, a genetic modification, a gene modification and modification of the nucleic acid sequence and is clearly understandable based on the context that the modification is a base editing modification. A base editing modification is therefore a modification at the nucleotide base level, for example as a result of the deaminase activity discussed throughout the disclosure, which then results in a change in the gene sequence and may affect the gene product.

In some embodiments, the modification, *e.g.*, single base edit results in about or at least about a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% reduction, or reduction to an undetectable level, of the gene targeted expression.

The disclosure provides adenosine deaminase variants (*e.g.*, ABE8 variants) that have increased efficiency and specificity. In particular, the adenosine deaminase variants described herein are more likely to edit a desired base within a polynucleotide and are less likely to edit bases that are not intended to be altered (*e.g.*, “bystanders”).

In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced bystander editing or mutations by at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%,

60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10.

In some embodiments, any of the ABE8 base editor variants described herein has higher base editing efficiency compared to the ABE7 base editors. In some embodiments, any
5 of the ABE8 base editor variants described herein have at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 310%,
10 320%, 330%, 340%, 350%, 360%, 370%, 380%, 390%, 400%, 450%, or 500% higher base editing efficiency compared to an ABE7 base editor, *e.g.*, ABE7.10.

The ABE8 base editor variants described herein may be delivered to a host cell via a plasmid, a vector, a LNP complex, or an mRNA. In some embodiments, any of the ABE8 base editor variants described herein is delivered to a host cell as an mRNA.

15 In some embodiments, the method described herein, for example, the base editing methods has minimum to no off-target effects. In some embodiments, the method described herein, for example, the base editing methods, has minimal to no chromosomal translocations.

In some embodiments, the base editing method described herein results in about 91%,
20 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of a cell population that have been successfully edited.

In some embodiments, the percent of viable cells in a cell population following a base editing intervention is greater than at least 60%, 70%, 80%, or 90% of the starting cell population at the time of the base editing event. In some embodiments, the percent of viable
25 cells in a cell population following editing is about 70%. In some embodiments, the percent of viable cells in a cell population following editing is about 75%. In some embodiments, the percent of viable cells in a cell population following editing is about 80%. In some embodiments, the percent of viable cells in a cell population as described above is about 85%. In some embodiments, the percent of viable cells in a cell population as described
30 above is about 90%, or about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the cells in the population at the time of the base editing event.

In embodiments, the cell population is a population of cells contacted with a base editor, complex, or base editor system of the present disclosure.

The number of intended mutations and indels can be determined using any suitable method, for example, as described in International PCT Application Nos. PCT/US2017/045381 (WO2018/027078) and PCT/US2016/058344 (WO2017/070632); Komor, A.C., *et al.*, “Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage” *Nature* 533, 420-424 (2016); Gaudelli, N.M., *et al.*, “Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage” *Nature* 551, 464-471 (2017); and Komor, A.C., *et al.*, “Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity” *Science Advances* 3:eaa04774 (2017); the entire contents of which are hereby incorporated by reference.

In some embodiments, to calculate indel frequencies, sequencing reads are scanned for exact matches to two 10-bp sequences that flank both sides of a window in which indels can occur. If no exact matches are located, the read is excluded from analysis. If the length of this indel window exactly matches the reference sequence the read is classified as not containing an indel. If the indel window is two or more bases longer or shorter than the reference sequence, then the sequencing read is classified as an insertion or deletion, respectively. In some embodiments, the base editors provided herein can limit formation of indels in a region of a nucleic acid. In some embodiments, the region is at a nucleotide targeted by a base editor or a region within 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of a nucleotide targeted by a base editor.

Multiplex Editing

In some embodiments, the base editor system provided herein is capable of multiplex editing of a plurality of nucleobase pairs in one or more genes or polynucleotide sequences. In some embodiments, the plurality of nucleobase pairs is located in the same gene or in one or more genes, wherein at least one gene is located in a different locus. In some embodiments, the multiplex editing comprises one or more guide polynucleotides. In some embodiments, the multiplex editing comprises one or more base editor systems. In some embodiments, the multiplex editing comprises one or more base editor systems with a single guide polynucleotide or a plurality of guide polynucleotides. In some embodiments, the multiplex editing comprises one or more guide polynucleotides with a single base editor system. It should be appreciated that the characteristics of the multiplex editing using any of the base editors as described herein can be applied to any combination of methods using any base editor provided herein. It should also be appreciated that the multiplex editing using any

of the base editors as described herein can comprise a sequential editing of a plurality of nucleobase pairs.

In some embodiments, the base editor system capable of multiplex editing of a plurality of nucleobase pairs in one or more genes comprises one of ABE7, ABE8, and/or ABE9 base editors.

DELIVERY SYSTEMS

Nucleic Acid-Based Delivery of Base Editor Systems

Nucleic acid molecules encoding a base editor system according to the present disclosure can be administered to subjects or delivered into cells *in vitro* or *in vivo* by art-known methods or as described herein. For example, a base editor system comprising a deaminase (*e.g.*, cytidine or adenine deaminase) can be delivered by vectors (*e.g.*, viral or non-viral vectors), or by naked DNA, DNA complexes, lipid nanoparticles, or a combination of the aforementioned compositions. A base editor system may be delivered to a cell using any methods available in the art including, but not limited to, physical methods (*e.g.*, electroporation, particle gun, calcium phosphate transfection), viral methods, non-viral methods (*e.g.*, liposomes, cationic methods, lipid nanoparticles, polymeric nanoparticles), or biological non-viral methods (*e.g.*, attenuated bacterial, engineered bacteriophages, mammalian virus-like particles, biological liposomes, erythrocyte ghosts, exosomes).

Nanoparticles, which can be organic or inorganic, are useful for delivering a base editor system or component thereof. Nanoparticles are well known in the art and any suitable nanoparticle can be used to deliver a base editor system or component thereof, or a nucleic acid molecule encoding such components. In one example, organic (*e.g.*, lipid and/or polymer) nanoparticles are suitable for use as delivery vehicles in certain embodiments of this disclosure. Non-limiting examples of lipid nanoparticles suitable for use in the methods of the present disclosure include those described in International Patent Application Publications No. WO2022140239, WO2022140252, WO2022140238, WO2022159421, WO2022159472, WO2022159475, WO2022159463, WO2021113365, and WO2021141969, and in International patent Application No. PCT/US23/27741, the disclosures of each of which is incorporated herein by reference in its entirety for all purposes.

Lipid Nanoparticle (LNP) Compositions

The pharmaceutical compositions for gene modification described herein may be encapsulated in a lipid nanoparticle (LNP). LNP compositions or formulations, as

contemplated herein, are typically sized on the order of micrometers or smaller and may include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (e.g., lipid vesicles), and lipoplexes. For example, a nanoparticle composition or formulation as contemplated herein may be a liposome having a lipid bilayer with a diameter

5 of 500 nm or less. A LNP as described herein may have a mean diameter of from about 20 nm to about 1000 nm, from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 50 nm to 90 nm, from about 55 nm to 85 nm, from about 55 nm to 75 nm, from about 50 nm to about 80 nm, from about 60 nm to about 80 nm, from about 70 nm to about 110 nm, from

10 about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, or from about 70 nm to about 80 nm. The LNPs described herein can have a mean diameter of about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm,

15 150 nm, or greater. In one embodiment the mean diameter of the LNP is about 70 nm +/- 10 nm, 70 nm +/- 5 nm, 65 nm +/- 10 nm, 65 nm +/- 5 nm, 60 nm +/- 10 nm, or 60 nm +/- 5 nm.

Described herein are LNP compositions comprising an ionizable lipid, a phospholipid, a conjugate-linker lipid (e.g., PEG lipid), a sterol (e.g., cholesterol), or a derivative thereof, a

20 payload, or any combination thereof. Each component is described in more detail below.

In the preparation of LNP compositions comprising the ionizable lipid, phospholipid, conjugate-linker lipid, and sterol, a desired molar ratio of the four excipients may be dissolved in a water miscible organic solvent, such as ethanol. The homogenous lipid solution may then be rapidly in-line mixed with an aqueous buffer containing nucleic acid payload to

25 form the lipid nanoparticle encapsulating the nucleic acid payload(s). After rapid in-line mixing, the LNPs thus formed may undergo further downstream processing including concentration and buffer exchange to achieve the final LNP pharmaceutical composition with near neutral pH for administration into cell line or animal diseases model for evaluation, or to administer to human subjects.

30 The LNP payload comprises a guide RNA targeting the TTR gene and an mRNA encoding a base editor protein. In some embodiments, the guide RNA to mRNA ratio in the acidic aqueous buffer and in the final formulation is 6:1, 5:1, 4:1, 3:1, 2.5:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:5 or 1:6 by weight. In some embodiments, the guide RNA to mRNA ratio in the acidic aqueous buffer and in the final formulation is about 1:1 by weight. In some

embodiment the mRNA encodes an adenosine base editor protein. In some other embodiments the mRNA encodes cytosine or a cytidine base editor protein.

Exemplary LNP compositions are described in Examples 1, 4, and 5 below.

5 **Ionizable lipids**

Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that comprise one or more ionizable lipids as described herein. An ionizable lipid as used herein comprises one or more ionizable nitrogen atoms or amine containing groups. In some embodiments, the ionizable nitrogen atom or amine
10 containing group is on the head group of the ionizable lipid.

For example, in some embodiments, compositions, preparations, nanoparticles, and/or nanomaterials having an ionizable lipid that is at about 50 mole percent or less, based on total moles of components of the lipid nanoparticle, may be useful and/or critical to functional activity of lipid nanoparticles such as desired tropisms, stabilization, and drug delivery
15 efficacy as described herein.

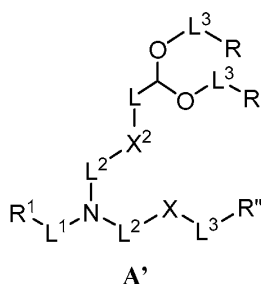
In some embodiments, an ionizable lipid is or comprises a compound described herein. In some embodiments, an ionizable lipid is present in a lipid nanoparticle (LNP) preparation from about 30 mole percent to about 70 mole percent, based on total moles of components of the lipid nanoparticle. In some embodiments, an ionizable lipid is present
20 from about 33 mole percent to about 60 mole percent, based on total moles of components of the lipid nanoparticle. In some embodiments, an ionizable lipid is present from about 34 mole percent to about 55 mole percent, based on total moles of components of the lipid nanoparticle. In some embodiments, an ionizable lipid is present from about 33 mole percent to about 51 mole percent, based on total moles of components of the lipid nanoparticle. In
25 some embodiments, an ionizable lipid is present at about 34.7 mole percent, based on total moles of components of the lipid nanoparticle. In some embodiments, an ionizable lipid is present at about 50 mole percent, based on total moles of components of the lipid nanoparticle. In some embodiments, an ionizable lipid is present at about 40 mole percent to about 60 mole percent or about 45 mole percent to about 55 mole percent, based on total
30 moles of components of the lipid nanoparticle.

Exemplary, non-limiting ionizable lipids suitable for the compositions described herein include those described herein.

Exemplary Lipids of WO2022140252

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022140252, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae A', A, I', I, II', II, III', III, I'-a, I'-a, I-a, I'-a-i, I'-a-ii, I'-a-iii, I'-b, I'-b, I-b, I'-b-i, I'-b-ii, I'-b-iii, I'-c, I'-c, I-c, I'-c-i, I'-c-ii, I'-c-iii, I'-d, I-d, I'-d-i, II-a, II-a-i, III-a, and III-a-i of WO2022140252, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids of **Table 1** of WO2022140252, including any of the lipids represented by Examples 7-1 to 7-253 and Examples 8-1 to 8-106, or a pharmaceutically acceptable salt or solvate thereof.

In some embodiments, an ionizable lipid is according to Formula A' of WO2022140252:



or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein

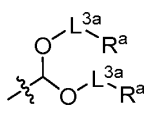
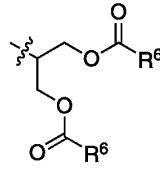
L¹ is absent, C₁₋₆ alkylenyl, or C₂₋₆ heteroalkylenyl;

each L² is independently optionally substituted C₂₋₁₅ alkylenyl, or optionally substituted C₃₋₁₅ heteroalkylenyl;

L is C₁₋₁₀ alkylenyl, or C₂₋₁₀ heteroalkylenyl;

X² is -OC(O)-, -C(O)O-, or -OC(O)O-;

X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;

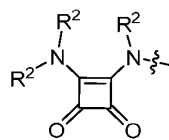
R'' is hydrogen, , , or an optionally substituted group selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged

bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

each of R and R^a is independently hydrogen, or an optionally substituted group selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl

each of L³ and L^{3a} is independently absent, optionally substituted C₁₋₁₀ alkylenyl, or optionally substituted C₂₋₁₀ heteroalkylenyl;

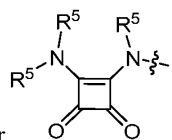
R¹ is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, -OR², -C(O)OR², -C(O)SR², -OC(O)R², -OC(O)OR², -CN, -N(R²)₂, -C(O)N(R²)₂, -S(O)₂N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR², -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -N(OR²)C(O)N(R²)₂, -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂, -C(NR²)R², -C(O)N(R²)OR², -C(R²)N(R²)₂C(O)OR², -CR²(R³)₂, -OP(O)(OR²)₂, or -P(O)(OR²)₂; or



R¹ is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R² or R³ groups;

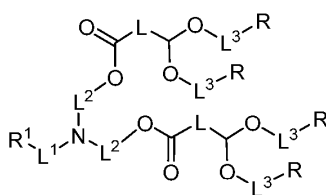
each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-

- membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or
- two occurrences of R^2 , taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional
- 5 heteroatom selected from nitrogen, oxygen, and sulfur;
- each R^3 is independently $-(CH_2)_n-R^4$; or
- two occurrences of R^3 , taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- 10 each R^4 is independently hydrogen, $-OR^5$, $-N(R^5)_2$, $-OC(O)R^5$, $-OC(O)OR^5$, $-CN$, $-C(O)N(R^5)_2$, $-NR^5C(O)R^5$, $-OC(O)N(R^5)_2$, $-N(R^5)C(O)OR^5$, $-NR^5S(O)_2R^5$, $-NR^5C(O)N(R^5)_2$,



- $-NR^5C(S)N(R^5)_2$, $-NR^5C(NR^5)N(R^5)_2$, or
- each R^5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or
- 15 two occurrences of R^5 , taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- each R^6 is independently C_{4-12} aliphatic; and
- each n is independently 0 to 4.

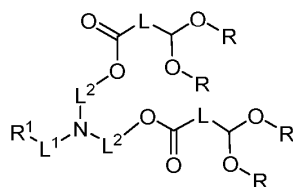
- 20 In some embodiments, an ionizable lipid is according to Formula III-a of WO2022140252:



III-a

- or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein each of R , R^1 , L , L^1 , L^2 , L^3 is as defined therein for any of Formulae A', A, III', and III, and described in
- 25 classes and subclasses above and herein, both singly and in combination. In embodiments of Formula III-a, each of R , R^1 , L , L^1 , L^2 , L^3 is as defined herein for Formula A' above.

In some embodiments, an ionizable lipid is according to Formula III-a-*i* of WO2022140252:

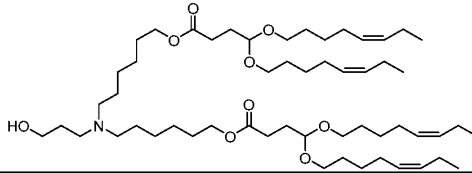
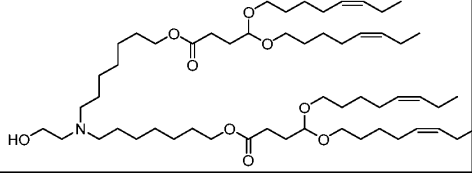
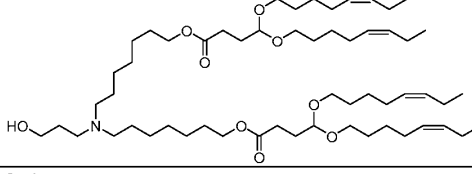
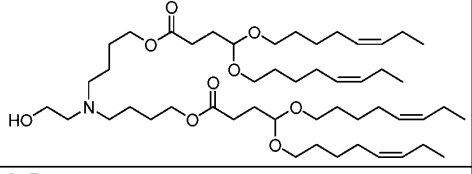
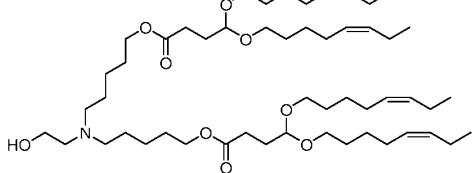
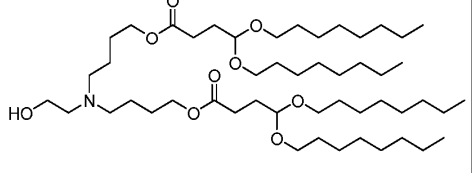
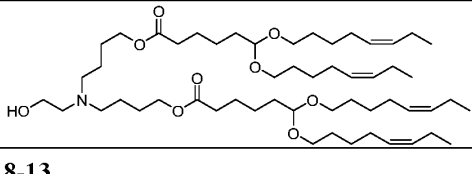
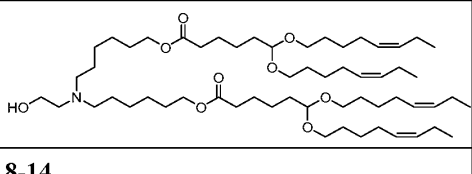
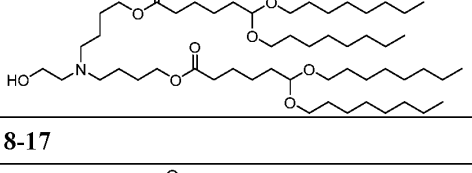
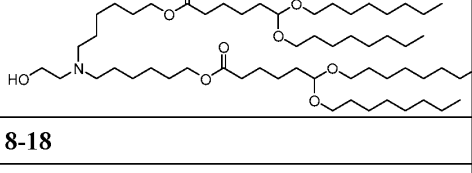
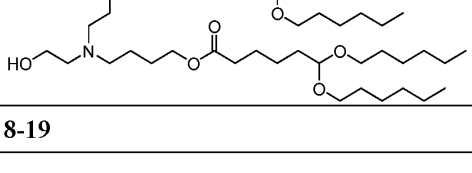
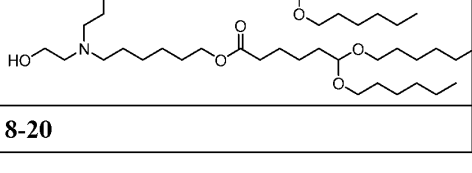


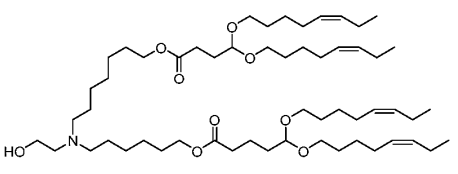
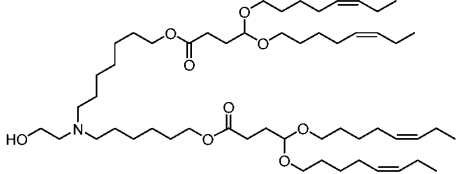
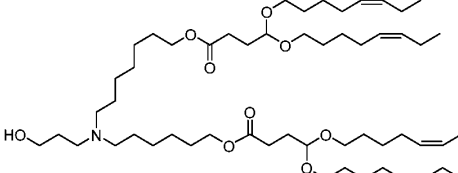
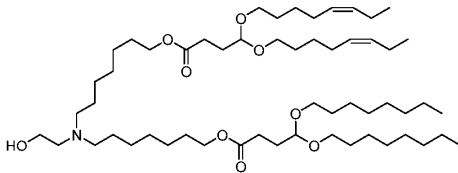
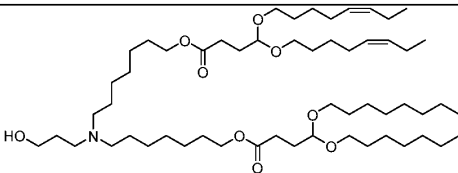
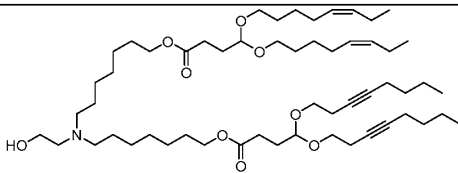
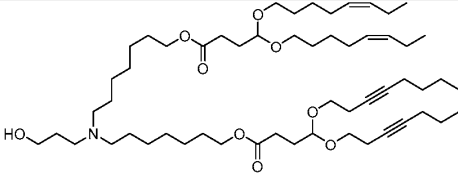
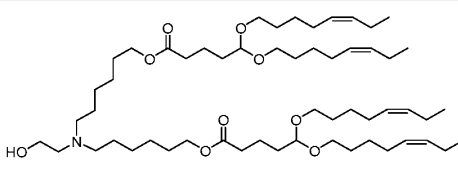
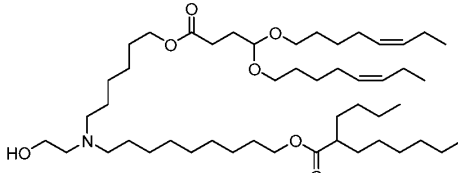
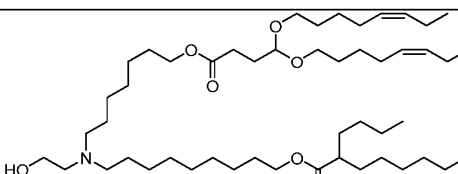
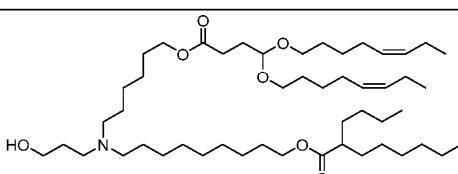
III-a-*i*

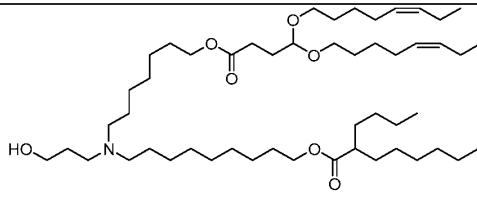
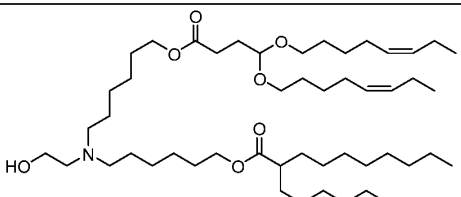
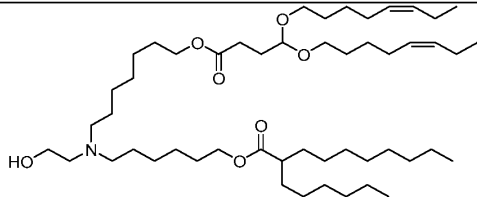
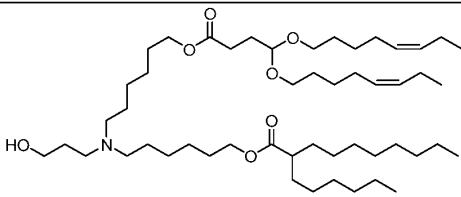
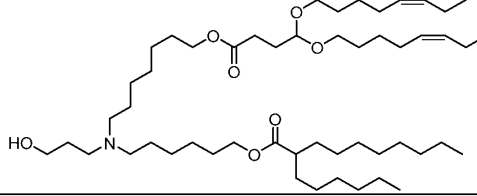
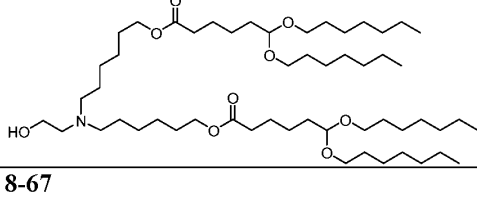
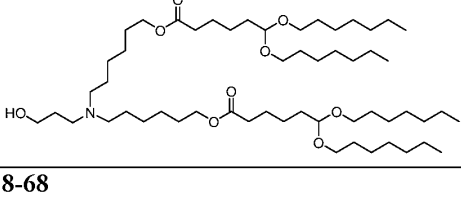
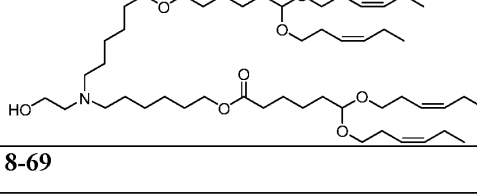
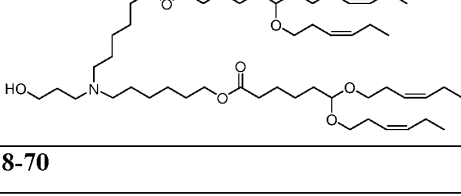
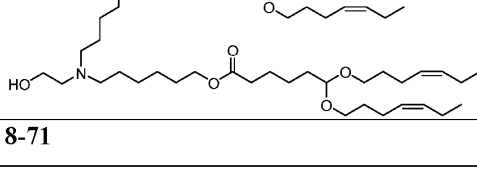
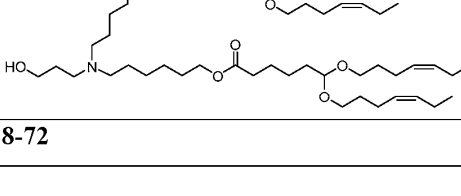
- 5 or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein each of R, R¹, L, L¹, and L² is as defined therein for any of Formulae A', A, III', and III, and described in classes and subclasses above and herein, both singly and in combination. In embodiments of Formula III-a-*i*, each of R, R¹, L, L¹, and L² is as defined herein for Formula A' above.

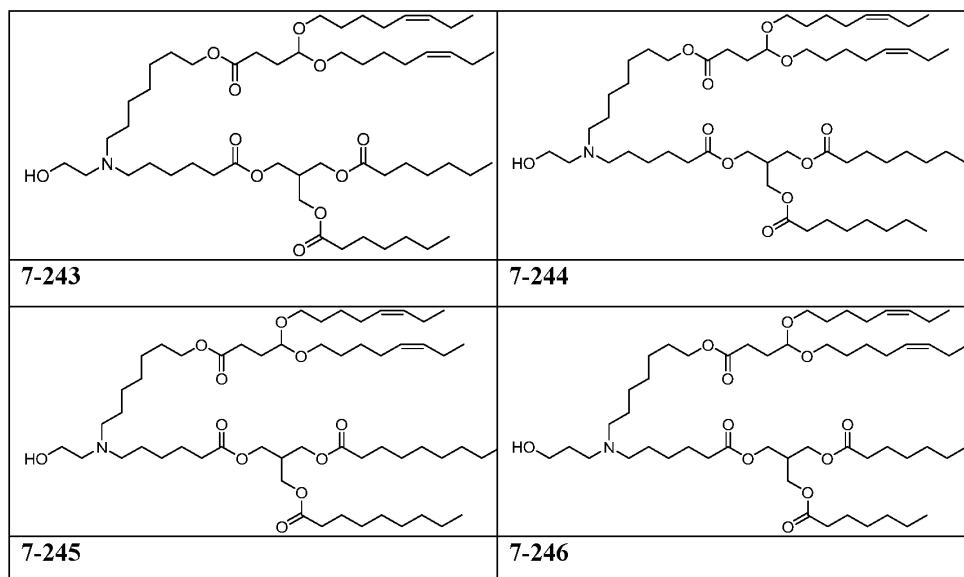
- 10 In some embodiments, an ionizable lipid is selected from any of the lipids described in **Table 1** of WO2022140252, or its N-oxide, or a pharmaceutically acceptable salt thereof. In embodiments, an ionizable lipid is selected from the group consisting of:

7-1	7-2
7-19	7-20
7-22	7-24

7-25	8-1
	
8-2	8-3
	
8-4	8-5
	
8-6	8-7
	
8-13	8-14
	
8-17	8-18
	
8-19	8-20

	
8-55	8-57
	
8-58	8-59
	
8-60	8-61
	
8-62	8-63
	
	7-232
	
7-233	7-234

	
7-235	7-236
	
7-237	7-238
	
7-239	
	
8-67	8-68
	
8-69	8-70
	
8-71	8-72



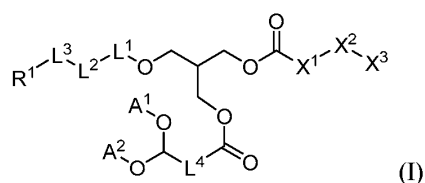
In some embodiments, an ionizable lipid is Example 7-1, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-2, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-19, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-20, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-22, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-24, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 7-25, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-1, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-2, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-3, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-4, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-5, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-13, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-14, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-17, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-18, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 8-19, or a pharmaceutically acceptable salt thereof. In some

[illegible]

Exemplary Lipids of WO2022159472

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022159472, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I, II, III, IIIA, IIIB, IIIC, IV, V, VA, VI, VIA, VII, and VIIA of WO2022159472, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids of **Table 1** of WO2022159472, including any of the lipids represented by Examples 4-1 to 4-86, or a pharmaceutically acceptable salt or solvate thereof.

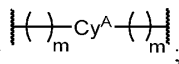
In some embodiments, an ionizable lipid is according to Formula I of WO2022159472:



or a pharmaceutically acceptable salt thereof, wherein:

L^1 is a covalent bond, $-C(O)-$, or $-OC(O)-$;

L^2 is a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or

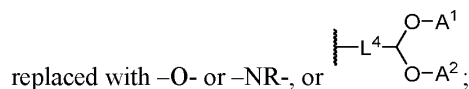


Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered saturated or partially unsaturated carbocyclene;

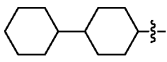
each m is independently 0, 1, or 2;

L^3 is a covalent bond, $-C(O)-$, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;

R^1 is $-(CH_2)_p-Cy^B-$, an optionally substituted saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain wherein 1-3 methylene units are optionally and independently



Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or partially

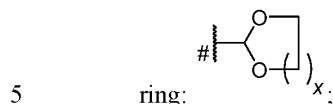
unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and phenyl;

p is 0, 1, 2, or 3;

each L^4 is independently a bivalent saturated or unsaturated, straight or branched C_1-C_6 hydrocarbon chain;

each A^1 and A^2 is independently an optionally substituted C_1-C_{20} aliphatic or $-L^5-R^5$;

or A^1 and A^2 , together with their intervening atoms, may form an optionally substituted



where

x is selected from 1 or 2; and

represents the point of attachment to L^4 ;

each L^5 is independently a bivalent saturated or unsaturated, straight or branched C_1-C_{20}

10 hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

each R^5 is independently an optionally substituted group selected from a 5- to 10-membered aryl ring or a 3- to 8-membered carbocyclic ring ;

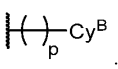
X^1 is a covalent bond, $-O-$, or $-NR-$;

15 X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C_1-C_{12} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$, $-NR-$, or $-Cy^C-$;

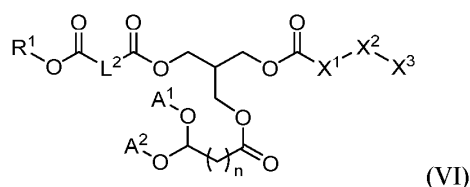
Cy^C is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated
20 heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially
25 unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C_1-C_6 aliphatic group;

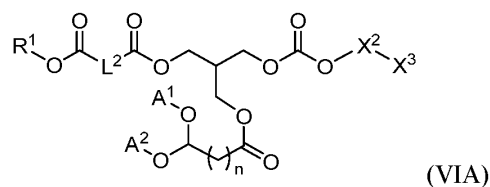
provided that when L^3 is a covalent bond, then R^1 must be .

