

TARGETING ONCOGENIC MUTATIONS WITH DUAL-CLEAVING ENDONUCLEASE

CROSS-REFERENCE

[1] This application claims the benefit of U.S. Provisional App. Ser. No. 63/166,763 filed on March 26, 2021, which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[2] Cancer is among the leading causes of death worldwide. In 2018, there were 18.1 million new cases and 9.5 million cancer-related deaths worldwide. By 2040, the number of new cancer cases per year is expected to rise to 29.5 million and the number of cancer-related deaths to 16.4 million. A proto-oncogene is a gene that has the potential to cause cancer. Once mutated, a proto-oncogene becomes an oncogene. In tumor cells, proto-oncogenes are often mutated, or expressed at high levels and can contribute to uncontrolled cell growth which is a hallmark of cancer. Many current therapeutics target the mutated protein expressed from an oncogene but there are no therapeutics that target the oncogene itself. It is therefore important to develop new technologies to disrupt an oncogene.

SUMMARY OF THE INVENTION

[3] Described herein are chimeric nucleases comprising an I-TevI domain (1), a Cas domain, and a guide RNA targeting the chimeric nuclease to an oncogenic mutation. Such chimeric nucleases advantageously allow for precise targeting *and* editing of the genome of a cell to restore a non-oncogenic function of an oncogene. Compared to use of Cas enzymes alone the inclusion of the I-TevI domain allows for more precise editing and replacement of oncogenic sequences in cancer cells.

[4] In an aspect, the present disclosure provides a composition comprising: a chimeric nuclease, wherein the chimeric nuclease comprises an I-TEVI nuclease domain, an RNA-guided nuclease Cas domain, and a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that targets an oncogenic mutation that is not a deletion in exon 19 of EGFR.

[5] In some embodiments, the oncogenic mutation is a single nucleotide polymorphism. In some embodiments, a sequence comprising the oncogenic mutation is selected from a mutation set forth in any one of SEQ ID NOs: 1- 683, or a combination thereof. In some embodiments, a sequence comprising the oncogenic mutation is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to a mutation set forth in any one of SEQ ID NOs: 1-683, or a combination thereof. In some embodiments, the oncogenic mutation comprises a mutation corresponding an EGFR

L858R mutation or an EGFR V769_D770insASV mutation. In some embodiments, the oncogenic mutation comprises a mutation corresponding to an EGFR L858R mutation. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 45, 130, or 141, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 45, 130, 141, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the oncogenic mutation comprises a mutation corresponding to an EGFR V769_D770insASV mutation. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 683, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1683 or 1684. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 683, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1683 or 1684. In some embodiments, the oncogenic mutation is an oncogenic mutation to a gene selected from any of Muc4, PIK3CA, KRAS, or a combination there. In some embodiments, the oncogenic mutation comprises a Muc4 mutation. In some embodiments, the Muc4 mutation is an in-frame deletion of exon 2 or an in-frame deletion of exon 3. In some embodiments, the Muc4 mutation comprises a mutation corresponding to any one of positions P1542, P1680, T1711, V1721, P1826, A1830, S3560, A1833, D2253, V2281, P3088, T3119, T3183, V3817, A3902 of human Muc4 protein, or a combination thereof. In some embodiments, the Muc4 mutation is selected from a mutation corresponding to any one of P1542L, P1680S, T1711I, V1721A, P1826H, A1830T, S3560S, A1833V, D2253H, V2281AM, P3088L, T3119T, T3183M, V3817A, A3902V of human Muc4 protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685. In some embodiments, the oncogenic mutation comprises a PIK3CA mutation. In some embodiments, the PIK3CA mutation comprises a mutation corresponding to any one of positions H1047, E542, E545, N345, C1636, G1624, G1633,

A3140, C3075, A1634, A1173 of human PIK3A protein, or a combination thereof. In some embodiments, the PIK3CA mutation is selected from a mutation corresponding to any one of H1047R, H1047L, E542K, E545K, N345K, C1636A, G1624A, G1633A, A3140T, A3140G, C3075T, A1634C, A1173G of human PIK3A protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210. In some embodiments, the oncogenic mutation comprises a KRAS mutation. In some embodiments, the KRAS mutation comprises a mutation selected from a mutation corresponding to any one of positions A59, D119, D33, G21, G12, G13, Q61, A146, K117 of human KRAS protein, or a combination thereof. In some embodiments, the KRAS mutation is selected from a mutation corresponding to any one of A59T, A59E, A59T, D119N, D33E, G21C, G12C, G12D, G12V, G12R, G12A, G12S, G13D, G13C, G13V, G13R, Q61R, Q61V, Q61L, Q61K, Q61H, Q61A, Q61P, Q61E, A146T, A146V, K117N, K117R of human KRAS protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077. In some embodiments, the guide RNA comprises one or more of: a non-natural internucleoside linkage, a nucleic acid mimetic, a modified sugar moiety, or a modified nucleobase. In some embodiments, the non-natural internucleoside linkage comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, or a boranophosphate. In some embodiments, the nucleic acid mimetic

comprises one or more of a peptide nucleic acid (PNA), morpholino nucleic acid, cyclohexenyl nucleic acid (CeNAs), or a locked nucleic acid (LNA). In some embodiments, the modified sugar moiety comprises one or more of 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, or 2'-fluoro. In some embodiments, the modified nucleobase comprises one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2- aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-halouracil; a 5-halocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-halo; an 8-amino; an 8-thiol; an 8-thioalkyl; an 8-hydroxyl; a 5-halo; a 5-bromo; a 5-trifluoromethyl; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-Fadenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7- deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deaza-adenine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; or a 5-propynylcytosine. In some embodiments, the composition further comprises a linker that is operably linked to the I-TEVI nuclease domain and the RNA-guided nuclease Cas domain. In some embodiments, the linker comprises an amino acid sequence as set forth in SEQ ID NO: 701, 702, 703, or 704. In some embodiments, the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof. In some embodiments, the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof. In some embodiments, the linker comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 701, 702, 703, or 704. In some embodiments, the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof. In some embodiments, the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas9 domain. In some embodiments, the RNA-guided nuclease Cas9 domain is any one of an RNA-guided nuclease *Staphylococcus aureus*

Cas9 domain, an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain, an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain, an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain, an RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain, or combination thereof. In some embodiments, the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Staphylococcus aureus* Cas9 domain. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K,

A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K,

H1045K, I0148M, K1050M or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to the D10E mutation. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 711. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E,

G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 711. In some embodiments, the RNA-guided nuclease *Staphylococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S,

N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 712. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E,

C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 712. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L,

L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 713. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581L, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751L, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971L, T972S, or a combination thereof. In some embodiments, the RNA-guided nuclease

Campylobacter jejuni Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 713. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581L, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751L, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971L, T972S, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an

amino acid sequence as set forth in SEQ ID NO: 714. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 714. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302,

N1316, T1346, I1374, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 715. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P,

G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 715. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Geobacillus thermodenitrificans T1* Cas9 domain. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans T1* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 716. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans T1* Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372,

T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 716. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation

corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas12 domain. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease CasX domain. In some embodiments, the I-TEVI nuclease domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 700. In some embodiments, the I-TEVI nuclease domain comprises a mutation at any one of positions corresponding to T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation selected from any one of corresponding to T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation. In some embodiments, the I-TEVI nuclease domain comprises an amino acid sequence as set forth in SEQ ID NO: 700. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to any one of positions T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation selected from a mutation corresponding to any one of T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation. In some embodiments, the chimeric nuclease further comprises a nuclear localization signal. In some embodiments, the nuclear localization signal comprises an SV40 nuclear localization signal. In some embodiments, the nuclear localization signal comprises a Nucleoplasmin nuclear localization signal. In some embodiments, the composition further comprises a donor nucleic

acid. In some embodiments, the donor nucleic acid restores a non-oncogenic function of a gene comprising the oncogenic mutation. In some embodiments, the donor nucleic acid comprises a non-oncogenic version of the oncogenic mutation. In some embodiments, the donor nucleic acid is DNA. In some embodiments, the donor nucleic acid comprises a blunt end and at least two nucleotide 3' overhang end. In some embodiments, the donor nucleic acid comprises a 5' and a 3' homology flanking the non-oncogenic version of the oncogenic mutation. In some embodiments, the composition does not comprise a donor nucleic acid. In some embodiments, the composition further comprises a pharmaceutically acceptable excipient, diluent or carrier. In some embodiments, the composition is encapsulated in a lipid nanoparticle. In some embodiments, the lipid nanoparticle comprises cationic or neutral lipids.

[6] In another aspect, the present disclosure provides a nucleic acid or plurality of nucleic acids encoding the chimeric nuclease or the guide RNA of the present disclosure. In some embodiments, the chimeric nuclease or the guide RNA is operably coupled to a eukaryotic promoter, an enhancer, a polyadenylation site, or a combination thereof. In some embodiments, the nucleic acid is an expression vector selected from a plasmid, a lentivirus vector, an adeno associated virus vector, or an adenovirus vector. In some embodiments, the nucleic acid or plurality of nucleic acids further comprise the donor nucleic acid portion.

[7] In another aspect, the present disclosure provides a method of targeting the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[8] In another aspect, the present disclosure provides a use of the composition of the present disclosure to the cell for targeting the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer

[9] In another aspect, the present disclosure provides a method of editing a genome in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[10] In another aspect, the present disclosure provides a use of the composition of the present disclosure for editing a genome in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[11] In another aspect, the present disclosure provides a method of deleting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[12] In another aspect, the present disclosure provides a use of the composition of the present disclosure for deleting at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[13] In another aspect, the present disclosure provides a method of silencing or disrupting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[14] In another aspect, the present disclosure provides a use of the composition of the present disclosure for silencing or disrupting at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[15] In another aspect, the present disclosure provides a method of replacing at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[16] In another aspect, the present disclosure provides a use of the composition of the present disclosure for replacing at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[17] In another aspect, the present disclosure provides a method of restoring a non-oncogenic function in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[18] In another aspect, the present disclosure provides a use of the composition of the present disclosure for restoring a non-oncogenic function in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[19] In another aspect, the present disclosure provides a method of treating cancer in an individual, comprising administering the composition of the present disclosure to the individual with cancer, thereby treating the cancer in the individual.

[20] In another aspect, the present disclosure provides a use of the composition of the present disclosure for treatment of cancer in an individual.

[21] In another aspect, the present disclosure provides a composition, comprising: a chimeric nuclease, wherein the chimeric nuclease comprises an I-TEVI nuclease domain, an RNA-guided nuclease Cas domain, and a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that targets an oncogenic mutation, wherein the oncogenic mutation is (i) an insertion of one or more nucleotides, or (ii) a substitution or deletion of 10 or less nucleotides.

[22] In some embodiments, the oncogenic mutation is a single nucleotide polymorphism. In some embodiments, a sequence comprising the oncogenic mutation is selected from a mutation set forth in any one of SEQ ID NOs: 1- 683, or a combination thereof. In some embodiments, a sequence comprising the oncogenic mutation is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to a mutation set forth in any one of SEQ ID NOs: 1-683, or a combination thereof. In some embodiments, the oncogenic mutation comprises a mutation corresponding an EGFR L858R mutation or an EGFR V769_D770insASV mutation. In some embodiments, the oncogenic mutation comprises a mutation corresponding to an EGFR L858R mutation. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 45, 130, or 141, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 45, 130, 141, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the oncogenic mutation comprises a mutation corresponding to an EGFR V769_D770insASV mutation. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 683, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1683 or 1684. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 683, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1683 or 1684. In some embodiments, the oncogenic mutation is an oncogenic mutation to a gene selected from any one of Muc4, PIK3CA, KRAS, or a combination thereof. In some embodiments, the oncogenic mutation comprises a Muc4 mutation. In some embodiments, the Muc4 mutation is an in-frame deletion of exon 2 or an in-frame deletion of exon 3. In some embodiments, the Muc4 mutation comprises a mutation corresponding to any one of positions P1542, P1680, T1711, V1721, P1826, A1830, S3560, A1833, D2253, V2281, P3088, T3119, T3183, V3817, A3902 of human Muc4 protein, or a combination thereof. In some embodiments, the Muc4 mutation is selected from a mutation corresponding to any one of P1542L, P1680S, T1711I, V1721A, P1826H, A1830T, S3560S, A1833V, D2253H, V2281AM, P3088L, T3119T, T3183M, V3817A, A3902V of human Muc4 protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685. In some embodiments, the guide RNA comprises a

nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685. In some embodiments, the oncogenic mutation comprises a PIK3CA mutation. In some embodiments, the PIK3CA mutation comprises a mutation corresponding to any one of positions H1047, E542, E545, N345, C1636, G1624, G1633, A3140, C3075, A1634, A1173 of human PIK3A protein, or a combination thereof. In some embodiments, the PIK3CA mutation is selected from a mutation corresponding to any one of H1047R, H1047L, E542K, E545K, N345K, C1636A, G1624A, G1633A, A3140T, A3140G, C3075T, A1634C, A1173G of human PIK3A protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210. In some embodiments, the oncogenic mutation comprises a KRAS mutation. In some embodiments, the KRAS mutation comprises a mutation selected from a mutation corresponding to any one of positions A59, D119, D33, G21, G12, G13, Q61, A146, K117 of human KRAS protein, or a combination thereof. In some embodiments, the KRAS mutation is selected from a mutation corresponding to any one of A59T, A59E, A59T, D119N, D33E, G21C, G12C, G12D, G12V, G12R, G12A, G12S, G13D, G13C, G13V, G13R, Q61R, Q61V, Q61L, Q61K, Q61H, Q61A, Q61P, Q61E, A146T, A146V, K117N, K117R of human KRAS protein, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077. In some embodiments, the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077. In some embodiments, the guide RNA comprises one or more of: a non-natural internucleoside linkage, a nucleic acid mimetic, a modified sugar moiety, or a modified nucleobase. In some embodiments, the non-natural internucleoside linkage comprises one or

more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, or a boranophosphate. In some embodiments, the nucleic acid mimetic comprises one or more of a peptide nucleic acid (PNA), morpholino nucleic acid, cyclohexenyl nucleic acid (CeNAs), or a locked nucleic acid (LNA). In some embodiments, the modified sugar moiety comprises one or more of 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, or 2'-fluoro. In some embodiments, the modified nucleobase comprises one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2- aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-halouracil; a 5-halocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-halo; an 8-amino; an 8-thiol; an 8-thioalkyl; an 8-hydroxyl; a 5-halo; a 5-bromo; a 5-trifluoromethyl; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-Fadenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7- deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deaza-adenine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; or a 5-propynylcytosine. In some embodiments, the composition further comprises a linker that is operably linked to the I-TEVI nuclease domain and the RNA-guided nuclease Cas domain. In some embodiments, the linker comprises an amino acid sequence as set forth in SEQ ID NO: 701, 702, 703, or 704. In some embodiments, the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof. In some embodiments, the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof. In some embodiments, the linker comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 701, 702, 703, or 704. In some embodiments, the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120,

K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof. In some embodiments, the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas9 domain. In some embodiments, the RNA-guided nuclease Cas9 domain is any one of an RNA-guided nuclease *Staphylococcus aureus* Cas9 domain, an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain, an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain, an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain, an RNA-guided nuclease *Geobacillus thermodenitrificans T1* Cas9 domain, or combination thereof. In some embodiments, the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Staphylococcus aureus* Cas9 domain. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q,

I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N,

D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to the D10E mutation. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 711. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A,

E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 711. In some embodiments, the RNA-guided nuclease *Staphylococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G,

Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 712. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H,

D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 712. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G,

Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 713. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581L, N587G, N596E, K600L,

Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751L, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971L, T972S, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 713. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581L, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I,

K723F, S736K, L739F, K742N, L747S, N751L, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971L, T972S, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 714. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 714. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327,

E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 715. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E,

R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 715. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof. In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino

acid sequence as set forth in SEQ ID NO: 716. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 716. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521,

V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas12 domain. In some embodiments, the RNA-guided nuclease Cas domain is a RNA-guided nuclease CasX domain. In some embodiments, the I-TEVI nuclease domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 700. In some embodiments, the I-TEVI nuclease domain comprises a mutation at any one of positions corresponding to T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation selected from any one of corresponding to T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation. In some embodiments, the I-TEVI nuclease domain comprises an amino acid sequence as set forth in SEQ ID NO: 700. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to any one of positions T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation selected from a mutation corresponding to any one of T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N,

C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof. In some embodiments, the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation. In some embodiments, the chimeric nuclease further comprises a nuclear localization signal. In some embodiments, the nuclear localization signal comprises an SV40 nuclear localization signal. In some embodiments, the nuclear localization signal comprises a Nucleoplasmin nuclear localization signal. In some embodiments, the composition further comprises a donor nucleic acid. In some embodiments, the donor nucleic acid restores a non-oncogenic function of a gene comprising the oncogenic mutation. In some embodiments, the donor nucleic acid comprises a non-oncogenic version of the oncogenic mutation. In some embodiments, the donor nucleic acid is DNA. In some embodiments, the donor nucleic acid comprises a blunt end and at least two nucleotide 3' overhang end. In some embodiments, the donor nucleic acid comprises a 5' and a 3' homology flanking the non-oncogenic version of the oncogenic mutation. In some embodiments, the composition does not comprise a donor nucleic acid. In some embodiments, the composition further comprises a pharmaceutically acceptable excipient, diluent or carrier. In some embodiments, the composition is encapsulated in a lipid nanoparticle. In some embodiments, the lipid nanoparticle comprises cationic or neutral lipids.

[23] In another aspect, the present disclosure provides a nucleic acid or plurality of nucleic acids encoding the chimeric nuclease or the guide RNA of the present disclosure. In some embodiments, the chimeric nuclease or the guide RNA is operably coupled to a eukaryotic promoter, an enhancer, a polyadenylation site, or a combination thereof. In some embodiments, the nucleic acid is an expression vector selected from a plasmid, a lentivirus vector, an adeno associated virus vector, or an adenovirus vector. In some embodiments, the nucleic acid or plurality of nucleic acids further comprise the donor nucleic acid portion.

[24] In another aspect, the present disclosure provides a method of targeting the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[25] In another aspect, the present disclosure provides a use of the composition of the present disclosure to the cell for targeting the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer

[26] In another aspect, the present disclosure provides a method of editing a genome in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[27] In another aspect, the present disclosure provides a use of the composition of the present disclosure for editing a genome in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[28] In another aspect, the present disclosure provides a method of deleting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[29] In another aspect, the present disclosure provides a use of the composition of the present disclosure for deleting at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[30] In another aspect, the present disclosure provides a method of silencing or disrupting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[31] In another aspect, the present disclosure provides a use of the composition of the present disclosure for silencing or disrupting at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[32] In another aspect, the present disclosure provides a method of replacing at least a portion of the oncogenic mutation in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[33] In another aspect, the present disclosure provides a use of the composition of the present disclosure for replacing at least a portion of the oncogenic mutation in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[34] In another aspect, the present disclosure provides a method of restoring a non-oncogenic function in a cell comprising contacting the composition of the present disclosure to the cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[35] In another aspect, the present disclosure provides a use of the composition of the present disclosure for restoring a non-oncogenic function in a cell. In some embodiments, the cell is a cell in an individual afflicted with cancer.

[36] In another aspect, the present disclosure provides a method of treating cancer in an individual, comprising administering the composition of the present disclosure to the individual with cancer, thereby treating the cancer in the individual.

[37] In another aspect, the present disclosure provides a use of the composition of the present disclosure for treatment of cancer in an individual.

[38] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[39] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[40] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and/or advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also “Figure” and “Fig.” herein), of which:

[41] **FIG. 1A** is a schematic representation of the I-TevI domain (1), linker domain (2), and the Cas domain (3) within the chimeric nuclease structure.

[42] **FIG. 1B** is a flow chart graphically depicting the steps undertaken by existing gene editors to produce a single cleavage that would be repaired through the error-prone non-homologous end-joining (NHEJ) pathway which may generate random indels.

[43] **FIG. 1C** is a flow chart graphically depicting the steps as to how the chimeric nuclease cuts DNA at two sites using its I-TevI and Cas domains generating defined-length deletions in cells. Defined-length deletions can be predicted based on the distance between the I-TevI and Cas domains.

[44] **FIG. 2A** shows the workflow of the algorithm where a dataset comprising pairs of wild type and mutant target sites are generated from databases of known disease-causing mutations.

- [45] **FIG. 2B** includes sample output data from the wild type Muc4 gene.
- [46] **FIG. 2C** includes sample output data from a mutant Muc4 gene. The bolded row is found in both the wild type and mutant copies of the Muc4 gene sequence. The remaining cells in **FIG. 2C** constitute putative allele-specific TevSaCas9 targets in the Muc4 gene.
- [47] **FIG. 3A** is a diagram depicting TevSaCas9's target site in an oncogene (in bold) that spans a large insertion (in light gray).
- [48] **FIG. 3B** is an agarose gel electrophoresis result showing TevSaCas9's cleavage products from an in vitro cleavage assay of the wild type (WT) and mutant (MUT) copies of the Muc4 gene. As shown, TevSaCas9 targeted with the guide RNA of SEQ ID NO: 1685 has successfully cut the MUT substrate, but not the WT Muc4.
- [49] **FIG. 4A**, in particular, is an illustration of the EGFR L858R target site. The position of the mutation is denoted by an asterisk ("*").
- [50] **FIG. 4B** is an agarose gel electrophoresis result showing the TevSaCas9 cleavage products from an in vitro cleavage assay of the wild type (WT) and mutant (MUT) copies of the EGFR gene. As shown, TevSaCas9 targeted with the guide RNA of SEQ ID NO: 1686 has preferentially cut the MUT EGFR L858R substrate over the WT EGFR substrate.
- [51] **FIG. 4C** is the result of a viability assay of treating the CLR-5908 cell line which contains mutant EGFR L858R and the NuLi-1 cell line which contains wild type EGFR with TevSaCas9 ribonucleoprotein complex targeted to EGFR L858R. As shown, TevSaCas9 target to EGFR L858R reduces the viability of the CRL-5908 cell line but not the NuLi-1 cell line demonstrating the allele-specific activity of TevSaCas9 in cells.
- [52] **FIG. 5** depict a diagram of the mechanism by which TevCas is complexed with multiple guide RNAs and electroporated into cells to disrupt genes encoding the oncogene and insert a sequence coding for modified version of the oncogene, thereby restoring wild-type function.

DETAILED DESCRIPTION OF THE DISCLOSURE

- [53] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed. As used herein, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Any reference to "or" herein is intended to encompass "and/or" unless otherwise stated.
- [54] Unless otherwise defined, all terms of art, notations and other scientific terminology

used herein are intended to have the meanings commonly understood by those of skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference; thus, the inclusion of such definitions herein should not be construed to represent a substantial difference over what is generally understood in the art.

[55] Within the framework of the present description and in the subsequent claims, except where otherwise indicated, all numbers expressing amounts, quantities, percentages, and so forth, are to be understood as being preceded in all instances by the term “about”. As used herein, the term “about” is defined as $\pm 5\%$. Also, all ranges of numerical entities include all the possible combinations of the maximum and minimum numerical values and all the possible intermediate ranges therein, in addition to those specifically indicated hereafter.

[56] The term “and/or” as used herein is defined as the possibility of having one or the other or both. For example, “A and/or B” provides for the scenarios of having just A or just B or a combination of A and B. If the claim reads A and/or B and/or C, the composition may include A alone, B alone, C alone, A and B but not C, B and C but not A, A and C but not B or all three A, B and C as components.

[57] For convenience, certain terms employed in the specification, examples and appended claims are collected here. These definitions should be read in light of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

[58] The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. The term “and/or” as used herein is defined as the possibility of having one or the other or both. For example, “A and/or B” provides for the scenarios of having just A or just B or a combination of A and B. If the claim reads A and/or B and/or C, the composition may include A alone, B alone, C alone, A and B but not C, B and C but not A, A and C but not B or all three A, B and C as components.

[59] The term “donor DNA”, as used herein, refers to a DNA that, in whole or in part, differs from the original target DNA sequence, and can be incorporated into an oncogene to restore wild type or non-oncogenic function to the oncogene.

[60] The term “flexible linker”, as used herein, refers to a situation when the RNA-guided Cas nuclease domain binds to the target DNA sequence, the amino acid linker domain ensures mobility of the I-TevI domain to allow for recognition, binding and cleaving of its target sequence under cell physiological conditions (typically: pH ~ 7.2 , temperature $\sim 37^\circ\text{C}$, $[\text{K}^+]$ ~ 140

mM, [Na⁺] ~5 – 15 mM, [Cl⁻] ~4 mM, [Ca⁺⁺] ~0.0001 mM). The length of the amino acid linker can influence how many nucleotides are preferred between the Cas target site and the I-TevI target site. Certain amino acids in the linker may also make specific contacts with the DNA sequence targeted by TevCas. These linker-DNA contacts can affect the flexibility of the I-TevI domain. Substituting amino acids in the linker domain may affect the ability of the linker domain to make contact with DNA.

[61] The term “including”, as used herein, is used to mean “including but not limited to”.

“Including” and “including but not limited to” are used interchangeably.

[62] The term “patient,” “individual,” “subject,” or “host” to be treated by the subject method may mean either a human or non-human animal. Non-human animals include companion animals (e.g. cats, dogs) and animals raised for consumption (i.e. food animals), such as cows, pigs, and chickens.

[63] The term “pharmaceutically acceptable carrier” refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof from one organ, or portion of the body, to another organ, or portion of the body. Each carrier can be “acceptable” in the sense of being compatible with the subject composition and its components and not injurious to the patient. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as dextrose, lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as microcrystalline cellulose, sodium carboxymethyl cellulose, methyl cellulose, ethyl cellulose, hydroxypropylmethyl cellulose (HPMC), and cellulose acetate; (4) glycols, such as propylene glycol; (5) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (6) esters, such as ethyl oleate, glyceryl behenate and ethyl laurate; (7) buffering agents, such as monobasic and dibasic phosphates, Tris/Borate/EDTA and Tris/Acetate/EDTA (8) pyrogen-free water; (9) isotonic saline; (10) Ringer's solution; (11) ethyl alcohol; (12) phosphate buffer solutions; (13) polysorbates; (14) polyphosphates; and (15) other non-toxic compatible substances employed in pharmaceutical formulations. The disclosed excipients may serve more than one function. For example, a solubilizing agent may also be a suspension aid, an emulsifier, a preservative, and the like.

[64] In certain preferred embodiments, the pharmaceutically acceptable excipient is a crystalline bulking excipient. The terms “crystalline bulking excipient” or “crystalline bulking agent” as used herein means an excipient which provides bulk and structure to the lyophilization cake. These crystalline bulking agents are inert and do not react with the protein or nucleic acid.

In addition, the crystalline bulking agents are capable of crystallizing under lyophilization conditions. Examples of suitable crystalline bulking agents include hydrophilic excipients, such as, water soluble polymers; sugars, such as mannitol, sorbitol, xylitol, glucitol, ducitol, inositol, arabinitol, arabitol, galactitol, iditol, allitol, maltitol, fructose, sorbose, glucose, xylose, trehalose, allose, dextrose, altrose, lactose, glucose, fructose, gulose, idose, galactose, talose, ribose, arabinose, xylose, lyxose, sucrose, maltose, lactose, lactulose, fucose, rhamnose, melezitose, maltotriose, raffinose, altritol, their optically active forms (D- or L-forms) as well as the corresponding racemates; inorganic salts, both mineral and mineral organic, such as, calcium salts, such as the lactate, gluconate, glycerylphosphate, citrate, phosphate monobasic and dibasic, succinate, sulfate and tartrate, as well as the same salts of aluminum and magnesium; carbohydrates, such as, the conventional mono- and di-saccharides as well as the corresponding polyhydric alcohols; proteins, such as, albumin; amino acids, such as glycine; emulsifiable fats and polyvinylpyrrolidone. Crystalline bulking agents may be selected from any one of glycine, mannitol, dextran, dextrose, lactose, sucrose, polyvinylpyrrolidone, trehalose, glucose, or combination thereof. Particularly useful bulking agents include dextran.

[65] The term “pharmaceutically-acceptable salts”, as used herein, is art-recognized and refers to the relatively non-toxic, inorganic and organic acid addition salts, or inorganic or organic base addition salts of compounds, including, for example, those contained in compositions of the present invention. Some examples of pharmaceutically-acceptable salts include: (1) calcium chlorides; (2) sodium chlorides; (3) sodium citrates; (4) sodium hydroxide; (5) sodium phosphates; (6) sodium ethylenediaminetetraacetic acid; (7) potassium chloride; (8) potassium phosphate; and (9) other non-toxic compatible substances employed in pharmaceutical formulations.

[66] The term “substitution”, as used herein, refers to the replacement of an amino acid in a sequence with a different amino acid. As used herein, the shorthand X¹⁰Y indicates that amino acid Y has been “substituted” for amino acid X found in the 10th position of the sequence. As an example, W26C denotes that *amino acid* Tryptophan-26 (Trp, W) is changed to a Cysteine (Cys). Similarly, the notation AA^X indicates that AA is an amino acid that replaced the amino acid found in the X position. As an example, Lys²⁶ denotes the replacement of the amino acid in the 26th position in a sequence with Lysine. Use of either shorthand is interchangeable. In addition, use of the one- or three- letter abbreviations for an amino acid is also interchangeable.

[67] The term “therapeutic agent”, as used herein refers to any chemical or biochemical moiety that is a biologically, physiologically, or pharmacologically active substance that acts locally or systemically in a subject. Examples of therapeutic agents, also referred to as “drugs,”

are described in well-known literature references such as the Merck Index, the Physician's Desk Reference, and The Pharmacological Basis of Therapeutics, and they include, without limitation, medicaments; vitamins; mineral supplements; substances used for the treatment, prevention, diagnosis, cure or mitigation of a disease or illness; substances which affect the structure or function of the body; or pro-drugs, which become biologically active or more active after they have been placed in a physiological environment.

[68] The term, "hybridization," as used herein, generally refers to and includes the capacity and/or ability of a first nucleic acid molecule to non-covalently bind (e.g., form Watson-Crick-base pairs and/or G/U base pairs), anneal, and/or hybridize to a second nucleic acid molecule under the appropriate or certain in vitro and/or in vivo conditions of temperature, pH, and/or solution ionic strength. Generally, standard Watson-Crick base pairing includes: adenine (A) pairing with thymidine (T); adenine (A) pairing with uracil (U); and guanine (G) pairing with cytosine (C). In some embodiments, hybridization comprises at least two nucleic acids comprising complementary sequences (e.g., fully complementary, substantially complementary, or partially complementary). In certain embodiments, hybridization comprises at least two nucleic acids comprising fully complementary sequences. In certain embodiments, hybridization comprises at least two nucleic acids comprising substantially complementary sequences (e.g., greater than about 75%, greater than about 80%, greater than about 85%, greater than about 90%, or greater than about 95% complementary). In certain embodiments, hybridization comprises at least two nucleic acids comprising partially complementary sequences (e.g., greater than about 40%, greater than about 50%, greater than about 60%, or greater than about 70% complementary). In certain embodiments, partially complementary sequences comprises one or more regions of fully or substantially complementary sequences. In certain embodiments, partially complementary sequences comprises one or more regions of fully or substantially complementary sequences, even if an overall complementarity is low (e.g., a total complementarity lower than about 50%, lower than about 40%, lower than about 30%, or lower than about 20%). The conditions appropriate for hybridization between two nucleic acids depend on the length of the nucleic acids and the degree of complementation, variables well known in the art. For example, the greater the degree of complementation between two nucleotide sequences, the greater the value of the melting temperature (T_m) for hybrids of nucleic acids having those sequences. For hybridizations between nucleic acids with short stretches of complementarity (e.g., complementarity over 35 or less, 30 or less, 25 or less, 22 or less, 20 or less, or 18 or less nucleotides) the position of mismatches becomes important (see Sambrook et al., *supra*, 11.7-11.8).

[69] The term “complementary” or “complementarity,” as used herein, generally refers to a polynucleotide that includes a nucleotide sequence capable of selectively annealing to an identifying region of a target polynucleotide under certain conditions. As used herein, the term substantially complementary and grammatical equivalents is intended to mean a polynucleotide that includes a nucleotide sequence capable of specifically annealing to an identifying region of a target polynucleotide under certain conditions. Annealing refers to the nucleotide base-pairing interaction of one nucleic acid with another nucleic acid that results in the formation of a duplex, triplex, or other higher-ordered structure. The primary interaction is typically nucleotide base specific, e.g., A:T, A:U, and G:C, by Watson-Crick and Hoogsteen-type hydrogen bonding. In certain embodiments, base-stacking and hydrophobic interactions can also contribute to duplex stability. Conditions under which a polynucleotide anneals to complementary or substantially complementary regions of target nucleic acids are well known in the art, e.g., as described in *Nucleic Acid Hybridization, A Practical Approach*, Hames and Higgins, eds., IRL Press, Washington, D.C. (1985) and Wetmur and Davidson, *Mol. Biol.* 31:349 (1968). Annealing conditions will depend upon the particular application and can be routinely determined by persons skilled in the art, without undue experimentation. Hybridization generally refers to process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide.

[70] The temperature and solution salt concentration are generally recognized as factors facilitating hybridization, and may be adjusted as necessary according to factors such as length of the region of complementation and the degree of complementarity. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual- Second Edition*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein; and Sambrook, J. and Russell, W., *Molecular Cloning: A Laboratory Manual, Third Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (2001). The conditions of temperature and ionic strength determine the stringency of the hybridization. In some embodiments, hybridization is measured at a physiological temperature (e.g., 37 degrees Celsius) and salt concentrations (e.g., 0.15 molar or 0.9% salt in solution).

[71] The term “treating”, as used herein, includes any effect, e.g., lessening, reducing, modulating, or eliminating, that results in the improvement of the condition, disease, disorder, and the like. As used herein, “treating” can include both prophylactic, and therapeutic treatment.

[72] A “buffer” as used herein is any acid or salt combination which is pharmaceutically acceptable and capable of maintaining the composition of the present invention within a desired

pH range. Buffers in the disclosed compositions maintain the pH in a range of about 2 to about 8.5, about 5.0 to about 8.0, about 6.0 to about 7.5, about 6.5 to about 7.5, or about 6.5. Suitable buffers include, any pharmaceutical acceptable buffer capable of maintaining the above pH ranges, such as, for example, acetate, tartrate phosphate or citrate buffers. In one embodiment, the buffer is a phosphate buffer. In another embodiment the buffer is an acetate buffer. In one embodiment the buffer is disodium hydrogen phosphate, sodium chloride, potassium chloride and potassium phosphate monobasic.

[73] In the disclosed compositions the concentration of buffer is typically in the range of about 0.1 mM to about 1000 mM, about 0.2 mM to about 200 mM, about 0.5 mM to about 50 mM, about 1 mM to about 10 mM or about 6.0 mM.

[74] As used herein, a stabilizer is a composition which maintains the chemical, biological or stability of the chimeric nuclease. Examples of stabilizing agent include polyols, which includes a saccharide, preferably a monosaccharide or disaccharide, e.g., glucose, trehalose, raffinose, or sucrose; a sugar alcohol such as, for example, mannitol, sorbitol or inositol, a polyhydric alcohol such as glycerin or propylene glycol or mixtures thereof and albumin.

[75] A pharmaceutically acceptable salt is a salt which is suitable for administration to a subject, such as, a human. The chimeric nuclease of the present invention can have one or more sufficiently acidic proton that can react with a suitable organic or inorganic base to form a base addition salt. Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like, and organic bases such as alkoxides, alkyl amides, alkyl and aryl amines, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, and the like. The chimeric nuclease of the present invention having a sufficiently basic group, such as an amine can react with an organic or inorganic acid to form an acid addition salt. Acids commonly employed to form acid addition salts from compounds with basic groups are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such salts include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propionate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate,

dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like.

[76] As used herein, a “cell” can generally refer to a biological cell. A cell can be the basic structural, functional and/or biological unit of a living organism. A cell can originate from any organism having one or more cells. Some non-limiting examples include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant, an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human, etc.), et cetera. Sometimes a cell may not originate from a natural organism (e.g., a cell can be synthetically made, sometimes termed an artificial cell). In certain embodiments cells refers to human cells.

[77] The terms “protein” and “polypeptide” can be used interchangeably to refer to a polymer of two or more amino acids joined by covalent bonds (e.g., an amide bond) that can adopt a three-dimensional conformation. In some embodiments, a protein or polypeptide comprises at least 10 amino acids, 15 amino acids, 20 amino acids, 30 amino acids or 50 amino acids joined by covalent bonds (e.g., amide bonds). In some embodiments, a protein comprises at least two amide bonds. In some embodiments, a protein comprises multiple amide bonds. In some embodiments, a protein comprises an enzyme, enzyme precursor proteins, regulatory protein, structural protein, receptor, nucleic acid binding protein, a biomarker, a member of a specific binding pair (e.g., a ligand or aptamer), or an antibody. In some embodiments, a protein can be a full-length protein (e.g., a fully processed protein having certain biological function). In some embodiments, a protein can be a variant or a fragment of a full-length protein. For example, in some embodiments, a Cas9 protein domain comprises an H840A amino acid substitution compared to a naturally occurring *S. pyogenes* Cas9 protein. A variant of a protein or enzyme, for example a variant reverse transcriptase, comprises a polypeptide having an amino acid sequence that is about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, about 99.5% identical, or about 99.9% identical to the amino acid sequence of a reference protein.

[78] In some embodiments, a protein comprises a functional variant or functional fragment of a full-length wild type protein. A “functional fragment” or “functional portion”, as used herein,

refers to any portion of a reference protein (e.g., a wild type protein) that encompasses less than the entire amino acid sequence of the reference protein while retaining one or more of the functions, e.g., catalytic or binding functions. For example, a functional fragment of a Cas or I-TevI protein can encompass less than the entire amino acid sequence of a wild type Cas or I-TevI protein, but retains the ability to catalyze the cleavage of a polynucleotide sequence. When the reference protein is a fusion of multiple functional domains, a functional fragment thereof can retain one or more of the functions of at least one of the functional domains. For example, a functional fragment of a Cas can encompass less than the entire amino acid sequence of a wild type Cas, but retains its DNA binding ability and lacks its nuclease activity partially or completely. In certain embodiments, functional fragments comprise one or more deletions from the N- or C-terminus of a protein, polypeptide or domain described herein. In certain embodiments, functional fragments comprise a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 amino acid deletions from the N- or C-terminus of a protein, polypeptide or domain described herein.

[79] A “functional variant” or “functional mutant”, as used herein, refers to any variant or mutant of a reference protein (e.g., a wild type protein) that encompasses one or more alterations to the amino acid sequence of the reference protein while retaining one or more of the functions, e.g., catalytic or binding functions. In some embodiments, the one or more alterations to the amino acid sequence comprises amino acid substitutions, insertions or deletions, or any combination thereof. In some embodiments, the one or more alterations to the amino acid sequence comprises amino acid substitutions. For example, a functional variant of a Cas or I-TevI protein can comprise one or more amino acid substitutions compared to the amino acid sequence of a wild type Cas or I-TevI protein, but retains the ability to catalyze the cleavage of a polynucleotide sequence. When the reference protein is a fusion of multiple functional domains, a functional variant thereof can retain one or more of the functions of at least one of the functional domains. For example, in some embodiments, a functional fragment of a Cas9 can comprise one or more amino acid substitutions in a nuclease domain, e.g., a H840A amino acid substitution, compared to the amino acid sequence of a wild type Cas9, but retains the DNA binding ability and lacks the nuclease activity partially or completely.

[80] The terms “homologous,” “homology,” or “percent homology” as used herein refer to the degree of sequence identity between an amino acid and a corresponding reference amino acid sequence, or a polynucleotide sequence and a corresponding reference polynucleotide sequence. “Homology” can refer to polymeric sequences, e.g., polypeptide or DNA sequences that are similar. Homology can mean, for example, nucleic acid sequences with at least about:

50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity. In other embodiments, a "homologous sequence" of nucleic acid sequences can exhibit 93%, 95% or 98% sequence identity to the reference nucleic acid sequence. For example, a "region of homology to a genomic region" can be a region of DNA that has a similar sequence to a given genomic region in the genome. A region of homology can be of any length that is sufficient to promote binding of a spacer, a primer binding site, or a protospacer sequence to the genomic region. For example, the region of homology can comprise at least 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100 or more bases in length such that the region of homology has sufficient homology to undergo binding with the corresponding genomic region.

[81] The term "identity," or "homology" as used interchangeable herein, may be to calculations of "identity," "homology," or "percent homology" between two or more nucleotide or amino acid sequences that can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first sequence). The nucleotides at corresponding positions may then be compared, and the percent identity between the two sequences may be a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100). For example, a position in the first sequence may be occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences may be a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In some embodiments, the length of a sequence aligned for comparison purposes may be at least about: 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of the length of the reference sequence. A BLAST® search may determine homology between two sequences. The two sequences can be genes, nucleotides sequences, protein sequences, peptide sequences, amino acid sequences, or fragments thereof. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A non-limiting example of such a mathematical algorithm may be described in Karlin, S. and Altschul, S., Proc. Natl. Acad. Sci. USA, 90- 5873-5877 (1993). Such an algorithm may be incorporated into the NBLAST and XBLAST programs (version 2.0),

as described in Altschul, S. et al., *Nucleic Acids Res.*, 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, any relevant parameters of the respective programs (e.g., NBLAST) can be used. For example, parameters for sequence comparison can be set at score= 100, word length= 12, or can be varied (e.g., W=5 or W=20). Other examples include the algorithm of Myers and Miller, CABIOS (1989), ADVANCE, ADAM, BLAT, and FASTA. In another embodiment, the percent identity between two amino acid sequences can be accomplished using, for example, the GAP program in the GCG software package (Accelrys, Cambridge, UK).

[82] When a percentage of sequence homology or identity is specified, in the context of two nucleic acid sequences or two polypeptide sequences, the percentage of homology or identity generally refers to the alignment of two or more sequences across a portion of their length when compared and aligned for maximum correspondence. When a position in the compared sequence can be occupied by the same base or amino acid, then the molecules can be homologous at that position. Unless stated otherwise, sequence homology or identity is assessed over the specified length of the nucleic acid, polypeptide or portion thereof. In some embodiments, the homology or identity is assessed over a functional portion or specified portion of the length.

[83] Alignment of sequences for assessment of sequence homology can be conducted by algorithms known in the art, such as the Basic Local Alignment Search Tool (BLAST) algorithm, which is described in Altschul et al, *J. Mol. Biol.* 215:403- 410, 1990. A publicly available, internet interface, for performing BLAST analyses is accessible through the National Center for Biotechnology Information. Additional known algorithms include those published in: Smith & Waterman, "Comparison of Biosequences", *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, "A general method applicable to the search for similarities in the amino acid sequence of two proteins" *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman "Improved tools for biological sequence comparison", *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; or by automated implementation of these or similar algorithms. Global alignment programs can also be used to align similar sequences of roughly equal size. Examples of global alignment programs include NEEDLE (available at www.ebi.ac.uk/Tools/psa/emboss_needle/) which is part of the EMBOSS package (Rice P et al., *Trends Genet.*, 2000; 16: 276-277), and the GGSEARCH program https://fasta.bioch.virginia.edu/fasta_www2/, which is part of the FASTA package (Pearson W and Lipman D, 1988, *Proc. Natl. Acad. Sci. USA*, 85: 2444-2448). Both of these programs are based on the Needleman-Wunsch algorithm which is used to find the optimum alignment (including gaps) of two sequences along their entire length. A detailed discussion of sequence

analysis can also be found in Unit 19.3 of Ausubel et al ("Current Protocols in Molecular Biology" John Wiley & Sons Inc, 1994-1998, Chapter 15, 1998).

[84] A skilled person understands that amino acid (or nucleotide) positions can be determined in homologous sequences based on alignment, for example, "H840" in a reference Cas9 sequence can correspond to H839, or another position in a Cas9 homolog.

[85] The term "polynucleotide" or "nucleic acid molecule" can be any polymeric form of nucleotides, including DNA, RNA, a hybridization thereof, or RNA-DNA chimeric molecules. In some embodiments, a polynucleotide comprises cDNA, genomic DNA, mRNA, tRNA, rRNA, or microRNA. In some embodiments, a polynucleotide is double-stranded, e.g., a double-stranded DNA in a gene. In some embodiments, a polynucleotide is single-stranded or substantially single-stranded, e.g., single-stranded DNA or an mRNA. In some embodiments, a polynucleotide is a cell-free nucleic acid molecule. In some embodiments, a polynucleotide circulates in blood. In some embodiments, a polynucleotide is a cellular nucleic acid molecule. In some embodiments, a polynucleotide is a cellular nucleic acid molecule in a cell circulating in blood.

