

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

07 September 2018 (07.09.2018)



(10) International Publication Number

WO 2018/160540 A1

(51) International Patent Classification:

A61K 38/20 (2006.01) C07K 14/54 (2006.01)

A61K 38/21 (2006.01) C07K 14/56 (2006.01)

C07K 14/535 (2006.01) C12N 15/67 (2006.01)

A61K 38/19 (2006.01)

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(21) International Application Number:

PCT/US2018/019878

(22) International Filing Date:

27 February 2018 (27.02.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/464,981 28 February 2017 (28.02.2017) US

17306089.8 23 August 2017 (23.08.2017) EP

62/597,527 12 December 2017 (12.12.2017) US

(71) Applicants: **SANOFI** [FR/FR]; 54 rue La Boétie, 75008 Paris (FR). **BIONTECH RNA PHARMACEUTICALS GMBH** [DE/DE]; An der Goldgrube 12, 55131 Mainz (DE).

(72) Inventors: **GIESEKE, Friederike**; c/o BioNTech AG, An der Goldgrube 12, 55131 Mainz (DE). **SAHIN, Ugur**; c/o BioNTech AG, An der Goldgrube 12, 55131 Mainz (DE). **WIEDERSCHAIN, Dmitri G.**; c/o Sanofi, 55 Corporate Drive, Mail Code: 55A-505A, Bridgewater, New Jersey 08807 (US). **WAGENAAR, Timothy R.**; c/o Sanofi, 55 Corporate Drive, Mail Code: 55A-505A, Bridgewater, New Jersey 08807 (US).

(74) Agent: **BAUR, Amelia Feulner** et al.; McNeill Baur PLLC, 125 Cambridge Park Drive, Suite 301, Cambridge, Massachusetts 02140 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

(54) Title: THERAPEUTIC RNA

(57) Abstract: This disclosure relates to the field of therapeutic RNAs for treatment of solid tumor cancers.



WO 2018/160540 A1

Therapeutic RNA

[0001] This application claims the benefit of priority to United States Provisional Application No. 62/464,981, filed February 28, 2017; United States Provisional Application No. 62/597,527, filed December 12, 2017; and European Patent Application No. 17306089.8, filed August 23, 2017; all of which are incorporated by reference in their entirety.

[0002] This disclosure relates to the field of therapeutic RNA to treat solid tumors. The National Cancer Institute defines solid tumors as abnormal masses of tissue that do not normally contain cysts or liquid areas. Solid tumors include benign and malignant (cancerous) sarcomas, carcinomas, and lymphomas, and can be physically located in any tissue or organ including the brain, ovary, breast, colon, and other tissues. Cancer is often divided into two main types: solid tumor cancer and hematological (blood) cancers. It is estimated that more than 1.5 million cases of cancer are diagnosed in the United States each year, and more than 500,000 people in the United States will die each year from cancer.

[0003] Solid tumor cancers are particularly difficult to treat. Current treatments include surgery, radiotherapy, immunotherapy and chemotherapy. Surgery alone may be an appropriate treatment for small localized tumors, but large invasive tumors and most metastatic malignancies are usually unresectable by surgery. Other common treatments such as radiotherapy and chemotherapy are associated with undesirable side effects and damage to healthy cells.

[0004] While surgery and current therapies sometimes are able to kill the bulk of the solid tumor, additional cells (including potentially cancer stem cells) may survive therapy. These cells, over time, can form a new tumor leading to cancer recurrence. In spite of multimodal conventional therapies, disease-free survival is less than 25% for many types of solid tumors. Solid tumors that are resistant to multi-modal therapy or that have recurred following therapy are even more difficult to treat, and long-term survival is less than 10%.

[0005] Disclosed herein are compositions, uses, and methods that can overcome present shortcomings in treatment of solid tumors. Administration of therapeutic RNAs disclosed herein can reduce tumor size, extend survival time, and/or protect against metastasis and/or recurrence of the tumor.

SUMMARY

Embodiment 1. A composition comprising RNA encoding an IL-12sc protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of

- SEQ ID NO: 14 and RNA encoding a GM-CSF protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 27.
- Embodiment 2. The composition of embodiment 1, further comprising RNA encoding an IL-15 sushi protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 24.
- Embodiment 3. The composition of embodiment 1, further comprising RNA encoding an IL-2 protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 9.
- Embodiment 4. The composition of embodiment 1, further comprising RNA encoding an IFN α 2b protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 19.
- Embodiment 5. A composition comprising:
- RNA encoding an IL-12sc protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 14;
 - RNA encoding a GM-CSF protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 27; and
 - RNA encoding an IFN α 2b protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 19.
- Embodiment 6. The composition of embodiment 5, further comprising RNA encoding an IL-15sushi protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 24.
- Embodiment 7. The composition of embodiment 5, further comprising RNA encoding an IL-2 protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 9.
- Embodiment 8. The composition of any one of embodiments 1-7, wherein at least one RNA comprises a modified nucleobase in place of at least one uridine.
- Embodiment 9. The composition of any one of embodiments 1-7, wherein each RNA comprises a modified nucleobase in place of each uridine.
- Embodiment 10. The composition of any one of embodiments 8-9, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).
- Embodiment 11. The composition of embodiment 10, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).

- Embodiment 12. The composition of any one of embodiments 1-11, wherein at least one RNA further comprises a 5' cap.
- Embodiment 13. The composition of any one of embodiments 1-11, wherein each RNA further comprises a 5' cap.
- Embodiment 14. The composition of any one of embodiments 12-13, wherein the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$.
- Embodiment 15. The composition of any one of embodiments 1-14, wherein at least one RNA further comprises a 5' UTR.
- Embodiment 16. The composition of any one of embodiments 1-14, wherein each RNA further comprises a 5' UTR.
- Embodiment 17. The composition of any one of embodiments 15-16, wherein the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6.
- Embodiment 18. The composition of any one of embodiments 1-17, wherein at least one RNA further comprises a 3' UTR.
- Embodiment 19. The composition of any one of embodiments 1-17, wherein each RNA further comprises a 3' UTR.
- Embodiment 20. The composition of any one of embodiments 18-19, wherein the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8.
- Embodiment 21. The composition of any one of embodiments 1-20, wherein at least one RNA further comprises a poly-A tail.
- Embodiment 22. The composition of any one of embodiments 1-20, wherein each RNA further comprises a poly-A tail.
- Embodiment 23. The composition of any one of embodiments 21-22, wherein the poly-A tail comprises at least 100 nucleotides.
- Embodiment 24. The composition of any one of embodiments 1-23, wherein at least one RNA comprises a 5' cap, 5' UTR, 3' UTR, and poly-A tail.
- Embodiment 25. The composition of any one of embodiments 1-23, wherein each RNA comprises a 5' cap, 5' UTR, 3' UTR, and poly-A tail.
- Embodiment 26. The composition of any one of embodiments 24-25, wherein
- a. the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$;

- b. the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6;
- c. the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8; and
- d. the poly-A tail comprises at least 100 nucleotides.

Embodiment 27. A method for treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject comprising administering the composition of any one of embodiments 1-26 to the subject.

Embodiment 28. A method for treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject comprising administering to the subject:

- a. an RNA encoding an IL-12sc protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 14, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 17 or 18; and
- b. an RNA encoding a GM-CSF protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 27, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NO: 29,

thereby treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in the subject.

Embodiment 29. The method of embodiment 28, further comprising administering RNA encoding an IFN α 2b protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 19, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 30. The method of embodiment 28, further comprising administering RNA encoding an IL-15 sushi protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 24, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 31. The method of embodiment 28, further comprising administering RNA encoding an IL-2 protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 9, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 32. The method of embodiment 28, further comprising administering RNA encoding

- a. an IL-15 sushi protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 24, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NO: 26; and
- b. an IFN α 2b protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 19, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 33. The method of embodiment 28, further comprising administering RNA encoding

- a. an IL-2 protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 9, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 12 or 13; and
- b. an IFN α 2b protein that is at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identical to the amino acids of SEQ ID NO: 19, and/or comprising nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 34. The method of any one of embodiments 27-33, wherein the cancer is a sarcoma, carcinoma, or lymphoma.

Embodiment 35. The method of any one of embodiments 27-33, wherein the cancer is a solid tumor.

Embodiment 36. The method of embodiment 35, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.

Embodiment 37. The method of embodiment 35, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma, testicular tumor, carcinoma, adenocarcinoma, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.

Embodiment 38. The method of any one of embodiments 27-37, wherein the composition is administered intra-tumorally or peri-tumorally.

Embodiment 39. The method of embodiment 38, wherein the injected tumor and a non-injected tumor are both reduced in size after intra- or peri- tumoral injection into or near the first tumor.

Embodiment 40. The method of any one of embodiments 27-39, wherein the subject is human.

Embodiment 41. The method of any one of embodiments 27-40, wherein another therapy is also administered.

Embodiment 42. The method of embodiment 41, wherein the other therapy is surgery to excise, resect, or debulk the tumor.

Embodiment 43. The method of embodiment 41, wherein the other therapy is immunotherapy, radiotherapy or chemotherapy.

Embodiment 44. The method of any one of embodiments 27-43, wherein at least one RNA comprises a modified nucleobase in place of at least one uridine.

Embodiment 45. The method of any one of embodiments 27-43, wherein each RNA comprises a modified nucleobase in place of each uridine.

Embodiment 46. The method of any one of embodiments 44-45, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$), or 5-methyl-uridine (m^5U).

Embodiment 47. The method of embodiment 46, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).

Embodiment 48. The method of any one of embodiments 27-47, wherein at least one RNA further comprises a 5' cap.

Embodiment 49. The method of any one of embodiments 27-47, wherein each RNA further comprises a 5' cap.