30 In some embodiments, an ionizable lipid is according to Formula VI of WO2022159472:



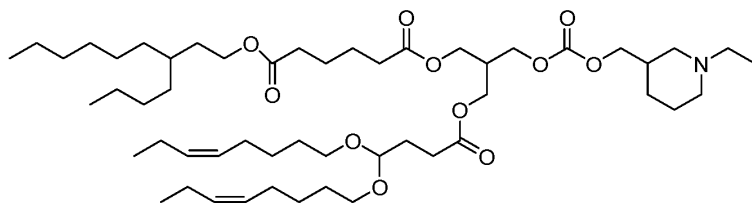
- or a pharmaceutically acceptable salt thereof, wherein n is 1, 2, 3 or 4, and L^2 , R^1 , A^1 , A^2 , X^1 , X^2 , and X^3 are as defined therein for Formula I and also described in classes and subclasses therein, both singly and in combination. In embodiments, L^2 , R^1 , A^1 , A^2 , X^1 , X^2 , and X^3 are as defined herein for Formula I above.

In some embodiments, an ionizable lipid is according to Formula VIA of WO2022159472:

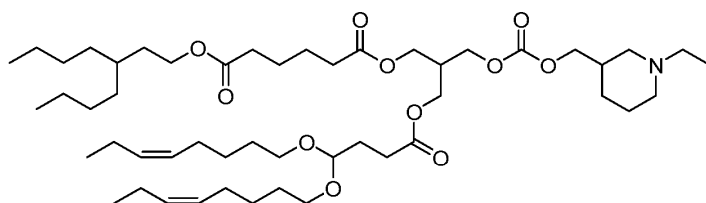


- or a pharmaceutically acceptable salt thereof, wherein n is 1, 2, 3 or 4, and L^2 , R^1 , A^1 , A^2 , X^2 , and X^3 are as defined therein for Formula I and also described in classes and subclasses therein, both singly and in combination. In embodiments, L^2 , R^1 , A^1 , A^2 , X^2 , and X^3 are as defined herein for Formula I above.

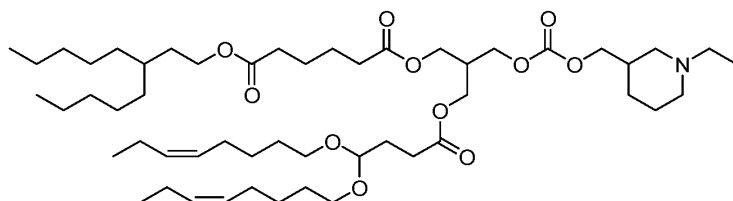
- In some embodiments, an ionizable lipid is selected from any of the lipids described in Table 1 of WO2022159472, or a pharmaceutically acceptable salt thereof. In embodiments, an ionizable lipid is selected from the group consisting of:



Example 4-62

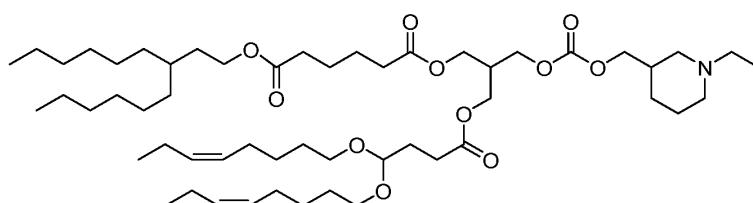


Example 4-63

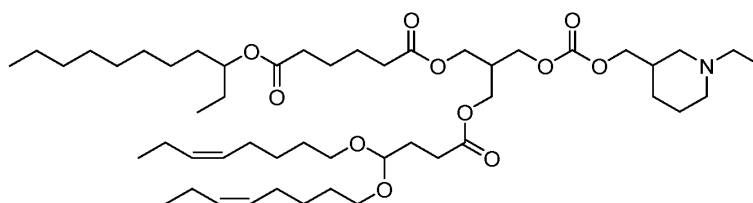


Example 4-64

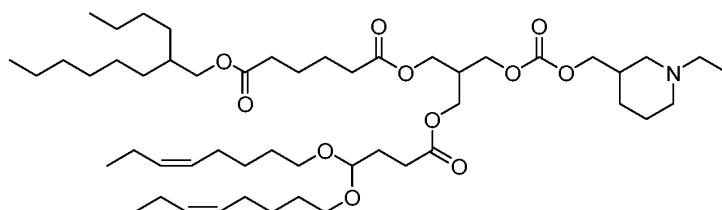
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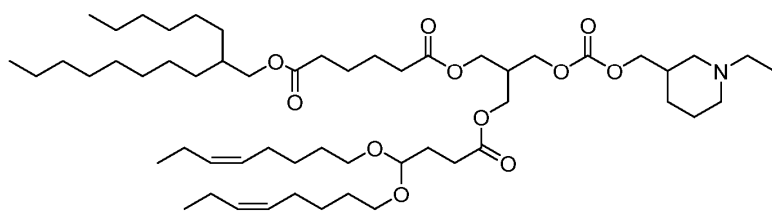
Example 4-65



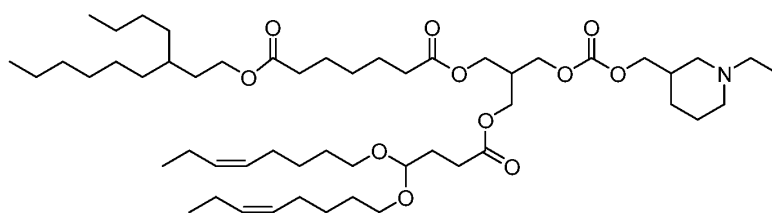
10 Example 4-66



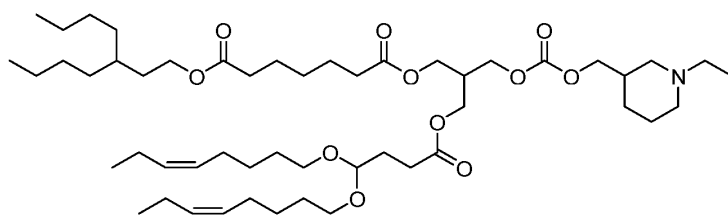
Example 4-67



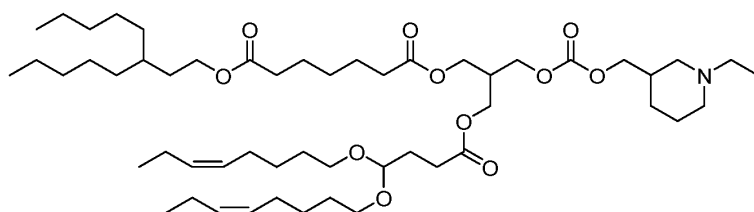
Example 4-68



5 Example 4-69

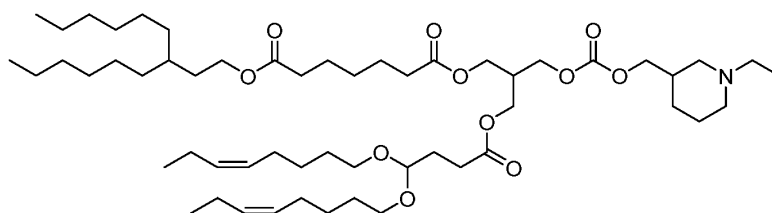


Example 4-70

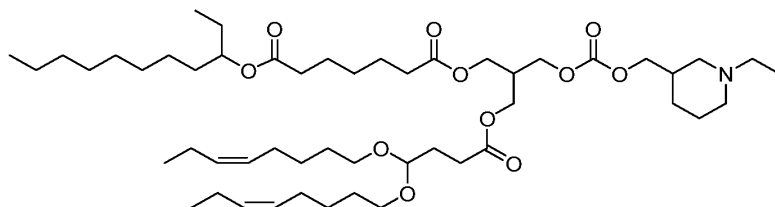


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Example 4-71

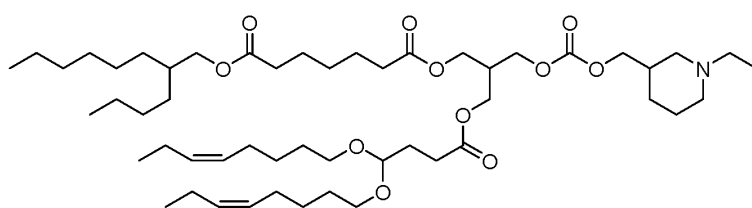


Example 4-72

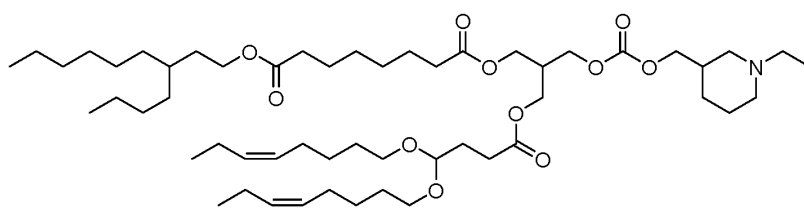


Example 4-73

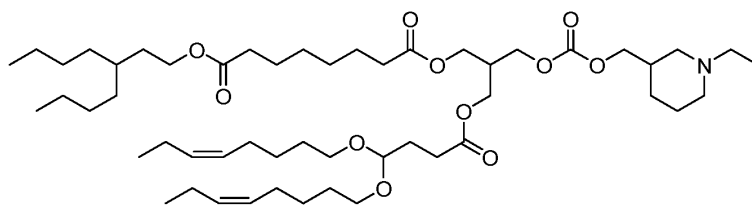
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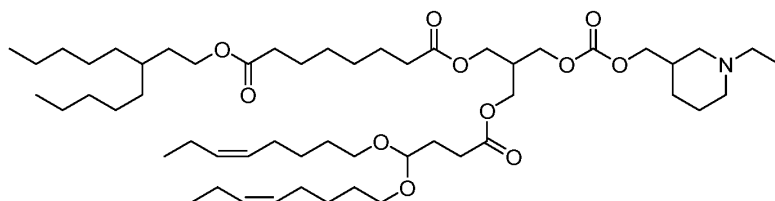
Example 4-74



10 Example 4-75

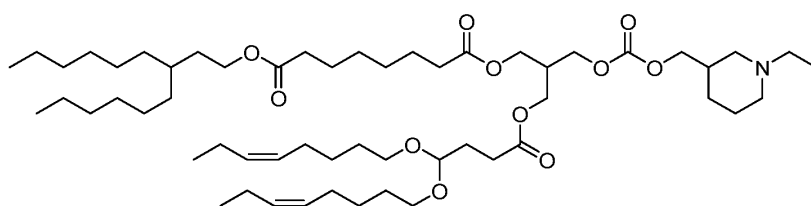


Example 4-76

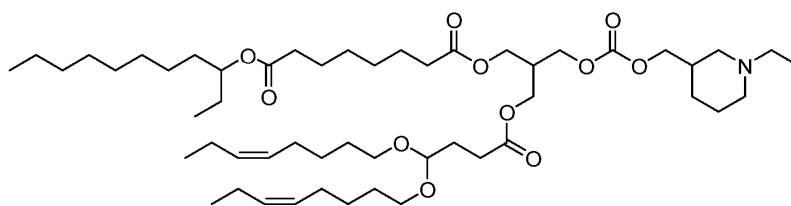


Example 4-77

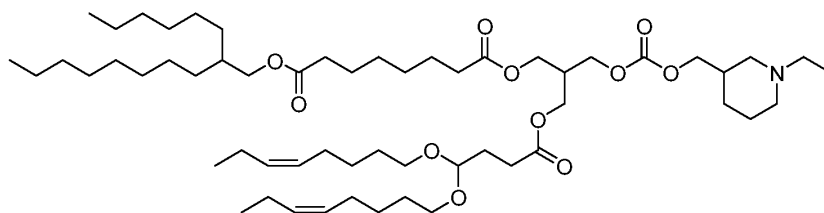
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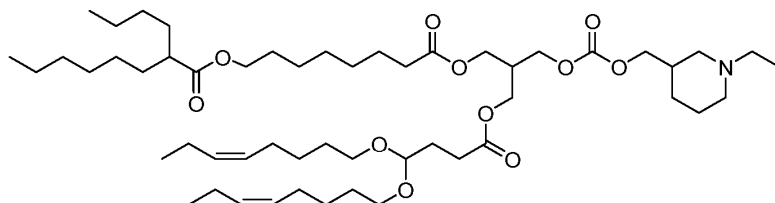
Example 4-78



10 Example 4-79

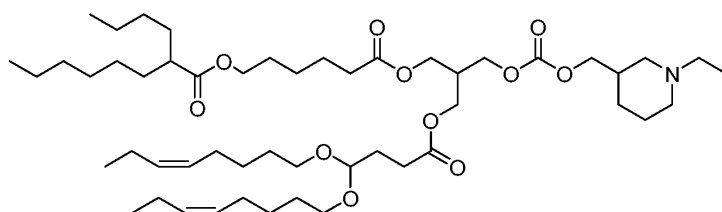


Example 4-80

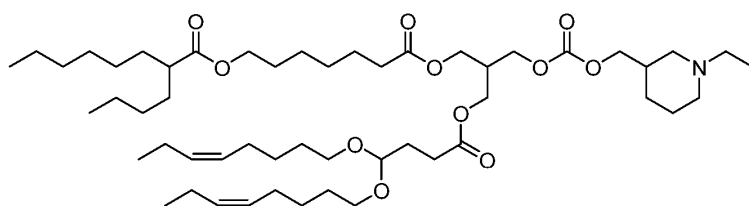


Example 4-81

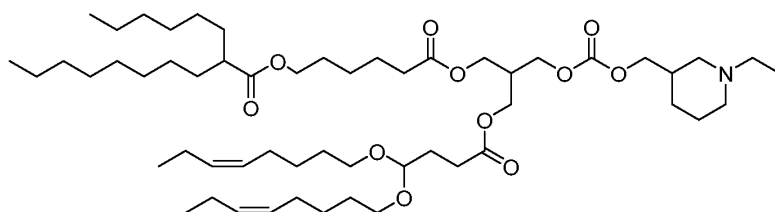
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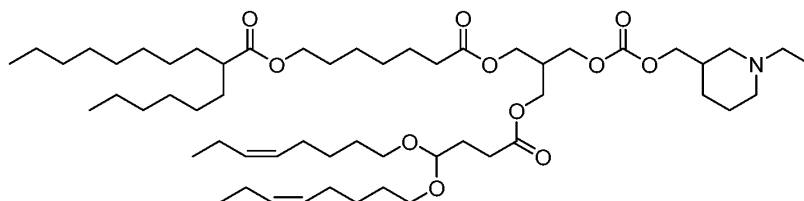


Example 4-82

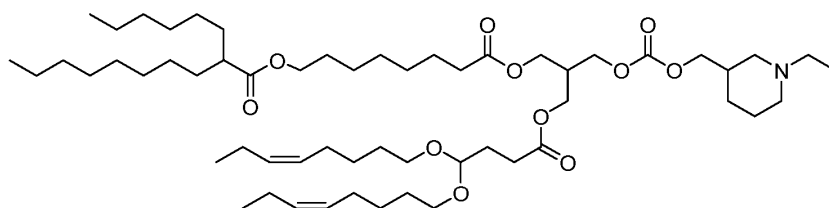


10 Example 4-83



Example 4-84**Example 4-85**

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**Example 4-86**

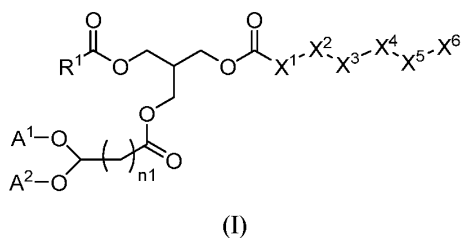
In some embodiments, an ionizable lipid is Example 4-62, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-63, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-64, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-65, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-66, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-67, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-68, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-69, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-70, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-71, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-72, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-73, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-74, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-75, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-76, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-77, or a pharmaceutically acceptable salt thereof. In some embodiments, an

ionizable lipid is Example 4-78, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-79, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-80, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-81, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-82, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-83, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-84, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-85, or a pharmaceutically acceptable salt thereof. In some embodiments, an ionizable lipid is Example 4-86, or a pharmaceutically acceptable salt thereof.

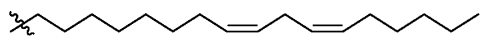
Exemplary Lipids of WO 2021141969

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2021141969, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I of WO2021141969, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of WO 2021141969.

In some embodiments, an ionizable lipid is according to a compound of Formula I of WO2021141969:



In various embodiments, the compound of Formula (I) is an ionizable lipid as described elsewhere herein. In various embodiments, R¹ in Formula (I) is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation. For example, in some embodiments R¹ in Formula (I) is C₉-C₂₀ alkenyl with 2 units of unsaturation, such as a C₁₇ alkenyl group of the formula



In various embodiments, X^1 and X^2 in Formula (I) are each independently absent or selected from $-\text{O}-$, $-\text{NR}^2-$, and $-\text{X}^7-$, wherein X^7 is $-(\text{CH})_a-$, wherein R^2 is hydrogen or $\text{C}_1\text{-C}_6$ alkyl, a is an integer between 1 and 6, X^7 is independently hydrogen, hydroxyl or $-\text{NR}^6\text{R}^7$, and R^6 and R^7 are each independently hydrogen or $\text{C}_1\text{-C}_6$ alkyl; or alternatively R^6 and R^7 join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen. In some embodiments, X^1 is absent, X^2 is absent, or both X^1 and X^2 are absent. As described elsewhere herein, $X^1\text{-X}^2\text{-X}^3\text{-X}^4$ does not contain any oxygen-oxygen, oxygen-nitrogen or nitrogen-nitrogen bonds to one another. Accordingly, X^1 and X^2 cannot both be $-\text{O}-$ and cannot both be $-\text{NR}^2-$. Similarly, X^1 and X^2 cannot be $-\text{O}-$ and $-\text{NR}^2-$, respectively, nor $-\text{NR}^2-$ and $-\text{O}-$, respectively.

In various embodiments, X^1 is $-\text{O}-$. In various embodiments, X^2 is $-\text{O}-$. In some embodiments, X^1 is $-\text{X}^7-$, such as $-(\text{CH}_2)_a-$, $-\text{CH}(\text{OH})-$ or $-(\text{CH}_2)_{a-1}\text{CH}(\text{OH})-$. In some embodiments, X^2 is $-\text{X}^7-$, such as $-(\text{CH}_2)_a-$, $-\text{CH}(\text{OH})-$ or $-(\text{CH}_2)_{a-1}\text{CH}(\text{OH})-$. In various embodiments, each a is independently 1, 2, 3, 4, 5 or 6. In various embodiments, X^1 is $-\text{NR}^6\text{R}^7$. In various embodiments, X^2 is $-\text{NR}^6\text{R}^7$. In some embodiments, R^6 is hydrogen or $\text{C}_1\text{-C}_6$ alkyl. In some embodiments, R^7 is hydrogen or $\text{C}_1\text{-C}_6$ alkyl. In other embodiments, R^6 and R^7 join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups. In some embodiments, the 4- to 7-membered heterocyclyl formed by the joining together of R^6 and R^7 optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen.

In various embodiments, X^3 and X^4 in Formula (I) are each independently absent or selected from:

- (1) 4- to 8-membered heterocyclyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups;
- (2) 5- to 6-membered heteroaryl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups;
- (3) 5- to 6-membered aryl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups;
- (4) 4- to 7-membered cycloalkyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ alkyl groups;

(5) $-\text{O}-$; or

(6) $-\text{NR}^3-$, wherein each R^3 is independently a hydrogen atom or $\text{C}_1\text{-C}_6$ alkyl.

In some embodiments, X^3 is absent, X^4 is absent, or both X^3 and X^4 are absent. As described elsewhere herein, $\text{X}^1\text{-X}^2\text{-X}^3\text{-X}^4$ does not contain any oxygen-oxygen, oxygen-nitrogen or nitrogen-nitrogen bonds to one another. Accordingly, X^2 and X^3 cannot both be $-\text{O}-$. When X^2 is $-\text{O}-$ or $-\text{NR}^2-$ then X^3 cannot be $-\text{NR}^3-$. Similarly, when X^3 is $-\text{O}-$ or $-\text{NR}^3-$ then X^2 cannot be $-\text{NR}^2-$. Likewise, X^3 and X^4 cannot both be $-\text{O}-$ and cannot both be $-\text{NR}^3-$. Similarly, X^3 and X^4 cannot be $-\text{O}-$ and $-\text{NR}^3-$, respectively, nor $-\text{NR}^3-$ and $-\text{O}-$, respectively.

In various embodiments, X^3 and X^4 in Formula (I) are each independently a 4- to 8-membered heterocyclyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ or $\text{C}_1\text{-C}_3$ alkyl groups. For example, in various embodiments X^3 and X^4 are each independently azetidiny, methylazetidiny, pyrrolidiny, methylpyrrolidiny, piperidiny, methylpiperidiny, piperaziny, methylpiperaziny, dimethylpiperaziny, morpholiny, diazepany, methyldiazepany, octahydro-2H-quinoliziny, azabicyclo[3.2.1]octyl, methylazabicyclo[3.2.1]octyl, diazaspiro[3.5]nonyl or methyldiazaspiro[3.5]nonyl.

In various embodiments, X^3 and X^4 in Formula (I) are each independently a 5- to 6-membered heteroaryl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ or $\text{C}_1\text{-C}_3$ alkyl groups. For example, in various embodiments X^3 and X^4 are each independently pyrrolyl, methylpyrrolyl, imidazolyl, methylimidazolyl, pyridiny, or methylpyridiny.

In various embodiments, X^3 and X^4 in Formula (I) are each independently a 5- to 6-membered aryl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ or $\text{C}_1\text{-C}_3$ alkyl groups. For example, in various embodiments X^3 and X^4 are each independently phenyl, methylphenyl, naphthyl or methylnaphthyl.

In various embodiments, X^3 and X^4 in Formula (I) are each independently a 4- to 7-membered cycloalkyl optionally substituted with 1 or 2 $\text{C}_1\text{-C}_6$ or $\text{C}_1\text{-C}_3$ alkyl groups. For example, in various embodiments X^3 and X^4 are each independently cyclopentyl, methylcyclopentyl, cyclohexyl, or methylcyclohexyl.

In various embodiments, X^3 in Formula (I) is $-\text{O}-$. In other embodiments, X^4 in Formula (I) is $-\text{O}-$. In various embodiments, X^3 is $-\text{NR}^3-$, wherein R^3 is a hydrogen atom or $\text{C}_1\text{-C}_6$ alkyl, such as a $\text{C}_1\text{-C}_3$ alkyl. For example, in various embodiments X^3 is $-\text{N}(\text{CH}_3)-$, $-\text{N}(\text{CH}_2\text{CH}_3)-$, or $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)-$. In other embodiments, X^4 is $-\text{NR}^3-$, wherein R^3 is a hydrogen atom or $\text{C}_1\text{-C}_6$ alkyl, such as a $\text{C}_1\text{-C}_3$ alkyl. For example, in various embodiments X^4 is $-\text{N}(\text{CH}_3)-$, $-\text{N}(\text{CH}_2\text{CH}_3)-$, or $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)-$.

In various embodiments, X^5 in Formula (I) is $-(CH_2)_b-$, wherein b is an integer between 0 and 6. In some embodiments, b is 0, in which case X^5 is absent. In other embodiments, b is 1, 2, 3, 4, 5 or 6.

In various embodiments, X^6 in Formula (I) is hydrogen, C_1 - C_6 alkyl, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C_1 - C_6 alkyl groups, or $-NR^4R^5$. In some embodiments, R^4 and R^5 are each independently hydrogen or C_1 - C_6 alkyl. Alternatively, in other embodiments R^4 and R^5 join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C_1 - C_6 alkyl groups, wherein the 4- to 7-membered heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen.

In various embodiments of Formula (I), at least one of X^1 , X^2 , X^3 , X^4 , and X^5 is present. For example, in various embodiments at least two of X^1 , X^2 , X^3 , X^4 , and X^5 are present in Formula (I). In other embodiments, at least three of X^1 , X^2 , X^3 , X^4 , and X^5 are present in Formula (I). For example, in some embodiments, at least four of X^1 , X^2 , X^3 , X^4 , and X^5 are present in Formula (I). In other embodiments, all of X^1 , X^2 , X^3 , X^4 , and X^5 are present in Formula (I).

In some embodiments, X^6 is hydrogen. In other embodiments, X^6 is C_1 - C_6 alkyl, such as C_1 - C_3 alkyl (e.g., methyl, ethyl or propyl). In other embodiments, X^6 is 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C_1 - C_6 alkyl groups. For example, in various embodiments X^6 is pyrrolyl, methylpyrrolyl, imidazolyl, methylimidazolyl, pyridinyl, or methylpyridinyl. In other embodiments, X^6 is $-NR^4R^5$. For example, in some embodiments X^6 is $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$, $-NHCH_2CH_2CH_3$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, or $-N(CH_2CH_2CH_3)_2$. Alternatively, in other embodiments, R^4 and R^5 join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl. The 4- to 7-membered heterocyclyl can be optionally substituted with 1 or 2 C_1 - C_6 alkyl groups, such as C_1 - C_3 alkyl, and/or the 4- to 7-membered heterocyclyl can optionally include an additional heteroatom selected from oxygen, sulfur, and nitrogen. For example, in some embodiments X^6 is azetidiny, methylazetidiny, pyrrolidinyl, methylpyrrolidinyl, piperidinyl, methylpiperidinyl, piperazinyl, methylpiperazinyl, dimethylpiperazinyl, morpholinyl, diazepanyl, or methyl diazepanyl.

In various embodiments, each X^7 in Formula (I) is hydrogen. In other embodiments, each X^7 is hydroxyl. In other embodiments, each X^7 is $-NR^6R^7$. For embodiments in which a is between 2 and 6, each X^7 can be the same or different. For example, in various embodiments X^7 is $-(CH_2)_{a-1}CH(X^7)-$, where a is 2, 3, 4, 5 or 6. In some embodiments for

which X^7 is $-NR^6R^7$, R^6 and R^7 are each independently hydrogen or C₁-C₆ alkyl, such as C₁-C₃ alkyl. For example, in some embodiments X^7 is $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$, $-NHCH_2CH_2CH_3$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, or $-N(CH_2CH_2CH_3)_2$. Alternatively, in some embodiments for which X^7 is $-NR^6R^7$, R^6 and R^7 join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups. Alternatively, in other embodiments for which X^7 is $-NR^6R^7$, the R^6 and R^7 can join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl. The 4- to 7-membered heterocyclyl can be optionally substituted with 1 or 2 C₁-C₆ alkyl groups, such as C₁-C₃ alkyl, and/or the 4- to 7-membered heterocyclyl can optionally include an additional heteroatom selected from oxygen, sulfur, and nitrogen. For example, in some embodiments X^6 is azetidiny, methylazetidiny, pyrrolidiny, methylpyrrolidiny, piperidiny, methylpiperidiny, piperaziny, methylpiperaziny, dimethylpiperaziny, morpholiny, diazepany, or methyldiazepany.

In various embodiments, A^1 and A^2 in Formula (I) are each independently selected from:

- (1) C₅-C₁₂ haloalkyl;
- (2) C₅-C₁₂ alkenyl;
- (3) C₅-C₁₂ alkynyl;
- (4) (C₅-C₁₂ alkoxy)-(CH₂)_{n2}-;
- (5) (C₅-C₁₀ aryl)-(CH₂)_{n3}- optionally ring substituted with one or two halo, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ alkoxy groups; and
- (6) (C₃-C₈ cycloalkyl)-(CH₂)_{n4}- optionally ring substituted with 1 or 2 C₁-C₆ alkyl groups;

or alternatively A^1 and A^2 join together with the atoms to which they are bound to form a 5- to 6-membered cyclic acetal substituted with 1 or 2 C₄-C₁₀ alkyl groups.

In various embodiments of Formula (I), n_1 , n_2 and n_3 are each individually an integer between 1 and 4 (i.e., 1, 2, 3 or 4), and n_4 is an integer between zero and 4 (i.e., 0, 1, 2, 3 or 4). In various embodiments, A^1 and A^2 have the same chemical structure.

In various embodiments of Formula (I), A^1 and A^2 are each independently a C₅-C₁₂ haloalkyl. For example, in various embodiments the C₅-C₁₂ haloalkyl is a C₅-C₁₂ fluoroalkyl such as a C₆ fluoroalkyl, a C₇ fluoroalkyl, a C₈ fluoroalkyl, a C₉ fluoroalkyl, a C₁₀ fluoroalkyl, a C₁₁ fluoroalkyl, or a C₁₂ fluoroalkyl. The number of halogen atoms attached to the C₅-C₁₂ haloalkyl can vary over a broad range, depending on the length of the alkyl chain and the degree of halogenation. For example, in various embodiments the C₅-C₁₂ haloalkyl contains

between 1 and 25 halogen atoms, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 halogen atoms. In various embodiments, the C₅-C₁₂ haloalkyl is a C₅-C₁₂ fluoroalkyl that comprises a fluorinated end group such as CF₃(CF₂)_{n5}–, where n₅ is an integer in the range of 0 to 5. For example in various embodiments the C₅-C₁₂ fluoroalkyl is CF₃(CF₂)_{n5}(CH₂)_{n6}–, where n₅ is an integer in the range of 0 to 5, n₆ is an integer in the range of 0 to 11, and n₅ + n₆ + 1 is equal to number of carbons in the C₅-C₁₂ fluoroalkyl.

In various embodiments of Formula (I), A¹ and A² are each independently a C₅-C₁₂ alkenyl. The position of the alkenyl double bond(s) can vary. For example, in various embodiments the C₅-C₁₂ alkenyl is CH₃CH₂CH=CH(CH₂)_{n7}–, where n₇ is an integer in the range of 1 to 8, such as CH₃CH₂CH=CH(CH₂)₄–. In some embodiments, the C₅-C₁₂ alkenyl is branched, such as, for example, (CH₃)₂C=CH(CH₂)_{n8}–CH(CH₃)–(CH₂)_{n9}– wherein n₈ and n₉ are each independently 1, 2 or 3.

In various embodiments of Formula (I), A¹ and A² are each independently a C₅-C₁₂ alkynyl. The position of the alkynyl triple bond(s) can vary. For example, in various embodiments the C₅-C₁₂ alkynyl is CH₃CH₂C≡C(CH₂)_{n10}–, where n₁₀ is an integer in the range of 1 to 8, such as CH₃CH₂C≡C(CH₂)₄–. In some embodiments, the C₅-C₁₂ alkynyl is branched, such as, for example, (CH₃)₂CHC≡C(CH₂)_{n11}–CH(CH₃)–(CH₂)_{n12}– wherein n₁₁ and n₁₂ are each independently 1, 2 or 3 and n₁₁ + n₁₂ is in the range of 2 to 5.