[86] Polynucleotides can have any three-dimensional structure. The following are nonlimiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), an exon, an intron, intergenic DNA (including, without limitation, heterochromatic DNA), messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), a ribozyme, cDNA, a recombinant polynucleotide, a branched polynucleotide, a plasmid, a vector, isolated DNA, isolated RNA, sgRNA, guide RNA, a nucleic acid probe, a primer, an snRNA, a long non-coding RNA, a snoRNA, a siRNA, a miRNA, a tRNA-derived small RNA (tsRNA), an antisense RNA, an shRNA, or a small rDNA-derived RNA (srRNA).

[87] In some embodiments, a polynucleotide comprises deoxyribonucleotides, ribonucleotides or analogs thereof. In some embodiments, a polynucleotide comprises modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component.

[88] In some embodiments, a polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. In some embodiments, the polynucleotide can comprise one or more other nucleotide bases, such as inosine (I), which is read by the translation machinery as guanine (G).

[89] In some embodiments, a polynucleotide can be modified. As used herein, the terms “modified” or “modification” refers to chemical modification with respect to the A, C, G, T and U nucleotides. In some embodiments, modifications can be on the nucleoside base and/or sugar portion of the nucleosides that comprise the polynucleotide. In some embodiments, the modification can be on the internucleoside linkage (e.g., phosphate backbone). In some embodiments, multiple modifications are included in the modified nucleic acid molecule. In some embodiments, a single modification is included in the modified nucleic acid molecule.

[90] The term “mutation” as used herein refers to a change and/or alteration in an amino acid sequence of a protein or a nucleic acid sequence of a polynucleotide. Such changes and/or alterations can comprise the substitution, insertion, deletion and/or truncation of one or more amino acids, in the case of an amino acid sequence, and/or nucleotides, in the case of nucleic acid sequence, compared to a reference amino acid or a reference nucleic acid sequence. In some embodiments, the reference sequence is a wild-type sequence. In some embodiments, a mutation in a nucleic acid sequence of a polynucleotide encodes a mutation in the amino acid sequence of a polypeptide. In some embodiments, the mutation in the amino acid sequence of the polypeptide or the mutation in the nucleic acid sequence of the polynucleotide is a mutation associated with a disease state.

[91] The term “subject” or “individual” can be used interchangeably. Its grammatical equivalents as used herein can refer to a human or a non-human. An individual can be a mammal. A human individual can be male or female. A human individual can be of any age. A individual can be a human embryo. A human individual can be a newborn, an infant, a child, an adolescent, or an adult. A human individual can be in need of treatment for a genetic disease or disorder. In some embodiments, a individual is suffering from, susceptible to, or at a risk of developing cancer.

[92] The term “oncogene” refers to a gene that upon mutation, disruption, or overexpression can lead to uncontrolled cell division leading to the formation of a tumor or cancer. Before mutation the oncogene is known as a proto-oncogene. An “oncogenic mutation” is any mutation that leads to the transformation of a proto-oncogene to an oncogene. Oncogenes can have a non-oncogenic function restored to by editing and reverting the function of the gene to a wild-type or non-pathogenic state. Such editing may restore a wild-type nucleotide sequence or amino acid sequence, or a sequence (amino acid or nucleotide) that differs from wild-type but restores a wild-type or non-pathogenic function.

[93] While specific embodiments of the disclosure’s embodiments have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will

become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

[94] The term “chimeric nuclease” refers to fusion protein comprising an I-TevI nuclease domain and a Cas nuclease domain. In some cases, the I-TevI nuclease domain and Cas nuclease domain are operably linked. A target gene of the chimeric nuclease can comprise a double stranded DNA molecule having two complementary strands: a first strand that can be referred to as a “coding strand”, and a second strand that can be referred to as a “non-coding strand.” In some embodiments, in a chimeric nuclease uses a guide RNA sequence that is complementary, substantially complementary to a specific sequence on the target strand. In some cases. The guide RNA hybridizes or substantially hybridizes to a specific sequence on the target strand. In some embodiments, the guide RNA sequence anneals with the target strand at the search target sequence.

[95] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention.

[96] The present disclosure describes chimeric nucleases and method of targeting oncogenes with the chimeric nucleases. For example, if the chimeric nuclease cleaves at two sites, it cleaves out precise lengths of DNA (for example, approximately 30 – 38 bases depending on the sites targeted by I-TevI domain and Cas domain). Thus, a target site can be selected in a gene to generate a precise-length deletion that will knock the gene out-of-frame in a cell. In one embodiment of the described nucleases, a nuclease targets and cleaves a target oncogene.

[97] The present disclosure describes methods of using the described nucleases to genetically engineer a cell population in order to target, contact, edit, silence, disrupt, restore, insert, modify, delete, or replace a nucleotides sequence at a genomic location. The disclosure describes forming a chimeric nuclease guide RNA complexes and administering the chimeric nuclease guide RNA complexes to cells. This administration can occur using one or more methods of electroporation or lipid mediated transfection (e.g., cationic lipids). Alternatively, a nucleic acid or plurality of nucleic acids encoding the guide RNA and/or the chimeric nuclease can be transferred into the cell using a method selected from electroporation, viral transduction, and or lipid mediated transfection can be utilized. In embodiments, where a genome sequence is

to be added to alter the genome a donor DNA can also be administered to affect the insertion or alteration. The donor DNA can be suitably provided as a linearized DNA, plasmid DNA or a viral vector.

[98] The methods described herein target oncogenes in a cell or an individual using a chimeric nuclease comprising an I-TevI nuclease domain and a Cas nuclease domain. As illustrated in **FIG. 5A**, purified chimeric nuclease (1) can be mixed complexed with multiple guide RNAs 2 to form ribonucleoprotein complex (3). Donor DNA (4) encoding an exogenous donor DNA containing complimentary ends to one or more of the sites targeted by the chimeric nuclease can be also mixed with the ribonucleoprotein complex (3). As shown in **FIG. 5B**, a population of cells (5) which encode for an oncogene expressed from the genomic DNA (7) can be exposed to the mixture of ribonucleoprotein complex (3) and donor DNA (4). An electrical pulse (8) can be applied to the mixture to permeabilize the cell membrane (9) (**FIG. 5C**). As depicted in **FIG. 5D**, the ribonucleoprotein complex (3) and donor DNA (4) can enter the cell through the permeabilized cell membrane. The ribonucleoprotein complex (3) can be targeted to the nucleus (10) through one or more nuclear localization sequences (“NLS”). As shown in **FIG. 5E**, the ribonucleoprotein complex (3) can bind to its target site on the genomic DNA (7). As depicted in **FIG. 5F**, the ribonucleoprotein complex (3) can cleave the genomic DNA (7) to create defined length deletions (12), or if compatible donor DNA (13) is present, can insert the donor DNA (13) into the cleaved site. The regions of the genomic DNA (7) deleted disrupt genes the oncogene (6) and the donor DNA (13) encodes for an modified version of the oncogene, thereby restoring wild-type function.

[99] In addition, this disclosure is directed to a method of targeted gene disruption (e.g., insertion, edit, delete, modification or replacement) of all or a portion of a DNA sequence in the genome of human cells to knock genes out-of-frame, comprising: (a) exposing cells to the nuclease *ex vivo*; (b) applying an electric current of between 1000 – 2500V to the cell population to permeabilize the membrane to allow for the passage of the claimed nuclease into the cells. Other ranges of electric currents between 1000 – 1500V, 1501 – 1700V, 1701 - 1900V, 1901 - 2100V or 2101 – 2500V may also be applied. The nuclease may also be delivered to the cell using lipofection or polymer-based transfection or the use of a viral vector such as adeno-associated virus or lentivirus. The nuclease may further be delivered as a ribonucleoprotein complex, a DNA encoding the nuclease or as messenger RNA encoding the nuclease. In eukaryotic cells, the chimeric nucleases of this disclosure can target the nuclei of the cells through one or more nuclear-localization sequences (“NLS”). For the application of generating knockouts of oncogenes in cells, a mixture of nucleases can be applied to target one or more

oncogenes in the population of cells. Specific guide RNAs to target the chimeric nuclease to a precise genomic location can be included with the nuclease, encoded by a nucleic acid, or a messenger RNA. For applications that target the replacement, repair, or insertion of a DNA into a genomic location, a donor nucleic acid may also be included either as an isolated and purified nucleic acid, by linear double stranded nucleic acid, by a plasmid or viral vector. A donor nucleic acid may be provided along with the nuclease and guide RNA or separately in separate formulation or delivered by a different method compared to the delivery of the nuclease and guide RNA. In the presence of a donor nucleic acid, the cell can insert the donor nucleic acid sequence (in whole or in part) between the two cleaved sites in the target genomic DNA using directed-ligation through non-homologous end joining.

[100] The present disclosure is directed to chimeric nucleases comprising different combinations of an I-TevI domain and a Cas domain. In some embodiments, the chimeric nuclease further comprises a linker domain. In some embodiments, the chimeric nuclease further comprises a guide RNA.

[101] Chimeric nucleases which target an oncogene can comprise (a) the I-TevI domain and the Cas domain; and (b) a guide RNA. In some embodiments, the guide RNA comprises an RNA sequence that hybridizes or is sufficiently complementary to at least a portion of a sequence selected from any one of SEQ ID NOs 1 – 683 or a combination thereof, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1001-1686 or a combination thereof. In some embodiments, chimeric nucleases which target KRAS comprise (a) a chimeric nuclease as described above; and (b) a guide RNA that comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs 37, 42, 51, 52, 62, 63, or 77. In some embodiments, chimeric nucleases which target PI3KCA comprise (a) a chimeric nuclease as described above; and (b) a guide RNA that comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs 5, 6, 7, 8, 33, 202, 204, 209 or 210. In some embodiments, chimeric nucleases which target MUC-4 comprise (a) a chimeric nuclease as described above; and (b) a guide RNA that comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs 676, 677, 678, 679 or 682. In some embodiments, chimeric nucleases which target EGFR comprise (a) a chimeric nuclease as described above; and (b) a guide RNA that comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NOs 45, 130, 141, or 683.

[102] Chimeric nucleases which target an oncogene comprise (a) the I-TevI domain and the Cas domain; and (b) a guide RNA. In some embodiments, the guide RNA comprises an RNA sequence with at least about 90%, 95%, 97%, 98%, or 99% identity or is identical to a sequence selected from any one of SEQ ID NOs 1001-1686, or a combination thereof. In some embodiments, chimeric nucleases which target KRAS comprise (a) a chimeric nuclease as described above; and (b) a guide RNA comprising an RNA sequence with at least about 90%, 95%, 97%, 98%, or 99% identity or is identical to a sequence selected from any one of SEQ ID NOs 1037, 1042, 1051, 1052, 1062, 1063, 1077, or a combination thereof. In some embodiments, chimeric nucleases which target PI3KCA comprise (a) a chimeric nuclease as described above; and (b) a guide RNA comprising an RNA sequence with at least about 90%, 95%, 97%, 98%, or 99% identity or is identical to a sequence selected from any one of SEQ ID NOs 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, 1210, or a combination thereof. In some embodiments, chimeric nucleases which target MUC-4 comprise (a) a chimeric nuclease as described above; and (b) a guide RNA comprising an RNA sequence with at least about 90%, 95%, 97%, 98%, or 99% identity or is identical to a sequence selected from any one of SEQ ID NOs 1676, 1677, 1678, 1679, 1682, or a combination thereof. Chimeric nucleases which target EGFR comprise (a) a chimeric nuclease as described above; and (b) a guide RNA comprising an RNA sequence with at least about 90%, 95%, 97%, 98%, or 99% identity or is identical to a sequence selected from any one of SEQ ID NOs 1683, 1684, or a combination thereof.

[103] In some embodiments, the chimeric nucleases are used to edit multiple genes simultaneously to generate multiple oncogene knockouts in a population of cells. For example, multiple chimeric nucleases can be used to target different oncogenes in an individual. In some embodiments, at least 1 chimeric nuclease is used to target an oncogenic mutation. In some embodiments, at least 2 chimeric nucleases is used to target an oncogenic mutation. In some embodiments, at least 3 chimeric nucleases is used to target an oncogenic mutation. In some embodiments, at least 4 chimeric nucleases is used to target an oncogenic mutation. In some embodiments, at least 5 chimeric nucleases is used to target an oncogenic mutation. In particular, the composition is directed to a mixture of the chimeric nucleases discussed above in the preceding paragraph in combination with a mixture of guide RNAs according to sequences SEQ ID NOs: 1001-1686 in an equimolar ratio to the chimeric nuclease. In another embodiment, the composition is directed to a mixture of the chimeric nucleases discussed above in the preceding paragraph in combination with a mixture of guide RNAs according to sequences SEQ ID NOs: 1001-1686 in an equimolar ratio to the chimeric nuclease.

[104] In some embodiments, a composition of other chimeric nucleases containing

different combinations of an I-TevI domain and an RNA-guided nuclease domain. In particular, the composition is directed to chimeric nucleases of SEQ ID NOs: 730-736, 740-755, or 756, wherein the chimeric nuclease comprises a wildtype I-TevI domain or variant thereof, and a wildtype Cas domain or variant thereof.

[105] The chimeric nucleases described herein can be formed from two different nucleases. The chimeric nucleases are useful for the *ex vivo* gene editing applications described herein and for *in vivo* applications.

Chimeric Nucleases

[106] The chimeric nuclease of the present disclosure may contain different combinations of an I-TevI domain and a Cas domain. In some embodiments, the Cas domain can be a Cas9 domain. In some embodiments, the Cas9 domain is derived from *Staphylococcus aureus*, *Streptococcus pyogenes*, *Neisseria meningitidis*, *Campylobacter jejuni*, *Streptococcus pasteurianus*, *Clostridium cellulolyticum*, or *Geobacillus thermodenitrificans T1*.

[107] In some embodiments, the chimeric nuclease further comprises a linker domain. In some embodiments, the chimeric nuclease further comprises a guide RNA, wherein the guide RNA targets an oncogenic mutation. In some embodiments, the chimeric nuclease can be used to target the oncogenic mutation. In some embodiments, the oncogenic mutation is a single polynucleotide polymorphism or SNP. In some embodiments, the oncogenic mutation is an insertion of one or more nucleotides. In some embodiments, the oncogenic mutation is a substitution or deletion of 10 or less nucleotides. In some embodiments, the oncogenic mutation comprises a deletion of 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotides. In some embodiments, the oncogenic mutation is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to a sequence set forth in any one of SEQ ID NOs: 1- 628, or a combination thereof. In some embodiments, the oncogenic mutation occurs in at least one of ABL1, AFF4/MLLT11, AKT2, ALK, ALK/NPM, RUNX1 (AML1), RUNX1/MTG8(ETO), AXL, BCL-2, BCL-3, BCL-6, BCR/ABL, MYC (c-MYC), MCF2 (DBL), DEK/NUP214, TCF3/PBX1, EGFR, MLLT11, ERG/FUS, ERBB2, ETS1, EWSR1/FLI1, CSF1R, FOS, FES, GLI1, GNAS (GSP), HER2/neu, TLX1, FGF4, IL3, FGF3 (INT-2), JUN, KIT, FGF4 (KS3), K-SAM, AKAP13, LCK, LMO1, LMO2, MYCL, LYL1, NFkB2, NFkB2/Cα1, MAS1, MDM2, MLLT11, MOS, MUC4, RUNX1T1, MYB, MYH11/CBFB, NEU, MYCN, MCF2L (OST), PAX-5, PBX1/E2A, PIM1, PIK3CA, CCND1, RAF1, RARA/PML, HRAS, KRAS, NRAS, REL/NRG, RET, RHOM1, RHOM2, ROS1, SKI, SIS (aka PDGFB), SET/CAN, SRC, TAL1, TAL2, NOTCH1 (TAN1), TIAM1, TSC2, or NTRK1. In some embodiments, the oncogenic mutation occurs in at least one of Muc4,

PIK3CA, EGFR, or KRAS.

[108] In some embodiments, the oncogenic mutation occurs in EGFR (e.g., UniProt accession number P00533). In some embodiments, the oncogenic mutation in EGFR is not a deletion in exon 19 of EGFR. In some embodiments, the oncogenic mutation in EGFR is at least one mutation corresponding to any one of P3L, S4A, G5A, T6A, A7P, A7D, L858R, V769_D770insASV, G8R, A10G, A13V, A16S, L18F, P20L, P20Q, A21S, A21T, A24T, K29T, T34M, T39M, D46E, R53K, E59K, E6K, Q18R, Q71R, T28A, T81A, Q83E, Q30E, E31Q, E84Q, E84D, E31D, A33V, A86V, L37F, L90F, N41S, N94S, V43M, V96M, R98Q, R45Q, Q52H, Q105H, R108G, R55G, R108K, R55K, G109A, G56A, M111T, M58T, or a combination thereof. In some embodiments, the oncogenic mutation comprises a mutation in EGFR corresponding to L858R. In some embodiments, the oncogenic mutation in EGFR is a mutation corresponding to V769_D770insASV.

[109] In some embodiments, the oncogenic mutation occurs in Muc4 (e.g., UniProt accession number Q99102). In some embodiments, Muc4 mutation is an in-frame deletion of exon 2 or an in-frame deletion of exon 3. In some embodiments, the Muc4 mutation is a mutation corresponding to any one of positions P1542, P1680, T1711, V1721, P1826, A1830, S3560, A1833, D2253, V2281, P3088, T3119, T3183, V3817, A3902, or any combination thereof. In some embodiments, the Muc4 mutation is selected from a mutation corresponding to P1542L, P1680S, T1711I, V1721A, P1826H, A1830T, S3560S, A1833V, D2253H, V2281AM, P3088L, T3119T, T3183M, V3817A, A3902V, or a combination thereof.

[110] In some embodiments, the oncogenic mutation occurs in KRAS (e.g., UniProt accession number P01116). In some embodiments, the KRAS mutation comprises a mutation corresponding to any one of positions A59, D119, D33, G21, G12, G13, Q61, A146, K117, or any combination thereof. In some embodiments, the KRAS mutation a mutation corresponding to any one of A59T, A59E, A59T, D119N, D33E, G21C, G12C, G12D, G12V, G12R, G12A, G12S, G13D, G13C, G13V, G13R, Q61R, Q61V, Q61L, Q61K, Q61H, Q61A, Q61P, Q61E, A146T, A146V, K117N, K117R, or a combination thereof.

[111] In some embodiments, the oncogenic mutation occurs in PIK3CA (e.g., UniProt accession number P42336). In some embodiments, the PIK3CA mutation is a mutation corresponding to positions H1047, E542, E545, N345, C1636, G1624, G1633, A3140, C3075, A1634, A1173, or a combination thereof. In some embodiments, the PIK3CA mutation is a mutation corresponding to any one of H1047R, H1047L, E542K, E545K, N345K, C1636A, G1624A, G1633A, A3140T, A3140G, C3075T, A1634C, A1173G, or a combination thereof.

[112] The present disclosure describes chimeric nucleases and methods of using the

chimeric nucleases to target oncogenic mutations. Cleavage with existing single-cut endonucleases can leave compatible DNA ends in the target site (**FIG. 1B**), whereas cleavage by a chimeric nuclease can leave a blunt end at the Cas site and a 3'-overhang at the I-TevI site (**FIG. 1C**). When the chimeric nuclease cleaves at two sites, it cleaves out precise lengths of DNA (for example, approximately 30 – 40 bases depending on the sites targeted by I-TevI and SaCas9). The methods described herein may generate precise deletions of at least about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or at most about 40 nucleotides from a genome. The chimeric nucleases described herein may generate precise deletions of at least about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or at most about 40 nucleotides from a genome. Thus, a target site can be selected in a gene to generate a precise-length deletion that will knock an oncogene out-of-frame in a cell or organism. In some embodiments, a chimeric nuclease of the present disclosure may require four elements in the target site to bind and cut: 1) an I-TevI site; 2) about 14 – 19 nucleotides of spacer sequence; 3) about 19- to 21-nucleotides of protospacer sequence and 4) a protospacer adjacent motif (PAM), where N is any nucleotide, R is the nucleotide A or G and K is the nucleotide T or G. Exemplary I-TevI motifs and PAMs are given in **Table 1** and **Table 2**, respectively. Given the unique target site requirement of the chimeric nuclease, target sites can be selected where an oncogenic mutation changes one of these elements to preferentially or selectively target the mutation. In one embodiment of the described chimeric nucleases generates a precise-length deletion in an oncogene but not in the wild type gene.

[113] **Table 1** describes different I-TevI variants. Different mutations to the I-TevI can alter the specificity of the binding site and changing the consensus sequence.

Oncogenic Mutations

[114] Oncogenic mutations other than those described can also be allele-specific targets of chimeric nucleases. Other allele-specific oncogenic mutations can be targeted as a result of a change in the binding site for the I-TevI domain, a change in the spacer DNA sequence between the I-TevI site and Cas site, a change in the Cas protospacer sequence or a change in the Cas protospacer adjacent motif sequence.

Epidermal Growth Factor- EGFR

[115] In some embodiments, the oncogenic mutation occurs in EGFR. In some embodiments, the oncogenic mutation in EGFR is not a deletion in exon 19 of EGFR. In some embodiments, the oncogenic mutation in EGFR is a mutation corresponding to L858R. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 45, 130, or

141, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the guide RNA comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 45, 130, or 141, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1045, 1130, 1141, or 1686. In some embodiments, the oncogenic mutation in EGFR is a mutation corresponding to V769_D770insASV. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 683, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1683 or 1684. In some embodiments, the guide RNA comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 683, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1683 or 1684.

Mucin 4 – Muc 4

[116] Mucin 4 (MUC-4) is a mucin protein that in humans is encoded by the MUC4 gene. Like other mucins, MUC-4 is a high-molecular weight glycoprotein. MUC-4 belongs to the human mucin family that is membrane-anchored and can range in molecular weight from 550 to 930 kDa for the actual protein, and up to 4,650 kDa with glycosylation. MUC4 can also be referred to as ASGP, HSA276359, MUC-4, or mucin 4.

[117] MUC4 is an O-glycoprotein that can reach up to 2 micrometers outside the cell. MUC4 mucin consists of a large extracellular alpha subunit that is heavily glycosylated and a beta subunit that is anchored in the cell membrane and extends into the cytosol. The beta subunit is considered an oncogene, whose role in cancer is increasingly being recognized particularly due to its involvement in signaling pathways, particularly with ErbB2 (Her2).

[118] The two subunits of MUC4 are transcribed from a single gene made of 25 exons and with its exon/intron structure identical to that of the mouse gene. Over 24 splice variants have been found for MUC4 using commercial mRNAs or total RNAs extracted from cancer cell lines.

[119] MUC-4 is thought to play a role in cancer progression by repressing apoptosis and consequently increasing tumor cell proliferation. The molecular mechanism is thought to be through a MUC-4 complex with ERBB2 receptors, which alters downstream signaling and down regulates CDKN1B. The beta subunit of MUC-4 appears to serve as a ligand that causes the phosphorylation of ErbB2, but does not activate the MAPK or AKT pathways. MUC-4 may also affect HER2 signaling, and result in its stabilization. As a mucin, MUC-4 also alters adhesive properties of the cell. When overexpressed, the disorganization of mucins may reduce adhesion to other cells as well as the extracellular matrix, promoting cancer cell migration and metastasis.

[120] The chimeric nuclease of the present disclosure can target an oncogenic mutation. Such as Muc-4. In some embodiments, the oncogenic mutation occurs in Muc4. In some embodiments, Muc4 mutation is an in-frame deletion of exon 2 or an in-frame deletion of exon 3. In some embodiments, the Muc4 mutation is a mutation corresponding to any one of positions P1542, P1680, T1711, V1721, P1826, A1830, S3560, A1833, D2253, V2281, P3088, T3119, T3183, V3817, A3902, or any combination thereof. In some embodiments, the Muc4 mutation is a mutation corresponding to P1542L, P1680S, T1711I, V1721A, P1826H, A1830T, S3560S, A1833V, D2253H, V2281AM, P3088L, T3119T, T3183M, V3817A, A3902V, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685. In some embodiments, the guide RNA comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685.

K-Ras - KRAS

[121] K-Ras is a part of the RAS/MAPK pathway. KRAS is also known as KRAS, C-K-RAS, CFC2, K-RAS2A, K-RAS2B, K-RAS4A, K-RAS4B, KI-RAS, KRAS1, KRAS2, NS, NS3, RALD, RASK2, K-ras, KRAS proto-oncogene, GTPase, c-Ki-ras2, OES, c-Ki-ras, K-Ras 2, 'C-K-RAS, K-Ras, Kirsten Rat Sarcoma virus, or Kirsten Rat Sarcoma virus. The protein relays signals from outside the cell to the cell's nucleus. These signals instruct the cell to grow and divide (proliferate) or to mature and take on specialized functions (differentiate). It is called KRAS because it was first identified as a viral oncogene in the Kirsten Rat Sarcoma virus. The oncogene identified was derived from a cellular genome, so KRAS, when found in a cellular genome, is called a proto-oncogene.

[122] KRAS acts as a molecular on/off switch. Once it is allosterically activated, it recruits and activates proteins necessary for the propagation of growth factors, as well as other cell signaling receptors like c-Raf and PI 3-kinase. KRAS upregulates the GLUT1 glucose transporter, thereby contributing to the Warburg effect in cancer cells. KRAS binds to GTP in its active state. It also possesses an intrinsic enzymatic activity which cleaves the terminal phosphate of the nucleotide, converting it to GDP. Upon conversion of GTP to GDP, KRAS is deactivated. The rate of conversion is usually slow but can be increased dramatically by an accessory protein of the GTPase-activating protein (GAP) class, for example RasGAP. In turn, KRAS can bind to proteins of the Guanine Nucleotide Exchange Factor (GEF) class (such as SOS1), which forces

the release of bound nucleotide (GDP). Subsequently, KRAS binds GTP present in the cytosol and the GEF is released from ras-GTP.

[123] In some embodiments, the oncogenic mutation occurs in KRAS. In some embodiments, the KRAS mutation comprises a mutation corresponding to any one of positions A59, D119, D33, G21, G12, G13, Q61, A146, K117, or any combination thereof. In some embodiments, the KRAS mutation is a mutation corresponding to any one of A59T, A59E, A59T, D119N, D33E, G21C, G12C, G12D, G12V, G12R, G12A, G12S, G13D, G13C, G13V, G13R, Q61R, Q61V, Q61L, Q61K, Q61H, Q61A, Q61P, Q61E, A146T, A146V, K117N, K117R, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1042, 1051, 1052, 1062, 1063, or 1077. In some embodiments, the guide RNA comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077.

Phosphatidylinositol 3-kinase catalytic subunit – PIK3CA

[124] Phosphatidylinositol 3-kinase catalytic subunit (PIK3CA) is one of the most common mutated genes in breast cancer and has been found to important in a number of cancer types. An integral part of the PI3K pathway, PIK3CA has long been described as an oncogene, with two main hotspots for activating mutations, the 542/545 region of the helical domain, and the 1047 region of the kinase domain.

[125] Phosphatidylinositol-4,5-bisphosphate 3-kinase (also called phosphatidylinositol 3-kinase (PI3K)) is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit (PIK3CA). The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate phosphatidylinositols (PtdIns), PtdIns4P and PtdIns(4,5)P2. PIK3CA can also be referred to as CLOVE, CWS5, MCAP, MCM, MCMTC, PI3K, p110-alpha, PI3K-alpha, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, CLAPO, CCM4.

[126] In some embodiments, the oncogenic mutation occurs in PIK3CA. In some embodiments, the PIK3CA mutation is a mutation corresponding to any one of positions H1047, E542, E545, N345, C1636, G1624, G1633, A3140, C3075, A1634, A1173, or a combination thereof. In some embodiments, the PIK3CA mutation is a mutation corresponding to any one of positions H1047R, H1047L, E542K, E545K, N345K, C1636A, G1624A, G1633A, A3140T, A3140G, C3075T, A1634C, A1173G, or a combination thereof. In some embodiments, the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 5, 6, 7, 8, 33,

202, 204, 209 or 210, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210. In some embodiments, the guide RNA comprises the nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210.

I-TevI Nuclease

[127] The chimeric nuclease of the present disclosure may comprise an I-TevI nuclease domain. An unmodified full-length I-TevI protein comprises the sequence according SEQ ID NO: 702.

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDGRKALYSKPGSKNGRWNPEHFKFCKGVRITSAYTCSKCRN (SEQ ID NO:
702).

[128] The sequence provided by SEQ ID NO: 700, 702, or 704 is a wild-type version of I-TevI except for a glycine insertion at position 2 that increases protein stability and prevents N-terminal degradation. With respect to specific substitutions referred to herein, the numbering corresponds to the wild-type version of the protein lacking the glycine stabilization. Thus, in the stabilized version of I-TevI the lysine at position 27 of SEQ ID NO: 700, 702, or 704 is referred to as K26 corresponding to the wild-type position without the glycine at position 2. There are several I-TevI substitutions to the I-TevI domain known to have little effect on I-TevI nuclease activity. Nuclease activity of I-TevI can be assayed for by mixing a chimeric nuclease containing the I-TevI domain with linear DNA containing a known I-TevI target and resolving the products of the cleavage reaction on an agarose gel. Products of the predicted size will be present if the I-TevI nuclease is active.

[129] The chimeric nuclease of the present disclosure can comprise an I-TevI nuclease domain. In some embodiments, the I-TevI nuclease domain is derived from Enterobacteria Phage T4. The I-TevI domain can comprise a 93-amino acid I-TevI domain of the Enterobacteria Phage T4 according to the following sequence:

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIA (SEQ ID NO: 700).

[130] In some embodiments, exemplary I-TevI nuclease domain are shown in SEQ ID NO: 700. In some embodiments, the mutation can correspond to any one of SEQ ID NO: 700.

[131] In some embodiments, the I-TevI nuclease domain can comprise at least one mutation as compared to SEQ ID NO: 700, 702, or 704. In some embodiments, the I-TevI nuclease domain comprises a mutation corresponding to any one of the positions selected from any one of T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, E81, or a combination thereof. In some embodiments, the I-TevI nuclease domain comprises a mutation corresponding to any one of T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof. In some embodiments, the I-TevI nuclease domain comprises at least a K26R mutation as compared to a wild-type sequence. In some embodiments, the I-TevI nuclease domain comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 700, 702, or 704.

[132] Other versions of the I-TevI nuclease domain might contain different combinations of mutations to alter the site targeted by the I-TevI domain or the activity of the I-TevI domain, including mutations that alter the sequence recognized by I-TevI, such as K26 and/or C39. Other versions of the nuclease might substitute the I-TevI domain with other GIY-YIG nuclease domains, such as I-BmoI, Eco29kI, etc. Some versions of I-TevI do not contain Met1 as a result of processing when expressed in *E. coli*.

[133] The unmodified full-length I-TevI nuclease comprises a nuclease domain, comprising position 1- 93 and a linker domain comprising position 94 -169. The positions of the mutations correspond to the positions according to the unmodified full-length I-TevI nuclease, or SEQ ID NO: 700, 702, or 704.

[134] Table 1 summarizes a list of exemplary wildtype I-TevI and its variants. In addition, the table notes the different cleavage motifs that are used by the different I-TevI proteins.

Table 1: Exemplary I-TevI Variants and Cleavage motifs

Protein Name	Variant	Motif
I-TevI	Wild-type	5'-CAACG-3' (SEQ ID NO: 800); 5'-CGAAG-3'(SEQ ID NO: 801)
I-TevI	K26R	5'-CAACG-3'(SEQ ID NO: 802); 5'-CAAGG-3'(SEQ ID NO: 803); 5'-CGAAG-3'(SEQ ID NO: 804)

I-TevI	T95S	5'-CAACG-3'(SEQ ID NO: 805); 5'-CAAGG-3'(SEQ ID NO: 806); 5'-CCCCG-3'(SEQ ID NO: 807); 5'-CGAAG-3'(SEQ ID NO: 808); 5'-CGCCG-3'(SEQ ID NO: 809); 5'-CGGAG-3'(SEQ ID NO: 810); 5'-CTGGG-3'(SEQ ID NO: 811)
I-TevI	Q158R	5'-CAACG-3' (SEQ ID NO: 812); 5'-CAAGG-3' (SEQ ID NO: 813); 5'-CCCCG-3' (SEQ ID NO: 814); 5'-CGAAG-3' (SEQ ID NO: 815); 5'-CGGAG-3' (SEQ ID NO: 816); 5'-TAACG-3' (SEQ ID NO: 817)
I-TevI	K26R/T95S	5'-CAACG-3'(SEQ ID NO: 818); 5'-CAAGG-3'(SEQ ID NO: 819); 5'-CCCCG-3'(SEQ ID NO: 820); 5'-CGAAG-3'(SEQ ID NO: 821); 5'-CGCCG-3'(SEQ ID NO: 822); 5'-CGGAG-3'(SEQ ID NO: 823); 5'-CTGGG-3'(SEQ ID NO: 824)
I-TevI	K26R/Q158R	5'-CAACG-3'(SEQ ID NO: 825); 5'-CAAGG-3'(SEQ ID NO: 826); 5'-CCAGG-3'(SEQ ID NO: 827); 5'-CCCCG-3'(SEQ ID NO: 828); 5'-CGAAG-3'(SEQ ID NO: 829); 5'-CGGAG-3'(SEQ ID NO: 830); 5'-CTGGG-3'(SEQ ID NO: 831); 5'-TAACG-3'(SEQ ID NO: 832)
I-TevI	T95S/Q158R	5'-CAACG-3'(SEQ ID NO: 833); 5'-CAAGG-3'(SEQ ID NO: 834); 5'-CACGG-3'(SEQ ID NO: 835); 5'-CCCAG-3'(SEQ ID NO: 836); 5'-CCCCG-3'(SEQ ID NO: 837); 5'-CGAAG-3'(SEQ ID NO: 838); 5'-CGCAG-3'(SEQ ID NO: 839); 5'-CGCCG-3'(SEQ ID NO: 840); 5'-CGCTG-3'(SEQ ID NO: 841); 5'-CGGAG-3'(SEQ ID NO: 842); 5'-CTCGG-3'(SEQ ID NO: 843); 5'-CTGGG-3'(SEQ ID NO: 844); 5'-AAACG-3'(SEQ ID NO: 845); 5'-GAACG-3'(SEQ ID NO: 846)
I-TevI	K26R/T95S/Q158R	5'-CAACG-3'(SEQ ID NO: 847); 5'-CAAGG-3'(SEQ ID NO: 848); 5'-CACGG-3'(SEQ ID NO:

		849); 5'-CCCAG-3'(SEQ ID NO: 850); 5'-CCCCG-3'(SEQ ID NO: 851); 5'-CGAAG-3'(SEQ ID NO: 852); 5'-CGCAG-3'(SEQ ID NO: 853); 5'-CGCCG-3'(SEQ ID NO: 854); 5'-CGCTG-3'(SEQ ID NO: 855); 5'-CGGAG-3'(SEQ ID NO: 856); 5'-CTGGG-3'(SEQ ID NO: 857); 5'-TAACG-3'(SEQ ID NO: 858)
Protein Name	Variant	Effect on I-TevI Function
I-TevI	V117F	Improved binding to cleavage site
I-TevI	V117F/K135R/N140S	Relaxes spacer sequence contact requirement

Linker Domain

[135] The chimeric nucleases of the present disclosure may further comprise a linker domain. The linker may comprise a flexible amino acid linker comprising from at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids. The linker may comprise a flexible amino acid linker comprising from no more than 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids. In some embodiments, the linker domains can be unstructured or comprise a Gly-Ser linker. Longer linkers generally can relax the 14-19 base pair I-TevI spacing requirement in the target site, whereas shorter linkers generally restrict it. Useful linkers include, but are not limited to, glycine-serine polymers, including for example (GS)_n (SEQ ID NO: 900), (GSGGS)_n (SEQ ID NO: 901), (GGGGS)_n (SEQ ID NO: 902), and (GGGS)_n (SEQ ID NO: 903), where n is an integer of at least one, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Exemplary, linkers for linking antibody fragments or single chain variable fragments can include AAEPKSS (SEQ ID NO: 904), AAEPKSSDKTHTCPPCP (SEQ ID NO: 904), GGGG (SEQ ID NO: 905), or GGGGDKTHTCPPCP (SEQ ID NO: 906). Alternatively, a variety of non-proteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers. In some embodiments, the I-TevI nuclease domain is joined to a Cas domain by a linker domain. The linker domain may comprise the I-TevI linker (amino acids 93 – 169 of SEQ ID NO: 701). In some embodiments, the linker comprises an amino acid sequence set forth

DATFGDTCSTHPLKEEIIKKRSETVKAKMLKLGPDKRKALYSKPGSKNGRWNPEETHKFCKCGVRIQTSAYTCSKCRNGGSGGS (SEQ ID NO: 703).

[136] In some embodiments, exemplary linkers are shown in SEQ ID NO: 702 or 704. In

some embodiments, the mutation can correspond to any one of SEQ ID NO: 702 or 704.

[137] In some embodiments, the linker comprises a mutation corresponding to a position selected from any one of T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof. In some embodiments, the linker comprises a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof. In some embodiments, the linker comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 701, 702, 703, or 704. In some embodiments, the linker comprises a mutation corresponding to K135R and/or N140S. In some embodiments, the linker comprises a mutation corresponding to V117F. In some embodiments, the linker comprises a mutation corresponding to V117F, K135R, and/or N140S.

Cas Protein Domains

[138] CRISPR systems generally contain two components: a guide RNA (gRNA or sgRNA) and a CRISPR-associated endonuclease (Cas protein). In nature, CRISPR/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. The CRISPR-Cas is an RNA-mediated adaptive defense system that relies on small RNA molecules for sequence-specific detection and silencing of foreign nucleic acids. CRISPR-Cas systems are composed of *cas* genes organized in operon(s) and CRISPR array(s) consisting of genome-targeting sequences (termed spacers).

[139] CRISPR-Cas systems can generally refer to and include an enzyme system that includes a guide RNA sequence that contains a nucleotide sequence complementary or substantially complementary to a region of a target polynucleotide (e.g., a template nucleic acid such a HSV genomic DNA), and a protein with nuclease activity. CRISPR-Cas systems can include Type I CRISPR-Cas system, Type II CRISPR-Cas system, Type III CRISPR-Cas system, and derivatives thereof. CRISPR-Cas systems include engineered and/or programmed nuclease systems derived from naturally accruing CRISPR-Cas systems. CRISPR-Cas systems may contain engineered and/or mutated Cas proteins. In certain embodiments, nucleases generally refer to enzymes capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids. In some embodiments, endonucleases are generally capable of cleaving the phosphodiester bond within a polynucleotide chain.

[140] In some embodiments, the CRISPR-Cas system used herein can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR-Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12, CasF, CasG, CasH, CasX, CasΦ, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CasX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966. In some embodiments, the CRISPR-Cas protein or endonuclease is Cas9. In some embodiments, the CRISPR-Cas protein or endonuclease is Cas12. In some embodiments, the CRISPR-Cas protein or endonuclease is CasX.

[141] In some embodiments, the Cas9 protein can be from or derived from: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycooides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas sp.*, *Crocospaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor beccii*, *Candidatus desulfurudis*, *Clostridium botulinum*, *Clostridium difficile*, *Fine goldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum the rmopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrogla mobilis*, *Thermosiphon africanus*, or *Acaryochloris marina*.

[142] In some embodiments, the CRISPR-Cas-like protein can be a wild type CRISPR-Cas protein, a modified CRISPR-Cas protein. In some embodiments, the CRISPR-Cas-like protein can be modified to increase nucleic acid binding affinity and/or specificity, alter an enzymatic activity, and/or change another property of the protein. For example, nuclease (i.e., DNase, RNase) domains of the CRISPR-Cas-like protein can be modified, deleted, or inactivated. Alternatively, in some embodiments, the CRISPR-Cas-like protein can be truncated to remove domains that are not essential for the function of the Cas protein. In some embodiments, the

CRISPR-Cas-like protein can also be truncated or modified to optimize the activity of the effector domain of the Cas protein.

[143] In some embodiments, the CRISPR-Cas-like protein can be derived from a wild type Cas protein or fragment thereof. In certain embodiments, the CRISPR-Cas-like protein is a modified Cas9 protein. For example, the amino acid sequence of the Cas9 protein can be modified to alter one or more properties (e.g., nuclease activity, affinity, stability, etc.) of the protein relative to wild-type or another Cas protein. Alternatively, in some embodiments, domains of the Cas9 protein not involved in RNA-guided cleavage can be eliminated from the protein such that the modified Cas9 protein is smaller than the wild-type Cas9 protein.

[144] The chimeric nuclease of the present disclosure can comprise a Cas domain. In some embodiments, the Cas domain is a Cas9 domain. In some embodiments, the Cas domain is a Cas12 domain.

[145] In some embodiments, the Cas9 domain is derived from a bacterial organism such as, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Neisseria meningitidis*, *Campylobacter jejuni*, *Streptococcus pasteurianus*, *Clostridium cellulolyticum*, or *Geobacillus thermodenitrificans T1*. In some embodiments, the Cas9 domain is derived from *Staphylococcus aureus* (SaCas9). In some embodiments, the Cas9 domain is derived from *Streptococcus pyogenes* (spCas9). In some embodiments, the Cas9 domain is derived from *Neisseria meningitidis* (NmCas9). In some embodiments, the Cas9 domain is derived from *Campylobacter jejuni* (CjCas9). In some embodiments, the Cas9 domain is derived from *Streptococcus pasteurianus* (SpCas9). In some embodiments, the Cas9 domain is derived from *Clostridium cellulolyticum* (CcCas9). In some embodiments, the Cas9 domain is derived from *Geobacillus thermodenitrificans T1* (GtCas9).

[146] In some embodiments, exemplary RNA-guided nuclease Cas9 domains is shown in SEQ ID NO: 710, 711, 712, 713, 714, 715, or 716. In some embodiments, the mutation or amino acid substitution can correspond to any one of SEQ ID NO: 710, 711, 712, 713, 714, 715, or 716.

[147] In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical, or is identical to any one of SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation or amino acid substitution corresponding to a position selected from any one of D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667,

R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation or substitution corresponding to any one or more of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof. In certain embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprise a mutation or substitution corresponding to D10E substitution. In certain embodiments, the modified I-TevI nuclease domain comprises SEQ ID NO: 700, the linker comprises any one of SEQ ID NOs: 701 or 703 and the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises any one of SEQ ID NO: 710-715, or 716. In certain embodiments, the modified I-TevI nuclease domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical, or is identical to any one of SEQ ID NO: 700, the linker domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical, or is identical to any one SEQ ID NOs: 701 or 703 and the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%,

90%, 95%, 97%, 98%, or 99% identical to any one SEQ ID NOs: 710-715, or 716.

[148] In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 710. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to position D10, H557, N580, H840, D1135, R1335, T1337, or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, or a combination thereof. In some embodiments, the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain (saCas9) comprises a mutation corresponding to D10E mutation. In some embodiments, the saCas9 comprises a mutation corresponding to a D10E and/or N580A mutation. In some embodiments, the saCas9 comprises a mutation corresponding to a D10A and/or N580A mutation. In some embodiments, the saCas9 comprises a mutation corresponding to a D10E D1135E, R1335Q, and/or T1337R mutation. In some embodiments, the saCas9 comprises a mutation corresponding to D10E, D1135E, R1335Q, T1337R, and/or H840A mutation. In some embodiments, the saCas9 comprises a mutation corresponding to D10E and/or H557A mutation. In some embodiments, the saCas9 comprises a mutation corresponding to a D10E, H840A, D1135E, R1335Q, and/or T1337R mutation.

[149] In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 711.

[150] In some embodiments, the RNA-guided nuclease *Staphylococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115,

G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 711. In some embodiments, the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 711.

In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain. In some embodiments, the RNA-guided nuclease *Neisseria*

meningitidis Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 712. Other *Neisseria meningitidis* Cas9 can be found at www.uniprot.org/uniprot/ with accession numbers C9X1G5, A1IQ68, E0NB23, A9M1K5, or C6S593. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 712. In some embodiments, the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain

comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 712.

[151] In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 713. Other *Campylobacter jejuni* Cas9 can be found at www.uniprot.org/uniprot/ with accession numbers Q0P897, A7H5P1, A0A2U0QR81, A0A5Y4VLH1, or A0A381CRM8. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581L, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751L, F756L, R763K, Q764E, E772N, K777H,

A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971L, T972S, or a combination thereof. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 713. In some embodiments, the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 713.

[152] In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 714. Other *Streptococcus pasteurianus* Cas9 can be found at www.uniprot.org/uniprot/ with accession number F5X275.

[153] In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V,

V1302A, N1316D, T1346N, I1374L, or a combination thereof. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 714. In some embodiments, the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 714.

