CLAIMS

~~What is claimed is:~~

1. A recombinant adeno-associated virus (rAAV) nucleic acid vector for delivering two or more transgenes into the heart of a subject, wherein said vector comprises, from 5' to 3', in order, a first adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence, two or more transgenes and a promoter operably linked to the two or more transgenes, a polyadenylation (polyA) signal, and a second AAV ITR sequence,

wherein the two or more transgenes encode an S100 family protein and an apoptotic inhibitor, respectively, and

wherein the transgene encoding an S100 family protein comprises a sequence that is at least 95%, at least 98%, or at least 99.5% identical to either of the nucleotide sequences of SEQ ID NOs: 25 and 26, optionally wherein the transgene encoding an S100 family protein comprises either of the nucleotide sequences of SEQ ID NOs: 25 and 26,

optionally wherein the transgene encoding an S100 family protein comprises a sequence having a reduced guanosine (G) and cytosine (C) (G/C) content, relative to any one of the nucleotide sequences of SEQ ID NOs: 5, 8, 19, and 20, optionally wherein the G/C content is reduced by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15%, relative to any one of the nucleotide sequences of SEQ ID NOs: 5, 8, 19, and 20,

optionally wherein the apoptotic inhibitor is cardiac Apoptosis Repressor with Caspase Recruitment Domain (ARC) or a variant thereof, and

optionally wherein an Internal Ribosome Entry Site (IRES) is present between the transgene encoding an S100 family protein and the transgene encoding an apoptotic inhibitor.~~.~~

~~2. The rAAV nucleic acid vector of claim 1, wherein the transgene encoding an S100 family protein comprises either of the nucleotide sequences of SEQ ID NOs: 25 and 26.~~

2~~3~~. The rAAV nucleic acid vector of claim 1~~ or claim 2~~, wherein the polyA signal comprises a nucleotide sequence that has at least 90%, 92.5%, 95%, 98%, or 99% identity to the nucleotide sequence of SEQ ID NO: 28, optionally wherein the polyA signal comprises the nucleotide sequence of SEQ ID NO: 28.~~.~~

~~4. The rAAV nucleic acid vector of any one of claims 1-3, wherein the polyA signal comprises the nucleotide sequence of SEQ ID NO: 28.~~

~~5. The rAAV nucleic acid vector of any one of claims 1-4, wherein the transgene encoding an S100 family protein comprises a sequence having a reduced guanosine (G) and cytosine (C) (G/C) content, relative to any one of the nucleotide sequences of SEQ ID NOs: 5, 8, 19, and 20.~~

~~6. The rAAV nucleic acid vector of claim 5, wherein the G/C content is reduced by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15%, relative to any one of the nucleotide sequences of SEQ ID NOs: 5, 8, 19, and 20.~~

~~7. The rAAV nucleic acid vector of any one of claims 1-6, wherein the apoptotic inhibitor is cardiac Apoptosis Repressor with Caspase Recruitment Domain (ARC) or a variant thereof.~~

3~~8~~. The rAAV nucleic acid vector of claim 1 or claim 2~~any one of claims 1-7~~, wherein the transgene encoding an apoptotic inhibitor comprises a sequence that is at least 90%, at least 95%, or at least 99.5% identical to any one of the nucleotide sequences of SEQ ID NOs: 3, 6, 7, and 15-18, optionally ~~.~~

~~9. The rAAV nucleic acid vector of any one of claims 1-8,~~ wherein the transgene encoding an apoptotic inhibitor comprises any one of the nucleotide sequences of SEQ ID NOs: 3, 6, 7, and 15-18.

~~10. The rAAV nucleic acid vector of any one of claims 1-9, wherein an Internal Ribosome Entry Site (IRES) is present between the transgene encoding an S100 family protein and the transgene encoding an apoptotic inhibitor.~~
~~11. The rAAV nucleic acid vector of any one of claims 1-10, wherein the transgenes are specific for *homo sapiens* or *canis lupus familiaris*.~~

4~~12~~. The rAAV nucleic acid vector of any one of claims 1-3~~11~~, wherein the promoter is a cardiac-restricted promoter, optionally~~.~~

~~13. The rAAV nucleic acid vector of claim 12,~~ wherein the promoter is a cardiac-restricted promoter selected from the group consisting of: cardiac troponin C, cardiac troponin I, and cardiac troponin T (cTnT), and optionally~~.~~

~~14. The rAAV nucleic acid vector of claim 12 or claim 13,~~ wherein the promoter is cTnT, optionally~~.~~

~~15. The rAAV nucleic acid vector of claim 12,~~ wherein the promoter is a cardiac-restricted promoter derived from a gene selected from the group consisting of: α-myosin heavy chain gene, 6-myosin heavy chain gene, myosin light chain 2v gene, myosin light chain 2a gene, CARP gene, cardiac α-actin gene, cardiac m2 muscarinic acetylcholine gene, ANF, cardiac sarcoplasmic reticulum Ca-ATPase gene, and skeletal α-actin; or is an artificial cardiac promoter derived from MLC-2v gene.

