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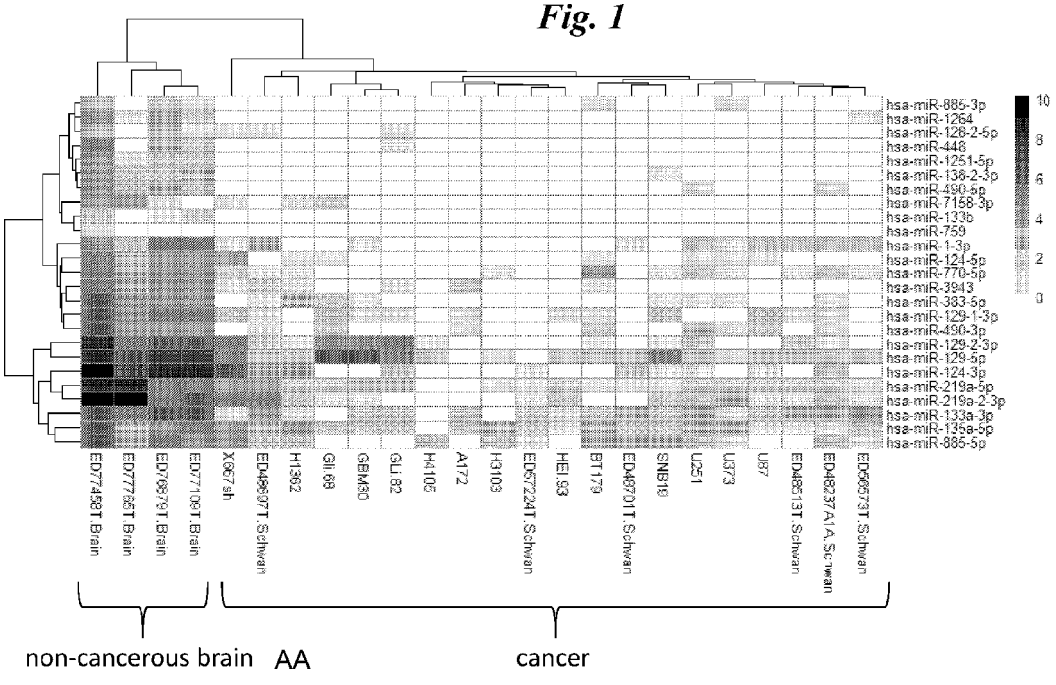
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(54) Title: ONCOLYTIC VIRAL VECTORS AND USES THEREOF

Fig. 1



(57) Abstract: The present disclosure relates to recombinant viral vectors for the treatment and prevention of cancer. Oncolytic viral vectors incorporate one or more of the following features: viral replication restriction by insertion of microRNA (miRNA) target sequences into the viral genome; disruption of oncogenic miRNA function; cancer microenvironment remodeling; and cancer cell targeting by incorporation of protease-activated antibodies into the viral particle.

[Continued on next page]

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ONCOLYTIC VIRAL VECTORS AND USES THEREOF

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/537,359, filed July 26, 2017; and U.S. Provisional Application No. 62/686,802, filed June 19, 2018, the disclosures of which are each incorporated herein by reference in their entireties.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is ONCR_010_02WO_ST25.txt. The text file is 500 KB, created on July 26, 2018, and is being submitted electronically via EFS-Web.

FIELD

[0003] The present disclosure relates to recombinant viral vectors for the treatment and prevention of cancer. In particular, the disclosure relates to oncolytic viral vectors.

BACKGROUND

[0004] Current targeted cancer therapeutics are efficacious in only a narrow range of cancers due to the heterogeneity of tumor protein expression profiles. Furthermore, many cancer treatments, including existing viral vectors, chemotherapy, radiation, and surgery lack the specificity to selectively treat cancerous cells, while maintaining the health and viability of normal, non-cancerous cells and can produce undesirable off-target effects. As such, there is a need in the art for cancer therapies that are broadly efficacious in multiple cancers and are capable of selectively eliminating cancerous cells.

[0005] Oncolytic viruses are viruses that preferentially infect cancer cells and have been used in multiple pre-clinical and clinical studies for cancer treatment. Use of oncolytic viruses carries the risk of non-specific viral infection of healthy cells, leading to the death of non-cancerous cells and tissues. However, genetic manipulation of the viruses to exploit pathways, proteins, and

genes that are differentially expressed in normal vs. cancerous tissue can increase the specificity of these viruses and limit off-target infection and cell death.

[0006] MicroRNAs (miRNAs or miRs) are small non-coding endogenous RNAs that regulate gene expression by directing their target messenger RNAs for degradation or translational repression. miRs are intimately associated with normal cellular processes and therefore, deregulation of miRNAs contributes to a wide array of diseases including cancer. Many miR genes are located in cancer associated genomic regions, or in fragile sites, further strengthening the evidence that miRs play a pivotal role in cancer. miRs are differentially expressed in cancer tissues compared to normal tissues and can have a causative relationship to tumorigenesis. By exploiting this differential miR expression in diverse tumor types, the cancer therapeutics described herein possess a broad spectrum safety and efficacy profile, wherein oncolytic viral replication is regulated based on the expression of a particular miR or group of miRs. Further, the oncolytic viruses described herein may also express proteins that facilitate viral spread throughout a tumor, such as those altering the expression of genes and proteins that regulate the extracellular matrix, thereby increasing their therapeutic efficacy.

[0007] There remains a need in the art for improved oncolytic viral vectors. The present disclosure provides such improved oncolytic viral vectors, and more.

SUMMARY

[0008] The present disclosure provides oncolytic viral vectors that exhibit improved technical effects compared to the prior art. The present inventors have designed various oncolytic viral vectors and performed extensive experiments, described herein, to identify oncolytic viral vectors with superior properties for clinical use in treatment of cancer.

[0009] The invention relates to recombinant viral vectors that are useful for the treatment and prevention of cancer. The oncolytic viral vectors described herein are capable of restricting viral vector replication to cancer or tumor cells by virtue of microRNA (miR) target sequences that are inserted into the viral genome. In particular embodiments described herein, the viral vectors comprise two, three, four or more copies of a miR target sequence incorporated into one or more essential viral genes. In further embodiments, the viral vectors comprise incorporation of one or more polynucleotide sequences into the viral genome whose product(s) disrupt the

function of an oncogenic miR and/or alter the extracellular matrix. In further embodiments, the viral vectors comprise protease-activated antibodies incorporated into the viral particle, thereby allowing forhighly selective targeting of the vectors to cancer/tumor cells. Compositions of the viral vectors and methods of use in killing of cancerous cells and the treatment of cancer are further provided herein.

[0010] In an embodiment, the present disclosure provides a recombinant oncolytic herpes simplex virus (HSV) comprising at least two micro-RNA (miRNA) target sequence inserted into a locus of one or more essential viral genes, wherein the one or more viral genes are selected from the group consisting of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, and UL42. In an embodiment, the replication of the recombinant HSV is reduced in a non-cancerous cell compared to the replication of the the recombinant HSV in a cancerous cell of the same cell type. In an embodiment, the one or more viral genes are selected from the group consisting of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, and UL42. In an embodiment, the one or more viral genes are selected from the group consisting of UL8, ICP8, and UL30. In an embodiment, the one or more viral genes are selected from the group consisting of ICP27 and ICP4. In an embodiment, the one or more viral genes are selected from the group consisting of ICP4, ICP27, UL8, UL42, and ICP34.5.

[0011] In an embodiment, the cell is selected from the group consisting of a neuronal cell, a cardiac cell, a muscle cell, and a liver cell. In an embodiment, the neuronal cell is a a central nervous system cell, a peripheral nervous system cell, a brain cell, or a spinal cord cell. In an embodiment, the muscle cell is a striated muscle cell or a smooth muscle cell. In an embodiment, the non-cancerous cell and the cancerous cell are brain cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-137, miR-219a, miR-124, miR-9, miR-487b, and miR-128. In an embodiment, the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-137, miR-219a, miR-124, and miR-128 In an embodiment, the non-cancerous cell and the cancerous cell are cardiac or striated muscle cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-208b, miR-1, miR-208a, miR-133a, miR-4284, miR-499a, miR-126, miR-30e, miR-378i, miR-30b, and miR-378. In an embodiment, the at least two miRNA target sequences are target sequences for an

miRNA selected from the group consisting of miR-208b, miR-1, and miR-208a In an embodiment, the non-cancerous cell and the cancerous cell are spinal cord cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-219a, miR-9, miR-204, miR-577, miR-99a, miR-100, miR-132, and miR-135. In an embodiment, the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-219a, miR-9, and miR-204. In an embodiment, the non-cancerous cell and the cancerous cell are peripheral nervous system cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-204, miR-1, miR-206, miR-9, miR-99a, miR-199b, miR-145, miR-100, miR-574. In an embodiment, the non-cancerous cell and the cancerous cell are liver cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-122 and miR-126. In an embodiment, the non-cancerous cell and the cancerous cell are smooth muscle cells, and the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-143 and miR-145.

[0012] In an embodiment, the two or more miR target sequences are incorporated into a miR-T cassette that is inserted in the 5' untranslated region (UTR) or 3' UTR of the one or more essential viral genes. In an embodiment, the miR-T cassette comprises a length of less than 1000 nucleotides. In an embodiment, the miR-T cassette comprises a length of between about 25 and about 500 nucleotides. In an embodiment, the miR-T cassette comprises a length of between about 100 and about 500 nucleotides.

[0013] In an embodiment, the present disclosure provides a recombinant oncolytic herpes simplex virus (HSV) comprising: (i) a first microRNA (miRNA) target sequence cassette (miR-TS cassette) inserted into a first viral gene and comprising at least 2 target sequences for each of miR-124, miR-1, and miR-143; (ii) a second miR-TS cassette inserted into a second viral gene and comprising at least 2 target sequences for each of miR-128, miR-219a, and miR-122; and (iii) a third miR-TS cassette inserted into a third viral gene and comprising at least 2 target sequences for each of miR-219a, miR-204, and miR-128. In an embodiment, the receombinant HSV further comprises a fourth miR-TS cassette inserted into a fourth viral gene, wherein the fourth miR-TS cassette comprises: (a) at least 2 target sequences for each of miR-137, miR-208b-3p, and miR-126; or (b) at least 2 target sequences for each of miR-137, miR-217, and miR-126.

[0014] In an embodiment, each of the miR-TS cassettes comprises 4 target sequences for each of the respectively miRNAs. In an embodiment, the first viral gene is ICP4. In an embodiment, the second viral gene is ICP27. In an embodiment, the third viral gene is ICP34.5. In an embodiment, the fourth viral gene is UL8. In an embodiment, the replication of the recombinant HSV is reduced in a non-cancerous cell compared to the replication of the the recombinant HSV in a cancerous cell of the same cell type, wherein the cell is selected from the group consisting of a neuronal cell, a cardiac cell, a muscle cell, and a liver cell.

[0015] In an embodiment, the first miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 852. In an embodiment, the first miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 852. In an embodiment, the second miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 853. In an embodiment, the second miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 853. In an embodiment, the third miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 854. In an embodiment, the third miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 854. In an embodiment, the fourth miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 855. In an embodiment, the fourth miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 855.

[0016] In an embodiment, the recombinant HSV further comprises a heterologous polynucleotide sequence encoding one or more payload molecules. In an embodiment, the heterologous polynucleotide sequence encodes a payload selected from the group consisting of IL-12, CCL4, and CXCL10. In an embodiment, the heterologous polynucleotide sequence encodes two or more payloads selected from the group consisting of IL-12, CCL4, and CXCL10. In an embodiment, the heterologous polynucleotide sequence encodes three payloads comprising IL-12, CCL4, and CXCL10.

[0017] In an embodiment, the present disclosure provides a recombinant oncolytic virus comprising one or more micro-RNA (miR) target sequences inserted into a locus of one or more viral genes required for viral replication, wherein the virus is herpes simplex virus (HSV), and wherein the one or more viral genes are selected from the group consisting of UL8, ICP34.5, UL42, UL19, ICP4, and ICP27. In an embodiment, the one or more miR target sequences is

incorporated into the 5' untranslated region (UTR) or 3' UTR of the one or more viral genes required for viral replication. In an embodiment, the miR target sequence is a target sequence for a miR selected from the group consisting of miR-122, miR-184, miR-34a, let7a, miR-145-5p, miR-199a-5p, miR-451a, miR-125a, miR-125a-5p, miR-126-3p, miR-233-3p, miR-143-3p, miR-1-3p, miR-133a-3p, miR-127a-3p, miR-133b, miR-134-3p, miR-124, miR-101, miR-125b, miR-145, miR-559, miR-213, miR-31-5p, and miR-205p.

[0018] In an embodiment, one or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes. In an embodiment, two, three, four, or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes. In an embodiment, replication of the virus is reduced or attenuated in a first cell compared to replication of the virus in a second cell, wherein the first cell has an increased expression of a miR capable of binding to the one or more miR target sequences compared to the expression of the miR in the second cell. In an embodiment, the expression level of the miR in the first cell is at least 5% greater than the expression level of the miR in the second cell. In an embodiment, the first cell is a non-cancerous cell. In an embodiment, the second cell has a reduced expression of a miR capable of binding to the one or more miR target sequences compared to the expression of the miR in the first cell. In an embodiment, the expression level of the miR in the second cell is at least 5% less than the expression level of the miR in the first cell. In an embodiment, the second cell is a cancerous cell.

[0019] In an embodiment, one copy of a miR-122 target sequence is inserted into the locus of one or more viral genes required for viral replication. In an embodiment, four copies of the miR-122 target sequence are inserted into the locus of one or more viral genes required for viral replication. In an embodiment, the one or more viral genes is ICP27. In an embodiment, one copy of a miR-125a target sequence inserted into the locus of one or more viral genes required for viral replication. In an embodiment, four copies of the miR-125a target sequence are inserted into the locus of one or more viral genes required for viral replication. In an embodiment, the one or more viral genes is UL42. In an embodiment, four copies of a miR-122 target sequence are inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42. In an embodiment, one copy of a miR-122 target sequence is inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42. In an

embodiment, one copy of a miR-122 target sequence is inserted into the locus of ICP27 and three copies of a miR-125a target sequence are inserted into the locus of UL42. In an embodiment, four copies of a miR-122 target sequence are inserted into the locus of ICP27 and four copies of a miR-125a target sequence are inserted into the locus of UL42.

[0020] In an embodiment, the present disclosure provides a recombinant oncolytic virus comprising: (a) one or more micro-RNA (miR) target sequences inserted into a locus of one or more viral genes required for viral replication; and (b) one or more polynucleotides encoding (i) one or more proteins or oligonucleotides, wherein the proteins or oligonucleotides reduce the expression or inhibit the function of a miR, a gene, or a tissue inhibitor of metalloproteinases (TIMP); or (ii) a protease-activated antibody; wherein the virus is an HSV, wherein the one or more viral genes are selected from the group consisting of UL42, UL19, ICP4, and ICP27. In an embodiment, the miR is an oncogenic miR or a microenvironment remodeling miR. In an embodiment, oncogenic miR is selected from the miRs listed in Table 4. In an embodiment,the gene is an oncogenic gene. In an embodiment, the oncogenic gene is selected from the genes listed in Table 7. In an embodiment, the microenvironment remodeling miR is selected from the miRs listed in Table 5. In an embodiment, the one or more miR target sequences is incorporated into the 5' untranslated region (UTR) or 3' UTR of the one or more viral genes required for viral replication. In an embodiment, the miR target sequence is a target sequence for a miR selected from the group consisting of miR-122, miR-184, miR-34a, let7a, miR-145-5p, miR-199a-5p, miR-451a, miR-125a, miR-125a-5p, miR-126-3p, miR-233-3p, miR-143-3p, miR-1-3p, miR-133a-3p, miR-127a-3p, miR-133b, miR-134-3p, miR-124, miR-101, miR-125b, miR-145, miR-559, miR-213, miR-31-5p, and miR-205p.

[0021] In an embodiment, one or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes. In an embodiment, two, three, four, or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes. In an embodiment, one copy of a miR-122 target sequence is inserted into the locus of one or more viral genes required for viral replication. In an embodiment, four copies of the miR-122 target sequence are inserted into the locus of one or more viral genes required for viral replication. In an embodiment, the one or more viral genes is ICP27. In an embodiment, one copy of a miR-125a target sequence is inserted into the locus of one or more viral genes required for

viral replication. In an embodiment, four copies of the miR-125a target sequence are inserted into the locus of one or more viral genes required for viral replication. In an embodiment, the one or more viral genes is UL42. In an embodiment, four copies of a miR-122 target sequence are inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42. In an embodiment, one copy of a miR-122 target sequence is inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42. In an embodiment, one copy of a miR-122 target sequence is inserted into the locus of ICP27 and three copies of a miR-125a target sequence are inserted into the locus of UL42. In an embodiment, four copies of a miR-122 target sequence are inserted into the locus of ICP27 and four copies of a miR-125a target sequence are inserted into the locus of UL42.

[0022] In an embodiment, the TIMP is selected from TIMP1, TIMP2, TIMP3 and TIMP4. In an embodiment, the oligonucleotide is an shRNA or a decoy oligonucleotide. In an embodiment, the protein is a nuclease, a bispecific T-cell engager (BiTE), an anti-immunosuppressive protein, or an immunogenic antigen. In an embodiment, the nuclease is selected from a Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease, a zinc-finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN). In an embodiment, the CRISPR-associated endonuclease is selected from SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, C2C1, C2C3, Cpf1, Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, Csx10, Csx16, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, and Csf4. In an embodiment, the recombinant virus further comprises a heterologous polynucleotide encoding an tracr-RNA (trRNA) and a crispr-RNA (crRNA), wherein the crRNA is targeted to a genomic DNA sequence encoding a miR or a TIMP and wherein the trRNA facilitates binding and activation of a CRISPR-associated endonuclease.

[0023] In an embodiment, the anti-immunosuppressive protein is an anti-regulatory T-cell (Treg) protein or an anti-myeloid-derived suppressor cell (MDSC) protein. In an embodiment, the anti-immunosuppressive protein is a VHH-derived blocker or a VHH-derived BiTE. In an embodiment, the protein induces an anti-tumor immune response. In an embodiment,the protein binds to an antigen expressed on a cell surface, wherein the antigen is

selected from the group consisting of EpCAM, CTLA-4, PD1, FGF2, FGFR/FGFR2b, endothelin B Receptor, and SEMA4D. In an embodiment, the protein is selected from, folate, IFNβ, , A2A, , CCL5, CD137, CD200, CD38, CD44, CSF-1R, CXCL10, CXCL13, IL-12, IL-15, IL-2, IL-21, IL-35, ISRE7, LFA-1, NG2 (also known as SPEG4), a SMAD protein, STING, TGFβ, and VCAM1. In an embodiment, the at least one protease-activated antibody is incorporated into a viral glycoprotein envelope. In an embodiment, the protease-activated antibody is activated by a protease selected from a cysteine cathepsin, an aspartic cathepsin, a kallikrein (hK), a serine protease, a caspase, a matrix metalloproteinase (MMP), and a disintegrin metalloproteinase (ADAM). In an embodiment, the protease is selected from cathepsin K, cathepsin B, cathepsin L, cathepsin E, cathepsin D, hK1, PSA (hK3), hK10, hK15, uPA, uPAR, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-18, MMP-19, MMP-20, MMP-21, MMP-23A, MMP-23B, MMP-24, MMP-25, MMP-26, MMP-27, MMP-28, or a protease listed in Table 6. In an embodiment, the protease-activated antibody binds to a protein expressed more highly by a cancer cell or in a cancer microenvironment than by a non-cancer cell or in a non-cancer microenvironment. In an embodiment, the protease-activated antibody binds NKG2D, c-met, HGFR, CD8, heparan sulfate, VSPG4 (also known as NG2), EGFR, EGFRvIII, CD133, CXCR4, carcinoembryonic antigen (CEA), CLC-3, annexin II, human transferrin receptor, or EpCAM.

[0024] In an embodiment,one or more polynucleotides are inserted into a gene locus of the viral genome, or inserted between two gene loci of the viral genome. In an embodiment, the viral gene loci are selected from the group consisting of the internal repeat joint region (comprising one copy each of the diploid genes ICP0, ICP34.5, LAT, ICP4, and the ICP47 promoter), ICP0, LAT, UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12.

[0025] In an embodiment, the present disclosure provides a nucleic acid molecule encoding an oncolytic virus described herein. In an embodiment, the present disclosure provides a viral stock comprising an oncolytic virus described herein. In an embodiment, the present

disclosure provides a composition comprising an oncolytic virus described herein and a pharmaceutically-acceptable carrier.

[0026] In an embodiment, the present disclosure provides a method of killing a cancerous cell, comprising exposing the cancerous cell to an oncolytic virus described herein or compositions thereof under conditions sufficient for the oncolytic virus to infect and replicate within said cancerous cell, and wherein replication of the oncolytic virus within the cancerous cell results in cell death. In an embodiment, the cancerous cell has a reduced expression of a miR capable of binding to the one or more miR-target sequences compared to the expression of the miR in a non-cancerous cell. In an embodiment, the expression level of the miR in the cancerous cell is at least 5% less than the expression level the miR in the non-cancerous cell. In an embodiment, replication of the oncolytic virus is increased or maintained in cancerous cells with a reduced expression of the miR capable of binding to the one or more miR-target sequences. In an embodiment, the viral replication is at least 5% greater in the cancerous cells compared to the viral replication in the non-cancerous cell. In an embodiment, the cell is in vivo. In an embodiment, the cell is within a tumor.

[0027] In an embodiment, the present disclosure provides a method of treating cancer in a subject in need thereof, comprising administering an oncolytic virus described herein or compositions thereof to a subject in need thereof. In an embodiment, the subject is a mouse, a rat, a rabbit, a cat, a dog, a horse, a non-human primate, or a human. In an embodiment, the oncolytic virus or compositions thereof are administered intravenously, subcutaneously, intratumorally, intramuscularly, or intranasally. In an embodiment, the cancer is selected from lung cancer, breast cancer, ovarian cancer, cervical cancer, prostate cancer, testicular cancer, colorectal cancer, colon cancer, pancreatic cancer, liver cancer, gastric cancer, head and neck cancer, thyroid cancer, malignant glioma, glioblastoma, melanoma, B-cell chronic lymphocytic leukemia, diffuse large B-cell lymphoma (DLBCL), and marginal zone lymphoma (MZL). In an embodiment, the lung cancer is small cell lung cancer or non-small cell lung cancer. In an embodiment, the liver cancer is hepatocellular carcinoma (HCC).

[0028] In an embodiment, a recombinant oncolytic virus described herein further comprises a payload molecule, wherein the payload molecule or protein is an anti-FAP/anti-CD3 bispecific T cell engager. In an embodiment, a recombinant oncolytic virus described herein

further comprises a payload molecule, wherein the payload molecule or protein is an anti-PD1-Fc-41BBL protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **Fig. 1** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous brain tissue corresponding to 25 selected miRNAs.

[0030] **Fig. 2** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous bladder tissue corresponding to 25 selected miRNAs.

[0031] **Fig. 3** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous breast tissue corresponding to 25 selected miRNAs.

[0032] **Fig. 4** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous colon tissue corresponding to 25 selected miRNAs.

[0033] **Fig. 5** illustrates a heat map of an miRNA expression profile in glioblastoma and non-cancerous brain tissue corresponding to 25 selected miRNAs.

[0034] **Fig. 6** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous head and neck tissue corresponding to 25 selected miRNAs.

[0035] **Fig. 7** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous lung tissue corresponding to 25 selected miRNAs.

[0036] **Fig. 8** illustrates a heat map of an miRNA expression profile in cancerous and non-cancerous pancreatic tissue corresponding to 25 selected miRNAs.

[0037] **Fig. 9** illustrates a heat map of an miRNA expression profile in schwannoma and non-schwannoma tissue corresponding to 25 selected miRNAs.

[0038] **Fig. 10A – Fig. 10C** illustrate an miRNA expression and attenuation reporter gene system described in Example 2. **Fig. 10A** shows a schematic of a pTetR tet repressor plasmid that induces expression of an miRNA expression plasmid. **Fig. 10B** shows a schematic of a pTF-002 miRNA expression plasmid containing a tet-inducible mCherry and miRNA expression cassette. **Fig. 10C** shows a schematic of a pTF-004 miRNA attenuation reporter enabling the read-out of destabilized GFP (dsGFP).

[0039] **Fig. 11** illustrates miR-122 expression and attenuation using the reporter system shown in **Fig. 10** and described in Example 2.

[0040] **Fig. 12** illustrates miR-122, miR-184, miR-34a, and Let7a-mediated GFP attenuation using the reporter system shown in **Fig. 10** and described in Example 2. Circled wells indicate reduced GFP expression levels.

[0041] **Fig. 13** illustrates expression of miR-122, miR-184, miR-34a, and Let7a, indicated by mCherry expression, using the reporter system shown in **Fig. 10** and described in Example 2.

[0042] **Fig. 14** illustrates miR-122, miR-124, miR-145, miR-199, and miR-451-mediated GFP attenuation using the reporter system shown in **Fig. 10** and described in Example 2. Circled wells indicate reduced GFP expression levels.

[0043] **Fig. 15** illustrates expression of miR-122, miR-124, miR-145, miR-199, and miR-451, indicated by mCherry expression, using the reporter system shown in **Fig. 10** and described in Example 2.

[0044] **Fig. 16** shows quantitation of miR-122 and miR-184-attenuated GFP fluorescence.

[0045] **Fig. 17** shows quantitation of miR-34a and miR-184-attenuated GFP fluorescence.

[0046] **Fig. 18** shows quantitation of Let7a and miR-184-attenuated GFP fluorescence.

[0047] **Fig. 19** shows quantitation of miR-124 and miR-184-attenuated GFP fluorescence.

[0048] **Fig. 20** shows quantitation of miR-145 and miR-184-attenuated GFP fluorescence.

[0049] **Fig. 21** shows quantitation of miR-199 and miR-451-attenuated GFP fluorescence.

[0050] **Fig. 22** shows quantitation of miR-125 and miR-451-attenuated GFP fluorescence.

[0051] **Fig. 23** shows quantitation of miR-126 and miR-451-attenuated GFP fluorescence.

[0052] **Fig. 24** shows quantitation of miR-127 and miR-451-attenuated GFP fluorescence.

[0053] **Fig. 25** shows quantitation of miR-133 and miR-451-attenuated GFP fluorescence.

[0054] **Fig. 26** shows quantitation of miR-223 and miR-451-attenuated GFP fluorescence.

[0055] **Fig. 27A – Fig. 27D** illustrate fluorescence-based quantitation of HSV attenuation by miR-125 in non-cancerous post-mitotic lung cells and cancerous A253 cells. **Fig. 27A** shows fluorescence-based quantitation of HSV attenuation in post-mitotic lung cells. **Fig. 27B** shows fluorescence-based quantitation of HSV attenuation in A253 cells. **Fig. 27C** shows qPCR-based quantitation of HSV attenuation in post-mitotic lung cells. **Fig. 27D** shows qPCR-based quantitation of HSV attenuation in A253 cells.

[0056] **Fig. 28A – Fig. 28B** illustrate fluorescence-based (**Fig. 28A**) and qPCR-based (**Fig. 28B**) quantitation of HSV attenuation by miR-145 in HCC1395 vs. A253 cells.

[0057] **Fig. 29A – Fig. 29B** illustrates fluorescence-based (**Fig. 29A**) and qPCR-based (**Fig. 29B**) quantitation of HSV attenuation by miR-199a-5p vs. miR-143-3p in normal lung cells.

[0058] **Fig. 30A – Fig. 30C** illustrate miRNA-125a (**Fig. 30A**) and miRNA-122 (**Fig. 30B**) expression and effect on viral replication (**Fig. 30C**) in A253, Huh7, and Hep3B cell lines.

[0059] **Fig. 31** shows a Western blot illustrating reduced viral spread and protein expression after infection with miR-T122 and/or miR-T125a containing HSV in cells expressing miR-122 and miR-125a.

[0060] **Fig. 32A – Fig. 32D** illustrate effects of miRNA expression on miR-attenuated HSV replication. **Fig. 32A – Fig. 32B** shows increased intracellular expression of miR-125a and mir-122 after transfection with a miR-125a mimic (**Fig. 32A**) or a miR-122 mimic (**Fig. 32B**). **Fig. 32C** shows attenuation of replication of oncolytic HSV vectors with cognate miR target sequences at distinct genetic loci shown by fluorescence images and quantification of GFP fluorescence (**Fig. 32D**).

[0061] **Fig. 33** illustrates a schematic of an ICP4-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences;

Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR)

[0062] **Fig. 34** shows a schematic of an ICP27-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP27:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the ICP27 gene (also may be placed in 5' UTR)

[0063] **Fig. 35** shows a schematic of a UL19-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; UL19:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the UL19 gene (also may be placed in 5' UTR).

[0064] **Fig. 36** shows a schematic of an UL19-TmiRNA and ICP27-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; UL19:TmiRNA & ICP27:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the UL19 and ICP27 genes (also may be placed in 5' UTR)

[0065] **Fig. 37** shows a schematic of an UL19-TmiRNA and ICP4-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; UL19:TmiRNA & ICP4:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the UL19 and ICP4 genes (also may be placed in 5' UTR).

[0066] **Fig. 38** shows a schematic of an UL19-TmiRNA, ICP27-TmiRNA, and ICP4-TmiRNA-attenuated HSV vector for the treatment of cancer or benign hyper-proliferative disorders. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; UL19:TmiRNA, ICP27:TmiRNA, & ICP4:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145, etc.) into the 3' UTR of the UL19, ICP27, and ICP4 genes (also may be placed in 5' UTR).

[0067] **Fig. 39** shows a schematic of an ICP4-TmiRNA-attenuated, genome-editing HSV vector for the treatment of cancer. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145) into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR); Pol II promoter: Constitutive (CAG, UbC, EF1a, PGK) or cell-specific (e.g. TRPV1, Nav1.7, hSYN); Endonuclease: CRISPR associated endonuclease (e.g. SpCas9, SaCas9, FnCpf1, FnCas9, etc.); Poly(A): polyadenylation signal (e.g. bGH); gRNA: Single crRNA-trRNA fusion (DR-crRNA-DR-trRNA); crRNA targeted to oncogenic microRNA (e.g. miR-17, miR-21, miR-155); **Pol III promoter:** E.g. U6, H1, 7SK.

[0068] **Fig. 40** shows a schematic of an ICP4-TmiRNA-attenuated, genome-editing, microenvironment-remodeling HSV vector for the treatment of cancer. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miRNA target sequences (e.g. let-7, miR-34a, miR-101, miR-125b, miR-145) into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR); Pol II promoter: Constitutive (CAG, UbC, EF1a, PGK) or cell-specific (e.g. TRPV1, Nav1.7, hSYN); Endonuclease: CRISPR associated endonuclease (e.g. SpCas9, SaCas9, FnCpf1, FnCas9, etc.); Poly(A): polyadenylation signal (e.g. bGH); gRNA2: crRNA targeted to microenvironment remodeling miRNA (e.g. miR-143, miR-218) or TIMP (e.g. TIMP1, TIMP2); Pol III promoter: E.g. U6, H1, 7SK.

[0069] **Fig. 41** shows a schematic of an ICP4-TmiRNA and ICP27-TmiRNA-attenuated HSV vector for the treatment of pancreatic, lung, and colon cancer. gB:NT: virus entry-

enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miR-124 target sequence cassette into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR); ICP27:TmiRNA: insertion of miR-451a, miR-143-3p, and miR-559 target sequence cassettes into the 3' UTR of the ICP-27 gene (also may be placed in 5' UTR).

[0070] **Fig. 42** shows a schematic of an ICP4-TmiRNA and ICP27-TmiRNA-attenuated HSV vector for the treatment of multiple cancer types. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miR-124 target sequence cassette into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR); ICP27:TmiRNA: insertion of miR-451a, miR-145-5p, and miR-559 target sequence cassettes into the 3' UTR of the ICP-27 gene (also may be placed in 5' UTR).

[0071] **Fig. 43** shows a schematic of an ICP4-TmiRNA and ICP27-TmiRNA-attenuated HSV vector for the treatment of schwannoma. gB:NT: virus entry-enhancing double mutation in gB gene; BAC: loxP-flanked choramphenicol-resistance and lacZ sequences; Δjoint: deletion of the complete internal repeat region including one copy of the ICP4 gene; ICP4:TmiRNA: insertion of miR-124 target sequence cassette into the 3' UTR of the remaining ICP4 gene (also may be placed in 5' UTR); ICP27:TmiRNA: insertion of miR-205p, miR-141-5p, and miR-31-5p target sequence cassettes into the 3' UTR of the ICP-27 gene (also may be placed in 5' UTR).

[0072] **Fig. 44** shows a schematic of a miR-attenuated HSV virus, wherein multiple copies of miR-122, miR-125a, and/or miR-124 target sites are inserted into ICP27, UL42 and/or ICP4.

[0073] **Fig. 45** shows a schematic of a miR-attenuated HSV virus (ONCR-157), wherein miR target site cassettes are inserted into UL8, ICP12, ICP4, and ICP34.5

[0074] **Fig. 46** shows a schematic of a miR-attenuated HSV virus (ONCR-159), wherein miR target site cassettes are inserted into UL8, ICP12, ICP4, and ICP34.5.

[0075] **Fig. 47A** shows GFP intensity generated by a reporter virus engineered to expression GFP in cells treated with pooled siRNAs against various viral genes. **Fig. 47B** shows results in the same assay for individual siRNAs.

[0076] **Figs. 48A** and **48B** shows GFP intensity generated by a reporter virus engineered to expression GFP in cells treated with pooled siRNAs against various viral genes using the HSV vector ONCR-003 (left) or ONCR-010 (right).

[0077] **Fig. 49** shows a Western blot to detect expression of viral proteins in cells infected with HSV vector and treated with siRNA against viral genes as indicated. Beta-actin is a positive control for the Western blot.

[0078] **Figs. 50A** and **50B** show Nanostring assay data for normal brain tissue compared to malignant tissue as ratio of miRNA expression (Fig. 50A) and normalized counts (Fig. 50B).

[0079] **Figs. 51A** and **51B** show Nanostring assay data for normal heart tissue compared to malignant tissue as ratio of miRNA expression (Fig. 51A) and normalized counts (Fig. 51B).

[0080] **Figs. 52A** shows Nanostring assay data for normal spinal-cord tissue compared to malignant tissue as ratio of miRNA expression. **Figs. 52A** and **52B** show Nanostring assay data for normal nerve or ganglion tissue compared to malignant tissue as ratio of miRNA expression (Fig. 52A) and normalized counts (Fig. 52C).

[0081] **Figs. 53A** and **53B** show luciferase assay testing of miR-TS cassettes for cassette 1 (Fig. 53A) or cassette 2 (Fig. 53B)

[0082] **Fig. 54A – Fig. 54G** show cytotoxicity of oncolytic HSV viral vectors ONCR-125, ONCR-131, ONCR-142, and ONCR-157 in cancer cell lies SW837 (Fig. 54A), SKMEL28 (Fig. 54B), COLO 205 (Fig. 54C), A375 (Fig. 54D), H446 (Fig. 54E), BXPC3 (Fig. 54F), and BT549 (Fig. 54G).

[0083] **Fig. 55** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor. ONCR-133 significantly inhibited tumor growth of injected tumors compared to vehicle treated controls ($p <$

0.0001). ONCR-133 treatment also significantly inhibited tumor growth of non-injected tumors ($p < 0.005$), indicating an enhanced abscopal effect.

[0084] **Fig. 56** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor. Mice treated with ONCR-133 + ONCR-007 (an HSV construct expressing ULBP3) or ONCR-133 + ONCR-002 (an HSV construct that does not express any additional payload molecules) both demonstrated a significant inhibition of tumor growth compared to vehicle treated controls.

[0085] **Fig. 57** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor. The additional expression of CXCL10 in the ONCR-106 + ONCR-113 treated group did not enhance the inhibition of tumor growth in either injected or non-injected tumors compared to mice treated with ONCR-031 + ONCR-113.

[0086] **Fig. 58** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor. the additional expression of CXCL10 in the ONCR-106 + ONCR-113 treated group did not enhance the inhibition of tumor growth in either injected or non-injected tumors compared to mice treated with ONCR-031 + ONCR-113.

[0087] **Fig. 59A – Fig. 59B** show replication of injected virus occurs only in the injected tumor, not in the non-injected tumor, suggesting that the anti-tumor effect observed in the non-injected tumor is immune-mediated. Data is ploted as raw genome copies per microgram DNA (Fig. 59A) or as normalized expression (Fig. 59B). HSV was detected in the injected tumors, but not in the non-injected tumors, indicating that the tumor growth inhibition observed in the non-injected tumors was not due to viral spread, but rather the abscopal effects of virus administration.

[0088] **Fig. 60A – Fig. 60C** show payload expression peaked in the injected tumors at 24-hours post-treatment and decreased thereafter.

[0089] **Fig. 61A – Fig. 61C** shows levels of the payloads in the serum of mice treated with ONCR-153, where only CXCL10 expression was observed.

[0090] **Fig. 62A** shows the expression level of interferon gamma in the injected and non-injected tumors. Treatment of mice with ONCR-153 induced an intra-tumoral IFNγ response in both injected and non-injected tumors. **Fig. 62B** shows the expression level of interferon gamma in the plasma of the host animal.

[0091] **Fig. 63** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor.

[0092] **Fig. 64** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor.

[0093] **Fig. 65** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor.

[0094] **Fig. 66A** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. Fig. 66B shows decreased incident of metasteses to the lung. **Fig. 66C** shows images of lung tissue from vehicle (PBS) control or vector-treated animals.

[0095] **Fig. 67** shows tumor growth inhibitor effect of vehicle compared to virus (as indicated in legend) in a mouse xenograph experiment. The increase in tumor volume of the injected tumor is compared to the increase in tumor volume of the non-injected tumor. Treatment with ONCR-152 and -139 demonstrated an enhanced effect in tumor growth inhibition compared to treatment with ONCR-139 alone.

DETAILED DESCRIPTION

[0096] In some aspects, the present invention utilizes differential miR expression profiles to effectively restrict viral vector replication to tumor cells by incorporating miR target sequences into one or more genes required for viral replication. In particular embodiments, the viral vectors described herein comprise two, three, four or more copies of a miR target sequence incorporated into one or more viral genes. In some embodiments, the viral vectors described herein also disrupt the expression of specific miRNAs for reduced tumor proliferation, metastasis, and/or

remodeling of the tumor microenvironment to enable enhanced viral spread. In some embodiments, the viral vectors described herein encompass the use of surface molecules on viral vectors to facilitate targeting to tumor cells. These aspects can be applied individually or in combination to develop viral vectors potentially capable of treating a wide array of cancer types with a single viral vector. As such, the invention further encompasses recombinant oncolytic viral vectors for use in the treatment and prevention of diseases and disorders (e.g., cancer). In some embodiments, this invention utilizes endogenous microRNA (miR) expression to enable a safe and efficacious recombinant viral vector suitable to treat a broad array of cancers.

[0097] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Definitions

[0098] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components unless otherwise indicated. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously. As used herein, "plurality" may refer to one or more components (e.g., one or more miRNA target sequences).

[0099] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00100] "Decrease" or "reduce" refers to a decrease or a reduction in a particular value of at least 5%, for example, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100% as compared to a reference value. A decrease or reduction in a particular value may also be represented as a fold-change in the value compared to a reference value, for example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold, or more, decrease as compared to a reference value.

[00101] "Increase" refers to an increase in a particular value of at least 5%, for example, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99, 100, 200, 300, 400, 500% or more as compared to a reference value. An increase in a particular value may also be represented as a fold-change in the value compared to a reference value, for example, at least 1-fold, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more, increase as compared to the level of a reference value.

[00102] The term "sequence identity" refers to the percentage of bases or amino acids between two polynucleotide or polypeptide sequences that are the same, and in the same relative position. As such one polynucleotide or polypeptide sequence has a certain percentage of sequence identity compared to another polynucleotide or polypeptide sequence. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. The term "reference sequence" refers to a molecule to which a test sequence is compared.

[00103] "Complementary" refers to the capacity for pairing, through base stacking and specific hydrogen bonding, between two sequences comprising naturally or non-naturally occurring (*e.g.*, modified as described above) bases (nucleosides) or analogs thereof. For example,

if a base at one position of a nucleic acid is capable of hydrogen bonding with a base at the corresponding position of a target, then the bases are considered to be complementary to each other at that position. Nucleic acids can comprise universal bases, or inert abasic spacers that provide no positive or negative contribution to hydrogen bonding. Base pairings may include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (*e.g.*, Wobble base pairing and Hoogsteen base pairing). It is understood that for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as such as 3-nitropyrrole or 5-nitroindole can hybridize to and are considered complementary to any A, C, U, or T. Nichols *et al.*, Nature, 1994;369:492-493 and Loakes *et al.*, Nucleic Acids Res., 1994;22:4039-4043. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U, or T. See Watkins and SantaLucia, Nucl. Acids Research, 2005; 33 (19): 6258-6267.

[00104] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a polynucleotide sequence if the promoter affects the transcription or expression of the polynucleotide sequence.

[00105] The term "subject" includes animals, such as *e.g.* mammals. In some embodiments, the mammal is a primate. In some embodiments, the mammal is a human. In some embodiments, subjects are livestock such as cattle, sheep, goats, cows, swine, and the like; or domesticated animals such as dogs and cats. In some embodiments (*e.g.*, particularly in research contexts) subjects are rodents (*e.g.*, mice, rats, hamsters), rabbits, primates, or swine such as inbred pigs and the like. The terms "subject" and "patient" are used interchangeably herein.

[00106] The term "effective amount" refers to the minimum amount of an agent or composition required to result in a particular physiological effect (*e.g.*, an amount required to increase, activate, and/or enhance a particular physiological effect). The effective amount of a particular agent may be represented in a variety of ways based on the nature of the agent, such as mass/volume, # of cells/volume, particles/volume, (mass of the agent)/(mass of the subject), # of cells/(mass of subject), or particles/(mass of subject). The effective amount of a particular agent may also be expressed as the half-maximal effective concentration (EC_{50}), which refers to the

concentration of an agent that results in a magnitude of a particular physiological response that is half-way between a reference level and a maximum response level.

[00107] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00108] As used herein "pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals.

[00109] As used herein, the term "oncolytic virus" refers to a virus that has been modified to, or naturally, preferentially infect cancer cells.

[00110] The terms "microRNA," "miRNA," and "miR" are used interchangeably herein and refer to small non-coding endogenous RNAs of about 21-25 nucleotides in length that regulate gene expression by directing their target messenger RNAs (mRNA) for degradation or translational repression.

[00111] "Essential viral gene" as used herein refers to a viral gene that is required for one or more essential viral function, such as viral replication, viral packaging, or viral infectivity.

[00112] The term "vector" is used herein to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA.

[00113] General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook *et al.*, HaRBor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel *et al.*

eds., John Wiley & Sons 1999); Protein Methods (Bollag *et al.*, John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner *et al.* eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference.

Oncolytic Viruses

[00114] In some embodiments, the present invention provides for recombinant oncolytic viruses, wherein one or more copies of one or more micro-RNA (miRNA) target sequences are inserted into a locus of one or more essential viral genes required for viral replication. Examples of oncolytic viruses are known in the art including, but not limited to, herpes simplex virus (HSV), an adenovirus, a polio virus, a vaccinia virus, a measles virus, a vesicular stomatitis virus, an orthomyxovirus, a parvovirus, a maraba virus or a coxsackievirus. In some embodiments, the oncolytic viruses described herein are referred to as recombinant viral vectors or oncolytic vectors.

[00115] In certain embodiments, an oncolytic virus described herein is a herpesvirus (for example, herpes simplex virus (e.g., HSV-1 or HSV-2)), an adenovirus, a polio virus, a vaccinia virus, a measles virus, a vesicular stomatitis virus, an orthomyxovirus, a parvovirus, a maraba virus or a coxsackievirus. In particular embodiments, the recombinant viral vector is an HSV capable of tumor-selective vector replication as described in International PCT Publication No. WO 2015/066042, which is incorporated by reference in its entirety.

[00116] HSV-based vectors and methods for their construction are described in, for example, U.S. Pat. Nos. 7,078,029, 6,261,552, 5,998,174, 5,879,934, 5,849,572, 5,849,571, 5,837,532, 5,804,413, and 5,658,724, and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583, which are incorporated herein by reference in their entireties. The sequence of HSV is published (NCBI Accession No. NC_001806; see also McGoech et al., J. Gen. Virol, 69 (PT 7), 1531-1574 (1988)), which may facilitate designing HSV-based vectors of the invention. In some cases, the oncolytic virus of the invention is a herpes simplex virus (HSV) and comprises a deletion of the internal repeat (joint) region comprising one

copy each of the diploid genes ICP0, ICP34.5, LAT, and ICP4 along with the promoter for the ICP47 gene.

[00117] In certain embodiments, the recombinant viral vector of the invention is an HSV that exhibits enhanced entry into cells, either through direct infection and/or lateral spread. In one aspect, HSV vectors of the present invention can directly infect cells through interaction with cell proteins other than typical mediators of HSV infection (e.g., other than nectin-1, HVEM, or heparan sulfate/chondroitin sulfate proteoglycans). In certain embodiments, the recombinant viral vector of the invention is an HSV and further comprises a mutation of the gB or gH gene that facilitates vector entry through non-canonical receptors. In another aspect, the invention provides an HSV vector further comprising mutant gH glycoproteins that exhibit lateral spread in cells typically resistant to HSV lateral spread, such as cells lacking gD receptors. In some embodiments, an HSV vector of the invention comprises one or more of the mutant gB or gH proteins as described in U.S. Patent Publication No. 2013/0096186, which is incorporated herein by reference in its entirety. In certain aspects, the mutant entry protein within an HSV vector is a glycoprotein involved with viral entry, such as gB, gH, and the mutant HSV vector can comprise mutated versions of both. However, the mutant entry protein can be any protein effecting entry of the HSV vector into cells. In certain embodiments, the mutant entry protein is other than gD, although the HSV vector can additionally comprise a mutant gD, such as containing a ligand or other desired mutation. Non-limiting mutations of gB or gH glycoprotein for use in the inventive HSV vector occur at one or more of the following residues: gB:D285, gB:A549, gB:S668, gH:N753, and gH:A778. In some embodiments, the inventive HSV vector comprises mutations at both gB:D285 and gB:A549, at both gH:N753 and gH:A778, and/or at each of gB:S668, gH:N753, and gH:A778. In certain embodiments, the HSV vector contains two or more of such mutations (e.g., 3 or more, 4 or more), and the HSV vector can comprise mutations in all five of these residues. In one embodiment, an HSV vector has mutations at gB:285, gB;549, gH:753, and gH:778. The mutations are referred to herein relative to the codon (amino acid) numbering of the gD, gB, and gH genes of the HSV-1 strain KOS derivative K26GFP. The sequences for gB and gH of K26GFP differ from the sequences for gB as disclosed in GenBank (#AF311740 (incorporated herein by reference)) and for gH (GenBank #X03896 (incorporated herein by reference)) as reflected in Table 9 below.

Table 9:

	Amino acid position	AF311740	K26GFP	Nucleotide position(s)	AF311740	K26GFP
gB	313	T	S	938-939	ACG	AGC
	315	A	T	943	GCC	ACC
	515	H	R	1,544	CAC	CGC
		X03896		X03896		
gH	12	I	L	1,011	ATT	CTT
	110	P	S	1,305	CCG	TCG
	127	T	I	1,357	ACC	ATC
	138	S	A	1,389	TCG	GCG
	150	A	T	1,425	GCC	ACC
	532	A	A	2,573	GCT	GCG
	633	R	R	2,876	CGT	CGC

[00118] However, K26GFP may contain additional differences in the region of the gene corresponding to nucleotides 2,079-2,102 of GenBank X03896. Thus, it will be understood that the sequence of either KOS derivative K26GFP or GenBank Accession No. AF311740 can serve as a reference sequence for the gB mutations discussed herein. Also, the sequence of either KOS derivative K26GFP or GenBank Accession No. X03896 can serve as a reference sequence for the gH mutations discussed herein. However, HSV vectors of the invention may include homologous mutations in gB and gH of any HSV strain.

[00119] In some aspects, the mutation of the entry protein for inclusion in an HSV vector is a substitution mutation; however, mutations are not limited to substitution mutants. In certain embodiments, mutant gB or gH glycoproteins for use in an HSV vector are selected from the group of substitution mutations consisting of gB:D285N, gB:A549T, gB:S668N, gH:N753K, gH:A778V. In certain aspects, an HSV vector includes combinations of these substitutions (such as two or more of such substitutions (e.g., 3 or more, 4 or more, or all)), with the gB:D285N/gB:A549T double mutant, the gH:N753K/gH:A778V double mutant, and the gB:S668N/gH:N753K/gH:A778V triple mutant being examples of embodiments. In one embodiment, an HSV vector comprises gB:D285N/gB:A549T/gH:N753K/gH:A778V.

[00120] In certain aspects, an HSV vector comprises a mutant gB and/or a mutant gH glycoprotein, wherein the mutations in the glycoproteins are substitution mutations in at least two residues, wherein, when the vector is HSV-1 K26GFP, the at least two residues are selected

from the group consisting of gB:D285, gB:A549, gB:S668, gH:N753, and gH:A778, or wherein when the vector is a homologous HSV, the at least two residues are selected from amino acids that correlate to gB:D285, gB:A549, gB:S668, gH:N753, and gH:A778 wherein the gB:D285 residue correlates to X in VYPYXEFVL (SEQ ID NO: 838), the gB:A549 residue correlates to X in KLNPNXIAS (SEQ ID NO: 839), the gB:S668 residue correlates to X in ITTVXTFID (SEQ ID NO: 840) the gH:N753 residue correlates to X in VDTDXTQQQ (SEQ ID NO: 841), and the gH:A778 residue correlates to X in VPSTXLLLF (SEQ ID NO: 842); and wherein the HSV vector is an HSV-1 or HSV-2 vector.

[00121] In some embodiments, the oncolytic HSV viruses described herein comprise one or more mutations in the UL37 gene that reduce HSV infection of neuronal cells, such as those described in International PCT Publication No. WO 2016/141320 and Richard *et al.*, Plos Pathogens, 2017, 13(12), e1006741.

miRNA-attenuated Oncolytic Viruses

[00122] miRs are differentially expressed in a broad array of disease states, including multiple types of cancer. Importantly, miRNAs are differentially expressed in cancer tissues compared to normal tissues, enabling them to serve as a targeting mechanism in a broad variety of cancers. miRNAs that are associated (either positively or negatively) with carcinogenesis, malignant transformation, or metastasis are known as "oncomiRs".

[00123] In some aspects, the expression level of a particular oncomiR is positively associated with the development or maintenance of a particular cancer. Such miRs are referred to herein as "oncogenic miRs." In some embodiments, the expression of an oncogenic miR is increased in cancerous cells or tissues compared to the expression level observed in non-cancerous controls cells (*i.e.*, normal or healthy controls) or is increased compared to the expression level observed in cancerous cells derived from a different cancer type. In some embodiments, the expression of an oncogenic miR is increased by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the expression of the oncogenic miR in a non-cancerous control cell or a cancerous cell derived from a different cancer type. In some aspects, a cancerous cell or tissue may express

an oncogenic miR that is not expressed in non-cancerous control cells or tissues. Examples of oncogenic miRNAs that are frequently over-expressed in cancer tissues include, but are not limited to, miR-21, miR-155 and miR-17-92. Additional examples of oncogenic miRs are listed in Table 4.

[00124] In some embodiments, the expression of a particular oncomiR is negatively associated with the development or maintenance of a particular cancer and/or metastasis. Such oncomiRs are referred to herein as "tumor-suppressor miRs" or "tumor-suppressive miRs," as their expression prevents or suppresses the development of cancer. In some embodiments, the expression of a tumor-suppressor miRNA is decreased in cancerous cells or tissues compared to the expression level observed in non-cancerous control cells (*i.e.*, normal or healthy controls), or is decreased compared to the expression level of the tumor-suppressor miRNA observed in cancerous cells derived from a different cancer type. For example, the expression of a tumor-suppressor miRNA in a cancerous cell may be decreased by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the expression of the tumor-suppressor miRNA in a non-cancerous control cell or a cancerous cell derived from a different cancer type. In some aspects, a non-cancerous control cell may express a tumor-suppressor miRNA that is not expressed in cancerous cells. Examples of tumor-suppressive miRNAs include, but are not limited to, miR-122, miR-184, miR-34a, let7a, miR-145-5p, miR-199a-5p, miR-451a, miR-125a, miR-125a-5p, miR-126-3p, miR-233-3p, miR-143-3p, miR-1-3p, miR-133a-3p, miR-127a-3p, miR-133b, miR-134-3p, miR-124, miR-101, miR-125b, miR-145, miR-559, miR-213, miR-31-5p, miR-205p, miR-15a, miR-16-1, miR-34, as well as miRNAs of the let-7 family. Additional examples of tumor-suppressive miRs are listed in Table 3 and Table 8.

[00125] Cancer pathogenesis is a heterogeneous and multigenic process. As such, activation of particular pathways and the expression of particular genes may lead to cancer development in one context, and result in distinct or opposing results when activated or expressed in a different context. Therefore, the characterization of a particular gene or miR as an "oncogene" or "oncogenic miR" or as a "tumor-suppressor" or "tumor-suppressive miR" is not a binary distinction and will vary according to the type of cancer. For example, the expression of one miRNA may be increased in a particular cancer and associated with the development of that cancer,

while the expression of the same miRNA may be decreased in a different cancer and associated with prevention of the development of that cancer. However, some miRNAs may function as oncogenic miRNAs independent of the type of cancer. For example, some miRNAs target mRNA transcripts of tumor suppressor genes for degradation, thereby reducing expression of the tumor suppressor protein. For example, miR-152b functions as an oncogenic miR in the vast majority of hematologic malignancies, but functions as a tumor-suppressive miR in many solid tumors. Further, a particular miR may be highly expressed in both cancerous and non-cancerous cells. For example, miR-155 is highly expressed in normal cells, playing an essential role in macrophage polarization, and is also highly expressed in cancer cells. As such, the development of the miR-attenuated, genome-editing, and microenvironment-remodeling oncolytic viruses described herein is based on the differential expression of a particular miR or group of miRs in one cell population or tissue compared to another cell population or tissue. One of skill in the art will understand that the term tumor-suppressive miR generally refers to a miR that is more highly expressed in a non-cancerous cell or tissue compared to a cancerous cell or tissue, and that the term oncogenic miR generally refers to a miR that is more highly expressed in a cancerous cell or tissue compared to a non-cancerous cell or tissue. One of skill in the art will further understand that a miR characterized as a tumor-suppressive miR in one type of cancer may or more may not function as a tumor-suppressive miR in a different type of cancer, and that a miR characterized as an oncogenic miR in one type of cancer may or more may not function as an oncogenic miR in a different type of cancer.

[00126] Table 1 shows the relationship between 12 select oncomiRs (9 tumor suppressors and 3 oncogenic miRNAs) and numerous cancers. A list of 3,410 oncomiR-cancer relationships is shown in Table 2. miRNAs regulate many transcripts of proteins that are involved in the control of cellular proliferation and apoptosis. Regulated proteins include conventional proto-oncoproteins and tumor suppressors such as Ras, Myc, Bcl2, PTEN and p53. Aberrant expression of miRNAs therefore often is involved in development of cancer and can therapeutically be corrected by either inhibiting oncogenic miRNAs or replacing the depleted tumor suppressor miRNA. Further, the differential expression of particular oncomiRs in cancerous vs. non-cancerous cells can be exploited as a means to target cancer therapeutics specifically to cancer cells. As such, in some embodiments, the oncolytic viral vectors described herein can

comprise the following properties individually or in combination: insertion of miRNA target sequences into the viral genome, thereby restricting viral vector replication to cancer or tumor cells; one or more polynucleotides incorporated into the viral genome whose product(s) disrupt the function of an oncogenic miRNA, modulate the cancer extracellular matrix, and/or enhance or activate an anti-cancer immune response; and/or protease-activated antibodies incorporated into the viral particle in order to selectively target the vectors to cancer and/or tumor cells.

[00127] One aspect of the invention comprises a recombinant oncolytic virus (or viral vector) comprising a plurality of copies of one or more miRNA target sequences inserted into a locus of one or more essential viral genes. In certain embodiments, a recombinant oncolytic virus may comprise miRNA target sequences inserted into a locus of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten essential viral genes. miRNAs expressed in normal (non-cancerous) cells can bind to such target sequences and suppress expression of the viral gene containing the miRNA target sequence, thereby limiting viral replication in healthy, non-cancerous cells. Such recombinant oncolytic viruses are referred to herein as "miR-attenuated" or "replication-restricted" as they demonstrate reduced or attenuated viral replication in cells that express one or more miRNAs capable of binding to the incorporated miR target sequences compared to cells that do not express, or have reduced expression of, the miR. By incorporating miRNA target sequences into key genes required for viral replication, viral replication can be conditionally suppressed in normal diploid cells expressing the miRNAs and can proceed normally in cells that do not express themiRNAs. In such embodiments, healthy, non-cancerous cells are protected from the normal cells from lytic effects of infection by the recombinant viral vector.

[00128] In certain embodiments, the one or more miRNA target sequences is incorporated into the 5' untranslated region (UTR) and/or 3' UTR of one or more essential viral genes. In some embodiments, the oncolytic virus is a herpes simplex virus (HSV), and the viral genes required for viral replication include any of UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP34.5, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and/or US12. In certain embodiments, the oncolytic virus is

HSV and comprises one or more miRNA target sequences incorporated into the 5' or 3' UTR of one or more essential viral genes. In some embodiments, the oncolytic virus is HSV, and the one or more miRNA target sequences is incorporated into one or more of ICP4, ICP27, UL8, UL42, UL19, and ICP34.5. In some embodiments, the oncolytic virus is HSV, and the one or more miRNA target sequences is incorporated into the 5' or 3' UTR of one or more of ICP4, ICP27, UL8, UL42, UL19, and ICP34.5

miRNA Target Sequence Cassettes

[00129] In animals, genes for miRNAs are transcribed to a primary miRNA (pri-miRNA), which is then processed in the nucleus by Drosha, a class 2 RNase III enzyme, to form a precursor miRNA (pre-miRNA) hairpin. The pre-miRNA hairpin are transported to the cytoplasm, where they are cleaved by the RNase III enzyme Dicer. This endoribonuclease interacts with 5' and 3' ends of the hairpin and cuts away the loop joining the 3' and 5' arms, yielding a duplex RNA molecule about 22 nucleotides in length. Although either strand of the duplex may potentially act as a functional miRNA, typically one strand of the miRNA is degraded and only one strand is loaded onto the Argonaute (Ago) protein to produce the effector RNA-induced silencing complex (RISC) where the miRNA and its mRNA target interact (Wahid *et al.*, 1803:11, 2010, 1231-1243).

[00130] Herein, the gene encoding a particular miRNA is referenced as "MIR" followed by the miRNA number. The intermediate hairpin pre-miRNA molecules are referenced as "mir-" followed by the miRNA number, while the mature single-stranded miRNA molecule is referenced as "miR-" followed by the miRNA numer. For example, "MIR122" refers to the gene encoding a hairpin mir-122 pre-miRNA molecule, which is then processed into a mature miR-122 molecule. Due to the hairpin structure of the pre-miRNA, it is possible that two mature microRNAs can originate from opposite arms of the same pre-miRNA. In some instances, expression data clearly indentify one strand as the predominantly expressed miRNA and the other as the minor product. In such instances, the mature miRNA sequences are assigned names of the form miR-## (the predominant product) and miR-##* (minor product from the opposite arm of the precursor). For example, the major and minor products of mir-56 are denoted as miR-56 and miR-56*, respectively. When the existing data are not sufficient to determine which sequence is the

predominant one, or when they are found in roughly similar amounts, the two mature miRNA products are denoted as miR-##-5p (from the 5' arm of the pre-miRNA hairpin) and miR-##-3p (from the 3' arm of the pre-miRNA hairpin). For example, the two mature miRNA products of mir-142 are denoted as miR-142-5p and miR-142-3p. Because they originate from opposite ends of the pre-miRNA hairpin, the -3p and -5p products of a particular miRNA will comprise different RNA sequences and will therefore recognize different target sequences.

[00131] Herein, miRNA target sequences are inserted into the locus of one or more essential viral genes in the form of a "miR target sequence cassette" or "miR-TS cassette." A miR-TS cassette which refers to a polynucleotide sequence comprising one or more miRNA target sequences and capable of being inserted into a specific locus of a viral gene. When transcribed, the mRNA transcripts of a viral gene comprising a miR-TS cassette will comprise one or more miRNA target sequences. In some embodiments, the miR-TS cassettes described herein comprise at least one miRNA target sequence. In some embodiments, the miR-TS cassettes described herein comprise a plurality of miRNA target sequences. For example, in some embodiments, the miR-TS cassettes described herein comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more miRNA target sequences. In such embodiments, wherein the miR-TS cassettes comprise two or more miRNA target sequences, the two or more target sequences are arranged such that the total length of the miR-TS cassette (m) is less than or equal to the average length of the miRNA target sequences (n) multiplied by the total number of miRNA target sequences in the cassette (y) plus the average length of a linker sequence (l) multiplied by the total number of miRNA target sequences in the cassette plus 1 ($y + 1$). Thus, the length of a miR-TS cassette (m) can be represented by the formula: $m \leq (n * y) + (l * (y+1))$, wherein n = the average length of the miRNA target sequences, l = the average length of the linker sequences, and y = the total number of target sequences in the miR-TS cassette). As an illustrative example, if a miR-TS cassettes comprises 4 miRNA target sequences (y) with an average length of 21 nt (n), and the average length of the linker sequences is between 4 and 25 nt (l), the length of the miR-TS cassette (m) is between about 104 nt and about 205 nt.

[00132] As used herein, the "length" of a miR-TS cassette is defined as the total number of nucleotides (basepairs for double-stranded polynucleotides) from the 5' nucleotide of the first miR-TS to the 3' nucleotide of the last miR-TS in the polynucleotide, inclusive of any

intervening sequences. For non-overlapping miR-TSs, the minimum length of a miR-TS cassette will be the sum of the lengths of the miR-TSs. Spacers increase the length. The choice of spacer length determines the number of additional nucleotides in the cassette. Longer spacers increase the length of the cassette more than shorter spacers. By recognizing that shorter spacers (as short as 0, 1, 2, 3, 4, 5, or 6 nt) can be used when miR-TSs are interleaved (minimizing the number of mi-TSs for the same miRNA that are adjacent to one another)—the interleaved miR-TSs serving to increase the space between the other miR-TSs—the present inventors have determined that it is possible to generate shorter miR-TS cassettes than is possible in miR-TS cassettes in which miR-TSs for the same miRNA are arrayed in tandem, *e.g.* four of one type followed by four of the next type. In some embodiments, the the length of the miR-TS cassette is less than 1000 nt. In some embodiments, the length of the miR-TS cassette is less than 900 nt, less than 800 nt, less than 700 nt, less than 600 nt, less than 500 nt, less than 400 nt, less than 300 nt, less than 200 nt, less than 100 nt, or less than 50 nt. In some embodiments, the length of the miR-TS cassette is less than 26, 27, 28, 29, or 30 nt times the number of miR-TS sites, less than about 30 nt times the number of miR-TS sites, less than about 35 nt times the number of miR-TS sites, or less than about 40 nt times the number of miR-TS sites.

[00133] In some embodiments, the miR-TS cassettes comprise a plurality miRNA target sequences, wherein each miRNA target sequence in the plurality is a target sequence for the same miRNA. For example, the miR-TS cassettes may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more copies of the same miR target sequence. In some embodiments, the miR-TS cassettes comprise between 2 to 6 copies of the same miR target sequence. In some embodiments, the miR-TS cassettes comprise 3 copies of the same miR target sequence. In some embodiments, the miR-TS cassettes comprise 4 copies of the same miR target sequence.

[00134] In some embodiments, the miR-TS cassettes described herein comprise a plurality of miRNA target sequences, wherein the plurality comprises at least two different miRNA target sequences. In some embodiments, the miR-TS cassettes described herein comprise 2, 3, 4, 5, 6, 7, 8, 9, or 10 different miRNA target sequences. For example, in some embodiments, the miR-TS cassette may one or more copies of a first miRNA target sequence and one or more copies of a second miRNA target sequence. In some embodiments, the miR-TS cassette comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a first miR target sequence and at least 2, 3, 4, 5, 6, 7, 8,

9, 10, or more copies of a second miR target sequence. In some embodiments, the miR-TS cassette comprises 3 or 4 copies of a first miR target sequence and 3 or 4 copies of a second miR target sequence. In some embodiments, the plurality of miRNA target sequences comprises at least 3 different miRNA target sequences. For example, in some embodiments, the miR-TS cassette comprises one or more copies of a first miR target sequence, one or more copies of a second miR target sequence, and one or more copies of a third miR target sequence. In some embodiments, the miR-TS cassette comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a first miR target sequence, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a second miR target sequence, and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a third miR target sequence. In some embodiments, the miR-TS cassette comprises 3 or 4 copies of a first miR target sequence, 3 or 4 copies of a second miR target sequence, and 3 or 4 copies of a third miR target sequence. In some embodiments, the plurality of miRNA target sequences comprises at least 4 different miRNA target sequences. For example, in some embodiments, the miR-TS cassette comprises one or more copies of a first miR target sequence, one or more copies of a second miR target sequence, one or more copies of a third miR target sequence, and one or more copies of a fourth miR target sequence. In some embodiments, the miR-TS cassette comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a first miR target sequence, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a second miR target sequence, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a third miR target sequence, and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of a fourth miR target sequence. In some embodiments, the miR-TS cassette comprises 3 or 4 copies of a first miR target sequence, 3 or 4 copies of a second miR target sequence, 3 or 4 copies of a third miR target sequence, and 3 or 4 copies of a fourth miR target sequence. In some embodiments, the miR-TS cassettes described herein comprise a plurality of miRNA target sequences, wherein

[00135] In some aspects, wherein the miR-TS cassettes comprise a plurality of miRNA target sequences, the plurality of miRNA target sequences may arranged in tandem, without any intervening nucleic acid sequences. In some aspects, the plurality of miRNA target sequences may be separated by a linker sequence. In some embodiments, the linker sequence comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more nucleotides. In some embodiments, the linker sequence comprises about 4 to about 20 nucleotides. In further embodiments, the linker sequence comprises about 4 to about 16 nucleotides. As an illustrative embodiment, a miR-TS cassette may

comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the following subunits: (a) a first miRNA target sequence – linker – a second miRNA target sequence, wherein adjacent subunits are separated by an additional linker sequence. In some embodiments, the first and the second miRNA target sequence are targets of the same miRNA. In some embodiments, the first and the second miRNA target sequence are targets of different miRNAs.

[00136] In some embodiments, miR-TS cassettes described herein comprise a miRNA target sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the reverse complement of a sequence selected from SEQ ID NOs: 1 – 803. In some embodiments, miR-TS cassettes described herein comprise a miRNA target sequence that comprises or consists of the reverse complement of a sequence selected from SEQ ID NOs: 1 – 803.

[00137] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-122-5p target sequences. In some embodiments, the miR-122-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 804. In some embodiments, the miR-122-5p target sequences comprise or consist of SEQ ID NO: 804. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-124-3p target sequences. In some embodiments, the miR-124-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 805. In some embodiments, the miR-124-3p target sequences comprise or consist of SEQ ID NO: 805. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-125a-5p target sequences. In some embodiments, the miR-125a-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 806. In some embodiments, the miR-125a-5p target sequences comprise or consist of SEQ ID NO: 806.

[00138] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-126-3p target sequences. In some embodiments, the miR-126-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 807 or SEQ ID NO: 808. In some embodiments, the miR-126-3p target sequences comprise or consist of SEQ ID NO: 807 or SEQ ID NO: 808. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at

least 4 miR-127a-3p target sequences. In some embodiments, the miR-127a-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 809. In some embodiments, the miR-127a-3p target sequences comprise or consist of SEQ ID NO: 809.

[00139] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-128-3p target sequences. In some embodiments, the miR-128-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 810 or SEQ ID NO: 811. In some embodiments, the miR-128-3p target sequences comprise or consist of SEQ ID NO: 810 or SEQ ID NO: 811. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-129-3p target sequences. In some embodiments, the miR-129-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 812. In some embodiments, the miR-129-3p target sequences comprise or consist of SEQ ID NO: 812.

[00140] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-129-5p target sequences. In some embodiments, the miR-129-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 813. In some embodiments, the miR-129-5p target sequences comprise or consist of SEQ ID NO: 813. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-130b-3p target sequences. In some embodiments, the miR-130b-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 814. In some embodiments, the miR-130b-3p target sequences comprise or consist of SEQ ID NO: 814. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-130b-5p target sequences. In some embodiments, the miR-130b-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 815. In some embodiments, the miR-130b-5p target sequences comprise or consist of SEQ ID NO: 815.

[00141] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-133a-3p target sequences. In some embodiments, the miR-133a-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 816. In some embodiments, the miR-133a-3p target sequences

comprise or consist of SEQ ID NO: 816. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-133b-3p target sequences. In some embodiments, the miR-133b-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 817. In some embodiments, the miR-133b-3p target sequences comprise or consist of SEQ ID NO: 817. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-134-3p target sequences. In some embodiments, the miR-134-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 818. In some embodiments, the miR-134-3p target sequences comprise or consist of SEQ ID NO: 818.