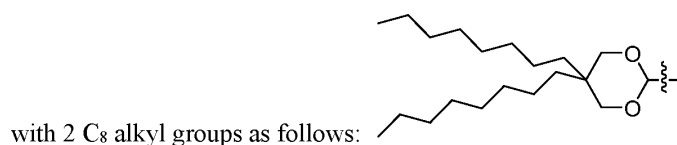
In various embodiments of Formula (I), A¹ and A² are each independently a (C₅-C₁₂ alkoxy)-(CH₂)_{n2}–. In various embodiments, each n₂ is independently an integer in the range of 1 to 4 (i.e., 1, 2, 3 or 4). The position of the oxygen(s) can vary. For example, in various embodiments the (C₅-C₁₂ alkoxy)-(CH₂)_{n2}– is CH₃O(CH₂)_{n13}–(CH₂)_{n2}–, where n₁₃ is an integer in the range of 1 to 11, such as CH₃O(CH₂)₇–. In other embodiments the (C₅-C₁₂ alkoxy)-(CH₂)_{n2}– is CH₃(CH₂)_{n14}–O–(CH₂)_{n15}–(CH₂)_{n2}–, wherein n₁₄ and n₁₅ are each independently integers between 1 and 8, and n₁₄ + n₁₅ is an integer in the range of 4 to 11, such as CH₃(CH₂)₇–O–(CH₂)₂–(CH₂)_{n2}–. In some embodiments, the C₅-C₁₂ alkoxy is branched, such as, for example, CH₃O(CH₂)_{n16}–CH(CH₃)–(CH₂)_{n17}–(CH₂)_{n2}–, wherein n₁₆ and n₁₇ are each independently 1, 2, 3, 4 or 5 and n₁₆ + n₁₇ is an integer in the range of 2 to 9.

In various embodiments of Formula (I), A¹ and A² are each independently a (C₅-C₁₀ aryl)-(CH₂)_{n3}– optionally ring substituted with one or two halo, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ alkoxy groups. In various embodiments, each n₃ is independently an integer between 1 and 4 (i.e., 1, 2, 3 or 4). In some embodiments, the C₅-C₁₀ aryl is a phenyl. For example, in various embodiments the (C₅-C₁₀ aryl)-(CH₂)_{n3}– is C₆H₅–(CH₂)_{n3}– optionally

ring substituted with one or two halo, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ alkoxy groups. In an embodiment, the optionally ring substituted (C₅-C₁₀ aryl)-(CH₂)_{n3}- is CF₃-C₆H₄-(CH₂)_{n3}-, such as CF₃-C₆H₄-CH₂- or CF₃-C₆H₄-(CH₂)₂-. In another embodiment, the optionally ring substituted (C₅-C₁₀ aryl)-(CH₂)_{n3}- is CH₃-(CH₂)_{n18}-C₆H₄-(CH₂)_{n2}-, wherein
 5 n18 is 1, 2 or 3 and n2 is 1, 2, 3 or 4, such as CH₃(CH₂)₃-C₆H₄-CH₂- or CH₃(CH₂)₃-C₆H₄-(CH₂)₂-.

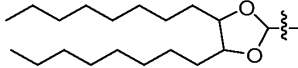
In various embodiments of Formula (I), A¹ and A² are each independently a (C₃-C₈ cycloalkyl)-(CH₂)_{n4}- optionally ring substituted with 1 or 2 C₁-C₆ alkyl groups. In various embodiments, each n4 is independently an integer between 0 and 4 (i.e., 0, 1, 2, 3 or 4). In
 10 some embodiments, the C₃-C₈ cycloalkyl is a cyclohexyl or cyclopentyl. For example, in various embodiments the (C₃-C₈ cycloalkyl)-(CH₂)_{n4}- is C₆H₁₁-(CH₂)_{n4}- optionally ring substituted with 1 or 2 C₁-C₆ alkyl groups, such as C₆H₁₁-(CH₂)₂-, C₆H₁₁-(CH₂)₃- or CH₃-C₆H₁₀-(CH₂)₃-.

Alternatively, in other embodiments of Formula (I), A¹ and A² join together with the
 15 atoms to which they are bound to form a 5- to 6-membered cyclic acetal substituted with 1 or 2 C₄-C₁₀ alkyl groups. For example, in an embodiment, A¹ and A² join together with the atoms to which they are bound to form a 6-membered cyclic acetal that is ring substituted



. In another embodiment, A¹ and A² join together with the atoms to which they are bound to form a 5-membered cyclic

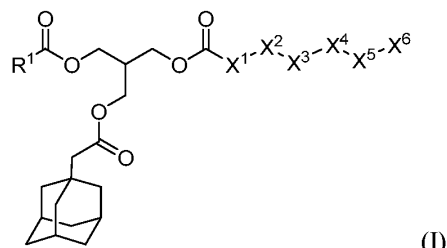
20 acetal that is ring substituted with 2 C₈ alkyl groups as follows:



Exemplary Lipids of WO2021113365

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication
 25 No. WO2021113365, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I of WO2021113365, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of WO2021113365.

In some embodiments, an ionizable lipid is according to a compound of Formula I of WO2021113365:



(I)

wherein:

5 R^1 is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation;

X^1 and X^2 are each independently absent or selected from -O-, NR², and $-(CH)_a-$,
 wherein R² is C₁-C₆ alkyl, and wherein X^1 and X^2 are not both -O- or NR²;

a is an integer between 1 and 6;

10 X^3 and X^4 are each independently absent or selected from the group consisting of: 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, and -NR³-,
 wherein each R³ is a hydrogen atom or C₁-C₆ alkyl;

X^5 is -(CH₂)_b-, wherein b is an integer between 0 and 6;

15 X^6 is hydrogen, C₁-C₆ alkyl, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, or -NR⁴R⁵, wherein R⁴ and R⁵ are each independently hydrogen or C₁-C₆ alkyl; or alternatively R⁴ and R⁵ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;

20 X^7 is hydrogen or -NR⁶R⁷, wherein R⁶ and R⁷ are each independently hydrogen or C₁-C₆ alkyl; or alternatively R⁶ and R⁷ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;

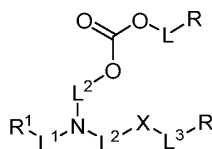
25 at least one of X^1 , X^2 , X^3 , X^4 , and X^5 is present; and

provided that when either X¹ or X² is -O-, neither X³ nor X⁴ is , and when either X¹ or X² is -O-, R⁴ and R⁵ are not both ethyl.

Exemplary Lipids of WO2022140239

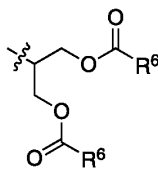
5 In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022140239, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I' of WO2022140239, or a pharmaceutically acceptable salt or solvate thereof. Exemplary
10 ionizable lipids also include any of the lipids represented by the Examples of WO2022140239.

In some embodiments, an ionizable lipid is according to a compound of Formula I' of WO2022140239:



I'

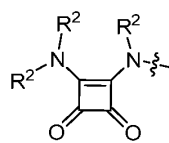
15 or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein
L¹ is absent, C₁₋₆ alkylenyl, or C₂₋₆ heteroalkylenyl;
each L² is independently optionally substituted C₂₋₁₅ alkylenyl, or optionally substituted C₃₋₁₅
heteroalkylenyl;
20 L is absent, optionally substituted C₁₋₁₀ alkylenyl, or optionally substituted C₂₋₁₀
heteroalkylenyl;
L³ is absent, optionally substituted C₁₋₁₀ alkylenyl, or optionally substituted C₂₋₁₀
heteroalkylenyl;
X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;



25 each R is independently hydrogen, , or an optionally substituted group
selected from C₆₋₂₀ aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered

bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

- R¹ is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3
 5 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, -OR², -C(O)OR², -C(O)SR², -OC(O)R², -OC(O)OR², -CN,
 10 -N(R²)₂, -C(O)N(R²)₂, -S(O)₂N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR², -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -N(OR²)C(O)N(R²)₂,
 -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂, -C(NR²)R², -C(O)N(R²)OR², -C(R²)N(R²)₂C(O)OR², -CR²(R³)₂, -OP(O)(OR²)₂, or
 15 -P(O)(OR²)₂; or

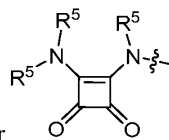


- R¹ is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R² or R³ groups;
 20 each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl comprising 1-3
 25 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or two occurrences of R², taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
 each R³ is independently -(CH₂)_n-R⁴; or

two occurrences of R^3 , taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

each R^4 is independently

- 5 hydrogen, $-OR^5$, $-N(R^5)_2$, $-OC(O)R^5$, $-OC(O)OR^5$, $-CN$, $-C(O)N(R^5)_2$, $-NR^5C(O)R^5$, $-OC(O)N(R^5)_2$, $-N(R^5)C(O)OR^5$, $-NR^5S(O)_2R^5$, $-NR^5C(O)N(R^5)_2$,



$-NR^5C(S)N(R^5)_2$, $-NR^5C(NR^5)N(R^5)_2$, or ;

each R^5 is independently hydrogen, or optionally substituted C_{1-6} aliphatic; or

two occurrences of R^5 , taken together with the atom(s) to which they are attached, form

- 10 optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

each R^6 is independently C_{4-12} aliphatic; and

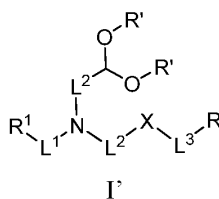
each n is independently 0 to 4.

15 Exemplary Lipids of WO2022140238

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022140238, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I' of

20 WO2022140238, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of WO2022140238.

In some embodiments, an ionizable lipid is according to a compound of Formula I' of WO2022140238:



25

or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein

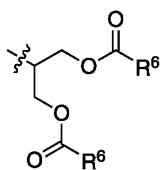
L^1 is absent, C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

each L^2 is independently optionally substituted C_{2-15} alkylene, or optionally substituted C_{3-15} heteroalkylene;

L^3 is absent, optionally substituted C_{1-10} alkylene, or optionally substituted C_{2-10} heteroalkylene;

5 X is absent, $-OC(O)-$, $-C(O)O-$, or $-OC(O)O-$;

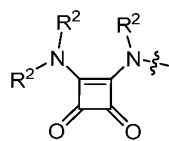
each R' is independently an optionally substituted group selected from C_{4-12} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

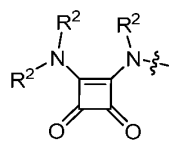


10 R is hydrogen, , or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

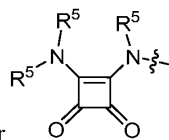
R^1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-OR^2$, $-C(O)OR^2$, $-C(O)SR^2$, $-OC(O)R^2$, $-OC(O)OR^2$, $-CN$, $-N(R^2)_2$, $-C(O)N(R^2)_2$, $-S(O)_2N(R^2)_2$, $-NR^2C(O)R^2$, $-OC(O)N(R^2)_2$, $-N(R^2)C(O)OR^2$, $-NR^2S(O)_2R^2$, $-NR^2C(O)N(R^2)_2$, $-NR^2C(S)N(R^2)_2$, $-NR^2C(NR^2)N(R^2)_2$, $-NR^2C(CHR^2)N(R^2)_2$, $-N(OR^2)C(O)R^2$, $-N(OR^2)S(O)_2R^2$, $-N(OR^2)C(O)OR^2$, $-N(OR^2)C(O)N(R^2)_2$, $-N(OR^2)C(S)N(R^2)_2$, $-N(OR^2)C(NR^2)N(R^2)_2$, $-N(OR^2)C(CHR^2)N(R^2)_2$, $-C(NR^2)N(R^2)_2$, $-C(NR^2)R^2$, $-C(O)N(R^2)OR^2$, $-C(R^2)N(R^2)_2C(O)OR^2$, $-CR^2(R^3)_2$, $-OP(O)(OR^2)_2$, or $-P(O)(OR^2)_2$; or

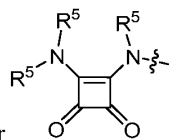
25



R¹ is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R² or R³ groups;

- 5 each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from
- 10 nitrogen, oxygen, and sulfur; or
- two occurrences of R², taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- each R³ is independently -(CH₂)_n-R⁴; or
- 15 two occurrences of R³, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;
- each R⁴ is independently hydrogen, -OR⁵, -N(R⁵)₂, -OC(O)R⁵, -OC(O)OR⁵, -CN, -C(O)N(R⁵)₂,
- 20 -NR⁵C(O)R⁵, -OC(O)N(R⁵)₂, -N(R⁵)C(O)OR⁵, -NR⁵S(O)₂R⁵, -NR⁵C(O)N(R⁵)₂,



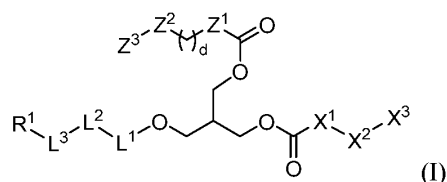
-NR⁵C(S)N(R⁵)₂, -NR⁵C(NR⁵)N(R⁵)₂, or  ;

- each R⁵ is independently hydrogen, or optionally substituted C₁₋₆ aliphatic; or
- two occurrences of R⁵, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional
- 25 heteroatom selected from nitrogen, oxygen, and sulfur;
- each R⁶ is independently C₄₋₁₂ aliphatic; and
- each n is independently 0 to 4.

Exemplary Lipids of WO2022159421

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022159421, which is hereby incorporated by reference in its entirety. In some
 5 embodiments, an ionizable lipid has a structure according to any of Formulae I of WO2022159421, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of WO2022159421.

In some embodiments, an ionizable lipid is according to a compound of Formula I of
 10 WO2022159421:



or a pharmaceutically acceptable salt thereof, wherein:

L^1 is a covalent bond, $-C(O)-$, or $-OC(O)-$;

15 L^2 is a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{Cy}^A \left(\text{---} \right)_{m'} \right]$;

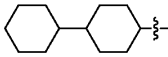
Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

20 L^3 is a covalent bond, $-C(O)-$, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;

R^1 is $\left[\left(\text{---} \right)_p \text{Cy}^B \right]$, or an optionally substituted saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or partially

25 unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and phenyl;

p is 0, 1, 2, or 3;

X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C_1 - C_{12} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O-, -NR-, or - Cy^C -;

Cy^C is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen or an optionally substituted C_1 - C_6 aliphatic group;

Z^1 is a covalent bond or -O-;

Z^2 is an optionally substituted group selected from 4- to 12-membered saturated or partially unsaturated carbocyclyl, phenyl, 1-adamantyl, and 2-adamantyl;

Z^3 is hydrogen, or an optionally substituted group selected from C_1 - C_{10} aliphatic, and 4- to 12-membered saturated or partially unsaturated carbocyclyl; and

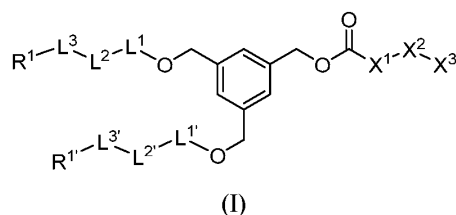
d is 0, 1, 2, 3, 4, 5, or 6;

provided that when L^3 is a covalent bond, then R^1 must be $\text{H} \left(\text{---} \right)_p \text{---} Cy^B$.

Exemplary lipids of WO2022159475

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022159475, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I of WO2022159475, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of WO2022159475.

In some embodiments, an ionizable lipid is according to a compound of Formula I of WO2022159475:



or a pharmaceutically acceptable salt thereof, wherein:

each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-C(O)O-$;

- 5 each L^2 and $L^{2'}$ is independently a covalent bond, an optionally substituted bivalent saturated

or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{Cy}^A \left(\text{---} \right)_m \right]$;

each Cy^A is independently an optionally substituted ring selected from phenylene and a 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

- 10 each L^3 and $L^{3'}$ is independently a covalent bond, $-C(O)O-$, $-OC(O)-$, $-O-$, or $-OC(O)O-$;

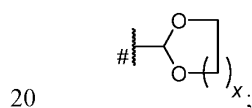
each R^1 and $R^{1'}$ is independently an optionally substituted group selected from a saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$, a 3- to 12-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, sterolyl,

- 15 phenyl, and $\left[\text{---} L^4 \begin{array}{l} \text{O}-A^1 \\ \text{O}-A^2 \end{array} \right]_x$;

each L^4 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_6 hydrocarbon chain;

each A^1 and A^2 is independently an optionally substituted C_1 - C_{20} aliphatic or $-L^5-R^5$;

or A^1 and A^2 , together with their intervening atoms, may form an optionally substituted ring:



wherein

x is selected from 1 or 2; and

$\#$ represents the point of attachment to L^4 ;

each L^5 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_{20}

- 25 hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

each R⁵ is independently an optionally substituted group selected from a 5- to 10-membered aryl ring and a 3- to 8-membered carbocyclic ring ;

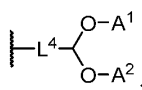
X¹ is a covalent bond, -O-, or -NR-;

X² is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

X³ is hydrogen or -Cy^B;

Cy^B is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

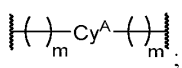
each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

provided that when X³ is hydrogen, at least one of R¹ or R^{1'} is .

In some embodiments, the present disclosure provides a compound of Formula I or a pharmaceutically acceptable salt thereof, wherein:

each L¹ and L^{1'} is independently -C(O)- or -C(O)O-;

each L² and L^{2'} is independently a covalent bond, an optionally substituted bivalent saturated

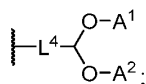
or unsaturated, straight or branched C₁-C₁₂ hydrocarbon chain, or .

each Cy^A is independently an optionally substituted ring selected from phenylene and a 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

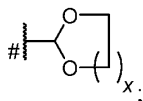
each L³ and L^{3'} is independently a covalent bond, -C(O)O-, -OC(O)-, -O-, or -OC(O)O-;

each R¹ and R^{1'} is independently an optionally substituted group selected from a saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, sterolyl, phenyl, and



each L⁴ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₆ hydrocarbon chain;

each A¹ and A² is independently an optionally substituted C₁-C₂₀ aliphatic or -L⁵-R⁵;
or A¹ and A², together with their intervening atoms, may form an optionally substituted ring:



wherein

5 x is selected from 1 or 2; and

represents the point of attachment to L⁴;

each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

10 each R⁵ is independently an optionally substituted group selected from a 5- to 10-membered aryl ring and a 3- to 8-membered carbocyclic ring ;

X¹ is a covalent bond, -O-, or -NR-;

X² is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and

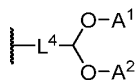
15 independently replaced with -O- or -NR-;

X³ is hydrogen or -Cy^B;

Cy^B is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

provided that when X³ is hydrogen, at least one of R¹ or R^{1'} is



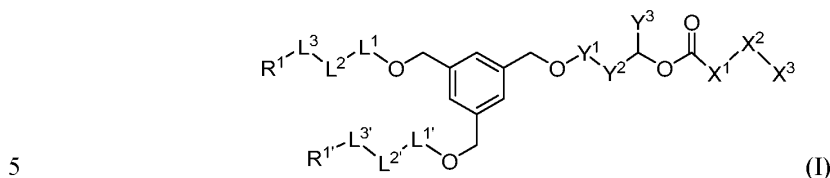
25 Exemplary Lipids of WO2022159463

In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Publication No. WO2022159463, which is hereby incorporated by reference in its entirety. In some embodiments, an ionizable lipid has a structure according to any of Formulae I of

30 WO2022159463, or a pharmaceutically acceptable salt or solvate thereof. Exemplary

ionizable lipids also include any of the lipids represented by the Examples of WO2022159463.

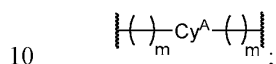
In some embodiments, an ionizable lipid is according to a compound of Formula I of WO2022159463:



or a pharmaceutically acceptable salt thereof, wherein:

each of L¹ and L^{1'} is independently a covalent bond, -C(O)-, or -OC(O)-;

each of L² and L^{2'} is independently a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or branched C₁-C₁₂ hydrocarbon chain, or

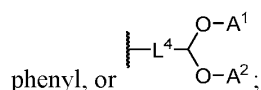


each Cy^A is independently an optionally substituted ring selected from phenylene or 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

each of L³ and L^{3'} is independently a covalent bond, -O-, -C(O)O-, -OC(O)-, or -OC(O)O-;

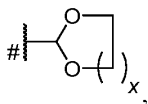
15 each of R¹ and R¹ is independently an optionally substituted group selected from saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, sterolyl,



20 each L⁴ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀
hydrocarbon chain;

each A¹ and A² is independently an optionally substituted C₁-C₂₀ aliphatic or -L⁵-R⁵,

or A¹ and A², together with their intervening atoms, may form an optionally substituted ring;



25 wherein

x is selected from 1 or 2; and

- # represents the point of attachment to L⁴;
- each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;
- 5 each R⁵ is independently an optionally substituted group selected from a 6- to 10-membered aryl ring or a 3- to 8-membered carbocyclic ring;
- Y¹ is a covalent bond, -C(O)-, or -C(O)O-;
- Y² is a bivalent saturated or unsaturated, straight or branched C₁-C₆ hydrocarbon chain, wherein 1-2 methylene units are optionally and independently replaced with
- 10 cyclopropylene, -O-, or -NR-;
- Y³ is an optionally substituted group selected from saturated or unsaturated, straight or branched C₁-C₁₄ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, or phenyl;
- 15 X¹ is a covalent bond, -O-, or -NR-;
- X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O-, -NR-, or -Cy^B-;
- each Cy^B is independently an optionally substituted ring selected from 3- to 7-membered
- 20 saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;
- X³ is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or
- 25 partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and
- each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group.
- 30

Exemplary Lipids of PCT/US23/27741

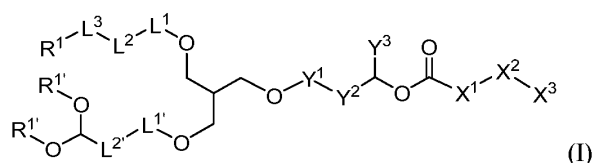
In another aspect, an ionizable lipid is according to any formula or structure, or a pharmaceutically acceptable salt or solvate thereof, as described in International Patent

Application No. PCT/US23/27741, which is hereby incorporated by reference in its entirety.

In some embodiments, an ionizable lipid has a structure according to any of Formulae I of PCT/US23/27741, or a pharmaceutically acceptable salt or solvate thereof. Exemplary ionizable lipids also include any of the lipids represented by the Examples of

5 PCT/US23/27741.

In some embodiments, an ionizable lipid is according to a compound of Formula I of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein:

- 10 each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-OC(O)-$;
 each L^2 and $L^{2'}$ is independently an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;
 L^3 is a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

R^1 is optionally substituted C_{1-20} aliphatic, $\text{---}L^{CyA}\text{---}Cy^A$, or $\text{---}L^{Ra}\text{---}C(O-R^a)_2$;

- 15 L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

- L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

each R^a and $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic;

Y^1 is $-C(O)-$ or $-C(O)O-$;

Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain;

- 25 Y^3 is optionally substituted C_{1-20} aliphatic;

X^1 is a covalent bond, $-O-$, or $-NR-$;

X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

X³ is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

5 and

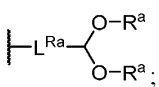
each R is independently hydrogen or optionally substituted C₁₋₆ aliphatic.

In some embodiments, the present disclosure provides a compound of Formula I of PCT/US23/27741, wherein:

each L¹ and L^{1'} is -C(O)-;

10 each L² and L^{2'} is independently a bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain;

L³ is a covalent bond, -C(O)O-, or -OC(O)-;

R¹ is C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, or ;

each L^{Ra} and R^{1'} is independently C₁₋₂₀ alkyl or C₂₋₂₀ alkenyl;

15 Y¹ is -C(O)- or -C(O)O-;

Y² is a bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain;

Y³ is C₁₋₂₀ alkyl or C₂₋₂₀ alkenyl;

X¹ is -O- or -NR-;

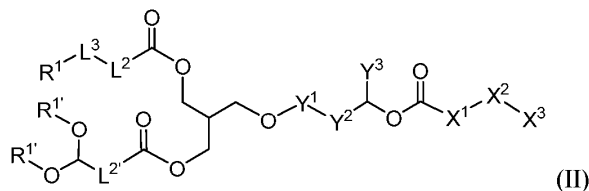
X² is a bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain,

20 wherein 1-3 methylene units are optionally and independently replaced with -NR-;

X³ is hydrogen or a 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur and optionally substituted with C₁₋₆ alkyl or C₂₋₆ alkenyl; and

each R is independently hydrogen or C₁₋₆ alkyl or C₂₋₆ alkenyl.

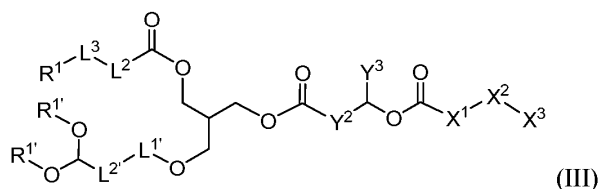
25 In some embodiments, the present disclosure provides a compound of Formula II of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^1 , Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

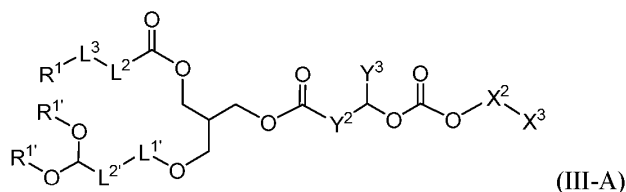
In some embodiments, the present disclosure provides a compound of Formula III of

5 PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

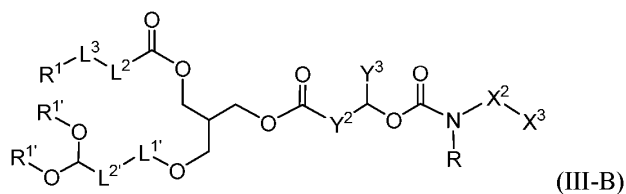
10 In some embodiments, the present disclosure provides a compound of Formula III-A of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and

15 in combination.

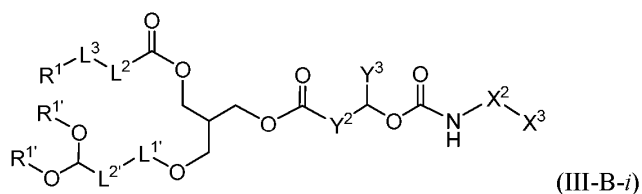
In some embodiments, the present disclosure provides a compound of Formula III-B of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and

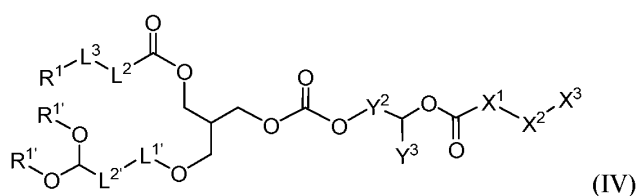
20 in combination.