In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 715. Other *Clostridium cellulolyticum* Cas9 can be found at www.uniprot.org/uniprot/ with accession number B8I085.

[154] In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K, or a combination thereof. In some embodiments, the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 715. In some embodiments, the

RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 715.

In some embodiments, the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 716. Other *Geobacillus thermodenitrificans* T1 Cas9 can be found at www.uniprot.org/uniprot/ with accession number A0A1W6VMQ3.

[155] In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions D8, D179, D282, D399, D685, D756, D1071. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T,

I1079V, or combination thereof. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions D8E, D179E, D282E, D399N, D685E, D756E, D1071H. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 716. In some embodiments, the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence having between 85-90%, 90-95%, 95-97%, 97-98%, or 98-99% sequence identity to SEQ ID NO: 716.

Table 2: Exemplary Cas9 domains and PAM Motifs

Cas Domain	PAM Motif
NmCas9 from <i>Neisseria meningitidis</i>	5'-NNNNGMTT-3' (M = A or C) (SEQ ID NO: 870)
SpCas9 from <i>Streptococcus pyogenes</i>	5'-NRG-3' (R = A or G) (SEQ ID NO: 871)
StCas9 from <i>Streptococcus thermophilus</i>	5'-NNAGAAW-3' (W = A or T) (SEQ ID NO: 872)
CjCas9 from <i>Campylobacter jejuni</i>	5'-NNNNRYAC-3' (R=A or G, Y= C or T) (SEQ ID NO: 873)
SpCas9 from <i>Streptococcus pasteurianus</i>	5'-NNGTGA-3' (SEQ ID NO: 874)
Nme2Cas9 from <i>Neisseria meningitidis</i>	5'-NNNNCC-3' (SEQ ID NO: 875)
CcCas9 from <i>Clostridium cellulolyticum</i>	5'-NNNNGNA-3' (SEQ ID NO: 876)
ThermoCas9 from <i>Geobacillus thermodenitrificans</i> T1	5'-NNNNCNR-3' (SEQ ID NO: 877)

[156] In some embodiments, the Cas domain is CasX domain. In some embodiments, the CasX domain is derived from *Planctomycetes bacterium*. In some embodiments, the CasX domain is from *Deltaproteobacteria*. In some embodiments, the CasX domain comprises an amino acid sequence at least about 85%, 90%, 95%, 97%, 98%, or 99% identical, or is identical to SEQ ID NO: 721. In some embodiments, the CasX domain comprises a mutation corresponding to any one of positions R11, R12, V14, K15, S17, N18, A22, G23, T25, P38, K41, E42, N46, L47, N53, I54, P57, T61, S62, R63, A64, E75, H82, Q89, P104, N106, I113, N199, S124, S125, C133, Y137, N145, D146, H151, S161, R165, N177, L180, R202, N205, G215, C219, V236, T241, L248, I254, S269, I290, E291, V297, Q299, I314, E318, Q323, L333, E359, D360, K362, Q366, N367, L368, A369, G370, Y371, H404, H409, G410, E411, Y417, V428, E429, S432, K433, L437, S443, A451, I464, A470, I502, L503, I531, G537, L540, N553, I559, S563, V571, N579, H589, S607, L608, L620, R623, R624, L644, S646, M652, I657, R679, L684, N686, H689, S696, T702, T737, L742, Y744, Q748, M751, I753, A771, R777, P792, S818, R823, V824, E826, K827, A832, T833, M836, I839, G841, V846, N860, V862, D864, V867, V877,

S883, S889, G890, S894, K908, N913, F916, T918, R936, Q938, Y940, K942, S963, R966, K967, K968, or any combination thereof. In some embodiments, the CasX domain comprises a mutation or substitution corresponding to any one or more of R11K, R12K, V14S, K15A, S17N, N18A, A22V, G23S, T25S, P38D, K41K, E42K, N46K, L47R, N53V, I54M, P57V, T61N, S62A, R63A, A64N, E75K, H82Q, Q89K, P104S, N106K, I113K, N199K, S124T, S125A, C133G, Y137F, N145S, D146E, H151Y, S161A, R165K, N177S, L180A, R202K, N205T, G215A, C219Y, V236I, T241S, L248I, I254V, S269G, I290V, E291D, V297I, Q299R, I314L, E318D, Q323L, L333V, E359D, D360M, K362R, Q366S, N367G, L368V, A369T, G370A, Y371E, H404Y, H409Y, G410A, E411G, Y417F, V428I, E429A, S432T, K433S, L437R, S443A, A451V, I464L, A470M, I502V, L503V, I531L, G537K, L540I, N553S, I559L, S563G, V571L, N579Q, H589T, S607L, L608I, L620I, R623K, R624K, L644V, S646P, M652V, I657V, R679E, L684S, N686G, H689D, S696G, T702A, T737S, L742F, Y744H, Q748H, M751V, I753V, A771T, R777K, P792T, S818T, R823G, V824M, E826V, K827R, A832S, T833D, M836A, I839L, G841N, V846A, N860T, V862E, D864E, V867A, V877G, S883K, S889R, G890D, S894F, K908Q, N913D, F916H, T918V, R936N, Q938N, Y940F, K942S, S963A, R966K, K967R, K968R, or a combination thereof.

[157] In some embodiments, the Cas12 domain is from *Acidaminococcus* sp. BV3L6. In some embodiments, the Cas12 domain comprises an amino acid sequence at least about 85%, 90%, 95%, 97%, 98%, or 99% identical, or is identical to SEQ ID NO: 720. In some embodiments, the Cas12 domain comprises a mutation corresponding to any one of positions T1, Q2, E4, G5, N8, L9, K28, H29, I30, Q31, E32, Q33, F35, I36, E37, E38, A41, N43, D44, H45, E48, I52, R55, T59, Y60, A61, D62, Q63, C64, Q66, L67, Q69, L70, N74, S76, A77, D80, S81, Y82, E85, E88, T90, R91, N92, A93, I95, E97, A99, T100, Y101, N103, A104, H106, D107, I110, R112, T113, D114, R159, S169, S185, A187, I192, D195, K201, T212, R218, N223, I228, S233, I236, E237, V239, F242, Q249, Y257, V279, I284, F305, N313, S324, I329, S331, T337, L338, L345, E349, S357, I358, N386, I393, L396, I400, S403, V408, Q409, G427, K428, Q436, L442, S468, Q469, S472, L473, L479, E487, S488, A497, L510, A516, K522, Q535, M536, S541, V545, K549, N550, G552, V557, N559, S586, Y596, A601, I604, A613, S628, E637, A657, K660, G663, Q665, C673, L683, L697, A711, L717, Q723, A733, E735, Y740, K751, K756, G766, I778, R793, L844, I858, S865, I874, H898, I903, I916, L931, K941, N945, V951, S958, V959, D965, I938, H984, A1009, C1024, G1037, T1049, G1055, T1056, Y1068, L1075, V1083, K1085, L1097, H1104, D1106, D1111, L1122, A1134, V1138, D1147, V1160, P1161, R1171, R1173, Y1176, N1205, D1207, S1220, V1221, A1230, N1237, L1243, M1259, Q1274, G1291, Q1295, A1299, or L1304. In some embodiments, the Cas12 domain comprises a mutation or substitution

corresponding to any one or more of T1S, Q2N, E4S, G5E, N8H, L9K, K28E, H29N, I30L, Q31T, E32A, Q33Y, F35M, I36V, E37N, E38D, A41L, N43S, D44E, H45N, E48K, I52V, R55K, T59Y, Y60F, A61I, D62E, Q63E, C64T, Q66K, L67H, Q69A, L70I, N74P, S76Y, A77K, D80T, S81A, Y82F, E85D, E88L, T90N, R91N, N92T, A93N, I95R, E97I, A99D, T100N, Y101C, N103K, A104S, H106A, D107G, I110E, R112K, T113V, D114P, R159K, S169V, S185A, A187S, I192L, D195E, K201I, T212K, R218N, N223T, I228T, S233G, I236L, E237D, V239I, F242V, Q249C, Y257F, V279T, I284V, F305Y, N313S, S324N, I329L, S331A, T337E, L338K, L345I, E349Q, S357L, I358A, N386D, I393V, L396A, I400L, S403N, V408I, Q409E, G427D, K428D, Q436A, L442I, S468V, Q469L, S472A, L473V, L479T, E487D, S488D, A497V, L510I, A516V, K522Q, Q535S, M536N, S541D, V545E, K549Q, N550Q, G552C, V557E, N559E, S586N, Y596Q, A601S, I604L, A613D, S628N, E637T, A657D, K660R, G663N, Q665K, C673H, L683V, L697V, A711G, L717F, Q723E, A733L, E735D, Y740F, K751E, K756A, G766A, I778V, R793P, L844F, I858V, S865T, I874L, H898N, I903V, I916A, L931F, K941N, N945Q, V951I, S958T, V959A, D965E, I938V, H984Q, A1009S, C1024Y, G1037S, T1049E, G1055R, T1056N, Y1068F, L1075A, V1083R, K1085G, L1097I, H1104K, D1106N, D1111N, L1122K, A1134D, V1138I, D1147A, V1160E, P1161F, R1171Q, R1173E, Y1176L, N1205T, D1207N, S1220L, V1221T, A1230E, N1237S, L1243I, M1259K, Q1274L, G1291A, Q1295N, A1299N, or L1304K.

[158] In some embodiments, the chimeric nuclease comprises a non-naturally occurring Cas domain. In some embodiments, the chimeric nuclease may comprise a Cas domain from other Class 1 or Class 2 CRISPR-Cas proteins, CRISPR-Cas3, CRISPR-Cascade, or Cas13d.

Nuclear Localization Signals

[159] In some embodiments, a chimeric nuclease further comprises one or more nuclear localization sequences (NLS). In some embodiments, the NLS helps promote translocation of a protein into the cell nucleus. In some embodiments, a chimeric nuclease comprises one or more NLSs. In some embodiments, the chimeric nuclease is fused to or linked to one or more NLSs.

[160] In some embodiments, a chimeric nuclease comprises at least one NLS. In some embodiments, a chimeric nuclease comprises at least two NLSs. In embodiments with at least two NLSs, the NLSs can be the same NLS, or they can be different NLSs.

[161] In some embodiments, a chimeric nuclease may further comprise at least one nuclear localization sequence (NLS). In some embodiments, a chimeric nuclease may further comprise 1 NLS. In some cases, a chimeric nuclease may further comprise 2 NLSs. In some embodiments, a chimeric nuclease may further comprise 3 NLSs. In some embodiments, a chimeric nuclease can

further comprise at least 4 NLSs. In some embodiments, a chimeric nuclease can further comprise at least 5 NLSs. In some embodiments, a chimeric nuclease can further comprise at least 6 NLSs. In some embodiments, a chimeric nuclease can further comprise at least 7 NLSs. In some embodiments, a chimeric nuclease can further comprise at least 8 NLSs. In some embodiments, a chimeric nuclease can further comprise at least 9 NLSs. In some embodiments, a chimeric nuclease can further comprise no more than 10 NLSs.

[162] In addition, the NLSs can be expressed as part of a chimeric nuclease. In some embodiments, a NLS can be positioned almost anywhere in a protein's amino acid sequence, and generally comprises a short sequence of three or more or four or more amino acids. The location of the NLS fusion can be at the N-terminus, the C-terminus, or positioned anywhere within a sequence of a chimeric nuclease or a component thereof (*e.g.*, inserted between the I-TevI domain and Cas domain of a chimeric nuclease, between the I-TevI domain and a linker domain, between a Cas domain polymerase and a linker domain or at the N-terminus or the C-terminus of the chimeric nuclease). In some embodiments, a chimeric nuclease comprises an NLS at the N terminus. In some embodiments, a chimeric nuclease comprises an NLS at the C terminus. In some embodiments, a chimeric nuclease comprises at least one NLS at both the N terminus and the C terminus. In some embodiments, a chimeric nuclease comprises two NLSs at the N terminus and/or the C terminus.

[163] Any NLSs that are known in the art are also contemplated herein. The NLSs may be any naturally occurring NLS, or any non-naturally occurring NLS (*e.g.*, an NLS with one or more mutations relative to a wild-type NLS). In some embodiments, the one or more NLSs of a chimeric nuclease comprise bipartite NLSs. In some embodiments, a nuclear localization signal (NLS) is predominantly basic. In some embodiments, the one or more NLSs of a chimeric nuclease are rich in lysine and arginine residues. In some embodiments, the one or more NLSs of a chimeric nuclease comprise proline residues. In some embodiments, a nuclear localization signal (NLS) comprises the sequence MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 742), KRTADGSEFESPKKKRKV (SEQ ID NO: 743), KRTADGSEFEPKKKKRKV (SEQ ID NO: 744), NLSKRPAAIKKAGQAKKKK (SEQ ID NO: 745), RQRRNELKRSF (SEQ ID NO: 746), or NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 747).

[164] In some embodiments, a NLS is a monopartite NLS. For example, in some embodiments, a NLS is a SV40 large T antigen NLS PKKKRKV (SEQ ID NO: 740). In some embodiments, a NLS is a bipartite NLS. In some embodiments, a bipartite NLS comprises two basic domains separated by a spacer sequence comprising a variable number of amino acids. In some

embodiments, a NLS is a bipartite NLS. In some embodiments, a bipartite NLS consists of two basic domains separated by a spacer sequence comprising a variable number of amino acids. In some embodiments, the spacer amino acid sequence comprises the sequence KRXXXXXXXXXXXXKKKL (*Xenopus nucleoplasmin* NLS) (SEQ ID NO: 748), wherein X is any amino acid. In some embodiments, the NLS comprises a nucleoplasmin NLS sequence KRPAATKKAGQAKKKK (SEQ ID NO: 741). In some embodiments, a NLS is a noncanonical sequences such as M9 of the hnRNP A1 protein, the influenza virus nucleoprotein NLS, and the yeast Gal4 protein NLS. In some embodiments, a NLS is a noncanonical sequences such as M9 of the hnRNP A1 protein, the influenza virus nucleoprotein NLS, and the yeast Gal4 protein NLS.

[165] In certain embodiments, said chimeric nuclease comprises a nuclear localization signal. In certain embodiments, the nuclear localization signal comprises an SV40 nuclear localization signal comprising the amino acid sequence SEQ ID NO: 740 (PKKKRKV). In certain embodiments, the nuclear localization signal comprises a Nucleoplasmin nuclear localization signal comprising the amino acid sequence SEQ ID NO: 741 (KRPAATKKAGQAKKKK).

[166] Non-limiting examples of NLS sequences are provided in **Table 3** below.

Table 3: Exemplary nuclear localization sequences

Description	Sequence	SEQ ID NO:
NLS of SV40 Large T-AG	PKKKRKV	880
NLS	MKRTADGSEFESPKKKRKV	881
NLS	MDSLLMNRKFLYQFKNVRWAKGRRETYLC	882
NLS of Nucleoplasmin	AVKRPAATKKAGQAKKKKLD	883
NLS of EGL-13	MSRRRKANPTKLSNAKKLAKEVEN	884
NLS of C-Myc	PAAKRVKLD	885
NLS of Tus-protein	KLKIKRPVK	886
NLS of polyoma large T-AG	VSRKRPRP	887
NLS of Hepatitis D virus antigen	EGAPPAKRAR	888
NLS of murine p53	PPQPKKKPLDGE	889
NLS of PE1 and PE2	SGGSKRTADGSEFESPKKKRKV	890

Donor Polynucleotide

[167] The chimeric nuclease of the present disclosure may be used or administered with a donor nucleic acid sequence. A nucleic acid sequence is a sequence that can be used as a template to replace a sequence excised by the chimeric nuclease. In some embodiments, the donor nucleic acid restores a non-oncogenic function of a gene comprising the oncogenic mutation (i.e., restoring wild-type function). In some embodiments, the donor nucleic acid is DNA.

[168] The methods and techniques described herein are useful for the genetic modification of a cell having an oncogene of a population of cells. The donor nucleic acid may be configured for incorporation by homologous recombination. Such donor DNAs for incorporation by homologous recombination may comprise a first flanking homology region, an exogenous polynucleotide sequence of interest, and a second flanking homology region. Alternatively, the exogenous donor DNA may be inserted into a genomic location by incorporation into a genomic location at a single double strand break or a dual double stranded break with the aid of non-homologous end joining.

[169] When a chimeric nuclease (e.g., TevSaCas9) cleaves a double stranded DNA, the Cas (e.g., Cas9) can leave a blunt end and the I-TevI can leave a 3' overhang. Donor DNAs supplied may include a blunt end and a nucleotide 3' overhang configured to bind the created 3' overhang in the chimeric nuclease (TevCas9) cleaved site. In some embodiments, the donor nucleic acid comprises a blunt end and a 3' overhang. In some embodiments, the 3' overhang is at least 1, 2, 3, 4, or 5 nucleotides in length. It would be understood that the length of the nucleotide overhang may be altered to accommodate the use of a Cas domain that generates an overhang not equal to two nucleotides.

[170] In some embodiments, the donor DNA comprises DNA sequences that are intended to be inserted into a site of a gene, such as an oncogene. In certain embodiments, the donor DNA comprises double-stranded DNA of the same length cleaved by the nuclease and also comprising complimentary DNA ends to those cleaved by the chimeric nuclease. In certain embodiments, the donor DNA comprises 5' ends of the DNA that are phosphorylated. In certain embodiments, the donor DNA comprises circular double-strand DNA comprising an I-TevI target site and Cas target site where the product cleaved from the double-strand DNA contains complimentary ends to those cleaved by the chimeric nuclease.

[171] The donor nucleic acid may comprise homology arms that flank either end of the DNA sequences to be inserted into a gene. In some embodiments, the homology arms comprise a 5'

and 3' homology arm that flank both ends of the DNA sequences to be inserted. In some embodiments, the 5' and 3' homology arms are identical or different in length.

[172] The double-stranded donor nucleic acid may contain different 5'-end chemical modifications such as biotin. Other versions of the donor DNA might include stability modifications to the 2' position of the ribose, including but not limited to 2'-fluoro, 2'-amino, and 2'-O-methyl. In some embodiments, the donor nucleic acid may contain 3'-end modifications such as an inverted dT or biotin. Other versions of the donor nucleic acid might include locked nucleic acids (LNAs) in which the 2'-O and 4'-C atoms of the ribose sugar are joined through a methylene bridge. Other versions of the double-stranded donor nucleic acid might include circular plasmid DNA containing a chimeric nuclease target site in which cleavage with the chimeric nuclease creates complementary DNA ends to those in the genome target. The double stranded donor nucleic acid may comprise a synthetic or amplified linear double stranded DNA. In certain embodiments the donor nucleic acid is supplied using a viral vector such as an adeno-associated virus or lentivirus.

Guide RNA

[173] The chimeric nuclease of the present disclosure can further comprise a guide RNA. A guide RNA might target the same region of DNA in the oncogenes but contain different sequences to account for genetic polymorphism in populations. Other versions of the guide RNA might target different oncogenes. Other versions might contain a mixture of guide RNAs to target multiple sequences within the same gene. Guide RNAs may comprise a single strand comprising all necessary elements for activity (e.g., target binding and nuclease binding). Alternatively guide RNAs may comprise two or more non-covalently bound nucleic acids that form a single moiety due to base pairing between the two or more nucleic acids.

[174] gRNAs are generally supported by a scaffold, wherein a scaffold refers to the portions of gRNA or crRNA molecules comprising sequences which are substantially identical or are highly conserved across natural biological species (e.g., not conferring target specificity). Scaffolds include the tracrRNA segment and the portion of the crRNA segment other than the polynucleotide-targeting guide sequence at or near the 5' end of the crRNA segment, excluding any unnatural portions comprising sequences not conserved in native crRNAs and tracrRNAs. In some embodiments, the gRNA comprises a CRISPR RNA (crRNA):trans activating crRNA (tracrRNA) duplex. In some embodiments, the gRNA comprises a stem-loop that mimics the natural duplex between the crRNA and tracrRNA. In some embodiments, the stem-loop comprises a nucleotide sequence comprising non-naturally occurring sequence. For example, in

some embodiments, the composition comprises a synthetic or chimeric guide RNA comprising a crRNA, stem, and tracrRNA.

[175] Generally, a protospacer adjacent motif (PAM) is also an important sequence element mediating enzymatic activity of a Cas nuclease. A PAM sequence or element also refers to and includes an approximately 2-6 base pair DNA sequence that is an important targeting component of a Cas nuclease. The PAM sequence further comprises, in certain instances, a DNA sequence that may be required for a Cas/sgRNA to form an R-loop to interrogate a specific DNA sequence through Watson-Crick pairing of its guide RNA with the genome. In certain instances, the PAM specificity can be a function of the DNA-binding specificity of the Cas protein (e.g., a PAM recognition domain of a Cas), wherein, a protospacer adjacent motif recognition domain refers to a Cas amino acid sequence that comprises a binding site to a DNA target PAM sequence.

[176] Typically, the PAM sequence is on either strand, and is downstream in the 5' to 3' direction of Cas9 cut site. The canonical PAM sequence (i.e., the PAM sequence that is associated with the Cas9 nuclease of *Streptococcus pyogenes* or SpCas9) is 5'-NGG-3' (SEQ ID NO: 760) wherein "N" is any nucleobase followed by two guanine ("G") nucleobases. Different PAM sequences can be associated with different Cas9 nucleases or equivalent proteins from different organisms. In addition, any given Cas9 nuclease, e.g., SpCas9, may be modified to alter the PAM specificity of the nuclease such that the nuclease recognizes alternative PAM sequence. In the CRISPR-Cas system derived from *S. pyogenes* (spCas9), the protospacer region DNA typically immediately precedes a 5'-NGG (SEQ ID NO: 761) or NAG (SEQ ID NO: 762) proto-spacer adjacent motif (PAM). Other Cas9 orthologs can have different PAM specificities. For example, Cas9 from *S. thermophilus* (stCas9) requires 5'-NNAGAA (SEQ ID NO: 763) for CRISPR 1 and 5'-NGGNG (SEQ ID NO: 764) for CRISPR3 and *Neisseria meningitidis* (nmCas9) requires 5'-NNNNGATT (SEQ ID NO: 765). Cas9 from *Staphylococcus aureus* subsp. *aureus* (saCas9) requires 5'-NNGRRT (SEQ ID NO: 766) (R=A or G). In some embodiments, Cas9 enzymes from different bacterial species (i.e., Cas9 orthologs) can have varying PAM specificities. For example, Cas9 from *Staphylococcus aureus* (SaCas9) recognizes NGRRT (SEQ ID NO: 767) or NGRRN (SEQ ID NO: 768). In addition, Cas9 from *Neisseria meningitidis* (NmCas) recognizes NNNNGATT (SEQ ID NO: 769). In another example, Cas9 from *Streptococcus thermophilus* (StCas9) recognizes NNAGAAW (SEQ ID NO: 770). In still another example, Cas9 from *Treponema denticola* (TdCas) recognizes NAAAAC (SEQ ID NO: 771). These are example are not meant to be limiting. It will be further appreciated that non-SpCas9s bind a variety of PAM sequences, which makes them useful when no suitable SpCas9 PAM

sequence is present at the desired target cut site. Furthermore, non-SpCas9s can have other characteristics that make them more useful than SpCas9.

[177] In some embodiments, the gRNA spacer sequence comprises about 15 nucleotides to about 28 nucleotides. In some embodiments, the gRNA comprises at least about 15 nucleotides. In some embodiments, the gRNA spacer sequence comprises at most about 28 nucleotides. In some embodiments, the gRNA spacer sequence comprises about 15 nucleotides to about 16 nucleotides, about 15 nucleotides to about 17 nucleotides, about 15 nucleotides to about 18 nucleotides, about 15 nucleotides to about 19 nucleotides, about 15 nucleotides to about 20 nucleotides, about 15 nucleotides to about 21 nucleotides, about 15 nucleotides to about 22 nucleotides, about 15 nucleotides to about 23 nucleotides, about 15 nucleotides to about 24 nucleotides, about 15 nucleotides to about 25 nucleotides, about 15 nucleotides to about 28 nucleotides, about 16 nucleotides to about 17 nucleotides, about 16 nucleotides to about 18 nucleotides, about 16 nucleotides to about 19 nucleotides, about 16 nucleotides to about 20 nucleotides, about 16 nucleotides to about 21 nucleotides, about 16 nucleotides to about 22 nucleotides, about 16 nucleotides to about 23 nucleotides, about 16 nucleotides to about 24 nucleotides, about 16 nucleotides to about 25 nucleotides, about 16 nucleotides to about 28 nucleotides, about 17 nucleotides to about 18 nucleotides, about 17 nucleotides to about 19 nucleotides, about 17 nucleotides to about 20 nucleotides, about 17 nucleotides to about 21 nucleotides, about 17 nucleotides to about 22 nucleotides, about 17 nucleotides to about 23 nucleotides, about 17 nucleotides to about 24 nucleotides, about 17 nucleotides to about 25 nucleotides, about 17 nucleotides to about 28 nucleotides, about 18 nucleotides to about 19 nucleotides, about 18 nucleotides to about 20 nucleotides, about 18 nucleotides to about 21 nucleotides, about 18 nucleotides to about 22 nucleotides, about 18 nucleotides to about 23 nucleotides, about 18 nucleotides to about 24 nucleotides, about 18 nucleotides to about 25 nucleotides, about 18 nucleotides to about 28 nucleotides, about 19 nucleotides to about 20 nucleotides, about 19 nucleotides to about 21 nucleotides, about 19 nucleotides to about 22 nucleotides, about 19 nucleotides to about 23 nucleotides, about 19 nucleotides to about 24 nucleotides, about 19 nucleotides to about 25 nucleotides, about 19 nucleotides to about 28 nucleotides, about 20 nucleotides to about 21 nucleotides, about 20 nucleotides to about 22 nucleotides, about 20 nucleotides to about 23 nucleotides, about 20 nucleotides to about 24 nucleotides, about 20 nucleotides to about 25 nucleotides, about 20 nucleotides to about 28 nucleotides, about 21 nucleotides to about 22 nucleotides, about 21 nucleotides to about 23 nucleotides, about 21 nucleotides to about 24 nucleotides, about 21 nucleotides to about 25 nucleotides, about 21 nucleotides to about 28 nucleotides, about 22 nucleotides to about 23

nucleotides, about 22 nucleotides to about 24 nucleotides, about 22 nucleotides to about 25 nucleotides, about 22 nucleotides to about 28 nucleotides, about 23 nucleotides to about 24 nucleotides, about 23 nucleotides to about 25 nucleotides, about 23 nucleotides to about 28 nucleotides, about 24 nucleotides to about 25 nucleotides, about 24 nucleotides to about 28 nucleotides, or about 25 nucleotides to about 28 nucleotides. In some embodiments, the gRNA spacer sequence comprises about 15 nucleotides, about 16 nucleotides, about 17 nucleotides, about 18 nucleotides, about 19 nucleotides, about 20 nucleotides, about 21 nucleotides, about 22 nucleotides, about 23 nucleotides, about 24 nucleotides, about 25 nucleotides, or about 28 nucleotides.

[178] In some embodiments, the guide RNA comprises different nucleobases for stability including, but not limited to, a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-halo adenine; an 8-amino adenine; an 8-thiol adenine; an 8-thioalkyl adenine; an 8-hydroxyl adenine; an 8-halo guanine; an 8-amino guanine; an 8-thiol guanine; an 8-thioalkyl guanine; an 8-hydroxyl guanine; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine. Other versions of guide RNA may include other nucleic acids such as bridged nucleic acids or locked nucleic acids.

[179] The guide RNAs described herein can further comprise one or more of a non-natural internucleoside linkage, a nucleic acid mimetic, a modified sugar moiety, and a modified nucleobase. In certain embodiments, the non-natural internucleoside linkage comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a

selenophosphate, and a boranophosphate. In certain embodiments, the nucleic acid mimetic comprises one or more of a peptide nucleic acid (PNA), morpholino nucleic acid, cyclohexenyl nucleic acid (CeNAs), or a locked nucleic acid (LNA). IN certain embodiments, the modified sugar moiety comprises one or more of 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, and 2'-fluoro. In certain embodiments the modified nucleobase comprises one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-halouracil; a 5-halocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-halo; an 8-amino; an 8-thiol; an 8-thioalkyl; an 8-hydroxyl; a 5-halo; a 5-bromo; a 5-trifluoromethyl; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deaza-adenine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine. Exemplary guide RNAs of the present disclosure can be found in **Table 4**.

Production of Chimeric Nucleases

[180] Chimeric nucleases described herein may be produced in many ways including using an *E. coli* expression system as described in WO2020225719A1. Alternatively, the chimeric nucleases may be produced by the target cell to be modified by supplying one or more genetic vectors that directs expression and production of the nucleases in the target cell. Additionally, the vector may provide sequences to direct expression of guide RNAs to target the chimeric nuclease to particular genomic region.

[181] An exemplary method for producing a genetically engineered cell as described herein is described below.

[182] A population of cells is grown in a T flask to 70 – 90% confluency. The cells are harvested by centrifugation and resuspended to 1.0×10^7 cells per milliliter (practical range $0.2 - 2 \times 10^7$ cells per milliliter) in Buffer T (Invitrogen, Carlsbad, California, US). Cells are electroporated with a chimeric nuclease described herein including those selected from SEQ ID

NOs: 750-756 and formulated in a Tris(hydroxymethyl)aminomethane or phosphate buffered saline with a Neon Transfection System (Thermo Fisher Scientific, Waltham, Massachusetts, US) at 2000 volts (practical range 1100 – 2500), 20 milliseconds (practical range 10 – 30 milliseconds) and 1 pulse (practical range 1 – 4). Cells are recovered in RPMI 1640 with 0.3 g/L glutamine and 2 g/L glucose (Sigma-Aldrich, Irvine, UK), 10% fetal bovine serum (Sigma-Aldrich, Oakville, Ontario, CA), 2mM L-glutamine, and 100 units penicillin and 0.1 mg streptomycin/mL (Sigma-Aldrich, St. Louis, Missouri, US) for 24 hours. Dead cells are removed using a Dead Cell Removal Kit (Miltenyi Biotec, Somerville, Massachusetts, US). Knockout efficiency is measured by amplifying the target genes by polymerase chain reaction and measuring the proportion of cells edited by targeted amplicon sequencing (GENEWIZ, South Plainfield, NJ, US). Amplicon sequencing is a method of targeted next generation sequencing that enables you to analyze genetic variation in specific genomic regions. This method uses PCR to create sequences of DNA called amplicons. Amplicons from different samples can be multiplexed, also called indexed or pooled, which involves adding a barcode (index) to samples so they can be identified. Before multiplexing, individual samples used for amplicon sequencing must be transformed into libraries by adding adapters and enriching target regions via PCR amplification. The adapters allows formation of indexed amplicons and allow the amplicons to adhere to the flow cell for sequencing. Amplicon sequencing is typically used for variant detection in a population of cells.

[183] Other methods to deliver the nuclease to the cell may be used, such as a lipid nanoparticle, polymer, viral vector or cell penetrating peptides. The chimeric nuclease or guide RNA may be delivered separately or in combination as DNA or RNA in either single-stranded or double-stranded form. Further, the chimeric nuclease may be delivered as RNA containing one or more of the following elements: a 5' cap, a 5' untranslated region, a coding sequence, a 3' untranslated region and a poly adenine (poly-A) tail. The RNA might include different nucleobases for stability including, but not limited to, a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-

methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

[184] In another embodiment, the chimeric nuclease may be delivered as an integrating vector including, but not limited to retrovirus vectors, lentivirus vectors, transposon vectors, and adeno-associated virus vectors. The chimeric nuclease may also be delivered by other electroporation systems, including but not limited to a Nucleofector™ (Lonza, Basel, Switzerland), MaxCyte (Gaithersburg, MD) or CliniMACS® (Bergisch Gladbach, Germany).

[185] The chimeric nucleases may further be included in a pharmaceutical composition comprising one or more of a pharmaceutically acceptable carrier, diluent, or excipient. The term “pharmaceutically acceptable excipient,” as used herein, refers to carriers and vehicles that are compatible with the active ingredient (for example, a compound of the invention) of a pharmaceutical composition of the invention (and preferably capable of stabilizing it) and not deleterious to the individual to be treated. For example, solubilizing agents that form specific, more soluble complexes with the compounds of the invention can be utilized as pharmaceutical excipients for delivery of the compounds. Suitable carriers and vehicles are known to those of extraordinary skill in the art. The term “excipient” as used herein will encompass all such carriers, adjuvants, diluents, solvents, or other inactive additives. Pharmaceutical formulations may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, glycerol, tetrahydrofuryl alcohol, and fatty acid esters of sorbitan, cyclodextrins, albumin, hyaluronic acid, chitosan and mixtures thereof. Polyethylene glycol (PEG) may be used to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. Representative examples of stabilizing components include polysorbate 80, L-arginine, polyvinylpyrrolidone, trehalose, and combinations thereof. Other excipients that may be employed, such as solution binders or anti-oxidants include, but are not limited to, butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben,

microcrystalline cellulose, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E (alpha-tocopherol), vitamin C and xylitol.

[186] The guide RNAs of the present disclosure hybridize to a target sequence. In some embodiments, the target sequence is an oncogenic mutation. In some embodiments, the oncogenic mutation is selected from any one of KRAS, PIK3CA, EGFR, Muc4, or a combination thereof.

Method of Targeting an Oncogenic Mutation

[187] In some embodiments, provided herein are methods for targeting an oncogenic mutation in a cell, wherein the method comprises contacting the chimeric nuclease of the present disclosure to the cell. In some embodiments, provided herein are methods for targeting an oncogenic mutation. In some embodiments, the method comprises administering the chimeric nuclease composition of the present disclosure to an individual with a disease or disorder, thereby treating the disease or disorder. In some embodiments, the disease or disorder is cancer.

[188] In some embodiments, the method comprises administering the chimeric nuclease composition of the present disclosure to an individual with cancer, thereby treating the cancer. The method comprising administering to an individual a therapeutically effective amount of a chimeric nuclease composition, or a pharmaceutical composition comprising a chimeric nuclease composition as described herein. In some embodiments, administration of the chimeric nuclease composition results in incorporation of one or more intended nucleotide edits in the target gene in the individual or cell. In some embodiments, administration of the chimeric nuclease results in correction of one or more oncogenic mutations, *e.g.*, point mutations, insertions, or deletions, associated with an oncogene in the individual or cell.

[189] The present disclosure provides a method of targeting an oncogenic mutation in a cell. The present disclosure also provides a method of targeting an oncogenic mutation in a individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual has cancer.

[190] The present disclosure also provide the use of the chimeric nuclease composition for targeting the oncogenic mutation in a cell. The present disclosure provides a method of editing a genome in a cell. The present disclosure also provides a method of editing a genome in an individual. The present disclosure also provide the use of the chimeric nuclease composition for targeting the oncogenic mutation in an individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has

cancer. In some embodiments, the individual is undergoing a treatment which may induce metastasis. In some embodiments, the treatment comprises surgery, radiation treatment and chemotherapy. In some embodiments, the individual is a human. In some embodiments, the cancer is a carcinoma or a sarcoma. In some embodiments, the carcinoma comprises breast cancer, lung cancer, colon cancer, or prostate cancer. In some embodiments, the sarcoma comprises an osteosarcoma or a soft tissue sarcoma. In some embodiments, the cancer is a glioblastoma.

[191] The present disclosure also provide the use of the chimeric nuclease composition for editing a genome in a cell. The present disclosure also provide the use of the chimeric nuclease composition for editing a genome in a individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[192] The present disclosure provides a method of deleting at least a portion of an oncogene in a cell. The present disclosure also provides a method of deleting at least a portion of an oncogene in a individual. In some embodiments. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[193] The present disclosure also provide the use of the chimeric nuclease composition for deleting at least a portion of an oncogene in a cell. The present disclosure also provide the use of the chimeric nuclease composition for deleting at least a portion of an oncogene in an individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[194] The present disclosure provides a method of silencing or disrupting at least a portion of an oncogene in a cell. The present disclosure also provides a method of silencing or disrupting at least a portion of an oncogene in a individual. In some embodiments. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[195] The present disclosure also provide the use of the chimeric nuclease composition for silencing or disrupting at least a portion of an oncogene in a cell. The present disclosure also provide the use of the chimeric nuclease composition for silencing or disrupting at least a portion of an oncogene in a individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[196] The present disclosure provides a method of replacing at least a portion of an oncogene in a cell. The present disclosure also provides a method of replacing at least a portion of an

oncogene in a individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[197] The present disclosure also provide the use of the chimeric nuclease composition for replacing at least a portion of an oncogene in a cell. The present disclosure also provide the use of the chimeric nuclease composition for replacing at least a portion of an oncogene in a individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[198] The present disclosure provides a method of restoring a non-oncogenic function in a cell. The present disclosure also provides a method restoring a non-oncogenic function in an individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[199] The present disclosure also provide the use of the chimeric nuclease composition for restoring a non-oncogenic function in a cell. The present disclosure also provide the use of the chimeric nuclease composition for restoring a non-oncogenic function in an individual. In some embodiments, the cell is a cell in an individual afflicted with cancer. In some embodiments, the individual is an individual who has cancer.

[200] Chimeric nucleases of this disclosure may be formulated for treating an individual (*e.g.*, a human) having a disorder associated with pathological angiogenesis (*e.g.*, cancer, such as breast cancer, ovarian cancer, renal cancer, colorectal cancer, liver cancer, gastric cancer, and lung cancer; obesity; macular degeneration; diabetic retinopathy; psoriasis; rheumatoid arthritis; cellular immunity; and rosacea).

[201] In some embodiments, the cancer treated with the chimeric nuclease is selected from any one of prostate cancer, liver cancer, colorectal cancer, ovarian cancer, endometrial cancer, breast cancer, triple negative breast cancer, pancreatic cancer, stomach (gastric) cancer, cervical cancer, head and neck cancer, thyroid cancer, testis cancer, urothelial cancer, lung cancer (small cell lung, non-small cell lung), sarcoma (soft tissue sarcoma and osteosarcoma), melanoma, non melanoma skin cancer (squamous and basal cell carcinoma), glioma, renal cancer, lymphoma (NHL or HL), Acute myeloid leukemia (AML), T cell Acute Lymphoblastic Leukemia (T-ALL), Diffuse Large B cell lymphoma, testicular germ cell tumors, mesothelioma, esophageal cancer, Merkel Cells cancer, MSI-high cancer, KRAS mutant tumors, adult T-cell leukemia/lymphoma, and Myelodysplastic syndromes (MDS). In some embodiments of the method, the cancer is selected from any one of cancer triple negative breast cancer, stomach (gastric) cancer, lung cancer (small cell lung, non-small cell lung), Merkel Cells cancer, MSI-

high cancer, KRAS mutant tumors, adult T-cell leukemia/lymphoma, Myelodysplastic syndromes (MDS), or a combination thereof. In some embodiments of the method, the cancer is selected from the group consisting of cancer triple negative breast cancer, stomach (gastric) cancer, lung cancer (small cell lung, non-small cell lung), Merkel Cells cancer, MSI-high cancer, or a combination thereof. In certain embodiments, the cancer includes a BRAF mutation (e.g., a BRAF V600E mutation), a BRAF wildtype, a KRAS wildtype or an activating KRAS mutation. The cancer may be at an early, intermediate or late stage.

[202] In some embodiments, the method provided herein comprises administering to an individual an effective amount of a chimeric nuclease composition. In some embodiments, the method comprises administering to the individual an effective amount of a chimeric nuclease composition described herein, for example, polynucleotides, vectors, or constructs that encode chimeric nuclease components, LNPs, and/or polypeptides comprising chimeric nuclease components. Chimeric nuclease compositions can be administered to target an oncogene in a individual. Identifying a individual in need of such treatment can be in the judgment of a individual or a health care professional and can be subjective (*e.g.* opinion) or objective (*e.g.* measurable by a test or diagnostic method).

[203] In some embodiments, the method comprises directly administering chimeric nuclease compositions provided herein to a individual. The chimeric nuclease compositions described herein can be delivered with in any form as described herein, *e.g.*, as LNPs, RNPs, polynucleotide vectors such as viral vectors, or mRNAs. The chimeric nuclease compositions can be formulated with any pharmaceutically acceptable carrier described herein or known in the art for administering directly to a individual. Components of a chimeric nuclease composition or a pharmaceutical composition thereof may be administered to the individual simultaneously or sequentially. For example, in some embodiments, the method comprises administering a chimeric nuclease composition, or pharmaceutical composition thereof, comprising a chimeric nuclease to a individual. In some embodiments, the method comprises administering a polynucleotide or vector encoding a chimeric nuclease to a individual with a donor nucleic acid.

[204] Suitable routes of administering the chimeric nuclease to an individual 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 compositions described are administered intraperitoneally, intravenously, or by direct injection or direct infusion. In some embodiments, the compositions described herein

are administered to a individual by injection, by means of a catheter, by means of a suppository, or by means of an implant.

[205] In some embodiments, the method comprises administering cells edited with a chimeric nuclease composition described herein to an individual.

[206] The specific dose administered can be a uniform dose for each individual. Alternatively, a individual's dose can be tailored to the approximate body weight of the individual. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, the severity of the disease, the route of administration, and the age, sex and medical condition of the patient.

[207] In embodiments wherein components of a chimeric nuclease composition are administered sequentially, the time between sequential administration can be at least 1 hour, at least 2 hours, at least 6 hours, at least 12 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 3 days, at least 4 days, at least 5 days, at least 7 days, at least 10 days, or at least 14 days.

[208] In some embodiments, a method of monitoring treatment progress is provided. In some embodiments, the method includes the step of determining a level of diagnostic marker, for example, correction of a mutation in proto-oncogene.

EXAMPLES

Example 1: Generation of disease-causing mutation datasets

[209] Provided herein, is a study utilizing a method to systematically scan a large dataset of mutant genes to identify putative allele-specific targets of chimeric nucleases using a computer algorithm is described. To increase the accuracy of Cas9 endonucleases, computer algorithms were developed to optimize guide RNA design and to predict potential target sites for nucleases have incorporated machine learning models to determine thermodynamic properties of target sites to better predict editing results. None of these algorithms identified allele-specific targets of chimeric nucleases.

[210] The present disclosure provides a method to systematically identify allele-specific targets by first generating a curated dataset of mutant and non-mutant alleles from databases of disease-causing mutations, for example the NCBI database, cancer genome atlas database. Here, the genomic location of the disease-causing mutations was pulled from the dataset using a custom Python script and was used as a query for the GRCh38.p13 human genome. The 50 nucleotides surrounding the mutation were called and printed into a table format and the location of the mutation annotated in the sequence. The result was a pair of sequences – one with the

wild type sequence and a second with the mutation annotated. The process was repeated for the subsequent mutations in the dataset.

Identification of allele-specific targets using a predictive algorithm

[211] The method was used computationally to predict allele-specific chimeric nuclease sites. Specifically, and as illustrated in **FIG. 2A**, a custom coded Python script was created that uses the pairwise mutant and wild type sequences generated as described above as an input. A user selects from a set of chimeric nuclease parameters that have associated position-weighted matrices for the binding and cleavage preference of the I-TevI site, linker and Cas9 site. The algorithm then inputs a pair of wild type and mutant sequences from the dataset and identifies and scores chimeric nuclease sites. The sites found in the mutant sequence and compared to the wild type sequence and those sites that are exclusive to the mutant sequence, or are allele-specific, are printed to a line in a table. The script then proceeds to the next set of wild type and mutant sequences in the dataset until all paired sequences have been compared. The output consists of a table of predicted chimeric nuclease sites in which the nuclease targets a mutant sequence but not the wild type.

[212] The development of the predictive algorithm allows for the systematic identification of allele-specific chimeric nuclease targets for potential therapeutic gene editing and replaces a previously manual process. In this example, 682 potential TevSaCas9 of SEQ ID Nos: 1- 682 have been identified from the top 1000 most frequent cancer-causing mutations in the **Genomic Data Commons (GDC) Data Portal** where a single nucleotide polymorphism, a deletion or an insertion are the oncogenic mutation. Each of these mutants may be targets for therapeutic gene editing as a treatment for cancer. On such insertion mutant was computationally predicted to contain allele-specific chimeric nuclease sites in a large in-frame insertion mutation of the mucin-4 (*Muc4*) gene (**FIG. 2B** and **FIG. 2C**).

[213] For the generation of **FIG. 2B**, a pair of wild type (WT) and mutant (MUT) sequences that includes 50 bases upstream and downstream of the mutation site are called from the GRCh38.p13 human genome sequence are input into the algorithm along with selected chimeric nuclease parameters, e.g., preferred I-TevI domain, linker and Cas9 domain. The sequence preferences consist of a position-weighted matrix of the known binding and cleavage preferences of each domain relative to the native preference from in vitro data. The algorithm will identify chimeric nuclease sites that are present in the mutant sequence but not in the wild type sequence and each site found is output to a line in a table with the position-weighted matrix

score. Once all sites are identified for the given pair of sequences, the algorithm will cycle to the next pair of mutant and wild type sequences until all pairs in the input dataset are analyzed.