[00142] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-137-3p target sequences. In some embodiments, the miR-137-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 819. In some embodiments, the miR-137-3p target sequences comprise or consist of SEQ ID NO: 819. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-1-3p target sequences. In some embodiments, the miR-1-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 820. In some embodiments, the miR-1-3p target sequences comprise or consist of SEQ ID NO: 820. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-143-3p target sequences. In some embodiments, the miR-143-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 821. In some embodiments, miR-143-3p target sequences comprise or consist of SEQ ID NO: 821.

[00143] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-145-3p target sequences. In some embodiments, the miR-145-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 822. In some embodiments, the miR-145-3p target sequences comprise or consist of SEQ ID NO: 822. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-145-5p target sequences. In some embodiments, the miR-145-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 823. In some embodiments, the miR-145-5p

target sequences comprise or consist of SEQ ID NO: 823. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-184-3p target sequences. In some embodiments, the miR-184-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 824. In some embodiments, the miR-184-3p target sequences comprise or consist of SEQ ID NO: 824.

[00144] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-199a-3p target sequences. In some embodiments, the miR-199a-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 825. In some embodiments, the miR-199a-3p target sequences comprise or consist of SEQ ID NO: 825. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-199a-5p target sequences. In some embodiments, the miR-199a-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 826. In some embodiments, the miR-199a-5p target sequences comprise or consist of SEQ ID NO: 826. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-204-5p target sequences. In some embodiments, the miR-204-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 827. In some embodiments, the miR-204-5p target sequences comprise or consist of SEQ ID NO: 827.

[00145] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-208b-3p target sequences. In some embodiments, the miR-208b-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 828. In some embodiments, the miR-208b-3p target sequences comprise or consist of SEQ ID NO: 828. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-214-3p target sequences. In some embodiments, the miR-214-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 829. In some embodiments, the miR-214-3p target sequences comprise or consist of SEQ ID NO: 829. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-217-5p target sequences. In some embodiments, the miR-217-5p target sequences are at least 95%, at least 96%,

at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 830. In some embodiments, the miR-217-5p target sequences comprise or consist of SEQ ID NO: 830.

[00146] In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-219a-5p target sequences. In some embodiments, the miR-219a-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 831. In some embodiments, the miR-219a-5p target sequences comprise or consist of SEQ ID NO: 831. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-223-3p target sequences. In some embodiments, the miR-223-3p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 832. In some embodiments, the miR-223-3p target sequences comprise or consist of SEQ ID NO: 832. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-34a-5p target sequences. In some embodiments, the miR-34a-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 833. In some embodiments, the miR-34a-5p target sequences comprise or consist of SEQ ID NO: 833.

[00147] n some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-451a target sequences. In some embodiments, the miR-451a target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 834. In some embodiments, the miR-451a target sequences comprise or consist of SEQ ID NO: 834. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-559-5p target sequences. In some embodiments, the miR-559-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 835. In some embodiments, the miR-559-5p target sequences comprise or consist of SEQ ID NO: 835. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-Let-7a-5p target sequences. In some embodiments, the miR-Let-7a-5p target sequences are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 836. In some embodiments, the miR-Let-7a-5p target sequences comprise or consist of SEQ ID NO: 836. In some embodiments, the miR-TS cassettes described herein comprise at least 1, at least 2, at least 3, or at least 4 miR-9-5p target sequences. In some embodiments, the miR-9-5p target sequences

are at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 837. In some embodiments, the miR-9-5p target sequences comprise or consist of SEQ ID NO: 837.

[00148] Table 10 below provides sequences of exemplary miRNAs that can bind to the miRNA target sequences in the oncolytic viruses described herein. Additional miRNA sequences are provided in SEQ ID NOs: 33 – 803.

Table 10: Exemplary miRNAs and Target Sequences

miRNA	miRNA Sequence	SEQ ID:	miR-TS	SEQ ID:
122-5p	uggagugugacaaugguguuug	1	caaacaccattgtcacactcca	804
124-3p	uaaggcacgcggugaaugcc	2	ggcattcaccgcgtgcctta	805
125a-5p	ucccugagacccuuuaaccuguga	3	tcacaggttaaagggtctcaggga	806
126-3p	ucguaccgugaguaauaaugcg	4	cgcattattactcacggtacga	807
			c**a**cattattactcacggtacga	808
127a-3p	ucggauccgucugagcuuggcu	5	agccaagctcagacggatccga	809
128-3p	ucacagugaaccggucucuuu	6	aaagagaccggttcactgtga	810
			aaagagaccggttcactgtg**g**	811
129-3p	aagcccuuaccccaaaaaguau	7	atacttttggggtaagggctt	812
129-5p	cuuuuugcggucugggcuugc	8	gcaagcccagaccgcaaaaag	813
130b-3p	cagugcaaugaugaaagggcau	9	atgcccttcatcattgcactg	814
130b-5p	acucuuucccuguugcacuac	10	gtagtgcaacagggaaagagt	815
133a-3p	uuuggucccccuucaaccagcug	11	cagctggttgaaggggaccaaa	816
133b-3p	uuuggucccccuucaaccagcua	12	tagctggttgaaggggaccaaa	817
134-3p	ccugugggccaccuagucaccaa	13	ttggtgactaggtggcccacagg	818
137-3p	uuauugcuuaagaauacgcguag	14	ctacgcgtattcttaagcaataa	819
1-3p	uggaauguaaagaaguauguau	15	atacatacttctttacattcca	820
143-3p	ugagaugaagcacuguagcuc	16	gagctacagtgcttcatctca	821
145-3p	ggauuccuggaaauacuguucu	17	agaacagtatttccaggaatcc	822
145-5p	guccaguuuucccaggaaucccu	18	agggattcctgggaaaactggac	823
184-3p	uggacggagaacugauaagggu	19	acccttatcagttctccgtcca	824
199a-3p	acaguagucugcacauugguua	20	taaccaatgtgcagactactgt	825
199a-5p	cccaguguucagacuaccuguuc	21	gaacaggtagtctgaacactggg	826
204-5p	uucccuuugucauccuaugccu	22	aggcataggatgacaaagggaa	827
208b-3p	auaagacgaacaaaaggguuugu	23	acaaacctttgttcgtcttat	828
214-3p	acagcaggcacagacaggcagu	24	actgcctgtctgtgcctgctgt	829
217-5p	uacugcaucaggaacugauugga	25	tccaatcagttcctgatgcagta	830
219a-5p	ugauuguccaaacgcaauucu	26	agaattgcgtttggacaatca	831
223-3p	ugucaguuugucaaauacccca	27	tggggtatttgacaaactgaca	832
34a-5p	uggcagugucuuagcugguugu	28	acaaccagctaagacactgcca	833

451a	aaaccguuaccauuacugaguu	29	aactcagtaatggtaacggttt	834
559-5p	uaaaguaaauaugcaccaaaa	30	ttttggtgcatatttacttta	835
Let7a-5p	ugagguaguagguuguauaguu	31	aactatacaacctactacctca	836
9-5p	ucuuugguuaucuagcuguauga	32	tcatacagctagataaccaaaga	837

[00149] In some embodiments, the miR-TS cassettes comprise one or more additional polynucleotide sequences that enable the cassette to be inserted into the locus of a viral gene. For example, a miR-TS cassette may further comprise short polynucleotide sequence on the 5' and 3' ends that are complementary to a nucleic acid sequence at a desired location in the viral genome. Such sequences are referred to herein as "homology arms" and facilitate the insertion of a miR-TS cassette into a specific locaton in the viral genome.

[00150] In some embodiments, the miR-TS cassettes disclosed comprise two or more pluralities of miR-TSs each corresponding to a different miRNA and the miR-TSs are selected to protect diverse cell types or organs from an oncolytic virus. In some embodiments, the pluralities of miR-TSs are interleaved rather than in tandem to one another. In some embodiments, the miR-TS cassettes have short (*e.g.*, 4 – 15 nt in length) spacers, resulting in a more compact cassette. In some embodiments, the miR-TS cassettes are free from (or have reduced) RNA secondary structures that inhibit activity of the miR-TSs. In some embodiments, the miR-TS cassettes are free from (or have reduced) seed sequences for miRNAs associated with carcinogenesis, malignant transformation, or metastasis (*i.e.*, "oncomiRs"). In some embodiments, the miR-TS cassettes are free from (or have reduced) polyadenylation sites.

Oncolytic viruses comprising miR-TS Cassettes

[00151] In some embodiments, a recombinant oncolytic virus may comprise one miR-TS cassette incorporated into a locus of one essential viral gene, wherein the miR-TS cassette comprises a plurality of miRNA target sequences, such that the recombinant oncolytic virus comprises a plurality of miRNA target sequences incorporated into a locus of one essential viral gene. In some aspects, the miR-TS cassette may comprise a plurality of miRNA target sequences, wherein each miRNA target sequence of the plurality is a target for the same miRNA, such that the recombinant oncolytic virus comprises a plurality (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) copies of the same miRNA target sequence incorporated into a locus of an essential viral gene. For

example, in some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising 2, 3, 4, 5, 6 or more target sequences inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising 2, 3, 4, or more target sequences inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising 2, 3, 4, 5, 6 or more target sequence inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00152] In some aspects, the plurality of miRNA target sequences comprises at least two different miRNA target sequences, at least three different miRNA target sequences, or at least four different miRNA target sequences, such that the recombinant oncolytic virus comprises one or more copies of at least 2, 3, or 4 different miRNA target sequence incorporated into a locus of an essential viral gene. For example, in some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-34a-5p, and miR-Let-7a-5p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-34a-5p, and miR-Let-7a-5p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-34a-5p, and miR-Let-7a-5p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-184-3p, and miR-Let-7a-5p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-184-3p, and miR-Let-7a-5p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p, miR-184-3p, and miR-Let-7a-5p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00153] In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p and miR-Let-7a-5p inserted

into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p and miR-Let-7a-5p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-122-5p and miR-Let-7a-5p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-145-5p, miR-199a-5p, and miR-599-5p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-145-5p, miR-199a-5p, and miR-599-5p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-145-5p, miR-199a-5p, and miR-599-5p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00154] In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-124-3p, miR-1-3p, and miR-124-3p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-124-3p, miR-1-3p, and miR-124-3p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-124-3p, miR-1-3p, and miR-124-3p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-219a-5p, miR-122-5p, and miR-128-3p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-219a-5p, miR-122-5p, and miR-128-3p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or

more target sequences for miR-219a-5p, miR-122-5p, and miR-128-3p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00155] In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-208b-3p, and miR-126-3p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-208b-3p, and miR-126-3p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-208b-3p, and miR-126-3p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-217-3p, and miR-126-3p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-217-3p, and miR-126-3p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-137-3p, miR-217-3p, and miR-126-3p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00156] In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-128-3p, miR-204-5p, and miR-219-5p inserted into one of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-128-3p, miR-204-5p, and miR-219-5p inserted into one of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, a recombinant oncolytic HSV may comprise a miR-TS cassette comprising one or more target sequences for miR-128-3p, miR-204-5p, and miR-219-5p inserted into one of ICP4, ICP27, ICP34.5, UL8, or UL9.

[00157] In some embodiments, a recombinant oncolytic virus may comprise one miR-TS cassette incorporated into the 3' or 5' untranslated region (UTR) of the viral genome. In

such embodiments, the miR-TS cassette may comprise one copy of a miRNA target sequence, such that the recombinant oncolytic virus comprises one copy of a miRNA target sequence incorporated into the 3' or 5' UTR of the viral genome. For example, in some embodiments, a recombinant polio virus, SVV, or Coxsackievirus may comprise a miR-TS cassette comprising a miRNA target sequence shown in Table 10 inserted into the 3' or 5' UTR of the viral genome. In some embodiments, a recombinant oncolytic virus may comprise one miR-TS cassette incorporated into the 3' or 5' UTR of the viral genome, wherein the miR-TS cassette comprises a plurality of miRNA target sequences shown in Table 10, such that the recombinant oncolytic virus comprises a plurality of miRNA target sequences incorporated into the 3' or 5' UTR of the viral genome.

[00158] In some aspects, the plurality of miRNA target sequences comprises at least two different miRNA target sequences, at least three different miRNA target sequences, or at least four different miRNA target sequences, such that the recombinant oncolytic virus comprises one or more copies of at least 2, 3, or 4 different miRNA target sequence incorporated into the 3' or 5' UTR of the viral genome. For example, in some embodiments, a recombinant polio virus, SVV, or Coxsackievirus may comprise a miR-TS cassette comprising one or more copies of at least 2, 3, or 4 different miRNA target sequences selected from Table 10 inserted into the 3' or 5' UTR of the viral genome.

[00159] In some embodiments, a recombinant oncolytic virus may comprise a miR-TS cassette incorporated into a locus of two or more essential viral genes. In some embodiments, the recombinant oncolytic virus is an HSV virus and the two or more essential viral genes are selected from the group consisting of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, the recombinant oncolytic virus is an HSV virus and the two or more essential viral genes are selected from the group consisting of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, or UL42. In some embodiments, the recombinant oncolytic virus is an HSV virus and the two or more essential viral genes are selected from the group consisting of ICP4, ICP27, ICP34.5, UL8, or UL9. In some embodiments, the recombinant oncolytic virus is an HSV virus and the two or more essential viral genes are selected from the group consisting of ICP27, ICP4, ICP34.5, UL8, and UL42.

[00160] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4 and a second miR-TS cassette comprising a plurality of miRNA target sequences into a locus of ICP27. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-124. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-124; 1, 2, 3, or 4 copies of a target sequence for miR-1-3p; and 1, 2, 3, or 4 copies of a target sequence for miR-143-3p. In some embodiments, the plurality of miRNA target sequences in the first miR-TS cassettes are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p);

(b) (124-3p) – (124-3p) – (124-3p) – (124-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p).

[00161] In some embodiments, the second miR-TS cassette is inserted into a locus of ICP27 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-1-3p; 1, 2, 3, or 4 copies of a target sequence for miR-145-5p; 1, 2, 3, or 4 copies of a target sequence for miR-199-5p; and 1, 2, 3, or 4 copies of a target sequence for miR-559. In some embodiments, the second miR-TS cassette is inserted into a locus of ICP27 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-219a-5p; 1, 2, 3, or 4 copies of a target sequence for miR-122-5p; and 1, 2, 3, or 4 copies of a target sequence for miR-128.

[00162] In some embodiments, the first miR-TS cassette comprises 4 copies of a target sequence for miR-124 and the second miR-TS cassette comprises 2, 3, or more copies of a target sequence for each of 1-3p, 145-5p, 199a-5p, and 559. In some embodiments, the first miR-TS cassette comprises 4 copies of a target sequence for miR-124 and the second miR-TS cassette comprises 4 copies of a target sequence for each of 219a-5p, 122-5p, 128T.

[00163] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4; and (ii) a second miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of UL42. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-124.

In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p).

[00164] In some embodiments, the second miR-TS cassette is inserted into a locus of UL42 comprises 1, 2, 3, or 4 copies of a target sequence for miR-122-5p. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged as follows:

(a) (122-5p) – (122-5p) – (122-5p).

[00165] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising 4 copies of a target sequence for miR-124 inserted into a locus of ICP4; and (ii) a second miR-TS cassette comprising 3 copies of a target sequence for miR-122-5p inserted into a locus of UL42.

[00166] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4; (ii) a second miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP27; and (iii) a third miR-TS cassette comprising a plurality of miRNA target sequences is inserted into a locus of UL42. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-124. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p).

[00167] In some embodiments, the second miR-TS cassette is inserted into a locus of ICP27 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-122. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged according to one of the following:

(a) (122-5p);

(b) (122-5p) – (122-5p) – (122-5p) – (122-5p);

(c) (122-5p) – (122-5p) – (122-5p).

[00168] In some embodiments, the third miR-TS cassette is inserted into a locus of UL42 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-125-5p. In some

embodiments, the plurality of miRNA target sequences in the third miR-TS cassettes are arranged according to one of the following:

(a) (122-5p);

(b) (122-5p) – (122-5p) – (122-5p) – (122-5p);

(c) (122-5p) – (122-5p) – (122-5p).

[00169] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for miRNA-124-3p; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 1, 2, 3, or 4 copies of a target sequence for miR-122-5p; and (iii) a third miR-TS cassette inserted into a locus of UL42 and comprising 4 copies of a target sequence for miR-125-5p. In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for miRNA-124; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 copies of a target sequence for miR-122; and (iii) a third miR-TS cassette inserted into a locus of UL42 and comprising 1, 2, 3, or 4 copies of a target sequence for miR-125-5p.. In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for miRNA-124; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 copies of a target sequence for miR-122-3p; and (iii) a third miR-TS cassette inserted into a locus of UL42 and comprising 4 copies of a target sequence for miR-125-5p. In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for miRNA-124-3p; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 3 copies of a target sequence for miR-122-3p; and (iii) a third miR-TS cassette inserted into a locus of UL42 and comprising 4 copies of a target sequence for miR-125-5p.

[00170] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4; (ii) a second miR-TS cassette a plurality of miRNA target sequences inserted into a locus of UL8. In some embodiments, the first miR-TS cassette is inserted into a locus of

ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for miR-124. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p).

[00171] In some embodiments, the second miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-208b-3p, and miR-126. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassettes are arranged as follows:

(a) (208b-3p) - (126-3p) - (137-3p) - (208b-3p) - (137-3p) - (126-3p) - (208b-3p) - (137-3p) - (126-3p) - (137-3p) - (126-3p) - (208b-3p).

[00172] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for miR-124; (ii) a second miR-TS cassette inserted into a locus of UL8 and comprising 4 copies of a miR-137 target sequence, 4 copies of a miR-208b-3p target sequence, and 4 copies of a miR-126-3p target sequence.

[00173] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4; (ii) a second miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP27; and (iii) a third miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of UL8. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-124, miR-1-3p, and miR-143-3p. In some embodiments, the plurality of miRNA target sequences in the first miR-TS cassette are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p).

[00174] In some embodiments, the second miR-TS cassette is inserted into a locus of ICP27 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassette are arranged as follows:

(a) (219a-5p) - (122-5p) - (128-3p) - (122-5p) - (219a-5p) - (128-3p) - (122-5p) - (128-3p) - (219a-5p) - (128-3p) - (122-5p) - (219a-5p).

[00175] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-208a, and miR-126. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (208b-3p) - (126-3p) - (137-3p) - (208b-3p) - (137-3p) - (126-3p) - (208b-3p) - (137-3p) - (126-3p) - (137-3p) - (126-3p) - (208b-3p).

[00176] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising 4 copies of a target sequence for each of miR-124, miR-1-3p, and miR-143-3p; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 copies of a target sequence for miR-219a-5p, 4 copies of a target sequence for miR-122-5p, and 4 copies of a target sequence for miR-128; and (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 copies of a target sequence for miR-137, 4 copies of a target sequence for miR-208a, and 4 copies of a target sequence for miR-126.

[00177] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP4; (ii) a second miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP27; (iii) a third miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of UL8; and (iv) a fourth miR-TS cassette comprising a plurality of miRNA target sequences inserted into a locus of ICP34.5. In some embodiments, the first miR-TS cassette is inserted into a locus of ICP4 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-124, miR-1-3p, and miR-143-3p. In some embodiments, the plurality of miRNA target sequences in the first miR-TS cassette are arranged as follows:

(a) (124-3p) – (124-3p) – (124-3p) – (124-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p) – (1-3p) – (143-3p).

[00178] In some embodiments, the second miR-TS cassette is inserted into a locus of ICP27 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miRNA 219a-5p, miRNA 122-5p, and miRNA 128. In some embodiments, the plurality of miRNA target sequences in the second miR-TS cassette are arranged as follows:

(a) (219a-5p) - (122-5p) - (128-3p) - (122-5p) - (219a-5p) - (128-3p) - (122-5p) - (128-3p) - (219a-5p) - (128-3p) - (122-5p) - (219a-5p).

[00179] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-208a, and miR-126. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (208b-3p) - (126-3p) - (137-3p) - (208b-3p) - (137-3p) - (126-3p) - (208b-3p) - (137-3p) - (126-3p) - (137-3p) - (126-3p) - (208b-3p).

[00180] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR137, miR-217-5p, and miR-126. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (137-3p) - (126-3p) - (217-5p) - (126-3p) - (217-5p) - (137-3p) - (217-5p) - (126-3p) - (137-3p) - (126-3p) - (217-5p) - (137-3p).

[00181] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-217-5p, and miR-127. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (137-3p) - (127-3p) - (217-5p) - (127-3p) - (217-5p) - (137-3p) - (217-5p) - (127-3p) - (137-3p) - (127-3p) - (217-5p) - (137-3p).

[00182] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-217-5p, and miR-128. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (137-3p) - (128-3p) - (217-5p) - (128-3p) - (217-5p) - (137-3p) - (217-5p) - (128-3p) - (137-3p) - (128-3p) - (217-5p) - (137-3p).

[00183] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-217-5p, and miR-129. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (137-3p) - (129-3p) - (217-5p) - (129-3p) - (217-5p) - (137-3p) - (217-5p) - (129-3p) - (137-3p) - (129-3p) - (219-5p) - (137-3p).

[00184] In some embodiments, the third miR-TS cassette is inserted into a locus of UL8 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miR-137, miR-217-5p, and miR-130. In some embodiments, the plurality of miRNA target sequences in the third miR-TS cassette are arranged as follows:

(a) (137-3p) - (130-3p) - (217-5p) - (130-3p) - (217-5p) - (130-3p) - (217-5p) - (127-3p) - (137-3p) - (130-3p) - (217-5p) - (137-3p).

[00185] In some embodiments, the fourth miR-TS cassette is inserted into a locus of ICP34.5 and comprises 1, 2, 3, or 4 copies of a target sequence for each of miRNA 128M, miRNA 204, and miRNA 219-3p. In some embodiments, the plurality of miRNA target sequences in the fourth miR-TS cassette are arranged as follows:

(a) (128-3p) - (219a-5p) - (204-5p) - (128-3p) - (219a-5p) - (204-5p) - (128-3p) - (219a-5p) - (204-5p) - (128-3p) - (219a-5p) - (204-5p).

[00186] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising4 of a target sequence for each of miR-137, miR-208b-3p, and miR-126; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising 4 of a target sequence for miR-128, 4 copies of a target sequence for miR-204, and 4 copies of a target sequence for miR-219-3p. In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; and (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 of a target sequence for each of miR-137, miR-208a, and miR-126.

[00187] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of

a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising4 of a target sequence for miR-137-3p, 4 of a target sequence for miR-217-5p, and 4 of a target sequence for miR-126-3p; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising 4 of a target sequence for miR-128, 4 copies of a target sequence for miR-204, and 4 copies of a target sequence for miR-219-3p.

[00188] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 of a target sequence for miR-137-3p, 4 of a target sequence for miR-217-5p, and 4 of a target sequence for miR-127; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising 4 of a target sequence for miR-128, 3 copies of a target sequence for miR-204, and 3 copies of a target sequence for miR-219-5p.

[00189] In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 of a target sequence for miR-137-3p, 4 of a target sequence for miR-217-5p, and4 of a target sequence for miR-128; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising 4 of a target sequence for miR-128, 3 copies of a target sequence for miR-204, and 3 copies of a target sequence for miR-219-5p. In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 of a target sequence for miR-137-3p, 4 of a target sequence for miR-

217-5p, and 4 of a target sequence for miR-129; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising4 of a target sequence for miR-128, 3 copies of a target sequence for miR-204, and 3 copies of a target sequence for miR-219-5p In some embodiments, the recombinant oncolytic virus is an HSV virus comprising (i) a first miR-TS cassette inserted into a locus of ICP4 and comprising four copies of a target sequence for each of miR-124, miR-1-3p, and miR-14; (ii) a second miR-TS cassette inserted into a locus of ICP27 and comprising 4 of a target sequence for each of miR-219a-5p, miR-122-5p, and miR-128; (iii) a third miR-TS cassette inserted into a locus of UL8 and comprising 4 of a target sequence for miR-137-3p, 4 of a target sequence for miR-217-5p, and 4 of a target sequence for miR-130; and (iv) a fourth miR-TS cassette inserted into a locus of ICP34.5 and comprising 4 of a target sequence for miR-128, 3 copies of a target sequence for miR-204, and 3 copies of a target sequence for miR-219-5p.

[00190] In some embodiments, the viral vectors described herein comprise one copy of a miR-125a target sequence incorporated into one essential viral gene. In some embodiments, the viral vectors described herein comprise one copy of a miR-125a target sequence incorporated into the UL42 locus. In some embodiments, the viral vectors described herein comprise one copy of a miR-122 target sequence incorporated into one essential viral gene. In some embodiments, the viral vectors described herein comprise one copy of a miR-122 target sequence incorporated into the ICP27 locus (*e.g.*, ONCR-036).

[00191] In further embodiments, the viral vectors described herein comprise 3 copies of a miR-125a target sequence incorporated a viral gene required for viral replication. In further embodiments, the viral vectors described herein may comprise 3 copies of a miR-125a target sequence incorporated into the UL42 locus. In some embodiments, 4 copies of a miR target sequence are incorporated into the 3' UTR of an essential viral gene. In further embodiments, the viral vectors described herein may comprise 4 copies of a miR-125a target sequence incorporated into an essential viral gene. In further embodiments, the viral vectors described herein may comprise 4 copies of a miR-125a target sequence incorporated into the UL42 locus. In some embodiments, the viral vectors described herein may comprise 4 copies of a miR-122 target sequence incorporated into an essential viral gene. In further embodiments, the viral vectors described herein may comprise 4 copies of a miR-122 target sequence incorporated into the ICP27 locus (*e.g.*, ONCR-063).

[00192] In some embodiments, 1 copy of a miR-122 target sequence is incorporated into the 3' UTR of a first essential viral gene, and 1 copy of a miR-125a target sequence is incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 1 copy of a miR-122 target sequence is incorporated into the 3' UTR of the ICP27 locus, and 1 copy of a miR-125a target sequence is incorporated into the 3' UTR of the UL42 locus (*e.g.*, ONCR-094).

[00193] In some embodiments, 1 copy of a miR-122 target sequence is incorporated into the 3' UTR of a first essential viral gene, and 3 copies of a miR-125a target sequence are incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 1 copy of a miR-122 target sequence is incorporated into the 3' UTR of the ICP27 locus, and 3 copies of a miR-125a target sequence are incorporated into the 3' UTR of the UL42 locus (*e.g.*, ONCR-095).

[00194] In some embodiments, 4 copies of a first miR target sequence are incorporated into the 3' UTR of a first essential viral gene, and 1 copy of a second miR target sequence is incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 4 copies of a miR-122 target sequence are incorporated into the 3' UTR of a first essential viral gene, and 1 copy of a miR-125a target sequence is incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 4 copies of a miR-122 target sequence are incorporated into the 3' UTR of the ICP27 locus, and 1 copy of a miR-125a target sequence is incorporated into the 3' UTR of the UL42 locus (*e.g.*, ONCR-093).

[00195] In some embodiments, 4 copies of a first miR target sequence are incorporated into the 3' UTR of a first essential viral gene, and 4 copies of a second miR target sequence are incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 4 copies of a miR-122 target sequence are incorporated into the 3' UTR of a first essential viral gene, and 4 copies of a miR-125a target sequence are incorporated into the 3' UTR of a second essential viral gene. In some embodiments, 4 copies of a miR-122 target sequence are incorporated into the 3' UTR of the ICP27 locus, and 4 copies of a miR-125a target sequence is incorporated into the 3' UTR of the UL42 locus (*e.g.*, ONCR-096).

[00196] In some embodiments, the miR-attenuated oncolytic viruses described herein result in reduced viral replication in a cell that expresses a miR capable of binding to one or more of the incorporated miR-target sequences. "Viral replication" refers to the total number of viral replication cycles that occur in a particular cell or population of cells during a given amount

of time. In some embodiments, viral replication can be measured directly by assessing the total viral titer present over the course of the given amount of time, or by assessing the number of viral genome copies present (*e.g.*, by sequencing). In some embodiments, the viral vector may additionally comprise a detectable label, such as a fluorescent reporter. In such embodiments, viral replication may be assessed by measuring the fluorescence intensity of the reporter, or the number of cells that express the reporter. In some embodiments, viral replication can be measured indirectly by assessing the number of viable cells over the course of the given amount of time. For example, the level of viral replication would be expected to inversely correlate with the number of viable cells over time.

[00197] "Reduced viral replication" as used herein, refers to a level of viral replication that is lower in a first cell or first population of cells compared to a second cell or a second population of cells. In some embodiments, the level of viral replication in the first cell or first population of cells is reduced by at least 5% compared to the level of viral replication in the second cell or population of cells. In some embodiments, the level of viral replication in the first cell or first population of cells is reduced by at least 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the level of viral replication in the second cell or population of cells. In some embodiments, viral replication in the first cell or first population of cells is completely inhibited compared to the viral replication in the second cell or population of cells.

[00198] In some embodiments, the reduced viral replication in the first cell or first population of cells correlates with the expression of a miR capable of binding to the one or more miR-target sequences incorporated into one or more viral genes required for replication. In some embodiments, expression of a miR corresponding to the incorporated miR-target sequence therefore inhibits or reduces the expression of the replication gene, thereby inhibiting or reducing viral replication. In some embodiments, the second cell or second population of cells does not express, or has a reduced expression level, of the t miR. In some embodiments, absent or reduced expression of a miR (*e.g.*, in a cancer cell) corresponding to the incorporated miR-target sequence allows for viral replication to proceed. In some embodiments, the expression level of the miR in the second cell or population of cells is at least 5% lower than the expression level of the miR in the first cell or population. In some embodiments, the expression level of the miR in the second

cell or population of cells is reduced at least 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the expression level of the miR in the first cell or population. In some embodiments, the second cell does not express the miR. In particular embodiments, the first cell is a non-cancerous cell and the second cell is a cancerous cell.

[00199] In some embodiments, a replication-restricted viral vector (*e.g.*, a miR-attenuated viral vector) comprises at least one let-7 target sequence and is used to treat lung cancer. In some embodiments, a replication-restricted viral vector comprises at least one miR-15a and/or at least one miR-16A target sequences and is used to treat B-cell chronic lymphocytic leukemia. In some embodiments, a replication-restricted viral vector comprises at least one miR-125b, at least one miR-145, at least one miR-21, and/or at least one miR-155 target sequences and is used to treat breast cancer. In other embodiments, a replication-restricted viral vector comprises at least one miR-143 and/or at least one miR-145 target sequences and is used to treat colorectal cancer. In certain embodiments, a replication-restricted viral vector comprises at least one miR-181a, at least one miR-181b, and/or at least one miR-181c target sequences and is used to treat glioblastoma. In some embodiments, a replication-restricted viral vector comprises at least one miR-199a*, at least one miR-195, at least one miR-199a, at least one miR-200a, and/or at least one miR-125a target sequences and is used to treat liver cancer (e.g., hepatocellular carcinoma).

[00200] In particular embodiments, a replication-restricted viral vector comprises at least one miR-451a target sequence, at least one miR-143-3p target sequence, at least one miR-559 target sequence, and at least one miR-124 target sequence and is used for the treatment of pancreatic, lung, and/or colon cancer. In such embodiments, the target sequences for miR-451a, miR-143-3p, miR-559, and miR-124 are incorporated into two or more genes required for viral replication (*e.g.*, ICP4 and ICP27). In further particular embodiments, a replication-restricted viral vector comprises at least one miR-451a target sequence, at least one miR-145-5p target sequence, at least one miR-559 target sequence, and at least one miR-124 target sequence and is used for the treatment of any type of cancer described herein. In such embodiments, the target sequences for miR-451a, miR-145-5p, miR-559, and miR-124 are incorporated into two or more genes required for viral replication (*e.g.*, ICP4 and ICP27). In further particular embodiments, a replication-restricted viral vector comprises at least one miR-205p target sequence, at least one miR-141-5p

target sequence, at least one miR-31-5p target sequence, and at least one miR-124 target sequence and is used for the treatment of schwannoma. In such embodiments, the target sequences for miR-205p, miR-141-5p, miR-31-5p, and miR-124 are incorporated into two or more genes required for viral replication (*e.g.*, ICP4 and ICP27).

[00201] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-136-3p, miR-432-5p, miR-1-3p, miR-127-3p, miR-379-5p, miR-493-5p, miR-223-5p, miR-223-5p, miR-136-5p, miR-451a, miR-487b-3p, miR-370-3p, miR-410-3p, miR-431-3p, miR-4485-3p, miR-4485-5p, miR-127-5p, miR-409-3p, miR-338-3p, miR-559, miR-411-5p, miR-133a-5p, miR-143-3p, miR-376b-3p, miR-758-3p, miR-1, miR-101, miR-1180, miR-1236, miR-124-3p, miR-125b, miR-126, miR-1280, miR-133a, miR-133b, miR-141, miR-143, miR-144, miR-145, miR-155, miR-16, miR-18a, miR-192, miR-195, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-214, miR-218, miR-23b, miR-26a, miR-29c, miR-320c, miR-34a, miR-370, miR-409-3p, miR-429, miR-451, miR-490-5p, miR-493, miR-576-3p, and/or miR-99a inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating bladder cancer.

[00202] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-1251-5p, miR-219a-5p, miR-219a-2-3p, miR-124-3p, miR-448, miR-138-2-3p, miR-490-5p, miR-129-1-3p, miR-1264, miR-3943, miR-490-3p, miR-383-5p, miR-133b, miR-129-2-3p, miR-128-2-5p, miR-133a-3p, miR-129-5p, miR-1-3p, miR-885-3p, miR-124-5p, miR-759, miR-7158-3p, miR-770-5p, miR-135a-5p, miR-885-5p, let-7g-5p, miR-100, miR-101, miR-106a, miR-124, miR-124a, miR-125a, miR-125a-5p, miR-125b, miR-127-3p, miR-128, miR-129, miR-136, miR-137, miR-139-5p, miR-142-3p, miR-143, miR-145, miR-146b-5p, miR-149, miR-152, miR-153, miR-195, miR-21, miR-212-3p, miR-219-5p, miR-222, miR-29b, miR-31, miR-3189-3p, miR-320, miR-320a, miR-326, miR-330, miR-331-3p, miR-340, miR-342, miR-34a, miR-376a, miR-449a, miR-483-5p, miR-503, miR-577, miR-663, miR-7, miR-7-5p, miR-873, let-7a, let-7f, miR-107, miR-122, miR-124-5p, miR-139, miR-146a, miR-146b, miR-15b, miR-16, miR-181a, miR-181a-1, miR-181a-2, miR-181b, miR-181b-1, miR-181b-2, miR-181c, miR-181d, miR-184, miR-185, miR-199a-3p, miR-200a, miR-200b, miR-203, miR-204, miR-205, miR-218, miR-23b, miR-26b, miR-27a, miR-29c, miR-

328, miR-34c-3p, miR-34c-5p, miR-375, miR-383, miR-451, miR-452, miR-495, miR-584, miR-622, miR-656, miR-98, miR-124-3p, miR-181b-5p, miR-200b, and/or miR-3189-3p inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating brain cancer. In certain embodiments, the brain cancer is astrocytoma, glioblastoma, or glioma.

[00203] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-10b-5p, miR-126-3p, miR-145-3p, miR-451a, miR-199b-5p, miR-5683, miR-3195, miR-3182, miR-1271-5p, miR-204-5p, miR-409-5p, miR-136-5p, miR-514a-5p, miR-559, miR-483-3p, miR-1-3p, miR-6080, miR-144-3p, miR-10b-3p, miR-6130, miR-6089, miR-203b-5p, miR-4266, miR-4327, miR-5694, miR-193b, let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-100, miR-107, miR-10a, miR-10b, miR-122, miR-124, miR-1258, miR-125a-5p, miR-125b, miR-126, miR-127, miR-129, miR-130a, miR-132, miR-133a, miR-143, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-149, miR-152, miR-153, miR-15a, miR-16, miR-17-5p, miR-181a, miR-1826, miR-183, miR-185, miR-191, miR-193a-3p, miR-195, miR-199b-5p, miR-19a-3p, miR-200a, miR-200b, miR-200c, miR-205, miR-206, miR-211, miR-216b, miR-218, miR-22, miR-26a, miR-26b, miR-300, miR-30a, miR-31, miR-335, miR-339-5p, miR-33b, miR-34a, miR-34b, miR-34c, miR-374a, miR-379, miR-381, miR-383, miR-425, miR-429, miR-450b-3p, miR-494, miR-495, miR-497, miR-502-5p, miR-517a, miR-574-3p, miR-638, miR-7, miR-720, miR-873, miR-874, miR-92a, miR-98, miR-99a, mmu-miR-290-3p, and/or mmu-miR-290-5p inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating breast cancer.

[00204] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-143, miR-145, miR-17-5p, miR-203, miR-214, miR-218, miR-335, miR-342-3p, miR-372, miR-424, miR-491-5p, miR-497, miR-7, miR-99a, miR-99b, miR-100, miR-101, miR-15a, miR-16, miR-34a, miR-886-5p, miR-106a, miR-124, miR-148a, miR-29a, and/or miR-375 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating cervical cancer.

[00205] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-133a-5p, miR-490-5p, miR-124-3p, miR-137, miR-655-3p, miR-376c-3p, miR-369-5p, miR-490-3p, miR-432-5p, miR-487b-3p, miR-342-3p, miR-223-3p, miR-136-3p, miR-136-3p, miR-143-5p, miR-1-3p, miR-214-3p, miR-143-3p, miR-199a-3p, miR-199b-3p, miR-451a, miR-127-3p, miR-133a-3p, miR-145-5p, miR-145-3p, miR-199a-5p, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-100, miR-101, miR-126, miR-142-3p, miR-143, miR-145, miR-192, miR-200c, miR-21, miR-214, miR-215, miR-22, miR-25, miR-302a, miR-320, miR-320a, miR-34a, miR-34c, miR-365, miR-373, miR-424, miR-429, miR-455, miR-484, miR-502, miR-503, miR-93, miR-98, miR-186, miR-30a-5p, miR-627, let-7a, miR-1, miR-124, miR-125a, miR-129, miR-1295b-3p, miR-1307, miR-130b, miR-132, miR-133a, miR-133b, miR-137, miR-138, miR-139, miR-139-5p, miR-140-5p, miR-148a, miR-148b, miR-149, miR-150-5p, miR-154, miR-15a, miR-15b, miR-16, miR-18a, miR-191, miR-193a-5p, miR-194, miR-195, miR-196a, miR-198, miR-199a-5p, miR-203, miR-204-5p, miR-206, miR-212, miR-218, miR-224, miR-24-3p, miR-26b, miR-27a, miR-28-3p, miR-28-5p, miR-29b, miR-30a-3p, miR-30b, miR-328, miR-338-3p, miR-342, miR-345, miR-34a-5p, miR-361-5p, miR-375, miR-378, miR-378a-3p, miR-378a-5p, miR-409-3p, miR-422a, miR-4487, miR-483, miR-497, miR-498, miR-518a-3p, miR-551a, miR-574-5p, miR-625, miR-638, miR-7, miR-96-5p, miR-202-3p, miR-30a, and/or miR-451 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating colon or colorectal cancer.

[00206] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-101, miR-130a, miR-130b, miR-134, miR-143, miR-145, miR-152, miR-205, miR-223, miR-301a, miR-301b, miR-30c, miR-34a, miR-34c, miR-424, miR-449a, miR-543, and/or miR-34b inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating endometrial cancer.

[00207] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-125b, miR-138, miR-15a, miR-15b, miR-16, miR-16-1, miR-16-1-3p, miR-16-2, miR-181a, miR-181b, miR-195, miR-223, miR-29b, miR-34b, miR-34c, miR-424, miR-10a, miR-146a, miR-150, miR-151, miR-155, miR-2278, miR-

26a, miR-30e, miR-31, miR-326, miR-564, miR-27a, let-7b, miR-124a, miR-142-3p, let-7c, miR-17, miR-20a, miR-29a, miR-30c, miR-720, miR-107, miR-342, miR-34a, miR-202, miR-142-5p, miR-29c, miR-145, miR-193b, miR-199a, miR-214, miR-22, miR-137, and/or miR-197 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating hematologic cancer. In some embodiments, the hematologic cancer is leukemia, lymphoma, or myeloma.

[00208] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-1, miR-145, miR-1826, miR-199a, miR-199a-3p, miR-203, miR-205, miR-497, miR-508-3p, miR-509-3p, let-7a, let-7d, miR-106a*, miR-126, miR-1285, miR-129-3p, miR-1291, miR-133a, miR-135a, miR-138, miR-141, miR-143, miR-182-5p, miR-200a, miR-218, miR-28-5p, miR-30a, miR-30c, miR-30d, miR-34a, miR-378, miR-429, miR-509-5p, miR-646, miR-133b, let-7b, let-7c, miR-200c, miR-204, miR-335, miR-377, and/or miR-506 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating kidney cancer.

[00209] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f, let-7f-1, let-7f-2, let-7g, let-7i, miR-1, miR-100, miR-101, miR-105, miR-122, miR-122a, miR-1236, miR-124, miR-125b, miR-126, miR-127, miR-1271, miR-128-3p, miR-129-5p, miR-130a, miR-130b, miR-133a, miR-134, miR-137, miR-138, miR-139, miR-139-5p, miR-140-5p, miR-141, miR-142-3p, miR-143, miR-144, miR-145, miR-146a, miR-148a, miR-148b, miR-150-5p, miR-15b, miR-16, miR-181a-5p, miR-185, miR-188-5p, miR-193b, miR-195, miR-195-5p, miR-197, miR-198, miR-199a, miR-199a-5p, miR-199b, miR-199b-5p, miR-200a, miR-200b, miR-200c, miR-202, miR-203, miR-204-3p, miR-205, miR-206, miR-20a, miR-21, miR-21-3p, miR-211, miR-212, miR-214, miR-217, miR-218, miR-219-5p, miR-22, miR-223, miR-26a, miR-26b, miR-29a, miR-29b-1, miR-29b-2, miR-29c, miR-302b, miR-302c, miR-30a, miR-30a-3p, miR-335, miR-338-3p, miR-33a, miR-34a, miR-34b, miR-365, miR-370, miR-372, miR-375, miR-376a, miR-377, miR-422a, miR-424, miR-424-5p, miR-433, miR-4458, miR-448, miR-450a, miR-451, miR-485-5p, miR-486-5p, miR-497, miR-503, miR-506, miR-519d, miR-520a, miR-520b, miR-520c-3p, miR-582-5p, miR-590-5p, miR-610, miR-612, miR-625, miR-

637, miR-675, miR-7, miR-877, miR-940, miR-941, miR-98, miR-99a, miR-132, and/or miR-31 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating liver cancer. In some embodiments, the liver cancer is hepatocellular carcinoma.

[00210] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-143-3p, miR-126-3p, miR-126-5p, miR-1266-3p, miR-6130, miR-6080, miR-511-5p, miR-143-5p, miR-223-5p, miR-199b-5p, miR-199a-3p, miR-199b-3p, miR-451a, miR-142-5p, miR-144, miR-150-5p, miR-142-3p, miR-214-3p, miR-214-5p, miR-199a-5p, miR-145-3p, miR-145-5p, miR-1297, miR-141, miR-145, miR-16, miR-200a, miR-200b, miR-200c, miR-29b, miR-381, miR-409-3p, miR-429, miR-451, miR-511, miR-99a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-1, miR-101, miR-133b, miR-138, miR-142-5p, miR-144, miR-1469, miR-146a, miR-153, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-182, miR-192, miR-193a-3p, miR-194, miR-195, miR-198, miR-203, miR-217, miR-218, miR-22, miR-223, miR-26a, miR-26b, miR-29c, miR-33a, miR-34a, miR-34b, miR-34c, miR-365, miR-449a, miR-449b, miR-486-5p, miR-545, miR-610, miR-614, miR-630, miR-660, miR-7515, miR-9500, miR-98, miR-99b, miR-133a, let-7a, miR-100, miR-106a, miR-107, miR-124, miR-125a-3p, miR-125a-5p, miR-126, miR-126*, miR-129, miR-137, miR-140, miR-143, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-154, miR-155, miR-17-5p, miR-181a-1, miR-181a-2, miR-181b, miR-181b-1, miR-181b-2, miR-181c, miR-181d, miR-184, miR-186, miR-193b, miR-199a, miR-204, miR-212, miR-221, miR-224, miR-27a, miR-27b, miR-29a, miR-30a, miR-30b, miR-30c, miR-30d, miR-30d-5p, miR-30e-5p, miR-32, miR-335, miR-338-3p, miR-340, miR-342-3p, miR-361-3p, miR-373, miR-375, miR-4500, miR-4782-3p, miR-497, miR-503, miR-512-3p, miR-520a-3p, miR-526b, miR-625*, and/or miR-96 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating lung cancer.

[00211] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for let-7b, miR-101, miR-125b, miR-1280, miR-143, miR-146a, miR-146b, miR-155, miR-17, miR-184, miR-185, miR-18b, miR-193b, miR-200c, miR-203, miR-204, miR-205, miR-206, miR-20a, miR-211, miR-218, miR-26a, miR-31, miR-33a, miR-34a, miR-34c, miR-376a, miR-376c, miR-573, miR-7-5p, miR-9, and/or miR-98

inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating melanoma.

[00212] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for let-7d, miR-218, miR-34a, miR-375, miR-494, miR-100, miR-124, miR-1250, miR-125b, miR-126, miR-1271, miR-136, miR-138, miR-145, miR-147, miR-148a, miR-181a, miR-206, miR-220a, miR-26a, miR-26b, miR-29a, miR-32, miR-323-5p, miR-329, miR-338, miR-370, miR-410, miR-429, miR-433, miR-499a-5p, miR-503, miR-506, miR-632, miR-646, miR-668, miR-877, and/or miR-9inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating oral cancer.

[00213] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for let-7i, miR-100, miR-124, miR-125b, miR-129-5p, miR-130b, miR-133a, miR-137, miR-138, miR-141, miR-145, miR-148a, miR-152, miR-153, miR-155, miR-199a, miR-200a, miR-200b, miR-200c, miR-212, miR-335, miR-34a, miR-34b, miR-34c, miR-409-3p, miR-411, miR-429, miR-432, miR-449a, miR-494, miR-497, miR-498, miR-519d, miR-655, miR-9, miR-98, miR-101, miR-532-5p, miR-124a, miR-192, miR-193a, and/or miR-7 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating ovarian cancer.

[00214] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-216a-5p, miR-802, miR-217, miR-145-3p, miR-143-3p, miR-451a, miR-375, miR-214-3p, miR-216b-3p, miR-432-5p, miR-216a-3p, miR-199b-5p, miR-199a-5p, miR-136-3p, miR-216b-5p, miR-136-5p, miR-145-5p, miR-127-3p, miR-199a-3p, miR-199b-3p, miR-559, miR-129-2-3p, miR-4507, miR-1-3p, miR-148a-3p, miR-101, miR-1181, miR-124, miR-1247, miR-133a, miR-141, miR-145, miR-146a, miR-148a, miR-148b, miR-150*, miR-150-5p, miR-152, miR-15a, miR-198, miR-203, miR-214, miR-216a, miR-29c, miR-335, miR-34a, miR-34b, miR-34c, miR-373, miR-375, miR-410, miR-497, miR-615-5p, miR-630, miR-96, miR-132, let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-126, miR-135a, miR-143, miR-144, miR-150, miR-16, miR-200a, miR-200b, miR-200c, miR-217, miR-218, miR-337, miR-494, and/or miR-98 inserted into the 5'

UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating pancreatic cancer.

[00215] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for let-7a-3p, let-7c, miR-100, miR-101, miR-105, miR-124, miR-128, miR-1296, miR-130b, miR-133a-1, miR-133a-2, miR-133b, miR-135a, miR-143, miR-145, miR-146a, miR-154, miR-15a, miR-187, miR-188-5p, miR-199b, miR-200b, miR-203, miR-205, miR-212, miR-218, miR-221, miR-224, miR-23a, miR-23b, miR-25, miR-26a, miR-26b, miR-29b, miR-302a, miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d, miR-30e, miR-31, miR-330, miR-331-3p, miR-34a, miR-34b, miR-34c, miR-374b, miR-449a, miR-4723-5p, miR-497, miR-628-5p, miR-642a-5p, miR-765, and/or miR-940 inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating prostate cancer.

[00216] In some embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences for miR-101, miR-183, miR-204, miR-34a, miR-365b-3p, miR-486-3p, and/or miR-532-5p inserted into the 5' UTR or 3' UTR of one or more viral genes required for viral replication. This oncolytic virus may be used in methods and compositions for treating retinoblastoma.

[00217] In some embodiments, an oncolytic virus described herein is a herpes simplex virus and wherein the one or more viral genes required for viral replication is selected from the group consisting of UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12.

Payload Molecules

[00218] In some embodiments, the oncolytic viruses described herein comprise a nucleic acid sequence encoding a payload molecule. As used herein, a "payload molecule" refers to a molecule capable of further enhancing the therapeutic efficacy of a virus. Payload molecules suitable for use in the present disclosure include antigen-binding molecules such as antibodies or

antigen binding fragments thereof, cytokines, chemokines, soluble receptors, cell-surface receptor ligands, bipartite peptides, enzymes, and nucleic acids (*e.g.*, shRNAs, siRNAs, antisense RNAs, antagomirs, ribozymes, apatamers, a decoy oligonucleotide, or an antagomir). The nature of the payload molecule will vary with the disease type and desired therapeutic outcome. In some embodiments, one or more miRNA target sequences is incorporated in to the 3' or 5' UTR of a polynucleotide sequence encoding a payload molecule. In such embodiments, translation and subsequent expression of the payload does not occur, or is substantially reduced, in cells where the corresponding miRNA is expressed. In some embodiments, one or more miRNA target sequences are inserted into the 3' and/or 5' UTR of the polynucleotide sequence encoding the therapeutic polypeptide.

[00219] In some embodiments, the recombinant oncolytic viruses described herein comprise at least one polynucleotide encoding a payload molecule that that reduces the expression or inhibits the function of an endogenous miRNA, a gene, or a tissue inhibitor of metalloproteinases (TIMP). Such recombinant oncolytic viruses are referred to herein as "genome-editing" or "microenvironment-remodeling" viruses or vectors. The encoded protein or oligonucleotide may reduce expression or inhibit the function of a miRNA, gene, or TIMP in any number of ways including targeting the protein (*e.g.*, a TIMP) for degradation (*e.g.*, by ubiquitination and proteosomal degradation or targeting for lysosomal degradation), blocking interactions with cognate receptors (*e.g.*, blocking antibodies or antigen binding fragments thereof or peptide inhibitors), degrading messenger RNA transcripts (*e.g.*, a short interfering RNA or short hairpin RNA), and/or altering the genomic DNA sequence encoding the specific miRNA, gene, or protein (*e.g.*, by an endonuclease).

[00220] In particular embodiments, the protein or oligonucleotide reduces the expression of a miR or a gene involved in carcinogenesis or metastasis (*e.g.*, an oncogenic miR or an oncogene). In some embodiments, a recombinant oncolytic virus comprises at least one polynucleotide encoding a payload molecule that reduces the expression or function of a miRNA that is an oncogenic miRNA (e.g., one or more of the miRNAs listed in Table 4). In some embodiments, the recombinant oncolytic virus comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more polynucleotides encoding for a protein or oligonucleotide that reduces the expression or function of an oncogenic miRNA. In some embodiments, the recombinant oncolytic virus comprises at least

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more polynucleotides encoding for a plurality of proteins or oligonucleotides that reduce the expression or function of a plurality of oncogenic miRNAs. In some embodiments, the protein or oligonucleotide reduces the expression of miR-17–92 and is used to treat lung cancer (e.g., small-cell lung cancer). In other embodiments, the protein or oligonucleotide reduces the expression of miR-221 and/or miR-21 and is used to treat glioblastoma. In certain embodiments, the protein or oligonucleotide reduces the expression of miR-155 and/or miR-17–92 and is used to treat lymphoma (e.g., Burkitt's lymphoma, diffuse large B cell lymphoma, marginal zone lymphoma, or chronic lymphocytic leukemia). In some embodiments, the protein or oligonucleotide reduces the expression of miR-221, miR-222, and/or miR-146 and is used to treat thyroid cancer. In some embodiments, the protein or oligonucleotide reduces the expression of miR-372 and/or miR-373 and is used to treat testicular cancer (e.g., testicular germ cell tumors). In some embodiments, the protein or oligonucleotide reduces the expression of miR-18 and/or miR-224 and is used to treat liver cancer (e.g., hepatocellular carcinoma).

[00221] In some embodiments, a recombinant viral vectors described herein comprise a polynucleotide encoding a payload molecule that degrades the tumor extracellular matrix (ECM), which in some aspects leads to enhanced viral spread. Matrix metalloproteinases (MMPs) are zinc-dependent proteases that are classified, based on their activity, into collagenases, gelatinases, stromelysins and matrilysins. These proteases are generally secreted as pro-enzymes (zymogens) and are activated by proteolytic removal of the pro-peptide pro-domain. The primary role that MMPs play in cancer is in the degradation of the ECM, which facilitates tumor invasion and metastasis. MMPs are also involved in tumor progression, epithelial to mesenchymal transition (EMT), and angiogenesis. MMPs are regulated by miRs as well as TIMPs, which comprise a family of four protease inhibitors (TIMP1, TIMP2, TIMP3, and TIMP4). A broad array of tumor microenvironments can be degraded by disrupting miRNAs or TIMPs that negatively regulate the MMP family with the recombinant viral vectors of the invention. Examples of miR/MMP interactions are shown in Table 5. Many of these interactions show that multiple MMPs are regulated by a single miRNA: e.g. let-7 regulates MMP-2, MMP-9, and MMP-14; miR-143 regulates MMP-2, MMP-9, and MMP-13; miR-218 regulates MMP-2, MMP-7, and MMP-9. Furthermore, the vast majority of MMPs may be regulated by a single TIMP master switch: e.g.

TIMP1 is known to inhibit most all of the known MMPs and also promotes cell proliferation in a wide range of cell types; TIMP2 interacts with MMP-14 and MMP-2.

[00222] In some embodiments, the recombinant oncolytic viruses described herin comprise at least one polynucleotide encoding a protein or an oligonucleotide that reduces the expression or function of a miRNA that is capable of altering the extracellular matrix or capable of modulating a pathway that alters the extracellular matrix, particularly in a tumor microenvironment (e.g., one or more of the miRNAs listed in Table 5). A microenvironment remodeling miR, as used herein, refers to a miR. In some embodiments, the protein or oligonucleotide reduces the expression or function of one microenvironment remodeling miR. In some embodiments, the protein or oligonucleotide reduces the expression or function of at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more microenvironment remodeling miRs. In some embodiments, the recombinant oncolytic virus comprises a plurality of polynucleotides encoding a plurality of protein or oligonucleotides that reduce the expression or function of a plurality of microenvironment remodeling miRs. In some embodiments, strategies described herein may be utilized by recombinant viral vectors of the present invention to knockdown or disrupt expression or function of miRs or TIMPs which negatively regulate MMPs. In some embodiments, a recombinant oncolytic virus reduces the expression of a TIMP selected from TIMP1, TIMP2, TIMP3 and TIMP4.

[00223] In some embodiments, the recombinant oncolytic viruses described herein comprise at least one polynucleotide encoding a protein or an oligonucleotide that reduces the expression or function of a gene in the host cell genome. In some aspects, the gene is an oncogenic gene (e.g., a gene selected from the genes listed in Table 7). In some aspects, the gene encodes an oncogenic miR (e.g., a miRNA listed in Table 4), a microenvironment remodeling miR (*e.g.*, a miRNA listed in Table 5), or a negative regulator of ECM-degradation (*e.g.*, a TIMP). Reduction of gene expression and/or function may be accomplished by at the level of transcription (*e.g.*, mutating, deleting, or silencing the genomic DNA sequence) or at the level of translation (*e.g.*, by inhibiting the production of the gene product through mRNA degradation). In some embodiments, the recombinant oncolytic viruses described herein comprise one or more polynucleotides that encode for nucleases that reduce the expression or function of a gene by enabling the mutation, deletion, or repression of transcription of a gene sequence. In specific embodiments, the nuclease

is selected from a Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease, a zinc-finger nuclease (ZFN) or a Transcription activator-like effector nuclease (TALEN). In non-limiting examples, a CRISPR-associated endonuclease is selected from SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, C2C1, C2C3, Cpf1, Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csx12), Cas10, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, Csx10, Csx16, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, and Csf4.

[00224] Recombinant viral vectors of the invention may utilize the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR Associated) nuclease system, which is an engineered nuclease system based on a bacterial system that can be used for mammalian genome engineering. Generally, the system comprises a Cas nuclease and a guide RNA (gRNA). The gRNA is comprised of two parts; a crispr-RNA (crRNA) that is specific for a target genomic DNA sequence, and a tracr RNA (trRNA) that facilitates Cas binding. The crRNA and trRNA may be present as separate RNA oligonucleotides, or may be present in the same RNA oligonucleotide, referred to as a single guide-RNA (sgRNA). As used herein, the term "guide RNA" or "gRNA" refers to either the combination of an individual trRNA and an individual crRNA or an sgRNA. See, e.g., Jinek *et al.* (2012) *Science* 337:816-821; Cong *et al.* (2013) *Science* 339:819-823; Mali *et al.* (2013) *Science* 339:823-826; Qi *et al.* (2013) *Cell* 152:1173-1183; Jinek *et al.* (2013), *eLife* 2:e00471; David Segal (2013) *eLife* 2:e00563; Ran *et al.* (2013) *Nature Protocols* 8(11):2281-2308; Zetsche *et al.* (2015) *Cell* 163(3):759-771; PCT Publication Nos. WO 2007/025097, WO 2008/021207, WO 2010/011961, WO 2010/054108, WO 2010/054154, WO 2012/054726, WO 2012/149470, WO 2012/164565, WO 2013/098244, WO 2013/126794, WO 2013/141680, and WO 2013/142578; U.S. Patent Publication Nos. 2010-0093617, 2013-0011828, 2010-0257638, 2010-0076057, 2011-0217739, 2011-0300538, 2013-0288251, and 2012-0277120; and U.S. Patent No. 8,546,553, each of which is incorporated herein by reference in its entirety.

[00225] Multiple class 1 CRISPR-Cas systems, which include the type I and type III systems, have been identified and functionally characterized in detail, revealing the complex architecture and dynamics of the effector complexes (Brouns *et al.*, 2008, Marraffini and

Sontheimer, 2008, Hale *et al.*, 2009, Sinkunas *et al.*, 2013, Jackson *et al.*, 2014, Mulepati *et al.*, 2014). In addition, several class 2-type II CRISPR-Cas systems that employ homologous RNA-guided endonucleases of the Cas9 family as effectors have also been identified and experimentally characterized (Barrangou et al., 2007, Garneau *et al.*, 2010, Deltcheva *et al.*, 2011, Sapranauskas *et al.*, 2011, Jinek *et al.*, 2012, Gasiunas *et al.*, 2012). A second, putative class 2-type V CRISPR-Cas system has been recently identified in several bacterial genomes. The putative type V CRISPR-Cas systems contain a large, ~1,300 amino acid protein called Cpf1 (CRISPR from Prevotella and Francisella 1).

[00226] In some embodiments, an oncolytic virus described herein further comprises at least one polynucleotide encoding a trRNA and crRNA targeted to the miRNA or the TIMP. In some cases, the at least one polynucleotide encoding a trRNA and crRNA is inserted into a locus on the viral genome. In some embodiments, the polynucleotide is an insulated sequence comprising a synthetic insulator or a native viral (e.g., HSV) insulator. In certain embodiments, an oncolytic virus is a herpes simplex virus and the at least one polynucleotide encoding an RNA binding site is inserted into or between one or more loci including the internal repeat joint region (comprising one copy each of the diploid genes ICP0, ICP34.5, LAT, ICP4, and the ICP47 promoter), ICP0, LAT, UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12. In one embodiment, an oncolytic virus is a herpes simplex virus (HSV) and the at least one polynucleotide encoding an RNA binding site is inserted into a locus between the UL3 and the UL4 open reading frames (*e.g.*, Fig. 39 and Fig. 40).

[00227] In some embodiments, the recombinant oncolytic virus comprises at least one polynucleotide encoding a payload molecule that activate or inhances an anti-tumor immune response. In some embodiments, the payload molecule is a cytokine, a chemokine, an antibody or antigen binding fragment thereof, a bispecific T-cell engager (BiTE). For example, in some embodiments, the payload molecule is an antibody or antigen binding fragments thereof that bind to and inhibit immune checkpoint receptors (*e.g.* CTLA4, LAG3, PD1, PDL1, and others). In some embodiments, the payload molecule is an anti-PD1 antibody or antigen-binding fragement thereof,

an anti-PDL1 antibody or antigen-binding fragement thereof, or an anti-CTLA4 antibody or antigen-binding fragement thereof.

[00228] In some embodiments, the payload molecule is a protein that binds to and activates a cell-surface receptor. For example, in some embodiments, payload molecule comprises an endogenous cell-surface ligand, such as the extracellular domain of 41BBL, the extracellular domain of CD40L, FLT3L. In some embodiments, the payload molecule is a cytokine (*e.g.*, IFNγ, IFNα, IFNβ, TNFα, IL-12, IL-2, IL-6, IL-8, IL-15, GM-CSF, IL-21, IL-35, TGFβ, and others) or chemokine (*e.g.*, CCL4, CXCL10, CCL5, CXCL13, or XCL1).

[00229] In some embodiments, the payload molecule is a protein that binding to and activate an activating receptor (*e.g.*, FcγRI, FcγIIa, FcγIIIa, costimulatory receptors, and others). In particular embodiments, the protein is selected from EpCAM, folate, A2A, anti-FGF2, anti-FGFR/FGFR2b, anti-SEMA4D, CD137, CD200, CD38, CD44, CSF-1R, endothelin B Receptor, ISRE7, LFA-1, NG2 (also known as SPEG4), SMADs, STING, , and VCAM1.

[00230] In certain embodiments, a polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an miRNA, a gene, or a TIMP is inserted into a locus on the viral genome of a recombinant oncolytic virus. In some embodiments, the polynucleotide is an insulated sequence comprising a synthetic insulator or a native viral (e.g., HSV) insulator. In certain embodiments, the oncolytic virus is a herpes simplex virus and the at least one polynucleotide encoding an RNA binding site is inserted into or between one or more loci including the internal repeat joint region (comprising one copy each of the diploid genes ICP0, ICP34.5, LAT, ICP4, and the ICP47 promoter), ICP0, LAT, UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12.. In one embodiment, the virus is a herpes simplex virus (HSV) and the at least one polynucleotide is inserted into a locus between the UL3 and the UL4 open reading frames (see, *e.g.*, Fig. 39 and Fig. 40).

[00231] In some embodiments, the recombinant oncolytic virus comprises at least one protease-activated antibody. Protease-activated antibodies, such as those described by Metz et al. (Protein Eng Des Sel, 25(10):571-80, 2012) are activated and bind only to targets following

protease cleavage of a protective cap. In some instances, tumor microenvironments possess an array of proteases that are well differentiated from surrounding healthy tissues. For example, the protease cathepsin B is overexpressed in numerous cancers, including breast, cervix, colon, colorectal, gastric, head and neck, liver, lung, melanoma, ovarian, pancreatic, prostate, and thyroid cancer. The human degradome, comprised of a complete list of proteases synthesized by human cells, is made up of at least 569 proteases that are distributed into five broad classes (in order from greatest to least number): metalloproteinases (MMPs), serine, cysteine, threonine, and aspartic proteases (Lopez-Otin et al., Nat Rev Cancer, 7(10):800-8, 2007). In particular, protease antibodies specifically cleaved by MMPs can serve as an excellent means of targeting the recombinant viral vectors described herein to the tumor microenvironment, as MMPs are found in the extracellular and pericellular areas of the cell. Table 6 summarizes proteases that are overexpressed in cancers which can be exploited to enable specific binding of recombinant viral vectors pseudotyped with protease-activated antibodies.

[00232] In certain embodiments, the protease-activated antibody is incorporated into the viral glycoprotein envelope. Protease-activated antibodies can be incorporated into the glycoprotein envelope of a recombinant viral vector of the invention (e.g., an HSV vector) to increase the therapeutic index and reduce off-target infection. In the case of an HSV vector, in some embodiments, the glycoprotein may be gC or gD. In some embodiments, the recombinant oncolytic viruses described herein comprise at least one polynucleotide encoding a protease-activated antibody. In certain embodiments, a protease-activated antibody is activated by a protease selected from a cysteine cathepsin, an aspartic cathepsin, a kallikrein (hK), a serine protease, a caspase, a matrix metalloproteinase (MMP), and a disintegrin and metalloproteinase (ADAM). In some embodiments, a protease is selected from cathepsin K, cathepsin B, cathepsin L, cathepsin E, cathepsin D, hK1, PSA (hK3), hK10, hK15, uPA, uPAR, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-18, MMP-19, MMP-20, MMP-21, MMP-23A, MMP-23B, MMP-24, MMP-25, MMP-26, MMP-27, MMP-28, or a protease listed in Table 6.

[00233] In some embodiments, the protease-activated antibody binds a protein expressed more highly by cancer cells or in cancer microenvironments than by non-cancer cells or in non-cancer microenvironments. In certain aspects, a protease-activated antibody binds NKG2D,

c-met, HGFR, CD8, heparan sulfate, VSPG4 (also known as NG2), EGFR, EGFRvIII, CD133, CXCR4, carcinoembryonic antigen (CEA), CLC-3, annexin II, human transferrin receptor, or EpCAM. In certain instances, multiple protease activated antibodies may be incorporated into a single viral vector particle to ensure that diverse tumor histotypes are targeted. For example, at least 1, 2, 3, 4, 6, 7, 8, 9, 10, or more protease activated antibodies may be incorporated into the viral glycoprotein envelope. In some embodiments, the recombinant oncolytic virus comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more polynucleotides that encodes for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more protease activated antibodies. In some embodiments, an oncolytic virus comprises a first protease-activated antibody that binds a first protein expressed more highly by cancer cells or in cancer microenvironments than by non-cancer cells or in non-cancer microenvironments, and a second protease-activated antibody that binds a second protein expressed more highly by cancer cells or in cancer microenvironments than by non-cancer cells or in non-cancer microenvironments. In further embodiments, an oncolytic virus comprises a plurality of protease-activated antibodies binding a plurality of protein expressed more highly by cancer cells or in cancer microenvironments than by non-cancer cells or in non-cancer microenvironments. An oncolytic virus comprises, for example, a protease-activated antibody that is a human antibody, a humanized antibody or a chimeric antibody. In some embodiments, an oncolytic virus comprises an antibody that is a full-length immunoglobulin, an scFv, a Fab, a Fab', an F(ab')2, an Fv, a diabody, a triabody, a minibody, a single-domain antibody, or a multispecific antibody.

[00234] In some embodiments, a recombinant oncolytic virus comprises one or more of: one or more micro-RNA (miR) target sequences inserted into a locus of one or more viral genes required for viral replication; one or more polynucleotides encoding one or more proteins or oligonucleotides, wherein the proteins or oligonucleotides reduce the expression or inhibit the function of a miR, a gene, or a TIMP; at least one protease-activated antibody; and/or a polynucleotide encoding at least one protease activated antibody. In some embodiments, a recombinant oncolytic virus comprises: a plurality of copies of one or more miRNA target sequences inserted into a locus of a viral gene required for viral replication in non-cancerous cells; and/or a first polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and/or a second polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or

a TIMP. In some embodiments, a recombinant oncolytic virus comprises: a plurality of copies of one or more miRNA target sequences inserted into a locus of a viral gene required for viral replication in non-cancerous cells; and/or a polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and/or at least one protease-activated antibody. In further embodiments, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences inserted into a locus of a viral gene required for viral replication in non-cancerous cells; and/or a polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or a TIMP; and/or at least one protease-activated antibody. In one embodiment, a recombinant oncolytic virus comprises a plurality of copies of one or more miRNA target sequences inserted into a locus of a viral gene required for viral replication in non-cancerous cells; and/or a first polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and/or a second polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or a TIMP; and/or at least one protease-activated antibody. In some specific embodiments, an oncolytic virus described in this paragraph is a herpes simplex virus and the viral gene required for viral replication in non-cancerous cells is UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12.

[00235] In certain aspects, the invention relates to a recombinant oncolytic virus comprising a first polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and a second polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or a TIMP. In other embodiments, a recombinant oncolytic virus comprises a polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and at least one protease-activated antibody. In some embodiments, a recombinant oncolytic virus comprises a polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or a

TIMP; and at least one protease-activated antibody. In one embodiment, a recombinant oncolytic virus comprises a first polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of an oncogenic miRNA or an oncogenic gene; and/or a second polynucleotide encoding a protein or an oligonucleotide targeted to reduce expression of a microenvironment remodeling miRNA or a TIMP; and/or at least one protease-activated antibody.

