We claim:

1. A composition for use in treating X-linked juvenile retinoschisis in a subject, comprising:

(a) a nucleic acid construct comprising a coding sequence for a retinoschisin protein or functional fragment thereof for integration into a target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) a nuclease agent comprising a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus, and

wherein the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct is to be expressed in a retinal cell,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site, and

wherein the integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct.

2. The composition for use of claim 1, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

3. The composition for use of claim 1 or 2, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

4. The composition for use of claim 1 or 2, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

5. The composition for use of claim 1 or 2, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

6. The composition for use of any one of claims 1-5, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

7. The composition for use of any one of claims 1-6, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

8. The composition for use of any one of claims 1-7, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the composition comprises a DNA encoding the Cas protein, and wherein the composition comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the composition comprises a messenger RNA encoding the Cas protein, wherein the composition comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

9. The composition for use of any one of claims 1-8, wherein the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis.

10. The composition for use of any one of claims 1-9, wherein the retinal cell is a human retinal cell.

11. Use of a nucleic acid construct and a nuclease agent in the manufacture of a medicament for treating X-linked juvenile retinoschisis in a subject, wherein:

(a) the nucleic acid construct comprises a coding sequence for a retinoschisin protein or functional fragment thereof for integration into a target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, and wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) the nuclease agent comprises a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site,

wherein the integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct, and

wherein (a) and (b) are to be administered sequentially, in any order.

12. The use of claim 11, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

13. The use of claim 11 or 12, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

14. The use of claim 11 or 12, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

15. The use of claim 11 or 12, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement

of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

16. The use of any one of claims 11-15, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

17. The use of any one of claims 11-16, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

18. The use of any one of claims 11-17, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the combination comprises a DNA encoding the Cas protein, and wherein the combination comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the combination comprises a messenger RNA encoding the Cas protein, wherein the combination comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

19. The use of any one of claims 11-18, wherein the endogenous *RS1* locus comprises a mutated *RS1* gene comprising a mutation that causes X-linked juvenile retinoschisis.

20. The use of any one of claims 11-19, wherein the retinal cell is a human retinal cell.

21. A combination for integrating a coding sequence for a retinoschisin protein or functional fragment thereof into a target genomic locus in a retinal cell, wherein the combination comprises:

(a) a nucleic acid construct comprising the coding sequence for the retinoschisin protein or functional fragment thereof for integration into the target genomic locus, wherein the coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the retinoschisin protein or functional fragment thereof, and wherein:

(I) the nucleic acid construct comprises a polyadenylation signal sequence located 3' of the coding sequence, and

(II) the nucleic acid construct comprises a splice acceptor site located 5' of the coding sequence, optionally wherein the splice acceptor site is from intron 1 of human *RS1*; and

(b) a nuclease agent comprising a Cas protein or a nucleic acid encoding the Cas protein and a guide RNA or a DNA encoding the guide RNA, wherein the nuclease agent targets a nuclease target sequence in the target genomic locus,

wherein the target genomic locus is in an endogenous *RS1* locus comprising an endogenous *RS1* gene,

wherein integration of the nucleic acid construct into the endogenous *RS1* locus prevents transcription of the endogenous *RS1* gene downstream of the integration site, and

wherein integration of the nucleic acid construct into the endogenous *RS1* locus in the retinal cell reduces or eliminates expression of endogenous retinoschisin protein and replaces it with expression of the retinoschisin protein or functional fragment thereof encoded by the nucleic acid construct.

22. The combination of claim 21, wherein the nucleic acid construct comprises a fragment or portion of the first intron of human *RS1* located 5' of the coding sequence.

23. The combination of claim 21 or 22, wherein the nucleic acid construct is for homology-independent targeted integration into the target genomic locus, wherein the nucleic

acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked on each side by a nuclease target sequence for a nuclease agent, optionally:

(I) wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation; or

(II) wherein the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and

wherein the nuclease target sequence in the target genomic locus is identical to the nuclease target sequence in the nucleic acid construct, and wherein the nuclease target sequence in the target genomic locus is destroyed if the nucleic acid construct is inserted into the target genomic locus in the correct orientation but is reformed if the nucleic acid construct is inserted into the target genomic locus in the opposite orientation.

