CLAIMS

1. A miRNA expression construct comprising, from 5' to 3', a promoter element, a spacer between 300 and 600 nucleotides in length, and a miRNA hairpin, wherein the spacer is at least 90% identical to SEQ ID NO: 48 or SEQ ID NO: 49.

2. The miRNA expression construct of claim 1, wherein the promoter is a eukaryotic Pol II or Pol III promoter.

3. The miRNA expression construct of claim 2, wherein the promoter has a sequence at least 90% or 95% identical to SEQ ID NO: 45.

4. The miRNA expression construct of claim 1, wherein the construct comprises at least 4, 5, 6, 7, 8, 9, or 10 miRNA hairpins.

5. The miRNA expression construct of claim 4, wherein at least two of the miRNA hairpins target different sequences.

6. The miRNA expression construct of any of claims 1-5, wherein the miRNA hairpin comprises from 5' to 3' and in the order from (a)-(g):

(a) a mir-16 flanking sequence comprising the sequence of SEQ ID NO: 25;

(b) a first lower stem sequence comprising the mir-16 sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30;

(c) an anti-sense target sequence 22 nucleotides in length;

(d) a mir-30 loop sequence comprising the sequence of SEQ ID NO: 31;

(e) a sense sequence wherein the sequence is complementary to the sequence of (c) except that the sequence comprises one or two mismatches relative to the sequence of (c), wherein the one or two mismatches comprise:

i) a mismatch located at the position 8 to 14 of the sense sequence; and/or

ii) a mismatch at the final 3' position (position 22) of the sense sequence;

(f) a second lower stem sequence wherein the sequence is complementary to the sequence of (b); and

(g) a second flanking sequence.

7. The miRNA expression construct of claim 6, wherein the sense sequence (e) of the miRNA hairpin comprises one mismatch relative to sequence (c) located at nucleotide position 11 of the sense sequence (e).

8. The miRNA expression construct of claim 6 or 7, wherein flanking sequence (g) is not complementary to the mir-16 flanking sequence (a).

9. The miRNA expression construct of any one of claims 1-8, further comprising a receptor-coding sequence.

10. The miRNA expression construct of claim 9, wherein the receptor-coding sequence is a chimeric antigen receptor-coding sequence and/or a T-cell receptor-coding sequence.

11. The miRNA expression construct of any one of claims 1-10, further comprising a selection gene and/or a suicide gene.

12. The miRNA expression construct of any one of claims 1-11, further comprising a peptide cleavage site.

13. The miRNA expression construct of claim 12, wherein the peptide cleavage site is a 2A peptide selected from the group comprising: 2A, P2A, T2A, E2A, F2A, BmCPV 2A, and BmIFV 2A.

14. The miRNA expression construct of any one of claims 1-13, wherein the nucleic acid molecule is RNA, or wherein the nucleic acid molecule is DNA.

15. An expression vector comprising the miRNA expression construct of any of claims 1-14.

16. A host cell comprising the miRNA expression construct of any one of claims 1-14 or the expression vector of claim 15.

17. The host cell of claim 16, wherein the host cell in an immune effector cell.

18. An in vitro method for reducing expression of a gene in a cell comprising expressing the miRNA expression construct of any one of claims 6-14 or expressing the miRNA

expression construct from the expression vector of claim 15, wherein the anti-sense target sequence (c) is complementary to the sense strand of the gene.

19. An in vitro method for preparing engineered immune effector cells comprising:

(a) transducing or transfecting immune effector cells with a chimeric antigen receptor or T cell receptor to generate modified immune effector cells; and

(b) transducing or transfecting the modified immune effector cells with the miRNA expression construct of any of claims 1-14 or the expression vector of claim 15 to generate engineered immune effector cells.

20. The method of claim 19, wherein the chimeric antigen receptor or T cell receptor is encoded by the miRNA expression construct or the expression vector.

21. The method of any one of claims 18-20, comprising:

(a) transfecting the cell with the miRNA expression construct or the expression vector; or

(b) inserting the miRNA expression construct or the expression vector into the genome of the cell by homologous recombination.

22. Use of the cell of claim 17, in the manufacture of a medicament for treating cancer.