[00236] In some embodiments, the oncolytic virus is an HSV virus comprising a first miR-TS cassette inserted into the 3' UTR of ICP4 comprising 4 target sequences for each of miR-124-3p, miR-1-3p, and miR-143-3p; a second miR-TS cassette inserted into the 3' UTR of ICP27 comprising 4 target sequences for each of miR-219a-5p, miR-122-5p, and miR128-3p; and a third miR-TS cassette inserted into the 3' UTR of UL8 comprising 4 target sequences for each of miR-137-3p, miR-208b-3p, and miR-126-3p. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and XCL1. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and CCL4. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and FLT3L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and 41BBL. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding 41BBL and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-CTLA4 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-PD1 or anti-PDL1 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus comprises a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 843,

844, 847, or 848. In some embodiments, the the oncolytic virus comprises or consists of the nucleic acid sequence of one of SEQ ID NOs: 843, 844, 847, or 848.

[00237] In some embodiments, the oncolytic virus is an HSV virus comprising a first miR-TS cassette inserted into the 3' UTR of ICP4 comprising 4 target sequences for each of miR-124-3p, miR-1-3p, and miR-143-3p; a second miR-TS cassette inserted into the 3' UTR of ICP27 comprising 4 target sequences for each of miR-219a-5p, miR-122-5p, and miR128-3p; a third miR-TS cassette inserted into the 3' UTR of UL8 comprising 4 target sequences for each of miR-137-3p, miR-208b-3p, and miR-126-3p. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and XCL1. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and CCL4. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and FLT3L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and 41BBL. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding 41BBL and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-CTLA4 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-PD1 or anti-PDL1 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus comprises a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 845 or 846. In some embodiments, the the oncolytic virus comprises or consists of the nucleic acid sequence of one of SEQ ID NOs: 845 or 846.

[00238] In some embodiments, the oncolytic virus is an HSV virus comprising a first miR-TS cassette inserted into the 3' UTR of ICP4 comprising 4 target sequences for each of miR-124-3p, miR-1-3p, and miR-143-3p; a second miR-TS cassette inserted into the 3' UTR of ICP27 comprising 4 target sequences for each of miR-219a-5p, miR-122-5p, and miR128-3p; a third miR-TS cassette inserted into the 3' UTR of UL8 comprising 4 target sequences for each of miR-137-3p, miR-208b-3p, and miR-126-3p; and a fourth miR-TS cassette inserted into the 3' UTR of ICP34.5 comprising 4 target sequences for each of miR-128-3p, miR-204-5p, and miR-219a-5p. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and XCL1. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and CCL4. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and FLT3L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and 41BBL. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding 41BBL and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-CTLA4 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-PD1 or anti-PDL1 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus comprises a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 850. In some embodiments, the the oncolytic virus comprises or consists of the nucleic acid sequence of SEQ ID NO: 850.

[00239] In some embodiments, the oncolytic virus is an HSV virus comprising a first miR-TS cassette inserted into the 3' UTR of ICP4 comprising 4 target sequences for each of miR-124-3p, miR-1-3p, and miR-143-3p; a second miR-TS cassette inserted into the 3' UTR of ICP27 comprising 4 target sequences for each of miR-219a-5p, miR-122-5p, and miR128-3p; a third miR-TS cassette inserted into the 3' UTR of UL8 comprising 4 target sequences for each of miR-137-3p, miR-217-5p, and miR-126-3p; and a fourth miR-TS cassette inserted into the 3' UTR of ICP34.5 comprising 4 target sequences for each of miR-128-3p, miR-204-5p, and miR-219a-5p. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and XCL1. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and CCL4. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and FLT3L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and 41BBL. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding 41BBL and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-CTLA4 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-PD1 or anti-PDL1 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus comprises a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 849. In some embodiments, the the oncolytic virus comprises or consists of the nucleic acid sequence of SEQ ID NO: 849.

[00240] In some embodiments, the oncolytic virus is an HSV virus comprising a first miR-TS cassette inserted into the 3' UTR of ICP4 comprising 4 target sequences for each of miR-124-3p, miR-1-3p, and miR-143-3p; a second miR-TS cassette inserted into the 3' UTR of ICP27 comprising 4 target sequences for each of miR-219a-5p, miR-122-5p, and miR128-3p; a third miR-TS cassette inserted into the 3' UTR of UL8 comprising 4 target sequences for each of miR-137-3p, miR-217-5p, and miR-126-3p; and a fourth miR-TS cassette inserted into the 3' UTR of ICP34.5 comprising 4 target sequences for each of miR-128-3p, miR-204-5p, and miR-219a-5p. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CXCL10. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding CXCL10 and MMP9. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and XCL1. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and CCL4. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12, CXCL10, and FLT3L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and 41BBL. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding IL-12 and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding 41BBL and CD40L. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-CTLA4 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus further comprises a polynucleotide sequence encoding an anti-PD1 or anti-PDL1 antibody or antigen binding fragment thereof. In some embodiments, the oncolytic virus comprises a nucleic acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 851. In some embodiments, the the oncolytic virus comprises or consists of the nucleic acid sequence of SEQ ID NO: 851.

[00241] The invention also encompasses a nucleic acid molecule encoding an oncolytic virus described herein.

Compositions and Methods of Use

[00242] Certain aspects of the invention relate to stocks and compositions comprising the oncolytic viruses described herein. In some aspects, the invention relates to a viral stock comprising an oncolytic virus described herein. In some embodiments, a viral stock is a homogeneous stock. The preparation and analysis of viral stocks is well known in the art. For example, a viral stock can be manufactured in roller bottles containing cells transduced with the viral vector. The viral stock can then be purified on a continuous nycodenze gradient, and aliquotted and stored until needed. Viral stocks vary considerably in titer, depending largely on viral genotype and the protocol and cell lines used to prepare them.

[00243] In particular embodiments, the titer of a viral stock (e.g., an HSV-based vector viral stock) contemplated herein is at least about 10^5 plaque-forming units (pfu), such as at least about 10^6 pfu or even more preferably at least about 10^7 pfu. In certain embodiments, the titer can be at least about 10^8 pfu, or at least about 10^9 pfu, and high titer stocks of at least about 10^{10} pfu or at least about 10^{11} pfu are most preferred.

[00244] The invention further contemplates a composition comprising an oncolytic virus or a nucleic acid molecule described herein and a pharmaceutically acceptable carrier. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a subject (e.g., a human). The term "composition" as used herein refers to a formulation of one or more oncolytic virus or a nucleic acid molecules described herein that is capable of being administered or delivered to a subject and/or a cell. Typically, formulations include all physiologically acceptable compositions including derivatives and/or prodrugs, solvates, stereoisomers, racemates, or tautomers thereof with any physiologically acceptable carriers, diluents, and/or excipients. A "therapeutic composition" or "pharmaceutical composition" (used interchangeably herein) is a composition of one or more agents capable of being administered or delivered to a patient and/or subject and/or cell for the treatment of a particular disease or disorder.

[00245] The compositions disclosed herein may be formulated in a neutral or salt form. "Pharmaceutically acceptable salt" includes both acid and base addition salts. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, *p*toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine. Upon formulation, solutions will be administered in a manner compatible with the dosage

formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

[00246] As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[00247] As used herein "pharmaceutically acceptable carrier" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible, including pharmaceutically acceptable cell culture media and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen- free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations. Except insofar as any conventional media and/or agent is incompatible with the agents of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[00248] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[00249] Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00250] In one embodiment, a composition comprising a carrier is suitable for parenteral administration, *e.g.,* intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with a viral vector or nucleic acid molecule, use thereof in the pharmaceutical compositions of the invention is contemplated.

[00251] The compositions of the invention may comprise one or more polypeptides, polynucleotides, vectors comprising same, infected cells, etc., as described herein, formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the invention may be administered in combination with other agents as well, such as, e.g., cytokines, growth factors, hormones, small molecules or various pharmaceutically-active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy.

[00252] In the pharmaceutical compositions of the invention, formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular

compositions described herein in a variety of treatment regimens. Upon formulation, solutions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective to result in an improvement or remediation of the symptoms. The formulations are easily administered in a variety of dosage forms such as ingestible solutions, drug release capsules and the like. Some variation in dosage can occur depending on the condition of the subject being treated. The person responsible for administration can, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations meet sterility, general safety and purity standards as required by FDA Center for Biologics Evaluation and Research standards. The route of administration will vary, naturally, with the location and nature of the disease being treated, and may include, for example intradermal, transdermal, subdermal, parenteral, nasal, intravenous, intramuscular, intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intratumoral, perfusion, lavage, direct injection, and oral administration.

[00253] In certain circumstances it will be desirable to deliver the compositions, recombinant viral vectors, and nucleic acid molecules disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described, for example, in U.S. Patent No. 5,543,158; U.S. Patent No. 5,641,515 and U.S. Patent No. 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00254] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Pat. No. 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable

mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabenes, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

[00255] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (*see*, *e.g.*, Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins, 2000). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

[00256] Sterile injectable solutions can be prepared by incorporating the active compounds in the required amount in the appropriate solvent with the various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated

above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00257] In certain embodiments, the compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering polynucleotides and peptide compositions directly to the lungs via nasal aerosol sprays has been described *e.g.*, in U.S. Pat. No. 5,756,353 and U.S. Pat. No. 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U.S. Pat. No. 5,780,045 (specifically incorporated herein by reference in its entirety).

[00258] In certain embodiments, the delivery may occur by use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, optionally mixing with CPP polypeptides, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, a nanoparticle or the like. The formulation and use of such delivery vehicles can be carried out using known and conventional techniques. The formulations and compositions of the invention may comprise one or more polypeptides, polynucleotides, and small molecules, as described herein, formulated in pharmaceutically-acceptable or physiologically-acceptable solutions (*e.g.*, culture medium) for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the invention may be administered in combination with other agents as well, such as, *e.g.*, cells, other proteins or polypeptides or various pharmaceutically-active agents.

[00259] In a particular embodiment, a formulation or composition according to the present invention comprises a cell contacted with a combination of any number of polynucleotides or viral vectors, as contemplated herein.

[00260] In certain aspects, the present invention provides formulations or compositions suitable for the delivery of viral vector systems.

[00261] Exemplary formulations for *ex vivo* delivery may also include the use of various transfection agents known in the art, such as calcium phosphate, electroporation, heat shock and various liposome formulations (*i.e.*, lipid-mediated transfection). Liposomes are lipid bilayers entrapping a fraction of aqueous fluid. DNA spontaneously associates to the external surface of cationic liposomes (by virtue of its charge) and these liposomes will interact with the cell membrane.

[00262] Particular embodiments of the invention may comprise other formulations, such as those that are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins, 2000.

[00263] In certain aspects, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically effective amount of one or more viral vectors or polynucleotides, as described herein, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents (*e.g.*, pharmaceutically acceptable cell culture medium). As used herein, a "therapeutically effective amount" refers to the amount of a composition or recombinant virus described herein required to achieve a desired physiologic and/or biological outcome. A "therapeutically effective amount" of a virus, a viral stock, or a composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the stem and progenitor cells to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the virus or transduced therapeutic cells are outweighed by the therapeutically beneficial effects. The term "therapeutically effective amount" includes an amount that is effective to "treat" a subject (e.g., a patient). The therapeutically effective amount may be quantified by the total number of plaque forming units (pfu) (*e.g.* at least $1e^{1}$ to at least $1e^{20}$, particularly about $1e^{4}$ to about $1e^{15}$, more particularly about $1e^{6}$ to about $1e^{12}$ pfu), or number of viral genomes (*e.g.* at least $1e^{1}$ to at least $1e^{20}$, particularly about $1e^{4}$ to about $1e^{15}$, more particularly about $1e^{6}$ to about $1e^{12}$ viral genomes). One of skill in the art will understand that the therapeutically effective amount will vary based on the type of virus being administered, nature of the formulation, route of administration,

nature and/or severity of the disease to be treated, and/or general health and well-being of the subject.

[00264] Some aspects of the invention encompass a method of killing a cancerous cell, comprising exposing the cancerous cell to an oncolytic virus described herein or compositions thereof under conditions sufficient for the oncolytic virus to infect and replicate within said cancerous cell, and wherein replication of the oncolytic virus within the cancerous cell results in cell death. In certain embodiments, the cancerous cell has a reduced expression of a miR compared to a non-cancerous cell. In some embodiments, a cancerous cell killed by this method is *in vivo*. In certain embodiments, a cancerous cell killed by this method is within a tumor.

[00265] The invention relates to a method of treating cancer in a subject in need thereof, comprising administering a prophylactically effective amount or a therapeutically effective amount of an oncolytic virus, a viral stock, or a composition as described herein to the subject. A "subject," as used herein, includes any animal that exhibits a symptom of a disease, disorder, or condition that can be treated with the recombinant viral vectors, compositions, and methods disclosed herein. Suitable subjects (e.g., patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals (such as horse or cow), and domestic animals or pets (such as cat or dog). Non-human primates and, preferably, human patients, are included.

[00266] "Administration" refers herein to introducing an oncolytic virus, a viral stock, or a composition thereof into a subject or contacting an oncolytic virus, a viral stock, or a composition thereof with a cell and/or tissue. Administration can occur by injection, irrigation, inhalation, consumption, electro-osmosis, hemodialysis, iontophoresis, and other methods known in the art. The route of administration will vary, naturally, with the location and nature of the disease being treated, and may include, for example auricular, buccal, conjunctival, cutaneous, dental, endocervical, endosinusial, endotracheal, enteral, epidural, interstitial, intra-articular, intra-arterial, intra-abdominal, intraauricular, intrabiliary, intrabronchial, intrabursal, intracavernous, intracerebral, intracisternal, intracorneal, intracronal, intracoronary, intracranial, intradermal, intradiscal, intraductal, intraduodenal, intraduodenal, intradural, intraepicardial, intraepidermal, intraesophageal, intragastric, intragingival, intrahepatic, intraileal, intralesional, intralingual, intraluminal, intralymphatic, intramammary, intramedulleray, intrameningeal, instramuscular, intranasal, intranodal, intraocular, intraomentum, intraovarian, intraperitoneal, intrapericardial,

intrapleural, intraprostatic, intrapulmonary, intraruminal, intrasinal, intraspinal, intrasynovial, intratendinous, intratesticular, intratracheal, intrathecal, intrathoracic, intratubular, intratumoral, intratympanic, intrauterine, intraperitoneal, intravascular, intraventricular, intravesical, intravestibular, intravenous, intravitreal, larangeal, nasal, nasogastric, oral, ophthalmic, oropharyngeal, parenteral, percutaneous, periarticular, peridural, perineural, periodontal, respiratory, retrotubular, rectal, spinal, subarachnoid, subconjunctival, subcutaneous, subdermal, subgingival, sublingual, submucosal, subretinal, topical, transdermal, transendocardial, transmucosal, transplacental, trantracheal, transtympanic, ureteral, urethral, and/or vaginal perfusion, lavage, direct injection, and oral administration.

[00267] The term "treating" and "treatment" as used herein refers to administering to a subject a therapeutically effective amount of a recombinant virus or composition thereof as described herein so that the subject has an improvement in a disease or condition, or a symptom of the disease or condition. The improvement is any improvement or remediation of the disease or condition, or symptom of the disease or condition. The improvement is an observable or measurable improvement, or may be an improvement in the general feeling of well-being of the subject. Thus, one of skill in the art realizes that a treatment may improve the disease condition, but may not be a complete cure for the disease. A “prophylactically effective amount” refers to an amount of a virus, a viral stock, or a composition effective to achieve the desired prophylactic result. As used herein, "prophylaxis" can mean complete prevention of the symptoms of a disease, a delay in onset of the symptoms of a disease, or a lessening in the severity of subsequently developed disease symptoms. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount is less than the therapeutically effective amount.

[00268] "Cancer" herein refers to or describes the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma, osteogenic sarcoma, angiosarcoma, endotheliosarcoma, leiomyosarcoma, chordoma, lymphangiosarcoma, lymphangioendotheliosarcoma, rhabdomyosarcoma, fibrosarcoma, myxosarcoma, chondrosarcoma), neuroendocrine tumors, mesothelioma, synovioma, schwannoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies.

More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, small cell lung carcinoma, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulvar cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, Ewing's tumor, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, myelodysplastic disease, heavy chain disease, neuroendocrine tumors, Schwannoma, and other carcinomas, as well as head and neck cancer.

[00269] In certain embodiments, an oncolytic virus (e.g., an HSV), a viral stock, or a composition as described herein are used to treat a cancer selected from lung cancer (e.g., small cell lung cancer or non-small cell lung cancer), breast cancer, ovarian cancer, cervical cancer, prostate cancer, testicular cancer, colorectal cancer, colon cancer, pancreatic cancer, liver cancer (e.g., hepatocellular carcinoma (HCC)), gastric cancer, head and neck cancer, thyroid cancer, malignant glioma, glioblastoma, melanoma, B-cell chronic lymphocytic leukemia, diffuse large B-cell lymphoma (DLBCL), and marginal zone lymphoma (MZL).

[00270] In certain aspects, the invention relates to an oncolytic viral vector as shown in any one of the figures or embodiments disclosed herein.

EXAMPLES

[00271] The following examples for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein, are exemplary, and are not intended as limitations on the scope of the invention. Alterations, modifications, and other changes to the described embodiments which are encompassed within the spirit of the invention as defined by the scope of the claims are specifically contemplated.

EXAMPLE 1 – MIR SEQUENCE ANALYSIS OF NORMAL AND MALIGNANT CELLS

[00272] Differential miR expression is a hallmark of many cancers (Lu et al, Nature, 2005). Experiments were performed to determine the miRs that were mostly highly differentially expressed in eight different cancer cells lines. Differential expression was determined by comparisons to non-cancerous control tissues. In total, 108 samples were sequenced. Sample details are provided in Table 11.

Table 11:

Cancer Type	**# of Cancer Cell Lines**	**# of Control Tissue Samples**
Bladder	8	4
Colon	8	3
Breast	12	4
Pancreatic	7	3
Lung	8	5
Head and Neck	6	6
Schwannoma	7	4*
Glioblastoma	14	4*
Additional Controls		
Normal Liver	3	
Normal Bone Marrow	3	

*Same control samples used for both Schwannoma and glioblastoma analysis

[00273] To facilitate the identification of appropriate miRNA target sequences suitable for HSV attenuation in select cell types, miRNA sequence profiling of cancer lines and non-cancer control tissue was performed. Sequencing libraries of dicer-processed RNAs were generated for cancer and non-cancer cells, including bladder, colon, breast, pancreas, lung, head

and neck, schwannoma, glioblastoma, brain, liver, and bone marrow. These miRNA sequencing libraries were normalized to total RNA, and sequenced using a HiSeq 2500 ultra-high throughput sequencing system with HiSeq V4 chemistry reagents for sequencing reads up to $3e^8$ reads/run (Illumina). FASTQ files from sequencing runs were analyzed using the miRNAs Analysis tool in Basespace (Illumina). Rankings were made by calculating the mean of normal, the mean of cancer and sorting the ratio of normal/cancer from high to low. Heat maps were generated with natural logarithmic values with zero and negative values converted to zero (scale: black is high, white is low expression). Normalized data across samples were expressed as normalized miRNA read counts in a given sample. Normalization is related to total number of reads in a given sample relative to other samples in the comparison.

[00274] Fig. 1 exemplifies the miRNA expression profile heat map in non-cancerous and cancerous brain tissue of twenty-five miRNAs. Additional examples of miRNA expression profile heat maps are shown for non-cancerous and cancerous bladder (Fig. 2), breast (Fig. 3), colon (Fig. 4), brain (Fig. 5), head and neck (Fig. 6), lung (Fig. 7), pancreas (Fig. 8), and schwannoma (Fig. 9) tissue corresponding to twenty-five miRNAs in each example. Table 12 shows a summary of the expression levels of particular miRs between cancerous and non-cancerous tissue. As shown, miR-451a levels are down regulated in all tumor types compared to non-cancerous tissue, representing a potential pan-tumor suppressor miRNA. miR-1-3p is down-regulated in all tumor types tested, present at moderate levels in non-cancerous tissue, and present at high levels in head and neck tissue. miR-559 is down-regulated in all tumor types tested, present generally at low levels in non-cancerous tissue, and present at high levels in non-cancerous lung tissue. miR-145-5p is down-regulated in all tumor types tested and present generally at high levels in the majority of non-cancerous tissue types tested. miR-143-3p is down-regulated in colon, lung, and pancreatic tumors, and is present at high levels in all normal tissue types and some breast tumor lines. miRNA data analysis revealed at least eleven miRNAs that represent novel and unexpected miRNA expression profiles not previously identified in the literature.

Table 12:

	miR-451a		**miR-1-3p**		**miR-559**		**miR-145-5p**		**miR-143-3p**	
Tissue	**Norm**	**Can**	**Norm**	**Can**	**Norm**	**Can**	**Norm**	**Can**	**Norm**	**Can**
Bladder	1402.9	21.1	175	0.8	14	0.4	971.5	39.8	489028.8	14904.6

Breast	2262.1	3.9	3	0.3	88.4	0.9	406.1	106.6	125943.9	**91543.1**
Colon	3606.9	22	149.3	1.6	40.8	2.1	1177.3	0.5	809955.6	193.9
Glioma	4269.7	16.7	75.2	1	162.7	0.4	2399.2	7.2	514114.8	1248.6
Head & Neck	11919.8	10.3	2846.6	2.6	71.2	2.2	690.9	30.4	331034.2	20706.2
Lung	31442	10.5	73.3	1.4	548.3	0.1	1547.5	1	436136.8	390.9
Pancreatic	1035.8	13.3	4.1	0.4	13.3	0.5	81.7	0.5	25557	269.8

[00275] Many of these identified miRNAs are pan- or multi-tumor specific. For example, expression of miR-451a, miR-559, miR-1-3p, miR-145-3p, and miR143-3p were generally down-regulated across all cancer cell lines tested compared to control tissues. This was particularly notable for miR-451a, which was highly expressed in all normal tissue type and substantially down-regulated in all cancer types, thus representing a pan-specific tumor-suppressive miRNA. The expression of miR-559 was lower in normal tissue types, except for normal lung tissue, and expression of miR-1-3p and mir-145-3p in normal tissue was variable. Despite the variability in the magnitude of differences and absolute expression levels, mean expression of each miR in cancer cells lines was substantially lower compared to levels in the corresponding normal tissues. These miRNAs are candidates for generating pan-tumor HSV virions that are capable of broadly treating a variety of cancer types. Although the mean expression for miR-451a, miR-559, miR-1-3p, miR-145-3p, and miR143-3p was lower in cancer cell lines compared to normal controls, the decreased expression was not fully penetrant across all cancer cell lines. For example, 2/3 of the normal bladder samples tested showed increased expression of miR-145-3p, while expression in the remaining sample was substantially similar to the average observed in the cancer cell lines. Similar results were observed in breast cancer cell lines. Although the average read count for all breast cancer samples was 106, 5/12 samples had a normalized read count of > 1000 counts, 2 of which were > 40,000 counts.

[00276] These data indicate the potential to generate a single miR-attenuated oncolytic virus capable of targeting a broad array of tumor types. For example, a construct comprising target sequences for miR-124, miR-451a, miR-559, miR-1, and miR-145-3p may be used in the treatment of all the tumor types tested (*e.g.*, bladder, colon, breast, pancreatic, lung, head and neck, Schwannoma, and glioblastoma). The variability in expression levels of miRs in different cancer types indicates the potential need to stratify patients by miR expression or through the use of an additional biomarker.

[00277] Additional miRNA profiling between cancerous and non-cancerous tissues was performed using a quantitative expression assay from Nanostring. The results of these experiments are shown for brain samples (Fig. 50), and demonstrate the identification of additional miRNAs that exhibit differential expression in cancerous and non-cancerous brain tissue that were not previously identified by the earlier studies outlined above, namely miR-9-5p, miR-128-3p, miR-137, miR-129-2-3p, and miR-487b-3p. Additional results are shown for heart samples (Fig. 51), demonstrating differential expression of miR-208b-3p, miR-1-3p, miR-208a-3p, miR133-3p, miR-4284, and miR-499a-5p between cancerous and non-cancerous heart tissue. Additional results are shown for peripheral nervous system samples (Fig. 52A-B), demonstrating differential expression of miR-204-5p, miR-1-3p, miR-206, miR-9-5p, miR-199b-5p, miR-145-5p, miR-100-5p, miR-574-3p. Specifically, miR-219a-2-3p, miR-9-5, miR-219a-5p, and miR-204-5p are differentially expressed in the spinal cord.

EXAMPLE 2 – IDENTIFICATION OF VIRAL GENES FOR MIR-T ATTENUATION

[00278] siRNA screens were performed to test the attenuation phenotype HSV genes. An siRNA screen is the ideal modality to test RISC attenuation phenotype of immediate early genes, and select early genes. siRNAs targeting the HSV genes ICP27, ICP4, ICP0, UL5, UL8, UL9, ICP8, ULC39/40, ICP22, UL30, UL42, and VP19 were transfected individually and in pools into A253 cells. 24 hours after siRNA transfection, cells were infected with ONCR-003 or ONCR-010 (described below in Table 13), each of which comprise a GFP cassette. Viral spread was measured 48 hours post-infection by quantifying GFP intensity. HSV genes identified as potential hits were validated by Western blots, viral titer measurements, and RT-PCR.

[00279] The results of these screen are shown in Fig. 47A and 47B. Individual siRNAs mediating >75% knockdown of the corresponding HSV gene are indicated by arrows in Fig. 47A and GFP intensity for a select subset of the siRNAs are shown in Fig. 47B. As shown in Fig. 48, siRNA-mediated knockdown of ICP4, UL5, UL8, ICP8, ICP22, and UL30 substantially reduced viral replication of ONCR-003 as indicated by a reduction in GFP intensity. Further, siRNA-mediated knockdown of ICP27, ICP4, UL5, UL8, UL9, ICP8, ICP22, and ICP30 substantially reduced viral replication of ONCR-010 as indicated by a reduction in GFP intensity. Cells infected with ONCR-010 were further analyzed by Western blot for expression of particular

viral proteins. As shown in Fig. 49, significant reduction in these HSV genes was also observed at the protein level.

EXAMPLE 3 – CONSTRUCTION AND USE OF A REPORTER SYSTEM TO RAPIDLY ASSAY MIRNA-BASED GENE ATTENUATION

[00280] A reporter system was developed to assess miRNA-based gene attenuation using virtually any miRNA target sequence and cognate miRNA. In this system (shown in Fig. 10), the target sequence recognized by miRNAs (i.e. hsa-miR-122) was inserted into the 3' UTR of de-stabilized green fluorescent protein (dsGFP). The cognate miRNA was then expressed via a tetracycline (tet) inducible promoter using mCherry as a control for miRNA expression. All expression vectors were cloned into a tet repressible vector pcDNA5 Frt/To that also expresses mCherry (pTF002). All miRNAs for expression generated by gene synthesis from human genomic DNA and were cloned into pTF002. To generate attenuation reporter vectors, dsGFP was cloned into cDNA3.1+, generating vector pTF004. Attenuation vectors contain four tandem repeats of the reverse complement of the miRNA sequence of interest separated by 8-16 nucleotides. Plasmids were constructed by insertion of synthetically generated oligonucleotides into the 3 'UTR of the dsGFP gene of pTF004 using standard molecular biology techniques. On day one, HEK293TetR cells were transfected with the miRNA attenuation and reporter expression plasmids (0.15 μg each, for a total of 0.3 μg of CMV promotor-containing plasmid) using Lipofectamine 2000 per manufacturers protocol (Invitrogen). On day two, cells were treated with Tetracycline at 5 ng/ml and allowed to incubate for up to 72 hours. After incubation, GFP and mCherry fluorescence signals were detected daily using a SpectraMax® i3x Minimax multi-mode microplate reader (Molecular Devices) and analyzed using Softmax Pro or Metamorph imaging software (Molecular Devices). Phase images were acquired with an exposure of 5-6 ms. Fluorescence images were acquired with a GFP (541nm channel) exposure of 10 ms, and an mCherry (713nm channel) of 200-1500ms.

[00281] Fig. 11 exemplifies miR-122 mediated attenuation of GFP expression upon induction of miR-122 expression via tet at 24 hours. The control miRNA mimics, miR-184, miR-34a, and Let7a, do not attenuate GFP levels, nor is GFP attenuation observed in the absence of tet. Fig. 12 shows the effect of miR-122, miR-184, miR-34a, and Let7a mimics on attenuation of GFP

cassettes with miR-TS cassettes comprising each miR target sequence individually and in cassette combinations of miR-122/Let7a, miR-122/Let7a/miR-34a, or miR-122/Let7a/miR-184. Decreased GFP is only observed when the appropriate miR and cognate target sequence are present together (circled wells). Fig. 13 serves as a non-attenuated control and shows mCherry expression as a measure of the expression of the miR-122, miR-184, miR-34a, and Let7a mimics. Fig. 14 shows the effects of miR-122, miR-124, miR-145, miR-199, and miR-451 mimic expression on attenuated-GFP cassettes with miR-TS cassettes comprising each miR target sequence individually (circled wells). Non-attenuated controls are shown in Fig. 15 and show miR-122, miR-124, miR-145, miR-199, and miR-451 expression and mCherry expression using each target sequence individually.

[00282] The ability of additional combinations of miR target sequence to attenuate GFP expression in the presence of cognate miR mimics are shown in Fig. 16 – Fig. 26. In each figure, GFP fluorescence is measured at 72 hours post-transfection. In each instance, insertion of the indicated miR target sequences resulted in attenuated GFP expression when the cognate miRs were also expression. The effects of insertion of miR-122 and miR-184 target sequences (Fig. 16), miR-34a and miR-184 target sequences (Fig. 17), Let-7a and miR-184 target sequences (Fig. 18), miR-124 and miR-184 target sequences (Fig. 19), miR-145 and miR-184 target sequences (Fig. 20), miR-199 and miR-451 target sequences (Fig. 21), miR-125 and miR-451 target sequences (Fig. 22), miR-126 and miR-451 target sequences (Fig. 23), miR-127 and miR-451 target sequences (Fig. 24), miR-133 and miR-451 target sequences (Fig. 25), and miR-223 and miR-451 target sequences (Fig. 26) are each shown.

[00283] As such, these data indicate that miR expression can result in the specific attenuation of genes expressing the cognate miR target sequence.

EXAMPLE 4 – GENERATION OF MIRNA-ATTENUATED HSV

[00284] Following reporter gene-based validation of miRNA target sequences and cognate miRNA pairs, HSV-based viruses comprising miR-TS cassettes were generated. A series of modifications were made in KOS-37 BAC, a full-length genomic clone of the KOS strain of HSV-1 on a bacterial artificial chromosome (BAC) as described (Mazzacurati et al., Mol Ther., 2015). The product, KG^{BAC}, was deleted for the internal repeat (joint) region containing one copy

each of the diploid genes ICP0, ICP34.5, LAT and ICP4 along with the promoter for the ICP47 gene. This deletion facilitates manipulation of the remaining copies of the 4 deleted genes, provides abundant space for the potential incorporation of transgenes that enhance the oncolytic activity of the virus, and increases tumor specificity by reducing expression of the neurovirulence factor ICP34.5; elimination of ICP47 expression benefits immune recognition of infected cancer cells by virus-specific T cells. KG^{BAC} also contains the GFP open reading frame (ORF) fused to the glycoprotein C (gC) ORF via a 2A peptide sequence to allow monitoring of late (post-replication) viral gene expression. Lastly, KG^{BAC} contains a pair of mutations in the gB gene shown to enhance HSV entry through non-canonical receptors (*See e.g.*, International PCT Publication No. WO 2011/130749). A miR-TS cassette comprising 4 repeats of a target sequence for miR-124-3p were recombined into the 3' UTR of ICP4 to generate the 2A5B vector *See e.g.*, International PCT Publication No. WO 2015/066042), and an expression cassette for MMP9 was inserted into the intergenic region between the UL3 and UL4 genes to generate the 2A5B-MMP9 vector (ONCR-003). Additional miRNA target sequence cassettes were recombined into the 3' UTR of the ICP4, ICP27, UL8, UL42, and/or ICP34.5 genes of ONCR-003 to generate the constructs shown in Table 13 below. All BAC constructs were converted to virus particles with simultaneous removal of the BAC sequences located between loxP sites by transfection of Vero-Cre cells. Following plaque purification, virus stocks were prepared and titered on Vero cells.

Table 13: Exemplary miRNA-attenuated HSV constructs

Construct	ICP27	UL8	ICP34.5	ICP4	UL42
ONCR-003	None	×	×	124-3p (4x)	×
ONCR-010	122-5p 34a-5p Let-7a-5p	×	×	124-3p (4x)	×
ONCR-011	125a-5p (1x)	×	×	124-3p (4x)	×
ONCR-012	143-3p (1x)	×	×	124-3p (4x)	×
ONCR-013	145-5p (1x)	×	×	124-3p (4x)	×
ONCR-014	199a-5p (1x)	×	×	124-3p (4x)	×
ONCR-015	1-3p (1x)	×	×	124-3p (4x)	×
ONCR-016	133a-3p (1x)	×	×	124-3p (4x)	×
ONCR-017	223-3p (1x)	×	×	124-3p (4x)	×
ONCR-018	451a# (1x)	×	×	124-3p (4x)	×
ONCR-019	126-3p (1x)	×	×	124-3p (4x)	×

ONCR-020	127a-3p (1x)	×	×	124-3p (4x)	×
ONCR-021	133b-3p (1x)	×	×	124-3p (4x)	×
ONCR-022	134-3p (1x)	×	×	124-3p (4x)	×
ONCR-030	199a-3p (1x)	×	×	124-3p (4x)	×
ONCR-035	214-3p (1x)	×	×	124-3p (4x)	×
ONCR-036	122-5p (1x)	×	×	124-3p (4x)	×
ONCR-039	122-5p (2x)	×	×	124-3p (4x)	×
ONCR-040	122-5p (3x)	×	×	124-3p (4x)	×
ONCR-043	137-3p	×	×	124-3p (4x)	×
ONCR-047	34a-5p	×	×	124-3p (4x)	×
ONCR-048	184-3p	×	×	124-3p (4x)	×
ONCR-053	Let7a-5p	×	×	124-3p (4x)	×
ONCR-054	122-5p 184-3p Let-7a-5p	×	×	124-3p (4x)	×
ONCR-055	145-3p	×	×	124-3p (4x)	×
ONCR-062	559-5p	×	×	124-3p (4x)	×
ONCR-063	122-5p	×	×	124-3p (4x)	×
ONCR-064	122-5p Let-7a-5p	×	×	124-3p (4x)	×
ONCR-081	×	×	×	124-3p (4x)	125a-5p (1x)
ONCR-082	×	×	×	124-3p (4x)	125a-5p (2x)
ONCR-083	×	×	×	124-3p (4x)	125a-5p (3x)
ONCR-084	×	×	×	124-3p (4x)	125a-5p (4x)
ONCR-092	122-5p (1x)	×	×	124-3p (4x)	125a-5p (4x)
ONCR-093	122-5p (4x)	×	×	124-3p (4x)	125a-5p (1x)
ONCR-094	122-5p (1x)	×	×	124-3p (4x)	125a-5p (1x)
ONCR-095	122-5p (1x)	×	×	124-3p (4x)	125a-5p (3x)
ONCR-096	122-5p (4x)	×	×	124-3p (4x)	125a-5p (4x)
ONCR-098 ONCR-099	1-3p 145-5p 199a-5p 559-5p	×	×	124-3p (4x)	×
ONCR-100	×	×	×	124-3p (4x)	122-5p (3x)
ONCR-103	122-5p (3x)	×	×	124-3p (4x)	125a-5p (1x)
ONCR-104	122-5p (3x)	×	×	124-3p (4x)	125a-5p (4x)
ONCR-129 ONCR-144	219a-5p (4x) 122-5p (4x) 128-3p (4x)	×	×	124-3p (4x), 1-3p (4x), 143-3p (4x)	×
ONCR-130	219a-5p (4x) 122-5p (4x) 128-3p (4x)	×	×	124-3p (4x)	×

ONCR-131 ONCR-136 ONCR-155	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 126-3p (4x)	×	124-3p (4x), 1-3p (4x), 143-3p (4x)	×
ONCR-132	×	137-3p (4x) 208b-3p (4x) 126-3p (4x)	×	124-3p (4x)	×

[#] miR-451a is non-canonically processed by Ago2 and does not have -3p and -5p arms

EXAMPLE 5 – VIRAL INFECTIVITY ASSAY USING MIRNA-ATTENUATED HSV

[00285] To assay for viral infectivity and replication in normal and cancerous cells, miRNA-attenuated HSV particles were tested in the following *in vitro* assay. On day one, for each cell type infected, HSV particles were introduced to achieve a multiplicity of infection (moi) of 0.01. On days two through five, viral infectivity was assayed by GFP detection using a SpectraMax® i3x Minimax multi-mode microplate reader (Molecular Devices) and analyzed using Softmax Pro or Metamorph imaging software (Molecular Devices). Phase images were acquired with an exposure of 5-6 ms. Fluorescence images were acquired with a GFP (541 nm channel) exposure of 10 ms and an mCherry (713 nm channel) exposure of 200-1500 ms to evaluated any potential nonspecific autofluorescence signal.

[00286] ONCR-011 replication was significantly attenuated in post-mitotic lung tissue due to the presence of the miR-T125 cassette in the ICP27 gene and high levels of miR-125a (>3000 counts, Table 14 below) in these cells, as shown in Fig. 27A (read out by GFP positive cell quantitation) and Fig. 27C (read out by quantitative PCR). Although ONCR-011 and the control virus, ONCR-003, contain miR-124 target sequences in the ICP4 gene, miR-124 is present at low levels (<100 counts, Table 14 below) which were insufficient to attenuate viral replication (Fig. 27B and Fig. 27D). Both ONCR-011 and ONCR-003 replicated freely in head and neck cancer cells (A253) because these cells contain low levels of both miR-125a and miR-124 (<100 counts) (Table 14).

Table 14: miR-125a and miR-124 counts in post-mitotic lung and A253 cells

Cell	miRNA 125a counts	miRNA 124 counts
PM-lung	> 3000	< 100
H&N CA	< 100	< 100

[00287] Fig. 28 shows replication of a miR-145 attenuated construct, ONCR-013, in HCC1395 and A253 cells. As shown in Fig. 28A (read out by GFP positive cell quantitation) and Fig. 28B (read out by quantitative PCR), replication of ONCR-013 was significantly attenuated in HCC1395 cells, but not in A253 cells due to the high expression of miR-145 in A253 and absence of expression in HCC1395 cells (Table 15).

Table 15: miR-145 counts in A253 and HCC1395 cells

Cell	miRNA 145 counts
A253	0
HCC1395	4487

[00288] Fig. 29 shows attenuation of a miR-143-3p attenuated construct (ONCR-012) and a miR-199a-5p attenuated construct (ONCR-014) in normal BEAS-2B lung cells. As shown, replication of ONCR-014 was significantly attenuated in non-cancerous lung tissue (Fig. 29A, read out by GFP positive cell quantitation and Fig. 29B, read out by quantitative PCR), indicating that miR-199a-5p target sequences can attenuate viral replication in normal lung cells.

EXAMPLE 6 – ATTENUATED REPLICATION OF OHSV COMPRISING MULTIPLE MIRNA TARGET SEQUENCES IN MULTIPLE GENE LOCI

[00289] Viral infectivity and replication of constructs comprising miR-TS cassettes in multiple genetic loci was assessed in A253, Hep3B, and Huh7 cells. Results for ONCR-036, ONCR-063, ONCR-093, ONCR-094, ONCR-095, and ONCR-096 miR-attenuated HSV constructs are provided herein. Each of these viruses comprised one or more miR-124 target sequences, one or more miR-122 target sequences, and/or one or more miR-125a target sequences inserted into the ICP4, ICP27 and/or UL42 loci. Expression of miR-122 and miR-125a in each of the cell lines was assessed by a TaqMan assay. Briefly, total RNA, including the small RNA fraction, were isolated from growing cells with miRNeasy columns. The RNA was then used as the substrate for miR-122 and miR-125 specific TaqMan assays, and a parallel TaqMan assay for the U6 snRNA was performed to normalize expression levels per cell type per the ΔΔCT method. The data are represented as a fold change relative to lowest cell line in question in each assay

(A253 cells, Fig. 30A; Hep3B, Fig. 30B). As shown, A253 cells do not express miR-122 or miR-125a. Hep3B cells express high levels of miR-125 and low levels of miR-122, and Huh7 cells express miR-125a and high levels of miR-122.

[00290] Viral infectivity and replication was assessed in an *in vitro* assay. Briefly, cells were plated at 45,000 cells/well in a 48-well dish and cultured overnight. On day one HSV particles were introduced into each cell type to achieve a multiplicity of infection (moi) of 0.01. 48 hours post-infection, viral infectivity was assessed by fluorescence microscopy. The results of this experiment are shown in Fig. 30C, and viral replication is indicated by eGFP levels. These data demonstrate enhanced attenuation of viral replication in cells that express intermediate to high levels of both miR-122 and miR-125a (Huh7 cells) compared to the attenuation observed in cells that express only one of the cognate miRs (Hep3B, expressing high levels of miR-125a) or compared to cells that express neither cognate miR (A253 cell, expressing neither miR-125a nor miR-122).

[00291] Viral spread and protein expression were also assessed. Briefly, cell lysates were harvested 72 hours post-infection and subjected to PAGE and Western blot analysis. An anti-HSV1 capsid (VP5) antibody was used to monitor viral spread/protein expression and B-actin antibody was used as a loading control. In A253 cells, where there is no miR-125a or miR-122 expression, a level of high viral replication was observed in all of the miR-attenuated viruses (Fig. 31, lanes 3 – 6) as compared to the non-attenuated control (Fig. 31, ONCR-003 as non-attenuated control). However, viral replication was reduced in Huh7 cells, which express both cognate miRNAs and especially high levels of miR-122. These data exemplify viral attenuation by specific miRs relative to control virus.

[00292] To further confirm that reduced viral replication of miR-attenuated HSV viruses was mediated by expression of specific miRs, A253 cells, which do not express endogenous miR-122 or miR-125a, were transfected with miR-122 and miR-125a mimics. Briefly, A253 cells were plated at 35,000 cells/well in a 48 well dish and cultured overnight. The cells were then transfected with Ambion miRNA mimics at 2.5 pM/well with Lipofectamine RNAiMAX. Total RNA, including the small RNA fraction, were isolated from growing cells with miRNeasy columns. These RNA samples wereused as the substrate for miR-122 and miR-125 specific TaqMan assays, and a parallel TaqMan assay for the U6 snRNA was performed to normalize

expression levels per cell type per the ΔΔCT method. The results are shown in Fig. 32. As shown in Fig. 32A and Fig. 32B, miR-122 and miR-125 mimics specifically increased intracellular miR-122 and miR-125 expression levels in A253 cells by orders of magnitude.

[00293] The subsequent day, cells were individually counted and infected with ONCR-036, ONCR-063, ONCR-093, ONCR-094, ONCR-095, or ONCR-096 miR-attenuated HSV particles and ONCR-003 non-attenuated controls at a MOI of 0.01; each of the oHSV viruses were added in 50 μL for 1 hr, followed by addition of complete media. eGFP expressed by productive viral infection was assessed by fluorescence microscopy images taken 48 hrs post-infection. All images were exposed and processed identically. The results of this experiment are shown in Fig. 32C and are quantified by GFP detection using a SpectraMax® i3x Minimax multi-mode microplate reader (Molecular Devices) and analyzed using Softmax Pro in Fig. 32D. These data exemplify attenuated viral replication in the presence of miR-122 mimics for all viruses with the exception of the ONCR-003 (WT). Further, the level of attenuation was proportional to the copy number of miR target sequences present in the HSV constructs, with the greatest reduction in GFP in ONCR-063, ONCR-093, ONCR-096, each comprising 4 miR-122 target sequence repeats in the ICP27 gene. Further, these data exemplify attenuated viral replication in the presence of miR-125a mimics for ONCR-093, -094, -095, and -096, relative to ONCR-003 (WT) and ONCR-036/ONCR-063 (each comprising only only miR-122 target sequences).

[00294] Similar experiments were performed to assess the viral replication of additional constructs comprising multiple miR-TS cassettes in two or more viral genes (*e.g.*, ONCR-129, ONCR-131, ONCR-125, ONCR-126, ONCR-128, ONCR-130). In each case, expression of one or more miRNAs was able to attenuate viral replication of a particular construct comprising a target sequence corresponding to the expressed miRNA (data not shown).

EXAMPLE 7 – COMPUTATIONAL METHOD FOR GENERATING MIR-TS CASSETTES

[00295] Based on the data described in the previous examples, miR-target sequence (miR-TS) cassettes were generated for insertion into particular HSV genes. miR target sequences exhibiting differential expression between cancerous and non-cancerous cells of different tissue types were selected to generate cassettes that are capable of attenuating viral replication in a broad

variety of healthy cells, while allowing viral replication in cancerous cells where expression of the cognate miRs is decreased.

[00296] This examples illustrates a method of generating candidate miR-TS cassettes and selected preferred candidates from the list. The method, implemented in the computer language Python, is depicted in FIG. 1. The inputs for the method include a list of seeds for exclusion—in this example, seed sequences for miRNAs that are expressed in cancers were excluded—and a list of miR-TSs to include in the cassette. The included miR-TSs used in this example are provided in Table 10, along with the parent miRNA sequence and length in nucleotides (nt) of each. miR-TSs tolerate imperfect matches between the microRNA and the miR-TS, so it will be understood that cassettes can be made with imperfect miR-TSs. miR-TS cassettes can be made with other miR-TSs, include miR-TSs based on any of the microRNAs known in the art or prospectively discovered.

[00297] For this example, we designed four miR-TS cassettes, one for each of four essential viral genes: ICP4, ICP27, ICP34.5, and UL8, as shown in Table 16. But in priniciple these cassettes could be used with other viral (or non-viral) genes. Because the viral genes are in the reverse complementary orientation in our favored vectors, in each case the reverse complementary sequence was used. The abbreviation "miR-126m" refers to a version of the miR-126 site mutagenized to improve the site by removing a seed match for the oncomiR miR-155. The abbreviation "miR-128m" refers to a version of the miR-128 site mutagenized to improve the site by removing a seed match for the oncomiR miR-27a-3p.

Table 16: Candidate miR-TS cassettes and target genes

Cassette	miR-T	HSV gene	Protected Tissue	Indication Specificity
1	miR-124-3p miR-1-3p miR-143-3p	ICP4	CNS/Brain/PNS, smooth muscle, striated muscle/heart	Lung, HnN
2	miR-128-3p miR-219a-5p miR-122-5p	ICP27	CNS/Brain/PNS/oligodendrocytes, liver	Lung, HnN
3	miR-137-3p miR-208b-3p miR-126-3p	UL8	CNS/Brain/PNS, heart, vasculature, hematopoietic stem cells	Lung, HnN, Bladder

4	miR-219a-5p miR-204-5p miR-128-3p	ICP34.5	Spine, PNS, CNS (Oligodendrocytes, Glial cells, Neurons)	All

[00298] The cassette designed for ICP4 can be used to down-regulate any gene, to which it is operatively linked, in smooth muscle (because of miR-143 target site) and striated muscale (because of miR-1 target site). The cassette designed for ICP27 can be used to down-regulate any gene, to which it is operatively linked, in healthy tissue because of miR-128m (expressed in cortical neurons), miR-122 (expressed in the liver), and miR-219 (expressed in the brain, spine and nerves) target sites. The cassette designed for ICP27 can be used to down-regulate any gene, to which it is operatively linked, in healthy tissue because of miR-128m, miR-204, and miR-219 target sites. The cassette designed for UL8 can be used to down-regulate any gene, to which it is operatively linked, in non-tumor tissue because of the mRNA target sites: miR-217, miR-137, and miR-126m.

[00299] The program was run, outputting 10,000-100,000 cassettes for each combination which match the criteria used for list example (each of which is optional): (1) four copies of each miR-TS sites (in reverse-complementary orientation) arranged in a random order; (2) except that the same miR-TS cannot repeat adjacent to itself; (3) seperated by 4 nucleotide spacers having random sequence; (4) no seeds from the excluded seed list; and (4) no polyadenylation sequence (AATAAA). The program also can, optionally, add 5' arm (CATGGACGAGCTGTACAAGTAAAGC) and 3' arm (GCGACCGGCTAGCGTACTAGCTTAG) sequences for Gibson assembly cloning (*Nat Methods* 2009;6(5):343-5).

[00300] The program next calculated a delta-G for folding of the candidate sequences using the "fold" subroutine of the ViennaRNA package (Lorenz et al. *Algorithms for Molecular Biology*, 6:1 26, 2011; available at https://www.tbi.univie.ac.at/RNA/) with a 40 nt sliding window. The values for this sliding window calculation were stored as a list for each candidate sequence, and the candidate sequences are sorted by the maximum value of the absolute values of all delta-G values in the list (*i.e.*, by the folding energy of the strongest secondary-structure element in the RNA). Sequences were further reviewed manually to eliminate candidate sequences with multiple, lower energy minima. Whenever possible, a candidate sequence with no local minima was chosen. In this manner, candidate sequences were identified in which there are

no strong RNA secondary structure (low max of abs of delta-G) and also no local minima, or few local minima, in predicted secondary-structure folding energy.

[00301] Finally, a microRNA target scanning algorithm (miranda v3.3a) was run on each candidate sequence to ensure that the desired miR-TSs were present and that no undesirable miR-TS were inserted by the program.

[00302] Example sequences generated by this method are provided in **Table 17**. The length of the cassette is defined as the number of nucleotides from the first miR-TS to the last miR-TS, inclusive of the first and last miR-TS. Because the program adds a 5' first spacer of 4 nt and a 3' last space not including the definition of length used herein, the length of the cassette is the length of the sequence output by the program minus 8 nt (= 2 * 4 nt first and last spacer).

Table 17:

Cassette	Sequence	SEQ ID	# of miR-TS	Length	Nucleotides/ miR-TS
miRT-1-143_1736	ccatatacatacttctttacattccatcctg agctacagtgcttcatctcattgcatacata cttctttacattccaacgtgagctacagtgc ttcatctcatccgatacatacttctttacat tccacggcgagctacagtgcttcatctcacc ttatacatacttctttacattccaaaaagag ctacagtgcttcatctcaccat	852	8	200	25
miRT-128m-122-219_6793	cacgagaattgcgtttggacaatcagacaca aacaccattgtcacactccatcttaaagaga ccggttcactgtggatgtcaaacaccattgt cacactccaacttagaattgcgtttggacaa tcaagggaaagagaccggttcactgtggcca gcaaacaccattgtcacactccaaaacaaag agaccggttcactgtggtacgagaattgcgt ttggacaatcagaaaaaagagaccggttcac tgtggaatacaaacaccattgtcacactcca acaaagaattgcgtttggacaatcaggtt	853	12	300	25
miRT-128m-204-219_9304	aagtaaagagaccggttcactgtggaataag aattgcgtttggacaatcaaggtaggcatag gatgacaaagggaacagcaaagagaccggtt cactgtggggctagaattgcgtttggacaat cacgtaaggcataggatgacaaagggaacga gaaagagaccggttcactgtgggggaagaat tgcgtttggacaatcatactaggcataggat gacaaagggaattagaaagagaccggttcac tgtggatttagaattgcgtttggacaatcat agaaggcataggatgacaaagggaattgt	854	12	300	25
miRT-217-137-126m_3163	tatgctacgcgtattcttaagcaataagact tccaatcagttcctgatgcagtacgaccaca ttattactcacggtacgaaagcctacgcgta ttcttaagcaataaccgccacattattactc	855	12	300	25

Cassette	Sequence	SEQ ID	# of miR-TS	Length	Nucleotides/ miR-TS
	acggtacgataaatccaatcagttcctgatg cagtaattactacgcgtattcttaagcaata actattccaatcagttcctgatgcagtaccc ccacattattactcacggtacgagaattcca atcagttcctgatgcagtacagtcacattat tactcacggtacgatcaactacgcgtattct taagcaataaccaa				

[00303] The ability of the miR-TS cassettes shown in Table 17 to attenuate viral replication is shown in Fig. 53A- Fig. 53B.

[00304] Additional constructs comprising miR-TS cassettes designed with this method are shown in Table 18 were constructed.

Table 18: HSV constructs comprising candidate miR-TS cassettes

Construct	ICP27	UL8	ICP34.5	ICP4	UL42
ONCR-142 ONCR-154	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 126-3p (4x)	128-3p^{M} (4x) 204-5p (4x)$^{\Psi}$ 219a-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-156	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 126-3p (4x)		124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-158	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 126-3p (4x)	128-3p^{M} (4x) 204-5p (4x) 219a-5p (4x)	124-3p (4x) 1-3p (4x) 143-3p (4x)	
ONCR-157	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 217-5p (4x) 126-3p (4x)	128-3p^{M} (4x) 204-5p (4x) 219a-5p (4x)	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-159 ONCR-165 ONCR-166 ONCR-167 ONCR-168 ONCR-169 ONCR-170 ONCR-171 ONCR-172 ONCR-173 ONCR-174 ONCR-175 ONCR-176 ONCR-177	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 217-5p (4x) 126-3p^{M} (4x)	128-3p^{M} (4x) 204-5p (4x) 219a-5p (4x)	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-160	219a-5p (4x) 122-5p (4x)	137-3p (4x) 208b-3p (4x)	128-3p^{M} 204-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x)	×

Construct	ICP27	UL8	ICP34.5	ICP4	UL42
	128-3p (4x)	126-3p (4x)	219a-5p (4x)$^{\Psi}$	143-3p (4x)	
ONCR-161	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 127 (4x)	128-3p^{M} (4x) 204-5p (4x)$^{\Psi}$ 219-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-162	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 128-3p (4x)	128-3p^{M} (4x) 204-5p (4x)$^{\Psi}$ 219-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-163	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 129 (4x)	128-3p^{M} (4x) 204-5p (4x)$^{\Psi}$ 219-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x) 143-3p (4x)	×
ONCR-164	219a-5p (4x) 122-5p (4x) 128-3p (4x)	137-3p (4x) 208b-3p (4x) 130 (4x)	128-3p^{M} (4x) 204-5p (4x)$^{\Psi}$ 219-5p (4x)$^{\Psi}$	124-3p (4x) 1-3p (4x) 143-3p (4x)	×

$^{\Psi}$ one of the 4 target sequences was non-functional due to cloning error
^{M} comprises a modified target sequence

EXAMPLE 8 – CYTOTOXICITY OF MIR-ATTENUATED HSV CONSTRUCTS

[00305] Experiments were performed to assess the in vitro cytotoxicity of select miR-attenuated HSV constructs, ONCR-125, ONCR-131, ONCR-142, and ONCR-157. Various cancer cell lines were infected with the indicated constructs at MOIs of 30, 10, 3.33, 1.11, 0.37, 0.12, 0.04, 0.13, 0.0045, and 0.0015 and cell viability was assessed at 72 hours post infection. Cell lines used include the colorectal adenocarcinoma cell lines SW837 and COLO205, the melanoma cell lines SKMEL28 and A375, small cell lung cancer cell line H446, pancreatic adenocarcinoma cell line BXPC3, and the breast cancer cell line BT 549. The IC50 of each construct was calculated and is shown below in Table 19. Results of each of these experiments are shown in Fig. 54A – Fig. 54E.

Table 19:

	ONCR-125	ONCR-131	ONCR-142	ONCR-157
SW837	0.07	0.04	0.05	0.07
COLO205	0.45	0.12	0.223	0.45
SKMEL28	0.12	0.04	0.12	0.25
A375	0.87	0.26	0.89	1.5
H446	0.13	0.05	0.15	0.15
BXPC3	0.08	0.002	0.08	0.07
BT549	0.45	0.17	0.6	1.0

EXAMPLE 9 – IL-12 ENAHNCES ABSCOPAL EFFICACY OF HSV

[00306] Experiments were performed to assess the effects of IL-12 on the absocpal effect of oncolytic HSV. Briefly, the ONCR-133 construct, comprising an ICP4 miR-TS cassette comprising 4 repeats of miR-124-3p and an expression cassette encoding murine IL-12 was administered to mice in an MC38 tumor model. As shown in Fig. 55, ONCR-133 significantly inhibited tumor growth of injected tumors compared to vehicle treated controls ($p < 0.0001$). ONCR-133 treatment also significantly inhibited tumor growth of non-injected tumors ($p < 0.005$), indicating an enhanced abscopal effect.

[00307] Surprisingly, expression of an additional immune-activating payload, ULBP3, did not further enhance the tumor growth inhibition effects of HSV expressing IL-12. As shown in Fig. 56, mice treated with ONCR-133 + ONCR-007 (an HSV construct expressing ULBP3) or ONCR-133 + ONCR-002 (an HSV construct that does not express any additional payload molecules) both demonstrated a significant inhibition of tumor growth compared to vehicle treated controls. However, there was no added benefit of ULBP3 expression in the inhibition of growth of injected or non-injected tumors. In fact, as shown in the table in Fig. 56, additional expression of ULBP3 slightly decreased the tumor growth inhibition observed with IL-12 expression alone.

[00308] Similarly, additional expression of CXCL10 did not further enhance and anti-tumor efficacy of HSV expressing IL-12. Mice treated with ONCR-113 (an HSV construct expressing IL-12 and MMP9) + ONCR-106 (an HSV construct expressing CXCL10 and MMP9) or ONCR-113 + ONCR-031 (an HSV construct expressing MMP9). As shown in Fig. 57, the additional expression of CXCL10 in the ONCR-106 + ONCR-113 treated group did not enhance the inhibition of tumor growth in either injected or non-injected tumors compared to mice treated with ONCR-031 + ONCR-113. In fact, as shown in the table in Fig. 57, additional expression of CXCL10 slightly decreased the tumor growth inhibition in both injected and non-injected tumors.

EXAMPLE 10 – CCL4 EXPRESSION FURTHER ENAHNCES ABSCOPAL EFFICACY OF HSV

[00309] Experiments were performed to assess the effects of CCL4 in tumor growth inhibition in the MC38 model described in Example F. Results of this experiment are shown in Fig. 58 and Table 20 below. Mice were treated with one of 4 constructs, ONCR-133 (expressing

IL-12), ONCR-151 (expressing IL-12, CXCL10, and XCL1), ONCR-152 (expressing IL-12, CXCL10, and FLT3 ligand), or ONCR-153 (expressing IL-12, CXCL10, and CCL4). As shown in Fig. 58, treatment with all of ONCR-133, -151, -152, and -153 significantly inhibited tumor growth of injected and non-injected tumors compared to vehicle controls. However, mice treated with ONCR-153 demonstrated an increase in tumor growth inhibition in the injected tumors and in the non-injected tumors compared to treatment with ONCR-133, whereas ONCR-151 or -152 demonstrated a slight decrease in efficacy compared to treatment with ONCR-133. These results demonstrate that CCL4 expression can increase the tumor growth inhibition and abscopal effect of oncolytic HSV above the effects observed with IL-12 expression alone.

Table 20:

Combo	Payloads	Injected			Non-injected		
		CR	% TGI	p	CR	% TGI	p
ONCR-133	IL-12	3/8	82	<0.0001	0/8	44	0.19
ONCR-151	IL-12, CXCL10, XCL1	3/8	76	<0.0001	0/8	33	0.35
ONCR-152	IL-12, CXCL10, FLT3L	1/8	81	<0.0001	0/8	34	0.3
ONCR-153	IL-12, CXCL10, CCL4	3/8	89	<0.0001	0/8	58	0.001

[00310] Tumors from mice treated with ONCR-153 were harvested and assessed for the presence of HSV by RT-PCR analysis of the gD gene. As shown in Fig. 59A and Fig 59B, HSV was detected in the injected tumors, but not in the non-injected tumors, indicating that the tumor growth inhibition observed in the non-injected tumors was not due to viral spread, but rather the abscopal effects of virus administration. Tumors were also assessed for the presence of the three payloads, IL-12, CXCL10, and CCL4. As shown in Fig. 60A-60C, payload expression peaked in the injected tumors at 24-hours post-treatment and decreased thereafter. Levels of the payloads were also assessed in the serum (Fig. 61A-61C) of mice treated with ONCR-153, where only CXCL10 expression was observed. However, as shown in Fig. 62, treatment of mice with ONCR-153 induced an intra-tumoral IFNγ response in both injected and non-injected tumors (left panel). Similarly, increased expression of IFNγ was observed in the serum of ONCR-153 treated mice. These data further indicate that the combination of IL-12 and CCL4 expression by HSV induce a localized immune response that may contribute to the abscopal effect observed with ONCR-153.

EXAMPLE 11 – FLT3 EXPRESSION FURTHER ENAHNCES ABSCOPAL EFFICACY OF HSV

[00311] Experiments were performed to assess the effects of FLT3 in tumor growth inhibition in the MC38 model described in Example F. Results of this experiment are shown in Fig. 63 and Table 21 below. Mice were treated with one of 3 combinations of constructs expressing different payloads as outlined in Table 21. As shown in Fig. 63, treatment with a combination of ONCR-152 and ONCR-140 enhanced the tumor growth inhibition in non-injected tumors, indicating that FLT3L expression can enhance the abscopal effect over that observed with IL-12, CXL10, and CCL4.

Table 21:

Combo	Payloads	Injected			Non-injected		
		CR	% TGI	p	CR	% TGI	p
152+140	IL12, CXCL10, CCL4	8/8	97	<0.0001	3/8	69	0.29
153+140	IL12, CXCL10, FLT3	8/8	99	<0.0001	3/8	58	0.22
152+153	IL12, CXCL10, CCL4, FLT3	8/8	97	<0.0001	3/8	75	0.005

EXAMPLE 13 – CD40L EXPRESSION FURTHER ENAHNCES ABSCOPAL EFFICACY OF HSV

[00312] Experiments were performed to assess the effects of CD40L in tumor growth inhibition in the MC38 model described in Example F. Results of this experiment are shown in Fig. 64 and Table 22 below. Mice were treated with constructs expressing different payloads as outlined in Table 22. As shown in Fig. 64, treatment with a combination of ONCR-153 and ONCR-147 enhanced the tumor growth inhibition in non-injected tumors, indicating that CD40L expression can enhance the abscopal effect over that observed with IL-12, CXL10, and CCL4.

Table 22:

Combo	Payloads	Injected			Non-injected		
		CR	% TGI	p	CR	% TGI	p
153	IL12, CXCL10, CCL4	1/8	65	0.0005	0/8	41	0.02
147	CD40L, 41BBL	0/8	14	0.35	0/8	--	0.83
153+147	IL12, CXCL10, CCL4, CD40L, 41BBL	2/8	73	<0.0001	0/8	59	0.0005

EXAMPLE 12– ANTI-CTLA4 EXPRESSION FURTHER ENAHNCES ABSCOPAL EFFICACY OF HSV

[00313] Experiments were performed to assess the effects of CTLA4 in tumor growth inhibition in the MC38 model described in Example F. Results of this experiment are shown in Fig. 66A – Fig. 66C and Table 23 below. Mice were treated with constructs expressing different payloads as outlined in Table 23. As shown in Fig. 66A – Fig. 66C, treatment with a combination of ONCR-149 and ONCR-139 enhanced the tumor growth inhibition in injected and non-injected tumors compared to treatment with ONCR-149 alone, indicating that anti-CTLA4 expression can enhance the anti-tumor effects and abscopal effect over that observed with IL-12.

Table 23:

Combo	Payloads	Injected			Non-injected		
		CR	% TGI	p	CR	% TGI	p
149	IL12	0/8	66	<0.0001	0/8	41	0.0002
149 + 139	IL12, anti-CTLA4	1/8	75	<0.0001	1/8	57	<0.0001

[00314] Similar experiments were performed to assess the effects of anti-CTLA4 over IL-12, CXCL10, FLT3L, and CCL4 expression in a 4T1-luc model. In brief, 1 x 10^6 4T1-luc cells in 100 µL of DPBS were injected subcutaneously into the right flank of Balb/c mice. When tumor volume reach an average of 100 mm^3, mice were intratumorally injected with HSV-1 at a dose of $3e^6$ PFU/injection. Dosing was repeated twice every third day for a total number of 3 doses (Q3Dx3). Tumor growth and body weight was monitor twice weekly. The experiment concluded at Day 22 when the first clinical symptoms of metastatic disease were observed. Lung metastases were visualized *ex vivo* using IVIS Lumina LT system and analyzed with Living Image Software. Mice were treated with a combination of constructs as shown in Table 24. Results of this experiment are shown in Fig. 67. As shown, treatment with ONCR-152 and -139 demonstrated an enhanced effect in tumor growth inhibition compared to treatment with ONCR-139 alone.

Table 24:

Combo	Payloads
152	IL12, CXCL10, FLT3L
139	anti-CTLA4

152 + 139	IL12, CXCL10, FLT3L, anti-CTLA4

EXAMPLE 13 – TREATMENT WITH ANTI-PD1 EXPRESSION FURTHER ENAHNCES EFFICACY OF HSV

[00315] Experiments were performed to assess the effects of anti-PD1 treatment on HSV-mediated tumor growth inhibition in the MC38 model described in Example F. Results of this experiment are shown in Fig. 68 and Table 25 below. Mice were treated with constructs expressing different payloads as outlined in Table 25. As shown in Fig. 68, treatment with a combination of ONCR-153 and anti-PD1 enhanced the tumor growth inhibition in injected tumors compared to that observed with ONCR-152 or anti-PD1 alone.

Table 25:

Combo	Payloads	Injected			Non-injected		
		CR	% TGI	p	CR	% TGI	p
153	IL12, CXCL10, CCL4	2/8	79	<0.0001	5/8	84	<0.0001
anti-PD1		5/8	65	0.0005	0/8	41	0.02
153+anti-PD1	IL12, CXCL10, CCL4, anti-PD1	2/8	95	<0.0001	0/8	83	<0.0001

EXAMPLE 14 – TREATMENT OF A PATIENT SUFFERING FROM PANCREATIC CANCER, LUNG CANCER, OR COLON CANCER

[00316] A patient suffering from pancreatic cancer, lung cancer, or colon cancer is treated using the compositions and methods disclosed herein. HSV-based viral stocks may be generated that are attenuated by incorporating one or more miRNA target sequences into UL19, ICP4, ICP27, or UL42 (or other viral genes) as shown in FIGs. 39-50. In some cases, genome-editing capabilities for tumor destruction and/or microenvironment remodeling are engineered into the virus in addition to miRNA target sequences, as shown in FIGs. 45-46. In a specific example, an HSV-based stock containing miR-124, miR-451a, miR-143-3p, and miR-559 attenuation cassettes incorporated into ICP4 and ICP27 is used. In another example, an HSV-based stock containing attenuation cassettes with one or more copies of miR-122 and miR-125a target sequences incorporated into ICP27 and/or UL42 genes. For any of these compositions, the HSV-based stock is generated according to the methods described in Example 3. The miRNA target

sequence cassettes are introduced into the 3' UTR of the ICP4, ICP27UL19, and/or UL42 genes. BAC constructs are converted to virus particles with simultaneous removal of the BAC sequences located between loxP sites by transfection of Vero-Cre cells. Following plaque purification, virus stocks are further purified, buffer exchanged, and titered on Vero cells. For *in vivo* administration to a patient suffering from pancreatic cancer, lung cancer, or colon cancer, HSV particles are prepared in phosphate buffered solution (PBS) along with pharmaceutically acceptable stabilizing agents. On the day of treatment, 10^9 vector genomes in a volume of 1.0 mL pharmaceutically acceptable carrier are administered via intra-tumoral infusion. The patient is monitored for tumor regression using standard of care procedures at an appropriate time interval based on that patient's particular prognosis.

EXAMPLE 15 – TREATMENT OF PATIENTS SUFFERING FROM BRAIN CANCER, BLADDER CANCER, BREAST CANCER, OR HEAD AND NECK CANCER

[00317] A patient suffering from brain cancer, bladder cancer, breast cancer, or head and neck cancer is treated using the compositions and methods disclosed herein. An HSV-based viral stock is generated containing miR-124, miR-451a, miR-145-3p, and miR-559 attenuation cassettes according to the methods described in Example 3. The miRNA target sequence cassettes are introduced into the 3' UTR of the ICP4 (miR-124) and ICP27 (miR-451a, miR-145-3p, miR-559) genes as shown in FIG. 48. BAC constructs are converted to virus particles with simultaneous removal of the BAC sequences located between loxP sites by transfection of Vero-Cre cells. Following plaque purification, virus stocks are further purified, buffer exchanged, and titered on Vero cells. For i*n vivo* administration to a patient suffering from brain cancer, bladder cancer, breast cancer, or head and neck cancer, HSV particles are prepared in phosphate buffered solution (PBS) along with pharmaceutically acceptable stabilizing agents. On the day of treatment, 10^9 vector genomes in a volume of 1.0 mL pharmaceutically acceptable carrier are administered via intra-tumoral infusion. The patient is monitored using standard of care procedures at an appropriate time interval based on that patient's particular prognosis. Potential outcomes of these experiments include partial or complete inhibition of tumor growth, inhibition of tumor metastasis, prolonged time in remission, and/or reduced rate of relapse compared to standard of care therapies.