In some embodiments, the present disclosure provides a compound of Formula III-B-i of PCT/US23/27741:



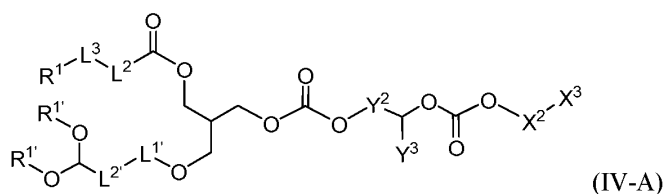
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- 5 In some embodiments, the present disclosure provides a compound of Formula IV of PCT/US23/27741:



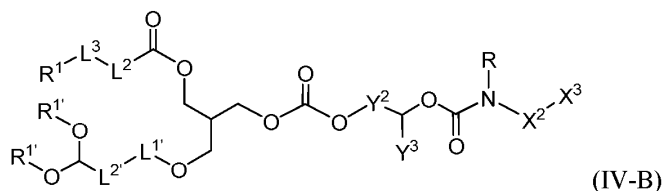
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- 10 In some embodiments, the present disclosure provides a compound of Formula IV-A of PCT/US23/27741:



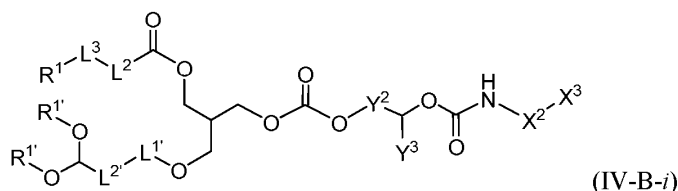
- 15 or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula IV-B of PCT/US23/27741:



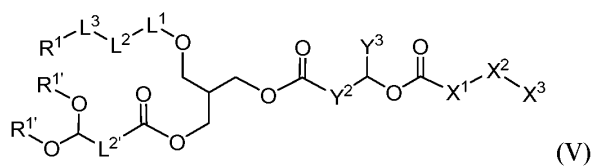
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , L^2' , L^3 , R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula IV-B-i of PCT/US23/27741:



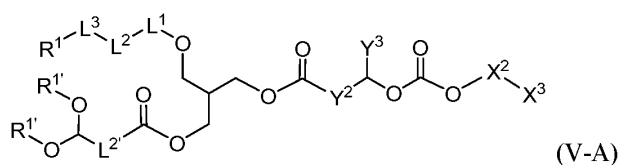
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , L^2' , L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula V of PCT/US23/27741:



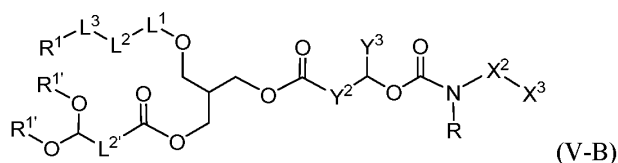
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , L^2' , L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula V-A of PCT/US23/27741:



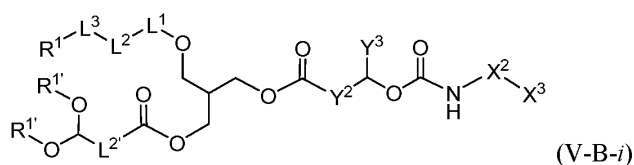
or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , L^2' , L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula V-B of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

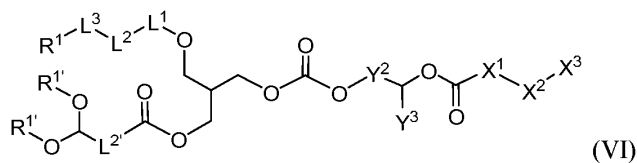
- 5 In some embodiments, the present disclosure provides a compound of Formula V-B-*i* of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

10

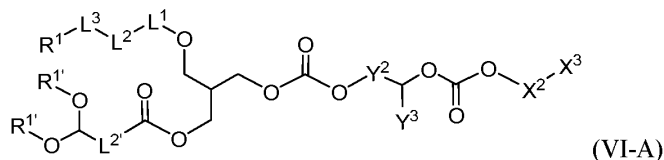
In some embodiments, the present disclosure provides a compound of Formula VI of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

15

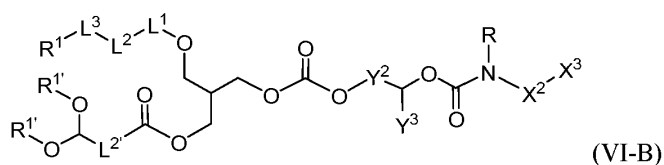
In some embodiments, the present disclosure provides a compound of Formula VI-A of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

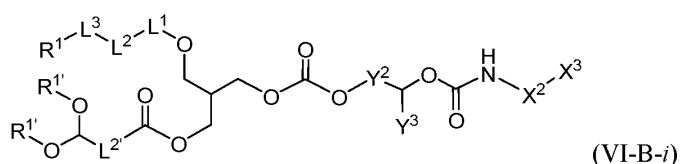
20

In some embodiments, the present disclosure provides a compound of Formula VI-B of PCT/US23/27741:



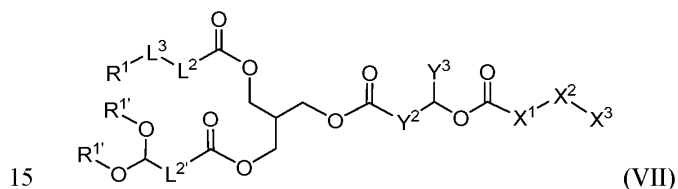
- or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula VI-B-*i* of PCT/US23/27741:



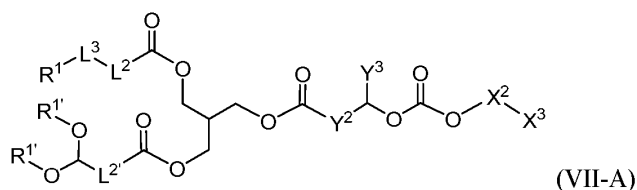
- or a pharmaceutically acceptable salt thereof, wherein each of L^1 , L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula VII of PCT/US23/27741:



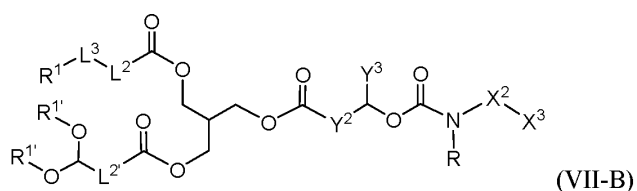
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- In some embodiments, the present disclosure provides a compound of Formula VII-A of PCT/US23/27741:



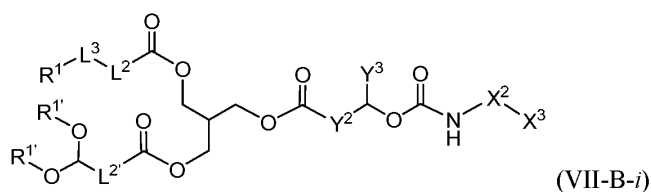
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- 5 In some embodiments, the present disclosure provides a compound of Formula VII-B of PCT/US23/27741:



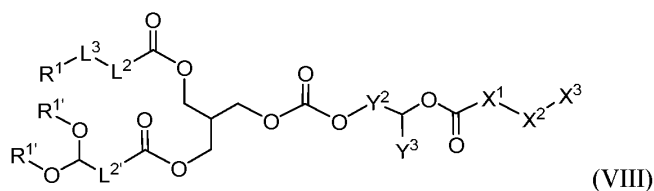
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- 10 In some embodiments, the present disclosure provides a compound of Formula VII-B-*i* of PCT/US23/27741:



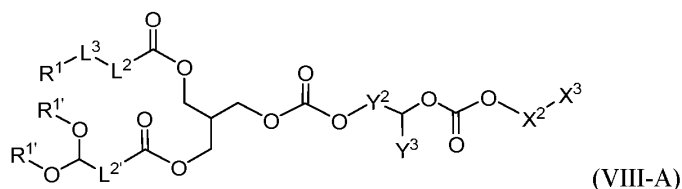
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, L^3 , R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

- 15 In some embodiments, the present disclosure provides a compound of Formula VIII of PCT/US23/27741:



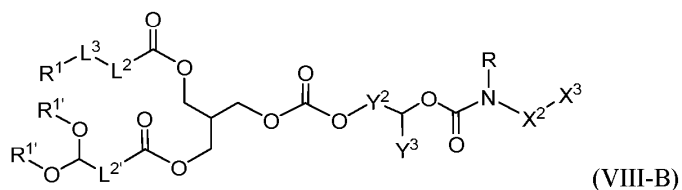
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , L^2 , L^3 , R^1 , R^1 , Y^2 , Y^3 , X^1 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula VIII-A
 5 of PCT/US23/27741:



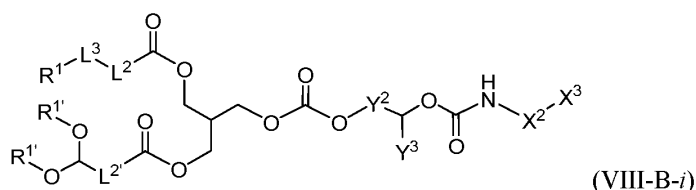
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , L^2 , L^3 , R^1 , R^1 , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

In some embodiments, the present disclosure provides a compound of Formula VIII-B
 10 of PCT/US23/27741:



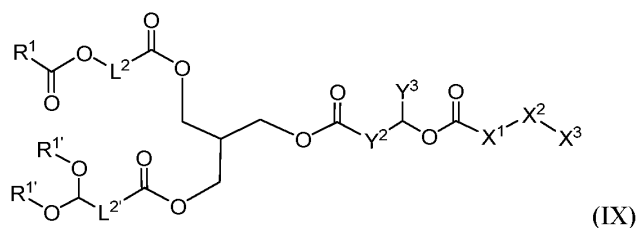
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , L^2 , L^3 , R^1 , R^1 , Y^2 , Y^3 , R , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and
 15 in combination.

In some embodiments, the present disclosure provides a compound of Formula VIII-B-*i* of PCT/US23/27741:



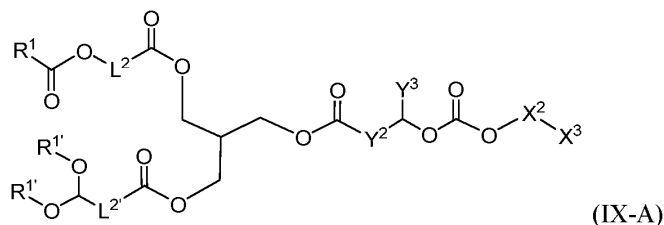
or a pharmaceutically acceptable salt thereof, wherein each of L^2 , L^2 , L^3 , R^1 , R^1 , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and
 20 in combination.

In some embodiments, the present disclosure provides a compound of Formula IX of PCT/US23/27741:



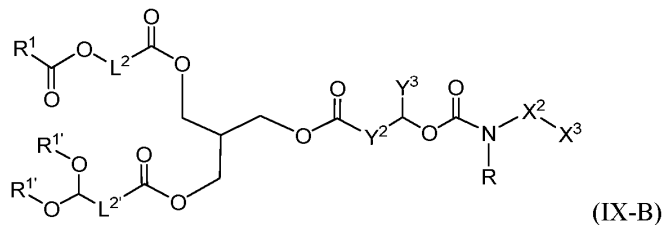
or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, Y², Y³, X¹, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

- 5 In some embodiments, the present disclosure provides a compound of Formula IX-A of PCT/US23/27741:



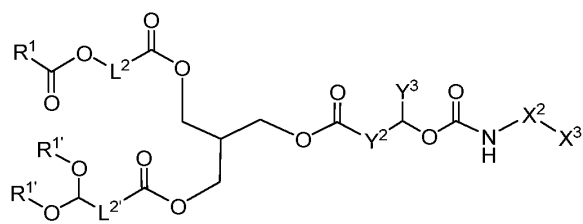
or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, Y², Y³, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

- 10 In some embodiments, the present disclosure provides a compound of Formula IX-B of PCT/US23/27741:



- 15 or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, R, Y², Y³, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

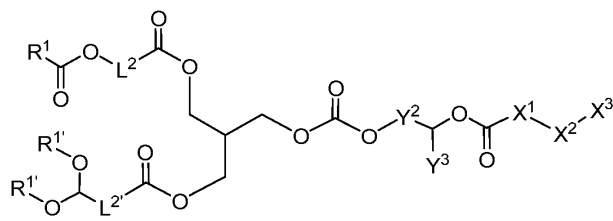
In some embodiments, the present disclosure provides a compound of Formula IX-B-i of PCT/US23/27741:



(IX-B-i)

or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, Y², Y³, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

- 5 In some embodiments, the present disclosure provides a compound of Formula X of PCT/US23/27741:

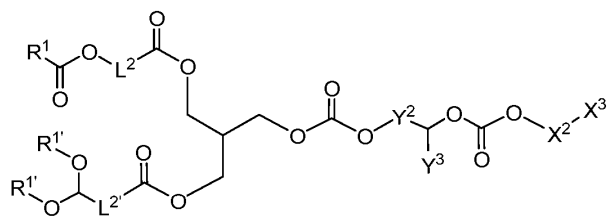


(X)

or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, Y², Y³, X¹, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

10

In some embodiments, the present disclosure provides a compound of Formula X-A of PCT/US23/27741:

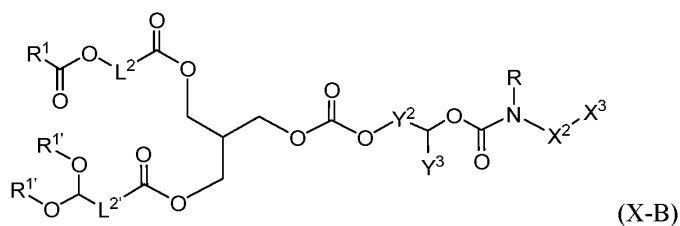


(X-A)

or a pharmaceutically acceptable salt thereof, wherein each of L², L^{2'}, R¹, R^{1'}, Y², Y³, X², and X³ is as described above and in classes and subclasses herein, both singly and in combination.

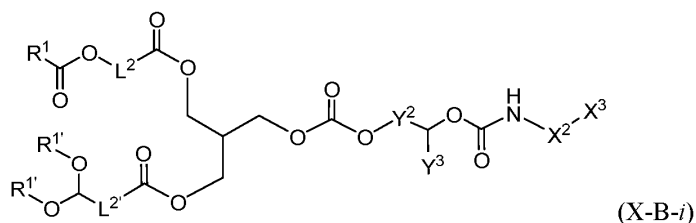
15

In some embodiments, the present disclosure provides a compound of Formula X-B of PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, R^1 , $R^{1'}$, R , Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

5 In some embodiments, the present disclosure provides a compound of Formula X-B-*i* PCT/US23/27741:



or a pharmaceutically acceptable salt thereof, wherein each of L^2 , $L^{2'}$, R^1 , $R^{1'}$, Y^2 , Y^3 , X^2 , and X^3 is as described above and in classes and subclasses herein, both singly and in combination.

10 In some embodiments of any Formulae of PCT/US23/27741 described herein, L^1 is -C(O)- or -OC(O)-. In some embodiments, L^1 is -C(O)-. In some embodiments, L^1 is -OC(O)-.

In some embodiments of any Formulae of PCT/US23/27741 described herein, $L^{1'}$ is -C(O)- or -OC(O)-. In some embodiments, $L^{1'}$ is -C(O)-. In some embodiments, $L^{1'}$ is -OC(O)-.

In some embodiments, the present disclosure provides a compound of Formula I of PCT/US23/27741, wherein: each L^1 and $L^{1'}$ is -C(O)-.

20 In some embodiments, the present disclosure provides a compound of Formula I of PCT/US23/27741, wherein: each L^2 and $L^{2'}$ is independently a bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain.

In some embodiments of any Formulae of PCT/US23/27741 described herein, L^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain. In some embodiments, L^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{4-8} hydrocarbon chain. In some embodiments, L^2 is an

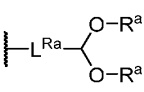
optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L² is an optionally substituted bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L² is an optionally substituted bivalent saturated, straight or branched C₄₋₈ hydrocarbon chain. In some embodiments, L² is an optionally substituted bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L² is a bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L² is a bivalent saturated, straight or branched C₄₋₈ hydrocarbon chain. In some embodiments, L² is a bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L² is -CH₂-. In some embodiments, L² is -(CH₂)₄- or -(CH₂)₅-. In some embodiments, L² is -(CH₂)₆- or -(CH₂)₇-.

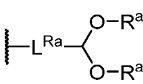
In some embodiments of any Formulae of PCT/US23/27741 described herein, L^{2'} is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L^{2'} is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L^{2'} is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, L^{2'} is an optionally substituted bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L^{2'} is an optionally substituted bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L^{2'} is an optionally substituted bivalent saturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, L^{2'} is a bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L^{2'} is a bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L^{2'} is a bivalent saturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, L^{2'} is -(CH₂)₂-.

In some embodiments of any Formulae of PCT/US23/27741 described herein, L³ is a covalent bond, -O-, -C(O)O-, -OC(O)-, or -OC(O)O-. In some embodiments, L³ is a covalent bond, -C(O)O-, or -OC(O)-. In some embodiments, L³ is a covalent bond. In some embodiments, L³ is -O-, -C(O)O-, -OC(O)-, or -OC(O)O-. In some embodiments, L³ is -C(O)O- or -OC(O)-. In some embodiments, L³ is -O-. In some embodiments, L³ is -C(O)O-. In some embodiments, L³ is -OC(O)-. In some embodiments, L³ is -OC(O)O-.

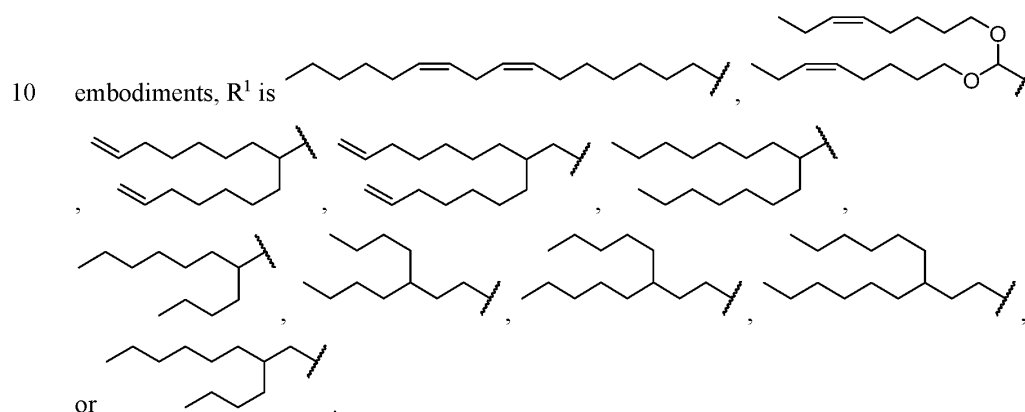
In some embodiments of any Formulae of PCT/US23/27741 described herein, R¹ is

optionally substituted C₁₋₂₀ aliphatic, $\text{---L}^{\text{CyA}}\text{---Cy}^{\text{A}}$, or $\text{---L}^{\text{Ra}}\text{---}\begin{array}{c} \text{O---R}^{\text{a}} \\ \text{O---R}^{\text{a}} \end{array}$. In some embodiments, R¹ is optionally substituted C₁₋₂₀ aliphatic. In some embodiments, R¹ is $\text{---L}^{\text{CyA}}\text{---Cy}^{\text{A}}$. In

some embodiments, R^1 is . In some embodiments, R^1 is C₁₋₂₀ aliphatic or

. In some embodiments, R^1 is optionally substituted C₆₋₁₂ aliphatic. In some embodiments, R^1 is optionally substituted C₆₋₁₂ alkyl. In some embodiments, R^1 is optionally substituted C₆₋₁₂ alkenyl. In some embodiments, R^1 is optionally substituted C₁₂₋₂₀ aliphatic.

- 5 In some embodiments, R^1 is optionally substituted C₁₂₋₂₀ alkyl. In some embodiments, R^1 is optionally substituted C₁₂₋₂₀ alkenyl. In some embodiments, R^1 is C₁₋₂₀ aliphatic. In some embodiments, R^1 is C₆₋₁₂ aliphatic. In some embodiments, R^1 is C₆₋₁₂ alkyl. In some embodiments, R^1 is C₆₋₁₂ alkenyl. In some embodiments, R^1 is C₁₂₋₂₀ aliphatic. In some embodiments, R^1 is C₁₂₋₂₀ alkyl. In some embodiments, R^1 is C₁₂₋₂₀ alkenyl. In some

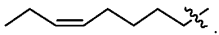


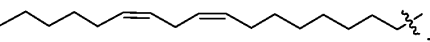
- In some embodiments of any Formulae of PCT/US23/27741 described herein, L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain.

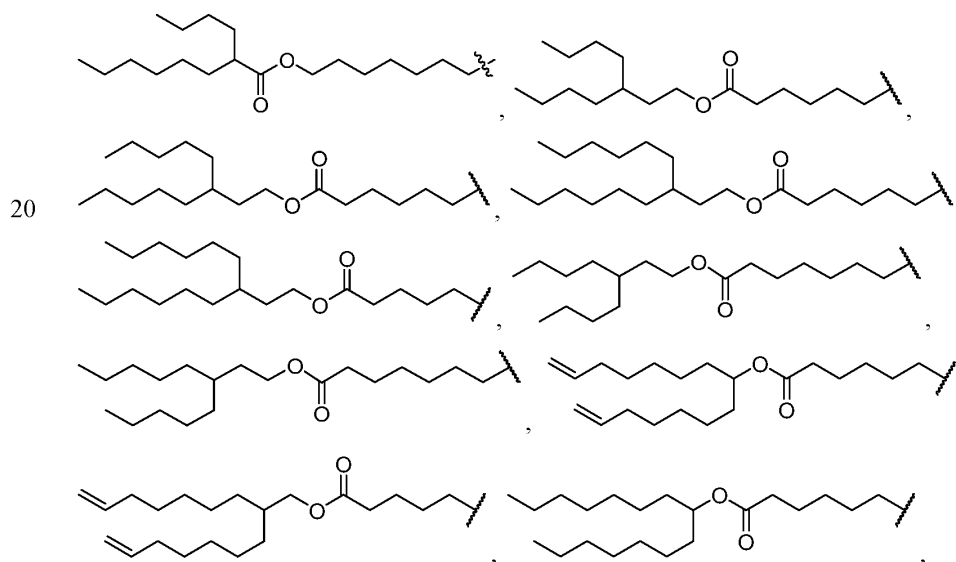
- In some embodiments of any Formulae of PCT/US23/27741 described herein, Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl.

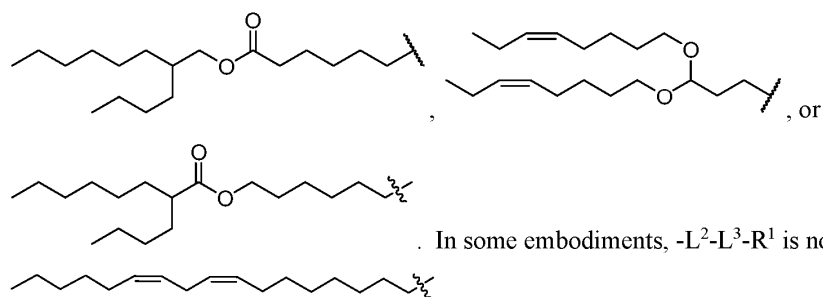
- 20 In some embodiments of any Formulae of PCT/US23/27741 described herein, L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L^{Ra} is a bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain. In some
- 25 embodiments, L^{Ra} is a bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, L^{Ra} is an optionally substituted bivalent saturated

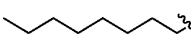
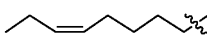
or unsaturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, L^{Ra} is a bivalent saturated or unsaturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, L^{Ra} is -CH₂- or -(CH₂)₂-.

In some embodiments of any Formulae of PCT/US23/27741 described herein, each R^a is independently optionally substituted C₁₋₂₀ aliphatic. In some embodiments, each R^a is independently optionally substituted C₁₋₁₂ aliphatic. In some embodiments, each R^a is independently optionally substituted C₁₋₁₂ alkyl. In some embodiments, each R^a is independently optionally substituted C₁₋₁₂ alkenyl. In some embodiments, each R^a is independently optionally substituted C₄₋₁₀ aliphatic. In some embodiments, R^a is optionally substituted C₆₋₁₂ aliphatic. In some embodiments, R^a is optionally substituted C₆₋₁₂ alkyl. In some embodiments, R^a is optionally substituted C₆₋₁₂ alkenyl. In some embodiments, R^a is optionally substituted C₇₋₉ aliphatic. In some embodiments, R^a is optionally substituted C₇₋₉ alkyl. In some embodiments, R^a is optionally substituted C₇₋₉ alkenyl. In some embodiments, each R^a is independently C₁₋₂₀ aliphatic. In some embodiments, R^a is C₆₋₁₂ aliphatic. In some embodiments, R^a is C₆₋₁₂ alkyl. In some embodiments, R^a is C₆₋₁₂ alkenyl. In some embodiments, R^a is C₇₋₉ aliphatic. In some embodiments, R^a is C₇₋₉ alkyl. In some embodiments, R^a is C₇₋₉ alkenyl. In some embodiments, R^a is .

In some embodiments, -L²-L³-R¹ is .





- In some embodiments of any Formulae of PCT/US23/27741 described herein, each
- 5 $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic. In some embodiments, $R^{1'}$ is optionally substituted C_{6-12} aliphatic. In some embodiments, $R^{1'}$ is optionally substituted C_{6-12} alkyl. In some embodiments, $R^{1'}$ is optionally substituted C_{6-12} alkenyl. In some
- embodiments, $R^{1'}$ is optionally substituted C_{7-9} aliphatic. In some embodiments, $R^{1'}$ is optionally substituted C_{7-9} alkyl. In some embodiments, $R^{1'}$ is optionally substituted C_{7-9}
- 10 alkenyl. In some embodiments, each $R^{1'}$ is independently C_{1-20} aliphatic. In some embodiments, $R^{1'}$ is C_{6-12} aliphatic. In some embodiments, $R^{1'}$ is C_{6-12} alkyl. In some embodiments, $R^{1'}$ is C_{6-12} alkenyl. In some embodiments, $R^{1'}$ is C_{7-9} aliphatic. In some embodiments, $R^{1'}$ is C_{7-9} alkyl. In some embodiments, $R^{1'}$ is C_{7-9} alkenyl. In some
- embodiments, $R^{1'}$ is  or .

- 15 In some embodiments of any Formulae of PCT/US23/27741 described herein, Y^1 is - $C(O)-$ or $-C(O)O-$. In some embodiments, Y^1 is $-C(O)-$. In some embodiments, Y^1 is $-C(O)O-$.

- In some embodiments of any Formulae of PCT/US23/27741 described herein, Y^2 is
- an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6}
- 20 hydrocarbon chain. In some embodiments, Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-3} hydrocarbon chain. In some embodiments, Y^2 is an optionally substituted bivalent saturated, straight or branched C_{1-6} hydrocarbon chain. In
- some embodiments, Y^2 is an optionally substituted bivalent saturated, straight or branched C_{1-3} hydrocarbon chain. In some embodiments, Y^2 is a bivalent saturated or unsaturated, straight
- 25 or branched C_{1-6} hydrocarbon chain. In some embodiments, Y^2 is a bivalent saturated, straight or branched C_{1-6} hydrocarbon chain. In some embodiments, Y^2 is a bivalent saturated, straight or branched C_{1-3} hydrocarbon chain. In some embodiments, Y^2 is $-CH_2-$ or $-(CH_2)_2-$.

In some embodiments of any Formulae of PCT/US23/27741 described herein, Y³ is optionally substituted C₁₋₂₀ aliphatic. In some embodiments, Y³ is C₁₋₂₀ aliphatic. In some embodiments, Y³ is optionally substituted C₁₋₁₂ aliphatic. In some embodiments, Y³ is optionally substituted C₁₋₁₂ alkyl. In some embodiments, Y³ is optionally substituted C₄₋₈ aliphatic. In some embodiments, Y³ is optionally substituted C₄₋₈ alkyl. In some embodiments, Y³ is optionally substituted C₁₋₆ alkyl. In some embodiments, Y³ is -CH₃, -CH₂CH₃, -(CH₂)₂CH₃, -(CH₂)₃CH₃, -(CH₂)₄CH₃, -(CH₂)₅CH₃, -(CH₂)₆CH₃, or -(CH₂)₇CH₃. In some embodiments, Y³ is -CH₃. In some embodiments, Y³ is -CH₂CH₃. In some embodiments, Y³ is -(CH₂)₂CH₃. In some embodiments, Y³ is -(CH₂)₃CH₃. In some embodiments, Y³ is -(CH₂)₄CH₃. In some embodiments, Y³ is -(CH₂)₅CH₃. In some embodiments, Y³ is -(CH₂)₆CH₃. In some embodiments, Y³ is -(CH₂)₇CH₃.

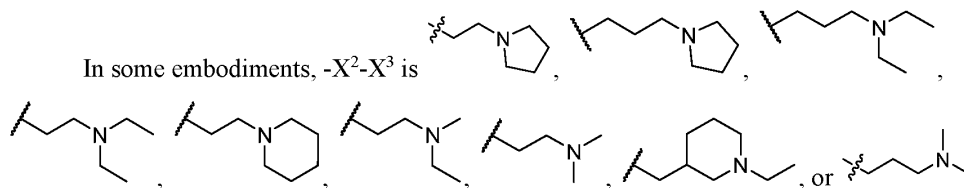
In some embodiments of any Formulae of PCT/US23/27741 described herein, X¹ is a covalent bond, -O-, or -NR-. In some embodiments, X¹ is a covalent bond. In some embodiments, X¹ is -O- or -NR-. In some embodiments, X¹ is -O-. In some embodiments, X¹ is -NR-.

In some embodiments of any Formulae of PCT/US23/27741 described herein, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-. In some embodiments, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain, wherein 1-2 methylene units are optionally and independently replaced with -O- or -NR-. In some embodiments, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, X² is a bivalent saturated, straight or branched C₁₋₁₂ hydrocarbon chain. In some embodiments, X² is a bivalent saturated, straight or branched C₁₋₆ hydrocarbon chain. In some embodiments, X² is a bivalent saturated, straight or branched C₁₋₃ hydrocarbon chain. In some embodiments, X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁₋₁₂ hydrocarbon chain, wherein 1-3 methylene units are independently

replaced with -O- or -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1-2 methylene units are independently replaced with -O- or -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{4-8} hydrocarbon chain, wherein 2 methylene units are independently replaced with -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1 methylene unit is replaced with -NR-. In some embodiments, X^2 is a bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -NR-. In some embodiments, X^2 is a bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain, wherein 1-2 methylene units are optionally and independently replaced with -NR-. In some embodiments, X^2 is a bivalent saturated or unsaturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1 methylene unit is replaced with -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated, straight or branched C_{1-12} hydrocarbon chain, wherein 1-3 methylene units are independently replaced with -O- or -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1-2 methylene units are independently replaced with -O- or -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated, straight or branched C_{4-8} hydrocarbon chain, wherein 2 methylene units are independently replaced with -NR-. In some embodiments, X^2 is an optionally substituted bivalent saturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1 methylene unit is replaced with -NR-. In some embodiments, X^2 is a bivalent saturated, straight or branched C_{4-8} hydrocarbon chain, wherein 1 methylene unit is replaced with -NR-.

In some embodiments of any Formulae of PCT/US23/27741 described herein, X^3 is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is hydrogen or a 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur and optionally substituted with C_{1-6} aliphatic. In some embodiments, X^3 is hydrogen. In some embodiments, X^3 is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered

heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is optionally substituted 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is optionally substituted 5- to 6-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some
5 embodiments, X^3 is 5- to 6-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is 5- to 6-membered heterocyclyl, having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, substituted with $-R^\circ$, wherein R° is C_{1-6} aliphatic (e.g., methyl or ethyl). In some
10 embodiments, X^3 is optionally substituted 5-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is 5-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is 5-membered heterocyclyl, having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, substituted with $-R^\circ$,
15 wherein R° is C_{1-6} aliphatic (e.g., methyl or ethyl). In some embodiments, X^3 is optionally substituted 5-membered heterocyclyl having 1-2 nitrogen atoms. In some embodiments, X^3 is 5-membered heterocyclyl having 1-2 nitrogen atoms. In some embodiments, X^3 is 5-membered heterocyclyl, having 1-2 nitrogen atoms, substituted with $-R^\circ$, wherein R° is C_{1-6} aliphatic (e.g., methyl or ethyl). In some embodiments, X^3 is optionally substituted 6-
20 membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is 6-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur. In some embodiments, X^3 is 6-membered heterocyclyl, having 1-2 heteroatoms independently selected from nitrogen, oxygen, and sulfur, substituted with $-R^\circ$, wherein R° is C_{1-6} aliphatic (e.g., methyl or ethyl). In some embodiments, X^3 is optionally substituted pyrrolidinyl,
25 piperidinyl, piperazinyl, or morpholinyl. In some embodiments, X^3 is pyrrolidinyl, piperidinyl, piperazinyl, or morpholinyl. In some embodiments, X^3 is pyrrolidinyl, piperidinyl, piperazinyl, or morpholinyl substituted with $-R^\circ$, wherein R° is C_{1-6} aliphatic (e.g., methyl or ethyl).



In some embodiments, at least one of X^2 and X^3 comprises at least one ionizable
 5 nitrogen atom.