- Embodiment 50. The method of any one of embodiments 48-49, wherein the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m_1^{7'}G(5')ppp(5')G$.
- Embodiment 51. The method of any one of embodiments 27-50, wherein at least one RNA further comprises a 5' UTR.
- Embodiment 52. The method of any one of embodiments 27-50, wherein each RNA further comprises a 5' UTR.
- Embodiment 53. The method of any one of embodiments 51-52, wherein the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6.
- Embodiment 54. The method of any one of embodiments 27-54, wherein at least one RNA further comprises a 3' UTR.
- Embodiment 55. The method of any one of embodiments 27-54, wherein each RNA further comprises a 3' UTR.
- Embodiment 56. The method of any one of embodiments 54-55, wherein the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8.
- Embodiment 57. The method of any one of embodiments 27-56, wherein at least one RNA further comprises a poly-A tail.
- Embodiment 58. The method of any one of embodiments 27-56, wherein each RNA further comprises a poly-A tail.
- Embodiment 59. The method of any one of embodiments 57-58, wherein the poly-A tail comprises at least 100 nucleotides.
- Embodiment 60. The method of any one of embodiments 27-59, wherein at least one RNA comprises a 5' cap, 5' UTR, 3' UTR, and poly-A tail.
- Embodiment 61. The method of any one of embodiments 27-59, wherein each RNA comprises a 5' cap, 5' UTR, 3' UTR, and poly-A tail.
- Embodiment 62. The method of any one of embodiments 60-61, wherein
- the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m_1^{7'}G(5')ppp(5')G$;
 - the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6;

- c. the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8; and
- d. the poly-A tail comprises at least 100 nucleotides.

Embodiment 63. A codon-optimized DNA comprising or consisting of contiguous nucleotides having at least 83% identity to SEQ ID NO: 11.

Embodiment 64. The DNA of embodiment 63, comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 11.

Embodiment 65. A codon-optimized RNA comprising or consisting of contiguous nucleotides having at least 83% identity to SEQ ID NO: 13.

Embodiment 66. The RNA of embodiment 65, comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 13.

Embodiment 67. An RNA produced from the DNA of any one of embodiments 63 or 64.

Embodiment 68. A codon-optimized DNA comprising or consisting of:

- a. contiguous nucleotides having at least 78% identity to nucleotides 1-984 of SEQ ID NO: 16;
- b. contiguous nucleotides having at least 81% identity to nucleotides 1027-1623 of SEQ ID NO: 16; and
- c. nucleotides encoding a linker between the nucleotides of a) and b).

Embodiment 69. The DNA of embodiment 68, wherein the linker comprises nucleotides 985-1026 of SEQ ID NO: 16.

Embodiment 70. The DNA of any one of embodiments 68 and 69, wherein part a) comprises contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% identity to nucleotides 1-984 of SEQ ID NO: 16; and part b) comprises contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 1027-1623 of SEQ ID NO: 16.

Embodiment 71. A codon-optimized RNA comprising or consisting of:

- a. contiguous nucleotides having at least 78% identity to nucleotides 1-984 of SEQ ID NO: 18;
- b. contiguous nucleotides having at least 81% identity to nucleotides 1027-1623 of SEQ ID NO: 18; and

- c. nucleotides encoding a linker between the nucleotides of a) and b).

Embodiment 72. The RNA of embodiment 71, wherein the linker comprises nucleotides 985-1026 of SEQ ID NO: 18.

Embodiment 73. The RNA of any one of embodiments 71 and 72, wherein part a) comprises contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% identity to nucleotides 1-984 of SEQ ID NO: 18; and part b) comprises contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 1027-1623 of SEQ ID NO: 18.

Embodiment 74. An RNA produced from the DNA of any one of embodiments 68-70.

Embodiment 75. A codon-optimized DNA comprising or consisting of contiguous nucleotides having at least 80% identity to SEQ ID NO: 21.

Embodiment 76. The DNA of embodiment 75, comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 21.

Embodiment 77. A codon-optimized RNA comprising or consisting of contiguous nucleotides having at least 80% identity to SEQ ID NO: 23.

Embodiment 78. The RNA of embodiment 77, comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 23.

Embodiment 79. An RNA produced from the DNA of any one of embodiments 75-76.

Embodiment 80. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 1-321 of SEQ ID NO: 25;
- b. contiguous nucleotides comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 382-729 of SEQ ID NO: 25; and
- c. nucleotides encoding a linker between the nucleotides of a) and b).

Embodiment 81. An RNA produced from the DNA of embodiment 80.

Embodiment 82. A RNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 1-321 of SEQ ID NO: 26;

- b. contiguous nucleotides comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to nucleotides 382-729 of SEQ ID NO: 26; and
- c. nucleotides encoding a linker between the nucleotides of a) and b).

Embodiment 83. A DNA comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 28.

Embodiment 84. An RNA produced from the DNA of embodiment 83.

Embodiment 85. An RNA comprising or consisting of contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 29.

Embodiment 86. The RNA of any one of embodiments 67, 74, 79, 81, and 84 wherein the RNA is transcribed from the DNA in vitro.

Embodiment 87. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-85, wherein at least one uridine is replaced with a modified nucleobase.

Embodiment 88. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-85, wherein each uridine is replaced with a modified nucleobase.

Embodiment 89. The RNA of embodiment 87 or 88, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$), or 5-methyl-uridine (m^5U).

Embodiment 90. The RNA of embodiment 89, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).

Embodiment 91. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-90, further comprising a 5' cap.

Embodiment 92. The RNA of embodiment 91, wherein the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$.

Embodiment 93. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-92, further comprising a 5' UTR.

Embodiment 94. The RNA of embodiment 93, wherein the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6.

Embodiment 95. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-94, further comprising a 3' UTR.

Embodiment 96. The RNA of embodiment 95, wherein the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8.

Embodiment 97. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-96, further comprising a poly-A tail.

Embodiment 98. The RNA of embodiment 97, wherein the poly-A tail comprises at least 100 nucleotides.

Embodiment 99. The RNA of any one of embodiments 65-67, 71-74, 78-79, 81-82, and 84-97, further comprising a 5' cap, 5' UTR, 3' UTR, and poly-A tail.

Embodiment 100. The RNA of embodiment 99, wherein

- a. the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$;
- b. the 5' UTR comprises or consists of the nucleotides of SEQ ID NOs: 2, 4, or 6, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NOs: 2, 4, or 6;
- c. the 3' UTR comprises or consists of the nucleotides of SEQ ID NO: 8, or nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, or 85% identity to SEQ ID NO: 8 and;
- d. the poly-A tail comprises at least 100 nucleotides.

Embodiment 101. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 83% identity to SEQ ID NO: 11; and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5, wherein when transcribed, the nucleotides of parts a) and b) form a single transcript.

Embodiment 102. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 78% identity to nucleotides 1-984 of SEQ ID NO: 16;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 81% identity to nucleotides 1027-1623 of SEQ ID NO: 16; and
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5, wherein when transcribed, the nucleotides of part a), b) and c) form a single transcript.

Embodiment 103. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 21;
and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5, wherein when transcribed, the nucleotides of part a) and b) form a single transcript.

Embodiment 104. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 28;
and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5, wherein when transcribed, the nucleotides of part a) and b) form a single transcript.

Embodiment 105. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 1-321 of SEQ ID NO: 25;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 382-729 of SEQ ID NO: 25; and
- c. contiguous nucleotides having at least 80% identity to SEQ ID NOs: 1, 3, or 5, wherein when transcribed, the nucleotides of part a), b), and c) form a single transcript.

Embodiment 106. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 83% identity to SEQ ID NO: 11;
and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a) and b) form a single transcript.

Embodiment 107. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 78% identity to nucleotides 1-984 of SEQ ID NO: 16;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 81% identity to nucleotides 1027-1623 of SEQ ID NO: 16; and
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b) and c) form a single transcript.

Embodiment 108. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 21; and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a) and b) form a single transcript.

Embodiment 109. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 28; and
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a) and b) form a single transcript.

Embodiment 110. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 1-321 of SEQ ID NO: 25;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 382-729 of SEQ ID NO: 25; and

- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b), and c) form a single transcript.

Embodiment 111. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 83% identity to SEQ ID NO: 11;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5; and
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b), and c) form a single transcript.

Embodiment 112. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 78% identity to nucleotides 1-984 of SEQ ID NO: 16;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or 81% identity to nucleotides 1027-1623 of SEQ ID NO: 16;
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5; and
- d. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b), c), and d) form a single transcript.

Embodiment 113. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 21;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3,

or 5, wherein the nucleotides of part b) regulate the expression of the nucleotides of part a); and

- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b) and c) form a single transcript.

Embodiment 114. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 28;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5; and
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b), and c) form a single transcript.

Embodiment 115. A DNA comprising or consisting of:

- a. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 1-321 of SEQ ID NO: 25;
- b. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to nucleotides 382-729 of SEQ ID NO: 25;
- c. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NOs: 1, 3, or 5; and
- d. contiguous nucleotides comprising or consisting of nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to SEQ ID NO: 7, wherein when transcribed, the nucleotides of part a), b), c), and d) form a single transcript.

Embodiment 116. An RNA produced from any one of the DNAs of embodiments 101-115.

Embodiment 117. The RNA of embodiment 116, wherein at least one uridine is replaced with a modified nucleobase.