Example 2: Activity of chimeric nucleases to selectively target an insertion oncogene

[214] In this study, a chimeric nuclease was designed to target a large in-frame oncogenic insertion in the mucin-4 (*Muc4*) gene termed or the chr3:g.195781031_195781032insACCGGTGGATGCCGAGGAAGCGTCGGTGACAGGAAGAGGGGTGGTGTACCTGTGGATACTGAGGAAAAGCTGGTGACAGGAAGAGGGGTGCGGTGACCTGTGGATACTGAGGAAGTGTCTGGTGACAGGAAGAGTCGTGGTGTG (SEQ ID NO: 780) mutation. Mucin-4 is implicated in a variety of cancers and in particular colon cancer. Selective disruption of an insertion mutation in *Muc4* using a chimeric nuclease to generate an out-of-frame deletion could provide a therapeutic benefit to patients. As illustrated in **FIG. 3A** the chimeric nuclease was targeted to *Muc4* using the guide RNA of SEQ ID NO: 1685 which targets the TevSaCas9 chimeric nuclease to the insertion mutation but does not target wild type *Muc4*. The TevSaCas9 chimeric nuclease was purified from *Escherichia coli* and complexed with the guide RNA of SEQ ID NO: 1685. The complex was mixed with DNA substrate in an *in vitro* cleavage assay that contains the *Muc4* insertion mutation in one reaction and wild type *Muc4* sequence in a second reaction. **FIG. 3B** shows the results of an *in vitro* cleavage assay as visualized using agarose gel electrophoresis. It demonstrates TevSaCas9 that is programmed to *Muc4* insertion preferentially cuts the oncogenic mutation sequence (presence of cleaved products), but not the wild type *Muc4* sequence (no cleaved products).

Example 3: Activity of chimeric nucleases to selectively eliminate cancer cells

[215] In another aspect of the invention, a chimeric nuclease is designed to target the *Egfr* L858R oncogenic activating mutation to selectively eliminate cancer cells. The *Egfr* L858R mutation is known to cause tumorigenesis and malignancy in non-small cell lung carcinoma (NSCLC). Targeting and eliminating the activating mutation could provide a therapeutic benefit for treating patients with cancer. As illustrated in **FIG. 4A**, the chimeric nuclease is targeted to the *Egfr* L858R mutation using the guide RNA of SEQ ID NO: 1686. The *Egfr* L858R mutation is located in the guide RNA binding site in the *Egfr* gene. Since precise binding of the guide RNA is required for TevSaCas9 activity, the mutant *Egfr* L858R gene can be discriminated from the wild type *Egfr* gene using the described nuclease. The TevSaCas9 chimeric nuclease is purified from *Escherichia coli* and complexed with the guide RNA of SEQ ID NO: 1686. The complex is mixed with DNA substrate in an *in vitro* cleavage assay amplified from cells

containing the *Egfr* L858R mutation and in a separate reaction from cells that are wild type for *Egfr*. FIG. 4B shows the results of the *in vitro* cleavage assay over time as visualized using agarose gel electrophoresis. TevSaCas9 that is programmed to *Egfr* L858R preferentially cuts the *Egfr* L858R mutation over the wild type *Egfr* substrate. The described chimeric nuclease selectively eliminates cancer cells. CRL-5908 cells (American Type Culture Collection, Manassas, Virginia, United States of America) are an immortalized lung epithelial cell line that contains the *Egfr* L858R activating mutation. A control lung epithelial cell line NuLi-1 is wild type for the *Egfr* L858 amino acid. FIG. 4C demonstrates that CRL-5908 cells lipofected with TevSaCas9 targeted to the *Egfr* L858R mutation (“TevSaCas9-EGFRL858R”) with guide RNA of SEQ ID NO: 1686 have reduced viability of as measured by CellTiter-Blue viability dye (Promega, Madison, Wisconsin, United State of America) compared to mock treated cells. NuLi-1 cell viability was not reduced by the same TevSaCas9-EGFRL858R complex nor was NuLi-1 cells or CRL-5908 cells treated with TevSaCas9 without guide RNA (“TevSaCas9”). Together, these data demonstrate allele-specific reduction in cell viability in human cells containing the *Egfr* L858R activating mutation by a chimeric nuclease targeted specifically to the mutation.

Site identification

[216] Other embodiments of the method to identify sites include using a coding language other than Python to encode the algorithm to identify allele-specific targets. In a further embodiment, the chimeric nuclease parameters may include the binding and cleavage preference of orthologs of the I-TevI and/or the Cas9 domain.

[217] Table 4 shows the output from the predictive algorithm of a set of putative TevSaCas9 sites in oncogenic mutations where nucleotide deletions are the driver mutation. Any of the sequences disclosed as a target site in Table 4 (SEQ ID NO: 1 to 683) can be targeted using the chimeric nucleases and methods described herein. Exemplary guide strands that can be used to target those target sites are also disclosed (SEQ ID NO: 1001 to 1686).

[218] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that

methods and structures within the scope of these claims and their equivalents be covered thereby.

Table 4: Sample output of Oncogenic mutations for use with the TevCas

Genome Location & Mutation	Target Site	SEQ ID NO:	Guide RNA sequence	SEQ ID NO:
Single Nucleotide Polymorphisms				
18:51065549G>A+	CTGTGGACATTGGAGAGTTGA CCCAAACAAAAGtGATCTCCTC CAGAAG	1	CCAAA CAAAA GtGATC TCCTC	1001
3:179199088G>A+	CAAAGTtGTCTTGTTCATCAA AAAATTCTTCCTTTCTGCTTCT TGAGT	2	AAATTC TTCCT TTCTGC TTC	1002
17:7674950A>C -	CGATGCTGAGGAGGGGCCAGA CCTAAGAGCAATCAGTGAGGA ATCAGAGG	3	TAAGA GCAAT CAGTG AGGAA T	1003
10:46131074C>T+	CAGCGGTGGCCCaCAGCAGCTG CTGGCCCAGGGACAGCTCAGT GCAGGGT	4	TGGCCC AGGGA CAGCTC AGTG	1004
3:179218294G>A+	CAAAGCAATTTCTACACGAGA TCCTCTCTCTAAAATCACTGAG CAGGAG	5	CCTCTC TCTAAA ATCACT GAG	1005
3:179218307A>G+	CAAAGCAATTTCTACACGAGA TCCTCTCTCTGAAATCACTGAG CGGGAG	6	CCTCTC TCTGAA ATCACT GAG	1006
3:179218303G>A+	CAAAGCAATTTCTACACGAGA TCCTCTCTCTGAAATCACTAAG CAGGAG	7	CCTCTC TCTGAA ATCACT AAG	1007
3:179218304A>C+	CAAAGCAATTTCTACACGAGA TCCTCTCTCTGAAATCACTGCG CAGGAG	8	CCTCTC TCTGAA ATCACT GCG	1008
17:4539347A>T-	CATGGTTTAATAAAAAAAAAA AAAAATAGGCGTCTCAGGCAG ATGGAGG	9	AAAAT AGGCG TCTCAG GCAGA	1009

3:195783009C> T-	CGTCGGTGACAGGAAGAGAGG TGGCGTGACCTGTGGATACTGA GGAAG	10	TGGCGT GACCT GTGGA TACTG	1010
3:195783008A> G-	CGTCGGTGACAGGAAGAGAGG TGGCGTGACCTGTGGGCACTG AGGAAG	11	TGGCGT GACCT GTGGG CACTG	1011
19:52212729C> T+	CTTGGCAAACCTCCCCAGCTTG GAGGCTGCGGCCCaCCGCACCA TGGGG	12	GAGGC TGCGG CCCaCC GCACC	1012
17:4539347A>T-	CATGGTTTAATAAAAAAAAAA AAAAATAGGCGTCTCAGGCAG ATGGAG	13	AAAAA TAGGC GTCTCA GGCAG	1013
17:7674947A>G -	CTCGGGTAAGATGCTGAGGAG GGGCCAGACCTAAGAGCAATC AGTGAGG	14	GGCCA GACCT AAGAG CAATC A	1014
17:7673704G>A -	CCAGGGAGCACTAAGtGAGGTA AGCAAGCAGGACAAGAAGCGG TGGAGG	15	AGCAA GCAGG ACAAG AAGCG G	1015
19:52212729C> T+	CTTGGCAAACCTCCCCAGCTTG GAGGCTGCGGCCCaCCGCACCA TGGGGG	16	AGGCT GCGGC CCaCCG CACCA	1016
7:56106490G>A -	CACCGAAGAGCTAAGCGACTT CTGAGGAGACCGGAAAATGGG AGGCGGGG	17	GAGGA GACCG GAAAA TGGGA G	1017
12:56085070G> A+	CCTGGGTCCCTCGCACCAAtGCG GAGGTTGGGCAATGGTAGAGT AGAGAAT	18	AGGTT GGGCA ATGGT AGAGT A	1018
10:121520163G >C-	CATCGCTCTGGTGGAGAGAGG GAAGAAAGGAGGAGTGGGGAT GGGAGAAT	19	AGAAA GGAGG AGTGG GGATG G	1019
10:46131074C> T+	CTGAGCTGTCCCTGGGCCAGCA GCTGCTGTGGGCCACCGCTGAT GAGG	20	AGCTG CTGTGG GCCAC CGCTG	1020

17:31350209C> T+	CACCGTTTTCCTTTTAGCTTTAC TTACAGTGTCTGAAGAAGTTTG AAG	21	ACTTAC AGTGTC TGAAG AAGT	1021
10:121520163G >C-	CATCGCTCTGGTGGAGAGAGG GAAGAAAGGAGGAGTGGGGAT GGGAG	22	GGAAG AAAGG AGGAG TGGGG A	1022
17:7674229C>A -	CATGGGCGtCATGAACCGGAGG CCCATCCTCACCATCATCACAC TGGAAG	23	CCATCC TCACCA TCATCA CAC	1023
6:26217357A>T +	CATGGGCAAGTGAAATGATTA CTAGTCAAATCCGTCAGTGATC CCGAGT	24	TAGTCA AATCC GTCAGT GATC	1024
19:49665874C> T+	CTGGGCTGTTCCCGCCCCTATG CCCTTTTTTGGGTTTTTCGGCCA GAGG	25	GCCCTT TTTTGG GTTTTC GGC	1025
17:4539348A>T-	CATGGTTTAAATAAAAAAAAAA AAAAATAGGCGTCTCAGGCAG ATGGAGG	26	AAAAT AGGCG TCTCAG GCAGA	1026
17:7674229C>T-	CATGGGCGaCATGAACCGGAG GCCCATCCTCACCATCATCACA CTGGAAG	27	CCATCC TCACCA TCATCA CAC	1027
17:7673704G>A -	CCAGGGAGCACTAAGtGAGGTA AGCAAGCAGGACAAGAAGCGG TGGAG	28	AAGCA AGCAG GACAA GAAGC G	1028
3:179199690G> A+	CACTGGCATGCCGATAGCAAA AtCTTTAATAAAATTACATAAA GAAT	29	AAtCTT TAATA AAATT ACATA	1029
3:41224633A>G +	CTCTGGAATCCATTCTGGTGCC ACTGCCACAGCTCCTTCTCTGA GT	30	GCCACT GCCAC AGCTCC TTCT	1030
3:41224622C>T +	CTCTGGAATCCATTTTGGTGCC ACTACCACAGCTCCTTCTCTGA GT	31	GCCACT ACCAC AGCTCC TTCT	1031
17:4539348A>T-	CATGGTTTAAATAAAAAAAAAA AAAAATAGGCGTCTCAGGCAG ATGGAG	32	AAAAA TAGGC GTCTCA GGCAG	1032

3:179218304A> C+	CGCAGGAGAAAGATTTTCTAT GGAGTCACAGGTAAGTGCTAA AATGGAG	33	GAGTC ACAGG TAAGT GCTAA A	1033
3:41224645T>C +	CTCTGGAATCCATTCTGGTGCC ACTACCACAGCTCCTCCTCTGA GT	34	GCCACT ACCAC AGCTCC TCCT	1034
17:7675190C>T-	CAGGGTAGGTCTTGGCCAGTTG GCAAAACATCTTGTTGAGGGC AGGGGAG	35	CAAAA CATCTT GTTGA GGGCA	1035
17:7675190C>T-	CGCGGGTGCCGGGCGGGGGTG TGGAATCAACCCACAGCTGCA CAGGGT	36	TGGAA TCAACC CACAG CTGCA	1036
12:25225628C> T-	CAGTGTTACTTACCTGTCTTGT CTTTGTTGATGTTTCAATAAAA GGAAT	37	CTTTGT TGATGT TTCAAT AAA	1037
6:26217357A>T +	CCATGGGCAAGTGAAATGATT ACTAGTCAAATCCGTCAGTGAT CCCGAGT	38	TAGTCA AATCC GTCAGT GATC	1038
17:7674945G>A -	CTCAGATAAGATGCTGAGGAG GGGCCAGACCTAAGAGCAATC AGTGAGG	39	GGCCA GACCT AAGAG CAATC A	1039
17:7675190C>T-	CAGGGTAGGTCTTGGCCAGTTG GCAAAACATCTTGTTGAGGGC AGGGG	40	GGCAA AACAT CTTGTT GAGGG	1040
10:45827364T> G-	CACAGGTTTCCACCGTGCACAT TATGAAGAAcAGAAATGGAGG TGGGAG	41	TATGA AGAAcA GAAAT GGAGG	1041
12:25227342T> C-	CTTGGATATTCTCGACACAGCA GGTCgAGAGGAGTACAGTGCA ATGAGG	42	GGTCgA GAGGA GTACA GTGCA	1042
17:7673704G>A -	CACCGCTTCTTGTCCTGCTTGC TTACCTCACTTAGTGCTCCCTG GGGG	43	CTTACC TCACTT AGTGCT CCC	1043
17:7674216C>A -	CATGGGCGGCATGAACCGGAGt CCCATCCTCACCATCATCACAC TGGAAG	44	CCATCC TCACCA TCATCA CAC	1044

7:55191822T>G +	CACCGCAGCATGTCAAGATCA CAGATTTTGGGCGGGCCAAAC TGCTGGGT	45	GATTTT GGGCG GGCCA AACTG	1045
17:39711955C> T+	CGAGGGTGCAGaATCCCACGTC CGTAGAAAGGTAGTTGTCTAA GGAG	46	TCCGTA GAAAG GTAGTT GTCT	1046
10:79514306C> T+	CTCTGCTTCTTTaCATCTCAGTT AGTCCCTTCTGAAATTCAGTGA GG	47	GTTAGT CCCTTC TGAAA TTCA	1047
3:195778903G> T-	CTGAGGAAAGGCTGGTGACAT GAAGAGGGGTGGCGTGACCTG TGGAT	48	TGAAG AGGGG TGGCGT GACCT	1048
17:7674220C>T-	CATGGGCGGCATGAACCgAGAG GCCCATCCTCACCATCATCACA CTGGAAG	49	CCATCC TCACCA TCATCA CAC	1049
17:7674221G>A -	CATGGGCGGCATGAACtGGAGG CCCATCCTCACCATCATCACAC TGGAAG	50	CCATCC TCACCA TCATCA CAC	1050
12:25227342T> A-	CTTGGATATTCTCGACACAGCA GGTCtAGAGGAGTACAGTGCAA TGAGG	51	GGTCtA GAGGA GTACA GTGCA	1051
12:25227341T> G-	CTTGGATATTCTCGACACAGCA GGTCaGAGGAGTACAGTGCA ATGAGG	52	GGTCaC GAGGA GTACA GTGCA	1052
10:45827364T> G-	CACAGGTTTCCACCGTGACAT TATGAAGAAcAGAAATGGAGG TGGGAGT	53	ATGAA GAAcAG AAATG GAGGT	1053
10:87957915C> T+	CAAAGTACATGAACTTGTCTTC CCGTCaTGTGGGTCCTGAATTG GAGG	54	CCCGTC aTGTGG GTCCTG AAT	1054
17:7673579G>A -	CCTAGCACTGCCCCAACAACAC CAGCTCCTCTCCcTAGCCAAAG AAG	55	ACCAG CTCCTC TCCcA GCCA	1055
17:7675994C>A -	CTCAGGGCAACTGACAGTGCA AGTCACAGACTTGGCTGTCCCA GAAT	56	AAGTC ACAGA CTTGGC TGTCC	1056
17:7673704G>A -	CACCGCTTCTTGTCTGCTTGC TTACCTCACTTAGTGCTCCCTG GGG	57	GCTTAC CTCACT	1057

			TAGTGC TCC	
17:7673767C>T-	CCTGGGAGAGACCGGCGCACA aAGGAAGAGAATCTCCGCAAG AAAGGGG	58	AGGAA GAGAA TCTCCG CAAGA	1058
17:7674953T>C-	CACTGATTGCTCTTAGGTCTGG CCCCTCCTCAGCgTCTTATCCG AGT	59	GGCCC CTCCTC AGCgTC TTAT	1059
17:7674950A>C -	CACTGATTGCTCTTAGGTCTGG CCCCTCCTCAGCATCgTATCCG AGT	60	GGCCC CTCCTC AGCAT CgTAT	1060
17:7673776G>A -	CCTGGGAGAGACtGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGG	61	AGGAA GAGAA TCTCCG CAAGA	1061
12:25227342T> C-	CACAGCAGGTCgAGAGGAGTA CAGTGCAATGAGGGACCAGTA CATGAGG	62	AGTGC AATGA GGGAC CAGTA C	1062
12:25227341T> G-	CACAGCAGGTCAcGAGGAGTA CAGTGCAATGAGGGACCAGTA CATGAGG	63	AGTGC AATGA GGGAC CAGTA C	1063
17:7675085C>A -	CCCAGCTGCTCACCATCGCTAT CTGAGCAGCGCTCATGGTGGG GGAAG	64	TCTGAG CAGCG CTCATG GTGG	1064
17:7673781C>T-	CTGGGAaAGACCGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGGAG	65	GAAGA GAATCT CCGCA AGAAA	1065
17:7673781C>T-	CTGGGAaAGACCGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGG	66	AGGAA GAGAA TCTCCG CAAGA	1066
3:195780140G> A-	CTGAGGAAAAGCTGGTGACAG GAAGAGGGGTGGCCTGACCTG TGGAT	67	GGAAG AGGGG TGGCCT GACCT	1067
10:79514306C> T+	CTCAGTTTCCTCACTGAATTTC AGAAGGGACTAACTGAGATGT AAAGAAG	68	GAAGG GACTA ACTGA GATGT A	1068

17:7674947A>G -	CACTGATTGCTCTTAGGTCTGG CCCCTCCTCAGCATCTTA _c CCG AGT	69	GGCCC CTCCTC AGCAT CTTA _c	1069
12:16040G>A-	CCCAGGGAAGTGGTtGACCCCT CCGGTGGCTGGGCCACTCTGCT AGAGT	70	CCGGT GGCTG GGCCA CTCTGC	1070
17:7674957G>A -	CACTGATTGCTCTTAGGTCTGG CCCCTCCTtAGCATCTTATCCGA GT	71	GGCCC CTCCTt AGCAT CTTAT	1071
17:7673781C>G -	CTGGGA _c AGACCGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGG	72	AGGAA GAGAA TCTCCG CAAGA	1072
1:247738656A> C+	CACAGATGATGGAATTCTTGCT TGTGAGCTTTACTGAGAATTGG GT	73	GCTTGT GAGCTT TACTGA GAA	1073
17:7673764C>T-	CCTGGGAGAGACCGGCGCACA GAGaAAGAGAATCTCCGCAAG AAAGGGG	74	AGaAAG AGAAT CTCCGC AAGA	1074
17:7673781C>G -	CTGGGA _c AGACCGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGGAG	75	GAAGA GAATCT CCGCA AGAAA	1075
12:16040G>A-	CCAGGGAAGTGGTtGACCCCTC CGGTGGCTGGGCCACTCTGCTA GAGT	76	CCGGT GGCTG GGCCA CTCTGC	1076
12:25227342T> A-	CACAGCAGGTctAGAGGAGTAC AGTGCAATGAGGGACCAGTAC ATGAGG	77	AGTGC AATGA GGGAC CAGTA C	1077
17:7674230C>T-	CATGGGCaGCATGAACCGGAG GCCCATCCTCACCATCATCACA CTGGAAG	78	CCATCC TCACCA TCATCA CAC	1078
17:7674957G>A -	CTCGGATAAGATGCTAAGGAG GGGCCAGACCTAAGAGCAATC AGTGAGG	79	GGCCA GACCT AAGAG CAATC A	1079
10:87957915C> T+	CAAAGTACATGAACCTTGCTTTC CCGTCaTGTGGGTCCTGAATTG GAG	80	TCCCGT CaTGTG GGTCCT GAA	1080

17:7673781C>G -	CCTGGGA _c AGACCGGCGCACA GAGGAAGAGAATCTCCGCAAG AAAGGGG	81	AGGAA GAGAA TCTCCG CAAGA	1081
17:7675139C>T-	CTGTGACTGCTTGTAGATGGCC ATGGCGTGGACGCGGGTGCCG GGCGGGG	82	TGGCGT GGACG CGGGT GCCGG	1082
17:7674953T>C-	CTCGGATAAGACGCTGAGGAG GGGCCAGACCTAAGAGCAATC AGTGAGG	83	GGCCA GACCT AAGAG CAATC A	1083
3:195779688A> G-	CTGAGGAACGGCTGGTGACAG GAAGAGAGGTGGCGTGCCCTG TGGAT	84	GGAAG AGAGG TGGCGT GGCCT	1084
17:7675139C>A -	CTGTGACTGCTTGTAGATGGCC ATGGCGAGGACGCGGGTGCCG GGCGGGG	85	TGGCG AGGAC GCGGG TGCCG G	1085
4:152326214C> T-	CAGAGTTGTTAGCGGTTCTCaA GATGCCACTCTTAGGGTTTGGG AT	86	CaAGAT GCCACT CTTAGG GTT	1086
17:7675143C>A -	CTGTGACTGCTTGTAGATGGCC ATGGCGCGGAAGCGGGTGCCG GGCGGGG	87	TGGCG CGGAA GCGGG TGCCG G	1087
19:52212718C> G+	CTGCGGCCCCGCCGCACCATG _c G GGTGTCTATCTGAGCACAGGTTC CGGAAG	88	GTGTCA TCTGAG CACAG GTTC	1088
17:7673781C>T-	CCTGGGA _a AGACCGGCGCACA GAGGAAGAGAATCTCCGCAAG AAAGGGG	89	AGGAA GAGAA TCTCCG CAAGA	1089
10:94402611C> T+	CCGAGCAGGCTCCGGCATCCTC CGGACCCACCATCTaGGGGTGG GT	90	CTCCGG ACCCA CCATCT aGGG	1090
10:94402613G> A+	CCGAGCAGGCTCCGGCATCCTC CGGACCCACCATtTGGGGGTGG GT	91	CTCCGG ACCCA CCATtT GGGG	1091
3:195783009C> T-	CTGAGGAAGCGTCGGTGACAG GAAGAGAGGTGGCGTGACCTG TGGAT	92	GGAAG AGAGG TGGCGT GACCT	1092

10:79513976C> T+	CACTGGCTTCTCATTTCaTTAA ACTGTGAACTCCTTTAGGGTGG GG	93	TAAACT GTGAA CTCCTT TAGG	1093
19:52212729C> T+	CCTTGGCAAACCTCCCCAGCTT GGAGGCTGCGGCCCaCCGCACC ATGGGG	94	GAGGC TGCGG CCCaCC GCACC	1094
17:7670685G>A -	CTCAGAACATCTCGAAGCGCTC ACGCCACGGATCTGCAGCAA CAGAGG	95	ACGCC CACGG ATCTGC AGCAA	1095
17:7675124T>C-	CATGGCCATCTgCAAGCAGTCA CAGCACATGACGGAGGTTGTG AGG	96	TCACA GCACA TGACG GAGGT T	1096
17:7674947A>G -	CTCAGCATCTTAcCCGAGTGGA AGGAAATTTGCGTGTGGAGTA TTTGGAT	97	GGAAA TTTGC TGTGG AGTAT	1097
10:87957915C> T+	CTGAGGGAACCTCAAAGTACAT GAACTTGTCTTCCCGTCaTGTG GGT	98	ATGAA CTTGTC TTCCCG TCaT	1098
1:144436988C> A-	CTTGGAGGTCCTGCCCCCTGGGA CTTGTCCGGCTCATAACGGAGTG AGGAG	99	CTTGTC CGGCTC ATACG GAGT	1099
17:7675085C>T-	CCCAGCTGCTCACCATCGCTAT CTGAGCAGCGCTCATGGTGGG GGT	100	TATCTG AGCAG CGCTCA TGGT	1100
19:52212729C> T+	CTGCGGCCCaCCGCACCATGGG GGTGTCTCTGAGCACAGGTTC CGGAAG	101	GTGTCA TCTGAG CACAG GTTC	1101
17:7675124T>C-	CCATGGCCATCTgCAAGCAGTC ACAGCACATGACGGAGGTTGT GAGG	102	TCACA GCACA TGACG GAGGT T	1102
19:52212729C> T+	CTCAGATGACACCCCATGGTG CGGTGGGCCGCAGCCTCCAAG CTGGGG	103	CGGTG GGCCG CAGCCT CCAAG	1103
3:195780075T> G-	CTGAGGAAGGGATGGTGACAG GAAGAGGGGTGGCGTGACCGG TGGAT	104	GGAAG AGGGG TGGCGT GACCG	1104

3:41224645T>C +	CACAGCTCCTCCTCTGAGTGGT AAAGGCAATCCTGAGGAAGAG GAT	105	GGTAA AGGCA ATCCTG AGGAA	1105
17:7674950A>C -	CTCAGCATCgTATCCGAGTGGA AGGAAATTTGCGTGTGGAGTA TTTGGAT	106	GGAAA TTTGCG TGTGG AGTAT	1106
17:7674917T>C-	CTCAGCATCTTATCCGAGTGGA AGGAAATTTGCGTGTGGAGTgT TTGGAT	107	GGAAA TTTGCG TGTGG AGTgT	1107
17:7674950A>C -	CTCGGATACGATGCTGAGGAG GGGCCAGACCTAAGAGCAATC AGTGAGG	108	GGCCA GACCT AAGAG CAATC A	1108
10:94402613G> A+	CTTTGCCTCCTGGGGCGGCCGC CACCCACCCCAAATGGTGGG TCCGGAG	109	ACCCA CCCCA AATGG TGGGT	1109
17:7674953T>C-	CTCAGCgTCTTATCCGAGTGGA AGGAAATTTGCGTGTGGAGTA TTTGGAT	110	GGAAA TTTGCG TGTGG AGTAT	1110
10:94402611C> T+	CTTTGCCTCCTGGGGCGGCCGC CACCCACCCCTAGATGGTGGGT CCGGAG	111	ACCCA CCCCTA GATGG TGGGT	1111
2:113595432G> A+	CTGTGGCGGGGGCGTCTCTGCA GGCCAGGGTCCTGGGCGCtCGT GAAG	112	AGGCC AGGGT CCTGG GCGCtC	1112
17:7674945G>A -	CTCAGCATCTTATCtGAGTGGA AGGAAATTTGCGTGTGGAGTA TTTGGAT	113	GGAAA TTTGCG TGTGG AGTAT	1113
2:113595432G> A+	CTGGGCGCtCGTGAAGATGGAG CCATATTCCTGCAGGCGCTCTG GAG	114	AGCCA TATTCC TGCAG GCGCT	1114
17:7675139C>A -	CCGCGTCCtCGCCATGGCCATC TACAAGCAGTCACAGCACATG ACGGAG	115	TACAA GCAGT CACAG CACAT G	1115
19:3118944A>T +	CTGTGTCCTTTTCAGGATGGTGG ATGTGGGGGGCCTGCGGTCGG AGCGGAG	116	TGTGG GGGGC CTGCG GTCGG A	1116

19:52212718C> G+	CTCAGATGACACCCGCATGGT GCGGCGGGCCGCAGCCTCCAA GCTGGGG	117	CGGCG GGCCG CAGCCT CCAAG	1117
19:52212729C> T+	CTCAGATGACACCCCCATGGTG CGGTGGGCCGCAGCCTCCAAAG CTGGGGG	118	GGTGG GCCGC AGCCTC CAAGC	1118
12:56085070G> A+	CATTGCCCAACCTCCGCATGGT GCGAGGGACCCAGGTCTACGA TGGAAG	119	CGAGG GACCC AGGTCT ACGAT	1119
17:7675139C>T-	CCGCGTCCaCGCCATGGCCATC TACAAGCAGTCACAGCACATG ACGGAG	120	TACAA GCAGT CACAG CACAT G	1120
3:195782159T> C-	CTGAGGAAGTGCCGGTGACAG GAAGAGCGGTGGCCTGACCTG TGGAT	121	GGAAG AGCGG TGGCCT GACCT	1121
17:7675124T>C-	CTGTGACTGCTTGACAGATGGCC ATGGCGCGGACGCGGGTGCCG GGCGGGG	122	TGGCG CGGAC GCGGG TGCCG G	1122
12:16040G>A-	CAGGGAAGTGGTtGACCCCTCC GGTGGCTGGGCCACTCTGCTAG AGT	123	CCGGT GGCTG GGCCA CTCTGC	1123
10:47461391G> A-	CAGAGAAGCGGGCGCGCGGGG GCGGGCGCGtGGGGCCTTGCCG GAG	124	GGGCG GGCGC GtGGGG CCTTG	1124
3:195783009C> T-	CACTGAGGAAGCGTCGGTGAC AGGAAGAGAGGTGGCGTGACC TGTGGAT	125	GGAAG AGAGG TGGCGT GACCT	1125
9:21971121G>A -	CCAGGAAGCCCTCCCGGGCAG CGTCGTGCACGGGTCaGGTGAG AGT	126	AGCGT CGTGC ACGGG TCaGGT	1126
17:7675139C>A -	CCGCGTCCtCGCCATGGCCATC TACAAGCAGTCACAGCACATG ACGGAGG	127	ACAAG CAGTC ACAGC ACATG A	1127
1:144436988C> A-	CTTGAGGTCTGCCCCCTGGGA CTTGTCGGGCTCATAACGGAGTG AGG	128	GACTTG TCCGGC TCATAC GGA	1128

17:7675139C>T- +	CCGCGTCCaCGCCATGGCCATC TACAAGCAGTCACAGCACATG ACGGAGG	129	ACAAG CAGTC ACAGC ACATG A	1129
7:55191822T>G +	CACAGATTTTGGGCGGGCCAA ACTGCTGGGTGCGGAAGAGAA AGAAT	130	AACTG CTGGGT GCGGA AGAGA	1130
11:1097397C>T +	CGGTGGGTaTTGGGGTTGGGGT CACCGTAGTGGTGGTGGTGAT GGGT	131	GTCACC GTAGT GGTGG TGGTG	1131
19:52212718C> G+	CTCAGATGACACCCGCATGGT GCGGCGGGCCGCAGCCTCCAA GCTGGGGG	132	GGCGG GCCGC AGCCTC CAAGC	1132
1:144436988C> A-	CTTGGAGGTCCTGCCCCTGGGA CTTGTCGGGCTCATACGGAGTG AGGAGG	133	TTGTCC GGCTC ATACG GAGTG	1133
19:52212729C> T+	CGGTGGGCGCGCAGCCTCCAAG CTGGGGGAGTTTGCCAAGGTG CTGGAG	134	CTGGG GGAGT TTGCCA AGGTG	1134
6:73519043A>G -	CTGAGCCACCCTACAGCCAGA AGAGATATGAGGAAATcGTTA AGGAAG	135	AGAGA TATGA GGAAA TcGTTA	1135
10:45826548G> A-	CTGGGTCTCACAGTCCACACAG TGGGCATTCCCACGCATGTTTT GGAT	136	GTGGG CATTCC CACGC ATGTT	1136
10:46131074C> T+	CGGTGGCCCaCAGCAGCTGCTG GCCAGGGACAGCTCAGTGCA GGGT	137	TGGCCC AGGGA CAGCTC AGTG	1137
12:56085070G> A+	CTGGGTCCCTCGCACCAcGCGG AGGTTGGGCAATGGTAGAGTA GAGAAT	138	AGGTT GGGCA ATGGT AGAGT A	1138
17:7675143C>A -	CTCCGTCATGTGCTGTGACTGC TTGTAGATGGCCATGGCGCGG AAG	139	TGCTTG TAGAT GGCCA TGGCG	1139
2:113595432G> A+	CGGGGGCGTCTCTGCAGGCCA GGGTCCTGGGCGCtCGTGAAGA TGGAG	140	GGGTC CTGGG CGCtCG TGAAG	1140

7:55191822T>G +	CGCAGCATGTCAAGATCACAG ATTTTGGGCGGGCCAAACTGCT GGGT	141	GATTTT GGGCG GGCCA AACTG	1141
17:7675124T>C-	CCGCGTCCGCGCCATGGCCATC TgCAAGCAGTCACAGCACATGA CGGAG	142	TgCAAG CAGTC ACAGC ACATG	1142
19:49665874C> T+	CGCTGGGCTGTTCCCGCCCCTA TGCCCTTTTTTGGGTTTTTCGGC CAGAGG	143	GCCCTT TTTTGG GTTTTC GGC	1143
17:7673579G>A -	CTTTGGCTAGGGAGAGGAGCT GGTGTGTTGGGCAGTGCTAGG AAAGAGG	144	TGTTGT TGGGC AGTGCT AGGA	1144
12:17513A>C-	CAGCGGGTGTCAgCTTGCCTGA CCCCATGTGCGCTCTGTAGGT AGAAG	145	CCCCCA TGTCGC CTCTGT AGG	1145
3:185152807G> A-	CCGCGCGGAGGGAGAGGAAAT GAGTGCGGCGGCCTtGCCGGCG TCGGAG	146	AGTGC GGCGG CCTtGC CGGCG	1146
22:21772875C> T-	CCCTGAAGCAGCAGCCAGGAA CATGAGCTCTTACCTTGTCACT CGGGT	147	CATGA GCTCTT ACCTTG TCAC	1147
17:7673802C>A -	CTTTGAGGTGctTGTTTGTGCCT GTCCTGGGAGAGACCGGCGCA CAGAGG	148	GTCCTG GGAGA GACCG GCGCA	1148
17:7670685G>A -	CTTCGAGATGTTctGAGAGCTG AATGAGGCCTTGGAActCAAG GAT	149	CTGAAT GAGGC CTTGGA ACTC	1149
17:7675124T>C-	CCGCGTCCGCGCCATGGCCATC TgCAAGCAGTCACAGCACATGA CGGAGG	150	gCAAGC AGTCA CAGCA CATGA	1150
17:7675190C>T-	CACAGGGTAGGTCTTGGCCAG TTGGCAAAACATCTTGTTGAGG GCAGGGG	151	GGCAA AACAT CTTGTT GAGGG	1151
17:7673796C>T-	CTTTGAGGTGCGTGTTTaTGCCCT GTCCTGGGAGAGACCGGCGCA CAGAGG	152	GTCCTG GGAGA GACCG GCGCA	1152
7:55165350G>T +	CATTGACGGCCCCCACTGCGTC AAGACCTGCCCGGCAGTAGTC ATGGGAG	153	AGACC TGCCCG	1153

			GCAGT AGTCA	
17:7673781C>G -	CTTTGAGGTGCGTGTTTGTGCC TGCCTGGGAGAGACCGGCGCA CAGAGG	154	GTCCTG GGAG ACCGG CGCA	1154
17:7673802C>T-	CTTTGAGGTGCaTGTTTGTGCCT GTCCTGGGAGAGACCGGCGCA CAGAGG	155	GTCCTG GGAGA GACCG GCGCA	1155
17:7673776G>A -	CTTTGAGGTGCGTGTTTGTGCC TGCCTGGGAGAGACtGGCGCA CAGAGG	156	GTCCTG GGAGA GACtGG CGCA	1156
3:41224633A>G +	CACTGCCACAGCTCCTTCTCTG AGTGGTAAAGGCAATCCTGAG GAAGAGG	157	GTGGT AAAGG CAATCC TGAGG	1157
11:1097434C>T +	CAGTGGGTGTTGGGGTTGGGG TCACCGTGGTGGTGGTGgTaAT GGGT	158	GTCACC GTGGT GGTGG TGGTa	1158
11:1097377T>C +	CGGTGGGTGTTGGGGTTGGGG TCACCGTgGTGGTGGTGGTGAT GGGT	159	GTCACC GTgGTG GTGGT GGTG	1159
20:58909365C> T+	CCTGGAACCTTGGTCTCAAAGAT TCCAGAAAGTCAGGACACaGCA GCGAAG	160	TCCAG AAGTC AGGAC ACaGCA	1160
17:7673781C>T-	CTTTGAGGTGCGTGTTTGTGCC TGCCTGGGAaAGACCGGCGCA CAGAGG	161	GTCCTG GGAaAG ACCGG CGCA	1161
17:7673806C>T-	CTTTGAGaTGCGTGTTTGTGCCT GTCCTGGGAGAGACCGGCGCA CAGAGG	162	GTCCTG GGAGA GACCG GCGCA	1162
3:41224633A>G +	CACTGCCACAGCTCCTTCTCTG AGTGGTAAAGGCAATCCTGAG GAAG	163	TGAGT GGTAA AGGCA ATCCTG	1163
3:185152807G> A-	CGACGCCGGCAAGGCCGCCGC ACTCATTTCCTCTCCCTCCGCG CGGAG	164	ACTCAT TTCCTC TCCCTC CGC	1164
2:113595432G> A+	CCTGGGCGCtCGTGAAGATGGA GCCATATTCTGCAGGCGCTCT GGAG	165	AGCCA TATTCC TGCAG GCGCT	1165

10:121520163G >C-	CTTGAGGATGGGCCGGTGAG GCCATCGCTCTGGTGGAGAGA GGGAAG	166	GCCATC GCTCTG GTGGA GAGA	1166
19:52212718C> G+	CCATGcGGGTGTCATCTGAGCA CAGGTTCCGGAAGTACCTGGG AGT	167	GCACA GGTTCC GGAAG TACCT	1167
3:195782483C> T-	CGGGGTGGCGTGACCGGTGGA TGTTGAGGAAGGGCTGGTGAC ATGAAG	168	TGTTGA GGAAG GGCTG GTGAC	1168
3:185152807G> A-	CGGAGGGAGAGGAAATGAGTG CGGCGGCCTtGCCGGCGTCGGA GCGGGG	169	GGCGG CCTtGC CGGCG TCGGA	1169
12:56085070G> A+	CGTAGACCTGGGTCCCTCGCAC CAtGCGGAGGTTGGGCAATGGT AGAGT	170	CAtGCG GAGGT TGGGC AATGG	1170
17:7673767C>T-	CTGGGAGAGACCGGCGCACAA AGGAAGAGAATCTCCGCAAGA AAGGGG	171	AGGAA GAGAA TCTCCG CAAGA	1171
17:7673767C>T-	CTGGGAGAGACCGGCGCACAA AGGAAGAGAATCTCCGCAAGA AAGGGGAG	172	GAAGA GAATCT CCGCA AGAAA	1172
17:7674216C>A -	CGGAGtCCCATCCTCACCATCA TCACACTGGAAGACTCCAGGT CAGGAG	173	TCACAC TGGAA GACTCC AGGT	1173
17:7673764C>T-	CTGGGAGAGACCGGCGCACAG AGaAAGAGAATCTCCGCAAGA AAGGGG	174	AGaAAG AGAAT CTCCGC AAGA	1174
10:46131074C> T+	CACTGAGCTGTCCCTGGGCCAG CAGCTGCTGTGGGCCACCGCTG ATGAGG	175	AGCTG CTGTGG GCCAC CGCTG	1175
12:17743A>G-	CCTCGGCCCTGCCTTCTGGCCA TACAGGTTCTCGGTGGTGTGA AG	176	CCATAC AGGTTT TCGGTG GTG	1176
17:7673764C>T-	CTGGGAGAGACCGGCGCACAG AGaAAGAGAATCTCCGCAAGA AAGGGGAG	177	aAAGAG AATCTC CGCAA GAAA	1177
3:185152807G> A-	CGGAGGGAGAGGAAATGAGTG CGGCGGCCTtGCCGGCGTCGGA GCGGGGT	178	GCGGC CTtGCC	1178

			GGCGT CGGAG	
17:7673776G>A -	CTGGGAGAGACtGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGG	179	AGGAA GAGAA TCTCCG CAAGA	1179
17:7673704G>A -	CTAAGtGAGGTAAGCAAGCAG GACAAGAAGCGGTGGAGGAGA CCAAGGGT	180	CAAGA AGCGG TGGAG GAGAC C	1180
17:7674953T>C-	CGCTGAGGAGGGGCCAGACCT AAGAGCAATCAGTGAGGAATC AGAGG	181	TAAGA GCAAT CAGTG AGGAA T	1181
17:7673776G>A -	CTGGGAGAGACtGGCGCACAG AGGAAGAGAATCTCCGCAAGA AAGGGGAG	182	GAAGA GAATCT CCGCA AGAAA	1182
3:41224622C>T +	CTCAGAGAAGGAGCTGTGGTA GTGGCACCAaAATGGATTCCAG AGT	183	TAGTG GCACC AaAATG GATTC	1183
3:41224645T>C +	CTCAGAGgAGGAGCTGTGGTA GTGGCACCCAGAATGGATTCCA GAGT	184	TAGTG GCACC AGAAT GGATTC	1184
17:39725079G> A+	CCCTGTGTAtGAGCCGCACATC CTCCAGGTAGCTCATCCCCTGG AAT	185	TCCTCC AGGTA GCTCAT CCCC	1185
17:7673704G>A -	CCCAGGGAGCACTAAGtGAGGT AAGCAAGCAGGACAAGAAGCG GTGGAG	186	AAGCA AGCAG GACAA GAAGC G	1186
3:41224633A>G +	CTCAGAGAAGGAGCTGTGGcA GTGGCACCCAGAATGGATTCCA GAGT	187	cAGTGG CACCA GAATG GATTC	1187
7:56106490G>A -	CGAAGAGCTAAGCGACTTCTG AGGAGACCGGAAAATGGGAGG CGGGG	188	GAGGA GACCG GAAAA TGGGA G	1188
16:15104542T> A-	CGCTGAGGGTGGAGCTGAGGG TGGAAGGGGAGTGAGCAGACA CACGGAGG	189	GAAGG GGAGT GAGCA GACAC A	1189

3:185152807G> A-	CGCGGAGGGAGAGGAAATGAG TGCGGCGGCCCTGCCGCGCTCG GAG	190	AGTGC GGCGG CCTtGC CGGCG	1190
5:112838934C> T+	CGGGGAGCCAATGGTTTCAGAA ACAAATTGAGTGGGTCTAATC ATGGAAT	191	AAATT GAGTG GGTTCT AATCA	1191
16:15104542T> A-	CGCTGAGGGTGGAGCTGAGGG TGGAAGGGGAGTGAGCAGACA CACGGAG	192	GGAAG GGGAG TGAGC AGACA C	1192
17:7673806C>T-	CTGGGACGGAACAGCTTTGAGa TGCGTGTTTGTGCCTGTCCTGG GAG	193	GaTGCG TGTTTG TGCCTG TCC	1193
17:7673803G>A -	CTGGGACGGAACAGCTTTGAG GTGtGTGTTTGTGCCTGTCCTGG GAG	194	GGTGtG TGTTTG TGCCTG TCC	1194
17:7673802C>A -	CTGGGACGGAACAGCTTTGAG GTGCtTGTTTGTGCCTGTCCTGG GAG	195	GGTGcT TGTTTG TGCCTG TCC	1195
17:7673802C>T-	CTGGGACGGAACAGCTTTGAG GTGCaTGTTTGTGCCTGTCCTG GGAG	196	GGTGCa TGTTTG TGCCTG TCC	1196
17:7673796C>T-	CTGGGACGGAACAGCTTTGAG GTGCGTGTTTaTGCCTGTCCTG GGAG	197	GGTGC GTGTTT aTGCCT GTCC	1197
3:195779688A> G-	CGTGGCCTGTGGATACTGAGG AAGTGTCGGTGACAGGAAGAG GGGT	198	GGAAG TGTCGG TGACA GGAAG	1198
10:121520163G >C-	CGGTGAGGCCATCGCTCTGGTG GAGAGAGGGAAGAAAGGAGG AGTGGGG	199	GAGAG AGGGA AGAAA GGAGG A	1199
16:15104542T> A-	CTGAGGGTGGAGCTGAGGGTG GAAGGGGAGTGAGCAGACACA CGGAG	200	GGAAG GGGAG TGAGC AGACA C	1200
16:15104542T> A-	CTGAGGGTGGAGCTGAGGGTG GAAGGGGAGTGAGCAGACACA CGGAGG	201	GAAGG GGAGT GAGCA	1201