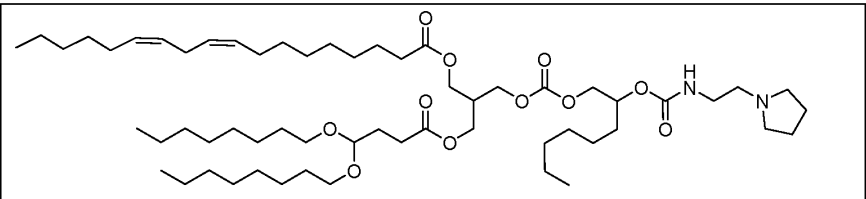
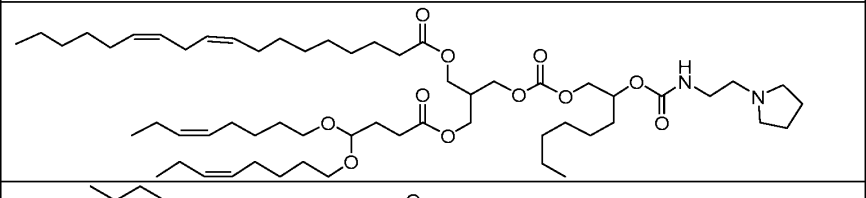
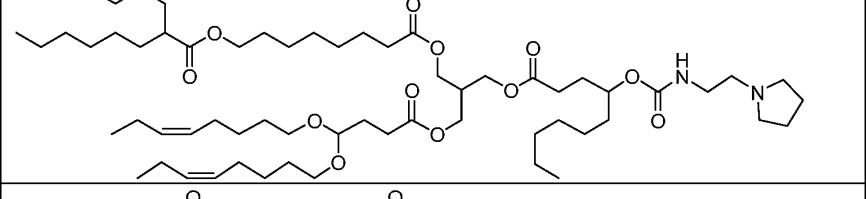
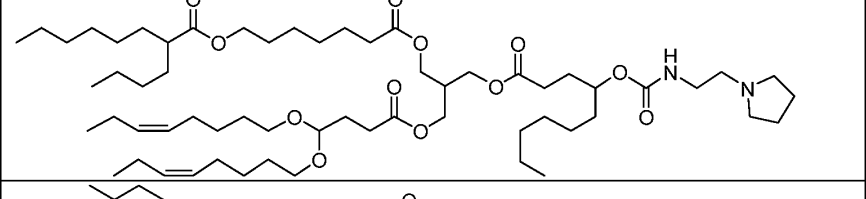
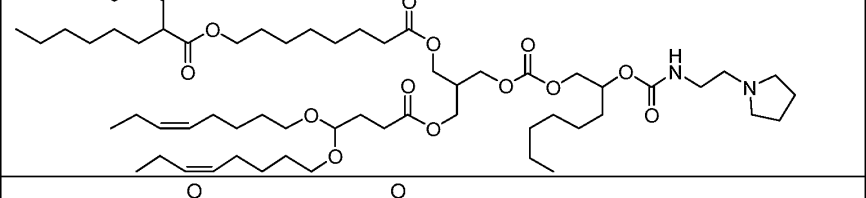
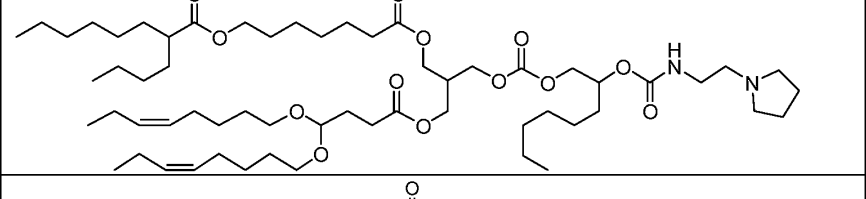
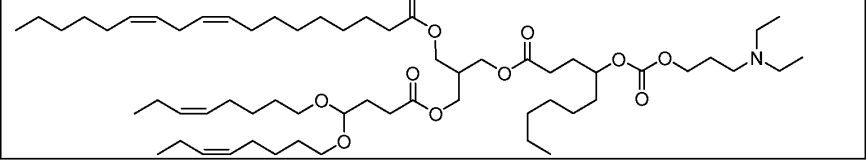
In some embodiments of any Formulae of PCT/US23/27741 described herein, each R
 is independently hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments,
 each R is independently hydrogen or C_{1-6} aliphatic. In some embodiments, R is hydrogen. In
 some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is C_{1-}
 10 6 aliphatic. In some embodiments, R is optionally substituted C_{1-3} aliphatic. In some
 embodiments, R is C_{1-3} aliphatic. In some embodiments, R is $-CH_3$.

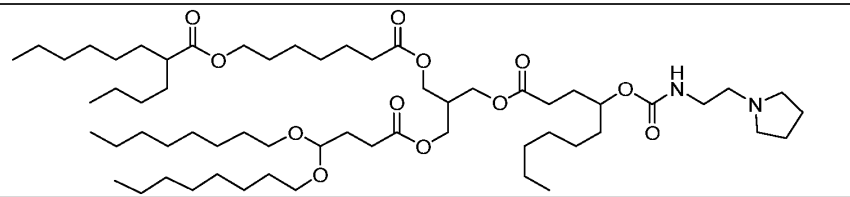
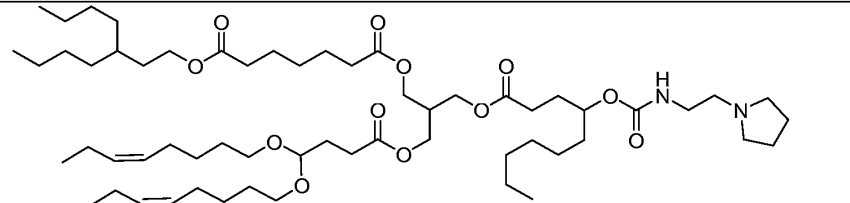
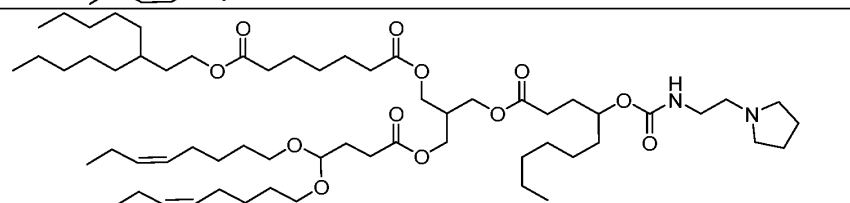
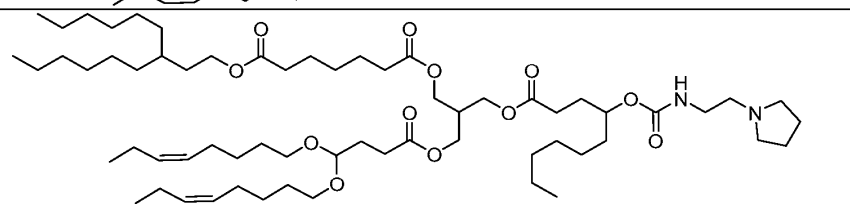
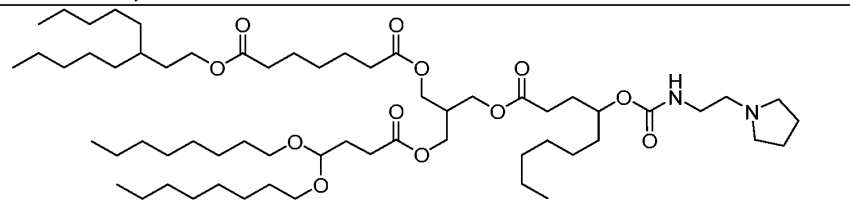
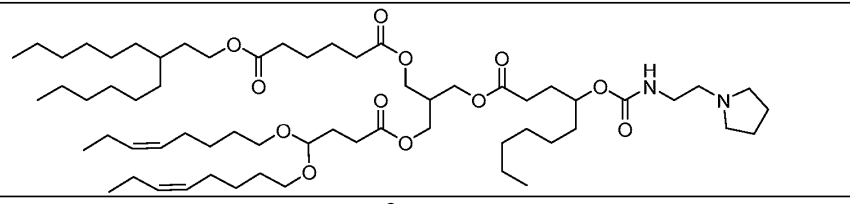
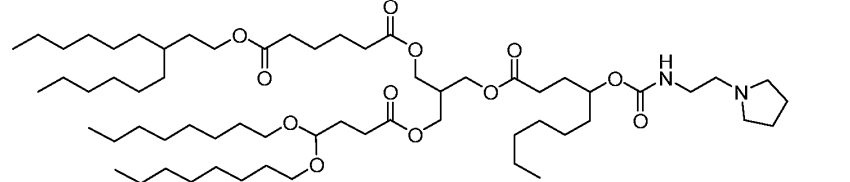
In some embodiments, R° is methyl. In some embodiments, R° is ethyl.

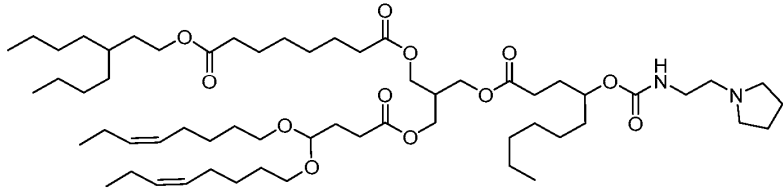
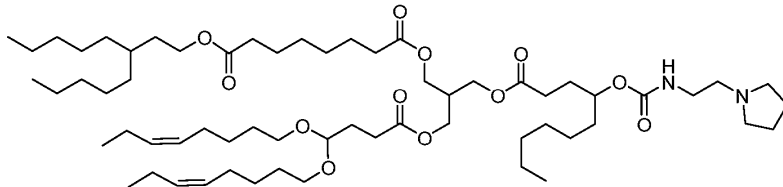
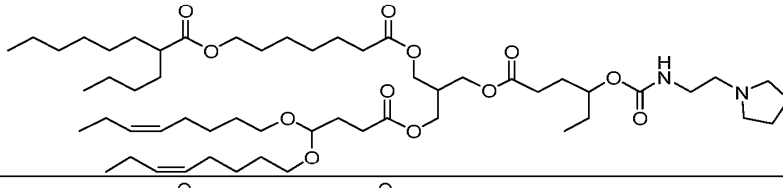
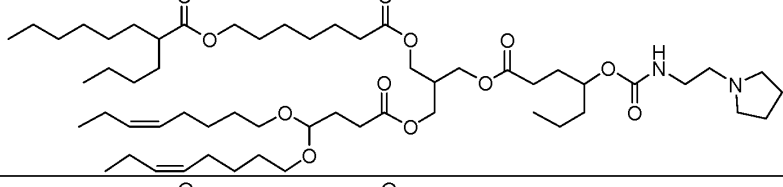
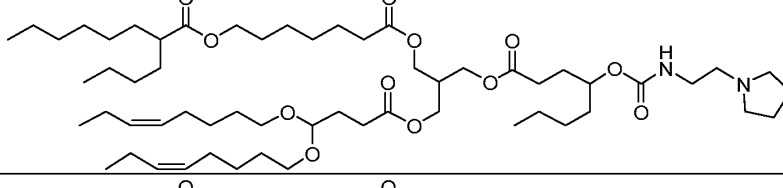
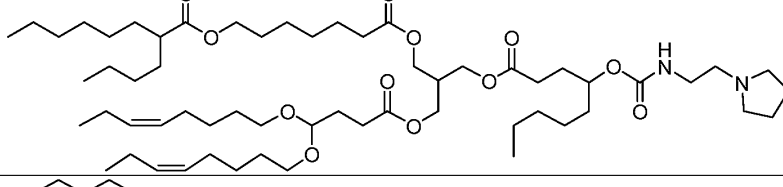
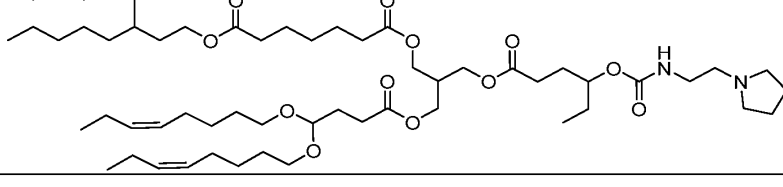
In some embodiments of any Formulae of PCT/US23/27741 described herein, the
 present disclosure provides compounds comprising an ionizable nitrogen atom, wherein the
 15 pKa of the conjugate acid thereof is between about 4 and about 12.

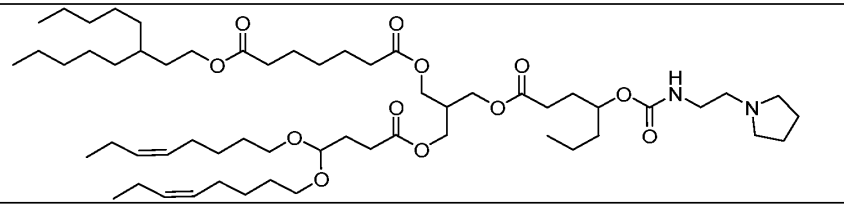
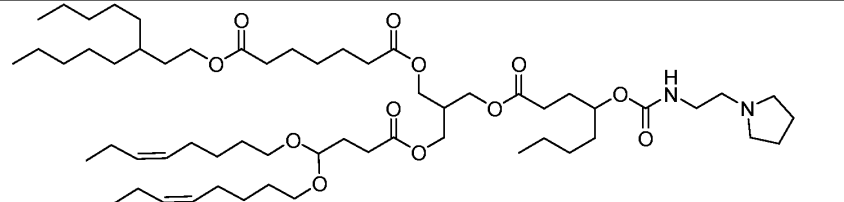
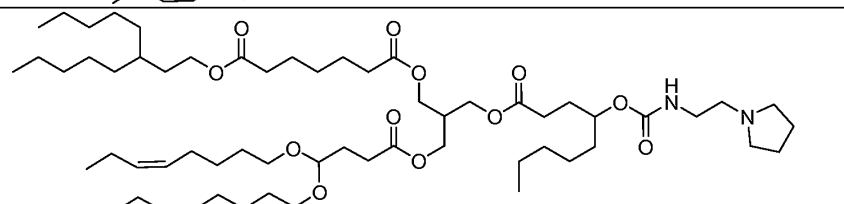
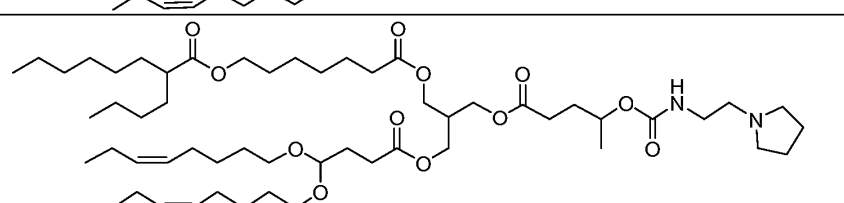
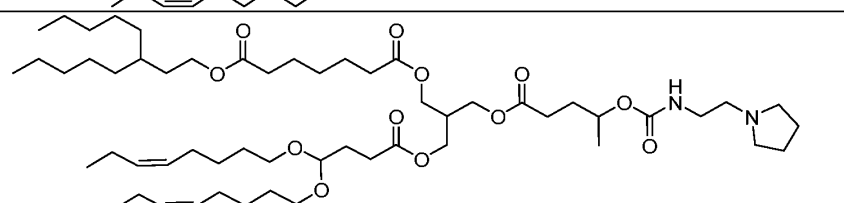
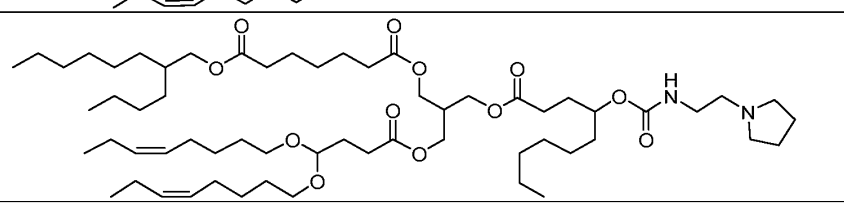
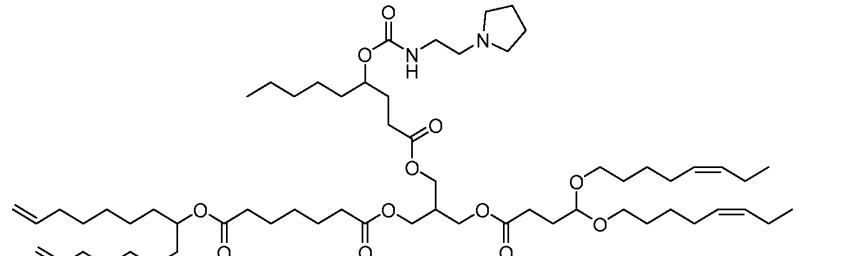
In some embodiments, the present disclosure provides a compound selected from
 Table 1 of PCT/US23/27741. In various embodiments, the compound is selected from the
 group consisting of:

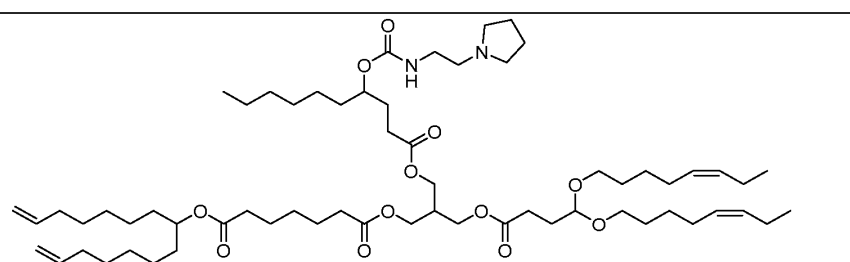
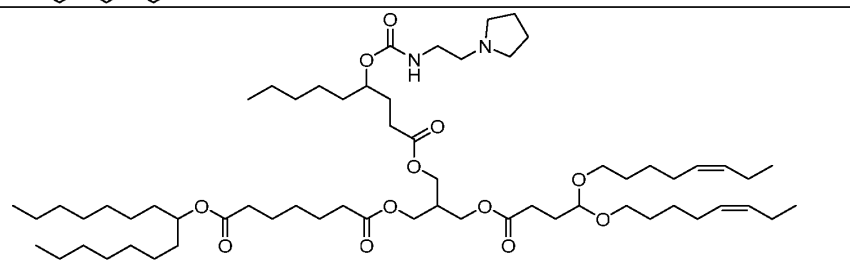
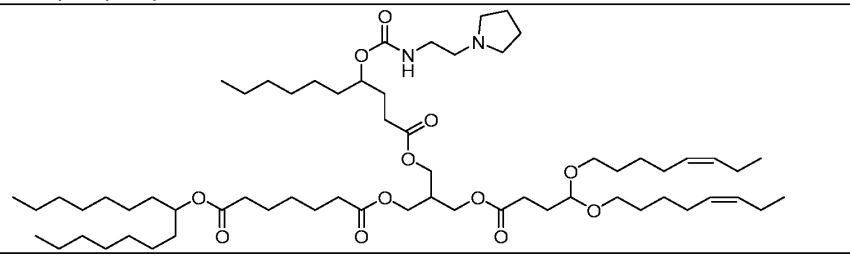
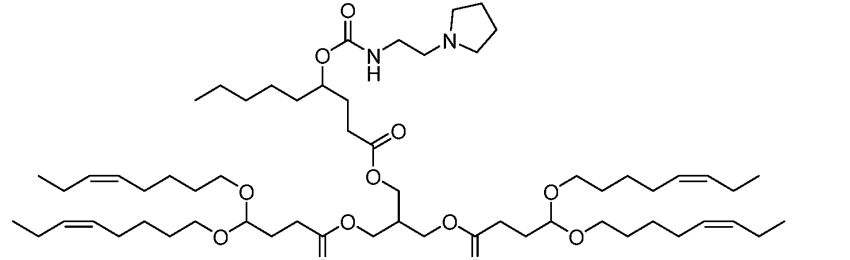
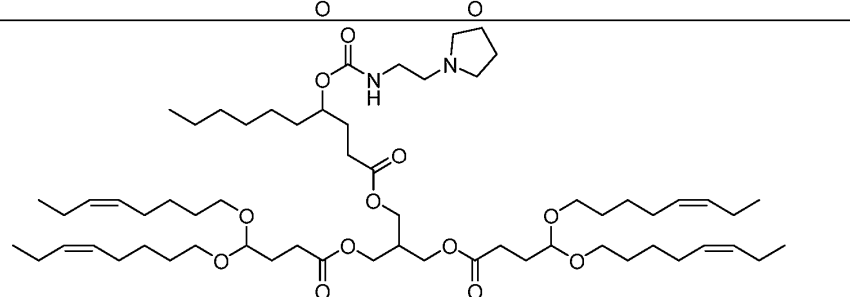
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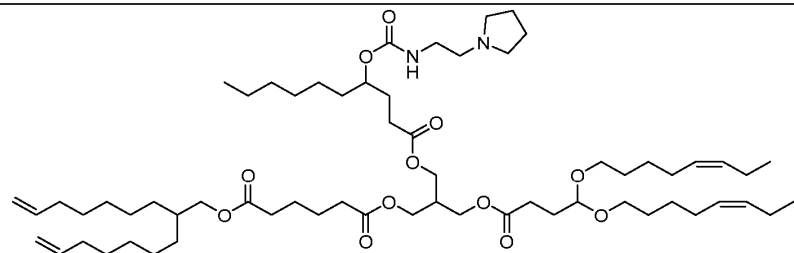
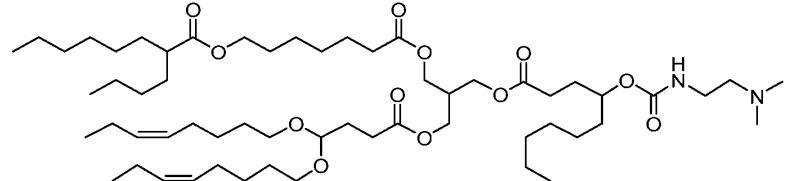
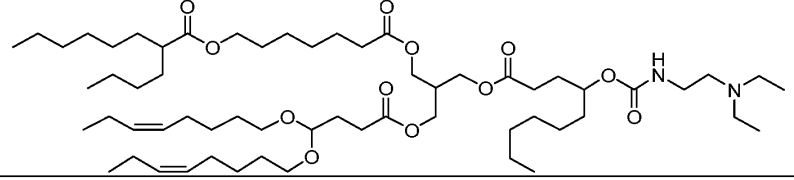
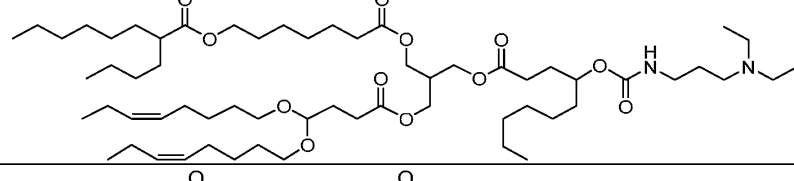
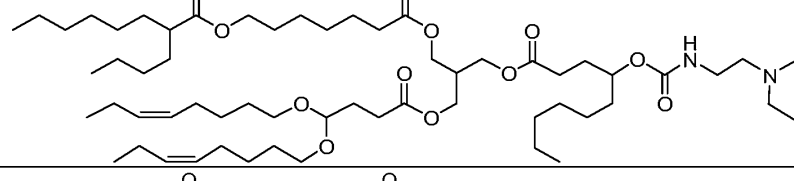
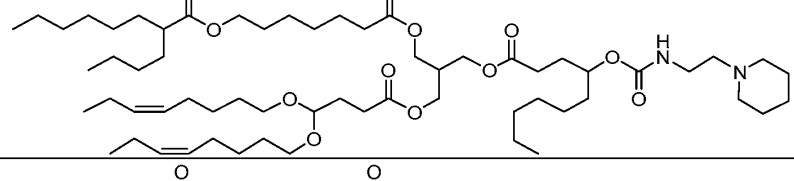
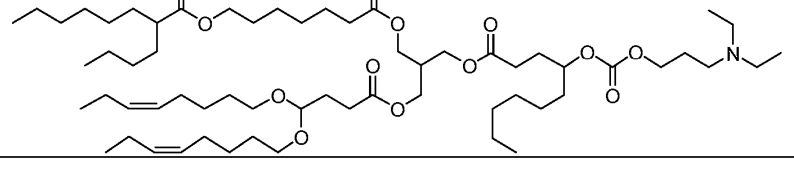
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or a pharmaceutically acceptable salt thereof.

It will be understood that, unless otherwise specified or prohibited by the foregoing definition of any of Formulae I, II, III, III-A, III-B, III-B-*i*, IV, IV-A, IV-B, IV-B-*i*, V, V-A, V-B, V-B-*i*, VI, VI-A, VI-B, VI-B-*i*, VII, VII-A, VII-B, VII-B-*i*, VIII, VIII-A, VIII-B, VIII-B-*i*, IX, IX-A, IX-B, IX-B-*i*, X, X-A, X-B, and X-B-*i* of PCT/US23/27741, embodiments of variables L¹, L^{1'}, L², L^{2'}, L³, L^{CyA}, Cy^A, L^{Ra}, R^a, R¹, R^{1'}, R, Y¹, Y², Y³, X¹, X², and X³ as defined above and described in classes and subclasses herein, apply to compounds of any of Formulae I, II, III, III-A, III-B, III-B-*i*, IV, IV-A, IV-B, IV-B-*i*, V, V-A, V-B, V-B-*i*, VI, VI-A, VI-B, VI-B-*i*, VII, VII-A, VII-B, VII-B-*i*, VIII, VIII-A, VIII-B, VIII-B-*i*, IX, IX-A, IX-B, IX-B-*i*, X, X-A, X-B, and X-B-*i* of PCT/US23/27741, both singly and in combination.

In some embodiments, provided compounds are provided and/or utilized in a salt form (e.g., a pharmaceutically acceptable salt form). Reference to a compound provided herein is understood to include reference to salts thereof, unless otherwise indicated.

It will be appreciated that throughout the present disclosure, unless otherwise indicated, reference to a compound of Formula I of PCT/US23/27741 is intended to also include any of Formulae II, III, III-A, III-B, III-B-*i*, IV, IV-A, IV-B, IV-B-*i*, V, V-A, V-B, V-

B-*i*, VI, VI-A, VI-B, VI-B-*i*, VII, VII-A, VII-B, VII-B-*i*, VIII, VIII-A, VIII-B, VIII-B-*i*, IX, IX-A, IX-B, IX-B-*i*, X, X-A, X-B, and X-B-*i* of PCT/US23/27741, and compound species of such formulae disclosed herein.

5 Conjugate-linker lipids

Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that comprise one or more conjugate-linker lipids as described herein.

In some embodiments, a conjugate-linker lipid is or comprises a polyethylene glycol (PEG)-lipid or PEG-modified lipid. In some embodiments, PEG or PEG-modified lipids may be alternately referred to as PEGylated lipids or PEG-lipids. Inclusion of a PEGylating lipid can be used to enhance lipid nanoparticle colloidal stability *in vitro* and circulation time *in vivo*. In some embodiments, the PEGylation is reversible in that the PEG moiety is gradually released in blood circulation. Exemplary PEG-lipids include but are not limited to PEG conjugated to saturated or unsaturated alkyl chains having a length of C6-C20. PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides (PEG-CER), PEG-modified dialkylamines, PEG-modified diacylglycerols (PEG-DAG), PEG-modified dialkylglycerols, and mixtures thereof. For example, in some embodiments, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPE, PEG-DSG or a PEG-DSPE lipid.

In some embodiments, a conjugate-linker lipid comprises a polyethylene glycol lipid. In some embodiments, the conjugate-linker lipid comprises DiMysterylGlycerol (DMG), 1,2-Dipalmitoyl-rac-glycerol, methoxypolyethylene Glycol (DPG-PEG), or 1,2-Distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG – PEG). In some embodiments, a conjugate-linker lipid has an average molecular mass from about 500 Da to about 5000 Da. In some embodiments, a conjugate-linker lipid has an average molecular mass of about 2000 Da.

In some embodiments, a LNP preparation comprises from about 0 mole percent to about 5 mole percent conjugate-linker lipid. In some embodiments, a LNP preparation comprises about 1.5 mole percent conjugate-linker lipid. In some embodiments, a LNP preparation comprises about 2.0 mole percent conjugate-linker lipid. In some embodiments, a LNP preparation comprises about 2.5 mole percent conjugate-linker lipid. In some embodiments, a LNP preparation comprises about 3 mole percent conjugate-linker lipid. In

some embodiments, a LNP preparation comprises about 3.5 mole percent conjugate-linker lipid.

Phospholipids

- 5 Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that comprise one or more phospholipids as described herein. In some embodiments, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that comprise one or more (poly)unsaturated lipids.
- 10 In some embodiments, one or more phospholipids may assemble into one or more lipid bilayers. In some embodiments, one or more phospholipids may include a phospholipid moiety. In some embodiments, one or more phospholipids may include one or more fatty acid moieties. In some embodiments, one or more phospholipids may include a phospholipid moiety and one or more fatty acid moieties. In some embodiments, a phospholipid moiety includes but is not limited to phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl
- 15 glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and sphingomyelin. In some embodiments, a fatty acid moiety includes but is not limited to lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.
- 20 Exemplary phospholipids include but are not limited to 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycerophosphocholine (DMPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-*sn*-glycerophosphocholine (DUPC), 1-palmitoyl-
- 25 2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-*sn*-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-*sn*-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-*sn*-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-*sn*-glycero-3-phosphocholine, 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-*sn*-glycero-3-phosphocholine, 1,2-diphytanoyl-*sn*-glycero-3-
- 30 phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dioleoyl-*sn*-glycero-3-phospho-

rac-(1 -glycerol) sodium salt (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyl-oleoylphosphatidylethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), 1-stearoyl-2-oleoyl-phosphatidyl ethanolamine (SOPE), 1-stearoyl-2-oleoylphosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine (LPE), or combinations thereof. In some embodiments, a phospholipid is DSPC. In some embodiments, a phospholipid is DMPC.

In some embodiments, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(succinyl) (succinyl-DPPE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), or a combination thereof.

In some embodiments, a LNP preparation comprises from about 5 mole percent to 25 mole percent of phospholipid. In some embodiments, a LNP preparation comprises about 5 mole percent to 15 mole percent of phospholipid. In some embodiments, a LNP preparation comprises about 9 mole percent to 11 mole percent of phospholipid. In some embodiments, a LNP preparation comprises about 10 mole percent of phospholipid.

Sterols

Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that comprise one or more sterols as described herein.

In some embodiments, a sterol is a cholesterol, or a variant or derivative thereof. In some embodiments, a cholesterol is modified. In some embodiments, a cholesterol is an oxidized cholesterol. In some embodiments, a cholesterol is esterified cholesterol. Unmodified cholesterol can be acted upon by enzymes to form variants that are side-chain or ring oxidized. In some embodiments, a cholesterol can be oxidized on the beta-ring structure or on the hydrocarbon tail structure. In some embodiments, a sterol is a phytosterol.

Exemplary sterols that are considered for use in the disclosed lipid nanoparticles include but are not limited to 25-hydroxycholesterol (25-OH), 20 α -hydroxycholesterol (20 α -OH), 27-hydroxycholesterol, 6-keto-5 α -hydroxycholesterol, 7-ketocholesterol, 7 β -hydroxycholesterol, 7 α -hydroxycholesterol, 7 β -25-dihydroxycholesterol, beta-sitosterol, stigmasterol,

brassicasterol, campesterol, or combinations thereof. In some embodiments, a side-chain oxidized cholesterol can enhance payload delivery relative to other cholesterol variants. In some embodiments, a cholesterol is an unmodified cholesterol.

In some embodiments, a LNP preparation comprises from about 25 mole percent to about 45 mole percent sterol. In some embodiments, a LNP preparation comprises from about 30 mole percent to about 45 mole percent sterol. In some embodiments, a LNP preparation comprises about 40 mole percent sterol. In some embodiments, a LNP preparation comprises about 35.75 mole percent sterol. In some embodiments, a LNP preparation comprises about 32.5 mole percent cholesterol.

LNP Formulations

Particular formulation of a nanoparticle composition comprising one or more described lipids is described herein. The present invention provides for compositions, preparations, nanoparticles, and/or nanomaterials that comprise lipid nanoparticles.

In some embodiments, a lipid nanoparticle preparation comprises about 30 mole percent to about 70 mole percent ionizable lipid, about 5 mole percent to about 25 mole percent phospholipid, about 25 mole percent to about 45 mole percent sterol, and about 0 mole percent to about 5 mole percent conjugate-linker lipid.

In some embodiments, a lipid nanoparticle preparation comprises about 47.5 mole percent ionizable lipid, about 10 mole percent phospholipid, about 40 mole percent sterol, and about 2.5 mole percent conjugate-linker lipid.

In some embodiments, a lipid nanoparticle preparation comprises about 51.25 mole percent ionizable lipid, about 10 mole percent phospholipid, about 35.75 mole percent sterol, and about 3 mole percent conjugate-linker lipid.

In some embodiments, the lipid nanoparticle preparation comprises about 55 mole percent ionizable lipid, about 10 mole percent phospholipid, about 32.5 mole percent sterol, and about 2.5 mole percent conjugate-linker lipid.