24. The combination of claim 21 or 22, wherein the nucleic acid construct is for homologous recombination with the target genomic locus, wherein the nucleic acid construct comprises the coding sequence for the retinoschisin protein or functional fragment thereof flanked by homology arms on each side, optionally wherein the coding sequence and homology arms are further flanked on each side by a nuclease target sequence for a nuclease agent, and optionally wherein each homology arm is between 25 nucleotides and 2.5 kb in length,

optionally wherein the retinoschisin protein or functional fragment thereof is a human retinoschisin protein or functional fragment thereof, the coding sequence for the retinoschisin protein or functional fragment thereof comprises complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*, and each homology arm is between 25 nucleotides and 2.5 kb in length.

25. The combination of claim 21 or 22, wherein the nucleic acid construct is bidirectional and comprises:

(a) a first segment comprising a first coding sequence for a first retinoschisin protein or functional fragment thereof, wherein the first coding sequence encodes the protein encoded by exons 2-6 of human *RS1*; and

(b) a second segment comprising a reverse complement of a second coding sequence for a second retinoschisin protein or functional fragment thereof, wherein the second coding sequence encodes the protein encoded by exons 2-6 of human *RS1*,

optionally:

(I) wherein the nucleic acid construct does not comprise a homology arm; or

(II) wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof, and wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence; or

(III) wherein the second segment is located 3' of the first segment,

wherein both the first retinoschisin protein or functional fragment thereof and the second retinoschisin protein or functional fragment thereof are a human retinoschisin protein or functional fragment thereof,

wherein the first retinoschisin protein or functional fragment thereof is identical to the second retinoschisin protein or functional fragment thereof,

wherein both the first coding sequence and the second coding sequence comprise complementary DNA (cDNA) that encodes the protein encoded by exons 2-6 of human *RS1*,

wherein the second coding sequence adopts a different codon usage from the codon usage of the first coding sequence,

wherein the first segment comprises a first polyadenylation signal sequence located 3' of the first coding sequence, and the second segment comprises a reverse complement of a second polyadenylation signal sequence located 5' of the reverse complement of the second coding sequence,

wherein the first segment comprises a first splice acceptor site located 5' of the first coding sequence, and the second segment comprises a reverse complement

of a second splice acceptor site located 3' of the reverse complement of the second coding sequence,

wherein the nucleic acid construct does not comprise a promoter that drives expression of the first retinoschisin protein or functional fragment thereof or the second retinoschisin protein or functional fragment thereof, and

wherein the nucleic acid construct does not comprise a homology arm.

26. The combination of any one of claims 21-25, wherein the nuclease target sequence in the target genomic locus is in the first intron in the endogenous *RS1* gene at the endogenous *RS1* locus.

27. The combination of any one of claims 21-26, wherein the nucleic acid construct is in a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) viral vector, and optionally wherein the AAV is selected from the group consisting of: AAV2, AAV5, AAV8, and AAV7m8.

28. The combination of any one of claims 21-27, wherein the Cas protein is a Cas9 protein, optionally wherein:

(I) the combination comprises a DNA encoding the Cas protein, and wherein the combination comprises the DNA encoding the guide RNA, and optionally wherein the DNA encoding the Cas protein and the DNA encoding the guide RNA are in one or more viral vectors; or

(II) the combination comprises a messenger RNA encoding the Cas protein, wherein the combination comprises the guide RNA in the form of RNA, and optionally wherein the guide RNA and the messenger RNA encoding the Cas protein are in a lipid nanoparticle.

29. The combination of any one of claims 21-28, wherein the nucleic acid construct and the nuclease agent are in the same delivery vehicle.

30. The combination of any one of claims 21-28, wherein the nucleic acid construct and the nuclease agent are in different delivery vehicles.