~~16. The rAAV nucleic acid vector of any one of claims 1-15, wherein the rAAV nucleic acid vector is single-stranded.~~
~~17. The rAAV nucleic acid vector of any one of claims 1-16, wherein the rAAV nucleic acid vector is self-complementary.~~

5~~18~~. The rAAV nucleic acid vector of any one of claims 1-4~~17~~, wherein the rAAV nucleic acid vector comprises a nucleotide sequence that is at least 90%, at least 95% or at least 99.5% identical to either of the nucleotide sequences of SEQ ID NOs: 22 and 23, optionally~~.~~

~~19. The rAAV nucleic acid vector of claim 18,~~ wherein the rAAV nucleic acid vector comprises the nucleotide sequence of SEQ ID NO: 22, optionally ~~.~~

~~20. The rAAV nucleic acid vector of claim 18,~~ wherein the rAAV nucleic acid vector comprises the nucleotide sequence of SEQ ID NO: 23.

6~~21~~. An rAAV particle comprising the rAAV nucleic acid vector of any one of claims 1-5~~20~~ encapsidated in an AAV capsid, optionally~~.~~

~~22. The rAAV particle of claim 21,~~ wherein the AAV capsid comprises a capsid protein derived from an AAV1, AAV2, AAV3, AAV6, AAV8, AAVrh.74, AAVrh.10, AAV2/6, or AAV9 serotype, and optionally ~~.~~

~~23. The rAAV particle of claim 21 or claim 22,~~ wherein the AAV capsid comprises a capsid protein derived from an AAVrh.10 serotype.

7~~24~~. A composition comprising the rAAV particle ~~of any one~~ of claim 6~~s 21-23~~.

825. Use of ~~A method of treatment of a subject suffering from a heart disease comprising administering to the subject the composition of claim 24 or~~ the rAAV particle ~~of any one~~ of claim ~~s 6~~21-23 in the preparation of a medicament for treating a heart disease in a subject, wherein the medicament is to be administered to the subject, optionally ~~.~~

~~26. The method of claim 25,~~ wherein the heart disease causes heart failure in the subject, optionally ~~.~~

~~27. The method of claim 25 or claim 26,~~ wherein the heart disease is cardiomyopathy, optionally ~~.~~

~~28. The method of any one of claims 25-27,~~ wherein the heart disease is hypertrophic cardiomyopathy or dilated cardiomyopathy, optionally ~~.~~

~~29. The method of claim 25 or claim 26,~~ wherein the heart disease is acute ischemia.

930. The use~~method~~ of claim 8~~any one of claims 25-29~~, wherein the composition is to be administered via injection into the heart of the subject or intravascular injection into the coronary arteries of the subject, optionally ~~.~~

~~31. The method of any one of claims 25-30,~~ wherein the ~~step of administering~~administration results in expression of the two or more transgenes in the subject's heart.

1032. The use~~method~~ of claim 8 or claim 9~~any one of claims 25-31~~, wherein the subject is a mammal, optionally wherein the mammal is a human or a companion animal, optionally wherein the companion animal is a dog or a cat.

~~33. The method of claim 32, wherein the mammal is a human.~~

~~34. The method of claim 32, wherein the mammal is a companion animal.~~

~~35. The method of claim 34, wherein the companion animal is a dog or a cat.~~

1136. The usemethod of any one of claims 8-1025-35, wherein the administrationstep of administering results in improved cardiac function in the subject, optionally wherein the administration results in improved cardiac function in the subject for more than 10 months.

37. The method of claim 36, wherein the step of administering results in improved cardiac function in the subject for more than 10 months.

1238. The rAAV nucleic acid vector of any one of claims 1-520, wherein the transgene encoding an S100 family protein is positioned 5′ to the transgene encoding an apoptotic inhibitor.

1339. The rAAV nucleic acid vector of any one of claims 1-5, 20, wherein the transgene encoding an apoptotic inhibitor is positioned 5′ to the transgene encoding an S100 family protein.

1440. The rAAV nucleic acid vector of any one of claims 1-520, 1238, and 1339, wherein the rAAV nucleic acid vector encodes a protein comprising an amino acid sequence at least 90%, at least 95%, or at least 99.5% identical to the amino acid sequence of SEQ ID NO: 13 or 14, optionally.

41. The rAAV nucleic acid vector of any one of claims 1-20 and 38-40, wherein the rAAV nucleic acid vector encodes a protein comprising the amino acid sequence of SEQ ID NO: 13 or 14.

1542. The rAAV nucleic acid vector of any one of claims 1-520 and 12-1438-41, wherein the rAAV nucleic acid vector encodes a protein comprising an amino acid sequence at least 90%, at least 95%, or at least 99.5% identical to the amino acid sequence of SEQ ID NO: 24 or 29, optionally .

43. The rAAV nucleic acid vector of any one of claims 1-20 and 38-42, wherein the rAAV nucleic acid vector encodes a protein comprising the amino acid sequence of SEQ ID NO: 24 or 29.