In some embodiments, a lipid nanoparticle preparation comprises about 45 mole percent to about 60 mole percent ionizable lipid of any provided compound, about 9 mole percent to about 11 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 1 mole percent to about 5 mole percent PEG₂₀₀₀-DMG, and about 30 mole percent to about 45 mole percent cholesterol, based on the total moles of these four ingredients.

In some embodiments, a lipid nanoparticle preparation is the formulation LNP1, LNP2, LNP3, or LNP4 in **Table 10A**.

5 In some embodiments, a lipid nanoparticle preparation comprises about 47.5 mole percent ionizable lipid IZ1, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 40 mole percent cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload comprising mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and guide RNA GA521
10 of **Table 1B**.

In some embodiments, a lipid nanoparticle preparation comprises about 55 mole percent ionizable lipid IZ2, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 32.5 mole percent cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a
15 payload comprising mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and guide RNA GA521 of **Table 1B**.

In some embodiments, a lipid nanoparticle preparation comprises about 51.25 mole
20 percent ionizable lipid IZ3, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 3 mole percent PEG₂₀₀₀-DMG, and about 35.75 mole percent cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload comprising mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the
25 combination of the amino acid alterations V82T, Y147T, and Q154S, and guide RNA GA521 of **Table 1B**.

In some embodiments, a lipid nanoparticle preparation comprises about 47.5 mole percent ionizable lipid IZ4, about 10 mole percent 1-2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.5 mole percent PEG₂₀₀₀-DMG, and about 40 mole percent
30 cholesterol, based on the total moles of these four ingredients, an N/P ratio of 6, and a payload comprising mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T or the combination of the amino acid alterations V82T, Y147T, and Q154S, and guide RNA GA521 of **Table 1B**.

The described lipid nanoparticle compositions are capable of delivering a therapeutic agent such as an RNA to a particular cell, tissue, organ, or system or group thereof in a mammal's body. Physiochemical properties of nanoparticle compositions may be altered in order to increase selectivity for particular bodily targets. For instance, particle sizes may be
5 adjusted based on the fenestration sizes of different organs.

Weight Ratio of Lipid Component to Therapeutic Agent

The amount of a therapeutic agent or drug substance (e.g., the mRNA that encodes for the base editor and the guide RNA) in an LNP composition may depend on the size,
10 composition, desired target and/or application, or other properties of the nanoparticle composition. For example, the amount of an RNA comprised in a nanoparticle composition may depend on the size, sequence, and other characteristics of the RNA. The relative amounts of a therapeutic agent and other elements (e.g., lipids) in a nanoparticle composition may also vary. In some embodiments, the wt/wt ratio of the lipid component to a therapeutic
15 agent in a nanoparticle composition may be from about 5:1 to about 60:1, such as about 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. For example, the wt/wt ratio of the lipid component to a therapeutic agent may be from about 10:1 to about 40:1. In certain embodiments, the wt/wt ratio is about 20:1. The amount of a therapeutic agent in a nanoparticle composition can be
20 measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

N/P Ratio

In some embodiments, an LNP formulation comprises one or more nucleic acids such as RNAs. In some embodiments, the one or more RNAs, lipids, and amounts thereof may be
25 selected to provide a specific N/P ratio. The N/P ratio can be selected from about 1 to about 30. In some embodiments, the N/P ratio is from about 2 to about 15, from about 2 to about 10, from about 2 to about 8, from about 2 to about 6, from about 3 to about 15, from about 3 to about 10, from about 3 to about 8, from about 3 to about 6, from about 4 to about 15, from about 4 to about 10, from about 4 to about 8, or from about 4 to about 6. In some
30 embodiments, the N/P ratio is about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 9, or about 10. In some embodiments, the N/P ratio is from about 5 to about 7. In some embodiments, the N/P ratio is

about 6. In each of the above N/P ratios and any other recitation of a single number for an N/P ratio, the moles or parts P is 1 and is not recited for brevity.

Polydispersity

5 Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that have a polydispersity index (PDI) of about 0.01 to about 0.3. In some embodiments, compositions, preparations, nanoparticles, and/or nanomaterials described herein have a PDI that is about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.2, 0.25, 0.3, or any range having endpoints defined by any two
10 of the aforementioned values. For example, in some embodiments, compositions, preparations, nanoparticles, and/or nanomaterials described herein have a PDI from about 0.05 to about 0.2, about 0.06 to about 0.1, or about 0.07 to about 0.09.

Encapsulation efficiency

15 Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials, wherein encapsulation efficiency of provided compositions, preparations, nanoparticles, and/or nanomaterials is from about 80% to about 100%. In some embodiments, encapsulation efficiency of compositions, preparations, nanoparticles, and/or nanomaterials described herein is about 80%, 85%, 90%, 91%, 92%,
20 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 100%, or any range having endpoints defined by any two of the aforementioned values. For example, in some embodiments, encapsulation efficiency of compositions, preparations, nanoparticles, and/or nanomaterials described herein is from about 90% to about 100%, about 95% to about 100%, about 95% to about 98%, or about 95.5% to about 97.5%. In some embodiments,
25 encapsulation efficiency of compositions, preparations, nanoparticles, and/or nanomaterials described herein is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

pKa

30 Among other things, the present disclosure describes compositions, preparations, nanoparticles, and/or nanomaterials that have a pKa from about 5 to about 9. In some embodiments, compositions, preparations, nanoparticles, and/or nanomaterials described herein have a pKa that is about 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, or any range having endpoints defined by any two of the aforementioned values. In some embodiments,

compositions, preparations, nanoparticles, and/or nanomaterials described herein have a pKa that is about 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, or any range having endpoints defined by any two of the aforementioned values.

5

Payload

The LNPs described herein can be designed to deliver a payload, such as one or more therapeutic agent(s) or drug substances(s) to a target cell or organ of interest. In some embodiments, a LNP described herein encloses one or more components of a base editor system as described herein. For example, a LNP may enclose one or more of a guide RNA, a nucleic acid encoding the guide RNA, a vector encoding the guide RNA, a base editor fusion protein, a nucleic acid encoding the base editor fusion protein, a programmable DNA binding domain, a nucleic acid encoding the programmable DNA binding domain, a deaminase, a nucleic acid encoding the deaminase, or all or any combination thereof. In some
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embodiments, the nucleic acid is a DNA. In some embodiments, the nucleic acid is a RNA, for example, a mRNA and/or a guide RNA. In some embodiments, the said nucleic acid(s) is/are chemically modified.

In some embodiments, the payload comprises one or more nucleic acid(s) (i.e., one or more nucleic acid molecular entities). In some embodiments, the nucleic acid is a single-
20
stranded nucleic acid. In some embodiments, single-stranded nucleic acid is an RNA. In some embodiments, the nucleic acid is a double-stranded nucleic acid. In some embodiments, the double-stranded nucleic acid is a DNA. In some embodiments, the nucleic acid is a messenger RNA (mRNA).

25 Methods of manufacturing LNPs

Methods of manufacturing lipid nanoparticles are known in the art. In some embodiments, the described compositions, preparations, nanoparticles, and/or nanomaterials are manufactured using microfluidics. For instance, exemplary methods of using microfluidics to form lipid nanoparticles are described by Leung, A.K.K, et al., *J Phys Chem*,
30
116:18440-18450 (2012), Chen, D., et al., *J Am Chem Soc*, 134:6947-6951 (2012), and Belliveau, N.M., et al., *Molecular Therapy- Nucleic Acids*, 1: e37 (2012), the disclosures of which are hereby incorporated by reference in their entireties.

Briefly, a payload, such as a payload described herein, is prepared in a first buffer solution. The other lipid nanoparticle components (such as ionizable lipid, conjugate-linker lipids, cholesterol, and phospholipid) are prepared in a second buffer solution. In some embodiments, a syringe pump introduces the two solutions into a microfluidic device. The two solutions come into contact within the microfluidic device to form lipid nanoparticles encapsulating the payload.

PHARMACEUTICAL COMPOSITIONS

In some aspects, the present disclosure provides a pharmaceutical composition comprising any of the cells, LNPs, polynucleotides, vectors, base editors, base editor systems, guide polynucleotides, fusion proteins, complexes, or the fusion protein-guide polynucleotide complexes described herein.

The pharmaceutical compositions of the present disclosure can be prepared in accordance with known techniques. See, *e.g.*, Remington, The Science And Practice of Pharmacy (21st ed. 2005). In general, the cell, or population thereof is admixed with a suitable carrier prior to administration or storage, and in some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers generally comprise inert substances that aid in administering the pharmaceutical composition to a subject, aid in processing the pharmaceutical compositions into deliverable preparations, or aid in storing the pharmaceutical composition prior to administration. Pharmaceutically acceptable carriers can include agents that can stabilize, optimize or otherwise alter the form, consistency, viscosity, pH, pharmacokinetics, solubility of the formulation. Such agents include buffering agents, wetting agents, emulsifying agents, diluents, encapsulating agents, and skin penetration enhancers. For example, carriers can include, but are not limited to, saline, buffered saline, dextrose, arginine, sucrose, water, glycerol, ethanol, sorbitol, dextran, sodium carboxymethyl cellulose, and combinations thereof.

In some embodiments, the pharmaceutical composition is formulated for delivery to a subject. Suitable routes of administering the pharmaceutical composition described herein include, without limitation: topical, subcutaneous, transdermal, intradermal, intralesional, intraarticular, intraperitoneal, intravesical, transmucosal, gingival, intradental, intracochlear, transtympanic, intraorgan, epidural, intrathecal, intramuscular, intravenous, intravascular, intraosseus, periocular, intratumoral, intracerebral, and intracerebroventricular administration.

In some embodiments, the pharmaceutical composition described herein is administered locally to a diseased site (*e.g.*, a liver). In some embodiments, the pharmaceutical composition described herein is administered to a subject by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being
5 of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber.

In some embodiments, any of the fusion proteins, gRNAs, and/or complexes described herein are provided as part of a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises any of the fusion proteins or
10 complexes provided herein. In some embodiments pharmaceutical composition comprises a gRNA, a nucleic acid programmable DNA binding protein, a cationic lipid, and a pharmaceutically acceptable excipient. In embodiments, pharmaceutical compositions comprise a lipid nanoparticle and a pharmaceutically acceptable excipient. In embodiments, the lipid nanoparticle contains a gRNA, a base editor, a complex, a base editor system, or a
15 component thereof of the present disclosure, and/or one or more polynucleotides encoding the same. Pharmaceutical compositions can optionally comprise one or more additional therapeutically active substances.

The compositions, as described above, can be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being
20 treated, and the desired outcome. It may also depend upon the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well-known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result.

In some embodiments, compositions in accordance with the present disclosure can be
25 used for treatment of any of a variety of diseases, disorders, and/or conditions.

METHODS OF TREATMENT

Some aspects of the present invention provide methods of treating a subject having or having a propensity to develop amyloidosis, the method comprising administering to a
30 subject in need an effective therapeutic amount of a pharmaceutical composition as described herein. In some embodiments, the methods of the invention comprise expressing or introducing into a cell of a subject a base editor polypeptide and one or more guide RNAs capable of targeting a nucleic acid molecule encoding a transthyretin polypeptide comprising a pathogenic mutation.

One of ordinary skill in the art would recognize that multiple administrations of the pharmaceutical compositions contemplated in particular embodiments may be required to affect the desired therapy. For example, a composition may be administered to the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more times over a span of 1 week, 2 weeks, 3 weeks, 1 month, 2
5 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 5 years, 10 years, or more.

In any of such methods, the methods may comprise administering to the subject an effective amount of an edited cell or a base editor system or polynucleotide encoding such system. In any of such methods, the methods may comprise administering one or more doses of an effective amount of the edited cells per day. In any of such methods, the methods may
10 comprise administering two or more doses of an effective amount of the modified cells per day. In any of such methods, the methods may comprise administering three or more doses of an effective amount of the edited cells per day. In any of such methods, the methods may comprise administering one or more doses of an effective amount of the edited cells per week. In any of such methods, the methods may comprise administering two or more doses
15 of an effective amount of the edited cells per week. In any of such methods, the methods may comprise administering three or more doses of an effective amount of the edited cells per week. In any of such methods, the methods may comprise administering one or more doses of an effective amount of the edited cells per month. In any of such methods, the methods may comprise administering two or more doses of an effective amount of the edited cells per
20 month. In any of such methods, the methods may comprise administering three or more doses of an effective amount of the edited cells per month.

Administration of the pharmaceutical compositions contemplated herein may be carried out using conventional techniques including, but not limited to, infusion, transfusion, or parenterally. In some embodiments, parenteral administration includes infusing or
25 injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally.

In some embodiments, the composition is administered over a period of 0.25 h, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, or 12 h. In another embodiment, the
30 composition is administered over a period of 0.25-2 h. In another embodiment, the composition is gradually administered over a period of 1 h. In another embodiment, the composition is gradually administered over a period of 2 h.

KITS

The invention provides kits for the treatment of amyloidosis in a subject. In some embodiments, the kit further includes a base editor system or a polynucleotide encoding a base editor system, wherein the base editor polypeptide system a nucleic acid programmable DNA binding protein (napDNAbp), a deaminase, and a guide RNA. In some embodiments, the napDNAbp is Cas9 or Cas12. In some embodiments, the polynucleotide encoding the base editor is a mRNA sequence. In some embodiments, the deaminase is a cytidine deaminase or an adenosine deaminase. In some embodiments, the kit comprises an edited cell and instructions regarding the use of such cell.

The kits may further comprise written instructions for using the base editor system and/or edited cell. In other embodiments, the instructions include at least one of the following: precautions; warnings; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. In a further embodiment, a kit can comprise instructions in the form of a label or separate insert (package insert) for suitable operational parameters. In yet another embodiment, the kit can comprise one or more containers with appropriate positive and negative controls or control samples, to be used as standard(s) for detection, calibration, or normalization. The kit can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as (sterile) phosphate-buffered saline, Ringer's solution, or dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The practice of embodiments of the present disclosure employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the disclosure, and, as such, may

be considered in making and practicing embodiments of the disclosure. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the disclosure, and are not intended to limit the scope of what the inventors regard as their invention.

Throughout the Examples, all of the guide polynucleotides contained the following polynucleotide sequence:

nsnsnsNNNNNNNNNNNNNNNNgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGU
 10 ccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususus (SEQ ID NO: 1235).

Where, “n” is a, c, g, or u; “N” is A, C, G, or U; A is adenosine; C is cytidine; G is guanosine; U is uridine; a is 2’-O-methyladenosine; c is 2’-O-methylcytidine; g is 2’-O-methylguanosine; u is 2’-O-methyluridine and s is phosphorothioate (PS) backbone linkage and wherein bold type represents the spacer sequence.

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EXAMPLES

Example 1: 15-Day In Vivo Non-human Primate (NHP) Study of Biodistribution and Base Editing

In this example, sgRNA (GA519; SEQ ID NO: 631) targeting a non-human primate transthyretin (TTR) gene site (i.e., “NHP surrogate sgRNA”) corresponding to the human TTR gene site targeted for base editing by sgRNA GA457 (SEQ ID NO: 637) was prepared and formulated with mRNA encoding an adenosine base editor (ABE) containing a Tada*8.20 adenosine deaminase domain with the amino acid alteration V82T (referred to throughout the figures as “ABE9.51”), encapsulated in lipid nanoparticles (LNP1, LNP2, LNP3, and LNP4), and intravenously dosed to non-human primates (NHPs).

25

The objectives of this study were to determine the targeted biodistribution and base editing of lipid nanoparticles (LNPs) encapsulating mRNA and a single guide RNA when given intravenously once on Day 1 to cynomolgus monkeys.

Each of LNP1 through LNP4 encapsulate the same sgRNA and ABE9.51 mRNA in a weight-to-weight ratio of 1:1. In other words, the LNPs were formulated with an equal amount by weight of guide RNA as mRNA. The resulting LNPs encapsulating the sgRNA and mRNA were filtered using 0.22 micron filters. The N/P ratio of the LNPs was 6. While the ratios of ionizable lipid, PEG-lipid, and sterol lipid differed between various LNP formulations, the most notable difference was in the ionizable lipid component in the LNP

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formulation, where LNP1 comprised Ionizable Lipid 1 (IZ1) and LNP2, LNP3, and LNP4 comprised IZ2, IZ3, and IZ4, respectively. The components of LNP1 through LNP4 are described in **Tables 10A and 10B** below.

5 **Table 10A. LNP Components**

Component	Description	LNP1 (mol%)	LNP2 (mol%)	LNP3 (mol%)	LNP4 (mol%)
Ionizable lipid	IZ1, IZ2, IZ3, or IZ4	47.5	55	51.25	47.5
Phospho lipid	1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)	10	10	10	10
PEG-lipid	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG ₂₀₀₀ -DMG)	2.5	2.5	3	2.5
Sterol	Cholesterol	40	32.5	35.75	40

Table 10B. Ionizable Lipids

Ionizable Lipid	Name	Source
IZ1	((3-hydroxypropyl)azanediyl)bis(heptane-7,1-diyl)bis(4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoate)	Example 8-4 in International Publication WO 2022/140252
IZ2	1-(3-((4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoyl)oxy)-2-((((1-ethylpiperidin-3-yl)methoxy)carbonyl)oxy)methyl)propyl 7-(3-pentyl)heptanedioate	Example 4-71 in International Publication WO 2022/159472
IZ3	3-((4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoyl)oxy)-2-(((7-((2-butyl)octanoyl)oxy)heptanoyl)oxy)methyl)propyl 4-(((4-(pyrrolidin-1-yl)butanoyl)oxy)decanoate)	Example 7-49 in PCT Application No. PCT/US23/27741
IZ4	3-((4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoyl)oxy)-2-(((7-((2-butyl)octanoyl)oxy)heptanoyl)oxy)methyl)propyl 4-(((2-(pyrrolidin-1-yl)ethyl)carbamoyl)oxy)decanoate	Example 7-7 in PCT Application No. PCT/US23/27741

It should be understood that the mol % of components in **Tables 10A** may be adjusted and that the mol % included in **Table 10A** are targeted excipient percentages of the LNP, which is intended to represent the aggregate mol % of all the LNPs formulated in a given batch and that specific LNPs within a batch may have varying mol %. Thus, it is contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 10A** may be adjusted, for example, by +/- 1-5%, +/- 5-10%, or +/- 10%-20%. It is

further contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 10A** with respect to a specific LNP formulated in a given batch of LNPs formulated in accordance with desired target excipient percentages, may vary from the targeted mol %, for example, by +/- 1-5%, +/- 5-10%, or +/-10%-20%, or even greater than +/- 20%. Further, it should be understood that additional LNP components, including non-lipid components, may be added to the LNP components set-forth in **Table 10A**.

Physical characteristics of the formulated LNPs are summarized in **Table 11** below.

Table 11. LNP Characterization

LNP	Z-average LNP diameter (nm)	PDI	RNA Encapsulation Efficiency (%)	pH
LNP1	62.87 nm	0.034	97.0%	7.37
LNP2	63.16 nm	0.042	97.0%	7.37
LNP3	70.52 nm	0.059	93.0%	7.34
LNP4	61.73 nm	0.022	97.0%	7.32

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One of ordinary skill in the art would understand that the average LNP size, PDI, RNA encapsulation efficiency, and pH values set forth in **Table 11** are subject to measurement error or accuracy. It is also contemplated herein that the LNP size, PDI, RNA encapsulation efficiency, and pH values set forth in **Table 11** may be varied by +/- 1-5%, +/- 5-10%, or +/- 10-20%.

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NHP Study Design

Naïve female cynomolgus monkeys of Cambodian origin were used as study animals. A premedication regimen comprising dexamethasone and antihistamines (diphenylhydramine and famotidine) was administered to all animals on day -1 and day 1 (predose), at 30 to 60 minutes prior to test article dose administration.

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Four groups of three monkeys each were dosed with LNP1 through LNP4 via a single IV infusion at a dose level of 1 mg of combined sgRNA and mRNA per kg of animal body weight, at a dose concentration of 0.167 mg per kg of animal body weight, and at a dose volume of 6 mL per kg of animal body weight.

25

Blood samples were collected from all animals predose for baseline measurements, including genotyping of the TTR target site via next-generation sequencing, and post-dose at various time points on days 1 through 15 to assess biomarkers, cytokines, pharmacokinetics,

and serum safety parameters. Necropsies were performed on all animals at day 15. Liver biopsy samples were collected to assess TTR gene editing.

Analysis of Editing Efficiency

- 5 The amount of gene editing in liver tissue of LNPs 1-4 was evaluated by next-generation sequencing (NGS) of the TTR target site. The NGS assay included the majority of Exon 1 of the TTR gene, which corresponded to the first 22 amino acids of the TTR protein. The editing target was in the start codon of the gene, specifically the second base on the reverse strand, which was an A.
- 10 Editing was assessed through single-plex, NGS amplicon sequencing using a two-step PCR process on genomic DNA isolated from liver tissue from each study animal. Analysis begins with isolation and QC of genomic DNA. Post isolation, first round PCR amplified the genomic specific target with primers carrying partial Illumina adapter sequences. PCR1 products were then used in a second PCR reaction which added on full length adapters as
- 15 well as dual index sequences. Purified, final products were pooled for NGS data acquisition on Illumina instrumentation. Final editing analysis was performed using the laboratory's existing computational pipeline.

In **Table 12**, editing was reported as the percent measure of the editing rate of the second base in the ATG start codon on the reverse strand (A to G conversion).

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Table 12. Hepatic TTR Editing Efficiency

Product	Dose (mg/kg)	Mean (%)	SD
LNP1	1	35	3.9
LNP2	1	33	11.0
LNP3	1	37	0.4
LNP4	1	46	9.7

Quantification of TTR protein expression in plasma

- 25 TTR protein expression in plasma was quantitated using LC-MS/MS. Plasma samples were denatured and proteolytically digested to generate proteotypic peptides, and then spiked with isotopically labeled peptides as internal standards for quantitation. Samples were analyzed with a targeted LC-MS/MS method that monitors four unique TTR peptide sequences. The measured TTR abundance at each timepoint for each animal was normalized against the pre-dose average TTR abundance for the same animal. Normalized percent

plasma cTTR (4-peptide average) at terminal (day 15) vs. pre-dose levels is provided in Table 13 below.

Table 13. cTTR Plasma Levels at Terminal vs. Pre-dose

Product	Dose (mg/kg)	Mean (%)	Variance between animals in the testing group (%)
LNP1	1	58	15
LNP2	1	58	8
LNP3	1	25	11
LNP4	1	38	11

5

Thus, infusion of LNP1 through LNP4 in NHPs resulted in editing of the TTR gene in the liver. LNP4 demonstrated greater editing than LNP1, LNP2, and LNP3, and LNP3 demonstrated greater reduction in plasma TTR concentrations than LNP1, LNP2 and LNP4.

10 *Example 2: Base editing of the human or non-human primate transthyretin (TTR) gene using base editor systems*

Experiments were undertaken to demonstrate that higher base editing rates were observed in human cells administered a base editor system for altering the TTR gene than in non-human primate cells administered a base editor system for altering the TTR gene. The base editor system contained a guide RNA targeting the TTR gene for base editing and mRNA encoding an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration. The amino acid sequence for the base editor (referred to as ABE9.51 in the figures of the disclosure) is provided below. The human TTR gene was targeted for base editing using the guide RNA gRNA2944 (human gRNA) and the cyno TTR gene was targeted for base editing using a cyno guide RNA (cyno gRNA; gRNA2945).

20 Amino acid sequence for the “ABE9.51” adenosine deaminase base editor used in the Examples of the present disclosure:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
25 LRQGGLVMQNYRLYDATLYTTFEPVCVMCAGAMHSRIGRVVFGVRNAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKK

HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDL
 NPDNSDVKLFQVLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKN
 LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSD
 AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
 5 GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
 LRRQEDFYFPLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEETITPWNFEVVDK
 GASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
 AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKI IKDKDFLD
 NEENEDILEDIVLTTLTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
 10 RDKQSGKTILDFLKSDGFANRNFQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
 IKKGILQTVKVVDELVKVMGRHKPENIVIEARENQTTQKGQKNSRERMKRIEEGIKELGSQ
 ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
 LTRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
 RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKREINN
 15 YHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNI
 MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKT EVQTGG
 FSKESILPKRNSDKLIARKKDWDPKYGGFDSPTVAYSVLVVAKEGKSKKLKSVKELLGI
 TIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
 PSKYVNFYLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKV
 20 LSAYNKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
 TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463).

An experiment was undertaken to measure any difference in base editing rates for the
 base editor systems that correlated with the gRNA polynucleotide used. HEK293T cells
 containing a polynucleotide containing both the human gRNA and cyno gRNA target
 25 sequences were administered base editor systems containing either the cyno gRNA or the
 human gRNA. The base editor systems were administered to the cells using the
 Lipofectamine™ MessengerMAX™ transfection reagent. The human gRNA was associated
 with base editing rates that were between about 1.28- and 2.65-fold (FIG. 1A) greater than
 those associated with the cyno gRNA.

30 An experiment was undertaken to compare base editing rates measured in primary
 human hepatocyte co-cultures and primary cyno hepatocyte co-cultures administered the base
 editor systems. Base editing was measured in the cells using 2-3 technical replicates. The
 base editor systems were administered to the primary human hepatocyte cells (PHH) or the
 primary cyno hepatocyte cells (PCH) using lipid nanoparticles containing the ionizable lipid

IZ1 (FIGs. 1B) or the ionizable lipid IZ4 (FIGs. 2A, 2B, 6A, and 6B). To alter a nucleobase in the TTR gene, the PHH were administered base editor systems containing the guide gRNA2944, and the PCH were administered base editor systems containing the guide gRNA2945. As a control, the cells were administered a base editor system containing the guide RNA sg23, which is known to be effective in targeting base editor systems to alter a nucleotide in an ALAS1 gene, and mRNA encoding the base editor ABE8.8 (FIG. 1B, right panel; FIG. 2B, and FIG. 6B). The base editing rates measured in the PHH at the TTR or ALAS1 gene locus were at least about 10- to 100-fold higher than those measured in the PCH. The base editor systems administered to the PHH were observed to be consistently more potent than base editor systems administered to the PCH (e.g., the EC50 value for the base editor system administered to the PHH was between 20- and 30-fold lower than that for the base editor system administered to the PCH (FIGs. 2A and 6A)). Similar differences in base editing rates were observed between the PHH and PCH cells across two different target loci and three different donors. Only minor and comparatively negligible differences in base editing rates were observed between cells from different donors. Representative images of the primary cyno hepatocytes used in the experiments are provided in FIGs. 5A and 5B. Without intending to be bound by theory, it is believed that the difference in base editing rates observed between the PHH and PCH is primarily driven mainly by differences between the two species from which the cells were derived.

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Example 3: Base editing of the transthyretin (TTR) gene in non-human primates using base editor systems administered using lipid nanoparticles

Experiments were undertaken to investigate base editing in non-human primates carried out using base editor systems administered using lipid nanoparticles and to investigate the pharmacology and toxicity of the lipid nanoparticles. The base editor systems contained a guide RNA targeting the TTR gene for base editing (cyno gRNA; gRNA2945) and mRNA encoding an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration. The lipid nanoparticles contained the ionizable lipid IZ1 and were administered to the non-human primates at doses of 0.5 mg/kg, 1 mg/kg, and 2 mg/kg.

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Liver enzyme levels were measured in the non-human primates at different timepoints before and after administration of the lipid nanoparticles. Administration of the lipid nanoparticles was not associated with any changes in alkaline phosphatase (ALP) levels in male or female non-human primates (FIGs. 3C and 3F). Administration of the lipid

nanoparticles was associated with minimal, transient, and generally non-dose dependent increases in alanine transaminase (ALT) and aspartate aminotransferase (AST) levels at day 2 or day 8 post-administration, and all ALT and AST levels were back to baseline by day 28 (FIGs. 3A, 3B, 3D, and 3E). Changes were observed in AST and ALT in control animals at a higher magnitude. There were also no changes in body weight or clinical signs in the non-human primates. All doses evaluated were well tolerated.

Example 4: A 4 Week In Vivo Non-human Primate (NHP) Study of Biodistribution and Base Editing

In this example, sgRNA (GA519; SEQ ID NO: 631) targeting a non-human primate transthyretin (TTR) gene site (i.e., “NHP surrogate sgRNA) corresponding to the human TTR gene site targeted for base editing by sgRNA GA457 (SEQ ID NO: 637) was prepared and formulated with mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alteration V82T (referred to throughout the figures as “ABE9.51”), encapsulated in a lipid nanoparticle (LNP1), and intravenously dosed to NHPs.

The objectives of this study were to determine the pharmacokinetics, pharmacodynamics and biodistribution of LNP1 for the treatment of ATTR amyloidosis when given intravenously (60 minutes infusion) as a single dose to cynomolgus monkeys.

LNP1 encapsulated the sgRNA and ABE9.51 mRNA in a weight-to-weight ratio of 1:1. In other words, the LNP was formulated with an equal amount by weight of guide RNA as mRNA. The resulting LNPs encapsulating the sgRNA and mRNA were filtered using 0.22 micron filters. The N/P ratio of the LNPs was 6. The components of LNP1 are described in Tables 14 and 15 below.

Table 14. LNP1 Components

Component	Description	Mol %
Ionizable lipid	IZ1	47.5
Phospholipid	1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)	10
PEG-lipid	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG ₂₀₀₀ -DMG)	2.5

Component	Description	Mol %
Sterol	Cholesterol	40

Table 15. Ionizable Lipid

Ionizable Lipid	Name	Source
IZ1	((3-hydroxypropyl)azanediy)bis(heptane-7,1-diyl) bis(4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoate)	Example 8-4 in International Publication WO 2022/140252

It should be understood that the mol % of components in **Table 14** may be adjusted and that the mol % included in **Table 14** are targeted excipient percentages of the LNP, which is intended to represent the aggregate mol % of all the LNPs formulated in a given batch and that specific LNPs within a batch may have varying mol %. Thus, it is contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 14** may be adjusted, for example, by +/- 1-5%, +/- 5-10%, or +/- 10%-20%. It is further contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 14** with respect to a specific LNP formulated in a given batch of LNPs formulated in accordance with desired target excipient percentages, may vary from the targeted mol %, for example, by +/- 1-5%, +/- 5-10%, or +/-10%-20%, or even greater than +/- 20%. Further, it should be understood that additional LNP components, including non-lipid components, may be added to the LNP components set-forth in **Table 14**.

Physical characteristics of the formulation LNP are summarized in **Table 16** below.

Table 16. LNP Characterization

LNP	Z-average LNP diameter (nm)	PDI	RNA Encapsulation Efficiency (%)	pH
1	67.445 nm	0.114	96.0%	7.46

One of ordinary skill in the art would understand that the average LNP size, PDI, RNA encapsulation efficiency, and pH values set forth in **Table 16** are subject to measurement error or accuracy. It is also contemplated herein that the LNP size, PDI, RNA

encapsulation efficiency, and pH values set forth in **Table 16** may be varied by +/- 1-5%, +/- 5-10%, or +/- 10-20%.

NHP Study Design

5 Naïve male and female cynomolgus monkeys of Cambodian origin were used as study animals. A premedication regimen comprising dexamethasone and antihistamines (diphenylhydramine and famotidine) was administered to all animals on day -1 and day 1 (predose), at 30 to 60 minutes prior to test article dose administration.