			GACAC A	
3:179218304A> C+	CTGTGACTCCATAGAAAATCTT TCTCCTGCgCAGTGATTTCAGA GAGAGG	202	CTCCTG CgCAGT GATTTC AGA	1202
17:7673704G>A -	CAGGGAGCACTAAGtGAGGTA AGCAAGCAGGACAAGAAGCGG TGGAGG	203	AGCAA GCAGG ACAAG AAGCG G	1203
3:179218303G> A+	CTGTGACTCCATAGAAAATCTT TCTCCTGCTtAGTGATTTCAGAG AGAGG	204	CTCCTG CTtAGT GATTTC AGA	1204
17:7673704G>A -	CAGGGAGCACTAAGtGAGGTA AGCAAGCAGGACAAGAAGCGG TGGAGGAG	205	CAAGC AGGAC AAGAA GCGGT G	1205
9:21971121G>A -	CGCCGACCCCGCCACTCTCACC tGACCCGTGCACGACGCTGCCC GGGAGG	206	GACCC GTGCA CGACG CTGCCC	1206
2:177234217G> C-	CTTGAGTAAGTgGAGAAGTAT TTGACTTCAGTCAGCGACGGA AAGAGT	207	TTGACT TCAGTC AGCGA CGGA	1207
17:7673704G>A -	CAGGGAGCACTAAGtGAGGTA AGCAAGCAGGACAAGAAGCGG TGGAG	208	AAGCA AGCAG GACAA GAAGC G	1208
3:179218307A> G+	CTGTGACTCCATAGAAAATCTT TCTCCcGCTCAGTGATTTCAGA GAGAGG	209	CTCCcG CTCAGT GATTTC AGA	1209
3:179218294G> A+	CTGTGACTCCATAGAAAATCTT TCTCCTGCTCAGTGATTtAGAG AGAGG	210	CTCCTG CTCAGT GATTt AGA	1210
17:7673803G>A -	CTTTGAGGTGtGTGTTTGTGCCT GTCCTGGGAGAGACCGGCGCA CAGAGG	211	GTCCTG GGAGA GACCG GCGCA	1211
17:7673704G>A -	CCCAGGGAGCACTAAGtGAGGT AAGCAAGCAGGACAAGAAGCG GTGGAGG	212	AGCAA GCAGG ACAAG AAGCG G	1212

12:16040G>A-	CGGAGGGGTCAACCACTTCCCT GGGAGCTCCCTGGACTGGAGC CGGGAGG	213	GGAGC TCCCTG GACTG GAGCC	1213
19:3118944A>T +	CGCTGTGTCCTTTCAGGATGGT GGATGTGGGGGGCCTGCGGTC GGAG	214	GTGGA TGTGG GGGGC CTGCG G	1214
6:73519043A>G -	CACTGAGCCACCCTACAGCCA GAAGAGATATGAGGAAATcGT TAAGGAAG	215	AGAGA TATGA GGAAA TcGTTA	1215
17:7675994C>A -	CTGGGACAGCCAAGTCTGTGA CTTGCAcGTCAGTTGCCCTGA GGGG	216	ACTTGC AcGTC AGTTGC CCT	1216
12:16040G>A-	CGGAGGGGTCAACCACTTCCCT GGGAGCTCCCTGGACTGGAGC CGGGAG	217	GGGAG CTCCCT GGACT GGAGC	1217
16:15104542T> A-	CACGGAGGTGTCTTGAGATTAT CATCCGCTGAGGGTGGAAGGG GAT	218	TATCAT CCGCTG AGGGT GGAA	1218
9:21971121G>A -	CGCCGACCCCGCCACTCTCACC tGACCCGTGCACGACGCTGCCC GGGAG	219	tGACCC GTGCA CGACG CTGCC	1219
		Deletions		
10:12829237del A+	CTATGGTGTGTTTTTTCATTAA TGACAAAAAAAAAAGTTTCA ACTGGAT	220	GACAA AAAAA AAAGG TTTCAA	1220
2:101891433del T+	CATTGCAGCCTTGATTTATTTT GGAGTAAGAAAAAAAAAAGA ATGGGGAT	221	GAGTA AGAAA AAAAA AAGAA T	1221
3:18348736delT-	CTTTGGAAACTTGCCCTTATT TAAAAAAAAAAGAAAAAAAAA AGAGT	222	TTTAAA AAAAA AAAGA AAAAA	1222
2:101891433del T+	CATTGCAGCCTTGATTTATTTT GGAGTAAGAAAAAAAAAAGA ATGGGG	223	TGGAG TAAGA AAAAA AAAAG A	1223
1:12725526delT +	CACTGCTCACCTGGAGTTTCAT CTGCTACTTTTTTTTCAAACC TGGAT	224	CTGCTA CTTTTT	1224

			TTCAA AAC	
11:64204409del T+	CATAGTTGGTTTTTTTTTATTTG GGGCAGTGGGCATGTTATGGG GAGG	225	TGGGG CAGTG GGCAT GTTATG	1225
14:41904405del T+	CAATGTAGGCTTTTTTCTTTTT TAAAAAAAATACTTGTAGGC CAGAAG	226	TAAAA AAAAA TACTTG TAGGC	1226
11:64204409del T+	CATAGTTGGTTTTTTTTTATTTG GGGCAGTGGGCATGTTATGGG GAGGGG	227	GGGCA GTGGG CATGTT ATGGG	1227
14:55684263del A+	CAGAGTTGGCTTTTTTTTCCTT CTTTAAATCAGTAGTCTAACAA GGAT	228	TCTTTA AATCA GTAGTC TAAC	1228
14:20992723del T+	CAGCGGAGATCCTCTTTAAAA AAAAATGCATATGCAATGTCC CAAGAAT	229	AAAAT GCATAT GCAAT GTCCC	1229
12:121238563del A-	CTTTGGTGTTCCTTCATTAAAA GAAAAAAAAAAGTCATCAATG TGGGT	230	AGAAA AAAAA AAGTC ATCAAT	1230
10:100827567del C+	CTGTGTTAACTTCCAGGTTCCC CTTATTATTATAGTGCCGCCCC CGGGG	231	CTTATT ATTATA GTGCC GCCC	1231
11:64204409del T+	CATAGTTGGTTTTTTTTTATTTG GGGCAGTGGGCATGTTATGGG GAG	232	TTGGG GCAGT GGGCA TGTTAT	1232
5:134727810del A+	CAATGGTCTGCATGGGTACAA ATGACTTTTTTTTTTTAACAG GAAT	233	ATGACT TTTTTT TTTTTT AAC	1233
6:17615262delT-	CAAAGTTGCCTAAATCCATTTG GAAATCTTTAAAAAAAATTG GGGAT	234	GGAAA TCTTTA AAAAA AAATT	1234
8:115411283del A-	CAATGCTAGTCGTTACTACTAT GTCTGTCTGAGAAAAAAA ATTGAAT	235	TCTGTC TGAGA AAAAA AAAAA	1235
4:157221000del A+	CAGAGGAAAACAGCCAAAGAA GGAAGAGGAGGAAAAGGAAA AAAAAGGGG	236	AAGAG GAGGA AAAGG AAAAA A	1236

X:108439959del T+	CTTGGGTGAAGAGAAAGAAGC TTTTTAAGAGTGGAAGAAAA AAAAGAAG	237	TTTAAG AGTGG AAGAA AAAAA	1237
11:129882601del T-	CTTGGCTGTCCTTAAAAAAGG TTAAGGAAAAAGAGGAAAAGA AGAAG	238	GTTAA GGAAA AAGAG GAAAA G	1238
7:44080815delG -	CTCTGTTAACCAGCTTATGTCC AGCAGAGCTGGGGGGGTGCAAC CCGGGG	239	AGCAG AGCTG GGGGG TGCAA C	1239
1:26780313delC +	CAATGTGGACCTGATTCTGGCC ACACCCCCTTCAGCCGCCTGGA GAAG	240	CACAC CCCCTT CAGCC GCCTG	1240
10:26173831del A+	CTGTGGAGAGTAACAACAGAG TGTATCAGACTCCAAAAAAT GAAT	241	AGTGT ATCAG ACTCCA AAAAA	1241
10:46809223del T-	CATGGGTTGTTTATTCTCGCTC TCTCTCTTTTTTTTTTTTGGAGA GG	242	TCTCTC TCTTTT TTTTTT TTT	1242
10:46809223del T-	CATGGGTTGTTTATTCTCGCTC TCTCTCTTTTTTTTTTTTGGAGA GGGAG	243	CTCTCT TTTTTT TTTTTT GAG	1243
12:12121051del C-	CAACGTAAAAATGTAAATATA AATTTGGTTGAGATCTGGAGG GGGGAGG	244	ATTTGG TTGAG ATCTGG AGGG	1244
3:185644316del T-	CTTGGTAAATTTCTACTTTCCT CCACATTTTTTTTTTAAAGAA AGGAAT	245	CACATT TTTTTT TTTAAA GAA	1245
13:26214104del A-	CCTTGCTCACTTTTTTTACTAA ATGAAAGTGATGATGATGATC GAAT	246	AAATG AAAGT GATGA TGATG A	1246
5:159203632del TC-	CTTTGCAGCCATGTTGTTTTTT TTTCTTTTTTTTTTCTTGAGAG AG	247	TTTTTC TTTTTT TTTTTC TTG	1247
1:45014295delT +	CTGAGGAAAGTAAATTTTTTT TTTAATTACTGGGTTTTTAGG GT	248	TTTTTT TAATTA CTGGGT TTT	1248

9:34088276delA -	CTCAGTTTCCTTGTTTCTTTTGA TTTTTTTTTCCCTAATTGTGTGAG G	249	TGATTT TTTTTT CCTAAT TGT	1249
4:144738016del A+	CACAGGAGTGTGTAGCAGGAT AACAGTCTTTTTTTTAAATGAC AGGAT	250	AACAG TCTTTT TTTTTA ATGA	1250
2:161992536del T-	CATTGTAAATGTGCTTTTAAAA AAAATACTGATGTTCCTAGTGA AAGAGG	251	AAATA CTGATG TTCCTA GTGA	1251
X:47635578delA -	CACTGTTTGTTTACTTCCCCA AAATGGACCTTTTTTTTCTAA AGAGT	252	AAATG GACCTT TTTTTT TCTA	1252
6:17615262delT-	CAAAGTTGCCTAAATCCATTTG GAAATCTTTAAAAAAAATTG GGG	253	TTGGA AATCTT TAAAA AAAAA	1253
7:44080815delG -	CTCTGTAAACCAGCTTATGTCC AGCAGAGCTGGGGGGTGCAAC CCGGGGG	254	GCAGA GCTGG GGGGT GCAAC C	1254
10:11331605del A+	CTTGTAACCTTTTTTTGTTTTG TTTTTTTTTTAAATATTAGGGA T	255	TTTGTT TTTTTT TTAAAT ATT	1255
4:56395207delA -	CCATGCTTATGTTTATAAGTTT TGAGATTTTTTTTTTCTGAAA AGGAT	256	TGAGA TTTTTT TTTTTC TGAA	1256
11:30012016del A-	CAAAGCTGGGGCGGTTCTGTC AAAAAATACTCATTGCGCAAA GGGT	257	TCAAA AAATA CTCATT GCGCA	1257
10:79920900del A+	CGGGGGTGAACAGGAATGGAG CATTGAGCTTTTGGGGAAAAA AAAAGAGT	258	TTGAGC TTTTGG GGAAA AAAA	1258
20:4727794delT +	CAATGCTTCTACTCATTTTCT ATACTTTTTTTTTTGAGGCAGAG T	259	TTCTAT ACTTTT TTTTTG AGG	1259
8:61499736delT +	CAAAGTTGGACAAAGACTTGA GAGATGCTTTTTTTTCCCCCAG TGAGGGG	260	GATGCT TTTTTT TCCCCC AGT	1260

8:61499736delT +	CAAAGTTGGACAAAGACTTGA GAGATGCTTTTTTTTCCCCCAG TGAGG	261	GAGAT GCTTTT TTTTCC CCCA	1261
5:95793485delA +	CTACGTAATTTTTTTTTTTAATC TAAGCATTCTTAACTGAGAGG GGT	262	TCTAAG CATTTT TTAACT GAG	1262
15:44711583del CT+	CTGTGCTCGCGCTATCTCTCTT TCTGGCCTGGAGGCTATCCAGC GTGAGT	263	CTGGCC TGGAG GCTATC CAGC	1263
5:95793485delA +	CTACGTAATTTTTTTTTTTAATC TAAGCATTCTTAACTGAGAGG GG	264	ATCTAA GCATTT CTTAAC TGA	1264
11:47175302del A-	CGCTGCTCTCTTCTTTGCTCTCC AGACGGCTTTTTTCGCCAACAT GGAT	265	CAGAC GGCTTT TTTCGC CAAC	1265
19:35732101del G+	CAGGGGAGGGCAGGGGGCCAA AGGAGACACCCCCAAGGGCCT CCGGGAT	266	GGAGA CACCCC CAAGG GCCTC	1266
1:66924743delT-	CGGAGGAGCTTGGGGGTACAG GGACTTCAGAGGCGGCCAAAA AAGGGGT	267	GACTTC AGAGG CGGCC AAAAA	1267
12:12121051del C-	CAACGTAAAAATGTAAATATA AATTTGGTTGAGATCTGGAGG GGGGAG	268	AATTTG GTTGA GATCTG GAGG	1268
9:32633586delT-	CTTTGCTAAAGCACATCAAAA AAAGGCCAAGATGAGAGAACA AGAGAGG	269	AAGGC CAAGA TGAGA GAACA A	1269
1:147259627del A+	CAAGGGATTTTTTTTTTAACAA AATGGGAAAGACCTATGCAGA AAGGAAG	270	ATGGG AAAGA CCTATG CAGAA	1270
7:20786861delT-	CAGAGGATCTTTTTTATATTGA TAAATCAGAGGCAGTGTTTTT TAGAGG	271	AAATC AGAGG CAGTGT TTTTT	1271
1:16135704delG -	CTCGGCTCTGCTGCGGCGGGG GATGCTCCAGGAGACGCTAAG CGAGG	272	GGATG CTCCAG GAGAC GCTAA	1272

7:55174772delG GAATTAAGAG AAGC+	CGGAGATGTTTTGATAGCGAC GGGAATTTTAACCTTCTCACCT TCTGGGAT	273	AATTTT AACTTT CTCACC TTC	1273
19:35719862del C+	CCTAGCAGATGTGGCTCCTACC CCCCAAAGACCCCTGCCCGGA AACGGGG	274	CCCAA AGACC CCTGCC CGGAA	1274
5:53884973delA -	CTATGTATCCTTCCTGATTCAT GACATTAAAAAAAAGCTTA AAGAAG	275	GACATT AAAAA AAAAA GCTTA	1275
12:49040709del G-	CTGGGCAGGGGTGGCTCCTGG GGCCTTAGGCCCAAGCCCGGG CTCTGGGG	276	CCTTAG GCCCA AGCCC GGGCT	1276
1:66924743delT-	CGGAGGAGCTTGGGGGTACAG GGACTTCAGAGGCGGCCAAAA AAGGGG	277	GGACTT CAGAG GCGGC CAAAA	1277
X:129805329del T-	CGAGGCTTAATGGAAGAACTG GTTAGCATTTTTTTTTTTGAGG GT	278	TGGTTA GCATTT TTTTTT TTT	1278
19:35719862del C+	CAGGGGTCTTTGGGGGGTAGG AGCCACATCTGCTAGGCGAGG AGGAGG	279	AGCCA CATCTG CTAGG CGAGG	1279
11:62882057del A+	CCAAGGAAGATTTTGACAGTCT CTTGCAATCGGCTAAAAAAG AGTGGGT	280	TTGCAA TCGGCT AAAAA AAGA	1280
2:147899472del A+	CTCTGCTTATTTATAGGACTGA TTGTGTAGAAAAAAGACAGC CCTGAAG	281	TGTGTA GAAAA AAAGA CAGCC	1281
17:50356606del C+	CGTAGGTGTTCTCCCAGTAGGC CTTGAGGGCTGGCGTGCCGGG GGGT	282	GCCTTG AGGGC TGGCGT GCCG	1282
10:844999delT-	CCTAGATAAGCAGAATTCCAA TGTTTTTTAAGTACTTCTCGGG GGT	283	AATGTT TTTTAA GTAATT CTC	1283
5:135334913del T-	CCCAGTAGGAGTGAAGGGGAT TTTTTTTTCTTTAACTGAAG GTGGGG	284	TTTTTT TCTTTT AAACT GAAG	1284
11:18210242del T+	CTCAGCAATGTCTTTTTTTTCG GCCTTCGAGGTCACGTTAAGG AG	285	TTCGGC CTTCGA	1285

			GGTCA CGTT	
2:1649188delG-	CCCTGCTTCTCTGTCATGATCC CCCCAATGACTCCCGGGCCAG GAG	286	TCCCC CAATG ACTCCC GGGC	1286
1:157697170del T-	CTCTGTACACGTATCTGAGATC TCAGGCTCCTTTTTTGATGCTG TGAGT	287	TCAGG CTCCTT TTTTGA TGCT	1287
7:100345930del T-	CTGAGTTAAAATGTGAAGGGA TTTTTTTTTTCAGATTACTGAG AGT	288	GATTTT TTTTTT CAGATT ACT	1288
15:75207463del C+	CTGTGGAAGAGACTGGCACTC CCGGGCACAGGGGGGCAGTGC TGGGGGGT	289	GGGCA CAGGG GGGCA GTGCTG	1289
X:80444260delT +	CCCTGGACTTTTCAAGCATTTT TTTTGACAATTAAATTGGGTTG GAT	290	TTTTTT GACAA TTAAAT TGGG	1290
22:16590897del T-	CAAAGAAACACCCACCTCCTG TGGAACAATAAATCCTTGG ATTGAAT	291	GGAAA CAAAA AAATC CTTGGA	1291
19:35719862del C+	CAGGGGTCTTTGGGGGGTAGG AGCCACATCTGCTAGGCGAGG AGGAG	292	GAGCC ACATCT GCTAG GCGAG	1292
11:105003284del T-	CTGTGTAAAAAATCATGATG AGGTGCTGTGCTCTCTGTATGA AATGGGG	293	GTGCTG TGCTCT CTGTAT GAA	1293
4:128948520del T-	CAAGGCCAAATTGAACGAGTC ATTAAGGAAAAAAGCAGTGG AAGAAG	294	ATTAA GGAAA AAAAG CAGTG G	1294
10:29471187del C-	CCAGGGAAAGGAGCCCCCTG TTTCCTGCAGTGTTTCCAGGGG GGAT	295	GTTTCC TGCAGT GTTTCC AGG	1295
5:177248268del A+	CCCGGGATGCCTGCCTCTAAAA AATGCAGGGTGAACGCGGTGG AGGAG	296	AAATG CAGGG TGAAC GCGGT G	1296
10:8069470delC A+	CACAGGACGTCCCTGCTCTCCT GGCTGCAGACTAGAGTGGGGA GAGAGG	297	GGCTG CAGAC TAGAG	1297

			TGGGG A	
12:48980565del G+	CAATGTTGTCGCTGCAGCCCCC CAGTGCCAGTCGGGGCCCCCG GGG	298	CCCCA GTGCC AGTCG GGGCC C	1298
8:10726136delG -	CAGGGGTGGCTACAGTGGAGA GGGCTTGGGGCGTACTCCGGT GAGT	299	GAGGG CTTGGG GCGTA CTCCG	1299
2:46187716delA +	CAAAGGTGAAAAACAATGCA TTCTTGCTTTAAAAAAG AAG	300	CATTCT TGCTTT AAAAA AAAA	1300
10:8069470delC A+	CCCTGCTCTCCTGGCTGCAGAC TAGAGTGGGGAGAGAGGAGAG GGT	301	GACTA GAGTG GGGAG AGAGG A	1301
12:6602380delT-	CTCGGGACCCTAAAATCCCTAA GAGCAAGCGCCAAAAAAGGAG GTGAGT	302	GAGCA AGCGC CAAAA AAGGA G	1302
X:133027177del A-	CACGGCTACTTTTTTAAAGAT ACCTATAATATAGAAATCAAG GAG	303	GATAC CTATAA TATAG AAATC	1303
1:155317840del A+	CAGAGCTGCTACTTTATATCTG TATATAGTTTTGCTTTTTTTGGT AGGGG	304	ATATA GTTTTG CTTTTT TTGG	1304
5:14711100delA -	CATCGTATTTTGTTCCCTTTTTT TGTTTTGTTTTGGTAATGAAAG AGG	305	TTTGTT TTGTTT TGGTA ATGA	1305
16:88624733del C+	CTCGGGAAGCCAGGAGGAAGG AGCGGCCAGCCAGGACCCCCC CAGGAGG	306	GCGGC CAGCC AGGAC CCCCC	1306
10:21518000del G-	CAGTGTAATTTTCATGGGGTTTC CCCCCCCCAATAATTCGCCTA GAGT	307	CCCCC CCCAAT AATTTC GCC	1307
18:36625553del C+	CACTGGAACACACGCTGGCCC TCCTCCGGTCCCGGCACCCACT GGGGGT	308	CCTCCG GTCCCG GCACC CACT	1308

5:53884973delA -	CTTTGAAACATCAAACACTTCT TTAAGCTTTTTTTTTTAATGTCA TGAAT	309	TAAGCT TTTTTT TTTAAT GTC	1309
20:37999211del G-	CTAAGCACTGCCAGGGGCGT CCCGTGTGAGTCGGTGAACGA GCGAGG	310	CCCGTG TGAGTC GGTGA ACGA	1310
1:16135704delG -	CCTCGCTTAGCGTCTCCTGGAG CATCCCCGCCGCAGCAGAGC CGAGT	311	GCATCC CCCCGC GCAGC AGAG	1311
6:31958429delG -	CCTCGCTCAGTCCGGGGGTATC ACCAACATGGTGGCTCCTAGTT CAGGGG	312	CCAAC ATGGT GGCTCC TAGTT	1312
10:29471187del C-	CCCTGTTTCCTGCAGTGTTTCC AGGGGGGATGGTGGTGCATC GGGGAG	313	AGGGG GGATG GTGGT GCACTC	1313
11:47175302del A-	CTGTGCATCCATGTTGGCGAAA AAAGCCGTCTGGAGAGCAAAG AAG	314	AAAAA AGCCG TCTGGA GAGCA	1314
4:7062246delA-	CCCTGCATTTTTTTTATGATGG CTCAACAGCAAAGGTGTCTGG AGGAG	315	GCTCA ACAGC AAAGG TGTCTG	1315
11:62882057del A+	CAAGGAAGATTTTGACAGTCTC TTGCAATCGGCTAAAAAAGA GTGGGT	316	TTGCAA TCGGCT AAAAA AAGA	1316
17:6787540delT +	CTGAGCTGGTCATGTTGTCATG GAGCTGACAAAAAAAAAAGTG GAGGGG	317	GAGCT GACAA AAAAA AAAGT G	1317
5:135334913del T-	CCCAGTAGGAGTGAAGGGGAT TTTTTTTCTTTTAAACTGAAG GTGGGGT	318	TTTTTT CTTTTA AACTG AAGG	1318
1:52055208delT-	CTCAGCACAAACCTCTACTTC CCAGATTTTTTTTTTCAAACCTCT GAAG	319	CCAGA TTTTTT TTTTCA AACT	1319
11:62882057del A+	CCAAGGAAGATTTTGACAGTCT CTTGCAATCGGCTAAAAAAG AGT	320	TCTCTT GCAAT CGGCT AAAAA	1320

1:40292750delT +	CCTGGCAGCATGTTCCAGCTCT TGATGTTTTTAACTTTTTTTAG AAG	321	TTGATG TTTTTA AACTTT TTT	1321
1:154584696del A-	CAAAGTTTTTCAGTATCACCAAT TATGGCTTAAAAAGAAAAAA AGGAG	322	TTATGG CTTAAA AAGAA AAAA	1322
5:37064968delA +	CCAGGTTCCACTTACTCTTCAT AAAAGTATTTTTTTTGCCAGA AT	323	CATAA AACTG ATTTTT TTTGC	1323
1:10637121delT-	CAATGTAGCAAAATCAAACCTT AAAAAAAAAAGAAGAAAAG AAGAAG	324	TAAAA AAAAA AAGAA GAAAA G	1324
2:186656358del G+	CAAGGGGGGACTGATGCAGTG TGAGGAATTGATAGCGTATCTG CGGGT	325	TGAGG AATTG ATAGC GTATCT	1325
16:88624733del C+	CTCGGGAAGCCAGGAGGAAGG AGCGGCCAGCCAGGACCCCCC CAGGAG	326	AGCGG CCAGC CAGGA CCCCC	1326
7:100345930del T-	CTCAGTAATCTGAAAAAAAAA ATCCCTTCACATTTTAACTCAG GAG	327	AAATC CCTTCA CATTTT AACT	1327
8:94519335delA -	CTCGGATCAATAAATTTGTTGC TTCATATTCCGACATAAAAAA GAAG	328	CTTCAT ATTCCG ACATA AAAA	1328
5:177248268del A+	CCCGGGATGCCTGCCTCTAAAA AATGCAGGGTGAACGCGGTGG AGG	329	AAAAA TGCAG GGTGA ACGCG G	1329
5:51393873delA +	CAATGATCTTAGAAGTACTGA AAAAAAGACGTTTTTAAAC GTAGAGG	330	AAAAA AGACG TTTTTA AAACG	1330
6:31536669delA -	CTCGGCAGGTTGCTGTTTTTTT GGTGGTCTGTCTATCAAGAAG GATGAAG	331	GTGGTC TGTCTA TCAAG AAGG	1331
4:25847636delT-	CTGAGTTCAGAAAGTAGCATTCC CGTGTACAAAAAAGGTGGAAG AAT	332	TCCCGT GTACA AAAAA GGTGG	1332

5:146511047del T+	CCGAGTTGGGGTTTGTGTTTGT TTGATTTTTTTTTTTAAAGCGG GT	333	GTTTTG ATTTTT TTTTTT AAA	1333
19:19110944del G+	CATGGAACGGTGGGGGTGCG CTTGATTCTACTTCAGGAGGCA CATGGGG	334	TGATTC TACTTC AGGAG GCAC	1334
19:35719862del C+	CCGGGCAGGGGTCTTTGGGGG GTAGGAGCCACATCTGCTAGG CGAGGAG	335	TAGGA GCCAC ATCTGC TAGGC	1335
17:30178149del A+	CTGTGTATGAATATGACAGTAT TTATGATGAAATGCAGAAAAA AAGGAG	336	TTATGA TGAAA TGCAG AAAAA	1336
15:42450759del A-	CAAAGAATATCTCAAAAAACA CCAAAACAAATTTTTTCCCT GGAG	337	ACCAA AACAA ATTTTT TTCCC	1337
17:4972443delG -	CAGAGGCTGGCAGAGGTGCAG GGGGGGACTGCCATCTGGGGC ACTAGAAT	338	GGGGA CTGCCA TCTGGG GCAC	1338
3:171101725del A-	CACAGTTCTATATAGAGCTTTT TTTTCTTGTTGCTTAAGCTGG AG	339	TTTTTT TTCTTG TTGCTT AAG	1339
2:238170406del A-	CATGGTTGTAAATAAAGGTTTC TCTTTTTTTCCTAGTCTTTTGA GT	340	TCTCTT TTTTTT CCTAGT CTT	1340
14:93241685del A-	CCCAGCTCCTAGGACTTATTAA AAAAAAATGACATTAGATTTA TGGAGG	341	AAAAA AATGA CATTAG ATTTA	1341
19:35719862del C+	CTGTGCCTTCCTCACCCCGTTT CCGGGCAGGGGTCTTTGGGGG GTAGGAG	342	CGGGC AGGGG TCTTTG GGGGG	1342
2:239052414del A-	CATCGGAAGATGCGAGTTTGT GCCTTTTTTTTATTGCTCTGGTG GAT	343	TGCCTT TTTTTT ATTGCT CTG	1343
12:107544001del C+	CAATGCCAAGCACACGGCAA TGGCACCCCTGCACCACAAG CAGGGGG	344	GGCAC CCCCTG CACCA CAAGC	1344
3:14488912delT +	CACAGTTGCTAGGGATTGGGA GATTAATTGGGTAAAAA AATGAAG	345	ATTAAT TGGGT	1345

			AAAAA AAAAA	
10:29471187del C-	CCCTGTTTCCTGCAGTGTTTCC AGGGGGGATGGTGGTGCACCTC GGGG	346	CCAGG GGGGA TGGTG GTGCA C	1346
1:16135704delG -	CCTCGTACTTCCACACTCGGCT CTGCTGCGGCGGGGGATGCTC CAGGAG	347	CTGCTG CGGCG GGGGA TGCTC	1347
10:29471187del C-	CCCTGTTTCCTGCAGTGTTTCC AGGGGGGATGGTGGTGCACCTC GGGGAGG	348	GGGGG GATGG TGGTGC ACTCG	1348
19:35719862del C+	CTTTGGGGGGTAGGAGCCACA TCTGCTAGGCGAGGAGGAGGA AGGGGGG	349	CTGCTA GGCGA GGAGG AGGAA	1349
19:35719862del C+	CCGGGCAGGGGTCTTTGGGGG GTAGGAGCCACATCTGCTAGG CGAGGAGG	350	AGGAG CCACAT CTGCTA GGCG	1350
8:107284561del A-	CTTTGTAATAGGTCTAGACAAA AAAAAAGAATTTTCATTTTGAA GGAT	351	AAAAA AAGAA TTTCA TTTTG	1351
8:66430512delT +	CGCCGTATATCCAACATTAATA AGAAAAAAAAAGGCTGTTTAAG AAT	352	AAAAG AAAAA AAAGG CTGTTT	1352
17:6787540delT +	CTGAGCTGGTCATGTTGTCATG GAGCTGACAAAAAAAAAAGTG GAGG	353	TGGAG CTGAC AAAAA AAAAA G	1353
8:144095973del C+	CAAGGAAGAGAGGAGGCCACG GTGAGACCACGGATAGCTGGG GGGT	354	CGGTG AGACC ACGGA TAGCTG	1354
1:204954905del C+	CACAGCTGCTCATCCAGAAGA TGACCGGGGATGGAAGTCCAG GCGGGGGT	355	ACCGG GGATG GAAGT CCAGG C	1355
3:50100281delT +	CCACGCTTAAAGTAACCATGC AACCGACTATAGTCAAAAAA AAAGGAG	356	ACCGA CTATAG TCAAA AAAAA	1356

5:51393873delA +	CTACGTTTTTAAAAACGTCTTTT TTTTCAGTACTTCTAAGATCAT TGAAT	357	TTTTCAGTACTT CTAAG ATCA	1357
11:112033459del A+	CACAGTTCAGAGATGGGAAAA AAAGTGGGTGAGAAGCTAAGT GAAGGAG	358	AAGTG GGTGA GAAGC TAAGT G	1358
14:93241685del A-	CCCAGCTCCTAGGACTTATTAA AAAAAAATGACATTAGATTTA TGGAG	359	AAAAA AAATG ACATTA GATTT	1359
17:70179777del A+	CTATGCCACACTTTTTTTTTCCC ACCTTAACATTATTAGACACAG AGT	360	CCACCT TAACAT TATTAG ACA	1360
1:27550358delT-	CAGCGGCCACCATGGCCATGC CAGAGGTAAAAACGACGGCG GCGGAAG	361	AGAGG TAAAA AACGA CGGCG G	1361
4:7062246delA-	CCCTGCATTTTTTTTATGATGG CTCAACAGCAAAGGTGTCTGG AGG	362	TGGCTC AACAG CAAAG GTGTC	1362
10:26173831del A+	CAGAGTGTATCAGACTCCAAA AAAATGAATAATGTGTATGAG GAAGAGG	363	AAATG AATAA TGTGTA TGAGG	1363
12:55028503del T+	CACAGTTGCCCATGGGAAAAA CCAATGGATTTTTTTTAAGCAA GATGAAT	364	AATGG ATTTTT TTTAAG CAAG	1364
3:100355352del T+	CAAGGCCGAATTAAAAA TCTTCATTAAAGAATTTAATAG GGAG	365	ATCTTC ATTAA AGAAT TTAAT	1365
8:13568072delT +	CACAGATTTGAGGACTAAGAA CTTTCACTTTTTTTCTATTAT AGAAG	366	TTTCAC TTTTTT TTCCTA TTA	1366
15:78104908del T+	CCCTGGTCACCCAGGACAGAG CGGAAAAAAAAAAGAGGTCA GGGT	367	AGCGG AAAAA AAAAA AGAGG T	1367
3:100355352del T+	CAAGGCCGAATTAAAAA TCTTCATTAAAGAATTTAATAG GGAGT	368	TCTTCA TTAAA GAATTT AATA	1368

19:35719862del C+	CCGGGCAGGGGTCTTTGGGGG GTAGGAGCCACATCTGCTAGG CGAGG	369	GGTAG GAGCC ACATCT GCTAG	1369
17:50356606del C+	CGTCGTAGGTGTTCTCCAGTA GGCCTTGAGGGCTGGCGTGCC GGGGGGT	370	GCCTTG AGGGC TGGCGT GCCG	1370
3:50100281delT +	CCACGCTTAAAGTAACCATGC AACCGACTATAGTCAAAAAA AAAGGAGT	371	CCGACT ATAGTC AAAAA AAAA	1371
20:59028741del T-	CATTGTCAATTTATGAACAAGA CAGGATTTTTTTTTTCCCATGG AAT	372	GACAG GATTTT TTTTTT CCCA	1372
12:107544001del C+	CACGGCAATGGCACCCCCTGC ACCACAAGCAGGGGGCACTGT ACTGGGAG	373	CACAA GCAGG GGGCA CTGTAC	1373
12:107544001del C+	CAATGCCAAGCACCCAGGCAA TGGCACCCCCTGCACCACAAG CAGGGG	374	TGGCA CCCCCT GCACC ACAAG	1374
6:31536669delA -	CTCGGCAGGTTGCTGTTTTTTT GGTGGTCTGTCTATCAAGAAG GAT	375	TTTGGT GGTCTG TCTATC AAG	1375
22:31089936del C+	CAGAGCCACCCCCAGCCCACC CAAGACCACCAGCCCTGAGCC TCAGGAG	376	AAGAC CACCA GCCCTG AGCCT	1376
19:35719862del C+	CTTTGGGGGGTAGGAGCCACA TCTGCTAGGCGAGGAGGAGGA AGGGGGGT	377	TGCTAG GCGAG GAGGA GGAAG	1377
19:35719862del C+	CTTTGGGGGGTAGGAGCCACA TCTGCTAGGCGAGGAGGAGGA AGGGGG	378	TCTGCT AGGCG AGGAG GAGGA	1378
15:64169061del A-	CCTTGCAGCTGGATTTGCGACT TTTTTTTGTCTTAAATTTTAC TGGAT	379	TTTTTT GTCTTA AAATTT TTA	1379
17:30178149del A+	CTGTGTATGAATATGACAGTAT TTATGATGAAATGCAGAAAAA AAGGAGG	380	TATGAT GAAAT GCAGA AAAAA	1380
5:128258372del A-	CAAGGAATATATGTTGTTGTTG TTGTTTTAAACCATTTTTTTTTT AGAAT	381	TGTTTT AAACC	1381

			CATTTT TTTT	
17:6787540delT +	CTGAGCTGGTCATGTTGTCATG GAGCTGACAAAAAAAAAAGTG GAG	382	ATGGA GCTGA CAAAA AAAAA A	1382
5:1074583delG-	CTACGCCCTGCTGCGCGTGGAG CACGGTCCCCCACCACCAAGA ACTGGAG	383	ACGGT CCCCC ACACC AAGAA	1383
19:47416569del C-	CTCTGCACCCCCCACCACCC CAGGGATGGGGCGTCAGGGAA GGAG	384	CCCAG GGATG GGGCG TCAGG G	1384
16:10773346del A-	CCATGATATGGATTGTGTTTTT TTTAGCACCTTATTTTCCTTGA AG	385	TTTTTT AGCAC CTTATT TTCC	1385
1:25563141delT +	CATGGCCAATCCCCCTTCCCCG TCAGGACTCACAGCTCTTCAAG GGGGG	386	TCAGG ACTCAC AGCTCT TCAA	1386
9:134154476del C+	CCAGGATGTATTTGCCGTTCCGG GGAGAACTTCACAAAAGACAC GGGGGGT	387	GAGAA CTTCAC AAAAG ACACG	1387
8:10726136delG -	CGGGGGACTGGCCAAGGGCCA GGGAGCCCAGGGGTGGCTACA GTGGAG	388	GGGAG CCCAG GGGTG GCTAC A	1388
19:45052871del C+	CCTGGCAACCCCTGGGAGGT GGCTTACATGGTGGTGAGCAG AGGGGGGT	389	CTTACA TGGTG GTGAG CAGAG	1389
19:45052871del C+	CCTGGGAGGTGGCTTACATGGT GGTGAGCAGAGGGGGGTGTAG TCGGGG	390	GGTGA GCAGA GGGGG GTGTA G	1390
11:55886134del A+	CTGAGATTTTCACAAGCTTTTT CCCATAAAGACTGCATTTTTTT AGGAG	391	CCCATA AAGAC TGCATT TTTT	1391
8:94880765delA +	CTTAGTCACACTAAATTAAAAA AAAAATTCCTTAGGGATATCTT AGAGT	392	AAAAA TTCCTT AGGGA TATCT	1392

3:48158363delT-	CCAGGGACTACCTCGGCTTTTA ATTTAAAAAAGAAAGTG GGT	393	TTAATT TAAAA AAAAA AAGAA	1393
22:31089936del C+	CAGAGCCACCCACGCCACC CAAGACCACCAGCCCTGAGCC TCAGGAGT	394	AGACC ACCAG CCCTGA GCCTC	1394
2:120294679del T+	CATGGGCTTTTTTTTGAATAAA AAAGCAGACAAATAGACTTTC TCGGGAT	395	AAGCA GACAA ATAGA CTTTCT	1395
19:35719862del C+	CTTTGGGGGGTAGGAGCCACA TCTGCTAGGCGAGGAGGAGGA AGGGG	396	ATCTGC TAGGC GAGGA GGAGG	1396
3:51394249delT +	CTAAGTTCTTAGATTTTGGGGG ATTTTTTTTAAACGATGAGA AG	397	GGGAT TTTTTT TTTAAA CGAT	1397
6:30189477delT-	CTGGGTTGGGAAACCCATTGCT CGAGTGTTAAAAAAGACCG GAGAAT	398	CGAGT GGTTA AAAAA AGACC G	1398
8:144095973del C+	CCAAGGAAGAGAGGAGGCCAC GGTGAGACCACGGATAGCTGG GGGGT	399	CGGTG AGACC ACGGA TAGCTG	1399
1:46609235delC A-	CACAGATGCCGGGGTGTGTGT GTGTGTGTGTATTTTCACTGTG GGGT	400	TGTGTG TGTGTA TTTCA CTG	1400
9:66920942delA +	CCAGGAAGACTGTGAGGGTTT TTCTTTTTTTTTTAAGGGCCAA GGGT	401	TTCTTT TTTTTT TTAAG GGCC	1401
1:204954905del C+	CAGTGCAACCCCGCCTGGACT TCCATCCCCGGTCATCTTCTGG AT	402	ACTTCC ATCCCC GGTCAT CTT	1402
5:1074583delG-	CCCTGCTGCGCGTGAGCACG GTCCCCCACACCAAGAACTG GAGG	403	CGGTCC CCCCAC ACCAA GAAC	1403
9:93660330delA +	CACAGTTTTGGGAGTCTTTTTT TGGACACTTCTCCAGGAGGG ATTGAGG	404	GGACA CTTTCT CCAGG AGGGA	1404

15:42450759del A-	CAGGGGAAAAAATTTGTTTT GGTGTTTTTTGAGATATTCTTT GGAT	405	TGGTGT TTTTTG AGATA TTCT	1405
2:238170406del A-	CCATGGTTGTAAATAAAGGTTT CTCTTTTTTTCCTAGTCTTTTG AGT	406	TCTCTT TTTTTT CCTAGT CTT	1406
15:75207463del C+	CACAGGGGGGCAGTGCTGGGG GGTACAGGGAAGTGGACTGTC TCGGAT	407	GGTAC AGGGA ACTGG ACTGTC	1407
14:20992723del T+	CTAGGATGGGGATTCTTGGA CATTGCATATGCATTTTTTTTA AAGAGG	408	TTGCAT ATGCAT TTTTTT TTA	1408
6:28534234delA +	CCATGCAGGAAGAGAGTGTGG TGAGAAGATGGGTATTCCTTTT TTTGGAG	409	AGAAG ATGGG TATTCC TTTTT	1409
1:52055208delT-	CCCAGATTTTTTTTTTCAAAC CTGAAGGAAGTGATGTTAGAC GGAT	410	CTCTGA AGGAA GTGAT GTTAG	1410
3:100355352del T+	CCTAGGAAGGACAAGGCCGAA TTAAAAAAAATCTTCATTA GAAT	411	ATTAA AAAAA AATCTT CATT	1411
19:47416569del C-	CAATGGGTCTCCTTCCCTGACG CCCCATCCCTGGGGTTGGTGGG GGGGT	412	CCCCAT CCCTGG GGTTG GTGG	1412
1:25563141delT +	CATGGCCAATCCCCCTTCCCCG TCAGGACTCACAGCTCTTCAAG GGG	413	CGTCA GGACT CACAG CTCTTC	1413
1:46609235delC A-	CACAGATGCCGGGGTGTGTGT GTGTGTGTGTATTTTCACTGTG GGG	414	GTGTGT GTGTGT ATTTTC ACT	1414
4:139266508del T-	CTTAGTTTAAAAAAAAGAC TTATTTTCTAGAAAACGTTAAT GGGT	415	CTTATT TTCTAG AAAAC GTTA	1415
1:204954905del C+	CCGGGGATGGAAGTCCAGGCG GGGGTTGCACTGGAGCGTCAA AGGAG	416	GGGGG TTGCAC TGGAG CGTCA	1416
10:26173831del A+	CAGAGTGTATCAGACTCCAAA AAAATGAATAATGTGTATGAG GAAG	417	AAAAA ATGAA	1417

			TAATGT GTATG	
12:45920608del A-	CAGGGATTAGGGATTGTTGGGTTT TTTTTTTTTCTCTTTTAAATACT AGAAT	418	TTTTTT TTCTCT TTTTAA TAC	1418
11:112033459del A+	CACAGTTCAGAGATGGGAAAA AAAGTGGGTGAGAAGCTAAGT GAAG	419	AAAAA GTGGG TGAGA AGCTA A	1419
9:35043653delC +	CTGGGTTCTGACAGAGAAGCT GGGGATTGGCAGGGGGTGGCA TCGGAGG	420	GGGAT TGGCA GGGGG TGGCAT	1420
11:70468159del T-	CACAGAAAAGAAAAAAAAAAAA GGAAAAAAAAATAAAGTGTGTG CCTTGGGT	421	AAAAA AAATA AAGTG TGTGCC	1421
1:25563141delT +	CATGGCCAATCCCCCTTCCCCG TCAGGACTCACAGCTCTTCAAG GGGG	422	GTCAG GACTC ACAGC TCTTCA	1422
19:47416569del C-	CATGGACCAGGTGGCCTTCTCT CTGCACCCCCCACCACCCCA GGGAT	423	CTGCAC CCCCC ACCAA CCCC	1423
11:44265074del G-	CAGAGCCAGGGGTGGGCATG AGGGGACATGCAGGCAGGCAC CGGGT	424	GAGGG GACAT GCAGG CAGGC A	1424
1:26780313delC +	CATAGTGCTATACAACCTTCTCC AGGCGGCTGAAGGGGGTGTGG CCAGAAT	425	GGCGG CTGAA GGGGG TGTGGC	1425
5:177248268del A+	CCGGGATGCCTGCCTCTAAAA AATGCAGGGTGAACGCGGTGG AGGAG	426	AAATG CAGGG TGAAC GCGGT G	1426
1:25563141delT +	CATGGCCAATCCCCCTTCCCCG TCAGGACTCACAGCTCTTCAAG GGGGGG	427	CAGGA CTCACA GCTCTT CAAG	1427
4:17624016delT +	CCAAGTAAGTATTTTTTTTTGT CTTTAGCAAAGTTTAGACTGTG AAT	428	GTCTTT AGCAA AGTTTA GACT	1428

