CLAIMS

1. A composition comprising a CRISPR complex comprising

a) a tracr mate RNA polynucleotide,

b) a tracr RNA polynucleotide,

c) a guide RNA polynucleotide capable of hybridizing to a target sequence in a eukaryotic cell or a cell in a multi-cellular eukaryotic organism; and

d) a Cas9 comprising one or more nuclear localization sequences,

for use in therapy.

2. A composition for use according to Claim 1, in which the CRISPR complex is provided by a CRISPR system that comprises:

I. a CRISPR polynucleotide sequence, in which the polynucleotide sequence encodes or comprises:

(a) a guide RNA polynucleotide capable of hybridizing to a target sequence in a eukaryotic cell or a cell in a multi-cellular eukaryotic organism,

(b) a tracr mate RNA polynucleotide, and

(c) a tracr RNA polynucleotide, and

II. a polynucleotide sequence encoding the Cas9

in which when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and in which the CRISPR complex comprises the Cas9 complexed with (1) the guide RNA polynucleotide that is capable of hybridizing to the target sequence, and (2) the tracr mate RNA polynucleotide that is hybridized to the tracr RNA polynucleotide and the polynucleotide sequence encoding a Cas9 is DNA or RNA.

3. A composition for use according to Claim 1, in which the CRISPR complex is provided by a CRISPR system that comprises:

I. polynucleotides comprising:

(a) a guide RNA polynucleotide capable of hybridizing to a target sequence in a eukaryotic cell or a cell in a multi-cellular eukaryotic organism, and

(b) at least one or more tracr mate RNA polynucleotides,

II. a polynucleotide sequence encoding the Cas9, and

III. a polynucleotide sequence comprising a tracr RNA polynucleotide,

in which when transcribed, the tracr mate RNA polynucleotide hybridizes to the tracr RNA polynucleotide and the guide RNA polynucleotide directs sequence-specific binding of a CRISPR complex to the target sequence, and in which the CRISPR complex comprises the Cas9 complexed with (I) the guide RNA polynucleotide that is capable of hybridizing to the target sequence, and (2) the tracr mate RNA polynucleotide that is hybridized to the tracr RNA polynucleotide, and the polynucleotide sequence encoding a Cas9 is DNA or RNA.

4. A composition for use according to Claim 1, in which the CRISPR complex is provided by a CRISPR system that comprises: one or more regulatory elements, one or more nucleotide sequences each encoding guide RNA polynucleotide, tracr mate RNA polynucleotide and tracr RNA polynucleotide and/or the Cas9, in which the one or more regulatory elements drive expression or transcription of the nucleotide sequences, and the guide RNA polynucleotide, tracr mate RNA polynucleotide and tracr RNA polynucleotide directs sequence-specific binding of a CRISPR complex to the target sequence in a eukaryotic cell or a cell in a multi-cellular eukaryotic organism, in which the CRISPR complex comprises the Cas9 complexed with (I) the guide RNA polynucleotide that is capable of hybridizing to the target sequence, and (2) the tracr mate RNA polynucleotide that is hybridized to the tracr RNA polynucleotide, in which a CRISPR complex is provided and when not supplied by nucleotide sequences expressed or transcribed the system includes one or more guide RNA and/or Cas9.

5. A composition for use according to Claim 1 or 2, in which the CRISPR complex is provided by a CRISPR system that comprises: one or more guide RNA polynucleotide, tracr mate RNA polynucleotide and tracr RNA polynucleotide and Cas9, in which the guide RNA directs sequence-specific binding of a CRISPR complex to the target sequence in a eukaryotic cell or a cell in a multi-cellular eukaryotic organism, in which the CRISPR complex comprises the Cas9 complexed with (I) the guide RNA polynucleotide that is capable of hybridizing to

the target sequence, and (2) the tracr mate RNA polynucleotide that is hybridized to the tracr RNA polynucleotide.

6. A composition for use according to any preceding claim, in which the CRISPR system is comprised within a vector system comprising one or more vectors.

7. A composition for use according to Claim 2, in which I and II are located on the same vector.

8. A composition for use according to Claim 3, in which I, II, and III are located on the same vector.

9. A composition for use according to Claim 4 in which nucleotide sequences are located on the same vector.

10. A composition for use according to any of Claims 6 to 9, in which the one or more vectors comprise one or more viral vectors.