10 Three groups of two monkeys each (one male and one female) were dosed. As described in **Table 17**, one group was dosed with vehicle as control, and the remaining three groups were dosed with LNP1 via a single IV infusion at a dose levels of 0.5 mg, 1 mg, and 2 mg of combined sgRNA and mRNA per kg of animal body weight, at dose concentrations of 0.083 mg, 0.167 mg, and 0.333 mg per kg of animal body weight, respectively, and at a dose volume of 6 mL per kg of animal body weight.

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Table 17. Dosing regimens.

Group No.	Test Article	Dose Level (mg/kg/day)	Dose Vol. (mL/kg)	Dose Conc. (mg/mL)	No. of Animals	
					Male	Female
1	Vehicle	0	6	0	1	1
2	LNP1	0.5	6	0.083	1	1
3	LNP1	1	6	0.167	1	1
4	LNP1	2	6	0.333	1	1

20 Blood samples were collected from all animals predose for baseline measurements, including genotyping of the TTR target site via next generation sequencing (NGS), and post-dose at various time points to assess biomarkers, cytokines, pharmacokinetics, and serum safety parameters. Necropsies were performed on all animals at day 28. Biopsy and other samples were collected to assess TTR gene editing as well as other aspects.

Analysis of Editing

25 The amount of gene editing in liver tissue was evaluated by NGS sequencing of targeted PCR amplicons at the TTR target site. The NGS assay included the majority of Exon 1 of the TTR gene, which corresponded to the first 22 amino acids of the TTR protein.

The editing target was in the start codon of the gene, specifically the second base on the reverse strand, which was an A.

Editing was assessed through single-plex, NGS amplicon sequencing using a two-step PCR process on genomic DNA isolated from liver tissue from each study animal. Analysis began with isolation and QC of genomic DNA. Post isolation, first round PCR amplified the genomic specific target with primers carrying partial Illumina adapter sequences. PCR1 products were then used in a second PCR reaction which added on full length adapters as well as dual index sequences. Purified, final products were pooled for NGS data acquisition on Illumina instrumentation. Final editing analysis was performed using the laboratory's existing computational pipeline.

In **Table 18**, editing was reported as the percent measure of the editing rate of the second base in the ATG start codon on the reverse strand (A to G conversion).

Table 18. Hepatic TTR Editing of LNP1 at Various Doses

	Group 1 (Vehicle)		Group 2 (0.5 mg/kg)		Group 3 (1 mg/kg)		Group 4 (2 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
% On-Target Editing (SD)	0.09% (0.01%)	0.12% (0.04%)	14.72% (1.07%)	26.92% (2.16%)	9.55% (2.23%)	57.76% (0.88%)	48.91% (4.98%)	56.29% (2.02%)

Quantification of TTR protein expression in plasma

TTR protein expression in plasma was quantitated using LC-MS/MS. Plasma samples were denatured and proteolytically digested to generate proteotypic peptides, and then spiked with isotopically labeled peptides as internal standards for quantitation. Samples were analyzed with a targeted LC-MS/MS method that monitors four unique TTR peptide sequences. The measured TTR abundance at each timepoint for each animal was normalized against the pre-dose average TTR abundance for the same animal. Normalized percent plasma cTTR (4-peptide average) at terminal timepoint is provided in **Table 19** below.

Table 19. cTTR Normalized Plasma Levels at Terminal Timepoint (Day 28)

	Group 1 (Vehicle)		Group 2 (0.5 mg/kg)		Group 3 (1 mg/kg)		Group 4 (2 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
% cTTR Knockdown (variance in measurements between the 4 surrogate peptides)	-2% (5%)	11% (4%)	33% (6%)	33% (2%)	32% (6%)	89% (1%)	77% (2%)	85% (1%)

Example 5: A 28-Day In Vivo Non-human Primate (NHP) Study of Biodistribution, and Base Editing

5 In this example, NHP surrogate sgRNA (GA519; SEQ ID NO: 631) targeting a non-human primate transthyretin (TTR) gene site (i.e., “NHP surrogate sgRNA) corresponding to the human TTR gene site targeted for base editing by sgRNA GA457 (SEQ ID NO: 637)) was prepared and formulated with mRNA encoding an adenosine base editor (ABE) containing a TadA*8.20 adenosine deaminase domain with the amino acid alterations V82T, Y147T, and Q154S (referred to throughout the figures as “ABE9.52”), encapsulated in a lipid nanoparticle (LNP1), and intravenously dosed to NHPs.

 The objectives of this study were to determine the pharmacokinetics, pharmacodynamics and biodistribution of LNP1 when given intravenously (60 minutes infusion) as a single dose to cynomolgus monkeys.

15 LNP1 encapsulated the sgRNA and mRNA encoding the ABE in a weight-to-weight ratio of 1:1. In other words, the LNP was formulated with an equal amount by weight of guide RNA as mRNA. The resulting LNP formulation was filtered using 0.22 micron filters. The N/P ratio of the LNP1 was 6. The components of LNP1 are described below in **Tables 20 and 21**.

Table 20. LNP1 Components

Component	Description	Mol %
Ionizable lipid	IZ1	47.5
Phospholipid	1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)	10

Component	Description	Mol %
PEG-lipid	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG ₂₀₀₀ -DMG)	2.5
Sterol	Cholesterol	40

Table 21. Ionizable Lipid

Ionizable Lipid	Name	Source
IZ1	((3-hydroxypropyl)azanediyl) bis(heptane-7,1-diyl) bis(4,4-bis(((Z)-oct-5-en-1-yl)oxy)butanoate)	Example 8-4 in International Publication WO 2022/140252

It should be understood that the mol % of components in **Table 20** may be adjusted and that the mol % included in **Table 20** are targeted excipient percentages of the LNP, which is intended to represent the aggregate mol % of all the LNPs formulated in a given batch and that specific LNPs within a batch may have varying mol %. Thus, it is contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 20** may be adjusted, for example, by +/- 1-5%, +/- 5-10%, or +/- 10%-20%. It is further contemplated herein that the mol % of one or more, or all of the LNP components set forth in **Table 20** with respect to a specific LNP formulated in a given batch of LNPs formulated in accordance with desired target excipient percentages, may vary from the targeted mol %, for example, by +/- 1-5%, +/- 5-10%, or +/-10%-20%, or even greater than +/- 20%. Further, it should be understood that additional LNP components, including non-lipid components, may be added to the LNP components set-forth in **Table 20**.

Physical characteristics of the LNP1 formulation are summarized in **Table 22** below.

Table 22. LNP1 Characterization

Z-average LNP diameter (nm)	PDI	RNA Encapsulation Efficiency (%)	pH
64.75	0.095	97.0	7.34

One of ordinary skill in the art would understand that the average LNP size, PDI, RNA encapsulation efficiency, and pH values set forth in **Table 22** are subject to measurement error or accuracy. It is also contemplated herein that the LNP size, PDI, RNA

encapsulation efficiency, and pH values set forth in **Table 22** may be varied by +/- 1-5%, +/- 5-10%, or +/- 10-20%.

NHP Study Design

5 Naïve male and female cynomolgus monkeys of Cambodian origin were used as study animals. A premedication regimen comprising dexamethasone and antihistamines (diphenylhydramine and famotidine) was administered to all animals on day -1 and day 1 (predose), at 30 to 60 minutes prior to test article dose administration.

10 Three groups of monkeys each were dosed. As described in **Table 23** below, one group was dosed with vehicle as control, and the remaining two groups were dosed with LNP1 via a single IV infusion at a dose levels of 0.5 mg and 1 mg of combined sgRNA and mRNA per kg of animal body weight, at a dose concentrations of 0.083 mg and 0.167 mg per kg of animal body weight, respectively, and at a dose volume of 6 mL per kg of animal body weight.

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Table 23. Dosing regimens.

Group No.	Test Article	Dose Level (mg/kg/day)	Dose Vol. (mL/kg)	Dose Conc. (mg/mL)	No. of Animals	
					Male	Female
1	Vehicle	0	6	0	1	1
2	LNP1	0.5	6	0.083	2	1
3	LNP1	1	6	0.167	1	2

20 Blood samples were collected from all animals predose for baseline measurements, including genotyping of the TTR target site via next generation sequencing, and post-dose at various time points to assess biomarkers, cytokines, pharmacokinetics, and serum safety parameters. Necropsies were performed on all animals at day 28. Biopsy and other samples were collected to assess TTR gene editing as well as other aspects.

Analysis of Editing

25 The amount of gene editing in liver tissue was evaluated by next-generation sequencing (NGS) of the TTR target site. The NGS assay included the majority of Exon 1 of the TTR gene, which corresponded to the first 22 amino acids of the TTR protein. The editing target was in the start codon of the gene, specifically the second base on the reverse strand, which was an A.

Editing was assessed through single-plex, NGS amplicon sequencing using a two-step PCR process on genomic DNA isolated from liver tissue from each study animal. Analysis began with isolation and QC of genomic DNA. Post isolation, first round PCR amplified the genomic specific target with primers carrying partial Illumina adapter sequences. PCR1 products were then used in a second PCR reaction which added on full length adapters as well as dual index sequences. Purified, final products were pooled for NGS data acquisition on Illumina instrumentation.

In **Table 24**, editing was reported as the percent measure of the editing rate of the second base in the ATG start codon on the reverse strand (A to G conversion).

10

Table 24. Hepatic TTR Editing of LNP1 At Various Doses

	Group 1 (Vehicle)	Group 2 (0.5 mg/kg)	Group 3 (1 mg/kg)
% On-Target Editing (SD)	0.02 (0.011)	27.68 (16.05)	43.72 (9.995)

Quantification of TTR protein expression in plasma

TTR protein expression in plasma was measured using quantitative peptide mapping LC-MS/MS. Plasma samples were denatured and proteolytically digested to generate proteotypic peptides, and then spiked with isotopically labeled peptides as internal standards for quantitation. Samples were analyzed with a targeted LC-MS/MS method that monitors four unique TTR peptide sequences. The measured TTR abundance at each timepoint for each animal was normalized against the pre-dose average TTR abundance for the same animal. Normalized percent plasma cTTR (4-peptide average) at the terminal timepoint is provided in **Table 25** below. Group 2 showed plasma TTR reduction of -41% at day 28 relative to pre-dose baseline, and Group 3 showed plasma TTR reduction of -70% at day 28 relative to pre-dose baseline.

25 **Table 25. cTTR Normalized Plasma Levels at Terminal Timepoint (Day 28)**

	Group 1 (Vehicle)	Group 2 (0.5 mg/kg)	Group 3 (1 mg/kg)
Terminal % TTR protein abundance (SD)	99% (3%)	59% (20%)	30% (15%)

Example 6: Assessment of off-target editing using ABE9.51

Experiments were undertaken to measure levels of gRNA-dependent off-target editing for base editor systems containing an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”) and a guide polynucleotide targeting the TTR gene for base editing, where the base editor systems were administered to cells using lipid nanoparticles at an EC₉₀_{editing} dose.

Only one gRNA-dependent off-target site was identified, namely location chr8:108362156-108362179 in the human genome, which corresponds to the middle of a 125kb intron in the *EIF3E* gene. The off-target site had the sequence CCCATCA-
GCCAAGAATGAGAGG (SEQ ID NO: 483), where the nucleotides in bold differ from the TTR target site, the term “-” indicates a deletion of a nucleotide relative to the TTR target site, and underlined text corresponds to the PAM sequence corresponding to the TTR HM17 target site. The *EIF3E* gene is expressed in the liver and spleen at > 1 transcript per million (TMP), and humans are tolerant of loss of function variants of the gene. Intentional base editing at the *EIF3E* off-target site did not affect *EIF3E* gene expression or splicing patterns in HepG2 cells.

Example 7: Assessment of on- and off-target editing for two different base editor systems in primary human hepatocytes

Experiments were undertaken to compare base editing rates associated with base editor systems containing an adenosine deaminase base editor containing a TadA*8.20 adenosine deaminase domain variant with a V82T amino acid alteration (referred to throughout the figures as “ABE9.51”) or a TadA*8.20 adenosine deaminase domain with the amino acid alterations V82T, Y147T, and Q154S (referred to throughout the figures as “ABE9.52”) and a guide polynucleotide targeting the human TTR gene for base editing (i.e., GA521). Primary human hepatocytes were contacted with mRNA encoding the adenosine deaminase base editor and the guide polynucleotide. The two base editor systems showed similar on- and off-target editing activity (FIG. 4). On- and off-target editing was assessed by amplicon-seq at the *TTR* (on-target) and *EIF3E* (off-target) loci.

OTHER EMBODIMENTS

From the foregoing description, it will be apparent that variations and modifications may be made to the aspects or embodiments described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

- 5 The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

- 10 All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference. The disclosure may be related to International Patent Applications No. PCT/US2022/030359, filed May 20, 2022, or PCT/US2022/029278, filed May 13, 2022, the disclosures of which are each incorporated herein by reference in their entireties for all purposes. The disclosure may be related to U.S.
- 15 Provisional Patent Applications No. 63/383,394, filed November 11, 2022, or to 63/385,004, filed November 25, 2022, the disclosures of which are each incorporated herein by reference in their entireties for all purposes.

CLAIMS

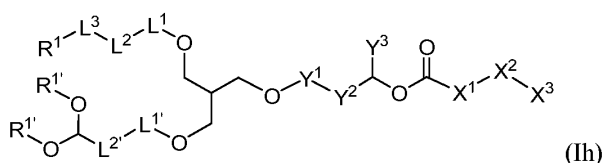
What is claimed:

1. A lipid nanoparticle (LNP) comprising:
 - 5 A) a guide RNA, or a polynucleotide encoding the guide RNA, wherein the guide RNA comprises a spacer comprising a nucleotide sequence selected from GCCAUC CUGCCAAGAAUGAG (SEQ ID NO: 472) and those listed in any of **Tables 1B, 1C, 1D, 1E, 2A, 2B, 2D, or 2E**;
 - B) an mRNA molecule, or a polynucleotide encoding the mRNA molecule, wherein
 - 10 the mRNA molecule encodes a base editor polypeptide comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises:
 - i) an amino acid sequence having at least 90% identity to the following TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:

15 MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAA
GSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD
(SEQ ID NO: 1), and
 - ii) a combination of amino acid alterations relative to the TadA*7.10 amino
 - 20 acid sequence selected from the group consisting of:
 - a) I76Y, V82T, Y123H, Y147R, and Q154R;
 - b) V82T, Y123H, D147R, and Q154R;
 - c) V82T, Y123H, D147T, and Q154S;
 - d) V82T and Q154R;
 - 25 e) V82T, Y147T, and Q154S;
 - f) I76Y, V82T, Y123H, Y147T, and Q154S; and
 - C) an ionizable lipid according to any one of the following formulas, or a pharmaceutically acceptable salt thereof:

i) a compound of Formula (Ih):

30



wherein:

each L^1 and $L^{1'}$ is independently -C(O)- or -OC(O)-;

each L^2 and $L^{2'}$ is independently an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

- 5 L^3 is a covalent bond, -O-, -C(O)O-, -OC(O)-, or -OC(O)O-;

R^1 is optionally substituted C_{1-20} aliphatic, $\text{---}L^{CyA}\text{---}Cy^A$, or $\text{---}L^{Ra}\text{---}\begin{matrix} O-R^a \\ O-R^a \end{matrix}$;

L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially

- 10 unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

each R^a and $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic;

Y^1 is -C(O)- or -C(O)O-;

- 15 Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain;

Y^3 is optionally substituted C_{1-20} aliphatic;

X^1 is a covalent bond, -O-, or -NR-;

X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12}

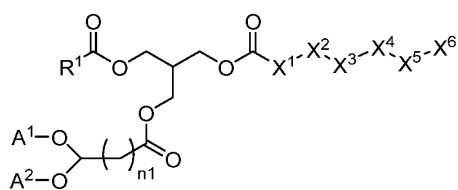
- 20 hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered

- 25 heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic;

ii) a compound of Formula (Ia):



Ia

wherein:

R¹ is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation;

- 5 X¹ and X² are each independently absent or selected from -O-, -NR²- and $\begin{matrix} -(\text{CH})_a- \\ \text{X}^7 \end{matrix}$,
 wherein each R² is independently hydrogen or C₁-C₆ alkyl;
 each a is independently an integer between 1 and 6;

- X³ and X⁴ are each independently absent or selected from the group consisting of: 4-
 to 8-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-
 10 membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered
 aryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 4- to 7-membered cycloalkyl
 optionally substituted with 1 or 2 C₁-C₆ alkyl groups, -O- and -NR³-, wherein each R³ is a
 independently a hydrogen atom or C₁-C₆ alkyl and wherein X¹-X²-X³-X⁴ does not contain
 any oxygen-oxygen, oxygen-nitrogen or nitrogen-nitrogen bonds;

- 15 X⁵ is -(CH₂)_b-, wherein b is an integer between 0 and 6;

- X⁶ is hydrogen, C₁-C₆ alkyl, 5- to 6-membered heteroaryl optionally substituted with
 1 or 2 C₁-C₆ alkyl groups, or -NR⁴R⁵, wherein R⁴ and R⁵ are each independently hydrogen or
 C₁-C₆ alkyl; or alternatively R⁴ and R⁵ join together with the nitrogen to which they are
 bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl
 20 groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from
 oxygen, sulfur, and nitrogen;

- each X⁷ is independently hydrogen, hydroxyl or -NR⁶R⁷, wherein R⁶ and R⁷ are each
 independently hydrogen or C₁-C₆ alkyl; or alternatively R⁶ and R⁷ join together with the
 nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally
 25 substituted with 1 or 2 C₁-C₆ alkyl groups, wherein the heterocyclyl optionally includes an
 additional heteroatom selected from oxygen, sulfur, and nitrogen;

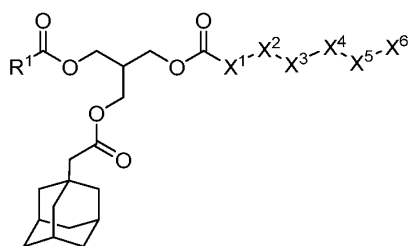
at least one of X¹, X², X³, X⁴, and X⁵ is present;

A¹ and A² are each independently selected from the group consisting of: C₅-C₁₂
 haloalkyl, C₅-C₁₂ alkenyl, C₅-C₁₂ alkynyl, (C₅-C₁₂ alkoxy)-(CH₂)_{n2}-, (C₅-C₁₀ aryl)-(CH₂)_{n3}-

optionally ring substituted with one or two halo, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ alkoxy groups, and (C₃-C₈ cycloalkyl)-(CH₂)_{n4}— optionally ring substituted with 1 or 2 C₁-C₆ alkyl groups; or alternatively A¹ and A² join together with the atoms to which they are bound to form a 5- to 6-membered cyclic acetal substituted with 1 or 2 C₄-C₁₀ alkyl groups;

- 5 n₁, n₂ and n₃ are each individually an integer between 1 and 4; and
 n₄ is an integer between zero and 4;

iii) A compound of Formula (Ib):



Ib

- 10 wherein:

R¹ is C₉-C₂₀ alkyl or C₉-C₂₀ alkenyl with 1-3 units of unsaturation;

X¹ and X² are each independently absent or selected from —O—, NR², and $\text{-(}\underset{|}{\text{CH}}\text{)}_a\text{—}$,
 wherein R² is C₁-C₆ alkyl, and wherein X¹ and X² are not both —O— or NR²;

a is an integer between 1 and 6;

- 15 X³ and X⁴ are each independently absent or selected from the group consisting of: 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, and —NR³—, wherein each R³ is a hydrogen atom or C₁-C₆ alkyl;

X⁵ is —(CH₂)_b—, wherein b is an integer between 0 and 6;

- 20 X⁶ is hydrogen, C₁-C₆ alkyl, 5- to 6-membered heteroaryl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, or —NR⁴R⁵, wherein R⁴ and R⁵ are each independently hydrogen or C₁-C₆ alkyl; or alternatively R⁴ and R⁵ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from
 25 oxygen, sulfur, and nitrogen;

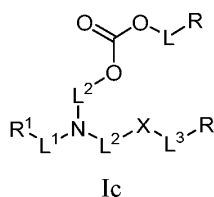
X⁷ is hydrogen or —NR⁶R⁷, wherein R⁶ and R⁷ are each independently hydrogen or C₁-C₆ alkyl; or alternatively R⁶ and R⁷ join together with the nitrogen to which they are bound to form a 4- to 7-membered heterocyclyl optionally substituted with 1 or 2 C₁-C₆ alkyl

groups, wherein the heterocyclyl optionally includes an additional heteroatom selected from oxygen, sulfur, and nitrogen;

at least one of X^1 , X^2 , X^3 , X^4 , and X^5 is present; and

provided that when either X^1 or X^2 is $-O-$, neither X^3 nor X^4 is , and when
 5 either X^1 or X^2 is $-O-$, R^4 and R^5 are not both ethyl;

iv) A compound of Formula (Ic):



or its N-oxide, or a salt thereof, wherein

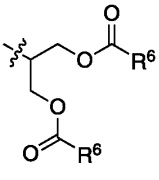
10 L1 is C1-6 alkylenyl, or C2-6 heteroalkylenyl;

each L2 is independently C2-10 alkylenyl, or C3-10 heteroalkylenyl;

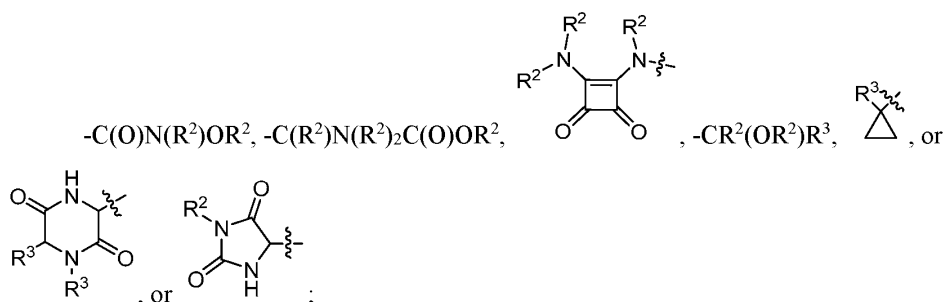
L is absent, C1-10 alkylenyl, or C2-10 heteroalkylenyl;

L3 is absent, C1-10 alkylenyl, or C2-10 heteroalkylenyl;

X is absent, $-OC(O)-$, $-C(O)O-$, or $-OC(O)O-$;

15 each R is independently hydrogen, , or an optionally substituted group selected from C6-20 aliphatic, C6-20 haloaliphatic, a 3- to 7-membered cycloaliphatic ring, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

R1 is hydrogen, a 3- to 7-membered cycloaliphatic ring, a 3- to 7-membered heterocyclic ring comprising 1-3 heteroatoms independently selected from nitrogen, oxygen,
 20 and sulfur, $-OR^2$, $-C(O)OR^2$, $-C(O)SR^2$, $-OC(O)R^2$, $-OC(O)OR^2$, $-CN$, $-N(R^2)_2$, $-C(O)N(R^2)_2$,
 $-NR^2C(O)R^2$, $-OC(O)N(R^2)_2$, $-N(R^2)C(O)OR^2$, $-NR^2S(O)_2R^2$, $-NR^2C(O)N(R^2)_2$,
 $-NR^2C(S)N(R^2)_2$, $-NR^2C(NR^2)N(R^2)_2$, $-NR^2C(CHR^2)N(R^2)_2$, $-N(OR^2)C(O)R^2$,
 $-N(OR^2)S(O)_2R^2$, $-N(OR^2)C(O)OR^2$, $-N(OR^2)C(O)N(R^2)_2$, $-N(OR^2)C(S)N(R^2)_2$,
 $-N(OR^2)C(NR^2)N(R^2)_2$, $-N(OR^2)C(CHR^2)N(R^2)_2$, $-C(NR^2)N(R^2)_2$, $-C(NR^2)R^2$,



each R^2 is independently

hydrogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{OR}^4$, $-\text{S}(\text{O})_2\text{R}^4$, $-\text{S}(\text{O})_2\text{N}(\text{R}^4)_2$, $-(\text{CH}_2)_n-\text{R}^4$, or an optionally substituted
 5 group selected from C_{1-6} aliphatic, a 3- to 7-membered cycloaliphatic ring, and a 3- to 7-
 membered heterocyclic ring comprising 1-3 heteroatoms independently selected from
 nitrogen, oxygen, and sulfur, or

two occurrences of R^2 , taken together with the atom(s) to which they are attached,
 form an optionally substituted 4- to 7-membered heterocyclic ring comprising 0-1 additional
 10 heteroatom selected from nitrogen, oxygen, and sulfur;

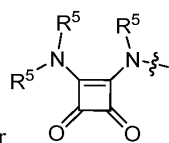
each R^3 is independently $-(\text{CH}_2)_n-\text{R}^4$, or

two occurrences of R^3 , taken together with the atoms to which they are attached, form
 an optionally substituted 5- to 6-membered heterocyclic ring comprising 0-1 additional
 heteroatom selected from nitrogen, oxygen, and sulfur;

15 each R^4 is independently

hydrogen, $-\text{OR}^5$, $-\text{N}(\text{R}^5)_2$, $-\text{OC}(\text{O})\text{R}^5$, $-\text{OC}(\text{O})\text{OR}^5$, $-\text{CN}$, $-\text{C}(\text{O})\text{N}(\text{R}^5)_2$,

$-\text{NR}^5\text{C}(\text{O})\text{R}^5$, $-\text{OC}(\text{O})\text{N}(\text{R}^5)_2$, $-\text{N}(\text{R}^5)\text{C}(\text{O})\text{OR}^5$, $-\text{NR}^5\text{S}(\text{O})_2\text{R}^5$, $-\text{NR}^5\text{C}(\text{O})\text{N}(\text{R}^5)_2$,



$-\text{NR}^5\text{C}(\text{S})\text{N}(\text{R}^5)_2$, $-\text{NR}^5\text{C}(\text{NR}^5)\text{N}(\text{R}^5)_2$, or

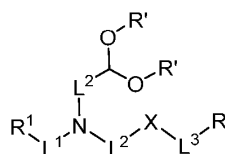
each R^5 is independently hydrogen, optionally substituted C_{1-6} aliphatic, or

20 two occurrences of R^5 , taken together with the atom(s) to which they are attached,
 form an optionally substituted 4- to 7-membered heterocyclic ring comprising 0-1 additional
 heteroatom selected from nitrogen, oxygen, and sulfur;

each R^6 is independently C_{4-12} aliphatic; and

n is 0 to 4;

25 **v) A compound of Formula (Id):**



Id

or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein

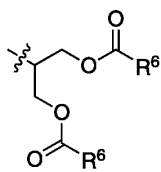
L1 is absent, C1-6 alkylenyl, or C2-6 heteroalkylenyl;

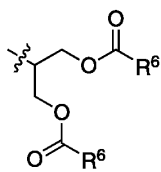
- 5 each L2 is independently optionally substituted C2-15 alkylenyl, or optionally substituted C3-15 heteroalkylenyl;

L3 is absent, optionally substituted C1-10 alkylenyl, or optionally substituted C2-10 heteroalkylenyl;

X is absent, -OC(O)-, -C(O)O-, or -OC(O)O-;

- 10 each R' is independently an optionally substituted group selected from C4-12 aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;



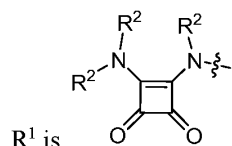
R is hydrogen, , or an optionally substituted group selected from C6-20

- 15 aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

R1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3

- 20 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, -OR², -C(O)OR², -C(O)SR², -OC(O)R², -OC(O)OR², -CN, -N(R²)₂, -C(O)N(R²)₂, -S(O)₂N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR², -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -N(OR²)C(O)N(R²)₂, -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂,

-C(NR²)R², -C(O)N(R²)OR², -C(R²)N(R²)₂C(O)OR², -CR²(R³)₂, -OP(O)(OR²)₂, or
-P(O)(OR²)₂; or



- R¹ is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally substituted with 1-4 R² or R³ groups;
- each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; or

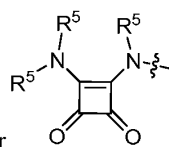
- two occurrences of R², taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

each R³ is independently -(CH₂)_n-R⁴; or

- two occurrences of R³, taken together with the atom(s) to which they are attached, form optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

- each R⁴ is independently hydrogen, -OR⁵, -N(R⁵)₂, -OC(O)R⁵, -OC(O)OR⁵, -CN, -C(O)N(R⁵)₂,

-NR⁵C(O)R⁵, -OC(O)N(R⁵)₂, -N(R⁵)C(O)OR⁵, -NR⁵S(O)₂R⁵, -NR⁵C(O)N(R⁵)₂,



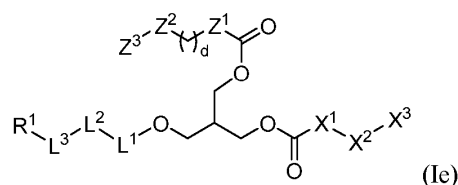
-NR⁵C(S)N(R⁵)₂, -NR⁵C(NR⁵)N(R⁵)₂, or

- each R⁵ is independently hydrogen, or optionally substituted C₁₋₆ aliphatic; or
- two occurrences of R⁵, taken together with the atom(s) to which they are attached, form optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional heteroatom selected from nitrogen, oxygen, and sulfur;

each R⁶ is independently C₄₋₁₂ aliphatic; and

each n is independently 0 to 4;

vi) A compound of Formula (Ic):



5 or a pharmaceutically acceptable salt thereof, wherein:

L^1 is a covalent bond, ---C(O)--- , or ---OC(O)--- ;

L^2 is a covalent bond, an optionally substituted bivalent saturated or unsaturated,

straight or branched $\text{C}_1\text{---C}_{12}$ hydrocarbon chain, or $\text{---}(\text{---})_m\text{---Cy}^A\text{---}(\text{---})_m\text{---}$;

Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered

10 saturated or partially unsaturated carbocyclene;

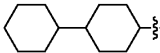
each m is independently 0, 1, or 2;

L^3 is a covalent bond, ---C(O)--- , ---C(O)O--- , ---OC(O)--- , ---O--- , or ---OC(O)O--- ;

R^1 is $\text{---}(\text{---})_p\text{---Cy}^B$, or an optionally substituted saturated or unsaturated, straight or branched

15 $\text{C}_1\text{---C}_{20}$ hydrocarbon chain wherein 1-3 methylene units are optionally and independently replaced with ---O--- or ---NR--- ;

Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or

partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and phenyl;

20 p is 0, 1, 2, or 3;

X^1 is a covalent bond, ---O--- , or ---NR--- ;

X^2 is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, $\text{C}_1\text{---C}_{12}$ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with ---O--- , ---NR--- , or $\text{---Cy}^C\text{---}$;

25 Cy^C is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated heterocyclene having 1-3 heteroatoms independently selected from nitrogen,

oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R is independently hydrogen or an optionally substituted C_1 - C_6 aliphatic group;

Z^1 is a covalent bond or -O-;

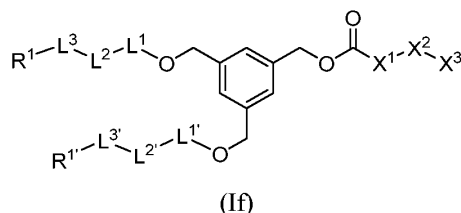
Z^2 is an optionally substituted group selected from 4- to 12-membered saturated or partially unsaturated carbocyclyl, phenyl, 1-adamantyl, and 2-adamantyl;