EXAMPLE 16 – TREATMENT OF A PATIENT SUFFERING FROM SCHWANNOMA

[00318] A patient suffering from schwannoma is treated using the compositions and methods disclosed herein. An HSV-based viral stock is generated containing miR-124-3p, miR-205-5p, miR-141-5p, and miR-31-5p attenuation cassettes according to the methods described in Example 3. The miRNA target sequence cassettes were recombined into the 3' UTR of the ICP4 (miR-124) and ICP27 (miR-205-5p, miR-141-5p, miR-31-5p) genes as shown in FIG. 49. BAC constructs are converted to virus particles with simultaneous removal of the BAC sequences located between loxP sites by transfection of Vero-Cre cells. Following plaque purification, virus stocks were further purified, buffer exchanged, and titered on Vero cells. For *in vivo* administration to a patient suffering from schwannoma, HSV particles are prepared in phosphate buffered solution (PBS) along with pharmaceutically acceptable stabilizing agents. On the day of treatment, 10^9 vector genomes in a volume of 1.0 mL pharmaceutically acceptable carrier are administered via intra-tumoral infusion. The patient is monitored using standard of care procedures at an appropriate time interval based on that patient's particular prognosis. Potential outcomes of these experiments include partial or complete inhibition of tumor growth, inhibition of tumor metastasis, prolonged time in remission, and/or reduced rate of relapse compared to standard of care therapies

[00319] While preferred embodiments of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions can be implemented by those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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INCORPORATION BY REFERENCE

[00320] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application

cited herein is not, and should not, be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Table 1: Summary of relationships between 12 select oncomiRs (9 tumor suppressors and 3 oncogenic miRNAs) and various cancers

	Down-regulated									Up-regulated		
Malignancy	**let-7**	**miR-15a**	**miR-16**	**miR-29a**	**miR-34a**	**miR-98**	**miR-101**	**miR-124**	**miR-202**	**miR-17**	**miR-21**	**miR-155**
acute lymphoblastic leukemia	X							X				
acute myeloid leukemia	X			X						X		X
acute promyelocytic leukemia	X											
adrenal cortical carcinoma											X	
anaplastic astrocytoma								X				
anaplastic large-cell lymphoma												X
astrocytoma								X				
B cell lymphoma					X					X		
bladder cancer			X		X		X	X		X		X
breast cancer	X	X	X	X	X	X		X		X		X
breast carcinoma										X		
bronchioloalveolar carcinoma	X					X						
cervical cancer								X		X		X
cervical carcinoma		X	X		X		X					
cervical squamous cell carcinoma				X				X				
cholangiocarcinoma					X		X			X		
chondrosarcoma	X											
chordoma					X							
choriocarcinoma					X							
chronic lymphocytic leukemia		X	X									X
chronic myelogenous leukemia			X									X
clear cell renal cell cancer					X							X
colon cancer	X				X	X	X					X
colorectal cancer	X	X	X	X	X		X	X	X	X		X
colorectal carcinoma										X		X
cutaneous T cell lymphoma												X
diffuse large B cell lymphoma												X
endometrial cancer					X		X					X
epithelial ovarian cancer								X				
esophageal cancer		X					X	X				
esophageal squamous cell carcinoma	X		X		X	X	X			X		
extrahepatic cholangiocarcinoma					X							
follicular lymphoma									X			
gallbladder carcinoma												X
gastric cancer	X			X	X	X	X	X	X	X		X
glioblastoma	X				X		X	X				
glioma	X		X		X	X		X		X		X
head and neck cancer												

Malignancy	Down-regulated									Up-regulated		
	let-7	miR-15a	miR-16	miR-29a	miR-34a	miR-98	miR-101	miR-124	miR-202	miR-17	miR-21	miR-155
head and neck squamous cell carcinoma	X		X	X	X						X	
hepatocellular carcinoma	X		X	X	X	X	X	X	X	X	X	X
hypopharyngeal squamous cell carcinoma											X	
kidney cancer											X	
laryngeal carcinoma			X								X	
laryngeal squamous cell carcinoma							X				X	
liver cancer							X				X	X
lung adenocarcinoma			X									X
lung cancer	X	X	X		X	X	X			X	X	X
malignant melanoma	X				X	X	X			X	X	X
malt lymphoma												X
mantle cell lymphoma				X				X		X		X
medulloblastoma								X		X		
mesenchymal cancer				X								
monocytic leukemia				X								
multiple myeloma											X	
nasopharyngeal cancer										X		
nasopharyngeal carcinoma	X					X	X	X			X	X
neuroblastoma	X	X	X	X	X	X		X				
non-small cell lung cancer	X	X	X	X	X		X	X		X	X	X
oral cancer	X				X						X	
oral squamous cell carcinoma				X				X			X	X
osteosarcoma	X	X	X		X		X	X	X	X	X	
ovarian cancer	X				X	X		X			X	X
ovarian carcinoma							X					
pancreatic adenocarcinoma					X						X	
pancreatic cancer		X					X	X		X	X	
pancreatic ductal adenocarcinoma	X	X	X		X	X					X	
papillary thyroid carcinoma	X		X		X		X				X	X
pituitary carcinoma										X		
prostate cancer	X	X	X		X		X	X			X	
rectal cancer					X						X	X
renal cell carcinoma	X		X		X						X	
renal clear cell carcinoma	X											X
retinoblastoma					X		X				X	
squamous carcinoma		X	X		X						X	X
T cell lymphoblastic lymphoma										X		
uveal melanoma					X							

Table 2: Summary of miRNA expression in cancer

Malignancy	Down-regulated miRs	Up-regulated miRs
breast cancer	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-100, mir-107, mir-10a, mir-10b, mir-122, mir-124, mir-1258, mir-125a-5p, mir-125b, mir-126, mir-127, mir-129, mir-130a, mir-132, mir-133a, mir-143, mir-145, mir-146a, mir-146b, mir-147, mir-148a, mir-149, mir-152, mir-153, mir-15a, mir-16, mir-17-5p, mir-181a, mir-1826, mir-183, mir-185, mir-191, mir-193a-3p, mir-193b, mir-195, mir-199b-5p, mir-19a-3p, mir-200a, mir-200b, mir-200c, mir-205, mir-206, mir-211, mir-216b, mir-218, mir-22, mir-26a, mir-26b, mir-300, mir-30a, mir-31, mir-335, mir-339-5p, mir-33b, mir-34a, mir-34b, mir-34c, mir-374a, mir-379, mir-381, mir-383, mir-425, mir-429, mir-450b-3p, mir-494, mir-495, mir-497, mir-502-5p, mir-517a, mir-574-3p, mir-638, mir-7, mir-720, mir-7515, mir-92a, mir-98, mir-99a, mmu-mir-290-3p, mmu-mir-290-5p	mir-10b, mir-125a, mir-135a, mir-140, mir-141, mir-142, mir-150, mir-155, mir-181a, mir-181b, mir-182, mir-18a, mir-18b, mir-191, mir-196a, mir-197, mir-19a, mir-19b, mir-200a, mir-200b, mir-200c, mir-203, mir-205, mir-20a, mir-20b, mir-21, mir-217, mir-221, mir-224, mir-23a, mir-24, mir-24-2-5p, mir-24-3p, mir-27a, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-373, mir-378, mir-423, mir-429, mir-495, mir-503, mir-510, mir-520c, mir-526b, mir-96
chondrosarcoma	let-7a, mir-100, mir-136, mir-145, mir-199a, mir-222, mir-30a, mir-335, mir-376a	
colorectal cancer	let-7a, mir-1, mir-100, mir-101, mir-124, mir-125a, mir-126, mir-129, mir-1295b-3p, mir-1307, mir-130b, mir-132, mir-133a, mir-133b, mir-137, mir-138, mir-139, mir-139-5p, mir-140-5p, mir-143, mir-145, mir-148a, mir-148b, mir-149, mir-150-5p, mir-154, mir-15a, mir-15b, mir-16, mir-18a, mir-191, mir-192, mir-193a-5p, mir-194, mir-195, mir-196a, mir-198, mir-199a-5p, mir-200c, mir-203, mir-204-5p, mir-206, mir-212, mir-215, mir-218, mir-22, mir-224, mir-24-3p, mir-26b, mir-27a, mir-28-3p, mir-28-5p, mir-29b, mir-30a-3p, mir-30b, mir-320a, mir-328, mir-338-3p, mir-342, mir-345, mir-34a, mir-34a-5p, mir-361-5p, mir-375, mir-378, mir-378a-3p, mir-378a-5p, mir-409-3p, mir-422a, mir-4487, mir-483, mir-497, mir-498, mir-518a-3p, mir-551a, mir-574-5p, mir-625, mir-638, mir-7, mir-96-5p	let-7a, mir-103, mir-106a, mir-10b, mir-1179, mir-1229, mir-1246, mir-125b-2*, mir-1269a, mir-130b, mir-133b, mir-135a, mir-135a-1, mir-135a-2, mir-135b, mir-139-3p, mir-145, mir-150, mir-150*, mir-155, mir-17, mir-181a, mir-182, mir-183, mir-18a, mir-191, mir-196a, mir-196b, mir-19a, mir-19b, mir-200b, mir-200c, mir-203, mir-204-5p, mir-20a, mir-20a-5p, mir-21, mir-210, mir-211, mir-221, mir-223, mir-224, mir-23a, mir-25, mir-27a, mir-29a, mir-301a, mir-31, mir-32, mir-320b, mir-326, mir-424, mir-429, mir-494, mir-497, mir-499-5p, mir-592, mir-630, mir-7-5p, mir-892a, mir-92, mir-92a, mir-93, mir-95, mir-96

Malignancy	Down-regulated miRs	Up-regulated miRs
esophageal squamous cell carcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-100, mir-101, mir-126, mir-1294, mir-133a, mir-133b, mir-138, mir-143, mir-145, mir-150, mir-185, mir-195, mir-200b, mir-203, mir-21, mir-210, mir-214, mir-218, mir-22, mir-27a, mir-29b, mir-29c, mir-302b, mir-34a, mir-375, mir-494, mir-518b, mir-655, mir-98, mir-99a	mir-100, mir-1179, mir-1290, mir-130b, mir-145, mir-16, mir-17, mir-183, mir-18a, mir-19a, mir-19b, mir-208, mir-20a, mir-21, mir-218, mir-223, mir-25, mir-30a-5p, mir-31, mir-330-3p, mir-373, mir-9, mir-92a, mir-942
gastric cancer	let-7a, let-7b, let-7g, mir-1, mir-101, mir-103a, mir-10a, mir-10b, mir-1207-5p, mir-122, mir-1228*, mir-124, mir-124-3p, mir-125a-3p, mir-126, mir-1266, mir-1271, mir-129-1-3p, mir-129-2-3p, mir-129-3p, mir-129-5p, mir-133a, mir-133b, mir-137, mir-141, mir-143, mir-144, mir-145, mir-146a, mir-146a-5p, mir-148a, mir-148b, mir-149, mir-152, mir-155, mir-155-5p, mir-181a, mir-181b, mir-182, mir-183, mir-185, mir-194, mir-195, mir-197, mir-199a-3p, mir-200b, mir-200c, mir-202-3p, mir-204, mir-204-5p, mir-205, mir-206, mir-210, mir-212, mir-217, mir-218, mir-22, mir-23b, mir-24, mir-26a, mir-29a, mir-29a-3p, mir-29b, mir-29b-1, mir-29b-2, mir-29c, mir-30a-5p, mir-30b, mir-31, mir-328, mir-329, mir-331-3p, mir-335-5p, mir-338, mir-338-3p, mir-34a, mir-34b, mir-34c, mir-361-5p, mir-367, mir-375, mir-378, mir-409-3p, mir-410, mir-429, mir-433, mir-449, mir-449a, mir-490-3p, mir-494, mir-497, mir-503, mir-506, mir-513b, mir-520d-3p, mir-542-3p, mir-622, mir-625, mir-638, mir-663, mir-7, mir-765, mir-9	mir-100, mir-103, mir-106a, mir-106b, mir-107, mir-10a, mir-10b, mir-1259, mir-125b, mir-126, mir-1274a, mir-1303, mir-130b*, mir-135a-5p, mir-135b, mir-138, mir-143, mir-146a, mir-147, mir-148a, mir-150, mir-17, mir-17-5p, mir-181a, mir-181a-2*, mir-181a-5p, mir-181c, mir-183, mir-185, mir-18a, mir-191, mir-192, mir-196a, mir-196a*, mir-196a-5p, mir-196b, mir-199a, mir-199a-3p, mir-199a-5p, mir-19a, mir-19b, mir-200b, mir-20a, mir-21, mir-214, mir-215, mir-221, mir-221*, mir-222, mir-223, mir-224, mir-23a, mir-23b, mir-27a, mir-27b, mir-296-5p, mir-301a, mir-302f, mir-337-3p, mir-340*, mir-34a, mir-362-3p, mir-370, mir-374a, mir-377, mir-421, mir-425, mir-500, mir-520c-3p, mir-544, mir-575, mir-601, mir-616*, mir-650, mir-92, mir-98, mir-99a
glioma	let-7a, let-7f, mir-106a, mir-107, mir-122, mir-124, mir-124-5p, mir-124a, mir-125b, mir-128, mir-136, mir-137, mir-139, mir-143, mir-145, mir-146a, mir-146b, mir-146b-5p, mir-152, mir-15b, mir-16, mir-181a, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-184, mir-185, mir-195, mir-199a-3p, mir-200a, mir-200b, mir-203, mir-204, mir-205, mir-218, mir-219-5p, mir-23b, mir-26b, mir-27a, mir-29c, mir-320, mir-326, mir-328, mir-34a, mir-34c-3p, mir-34c-5p, mir-	mir-106b, mir-106b-5p, mir-10b, mir-125b, mir-132, mir-155, mir-17, mir-181a, mir-182, mir-183, mir-193b, mir-19a, mir-19b, mir-20a, mir-210, mir-214, mir-221, mir-222, mir-224, mir-23a, mir-24, mir-24-3p, mir-25, mir-26a, mir-27a-3p, mir-27b, mir-30a-5p, mir-30e, mir-30e*, mir-328, mir-335, mir-33a, mir-372, mir-486, mir-494, mir-497, mir-566, mir-603, mir-650, mir-675, mir-9, mir-92b, mir-93, mir-96

Malignancy	Down-regulated miRs	Up-regulated miRs
	375, mir-383, mir-451, mir-452, mir-483-5p, mir-495, mir-584, mir-622, mir-656, mir-7, mir-98	
nasopharyngeal carcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-101, mir-124, mir-138, mir-143, mir-145, mir-148a, mir-200b, mir-204, mir-216b, mir-29c, mir-320a, mir-324-3p, mir-34c, mir-375, mir-378, mir-451, mir-506, mir-9, mir-98	mir-10b, mir-144, mir-149, mir-155, mir-18a, mir-21, mir-214, mir-24, mir-421, mir-663, mir-7-5p, mir-93
non-small cell lung cancer	let-7a, let-7c, mir-1, mir-100, mir-101, mir-106a, mir-107, mir-124, mir-125a-3p, mir-125a-5p, mir-126*, mir-129, mir-133a, mir-137, mir-138, mir-140, mir-143, mir-145, mir-146a, mir-146b, mir-148a, mir-148b, mir-149, mir-152, mir-153, mir-154, mir-155, mir-15a, mir-16, mir-17-5p, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-184, mir-186, mir-193b, mir-195, mir-199a, mir-204, mir-212, mir-221, mir-224, mir-26b, mir-27a, mir-27b, mir-29a, mir-29b, mir-29c, mir-30a, mir-30b, mir-30c, mir-30d, mir-30d-5p, mir-30e-5p, mir-32, mir-335, mir-338-3p, mir-340, mir-342-3p, mir-34a, mir-34b, mir-361-3p, mir-365, mir-373, mir-375, mir-429, mir-449a, mir-4500, mir-451, mir-4782-3p, mir-497, mir-503, mir-512-3p, mir-520a-3p, mir-526b, mir-625*, mir-96, mir-99a	mir-10b, mir-125a-5p, mir-1280, mir-136, mir-140, mir-141, mir-142-3p, mir-145, mir-146a, mir-150, mir-18a, mir-196a, mir-19a, mir-200a, mir-200c, mir-205, mir-205-5p, mir-21, mir-212, mir-22, mir-221, mir-222, mir-24, mir-25, mir-29c, mir-31, mir-328, mir-330-3p, mir-339, mir-34a, mir-375, mir-494, mir-675-5p, mir-9, mir-92b, mir-93, mir-95
osteosarcoma	let-7a, mir-1, mir-100, mir-101, mir-122, mir-124, mir-125b, mir-126, mir-127-3p, mir-132, mir-133a, mir-141, mir-142-3p, mir-142-5p, mir-143, mir-144, mir-145, mir-153, mir-16, mir-183, mir-194, mir-195, mir-199a-3p, mir-204, mir-212, mir-217, mir-218, mir-22, mir-23a, mir-24, mir-26a, mir-26b, mir-29b, mir-32, mir-320, mir-335, mir-33b, mir-340, mir-34a, mir-34b, mir-34c, mir-375, mir-376c, mir-382, mir-3928, mir-424, mir-429, mir-449a, mir-451, mir-454, mir-503, mir-519d, mir-646	mir-128, mir-151-3p, mir-17, mir-181a, mir-181b, mir-181c, mir-18a, mir-191, mir-195-5p, mir-199a-3p, mir-19a, mir-19b, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-27a, mir-300, mir-320a, mir-374a-5p, mir-720, mir-9, mir-92a
pancreatic ductal adenocarcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-126, mir-135a, mir-143, mir-144, mir-145, mir-148a, mir-150, mir-15a, mir-16, mir-200a, mir-	mir-10b, mir-186, mir-18a, mir-192, mir-194, mir-196a, mir-198, mir-203, mir-21, mir-212, mir-30b-5p, mir-31, mir-34a, mir-369-5p, mir-376a, mir-541

Malignancy	Down-regulated miRs	Up-regulated miRs
	200b, mir-200c, mir-217, mir-218, mir-337, mir-375, mir-494, mir-615-5p, mir-98	
renal cell carcinoma	let-7a, let-7d, mir-1, mir-106a*, mir-126, mir-1285, mir-129-3p, mir-1291, mir-133a, mir-133b, mir-135a, mir-138, mir-141, mir-143, mir-145, mir-182-5p, mir-199a-3p, mir-200a, mir-205, mir-218, mir-28-5p, mir-30a, mir-30c, mir-30d, mir-34a, mir-378, mir-429, mir-509-3p, mir-509-5p, mir-646	mir-100, mir-1233, mir-1260b, mir-146a, mir-146b, mir-16, mir-193a-3p, mir-203a, mir-21, mir-210, mir-27a, mir-362, mir-572, mir-7
bronchioloalveolar carcinoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-98	
colon cancer	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-100, mir-101, mir-126, mir-142-3p, mir-143, mir-145, mir-192, mir-200c, mir-21, mir-214, mir-215, mir-25, mir-302a, mir-320, mir-320a, mir-34a, mir-34c, mir-365, mir-373, mir-424, mir-429, mir-455, mir-484, mir-502, mir-503, mir-93, mir-98	mir-1290, mir-145, mir-155, mir-181a, mir-18a, mir-200c, mir-31, mir-675
hepatocellular carcinoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-100, mir-101, mir-105, mir-122, mir-122a, mir-1236, mir-124, mir-125b, mir-126, mir-127, mir-1271, mir-128-3p, mir-129-5p, mir-130a, mir-130b, mir-133a, mir-134, mir-137, mir-138, mir-139, mir-139-5p, mir-140-5p, mir-141, mir-142-3p, mir-143, mir-144, mir-145, mir-146a, mir-148a, mir-148b, mir-150-5p, mir-15b, mir-16, mir-181a-5p, mir-185, mir-188-5p, mir-193b, mir-195, mir-195-5p, mir-197, mir-198, mir-199a, mir-199a-5p, mir-199b, mir-199b-5p, mir-200a, mir-200b, mir-200c, mir-202, mir-203, mir-204-3p, mir-205, mir-206, mir-20a, mir-21, mir-21-3p, mir-211, mir-212, mir-214, mir-217, mir-218, mir-219-5p, mir-22, mir-26a, mir-26b, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-302b, mir-302c, mir-30a, mir-30a-3p, mir-335, mir-338-3p, mir-33a, mir-34a, mir-34b, mir-365, mir-370, mir-372, mir-375, mir-376a, mir-377, mir-422a, mir-424, mir-424-5p, mir-433, mir-4458, mir-448, mir-450a, mir-451, mir-	mir-106b, mir-10b, mir-122, mir-1228, mir-1269, mir-128a, mir-130a, mir-130b, mir-146a, mir-153, mir-155, mir-17-5p, mir-181a, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-183, mir-184, mir-190b, mir-191, mir-20a, mir-20b, mir-21, mir-210, mir-214, mir-215, mir-216a, mir-217, mir-221, mir-222, mir-223, mir-224, mir-23a, mir-24, mir-25, mir-27a, mir-301a, mir-30d, mir-31, mir-3127, mir-32, mir-331-3p, mir-362-3p, mir-371-5p, mir-372, mir-373, mir-423, mir-429, mir-452, mir-483-3p, mir-483-5p, mir-485-3p, mir-490-3p, mir-494, mir-495, mir-500, mir-501-5p, mir-519d, mir-520g, mir-574-3p, mir-590-5p, mir-630, mir-650, mir-657, mir-664, mir-885-5p, mir-9, mir-92a, mir-96

Malignancy	Down-regulated miRs	Up-regulated miRs
	485-5p, mir-486-5p, mir-497, mir-503, mir-506, mir-519d, mir-520a, mir-520b, mir-520c-3p, mir-582-5p, mir-590-5p, mir-610, mir-612, mir-625, mir-637, mir-675, mir-7, mir-877, mir-940, mir-941, mir-98, mir-99a	
lung cancer	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-101, mir-133b, mir-138, mir-142-5p, mir-144, mir-145, mir-1469, mir-146a, mir-153, mir-15a, mir-15b, mir-16-1, mir-16-2, mir-182, mir-192, mir-193a-3p, mir-194, mir-195, mir-198, mir-203, mir-217, mir-218, mir-22, mir-223, mir-26a, mir-26b, mir-29c, mir-33a, mir-34a, mir-34b, mir-34c, mir-365, mir-449a, mir-449b, mir-486-5p, mir-545, mir-610, mir-614, mir-630, mir-660, mir-7-5p, mir-9500, mir-98, mir-99b	mir-10b, mir-135b, mir-150, mir-155, mir-17, mir-182, mir-183-3p, mir-18a, mir-197, mir-19a, mir-19b, mir-205, mir-20a, mir-21, mir-210, mir-24, mir-30d, mir-4423, mir-5100, mir-570, mir-663, mir-7, mir-92a
neuroblastoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-124, mir-137, mir-145, mir-181c, mir-184, mir-200a, mir-29a, mir-335, mir-338-3p, mir-34a, mir-449a, mir-885-5p, mir-98	mir-125b, mir-15a, mir-15b, mir-16-1, mir-16-2, mir-18a, mir-195, mir-19a, mir-23a, mir-421, mir-92
prostate cancer	let-7a-3p, let-7c, mir-100, mir-101, mir-105, mir-124, mir-128, mir-1296, mir-130b, mir-133a-1, mir-133a-2, mir-133b, mir-135a, mir-143, mir-145, mir-146a, mir-154, mir-15a, mir-187, mir-188-5p, mir-199b, mir-200b, mir-203, mir-205, mir-212, mir-218, mir-221, mir-224, mir-23a, mir-23b, mir-25, mir-26a, mir-26b, mir-29b, mir-302a, mir-30a, mir-30b, mir-30c-1, mir-30c-2, mir-30d, mir-30e, mir-31, mir-330, mir-331-3p, mir-34a, mir-34b, mir-34c, mir-374b, mir-449a, mir-4723-5p, mir-497, mir-628-5p, mir-642a-5p, mir-720, mir-940	mir-125b, mir-141, mir-153, mir-155, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-182-5p, mir-183, mir-18a, mir-204, mir-20a, mir-21, mir-221, mir-223-3p, mir-31, mir-429, mir-96
acute lymphoblastic leukemia	let-7b, mir-124a, mir-142-3p	mir-128
malignant melanoma	let-7b, mir-101, mir-125b, mir-1280, mir-143, mir-146a, mir-146b, mir-155, mir-17, mir-184, mir-185, mir-18b, mir-193b, mir-200c, mir-203, mir-204, mir-205, mir-206, mir-20a, mir-211, mir-218, mir-26a, mir-31, mir-33a, mir-34a, mir-34c, mir-376a, mir-376c, mir-573, mir-7, mir-9, mir-98	mir-126, mir-141, mir-15b, mir-17, mir-17-5p, mir-182, mir-18a, mir-193b, mir-200a, mir-200b, mir-200c, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-222, mir-429, mir-455-5p, mir-532-5p, mir-638, mir-92a

Malignancy	Down-regulated miRs	Up-regulated miRs
renal clear cell carcinoma	let-7b, let-7c, mir-138, mir-141, mir-200c, mir-204, mir-218, mir-335, mir-377, mir-506	mir-122, mir-155, mir-630
acute myeloid leukemia	let-7c, mir-17, mir-181a, mir-20a, mir-223, mir-26a, mir-29a, mir-30c, mir-7	mir-125b, mir-126-5p, mir-128, mir-155, mir-29a, mir-32, mir-331, mir-370, mir-378
acute promyelocytic leukemia	let-7c, mir-107, mir-342	mir-181a, mir-181b, mir-92a
head and neck squamous cell carcinoma	let-7d, mir-1, mir-107, mir-128, mir-133a, mir-138, mir-149, mir-200c, mir-205, mir-218, mir-27a*, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-300, mir-34a, mir-363, mir-375, mir-874	mir-106b, mir-134, mir-16, mir-184, mir-196a, mir-21, mir-25, mir-30a-5p, mir-31, mir-372, mir-93
oral cancer	let-7d, mir-218, mir-34a, mir-375, mir-494	mir-10b, mir-196a-1, mir-196a-2, mir-196b, mir-21
papillary thyroid carcinoma	mir-101, mir-130b, mir-138, mir-146a, mir-16, mir-195, mir-199a-3p, mir-204-5p, mir-219-5p, mir-26a, mir-34b, mir-613	let-7e, mir-146b, mir-146b-5p, mir-151-5p, mir-155, mir-181a-1, mir-181a-2, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-183, mir-199b-5p, mir-21, mir-221, mir-222, mir-339-5p, mir-34a
glioblastoma	let-7g-5p, mir-100, mir-101, mir-106a, mir-124, mir-124a, mir-125a, mir-125a-5p, mir-125b, mir-127-3p, mir-128, mir-129, mir-136, mir-137, mir-139-5p, mir-142-3p, mir-143, mir-145, mir-146b-5p, mir-149, mir-152, mir-153, mir-195, mir-21, mir-212-3p, mir-219-5p, mir-222, mir-29b, mir-31, mir-3189-3p, mir-320, mir-320a, mir-326, mir-330, mir-331-3p, mir-340, mir-342, mir-34a, mir-376a, mir-449a, mir-483-5p, mir-503, mir-577, mir-663, mir-7, mir-744	mir-10b, mir-125b, mir-127-3p, mir-148a, mir-18a, mir-196a, mir-196a-1, mir-196a-2, mir-196b, mir-21, mir-210, mir-210-3p, mir-223, mir-340, mir-576-5p, mir-626, mir-92b
ovarian cancer	let-7i, mir-100, mir-124, mir-125b, mir-129-5p, mir-130b, mir-133a, mir-137, mir-138, mir-141, mir-145, mir-148a, mir-152, mir-153, mir-155, mir-199a, mir-200a, mir-200b, mir-200c, mir-212, mir-335, mir-34a, mir-34b, mir-34c, mir-409-3p, mir-411, mir-429, mir-432, mir-449a, mir-494, mir-497, mir-498, mir-519d, mir-655, mir-9, mir-98	mir-106a, mir-141, mir-148b, mir-181b, mir-182, mir-200a, mir-200c, mir-205, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-224-5p, mir-23b, mir-25, mir-26a, mir-27a, mir-27b, mir-346, mir-378, mir-424, mir-503, mir-572, mir-9, mir-96
bladder cancer	mir-1, mir-101, mir-1180, mir-1236, mir-124-3p, mir-125b, mir-126, mir-1280, mir-133a, mir-133b, mir-141, mir-143, mir-144, mir-145, mir-155, mir-16, mir-18a, mir-192, mir-195, mir-200a, mir-200b, mir-200c, mir-203, mir-205, mir-	mir-103a-3p, mir-10b, mir-135a, mir-137, mir-141, mir-155, mir-17-5p, mir-182, mir-182-5p, mir-183, mir-185, mir-19a, mir-203, mir-205, mir-210, mir-221, mir-

Malignancy	Down-regulated miRs	Up-regulated miRs
	214, mir-218, mir-23b, mir-26a, mir-29c, mir-320c, mir-34a, mir-370, mir-409-3p, mir-429, mir-451, mir-490-5p, mir-493, mir-576-3p, mir-99a	222, mir-223, mir-23a, mir-23b, mir-26b, mir-639, mir-96
chordoma	mir-1, mir-222, mir-31, mir-34a, mir-608	mir-140-3p, mir-148a
kidney cancer	mir-1, mir-145, mir-1826, mir-199a, mir-199a-3p, mir-203, mir-205, mir-497, mir-508-3p, mir-509-3p	mir-183, mir-21, mir-210, mir-223
cervical carcinoma	mir-100, mir-101, mir-15a, mir-16, mir-34a, mir-886-5p, mir-99a, mir-99b	mir-133b, mir-21, mir-25, mir-373
mesenchymal cancer	mir-100, mir-141, mir-199b-5p, mir-200a, mir-200b, mir-200c, mir-29a, mir-29b-1, mir-29b-1-5p, mir-29b-2, mir-29c, mir-335, mir-429, mir-99a	mir-125b-1-3p, mir-182
oral squamous cell carcinoma	mir-100, mir-124, mir-1250, mir-125b, mir-126, mir-1271, mir-136, mir-138, mir-145, mir-147, mir-148a, mir-181a, mir-206, mir-220a, mir-26a, mir-26b, mir-29a, mir-32, mir-323-5p, mir-329, mir-338, mir-370, mir-410, mir-429, mir-433, mir-499a-5p, mir-503, mir-506, mir-632, mir-646, mir-668, mir-877, mir-9	mir-125b, mir-126, mir-146a, mir-146b, mir-155, mir-181b, mir-196a-1, mir-196a-2, mir-196b, mir-21, mir-221, mir-222, mir-24, mir-27b, mir-31, mir-345
ovarian carcinoma	mir-100, mir-101, mir-34b, mir-34c, mir-532-5p	mir-148b, mir-182
cholangiocarcinoma	mir-101, mir-144, mir-200b, mir-200c	mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-21, mir-26a, mir-92a
endometrial cancer	mir-101, mir-130a, mir-130b, mir-134, mir-143, mir-145, mir-152, mir-205, mir-223, mir-301a, mir-301b, mir-30c, mir-34a, mir-34c, mir-424, mir-449a, mir-543	mir-106a, mir-145, mir-155, mir-182, mir-200b, mir-200c, mir-205, mir-21, mir-222-3p, mir-25, mir-93
esophageal cancer	mir-124, mir-126, mir-140, mir-197, mir-203, mir-218, mir-223, mir-30b, mir-375, mir-454, mir-486, mir-574-3p	mir-101, mir-10b, mir-130a, mir-141, mir-143, mir-146b, mir-15a, mir-183, mir-196b, mir-200a, mir-203, mir-205, mir-21, mir-210, mir-221, mir-27a, mir-28-3p, mir-31, mir-452, mir-96, mir-99b
liver cancer	mir-101, mir-122, mir-132, mir-140-5p, mir-145, mir-148b, mir-31, mir-338-3p, mir-433	mir-1301, mir-155, mir-21, mir-221, mir-27a, mir-525-3p
pancreatic cancer	mir-101, mir-1181, mir-124, mir-1247, mir-133a, mir-141, mir-145, mir-146a, mir-148a, mir-148b, mir-150*, mir-150-5p, mir-152, mir-15a, mir-198, mir-203, mir-214, mir-216a, mir-29c, mir-335, mir-34a, mir-34b, mir-34c, mir-373, mir-	mir-10a, mir-10b, mir-132, mir-15a, mir-17-5p, mir-181a, mir-18a, mir-191, mir-196a, mir-21, mir-212, mir-214, mir-222, mir-27a, mir-301a, mir-301a-3p, mir-

Malignancy	Down-regulated miRs	Up-regulated miRs
	375, mir-410, mir-497, mir-615-5p, mir-630, mir-96	367, mir-424-5p, mir-7, mir-92, mir-99a
retinoblastoma	mir-101, mir-183, mir-204, mir-34a, mir-365b-3p, mir-486-3p, mir-532-5p	mir-181b, mir-21
cervical squamous cell carcinoma	mir-106a, mir-124, mir-148a, mir-214, mir-218, mir-29a, mir-375	mir-205
clear cell renal cell cancer	mir-106a-5p, mir-135a-5p, mir-206	mir-142-5p, mir-155, mir-21-5p
laryngeal carcinoma		mir-106b, mir-16, mir-21, mir-27a, mir-423-3p
medulloblastoma	mir-124, mir-128a, mir-199b-5p, mir-206, mir-22, mir-31, mir-383	mir-106b, mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-30b, mir-30d, mir-92
pituitary carcinoma		mir-106b, mir-122, mir-20a, mir-493
prostate carcinoma	mir-107	
cervical cancer	mir-143, mir-145, mir-17-5p, mir-203, mir-214, mir-218, mir-335, mir-342-3p, mir-372, mir-424, mir-491-5p, mir-497, mir-7, mir-99a, mir-99b	mir-10a, mir-155, mir-181a, mir-181b, mir-196a, mir-19a, mir-19b, mir-205, mir-20a, mir-21, mir-215, mir-224, mir-31, mir-494, mir-590-5p, mir-92a, mir-944
chronic myelogenous leukemia	mir-10a, mir-146a, mir-150, mir-151, mir-155, mir-2278, mir-26a, mir-30e, mir-31, mir-326, mir-564	mir-424, mir-96
gastrointestinal cancer	mir-122a, mir-148a, mir-152	
anaplastic astrocytoma	mir-124, mir-137	
astrocytoma	mir-124-3p, mir-181b-5p, mir-200b, mir-3189-3p	mir-335
epithelial ovarian cancer	mir-124a, mir-192, mir-193a, mir-7	mir-372, mir-373
mantle cell lymphoma	mir-142-3p, mir-142-5p, mir-150, mir-223, mir-29a, mir-29b, mir-29c	mir-124a, mir-155, mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-92a
chronic lymphocytic leukemia	mir-125b, mir-138, mir-15a, mir-15b, mir-16, mir-16-1, mir-16-1-3p, mir-16-2, mir-181a, mir-181b, mir-195, mir-223, mir-29b, mir-34b, mir-34c, mir-424	mir-150, mir-155
follicular cancer	NA	mir-125b
malignant mesothelioma	mir-126	
small cell lung cancer	mir-126, mir-138, mir-27a	mir-25
meningioma	mir-128, mir-200a	mir-224, mir-335
laryngeal squamous cell carcinoma	mir-129-5p, mir-203, mir-205, mir-206, mir-24, mir-370, mir-375	mir-21, mir-9, mir-93
medullary thyroid carcinoma	mir-129-5p	mir-183

Malignancy	Down-regulated miRs	Up-regulated miRs
lung adenocarcinoma	mir-1297, mir-141, mir-145, mir-16, mir-200a, mir-200b, mir-200c, mir-29b, mir-381, mir-409-3p, mir-429, mir-451, mir-511, mir-99a	mir-150, mir-155, mir-31
pancreatic carcinoma	mir-132, mir-375	mir-301b
lung squamous cell carcinoma	mir-133a, mir-218	
multiple myeloma	mir-137, mir-197, mir-214	mir-21
squamous carcinoma	mir-15a, mir-16, mir-203, mir-205, mir-375	mir-137, mir-155, mir-184, mir-196a, mir-203, mir-21, mir-221, mir-27a, mir-34a
uveal melanoma	mir-137, mir-144, mir-145, mir-182, mir-34a, mir-34b, mir-34c, mir-9	NA
anaplastic thyroid carcinoma	mir-138	mir-146b, mir-221, mir-222
colorectal carcinoma	mir-139, mir-143, mir-145, mir-202-3p, mir-30a, mir-338-3p, mir-429, mir-451, mir-93	mir-17, mir-182, mir-191, mir-21, mir-95
malt lymphoma		mir-142-5p, mir-155
thyroid cancer	mir-144, mir-886-3p	
primary cns lymphomas	mir-145, mir-193b, mir-199a, mir-214	
follicular thyroid carcinoma	mir-199b	mir-146b, mir-183, mir-197, mir-221, mir-346
gallbladder carcinoma	mir-146b-5p	mir-155, mir-182
adult t-cell leukemia		mir-150
anaplastic large-cell lymphoma		mir-155
cutaneous t-cell lymphoma		mir-155
diffuse large B-cell lymphoma		mir-155, mir-21
rectal cancer		mir-155, mir-200c, mir-21-5p, mir-34a
tongue cancer	mir-15b, mir-200b	
b-cell lymphoma	mir-34a	mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-92a
breast carcinoma		mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-24, mir-92a
nasopharyngeal cancer	mir-218, mir-223, mir-29c	mir-17, mir-20a
gastric adenocarcinoma	mir-181b, mir-182, mir-200a, mir-302b, mir-449a, mir-9	mir-23a, mir-27a, mir-373
colorectal adenocarcinoma		mir-182
colon carcinoma	mir-186, mir-30a-5p	mir-221, mir-23a
adrenal cortical carcinoma	mir-195, mir-1974, mir-335, mir-497	mir-21, mir-210, mir-483-3p, mir-483-5p

Malignancy	Down-regulated miRs	Up-regulated miRs
esophageal adenocarcinoma	mir-203	mir-196a, mir-199a-3p, mir-199a-5p, mir-199b-3p, mir-200a, mir-223
gastrointestinal stromal tumor	mir-218, mir-221, mir-222	mir-196a
uterine leiomyoma	mir-197	
choriocarcinoma	mir-199b, mir-218, mir-34a	
follicular lymphoma	mir-202	
basal cell carcinoma	mir-203	
hypopharyngeal cancer		mir-203
pancreatic adenocarcinoma		mir-203, mir-301a
rhabdomyosarcoma	mir-203	
head and neck cancer	NA	mir-21
hypopharyngeal squamous cell carcinoma	mir-451a, mir-504	mir-21
t-cell lymphoma	mir-22	
thyroid carcinoma		mir-221, mir-222
splenic marginal zone lymphoma	mir-223	
laryngeal cancer		mir-23a
primary thyroid lymphoma	mir-26a	
acute leukemia	mir-27a	
monocytic leukemia	mir-29a, mir-29b	
oral carcinoma	mir-375	mir-31
primary gallbladder carcinoma	mir-335	
endometrial serous adenocarcinoma	mir-34b	
esophageal carcinoma	mir-451	
hepatoblastoma		mir-492
colonic adenocarcinoma	mir-627	

Table 4: Exemplary oncogenic miRs

Cancer	miRNA
colorectal cancer	let-7a, mir-103, mir-106a, mir-10b, mir-1179, mir-1229, mir-1246, mir-125b-2*, mir-1269a, mir-130b, mir-133b, mir-135a, mir-135a-1, mir-135a-2, mir-135b, mir-139-3p, mir-145, mir-150, mir-150*, mir-155, mir-17, mir-181a, mir-182, mir-183, mir-18a, mir-191, mir-196a, mir-196b, mir-19a, mir-19b, mir-200b, mir-200c, mir-203, mir-

Cancer	miRNA
	204-5p, mir-20a, mir-20a-5p, mir-21, mir-210, mir-211, mir-221, mir-223, mir-224, mir-23a, mir-25, mir-27a, mir-29a, mir-301a, mir-31, mir-32, mir-320b, mir-326, mir-424, mir-429, mir-494, mir-497, mir-499-5p, mir-592, mir-630, mir-720, mir-892a, mir-92, mir-92a, mir-93, mir-95, mir-96
papillary thyroid carcinoma	let-7e, mir-146b, mir-146b-5p, mir-151-5p, mir-155, mir-181a-1, mir-181a-2, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-183, mir-199b-5p, mir-21, mir-221, mir-222, mir-339-5p, mir-34a
esophageal squamous cell carcinoma	mir-100, mir-1179, mir-1290, mir-130b, mir-145, mir-16, mir-17, mir-183, mir-18a, mir-19a, mir-19b, mir-208, mir-20a, mir-21, mir-218, mir-223, mir-25, mir-30a-5p, mir-31, mir-330-3p, mir-373, mir-9, mir-92a, mir-942
gastric cancer	mir-100, mir-103, mir-106a, mir-106b, mir-107, mir-10a, mir-10b, mir-1259, mir-125b, mir-126, mir-1274a, mir-1303, mir-130b*, mir-135a-5p, mir-135b, mir-138, mir-143, mir-146a, mir-147, mir-148a, mir-150, mir-17, mir-17-5p, mir-181a, mir-181a-2*, mir-181a-5p, mir-181c, mir-183, mir-185, mir-18a, mir-191, mir-192, mir-196a, mir-196a*, mir-196a-5p, mir-196b, mir-199a, mir-199a-3p, mir-199a-5p, mir-19a, mir-19b, mir-200b, mir-20a, mir-21, mir-214, mir-215, mir-221, mir-221*, mir-222, mir-223, mir-224, mir-23a, mir-23b, mir-25, mir-27a, mir-27b, mir-296-5p, mir-301a, mir-302f, mir-337-3p, mir-340*, mir-34a, mir-362-3p, mir-370, mir-374a, mir-377, mir-421, mir-425, mir-500, mir-520c-3p, mir-544, mir-575, mir-601, mir-616*, mir-650, mir-92, mir-98, mir-99a
renal cell carcinoma	mir-100, mir-1233, mir-1260b, mir-146a, mir-146b, mir-16, mir-193a-3p, mir-203a, mir-21, mir-210, mir-27a, mir-362, mir-572, mir-7
esophageal cancer	mir-101, mir-10b, mir-130a, mir-141, mir-143, mir-146b, mir-15a, mir-183, mir-196b, mir-200a, mir-203, mir-205, mir-21, mir-210, mir-221, mir-27a, mir-28-3p, mir-31, mir-452, mir-96, mir-99b
bladder cancer	mir-103a-3p, mir-10b, mir-135a, mir-137, mir-141, mir-155, mir-17-5p, mir-182, mir-182-5p, mir-183, mir-185, mir-19a, mir-203, mir-205, mir-210, mir-221, mir-222, mir-223, mir-23a, mir-23b, mir-26b, mir-639, mir-96
endometrial cancer	mir-106a, mir-145, mir-155, mir-182, mir-200b, mir-200c, mir-205, mir-21, mir-222-3p, mir-25, mir-93
ovarian cancer	mir-106a, mir-141, mir-148b, mir-181b, mir-182, mir-200a, mir-200c, mir-205, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-224-5p, mir-23b, mir-25, mir-26a, mir-27a, mir-27b, mir-346, mir-378, mir-424, mir-503, mir-572, mir-9, mir-96
glioma	mir-106b, mir-106b-5p, mir-10b, mir-125b, mir-132, mir-155, mir-17, mir-181a, mir-182, mir-183, mir-193b, mir-19a, mir-19b, mir-20a, mir-210, mir-214, mir-221, mir-222, mir-224, mir-23a, mir-24, mir-24-3p, mir-25, mir-26a, mir-27a-3p, mir-27b, mir-30a-5p, mir-30e, mir-30e*, mir-328, mir-335, mir-33a, mir-372, mir-486, mir-494, mir-497, mir-566, mir-603, mir-650, mir-675, mir-9, mir-92b, mir-93, mir-96
head and neck squamous cell carcinoma	mir-106b, mir-134, mir-16, mir-184, mir-196a, mir-21, mir-25, mir-30a-5p, mir-31, mir-372, mir-93

Cancer	miRNA
hepatocellular carcinoma	mir-106b, mir-10b, mir-122, mir-1228, mir-1269, mir-128a, mir-130a, mir-130b, mir-146a, mir-153, mir-155, mir-17-5p, mir-181a, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-183, mir-184, mir-190b, mir-191, mir-20a, mir-20b, mir-21, mir-210, mir-214, mir-215, mir-216a, mir-217, mir-221, mir-222, mir-223, mir-224, mir-23a, mir-24, mir-25, mir-27a, mir-301a, mir-30d, mir-31, mir-3127, mir-32, mir-331-3p, mir-362-3p, mir-362-5p, mir-371-5p, mir-372, mir-373, mir-423, mir-429, mir-452, mir-483-3p, mir-483-5p, mir-485-3p, mir-490-3p, mir-494, mir-495, mir-500, mir-501, mir-501-5p, mir-519d, mir-520g, mir-574-3p, mir-590-5p, mir-630, mir-650, mir-657, mir-664, mir-885-5p, mir-9, mir-92a, mir-96
laryngeal carcinoma	mir-106b, mir-16, mir-21, mir-27a, mir-423-3p
medulloblastoma	mir-106b, mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-30b, mir-30d, mir-92
pituitary carcinoma	mir-106b, mir-122, mir-17-5p, mir-20a, mir-493
cervical cancer	mir-10a, mir-155, mir-181a, mir-181b, mir-196a, mir-19a, mir-19b, mir-205, mir-20a, mir-21, mir-215, mir-224, mir-31, mir-494, mir-590-5p, mir-92a, mir-944
pancreatic cancer	mir-10a, mir-10b, mir-132, mir-15a, mir-17-5p, mir-181a, mir-18a, mir-191, mir-196a, mir-21, mir-212, mir-214, mir-221, mir-222, mir-27a, mir-301a, mir-301a-3p, mir-367, mir-424-5p, mir-7, mir-92, mir-99a
breast cancer	mir-10b, mir-125a, mir-135a, mir-140, mir-141, mir-142, mir-150, mir-155, mir-17, mir-17-5p, mir-181a, mir-181b, mir-182, mir-18a, mir-18b, mir-191, mir-196a, mir-197, mir-19a, mir-19b, mir-200a, mir-200b, mir-200c, mir-203, mir-205, mir-20a, mir-20b, mir-21, mir-217, mir-221, mir-222, mir-224, mir-23a, mir-24, mir-24-2-5p, mir-24-3p, mir-27a, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-373, mir-378, mir-423, mir-429, mir-495, mir-503, mir-510, mir-520c, mir-526b, mir-96
glioblastoma	mir-10b, mir-125b, mir-127-3p, mir-148a, mir-18a, mir-196a, mir-196a-1, mir-196a-2, mir-196b, mir-21, mir-210, mir-210-3p, mir-223, mir-340, mir-576-5p, mir-626, mir-92b
lung cancer	mir-10b, mir-135b, mir-150, mir-155, mir-17, mir-182, mir-183-3p, mir-18a, mir-197, mir-19a, mir-19b, mir-205, mir-20a, mir-21, mir-210, mir-24, mir-30d, mir-4423, mir-5100, mir-570, mir-663, mir-7, mir-92a
nasopharyngeal carcinoma	mir-10b, mir-144, mir-149, mir-155, mir-18a, mir-21, mir-214, mir-24, mir-421, mir-663, mir-744, mir-93
non-small cell lung cancer	mir-10b, mir-125a-5p, mir-1280, mir-136, mir-140, mir-141, mir-142-3p, mir-145, mir-146a, mir-150, mir-18a, mir-196a, mir-19a, mir-200a, mir-200c, mir-205, mir-205-3p, mir-205-5p, mir-21, mir-212, mir-22, mir-221, mir-222, mir-24, mir-25, mir-29c, mir-31, mir-328, mir-330-3p, mir-339, mir-34a, mir-375, mir-494, mir-675-5p, mir-9, mir-92b, mir-93, mir-95
oral cancer	mir-10b, mir-196a-1, mir-196a-2, mir-196b, mir-21
pancreatic ductal adenocarcinoma	mir-10b, mir-186, mir-18a, mir-192, mir-194, mir-196a, mir-198, mir-203, mir-21, mir-212, mir-30b-5p, mir-31, mir-34a, mir-369-5p, mir-376a, mir-541
renal clear cell carcinoma	mir-122, mir-155, mir-210, mir-630
mantle cell lymphoma	mir-124a, mir-155, mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-92a

Cancer	miRNA
acute myeloid leukemia	mir-125b, mir-126-5p, mir-128, mir-155, mir-29a, mir-32, mir-331, mir-370, mir-378
follicular cancer	mir-125b
neuroblastoma	mir-125b, mir-15a, mir-15b, mir-16-1, mir-16-2, mir-18a, mir-195, mir-19a, mir-23a, mir-421, mir-92
oral squamous cell carcinoma	mir-125b, mir-126, mir-146a, mir-146b, mir-155, mir-181b, mir-196a-1, mir-196a-2, mir-196b, mir-21, mir-221, mir-222, mir-24, mir-27b, mir-31, mir-345
prostate cancer	mir-125b, mir-141, mir-153, mir-155, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-182, mir-182-5p, mir-183, mir-18a, mir-204, mir-20a, mir-21, mir-221, mir-223-3p, mir-31, mir-429, mir-96
mesenchymal cancer	mir-125b-1-3p, mir-182
malignant melanoma	mir-126, mir-141, mir-15b, mir-17, mir-17-5p, mir-182, mir-18a, mir-193b, mir-200a, mir-200b, mir-200c, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-222, mir-429, mir-455-5p, mir-532-5p, mir-638, mir-92a
acute lymphoblastic leukemia	mir-128
osteosarcoma	mir-128, mir-151-3p, mir-17, mir-181a, mir-181b, mir-181c, mir-18a, mir-191, mir-195-5p, mir-199a-3p, mir-19a, mir-19b, mir-20a, mir-21, mir-210, mir-214, mir-221, mir-27a, mir-300, mir-320a, mir-374a-5p, mir-802, mir-9, mir-92a
colon cancer	mir-1290, mir-145, mir-155, mir-181a, mir-18a, mir-200c, mir-31, mir-675
liver cancer	mir-1301, mir-155, mir-21, mir-221, mir-27a, mir-525-3p
cervical carcinoma	mir-133b, mir-21, mir-25, mir-373
squamous carcinoma	mir-137, mir-155, mir-184, mir-196a, mir-203, mir-21, mir-221, mir-27a, mir-34a
chordoma	mir-140-3p, mir-148a
clear cell renal cell cancer	mir-142-5p, mir-155, mir-21-5p
malt lymphoma	mir-142-5p, mir-155
anaplastic thyroid carcinoma	mir-146b, mir-221, mir-222
follicular thyroid carcinoma	mir-146b, mir-183, mir-197, mir-221, mir-346
primary thyroid lymphoma	mir-146b
ovarian carcinoma	mir-148b, mir-182
adult t-cell leukemia	mir-150
chronic lymphocytic leukemia	mir-150, mir-155
lung adenocarcinoma	mir-150, mir-155, mir-31
anaplastic large-cell lymphoma	mir-155

Cancer	miRNA
cutaneous t-cell lymphoma	mir-155
diffuse large B-cell lymphoma	mir-155, mir-21
gallbladder carcinoma	mir-155, mir-182
rectal cancer	mir-155, mir-200c, mir-21-5p, mir-34a
b-cell lymphoma	mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-92a
breast carcinoma	mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-24, mir-92a
cholangiocarcinoma	mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-21, mir-26a, mir-92a
colorectal carcinoma	mir-17, mir-182, mir-191, mir-21, mir-95
nasopharyngeal cancer	mir-17, mir-20a
acute promyelocytic leukemia	mir-181a, mir-181b, mir-92a
retinoblastoma	mir-181b, mir-21
colorectal adenocarcinoma	mir-182
kidney cancer	mir-183, mir-21, mir-210, mir-223
medullary thyroid carcinoma	mir-183
esophageal adenocarcinoma	mir-196a, mir-199a-3p, mir-199a-5p, mir-199b-3p, mir-200a, mir-223
gastrointestinal stromal tumor	mir-196a
hypopharyngeal cancer	mir-203
pancreatic adenocarcinoma	mir-203, mir-301a
cervical squamous cell carcinoma	mir-205
adrenal cortical carcinoma	mir-21, mir-210, mir-483-3p, mir-483-5p
head and neck cancer	mir-21
hypopharyngeal squamous cell carcinoma	mir-21
laryngeal squamous cell carcinoma	mir-21, mir-9, mir-93
multiple myeloma	mir-21
colon carcinoma	mir-221, mir-23a
thyroid carcinoma	mir-221, mir-222
meningioma	mir-224, mir-335

Cancer	miRNA
gastric adenocarcinoma	mir-23a, mir-27a, mir-373
laryngeal cancer	mir-23a
small cell lung cancer	mir-25
pancreatic carcinoma	mir-301b
oral carcinoma	mir-31
astrocytoma	mir-335
epithelial ovarian cancer	mir-372, mir-373
chronic myelogenous leukemia	mir-424, mir-96
hepatoblastoma	mir-492

Table 3: Exemplary tumor suppressive miRs

Cancer	Down regulated tumor suppressive miR
acute leukemia	mir-27a
acute lymphoblastic leukemia	let-7b, mir-124a, mir-142-3p
acute myeloid leukemia	let-7c, mir-17, mir-181a, mir-20a, mir-223, mir-26a, mir-29a, mir-30c, mir-720
acute promyelocytic leukemia	let-7c, mir-107, mir-342
adrenal cortical carcinoma	mir-195, mir-1974, mir-335, mir-497
anaplastic astrocytoma	mir-124, mir-137
anaplastic thyroid carcinoma	mir-138
astrocytoma	mir-124-3p, mir-181b-5p, mir-200b, mir-3189-3p
basal cell carcinoma	mir-203
b-cell lymphoma	mir-34a
bladder cancer	mir-1, mir-101, mir-1180, mir-1236, mir-124-3p, mir-125b, mir-126, mir-1280, mir-133a, mir-133b, mir-141, mir-143, mir-144, mir-145, mir-155, mir-16, mir-18a, mir-192, mir-195, mir-200a, mir-200b, mir-200c, mir-203, mir-205, mir-214, mir-218, mir-23b, mir-26a, mir-29c, mir-320c, mir-34a, mir-370, mir-409-3p, mir-429, mir-451, mir-490-5p, mir-493, mir-576-3p, mir-99a
breast cancer	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-100, mir-107, mir-10a, mir-10b, mir-122, mir-124, mir-1258, mir-125a-5p, mir-125b, mir-126, mir-127, mir-129, mir-130a, mir-132, mir-133a, mir-143, mir-145, mir-146a, mir-146b, mir-147, mir-148a, mir-149, mir-152, mir-153, mir-15a, mir-16, mir-17-5p, mir-181a, mir-1826, mir-183, mir-185, mir-191, mir-193a-3p, mir-193b, mir-195, mir-199b-5p, mir-19a-3p, mir-200a, mir-200b, mir-200c, mir-205, mir-206, mir-211, mir-216b, mir-218, mir-22, mir-26a, mir-26b, mir-300, mir-30a, mir-31, mir-335, mir-339-5p, mir-33b, mir-34a, mir-34b, mir-34c, mir-374a, mir-379, mir-381, mir-383, mir-425, mir-429, mir-450b-

Cancer	Down regulated tumor suppressive miR
	3p, mir-494, mir-495, mir-497, mir-502-5p, mir-517a, mir-574-3p, mir-638, mir-7, mir-720, mir-873, mir-874, mir-92a, mir-98, mir-99a, mmu-mir-290-3p, mmu-mir-290-5p
bronchioloalveolar carcinoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-98
cervical cancer	mir-143, mir-145, mir-17-5p, mir-203, mir-214, mir-218, mir-335, mir-342-3p, mir-372, mir-424, mir-491-5p, mir-497, mir-7, mir-99a, mir-99b
cervical carcinoma	mir-100, mir-101, mir-15a, mir-16, mir-34a, mir-886-5p, mir-99a, mir-99b
cervical squamous cell carcinoma	mir-106a, mir-124, mir-148a, mir-214, mir-218, mir-29a, mir-375
cholangiocarcinoma	mir-101, mir-144, mir-200b, mir-200c
chondrosarcoma	let-7a, mir-100, mir-136, mir-145, mir-199a, mir-222, mir-30a, mir-335, mir-376a
chordoma	mir-1, mir-222, mir-31, mir-34a, mir-608
choriocarcinoma	mir-199b, mir-218, mir-34a
chronic lymphocytic leukemia	mir-125b, mir-138, mir-15a, mir-15b, mir-16, mir-16-1, mir-16-1-3p, mir-16-2, mir-181a, mir-181b, mir-195, mir-223, mir-29b, mir-34b, mir-34c, mir-424
chronic myelogenous leukemia	mir-10a, mir-138, mir-146a, mir-150, mir-151, mir-155, mir-16, mir-2278, mir-26a, mir-30e, mir-31, mir-326, mir-564
clear cell renal cell cancer	mir-106a-5p, mir-135a-5p, mir-206
colon cancer	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-100, mir-101, mir-126, mir-142-3p, mir-143, mir-145, mir-192, mir-200c, mir-21, mir-214, mir-215, mir-22, mir-25, mir-302a, mir-320, mir-320a, mir-34a, mir-34c, mir-365, mir-373, mir-424, mir-429, mir-455, mir-484, mir-502, mir-503, mir-93, mir-98
colon carcinoma	mir-186, mir-30a-5p
colonic adenocarcinoma	mir-627
colorectal cancer	let-7a, mir-1, mir-100, mir-101, mir-124, mir-125a, mir-126, mir-129, mir-1295b-3p, mir-1307, mir-130b, mir-132, mir-133a, mir-133b, mir-137, mir-138, mir-139, mir-139-5p, mir-140-5p, mir-143, mir-145, mir-148a, mir-148b, mir-149, mir-150-5p, mir-154, mir-15a, mir-15b, mir-16, mir-18a, mir-191, mir-192, mir-193a-5p, mir-194, mir-195, mir-196a, mir-198, mir-199a-5p, mir-200c, mir-203, mir-204-5p, mir-206, mir-212, mir-215, mir-218, mir-22, mir-224, mir-24-3p, mir-26b, mir-27a, mir-28-3p, mir-28-5p, mir-29b, mir-30a-3p, mir-30b, mir-320a, mir-328, mir-338-3p, mir-342, mir-345, mir-34a, mir-34a-5p, mir-361-5p, mir-375, mir-378, mir-378a-3p, mir-378a-5p, mir-409-3p, mir-422a, mir-4487, mir-483, mir-497, mir-498, mir-518a-3p, mir-551a, mir-574-5p, mir-625, mir-638, mir-7, mir-96-5p
colorectal carcinoma	mir-139, mir-143, mir-145, mir-202-3p, mir-30a, mir-338-3p, mir-429, mir-451, mir-93
endometrial cancer	mir-101, mir-130a, mir-130b, mir-134, mir-143, mir-145, mir-152, mir-205, mir-223, mir-301a, mir-301b, mir-30c, mir-34a, mir-34c, mir-424, mir-449a, mir-543
endometrial serous adenocarcinoma	mir-34b
epithelial ovarian cancer	mir-124a, mir-192, mir-193a, mir-7

Cancer	Down regulated tumor suppressive miR
esophageal adenocarcinoma	mir-203
esophageal cancer	mir-124, mir-126, mir-140, mir-197, mir-203, mir-218, mir-223, mir-30b, mir-375, mir-454, mir-486, mir-574-3p
esophageal carcinoma	mir-451
esophageal squamous cell carcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-100, mir-101, mir-126, mir-1294, mir-133a, mir-133b, mir-138, mir-143, mir-145, mir-150, mir-185, mir-195, mir-200b, mir-203, mir-21, mir-210, mir-214, mir-218, mir-22, mir-27a, mir-29b, mir-29c, mir-302b, mir-34a, mir-375, mir-494, mir-518b, mir-655, mir-98, mir-99a
follicular lymphoma	mir-202
follicular thyroid carcinoma	mir-199b
gallbladder carcinoma	mir-146b-5p
gastric adenocarcinoma	mir-181b, mir-182, mir-200a, mir-302b, mir-449a, mir-9
gastric cancer	let-7a, let-7b, let-7g, mir-1, mir-101, mir-103a, mir-10a, mir-10b, mir-1207-5p, mir-122, mir-1228*, mir-124, mir-124-3p, mir-125a-3p, mir-126, mir-1266, mir-127, mir-1271, mir-129-1-3p, mir-129-2-3p, mir-129-3p, mir-129-5p, mir-133a, mir-133b, mir-137, mir-141, mir-143, mir-144, mir-145, mir-146a, mir-146a-5p, mir-148a, mir-148b, mir-149, mir-152, mir-155, mir-155-5p, mir-181a, mir-181b, mir-182, mir-183, mir-185, mir-194, mir-195, mir-197, mir-199a-3p, mir-200b, mir-200c, mir-202-3p, mir-204, mir-204-5p, mir-205, mir-206, mir-210, mir-212, mir-217, mir-218, mir-22, mir-23b, mir-24, mir-26a, mir-29a, mir-29a-3p, mir-29b, mir-29b-1, mir-29b-2, mir-29c, mir-30a-5p, mir-30b, mir-31, mir-328, mir-329, mir-331-3p, mir-335-5p, mir-338, mir-338-3p, mir-34a, mir-34b, mir-34c, mir-361-5p, mir-367, mir-375, mir-378, mir-409-3p, mir-410, mir-429, mir-433, mir-449, mir-449a, mir-490-3p, mir-494, mir-497, mir-503, mir-506, mir-513b, mir-520d-3p, mir-542-3p, mir-622, mir-625, mir-638, mir-663, mir-7, mir-874, mir-9
gastrointestinal cancer	mir-122a, mir-148a, mir-152
gastrointestinal stromal tumor	mir-218, mir-221, mir-222
glioblastoma	let-7g-5p, mir-100, mir-101, mir-106a, mir-124, mir-124a, mir-125a, mir-125a-5p, mir-125b, mir-127-3p, mir-128, mir-129, mir-136, mir-137, mir-139-5p, mir-142-3p, mir-143, mir-145, mir-146b-5p, mir-149, mir-152, mir-153, mir-195, mir-21, mir-212-3p, mir-219-5p, mir-222, mir-29b, mir-31, mir-3189-3p, mir-320, mir-320a, mir-326, mir-330, mir-331-3p, mir-340, mir-342, mir-34a, mir-376a, mir-449a, mir-483-5p, mir-503, mir-577, mir-663, mir-7, mir-7-5p, mir-873
glioma	let-7a, let-7f, mir-106a, mir-107, mir-122, mir-124, mir-124-5p, mir-124a, mir-125b, mir-128, mir-136, mir-137, mir-139, mir-143, mir-145, mir-146a, mir-146b, mir-146b-5p, mir-152, mir-15b, mir-16, mir-181a, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-184, mir-185, mir-195, mir-199a-3p, mir-200a, mir-200b, mir-203, mir-204, mir-205, mir-218, mir-219-5p, mir-23b, mir-26b, mir-27a, mir-29c, mir-320, mir-326, mir-328, mir-34a, mir-34c-3p, mir-34c-5p, mir-375, mir-383, mir-451, mir-452, mir-483-5p, mir-495, mir-584, mir-622, mir-656, mir-7, mir-98

Cancer	Down regulated tumor suppressive miR
head and neck squamous cell carcinoma	let-7d, mir-1, mir-107, mir-128, mir-133a, mir-138, mir-149, mir-200c, mir-205, mir-218, mir-27a*, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-300, mir-34a, mir-363, mir-375, mir-874
hepatocellular carcinoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-100, mir-101, mir-105, mir-122, mir-122a, mir-1236, mir-124, mir-125b, mir-126, mir-127, mir-1271, mir-128-3p, mir-129-5p, mir-130a, mir-130b, mir-133a, mir-134, mir-137, mir-138, mir-139, mir-139-5p, mir-140-5p, mir-141, mir-142-3p, mir-143, mir-144, mir-145, mir-146a, mir-148a, mir-148b, mir-150-5p, mir-15b, mir-16, mir-181a-5p, mir-185, mir-188-5p, mir-193b, mir-195, mir-195-5p, mir-197, mir-198, mir-199a, mir-199a-5p, mir-199b, mir-199b-5p, mir-200a, mir-200b, mir-200c, mir-202, mir-203, mir-204-3p, mir-205, mir-206, mir-20a, mir-21, mir-21-3p, mir-211, mir-212, mir-214, mir-217, mir-218, mir-219-5p, mir-22, mir-223, mir-26a, mir-26b, mir-29a, mir-29b-1, mir-29b-2, mir-29c, mir-302b, mir-302c, mir-30a, mir-30a-3p, mir-335, mir-338-3p, mir-33a, mir-34a, mir-34b, mir-365, mir-370, mir-372, mir-375, mir-376a, mir-377, mir-422a, mir-424, mir-424-5p, mir-433, mir-4458, mir-448, mir-450a, mir-451, mir-485-5p, mir-486-5p, mir-497, mir-503, mir-506, mir-519d, mir-520a, mir-520b, mir-520c-3p, mir-582-5p, mir-590-5p, mir-610, mir-612, mir-625, mir-637, mir-675, mir-7, mir-877, mir-940, mir-941, mir-98, mir-99a
hypopharyngeal squamous cell carcinoma	mir-451a, mir-504
kidney cancer	mir-1, mir-145, mir-1826, mir-199a, mir-199a-3p, mir-203, mir-205, mir-497, mir-508-3p, mir-509-3p
laryngeal squamous cell carcinoma	mir-129-5p, mir-203, mir-205, mir-206, mir-24, mir-370, mir-375
liver cancer	mir-101, mir-122, mir-132, mir-140-5p, mir-145, mir-148b, mir-31, mir-338-3p, mir-433
lung adenocarcinoma	mir-1297, mir-141, mir-145, mir-16, mir-200a, mir-200b, mir-200c, mir-29b, mir-381, mir-409-3p, mir-429, mir-451, mir-511, mir-99a
lung cancer	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-101, mir-133b, mir-138, mir-142-5p, mir-144, mir-145, mir-1469, mir-146a, mir-153, mir-15a, mir-15b, mir-16-1, mir-16-2, mir-182, mir-192, mir-193a-3p, mir-194, mir-195, mir-198, mir-203, mir-217, mir-218, mir-22, mir-223, mir-26a, mir-26b, mir-29c, mir-33a, mir-34a, mir-34b, mir-34c, mir-365, mir-449a, mir-449b, mir-486-5p, mir-545, mir-610, mir-614, mir-630, mir-660, mir-7515, mir-9500, mir-98, mir-99b
lung squamous cell carcinoma	mir-133a, mir-218
malignant melanoma	let-7b, mir-101, mir-125b, mir-1280, mir-143, mir-146a, mir-146b, mir-155, mir-17, mir-184, mir-185, mir-18b, mir-193b, mir-200c, mir-203, mir-204, mir-205, mir-206, mir-20a, mir-211, mir-218, mir-26a, mir-31, mir-33a, mir-34a, mir-34c, mir-376a, mir-376c, mir-573, mir-7-5p, mir-9, mir-98
malignant mesothelioma	mir-126
mantle cell lymphoma	mir-142-3p, mir-142-5p, mir-150, mir-223, mir-29a, mir-29b, mir-29c
medullary thyroid carcinoma	mir-129-5p

Cancer	Down regulated tumor suppressive miR
medulloblastoma	mir-124, mir-128a, mir-199b-5p, mir-206, mir-22, mir-31, mir-383
meningioma	mir-128, mir-200a
mesenchymal cancer	mir-100, mir-141, mir-199b-5p, mir-200a, mir-200b, mir-200c, mir-29a, mir-29b-1, mir-29b-1-5p, mir-29b-2, mir-29c, mir-335, mir-429, mir-99a
monocytic leukemia	mir-29a, mir-29b
multiple myeloma	mir-137, mir-197, mir-214
nasopharyngeal cancer	mir-218, mir-223, mir-29c
nasopharyngeal carcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-1, mir-101, mir-124, mir-138, mir-143, mir-145, mir-148a, mir-200b, mir-204, mir-216b, mir-223, mir-29c, mir-320a, mir-324-3p, mir-34c, mir-375, mir-378, mir-451, mir-506, mir-9, mir-98
neuroblastoma	let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-124, mir-137, mir-145, mir-181c, mir-184, mir-200a, mir-29a, mir-335, mir-338-3p, mir-34a, mir-449a, mir-885-5p, mir-98
non-small cell lung cancer	let-7a, let-7c, mir-1, mir-100, mir-101, mir-106a, mir-107, mir-124, mir-125a-3p, mir-125a-5p, mir-126, mir-126*, mir-129, mir-133a, mir-137, mir-138, mir-140, mir-143, mir-145, mir-146a, mir-146b, mir-148a, mir-148b, mir-149, mir-152, mir-153, mir-154, mir-155, mir-15a, mir-16, mir-17-5p, mir-181a-1, mir-181a-2, mir-181b, mir-181b-1, mir-181b-2, mir-181c, mir-181d, mir-184, mir-186, mir-193b, mir-195, mir-199a, mir-204, mir-212, mir-221, mir-224, mir-26b, mir-27a, mir-27b, mir-29a, mir-29b, mir-29c, mir-30a, mir-30b, mir-30c, mir-30d, mir-30d-5p, mir-30e-5p, mir-32, mir-335, mir-338-3p, mir-340, mir-342-3p, mir-34a, mir-34b, mir-361-3p, mir-365, mir-373, mir-375, mir-429, mir-449a, mir-4500, mir-451, mir-4782-3p, mir-497, mir-503, mir-512-3p, mir-520a-3p, mir-526b, mir-625*, mir-96, mir-99a
oral cancer	let-7d, mir-218, mir-34a, mir-375, mir-494
oral carcinoma	mir-375
oral squamous cell carcinoma	mir-100, mir-124, mir-1250, mir-125b, mir-126, mir-1271, mir-136, mir-138, mir-145, mir-147, mir-148a, mir-181a, mir-206, mir-220a, mir-26a, mir-26b, mir-29a, mir-32, mir-323-5p, mir-329, mir-338, mir-370, mir-410, mir-429, mir-433, mir-499a-5p, mir-503, mir-506, mir-632, mir-646, mir-668, mir-877, mir-9
osteosarcoma	let-7a, mir-1, mir-100, mir-101, mir-122, mir-124, mir-125b, mir-126, mir-127-3p, mir-132, mir-133a, mir-141, mir-142-3p, mir-142-5p, mir-143, mir-144, mir-145, mir-153, mir-16, mir-183, mir-194, mir-195, mir-199a-3p, mir-204, mir-212, mir-217, mir-218, mir-22, mir-23a, mir-24, mir-26a, mir-26b, mir-29b, mir-32, mir-320, mir-335, mir-33b, mir-340, mir-34a, mir-34b, mir-34c, mir-375, mir-376c, mir-382, mir-3928, mir-424, mir-429, mir-449a, mir-451, mir-454, mir-503, mir-519d, mir-646
ovarian cancer	let-7i, mir-100, mir-124, mir-125b, mir-129-5p, mir-130b, mir-133a, mir-137, mir-138, mir-141, mir-145, mir-148a, mir-152, mir-153, mir-155, mir-199a, mir-200a, mir-200b, mir-200c, mir-212, mir-335, mir-34a, mir-34b, mir-34c, mir-409-3p, mir-411, mir-429, mir-432, mir-449a, mir-494, mir-497, mir-498, mir-519d, mir-655, mir-9, mir-98
ovarian carcinoma	mir-100, mir-101, mir-34b, mir-34c, mir-532-5p