14:73739070del G-	CCTTGCGCAGTTCTGGGTTCAT ATCTGGGTTGGGGGAAGGGG TAGGGT	429	ATCTGG GTTGG GGGA AGGGG	1429
6:80010913delA +	CCCTGCGGAATTTAAACCTCCA AAAAAGCAGCTGCTTTCAGAG GAGG	430	CAAAA AAGCA GCTGCT TTCAG	1430
10:29471187del C-	CAGTGTTTCCAGGGGGGATGG TGGTGCACCTCGGGAGGCGGG AAGAGG	431	TGGTGC ACTCG GGGAG GCGGG	1431
2:239081127del G-	CAACGTCAACATGGCTTTCACC GGCGGCCTGGACCCCCCATGG GAG	432	ACCGG CGGCCT GGACC CCCCA	1432
22:37373064del G-	CCCAGCAGAAGCTGTGACCCC CCCTTCCTCCCTGGTGAGGTCG GAG	433	CCCCCT TCCTCC CTGGTG AGG	1433
4:92304602delA +	CACCGTGACCTCAAACCTCTTTG GACTGTTTGAAAAAATAAT TGGAAG	434	GACTGT TTGAA AAAAA AAAAT	1434
16:28836029del G+	CTCTGGGGCACCAGCGCCTTGGG GGGGCCCCCATGACTCTGGGG TGGGT	435	GGGGG CCCCCA TGA CTC TGGG	1435
8:22082312delC +	CTGGGGGGCCCCAATAAATTA CATTCTTGAGAGAGCATAGTGT GTGGGG	436	ATTCTT GAGAG AGCAT AGTGT	1436
1:100872563del A-	CTTTGTGCATTTAGTTCCGCAT ATGATGGTTTTTTTTTACATTA AAGAGT	437	TGATG GTTTTT TTTTAC ATTA	1437
5:140669517del A+	CTGCGAGCTGTGGTGGTGGAT GACTACCGTCGGCGCAAAAAA AGGGAGG	438	ACTACC GTCGG CGCAA AAAAA	1438
12:122603073del A+	CTCTGTACGTGTCTACAGCAAA ACACGTTTTTCGAAAAAACTG AAG	439	AAAAC ACGTTT TCGAA AAAAA	1439
5:37064968delA +	CCCAGGTTCCACTTACTCTTCA TAAACTGATTTTTTTTGCCAG AAT	440	CATAA AACTG ATTTTT TTTGC	1440
12:7089414delG -	CCTCGGTCCTACCCCTGACCT GCGCTGCAACTACAGCATCCG GGTGGAG	441	CGCTGC AACTA	1441

			CAGCA TCCGG	
19:4816453delG -	CTGTGGGGAGGGCGGTGGGGG GTGCCAGCCTGCCATGCGTGCA GGGG	442	GGTGC CAGCCT GCCAT GCGTG	1442
4:38689855delC +	CGGGGGGACACTGAGTTCATT AAGGGGGGTGACATTTCTCA GGAT	443	TTAAG GGGGG TGACAT TTCTT	1443
2:239081127del G-	CATGGCTTTCACCGGCGGCCTG GACCCCCCATGGGAGACGCTG AGT	444	CTGGA CCCCC ATGGG AGACG	1444
2:205301574del A+	CATGGAAAATAAAGCCAGGAA AGTCAAAAAACGAAAGAGAAG GAGAAG	445	AGTCA AAAAA CGAAA GAGAA G	1445
19:4816453delG -	CTGTGGGGAGGGCGGTGGGGG GTGCCAGCCTGCCATGCGTGCA GGGGT	446	GTGCC AGCCT GCCAT GCGTG C	1446
9:32633586delT-	CTTGGCCTTTTTTTGATGTGCTT TAGCAAAGGTTGGACTGAATG GGG	447	CTTTAG CAAAG GTTGG ACTGA	1447
1:27550358delT-	CCATGGCCATGCCAGAGGTAA AAAACGACGGCGGCGGAAGCA GAAG	448	AAAAA ACGAC GGCGG CGGAA G	1448
20:32434639del G+	CATAGAGAGGCGGCCACCACT GCCATCGGAGGGGGGGTGGCC CGGGT	449	TGCCAT CGGAG GGGGG GTGGC	1449
8:22082312delC +	CTGGGGGGCCCCAATAAATTA CATTCTTGAGAGAGCATAGTGT GTGGGGG	450	TTCTTG AGAGA GCATA GTGTG	1450
16:88624733del C+	CCTGGGGGGGTCTGGCTGGC CGCTCCTTCCTCCTGGCTTCCC GAGGGT	451	GCTCCT TCCTCC TGGCTT CCC	1451
5:140669517del A+	CTGCGAGCTGTGGTGGTGGAT GACTACCGTCGGCGCAAAAAA AGGGAG	452	GACTA CCGTCG GCGCA AAAAA	1452

6:30653271delT-	CTAAGAAAATGCCCAAAAAAT AGGCAAAACACGAGAAGAGCT AGGGT	453	TAGGC AAAAC ACGAG AAGAG C	1453
14:20992723del T+	CGGAGATCCTCTTTAAAAAAA AATGCATATGCAATGTCCCAA GAAT	454	AAAAT GCATAT GCAAT GTCCC	1454
X:129805329del T-	CTCAGCCGAGGCTTAATGGAA GAACTGGTTAGCATTTTTTTT TTTGAGG	455	ACTGGT TAGCAT TTTTT TTT	1455
3:127016548del C+	CTCTGCCCCGGGGGTGTCAGGC ACCGGATCTCACGGGAGTTCCT CCTGGAG	456	CGGAT CTCACG GGAGT TCCTC	1456
10:29471187del C-	CCAGGGGGGATGGTGGTGCAC TCGGGGAGGCGGGAAGAGGAA GAAG	457	ACTCG GGGAG GCGGG AAGAG G	1457
3:51380173delC +	CTGGGGGGTATCACCCAGAGG GTGGTGGAAGGCGTCAAAGTG TAGGGAG	458	TGGTG GAAGG CGTCA AAGTG T	1458
1:1354730delG-	CCCCGGGGGGCGGCTCCGCGT GGGGTTCGGCGACCGTCAGGT GGAAG	459	TGGGG TTCGGC GACCG TCAGG	1459
9:35043653delC +	CTGGGTTCTGACAGAGAAGCT GGGGATTGGCAGGGGGTGGCA TCGGAG	460	GGGGA TTGGCA GGGGG TGGCA	1460
12:6602380delT-	CCTCGGGACCCTAAAATCCCTA AGAGCAAGCGCCAAAAAAGGA GGTGAGT	461	GAGCA AGCGC CAAAA AAGGA G	1461
7:997675delG-	CCCTGATGCGGGAGCTGGATG AGGAGGGCTCTGATCCCCCTG CCGGGG	462	GGAGG GCTCTG ATCCCC CCTG	1462
6:30189477delT-	CTGGGTTGGGAAACCCATTGCT CGAGTGGTTAAAAAAGACCG GAG	463	GCTCG AGTGG TTAAA AAAAG A	1463

20:32434639del G+	CGGAGGGGGGGTGGCCCGGGT GGAGGTGGCGGCGGGGCCACC GATGAGG	464	GAGGT GGCGG CGGGG CCACC G	1464
16:28836029del G+	CAGAGTCATGGGGGCCCCCCC AAGGCGCGGTGCCCCAGAGTG GGGT	465	CCAAG GCGCG GTGCCC CAGAG	1465
22:37373064del G-	CTGTGACCCCCCTTCCTCCCT GGTGAGGTCGGAGCCAGAGGG CTGGGG	466	GGTGA GGTCG GAGCC AGAGG G	1466
19:45052871del C+	CCCTGGGAGGTGGCTTACATG GTGGTGAGCAGAGGGGGGTGT AGTCGGGG	467	GGTGA GCAGA GGGGG GTGTA G	1467
6:80010913delA +	CCCTGCGGAATTTAAACCTCCA AAAAAGCAGCTGCTTTCAGAG GAG	468	CCAAA AAAGC AGCTG CTTTCA	1468
19:49347216del G-	CCAGGAAGAAGGCATGGGGGG GCCACGATCATATAGCTCTCGG AGG	469	GGGCC ACGAT CATATA GCTCT	1469
11:2130529delG -	CATGGGGGGGGGTTTAATTTG GTTTCTGAGCGCATAAAGCTAA GGAGGGG	470	TTCTGA GCGCA TAAAG CTAAG	1470
20:32453753del G-	CGTAGCTCCCAGAGCTGTAGG GGGGGACTAAAAGGAGGGCAA GAGG	471	GGGGG GGACT AAAAG GAGGG C	1471
11:2130529delG -	CATGGGGGGGGGTTTAATTTG GTTTCTGAGCGCATAAAGCTAA GGAGG	472	GTTTCT GAGCG CATAA AGCTA	1472
11:44265074del G-	CGGTGCCTGCCTGCATGTCCCC TCATGCCCACCCCCTGGCTCTG GGG	473	CCTCAT GCCCA CCCCCT GGCT	1473
15:89200730del A+	CAAAGCATGCAGAGTGCTATTT CTTTTTTTTTCTCTTGACCAGAA G	474	TTTCTT TTTTTT TCTCTT GAC	1474
17:36943098del C+	CCGTGCGAGACCCCGCTACCA CACGGCCGCTCGTTCATTTG GGGGGT	475	ACGGC CGCCTC	1475

			GTTCAT TTCG	
8:66430512delT +	CCTCGCCGTATATCCAACATTA AAAAGAAAAAAAAGGCTGTTT AAGAAT	476	AAAAG AAAAA AAAGG CTGTTT	1476
5:140852886del T+	CTATGTCATCAATAATCATAAA ACGTATTTTTTTTTTGAGTCAG AGT	477	AAACG TATTTT TTTTTT GAGT	1477
3:51380173delC +	CTGGGGGGTATCACCCAGAGG GTGGTGGAAGGCGTCAAAGTG TAGGGAGT	478	GGTGG AAGGC GTCAA AGTGT A	1478
11:112033459del A+	CCATGACCATGGGACAGTTC AGAGATGGGAAAAAAGTGGG TGAGAAG	479	GAGAT GGGAA AAAAA GTGGG T	1479
9:137838509del C+	CAGGGGGCTCTGCTCTCCCTTG CCTGACAGGAGACGCACTCGG CCCGAGT	480	CTGAC AGGAG ACGCA CTCGGC	1480
10:46809223del T-	CAGAGTGAGACTCCCTCTCAA AAAAAAAAAAGAGAGAGAG CGAGAAT	481	AAAAA AAAAA AGAGA GAGAG C	1481
X:12977149delT +	CAGAGTGCCATTTTTTTTTTGTT CAAATGATTTTAATTATTGGAA T	482	TGTTCA AATGA TTTTAA TTAT	1482
1:71404165delT-	CAGGGAAAAAATAATATATA TATATATAAATACCCCTACATT TGAAG	483	TATATA TAAAT ACCCCT ACAT	1483
1:156745312del A-	CCAAGTCTTTTTTCGGGACCC ACGAGACGTGAGTGGAGGCCA AAGGGG	484	ACGAG ACGTG AGTGG AGGCC A	1484
18:36625553del C+	CTCAGGCACGAGGATGGCGAT GAGACCACGGAGCCACCCCA GTGGGT	485	GAGAC CACGG AGCCA CCCCCA	1485
2:186656358del G+	CCAAGAACATGACTATTTCAA GGGGGGACTGATGCAGTGTGA GGAAT	486	AGGGG GGACT GATGC AGTGT G	1486

16:88624733del C+	CCTCGGGAAGCCAGGAGGAAG GAGCGGCCAGCCAGGACCCCC CCAGGAG	487	AGCGG CCAGC CAGGA CCCCC	1487
2:1649188delG-	CCTGGCCCCGGGAGTCATTGGG GGGATCATGACAGAGAAGCAG GGGGGGT	488	GGATC ATGAC AGAGA AGCAG G	1488
20:49636246del A-	CACCGCCACCAAGAAAGCAGT CCGATGAGATTTTTTTGGAGG GGGGAG	489	CGATG AGATTT TTTTTG GAGG	1489
12:109581435del C+	CCCTGCCGAGCCTGGATATCGT AGTGTGGTCGGAGCTGCCCCC GGGG	490	GTAGT GTGGTC GGAGC TGCCC	1490
X:154409207del A-	CACAGCCTCTTCCTCTTTTTTTC CCCTCCTAGCCCTATTGAGGCA GGAG	491	CCCCTC CTAGCC CTATTC AGG	1491
22:37373064del G-	CTGTGACCCCCCTTCCTCCCT GGTGAGGTCGGAGCCAGAGGG CTGGGGG	492	GTGAG GTCGG AGCCA GAGGG C	1492
15:44711583del CT+	CTCCGTGGCCTTAGCTGTGCTC GCGCTATCTCTTTCTGGCCT GGAGG	493	GCGCT ATCTCT CTTTCT GGCC	1493
12:7089414delG -	CCTCGGTCCTACCCCCTGACCT GCGCTGCAACTACAGCATCCG GGT	494	CCTGCG CTGCA ACTAC AGCAT	1494
11:66271672del A+	CAGAGTGAGACCTTATTGCTAA AAAAAAATAAAAAATAAACCAA GGGAT	495	AAAAA AAATA AAAAT AAACC A	1495
1:154963387del G-	CAAAGGCACAAAGTTTAAACA TGGGGGGGCGGGTGTTGAGAG GGGT	496	CATGG GGGGG CGGGT GTTGA G	1496
19:47416569del C-	CCAGGTGGCCTTCTCTCTGCAC CCCCCACCAACCCAGGGAT GGGG	497	ACCCCC CCACC AACCC CAGGG	1497
2:200818782del A+	CAAGGAGGAGATGAAAAAAC AACATCCCAGAGCCAGTTGTC ATCGGAAT	498	CATCCC AGAGC	1498

			CAGTTG TCAT	
3:100355352del T+	CTAGGAAGGACAAGGCCGAAT TAAAAAAAAAATCTTCATTAA GAAT	499	ATTAA AAAAA AATCTT CATTA	1499
8:10726136delG -	CCAAGGGCCAGGGAGCCCAGG GGTGGCTACAGTGGAGAGGGC TTGGGG	500	GGTGG CTACA GTGGA GAGGG C	1500
11:2130529delG -	CATGGGGGGGGGTTTAATTTG GTTTCTGAGCGCATAAAGCTAA GGAG	501	GGTTTC TGAGC GCATA AAGCT	1501
12:107544001del C+	CCCTGCTTGTGGTGCAGGGGGT GCCATTGCCGTGGTGGCTTGGA TTGAGG	502	CCATTG CCGTG GTGCTT GGCA	1502
1:157697170del T-	CACAGCATCAAAAAAGGAGCC TGAGATCTCAGATACGTGTACA GAGT	503	CTGAG ATCTCA GATAC GTGTA	1503
16:28836029del G+	CTGGGGCACCGCGCCTTGGGG GGGCCCCCATGACTCTGGGGT GGGT	504	GGGGG CCCCCA TGACTC TGGG	1504
16:88624733del C+	CCTGGGGGGGTCTGGCTGGC CGCTCCTTCCTCCTGGCTTCCC GAGG	505	CCGCTC CTTCCT CCTGGC TTC	1505
15:44711583del CT+	CTCCGTGGCCTTAGCTGTGCTC GCGCTATCTCTTTCTGGCCT GGAG	506	CGCGCT ATCTCT CTTTCT GGC	1506
17:61482984del C+	CGTAGGGAGGGGGGAACGGAA ATAGTGATCCTCCCCACCGAA GAGGGG	507	TAGTG ATCCTC CCCCAC CGAA	1507
1:156673012del G-	CTCTGCATCTACAGCAGGAGA GGGTGCCTGAGGTGTGGGGGG ATGGGGG	508	GGTGC CTGAG GTGTG GGGGG A	1508
16:88624733del C+	CCTCGGGAAGCCAGGAGGAAG GAGCGGCCAGCCAGGACCCCC CCAGGAGG	509	GCGGC CAGCC AGGAC CCCCC	1509
8:10726136delG -	CAAGGGCCAGGGAGCCCAGGG GTGGCTACAGTGGAGAGGGCT TGGGG	510	GGTGG CTACA GTGGA	1510

			GAGGG C	
2:1649188delG-	CCTGGCCCGGGAGTCATTGGG GGGATCATGACAGAGAAGCAG GGGGGG	511	GGGAT CATGA CAGAG AAGCA G	1511
19:38730431del T+	CCAAGAAAAAAAAAATCAATCAG AATAAACTCAAAAAAAAAAGGT AGGGGG	512	AATAA ACTCA AAAAA AAAGG T	1512
X:91436820delT +	CTTTGTGATAAGGGGTTATTTT ATGCTAATTCACAAGTTTTTTT TGAAG	513	ATGCTA ATTCAC AAGTTT TTT	1513
19:38730431del T+	CCAAGAAAAAAAAAATCAATCAG AATAAACTCAAAAAAAAAAGGT AGGGGGGAG	514	TAAACT CAAAA AAAAA GGTAG	1514
8:66453963delT +	CTTAGGGAAAAGATATGGTGAA AAAAAAGAAATGCTACTCGGT AGGAAG	515	AAAAA AGAAA TGCTAC TCGGT	1515
7:44080815delG -	CCCAGGCTCTCTGTTAACCAGC TTATGTCCAGCAGAGCTGGGG GGT	516	AGCTTA TGTCCA GCAGA GCTG	1516
19:38730431del T+	CCAAGAAAAAAAAAATCAATCAG AATAAACTCAAAAAAAAAAGGT AGGGG	517	GAATA AACTC AAAAA AAAAG G	1517
12:7089414delG -	CCCGGATGCTGTAGTTGCAGCG CAGGTCAGGGGGTAGGACCGA GGGT	518	CGCAG GTCAG GGGGT AGGAC C	1518
20:57652293del G-	CGTAGCACGTGGCGCTGATGC CCGAGTTACTGCTGGGGGGCA GGGG	519	GCCCG AGTTAC TGCTGG GGGG	1519
1:156745312del A-	CCAAGTCTTTTTTTCGGGACCC ACGAGACGTGAGTGGAGGCCA AAGGGGT	520	CGAGA CGTGA GTGGA GGCCA A	1520
20:49636246del A-	CACCGCCACCAAGAAAGCAGT CCGATGAGATTTTTTTTGGAGG GGGGAGG	521	GATGA GATTTT TTTTGG AGGG	1521

10:100827567del C+	CAGCGGCAGGGGCGGAGCCCC GGGGGCGGCACTATAATAATA AGGGG	522	CGGGG GCGGC ACTATA ATAAT	1522
19:11453958del T-	CAGTGGGGCTGGAAGCAGAAA CAAAATGAAAAAAAAGGGGGG TGGGAGG	523	AAAAT GAAAA AAAAG GGGGG T	1523
X:130056036del C+	CCTGGAGGCTTGGACGACAGA TCCCCCAGGCTCCTCTGAGAC TGTGGAG	524	CCCCA GGCTCC TCTGAG ACT	1524
11:44265074del G-	CCAGGGGGTGGGCATGAGGGG ACATGCAGGCAGGCACCGGGT CGCAGGGG	525	ATGCA GGCAG GCACC GGGTC G	1525
20:49636246del A-	CACCGCCACCAAGAAAGCAGT CCGATGAGATTTTTTTTGGAGG GGGG	526	TCCGAT GAGAT TTTTTT TGGA	1526
19:19110944del G+	CTGGGCCCATGGAACGGTGGG GGGTGCGCTTGATTCTACTTCA GGAG	527	GGGGT GCGCTT GATTCT ACTT	1527
2:66568967delT T+	CAAAGTCAACAGATAGTGCCA AAAGACCCTTAAAAAAAACA GGAT	528	CAAAA GACCCT TAAAA AAAAA	1528
2:66568967delT +	CAAAGTCAACAGATAGTGCCA AAAGACCCTTAAAAAAAACA GGAT	529	CAAAA GACCCT TAAAA AAAAA	1529
20:59945733del T+	CTCTGACATGGTTTTTTTTTCTT TTTTGAGGGGCATTTTAAACTT AGAGG	530	TTTTGA GGGGC ATTTTA AACT	1530
6:31958429delG -	CCCTGAACTAGGAGCCACCAT GTTGGTGATACCCCGGACTGA GCGAGG	531	TTGGTG ATACCC CCGGA CTGA	1531
8:10726136delG -	CCCAGGGGTGGCTACAGTGGA GAGGGCTTGGGGCGTACTCCG GTGAGT	532	GAGGG CTTGGG GCGTA CTCCG	1532
19:19110944del G+	CTGGGCCCATGGAACGGTGGG GGGTGCGCTTGATTCTACTTCA GGAGG	533	GGGTG CGCTTG ATTCTA CTTC	1533

2:1649188delG-	CCCCGCTCCTGGCCCCGGGAGTC ATTGGGGGGATCATGACAGAG AAG	534	GTCATT GGGGG GATCAT GACA	1534
4:157221000del A+	CAAAGAAGGAAGAGGAGGAA AAGGAAAAAAAAAGGGGTATAT TGTGGAT	535	AGGAA AAAAA AGGGG TATATT	1535
17:61482984del C+	CGTAGGGAGGGGGGAACGGAA ATAGTGATCCTCCCCACCGAA GAGGGGG	536	AGTGA TCCTCC CCCACC GAAG	1536
17:61482984del C+	CGTAGGGAGGGGGGAACGGAA ATAGTGATCCTCCCCACCGAA GAGG	537	AATAG TGATCC TCCCC ACCG	1537
3:114339156del G-	CCTGGGGGGGCCAGCGCGGGCA CCTGGGGGTGTGCCTGCAGGG GGGT	538	CACCTG GGGGT GTGCCT GCAG	1538
16:88624733del C+	CTGGGGGGGTCTGGCTGGCC GCTCCTTCCTCTGGCTTCCCG AGGGT	539	GCTCCT TCCTCC TGGCTT CCC	1539
5:159203632del TC-	CCAAGAAAAAAAAAAAAAGAAA AAAAAAACAACATGGCTGCAA AGGAG	540	AAAAA AAACA ACATG GCTGC A	1540
19:2038032delC-	CACTGCCCATATCTGTGGACTG CCCCTTCCAAAGACCCCTGGGG GGGT	541	GCCCCT TCCAA AGACC CCTGG	1541
11:72237704del C+	CCGAGGGCAGTCCCCGGGGGG CTGCAGCTCCAGGGGGCCTGG GAGGAG	542	CTGCA GCTCCA GGGGG CCTGG	1542
2:1649188delG-	CCTGGCCCCGGGAGTCATTGGG GGGATCATGACAGAGAAGCAG GGGGG	543	GGGGA TCATGA CAGAG AAGCA	1543
9:135487197del G+	CGCAGCCAGAGGGCCAGGGGG TCCCACATCTGGCCGAAGGGCT TCGAGG	544	CCCAC ATCTGG CCGAA GGGCT	1544
22:21492040del C-	CAGAGTGCCAAGGGCCCAGAC ACCATGTGAGCAGCAGCCAGC GGGGGGG	545	CCATGT GAGCA GCAGC CAGCG	1545

3:127016548del C+	CCCGGGGGTGTACAGGCACCGG ATCTCACGGGAGTTCCTCCTGG AGG	546	GGATCT CACGG GAGTTC CTCC	1546
1:156673012del G-	CTCTGCATCTACAGCAGGAGA GGGTGCCTGAGGTGTGGGGGG ATGGGG	547	GGGTG CCTGA GGTGT GGGGG G	1547
20:49636246del A-	CACCGCCACCAAGAAAGCAGT CCGATGAGATTTTTTTTGGAGG GGG	548	GTCCG ATGAG ATTTTT TTTGG	1548
15:75207463del C+	CAGGGGGGCAGTGCTGGGGGG TACAGGGAAGTGGACTGTCTC GGAT	549	GGTAC AGGGA ACTGG ACTGTC	1549
19:11453958del T-	CAGTGGGGCTGGAAGCAGAAA CAAAATGAAAAAAAAAGGGGGG TGGGAG	550	CAAAA TGAAA AAAAA GGGGG G	1550
12:7089414delG -	CTCGGTCCTACCCCCTGACCTG CGCTGCAACTACAGCATCCGG GTGGAG	551	CGCTGC AACTA CAGCA TCCGG	1551
1:156673012del G-	CTCTGCATCTACAGCAGGAGA GGGTGCCTGAGGTGTGGGGGG ATGGGGGT	552	GTGCCT GAGGT GTGGG GGGAT	1552
10:100827567del C+	CGGGGGCGGCACTATAATAAT AAGGGGAACCTGGAAGTTAAC ACAGGAG	553	AGGGG AACCT GGAAG TTAACA	1553
15:78104908del T+	CCCTGACCTCTTTTTTTTTTCC GCTCTGTCCTGGGTGACCAGGG T	554	TTCCGC TCTGTC CTGGGT GAC	1554
8:11305000delA +	CATGGAGGGCGCTTCCCAGTA CTAAGCTATTACCACAAAAAA ATGGGAT	555	TAAGCT ATTACC ACAAA AAAA	1555
2:1649188delG-	CCTGGCCCCGGGAGTCATTGGG GGGATCATGACAGAGAAGCAG GGGG	556	GGGGG ATCATG ACAGA GAAGC	1556
11:72237704del C+	CCGAGGGCAGTCCCCGGGGGG CTGCAGCTCCAGGGGGCCTGG GAGG	557	GGCTG CAGCTC CAGGG GGCCT	1557

1:225403186del A-	CACTGACAGGGTCTGTACTTTT TTTTCTTTTGTAGTCAGGACT ATGGAG	558	TTTTCT TTTTGA GTCAG GACT	1558
4:62071046delT +	CTCAGACTTTTTTTTTTTAATGG GATTTTATAGGTCAGCCCAGGGG AG	559	TGGGA TTTTTA GGTCA GCCCA	1559
14:93241685del A-	CTCAGCCTCCATAAATCTAATG TCATTTTTTTTTTAATAAGTCCTA GGAG	560	TCATTT TTTTTT AATAA GTCC	1560
9:89377969delA -	CAAGGAATAAAGTTAAAAAAA AAAAAGAAAAAGAAAAAAGG TGAGT	561	AAAAA AAGAA AAAGA AAAAA G	1561
12:107544001del C+	CAGGGCCTCGGGCTCCAGTA CAGTGCCCCCTGCTTGTGGTGC AGGGGGT	562	GTGCCC CCTGCT TGTGGT GCA	1562
19:42334146del G+	CAGTGCCAGCCACCGGGTGTG TGTGCCTGCGAGCCGGGCTGG GGGGT	563	GTGTGC CTGCG AGCCG GGCTG	1563
8:10726136delG -	CATGGAGACGCCGGGGGACTG GCCAAGGGCCAGGGAGCCCAG GGGT	564	TGGCC AAGGG CCAGG GAGCC C	1564
15:42450759del A-	CCAGGGGAAAAAAATTTGTTTT GGTGTTTTTTGAGATATTCTTT GGAT	565	TGGTGT TTTTTG AGATA TTCT	1565
20:23085832del C-	CTGGGTGGGCGGGGGGAGGAC ACGCCTTACTCTAACTGGCACA AGGAG	566	ACGCCT TACTCT AACTG GCAC	1566
16:88624733del C+	CTGGGGGGGTCTCTGGCTGGCC GCTCCTTCCTCCTGGCTTCCCG AGG	567	CCGCTC CTTCCT CCTGGC TTC	1567
1:11024277delT +	CATTGGCCAAAGTGAAAATTTT TTTTTCTTTTGAAATCTAGTTT TGAAT	568	TTTTTC TTTTGA AATCTA GTT	1568
1:160371891del A+	CTGTGTGTCACTAGAGAAAAA AAAAACAAAAACCTAGATTCC GGAT	569	AAAAA AACAA AAACC TAGATT	1569

10:29471187del C-	CCGAGTGCACCACCATCCCCC TGGAAACACTGCAGGAAACAG GGGGG	570	CTGGA AACAC TGCAG GAAAC A	1570
3:127016548del C+	CGCTGCCAGGGCTCTGCCCCG GGGTGTCAGGCACCGGATCTC ACGGGAG	571	GGTGT AGGCA CCGGA TCTCA	1571
7:50463377delT-	CATGGAACCAAGTGGATTTTT GGCACTGTTTATTCTTTGCAGA AG	572	TTTGG ACTGT TATTCT TTG	1572
15:44711583del CT+	CCGTGGCCTTAGCTGTGCTCGC GCTATCTCTCTTTCTGGCCTGG AGG	573	GCGCT ATCTCT CTTTCT GGCC	1573
22:21492040del C-	CAGAGTGCCAAGGGCCCAGAC ACCATGTGAGCAGCAGCCAGC GGGGGG	574	ACCAT GTGAG CAGCA GCCAG C	1574
2:44209432delA +	CCTGGGCGACACACCAAGGCT CTGTCTCAAAAAAAAAAATT TAGAGAGG	575	GTCTCA AAAAA AAAAA ATTTA	1575
15:82436430del A+	CTAAGAGCGGGTCAAGAAATT GAAAAAAAAAACAAACATTT AAGGGGT	576	AAAAA AAAAA CAAAA CATTTA	1576
22:21492040del C-	CAGAGTGCCAAGGGCCCAGAC ACCATGTGAGCAGCAGCCAGC GGGGGGGG	577	CATGTG AGCAG CAGCC AGCGG	1577
15:44711583del CT+	CCGTGGCCTTAGCTGTGCTCGC GCTATCTCTCTTTCTGGCCTGG AG	578	CGCGCT ATCTCT CTTTCT GGC	1578
11:72237704del C+	CGAGGGCAGTCCCCGGGGGGC TGCAGCTCCAGGGGGCCTGGG AGGAG	579	CTGCA GCTCCA GGGGG CCTGG	1579
22:21492040del C-	CAGAGTGCCAAGGGCCCAGAC ACCATGTGAGCAGCAGCCAGC GGGGG	580	CACCAT GTGAG CAGCA GCCAG	1580
20:32453753del G-	CTCTGCCTCTTGCCCTCCTTTTA GTCCCCCCTACAGCTCTGGGA G	581	TTTAGT CCCCC CTACA GCTC	1581

3:51380173delC +	CCTTGCGCAGGGTCCGGGCAG GGAGGGCTGGGGGGTATCACC CAGAGG	582	GGAGG GCTGG GGGGT ATCACC	1582
X:130056036del C+	CCTGGGGGGATCTGTCGTCCAA GCCTCCAGGCCTCTCGGCAGG GGT	583	CAAGC CTCCAG GCCTCT CGGC	1583
10:29471187del C-	CAGGGAAGGAGCCCCCTGT TTCCTGCAGTGTTCAGGGGG GAT	584	GTTTCC TGCAGT GTTTCC AGG	1584
5:55164285delT +	CACTGTCTCCAAAAAAAATG TTTAAAATGAGACCAAACCCTC ATGGAG	585	TTAAA ATGAG ACCAA ACCCTC	1585
20:32434639del G+	CACTGCCATCGGAGGGGGGGT GGCCCGGGTGGAGGTGGCGGC GGGG	586	GTGGC CCGGG TGGAG GTGGC G	1586
1:16922012delC +	CACAGCCTCCCCCATGAGCTT GGGGCTGGCGGGGGCACAGGA GGTGGAG	587	GGGCT GGCGG GGGCA CAGGA G	1587
12:57466292del G+	CACGGGGAGCGGAAGGAGTTC GTGTGCCACTGGGGGGCTGCTC CAGGGAG	588	GTGCC ACTGG GGGGC TGCTCC	1588
3:51380173delC +	CCTTGCGCAGGGTCCGGGCAG GGAGGGCTGGGGGGTATCACC CAGAGGGT	589	AGGGC TGGGG GGTATC ACCCA	1589
3:127016548del C+	CGCTGCCAGGGCTCTGCCCCG GGGTGTCAGGCACCGATCTC ACGGGAGT	590	GTGTCA GGCAC CGGAT CTCAC	1590
10:100827567del C+	CCGGGGGCGGCACTATAATAA TAAGGGGAACCTGGAAGTTAA CACAGGAG	591	AGGGG AACCT GGAAG TTAACA	1591
10:29471187del C-	CCGAGTGCACCACCATCCCCC TGGAACACTGCAGGAAACAG GGGG	592	CCTGG AAACA CTGCA GGAAA C	1592
19:47416569del C-	CCCTGACGCCCCATCCCTGGGG TTGGTGGGGGGGTGCAGAGAG AAG	593	GGGTT GGTGG GGGGG	1593

			TGCAG A	
8:10726136delG -	CCAGGGGTGGCTACAGTGGAG AGGGCTTGGGGCGTACTCCGG TGAGT	594	GAGGG CTTGGG GCGTA CTCCG	1594
11:105003284del T-	CAGAGAGCACAGCACCTCATC ATGATTTTTTTACACAGTCTCA GGAAT	595	ATGATT TTTTTA CACAG TCTC	1595
7:93131425delT-	CTTTGACTTCATTTTTTTCCACA CATCCCCACTGTGCCAGAGGG AAT	596	ACACA TCCCCA CTGTGC CAGA	1596
12:121804752del C+	CGAAGGGCCGGGCTGCGGCGG GGGCTGCTGGTGGTGGTGGTG GGGGGT	597	GGCTG CTGGTG GTGGT GGTGG	1597
17:50356606del C+	CCTTGAGGGCTGGCGTGCCGG GGGGTAGCTGCCATACAGGTG GAAG	598	GGGGG GTAGCT GCCAT ACAGG	1598
18:36625553del C+	CGATGAGACCACGGAGCCACC CCCAGTGGGTGCCGGGACCGG AGGAGG	599	CCCAGT GGGTG CCGGG ACCGG	1599
11:44265074del G-	CAGGGGGTGGGCATGAGGGGA CATGCAGGCAGGCACCGGGTC GCAGGGG	600	ATGCA GGCAG GCACC GGGTC G	1600
4:62071046delT +	CCTGGGCTGACCTAAAAATCCC ATTAAAAAAGTCTGAG AGT	601	CCCATT AAAAA AAAAA AGTCT	1601
15:82436430del A+	CTAAGAGCGGGTCAAGAAATT GAAAAAACAACAAACATTT AAGGGG	602	GAAAA AAAAA ACAAA ACATTT	1602
X:129805329del T-	CCGAGGCTTAATGGAAGAACT GGTTAGCATTTTTTTTTTTGAG GGT	603	TGGTTA GCATTT TTTTTT TTT	1603
X:21994597delC +	CACAGAGAAATAAAAAGGAAC AAAAATCACATTCTAATGGGG GGGT	604	ACAAA AATCA CATTCT AATGG	1604
1:154963387del G-	CTAAGAGATGGTCAAAGGCAC AAAGTTTAAACATGGGGGGGC GGGT	605	ACAAA GTTTAA ACATG GGGGG	1605

X:80929822delA +	CCCTGTCTTGATTTTAGCATTTT TTTCCCAGTGTTAGGTGAAAAG GAT	606	TTTTTC CCAGT GTTAG GTGAA	1606
15:44711583del CT+	CCAGGCCAGAAAGAGAGATAG CGCGAGCACAGCTAAGGCCAC GGAG	607	AGCGC GAGCA CAGCT AAGGC C	1607
17:75494982del C+	CTGGGGCTGGAGGGGGGATCT CGGAGCCAGGCATGTCACCAT TGGGT	608	TCGGA GCCAG GCATGT CACCA	1608
17:58357800del C-	CGGAGGGACCCCCCGCCTTTTC CTCTGTGGGTGTGCGGCAGAG AGG	609	TTCCTC TGTGG GTGTCTG GGCA	1609
1:204954905del C+	CGGGGATGGAAGTCCAGGCGG GGGTTGCACTGGAGCGTCAAA GGAG	610	GGGGG TTGCAC TGGAG CGTCA	1610
2:44209432delA +	CTGGGCGACACACCAAGGCTC TGTCTCAAAAAAAAAAAAAATT AGAGAGG	611	GTCTCA AAAAA AAAAA ATTTA	1611
15:64675048del A+	CCTTGACTCCAGCCAAGGACA AGAAAAAGAAAGACAAAAAA AGAAG	612	CAAGA AAAAG AAAGA CAAAA A	1612
15:64675048del A+	CCTTGACTCCAGCCAAGGACA AGAAAAAGAAAGACAAAAAA AGAAGGAAT	613	AAAAA GAAAG ACAAA AAAAG A	1613
X:37453358delC +	CCTGGAGACAATCCACTGCTGT CAAACACTTCATCTGGTGGGG GGGT	614	GTCAA ACACTT CATCTG GTGG	1614
1:25563141delT +	CCAAGACTGCACCCCCCTTGA AGAGCTGTGAGTCCTGACGGG GAAGGGG	615	GAGCT GTGAG TCCTGA CGGGG	1615
12:57466292del G+	CGGGGAGCGGAAGGAGTTCGT GTGCCACTGGGGGGCTGCTCC AGGGAG	616	GTGCC ACTGG GGGGC TGCTCC	1616
1:25563141delT +	CCAAGACTGCACCCCCCTTGA AGAGCTGTGAGTCCTGACGGG GAAG	617	GAAGA GCTGTG AGTCCT GACG	1617

5:177248268del A+	CCTTGAGTGCAGCTCCTCCACC GCGTTCACCCTGCATTTTTTAG AGG	618	CCGCGT TCACCC TGCATT TTT	1618
10:29471187del C-	CCCCGAGTGCACCACCATCCCC CCTGGAAACACTGCAGGAAAC AGGGG	619	CCCTGG AAACA CTGCA GGAAA	1619
10:29471187del C-	CCCCGAGTGCACCACCATCCCC CCTGGAAACACTGCAGGAAAC AGGGGGG	620	CTGGA AACAC TGCAG GAAAC A	1620
10:29471187del C-	CCCCGAGTGCACCACCATCCCC CCTGGAAACACTGCAGGAAAC AGGGGG	621	CCTGG AAACA CTGCA GGAAA C	1621
22:21492040del C-	CCAAGGGCCCAGACACCATGT GAGCAGCAGCCAGCGGGGGGG GGG	622	GTGAG CAGCA GCCAG CGGGG G	1622
1:13392592delT +	CTAGGACACAGGTGGGTTTTTT TGTTTTTTTGTTTTTTTTIGATG GAG	623	TTGTTT TTTTGT TTTTTT TTG	1623
19:4816453delG -	CAGTGACTGTGGAAAGGCTGC TGGCTGTGGGGAGGGCGGTGG GGGT	624	CTGGCT GTGGG GAGGG CGGTG	1624
8:61499736delT +	CAAAGACTTGAGAGATGCTTTT TTTTCCCCCAGTGAGGGGACTG GAG	625	TTTTTT CCCCCA GTGAG GGGA	1625
8:61499736delT +	CAAAGACTTGAGAGATGCTTTT TTTTCCCCCAGTGAGGGGACTG GAGG	626	TTTTTC CCCCA GTGAG GGGAC	1626
19:35732101del G+	CAGGGGGCCAAAGGAGACACC CCCAAGGGCCTCCGGGATGGC GAGT	627	CCCCCA AGGGC CTCCGG GATG	1627
11:31790769del G-	CGAGGTGCCCATTTGGCTGACTG TTCATGTGTGTCTGCATATGTG GGGGGT	628	TCATGT GTGTCT GCATAT GTG	1628
3:48158363delT-	CCCAGGGACTACCTCGGCTTTT AATTTAAAAAAGAAAGT GGGT	629	TTAATT TAAAA AAAAA AAGAA	1629

11:44265074del G-	CCCAGAGCCAGGGGGTGGGCA TGAGGGGACATGCAGGCAGGC ACCGGGT	630	GAGGG GACAT GCAGG CAGGC A	1630
8:61499736delT +	CAAAGACTTGAGAGATGCTTTT TTTCCCCCAGTGAGGGGACTG GAGGAT	631	TTTCCC CCAGT GAGGG GACTG	1631
1:13392592delT +	CTAGGACACAGGTGGGTTTTTT TGTTTTTTTGTTTTTTTTGATG GAGT	632	TGTTTT TTTGTT TTTTTT TGA	1632
8:10726136delG -	CCGGGGGACTGGCCAAGGGCC AGGGAGCCCAGGGGTGGCTAC AGTGGAG	633	GGGAG CCCAG GGGTG GCTAC A	1633
16:28836029del G+	CCCAGAGTCATGGGGGCCCCC CCAAGGCGCGGTGCCCCAGAG TGGGG	634	CCCAA GGCGC GGTGC CCCAG A	1634
1:16135704delG -	CCTGGAGCATCCCCGCCGCA GCAGAGCCGAGTGTGGAAGTA CGAGG	635	AGCAG AGCCG AGTGT GGAAG T	1635
15:44711583del CT+	CGTGGCCTTAGCTGTGCTCGCG CTATCTCTCTTTCTGGCCTGGA GG	636	GCGCT ATCTCT CTTTCT GGCC	1636
17:58357800del C-	CTCGGAGGGACCCCCGCCTTT TCCTCTGTGGGTGTGCGGCAGA GAGG	637	TTCCTC TGTGG GTGTCG GGCA	1637
11:558069delG-	CTCCGAGAGGGCCTGTGGTTG GTGGTGGGGGGTGTCTTCTGCA GAAG	638	GGTGG TGGGG GGTGTC TTCTG	1638
2:70964443delC +	CCGCGACAGGGAAGGGAGCAC GTTGATGGGGGGTAGATCTGA GGGAG	639	CGTTGA TGGGG GGTAG ATCTG	1639
1:154582079del T-	CAGTGACTTAACAATATACATT CCTCATAAATAAAAAAAAAACA AGAAT	640	TCCTCA TAAAT AAAAA AAAAC	1640
16:28836029del G+	CCCAGAGTCATGGGGGCCCCC CCAAGGCGCGGTGCCCCAGAG TGGGGT	641	CCAAG GCGCG	1641

			GTGCCC CAGAG	
7:10982799delA +	CAGCGAGCCAAAAAATGGAAC CTTCGACGAAACCGACCACTTC TGGAT	642	CTTCGA CGAAA CCGAC CACTT	1642
14:50976115del T-	CCACGCCTTAAAAAATTGACAGT TGAAAAAAGAGTGACCAG AGG	643	AGTTG AAAAA AAAAG AGTGA C	1643
20:4727794delT +	CTCTGCCTCAAAAAAAGTA TAGAAAAATGAGTAGAAAGCA TTGAAT	644	TAGAA AAATG AGTAG AAAGC A	1644
11:44265074del G-	CCCGGTGCCTGCCTGCATGTCC CCTCATGCCCCACCCCTGGCTC TGGGG	645	CCTCAT GCCCA CCCCCT GGCT	1645
1:160371891del A+	CCCTGTGTGTCCTAGAGAAA AAAAAACAAAAACCTAGATT CCGGAT	646	AAAAA AACAA AAACC TAGATT	1646
1:204259283del C-	CAGTGGGTGAATCTGCGCCGG GGGTACCCCCGCTGAAGACC TTCGGAGG	647	GTACCC CCGCCT GAAGA CCTT	1647
2:70964443delC +	CCGCGACAGGGAAGGGAGCAC GTTGATGGGGGGTAGATCTGA GGGAGAAG	648	TGATG GGGGG TAGATC TGAGG	1648
4:92304602delA +	CCGTGACCTCAAACCTCTTTGGA CTGTTTGAAAAAATAATTG GAAG	649	GACTGT TTGAA AAAAA AAAAT	1649
4:62071046delT +	CCCTGGGCTGACCTAAAAATCC CATTAAAAAATAAGTCTGA GAGT	650	CCCATT AAAAA AAAAA AGTCT	1650
19:35732101del G+	CCAGGGGAGGGCAGGGGGCCA AAGGAGACACCCCAAGGGCC TCCGGGAT	651	GGAGA CACCCC CAAGG GCCTC	1651
X:80444260delT +	CCTGGACTTTTCAAGCATTTTT TTTGACAATTAAATTGGGTTGG AT	652	TTTTTT GACAA TTAAAT TGGG	1652
1:16135704delG -	CTTAGCGTCTCCTGGAGCATCC CCCGCCGCAGCAGAGCCGAGT GTGGAAG	653	CCGCC GCAGC AGAGC	1653

			CGAGT G	
1:25563141delT +	CCTGGGGGCCCCGCCAAGACTG CACCCCCCCTTGAAGAGCTGTG AGT	654	TGCACC CCCCCT TGAAG AGCT	1654
1:225403186del A-	CAGGGTCTGTACTTTTTTTTCT TTTTGAGTCAGGACTATGGAGC CGAGT	655	TTTTGA GTCAG GACTAT GGAG	1655
2:85544939delT-	CATGGTGTTGAGAGAAAAAA AAAATCTTTTAAAAGCTGCCAT CTGAGG	656	AAATCT TTTAAA AGCTG CCAT	1656
12:57466292del G+	CCTGGAGCAGCCCCCAGTGG CACACGAACTCCTTCCGCTCCC CGTGGAT	657	CACGA ACTCCT TCCGCT CCCC	1657
5:132815809del T+	CAAAGTGCTTAGACATTTTCAA TTTTTTTTTGCTAAATACTTTGG AAT	658	ATTTTT TTTTGC TAAAT ACTT	1658
2:97083988delG -	CCTTGAGAAAGACAGGAGGTT TCCTGAATACACCGACACCTGG GGGGT	659	TCCTGA ATACA CCGAC ACCTG	1659
X:63754409delT T-	CCCTGTCTCTGTCTGTGATTTT TTTTTCTCGGTGGCTCTCGGG AT	660	TTTTTT TTTCTC GGTGG CTCT	1660
6:31958429delG -	CTAGGAGCCACCATGTTGGTG ATACCCCGGACTGAGCGAGG AAGAGGAG	661	ACCCCC GGACT GAGCG AGGAA	1661
6:31958429delG -	CTAGGAGCCACCATGTTGGTG ATACCCCGGACTGAGCGAGG AAGAGG	662	ATACCC CCGGA CTGAG CGAGG	1662
1:1354730delG-	CCCGGGGGGCGGCTCCGCGTG GGGTTCGGCGACCGTCAGGTG GAAG	663	TGGGG TTCGGC GACCG TCAGG	1663
20:32453753del G-	CCCAGAGCTGTAGGGGGGGAC TAAAAGGAGGGCAAGAGGCAG AGGGT	664	CTAAA AGGAG GGCAA GAGGC A	1664
X:63754409delT -	CCCTGTCTCTGTCTGTGATTTT TTTTTCTCGGTGGCTCTCGGG AT	665	TTTTTT TTTCTC GGTGG CTCT	1665

11:18210242del T+	CGAAGGCCGAAAAAAAAGAC ATTGCTGAGTCCATTCTGAAA AGAAT	666	ATTGCT GAGTC CATTCT GGAA	1666
1:204259283del C-	CAGTGGGTGAATCTGCGCCGG GGGTACCCCCGCCTGAAGACC TTCGGAG	667	GGTAC CCCCGC CTGAA GACCT	1667
6:111661353del T-	CAAAGAGGCCACTTTTGGAAA ATAATACTTTTTTTTTTAGTTG AAT	668	AATAA TACTTT TTTTTT TTAG	1668
12:49040709del G-	CTTGGAGGAGAAGGTGCCAAA GCCTGGGCAGGGGTGGCTCCT GGGG	669	AAGCC TGGGC AGGGG TGGCTC	1669
3:42908691delT +	CAGTGTCTTCAGGGGTAGGAG GGGAAAAAACGGAATAACTA GGAAG	670	GGGGA AAAAA CGGAA ATAACT	1670
18:36625553del C+	CGATGAGACCACGGAGCCACC CCCAGTGGGTGCCGGGACCGG AGGAG	671	CCCCA GTGGG TGCCG GGACC G	1671
19:45052871del C+	CTGGGAGGTGGCTTACATGGT GGTGAGCAGAGGGGGGTGTAG TCGGGG	672	GGTGA GCAGA GGGGG GTGTA G	1672
2:131263505del A+	CTAAGAGAAAAGAAATATTTG GAGGATATTGAAAGTGTGAAA AAAAGAAT	673	GGATA TTGAA AGTGT GAAAA A	1673
10:29471187del C-	CCGAGTGCACCACCATCCCCC TGGAACACTGCAGGAAACAG GGG	674	CCCTGG AAACA CTGCA GGAAA	1674
19:45052871del C+	CTGGGAGGTGGCTTACATGGT GGTGAGCAGAGGGGGGTGTAG TCGGGGAT	675	TGAGC AGAGG GGGGT GTAGTC	1675
Insertions				
3:195781031_19 5781032insACC GGTGGATGCC GAGGAAGCG TCGGTGACAG GAAGAGGGG TGGTGTCACC	CTGAGGAAAAGCTGGTGACAG GAAGAGGGGTGGCGTGACCTG TGGAT	676	GGAAG AGGGG TGCGGT GACCT	1676

TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-				
3:195781031_19 5781032insACC GGTGGATGCC GAGGAAGCG TCGGTGACAG GAAGAGGGG TGGTGTACAC TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-	CGAGGAAGCGTCGGTGACAGG AAGAGGGGTGGTGTACCTGT GGAT	677	GGAAG AGGGG TGGTGT CACCT	1677
3:195781031_19 5781032insACC GGTGGATGCC GAGGAAGCG TCGGTGACAG GAAGAGGGG TGGTGTACAC TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-	CCGAGGAAGCGTCGGTGACAG GAAGAGGGGTGGTGTACCTG TGGAT	678	GGAAG AGGGG TGGTGT CACCT	1678
3:195781031_19 5781032insACC GGTGGATGCC GAGGAAGCG TCGGTGACAG GAAGAGGGG TGGTGTACAC TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-	CTGAGGAAGTGTCTGGTGACAG GAAGAGTCGTGGTGTACCGG TGGAT	679	GGAAG AGTCGT GGTGTG ACCG	1679

TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-				
6:167976333_16 7976334insA+	CAGGGGGAATGACCCCCACTG TCTTCTCCTtCCCCACACACTGC AGGGG	680	CTTCTC CTtCCC CACAC ACTG	1680
6:167976333_16 7976334insA+	CAGGGGGAATGACCCCCACTG TCTTCTCCTtCCCCACACACTGC AGGGGG	681	TTCTCC TtCCCC ACACA CTGC	1681
3:195781031_19 5781032insACC GGTGGATGCC GAGGAAGCG TCGGTGACAG GAAGAGGGG TGGTGTCACC TGTGGATACT GAGGAAAAG CTGGTGACAG GAAGAGGGG TGGCGTGACC TGTGGATACT GAGGAAGTGT CGGTGACAGG AAGAGTCGTG GTGTC-	CGTGGTGTCACCGGTGGATGCT GAGGAAGCGCCGGTGACAGGA AGAGT	682	TGAGG AAGCG CCGGT GACAG G	1682

The + indicates the target sequence in on the coding strand in the genome. The – indicates it is on the non-coding strand.