- Embodiment 118. The RNA of embodiment 116, wherein each uridine is replaced with a modified nucleobase.
- Embodiment 119. The RNA of any one of embodiments 117-118, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$), or 5-methyl-uridine (m^5U).
- Embodiment 120. The RNA of embodiment 119, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).
- Embodiment 121. The RNA of any one of embodiments 116-120, wherein the RNA further comprises a 5' cap.
- Embodiment 122. The RNA of embodiment 121, wherein the 5' cap is $m_2^{7,3'}\text{-}^OGppp(m_1^{2'}\text{-}O)ApG$ or $3'\text{-}O\text{-Me-}m^7G(5')ppp(5')G$.
- Embodiment 123. The RNA of any of embodiments 116-122, wherein the RNA is substantially free of double-stranded RNA.
- Embodiment 124. The RNA of any of embodiments 116-122, wherein double stranded RNA has been removed from the RNA.
- Embodiment 125. The RNA of any one of embodiments 123-124, wherein the RNA has been purified via HPLC or cellulose-based chromatography.
- Embodiment 126. A pharmaceutical formulation comprising any one of the DNA or RNAs of embodiments 63-125 and a pharmaceutically acceptable excipient.
- Embodiment 127. A method for treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject comprising administering any one or more of the RNAs or DNAs of embodiments 63-125, or the pharmaceutical formulation of embodiment 126.
- Embodiment 128. A method of producing a polypeptide encoding IL-2, IL-12sc, IL-15 sushi, GM-CSF and IFN α 2b *in vivo* comprising administering to a subject one or more of the DNAs or RNAs of any one of embodiment 63-125, the composition of any one of embodiments 1-26, or the pharmaceutical formulation of embodiment 126.
- Embodiment 129. A composition comprising at least two RNAs, wherein the RNAs encode different proteins, and wherein the RNAs are selected from the RNAs of any one of embodiments 65-67, 71-74, 78-79, 81-82, 84-100, and 116-125.

Embodiment 130. The composition of embodiment 129, wherein the composition comprises two RNAs encoding GM-CSF and IL-12sc.

Embodiment 131. The composition of embodiment 129, wherein the composition comprises three RNAs encoding GM-CSF, IL-12sc, and IFN α 2b.

Embodiment 132. The composition of embodiment 129, wherein the composition comprises three RNAs encoding GM-CSF, IL-2, and IFN α 2b.

Embodiment 133. The composition of embodiment 129, wherein the composition comprises four RNAs encoding GM-CSF, IL-12sc, IL-2 and IFN α 2b.

Embodiment 134. The composition of embodiment 129, wherein the composition comprises four RNAs encoding GM-CSF, IL-12sc, IL-15 sushi and IFN α 2b.

Embodiment 135. The composition of any one of embodiments 130-134, wherein the RNA encoding GM-CSF is selected from the RNA of any one of embodiments 84-85.

Embodiment 136. The composition of any one of embodiments 130-134, wherein the RNA encoding IL-12sc is selected from the RNA of any one of embodiments 71-74.

Embodiment 137. The composition of any one of embodiments 131-134, wherein the RNA encoding IFN α 2b is selected from the RNA of any one of embodiments 77-79.

Embodiment 138. The composition of embodiment 134, wherein the RNA encoding IL-15 sushi is selected from the RNA of any one of embodiments 81-82.

Embodiment 139. The composition of any one of embodiments 132-133, wherein the RNA encoding IL-2 is selected from the RNA of any one of embodiments 65-67.

Embodiment 140. A method of treating or preventing solid tumor cancer comprising administering a therapeutically effective amount of each of the following RNAs directly into a tumor:

- a. an RNA comprising an RNA encoding IL-12sc (SEQ ID NOs: 17 or 18);
- b. an RNA comprising an RNA encoding GM-CSF (SEQ ID NO: 29); and
- c. an RNA comprising an RNA encoding IFN α 2b (SEQ ID NOs: 22 or 23),

wherein each RNA comprises a modified nucleobase in place of each uridine, and wherein each RNA comprises a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 141. A method of treating or preventing solid tumor cancer comprising administering a therapeutically effective amount of each of the following RNAs directly into a tumor:

- a. an RNA comprising an RNA encoding IL-12sc (SEQ ID NOs: 17 or 18);
- b. an RNA comprising an RNA encoding GM-CSF (SEQ ID NO: 29);
- c. an RNA comprising an RNA encoding IFN α 2b (SEQ ID NOs: 22 or 23); and
- d. an RNA comprising an RNA encoding IL-2 (SEQ ID NOs: 12 or 13),

wherein each RNA comprises a modified nucleobase in place of each uridine, and wherein each RNA comprises a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 142. A method of treating or preventing solid tumor cancer comprising administering a therapeutically effective amount of each of the following RNAs directly into a tumor:

- a. an RNA comprising an RNA encoding IL-12sc (SEQ ID NOs: 17 or 18);
- b. an RNA comprising an RNA encoding GM-CSF (SEQ ID NO: 29);
- c. an RNA comprising an RNA encoding IFN α 2b (SEQ ID NOs: 22 or 23); and
- d. an RNA comprising an RNA encoding IL-15 sushi (SEQ ID NO: 26),

wherein each RNA comprises a modified nucleobase in place of each uridine, and wherein each RNA comprises a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 143. A composition comprising an RNA encoding an IL-2 protein having at least 95% identity to the amino acids of SEQ ID NO: 9, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 144. A composition comprising an RNA encoding an IL-12sc protein having at least 95% identity to the amino acids of SEQ ID NO: 14, wherein

each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 145. A composition comprising an RNA encoding a GM-CSF protein having at least 95% identity to the amino acids of SEQ ID NO: 27, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 146. A composition comprising an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acids of SEQ ID NO: 19, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 147. A composition comprising an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acids of SEQ ID NO: 24, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 148. An IL-12sc RNA composition comprising or consisting of nucleotides having at least 95% identity to SEQ ID NOs: 17 or 18, wherein each uridine is replaced with a modified nucleobase and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 149. A GM-CSF RNA composition comprising or consisting of nucleotides having at least 95% identity to SEQ ID NO: 29, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 150. An IFN α 2b RNA composition comprising or consisting of nucleotides having at least 95% identity to SEQ ID NOs: 22 or 23, wherein each uridine is replaced with a uridine analog, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 151. An IL-15 sushi RNA composition comprising or consisting of nucleotides having at least 95% identity to SEQ ID NO: 26, wherein each uridine is

replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 152. An IL-2 RNA composition comprising or consisting of nucleotides having at least 95% identity to SEQ ID NOs: 12 or 13, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 153. An IL-12sc RNA composition comprising or consisting of the nucleotides of SEQ ID NOs: 17 or 18, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 154. A GM-CSF RNA composition comprising or consisting of the nucleotides of SEQ ID NO: 29, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 155. An IFN α 2b RNA composition comprising or consisting of the nucleotides of SEQ ID NOs: 22 or 23, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 156. An IL-15 sushi RNA composition comprising or consisting of the nucleotides of SEQ ID NO: 26, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 157. An IL-2 RNA composition comprising or consisting of the nucleotides of SEQ ID NOs: 12 or 13, wherein each uridine is replaced with a modified nucleobase, and further comprising a 5' UTR (SEQ ID NOs: 2, 4, or 6), a 3' UTR (SEQ ID NO: 8), a 5' cap, and a poly-A tail.

Embodiment 158. The composition of any of embodiments 143-157, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$), or 5-methyl-uridine (m^5U).

Embodiment 159. A composition comprising an RNA encoding IFN α 2b, wherein the RNA is altered to have reduced immunogenicity as compared to un-altered RNA.

- Embodiment 160. The composition of embodiment 159, wherein the alteration comprises substitution of at least one uridine with a modified nucleobase.
- Embodiment 161. The composition of embodiment 159, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$), or 5-methyl-uridine (m^5U).
- Embodiment 162. The composition of embodiment 161, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).
- Embodiment 163. The composition of any one of embodiments 159-162, wherein the alteration comprises a reduction in the amount of double-stranded RNA.
- Embodiment 164. The composition of embodiment 163, wherein the reduction in double-stranded RNA is the result of purification via HPLC or cellulose-based chromatography.
- Embodiment 165. The composition of any one of embodiments 159-164, wherein the alteration reduces RNA recognition by an innate immune system as compared to un-altered RNA.
- Embodiment 166. The composition of any one of embodiments 159-165, wherein the alteration comprises addition of a 5' cap to the RNA.
- Embodiment 167. The composition of embodiment 166, wherein the 5' cap is $m_2^{7,3'}\text{-}^0\text{Gppp}(m_1^{2'}\text{-}^0)\text{ApG}$ or $3'\text{-O-Me-}m^7\text{G}(5')\text{ppp}(5')\text{G}$.
- Embodiment 168. The composition of any one of embodiments 159-167, further comprising a second RNA encoding a peptide or protein of interest.
- Embodiment 169. The composition of embodiment 168, wherein the peptide or protein of interest is a peptide or protein selected or derived from cytokines, chemokines, suicide gene products, immunogenic proteins or peptides, apoptosis inducers, angiogenesis inhibitors, heat shock proteins, tumor antigens, β -catenin inhibitors, activators of the STING pathway, activators of the retinoic inducible gene (RIG)-I pathway, agonists of toll-like receptor (TLR) pathways, checkpoint modulators, innate immune activators, antibodies, dominant negative receptors and decoy receptors, inhibitors of myeloid derived suppressor cells (MDSCs), IDO pathway inhibitors, and proteins or peptides that bind inhibitors of apoptosis.

