Icam-l, Tnfα, Ncf, Il6, Ifn-α, Ifn-β, Ifn-γ, and Stat4 and administering to the subject additional doses of the synthetic repression system composition until the level of the at least one immune related marker is reduced.

[0012] In a fifth aspect, provided herein is a method of treating Waldenstrom macroglobulinemia in a subject in need thereof comprising administering to a subject with Waldenstrom macroglobulinemia a therapeutically effective amount of a composition comprising the synthetic repression system described herein. In some embodiments, the method additionally comprises administering to the subject a composition comprising a gRNA configured to cleave MyD88 at L265 locus and a homology directed repair template encoding a portion of wild-type MyD88 at the L265 locus, whereby levels of endogenous L265P mutant MyD88 are reduced in the subject.

[0012a] In a sixth aspect, provided herein is a use of a therapeutically effective amount of the composition as described herein in the manufacture of a medicament for treating a subject with septicemia, wherein the composition is to be administered to the subject with septicemia.

[0012b] In a seventh aspect, provided herein is a use of a therapeutically effective amount of the composition as described herein in the manufacture of a medicament for preventing an adverse immune response in a subject, wherein the composition is to be administered to the subject prior to or concurrently with administration of an AAV vector based gene therapy to the subject, wherein the level of an immune related marker selected from the group consisting of Icam-1, Tnfα, Ncf, Il6, Ifn-α, Ifn-β, Ifn-γ, and Stat4 is reduced in the subject relative to subject who received the AAV vector based gene therapy but did not receive the therapeutic synthetic repression system composition.

[0012c] In an eighth aspect, provided herein is a use of a therapeutically effective amount of the composition as described herein in the manufacture of a medicament for treating Waldenström macroglobulinemia in a subject in need thereof, wherein the composition is to be administered to a subject with Waldenström macroglobulinemia.

[0012d] In a ninth aspect, provided herein is the composition as described herein for use in therapy.

BRIEF DESCRIPTION OF DRAWINGS

[0013] FIGS. 1A-1H show CRISPR-based targeted Myd88 repression in vitro and in vivo demonstrates efficacy in modulating host response against AAV-based gene therapies. (FIG.1A) Top: Schematic representation of the gRNA binding sites targeted to the promoter

of Myd88 locus; Bottom: Mouse Neuroblastoma (N2A) cell lines were transfected with MyD88 gRNA pairs or mock gRNA control together with Cas9 nuclease and either MS2-KRAB or MS2-HPlaKRAB cassettes. 72 hours post-transfection cells were treated with Lipopolysaccharides (E . coli LPS, serotype 011 EB4) at a concentration of 10 pg/ml to induce Myd88 expression. (FIG. 1B) qRT-PCR analysis of Myd88 expression mRNA level post LPS treatment. Fold changes were quantified relative to the expression level of cells receiving non-targeting Mockg RNA (N = 4 independent transfections). (FIG. 1C) Schematic of experiments involving retro-orbital injection of AAV-MyD88 or Mock repressors to Cas9 nuclease transgenic mice. (FIG. 1D) qRT-PCR analysis of Myd88 expression level in lung, blood, and bone marrow of Cas9 transgenic mice 3 weeks post retro-orbital injections of 1E+12 GC of AAV/Myd88 or AAV/Mock vectors carrying MS2- HP la -KRAB or MS2-KRAB (N = 4-5 for injected groups and N = 2 for Not Injected group). Fold changes are relative to universal control (FIGS. 1E and 1F) Fold-change in the expression level of Icam-1 (FIG. 1E), and Tnfa (FIG. 1F) mRNAs relative to the universal control. (N = 4-5 for injected groups and N = 2 for Not Injected group). Universal control is the level of the desired transcript in the blood sample collected from a not injected mouse, which did

CLAIMS

We claim:

1. A synthetic repression system for repressing myeloid differentiation primary response 88 (MyD88) expression *in vivo* comprising a single amplicon comprising:

(a) a guide RNA (gRNA) comprising (i) a guide sequence complementary to a portion of MyD88 and (ii) an aptamer target site specific for an RNA binding protein;

(b) a nucleotide sequence encoding the aptamer RNA binding protein fused to one or more repression domain; and

(c) a nucleotide sequence encoding a multifunctional Cas nuclease;

wherein the single amplicon is packaged in a vector for DNA-based viral delivery.

2. The synthetic repression system of claim 1, wherein the gRNA guide sequence is 15 or fewer nucleotides in length.

3. The synthetic repression system of claim 1, wherein the gRNA guide sequence targets within 100 base pairs (bp) upstream of TATA box region of MyD88.

4. The synthetic repression system of claim 1, wherein gRNA guide sequence is selected from the group consisting of SEQ ID NOs: 1-4.