SEQ ID NO 683 (EGFR V769_D770insASV Target sequence)

CcacgctggcCACGCTGGCCATCACGTAGGCTTCCTGGAGGGAGGGAGAGG

SEQ ID NO: 1683: (EGFR guide RNA sequence)

CGTAGGCTTCCTGGAGGGAGG

SEQ ID NO: 1684: (EGFR guide RNA sequence)

TCACGTAGGCTTCCTGGAGGG

SEQ ID NO: 1685 (Muc4 guide RNA sequence)

GAAGAGTCGTGGTGTCACCG

SEQ ID NO: 1686 (EGFR L858R guide RNA sequence)

GATTTTGGGCgGGCCAAACTG

SEQ ID NO: 700MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHF KDLEKGCHSSIKLQRSFNKHG NVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIA**LENGTH:** 93**TYPE:** AMINO ACID**FEATURE:** I-TEVI DOMAIN**SEQ ID NO: 701**DATFGDTCSTHPLKEEIIKKRSETVKAKMLKLGP DGRKALYSKPGSKNGRWN PETHKF
CKCGVRIQTSAYTCSKCRN**LENGTH:** 77**TYPE:** AMINO ACID**FEATURE:** LINKER DOMAIN**SEQ ID NO: 702 [I-TEVI WT NUCLEASE DOMAIN AND LINKER DOMAIN]**MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHF KDLEKGCHSSIKLQRSFNKHG NVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIA DATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGP DGRKALYSKPGSKNGRWN PETHKFCKCGVRIQTSAYTCSKCRN**SEQ ID NO: 703**DATFGDTCSTHPLKEEIIKKRSETVKAKMLKLGP DGRKALYSKPGSKNGRWN PETHKF
CKCGVRIQTSAYTCSKCRN GGSGGS**LENGTH:** 83**TYPE:** AMINO ACID**FEATURE:** LINKER DOMAIN with GGSGGS

SEQ ID NO: 704 [I-TEVI WT NUCLEASE DOMAIN AND LINKER DOMAIN with GGSGGS]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNGGSGGS

SEQ ID NO: 710

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRR
RRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGV
HNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYV
KEAKQLLKVKAYHQDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMG
HCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYEFQIENVFQKQKKP
TLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEELDQIAKILT
IYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIF
NRLKLVKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELARE
KNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEA
IPLEDLLNNPFNYEVDHIIPRSVSFDNSFNKVLVKQEENSKKGNRTPFQYLSSSDSKISY
ETFKKHILNLAAGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNL
RSYFRVNNLDVKVK SINGGFTSFLRRKWKFKKERNKGYKHHAEALIANADFIFKEW
KKLDKAKKVMENQMFEKQAESMPEIETEYKEYEKFITPHQIKHIKDFKDYKYSHRVDK
KPNRELINDTLYSTRKDDKGNTLIVNNLNLGLYDKDNDKLKLINKSPEKLLMYHHPQ
TYQKLKLIMEQYGDEKNPLYKYEEETGNYLTKYSKKDNGPVIKKIKYYGNKLNALHLDI
TDDYPNSRNKVVKLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEA
KKLKKISNQAEFASFYNNDLIKINGELYRVIGVNNDLLNRIEVMIDITYREYLENMND
KRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG

LENGTH: 1,053

TYPE: AMINO ACID

FEATURE: *Staphylococcus aureus* Cas9

SEQ ID NO: 711

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
E
ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFG
NIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRILIYLA LAHMIKFRGHFLIEGDLNPDNS
D
VDKLFQQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGN
LIALSLGLTPNFKSNFDLAEDAKLQLSKD TYDDLDNLLAQIGDQYADLFLAAKNLSDA
ILLS DILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGY
A
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH
AILRRQEDFYPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEETITPWNFEE

VVDKGASASQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA
FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL
LKIHKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYT
GWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIAMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK
LITQRKFDNLTKAERGGSELDKAGFIKRQLVETRQITKHVAQILD SRMNTKYDENDKL
IREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEF
VYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
TGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW
DPKKYGGFDSPTVAYSVLVVAKEVGKSKKLKSVKELLGITIMERSSSFENPIDFLEAK
GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEK
LKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKH RDKPIRE
QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQL
GGD

LENGTH: 1,368

TYPE: AMINO ACID

FEATURE: *Streptococcus pyogenes* Cas9

SEQ ID NO: 712

MAAFKPNPINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGV RVFERAEV PKTGDSLAM
ARRLARSVRRLTRRAHRLRLARLLKREGVLQAADFDENGLIKSLPNTPWQLRAAAL
DRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVANNAHALQTGDFR
TPAELALNKFEEKESGHIRNQRGDYSHTFSRKDLQAE LILLFEKQKEFGNPHVSGGLKEGI
ETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKNTYTAERFIWLTCLNLRILEQGS
RPLTDTERATLMDEPYRKSCLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMK
AYHAISRALEKEGLKDKKSPLNLSSELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALL
KHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADKIR
NPVVLRLALSQARKVINGVVRRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREK
AAAKFREYFPNFVGEPSKDILKLRLYEQQHGKCLYSGKEINL VRLNEKGYVEIDHALP
FSRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKK
QRILLQKFDEDEGFKECNLNDTRYVNRFLCQFVADHILLTGKGKRRVFASNGQITNLLRG
FWGLRKVRAENDRRHHALDAVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGK
VLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSRPEAVHE
YVTPLFVSRAPNRKMSGAHKDTLRSARFVKHNEKISVKRVWLTEIKLADLENMVNY
KNGREIELYEALKARLEAYGGNAKQAFDPKDNPFYKKGQLVKAVRVEKTQESGVLL
NKKNAYTIADNGDMVRVDVFCKVDKKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDD
SYTFCFSLHKYDLIAFQKDEKSKVEFAYYINCDSSNGRFYLA WHDKGSKEQQFRISTQN
LVLIQKYQVNELGKEIRPCRLKKRPPVR

LENGTH: 1082

TYPE: AMINO ACID

FEATURE: *Neisseria meningitidis* Cas9

SEQ ID NO: 713

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKAENPKTGESLALPRRLARSARKRLAR
RKARLNHLKHLIANEFKLNIEDYQSFDESLAKAYKGLISPYELRFRALNELLISKQDFA
RVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEYLYKEYFQKFKENS
KEFTNVRNKKESYERCIAQSFLKDGLKLIFKKQREFGFSFSKKFEEVLVSAFYKRALKD
FSHLVGNCSSFTDEKRAPKNSPLAFMFVALTRIINLLNNLNKNTGILYTKDDLNTLLNEV
LKNGLTYKQTKKLLGLSDDYEFKREKGTIFYIEFKKYKEFIKALGEHNLSQDDLNEIAK
DITLIKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNISFKALKLITPLMLEGKKYDEA
CNELNLKVAINEDKKDFLPAFNETYYKDEVTPVVLRAIKEYRKVLNALLKKYGVHK
INIELAREVGKNHSQRAKIEKEQNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKE
FCAYSGEKIKISDLQDEKMLEIDHIYPYSRSFDDSYMNVLVFTKQNEKLNKTPFEAFG
NDSAKWQKIEVLAKNLPKKQKRILDKNYKDKEQKDFKDRNLNDTRYIARLVNLYTK
DYLDLPLSDDENTKLNLTQKGSKVHVEAKSGMLTSALRHTWGFSTKDRNNHLHHAI
DAVIAAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLKD
IDEIFVSKPERKKPSGALHEETFRKEEFYQSYGGKEGVKALELGKIRKVNGKIVKNGD
MFRVDIFKHKKTNKFYAVPIYTMDFAKLVLPNKAVARSKKGEIKDWILMDENYEFCS
LYKDSLILIQTKDMQEPEFVYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVI
AKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK

LENGTH: 984**TYPE:** AMINO ACID**FEATURE:** *Campylobacter jejuni* Cas9**SEQ ID NO: 714**

MTKKNYSIGLDIGTNSVGWAVITDDYKVPKMKVVLGNTDKKYIKKNLLGALLFDSG
ETAETRLKRTARRRYTRRNRLRYLQEIFAEMTKVDESFFYRLDESFLTDEKDFER
HPIFGNKADEIKYHQEFPTIYHLRKHLADSSEKADLRLVYLALAHMIKFRGHFLIEGELN
AENTDVQKIFADFGVYDRFTFDDSHLSEITVDAASILTEKISKSRLENLIKYYPTTEKNT
LFGNLIALALGLQPNFKMNFKLSEDAKLQFSKDSYNEDLEELLGKIGDDYADLFTSAKN
LYDAILLSGILTVDDNSTKAPLSASMIKRYAEHHEDLEKLKEFIKANKSELYHDIFKDET
KNGYAGYIENGVKQDEFYKYLKNTLSKIAGSDYFLDKIEREDFLRKQRTFDNGSIPHQIH
LQEMHAILRRQGDYYPFLKENQDRIEKILTFRIPYYVGPLARKDSRFSWAEYHSDEKITP
WNFDKVIDKEKSAEKFITRMTLNDLYLPEEKVLPKSHVYETYAVYNELTKIKYVNEQ
GKDSFFDSNMKQEIFDHVFKENRKVTKEKLLNYLNKEFPEYRIKDLIGLDKENKSFNAS
LGTYHDLKKILDKAFLDDKVNEEVIEDIITLTLFEDKDMIHERLQKYSIDFTADQLKKL
ERRHYTGWGRLSYKLLINGIRNKENNTILDYLIIDGGSANRNFMLINDDTLPFKQIIQS
QVVGDVDDIEAVVHDLPGSPAIIKKGILQSVKIVDELVKVMGDNPDNIVIAMARENQTTN
RGRSQSQRLKKLQNSLKLGSNILNEEKPSYIEDKVENSHLQNDQLFLYYIQNGKDMY
TGDELIDHLSYDIDHIIPQAFIKDDSIDNRVLTSSAKNRGKSDDVPSLDIVRARKAEW
VRLYKSGLISKRKFDNLTKAERGGTTEADKAGFIKRQLVETRQITKHVAQILDARFNTE
DENDKVIRDVKVITLKSNLVSQFRKDFEFYKVBREINDYHHAHDAYLNAVVGTTALLKKY
PKLASEFVYGEYKKYDVHKLIAKSSDDHSEMGAATAKYFFYSNLMNFFKRVIRYSNGK
VIVRPVVEYSKDTEDIAWDKKSNFRTICKVLSYPQVNIVKKVETQTGGFSKESILPKGDS
DKLIPRKTKKAYWDTKKYGGFDSPTVAYSVFVADVEKGKAKKLKTVKELVGISIMER
SFFEENPVEFLENKGYHNIREDKLIKLPKYSLFEFEGGKRLLASASELQKGNEMVIPGH
LVKLLYHAQRINSFNSTKYLDYVSAHKKEFEKVLSCVEDFANLYVDVEKNLSKIRAVA

DSMDNFSIEEISNSFINLLTLTALGAPADFNFLGEKIPRKRYTSTKECLNATLIHQSIITGLY
ETRIDL SKIGEE

LENGTH: 1377

TYPE: AMINO ACID

FEATURE: *Streptococcus pasteurianus* Cas9

SEQ ID NO: 715

MKYTLGLDVGIA SVGWAVIDKDNNKIIDLGVRCFDKAEESKTGESLATARRIARGMRR
RISRRSQRLRLVKKLFVQYEIIKDSSEFNRI FDTSRD GWKDPWELRYNALSRILKPYELV
QVLTHITKRRGFKSNRKEDLSTTKEGVVITSIKNNSEMLRTKNYRTIGEMIFMETPENS
NKRNVDEYIHTIAREDLLNEIKYIFSIQRKLGSPFVTEKLEHDFLNIWEFQRPFASGDSILS
KVGKCTLLKEELRAPTSCTSEYFGLLQSINNVLVEDNNTLTLNNDQRAKIIIEYAHFK
NEIKYSEIRKLLDIEPEILFKAHNLTHKNPSGNNEKSKFYEMKSYHKLKSTLPTDIWGKL
HSNKESLDNLFYCLTVYKNDNEIKDYLAQANNLDYIEYIAKLPTFNKFKHLSLVAMKRRI
PFMEKGYKYSDACNMAELDFTGSSKLEKCNKLTVEPIIENVTPV VIRALTQARKVINAI
IQKYGLPYMVNIELAREAGMTRQDRDNLKKEHENNRKAREKISDLIRQNGRVASGLDIL
KWRLWEDQGGRCAYSGKPIPVCDLLNDSL TQIDHIYPYSRSMDDSYMNKVLVLTDENQ
NKRSTPYEVWGSTEKWEDFEARIYSMHL PQSKEKRLNRNFITKDLD SFISRNLDNR
YISRFLKNYIESYLQFSNDSPKSCVVCVNGQCTAQLRSRWGLNKNREESDLHHALDAA
VIACADRKIIKEITNYNERENHNYKV KYPLPWHSFRQDLMETLAGVFISRAPRRKITGP
AHDETIRSPKHFNKGLTSVKIPLTTVTLEKLETMVKNTKGGISDKAVYNVLKNRLIEHN
NKPLKAF AEKIYKPLKNGTNGAIIRSIRVETPSYTG VFRNEGKGISDNLMVRVDVFKKK
DKYYLVPIYVAHMIKKELPSKAIVPLKPESQWELIDSTHEFLFSLYQNDYLVIKTKKGITE
GYRSCHRGTGSLSLMPHFANNKNVKIDIGVRTAISIEKYNVDILGNKSIVKGEPRRGME
KYN SFKSN

LENGTH: 1021

TYPE: AMINO ACID

FEATURE: *Clostridium cellulolyticum* Cas9

SEQ ID NO: 716

MKYKIGLDIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLARSARRRLR
RRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNDELARILLHL
AKRRGFRSNRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDPKFSLHKRNKEDN
YTNTVARDDLREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRPFASKDDIEKKVGFC
TFEPKEKRAPKATYTFQSFTVWEHINKLRLVSPGGIRALTDDERRLIYKQAFHKNKITFH
DVRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFL ELGAYHKIRKAIDSVYGGKAASF
RPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLADKVYDEELIEELLNLSFSK
FGHLSLKALRNILPYMEQGEVYSTACERAGYTFTGPKKKQKT VLLPNIPPIANPVVMRA
LTQARKVVNAIIKKYGPVSIHIELARELSQSFDERRKMQKEQEGRKKNETAIRQLVEY
GLTLNPTGLDIVKFLWSEQNGKCAYS LQPIEIERLLEPGYTEVDHVIPYSRSLDDSYTN
KVLVLTKENREKGNRTPAEYLG LGSERWQQFETVLTN KQFSKKRDRLLRLHYDENE
ENEFKNRNLDTRYISRFLANFIREHLKFADSDDKQKVYTVNGRITAHLSRWNFNKNR
EESNLHHA VDAAIVACTTPSDIARVTAFYQRREQNKELSKKTD PQFPQWPWFAD ELQA

RLSKNPKESIKALNLGNYDNEKLES LQPVFVSRMPKRSITGA AHQETLRRYIGIDERSGK
IQT VVKKKLSEIQ LDKTGHFPMY GKE SDPRTYEAIRQR LLEHNNDPKKAFQEPLYKPKK
NGELGPIIRTIKIIDTTNQVIPLNDGKTVA YNSNIVRV DVFEKDGKYYCVPIYTIDMMKGI
LPNKAIEPNKPYSEWKEMTEDYTRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYY
QTIDSSNGGLSLVSHDNNFSLRSIGSR TLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHS
KAGETIRPL

LENGTH: 1082

TYPE: AMINO ACID

FEATURE: *Geobacillus thermodenitrificans*T1 Cas9

SEQ ID NO: 720 [Cas12a]

TQFEGFTNLYQVSKTLRFELIPQGKTLKHIQE QGFIEEDKARNDHYKELKPIIDRIYKTYA
DQCLQLVQLDWENLSAAIDSYRKEKTEETR NALIEEQATYRNAIH DYFIGRTDNL TDAI
NKRHAEIYKGLFKAE LFNGKVLKQLGT VTTTEHENALLRSFDKFTTYFSGFYENRKNVF
SAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFP
FYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLF
KQILSDRNTLSFILEEFKSDEEVIQSFCYK TLLRNENVLETA EALFNELNSIDLTHIFISH
KKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQ RSLKHEDINLQEII SAAGK
ELSEAFKQKTSEILSHAHAA LDQPLPTTLKKQEEKEILKSQ LDSLGLYHLLDWFAVDES
NEVDPEFSARLTGIKLEMEPSLSFY NKARNYATKKPYSVEKF KLNFMPTLASGWDVN
KEKNNGAILFVKNGLYYL GIMPKQKG RYKALSFEPT EKTSEGF DKMYDYFPDA AKMI
PKCSTQLKAVTAHFQTH TTPILLSNNFIEPLEITKEIYDLN NPEKEPKKFQTAYAKKTGDQ
KGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFORIA
EKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQA
ELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPD TLYQELYDYVNHRLSHDLSDEA
RALLPNVITKEVSHEI IKDRRFTSDKFFFHVPITLNYQA ANSPSKFNQ RVNAYLKEHPETP
IIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREK ERVAARQAWSVVG TI
KDLKQGYLSQVIHEIVDLMIHYQAVVLENLNF GFKSKRTGIAEKAVYQQFEKMLIDKL
NCLVLKDYP AEKVGGVLN PYQLTDQFTSFAKMG TQSGFLFYVPAPYTSKIDPLTGFVDP
FVWKTIKNHESRKHFLEGFDFLHYDVKTGD FILHFKMNRNLSFQRGLPGFMPAWDIVFE
KNETQFDAKGTPFIAGKRIVPV IENHRFTGRYRDLYPAN ELIALLEEKGIVFRDGSNILPK
LLEND DSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVC FDSRFQNP EWPM
DADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAIQELRN

SEQ ID NO: 721 [CasX]

QEIKRINKIRRR LVKDSNTKKAGKTGPMK TLLVRVMTPDLRERLENLRKKPENIPQ PISN
TSRANLNKLLTDYTEMKKAILHVYWE EFQKDPVGLMSRVAQ PAKNIDQRKLIPVKDG
NERLTSSGFAC SQCCQPLYVYKLEQVNDKGK PHTNYFGRCNVSEHERLILLSPHKPEAN
DELVTYSLGKFGQRALDFYSIHVTRESNHPVKPLEQIGGNSC ASGPVGKALSDACMGA
VASFLT KYQDIILEHQKVIKKNEKRLANLKD IASANGLAFPKITLPPQPHTKEGIEAYNN
VVAQIVIWNLNLWQKLKIGRDEAKPLQRLKGFP SFPLVERQANEVDWWD MVCNVK
KLINEKKEDGK VFWQNLAGYKRQEALLPYLSSEEDRKKGKKFARYQFGDLLLHLEKK
HGEDWGKVYDEAWERIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEG
LKEADKDEFRC ELKLQKWYGD LRGPFAIEAENSILDISGFSKQYNCAFIWQKDG VKK
LNLYLII NYFKGGKLRFKKIKPEAFEANRFYTVINKKSGEIVPMEVNFNFDDPNL IILPLAF
GKRQGREFIWNDLLSLETGSLKL ANGRVIEKTLYNRRTRQDEPALFVALTFERREVLDS

SNIKPMNLIGIDRGENIPAVIALTDPEGCPLSRFKDSLGNPTHILRIGESYKEKQRTIQAAK
 EVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFENLSRGFGRQG
 KRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTCSNCGFTITSADYDR
 VLEKLKKTATGWMTTINGKELKVEGQITYYNYRKYRQNVVKDLSVELDRLSEESVNNDI
 SSWTKGRSGEALSLLKKRFSHRPVQEKFVCLNCGFETHADEQAALNIARSWLFLRSQEY
 KKYQTNKTTGNTDKRAFVETWQSFYRKKLKEVWKPAV

SEQ ID NO: 730 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Staphylococcus aureus* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDGRKALYSKPGSKNGRWNPEHFKFCKCGVRIQTSAYTCSKCRNMKRNYILG
 LDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVK
 KLLFDYNLLTDHSELGINPYEARVKGLSQKLSSEEFSAALLHLAKRRGVHNVNEVEED
 TGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYVKEAKQLLKV
 QKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEEL
 RSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYEFQIIENVFKQKKKPTLKQIAKEI
 LVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTIYQSSEDIQ
 EELTNLSELQTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVK
 KVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSKDAQK
 MINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLN
 PFNYEVDHIIPRSVSFDNSFNKVLVKQEENSCKGNRTPFQYLSSSDSKISYETFKKHILN
 LAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNL
 DVKKSINGGFTSFLRRKWKFKKERNKGYKHAEDALIINANADFIFKEWKKLDAKKV
 MENQMFEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDT
 LYSTRKDDKGNTLIVNNLNGLYDKDNDKLLKLINKSPEKLLMYHHDHPQTYQKLKLIME
 QYGDEKNPLYKYEEETGNLYTKYSKKDNGPVIKKIKYYGNKLNALHDITDDYPNSRNK
 VVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKISNQAE
 FIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASK
 TQSIKKYSTDILGNLYEVKSKKHPQIIKKG

SEQ ID NO: 750 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and *Staphylococcus aureus* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDGRKALYSKPGSKNGRWNPEHFKFCKCGVRIQTSAYTCSKCRNGGSGGSMK
 RNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRH
 RIQRVKKLLFDYNLLTDHSELGINPYEARVKGLSQKLSSEEFSAALLHLAKRRGVHNV
 NEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYVKEA
 KQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCT
 YFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYEFQIIENVFKQKKKPTLK
 QIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTIYQ
 SSEDIEELTNLSELQTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRL
 KLVKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNS
 KDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLE
 DLLNPFNYEVDHIIPRSVSFDNSFNKVLVKQEENSCKGNRTPFQYLSSSDSKISYETF
 KKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSY

FRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIKKEWKKL
DKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKN
RELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLLINKSPEKLLMYHHDHPQTYQ
KLKLIMEQYGDEKNPLYKYEEETGNLYTKYSKKDNGPVIKKIKYYGNKLNALHDITDD
YPNSRNKVVKLKLPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKL
KKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRP
PRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG

SEQ ID NO: 731 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Streptococcus pyogenes* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNMDKKYSIG
LDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRT
ARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLG
LTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDIL
RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDG
GASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRR
QEDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFEVVDKG
ASAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGE
QKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD
KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGR
LSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLH
EHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRER
MKRIEELGELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDV
DHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQLLNAKLITQRK
FDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKV
ITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDY
KVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY
GGFDSPTVAYSVLVVAKEVGKSKKLKSVKELLGITIMERSSEFEKNPIDFLEAKGYKEV
KKDLIIKLPKYSLEFENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGP
EDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHHRDKPIREQAENII
HLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDA TLIHQSITGLYETRIDLSQLGGD

SEQ ID NO: 751 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and *Streptococcus pyogenes* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNGGSGGSM
KKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNI
VDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV
DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLI
ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAIL

LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG
YIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVV
DKGASASQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFL
SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGW
GRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDS
LHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDY
DVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREV
KVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYG
DYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETGEI
VWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPK
YGGFDSPTVAYSVLVVAKEVGKSKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLKDVL SAYNKHRDKPIREQAEN
IIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLHQSITGLYETRIDLSQLGGD

SEQ ID NO: 732 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Neisseria meningitidis* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDRKALYSKPGSKNGRWNPEHFKCKCGVRIQTSAYTCSKCRNMAAFKPNPI
NYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEPKTDGSLAMARRLARSVR
RLTRRRARHLLRARLLKREGVLQAADFDENGLIKSLPNTPWQLRAAALDRKLTPLEW
SAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVANNAHALQTGDFRTPAELALNKF
EKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRP
LSGDAVQKMLGHCTFEPAEPKAAKNTYTAERFIWLTCLNNLRILEQGSERPLTDTERAT
LMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYHAISRALE
KEGLKDKKSPLNLSSELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDKFVQ
ISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTTEKIYLPPIPADKIRNPVLRALS
QARKVINGVVRRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYF
PNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLVRNEKGYVEIDHALPFSRTWDDSF
NNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKQRILLQKFD
EDGFKECNLNDTRYVNRFLCQFVADHILLTGKGKRRVFASNGQITNLLRGFWGLRKVR
AENDRHHALDAVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKVLHQKTHFP
QPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSR
APNRKMSGAHKDTLRSKRFRVKNHNEKISVKRVWLTEIKLADLENMVNYKNGREIELYE
ALKARLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQESGVLLNKKNAYTIAD
NGDMVRVDVFCVKDKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSYTFCSLHK
YDLIAFQKDEKSKVEFAYYINCDSSNGRFYLAWHDKGSKEQQFRISTQNLVLIQKYQVN
ELGKEIRPCRLKKRPPVR

SEQ ID NO: 752 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and *Neisseria meningitidis* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNNGSGGSMA
 AFKPNPINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEVPKTGDSLAMAR
 RLARSVRRLTRRRARHLLRARRLKREGVLQAADFENGLIKSLPNTPWQLRAAALDR
 KLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVANNAHALQTGDFRTPA
 ELALNKFEEKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETL
 LMTQRPALSGDAVQKMLGHCTFEPAPKAAKNTYTAERFIWLTKLNNLRILEQGSERPL
 TDTERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYH
 AISRALEKEGLKDKKSPNLSSSELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHIS
 FDKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIADKIRNPV
 VLRALSQARKVINGVVRRYGSPARIHIETAREVGKSKDRKEIEKRQEENRKDREKAAA
 KFREYFPNFVGEPSKDILKLRLYEQQHGKCLYSKKEINLVRLEKGYVEIDHALPFSRT
 WDDSFNNKVLVLGSENQNGNQTPEYFNGKDNSREWQEFKARVETSRFPRSKKQRIL
 LQKFDEDDGFEKCNLNDTRYVNRFLCQFVADHILLTGKGKRRVFAASNGQITNLLRGFWG
 LRKVRANDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKVLHQ
 KTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTPEKLRLLAEKLSRPEAVHEYVTP
 LFVSRAPNRKMSGAHKDTLRSARFVKHNEKISVKRVWLTEIKLADLENMVNYKNGR
 EIELYEALKARLEAYGGNAKQAFDPKDNPFYKKGQGVKAVRVEKTQESGVLLNKKN
 AYTADNGDMVRVDVFCKVDKKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSYTF
 CFSLHKYDLIAFQKDEKSKVEFAYYINCSSNGRFYLAWHDKGSKEQQFRISTQNLVLI
 QKYQVNELGKEIRPCRLKKRPPVR

SEQ ID NO: 733 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Campylobacter jejuni* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNMARILAFDI
 GISSIGWAFSENDELKDCGVRIFTKAENPKTGESLALPRRLARSARKRLARRKARLNHL
 KHLIANEFKLNIEDYQSFDSESLAKAYKGLISPYELRFRALNELLSKQDFARVILHIAKR
 RGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEYLYKEYFQKFKENSKEFTNVRN
 KKESYERCIAQSFLKDGLKLIFKKQREFGFSFSKKEEEVLSVAFYKRALKDFSHLVGNC
 SFFTDEKRAPKNSPLAFMFVALTRIINLLNNLNKNTGILYTKDDLNTLLNEVLKNGTLTY
 KQTKLLGLSDDYEFKREKGTIFYIEFKKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIK
 LKKALAKYDLNQNQIDSLSKLEFKDHLNISFKALKLITPLMLEGKKYDEACNELNLKVA
 INEDKKDFLPAFNETYYKDEVTPVVLRAIKEYRKVLNALLKKYGVHVKINIELAREVG
 KNHSQRAKIEKEQNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKI
 KISDLQDEKMLEIDHIYPYSRSDDSYMNVLVFTKQKQEKLNKTPFEAFGNDSAKWQ
 KIEVLAKNLPTKKQKRILDKNYKDKEQKDFKDRNLNDTRYIARLVNLYTKDYLDLPL
 SDDENTKLNDTQKGSKVHVEAKSGMLTSALRHTWGFSTKDRNNHLHHAIDAVIIAYAN
 NSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKIDEIFVSKPE
 RKKPSGALHEETFRKEEEFYQSYGGKEGVKALELGKIRKVNGKIVKNGDMFRVDIFK
 HKKTNKFYAVPIYTMDFALKVLPNKAVARSKKGEIKDWILMDENYEFCSLYKDSLILI
 QTKDMQEPEFVYYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVIAKSIGIQN
 LKVFEKYIVSALGEVTKAEFRQREDFKK

SEQ ID NO: 753 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and *Campylobacter jejuni* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNNGSGGSMA
RILAFDIGISSIGWAFSENDELKDCGVRIPTKAENPKTGESLALPRRLARSARKRLARRKA
RLNHLKHLIANEFKLNIEDYQSFDESLAKAYKGLISPYELRFRALNELLSKQDFARVIL
HIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEYLYKEYFQKFKEKSKEFT
NVRNKKESYERCIASFLKDGLKLIFKKQREFGFSFSKKFEEVLSVAFYKRALKDFSHL
VGNCSTFTDEKRAPKNSPLAFMFVALTRIINLLNNLKNTEGILYTKDDLNTLLNEVLKN
GTLTYKQTKKLLGLSDDYEFKREKGTIFYEFKKYKEFIKALGEHNLSQDDLNEIAKDITL
IKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNISFKALKLITPLMLEGKKYDEACNEL
NLKVAINEDKKDFLPAFNETYYKDEVTPVVLRAIKEYRKVLNALLKKYGVHKINIEL
AREVGKNHSQRAKIEKEQNENYKAKKDAELECEKLGLKINSKNILKRLFKKEQKEFCA
YSGEKIKISDLQDEKMLEIDHIYPYSRSDDSYMNKVLVFTKQKQEKLNKTPFEAFGND
SAKWQKIEVLAKNLPKKQKRILDKNYKDKEQKDFKDRNLNDTRYIARLVNNTKDY
LDLPLSDDENTKLNLTQKGSKVHVEAKSGMLTSALRHTWGFSTKDRNNHLHHAIDA
VIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFEPFSGFRQKVLDKID
EIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNKIVKNGDM
FRVDIFKHKKTNKFYAVPIYTMDFAKVLPNKAVARSKKGEIKDWILMDENYEFCSLY
KDSLILIQTKDMQEPEFVYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVIA
KSIQIQLNKVFEKYIVSALGEVTKAEFRQREDFKK

SEQ ID NO: 734 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Streptococcus pasteurianus* Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHGNVFE
CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
MLKLGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAYTCSKCRNMTKKNYSI
GLDIGTNSVGWAVITDDYKVPKMKVLGNTDKKYIKKNLLGALLFDSGETAEATRL
KRTARRRYTRRKNRLRYLQEFAEEMTKVDESFFYRLDESFLTDEKDFERHPIFGNKA
DEIKYHQEFPTIYHLRKHLADSSEKADLRLVYLALAHMIKFRGHFLIEGELNAENTDVQ
KIFADFGVYDRTFDDSHLSEITVDAASILTEKISKSRLENLIKYYPTKEKNTLFGNLIA
LALGLQPNFKMNFKLSEDAKLQFSKDSYNEDLEELLGKIGDDYADLFTSAKNLYDAILL
SGILTVDDNSTKAPLSASMIKRYAEHHEDLEKLKEFIKANKSEL YHDIFKDETKNGYAG
YIENGVKQDEFYKYLKNTLSKIAGSDYFLDKIEREDFLRKQRTFDNGSIPHQIHLQEMHA
ILRRQGDYYPFLKENQDRIEKILTFRIPYYVGPLARKDSRFSWA EYHSDEKITPWNFDKV
IDKEKSAEKFITRMTLNDLYLPEEKVLPKHSHVYETYAVYNELTKIKYVNEQGKDSFFD
SNMKQEIFDHVFKENRKVTKEKLLNYLNKEFPEYRIKDLIGLDKENKSFNASLGTYHDL
KKILDKAFLDDKVNEEVIEDIITLTLFEDKDMIHERLQKYS DIFTADQLKKLERRHYTG
WGRLSYKLINGIRNKENKNTILDYLIDDGSANRNFMLINDDTLPFKQIIQKSQVVGDV
DDIEAVVHDLPGSPAIIKKGILQSVKIVDELVKVMGDNPDNIVIAMARENQTTNRGRSQS
QQRLKKLQNSLKELGSNILNEEKPSYIEDKVENSHLQNDQLFLYYIQNGKDMYTGDELD
IDHLSDYDIDHIIPQAFIKDDSIDNRVL TSSAKNRGKSDDVPSLDIVRARKAEWVRLYKS
GLISKRKFDNLTKAERGGTLEADKAGFIKQQLVETRQITKHVAQILDARFNTESDENDK
VIRDVKVITLKSNLVSQFRKDFEFYKVREINDYHHAHDAYLNAVVG TALLKKYPKLAS
EFVYGEYKKYDVHKLIAKSSDDHSEM GKATAKYFFYSNLMNFFKRVIRYSNGKVIVRP

VVEYSKDTEDIAWDKKS NFRTICKVLSYPQVNIVKKVETQTGGFSKESILPKGDSDKLIP
 RKTKKAYWDTKKYGGFDSPTVAYSVFVADVEKGKAKKLKTVKELVGISIMERSFFEE
 NPVEFLENKGYHNIREDKLIKLPKYSLFEFEGGKRRLASASELQKGNEMVIPGHLVKLL
 YHAQRINSFNSTKYLDYVSAHKKEFEKVLSCVEDFANLYVDVEKNLSKIRAVADSM DN
 FSIEEISNSFINLLTLTALGAPADFNFLGEKIPRKRYTSTKECLNATLIHQ SITGLYETRIDL
 SKIGEE

SEQ ID NO: 754 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and
Streptococcus pasteurianus Cas9]

MGKSGIYQIKNTLNNKVYVGS AKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHG NVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGP DGRKALYSKPGSKNGRWN PETHKFCCKGVRIQTSAYTCSKCRNGGSGGSM T
 KKNYSIGLDIGTNSVGWAVITDDYKVP AKKMKVLGNTDKKYIKKNLLGALLFDSGETA
 EATRLKRTARRRYTRRKNRLRYLQEIFA EEMTKVDESFFYRLDESFLT TDEKDFERHPIF
 GNKADEIKYHQEFPTIYHLRKHLADSSEKADLRLVYLALAHMIKFRGHFLIEGELNAEN
 TDVQKIFADFGVGYDRTFDDSHLSEITVDAASILTEKISKSRLENLIKYYPT EKKNTLFG
 NLIALALGLQPNFKMNFKLSEDAKLQFSKDSYNEDLEELLGKIGDDYADLFTSAKNLYD
 AILLSGILTVDDNSTKAPLSASMIKRYAEHHEDLEKLKEFIKANKSELYHDIFKDET KNG
 YAGYIENG VKQDEFYKYLKNTLSKIAGSDYFLDKIEREDFLRKQRTFDNGSIPHQIHLQE
 MHAILRRQGDYYPFLKENQDRIEKILTFRIPYYVGPLARKDSRFSWA EYHSDEKITPWNF
 DKVIDKEKSAEFITRMTLNDLYLPEEKVLPKHSVYETYAVYNELTKIKYVNEQGKDS
 FFDSNMKQEIFDHVFKENRKVTKEKLLNYLNKEFPEYRIKDLIGLDKENKSFNASLGTY
 HDLKKILDKAFLDDKVNEEVIEDIIKTLTFEDKDMIHERLQKYS DIFTADQLKKLERRH
 YTGWGRLSYKLINGIRNKENNK TILDYLI DDGSANRNFMQLINDDTLPFKQIIQKSQVVG
 DVDDIEAVVHDLPGSPA IKGILQSVKIVDELVKVMGDNPDNIVIEMARENQTTNRGRS
 QSQQRLKKLQNSLKELGSNILNEEKPSYIEDKVENSHLQNDQLFLYYIQNGKDMYTGD E
 LDIDHLSDYDIDHIIPQAFIKDDSIDNRVLTSSAKNRGKSDDVPSLDIVRARKAEWVRLY
 KSGLISKRKFDNLTKAERGGLTEADKAGFIK RQLVETRQITKHVAQILDARFNTESDEN
 DKVIRDVKVITLKS NLVSQFRKDFEFYK VREINDYHHAHDAYLNAVVG TALLKKYPKL
 ASEFVYGEYKKYDVHKLIAKSSDDHSEM GKATAKYFFYSNLMNFFKRVIRYSNGKVIV
 RPVVEYSKDTEDIAWDKKS NFRTICKVLSYPQVNIVKKVETQTGGFSKESILPKGDSDKL
 IPRKTKKAYWDTKKYGGFDSPTVAYSVFVADVEKGKAKKLKTVKELVGISIMERSFF
 EENPVEFLENKGYHNIREDKLIKLPKYSLF EFEGGKRRLASASELQKGNEMVIPGHLVK
 LLYHAQRINSFNSTKYLDYVSAHKKEFEKVLSCVEDFANLYVDVEKNLSKIRAVADSM
 DNFSIEEISNSFINLLTLTALGAPADFNFLGEKIPRKRYTSTKECLNATLIHQ SITGLYETRI
 DLSKIGEE

SEQ ID NO: 735 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Clostridium*
cellulolyticum Cas9]

MGKSGIYQIKNTLNNKVYVGS AKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHG NVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGP DGRKALYSKPGSKNGRWN PETHKFCCKGVRIQTSAYTCSKCRNMKYTLGLD
 VGIASVGWAVIDKDN NKIIDLGVR CFDKAEESKTGESLATARRIARGMRRRISRRSQRL
 RLVKKLFVQY EIIKDSSEFN RIFDTSRDGWKDPWELRYNALSRLKPYELVQVLTHITKR
 RGFKSNRKEDLSTTKEGVVITSIKNNSEMLRTKNYRTIGEMIFMETPENS NKRNKVDEYI
 HTIAREDLLNEIKYIFSIQRKLGS PFVTEKLEHDFLNIWEFQRPFASGDSILSKVGKCTLLK
 EELRAP TSCYTSEYFGLLQSINNVLVEDNNTLTLNNDQRAKII EYAHFKNEIKYSEIRKL
 LDIEPEILFKAHNLT HKNPSGNNESKKFYEMKSYHKLKSTLPTDIWGKLHSNKESLDNLF