- Embodiment 170. The composition of embodiment 168 or 169, wherein the second RNA is altered to have reduced immunogenicity as compared to un-altered RNA.
- Embodiment 171. The composition of embodiment 170, wherein the alteration comprises substitution of at least one uridine with a modified nucleobase.
- Embodiment 172. The composition of embodiment 171, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).
- Embodiment 173. The composition of embodiment 172, wherein the modified nucleobase is N1-methyl-pseudouridine ($m^1\psi$).
- Embodiment 174. The composition of any one of embodiments 168-173, wherein the alteration comprises a reduction in the amount of double-stranded RNA.
- Embodiment 175. The composition of embodiment 174, wherein the reduction in double-stranded RNA is the result of purification via HPLC or cellulose-based chromatography.
- Embodiment 176. The composition of any one of embodiments 168-173, wherein the alteration comprises addition of a 5' cap to the RNA.
- Embodiment 177. The composition of embodiment 176, wherein the 5' cap is $m_2^{7,3'}\text{-}^0\text{Gppp}(m_1^{2'}\text{-}^0)\text{ApG}$ or $3'\text{-O-Me-}m^7\text{G}(5')\text{ppp}(5')\text{G}$.
- Embodiment 178. The composition of any one of embodiments 168-177, wherein the second RNA comprises:
- an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
 - an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
 - an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; or

- d. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 179. A method for treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject comprising administering any one of the compositions of embodiments 159-178.

Embodiment 180. The composition of any one of embodiments 159-178 for use in a method of treating or preventing cancer, reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis.

Embodiment 181. A composition comprising an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14.

Embodiment 182. A composition comprising an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27.

Embodiment 183. A composition comprising an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19.

Embodiment 184. A composition comprising an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24.

Embodiment 185. A composition comprising an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9.

Embodiment 186. A composition comprising an RNA encoding an IL-12sc protein, wherein the RNA comprises nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18.

Embodiment 187. A composition comprising an RNA encoding a GM-CSF protein, wherein the RNA comprises nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29.

Embodiment 188. A composition comprising an RNA encoding an IFN α 2b protein, wherein the RNA comprises nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 189. A composition comprising an RNA encoding an IL-15 sushi protein, wherein the RNA comprises nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 190. A composition comprising an RNA encoding an IL-2 protein, wherein the RNA comprises nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 191. A composition comprising any two of the following RNAs:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- d. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- e. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 192. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18; and
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29.

Embodiment 193. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18; and
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 194. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18; and
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 195. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18; and
- b. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 196. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 197. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 198. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and
- b. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 199. A composition comprising:

- a. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 200. A composition comprising:

- a. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and
- b. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 201. A composition comprising:

- a. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- b. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 202. A composition comprising any three of the following:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- d. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- a. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 203. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and

- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23.

Embodiment 204. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and
- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 205. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29; and
- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 206. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and

- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 207. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and
- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 208. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 209. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and

- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 210. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and
- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 211. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 212. A composition comprising:

- a. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- b. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and

- c. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 213. A composition comprising any four of the following:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- d. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- e. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 214. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and

- d. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26.

Embodiment 215. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23; and
- d. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 216. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- d. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 217. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- d. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 218. A composition comprising:

- a. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- b. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- c. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- d. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 219. A composition comprising:

- a. an RNA encoding an IL-12sc protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 14, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 17 or 18;

- b. an RNA encoding a GM-CSF protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 27, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 29;
- c. an RNA encoding an IFN α 2b protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 19, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 22 or 23;
- d. an RNA encoding an IL-15 sushi protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 24, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NO: 26; and
- e. an RNA encoding an IL-2 protein having at least 95% identity to the amino acid sequence of SEQ ID NO: 9, and/or comprising nucleotides having at least 95% identity to the nucleotides of SEQ ID NOs: 12 or 13.

Embodiment 220. A pharmaceutical formulation comprising any one of the compositions of embodiments 181-219.

Embodiment 221. A pharmaceutical formulation comprising any one of the compositions of embodiments 181-219 and a pharmaceutically acceptable excipient.

Embodiment 222. The composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221, for use in a method of treating or preventing cancer.

Embodiment 223. The composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221 for use in a method of reducing the size of a tumor.

Embodiment 224. The composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221 for use in a method of preventing the reoccurrence of cancer in remission.

Embodiment 225. The composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221 for use in a method of preventing cancer metastasis.

Embodiment 226. A method for treating or preventing cancer comprising administering the composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221.

Embodiment 227. A method for reducing the size of a tumor comprising administering the composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221.

Embodiment 228. A method for preventing the reoccurrence of cancer in remission comprising administering the composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221.

Embodiment 229. A method for preventing cancer metastasis comprising administering the composition of any one of embodiments 181-219, or the pharmaceutical formulation of embodiment 220 or 221.

[0006] Further embodiments of the present invention are as follows:

Embodiment A 1. A medical preparation comprising RNA encoding an IL-12sc protein and RNA encoding a GM-CSF protein.

Embodiment A 2. The medical preparation of embodiment A 1, further comprising RNA encoding an IL-15 sushi protein.

Embodiment A 3. The medical preparation of embodiment A 1 or 2, further comprising RNA encoding an IL-2 protein.

Embodiment A 4. The medical preparation of any one of embodiments A 1 to 3, further comprising RNA encoding an IFN α protein.

Embodiment A 5. The medical preparation of embodiment A 4, wherein the IFN α protein is an IFN α 2b protein.

Embodiment A 6. The medical preparation of embodiment A 2, comprising RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IL-15 sushi protein.

Embodiment A 7. The medical preparation of embodiment A 3, comprising RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IL-2 protein.

Embodiment A 8. The medical preparation of embodiment A 4 or 5, comprising RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IFN α protein.

Embodiment A 9. The medical preparation of embodiment A 4 or 5, comprising RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, RNA encoding an IL-15 sushi protein, and RNA encoding an IFN α protein.

Embodiment A 10. The medical preparation of embodiment A 4 or 5, comprising RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, RNA encoding an IL-2 protein, and RNA encoding an IFN α protein.

Embodiment A 11. The medical preparation of any one of embodiments A 1-10, wherein (i) the RNA encoding an IL-12sc protein comprises the nucleotide sequence of SEQ ID NO: 17 or 18, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17 or 18 and/or (ii) the IL-12sc protein comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14.

Embodiment A 12. The medical preparation of any one of embodiments A 1-11, wherein (i) the RNA encoding a GM-CSF protein comprises the nucleotide sequence of SEQ ID NO: 29, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 29 and/or (ii) the GM-CSF protein comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

Embodiment A 13. The medical preparation of any one of embodiments A 2-12, wherein (i) the RNA encoding an IL-15 sushi protein comprises the nucleotide sequence of SEQ ID NO: 26, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 26 and/or (ii) the IL-15 sushi protein comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 24.

Embodiment A 14. The medical preparation of any one of embodiments A 3-13, wherein (i) the RNA encoding an IL-2 protein comprises the nucleotide sequence of SEQ ID NO: 12 or 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12 or 13 and/or (ii) the IL-2 protein comprises the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 9.

Embodiment A 15. The medical preparation of any one of embodiments 4-14, wherein (i) the RNA encoding an IFN α protein comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%,

85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 22 or 23 and/or (ii) the IFN α protein comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 19.

Embodiment A 16. The medical preparation of any one of embodiments A 1-15, wherein at least one RNA comprises a modified nucleobase in place of at least one uridine.

Embodiment A 17. The medical preparation of any one of embodiments A 1-16, wherein each RNA comprises a modified nucleobase in place of at least one uridine.

Embodiment A 18. The medical preparation of any one of embodiments A 1-17, wherein each RNA comprises a modified nucleobase in place of each uridine.

Embodiment A 19. The medical preparation of any one of embodiments A 16-18, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ) or 5-methyl-uridine (m5U).

Embodiment A 20. The medical preparation of embodiment A 19, wherein the modified nucleobase is N1-methyl-pseudouridine (m1 ψ).

Embodiment A 21. The medical preparation of any one of embodiments A 1-20, wherein at least one RNA comprises a 5' cap.

Embodiment A 22. The medical preparation of any one of embodiments A 1-21, wherein each RNA comprises a 5' cap.

Embodiment A 23. The medical preparation of embodiment A 21 or 22, wherein the 5' cap is m₂^{7,3'}-O-Gppp(m₁^{2'-O})ApG or 3'-O-Me-m⁷G(5')ppp(5')G.

Embodiment A 24. The medical preparation of any one of embodiments A 1-23, wherein at least one RNA comprises a 5' UTR.

Embodiment A 25. The medical preparation of any one of embodiments A 1-24, wherein each RNA comprises a 5' UTR.

Embodiment A 26. The medical preparation of embodiment A 24 or 25, wherein the 5' UTR comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.

Embodiment A 27. The medical preparation of any one of embodiments A 1-26, wherein at least one RNA comprises a 3' UTR.

Embodiment A 28. The medical preparation of any one of embodiments A 1-27, wherein each RNA comprises a 3' UTR.

- Embodiment A 29. The medical preparation of embodiment A 27 or 28, wherein the 3' UTR comprises the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
- Embodiment A 30. The medical preparation of any one of embodiments A 1-29, wherein at least one RNA comprises a poly-A tail.
- Embodiment A 31. The medical preparation of any one of embodiments A 1-30, wherein each RNA comprises a poly-A tail.
- Embodiment A 32. The medical preparation of embodiment A 30 or 31, wherein the poly-A tail comprises at least 100 nucleotides.
- Embodiment A 33. The medical preparation of any one of embodiments A 1-32, wherein at least one RNA comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail.
- Embodiment A 34. The medical preparation of any one of embodiments A 1-33, wherein each RNA comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail.
- Embodiment A 35. The medical preparation of embodiment A 33 or 34, wherein
- a. the 5' cap is $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$;
 - b. the 5' UTR comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6;
 - c. the 3' UTR comprises the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8; and
 - d. the poly-A tail comprises at least 100 nucleotides.
- Embodiment A 36. The medical preparation of any one of embodiments A 1 to 35, wherein the RNA is mRNA.
- Embodiment A 37. The medical preparation of any one of embodiments A 1 to 36, which comprises a further therapeutic agent.
- Embodiment A 38. The medical preparation of embodiment A 37, wherein the further therapeutic agent is an anti-cancer therapeutic agent.
- Embodiment A 39. The medical preparation of embodiment A 37 or 38, wherein the further therapeutic agent is a checkpoint modulator.