Cancer	Down regulated tumor suppressive miR
pancreatic cancer	mir-101, mir-1181, mir-124, mir-1247, mir-133a, mir-141, mir-145, mir-146a, mir-148a, mir-148b, mir-150*, mir-150-5p, mir-152, mir-15a, mir-198, mir-203, mir-214, mir-216a, mir-29c, mir-335, mir-34a, mir-34b, mir-34c, mir-373, mir-375, mir-410, mir-497, mir-615-5p, mir-630, mir-96
pancreatic carcinoma	mir-132, mir-375
pancreatic ductal adenocarcinoma	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, mir-126, mir-135a, mir-143, mir-144, mir-145, mir-148a, mir-150, mir-15a, mir-16, mir-200a, mir-200b, mir-200c, mir-217, mir-218, mir-337, mir-375, mir-494, mir-615-5p, mir-98
papillary thyroid carcinoma	mir-101, mir-130b, mir-138, mir-146a, mir-16, mir-195, mir-199a-3p, mir-204-5p, mir-219-5p, mir-26a, mir-34b, mir-613
primary cns lymphomas	mir-145, mir-193b, mir-199a, mir-214
primary gallbladder carcinoma	mir-335
primary thyroid lymphoma	mir-26a
prostate cancer	let-7a-3p, let-7c, mir-100, mir-101, mir-105, mir-124, mir-128, mir-1296, mir-130b, mir-133a-1, mir-133a-2, mir-133b, mir-135a, mir-143, mir-145, mir-146a, mir-154, mir-15a, mir-187, mir-188-5p, mir-199b, mir-200b, mir-203, mir-205, mir-212, mir-218, mir-221, mir-224, mir-23a, mir-23b, mir-25, mir-26a, mir-26b, mir-29b, mir-302a, mir-30a, mir-30b, mir-30c-1, mir-30c-2, mir-30d, mir-30e, mir-31, mir-330, mir-331-3p, mir-34a, mir-34b, mir-34c, mir-374b, mir-449a, mir-4723-5p, mir-497, mir-628-5p, mir-642a-5p, mir-765, mir-940
prostate carcinoma	mir-107
renal cell carcinoma	let-7a, let-7d, mir-1, mir-106a*, mir-126, mir-1285, mir-129-3p, mir-1291, mir-133a, mir-135a, mir-138, mir-141, mir-143, mir-145, mir-182-5p, mir-199a-3p, mir-200a, mir-205, mir-218, mir-28-5p, mir-30a, mir-30c, mir-30d, mir-34a, mir-378, mir-429, mir-509-3p, mir-509-5p, mir-646
renal clear cell carcinoma	let-7b, let-7c, mir-138, mir-141, mir-200c, mir-204, mir-218, mir-335, mir-377, mir-506
retinoblastoma	mir-101, mir-183, mir-204, mir-34a, mir-365b-3p, mir-486-3p, mir-532-5p
rhabdomyosarcoma	mir-203
small cell lung cancer	mir-126, mir-138, mir-27a
splenic marginal zone lymphoma	mir-223
squamous carcinoma	mir-15a, mir-16, mir-203, mir-205, mir-375
t-cell lymphoma	mir-22
thyroid cancer	mir-144, mir-886-3p
tongue cancer	mir-15b, mir-200b
uterine leiomyoma	mir-197
uveal melanoma	mir-137, mir-144, mir-145, mir-182, mir-34a, mir-34b, mir-34c, mir-9

Table 5. Summary of microRNA/MMP linked interactions in cancer.

microRNA	MMP Type and target molecule	Cancer type	Phenotype	Pathway
let-7	MMP-9	Melanoma	Cell proliferation and migration	-
	MMP-14, ERK1/2 activation	Pancreatic ductal adenocarcinoma	NA	ERK1/2 activation, TGF-β1 signaling
	Focal adhesion kinase (FAK), AKT, ERK, MMP-2 and MMP-9	Glioblastoma	Migration and invasion	AKT and ERK
miR-9	MMP-2, MMP-9 and VEGFA	Uveal melanoma	Migration and invasion	NF-κB1 signaling
	MMP-14	Neuroblastoma	Invasion, metastasis, and angiogenesis	-
miR-10b	MMP-9, E-cadherin and vimentin	Nasopharyngeal carcinoma cells	Proliferation, migration, invasion	-
	MMP-14 and uPAR	Glioma	Cell invasiveness	-
	MMP-2, EGFR.	Glioblastoma multiforme	Apoptosis invasion and migration	EGFR pathways
miR-15b	MMP-3	Glioma	Cell invasiveness	MEK-ERK pathway
miR-17	MMP-3	Hepatocellular carcinoma	Migration and invasion	p-AKT
miR-21	RECK, MMP-9	Prostate cancer	NA	-
	Phospho-c-Jun, MMP-2, MMP-9	Hepatocellular carcinoma	Migration and invasion	-
	RECK, MMP-2	Glioma	Apoptosis, migration, and invasiveness	-
	MMP-2, EGFR.	Glioblastoma multiforme	Apoptosis invasion and migration	EGFR pathways
miR-26a	MMP-2	Lung cancer	Migration, invasion and metastasis	AKT phosphorylation
miR-29b	MMP-2	Colon cancer	Migration	-
	MMP-2	Hepatocellular carcinoma	Tumor angiogenesis, invasion, and metastasis	VEGFR-2-signaling
	MMP-2, Mcl-1, COL1A1, and COL4A1	Prostate cancer	invasion and metastasis	-
miR-29c	MMP-2	Nerve sheath tumours	Cell invasion and migration	-
miR-30d	SOCS1, phospho-STAT3, MMP-2 and MMP-9	Prostate cancer	Proliferation and invasion	STAT3 signalling
miR-34a	Fra-1, p53 MMP-1 and MMP-9	Colon cancer	Migration and invasion	-
miR-92a	MMP-2 and -9	Lung cancer	Migration and invasion	STAT3 signaling
miR-101	Enhancer of zeste homolog 2 (EZH2), CDH1 and MMP-2	Lung cancer	Cell invasiveness	-
miR-106b	MMP-2	Breast cancer	Migration and invasion	ERK signaling cascade
miR-125b	MMP-2 and MMP-9	Glioblastoma	Invasion	-
miR-133	MMP-14	Lung cancer	Cell proliferation, migration and invasion	-
miR-138	RhoC, MMP-2 and MMP-9	Cholangiocarcinoma	Proliferation, migration and invasion	p-ERK signaling

microRNA	MMP Type and target molecule	Cancer type	Phenotype	Pathway
miR-139	IGF-IR and MMP-2	Colorectal cancer	Migration, invasion and metastasis	IGF-IR/MEK/ERK signaling
miR-143	MMP-13	Prostate cancer	Migration and invasion	-
	MMP-2 and MMP-9	Pancreatic cancer	Migration and invasion	-
	MMP-13	Osteosarcoma	Cell invasiveness	-
miR-145	Ets1, MMP-1 and -9	Gastric cancer	Invasion, metastasis, and angiogenesis	-
miR-146a	MMP-1, uPA, and uPAR	Brain cancer	Migration, invasion and metastasis	-
	MMP-16	Colon cancer	Invasion	-
miR-149	MMP-2 and CyclinD1	Glioma	Proliferation and invasion	AKT signaling
miR-152	MMP-3	Glioma	Cell invasiveness	MEK-ERK pathway
miR-181b	MMP-2 and MMP-9	Hepatocellular carcinomas	Migration and invasion	TGF- β, Smad signaling
miR-182	MMP-9, RECK	Breast cancer	cell invasion and colony formation ability	-
miR-196b	Vimentin, MMP-2 and MMP-9	Gastric cancer	Migration and invasion	-
miR-203	MMP-9 and Robo1	Glioblastoma	Proliferation, migration, and invasion	ERK phosphorylation
miR-206	MMP-2 and MMP-9	Breast cancer	Invasion and migration	-
miR-211	MMP-9	Glioblastoma multiforme	Cell invasion and migration	-
miR-218	LEF1, MMP-2, -7 and -9	Glioblastoma multiforme	Invasion	-
miR-218	MMP-9	Gliomas	Cell invasiveness	IKK-β/NF-κB pathway
miR-224	MMP-9 via targeting HOXD10	Human hepatocellular carcinoma	Migration and invasion	-
miR-338-3p	SMO and MMP-9	Hepatocellular carcinoma	Invasion and metastasis	-
miR-340	MMP-2 and MMP-9	Breast cancer	Tumor cell growth, migration, and invasion	-
miR-430	ERK, MMP-2 and MMP-9	Bladder cancer	Proliferation, migration and colony formation ablility	-
miR-451	Akt1, CyclinD1, MMP-2, MMP-9 and Bcl-2	Glioblastoma	Proliferation, invasion and apoptosis	PI3K/AKT signaling
miR-491	MMP-9	Hepatocellular carcinoma	Migration	-
miR-491-5p	MMP-9	Glioblastoma multiforme	Invasion	-
miRNA-590-3p	PI3K, Akt, MMP-2 and MMP-9	Bladder cancer	Proliferation, migration and colony-formation	PI3K, Akt signaling
miR-874	MMP-2 and -9, Aquaporin-3	Human gastric cancer	Cell migration and invasion assays and in vivo tumorigenicity	-
miR-874	MMP-2 and uPA	Non-small cell lung cancer	Tumor cell invasiveness and in vivo tumor growth	-
miR-885-5p	MMP-9	Glioblastoma multiforme	Invasion	-

Table 6. Target proteases and cancers associated with their overexpression.

Family	Protease	Location	Cancer
Cysteine Cathepsins	General	Intracellular, lysosomes	Most
	Cathepsin K	Extracellular, bone	Breast
	Cathepsin B	Extracellular and pericellular under pathological conditions	Breast, cervix, colon, colorectal, gastric, head and neck, liver, lung, melanoma, ovarian, pancreatic, prostate, thyroid
	Cathepsin L		Breast, colorectal
Aspartic Cathepsins	Cathepsin E	Endosomal structures, ER, Golgi	Cervical, gastric, lung, pancreas adenocarcinomas
	Cathepsin D	Lysosome	Breast, colorectal, ovarian
Kallikreins (hK)	General	Intracellular, secreted	Most
	hK1		
	PSA (hK 3)		Prostate, ovarian
	hK10		Colon, ovarian, pancreatic, head and neck
	hK15		Ovarian, prostate
Serine Proteases	uPA, uPAR	Membrane, Pericellular	Cervical, colorectal, gastric, prostate
Caspases		Intracellular	
MMPs	General	Extracellular	Most
	MMP-1, -8, -13		Breast
	MMP-2, -9		Breast, colorectal, lung, malignant gliomas, ovarian
	MMP-14	Membrane	Breast
ADAM		Extracellular	

Table 7. List of selected oncogenes associated with human malignancy

Gene Name	Gene Locus	Malignancies associated with	Comments
ABL1 (ABL)	9q34.1	Chronic myeloid leukemia	see tyrosine kinase Abelson murine leukemia protein
ABL2 (ABLL,ARG)	1q24-q25	acute myeloid leukemia	Member of the tyrosine kinase family. Important for synapse assembly and remodeling
AKAP13 (HT31, LBC. BRX)	15q24-q25	breast cancer	Blast crisis oncogene
ARAF1	Xp11.4-p11.2	angioimmunoblastic lymphadenopathy with dysproteinemia	Serine/threonine kinase
ARHGEF5 (TIM)	7q33-q35	Breast cancer	Codes for protein that controls cytoskeletal organization through regulation of small GTP-binding proteins
ATF1	12q13	ATF1/EWS fusion gene associated with malignant melanoma of soft parts	Codes for cAMP-dependent transcription factor-1

Gene Name	Gene Locus	Malignancies associated with	Comments
		(MMSP) ATF1/FUS with histiocytoma.	
AXL	19q13.1-q13.2	Chronic myelogenous leukemia	transforming gene to acute leukemia
BCL2	18q21.3	Burkitt lymphoma, follicular lymphoma	Mediator of apoptosis. Translocation is marker of poorer therapeutic response
BRAF (BRAF1, RAFB1)	7q34	Hairy cell leukemia, Malignant melanoma, thyroid papillary cancer, thyroid anaplastic carcinoma, bowel cancer, adenocarcinoma of lung, non-Hogkins lymphoma	see proto-oncogenes
BRCA1	17q21	Hereditary breast-ovarian cancer syndrome. Familial Breast cancer, Papillary serous carcinoma of the peritoneum (PSCP), Prostate cancer	see BRCA1.
BRCA2(FANCD1)	13q12.3	Familial Breast cancer, prostate cancer, pancreatic cancer	see BRCA2
BRIP1	17q22.2	Ovarian cancer, breast cancer	BRCA1 interacting protein C-terminal helicase 1 which is important in normal double-strand break repair
CBL (CBL2)	11q23.3		see proto-oncogenes
CSF1R (CSF-1, FMS, MCSF)	5q33.2-q33.3	Type M4 acute myeloblastic leukemia and chronic myelomonocytic leukemia	Codes for colony-stimulating factor-1 receptor, otherwise known as macrophage colony-stimulating factor
DAPK1 (DAPK)	9q34.1	Bladder cancer	Codes for death-associated protein kinase a positive mediators of apoptosis induced by gamma-interferon.
DEK (D6S231E)	6p23	DEK/NUP214(DEK/CAN) fusion gene associated with acute myeloid leukemia	Codes for DNA binding protein involved in transcriptional regulation and signal transduction as a component of the splicing complex that remains associated with spliced exons.
DUSP6 (MKP3,PYST1)	12q22-q23	Non-small cell lung cancer, pancreatic cancer	Codes for member of mitogen-activated protein (MAP) kinase family and has key role in cellular signal transduction
EGF			see proto-oncogenes
EGFR (ERBB, ERBB1)			see proto-oncogenes

Gene Name	Gene Locus	Malignancies associated with	Comments
ERBB3 (HER3)	12q13	Non-small cell lung cancer	elevated ERBB3 mRNA levels in breast cancer
ERG			see proto-oncogenes
ETS1			see proto-oncogenes
ETS2		Acute myeloid leukemia	Codes for a transcription factor
EWSR1 (EWS, ES, PNE,)	22q12	EWS/ERG in Ewing sarcoma, esthesioneuroblastoma EWS/FEV fusion gene in Ewing sarcoma, EWS/ZNF278 in small round cell sarcoma, EWS/FLI1 in Ewing sarcoma , EWS/ATF1 in malignant melanoma of soft parts (MMSP) EWS/WT1 in desmoplastic small round cell tumor	Ewing sarcoma breakpoint 1 gene
FES (FPS)	15q26.1	B cell lymphoma, acute promyelocytic leukemia, bladder carcinoma, lung cancer, breast cancer, colon cancer, neuroblastoma, pre-B lymphocyte neoplasm, plasmacytoma, multiple myeloma, T cell lymphoma, sarcoma	Codes for a tyrosine-specific protein kinase with a role in regulating immune response
FGF4 (HSTF1, KFGF)	11q13	Stomach cancer, kaposi sarcoma	A fibroblast growth factor Important in limb development.
FGFR1			see proto-oncogenes
FGFR10P (FOP)	6q27	FGFR1/FGFR1OP2 fusion gene in non-Hodgkin lymphoma	
FLCN	17p11.2	Renal cancer, bowel cancer	see FFCN
FOS (c-fos)	14q24.3		see proto-oncogenes
FRAP1			see tumor suppressors
FUS (TLS)	16p11.2		see proto-oncogenes
HRAS	11p15.5		see proto-oncogenes.
GLI1	12q13.2-q13.3	Glioma, myxoid liposarcoma, salivary gland tumor	Codes for a Kruppel (Kr) zinc finger protein
GLI2	2q14	Glioma	Codes for a Kruppel (Kr) zinc finger protein
GPC3	Xq26	Germ cell cancer, Hepatocellular cancer	see GPC3
HER2 (ERBB2, TKR1, NEU)	17q21.1	Breast cancer, lung cancer	see HER2. Targeted by Trastuzumab.

Gene Name	Gene Locus	Malignancies associated with	Comments
HGF (SF)	7q21.1	Prostate cancer, renal cancer	Codes for hepatocyte growth factor (hepatopoietin A, scatter factor) which is upregulated in many malignancies
IRF4 (LSIRF, MUM1)	6p25-p23	B-cell lymphoma, B-cell leukemia, Multiple myeloma	Codes for an interferon regulatory factor essential for lymphocyte function
JUNB	19p13.2		see proto-oncogenes
KIT(SCFR)	4q12	Gastrointestinal stromal tumor (GISTs), mast cell leukemia, mastocytosis, seminoma and dysgerminoma	Transmembrane tyrosine kinase receptor for stem cell factor (SCFR) is required for haematopoiesis, melanogenesis and gametogenesis. Mutations cause piebaldism.
KRAS2 (RASK2)	12p12.1		see proto-oncogenes.
LCK	1p35-p34.3	Non-small cell lung cancer, Neuroblastoma, non-Hodgkin lymphoma	codes for lymphocyte specific protein tyrosine kinase
LCO	2q14-q21	Hepatocellular carcinoma	
MAP3K8(TPL2, COT, EST)	10p11.2	Ewings sarcoma, adenocarcinoma of lung, thyroid carcinoma	Codes for a serine-threonine protein kinase.
MCF2 (DBL)	Xq27	Breast cancer	Codes for a GDP-GTP exchange factor that modulates the activity of small GTPases of the Rho family
MDM2	12q14.3-q15	Multiple	MDM2 acts as a major regulator of the tumor suppressor p53 by targeting its destruction. Direct association of p53 with the protein MDM2 results in ubiquitination and subsequent degradation of p53
MET(HGFR, RCCP2)	7q31		see proto-oncogenes
MLH type genes			see proto-oncogenes
MMD	17q	Non small cell lung cancer, hepatocellular carcinoma, colon cancer	Codes for monocyte to macrophage differentiation associated protein.
MOS (MSV)	8q11	Burkitt lymphoma, acute myeloblastic leukemia	Function in man unknown. Above associations indirect but analogous gene to Moloney murine sarcoma virus.
MRAS (RRAS3)	3q22.3	Activated in many tumors	Codes for a RAS GTP-binding protein membrane-anchored, intracellular signal transducer
MSH type genes			see proto-oncogenes

Gene Name	Gene Locus	Malignancies associated with	Comments
MYB (AMV)	6q22	Alterations found in more than a third of human solid tumor lines	Encodes for proteins critical to hematopoietic cell proliferation and development
MYC	8q24.12-q24.13	Burkitt lymphoma Over expression in many malignancies, possibly associated with angiogenic, invasive promoting properties in excess.	A transcription factor that promotes cell proliferation
MYCL1 (LMYC)	1p34.3	Small cell lung cancer, adenocarcinoma of lung, neuroblastoma	
MYCN	2p24.1	Neuroblastomas	Overlaps with NMYC and is transcribed from opposite DNA strand
NCOA4 (ELE1, ARA70, PTC3)	10q11.2	Prostate cancer	Interacts with the androgen receptor in presence of dihydrotestosterone.
NF1 type genes			see tumor suppressors
NMYC	2p24	Neuroblastomas, retinoblastoma	Overlaps with MYCN and is transcribed from opposite DNA strand. Probably a DNA-binding protein.
NRAS	1p13.2		see proto-oncogenes.
NTRK1 (TRK, TRKA)	1q21-q22		see proto-oncogenes.
NUP214 (CAN, D9S46E)	9q34.1	NUP214/DEK fusion gene associated with acute myeloid leukemia, NUP214/ABL1 associated with T-cell acute lymphoblastic leukemia (T-ALL).	Codes for nucleoporin component of the vertebrate nuclear pore complex.
OVC	9p24	Ovarian adenocarcinoma	Abnormal in about 40% ovarian adenocarcinoma
TP53 (P53)	17p13.1		see tumor suppressors
PALB2	16p12	Breast cancer	see PALB2
PAX3 (HUP2) STAT1	2q35	Alveolar rhabdomyosarcoma	Transcriptions factor, causes some forms of Waardenburg syndrome and regulates RET.
PDGFB (SIS)			see proto-oncogenes
PIM genes			see proto-oncogenes
PML (MYL)	15q22		see tumour suppressors
PMS (PMSL) genes			see tumour suppressors
PPM1D (WIP1)	17q22-q23	Breast cancer, Osteosarcoma	Codes for a serine/threonine protein phosphatase that attenuates apoptosis and facilitates transformation of

Gene Name	Gene Locus	Malignancies associated with	Comments
			primary cells in cooperation with RAS
PTEN (MMAC1)	10q23.31		see tumor suppressors
PVT1	8q24	Burkitt lymphoma	
RAF1 (CRAF)	3p25	Stomach cancer, renal cancer, glioblastoma, laryngeal cancer	A regulator of endothelial cell survival during angiogenesis. Activated RAF counteracts apoptosis by suppressing the activation of mammalian sterile 20-like kinase (MST2).
RB1 (RB)	13q14.1-q14.2	Retinoblastoma, osteogenic sarcoma, small cell carcinoma of lung, bladder cancer	see RB1
RET	10q11.2	Multiple endocrine neoplasia type 2a and 2b and Medullary thyroid carcinoma	see RET
RRAS2 (TC21)	11pter-p15.5	Teratocarcinoma, ovarian cancer	Single point mutation activates its oncogene potential
ROS1 (ROS, MCF3)	6q22	Glioblastoma and probably others	ROS1/FIG fusion protein is a tyrosine kinase found in astrocytoma
SMAD type genes			see tumor suppressors
SMARCB1 (SNF5, INI1)	22q11		see tumor suppressors
SMURF1	7q21.1-q31.1	Pancreatic cancer	Codes for a HECT domain E3 ubiquitin ligase that regulates tumor cell plasticity and motility through degradation of RhoA
SRC (AVS)	20q12-q13	hepatic metastatic bowel cancer, colon cancer, leukemia	Intracellular communication regulator protein. Mutations are activating, transforming, tumorigenic, and metastasis-promoting
STAT1	2q32.2-q32.3	Non-small cell lung cancer	see STAT1
STAT3	17q21	Epithelial cancers	Codes signal protein that induces cell transformation through a combined inhibition of apoptosis and cell-cycle activation
STAT5	17q11.2	Permissive for a wide range of malignancies	Codes signal protein that induces cell transformation through a combined inhibition of apoptosis and cell-cycle activation
TDGF1 (CRGF)	3p23-p21	teratocarcinoma	Probably codes for signaling protein for mesoderm development
TGFBR2	3p22		see proto-oncogenes
THRA (ERBA, EAR7 etc)			see proto-oncogenes

Gene Name	Gene Locus	Malignancies associated with	Comments
TFG (TRKT3)	3q11-q12	Papillary thyroid carcinoma	Chimeric oncogene with NTRK1 proto-oncogene
TIF1 (TRIM24, TIF1A)	7q32-q34	Fusion genes associated with papillary thyroid carcinoma and myleoproliferative disorder.	Codes for transcriptional intermediary factor 1
TNC (TN, HXB)	9q33	Neurofibromatosis type 1, Pancreatic cancer	see TNC
TRK	1q21-q22		see proto-oncogenes
TUSC3	8p22		see tumor suppressors
USP6 (TRE2)	17p13	Multiple cancers	Codes for a ubiquitin-specific protease found only in primates
WNT1 (INT1)	12q12-q13		see proto-oncogenes
WT1	11p13	Wilms tumour, over expressed in breast and lung cancer, myelodysplastic syndrome and acute myeloid leukemia	A zinc finger DNA-binding protein acting as a transcriptional activator or repressor depending on intracellular context
VHL	3p26-p25		see tumor suppressors

Table 8. Tumor suppresor miRs that are downregulated in specific cancer types

Cancer	miRNA
Bladder	mir-1; mir-101; mir-1180; mir-1236; mir-124-3p; mir-125b; mir-126; mir-1280; mir-133a; mir-133b; mir-141; mir-143; mir-144; mir-145; mir-155; mir-16; mir-18a; mir-192; mir-195; mir-200a; mir-200b; mir-200c; mir-203; mir-205; mir-214; mir-218; mir-23b; mir-26a; mir-29c; mir-320c; mir-34a; mir-370; mir-409-3p; mir-429; mir-451; mir-490-5p; mir-493; mir-576-3p; mir-99a
Brain (Astrocytoma, Glioblastoma, Glioma)	let-7g-5p; mir-100; mir-101; mir-106a; mir-124; mir-124a; mir-125a; mir-125a-5p; mir-125b; mir-127-3p; mir-128; mir-129; mir-136; mir-137; mir-139-5p; mir-142-3p; mir-143; mir-145; mir-146b-5p; mir-149; mir-152; mir-153; mir-195; mir-21; mir-212-3p; mir-219-5p; mir-222; mir-29b; mir-31; mir-3189-3p; mir-320; mir-320a; mir-326; mir-330; mir-331-3p; mir-340; mir-342; mir-34a; mir-376a; mir-449a; mir-483-5p; mir-503; mir-577; mir-663; mir-7; mir-7-5p; mir-873; let-7a; let-7f; mir-107; mir-122; mir-124-5p; mir-139; mir-146a; mir-146b; mir-15b; mir-16; mir-181a; mir-181a-1; mir-181a-2; mir-181b; mir-181b-1; mir-181b-2; mir-181c; mir-181d; mir-184; mir-185; mir-199a-3p; mir-200a; mir-200b; mir-203; mir-204; mir-205; mir-218; mir-23b; mir-26b; mir-27a; mir-29c; mir-328; mir-34c-3p; mir-34c-5p; mir-375; mir-383; mir-451; mir-

Cancer	miRNA
	452; mir-495; mir-584; mir-622; mir-656; mir-98; mir-124-3p; mir-181b-5p; mir-200b; mir-3189-3p
Breast	mir-193b; let-7a; let-7a-1; let-7a-2; let-7a-3; let-7b; let-7c; let-7d; let-7e; let-7f-1; let-7f-2; let-7g; let-7i; mir-100; mir-107; mir-10a; mir-10b; mir-122; mir-124; mir-1258; mir-125a-5p; mir-125b; mir-126; mir-127; mir-129; mir-130a; mir-132; mir-133a; mir-143; mir-145; mir-146a; mir-146b; mir-147; mir-148a; mir-149; mir-152; mir-153; mir-15a; mir-16; mir-17-5p; mir-181a; mir-1826; mir-183; mir-185; mir-191; mir-193a-3p; mir-195; mir-199b-5p; mir-19a-3p; mir-200a; mir-200b; mir-200c; mir-205; mir-206; mir-211; mir-216b; mir-218; mir-22; mir-26a; mir-26b; mir-300; mir-30a; mir-31; mir-335; mir-339-5p; mir-33b; mir-34a; mir-34b; mir-34c; mir-374a; mir-379; mir-381; mir-383; mir-425; mir-429; mir-450b-3p; mir-494; mir-495; mir-497; mir-502-5p; mir-517a; mir-574-3p; mir-638; mir-7; mir-720; mir-873; mir-874; mir-92a; mir-98; mir-99a; mmu-mir-290-3p; mmu-mir-290-5p
Cervical	mir-143; mir-145; mir-17-5p; mir-203; mir-214; mir-218; mir-335; mir-342-3p; mir-372; mir-424; mir-491-5p; mir-497; mir-7; mir-99a; mir-99b; mir-100; mir-101; mir-15a; mir-16; mir-34a; mir-886-5p; mir-106a; mir-124; mir-148a; mir-29a; mir-375
Colon/Colorectal	let-7a-1; let-7a-2; let-7a-3; let-7b; let-7c; let-7d; let-7e; let-7f-1; let-7f-2; let-7g; let-7i; mir-100; mir-101; mir-126; mir-142-3p; mir-143; mir-145; mir-192; mir-200c; mir-21; mir-214; mir-215; mir-22; mir-25; mir-302a; mir-320; mir-320a; mir-34a; mir-34c; mir-365; mir-373; mir-424; mir-429; mir-455; mir-484; mir-502; mir-503; mir-93; mir-98; mir-186; mir-30a-5p; mir-627; let-7a; mir-1; mir-124; mir-125a; mir-129; mir-1295b-3p; mir-1307; mir-130b; mir-132; mir-133a; mir-133b; mir-137; mir-138; mir-139; mir-139-5p; mir-140-5p; mir-148a; mir-148b; mir-149; mir-150-5p; mir-154; mir-15a; mir-15b; mir-16; mir-18a; mir-191; mir-193a-5p; mir-194; mir-195; mir-196a; mir-198; mir-199a-5p; mir-203; mir-204-5p; mir-206; mir-212; mir-218; mir-224; mir-24-3p; mir-26b; mir-27a; mir-28-3p; mir-28-5p; mir-29b; mir-30a-3p; mir-30b; mir-328; mir-338-3p; mir-342; mir-345; mir-34a-5p; mir-361-5p; mir-375; mir-378; mir-378a-3p; mir-378a-5p; mir-409-3p; mir-422a; mir-4487; mir-483; mir-497; mir-498; mir-518a-3p; mir-551a; mir-574-5p; mir-625; mir-638; mir-7; mir-96-5p; mir-202-3p; mir-30a; mir-451
Endometrial	mir-101; mir-130a; mir-130b; mir-134; mir-143; mir-145; mir-152; mir-205; mir-223; mir-301a; mir-301b; mir-30c; mir-34a; mir-34c; mir-424; mir-449a; mir-543; mir-34b
Hematologic (Leukemia,	mir-125b; mir-138; mir-15a; mir-15b; mir-16; mir-16-1; mir-16-1-3p; mir-16-2; mir-181a; mir-181b; mir-195; mir-223; mir-29b; mir-34b; mir-34c; mir-424; mir-10a; mir-146a; mir-150; mir-151; mir-155; mir-2278; mir-26a; mir-30e;

Cancer	miRNA
Lymphoma, Myeloma)	mir-31; mir-326; mir-564; mir-27a; let-7b; mir-124a; mir-142-3p; let-7c; mir-17; mir-20a; mir-29a; mir-30c; mir-720; mir-107; mir-342; mir-34a; mir-202; mir-142-5p; mir-29c; mir-145; mir-193b; mir-199a; mir-214; mir-22; mir-137; mir-197
Kidney	mir-1; mir-145; mir-1826; mir-199a; mir-199a-3p; mir-203; mir-205; mir-497; mir-508-3p; mir-509-3p; let-7a; let-7d; mir-106a*; mir-126; mir-1285; mir-129-3p; mir-1291; mir-133a; mir-135a; mir-138; mir-141; mir-143; mir-182-5p; mir-200a; mir-218; mir-28-5p; mir-30a; mir-30c; mir-30d; mir-34a; mir-378; mir-429; mir-509-5p; mir-646; mir-133b; let-7b; let-7c; mir-200c; mir-204; mir-335; mir-377; mir-506
Liver (Hepatocellular Carcinoma)	mir-137; mir-138; mir-139; mir-139-5p; mir-140-5p; mir-141; mir-142-3p; mir-143; mir-144; mir-145; mir-146a; mir-148a; mir-148b; mir-150-5p; mir-15b; mir-16; mir-181a-5p; mir-185; mir-188-5p; mir-193b; mir-195; mir-195-5p; mir-197; mir-198; mir-199a; mir-199a-5p; mir-199b; mir-199b-5p; mir-200a; mir-200b; mir-200c; mir-202; mir-203; mir-204-3p; mir-205; mir-206; mir-20a; mir-21; mir-21-3p; mir-211; mir-212; mir-214; mir-217; mir-218; mir-219-5p; mir-22; mir-223; mir-26a; mir-26b; mir-29a; mir-29b-1; mir-29b-2; mir-29c; mir-302b; mir-302c; mir-30a; mir-30a-3p; mir-335; mir-338-3p; mir-33a; mir-34a; mir-34b; mir-365; mir-370; mir-372; mir-375; mir-376a; mir-377; mir-422a; mir-424; mir-424-5p; mir-433; mir-4458; mir-448; mir-450a; mir-451; mir-485-5p; mir-486-5p; mir-497; mir-503; mir-506; mir-519d; mir-520a; mir-520b; mir-520c-3p; mir-582-5p; mir-590-5p; mir-610; mir-612; mir-625; mir-637; mir-675; mir-7; mir-877; mir-940; mir-941; mir-98; mir-99a; mir-132; mir-31
Lung	mir-1297; mir-141; mir-145; mir-16; mir-200a; mir-200b; mir-200c; mir-29b; mir-381; mir-409-3p; mir-429; mir-451; mir-511; mir-99a; let-7a-1; let-7a-2; let-7a-3; let-7b; let-7c; let-7d; let-7e; let-7f-1; let-7f-2; let-7g; let-7i; mir-1; mir-101; mir-133b; mir-138; mir-142-5p; mir-144; mir-1469; mir-146a; mir-153; mir-15a; mir-15b; mir-16-1; mir-16-2; mir-182; mir-192; mir-193a-3p; mir-194; mir-195; mir-198; mir-203; mir-217; mir-218; mir-22; mir-223; mir-26a; mir-26b; mir-29c; mir-33a; mir-34a; mir-34b; mir-34c; mir-365; mir-449a; mir-449b; mir-486-5p; mir-545; mir-610; mir-614; mir-630; mir-660; mir-7515; mir-9500; mir-98; mir-99b; mir-133a; let-7a; mir-100; mir-106a; mir-107; mir-124; mir-125a-3p; mir-125a-5p; mir-126; mir-126*; mir-129; mir-137; mir-140; mir-143; mir-146b; mir-148a; mir-148b; mir-149; mir-152; mir-154; mir-155; mir-17-5p; mir-181a-1; mir-181a-2; mir-181b; mir-181b-1; mir-181b-2; mir-181c; mir-181d; mir-184; mir-186; mir-193b; mir-199a; mir-204; mir-212; mir-221; mir-224; mir-27a; mir-27b; mir-29a; mir-30a; mir-30b; mir-30c; mir-30d; mir-30d-5p; mir-30e-5p; mir-32; mir-335; mir-338-3p; mir-340; mir-342-3p;

Cancer	miRNA
	mir-361-3p; mir-373; mir-375; mir-4500; mir-4782-3p; mir-497; mir-503; mir-512-3p; mir-520a-3p; mir-526b; mir-625*; mir-96
Melanoma	let-7b; mir-101; mir-125b; mir-1280; mir-143; mir-146a; mir-146b; mir-155; mir-17; mir-184; mir-185; mir-18b; mir-193b; mir-200c; mir-203; mir-204; mir-205; mir-206; mir-20a; mir-211; mir-218; mir-26a; mir-31; mir-33a; mir-34a; mir-34c; mir-376a; mir-376c; mir-573; mir-7-5p; mir-9; mir-98
Oral Cancer	let-7d; mir-218; mir-34a; mir-375; mir-494; mir-100; mir-124; mir-1250; mir-125b; mir-126; mir-1271; mir-136; mir-138; mir-145; mir-147; mir-148a; mir-181a; mir-206; mir-220a; mir-26a; mir-26b; mir-29a; mir-32; mir-323-5p; mir-329; mir-338; mir-370; mir-410; mir-429; mir-433; mir-499a-5p; mir-503; mir-506; mir-632; mir-646; mir-668; mir-877; mir-9
Ovarian	let-7i; mir-100; mir-124; mir-125b; mir-129-5p; mir-130b; mir-133a; mir-137; mir-138; mir-141; mir-145; mir-148a; mir-152; mir-153; mir-155; mir-199a; mir-200a; mir-200b; mir-200c; mir-212; mir-335; mir-34a; mir-34b; mir-34c; mir-409-3p; mir-411; mir-429; mir-432; mir-449a; mir-494; mir-497; mir-498; mir-519d; mir-655; mir-9; mir-98; mir-101; mir-532-5p; mir-124a; mir-192; mir-193a; mir-7
Pancreatic	mir-101; mir-1181; mir-124; mir-1247; mir-133a; mir-141; mir-145; mir-146a; mir-148a; mir-148b; mir-150*; mir-150-5p; mir-152; mir-15a; mir-198; mir-203; mir-214; mir-216a; mir-29c; mir-335; mir-34a; mir-34b; mir-34c; mir-373; mir-375; mir-410; mir-497; mir-615-5p; mir-630; mir-96; mir-132; let-7a; let-7a-1; let-7a-2; let-7a-3; let-7b; let-7c; let-7d; let-7e; let-7f-1; let-7f-2; let-7g; let-7i; mir-126; mir-135a; mir-143; mir-144; mir-150; mir-16; mir-200a; mir-200b; mir-200c; mir-217; mir-218; mir-337; mir-494; mir-98
Prostate	let-7a-3p; let-7c; mir-100; mir-101; mir-105; mir-124; mir-128; mir-1296; mir-130b; mir-133a-1; mir-133a-2; mir-133b; mir-135a; mir-143; mir-145; mir-146a; mir-154; mir-15a; mir-187; mir-188-5p; mir-199b; mir-200b; mir-203; mir-205; mir-212; mir-218; mir-221; mir-224; mir-23a; mir-23b; mir-25; mir-26a; mir-26b; mir-29b; mir-302a; mir-30a; mir-30b; mir-30c-1; mir-30c-2; mir-30d; mir-30e; mir-31; mir-330; mir-331-3p; mir-34a; mir-34b; mir-34c; mir-374b; mir-449a; mir-4723-5p; mir-497; mir-628-5p; mir-642a-5p; mir-765; mir-940
Retinoblastoma	mir-101; mir-183; mir-204; mir-34a; mir-365b-3p; mir-486-3p; mir-532-5p

CLAIMS:

1. A recombinant oncolytic herpes simplex virus (HSV) comprising at least two micro-RNA (miRNA) target sequence inserted into a locus of one or more essential viral genes, wherein the one or more viral genes are selected from the group consisting of ICP4, ICP27, ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, and UL42,

wherein the replication of the recombinant HSV is reduced in a non-cancerous cell compared to the replication of the the recombinant HSV in a cancerous cell of the same cell type.

2. The onycolytic virus of claim 1, wherein the one or more viral genes are selected from the group consisting of ICP8, ICP22, ICP34.5, UL5, UL8, UL9, UL30, UL39/40, and UL42.

3. The onycolytic virus of claim 2, wherein the one or more viral genes are selected from the group consisting of UL8, ICP8, and UL30.

4. The onycolytic virus of claim 1, wherein the one or more viral genes are selected from the group consisting of ICP27 and ICP4.

5. The onycolytic virus of claim 1, wherein the one or more viral genes are selected from the group consisting of ICP4, ICP27, UL8, UL42, and ICP34.5.

6. The onycolytic virus of claim 1, wherein the cell is selected from the group consisting of a neuronal cell, a cardiac cell, a muscle cell, and a liver cell.

7. The onycolytic virus of claim 6, wherein the neuronal cell is a a central nervous system cell, a peripheral nervous system cell, a brain cell, or a spinal cord cell.

8. The onycolytic virus of claim 6, wherein the muscle cell is a striated muscle cell or a smooth muscle cell.

9. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are brain cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-137, miR-219a, miR-124, miR-9, miR-487b, and miR-128.

10. The onycolytic virus of claim 9, wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-137, miR-219a, miR-124, and miR-128

11. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are cardiac or striated muscle cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-208b, miR-1, miR-208a, miR-133a, miR-4284, miR-499a, miR-126, miR-30e, miR-378i, miR-30b, and miR-378.

12. The onycolytic virus of claim 11, wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-208b, miR-1, and miR-208a

13. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are spinal cord cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-219a, miR-9, miR-204, miR-577, miR-99a, miR-100, miR-132, and miR-135.

14. The onycolytic virus of claim 13, wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-219a, miR-9, and miR-204

15. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are peripheral nervous system cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-204, miR-1, miR-206, miR-9, miR-99a, miR-199b, miR-145, miR-100, miR-574.

16. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are liver cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-122 and miR-126.

17. The onycolytic virus of claim 1, wherein the non-cancerous cell and the cancerous cell are smooth muscle cells, and wherein the at least two miRNA target sequences are target sequences for an miRNA selected from the group consisting of miR-143 and miR-145.

18. The oncolytic virus of any one of claims 1 – 17, wherein the two or more miR target sequences is incorporated into a miR-T cassette that is inserted in the 5' untranslated region (UTR) or 3' UTR of the one or more essential viral genes.

19. The oncolytic virus of claim 18, wherein the miR-T cassette comprises a length of less than 1000 nucleotides.

20. The oncolytic virus of claim 19, wherein the miR-T cassette comprises a length of between about 25 and about 500 nucleotides.

21. The oncolytic virus of claim 18, wherein the miR-T cassette comprises a length of between about 100 and about 500 nucleotides.

22. A recombinant oncolytic herpes simplex virus (HSV) comprising:

(i) a first microRNA (miRNA) target sequence cassette (miR-TS cassette) inserted into a first viral gene and comprising at least 2 target sequences for each of miR-124, miR-1, and miR-143;

(ii) a second miR-TS cassette inserted into a second viral gene and comprising at least 2 target sequences for each of miR-128, miR-219a, and miR-122; and

(iii) a third miR-TS cassette inserted into a third viral gene and comprising at least 2 target sequences for each of miR-219a, miR-204, and miR-128.

23. The recombinant HSV of claim 22, further comprising a third miR-TS cassette inserted into a third viral gene, wherein the third miR-TS cassette comprises:

(a) at least 2 target sequences for each of miR-137, miR-208b-3p, and miR-126; or

(b) at least 2 target sequences for each of miR-137, miR-217, and miR-126.

24. The recombinant HSV of claim 22 or 23, wherein each of the miR-TS cassettes comprises 4 target sequences for each of the respectively miRNAs

25. The recombinant HSV of any one of claims 22 – 23, wherein the first viral gene is ICP4.

26. The recombinant HSV of any one of claims 22 – 23, wherein the second viral gene is ICP27.

27. The recombinant HSV of any one of claims 22 – 23, wherein the third viral gene is ICP34.5.

28. The recombinant HSV of claim 23, wherein the fourth viral gene is UL8.

29. The recombinant HSV of any one of claims 22 – 28, wherein the replication of the recombinant HSV is reduced in a non-cancerous cell compared to the replication of the the recombinant HSV in a cancerous cell of the same cell type, wherein the cell is selected from the group consisting of a neuronal cell, a cardiac cell, a muscle cell, and a liver cell.

30. The recombinant HSV of any one of claims 22 – 29, wherein the first miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 852.

31. The recombinant HSV of claim 30, wherein the first miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 852.

32. The recombinant HSV of any one of claims 22 – 29, wherein the second miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 853.

33. The recombinant HSV of claim 32, wherein the second miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 853.

34. The recombinant HSV of any one of claims 22 – 29, wherein the third miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 854.

35. The recombinant HSV of claim 34, wherein the third miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 854.

36. The recombinant HSV of any one of claims 23 – 29, wherein the fourth miR-TS cassette comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 855.

37. The recombinant HSV of claim 36, wherein the fourth miR-TS cassette comprises or consists of the nucleic acid sequence of SEQ ID NO: 855.

38. The recombinant HSV of any one of claims 1 – 37, further comprising a heterologous polynucleotide sequence encoding one or more payload molecules.

39. The recombinant HSV of claim 38, wherein the heterologous polynucleotide sequence encodes a payload selected from the group consisting of IL-12, CCL4, and CXCL10.

40. The recombinant HSV of claim 38, wherein the heterologous polynucleotide sequence encodes two or more payloads selected from the group consisting of IL-12, CCL4, and CXCL10.

41. The recombinant HSV of claim 38, wherein the heterologous polynucleotide sequence encodes three payloads comprising IL-12, CCL4, and CXCL10.

42. A recombinant oncolytic virus comprising one or more micro-RNA (miR) target sequences inserted into a locus of one or more viral genes required for viral replication, wherein

the virus is herpes simplex virus (HSV), and wherein the one or more viral genes are selected from the group consisting of UL8, ICP34.5, UL42, UL19, ICP4, and ICP27.

43. The oncolytic virus of claim 42, wherein the one or more miR target sequences is incorporated into the 5' untranslated region (UTR) or 3' UTR of the one or more viral genes required for viral replication.

44. The oncolytic virus of claim 42 or 43, wherein the miR target sequence is a target sequence for a miR selected from the group consisting of miR-122, miR-184, miR-34a, let7a, miR-145-5p, miR-199a-5p, miR-451a, miR-125a, miR-125a-5p, miR-126-3p, miR-233-3p, miR-143-3p, miR-1-3p, miR-133a-3p, miR-127a-3p, miR-133b, miR-134-3p, miR-124, miR-101, miR-125b, miR-145, miR-559, miR-213, miR-31-5p, and miR-205p.

45. The oncolytic virus of any one of claims 42-44, wherein one or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes.

46. The oncolytic virus of claim 45, wherein two, three, four, or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes.

47. The oncolytic virus of any one of claims 42-46, wherein replication of the virus is reduced or attenuated in a first cell compared to replication of the virus in a second cell, wherein the first cell has an increased expression of a miR capable of binding to the one or more miR target sequences compared to the expression of the miR in the second cell.

48. The oncolytic virus of claim 47, wherein the expression level of the miR in the first cell is at least 5% greater than the expression level of the miR in the second cell.

49. The oncolytic virus of claim 47, wherein the first cell is a non-cancerous cell.

50. The oncolytic virus of claim 47, wherein the second cell has a reduced expression of a miR capable of binding to the one or more miR target sequences compared to the expression of the miR in the first cell.

51. The oncolytic virus of claim 50, wherein the expression level of the miR in the second cell is at least 5% less than the expression level of the miR in the first cell.

52. The oncolytic virus of claim 47, wherein the second cell is a cancerous cell.

53. The oncolytic virus of claim 42, wherein one copy of a miR-122 target sequence is inserted into the locus of one or more viral genes required for viral replication.

54. The oncolytic virus of claim 53, wherein four copies of the miR-122 target sequence are inserted into the locus of one or more viral genes required for viral replication.

55. The oncolytic virus of claim 53 or 54, wherein the one or more viral genes is ICP27.

56. The oncolytic virus of claim 53, further comprising one copy of a miR-125a target sequence inserted into the locus of one or more viral genes required for viral replication.

57. The oncolytic virus of claim 56, wherein four copies of the miR-125a target sequence are inserted into the locus of one or more viral genes required for viral replication.

58. The oncolytic virus of claim 56 or 58, wherein the one or more viral genes is UL42.

59. The oncolytic virus of any of claims 42-52, wherein four copies of a miR-122 target sequence are inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42.

60. The oncolytic virus of any of claims 42-52, wherein one copy of a miR-122 target sequence is inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42.

61. The oncolytic virus of any of claims 42-52, wherein one copy of a miR-122 target sequence is inserted into the locus of ICP27 and three copies of a miR-125a target sequence are inserted into the locus of UL42.

62. The oncolytic virus of any of claims 42-52, wherein four copies of a miR-122 target sequence are inserted into the locus of ICP27 and four copies of a miR-125a target sequence are inserted into the locus of UL42.

63. A recombinant oncolytic virus comprising:

(a) one or more micro-RNA (miR) target sequences inserted into a locus of one or more viral genes required for viral replication; and

(b) one or more polynucleotides encoding

(i) one or more proteins or oligonucleotides, wherein the proteins or oligonucleotides reduce the expression or inhibit the function of a miR, a gene, or a tissue inhibitor of metalloproteinases (TIMP); or

(ii) a protease-activated antibody;

wherein the virus is an HSV, wherein the one or more viral genes are selected from the group consisting of UL42, UL19, ICP4, and ICP27.

64. The oncolytic virus of claim 63, wherein the miR is an oncogenic miR or a microenvironment remodeling miR.

65. The oncolytic virus of claim 64, wherein the oncogenic miR is selected from the miRs listed in Table 4.

66. The oncolytic virus of claim 63, wherein the gene is an oncogenic gene.

67. The oncolytic virus of claim 66, wherein the oncogenic gene is selected from the genes listed in Table 7.

68. The oncolytic virus of claim 63, wherein the microenvironment remodeling miR is selected from the miRs listed in Table 5.

69. The oncolytic virus of claim 22, wherein the one or more miR target sequences is incorporated into the 5' untranslated region (UTR) or 3' UTR of the one or more viral genes required for viral replication.

70. The oncolytic virus of claim 69, wherein the miR target sequence is a target sequence for a miR selected from the group consisting of miR-122, miR-184, miR-34a, let7a, miR-145-5p, miR-199a-5p, miR-451a, miR-125a, miR-125a-5p, miR-126-3p, miR-233-3p, miR-143-3p, miR-1-3p, miR-133a-3p, miR-127a-3p, miR-133b, miR-134-3p, miR-124, miR-101, miR-125b, miR-145, miR-559, miR-213, miR-31-5p, and miR-205p.

71. The oncolytic virus of claim 63, wherein one or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes.

72. The oncolytic virus of claim 71, wherein two, three, four, or more copies of the one or more miR target sequences are inserted into a locus of one or more viral genes.

73. The oncolytic virus of any of claims 63-72, wherein one copy of a miR-122 target sequence is inserted into the locus of one or more viral genes required for viral replication.

74. The oncolytic virus of claim 73, wherein four copies of the miR-122 target sequence are inserted into the locus of one or more viral genes required for viral replication.

75. The oncolytic virus of claim 73 or 74, wherein the one or more viral genes is ICP27.

76. The oncolytic virus of claim 73, further comprising one copy of a miR-125a target sequence is inserted into the locus of one or more viral genes required for viral replication.

77. The oncolytic virus of claim 76, wherein four copies of the miR-125a target sequence are inserted into the locus of one or more viral genes required for viral replication.

78. The oncolytic virus of claim 76 or 77, wherein the one or more viral genes is UL42.

79. The oncolytic virus of claim 63, wherein four copies of a miR-122 target sequence are inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42.

80. The oncolytic virus of claim 63, wherein one copy of a miR-122 target sequence is inserted into the locus of ICP27 and one copy of a miR-125a target sequence is inserted into the locus of UL42.

81. The oncolytic virus of claim 63, wherein one copy of a miR-122 target sequence is inserted into the locus of ICP27 and three copies of a miR-125a target sequence are inserted into the locus of UL42.

82. The oncolytic virus of claim 63, wherein four copies of a miR-122 target sequence are inserted into the locus of ICP27 and four copies of a miR-125a target sequence are inserted into the locus of UL42.

83. The oncolytic virus of claim 63, wherein the TIMP is selected from TIMP1, TIMP2, TIMP3 and TIMP4.

84. The oncolytic virus of claim 63, wherein the oligonucleotide is an shRNA or a decoy oligonucleotide.

85. The oncolytic virus of claim 63, wherein the protein is a nuclease, a bispecific T-cell engager (BiTE), an anti-immunosuppressive protein, or an immunogenic antigen.

86. The oncolytic virus of claim 85, wherein the nuclease is selected from a Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease, a zinc-finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN).

87. The oncolytic virus of claim 86, wherein the CRISPR-associated endonuclease is selected from SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, C2C1, C2C3, Cpf1, Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, Csx10, Csx16, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, and Csf4.

88. The oncolytic virus of claim 87, further comprising a heterologous polynucleotide encoding an tracr-RNA (trRNA) and a crispr-RNA (crRNA), wherein the crRNA is targeted to a genomic DNA sequence encoding a miR or a TIMP and wherein the trRNA facilitates binding and activation of a CRISPR-associated endonuclease.

89. The oncolytic virus of claim 85, wherein the anti-immunosuppressive protein is an anti-regulatory T-cell (Treg) protein or an anti-myeloid-derived suppressor cell (MDSC) protein.

90. The oncolytic virus of claim 85, wherein the anti-immunosuppressive protein is a VHH-derived blocker or a VHH-derived BiTE.

91. The oncolytic virus of any one of claims 63 - 90, wherein the protein induces an anti-tumor immune response.

92. The oncolytic virus of claim 91, wherein the protein binds to an antigen expressed on a cell surface, wherein the antigen is selected from the group consisting of EpCAM, CTLA-4, PD1, FGF2, FGFR/FGFR2b, endothelin B Receptor, and SEMA4D.

93. The oncolytic virus of claim 91, wherein the protein is selected from, folate, IFNβ, , A2A, , CCL5, CD137, CD200, CD38, CD44, CSF-1R, CXCL10, CXCL13, IL-12, IL-15, IL-2, IL-21, IL-35, ISRE7, LFA-1, NG2 (also known as SPEG4), a SMAD protein, STING, TGFβ, and VCAM1.

94. The oncolytic virus of claim 63, wherein the at least one protease-activated antibody is incorporated into a viral glycoprotein envelope.

95. The oncolytic virus of claim 63 or 94, wherein the protease-activated antibody is activated by a protease selected from a cysteine cathepsin, an aspartic cathepsin, a kallikrein (hK), a serine protease, a caspase, a matrix metalloproteinase (MMP), and a disintegrin metalloproteinase (ADAM).

96. The oncolytic virus of claim 95, wherein the protease is selected from cathepsin K, cathepsin B, cathepsin L, cathepsin E, cathepsin D, hK1, PSA (hK3), hK10, hK15, uPA, uPAR, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-18, MMP-19, MMP-20, MMP-21, MMP-23A, MMP-23B, MMP-24, MMP-25, MMP-26, MMP-27, MMP-28, or a protease listed in Table 6.

97. The oncolytic virus of claim 63 or 94, wherein the protease-activated antibody binds to a protein expressed more highly by a cancer cell or in a cancer microenvironment than by a non-cancer cell or in a non-cancer microenvironment.

98. The oncolytic virus of claim 97, wherein the protease-activated antibody binds NKG2D, c-met, HGFR, CD8, heparan sulfate, VSPG4 (also known as NG2), EGFR, EGFRvIII, CD133, CXCR4, carcinoembryonic antigen (CEA), CLC-3, annexin II, human transferrin receptor, or EpCAM.

99. The oncolytic virus of any of claims 63-98, wherein the one or more polynucleotides are inserted into a gene locus of the viral genome, or inserted between two gene loci of the viral genome.

100. The oncolytic virus of claim 99, wherein the viral gene loci are selected from the group consisting of the internal repeat joint region (comprising one copy each of the diploid genes ICP0, ICP34.5, LAT, ICP4, and the ICP47 promoter), ICP0, LAT, UL1, UL5, UL6, UL7, UL8, UL9, UL11, UL12, UL14, UL15, UL17, UL18, UL19, UL20, UL22, UL25, UL26, UL26.5, UL27, UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL36, UL37, UL38, UL39, UL40, UL42, UL48, UL49, UL52, UL53, UL54, ICP0, ICP4, ICP22, ICP27, ICP47, gamma-34.5, US3, US4, US5, US6, US7, US8, US9, US10, US11, and US12.

101. A nucleic acid molecule encoding the oncolytic virus of any of the preceding claims.

102. A viral stock comprising the oncolytic virus of any of claims 1-100.

103. A composition comprising the oncolytic virus of any of claims 42-100 and a pharmaceutically-acceptable carrier.

104. A method of killing a cancerous cell, comprising exposing the cancerous cell to the oncolytic virus of any one of claims 1-100 or compositions thereof under conditions sufficient for the oncolytic virus to infect and replicate within said cancerous cell, and wherein replication of the oncolytic virus within the cancerous cell results in cell death.

105. The method of claim 104, wherein the cancerous cell has a reduced expression of a miR capable of binding to the one or more miR-target sequences compared to the expression of the miR in a non-cancerous cell.

106. The method of claim 105, wherein the expression level of the miR in the cancerous cell is at least 5% less than the expression level the miR in the non-cancerous cell.

107. The method of any one of claims 104 – 106, wherein replication of the oncolytic virus is increased or maintained in cancerous cells with a reduced expression of the miR capable of binding to the one or more miR-target sequences.

108. The method of claim 107, wherein the viral replication is at least 5% greater in the cancerous cells compared to the viral replication in the non-cancerous cell.

109. The method of any one of claims 104 – 108, wherein the cell is *in vivo*.

110. The method of claim 109, wherein the cell is within a tumor.

111. A method of treating cancer in a subject in need thereof, comprising administering the oncolytic virus of any of claims 1-100 or compositions thereof to a subject in need thereof.

112. The method of claim 111, wherein the subject is a mouse, a rat, a rabbit, a cat, a dog, a horse, a non-human primate, or a human.

113. The method of claim 111 or 112, wherein the oncolytic virus or compositions thereof are administered intravenously, subcutaneously, intratumorally, intramuscularly, or intranasally.

114. The method of claim 111, wherein the cancer is selected from lung cancer, breast cancer, ovarian cancer, cervical cancer, prostate cancer, testicular cancer, colorectal cancer, colon cancer, pancreatic cancer, liver cancer, gastric cancer, head and neck cancer, thyroid cancer, malignant glioma, glioblastoma, melanoma, B-cell chronic lymphocytic leukemia, diffuse large B-cell lymphoma (DLBCL), and marginal zone lymphoma (MZL).

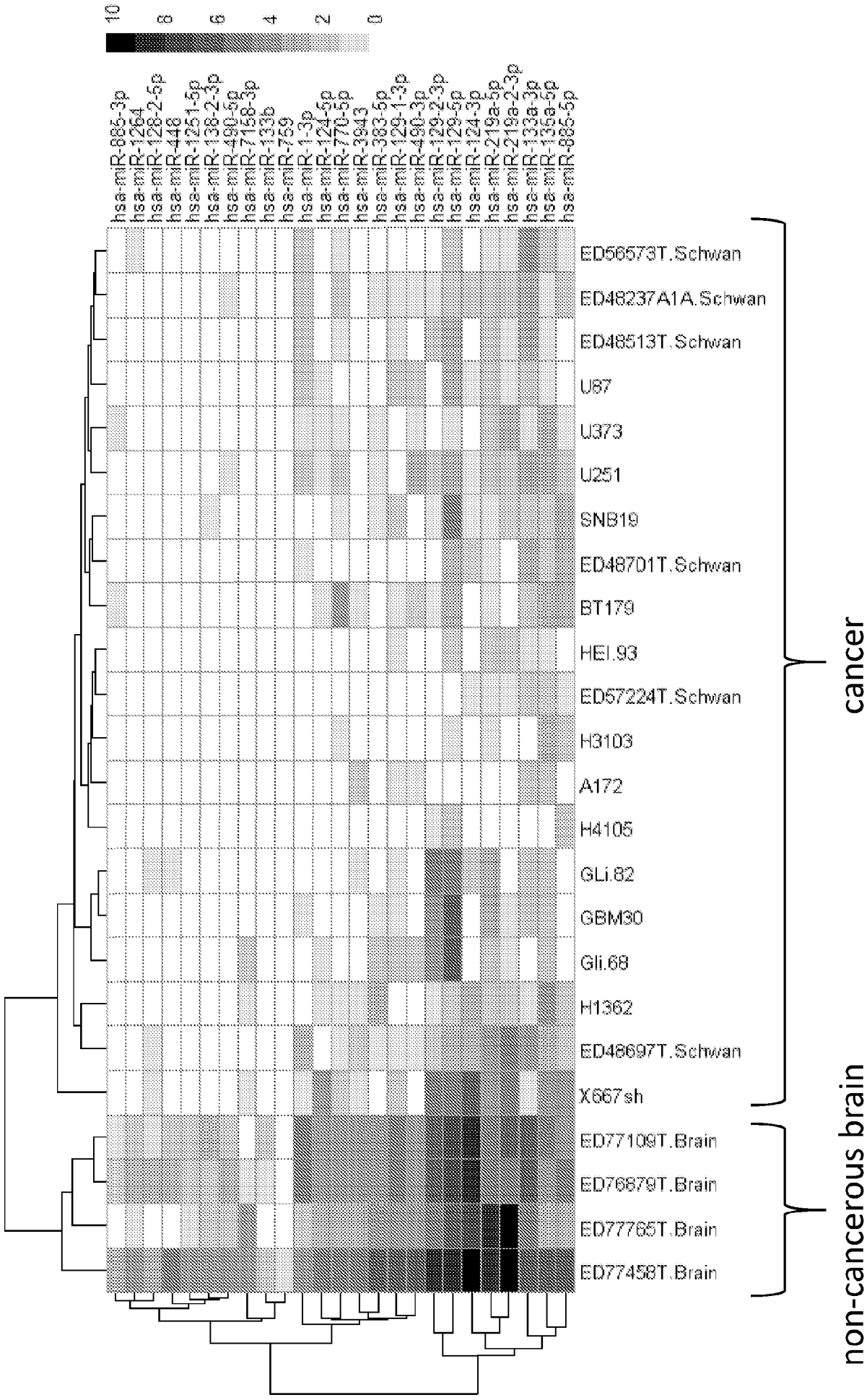
115. The method of claim 114, wherein the lung cancer is small cell lung cancer or non-small cell lung cancer.

116. The method of claim 114, wherein the liver cancer is hepatocellular carcinoma (HCC).

117. The recombinant HSV of claim 38 or the recombinant oncolytic virus of any one of claims 63-90 wherein the payload molecule or protein is an anti-FAP/anti-CD3 bispecific T cell engager.

118. The recombinant HSV of claim 38 or the recombinant oncolytic virus of any one of claims 63-90 wherein the payload molecule or protein is an anti-PD1-Fc-41BBL protein.