YCLTVYKNDNEIKDYLQANNLDYLIEYIAKLPTFNKFKHLSLVAMKRIIPFMEKGYKYS
 DACNMAELDFTGSSKLEKCNKLTVEPIIENVTPVIRALTQARKVINAIQKYGLPYMV
 NIELAREAGMTRQDRDNLKKEHENNRKAREKISDLIRQNGRVASGLDILKWRLWEDQG
 GRCAYSKGPIVCDLLNDSLTDIDHIYPYSRSMDDSYMKNVLVLTENQNKRSYTPYEV
 WGSTEKWEDFEARIYSMHLPQSKEKRLNRFITKDLDSEFISRNLDTRYISRFLKNYIE
 SYLQFSNDSPKSCVVCVNGQCTAQLRSRWGLNKNREESDLHHALDAAVIACADRKKIK
 EITNYYNERENHNYKVKYPLPWHSFRQDLMETLAGVFISRAPRRKITGPAHDETIRSPK
 HFNKGLTSVKIPLTTVTLEKLETMVKNKTKGGISDKAVYNVLKNRLIEHNNKPLKAFAEK
 IYKPLKNGTNGAIIRSIRVETPSYTGVRNEGKGISDNLSLMVRVDVFKKKDKYYLVPIYV
 AHMIKKELPSKAIVPLKPESQWELIDSTHEFLFSLYQNDYLVKTKKGITEGYRSCHRG
 TGSLSLMPHFANNKNVKIDIGVRTAISIEKYNVDILGNKSIVKGEPRRGMEKYN SFKSN

SEQ ID NO: 755 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and
Clostridium cellulolyticum Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDRKALYSKPGSKNGRWNPEHFKFCKCGVRIQTSAYTCSKCRNGSGGSMK
 YTLGLDVGIAVGVAVIDKDNKIIDLGVRCFDKAEESKTGESLATARRIARGMRRRIS
 RRSQRLRLVKKLFVQYEIKDSSEFNRIFDTSRDGWKDPWELRYNALSRILKPYELVQVL
 THITKRRGFKSNRKEDLSTTKEGVVITSIKNNSEMLRTKNYRTIGEMIFMETPENSNNRN
 KVDEYIHTIAREDLLNEIKYIFSIQRKLGSPFVTEKLEHDFLNIWFEQRPFASGDSILSKVG
 KCTLLKEELRAPTSCTSEYFGLLQSINNLVLVEDNNTLTLNNDQRAKIEYAHFKNEIK
 YSEIRKLLDIEPEILFAHNLTHKNPSGNNESSKFFYEMKSYHKLKSTLPTDIWGLHSNK
 ESLDNLFYCLTVYKNDNEIKDYLQANNLDYLIEYIAKLPTFNKFKHLSLVAMKRIIPFME
 KGYKYSACNMAELDFTGSSKLEKCNKLTVEPIIENVTPVIRALTQARKVINAIQKY
 GLPYMVNIELAREAGMTRQDRDNLKKEHENNRKAREKISDLIRQNGRVASGLDILKWR
 LWEDQGGRCAYSGKPIVCDLLNDSLTDIDHIYPYSRSMDDSYMKNVLVLTENQNK
 SYTPYEVWGSTEKWEDFEARIYSMHLPQSKEKRLNRFITKDLDSEFISRNLDTRYISR
 FLKNYIESYLQFSNDSPKSCVVCVNGQCTAQLRSRWGLNKNREESDLHHALDAAVIAC
 ADRKKIEITNYYNERENHNYKVKYPLPWHSFRQDLMETLAGVFISRAPRRKITGPAHD
 ETIRSPKHFNKGLTSVKIPLTTVTLEKLETMVKNKTKGGISDKAVYNVLKNRLIEHNNKPL
 KAFAEKIYKPLKNGTNGAIIRSIRVETPSYTGVRNEGKGISDNLSLMVRVDVFKKKDKY
 YLVPIYVAHMIKKELPSKAIVPLKPESQWELIDSTHEFLFSLYQNDYLVKTKKGITEGY
 RSCHRG TGSLSLMPHFANNKNVKIDIGVRTAISIEKYNVDILGNKSIVKGEPRRGMEKYN
 SFKSN

SEQ ID NO: 736 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN, and *Geobacillus*
thermodenitrificans T1 Cas9]

MGKSGIYQIKNTLNNKVYVGSAKDFEKRWRHFKDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGPDRKALYSKPGSKNGRWNPEHFKFCKCGVRIQTSAYTCSKCRNMKYKIGLDI
 GITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLARSARRRLRRRKHRLERI
 RRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNDELARILLHLAKRRGFRS
 NRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDPKFSLHKRNKEDNYTNTVAR
 DDLEREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRPFASKDDIEKKVGFCFTEPKEKR
 APKATYTFQSFTVWEHINKLRLVSPGGIRALTDDERRLIYKQAFHKNKITFHDVRTLLNL
 PDDTRFKGLLYDRNTTLKENEKVRFLELGAYHKIRKAIDSVYGGKGAAKSFRPIDFDTFG
 YALTMFKDDTDIRSILRNEYEQNGKRMENLADKVYDEELIEELLNLSFSKFGHLKAL

RNILPYMEQGEVYSTACERAGYTFTGPKKKQKTVLLPNIPPIANPVVMRALTQARKVV
 NAIKKYGGSPVSIHIELARELSQSFDERRKMQKEQEGNRKKNETAIRQLVEYGLTLNPTG
 LDIVKFKLWSEQNGKCAYSLQPIEIERLLEPGYTEVDHVIPYSRSLDDSYTNKVLVLTK
 NREKGNRTPAEYLGSGSERWQQFETFVL TNKQFSKKKRDRLRLHYDENEENEFKNRN
 LNDTRYISRFLANFIREHLKFADSDDKQKVYTVNGRITAHLSRWNFNKNREESNLHHA
 VDAIVACTTPSDIARVTA FYQRREQNKELSKKTDPPQFPQWPHFADELQARLSKNPKE
 SIKALNLGNYDNEKLESLOPVFVSRMPKRSITGAHQETLRRYIGIDERSGKIQT VVKKK
 LSEIQDKTGHPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGELGPIIR
 TIKIIDTTNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPIYTIDMMKGILPNKAIEPN
 KPYSEWKEMTEDYTFRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYYQTIDSSNGG
 LSLVSHDNNFSLRSIGSRTLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHSKAGETIRPL

SEQ ID NO: 756 [I-TEVI WT NUCLEASE DOMAIN, LINKER DOMAIN with GGSGGS, and
Geobacillus thermodenitrificans T1 Cas9]

MGKSGIYQIKNTLNNKVYVGS AKDFEKRWKRHF KDLEKGCHSSIKLQRSFNKHGNVFE
 CSILEEIPYEKDLIERENFWIKELNSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAK
 MLKLGP DGRKALYSKPGSKNGRWN PETHKFCCKGVRIQTSAYTCSKCRNGSGGSMK
 YKIGLDIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLARSARRRLRRR
 HRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNNDELARILLHLAK
 RRGFRSNRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDPKFSLHKRNKEDNYT
 NTVARDDLEREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRPFASKDDIEKKVGFCTFE
 PKEKRAPKATYTFQSFTVWEHINKLRLVSPGGIRALTDDERRLIYKQAFHKNKITFHDV
 RTLLNLPDDTRFKGLLYDRNTTLKENEKVRFELEGAYHKIRKAIDSVYKGGA AKSFRPI
 DFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLADKVYDEELIEELLNLSFSKFGH
 LSLKALRNILPYMEQGEVYSTACERAGYTFTGPKKKQKTVLLPNIPPIANPVVMRALTQ
 ARKVVNAIKKYGGSPVSIHIELARELSQSFDERRKMQKEQEGNRKKNETAIRQLVEYGLT
 LNPTGLDIVKFKLWSEQNGKCAYSLQPIEIERLLEPGYTEVDHVIPYSRSLDDSYTNKVL
 VLTKENREKGNRTPAEYLGSGSERWQQFETFVL TNKQFSKKKRDRLRLHYDENEENE
 FKNRNLNDTRYISRFLANFIREHLKFADSDDKQKVYTVNGRITAHLSRWNFNKNREES
 NLHHA VDAIVACTTPSDIARVTA FYQRREQNKELSKKTDPPQFPQWPHFADELQARLS
 KNPKE SIKALNLGNYDNEKLESLOPVFVSRMPKRSITGAHQETLRRYIGIDERSGKIQT
 VVKKKLSEIQDKTGHPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNG
 ELGPIIRTIKIIDTTNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPIYTIDMMKGILP
 NKAIEPNKPYSEWKEMTEDYTFRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYYQT
 IDSSNGGLSLVSHDNNFSLRSIGSRTLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHSK
 AGETIRPL

SEQ ID NO: 740 [SV40 nuclear localization sequence]

PKKKRKV

SEQ ID NO: 741 [nucleoplasmin nuclear localization sequence]

KRPAATKKAGQAKKKK

CLAIMS

WHAT IS CLAIMED IS:

1. A composition, comprising: a chimeric nuclease, wherein the chimeric nuclease comprises an I-TEVI nuclease domain, an RNA-guided nuclease Cas domain, and a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that targets an oncogenic mutation that is not a deletion in exon 19 of EGFR.
2. A composition, comprising: a chimeric nuclease, wherein the chimeric nuclease comprises an I-TEVI nuclease domain, an RNA-guided nuclease Cas domain, and a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that targets an oncogenic mutation, wherein the oncogenic mutation is (i) an insertion of one or more nucleotides, or (ii) a substitution or deletion of 10 or less nucleotides.
3. The composition of claim 1 or 2, wherein the oncogenic mutation is a single nucleotide polymorphism.
4. The composition of claim 1-3, wherein a sequence comprising the oncogenic mutation is selected from a mutation set forth in any one of SEQ ID NOs: 1-683, or a combination thereof.
5. The composition of any one of claims 1-3, wherein a sequence comprising the oncogenic mutation is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to a mutation set forth in any one of SEQ ID NOs: 1- 683, or a combination thereof.
6. The composition of any one of claims 1-5, wherein the oncogenic mutation comprises a mutation corresponding to an EGFR L858R mutation or an EGFR V769_D770insASV mutation.
7. The composition of any one of claims 1-6, wherein the oncogenic mutation comprises a mutation corresponding to an EGFR L858R mutation.
8. The composition of any one of claims 1-7, wherein the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 45, 130, or 141, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1045, 1130, 1141, or 1686.
9. The composition of any one of claims 1-7, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 45, 130, 141, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1045, 1130, 1141, or 1686.
10. The composition of any one of claims 1-9, wherein the oncogenic mutation comprises a mutation corresponding to an EGFR V769_D770insASV mutation.
11. The composition of any one of claims 1-10, wherein the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 683, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1683 or 1684.
12. The composition of any one of claims 1-10, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 683, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1683 or 1684.

13. The composition of any one of claims 1-12, wherein the oncogenic mutation is an oncogenic mutation to a gene selected from any one of Muc4, PIK3CA, KRAS, or a combination thereof.
14. The composition of any one of claims 1-13, wherein the oncogenic mutation comprises a Muc4 mutation.
15. The composition of claim 14, wherein the Muc4 mutation is an in-frame deletion of exon 2 or an in-frame deletion of exon 3.
16. The composition of claim 14 or 15, wherein the Muc4 mutation comprises a mutation corresponding to any one of positions P1542, P1680, T1711, V1721, P1826, A1830, S3560, A1833, D2253, V2281, P3088, T3119, T3183, V3817, A3902 of human Muc4 protein, or a combination thereof.
17. The composition of any one of claims 14-16, wherein the Muc4 mutation is selected from a mutation corresponding to any one of P1542L, P1680S, T1711I, V1721A, P1826H, A1830T, S3560S, A1833V, D2253H, V2281AM, P3088L, T3119T, T3183M, V3817A, A3902V of human Muc4 protein, or a combination thereof.
18. The composition of any one of claims 1-17, wherein the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685.
19. The composition of any one of claims 1-17, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 676, 677, 678, 679 or 682, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1676, 1677, 1678, 1679, 1682, or 1685.
20. The composition of any one of claims 1-19, wherein the oncogenic mutation comprises a PIK3CA mutation.
21. The composition of claim 20, wherein the PIK3CA mutation comprises a mutation corresponding to any one of positions H1047, E542, E545, N345, C1636, G1624, G1633, A3140, C3075, A1634, A1173 of human PIK3A protein, or a combination thereof.
22. The composition of claim 20 or 21, wherein the PIK3CA mutation is selected from a mutation corresponding to any one of H1047R, H1047L, E542K, E545K, N345K, C1636A, G1624A, G1633A, A3140T, A3140G, C3075T, A1634C, A1173G of human PIK3A protein, or a combination thereof.
23. The composition of any one of claims 1-22, wherein the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210.
24. The composition of any one of claims 1-22, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 5, 6, 7, 8, 33, 202, 204, 209 or 210, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1005, 1006, 1007, 1008, 1033, 1202, 1204, 1209, or 1210.

25. The composition of any one of claims 1-24, wherein the oncogenic mutation comprises a KRAS mutation.
26. The composition of claim 25, wherein the KRAS mutation comprises a mutation selected from a mutation corresponding to any one of positions A59, D119, D33, G21, G12, G13, Q61, A146, K117 of human KRAS protein, or a combination thereof.
27. The composition of claim 25 or 26, wherein the KRAS mutation is selected from a mutation corresponding to any one of A59T, A59E, A59T, D119N, D33E, G21C, G12C, G12D, G12V, G12R, G12A, G12S, G13D, G13C, G13V, G13R, Q61R, Q61V, Q61L, Q61K, Q61H, Q61A, Q61P, Q61E, A146T, A146V, K117N, K117R of human KRAS protein, or a combination thereof.
28. The composition of any one of claims 1-27, wherein the guide RNA hybridizes to a target nucleotide sequence set forth in SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence as set forth in SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077.
29. The composition of any one of claims 1-27, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to a target nucleic acid sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 37, 42, 51, 52, 62, 63, or 77, or comprises a nucleotide sequence at least 85%, 90%, 95%, 97%, 98%, or 99% identical to any one of SEQ ID NO: 1037, 1042, 1051, 1052, 1062, 1063, or 1077.
30. The composition of any one of claims 1-29, wherein the guide RNA comprises one or more of: a non-natural internucleoside linkage, a nucleic acid mimetic, a modified sugar moiety, or a modified nucleobase.
31. The composition of claim 30, wherein the non-natural internucleoside linkage comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, or a boranophosphate.
32. The composition of claim 30 or 31, wherein the nucleic acid mimetic comprises one or more of a peptide nucleic acid (PNA), morpholino nucleic acid, cyclohexenyl nucleic acid (CeNAs), or a locked nucleic acid (LNA).
33. The composition of any one of claims 30-32, wherein the modified sugar moiety comprises one or more of 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, or 2'-fluoro.
34. The composition of any one of claims 30-33, wherein the modified nucleobase comprises one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-halouracil; a 5-halocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-halo; an 8-amino; an 8-thiol; an 8-thioalkyl; an 8-hydroxyl; a 5-halo; a 5-bromo; a 5-trifluoromethyl; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-Fadenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-

deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deaza-adenine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; or a 5-propynylcytosine.

35. The composition of any one of claims 1-34, further comprising a linker that is operably linked to the I-TEVI nuclease domain and the RNA-guided nuclease Cas domain.

36. The composition of claim 35, wherein the linker comprises an amino acid sequence as set forth in SEQ ID NO: 701, 702, 703, or 704.

37. The composition of claim 35 or 36, wherein the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof.

38. The composition of any one of claims 35-37, wherein the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof.

39. The composition of claim 35, wherein the linker comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 701, 702, 703, or 704.

40. The composition of claim 35 or 39, wherein the linker comprises a mutation corresponding to any one of positions T95, S101, A119, K120, K135, P126, D127, N140, T147, Q158, A161, V117, S165, or a combination thereof.

41. The composition of any one of claims 35, 39, or 40, wherein the linker comprises a mutation selected from a mutation corresponding to any one of T95S, S101Y, A119D, K120N, K135N, K135R, P126S, D127K, N140S, T147I, Q158R, A161V, V117F, S165G, or a combination thereof.

42. The composition of any one of claims 1-41, wherein the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas9 domain.

43. The composition of claim 42, wherein the RNA-guided nuclease Cas9 domain is any one of an RNA-guided nuclease *Staphylococcus aureus* Cas9 domain, an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain, an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain, an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain, an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain, an RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain, or combination thereof.

44. The composition of claim 42 or 43, wherein the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Staphylococcus aureus* Cas9 domain.

45. The composition of any one of claims 42-44, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 710.

46. The composition of any one of claims 42-45, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of

positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof.

47. The composition of any one of claims 42-46, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof.

48. The composition of any one of claims 42-44, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 710.

49. The composition of any one of claims 42-44 or 48, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to any one of positions D10, H557, N580, H840, D1135, R1335, T1337, T267, L325, V327, D333, A336, I341, E345, D348, K352, S360, T368, N369, N371, S372, E373, K386, N393, H408, N410, I414, A415, T438, Y467, N471, D485, M489, E506, R409, T510, N515, Y518, A539, F550, N551, S596, T602, A611, I617, T620, G654, N667, R685, K695, I706, K722, A723, K724, M731, F732, K735, S739, P741, E742, E746, Q747, I754, T755, H757, K760, H761, P778, E781, I783, N784, D785, T786L, L787, Y788, K792, D794, T798, L799, V801, N803, L804, N805, G806, D813, K814, L818, I819, S822, E824, L841, G847, D848, Y857, V875, I876, N884, A888, L890, D894, D895, P897, V903, G920, F924, N929, E936, N937, V941, N942, S943, C945, E947, K951, L952, S956, N957, Q958, A959, N974, G975, V983, N984, N985, D986, I991, V993, M995, I996, T999, Y1000, R1001, E1002, L1004, E1005, N1006, M1007, D1009, K1010, R1011, P1012, P1013, I1015, I1016, A1020, S1021, Q1024, K1027, E1039, H1045, I0148, K1050 or a combination thereof.

50. The composition of any one of claims 42-44, 48, or 49, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation selected from a mutation

corresponding to any one of D10A, D10E, H557A, N580A, H840A, D1135E, R1335Q, T1337R, T267A, L325F, V327I, D333G, A336S, I341L, E345D, D348N, K352E, S360A, T368A, N369E, N371E, S372P, E373K, K386T, N393R, H408N, N410S, I414M, A415T, T438S, Y467F, N471K, D485E, M489F, E506K, R409K, T510E, N515K, Y518F, A539P, F550Y, N551H, S596A, T602I, A611S, I617V, T620K, G654E, N667D, R685K, K695Q, I706V, K722T, A723T, K724N, M731T, F732V, K735Q, S739N, P741L, E742G, E746D, Q747D, I754D, T755I, H757R, K760Q, H761S, P778I, E781K, I783V, N784D, D785E, T786L, L787V, Y788H, K792E, D794T, T798R, L799I, V801I, N803S, L804I, N805K, G806N, D813G, K814E, L818I, I819F, S822P, E824G, L841T, G847S, D848N, Y857H, V875I, I876V, N884K, A888V, L890R, D894G, D895H, P897L, V903I, G920D, F924L, N929Y, E936D, N937G, V941I, N942D, S943L, C945A, E947K, K951R, L952Q, S956N, N957E, Q958K, A959S, N974D, G975K, V983A, N984S, N985D, D986G, I991V, V993L, M995F, I996V, T999N, Y1000K, R1001E, E1002D, L1004I, E1005K, N1006M, M1007N, D1009L, K1010S, R1011T, P1012S, P1013F, I1015L, I1016R, A1020G, S1021K, Q1024K, K1027S, E1039K, H1045K, I0148M, K1050M or a combination thereof.

51. The composition of any one of claims 42-50, wherein the RNA-guided nuclease *Staphylococcus aureus* Cas9 domain comprises a mutation corresponding to the D10E mutation.

52. The composition of claim 42 or 43, wherein the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain.

53. The composition of any one of claims 42, 43, or 52, wherein the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 711.

54. The composition of any one of claims 42, 43, 52, or 53, wherein the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof.

55. The composition of any one of claims 42, 43, or 52-54, wherein the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T,

S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W, E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof.

56. The composition of any one of claims 42, 43, or 52, wherein the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 711.

57. The composition of any one of claims 42, 43, 52, or 56, wherein the RNA-guided nuclease *Staphylococcus pyogenes* Cas9 domain comprises a mutation corresponding to any one of positions D10, S29, F32, D39, R40, H41, S42, I48, C80, S87, K112, H113, K132, K141, D147, L158, E171, P176, I186, V189, Q190, Q194, N199, I201, N202, A203, S204, R205, A210, Q228, L229, G231, S245, T249, S254, D261, T270, N295, T300, D304, V308, N309, I312, T333, A337, E345, F352, Q354, S355, K356, G366, A367, E396, L398, I414, D428, F429, D435, K468, S469, E470, T472, E480, A486, S490, F498, K500, N501, N504, K528, V530, E532, G533, A538, T555, K570, F575, D605, E611, R629, E634, T638, R655, R664, R671, K705, E706, Q709, K710, S714, G7115, G717, H721, H723, A725, N726, V743, L747, V748, K772, K775, N776, I788, G792, K797, Y799, T804, N808, L811, R820, N831, R832, V842, L847, N869, E874, N881, Q885, N888, T893, L911, Y945, D946, L949, E952, A1023, Y1036, G1067, G1077, R1078, N1093, R1114, N1115, D1117, A1121, D1125, P1128, K1129, V1146, S1154, S1159, L1164, S1172, N1177, P1178, I1179, D1180, K1211, M1213, G1218, N1234, E1243, K1244, E1253, E1260, K1263, H1264, E1271, Q1272, E1275, V1290, L1291, S1292, A1293, N1295, H1297, R1298, D1299, K1300, R1303, E1307, N1308, I1309, I1310, H1311, L1312, L1315, T1316, N1317, Y1326, D1328, V1342, A1345, I1360, S1363, or a combination thereof.

58. The composition of any one of claims 42, 43, 52, 56, or 57, wherein the RNA-guided nuclease *Streptococcus pyogenes* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D10E, D10A, S29T, F32M, D39N, R40K, H41Q, S42T, I48L, C80R, S87A, K112D, H113N, K132N, K141E, D147E, L158V, E171Q, P176S, I186K, V189L, Q190H, Q194E, N199R, I201L, N202E, A203E, S204I, R205K, A210G, Q228A, L229F, G231N, S245A, T249M, S254A, D261N, T270S, N295K, T300I, D304G, V308A, N309D, I312V, T333A, A337V, E345K, F352S, Q354K, S355T, K356T, G366K, A367T, E396D, L398F, I414V, D428A, F429Y, D435E, K468Q, S469R, E470N, T472A, E480D, A486T, S490L, F498V, K500E, N501H, N504T, K528R, V530I, E532D, G533E, A538E, T555A, K570Q, F575C, D605E, E611D, R629K, E634K, T638K, R655H, R664K, R671K, K705V, E706D, Q709K, K710A, S714F, G7115E, G717K, H721K, H723Q, A725S, N726A, V743I, L747I, V748I, K772Q, K775R, N776R, I788M, G792R, K797E, Y799H, T804A, N808D, L811R, R820K, N831D, R832H, V842I, L847I, N869D, E874A, N881S, Q885R, N888K, T893S, L911A, Y945H, D946G, L949P, E952A, A1023G, Y1036R, G1067E, G1077E, R1078K, N1093T, R1114G, N1115E, D1117A, A1121P, D1125G, P1128T, K1129T, V1146I, S1154T, S1159P, L1164V, S1172N, N1177D, P1178S, I1179V, D1180S, K1211R, M1213L, G1218T, N1234H, E1243D, K1244T, E1253K, E1260D, K1263Q, H1264Y, E1271D, Q1272W,

E1275H, V1290L, L1291R, S1292A, A1293T, N1295E, H1297N, R1298T, D1299H, K1300L, R1303S, E1307D, N1308S, I1309M, I1310L, H1311N, L1312A, L1315F, T1316S, N1317R, Y1326F, D1328N, V1342I, A1345S, I1360L, S1363N, or a combination thereof.

59. The composition of claim 42 or 43, wherein the RNA-guided nuclease Cas9 domain is an RNA-guided nuclease *Neisseria meningitidis* Cas9 domain.

60. The composition of any one of claims 42, 43, or 59, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 712.

61. The composition of any one of claims 42, 43, 59, or 60, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919, L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof.

62. The composition of any one of claims 42, 43, or 59-61, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof.

63. The composition of any one of claims 42, 43, or 59, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 712.

64. The composition of any one of claims 42, 43, 59, or 63, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation corresponding to any one of positions I9, D16, D30, E31, A94, I103, P124, N164, I213, G229, T241, S376, E393, G454, K471, G490, D660, C665, K764, T770, P803, A841, H842, K843, D844, L846, R847, K854, H855, N856, K858, K862, W865, E868, I869, A872, D873, N876, Y880, G883, I886, E887, E890, R895, A898, Y899, G900, G901, N902, A903, K904, Q905, D908, N912, K917, G919,

L921, V927, K929, T930, E932, S933, L936, L937, N938, K939, K940, Y943, T944, G949, D950, C958, K965, N966, Q967, F969, A975, E980, N981, I986, D987, C988, K989, G990, Y991, R992, I993, D994, Y997, T998, C1000, S1002, H1004, K1005, Y1006, A1010, F1011, Q1012, K1013, D1014, E1015, K1018, V1019, E1020, F1021, A1022, Y1024, I1025, N1026, C1027, D1028, S1029, S1030, N1031, R1033, F1034, Y1035, L1036, A1037, W1038, K1041, G1042, K1044, E1045, Q1046, Q1047, F1048, R1049, I1050, S1051, T1052, Q1053, N1054, L1055, V1056, L1057, I1058, Y1061, V1063, N1064, or a combination thereof.

65. The composition of any one of claims 42, 43, 59, 63, or 64, wherein the RNA-guided nuclease *Neisseria meningitidis* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of I9M, D16E, D30E, E31K, A94D, I103V, P124C, N164D, I213N, G229D, T241A, S376T, E393K, G454C, K471E, G490C, D660E, C665R, K764E, T770A, P803S, A841Q, H842G, K843H, D844E, L846V, R847K, K854R, H855L, N856D, K858G, K862L, W865P, E868Q, I869L, A872K, D873G, N876K, Y880R, G883E, I886P, E887K, E890E, R895Q, A898T, Y899H, G900K, G901D, N902D, A903P, K904T, Q905K, D908A, N912E, K917Y, G919T, L921Q, V927I, K929Q, T930V, E932K, S933T, L936W, L937V, N938R, K939N, K940H, Y943N, T944G, G949A, D950T, C958E, K965G, N966G, Q967K, F969Y, A975S, E980K, N981G, I986R, D987A, C988V, K989V, G990A, Y991F, R992K, I993D, D994E, Y997F, T998E, C1000R, S1002I, H1004Y, K1005A, Y1006N, A1010K, F1011L, Q1012T, K1013A, D1014K, E1015K, K1018N, V1019E, E1020F, F1021L, A1022G, Y1024F, I1025V, N1026S, C1027L, D1028N, S1029R, S1030A, N1031T, R1033A, F1034I, Y1035D, L1036I, A1037R, W1038T, K1041T, G1042D, K1044T, E1045K, Q1046G, Q1047E, F1048Q, R1049S, I1050V, S1051G, T1052V, Q1053K, N1054T, L1055A, V1056L, L1057S, I1058F, Y1061N, V1063I, N1064D, or a combination thereof.

66. The composition of claim 42 or 43, wherein the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Campylobacter jejuni* Cas9 domain.

67. The composition of any one of claims 42, 43, or 66, wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 713.

68. The composition of any one of claims 42, 43, 66, or 67, wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A, N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof.

69. The composition of any one of claims 42, 43, 66-68, wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K,

L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581I, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751I, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971I, T972S, or a combination thereof.

70. The composition of any one of claims 42, 43, or 66, wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 713.

71. The composition of any one of claims 42, 43, 66, or 70, wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation corresponding to any one of positions L5, A6, D8, I9, S12, S13, F18, S19, L24, K25, I31, T40, E42, L50, L58, A59, R61, L58, L65, H67A N74, K77, L98, I99, P101, N110, L113, A119, A126, R128, I134, K140, A144, K147, Q151, L156, V184, S190, F199, D202, G203, R212, F214, K221, E223, Y232, A235, V243, S247, D251, P256, L261, T269, N276, N277, L285, T287, L291, K300, T305, Q308, L312, G314, Y335, K336, I339, H345, D351, N353, E354, I362, K370, D383E, S384, K391, I396, L403, T405, K413, N419, L421, D430, K432, A437, L453, K457, V462, A465, K472, N477, A492, E495, L525, K526, L527, K531, E532, E542, Q550, E556, H559, Y561, S564, M572, V577, Q581, N587, N596, K600, Q602, K603, Q616, K617, N623, Y624, K633, D634, Y642, N649, D656, L660, D662, K667, V677, E680, K682, L686, H692, T693, V712, I714, V722, K723, S736, L739, K742, L747, N751, F756, R763, Q764, E772, K777, A786, E790, F792, Q800, S801, G804, L812, E813, V833, I835, T841, Y845, A855, L856, A863, V864, D879, E883, D900, Q902, K927, F928, V971, T972, or a combination thereof.

72. The composition of any one of claims 42, 43, 66, 70, or 71 wherein the RNA-guided nuclease *Campylobacter jejuni* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of L5I, A6G, D8N, D8E, I9L, S12A, S13N, F18L, S19R, L24I, K25I, I31V, T40N, E42N, L50E, L58V, A59K, R61K, L58V, L65M, H67A, N74K, K77N, L98T, I99Q, P101I, N110S, L113I, A119S, A126V, R128H, I134S, K140N, A144T, K147E, Q151K, L156M, V184I, S190D, F199L, D202Q, G203E, R212K, F214L, K221K, E223K, Y232F, A235P, V243I, S247I, D251N, P256A, L261S, T269G, N276K, N277S, L285V, T287E, L291I, K300D, T305S, Q308K, L312I, G314N, Y335L, K336N, I339K, H345T, D351I, N353D, E354S, I362T, K370E, D383E, S384K, K391N, I396L, L403Q, T405I, K413R, N419E, L421C, D430E, K432S, A437L, L453I, K457C, V462L, A465D, K472S, N477H, A492K, E495I, L525Q, K526I, L527V, K531E, E532D, E542L, Q550D, E556V, H559Y, Y561R, S564N, M572S, V577T, Q581I, N587G, N596E, K600L, Q602A, K603E, Q616R, K617F, N623F, Y624F, K633T, D634E, Y642W, N649S, D656S, L660I, D662E, K667A, V677Q, E680V, K682S, L686I, H692N, T693F, V712I, I714V, V722I, K723F, S736K, L739F, K742N, L747S, N751I, F756L, R763K, Q764E, E772N, K777H, A786T, E790L, F792P, Q800N, S801T, G804D, L812V, E813K, V833S, I835L, T841K, Y845H, A855S, L856T, A863T, V864P, D879N, E883N, D900G, Q902K, K927N, F928Y, V971I, T972S, or a combination thereof.

73. The composition of claim 42 or 43, wherein the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain.

74. The composition of any one of claims 42, 43, or 73, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 714.

75. The composition of any one of claims 42, 43, 73, or 74, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof.

76. The composition of any one of claims 42, 43, or 73-75, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D, I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof.

77. The composition of any one of claims 42, 43, or 73, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 714.

78. The composition of any one of claims 42, 43, 73, or 77, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation corresponding to any one of positions D11, E85, A88, T92, E96, Y100, T109, D110, D113, E115, R116, D125, I127, K128, E132, S147, I185, A187, K228, Y229, T232, M255, S271, N273, A294, A327, E355, K357, N379, T380, S382, A385, D439, R440, S464, H469, Y519, I528, N569, I581, A607, K632, D633, H635, E636, A647, D648, T703, P705, K712, S713, A724, V750, D882, S951, D977, E979, S1014, H1027, I1030, E1081, D1082, D1086, K1088, S1089, N1090, R1092, T1093, I1094, C1095, A1138, Y1139, D1141, T1142, F1158, A1168, E1190, E1198, H1202, I1204, R1205, I1210, K1224, S1232, M1240, V1241, I1242, P1243, G1424, K1248, Q1254, N1257, S1258, T1262, K1263, Y1264, D1266, A1270, K1277, D1284, L1288, V1302, N1316, T1346, I1374, or a combination thereof.

79. The composition of any one of claims 42, 43, 73, 77, or 78, wherein the RNA-guided nuclease *Streptococcus pasteurianus* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of D11E, D11A, E85D, A88T, T92A, E96D, Y100Q, T109D, D110N, D113N, E115D, R116S, D125E, I127D, K128A, E132K, S147T, I185L, A187T, K228N, Y229N, T232K, M255T, S271T, N273E, A294S, A327V, E355K, K357Q, N379G, T380I, S382T, A385N, D439E, R440E, S464A, H469R, Y519F, I528V, N569D,

I581V, A607S, K632R, D633E, H635Q, E636Q, A647K, D648Q, T703A, P705S, K712E, S713A, A724T, V750I, D882G, S951R, D977E, E979K, S1014P, H1027R, I1030V, E1081G, D1082E, D1086N, K1088R, S1089T, N1090D, R1092E, T1093K, I1094V, C1095R, A1138V, Y1139L, D1141E, T1142P, F1158L, A1168T, E1190K, E1198K, H1202Q, I1204V, R1205Q, I1210M, K1224R, S1232T, M1240I, V1241M, I1242L, P1243S, G1424A, K1248A, Q1254H, N1257G, S1258N, T1262A, K1263E, Y1264H, D1266K, A1270E, K1277E, D1284N, L1288V, V1302A, N1316D, T1346N, I1374L, or a combination thereof.

80. The composition of claim 42 or 43, wherein the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain.

81. The composition of any one of claims 42, 43, or 80, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 715.

82. The composition of any one of claims 42, 43, 80, or 81, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850, G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof.

83. The composition of any one of claims 42, 43, or 80-82, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof.

84. The composition of any one of claims 42, 43, or 80, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 715.

85. The composition of any one of claims 42, 43, 80, or 84, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation corresponding to any one of positions T4, D10, V9, D20, K21, I27, C33, K36, A47, A49, S64, Q65, E102, L103, T122, I124, K131, D137, R163, G166, I169, F170, V183, D184, I187, E193, K200, K208, L209, D221, N224, E227, F228, S234, V242, K244, L252, T256, C258, S261, V413, M415, K416, R417, K424, Y426, K427, S429, D430, A468, T470, A472, A478, Q481, K482, L485, A497, L535, W540, R541, E544, G554, P556, I570, Y574, M580, Y584, M585, T592, D593, V606, W607, I647, N650, S693, L697, E702, S704, A713, V714, I715, D776, L847, G850,

G853, A854, R860, I900, H904, M905, I906, E921, Q923, S929, T930, H931, Q939, N994, I997, N1000, K1001, S1002, I1003, K1005, P1008, or a combination thereof.

86. The composition of any one of claims 42, 43, 80, 84, or 85, wherein the RNA-guided nuclease *Clostridium cellulolyticum* Cas9 domain comprises a mutation selected from a mutation corresponding to any one of T4S, D10E, V9I, D20N, K21E, I27E, C33I, K36V, A47S, A49P, S64R, Q65H, E102L, L103V, T122V, I124F, K131Q, D137E, R163Q, G166S, I169L, F170L, V183G, D184G, I187T, E193S, K200Q, K208A, L209Y, D221K, N224Q, E227S, F228S, S234T, V242I, K244N, L252K, T256K, C258T, S261F, V413K, M415L, K416R, R417N, K424Q, Y426I, K427P, S429H, D430Q, A468S, T470S, A472V, A478G, Q481K, K482R, L485S, A497M, L535H, W540Y, R541K, E544Q, G554F, P556S, I570V, Y574I, M580F, Y584N, M585N, T592A, D593A, V606W, W607F, I647R, N650H, S693K, L697F, E702Q, S704N, A713V, V714I, I715V, D776E, L847A, G850P, G853A, A854P, R860K, I900V, H904D, M905V, I906L, E921Y, Q923E, S929D, T930E, H931Y, Q939P, N994Q, I997P, N1000R, K1001M, S1002N, I1003K, K1005H, P1008K or a combination thereof.

87. The composition of claim 42 or 43, wherein the RNA-guide nuclease Cas9 domain is an RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain.

88. The composition of any one of claims 42, 43, or 87, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence as set forth in SEQ ID NO: 716.

89. The composition of any one of claims 42, 43, 87, or 88, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof.

90. The composition of any one of claims 42, 43, 87-89, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof.

91. The composition of any one of claims 42, 43, or 87, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 716.

92. The composition of any one of claims 42, 43, 87, or 91, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation corresponding to any one of positions K2, D8, I14, D35, K41, F74, V75, K91, I117, R128, T136, Q151, S152, S156, A161, V164, S171, E178, D179, V185, R192, K195, A199, Y204, I207, V208, A212, H215, S219, F227, T260, V261, V271, G274, I276, A278, L279, D282, I287, K289, H293, F299, V302, N307, R313, L317, L318, V331, G337, K341, S348, A354, A355, K356, R359, M372, T377, R380, E395, D399, E404, S416, T441, R445, N464, E504, S508, M515, Q516, E520, G521, V534, L545, K559, T578, K603, T612, L619, S621, N656, N660, L673, D685, I699, N708, N717, R737, V738, S752, D756, Q771, N777, N792, E793, I811, I824, K839, Q845, K848, T849, L895, I902, T908, V929, I943, I946, M948, F990, T995, V1000, Q1014, D1017, S1019, N1020, G1021, S1024, N1030, N1031, R1035, S1036, I1037, V1067, S1071, A1075, I1079, or a combination thereof.

93. The composition of any one of claims 42, 43, 87, 91, or 92, wherein the RNA-guided nuclease *Geobacillus thermodenitrificans* T1 Cas9 domain comprises a mutation selected from a mutation corresponding to any one of K2R, D8E, D8A, I14V, D35E, K41Q, F74V, V75I, K91E, I117V, R128K, T136S, Q151R, S152A, S156G, A161G, V164I, S171A, E178G, D179E, V185I, R192H, K195R, A199S, Y204F, I207M, V208S, A212K, H215N, S219T, F227V, T260I, V261A, V271I, G274S, I276A, A278G, L279P, D282E, I287L, K289E, H293Q, F299Y, V302I, N307R, R313Y, L317I, L318V, V331I, G337D, K341Q, S348K, A354K, A355S, K356S, R359L, M372L, T377A, R380H, E395P, D399N, E404N, S416T, T441S, R445K, N464T, E504D, S508T, M515T, Q516K, E520D, G521E, V534M, L545H, K559R, T578V, K603R, T612I, L619V, S621T, N656M, N660S, L673F, D685E, I699V, N708E, N717D, R737K, V738I, S752A, D756E, Q771R, N777H, N792D, E793Q, I811V, I824V, K839T, Q845K, K848A, T849S, L895P, I902V, T908K, V929V, I943V, I946M, M948I, F990L, T995I, V1000G, Q1014K, D1017H, S1019G, N1020T, G1021A, S1024E, N1030C, N1031S, R1035S, S1036G, I1037V, V1067L, S1071A, A1075T, I1079V, or a combination thereof.

94. The composition of any one of claims 1-41, wherein the RNA-guided nuclease Cas domain is a RNA-guided nuclease Cas12 domain.

95. The composition of any one of claims 1-41, wherein the RNA-guided nuclease Cas domain is a RNA-guided nuclease CasX domain.

96. The composition of any one of claims 1-95, wherein the I-TEVI nuclease domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NO: 700.

97. The composition of any one of claims 1-96, wherein the I-TEVI nuclease domain comprises a mutation at any one of positions corresponding to T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof.

98. The composition of any one of claims 1-97, wherein the I-TEVI nuclease domain comprises a mutation selected from any one of corresponding to T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof.

99. The composition of any one of claims 1-98, wherein the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation.
100. The composition of any one of claims 1-95, wherein the I-TEVI nuclease domain comprises an amino acid sequence as set forth in SEQ ID NO: 700.
101. The composition of any one of claims 1-95 or 100, wherein the I-TEVI nuclease domain comprises a mutation corresponding to any one of positions T11, V16, N14, E25, K26, R27, E36, K37, G38, C39, S41, L45, F49, I60, and E81, or a combination thereof.
102. The composition of any one of claims 1-95, 100 or 101, wherein the I-TEVI nuclease domain comprises a mutation selected from a mutation corresponding to any one of T11V, V16I, N14G, E25D, K26R, R27A, E36S, K37N, G38N, C39V, S41H, L45F, F49Y, I60V, E81I, or a combination thereof.
103. The composition of any one of claims 1-95, or 100-102, wherein the I-TEVI nuclease domain comprises a mutation corresponding to a K26R mutation.
104. The composition of any one of claims 1-103, wherein the chimeric nuclease further comprises a nuclear localization signal.
105. The composition of claim 104, wherein the nuclear localization signal comprises an SV40 nuclear localization signal.
106. The composition of claim 104 or 105, wherein the nuclear localization signal comprises a Nucleoplasmin nuclear localization signal.
107. The composition of any one of claims 1-106, wherein the composition further comprises a donor nucleic acid.
108. The composition of claim 107, wherein the donor nucleic acid restores a non-oncogenic function of a gene comprising the oncogenic mutation.
109. The composition of claim 107 or 108, wherein the donor nucleic acid comprises a non-oncogenic version of the oncogenic mutation.
110. The composition of any one of claims 107-109, wherein the donor nucleic acid is DNA.
111. The composition of any one of claims 107-110, wherein the donor nucleic acid comprises a blunt end and at least two nucleotide 3' overhang end.
112. The composition of any one of claims 109-111, wherein the donor nucleic acid comprises a 5' and a 3' homology flanking the non-oncogenic version of the oncogenic mutation.
113. The composition of any one of claims 1-106, wherein the composition does not comprise a donor nucleic acid.
114. The composition of any one of claims 1-113, further comprising a pharmaceutically acceptable excipient, diluent or carrier.
115. The composition of any one of claims 1-114, wherein the composition is encapsulated in a lipid nanoparticle.

116. The composition of claim 115, wherein the lipid nanoparticle comprises cationic or neutral lipids.

117. A nucleic acid or plurality of nucleic acids encoding the chimeric nuclease or the guide RNA of any one of claims 1-116.

118. The nucleic acid or plurality of nucleic acids of claim 117, wherein the chimeric nuclease or the guide RNA is operably coupled to a eukaryotic promoter, an enhancer, a polyadenylation site, or a combination thereof.

119. The nucleic acid or plurality of nucleic acids of claim 117 or 118, wherein the nucleic acid is an expression vector selected from a plasmid, a lentivirus vector, an adeno associated virus vector, or an adenovirus vector.

120. The nucleic acid or plurality of nucleic acids of any one of claims 117-119, further comprising the donor nucleic acid portion.

121. A method of targeting the oncogenic mutation in a cell comprising contacting the composition of any one of claims 1-116 to the cell.

122. The method of claim 121, wherein the cell is a cell in an individual afflicted with cancer.

123. Use of the composition of any one of claims 1-116 to the cell for targeting the oncogenic mutation in a cell.

124. The use of claim 123, wherein the cell is a cell in an individual afflicted with cancer

125. A method of editing a genome in a cell comprising contacting the composition of any one of claims 1-116 to the cell.

126. The method of claim 125, wherein the cell is a cell in an individual afflicted with cancer.

127. Use of the composition of any one of claims 1-116 for editing a genome in a cell.

128. The use of claim 127, wherein the cell is a cell in an individual afflicted with cancer.

129. A method of deleting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of any one of claims 1-116 to the cell.

130. The method of claim 129, wherein the cell is a cell in an individual afflicted with cancer.

131. Use of the composition of any one of claims 1-116 for deleting at least a portion of the oncogenic mutation in a cell.

132. The use of claim 131, wherein the cell is a cell in an individual afflicted with cancer.

133. A method of silencing or disrupting at least a portion of the oncogenic mutation in a cell comprising contacting the composition of any one of claims 1-116 to the cell.

134. The method of claim 133, wherein the cell is a cell in an individual afflicted with cancer.

135. Use of the composition of any one of claims 1-116 for silencing or disrupting at least a portion of the oncogenic mutation in a cell.

136. The use of claim 135, wherein the cell is a cell in an individual afflicted with cancer.
137. A method of replacing at least a portion of the oncogenic mutation in a cell comprising contacting the composition of any one of claims 1-116 to the cell.
138. The method of claim 137, wherein the cell is a cell in an individual afflicted with cancer.
139. Use of the composition of any one of claims 1-116 for replacing at least a portion of the oncogenic mutation in a cell.
140. The use of claim 139, wherein the cell is a cell in an individual afflicted with cancer.
141. A method of restoring a non-oncogenic function in a cell comprising contacting the composition of any one of claims 1-116 to the cell.
142. The method of claim 141, wherein the cell is a cell in an individual afflicted with cancer.
143. Use of the composition of any one of claims 1-116 for restoring a non-oncogenic function in a cell.
144. The use of claim 143, wherein the cell is a cell in an individual afflicted with cancer.
145. A method of treating cancer in an individual, comprising administering the composition of any one of claims 1-116 to the individual with cancer, thereby treating the cancer in the individual.
146. Use of the composition of any one of claims 1-116 for treatment of cancer in an individual.