11. A composition for use according to Claim 10, in which the one or more viral vectors comprise one or more lentiviral, baculoviral, adenoviral or adeno-associated viral (AAV) vectors.

12. A composition for use according to Claim 11, in which the AAV vector(s) comprise AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8 or AAV9 or any combination thereof.

13. A composition for use according to Claim 11 or 12, in which the AAV vector(s) comprise AAV serotypes 1, 2, 5 or a hybrid or capsid AAV1, AAV2, AAV5 or any combination thereof for targeting brain or neuronal cells.

14. A composition for use according to Claim 11 or 12, in which the AAV vector(s) comprise AAV4 for targeting cardiac tissue.

15. A composition for use according to Claim 11 or 12, in which the AAV vector(s) comprise AAVl, AAV5, AAV6, or AAV9 for targeting lung epithelium.

16. A composition for use according to Claim 11 or 12, in which the AAV vector(s) comprise AAV8 for delivery to the liver.

17. A composition for use according to any of Claims 6 to 16, in which the vector comprises a promoter for driving Cas9 coding nucleic acid molecule expression, and in which the promoter is for either ubiquitous expression or tissue specific expression.

18. A composition for use according to Claim 17, in which the promoter is for ubiquitous expression and comprises a CMV promoter, a CAG promoter, a CBh promoter, a PGK promoter, or a SV40 promoter, or Ferritin heavy or light chains promoter.

19. A composition for use according to Claim 17, in which the promoter is for tissue specific expression and which directs expression in a tissue comprising muscle, neuron, bone, skin, blood, liver, pancreas, or lymphocytes.

20. A composition for use according to Claim 17, in which the promoter is for tissue specific expression and comprises synapsin I for neurons, CaMKIIalpha for excitatory neurons, GAD67 or GAD65 or VGAT for GABAergic neurons, albumin promoter for liver expression, SP-B for lung expression, ICAM for endothelial cells, IFNbeta or CD45 for hematopoietic cells, or OG-2 for osteoblasts.

21. A composition for use according to any preceding claim, in which the Cas9 is or is derived from an organism in a genus comprising *Corynebacter, Sutterella, Legionella, Treponema, Filifactor, Eubacterium, Streptococcus, Lactobacillus, Mycoplasma, Bacteroides, Flaviivola, Flavobacterium, Sphaerochaeta, Azospirillum, Gluconacetobacter, Neisseria, Roseburia, Parvibaculum, Staphylococcus, Nitratifractor, Mycoplasma* or *Campylobacter.*

22. A composition for use according to Claim 21, in which the Cas9 is or is derived from a *Streptococcus pyogenes,* a *Staphylococcus aureus* or a *Streptococcus thermophilus*.

23. A composition for use according to any preceding claim, in which the therapy comprises *ex vivo* therapy.

24. A composition for use according to Claim 23, in which the *ex vivo* therapy is carried out on stem cells or iPS cells.

25. A composition for use according to Claim 24, in which the stem cells are HSCs (Hematopoietic Stem Cells).

26. A composition for use according to any preceding claim, in which the therapy comprises treating or inhibiting a condition caused by a gene defect in a genomic locus of interest.

27. A composition for use according to any preceding claim, in which the therapy comprises treating: blood and coagulation disease or disorder; cell dysregulation and oncology disease or disorder; inflammation and immune related disease or disorder; metabolic, liver, lung, kidney, or protein disease or disorder; muscular / skeletal disease or disorder; neurological or neuronal disease or disorder; or ocular disease or disorder.

28. A composition for use according to Claim 27, in which the blood and coagulation diseases and disorders comprise: anemia; Bare lymphocyte syndrome; bleeding disorder; factor H and factor H-like 1; factor V and factor VIII; factor VII deficiency; factor X deficiency; factor XI deficiency; factor XII deficiency; factor XIIIA deficiency; factor XIIIB deficiency; Fanconi anemia; hemophagocytic lymphohistiocytosis disorder; hemophilia A; hemophilia B; hemorrhagic disorder; leukocyte deficiency or disorder; sickle cell anemia; or thalassemia.