Fig. 1


10
8
6
4
2
0
hsa-miR-885-3p
hsa-miR-1264
hsa-miR-128-2-5p
hsa-miR-448
hsa-miR-1251-5p
hsa-miR-136-2-3p
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U373
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H1362
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cancer
non-cancerous brain

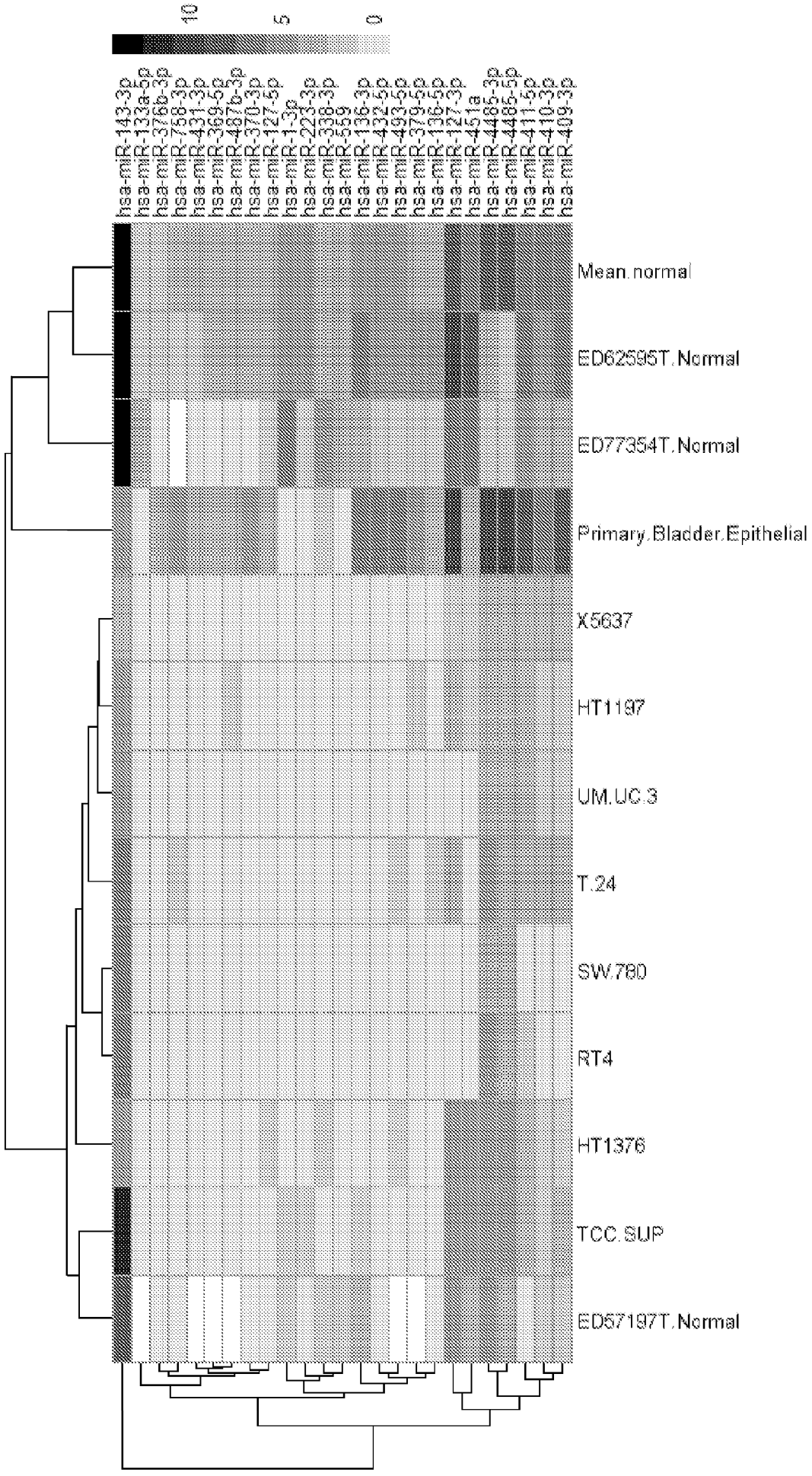

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hsa-miR-410-3p
hsa-miR-409-3p
Mean.normal
ED62595T.Normal
ED77354T.Normal
Primary.Bladder.Epithelial
X5637
HT1197
UM.UC.3
T.24
SW.780
RT4
HT1376
TCC.SUP
ED57197T.Normal

Fig. 2

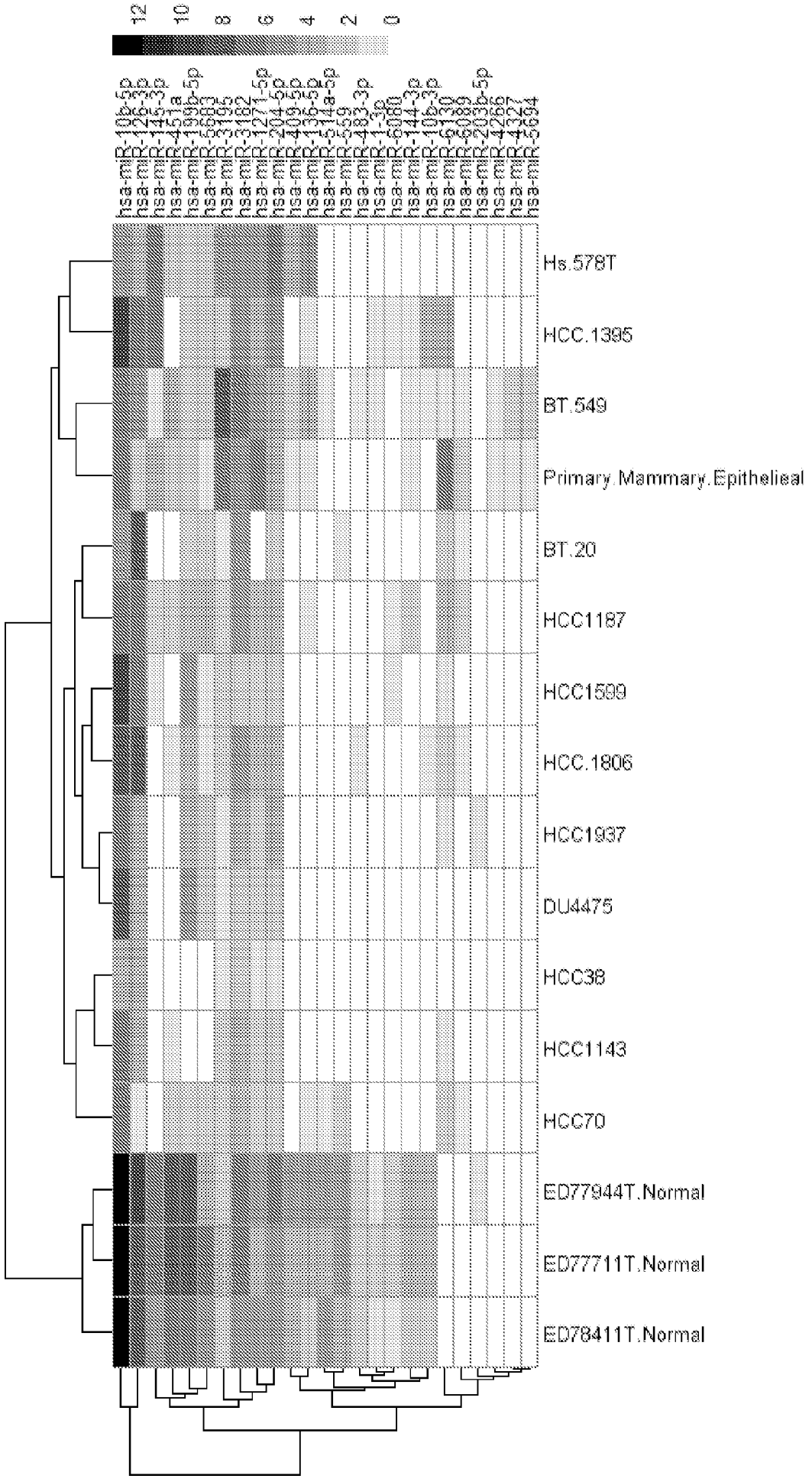

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Fig. 3

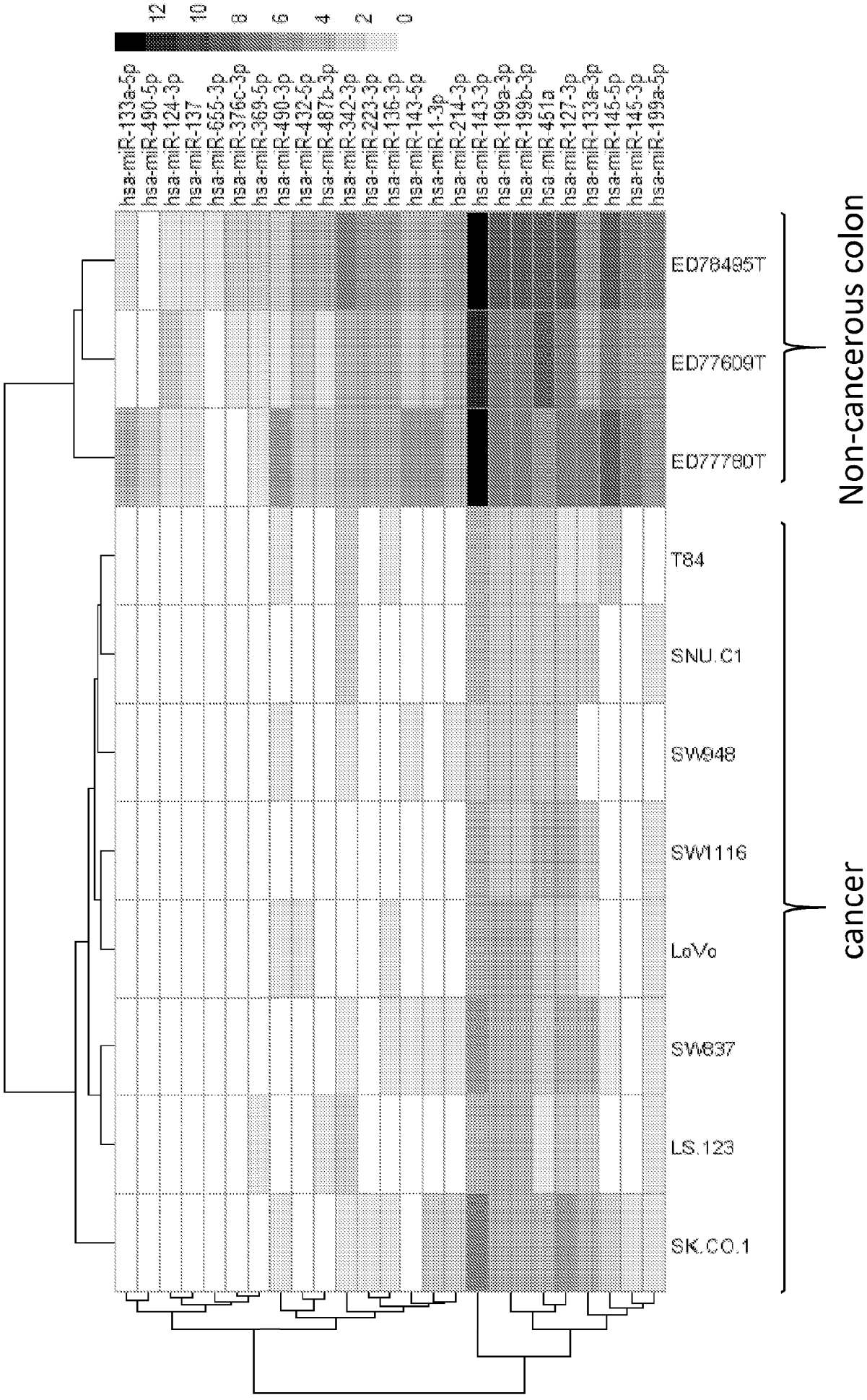

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Non-cancerous colon
cancer

Fig. 4

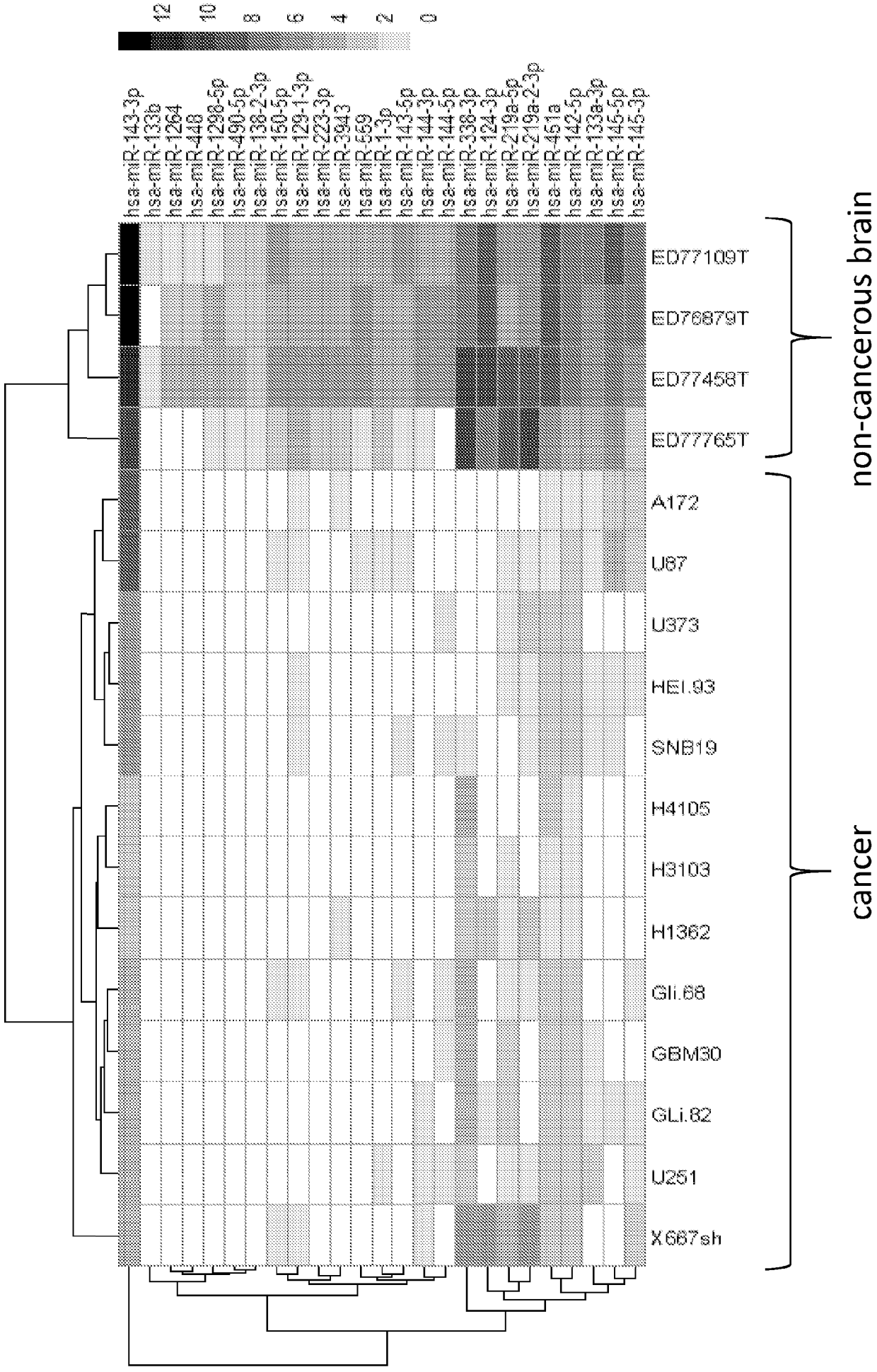

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hsa-miR-1264
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hsa-miR-490-5p
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hsa-miR-150-5p
hsa-miR-129-1-3p
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hsa-miR-133a-3p
hsa-miR-145-5p
hsa-miR-145-3p
ED77109T
ED76879T
ED77458T
ED77765T
A172
U87
U373
HEI.93
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H1362
Gli.68
GBM30
GLi.82
U251
X667sh
non-cancerous brain
cancer

Fig. 5

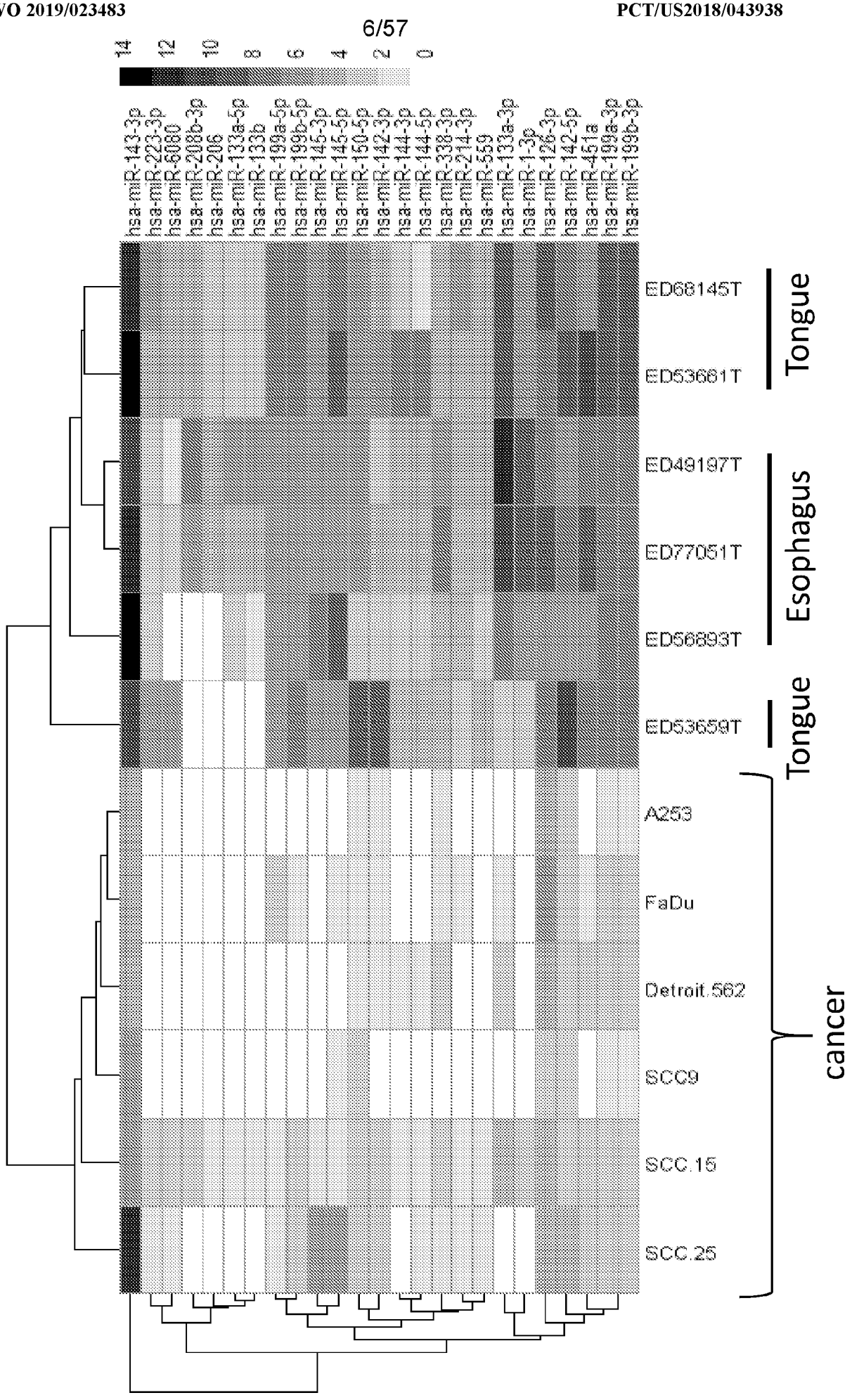

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hsa-miR-199b-5p
hsa-miR-145-3p
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hsa-miR-142-3p
hsa-miR-144-3p
hsa-miR-144-5p
hsa-miR-338-3p
hsa-miR-214-3p
hsa-miR-559
hsa-miR-133a-3p
hsa-miR-1-3p
hsa-miR-126-3p
hsa-miR-142-5p
hsa-miR-451a
hsa-miR-199a-3p
hsa-miR-199b-3p
ED68145T
ED53681T
ED49197T
ED77051T
ED56893T
ED53659T
A253
FaDu
Detroit.562
SCC9
SCC.15
SCC.25
Tongue
Esophagus
Tongue
cancer

Fig. 6

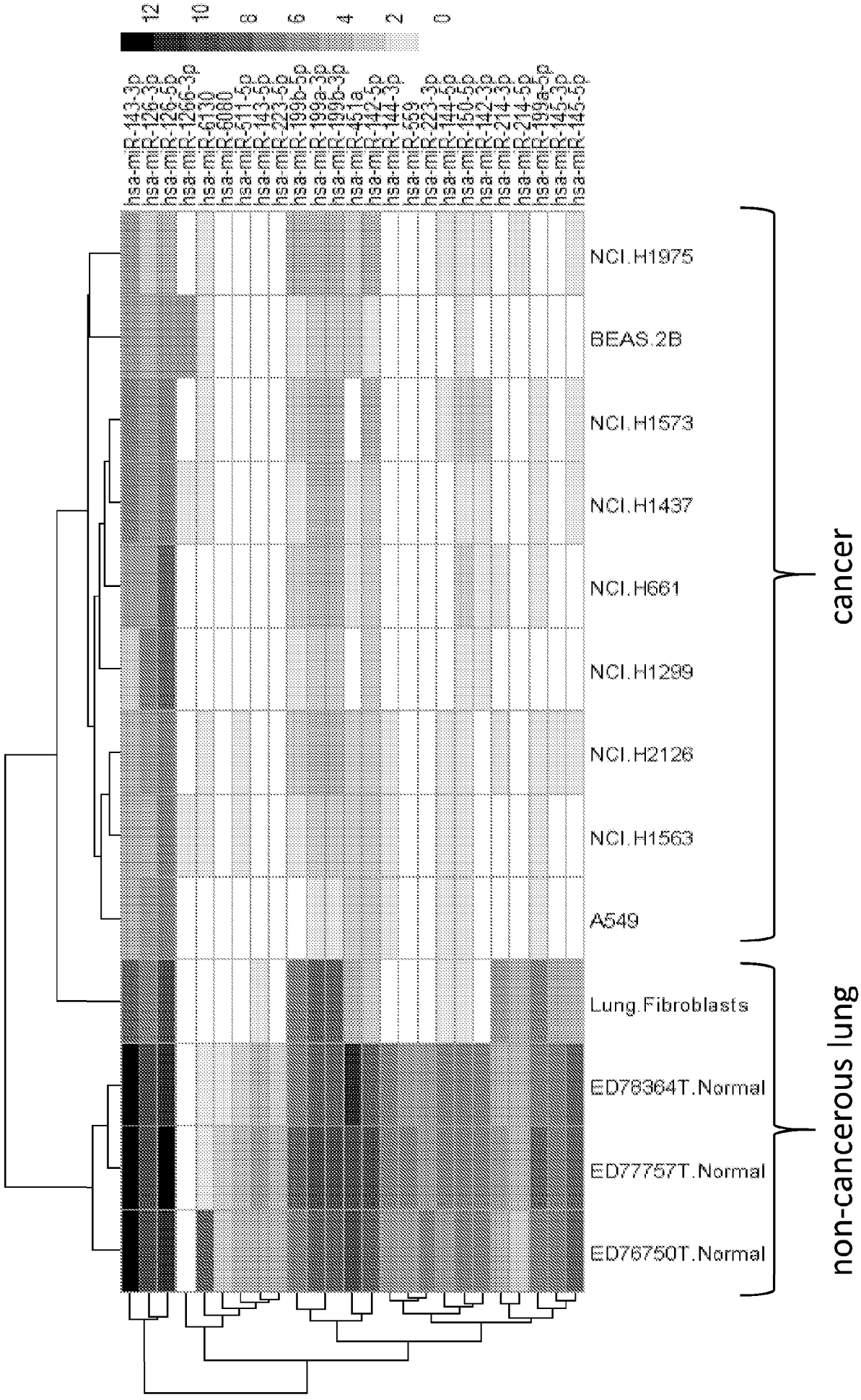
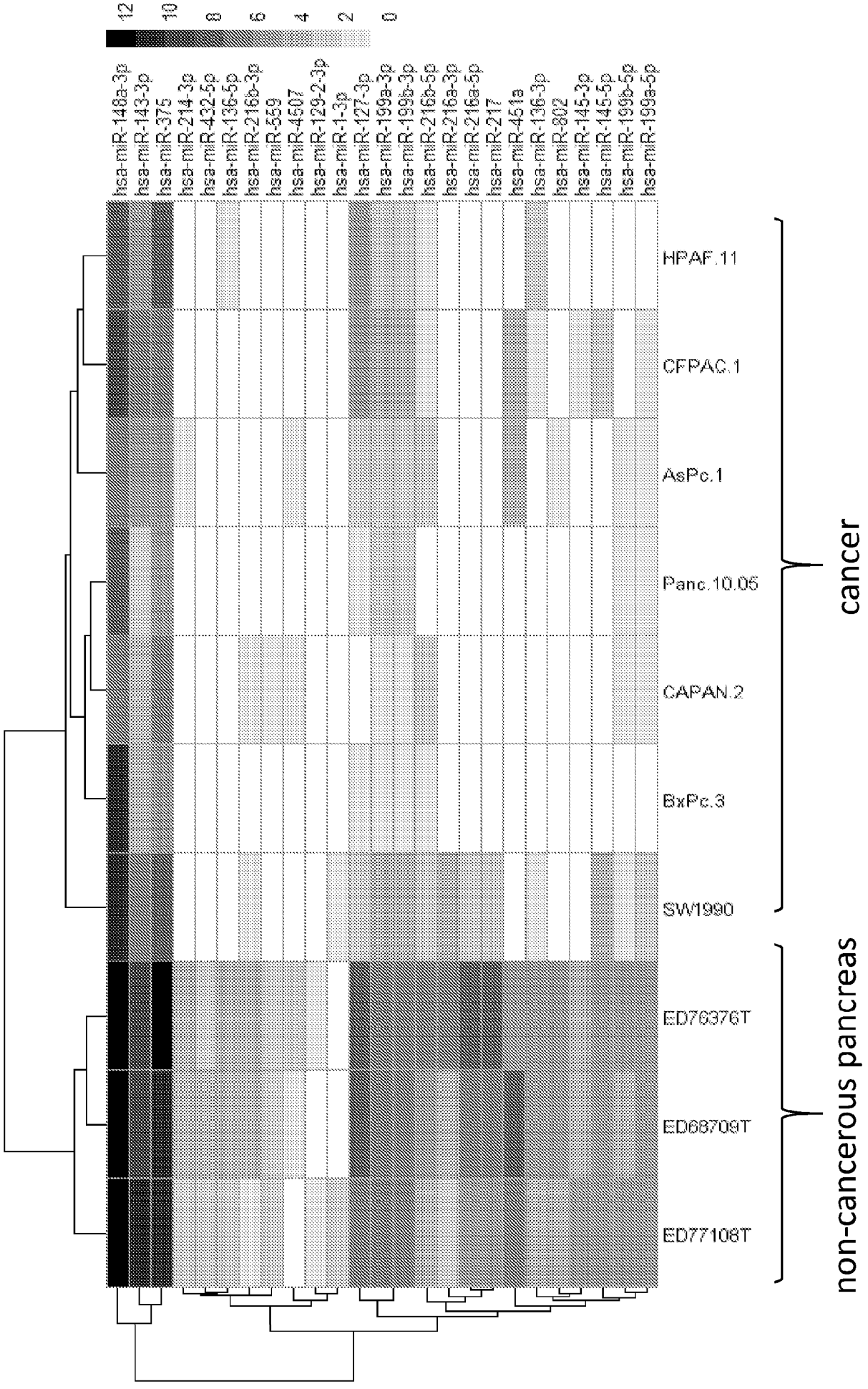

12
10
8
6
4
2
0
hsa-miR-143-3p
hsa-miR-126-3p
hsa-miR-126-5p
hsa-miR-126-3p
hsa-miR-6130
hsa-miR-6080
hsa-miR-511-5p
hsa-miR-143-5p
hsa-miR-223-5p
hsa-miR-199a-5p
hsa-miR-199a-3p
hsa-miR-199b-3p
hsa-miR-451a
hsa-miR-142-5p
hsa-miR-144-3p
hsa-miR-150-3p
hsa-miR-223-3p
hsa-miR-144-5p
hsa-miR-150-5p
hsa-miR-142-3p
hsa-miR-214-3p
hsa-miR-214-5p
hsa-miR-199a-5p
hsa-miR-145-3p
hsa-miR-145-5p
NCI.H1975
BEAS.2B
NCI.H1573
NCI.H1437
NCI.H661
NCI.H1299
NCI.H2126
NCI.H1563
A549
Lung.Fibroblasts
ED78364T.Normal
ED77757T.Normal
ED76750T.Normal
cancer
non-cancerous lung

Fig. 7

Fig. 8


12
10
8
6
4
2
0
hsa-miR-148a-3p
hsa-miR-143-3p
hsa-miR-375
hsa-miR-214-3p
hsa-miR-432-5p
hsa-miR-136-5p
hsa-miR-216b-3p
hsa-miR-559
hsa-miR-4507
hsa-miR-129-2-3p
hsa-miR-1-3p
hsa-miR-127-3p
hsa-miR-199a-3p
hsa-miR-199b-3p
hsa-miR-216b-5p
hsa-miR-216a-3p
hsa-miR-216a-5p
hsa-miR-217
hsa-miR-451a
hsa-miR-136-3p
hsa-miR-802
hsa-miR-145-3p
hsa-miR-145-5p
hsa-miR-199b-5p
hsa-miR-199a-5p
HPAF.11
CFPAC.1
AsPc.1
Panc.10.05
CAPAN.2
BxPc.3
SW1990
ED76376T
ED68709T
ED77108T
cancer
non-cancerous pancreas

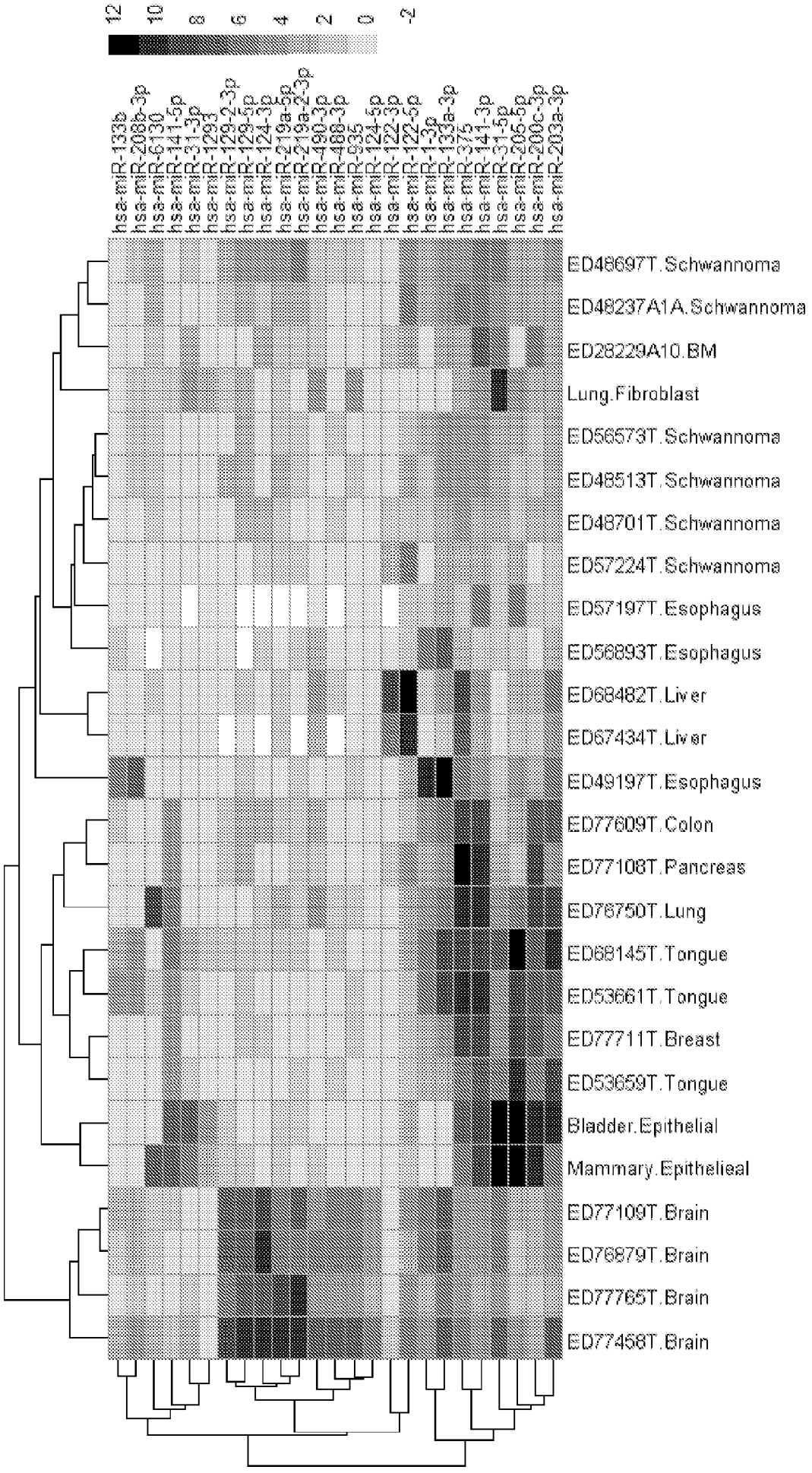


Fig. 9

All non-Schwannoma = non-cancerous tissue
BM = non-cancerous bone marrow

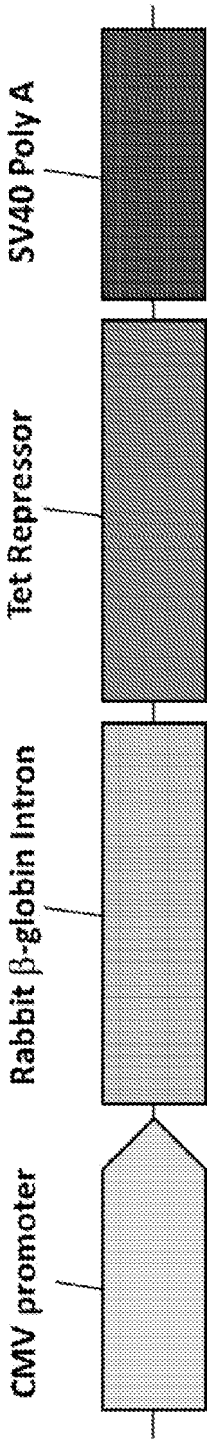


Fig. 10A

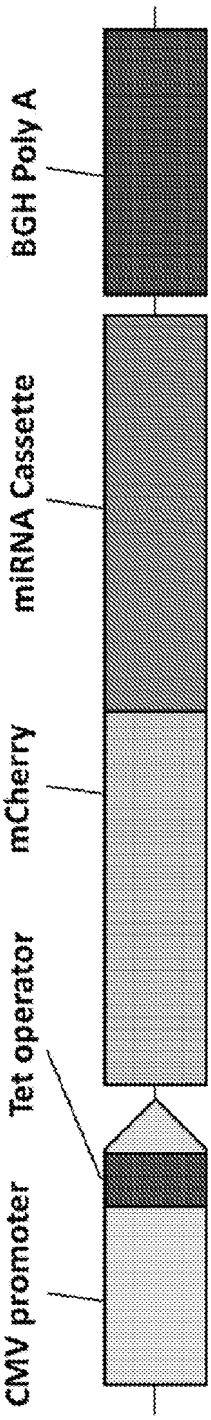


Fig. 10B

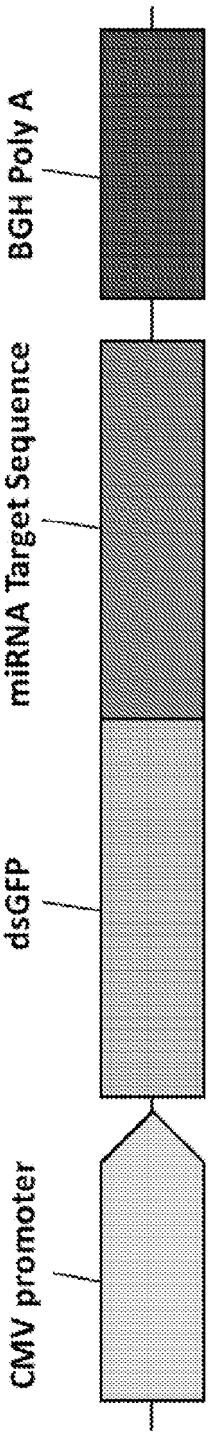


Fig. 10C

Fig. 11

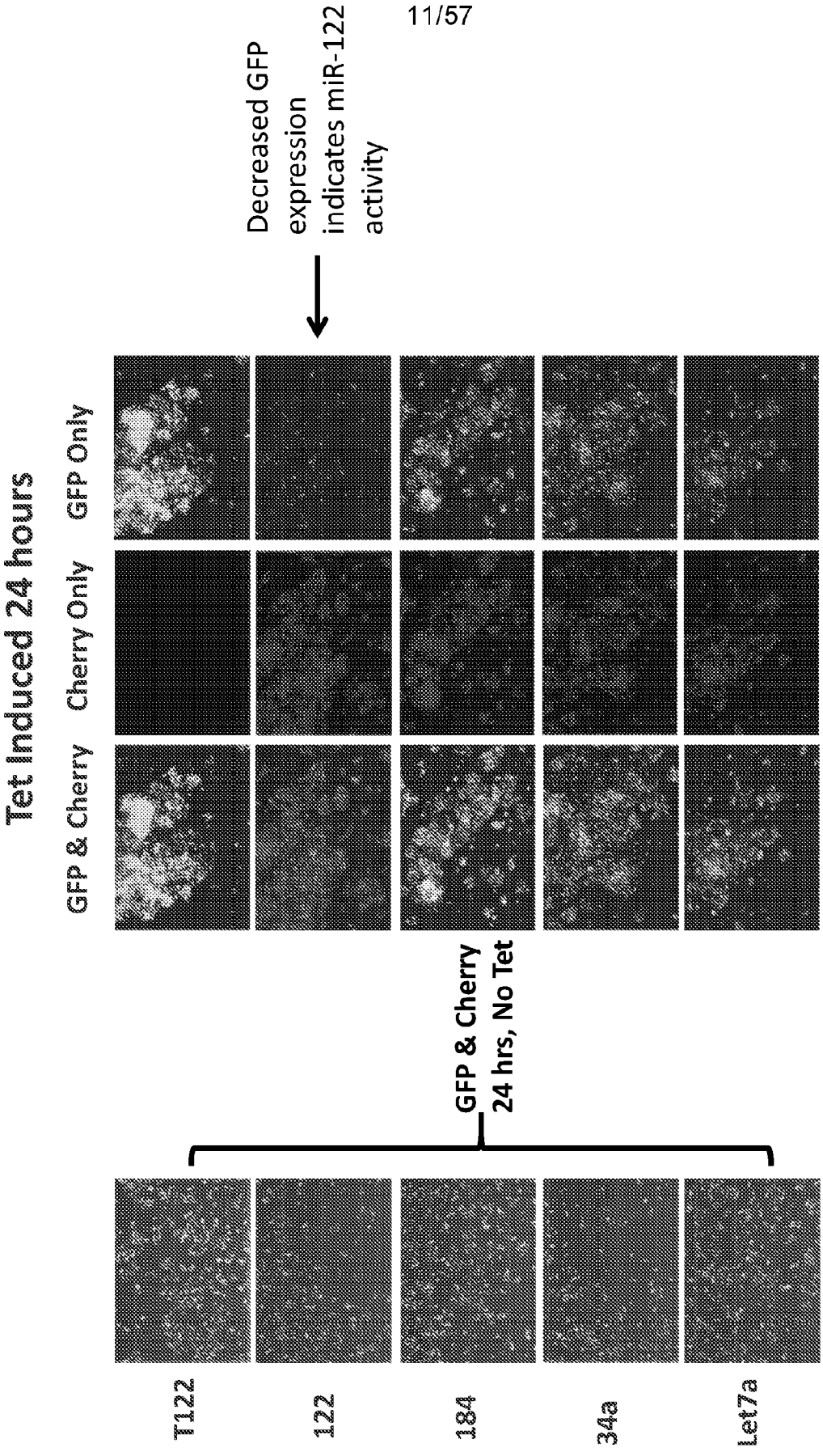
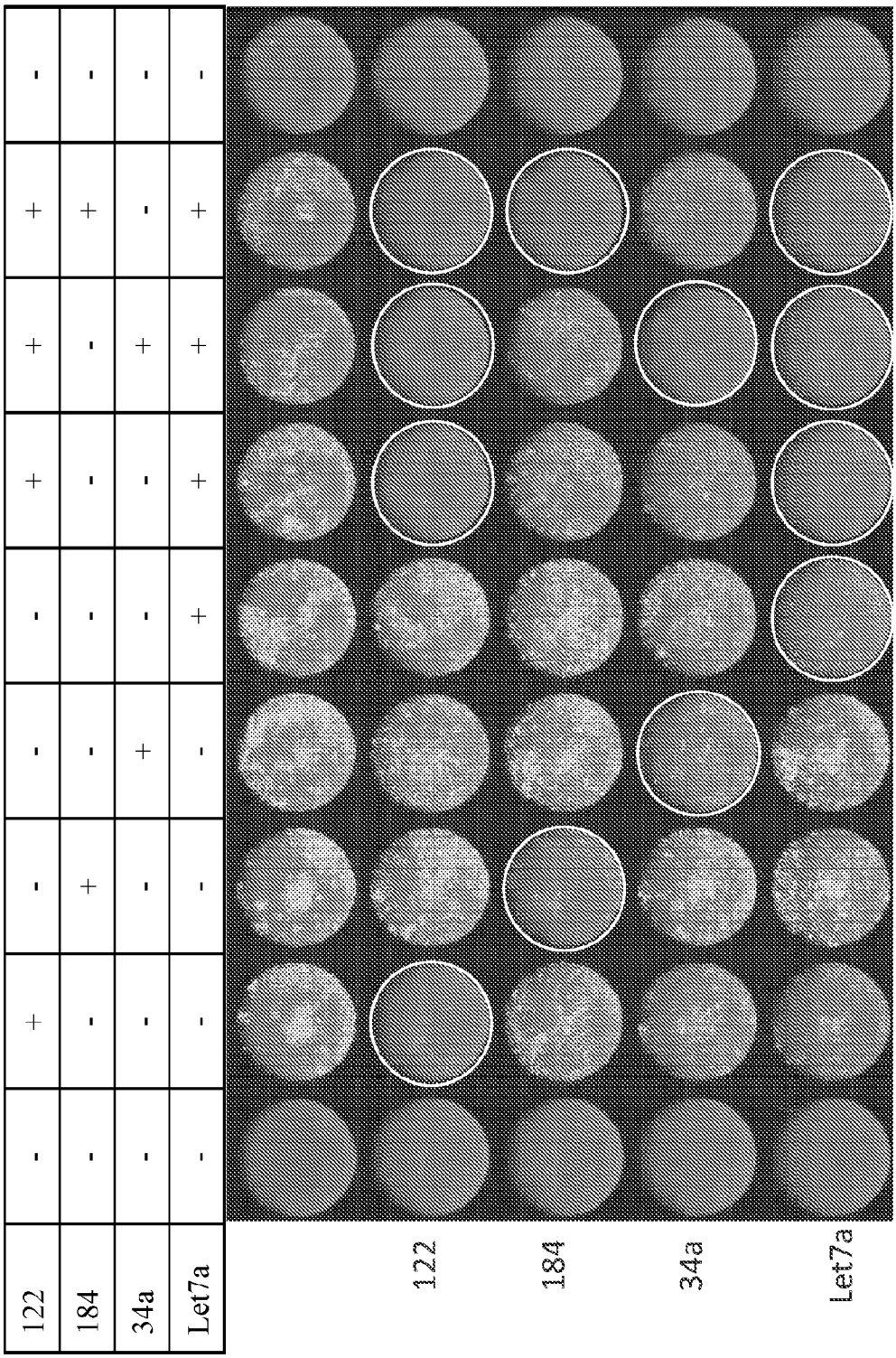

Decreased GFP expression indicates miR-122 activity
Tet Induced 24 hours
GFP & Cherry
Cherry Only
GFP Only
GFP & Cherry
24 hrs, No Tet
T122
122
184
34a
Let7a

Fig. 12



122	-	+	-	-	-	+	+	+	-
184	-	-	+	-	-	-	-	+	-
34a	-	-	-	+	-	-	+	-	-
Let7a	-	-	-	-	+	+	+	+	-

Fig. 13

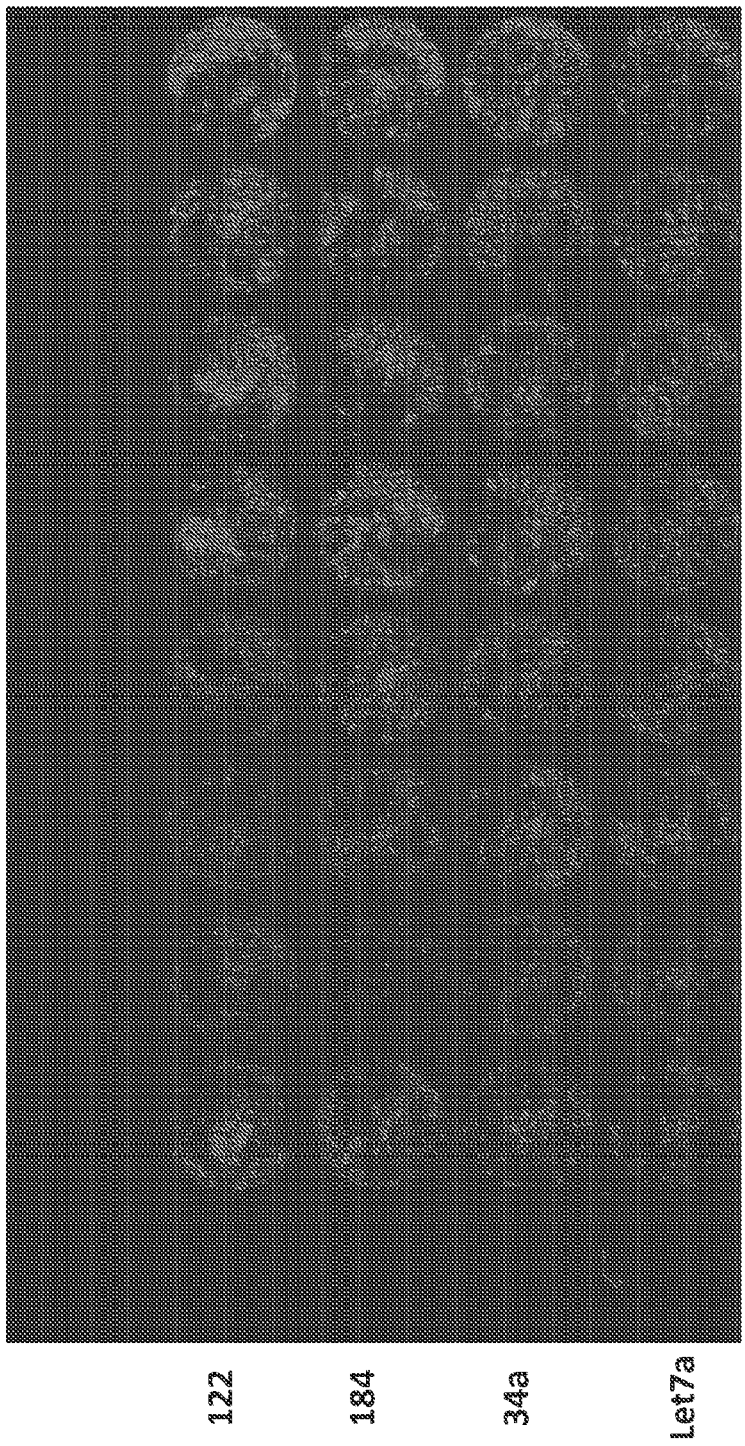

T122
T184
T34a
TLet7a
T122
TLet7a
T122, T34a
TLet7a
T122, T184
TLet7a
122
184
34a
Let7a

Fig. 14

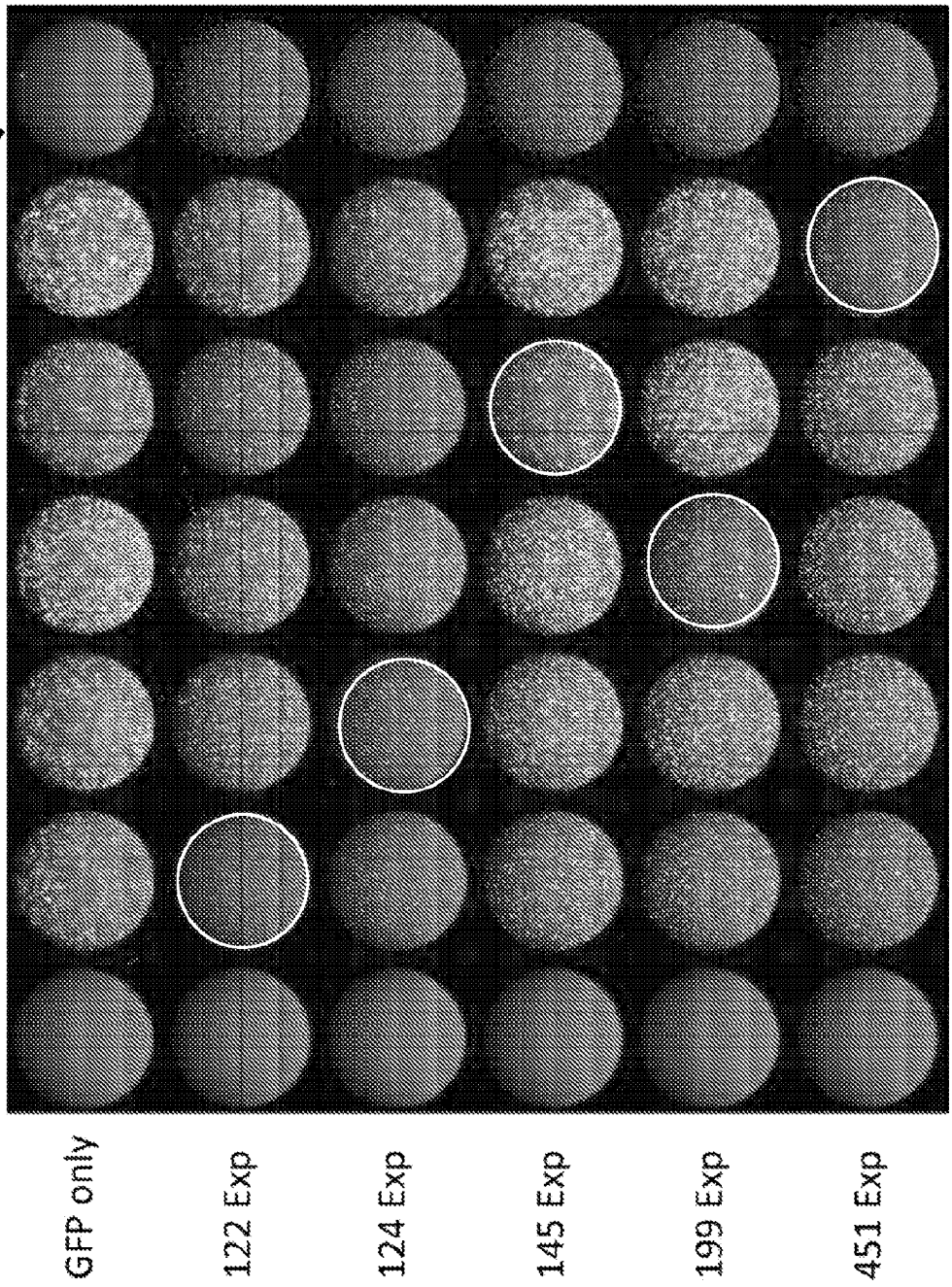
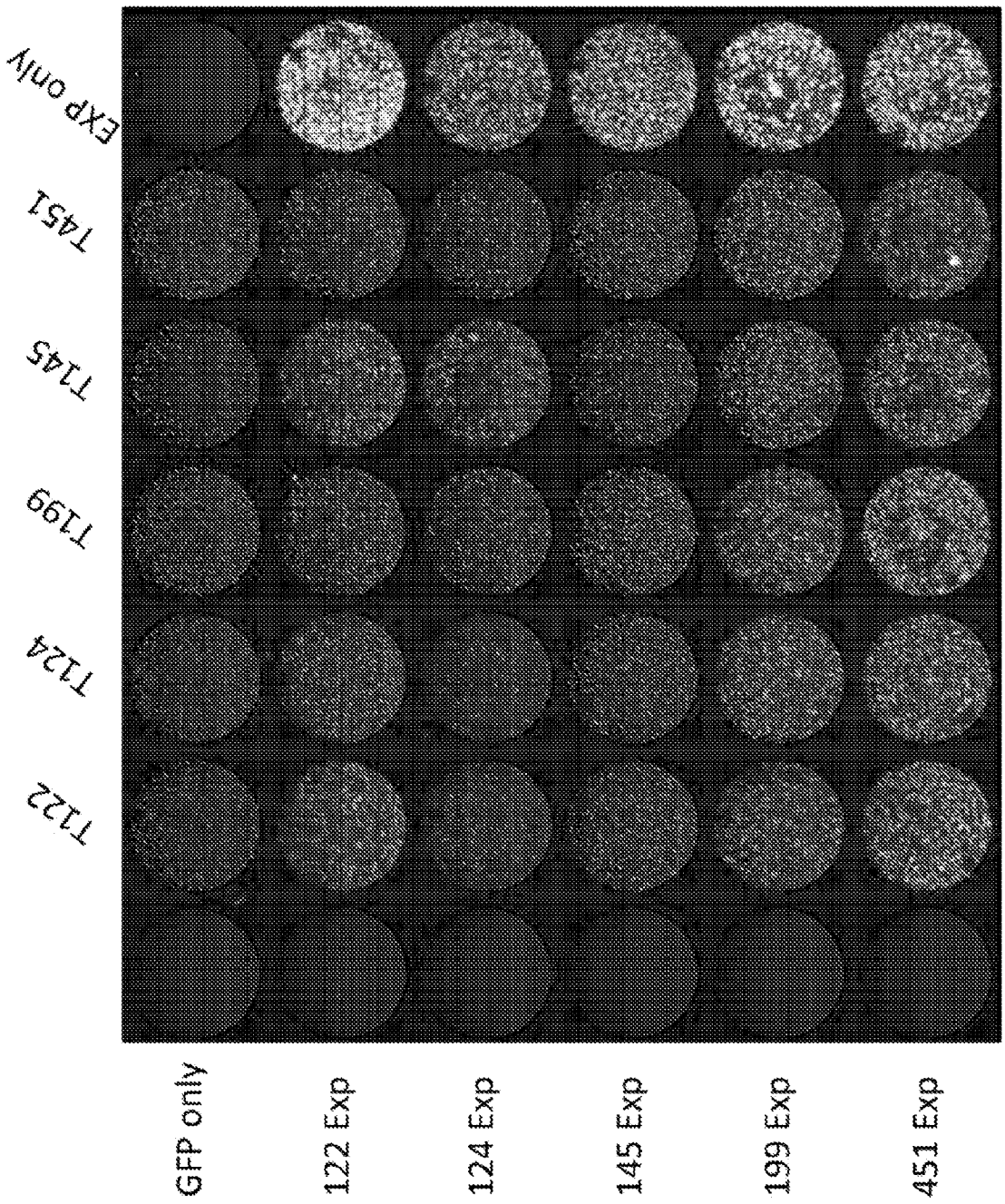

T122
T124
T199
T145
T451
EXP only
GFP only
122 Exp
124 Exp
145 Exp
199 Exp
451 Exp

Fig. 15


GFP only
122 Exp
124 Exp
145 Exp
199 Exp
451 Exp
T22
T24
T99
T45
T451
EXP only

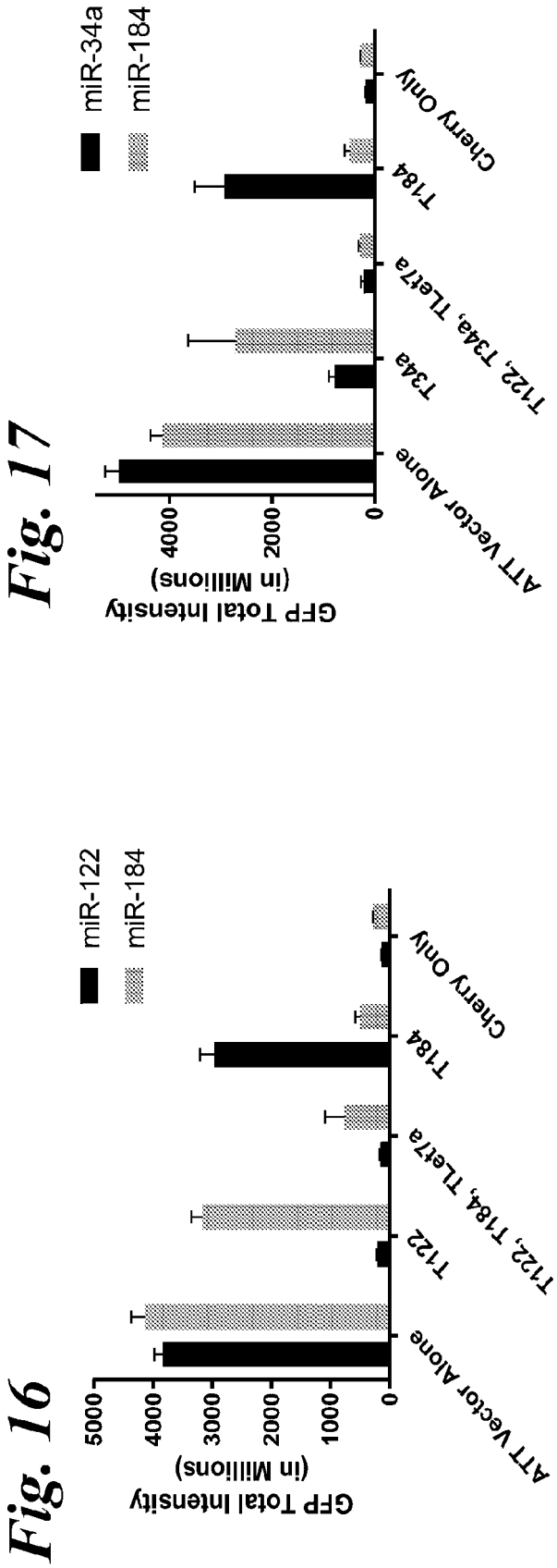
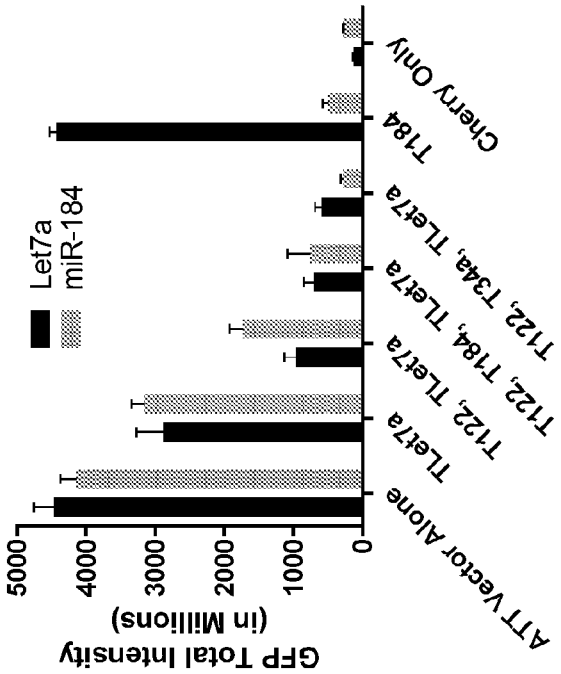

Fig. 16
GFP Total Intensity (in Millions)
5000
4000
3000
2000
1000
0
miR-122
miR-184
ATT Vector Alone
T122
T122, T184, TLet7a
T184
Cherry Only

Fig. 17
GFP Total Intensity (in Millions)
4000
2000
0
miR-34a
miR-184
ATT Vector Alone
T34a
T122, T34a, TLet7a
T184
Cherry Only


Fig. 18
GFP Total Intensity (in Millions)
5000
4000
3000
2000
1000
0
Let7a
miR-184
ATT Vector Alone
TLet7a
T122, TLet7a
T122, T184, TLet7a
T122, T34a, TLet7a
T184
Cherry Only

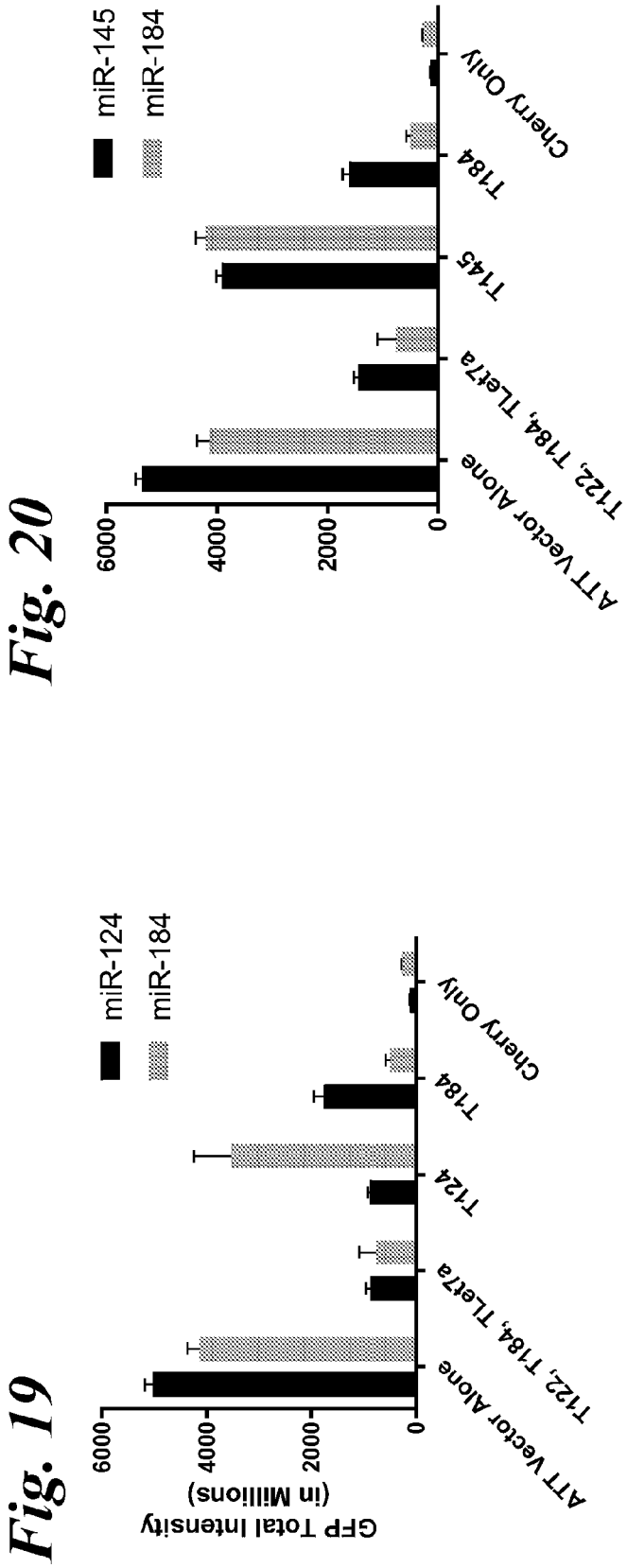


Fig. 19

Fig. 20

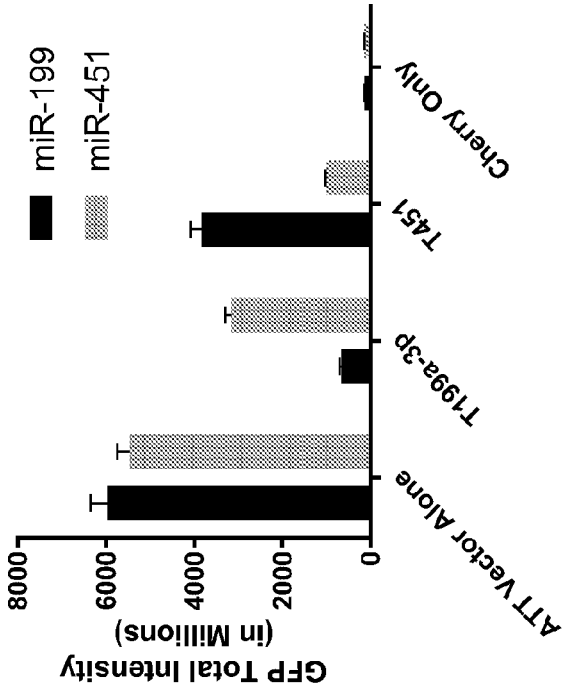


Fig. 21

Fig. 23

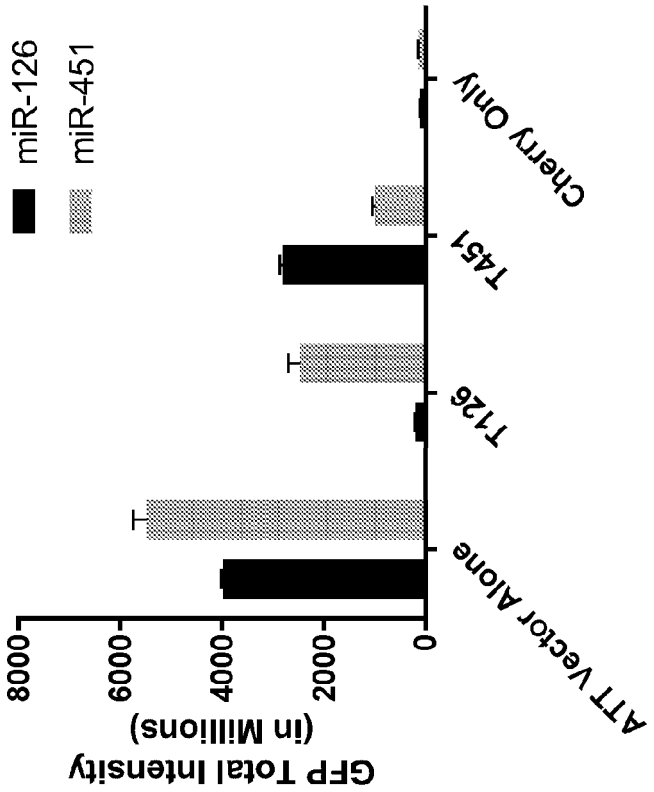

miR-126
miR-451
GFP Total Intensity
(in Millions)
8000
6000
4000
2000
0
ATT Vector Alone
T126
T451
Cherry Only

Fig. 22

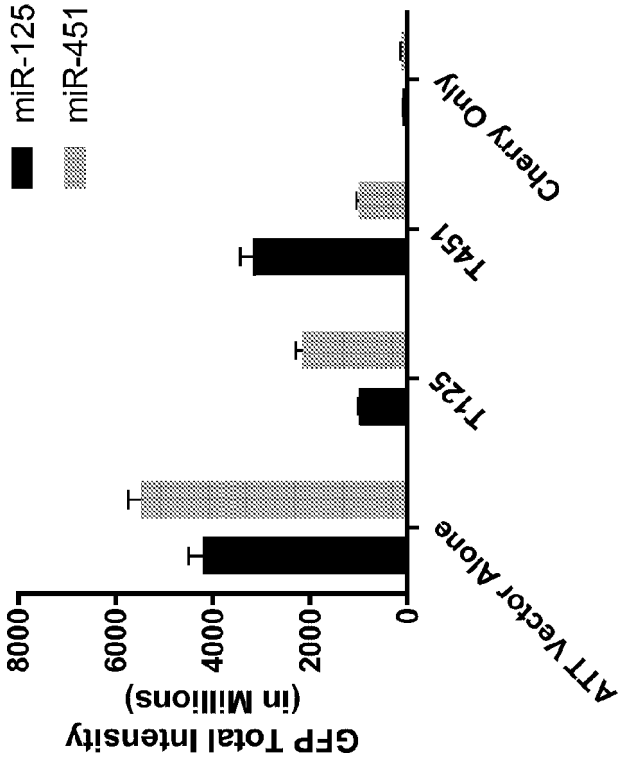

miR-125
miR-451
GFP Total Intensity
(in Millions)
8000
6000
4000
2000
0
ATT Vector Alone
T125
T451
Cherry Only