- Embodiment A 40. The medical preparation of embodiment A 39, wherein the checkpoint modulator is an anti-PD1 antibody, an anti-CTLA-4 antibody, or a combination of an anti-PD1 antibody and an anti-CTLA-4 antibody.
- Embodiment A 41. The medical preparation of any one of embodiments A 1 to 40, which is a kit comprising at least two containers, each container comprising at least one of said RNAs.
- Embodiment A 42. The medical preparation of embodiment A 41, which comprises each RNA in a separate container.
- Embodiment A 43. The medical preparation of embodiment A 41 or 42, wherein the further therapeutic agent is in a container not comprising the RNA.
- Embodiment A 44. The medical preparation of any one of embodiments A 41-43, further comprising instructions for use of the medical preparation for treating or preventing cancer.
- Embodiment A 45. The medical preparation of any one of embodiments A 1 to 40, which is a pharmaceutical composition comprising the RNAs.
- Embodiment A 46. The medical preparation of embodiment A 45, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.
- Embodiment A 47. The medical preparation of any one of embodiments A 1 to 46, wherein the RNA is present in a form selected from a liquid form, a solid form, or a combination thereof.
- Embodiment A 48. The medical preparation of embodiment A 47, wherein the solid form is a frozen form or a dehydrated form.
- Embodiment A 49. The medical preparation of embodiment A 48, wherein the dehydrated form is a freeze-dried or spray-dried form.
- Embodiment A 50. The medical preparation of any one of embodiments A 1 to 49 for pharmaceutical use.
- Embodiment A 51. The medical preparation of embodiment A 50, wherein the pharmaceutical use comprises a therapeutic or prophylactic treatment of a disease or disorder.
- Embodiment A 52. The medical preparation of any one of embodiments A 1 to 51 for use in a method for treating or preventing cancer.
- Embodiment A 53. The medical preparation of any one of embodiments A 38-52, wherein the cancer is a sarcoma, carcinoma, or lymphoma.

Embodiment A 54. The medical preparation of any one of embodiments A 38-53, wherein the cancer is a solid tumor.

Embodiment A 55. The medical preparation of embodiment A 54, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.

Embodiment A 56. The medical preparation of embodiment A 54 or 55, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.

Embodiment A 57. The medical preparation of any one of embodiments A 1-56, wherein the RNA is for intra-tumoral or peri-tumoral administration.

Embodiment A 58. The medical preparation of any one of embodiments A 37-57, wherein the further therapeutic agent is for systemic administration.

Embodiment A 59. The medical preparation of any one of embodiments A 1-58, which is for administration to a human.

Embodiment A 60. The medical preparation of any one of embodiments A 44 and 47-59, wherein treating or preventing cancer comprises reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject.

[0007] Further embodiments of the present invention are as follows:

Embodiment B 1. RNA for use in a method for treating or preventing cancer in a subject, wherein the method comprises administering RNA encoding an IL-12sc protein and RNA encoding a GM-CSF protein.

Embodiment B 2. The RNA of Embodiment B 1, wherein the method further comprises administering RNA encoding an IL-15 sushi protein.

Embodiment B 3. The RNA of Embodiment B 1 or 2, wherein the method further comprises administering RNA encoding an IL-2 protein.

- Embodiment B 4. The RNA of any one of embodiments B 1 to 3, wherein the method further comprises administering RNA encoding an IFN α protein.
- Embodiment B 5. The RNA of Embodiment B 4, wherein the IFN α protein is an IFN α 2b protein.
- Embodiment B 6. The RNA of Embodiment B 2, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IL-15 sushi protein.
- Embodiment B 7. The RNA of Embodiment B 3, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IL-2 protein.
- Embodiment B 8. The RNA of Embodiment B 4 or 5, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, and RNA encoding an IFN α protein.
- Embodiment B 9. The RNA of Embodiment B 4 or 5, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, RNA encoding an IL-15 sushi protein, and RNA encoding an IFN α protein.
- Embodiment B 10. The RNA of Embodiment B 4 or 5, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding a GM-CSF protein, RNA encoding an IL-2 protein, and RNA encoding an IFN α protein.
- Embodiment B 11. The RNA of any one of embodiments B 1-10, wherein (i) the RNA encoding an IL-12sc protein comprises the nucleotide sequence of SEQ ID NO: 17 or 18, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17 or 18 and/or (ii) the IL-12sc protein comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14.
- Embodiment B 12. The RNA of any one of embodiments B 1-11, wherein (i) the RNA encoding a GM-CSF protein comprises the nucleotide sequence of SEQ ID NO: 29, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 29 and/or (ii) the GM-CSF protein comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

Embodiment B 13. The RNA of any one of embodiments B 2-12, wherein (i) the RNA encoding an IL-15 sushi protein comprises the nucleotide sequence of SEQ ID NO: 26, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 26 and/or (ii) the IL-15 sushi protein comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 24.

Embodiment B 14. The RNA of any one of embodiments B 3-13, wherein (i) the RNA encoding an IL-2 protein comprises the nucleotide sequence of SEQ ID NO: 12 or 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12 or 13 and/or (ii) the IL-2 protein comprises the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 9.

Embodiment B 15. The RNA of any one of embodiments B 4-14, wherein (i) the RNA encoding an IFN α protein comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 22 or 23 and/or (ii) the IFN α protein comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 19.

Embodiment B 16. The RNA of any one of embodiments B 1-15, wherein at least one RNA comprises a modified nucleobase in place of at least one uridine.

Embodiment B 17. The RNA of any one of embodiments B 1-16, wherein each RNA comprises a modified nucleobase in place of at least one uridine.

Embodiment B 18. The RNA of any one of embodiments B 1-17, wherein each RNA comprises a modified nucleobase in place of each uridine.

Embodiment B 19. The RNA of any one of embodiments B 16-18, wherein the modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ) or 5-methyl-uridine (m5U).

Embodiment B 20. The RNA of Embodiment B 19, wherein the modified nucleobase is N1-methyl-pseudouridine (m1 ψ).

Embodiment B 21. The RNA of any one of embodiments B 1-20, wherein at least one RNA comprises a 5' cap.

- Embodiment B 22. The RNA of any one of embodiments B 1-21, wherein each RNA comprises a 5' cap.
- Embodiment B 23. The RNA of Embodiment B 21 or 22, wherein the 5' cap is $m_2^{7,3'}\text{-}^OGppp(m_1^{2'}\text{-}^O)ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$.
- Embodiment B 24. The RNA of any one of embodiments B 1-23, wherein at least one RNA comprises a 5' UTR.
- Embodiment B 25. The RNA of any one of embodiments B 1-24, wherein each RNA comprises a 5' UTR.
- Embodiment B 26. The RNA of Embodiment B 24 or 25, wherein the 5' UTR comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.
- Embodiment B 27. The RNA of any one of embodiments B 1-26, wherein at least one RNA comprises a 3' UTR.
- Embodiment B 28. The RNA of any one of embodiments B 1-27, wherein each RNA comprises a 3' UTR.
- Embodiment B 29. The RNA of embodiment B 27 or 28, wherein the 3' UTR comprises the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
- Embodiment B 30. The RNA of any one of embodiments B 1-29, wherein at least one RNA comprises a poly-A tail.
- Embodiment B 31. The RNA of any one of embodiments B 1-30, wherein each RNA comprises a poly-A tail.
- Embodiment B 32. The RNA of embodiment B 30 or 31, wherein the poly-A tail comprises at least 100 nucleotides.
- Embodiment B 33. The RNA of any one of embodiments B 1-32, wherein at least one RNA comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail.
- Embodiment B 34. The RNA of any one of embodiments B 1-33, wherein each RNA comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail.
- Embodiment B 35. The RNA of embodiment B 33 or 34, wherein
- a. the 5' cap is $m_2^{7,3'}\text{-}^OGppp(m_1^{2'}\text{-}^O)ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$;

- b. the 5' UTR comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6;
- c. the 3' UTR comprises the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO:8; and
- d. the poly-A tail comprises at least 100 nucleotides.

Embodiment B 36. The RNA of any one of embodiments B 1 to 35, wherein the RNA is mRNA.

Embodiment B 37. The RNA of any one of embodiments B 1 to 36, wherein the method further comprises administering a further therapy.

Embodiment B 38. The RNA of embodiment B 37, wherein the further therapy comprises one or more selected from the group consisting of: (i) surgery to excise, resect, or debulk a tumor, (ii) immunotherapy, (iii) radiotherapy, and (iv) chemotherapy.

Embodiment B 39. The RNA of embodiment B 37 or 38, wherein the further therapy comprises administering a further therapeutic agent.

Embodiment B 40. The RNA of embodiment B 39, wherein the further therapeutic agent is an anti-cancer therapeutic agent.

Embodiment B 41. The RNA of embodiment B 39 or 40, wherein the further therapeutic agent is a checkpoint modulator.

Embodiment B 42. The RNA of embodiment B 41, wherein the checkpoint modulator is an anti-PD1 antibody, an anti-CTLA-4 antibody, or a combination of an anti-PD1 antibody and an anti-CTLA-4 antibody.

Embodiment B 43. The RNA of any one of embodiments B 1-42, wherein the cancer is a sarcoma, carcinoma, or lymphoma.

Embodiment B 44. The RNA of any one of embodiments B 1-43, wherein the cancer is a solid tumor.

Embodiment B 45. The RNA of embodiment B 44, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.

Embodiment B 46. The RNA of embodiment B 44 or 45, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor,

ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.