5. The synthetic repression system of claim 1, wherein the aptamer target site is an MS2 aptamer target site and the RNA binding protein is bacteriophage MS2 coat protein.

6. The synthetic repression system of claim 5, wherein the gRNA comprises the MS2 aptamer target site and the guide sequence is 15 or fewer nucleotides in length.

7. The synthetic repression system of claim 5, wherein the repression domain is selected from the group consisting of a Kruppel associated box (KRAB) domain, methyl-CpG (mCpG) binding domain 2 (meCP2), Switch independent 3 transcription regulator family member A (SIN3A), histone deacetylase HDT1 (HDT1), n-terminal truncation of methyl-CpG-binding

domain containing protein 2 (MBD2B), nuclear inhibitor of protein phosphatase- 1 (NIPP1), and heterochromatin protein 1 (HP1A).

8. The synthetic repression system of claim 7, wherein the nucleotide of (b) encodes MS2 fused to at least two repression domains.

9. The synthetic repression system of claim 8, wherein the nucleotide sequence encoding MS2 fused to a repression domain comprises a sequence selected from the group consisting of SEQ ID NOs: 55, 59, 61, and 63.

10. The synthetic repression system of claim 1, wherein the system comprises a nucleotide sequence encoding a multifunctional Cas nuclease fused to the aptamer RNA binding protein and a repression domain.

11. The synthetic repression system of claim 1, wherein the Cas nuclease is an *S. aureus* Cas9 nuclease or an *S. pyrogenes* Cas9 nuclease.

12. The synthetic repression system of claim 1, wherein the vector is an AAV2/1 delivery vector.

13. A pharmaceutical composition comprising the synthetic repression system of claim 1 and a pharmaceutically acceptable delivery vehicle.

14. ~~A method of treating a subject with septicemia comprising administering to the subject with septicemia~~ Use of a therapeutically effective amount of the composition of claim 13 in the manufacture of a medicament for treating a subject with septicemia, wherein the composition is to be administered to the subject with septicemia.

15. The ~~method~~ use of claim 14, wherein levels of immune related markers Icam-1, Tnfα, Ncf, Il6, Ifn-α, Ifn-β, Ifn-γ, and Stat4 are reduced in the subject following administration.

16. The ~~method~~ use of claim 14, wherein ~~additionally comprising the step of monitoring~~ the level of at least one immune related marker selected from the group consisting of Icam-1, Tnfα, Ncf, Il6, Ifn-

α, Ifn-β, Ifn-γ, and Stat4 is to be monitored and ~~administering to the subject~~ additional doses of the synthetic repression system composition is to be administered to the subject until the level of the at least one immune related marker is reduced.

17. The ~~method~~ use of claim 14, wherein serum lactate is reduced in the subject following administration.

18. ~~A method of preventing an adverse immune response in a subject comprising administering to the subject~~ Use of a therapeutically effective amount of the composition of claim 13 in the manufacture of a medicament for preventing an adverse immune response in a subject, wherein the composition is to be administered to the subject prior to or concurrently with administration of an AAV vector based gene therapy to the subject, wherein the level of an immune related marker selected from the group consisting of Icam-1, Tnfα, Ncf, Il6, Ifn-α, Ifn-β, Ifn-γ, and Stat4 is reduced in the subject relative to subject who received the AAV vector based gene therapy but did not receive the therapeutic synthetic repression system composition.

19. The ~~method~~ use of claim 18, wherein the subject previously received at least one AAV vector based gene therapy.

20. The ~~method~~ use of claim 18, wherein~~additionally comprising the step of monitoring~~ the level of at least one immune related marker selected from the group consisting of Icam-1, Tnfα, Ncf, Il6, Ifn-α, Ifn-β, Ifn-γ, and Stat4 is to be monitored and ~~administering to the subject~~ additional doses of the synthetic repression system composition is to be administered to be subject until the level of the at least one immune related marker is reduced.

21. ~~A method of treating Waldenström macroglobulinemia in a subject in need thereof comprising administering to a subject with Waldenström macroglobulinemia~~ Use of a therapeutically effective amount of the composition of claim 13 in the manufacture of a medicament for treating Waldenström macroglobulinemia in a subject in need thereof, wherein the composition is to be administered to a subject with Waldenström macroglobulinemia.

22. The ~~method~~ use of treatment of claim 21, wherein ~~additionally comprising administering to the subject~~ a composition comprising a gRNA configured to cleave MyD88 at L265 locus and a homology directed repair template encoding a portion of wild-type MyD88 at the L265 locus is to be further administered to the subject, whereby levels of endogenous L265P mutant MyD88 are reduced in the subject.

23. The composition of claim 13 for use in therapy.