29. A composition for use according to Claim 27, in which the cell dysregulation and oncology diseases and disorders comprise: B-cell non-Hodgkin lymphoma or Leukemia.

30. A composition for use according to Claim 27, in which the inflammation and immune related diseases and disorders comprise AIDS; autoimmune lymphoproliferative syndrome; combined immunodeficiency; HIV-1; HIV susceptibility or infection; immunodeficiency; inflammation; or severe combined immunodeficiency (SCIDs).

31. A composition for use according to Claim 27, in which the metabolic, liver, kidney, and protein diseases and disorders comprise: amyloid neuropathy; amyloidosis; TTR amyloidosis (specifically ATTR), cystic fibrosis; glycogen storage disease; hepatic adenoma; hepatic failure; neurologic disorder; hepatic lipase deficiency; hepatoblastoma, cancer or carcinoma; medullary cystic kidney disease; phenylketonuria; polycystic kidney; or hepatic disease.

32. A composition for use according to Claim 27, in which the muscular / skeletal diseases and disorders comprise: muscular dystrophy; osteoporosis; or muscular atrophy.

33. A composition for use according to Claim 27, in which the neurological and neuronal diseases and disorders comprise: ALS; Alzheimer disease; autism; fragile X syndrome; Huntington's disease and disease like disorders; Parkinson disease; schizophrenia; secretase related disorders; trinucleotide repeat disorders; Kennedy's disease; Friedrich's ataxia;

Machado-Joseph's disease; spinocerebellar ataxia; myotonic dystrophy; Dentatorubral Pallidoluysian Atrophy (DRPLA) disease; or creb BP global instability.

34. A composition for use according to Claim 27, in which the ocular diseases and disorders comprise: age related macular degeneration, corneal clouding and dystrophy; cornea plana congenital; glaucoma; leber congenital amaurosis; retinitis pigmentosa; and/or macular dystrophy.

35. A composition for use according to any of Claims 1 to 26, in which the therapy comprises treating a post-mitotic disorder.

36. A composition for use according to Claim 35, in which the post-mitotic disorder comprises a neuronal disorder.

37. A composition for use according to any of Claims 1 to 26, in which the therapy comprises: treating a metabolic disorder, amyloidosis or protein-aggregation related disease, cellular transformation arising from genetic mutation or translocation, dominant negative effects of gene mutation, or latent viral infection, or targeting a chromosomal sequence comprising SERPINA1 (serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, anti trypsin), member 1).

38. A composition for use according to any preceding claim, in which the Cas9 comprises one or more mutations.

39. A composition for use according to Claim 38, in which the one or more mutations comprises one or more mutations in a RuvC1 domain of the Cas9.

40. A composition for use according to Claim 39, in which the Cas9 comprises a nickase directing cleavage at the location of the target sequence.

41. A composition for use according to Claim 40, in which the Cas9 introduces double strand breaks using multiplexed nickase activity.

42. A composition for use according to any preceding claim, in which the CRISPR system comprises a multiplexed Cas9 system comprising two or more guide RNA polynucleotides.

43. A composition for use according to any preceding claim, in which the Cas9 has altered nuclease activity compared with the wild type Cas9.

44. A composition for use according to any preceding claim, in which the Cas9 comprises one or more heterologous functional domains or two or more heterologous functional domains.

45. A composition for use according to Claim 44, in which the Cas9 coding sequence encodes the one or more or the two or more heterologous functional domains.

46. A composition for use according to Claim 44 or 45, in which the Cas9 coding sequence encodes heterologous functional domain(s) fused to the Cas9.

47. A composition for use according to any of Claims 44 to 46, in which the functional domain(s) is / are fused to the Cas9.

48. A composition for use according to any of Claims 44 to 46, comprising a linker between the Cas9 and the domain.

49. A composition for use according to any of Claims 44 to 48, in which the one or more functional domains have one or more of the following activities: methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity, nuclear localization activity or nucleic acid binding activity.

50. A composition for use according to any preceding claim, in which the complex comprises a nuclease directing cleavage of one or both strands at the location of the target sequence.

51. A composition for use according to any preceding claim, including a recombination template.