Embodiment B 47. The RNA of any one of embodiments B 1-46, wherein the RNA is administered intra-tumorally or peri-tumorally.

Embodiment B 48. The RNA of any one of embodiments B 39 to 47, wherein the further therapeutic agent is administered systemically.

Embodiment B 49. The RNA of any one of embodiments B 1-48, wherein the subject is a human.

Embodiment B 50. The RNA of any one of embodiments B 1-49, wherein the RNAs are administered at the same time.

Embodiment B 51. The RNA of any one of embodiments B 1-50, wherein the RNAs are administered by administering a composition comprising a combination of the RNAs.

Embodiment B 52. The RNA of any one of embodiments B 1-49, wherein at least two of the RNAs are administered at different times.

Embodiment B 53. The RNA of any one of embodiments B 1-49 and 52, wherein the RNAs are administered by administering at least two compositions, each composition comprising at least one of said RNAs.

Embodiment B 54. The RNA of any one of embodiments B 1 to 53, wherein treating or preventing cancer comprises reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject.

Embodiment B 55. The RNA of any one of embodiments B 1 to 54, which is or comprises one or more of the RNAs administered in said method.

Embodiment B 56. The RNA of embodiment B 55, which is or comprises one or more selected from the group consisting of the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, the RNA encoding an IL-15 sushi protein, the RNA encoding an IL-2 protein, and the RNA encoding an IFN α protein.

Embodiment B 57. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein.

Embodiment B 58. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding a GM-CSF protein.

Embodiment B 59. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-15 sushi protein.

Embodiment B 60. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-2 protein.

Embodiment B 61. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IFN α protein.

Embodiment B 62. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein and the RNA encoding a GM-CSF protein.

Embodiment B 63. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, and the RNA encoding an IL-15 sushi protein.

Embodiment B 64. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, and the RNA encoding an IL-2 protein.

Embodiment B 65. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, and the RNA encoding an IFN α protein.

Embodiment B 66. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, the RNA encoding an IL-15 sushi protein, and the RNA encoding an IFN α protein.

Embodiment B 67. The RNA of embodiment B 55 or 56, which is or comprises the RNA encoding an IL-12sc protein, the RNA encoding a GM-CSF protein, the RNA encoding an IL-2 protein, and the RNA encoding an IFN α protein.

FIGURE LEGENDS

[0008] FIGS 1A – 1G shows results of experiments where B16F10 tumor bearing mice were injected intratumorally with mRNA on days 8, 10, 12, 14 and individual tumor growth was monitored to day 41. FIG 1A and FIG 1D show results when using IL-2, IL-12sc, and GM-CSF (ModA) mRNA. FIG 1B and FIG 1E show IL-2, IL-12sc, and GM-CSF (ModB) mRNA. FIG 1C and FIG 1F show results when using luciferase mRNA (ModA). FIG 1G shows results when using luciferase mRNA (ModB). (N=10 mice/group for A-C and N= 9 mice/group for D-G).

[0009] FIGS 2A – 2D show results of experiments where CT26 tumor bearing mice were injected intratumorally with mRNA on day 19, 21, 24, 26, 28 and 31 and individual

tumor growth was monitored to day 48. FIG 2A shows GM-CSF, IL-2, IL-12sc (ModA). FIG 2B shows GM-CSF, IL-2, IL-12sc (ModB). FIG 2C shows luciferase mRNA (ModA) as a control. FIG 2D shows Ringer's solution as a control.

[0010] FIGS 3A – 3C show results of experiments where CT26 tumor bearing mice were injected intratumorally with mRNA on day 13, 15, 18, 20 and 22 and tumor growth was monitored to day 42. FIG 3A shows IL-2, GM-CSF, IL-12sc (ModB). FIG 3B shows IL-15 sushi, GM-CSF, IL-12sc (ModB). FIG 3C shows luciferase mRNA (ModB) as a control.

[0011] FIGS 4A – 4F show results of experiments where B16F10 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 11, 13, 15, 17 and individual tumor growth was monitored to day 45. FIG 4A shows IL-2, IL-12sc, and GM-CSF (ModA). FIG 4B is a duplicate in the same experiment as described in FIG 5A, showing IL-2, IL-12sc, and GM-CSF (ModB). FIG 4C shows IL-15 sushi, IL-12sc, and GM-CSF (ModA). FIG 4D is a duplicate in the same experiment as described in FIG 5B, showing IL-15 sushi, IL-12sc, and GM-CSF (ModB). FIG 4E shows control luciferase mRNA (ModA). FIG 4F is a duplicate in the same experiment as described in FIG 5D and 6D showing control luciferase mRNA (ModB). (N=8 mice/group).

[0012] FIGS 5A – 5D show results of experiments where B16F10 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 11, 13, 15, 17 and individual tumor growth was monitored to day 45. FIG 5A is a duplicate in the same experiment as described in FIG 4B, showing IL-2, IL-12sc, and GM-CSF (ModB). FIG 5B is a duplicate in the same experiment as described in FIG 4D, showing IL-15 sushi, IL-12sc, and GM-CSF (ModB). FIG 5C is a duplicate in the same experiment as described in FIG 6C, showing IL-2, IL-12sc, GM-CSF, and IFN α (ModB). FIG 5D is a duplicate in the same experiment as described in FIG 4F and 6D, showing luciferase mRNA (ModB) as control. (N=8 mice/group).

[0013] FIGS 6A and 6B show results of experiments where CT26 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 13, 15, 17, 19, 21, 23 and individual tumor growth was plotted. FIG 6A is a duplicate in the same experiment as described in FIG 7A, showing GM-CSF, IL-2, IL-12sc, IFN α (ModB). FIG 6B is a duplicate in the same experiment as described in FIG 7C, showing luciferase mRNA (ModB). N=8 mice/group.

[0014] FIGS 6C and 6D show results of experiments where B16F10 tumor bearing mice were injected intratumorally with mRNA on days 11, 13, 15, 17 and individual tumor growth was plotted. FIG 6C is a duplicate in the same experiment as described in FIG 5C,

showing GM-CSF, IL-2, IL-12sc, IFN α (ModB). FIG 6D is a duplicate in the same experiment as described in FIG 4F and 5D, showing luciferase mRNA (ModB). N=8 mice/group.

[0015] FIG 6E and 6F show results of experiments where MC38 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 11, 15, 19, 23 and individual tumor growth was plotted. FIG 6E shows GM-CSF, IL-2, IL-12sc, IFN α (ModB). FIG 6F shows luciferase mRNA (ModB). N=5 mice/group.

[0016] FIGS 7A – 7F show results of experiments where CT26 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 13, 15, 17, 19, 21, 23 and individual tumor growth was plotted. FIG 7A is a duplicate in the same experiment as described in FIG 6A, showing IL-2, IL-12sc, GM-CSF, IFN α (ModB). FIG 7B shows IL-15 sushi, IL-12sc, GM-CSF, IFN α (ModB). FIG 7C is a duplicate in the same experiment as described in FIG 6B, showing a luciferase mRNA (ModB) control. In a repeat study of similar design, CT26 tumor bearing mice were injected intratumorally with cytokine mRNA mixtures on days 19, 21, 23, 26, 28 and 30 and individual tumor growth was plotted. FIG 7D is a duplicate of the same experiment as described in FIG 9A, showing IL-2, IL-12sc, GM-CSF, IFN α (ModB). FIG 7E shows IL-15 sushi, IL-12sc, GM-CSF, IFN α (ModB). FIG 7F is a duplicate of the same experiment as described in FIG 9F, showing a luciferase mRNA (ModB) control. N=8 mice/group for Figures A-C and N=10-11 mice/group for Figures D-F.

[0017] FIGS 8A – 8H show results of experiments where CT26 tumor bearing mice were injected intratumorally with mRNA on days 12, 15, 19 and 22 and individual tumor growth was monitored and plotted to day 35. FIG 8A shows IL-15 sushi, IL-12sc, GM-CSF, IFN α (ModB). FIG 8B shows IL-15 sushi, IL-12sc, IFN α (ModB). FIG 8C shows IL-15 sushi, GM-CSF, IFN α (ModB). FIG 8D shows GM-CSF, IL-12sc, IFN α (ModB). FIG 8E shows IL-15 sushi, GM-CSF, IL-12sc (ModB). FIG 8F shows a luciferase mRNA (ModB) control. (N=10/group). FIGS 8G and 8H show tumor growth kinetics of the study shown in FIGS 8A-8F. FIG 8G shows mean tumor volumes up to day 33 for all treatment groups. FIG 8H shows tumor growth repression. T/C (Tumor/Control based on mean tumor volume) was calculated up to day 19.

[0018] FIGS 9A – 9F show experiments where CT26 tumor bearing mice were injected intratumorally with mRNA on days 19, 21, 23, 26, 28 and 30 and tumor growth was monitored to day 50. FIG 9A is a duplicate in the same experiment as described in FIG 7D, showing GM-CSF, IL-2, IL-12sc, IFN α (ModB). FIG 9B shows IL-2, IL-12sc, IFN α (ModB). FIG 9C shows GM-CSF, IL-2, IFN α (ModB). FIG 9D shows GM-CSF, IL-12sc,

IFN α (ModB). FIG 9E shows GM-CSF, IL-2, IL-12sc (ModB). FIG 9F is a duplicate in the same experiment as described in FIG 7F, showing shows luciferase mRNA (ModA) as control. (N=11/group for FIG 9A-E; luciferase mRNA group N=10 for FIG 9F).

[0019] FIGS 10A – 10B shows tumor growth kinetics of the study shown in Figure 9. FIG 10A shows mean tumor volumes up to day 36 for all treatment groups. FIG 10B shows tumor growth repression. T/C (Tumor/Control based on mean tumor volume) was calculated up to day 30.

