What is claimed is:

1. An shRNA expression cassette, which is expressed by using an adeno-associated virus (AAV) vector, wherein in an order of 5’ to 3’, the shRNA expression cassette sequentially comprises a DNA coding sequence of the shRNA and a stuffer sequence; and the stuffer sequence is a human non-coding sequence, and wherein the stuffer sequence is all located at 3’end of the DNA coding sequence of the shRNA,

wherein the shRNA expression cassette is expressed by using a single-stranded AAV viral vector, and a sequence length of the shRNA expression cassette is 3.2 kb to 5.2 kb; or the shRNA expression cassette is expressed by using a double-stranded AAV viral vector, and a sequence length of the shRNA expression cassette expressed by using the double-stranded AAV viral vector is half of a sequence length of the shRNA expression cassette expressed by using a single-stranded AAV viral vector.

2. The shRNA expression cassette of claim 1, wherein the human non-coding sequence is selected from a sequence fragment or a combination of a plurality of sequence fragments of an intron sequence of human factor IX, a sequence of human cosmid C346 or an HPRT-intron sequence.

3. The shRNA expression cassette of claim 1 or 2, wherein the human non-coding sequence is a sequence fragment of the HPRT-intron sequence.

4. The shRNA expression cassette of any one of claims 1 to 3, wherein the shRNA expression cassette is expressed by using a single-stranded AAV viral vector, and wherein a sequence length of the shRNA expression cassette is 3.8 kb to 5.1 kb.

5. The shRNA expression cassette of any one of claims 1 to 4, wherein a 5’ end of the DNA coding sequence of the shRNA in the shRNA expression cassette comprises a promoter.

6. The shRNA expression cassette of claim 5, wherein the promoter comprises one or more selected from an RNA polymerase II promoter and an RNA polymerase III promoter.

7. The shRNA expression cassette of claim 6, wherein the RNA polymerase II promoter is a tissue-specific promoter; optionally, the RNA polymerase II promoter is a liver-specific promoter; optionally the liver-specific promoter is LP1 promoter, ApoE/hAAT promoter, DC172 promoter, DC190 promoter, ApoA-I promoter, TBG promoter, LSP1 or HD-IFN promoter.

8. The shRNA expression cassette of claim 6, wherein the RNA polymerase III promoter is a U6 promoter, an H1 promoter, or a 7SK promoter; optionally, the RNA polymerase III promoter is an H1 promoter.

9. The shRNA expression cassette of any one of claims 1 to 8, wherein the DNA coding sequence of the shRNA is one or more selected from SEQ ID No: 1 to SEQ ID No: 3 in the Sequence Listing.

10. An shRNA, expressed by the shRNA expression cassette of any one of claims 1 to 8, wherein the DNA coding sequence of the shRNA is one or more selected from SEQ ID No: 1 to SEQ ID No: 2 in the Sequence Listing.

11. A polynucleotide sequence, carrying the shRNA expression cassette of any one of claims 1 to 9, wherein both ends of the shRNA expression cassette are Adeno-Associated Virus (AAV) terminal inverted repeat sequences, respectively.

12. The polynucleotide sequence of claim 11, wherein the AAV terminal inverted repeat sequences at each end of the shRNA expression cassette are from different serotypes of AAVs, optionally the AAV terminal inverted repeat sequence is selected from any serotype of AAVs in clades A-F or AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 or any of hybrid/chimeric types thereof, optionally the AAV terminal inverted repeat sequence is derived from the AAV2 serotype.

13. The polynucleotide sequence of claim 12, wherein the sequence length between the two terminal inverted repeat sequences in the polynucleotide sequence is

3.2 kb to 5.2 kb; when trs in one inverted terminal repeat sequence is modified by insertion, deletion, or substitution, resulting in a Rep protein mutant cleavage site which cannot be efficiently cleaved, the sequence length between the two inverted repeat sequences is half of 3.2 kb to 5.2 kb.