Z^3 is hydrogen, or an optionally substituted group selected from C_1 - C_{10} aliphatic, and 4- to 12-membered saturated or partially unsaturated carbocyclyl; and

d is 0, 1, 2, 3, 4, 5, or 6;

provided that when L^3 is a covalent bond, then R^1 must be $\text{---} \left(\text{---} \right)_p \text{---} \text{Cy}^B$;

vii) A compound of Formula (If):



or a pharmaceutically acceptable salt thereof, wherein:

each L^1 and $L^{1'}$ is independently -C(O)- or -C(O)O-;

each L^2 and $L^{2'}$ is independently a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or $\text{---} \left(\text{---} \right)_m \text{---} \text{Cy}^A \text{---} \left(\text{---} \right)_m \text{---}$;

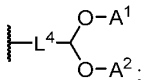
each Cy^A is independently an optionally substituted ring selected from phenylene or a 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

each L^3 and $L^{3'}$ is independently a covalent bond, -C(O)O-, -OC(O)-, -O-, or -OC(O)O-;

each R^1 and $R^{1'}$ is independently an optionally substituted group selected from a saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain, wherein 1-3

methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 12-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl,

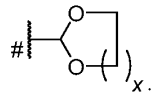
steroyl, phenyl, and ;

each L⁴ is independently a bivalent saturated or unsaturated, straight or branched C₁-

5 C₆ hydrocarbon chain;

each A¹ and A² is independently an optionally substituted C₁-C₂₀ aliphatic or -L⁵-R⁵;

or A¹ and A², together with their intervening atoms, may form an optionally

substituted ring: ;

where

10 x is selected from 1 or 2; and

represents the point of attachment to L⁴;

each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

15 each R⁵ is independently an optionally substituted group selected from a 5- to 10-membered aryl ring and a 3- to 8-membered carbocyclic ring ;

X¹ is a covalent bond, -O-, or -NR-;

X² is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally

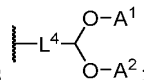
20 and independently replaced with -O- or -NR-;

X³ is hydrogen or -Cy^B;

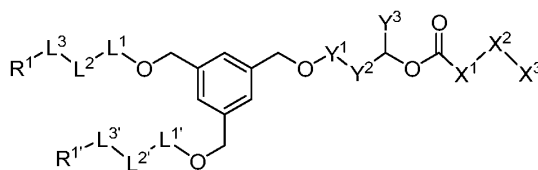
Cy^B is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from

25 nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

provided that when X³ is hydrogen, at least one of R¹ or R^{1'} is ;

viii) a compound of Formula (Ig):



(Ig)

or a pharmaceutically acceptable salt thereof, wherein:

each of L^1 and $L^{1'}$ is independently a covalent bond, $-C(O)-$, or $-OC(O)-$;

5 each of L^2 and $L^{2'}$ is independently a covalent bond, an optionally substituted bivalent

saturated or unsaturated, straight or branched C_1 - C_{12} hydrocarbon chain, or $\left[\left(\text{---} \right)_m \text{---} \text{Cy}^A \text{---} \left(\text{---} \right)_m \right]$;

each Cy^A is independently an optionally substituted ring selected from phenylene or 3- to 7-membered saturated or partially unsaturated carbocyclene;

each m is independently 0, 1, or 2;

10 each of L^3 and $L^{3'}$ is independently a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

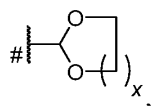
each of R^1 and $R^{1'}$ is independently an optionally substituted group selected from saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl,

15 sterolyl, phenyl, or $\left[\text{---} L^4 \text{---} \begin{array}{c} O-A^1 \\ O-A^2 \end{array} \right]$;

each L^4 is independently a bivalent saturated or unsaturated, straight or branched C_1 - C_{20} hydrocarbon chain;

each A^1 and A^2 is independently an optionally substituted C_1 - C_{20} aliphatic or $-L^5-R^5$,

20 or A^1 and A^2 , together with their intervening atoms, may form an optionally substituted ring:



wherein

x is selected from 1 or 2; and

25 # represents the point of attachment to L^4 ;

each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

each R⁵ is independently an optionally substituted group selected from a 6- to 10-membered aryl ring or a 3- to 8-membered carbocyclic ring;

Y¹ is a covalent bond, -C(O)-, or -C(O)O-;

Y² is a bivalent saturated or unsaturated, straight or branched C₁-C₆ hydrocarbon chain, wherein 1-2 methylene units are optionally and independently replaced with cyclopropylene, -O-, or -NR-;

Y³ is an optionally substituted group selected from saturated or unsaturated, straight or branched C₁-C₁₄ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-, a 3- to 7-membered saturated or partially unsaturated carbocyclic ring, 1-adamantyl, 2-adamantyl, or phenyl;

X¹ is a covalent bond, -O-, or -NR-;

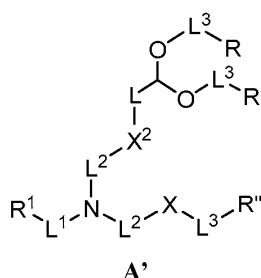
X² is an optionally substituted bivalent saturated or unsaturated, straight or branched C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O-, -NR-, or -Cy^B-;

each Cy^B is independently an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

X³ is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

ix) a compound of formula A':



or its N-oxide, or a pharmaceutically acceptable salt thereof, wherein

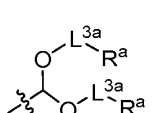
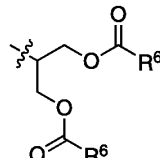
L^1 is absent, C_{1-6} alkylenyl, or C_{2-6} heteroalkylenyl;

- 5 each L^2 is independently optionally substituted C_{2-15} alkylenyl, or optionally substituted C_{3-15} heteroalkylenyl;

L is C_{1-10} alkylenyl, or C_{2-10} heteroalkylenyl;

X^2 is $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, or $-\text{OC}(\text{O})\text{O}-$;

X is absent, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, or $-\text{OC}(\text{O})\text{O}-$;

- 10 R'' is hydrogen, , , or an optionally substituted group selected from C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

each of R and R^a is independently hydrogen, or an optionally substituted group selected from

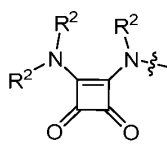
- 15 C_{6-20} aliphatic, 3- to 12-membered cycloaliphatic, 7- to 12-membered bridged bicyclic comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl

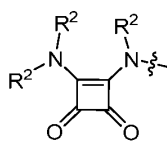
each of L^3 and L^{3a} is independently absent, optionally substituted C_{1-10} alkylenyl, or optionally substituted C_{2-10} heteroalkylenyl;

- 20 R^1 is hydrogen, optionally substituted phenyl, optionally substituted 3- to 7-membered cycloaliphatic, optionally substituted 3- to 7-membered heterocyclyl comprising 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 5- to 6-membered monocyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, optionally substituted 8- to 10-
- 25 membered bicyclic heteroaryl comprising 1-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, $-\text{OR}^2$, $-\text{C}(\text{O})\text{OR}^2$, $-\text{C}(\text{O})\text{SR}^2$, $-\text{OC}(\text{O})\text{R}^2$, $-\text{OC}(\text{O})\text{OR}^2$, $-\text{CN}$,

-N(R²)₂, -C(O)N(R²)₂, -S(O)₂N(R²)₂, -NR²C(O)R², -OC(O)N(R²)₂, -N(R²)C(O)OR²,
 -NR²S(O)₂R², -NR²C(O)N(R²)₂, -NR²C(S)N(R²)₂, -NR²C(NR²)N(R²)₂, -
 NR²C(CHR²)N(R²)₂, -N(OR²)C(O)R², -N(OR²)S(O)₂R², -N(OR²)C(O)OR², -
 N(OR²)C(O)N(R²)₂,

- 5 -N(OR²)C(S)N(R²)₂, -N(OR²)C(NR²)N(R²)₂, -N(OR²)C(CHR²)N(R²)₂, -C(NR²)N(R²)₂,
 -C(NR²)R², -C(O)N(R²)OR², -C(R²)N(R²)₂C(O)OR², -CR²(R³)₂, -OP(O)(OR²)₂, or
 -P(O)(OR²)₂; or



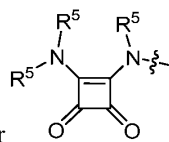
- R¹ is , or a ring selected from 3- to 7-membered cycloaliphatic and 3- to 7-
 membered heterocyclyl comprising 1-3 heteroatoms independently selected from
 10 nitrogen, oxygen, and sulfur, wherein the cycloaliphatic or heterocyclyl ring is optionally
 substituted with 1-4 R² or R³ groups;

- each R² is independently hydrogen, oxo, -CN, -NO₂, -OR⁴, -S(O)₂R⁴, -S(O)₂N(R⁴)₂, -(CH₂)_n-
 R⁴, or an optionally substituted group selected from C₁₋₆ aliphatic, phenyl, 3- to 7-
 membered cycloaliphatic, 5- to 6-membered monocyclic heteroaryl comprising 1-4
 15 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 3- to 7-
 membered heterocyclyl comprising 1-3 heteroatoms independently selected from
 nitrogen, oxygen, and sulfur; or

- two occurrences of R², taken together with the atom(s) to which they are attached, form
 optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional
 20 heteroatom selected from nitrogen, oxygen, and sulfur;

- each R³ is independently -(CH₂)_n-R⁴; or
 two occurrences of R³, taken together with the atom(s) to which they are attached, form
 optionally substituted 5- to 6-membered heterocyclyl comprising 0-1 additional
 heteroatom selected from nitrogen, oxygen, and sulfur;

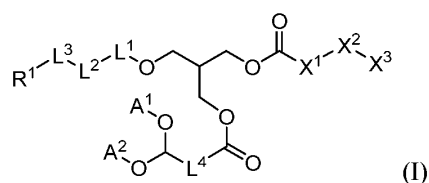
- 25 each R⁴ is independently hydrogen, -OR⁵, -N(R⁵)₂, -OC(O)R⁵, -OC(O)OR⁵, -CN, -
 C(O)N(R⁵)₂,
 -NR⁵C(O)R⁵, -OC(O)N(R⁵)₂, -N(R⁵)C(O)OR⁵, -NR⁵S(O)₂R⁵, -NR⁵C(O)N(R⁵)₂,



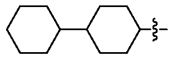
-NR⁵C(S)N(R⁵)₂, -NR⁵C(NR⁵)N(R⁵)₂, or ;

- each R⁵ is independently hydrogen, or optionally substituted C₁₋₆ aliphatic; or
 two occurrences of R⁵, taken together with the atom(s) to which they are attached, form
 optionally substituted 4- to 7-membered heterocyclyl comprising 0-1 additional
 heteroatom selected from nitrogen, oxygen, and sulfur;
- 5 each R⁶ is independently C₄₋₁₂ aliphatic; and
 each n is independently 0 to 4; or

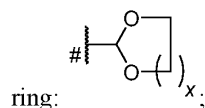
x) a compound of Formula I:



or a pharmaceutically acceptable salt thereof, wherein:

- 10 L¹ is a covalent bond, -C(O)-, or -OC(O)-;
 L² is a covalent bond, an optionally substituted bivalent saturated or unsaturated, straight or
 branched C₁-C₁₂ hydrocarbon chain, or $\left[\text{---} \right]_m \text{Cy}^A \text{---} \left[\text{---} \right]_m$;
 Cy^A is an optionally substituted ring selected from phenylene and 3- to 7-membered saturated
 or partially unsaturated carbocyclene;
- 15 each m is independently 0, 1, or 2;
 L³ is a covalent bond, -C(O)-, -C(O)O-, -OC(O)-, -O-, or -OC(O)O-;
 R¹ is $\left[\text{---} \right]_p \text{Cy}^B$, an optionally substituted saturated or unsaturated, straight or branched
 C₁-C₂₀ hydrocarbon chain wherein 1-3 methylene units are optionally and independently
 replaced with -O- or -NR-, or $\left[\text{---} \right]_p \text{---} \text{O---A}^1 \text{---} \text{O---A}^2 \text{---}$;
- 20 Cy^B is an optionally substituted ring selected from 3- to 12-membered saturated or partially
 unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, , sterolyl, and
 phenyl;
 p is 0, 1, 2, or 3;
 each L⁴ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₆
 hydrocarbon chain;
- 25 each A¹ and A² is independently an optionally substituted C₁-C₂₀ aliphatic or -L⁵-R⁵;

or A¹ and A², together with their intervening atoms, may form an optionally substituted



where

x is selected from 1 or 2; and

5 # represents the point of attachment to L⁴;

each L⁵ is independently a bivalent saturated or unsaturated, straight or branched C₁-C₂₀ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O- or -NR-;

each R⁵ is independently an optionally substituted group selected from a 5- to 10-membered aryl ring or a 3- to 8-membered carbocyclic ring ;

X¹ is a covalent bond, -O-, or -NR-;

X² is a covalent bond or an optionally substituted, bivalent saturated or unsaturated, straight or branched, C₁-C₁₂ hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with -O-, -NR-, or -Cy^C-;

15 Cy^C is an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclene, phenylene, 3- to 7-membered saturated or partially unsaturated heterocyclene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, and 5- to 6-membered heteroarylene having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

20 X³ is hydrogen or an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered saturated or partially unsaturated heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

25 each R is independently hydrogen or an optionally substituted C₁-C₆ aliphatic group;

provided that when L³ is a covalent bond, then R¹ must be $\text{---} \left(\text{---} \right)_p \text{---} \text{Cy}^B$.

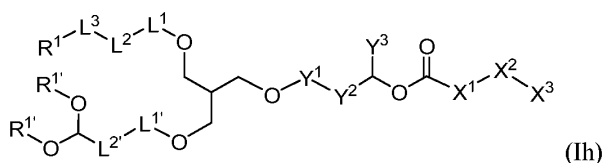
2. A lipid nanoparticle (LNP) comprising:

30 A) a guide RNA, or a polynucleotide encoding the guide RNA, wherein the guide RNA is capable of directing a base editor polypeptide to alter a nucleotide in a transthyretin (TTR) polynucleotide;

B) an mRNA molecule, or a polynucleotide encoding the mRNA molecule, wherein the mRNA molecule encodes a base editor polypeptide comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises:

- 5 i) an amino acid sequence having at least 90% identity to the following
TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAA
GSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD
- 10 (SEQ ID NO: 1), and
- ii) a combination of amino acid alterations relative to the TadA*7.10 amino
acid sequence selected from the group consisting of:
 - a) I76Y, V82T, Y123H, Y147R, and Q154R;
 - b) V82T, Y123H, D147R, and Q154R;
 - 15 c) V82T, Y123H, D147T, and Q154S;
 - d) V82T and Q154R;
 - e) V82T, Y147T, and Q154S;
 - f) I76Y, V82T, Y123H, Y147T, and Q154S;
 - g) Y123H, Y147R, Q154R;
 - 20 h) I76Y, Y147R, and Q154R;
 - i) Y147R, Q154R, and T166R;
 - j) Y147T and Q154R;
 - k) Y147T and Q154S;
 - l) I76Y, Y123H, Y147R, and Q145R;
 - 25 m) I76Y and V82S;
 - n) V82S and Y147R;
 - o) V82S, Y123H, and Y147R;
 - p) V82S and Q154R;
 - q) V82S, Y123H, and Q154R;
 - 30 r) V82S, Y123H, Y147R, and Q154R;
 - s) I76Y, V82S, Y123H, Y147R, and Q154R; and

C) an ionizable lipid according to Formula (I), or a pharmaceutically acceptable salt thereof:



wherein:

each L^1 and $L^{1'}$ is independently $-C(O)-$ or $-OC(O)-$;

each L^2 and $L^{2'}$ is independently an optionally substituted bivalent saturated or unsaturated,

5 straight or branched C_{1-12} hydrocarbon chain;

L^3 is a covalent bond, $-O-$, $-C(O)O-$, $-OC(O)-$, or $-OC(O)O-$;

R^1 is optionally substituted C_{1-20} aliphatic, $\text{---}L^{CyA}\text{---}Cy^A$, or $\text{---}L^{Ra}\text{---}\begin{matrix} O-R^a \\ | \\ O-R^a \end{matrix}$;

L^{CyA} is a covalent bond or an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

10 Cy^A is an optionally substituted ring selected from 3- to 7-membered saturated or partially unsaturated carbocyclyl, 1-adamantyl, 2-adamantyl, sterolyl, and phenyl;

L^{Ra} is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain;

each R^a and $R^{1'}$ is independently optionally substituted C_{1-20} aliphatic;

15 Y^1 is $-C(O)-$ or $-C(O)O-$;

Y^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain;

Y^3 is optionally substituted C_{1-20} aliphatic;

X^1 is a covalent bond, $-O-$, or $-NR-$;

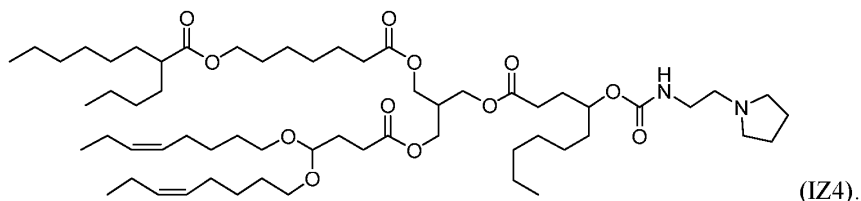
20 X^2 is an optionally substituted bivalent saturated or unsaturated, straight or branched C_{1-12} hydrocarbon chain, wherein 1-3 methylene units are optionally and independently replaced with $-O-$ or $-NR-$;

X^3 is hydrogen or an optionally substituted ring selected from 3- to 7- membered saturated or partially unsaturated carbocyclyl, phenyl, 3- to 7-membered heterocyclyl having 1-3

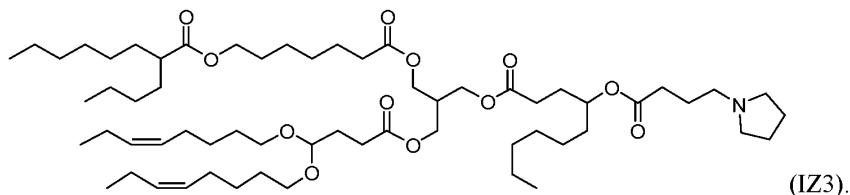
25 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or 5- to 6-membered heteroaryl having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

3. The LNP of claim 1 or claim 2, wherein the lipid nanoparticle comprises an ionizable lipid having the following structure, or a pharmaceutically acceptable salt thereof:



5 4. The LNP of claim 1 or claim 2, wherein the lipid nanoparticle comprises an ionizable lipid having the following structure, or a pharmaceutically acceptable salt thereof:



10

5. The LNP of claim 1 or claim 2, wherein the guide RNA comprises a scaffold with the following nucleotide sequence:

gUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugG
caccgagucggugcuusus (SEQ ID NO: 478), wherein A is adenosine; C is cytidine; G
is guanosine; U is uridine; a is 2'-O-methyladenosine; c is 2'-O-methylcytidine; g is 2'-O-
methylguanosine; u is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone
linkage.

6. The LNP of claim 1, 2, or 5, wherein the guide RNA comprises a spacer comprising
20 the following nucleotide sequence: GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472).

7. The LNP of claim 6, wherein the guide RNA comprises the following nucleotide sequence:

25 mGsmCsmCsAUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAm
AGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUmAmUmCAAmCmUmUGmAmAmAmAmAmGmU
mGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUsmUsmUsmU (SEQ ID NO: 477), wherein

A is adenosine; C is cytidine; G is guanosine; U is uridine; mA is 2'-O-methyladenosine; mC is 2'-O-methylcytidine; mG is 2'-O-methylguanosine; mU is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage.

- 5 8. The LNP of claim 2 or claim 5, wherein the guide RNA comprises a spacer selected from those listed in any of **Tables 1B, 1C, 1D, 1E, 2A, 2B, 2D, or 2E**.
9. The LNP of claim 1 or claim 2, wherein the guide RNA comprises 2-5 contiguous 2'-O-methylated nucleobases at the 3' end and at the 5' end.
- 10 10. The LNP of claim 1 or claim 2, wherein the guide RNA comprises 2-5 contiguous nucleobases at the 3' end and at the 5' end that comprise phosphorothioate internucleotide linkages.
- 15 11. The LNP of claim 1 or claim 2, wherein the napDNAbp domain comprises a Cas9 polypeptide.
12. The LNP of claim 1 or claim 2, wherein the napDNAbp is a nickase.
- 20 13. The LNP of claim 1 or claim 2, wherein the base editor polypeptide comprises an amino acid sequence with at least about 90% identity to the following amino acid sequence:
ABE9.51
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGGLVMQNYRLYDATLYTTFPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHP
25 GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSES
ATPESGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEAKVDDSFHRLAESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEGDL
NPDNSDVKLF IQLVQTYNQLFEENPINASGVDKAIL SARLSKSRLENLIAQLPGEKKN
30 LFGNLI ALSGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVVDK
GASAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK

AIVDLLFKNRKTVPKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKLIKDKDFLD
 NEENEDILEDIVLTTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
 RDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
 IKKGILQTVKVVDLVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEDIKELGSQ
 5 ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
 LTRSDKNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
 RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINN
 YHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNI
 MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKKEVQTGG
 10 FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLKSVELLGI
 TIMERSSFENPIDFLEAKGYKEVKDLIIKLPKYSLEFENGRKRMLASAGELQKGNELAL
 PSKYVNFYLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIEQISEFSKRVILADANLDKV
 LSAYNKHDKPIREQAENIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSI
 TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463).

15

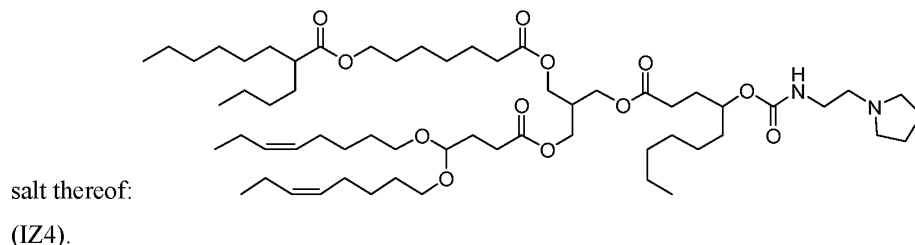
14. A lipid nanoparticle (LNP) comprising:

A) a guide RNA having the following nucleotide sequence:

mGsmCsmCsAUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAm
 AGUUmAAmAAmUAmAmGmGmUmAGUmCmCGUUAmUmCAAmCmUmUGmAmAmAmAmGmU
 20 mGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUsmUsmUsmU (SEQ ID NO: 477), wherein
 A is adenosine; C is cytidine; G is guanosine; U is uridine; mA is 2'-O-methyladenosine; mC
 is 2'-O-methylcytidine; mG is 2'-O-methylguanosine; mU is 2'-O-methyluridine, and s is a
 phosphorothioate (PS) backbone linkage;

B) a base editor polypeptide, or a polynucleotide encoding the base editor
 25 polypeptide, wherein the base editor polypeptide comprises a nucleic acid programmable
 DNA binding protein (napDNAbp) domain and an adenosine deaminase domain, wherein the
 adenosine deaminase domain comprises an amino acid sequence with at least 90% identity to
 the following amino TadA*7.10 amino acid sequence, or a truncation thereof lacking only the
 first M:
 30 MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMA
 LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
 GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 1), and
 comprises the following combination of amino acid alterations relative to the TadA*7.10
 amino acid sequence: I76Y, V82T, Y123H, Y147R, and Q154R; and

C) an ionizable lipid having the following structure, or a pharmaceutically acceptable



- 5 15. The lipid nanoparticle of claim 14, wherein the base editor polypeptide comprises an amino acid sequence with at least about 90% identity to the following amino acid sequence: ABE9.51
- MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLYDATLYTTFEPVCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHP
10 GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSSGSSGGSSGSETPGTSES
ATPESGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLLIYLALAHMIKFRGHFLIEGDL
NPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKN
15 LFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSEETITPWNFEEVVDK
GASAQSFIERM TNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
20 AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKI IKDKDFLD
NEENEDILEDIVLTTLTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQDSLHEHIANLAGSPA
IKKGILQTVKVVDDELVKVMGRHKPENIVIE MARENQTTQKGQKNSRERMKRIEEGKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
25 LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYK VREINN
YHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNI
MNF FKT EITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLSVKELLGI
30 TIMERSSEFKNPIDFLEAKGYKEVKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL

PSKYVNFLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSCRVLADANLDKV
LSAYNKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463).

5 16. A base editor system comprising:

A) a guide RNA, or a polynucleotide encoding the guide RNA, wherein the guide RNA is capable of directing a base editor polypeptide to alter a nucleotide in a transthyretin (TTR) polynucleotide; and

10 B) a base editor polypeptide, or one or more polynucleotide encoding the base editor polypeptide, wherein the base editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises:

i) an amino acid sequence with at least 90% identity to the following amino TadA*7.10 amino acid sequence, or a truncation thereof lacking only the first M:

15 MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAA
GSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

(SEQ ID NO: 1), and

20 ii) a combination of amino acid alterations relative to the TadA*7.10 amino acid sequence selected from the group consisting of:

a) V82T, Y123H, D147R, and Q154R;

b) V82T, Y123H, D147T, and Q154S;

c) V82T and Q154R;

d) V82T, Y147T, and Q154S;

25 e) I76Y, V82T, Y123H, Y147R, and Q154R; and

f) I76Y, V82T, Y123H, Y147T, and Q154S.

17. The base editor system of claim 16, wherein the guide RNA comprises a spacer selected from those listed in any of **Tables 1B, 1C, 1D, 1E, 2A, 2B, 2D, or 2E**.

30

18. The base editor system of claim 16, wherein the guide RNA comprises a scaffold with the following nucleotide sequence:

gUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugG
caccgagucggugcuusus (SEQ ID NO: 478), wherein A is adenosine; C is cytidine; G

is guanosine; U is uridine; a is 2'-O-methyladenosine; c is 2'-O-methylcytidine; g is 2'-O-methylguanosine; u is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage.

5 19. The base editor system of claim 16 or claim 18, wherein the guide RNA comprises a spacer with the following nucleotide sequence: GCCAUCCUGCCAAGAAUGAG (SEQ ID NO: 472).

20. The base editor system of claim 16, wherein the guide RNA comprises 2-5 contiguous
10 2'-O-methylated nucleobases at the 3' end and at the 5' end.

21. The base editor system of claim 16, wherein the guide RNA comprises 2-5 contiguous nucleobases at the 3' end and at the 5' end that comprise phosphorothioate internucleotide linkages.

15

22. The base editor system of claim 16, wherein the guide RNA comprises the following nucleotide sequence:

mGsmCsmCsAUCCUGCCAAGAAUGAGmGUUUUAGmAmGmCmUmAGmAmAmAmUmAmGmCmAm
AGUUmAAmAAmUAmAmGmGmCmUmAGUmCmCGUUAmUmCAAmCmUmUGmAmAmAmAmAmGmU

20 mGGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmUsmUsmUsmU (SEQ ID NO: 477), wherein A is adenosine; C is cytidine; G is guanosine; U is uridine; mA is 2'-O-methyladenosine; mC is 2'-O-methylcytidine; mG is 2'-O-methylguanosine; mU is 2'-O-methyluridine, and s is a phosphorothioate (PS) backbone linkage.

25 23. The base editor system of claim 16, wherein the napDNAbp domain comprises a Cas9 polypeptide.

24. The base editor system of claim 16, wherein the napDNAbp is a nickase.

30 25. The base editor system of claim 16, wherein the base editor polypeptide comprises an amino acid sequence with at least about 90% identity to the following amino acid sequence: ABE9.51

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLYDATLYTTTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHP

GMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSES
 ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVP SKKFKVLGNTDRHSIKKNLIGA
 LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKK
 HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDL
 5 NPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKN
 LFGNLIALSLGLTPNFKSNFDLAEDAKQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSD
 AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
 GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
 LRRQEDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDK
 10 GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
 AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKI IKDKDFLD
 NEENEDILEDIVLTTLTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
 RDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
 IKKGILQTVKVVDELVKVMGRHKPENIVIEARENQTTQKGQKNSRERMKRIEEGIKELGSQ
 15 ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
 LTRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGSELKAGFIK
 RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVINN
 YHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNI
 MNFFKTEITLANGEIRKRPLIETNGETGEIWDKGRDFATVRKVL SMPQVNIVKKTEVQTGG
 20 FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLKSVKELLGI
 TIMERSSEFKNPIDFLEAKGYKEVKDLIIKLPKYSLEFELENGRKRMLASAGELQKGNELAL
 PSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIEQISEFSKRVLADANLDKV
 LSAYNKHDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSI
 TGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 463).

25

26. A polynucleotide or set of polynucleotides encoding the base editor system of any one of claims 16-25.

27. A cell comprising the base editor system of any one of claims 16-25 or the
 30 polynucleotide or set of polynucleotides of claim 26.

28. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 1-15, the base editor system of any one of claims 16-25, the polynucleotide or set of

polynucleotides of claim 26, or the cell of claim 27, and a pharmaceutically acceptable carrier or excipient.

29. A kit containing the lipid nanoparticle of any one of claims 1-15, the base editor
5 system of any one of claims 16-25, the polynucleotide or set of polynucleotides of claim 26, the cell of claim 27, or the pharmaceutical composition of claim 28, and a container.

30. A method for modifying a target nucleobase in a transthyretin (TTR) polynucleotide
10 in a cell, the method comprising contacting the cell with the lipid nanoparticle of any one of claims 1-15 or the base editor system of any one of claims 16-25, thereby modifying the target nucleobase in the *TTR* polynucleotide.

31. The method of claim 30, wherein the cell is in a subject.

15 32. A method of treating a disease in a subject in need thereof, wherein the disease is associated with a pathogenic mutation in a transthyretin (*TTR*) polynucleotide in the subject, the method comprising administering to the subject the lipid nanoparticle of any one of claims 1-15 or the base editor system of any one of claims 16-25, thereby altering a target nucleobase in the *TTR* polynucleotide.

20

33. The method of claim 31 or claim 32, wherein an ionizable lipid of the lipid nanoparticle has a half-life in the liver of the subject that is less than 14 days.

34. The method of claim 31 or claim 32, wherein the target nucleobase is altered with an
25 editing efficiency of at least about 50% or 60% in the cell and/or in the liver of the subject.

35. The method of claim 31 or claim 32, wherein altering the target nucleobase results in a reduction in TTR polypeptide levels in the cell and/or in the subject.

30 36. The method of claim 35, wherein TTR polypeptide levels are reduced by at least about 80% or 90%.

37. The method of claim 32, wherein the method is associated with only a transient increase or no increase in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate aminotransferase (AST) levels in the subject.
- 5 38. The method of claim 37, wherein the method is associated with no increase in ALP levels in the subject.
39. The method of claim 32, wherein the disease is selected from the group consisting of amyloidosis, cardiomyopathy, familial amyloid polyneuropathy (FAP), familial amyloid
- 10 cardiomyopathy (FAC), familial transthyretin amyloidosis (FTA), senile systemic amyloidosis (SSA), and transthyretin amyloidosis.