Fig. 24

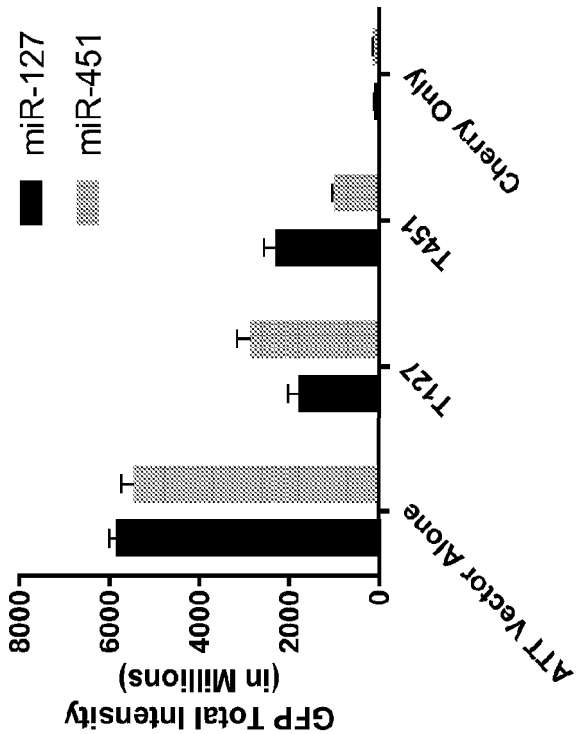


Fig. 25

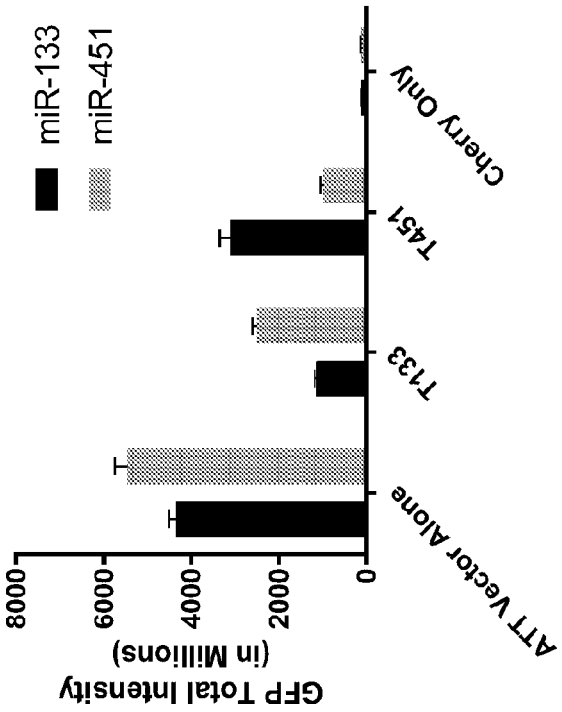


Fig. 26

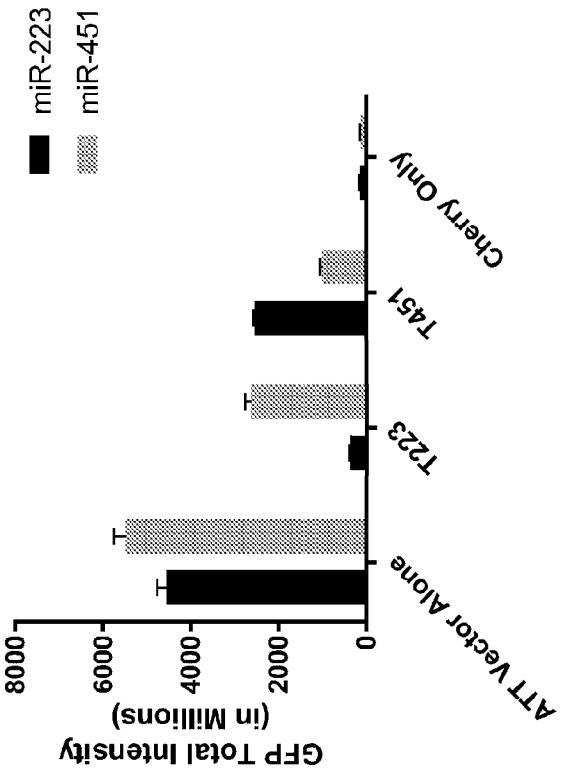


Fig. 27A

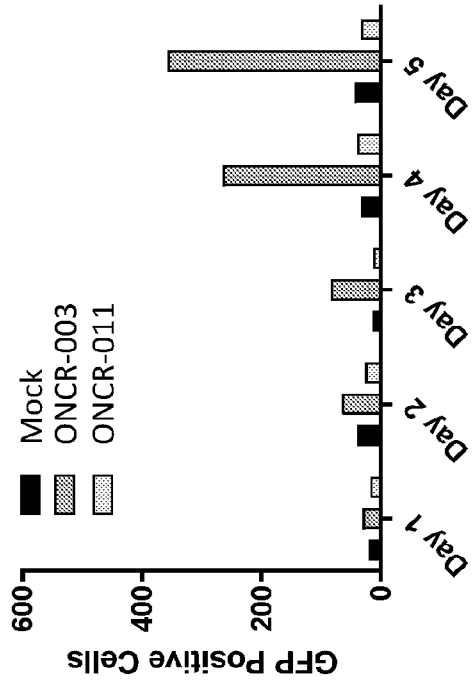


Fig. 27B

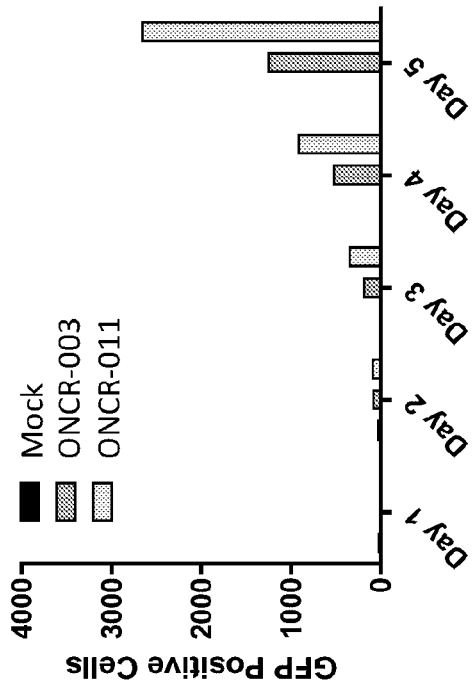


Fig. 27C

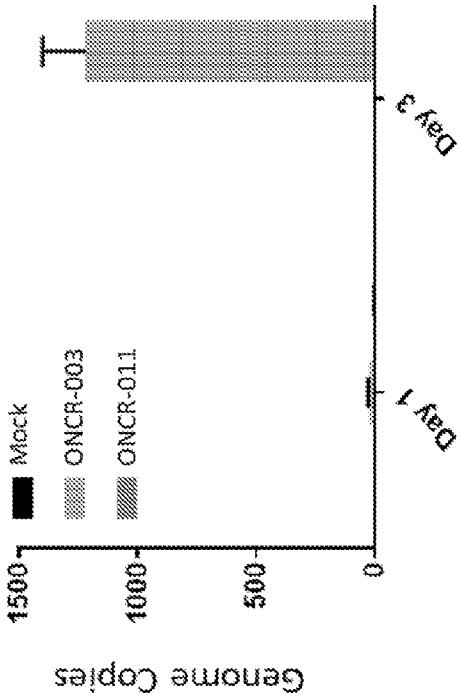


Fig. 27D

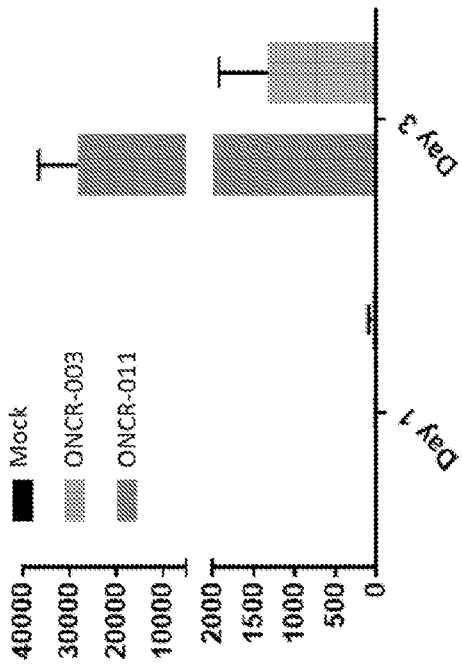


Fig. 28B

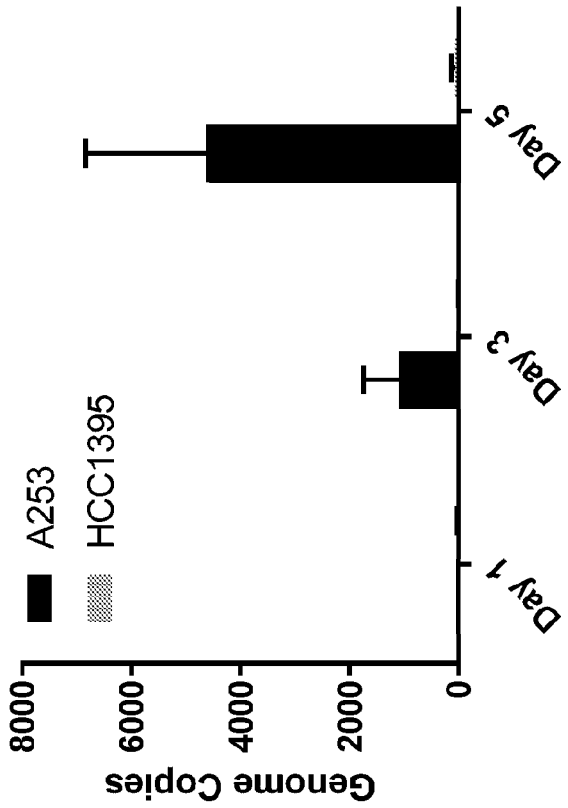


Fig. 28A

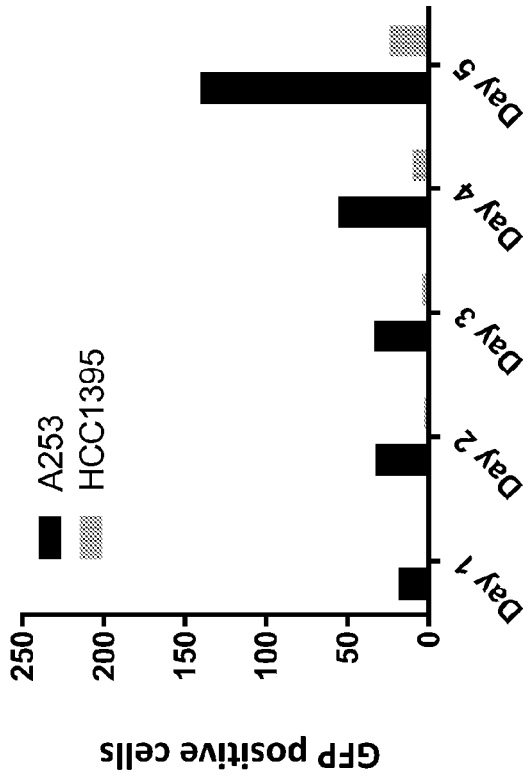


Fig. 29B

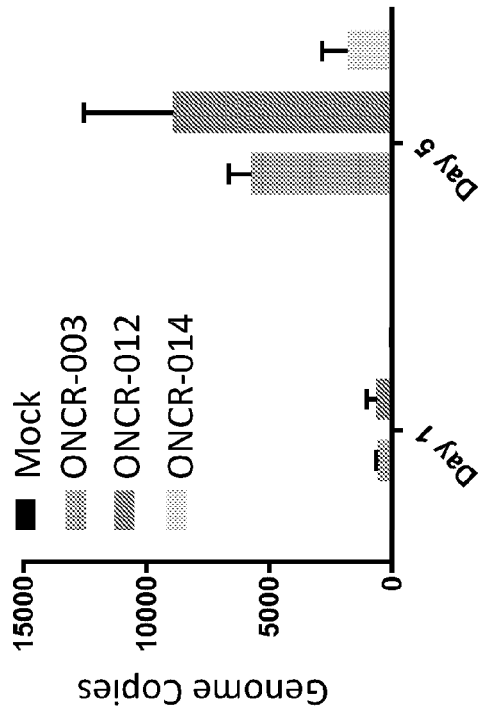


Fig. 29A

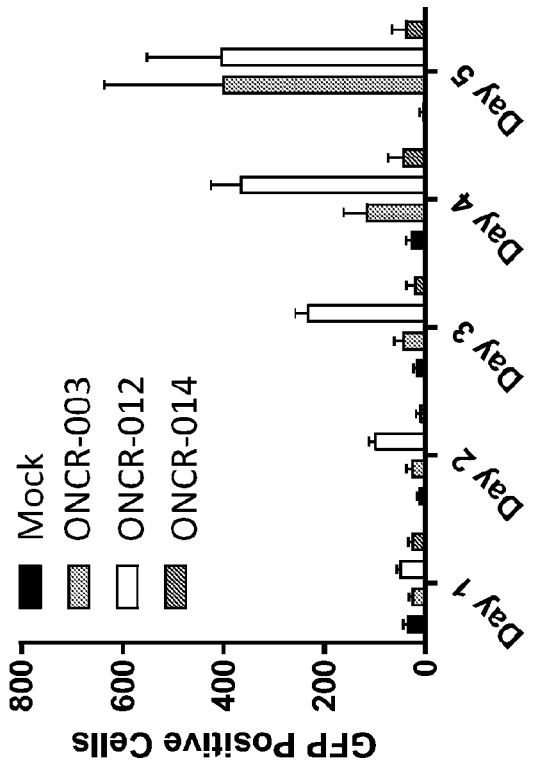


Fig. 31C

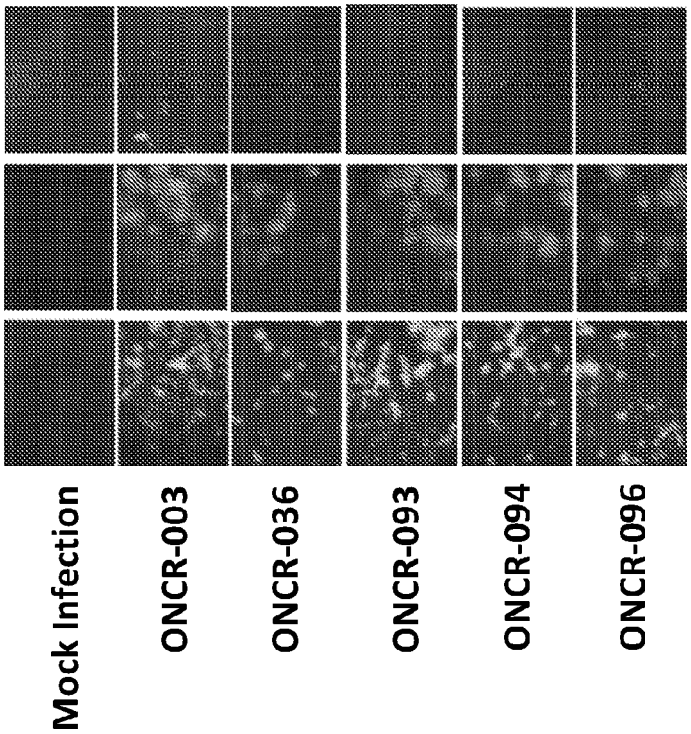


Fig. 30A

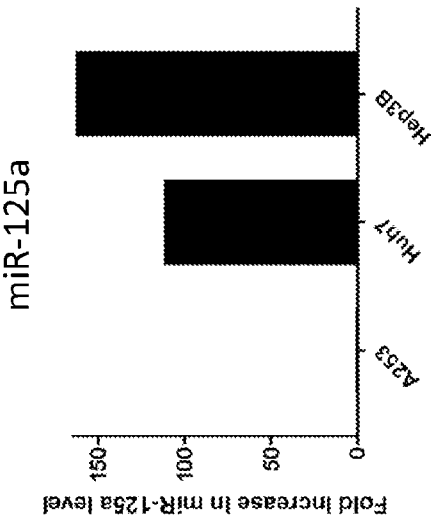


Fig. 30B

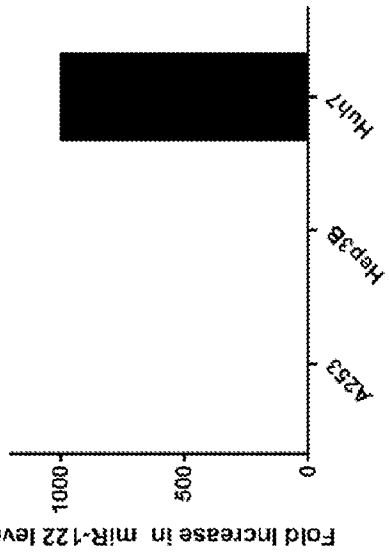


Fig. 31

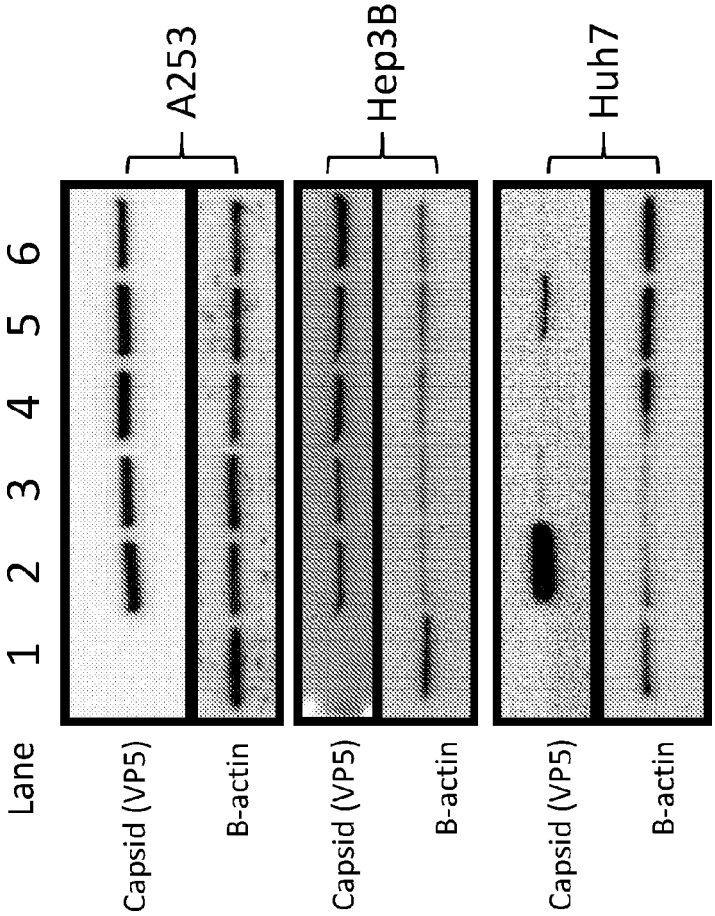

Lane 1: Mock
Lane 2: ONCR-003 (Control)
Lane 3: ONCR-036
Lane 4: ONCR-093
Lane 5: ONCR-094
Lane 6: ONCR-096
A253
Hep3B
Huh7
Lane
1 2 3 4 5 6
Capsid (VP5)
B-actin
Capsid (VP5)
B-actin
Capsid (VP5)
B-actin

Fig. 32A

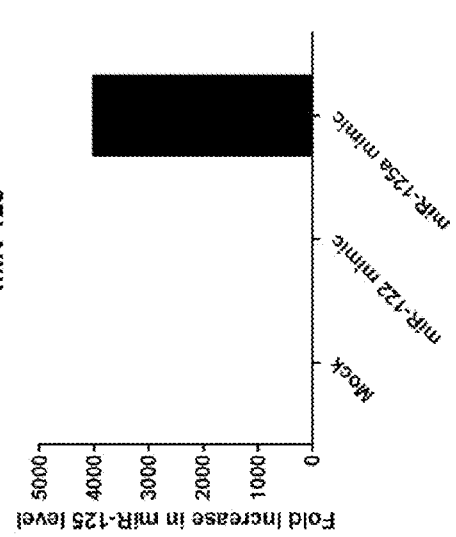


Fig. 32B

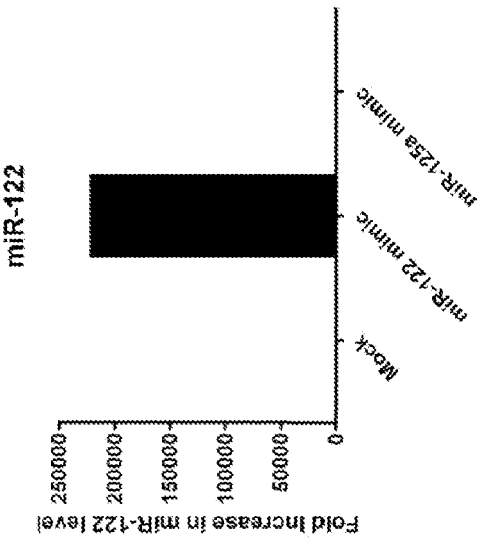


Fig. 32C

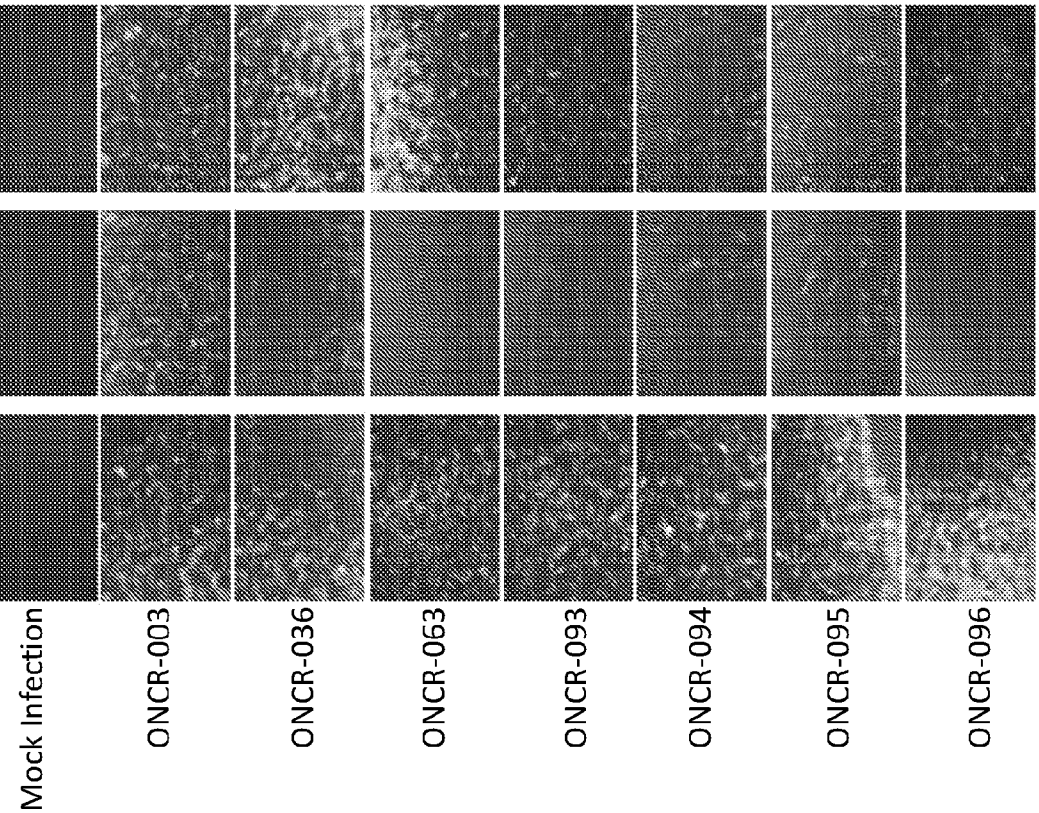
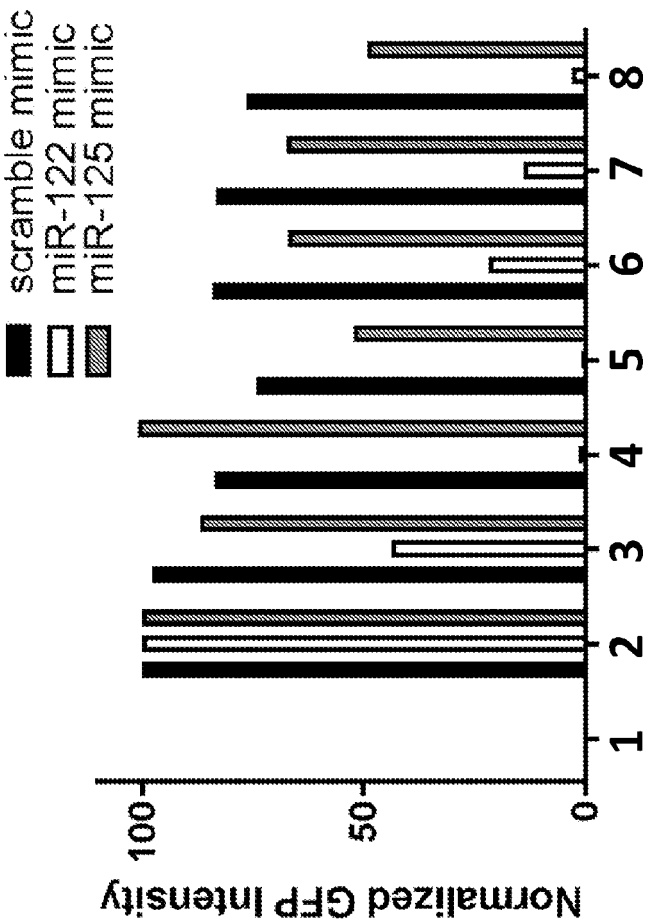


Fig. 32D


scramble mimic
miR-122 mimic
miR-125 mimic
Normalized GFP Intensity
100
50
0
1
2
3
4
5
6
7
8
1: Mock
2: ONCR-003 (Control)
3: ONCR-036
4: ONCR-063
5: ONCR-093
6: ONCR-094
7: ONCR-095
8: ONCR-096

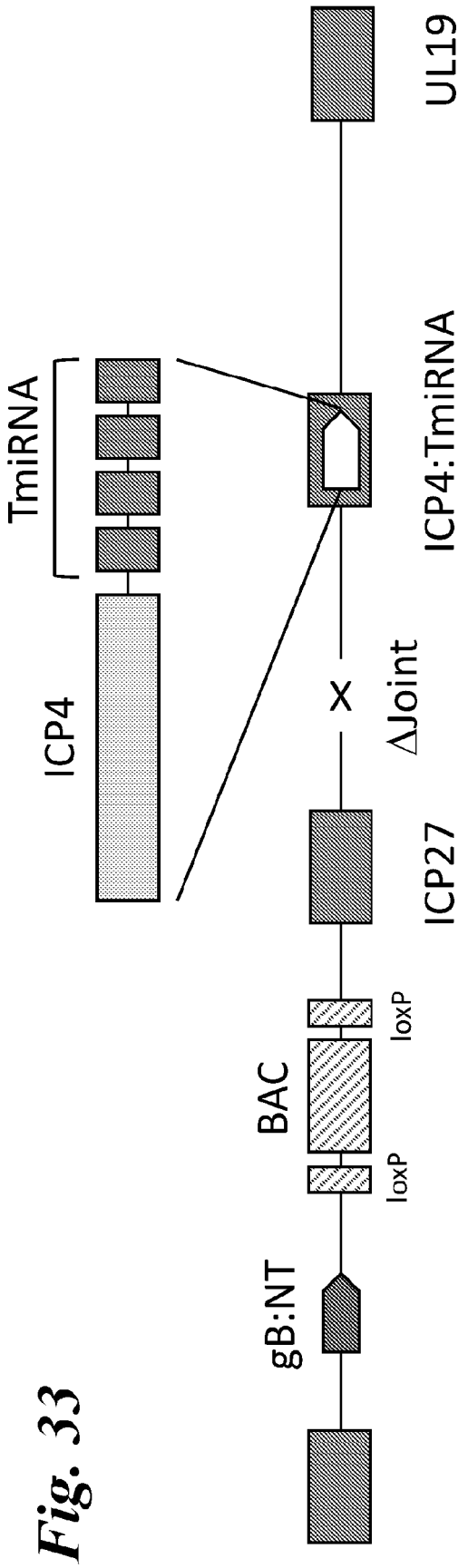

TmiRNA
ICP4
UL19
ICP4:TmiRNA
ΔJoint
X
ICP27
BAC
loxP
loxP
gB:NT

Fig. 33

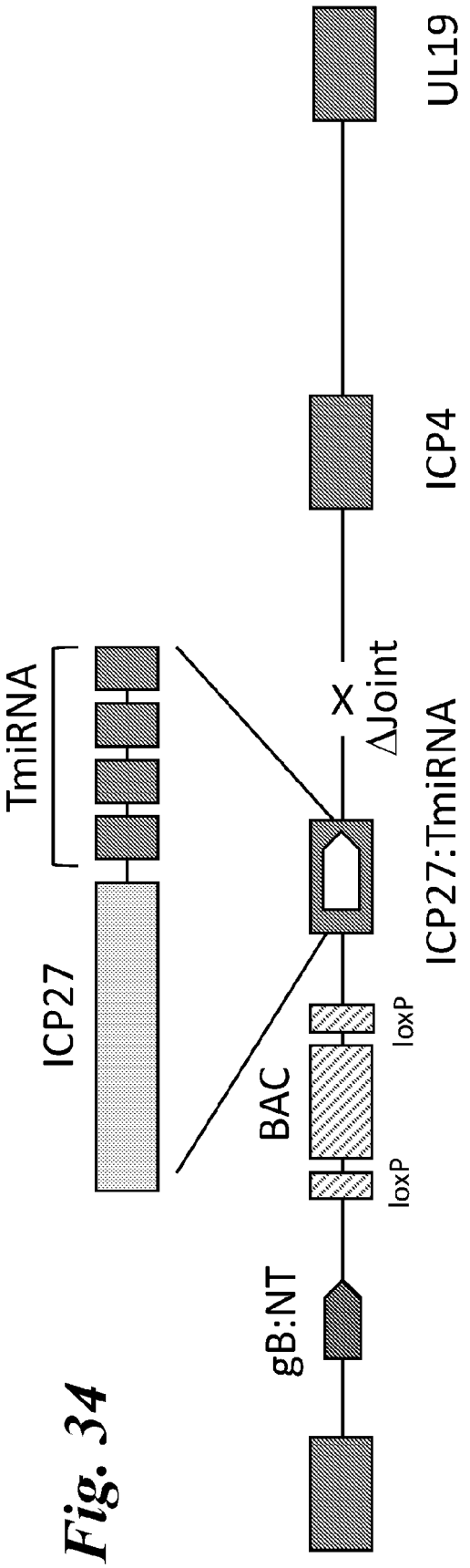

TmiRNA
ICP27
UL19
ICP4
ΔJoint
X
ICP27:TmiRNA
BAC
loxP
loxP
gB:NT

Fig. 34

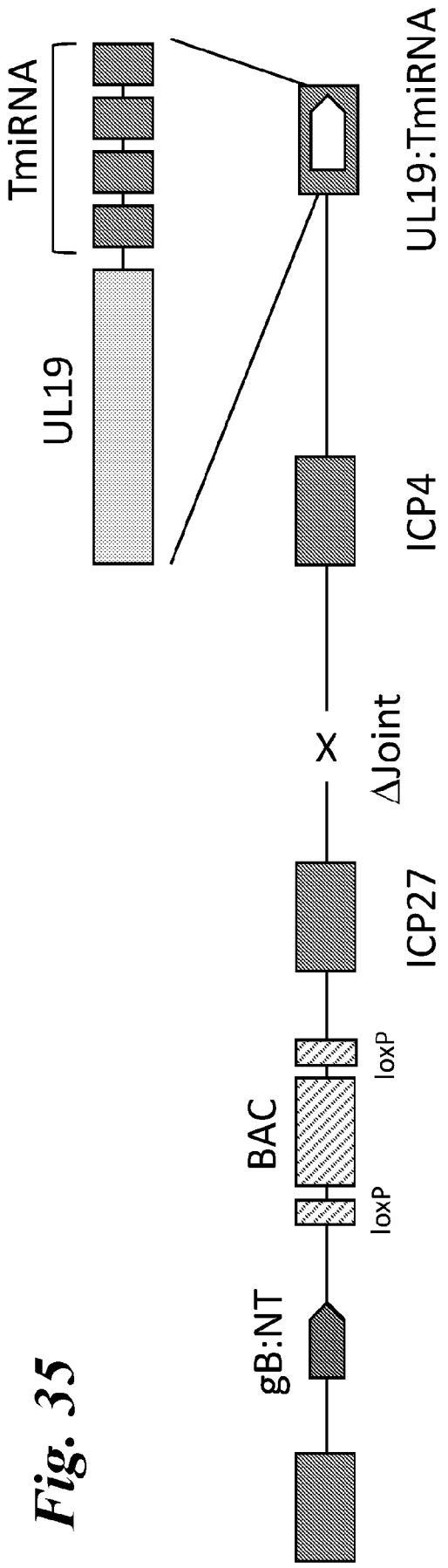

TmiRNA
UL19
UL19:TmiRNA
ICP4
ΔJoint
ICP27
BAC
loxP
loxP
gB:NT

Fig. 35

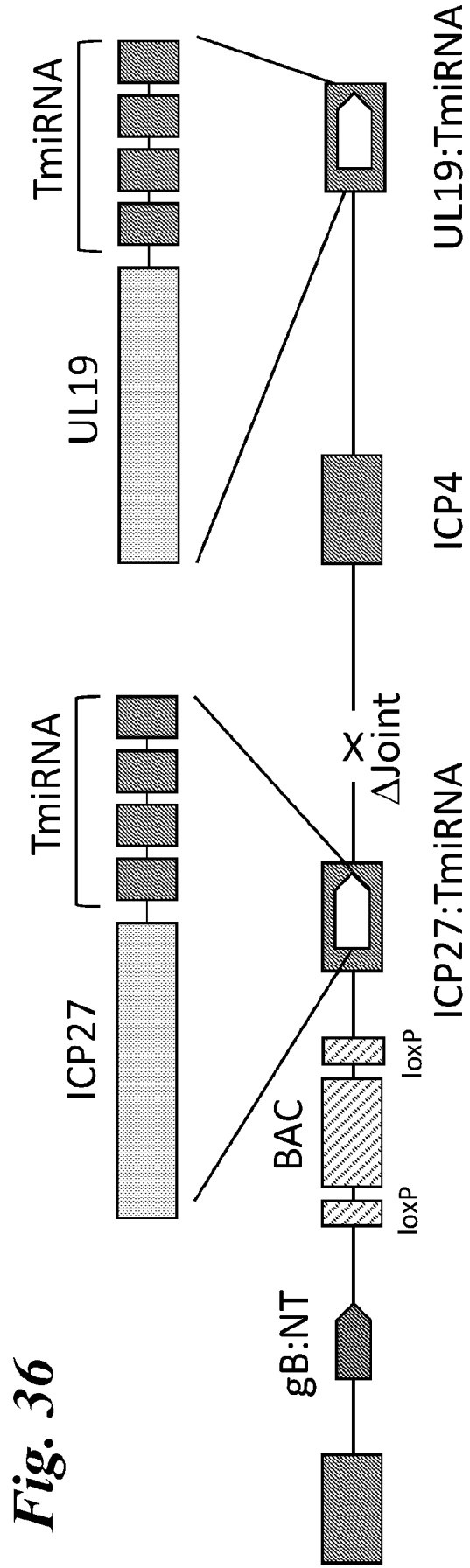

TmiRNA
UL19
UL19:TmiRNA
ICP4
ΔJoint
TmiRNA
ICP27
ICP27:TmiRNA
BAC
loxP
loxP
gB:NT

Fig. 36

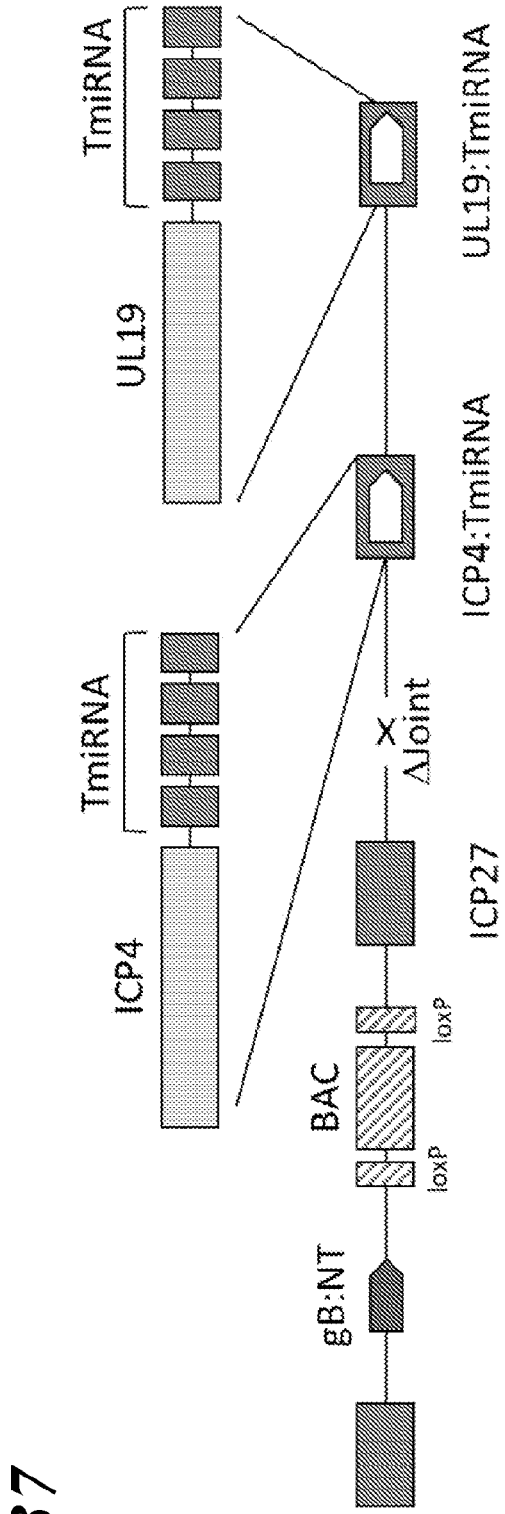

TmiRNA
UL19
TmiRNA
ICP4
BAC
loxP
loxP
gB:NT
X
ΔJoint
ICP27
ICP4:TmiRNA
UL19:TmiRNA

Fig. 37

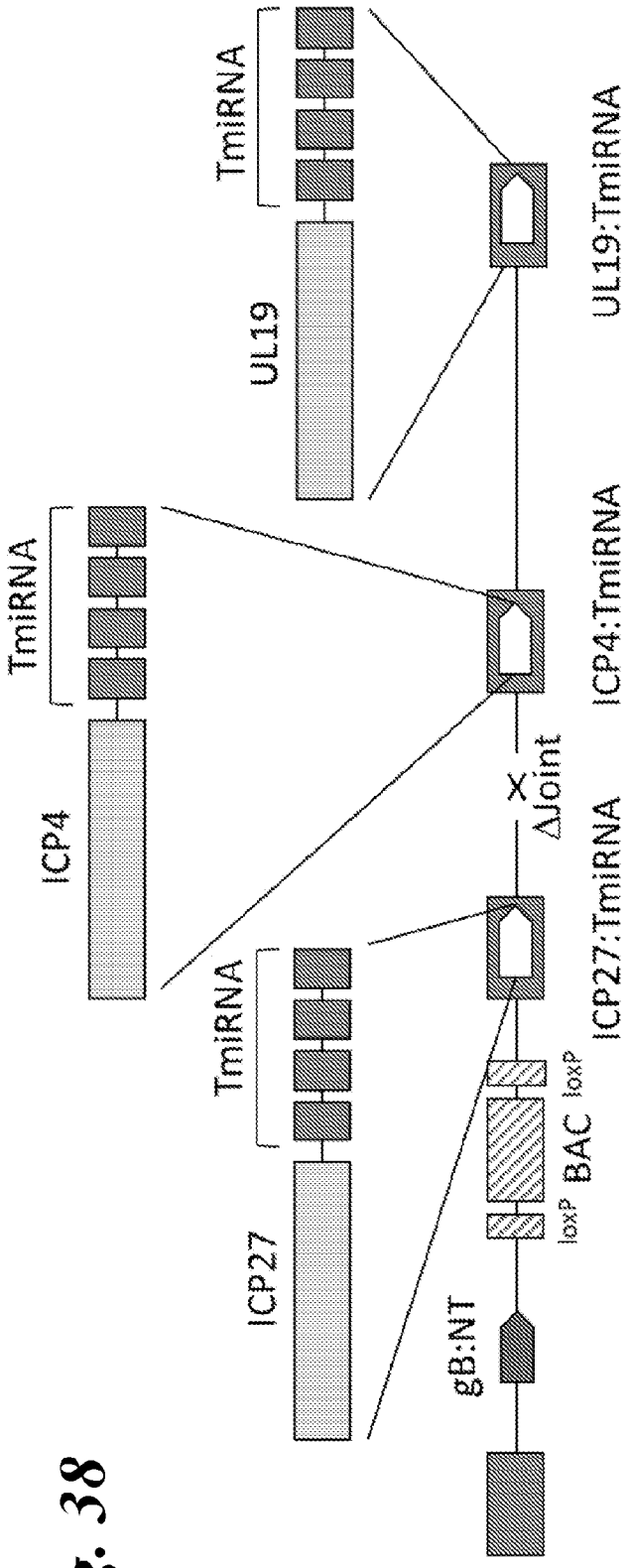

TmiRNA
UL19
TmiRNA
ICP4
TmiRNA
ICP27
BAC
loxP
loxP
gB:NT
X
ΔJoint
ICP27:TmiRNA
ICP4:TmiRNA
UL19:TmiRNA

Fig. 38

Fig. 39

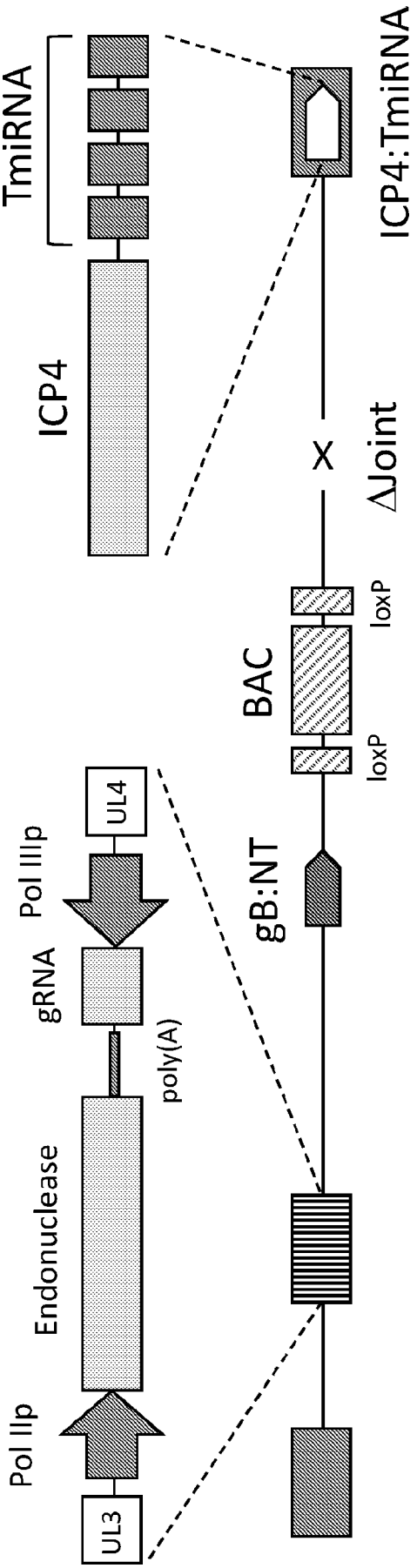

TmiRNA
ICP4
ICP4:TmiRNA
ΔJoint
X
BAC
loxP
loxP
gB:NT
Pol IIIp
UL4
gRNA
poly(A)
Endonuclease
Pol IIp
UL3

Fig. 40

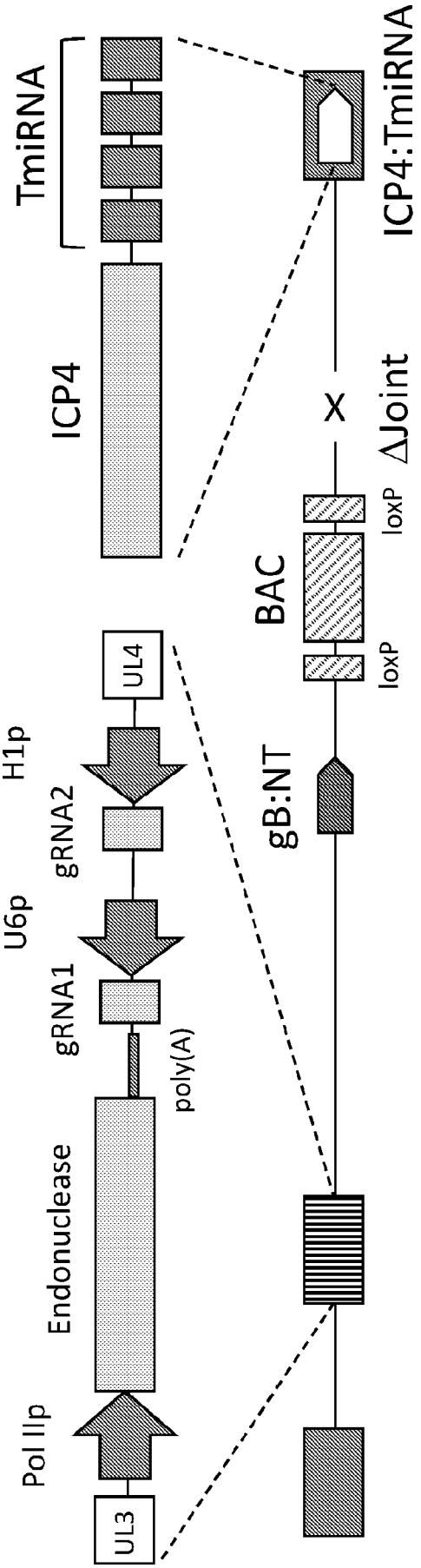

TmiRNA
ICP4
ICP4:TmiRNA
ΔJoint
X
BAC
loxP
loxP
gB:NT
H1p
UL4
gRNA2
U6p
gRNA1
poly(A)
Endonuclease
Pol IIp
UL3

Fig. 41

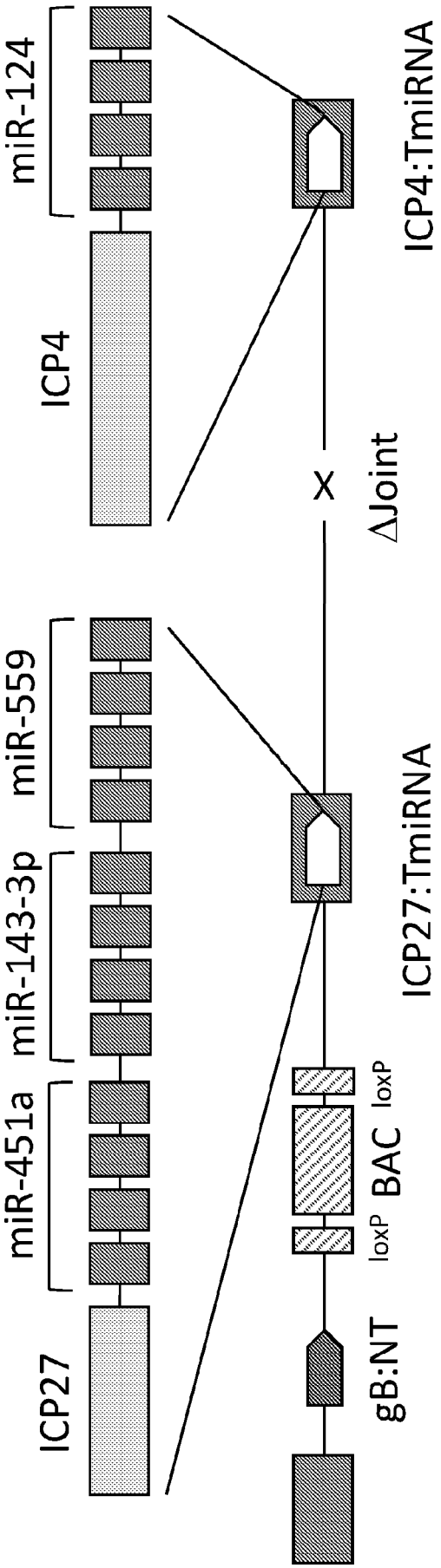
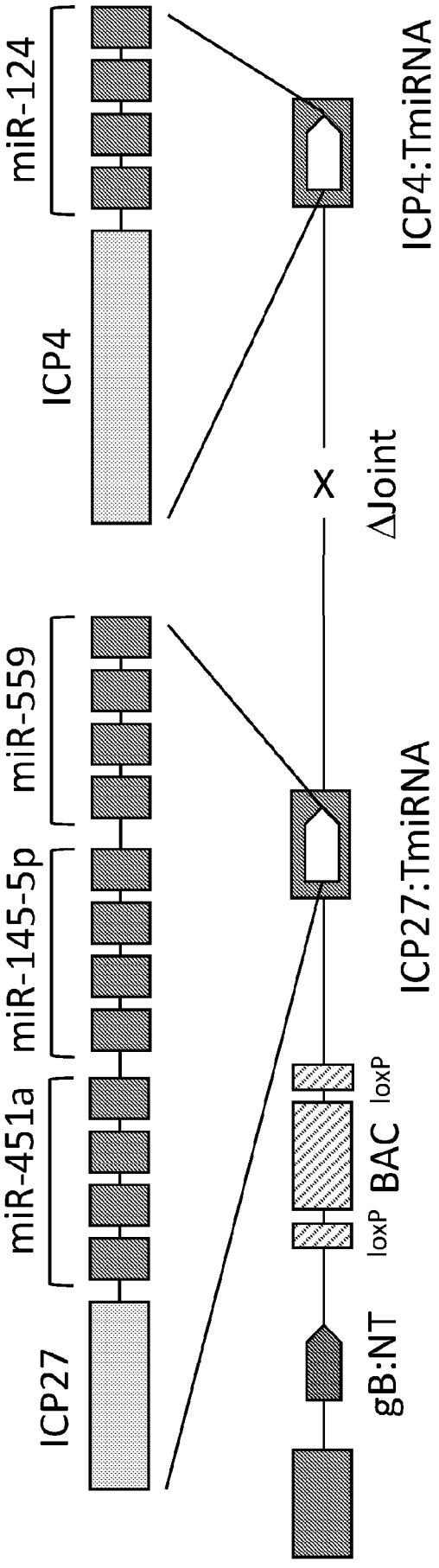

ICP27
miR-451a
miR-143-3p
miR-559
gB:NT
loxP
BAC
loxP
ICP27:TmiRNA
X
ΔJoint
ICP4
miR-124
ICP4:TmiRNA

Fig. 42


ICP27
miR-451a
miR-145-5p
miR-559
gB:NT
loxP
BAC
loxP
ICP27:TmiRNA
X
ΔJoint
ICP4
miR-124
ICP4:TmiRNA

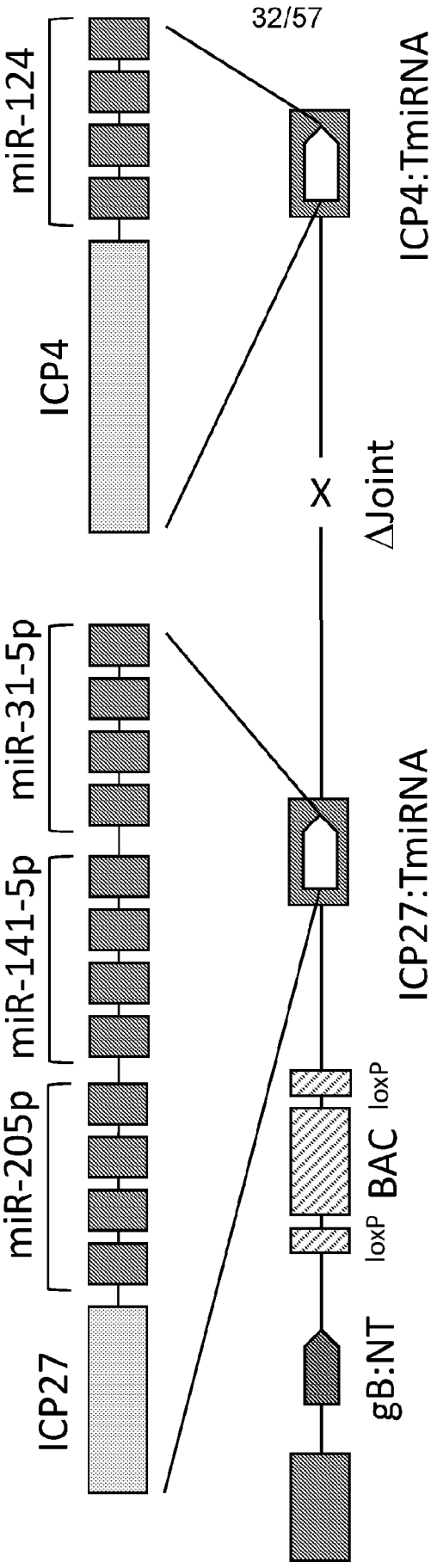

miR-124
ICP4
ICP4:TmiRNA
X
ΔJoint
miR-31-5p
miR-141-5p
miR-205p
ICP27
ICP27:TmiRNA
gB:NT
loxP
BAC
loxP

Fig. 43

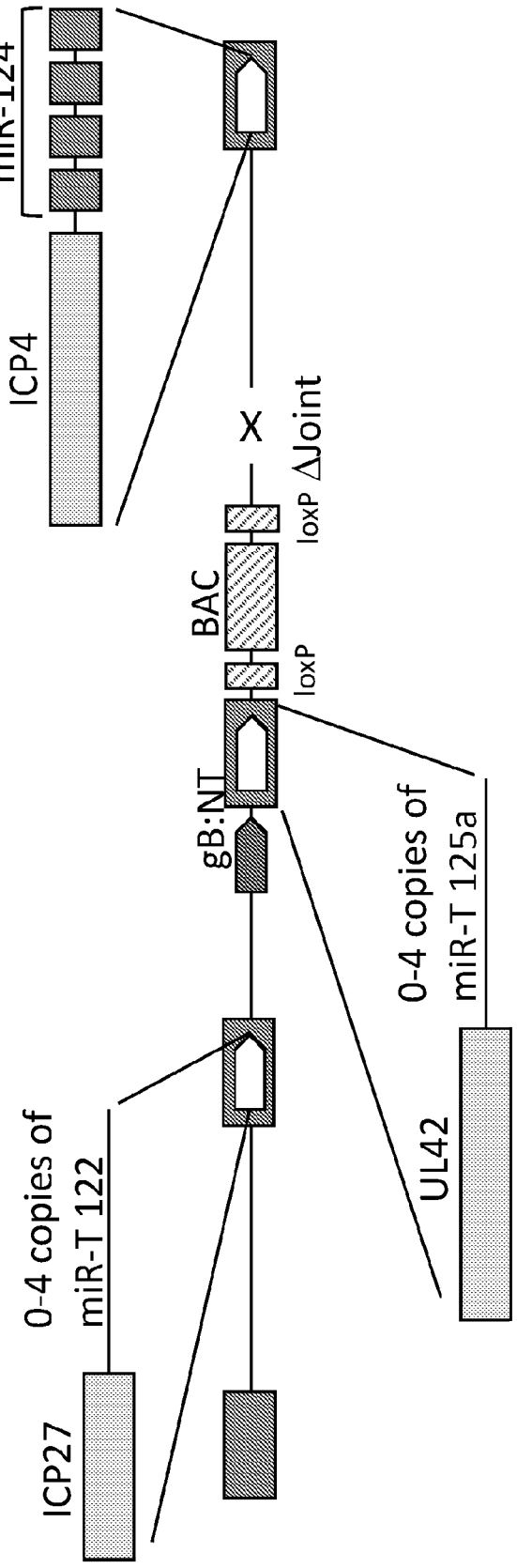

miR-124
ICP4
X
loxP ΔJoint
BAC
loxP
gB:NT
0-4 copies of miR-T 125a
UL42
0-4 copies of miR-T 122
ICP27

Fig. 44

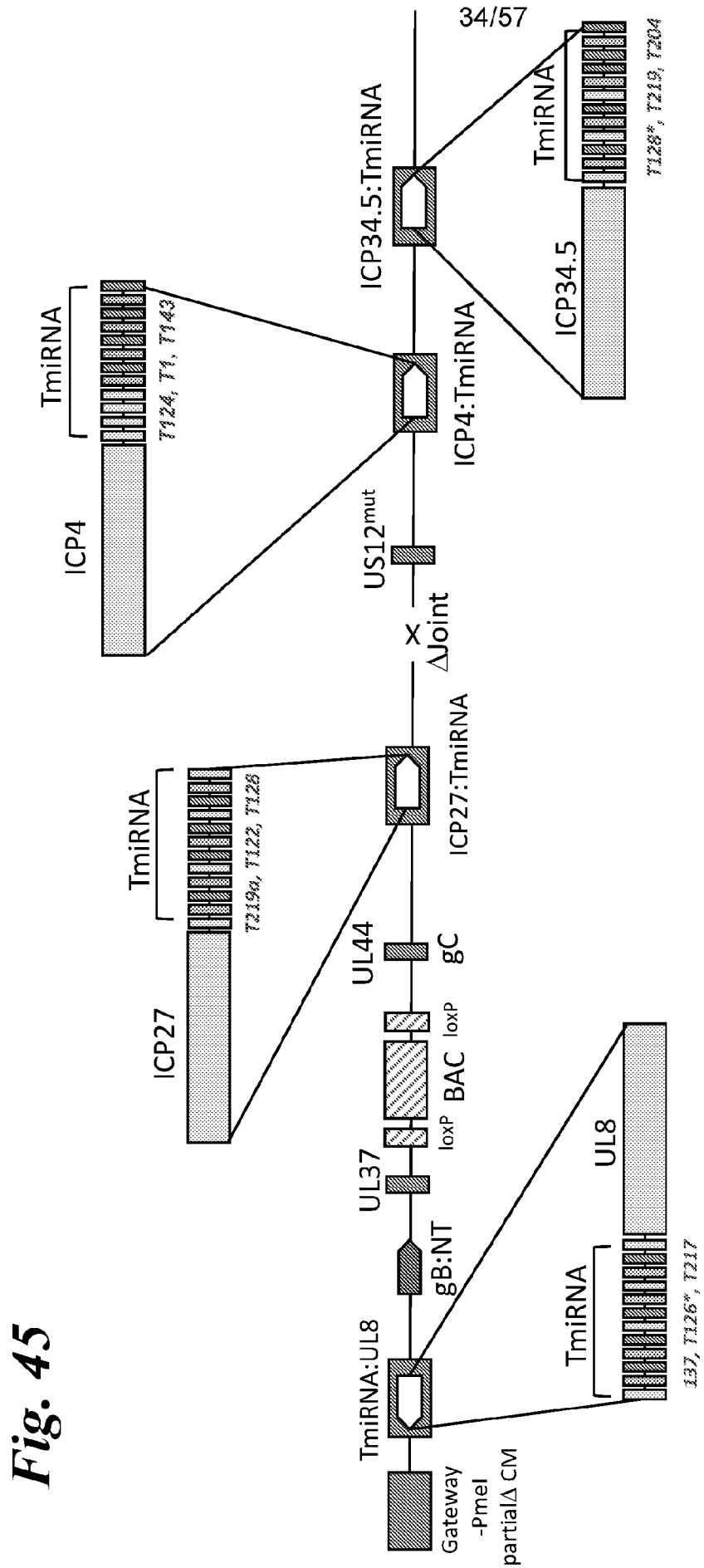

TmiRNA
T124, T1, T143
ICP4
ICP34.5:TmiRNA
TmiRNA
T128*, T219, T204
ICP34.5
ICP4:TmiRNA
US12mut
X
ΔJoint
TmiRNA
T219a, T122, T126
ICP27
ICP27:TmiRNA
UL44
gC
loxP
BAC
loxP
UL37
gB:NT
TmiRNA:UL8
TmiRNA
I32, T126*, T217
UL8
Gateway
-PmeI
partialΔ CM

Fig. 45

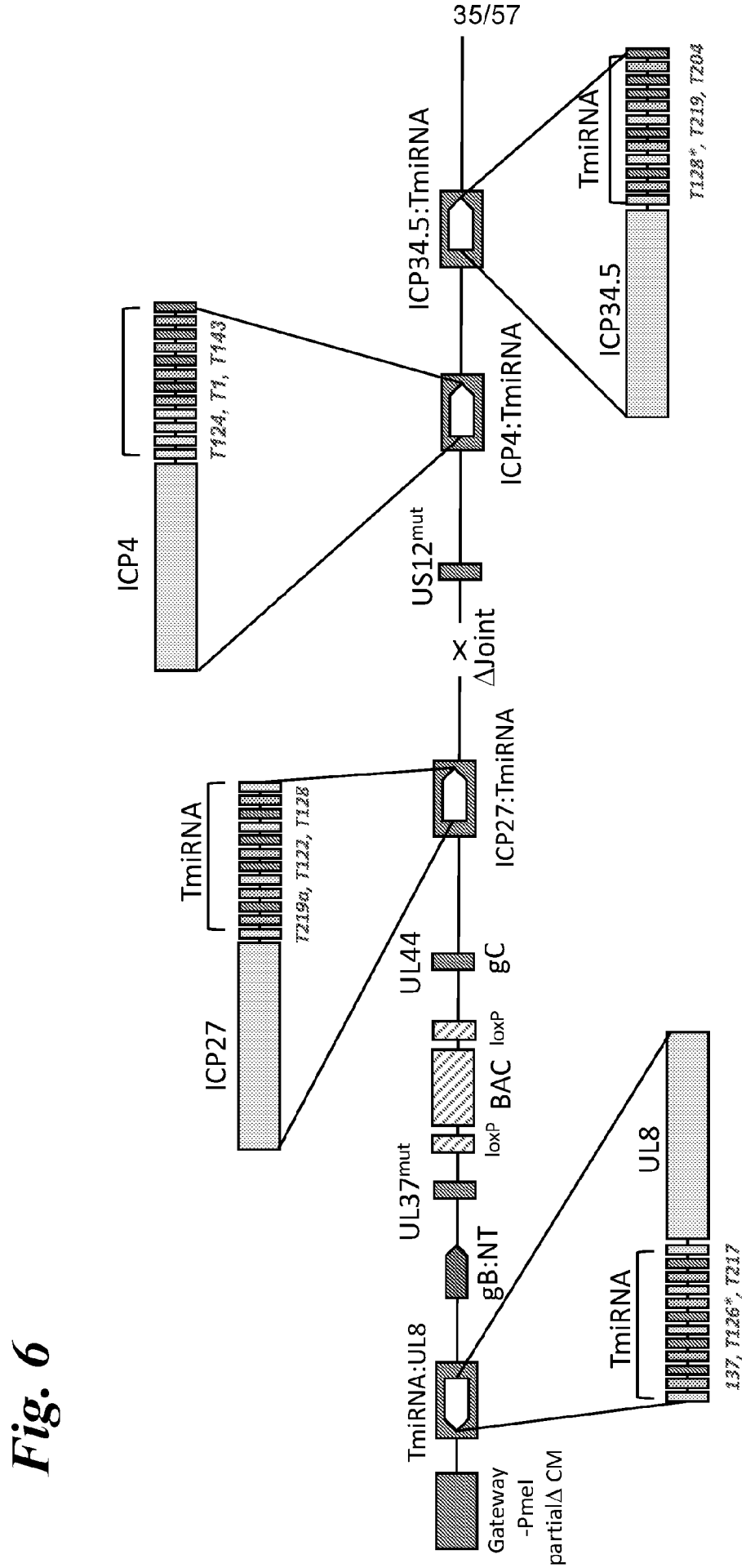

ICP4
T124, T1, T143
ICP34.5:TmiRNA
TmiRNA
T128*, T219, T204
ICP34.5
ICP4:TmiRNA
US12mut
ΔJoint
TmiRNA
T219a, T122, T128
ICP27
ICP27:TmiRNA
UL44
gC
loxP
BAC
loxP
UL37mut
gB:NT
TmiRNA:UL8
Gateway
-PmeI
partialΔ CM
TmiRNA
T137, T126*, T217
UL8

Fig. 6

Fig. 47A

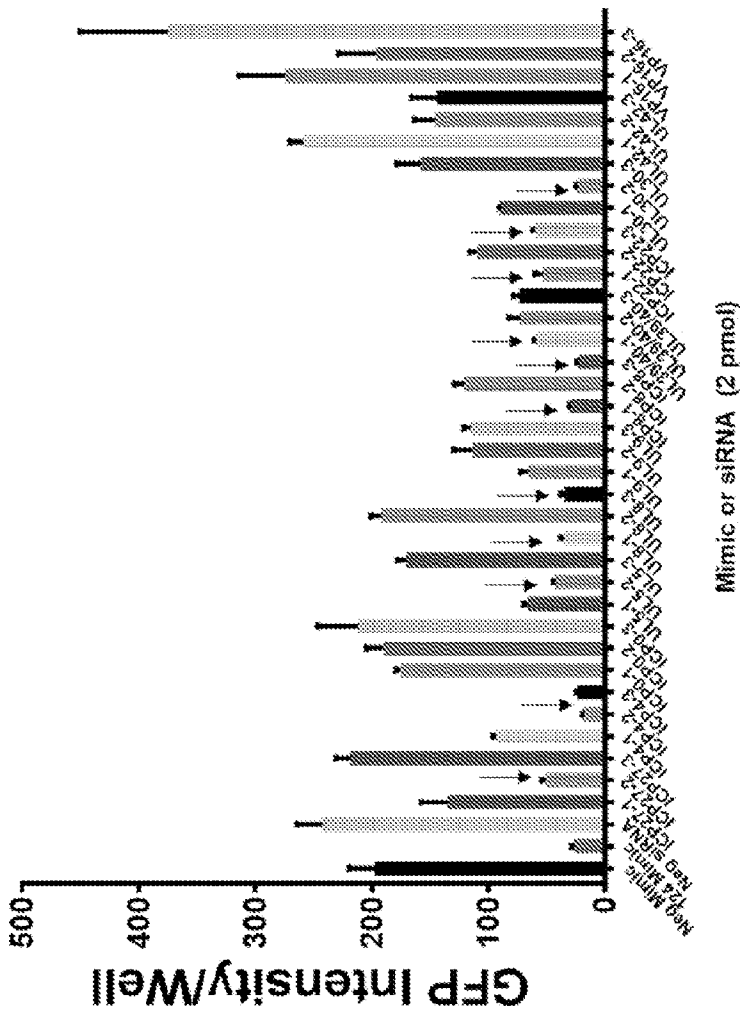


Fig. 47B

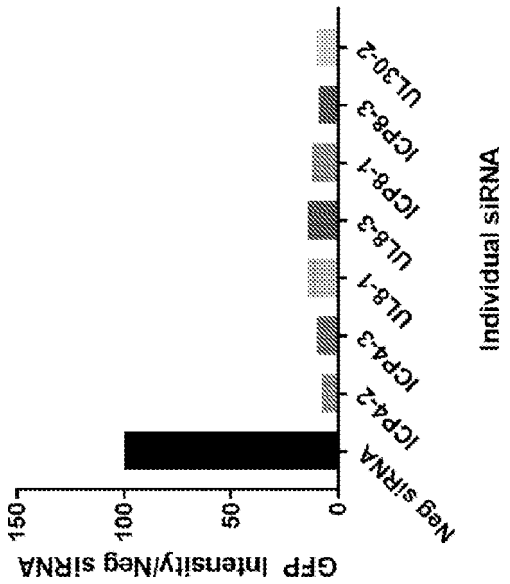


Fig. 48A

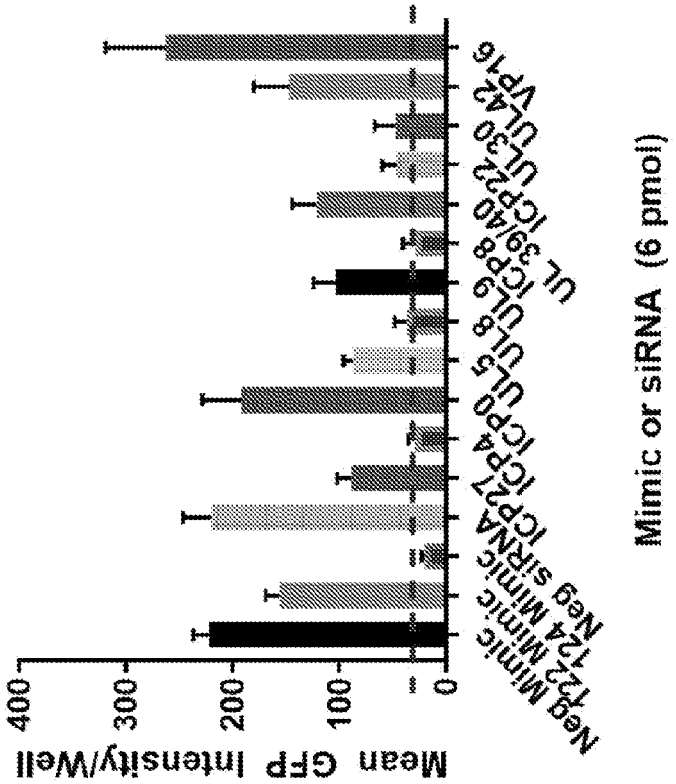
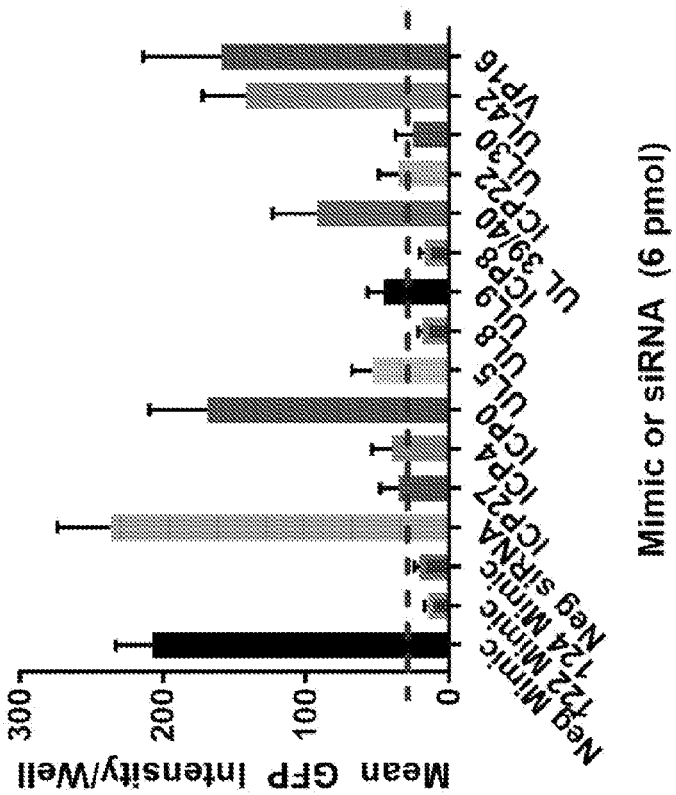