14. The polynucleotide sequence of any one of claims 11 to 13, wherein a 5’ end inverted repeat sequence in the polynucleotide sequence has deletion of D sequence, and the sequence length between the two terminal inverted repeat sequences in the polynucleotide sequence is half of 4.6 kb to 5.1 kb.

15. A recombinant adeno-associated virus (AAV) vector plasmid, carrying the shRNA expression cassette of any one of claims 1 to 9 or the polynucleotide sequence of any one of claims 11 to 14.

16. The recombinant adeno-associated virus (AAV) vector plasmid of claim 15, wherein the adeno-associated virus vector is of the AAV2/8 type, in which a capsid protein of the adeno-associated virus vector is from serotype VIII, and a terminal inverted repeat sequence of the adeno-associated virus vector is from serotype II.

17. A host comprising the recombinant adeno-associated virus (AAV) vector plasmid of any one of claims 15 to 16.

18. The host of claim 17, wherein the host is selected from one or more of *Escherichia coli*, HEK293 cell line, HEK293T cell line, HEK293A cell line, HEK293S cell line, HEK293FT cell line, HEK293F cell line, HEK293H cell line, HeLa cell line, SF9 cell line, SF21 cell line, SF900 cell line, and BHK cell line.

19. A viral particle, packaged by the recombinant adeno-associated virus (AAV) vector plasmid of any one of claims 15 to 16, or produced by the host of any one of claims 17 to 18.

20. The viral particle of claim 19, wherein the viral particle is non-selectively or selectively expressed in liver tissue or liver cancer cell.

21. An isolated and engineered cell, wherein the cell expresses or comprises the shRNA expression cassette of any one of claims 1 to 9, the polynucleotide sequence of any one of claims 11 to 14, the recombinant adeno-associated virus (AAV) vector plasmid of any one of claims 15 to 16, or the viral particle of claim 18 or 20.

22. The engineered cell of claim 21, wherein the cell is a mammalian cell, optionally a human cell, or even optionally a human stem cell or hepatocyte.

23. A pharmaceutical composition, comprising an active ingredient and a pharmaceutically acceptable excipient, wherein the active ingredient is selected from

one or more of the recombinant adeno-associated virus (AAV) vector plasmid of any one of claims 15 to 16, the shRNA of claim 10, the viral particle of claim 19 or 20, and the isolated and engineered cell of claim 21 or 22.

24. The pharmaceutical composition of claim 23, wherein the pharmaceutical composition is an injection comprising a pharmaceutically acceptable excipient and the active ingredient.

25. Use of the recombinant adeno-associated virus (AAV) vector plasmid of any one of claims 15-16, or the shRNA of claim 10, or the virus particle ofclaim 19 or 20, or the isolated and engineered cell of claim 21 or 22 in preparation of a medicament for prevention and treatment of hepatitis B, acquired immunodeficiency syndrome, for treatment of Duchenne muscular dystrophy (DMD), and for treatment of hypercholesterolemia.

26. A vector preparation system for packaging the recombinant adeno-associated virus (AAV) particle of claim 19 or 20, wherein the vector preparation system is a conventional AAV vector preparation system comprising a two-plasmid packaging system, a three-plasmid packaging system, a baculovirus packaging system, and an AAV packaging system using Ad or HSV as a helper virus, and wherein the vector preparation system comprises the shRNA expression cassette of any one of claims 1 to 9.

27. The vector preparation system of claim 26, wherein the three-plasmid packaging system comprises a plasmid pscAAV-H1-shRNA-Stuffer, a plasmid pHelper, and a plasmid pAAV-R2CX; wherein

the plasmid pHelper provides E2A, E4 and VA regions of adenovirus;

the plasmid pAAV-R2CX provides a sequence comprising a rep gene and a cap gene;

the plasmid pscAAV-H1-shRNA-Stuffer comprises the polynucleotide sequence of any one of claims 11 to 14, X refers to an AAV serotype name corresponding to a source of Cap gene constituting the pAAV-R2CX recombinant vector plasmid.