

We claim:

1. An ex-vivo method for correcting a mutant allele of a gene of interest in a primate cell, comprising:

5 a) introducing a non-naturally occurring targeted nuclease and site-specific nucleotide-binding guide that act together to introduce double-stranded breaks in the mutant allele into the primate cell, wherein:

i) the primate cell is undergoing mitotic cell division;

10 ii) the primate cell comprises a genome that is heterozygous for the mutant allele, such that the genome comprises one copy of the mutant allele and one copy of a wild-type allele;

iii) single-stranded oligonucleotides homologous to the wild-type allele are not introduced into the primate cell; and

15 b) allowing the primate cell to activate homology-directed repair of the double-stranded DNA breaks in the mutant allele,

thereby correcting the mutant allele using the normal wild-type allele as a repair template and producing a primate cell that is homozygous for the wild-type allele.

2. The ex-vivo method of claim 1, wherein the primate cell is an embryo.

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3. The ex-vivo method of claim 2, further comprising generating the embryo prior to step (a).

4. The ex-vivo method of claim 2 or claim 3, wherein the embryo is a one-cell embryo.

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5. The ex-vivo method of any one of claims 2-4, further comprising:

selecting a primate oocyte comprising a genome having a mutant allele or a wild-type allele of a gene of interest from a primate species;

30 fertilizing the primate oocyte with a sperm from the same primate species, wherein the sperm comprises a wild-type allele or a mutant allele of the gene of interest, respectively, thereby forming a one-cell primate embryo, wherein the primate embryo is heterozygous and comprises the one copy of the wild-type allele and the one copy of the mutant allele.

6. The ex-vivo method of claim 5, wherein the targeted nuclease and the site-specific nucleotide-binding guide are introduced into the primate oocyte simultaneously with fertilizing the primate oocyte.
- 5 7. The ex-vivo method of claim 5 or claim 6, wherein fertilizing the primate oocyte comprises intracytoplasmic sperm injection (ICSI).
8. The ex-vivo method of any one of claims 5-7, wherein the primate oocyte is at metaphase II when the targeted nuclease and the site-specific nucleotide-binding guide are
10 introduced.
9. The ex-vivo method of any one of claims 2-4, further comprising culturing the embryo to form a multi-cell embryo *in vitro*.
- 15 10. The ex-vivo method of claim 9, wherein the multi-cell embryo is not mosaic for cells comprising the mutant allele.
11. The ex-vivo method of any one of claims 1-10, wherein the targeted nuclease is clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas)9, zinc finger
20 nuclease (ZFN), or transcription activator-like effector nuclease (TALEN).
12. The ex-vivo method of claim 11, wherein the targeted nuclease is CRISPR-Cas9, and the site-specific nucleotide-binding guide is a nucleic acid guide RNA.
- 25 12. The ex-vivo method of any one of claims 1-11, further comprising:
c) assaying for successful correction of the mutant allele.
13. The ex-vivo method of claim 12, wherein assaying for successful correction comprises the use of Sanger sequencing.
- 30 14. The ex-vivo method of any one of claims 1-12, further comprising assaying for off-target effects.

15. The ex-vivo method of claim 13, wherein assaying for off-target effects comprises whole-genome sequencing.

5 16. The ex-vivo method of any one of claim 1 or claim 11-15, wherein the primate cell is a somatic cell.

17. The ex-vivo method of claim 16, wherein the somatic cell is a mesoderm, endoderm, or ectoderm cell.

10 18. The ex-vivo method of claim 17, wherein the somatic cell is a cardiac cell, skin cell, white blood cell, liver cell, pancreatic cell, kidney cell, ovarian cell, testicular cell, prostatic cells breast cell, muscle cell, cell of the digestive system cell of the respiratory system, or an osteogenic cell.

15 19. The ex-vivo method of any one of claims 1 or 11-15, wherein the primate cell is a pluripotent or multipotent stem cell.

20 20. The ex-vivo method of claim 11 or claim 12, wherein the stem cell is a bone marrow stem cell, hematopoietic stem cell, mesenchymal stem cell, intestinal stem cell, neuronal stem cell, or dental stem cell.