[0020] FIG 11 shows a bar graph of data from the experiments shown in FIG 9 showing mRNA mixtures with significant reduction in tumor volume, where the number of mice in each of the treatment groups with significant tumor reduction was compared to the luciferase control group based on Z score of tumor volume and the ratio between tumor volume change and the mean of the control group.

[0021] FIGS 12A – 12D show the results of experiments where mice that were 1) tumor naïve, or 2) had been previously injected subcutaneously with 5×10^5 B16F10 cells and rejected the original tumor following intratumoral cytokine mRNA treatment. Both groups were re-challenged with B16F10 tumors. FIG 12A shows tumor naïve host mice. FIG 12B shows mice that had previously rejected B16F10 tumors following intratumoral cytokine mRNA treatment with GM-CSF, IL-15sushi, IL-12sc, IFN α (ModB). Mice were monitored for 55 days following B16F10 injection and tumor growth for each mouse was plotted. All nine naïve mice engrafted with B16F10 cells developed tumors (FIG 12A), whereas all eight tumor-free mice rejected the B16F10 cells and did not exhibit growth of B16F10 tumors (FIG 12B). The graph in FIG 12B has no visible data trace because all observations were zero, i.e., overlapping the horizontal axis. FIG 12C shows an example of localized vitiligo at the tumor site. FIG 12D shows the results of experiments where mice that were tumor naïve (triangle symbol), or had been previously injected subcutaneously with CT26 tumor cells and rejected the original tumor following intratumoral cytokine mRNA treatment (circle symbol). Both groups were re-challenged with either CT26 tumor cells (CT26-WT) or with CT26- Δ gp70 tumor cells, in which the gp70-epitope had been knocked out. Mice were monitored for 21 days following tumor cell injection. All nine but one naïve mice engrafted with CT26-WT cells and all naïve mice engrafted with CT26- Δ gp70 cells developed tumors, whereas all three tumor-free mice rejected the CT26 tumor cells and did not exhibit growth of CT26 and CT26- Δ gp70 tumors, respectively.

[0022] FIGS 13A – 13D show the results of experiments where mice were implanted with B16F10 tumor cells on day 0 on the right (injected) and left flanks (uninjected) (FIG 13A). Mice received a series of 4 intratumoral injections with ModB cytokine mRNA (IL-15 sushi, IL-12sc, GM-CSF and IFN α) or ModB control mRNA (luciferase) in the right tumor on days 11, 15, 19, and 23. Mean tumor volumes +/- SEM (n=12) are shown for the injected (FIG 13B) and the contralateral uninjected tumors (FIG 13C). Median survival is shown in FIG 13D.

[0023] FIGS 14A – 14F show results of experiments where human HEK293 (FIG 14B) and melanoma cell lines (A101D (FIG 14C), A2058 (FIG 14D), A375 (FIG 14E), and Hs294T (FIG 14F)) were transfected with human cytokine mRNA mixture (IL-12sc, GM-CSF, IL-15 sushi and IFN α 2b) in a range of mRNA doses. Supernatants were collected 24 hrs after transfection and protein concentrations were determined with cytokine specific ELISAs. FIG 14A shows a schematic of the experiment.

[0024] FIGS 15A – 15B show a schematic (FIG 15A) and results (FIG 15B) from a study where a human cytokine mRNA mixture encoding IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b, or individual cytokine mRNAs, were transfected in HEK293 cells and the conditioned media was collected at 24 hrs, diluted and added to human PBMCs. IFN γ was measured in the PBMC culture supernatant at 24 hrs. (N=6 donors, Mean).

[0025] FIGS 16A – 16E show the results of experiments where immune compromised mice bearing human A375 tumor xenografts received a single injection with the ModB mRNA mixture encoding the human cytokines (IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b; “the IL15 sushi mixture”) or (IL-2, IL-12sc, GM-CSF and IFN α 2b; “the IL2 mixture”). Tumor cell lysates were prepared at 2 hrs, 4 hrs, 8 hrs, 24 hrs, 48 hrs, and 72 hrs after injection and the concentration of each cytokine was measured with respect to the total protein in the tumor lysates (n=3 mice/time point, +/- SEM). FIG 16A shows IFN α 2b, FIG 16B shows IL-2, FIG 16C shows IL-12sc, FIG 16D shows IL-15 sushi, and FIG 16E shows GM-CSF.

[0026] FIGS 17A – 17C show the results of experiments where mRNA was isolated from A375 tumors at 2 hrs, 4 hrs, 8 hrs, 24 hrs, 48 hrs, and 72 hrs after injection of ModB cytokine mRNA mixture (IL-15 sushi, IL-12sc, GM-CSF, IFN α 2b) or (IL-2, IL-12sc, GM-CSF, IFN α 2b). Expression of interferon alpha response genes were monitored by qPCR. FIG 17A shows human ISG15, FIG 17B shows human ISG54, and FIG 17C shows human MX1.

[0027] FIGS 18A – 18E show the results of experiments where mice were implanted with B16F10 tumor cells and treated with mRNA mixtures (FLT3L, IL-2, 41BBL, and

CD27L-CD40L) with or without IFN α . mRNA mixtures without IFN α in standard (ModA, FIG 18B) and modified forms (ModB, FIG 18C) were compared to those including IFN α in standard (ModA, FIG 18D) and modified forms (ModB, FIG 18E). FIG 18A is a negative control where Ringer's media without mRNA was provided.

[0028] FIGS 19A – 19E show the results of experiments where mice were implanted with tumors on one flank and received an IV injection of luciferase-expressing tumor cells that homed to the lung (FIG 19A). Mice in the treatment group received intratumoral injections of mRNA mixtures IL-15 sushi, IL-12sc, GM-CSF and IFN α into the flank tumor only while tumors in the lung were untreated. FIG 19B shows exemplarily bioluminescence measurements in lungs and pictures of the according lungs taken out on the same day (day 20); tumor nodes are visual as black marks; FIG 19C shows mean tumor volume of flank tumors as determined by caliper measurements; FIG 19D shows total flux analysis of bioluminescence measurements on day 20; FIG 19E shows lung weights.

[0029] FIGS 20A – 20G show the results of experiments designed to assess the effect of intratumoral injection of mRNA mixtures in combination with systemic administration of antibodies in dual flank tumor models. Mice implanted with either the B16F10 tumor on the left and right flank or MC38 tumors on the left and right flank received intratumoral injections with an mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (Mod B) into only one flank tumor, while the other flank tumor was untreated. Mice also received intraperitoneal (systemic) injection of an anti-PD1 antibody. FIG 20 shows overall survival in the B16F10 (FIG 20A) and MC38 (FIG 20B) tumor models. FIGS 20C-G show the results of an experiment evaluating the anti-PD-1 antibody where mice were implanted with B16F10 tumors on one flank and received an IV injection of luciferase-expressing B16F10 tumor cells that homed to the lung. Mice received three intratumoral injections with an mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α and also received three intraperitoneal (systemic) injection of an anti-PD-1 antibody. Tumor growth of the SC tumors is depicted in FIG 20C-F. FIG 20C shows control mRNA and control antibody; FIG 20D shows control mRNA plus anti-PD1 antibody; FIG 20E shows cytokine mRNA mixture plus isotype control antibody. FIG 20F shows cytokine mRNA plus anti-PD-1 antibody. FIG 20G shows percent survival of all four treatment groups until day 70 after IV tumor inoculation; the treatment group that received mRNA plus anti-PD-1 antibody showed strongest anti-tumoral activity with 6 out of 15 mice being tumor-free on day 40 after tumor inoculation.

[0030] FIGS 21A – 21I show the results of additional experiments designed to assess the effect of intratumoral injection of mRNA mixtures in combination with systemic administration of antibodies. Mice bearing CT26 tumors received intratumoral injections with an mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α . Mice also received intraperitoneal (systemic) injection of an anti-CTLA-4 antibody. FIG 21A shows that the combination therapy of intratumoral cytokine mRNA and IP-injected anti-CTLA-4 resulted in strongest anti-tumoral activity with 12 out of 16 mice being tumor-free on day 55 after tumor inoculation. FIG 21B shows cytokine mRNA mixture plus isotype control antibody; FIG 21C shows control mRNA plus anti-CTLA-4 antibody; FIG 21D shows control mRNA and control antibody. FIGS 21E-21I show the results of additional experiments designed to assess the effect of intratumoral injection of mRNA mixtures in combination with anti-CTLA-4 antibody in B16F10 tumor model. Mice bearing B16F10 tumors received intratumoral injections with an mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α . Mice also received intraperitoneal (systemic) injection of an anti-CTLA-4 antibody. FIG 21E shows that the combination therapy of intratumoral cytokine mRNA and IP-injected anti-CTLA-4 resulted in strongest anti-tumoral activity with 6 out of 9 mice being tumor-free on day 70 after tumor inoculation. FIG 21F shows cytokine mRNA mixture plus isotype control antibody; FIG 21G shows control mRNA plus anti-CTLA-4 antibody; FIG 21H shows control mRNA and control antibody. FIG 21I shows percent survival of all four treatment groups until day 70 after tumor inoculation.

[0031] FIGS 22A – 22D shows the results of experiments designed to evaluate the effect of intratumoral injection of cytokine mRNA (Mod B) in human tumor xenografts of different human cancers. Intratumoral expression of each of the 4 mRNA encoded cytokines is shown: IL-12sc (FIG 22A), IFN α 2b (FIG 22B), GM-CSF (FIG 22C), and IL-15 sushi (FIG 22D).

[0032] FIGS 23A – 23D show the results of experiments designed to evaluate the effect of different intratumoral mRNA doses on the expression of the encoded cytokines: IL-15 sushi (FIG 23A), IL-12sc (FIG 23B), GM-CSF (FIG 23C) and IFN α 2b (FIG 23D).