Fig. 48B



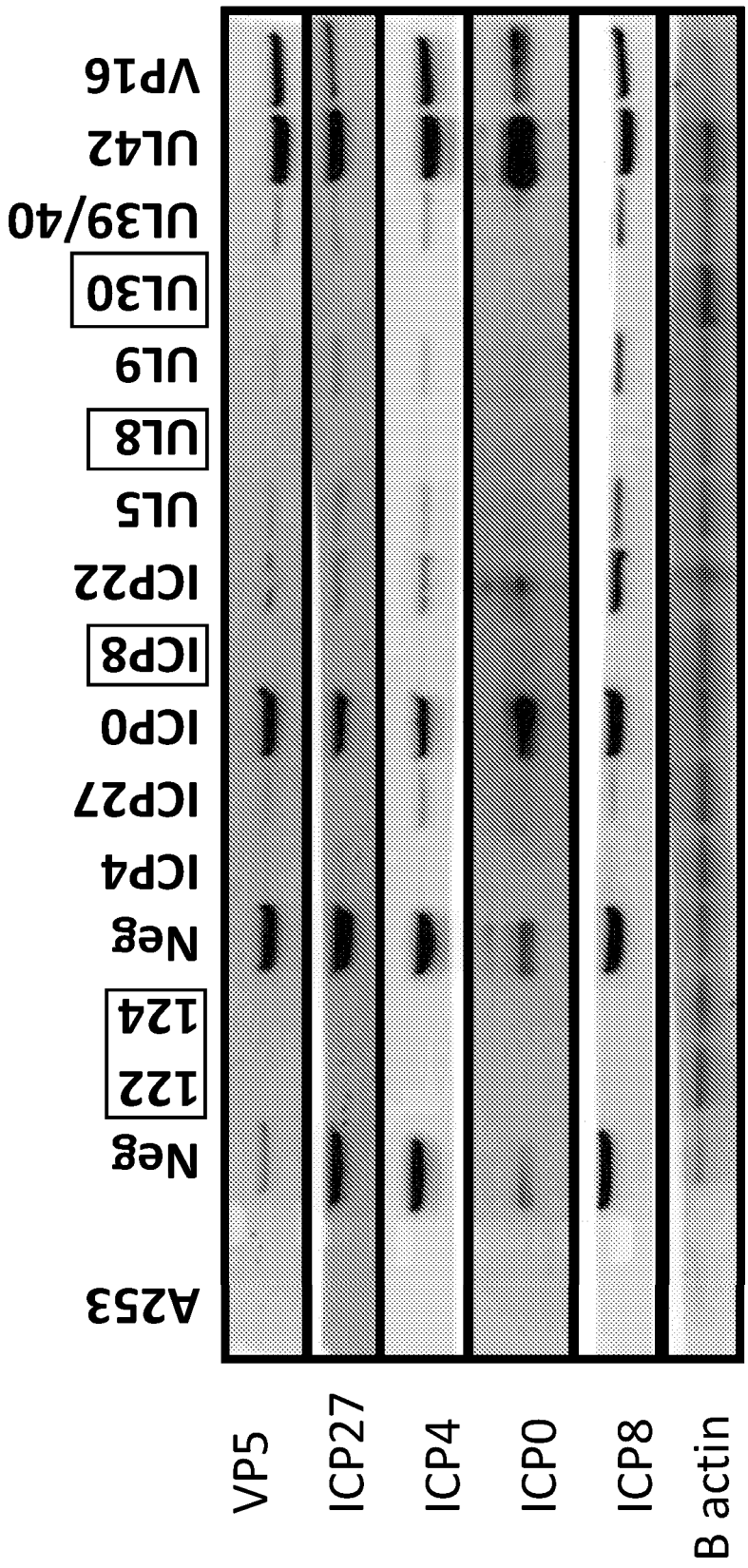

A253
Neg
122
124
Neg
ICP4
ICP27
ICP0
ICP8
ICP22
UL5
UL8
UL9
UL30
UL39/40
UL42
VP16
VP5
ICP27
ICP4
ICP0
ICP8
B actin

Fig. 49

Fig. 50

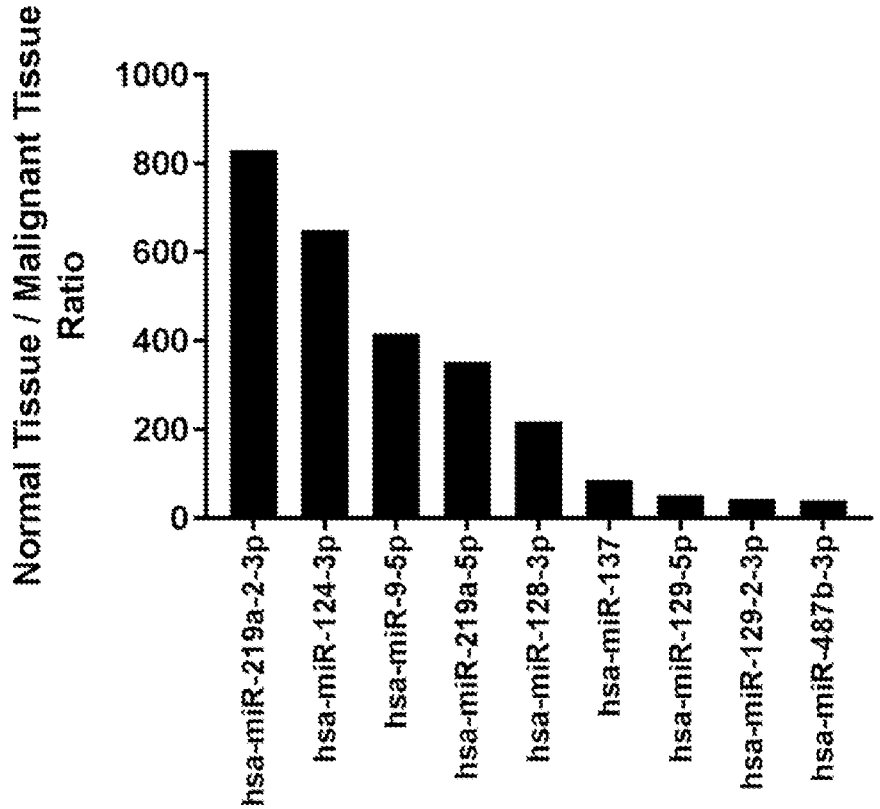


Fig. 50B

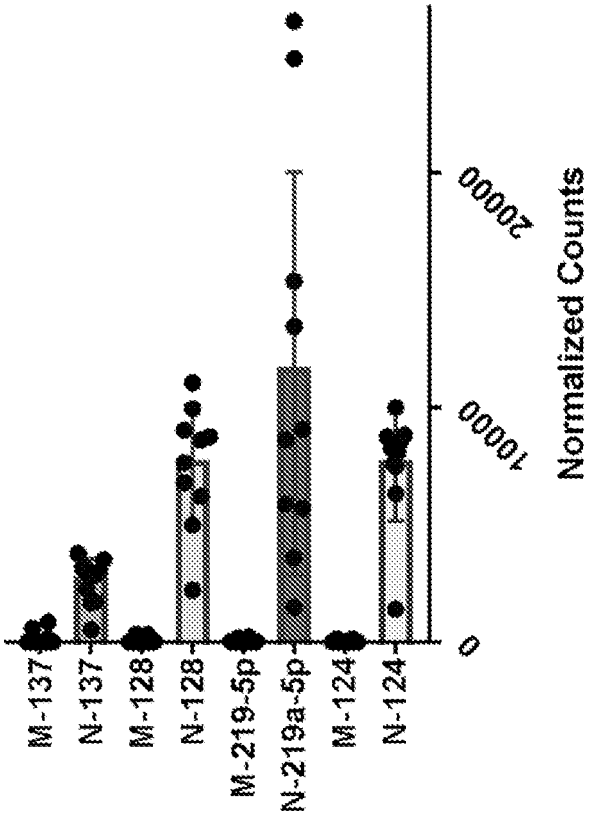


Fig. 51A

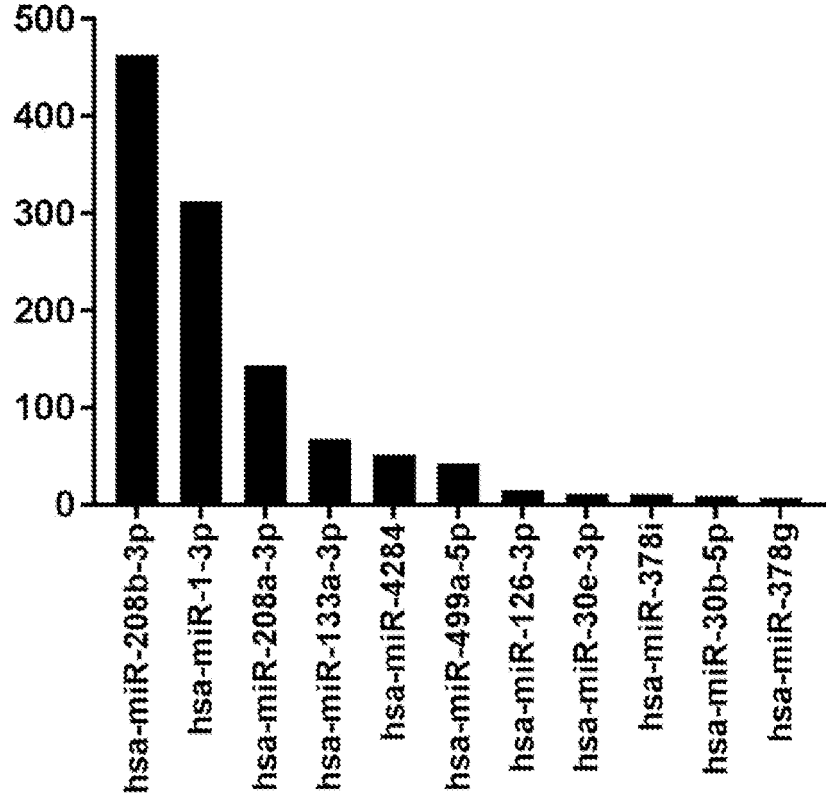


Fig. 51B

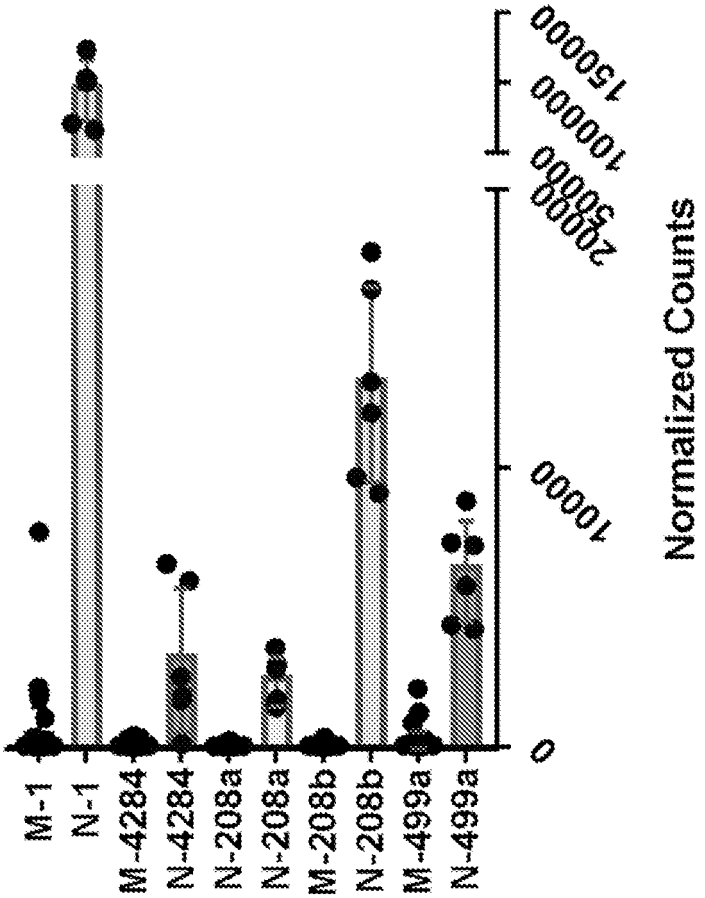


Fig. 52A

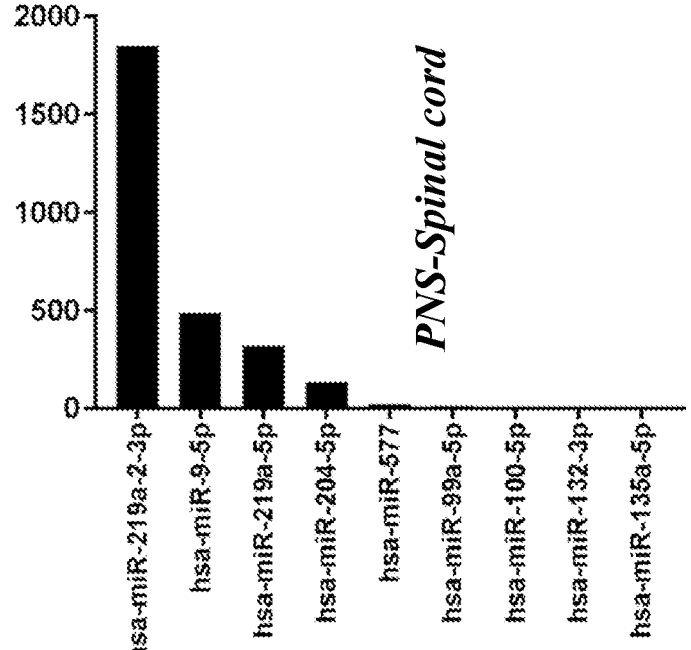


Fig. 52B

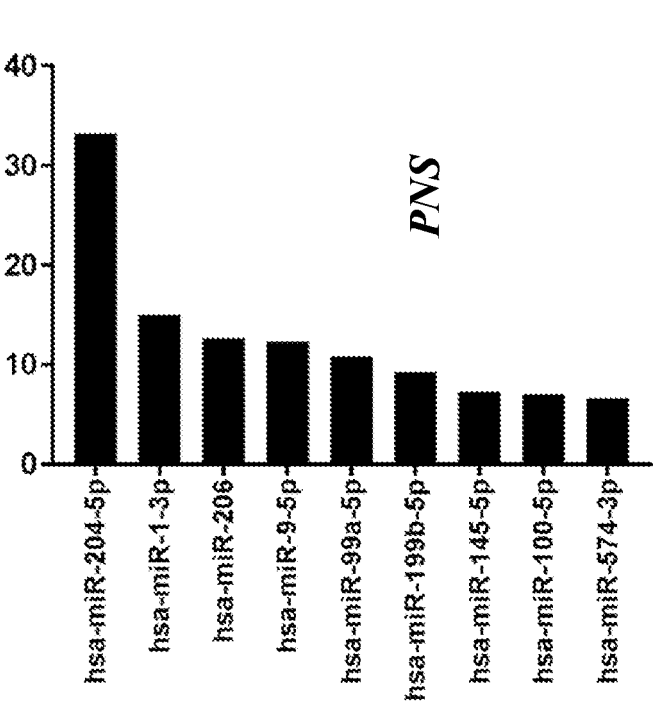


Fig. 52C

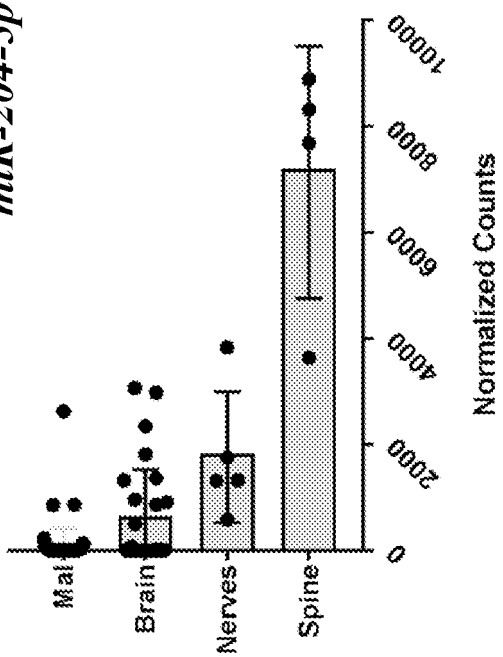


Fig. 53A

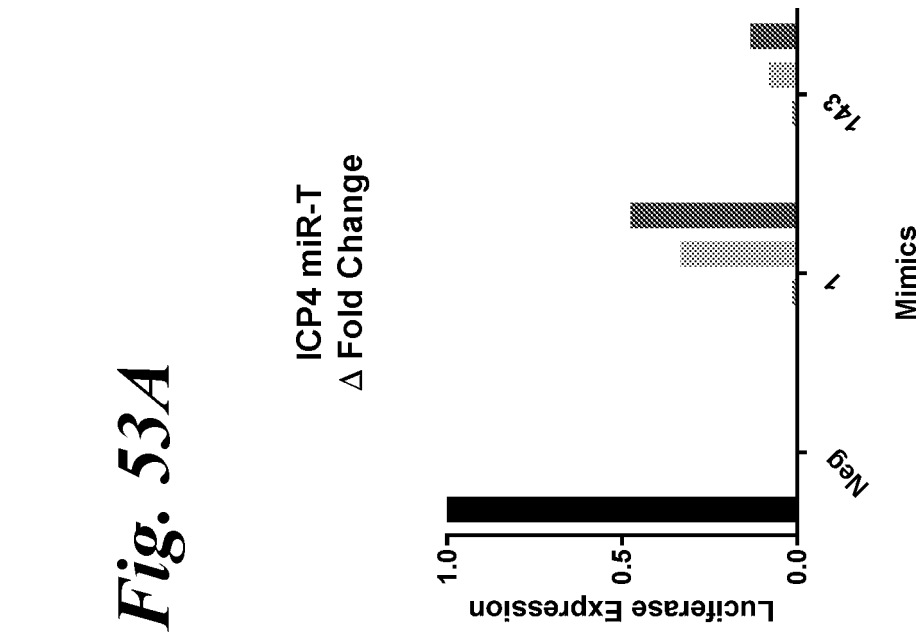


Fig. 53B

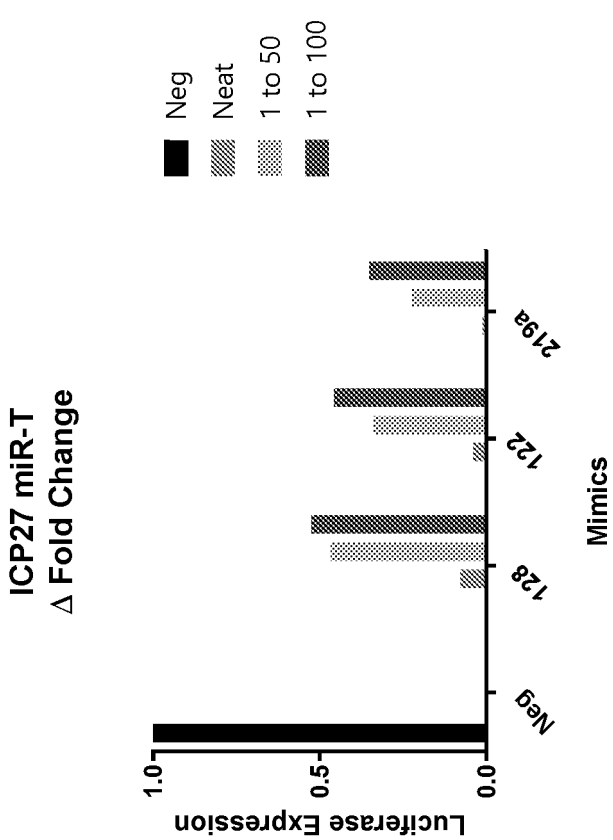


Fig. 54A

COLORECTAL ADENOCARCINOMA

SW837

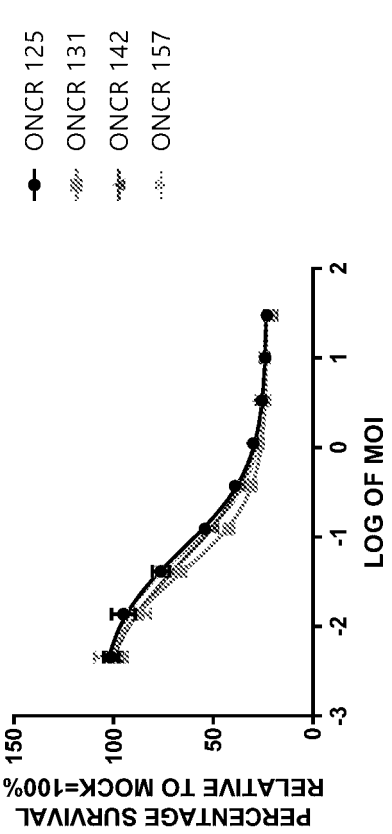


Fig. 54B

MELANOMA

SKMEL28

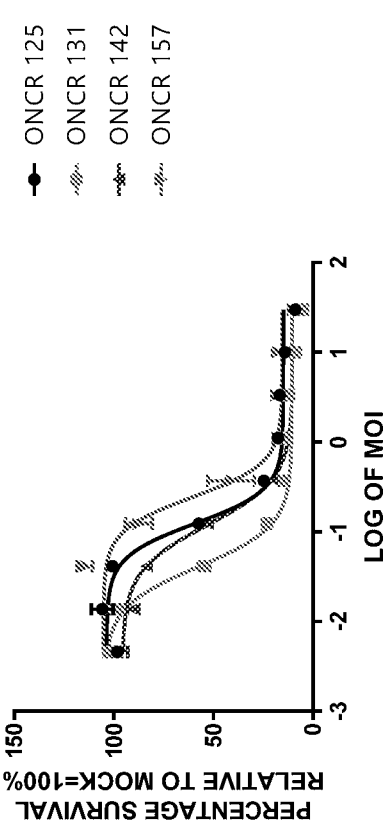


Fig. 54C

COLO 205

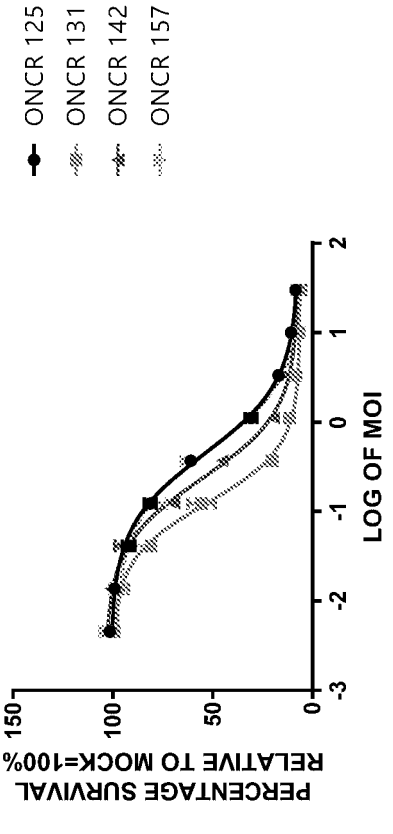


Fig. 54D

A375

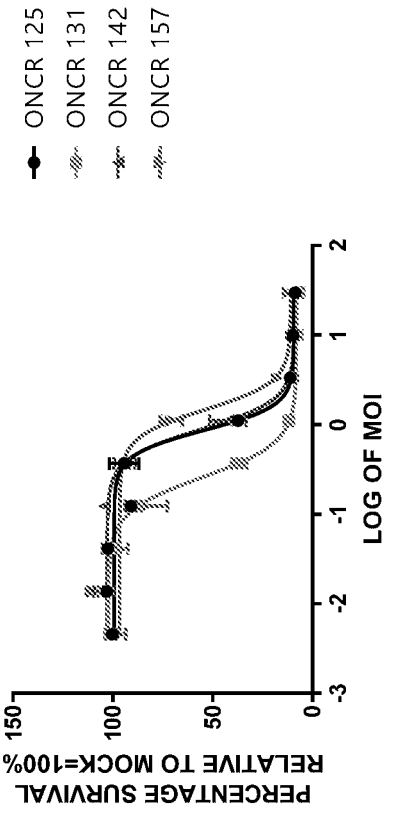


Fig. 54E

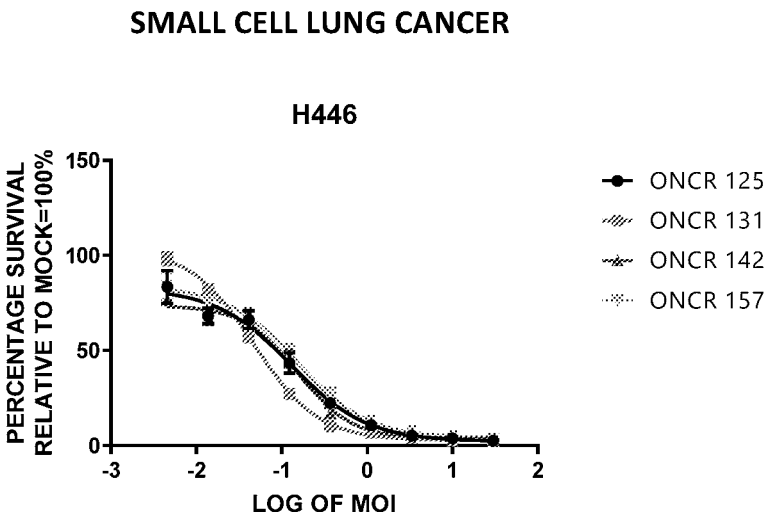


Fig. 54F

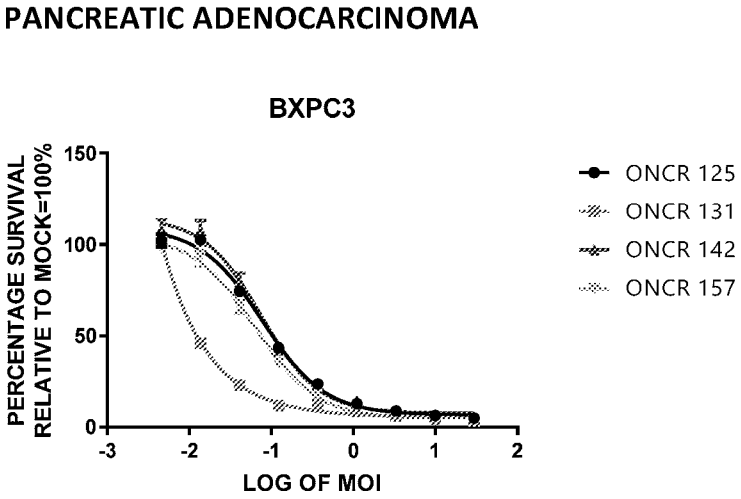


Fig. 54G

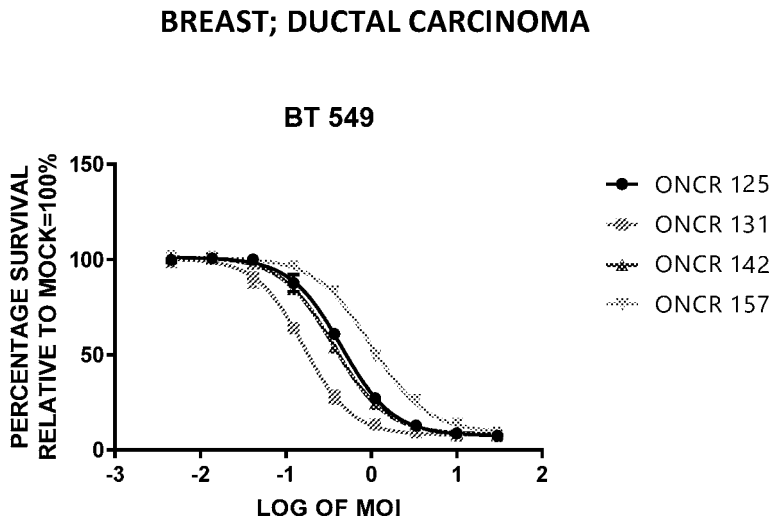
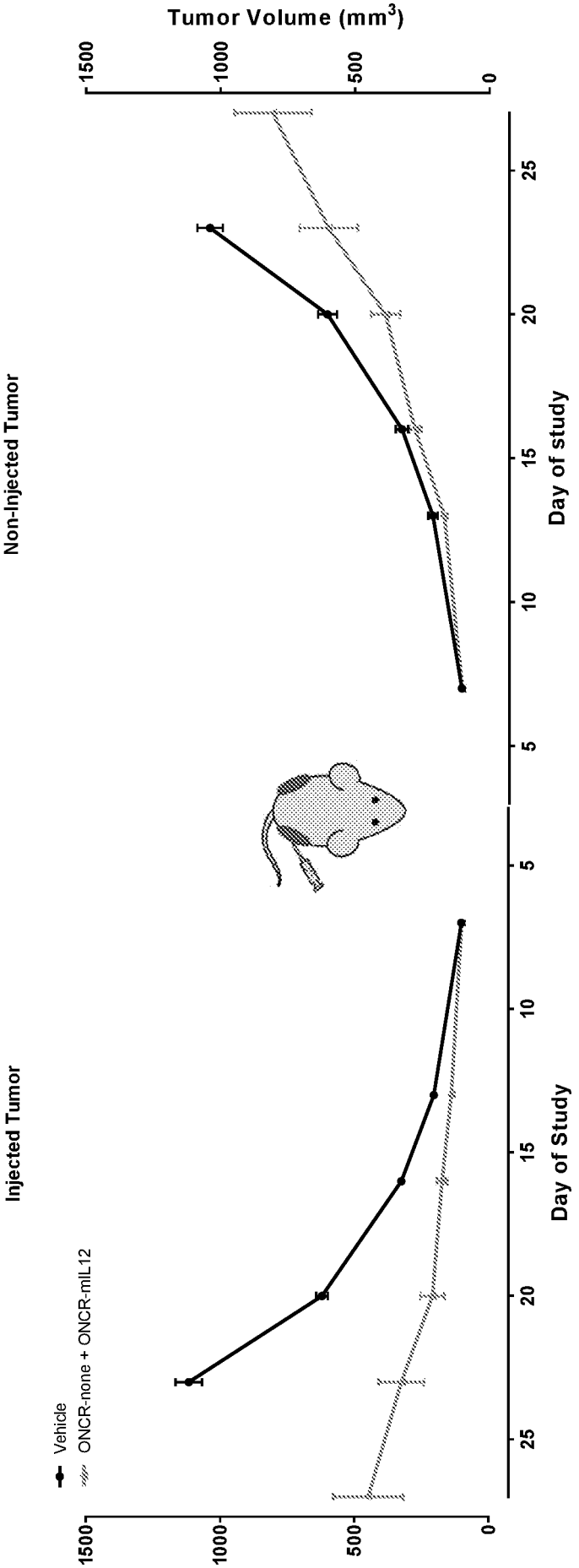
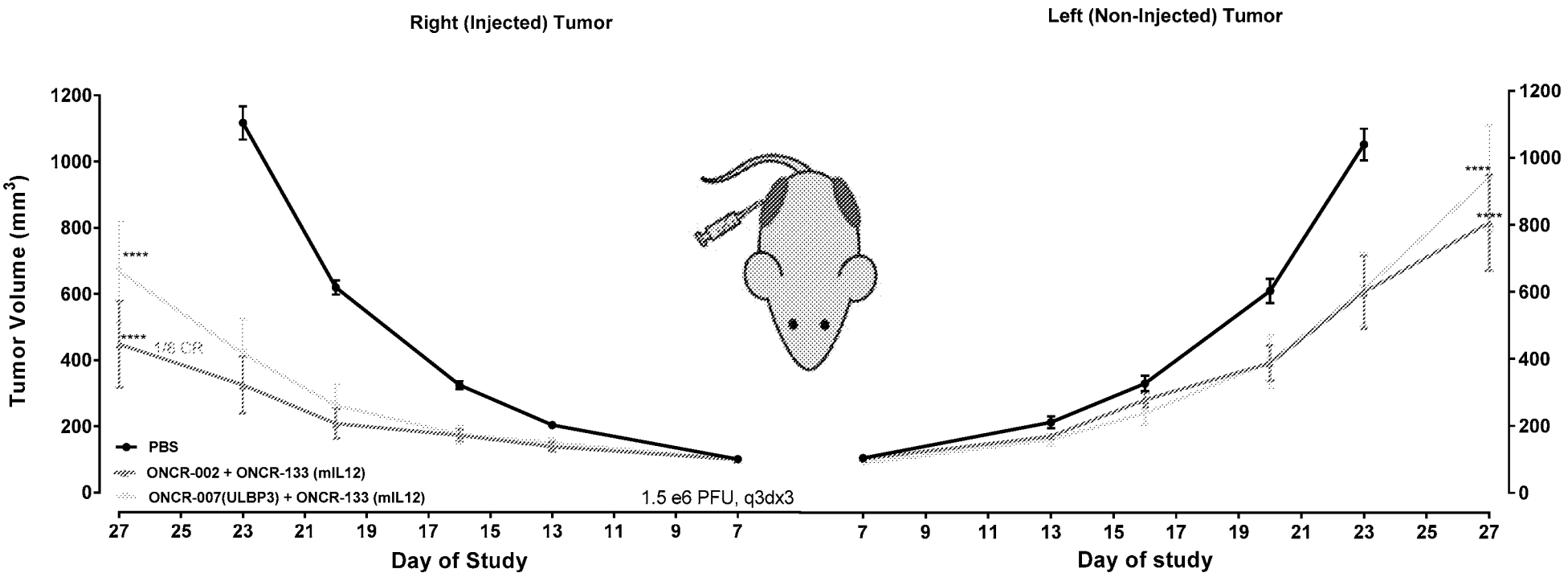


Fig. 55



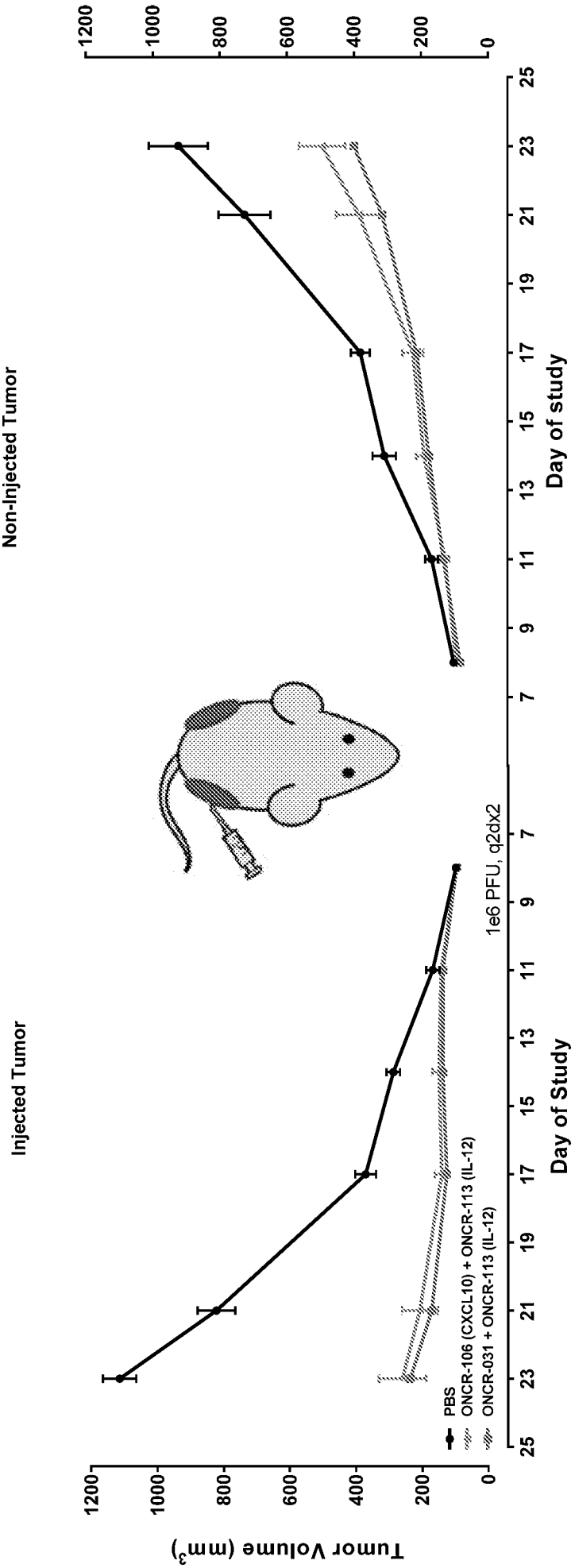
	Injected		Non-injected	
	%TGI	p	%TGI	p
ONCR-133	71	<0.0001	42	0.005

Fig. 56



	Injected			Non-injected		
	CR	%TGI	p	CR	%TGI	p
ONCR-2 + ONCR-133	1/8	66	<0.0001	0/8	35	0.005
ONCR-7 + ONCR-133	0/8	57	<0.0001	0/8	35	0.01

Fig. 57



	Injected			Non-injected		
	CR	%TGI	p	CR	%TGI	p
ONCR-106 + ONCR-113	3/8	75	<0.0001	0/8	47	0.001
ONCR-031 + ONCR-113	3/8	79	<0.0001	1/8	57	0.001

Fig. 58

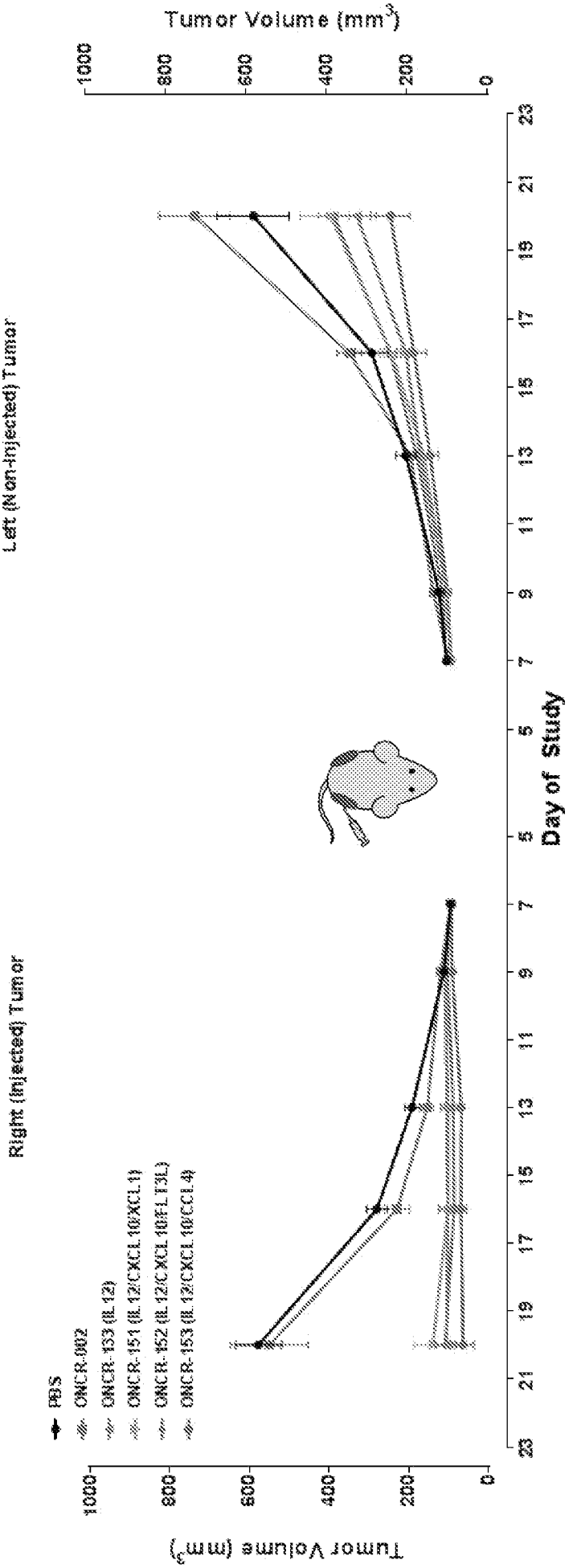

Tumor Volume (mm3)
1000
800
600
400
200
0
Left (Non-Injected) Tumor
Right (Injected) Tumor
PBS
ONCR-002
ONCR-153 (IL12)
ONCR-151 (IL12/CXCL10/XCL1)
ONCR-152 (IL12/CXCL10/FLT3L)
ONCR-153 (IL12/CXCL10/CCL4)
1
3
5
7
9
11
13
15
17
19
21
23
Day of Study

Fig. 59A

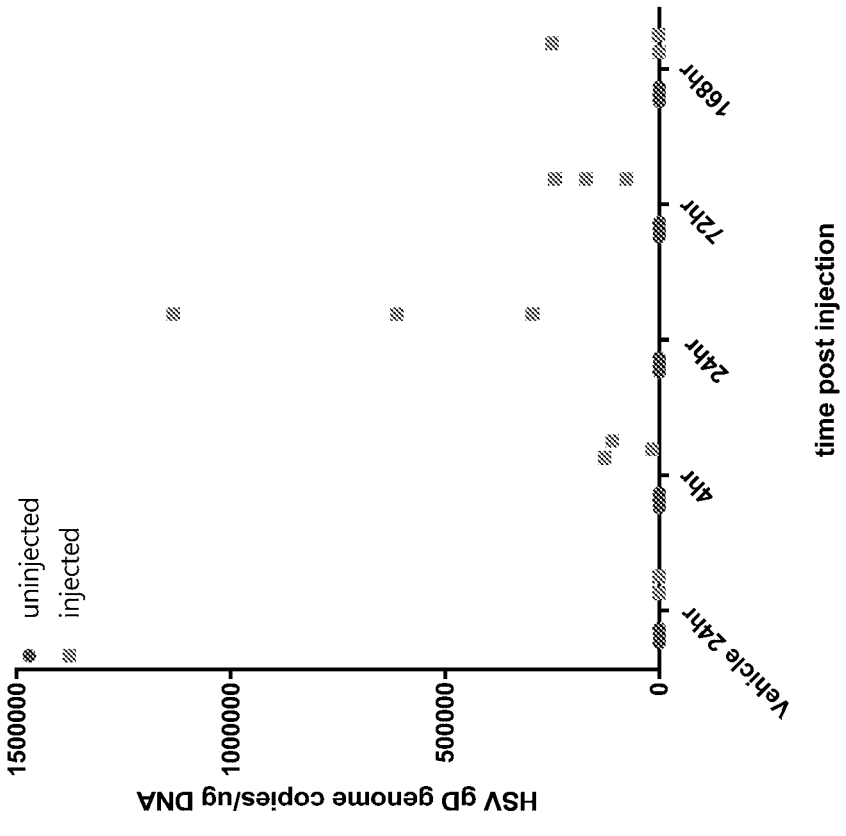


Fig. 59B

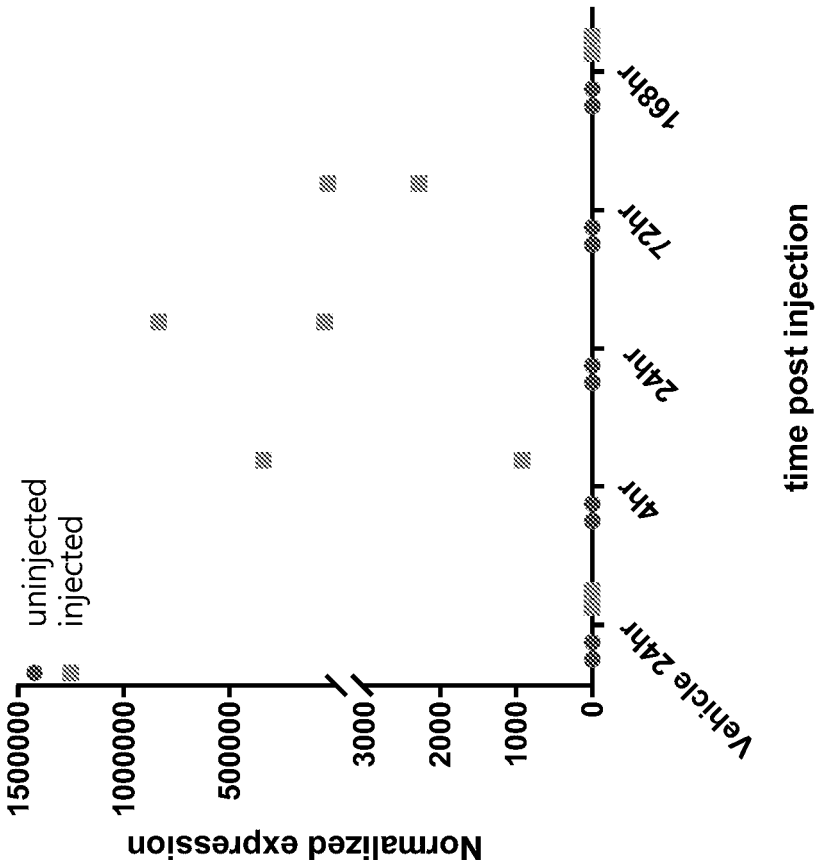


Fig. 60A

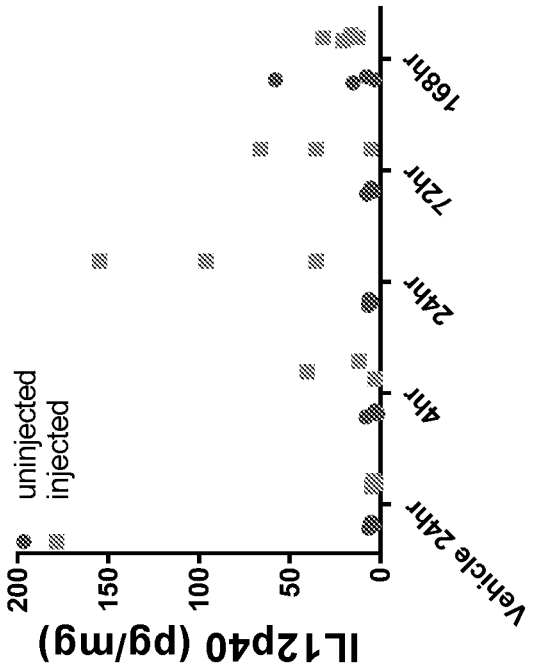


Fig. 60B

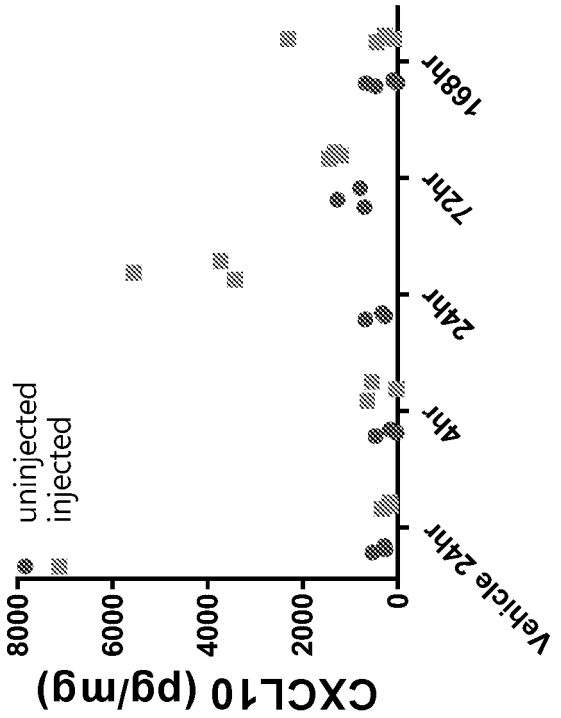
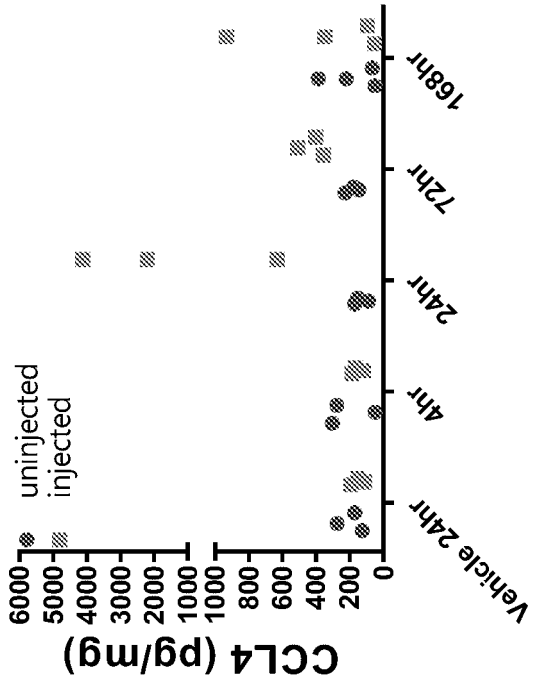


Fig. 60C



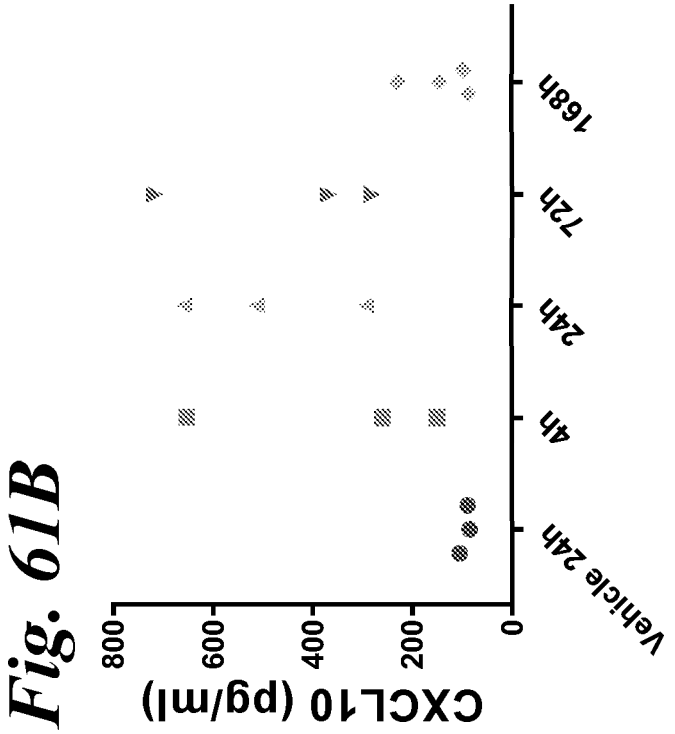


Fig. 61A

Fig. 61B

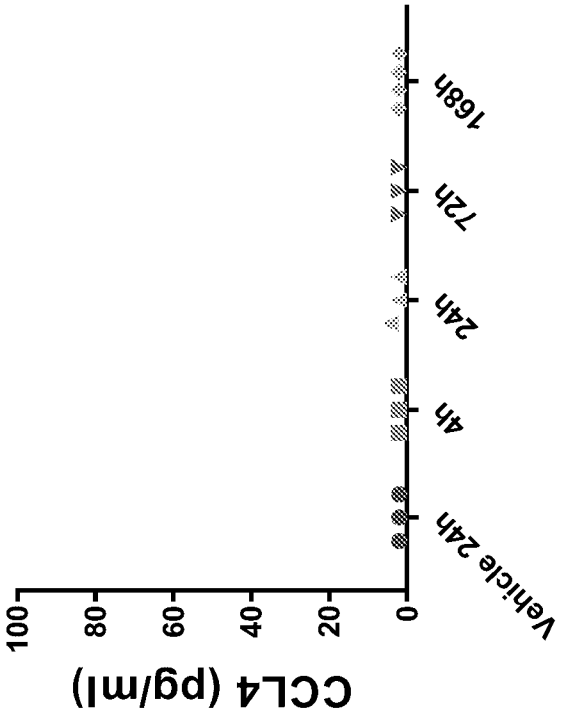


Fig. 61C

Fig. 62A

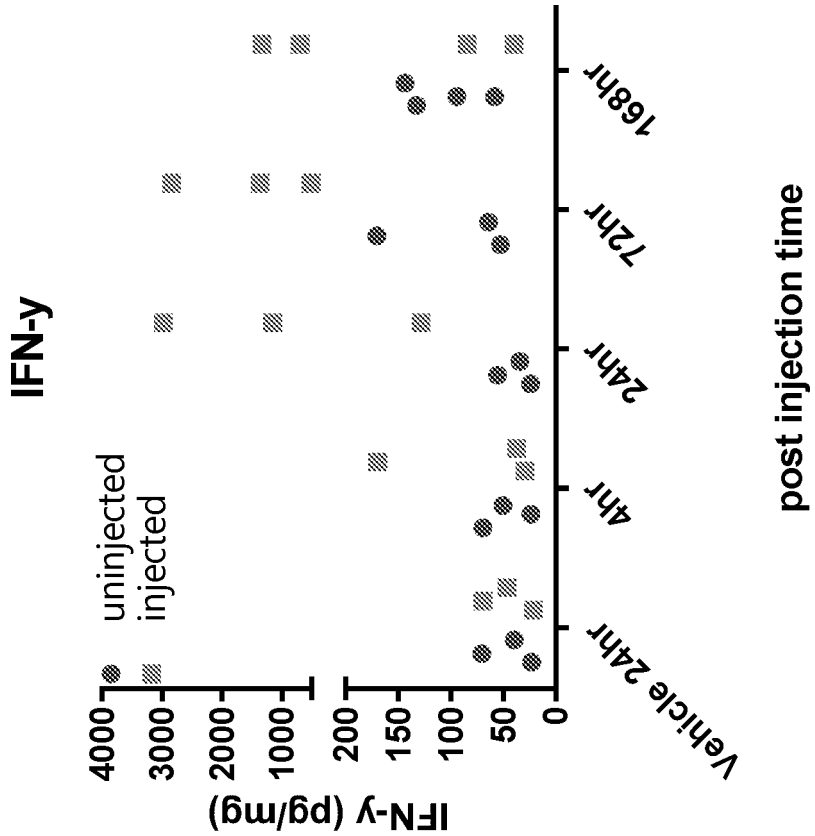


Fig. 62B

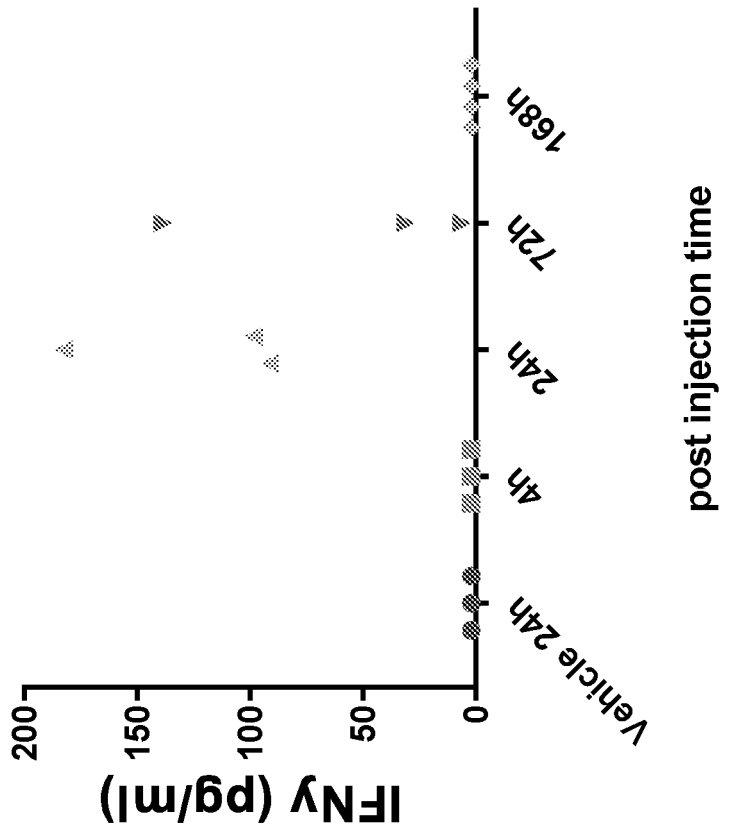


Fig. 63

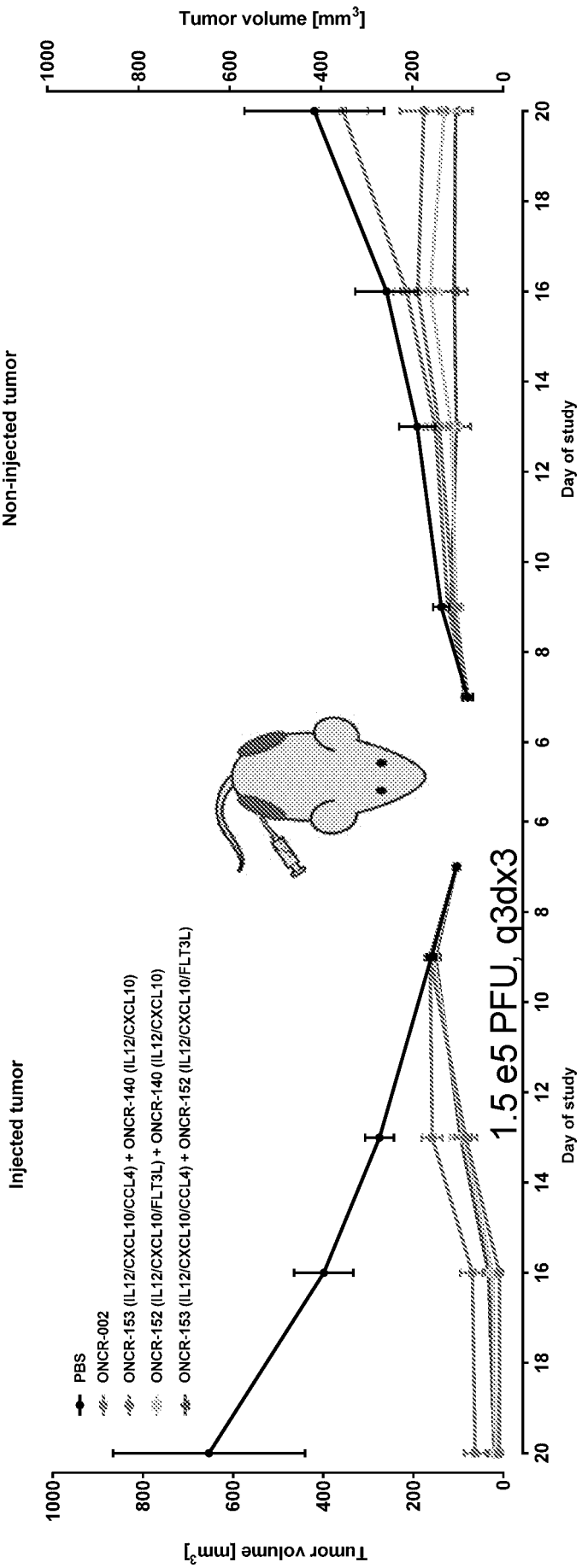

Tumor volume [mm3]
1000
800
600
400
200
0
20
18
16
14
12
10
8
6
4
2
0
Day of study
Injected tumor
Non-injected tumor
PBS
ONCR-002
ONCR-153 (IL12/CXCL10/CCL4) + ONCR-140 (IL12/CXCL10)
ONCR-152 (IL12/CXCL10/FLT3L) + ONCR-140 (IL12/CXCL10)
ONCR-153 (IL12/CXCL10/CCL4) + ONCR-152 (IL12/CXCL10/FLT3L)
1.5 e5 PFU, q3dx3

Fig. 64

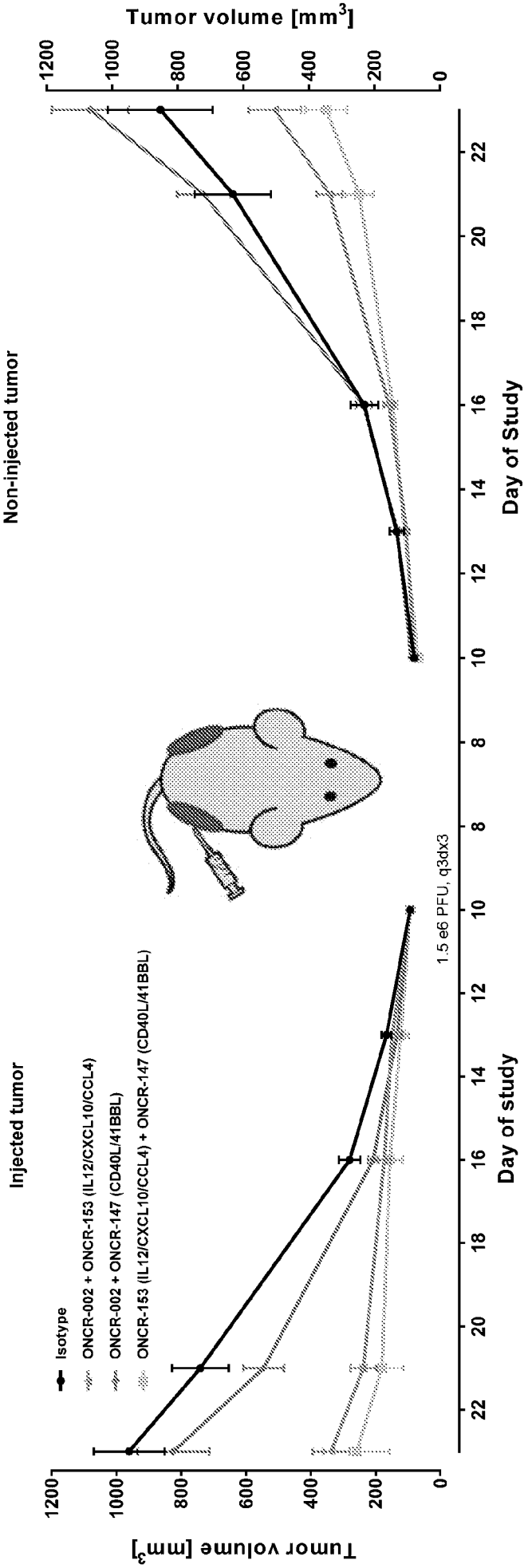

Tumor volume [mm3]
1200
1000
800
600
400
200
0
Isotype
ONCR-002 + ONCR-153 (IL12/CXCL10/CCL4)
ONCR-002 + ONCR-147 (CD40L/41BBL)
ONCR-153 (IL12/CXCL10/CCL4) + ONCR-147 (CD40L/41BBL)
Injected tumor
Non-injected tumor
1.5 e6 PFU, q3dx3
Day of study
Day of Study
2
4
6
8
10
12
14
16
18
20
22

Fig. 65

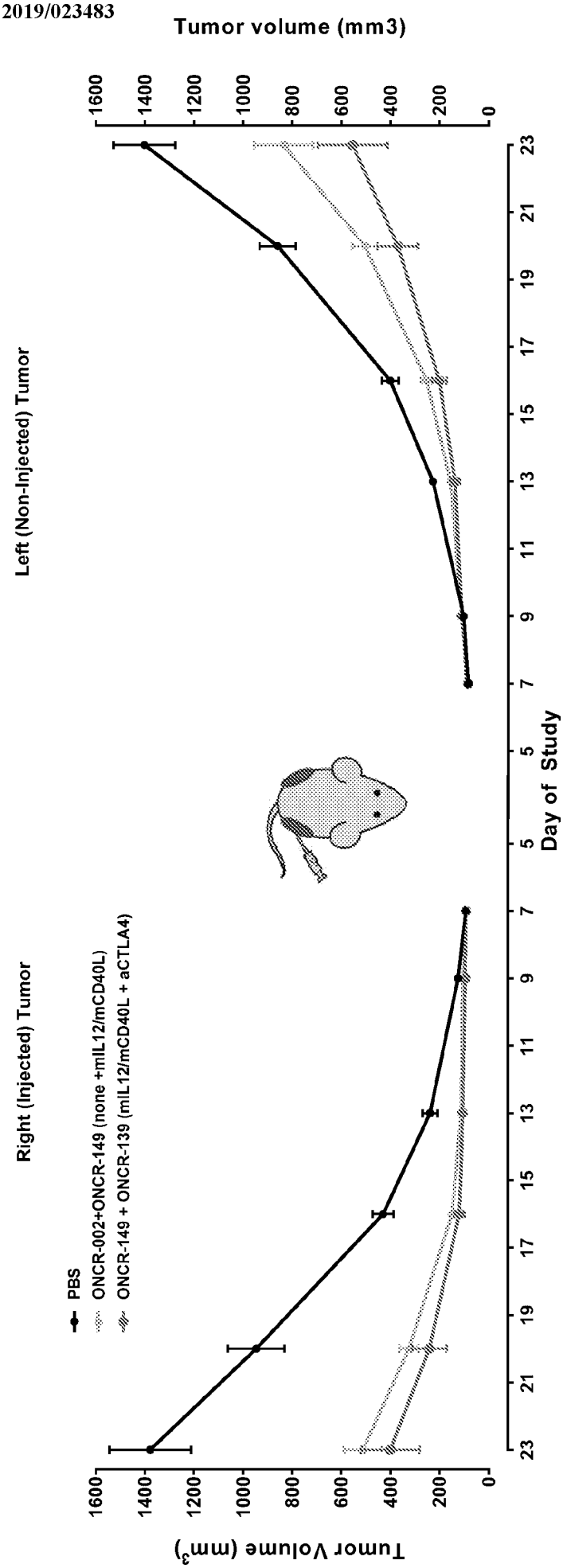

Tumor volume (mm3)
Left (Non-Injected) Tumor
Right (Injected) Tumor
PBS
ONCR-002+ONCR-149 (none +mIL12/mCD40L)
ONCR-149 + ONCR-139 (mIL12/mCD40L + aCTLA4)
Tumor Volume (mm³)
Day of Study
0
200
400
600
800
1000
1200
1400
1600
1
5
7
9
11
13
15
17
19
21
23

Fig. 66A

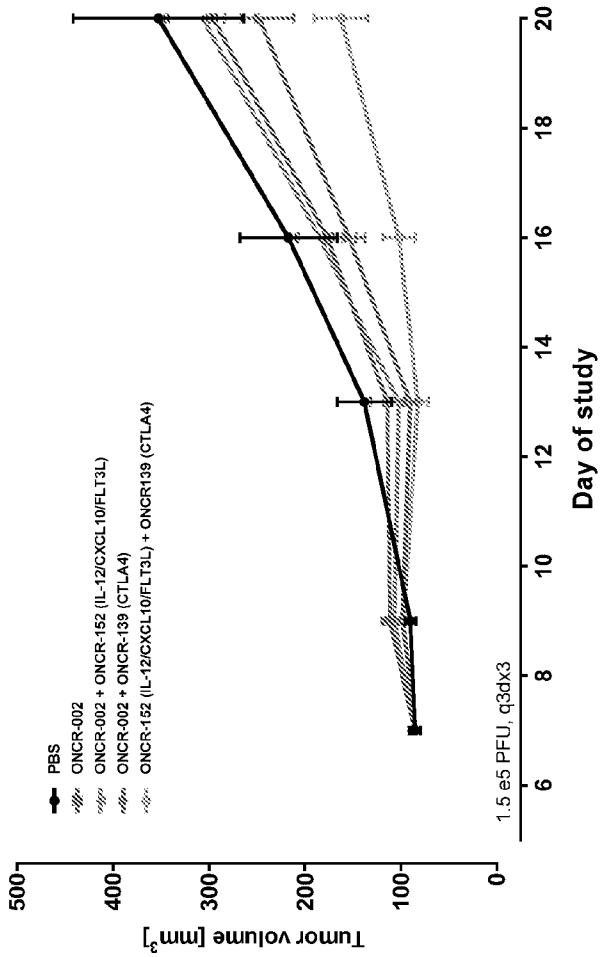


Fig. 66B

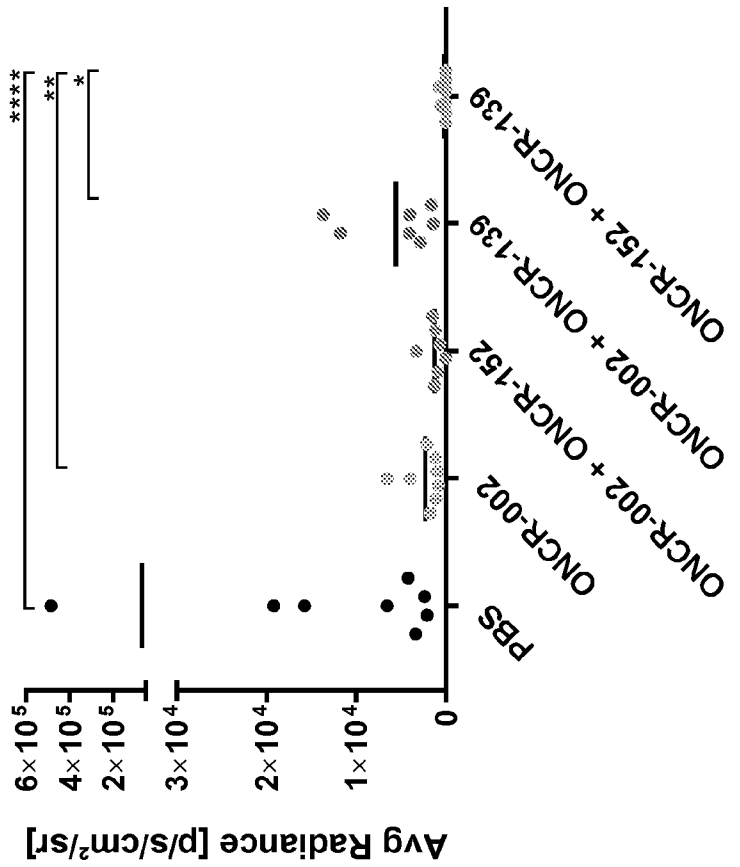


Fig. 66C

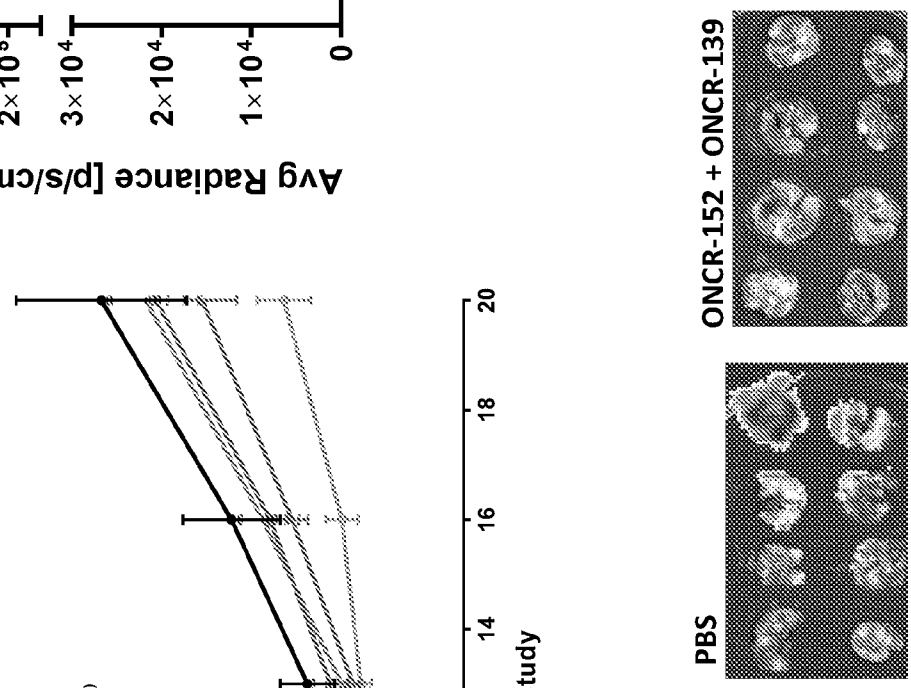
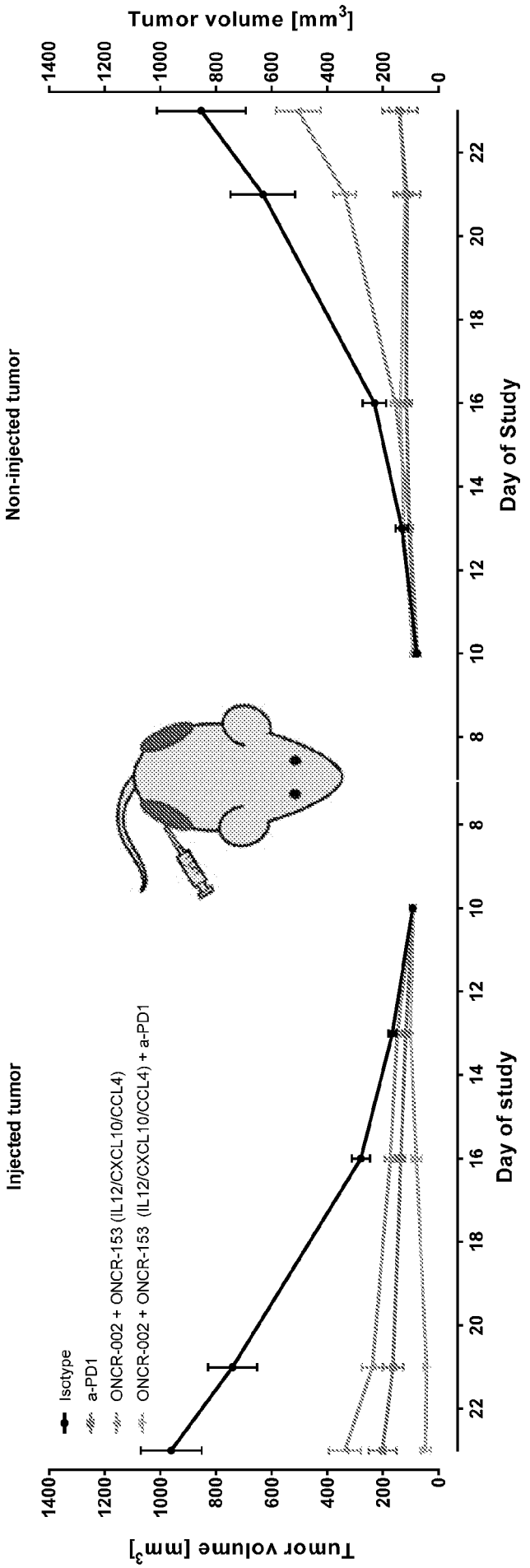


Fig. 67


Tumor volume [mm3]
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1200
1000
800
600
400
200
0
Injected tumor
Isotype
a-PD1
ONCR-002 + ONCR-153 (IL12/CXCL10/CCL4)
ONCR-002 + ONCR-153 (IL12/CXCL10/CCL4) + a-PD1
Day of study
Non-injected tumor
Tumor volume [mm3]
Day of Study

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Lerner, Lorena
Queva, Christophe

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