21. The ex-vivo method of any one of claims 1-20 , wherein the primate is a human.

25 22. The ex-vivo method of any one of claims 1-21, wherein the mutant allele comprises a deletion or an insertion as compared to the wild-type allele.

23. The ex-vivo method of any one of claims 1-22, wherein the mutant allele comprises a base pair substitution as compared to the wild-type allele.

30 24. The ex-vivo method of any one of claims 1-23, wherein the mutant allele comprises a frame shift mutation as compared to the wild-type allele.

25. The ex-vivo method of any one of claims 1-24, wherein the gene of interest is myosin binding protein C (MYBPC3), fibroblast growth factor receptor 3 (FGFR3), serpin family A

member 1 (SERPINA1), protein kinase D1 (PKD), breast cancer 1 (BRCA1), breast cancer 2 (BRCA2), glycyl-tRNA synthetase (GARS), WNT signaling pathway regulator (APC), cystic fibrosis transmembrane conductance regulator (CFTR), chimerin 1 (CHN1), dystrophin (DMD), coagulation factor V (F5), fragil X mental retardation 1 (FMR1), glucosylceramidase beta (GBA),
5 homeostatic iron regulator (HFE), coagulation factor IX (FIX), huntingtin (HD), fibrillin 1 (FBN1), dystrophia myotonica protein kinase (DMPK), cellular nucleic acid binding protein (CNBP), protein tyrosine phosphatase, non-receptor type 11 (PTPN11), Ras/Rac guanine nucleotide exchange factor 1 (SOS1), Raf proto-oncogene serine/threonine kinase (RAF1), Kras proto-oncogene GTPase (KRAS), collagen type alpha 1 chain (COL1A1), collagen type alpha 2 chain
10 (COL1A2), synuclein alpha (SNCA), ubiquitin C-terminal hydrolase L1 (UCHL1), leucine rich repeat kinase 2 (LRRK2), Parkinson disease 3 (PARK3), parkin RBR E3 ubiquitin protein ligase (PARK2), parkinsonism associated deglycase (PARK7), PTEN induced putative kinase 1 (PARK6), apolipoprotein B (APOB), low density lipoprotein receptor (LDLR), low density lipoprotein receptor adaptor protein 1 (LDLRAP1), proprotein convertase subtilisin/kexin type 9
15 (PCSK9), actin alpha cardiac muscle 1 (ACTC1), actinin alpha2 (ACTN2), calreticulin 3 (CALR3), cysteine and glycine rich protein 3 (CSRP3), junctophilin2 (JPH2), myosin heavy chain 7 (MYH7), myosin light chain 2 (MYL2), myosin light chain 3 (MyL3), myozenin 2 (MYOZ2), nexilin F-actin binding protein (NEXN), phospholamban (PLN), protein kinase AMP-activated non-catalytic subunit gamma 2 (PRKAG2), titin-cap (TCAP), troponin I3 cardiac type (TNNI3), troponin T2
20 cardiac type (TNNT2), tropomyosin 1 (TPM1), titin (TTN), and/or vinculin (VCL).

26. The ex-vivo method of claim 18, wherein (a) the somatic cell is from a human subject that has breast cancer, (b) the somatic cell is a breast cell, and (c) wherein the gene of interest is BRCA1 or BRCA 2.

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27. The ex-vivo method of claim 18, wherein:

(a) the somatic cell is from a human subject that has familial cardiomyopathy,

(b) the cell is a cardiac cell, and

(c) the gene of interest is MYBPC3, ACTC1, ACTN2, CALR3, CSRP3, JPH2, MYH7,
30 MYL2, MyL3, MYOZ2, NEXN, PLN, PRKAG2, TCAP, TNNI3, TNNT2, TPM1, TTN, and/or VCL.

28. The ex-vivo method of claim 18, wherein:

- (a) the somatic cell is from a human subject that has familial hypercholesterolemia,
- (b) the cell is a cardiac cell, and/or,
- (c) the gene of interest is APOB, LDLR, LDLRAP1, and/or PCSK9.