[0033] FIGS 24A – 24G show the results of experiments where mice were implanted with B16F10 tumor, and treated with four intratumoral injections of cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF, IFN α (ModB) or mRNA encoding a single cytokine. Tumor volume out to approximately day 70 was measured. FIG 24A shows luciferase control; FIG 24B shows the four-cytokine mixture; FIG 24C shows IL-12sc mRNA only; FIG 24D shows GM-CSF mRNA only; FIG 24E shows IFN α mRNA only; and FIG 24F shows IL-15 sushi only. FIG 24G shows overall survival of B16F10 tumors treated with cytokine mRNA

mixture or individual mRNA encoded cytokines. Survival data is from experiment presented in Figure 24A-F.

[0034] FIG 25 shows CD8+ immune cell infiltrate in subcutaneous tumors after control mRNA (“placebo”) and cytokine mRNA treatment.

[0035] FIGS 26A – 26C show results of measurements of CD8+ T cells specific for the gp70 tumor antigen of gp70 in blood of CT26 tumor bearing mice that had received intratumoral administration of cytokine mRNA treatment and control mRNA, respectively. FIG 26C shows exemplarily a FACS histogram of CD8+ T cells stained with anti-mouse CD8 antibody and with the gp70-specific tetramer derived from an animal that had received control mRNA and FIG 26B shows the example from one animal treated with cytokine mRNA. FIG 26C shows the analysis of percentage of gp70-specific CD8+ T-cells in blood 13 days after treatment start from 9 mice that had received four injections of control mRNA and 10 mice that had received 4 injections of cytokine mRNA.

[0036] FIGS 27A-27C show experiments where mice bearing B16F10 tumors on the left and right flanks received a single intratumoral mRNA injection with cytokine mRNA or control mRNA in only one tumor. On day 7 following the mRNA injection the left and right tumors were collected and subjected to RNA sequencing. FIG 27B shows the results of ingenuity pathway analysis comparing the gene expression changes between the cytokine mRNA treatment vs control mRNA treated tumors. Causal network analysis for treated tumor side (Column 1) and untreated tumor side (Column 2) was performed and Activation Z score (Top half) and Inhibition Z score (Lower half) was analyzed to define pathways up and down regulated, respectively. FIG 27 shows cluster analysis of injected and non-injected tumors was performed based on 327 interferon gamma regulated genes. Both the injected and non-injected tumors of mice treated with cytokine mRNA showed upregulation of multiple IFN gamma genes in comparison to mice treated with control mRNA.

[0037] FIGS 28A – 28D show fluorescence micrographs of cells from a B16F10 dualtumor model. Panel A shows the injected tumor treated with cytokine mRNA and panel B shows the corresponding uninjected tumor. Panel C shows the injected tumor treated with control mRNA and panel D shows the corresponding uninjected tumor. The slides were stained for CD4+, CD8+, and FoxP3+ cells.

[0038] FIGS 28E-G show frequency of CD4+, CD8+ and FOXP3+ cells quantified in the immunofluorescent images. The frequency of CD4+ and CD8+ cells/mm² is presented in FIG 28E and 28F. The ratio of the CD8+ frequency divided by FOXP3+ frequency is presented in FIG 28G.

[0039] FIGS 29A – 29G show mice with a single B16F10 tumor received a single injection with either mRNA encoding the Thy1.1 cell surface protein or vehicle alone (Ringer's solution). At approximately 16-18 hours following intratumoral injection the tumor was excised, digested, stained with a panel of antibodies and analyzed by flow cytometry. The cell type and frequency of cells expressing Thy1.1 were characterized.

[0040] FIGS 30A – 30F show expression of the indicated proteins following various doses of cytokine mRNA or luciferase control mRNA detected in tumor lysates as described in Example 15. "IFN γ " in FIG 30E indicates IFN γ .

[0041] FIGS 31A – 31B show flow cytometry results for CD8 $^{+}$ and FOXP3 $^{+}$ (Treg) cells following control or cytokine mRNA treatments as described in Example 15. The observed ratio of CD8 $^{+}$ to Treg cells is shown in each panel.

[0042] FIGS 31C – 31D show flow cytometry results for polyfunctional CD8 $^{+}$ T cells following control or cytokine mRNA treatments as described in Example 15. The proportion of polyfunctional CD8 $^{+}$ T cells is shown in each panel.

[0043] FIG 31E shows the level of PD-L1 on infiltrating myeloid cells following control or cytokine mRNA treatments as described in Example 15.

[0044] FIG 31F shows the level of PD-1 on infiltrating CD8 $^{+}$ cells following control or cytokine mRNA treatments as described in Example 15.

[0045] FIGS 31G-H show the frequency of intratumoral Granzyme B CD8 $^{+}$ T cells following control or cytokine mRNA treatments as described in Example 15.

[0046] FIGS 32A – 32B show luciferase expression in various tissues following intratumoral injection of 50 μ g mRNA encoding firefly luciferase as described in Example 16.

[0047] FIGS 33 shows data relating to an experiment essentially as shown in Figure 12.

[0048] FIG 34 shows the effect of depleting CD8 $^{+}$ T cells, CD4 $^{+}$ T cells or NK cells before treatment with cytokine mRNAs on survival in mice bearing B16F10 tumors as described in Example 17.

[0049] FIG 35 shows survival of WT and IFN γ KO mice implanted with B16F10 tumor cells as described in Example 1 and treated with control or cytokine mRNAs as described in Example 18.

DESCRIPTION OF THE SEQUENCES

[0050] Tables 1 and 2 provide a listing of certain sequences referenced herein.

[illegible]

15	Human non-optimized IL-12sc (CDS DNA) Sequence annotation CAPS: p40 domain; CAPS: linker; CAPS: p35.	ATGTGTACACGACGTTGGTCATCTCTTTGGTTTTCCCTGGTTTTTTCTGGCATCTCCCTCGTGGCCATATGGAACTGAAGAAAGATG TTTTATGTCGTAGAAATTTGGATGGTATCCGGATGCCCTGGAGAAATGGTGGTCTTCACTGTGACACCCCTGAAAGAGATGGTATCAC CTGGACCTTTGGACGAGCAGTGAGGTCTTAGGCTCTGGCAAAACCCCTGACCATCAAGTCAAAGAGTTTGGAGATGCTGGCCAGTAC ACCTGTCAAAAAGGAGCGAGGTTCTAAGGCCATTCGCTCTGTCTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATATTTAA AGGACAGAAAGAACCCCAAAATAAGACCTTTCTAAGATCGAGGCCAAGAAATATTTCTGGACGTTTCACTGCTGGTGGCTGACGAC AATCAGTACTGATTTGACATCAGTGTCAAAAGCAGAGAGGCTCTTTCTGACCCCAAGGGGTGA CGTGGGAGCTGCTACACTCTCT GCAGAGAGTGCAGAGGGGCAAAAGGATAGAGTACTGAGTACTGAGTGCAGGAGGACCTGCTGCTGCCACAGCTGTGAGGAGATC TGCCCATTTGAGGTGATGTTGGATGCCGTTTCAACAAGCTCAAGTATGAAATCTACACAGCAGCTTCTTCAATCAGGGACATCATCAAAC TGACCCACCCAAAGAACTTGACGTGAAGCCATTAAAGAAATCTCGGCAAGTGGAGTCACTGGAGTACCTGACACCTGGAGTACT CCACATTTCTACTTCTCCCTGACATTTCTGCTTCAGGTCAGGGCAAGAGCAAGAGAAAAAGAAATAGATCTTTCACGGACAAAGA CCTCAGCCACGGTCTATCTGCCGCAAAAATGCCAGCATTAGCGTGGGGCCCGAGACCGCTACTATAGCTCATCTTTGGAGCGAATGGGC ATCTGTGCCCTGCAGTGGCTTAGCGGAGGGGAGGCTCTCTGGCGGGGATCTAGCAGAAACCTCCCGTGGCCACTCCAGACCCCA GGAATGTTCCCATGCTTCCACCTCCCAAAACCTGCTGAGGGCCGTCAGCAACATGCTCCAGAAAGGCCAGCAAACTCTAGAAATTTT ACCTTGTCACCTTCTGAGGAAATGATCATGAAGATATCAAAAAGATATAAACAGCAGCAGTGGAGGCTGTTTACCATTTGAAATTAAC CAAGATGAGAGTTGCCATAATTCAGAGAGACCTCTTTTCACTAATAATGGAGTTGCCCTCCAGAAAGACCTCTTTTATGATG GCCCTGTGCCCTTAGTAGTATTTATGAAGACTTTGAAGATGTACCCAGGTGGATTCAGAAAGCTTCTGATGGATCCCTA AGAGGCAGATCTTTCTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCGCCCTGAAATTTTCAACAGTGAGACTGTGCCACA AAAATCCTCCCTTGAAAGAACCGGATTTTATAAAAATCAAGCTCTGCACTCTTCTCAATGCTTTTCAAGAAATTCGGGCACTGACT ATTGATAGAGTGTGAGCTATCTGAAATGCTTCCCTGATGA
16	Human optimized IL-12sc (CDS DNA) Sequence annotation CAPS: p40 domain; CAPS: linker; CAPS: p35.	ATGTGTACACGACGCTGGTGTATCTCATGGTTCTCCCTGGTATTTCTGGCATCTCTCTTGTGCAATCTGGAACTGAAGAAAGACG TGATATGTCGTTGAGCTCGACTGGTATCCGGATCGGCTGGCGAGATGGTGGTGTGACCTGTGACACCCAGAGAGGATGGATCAC TTTGACCCCTTGATCAATCCTCGAAGTGCTCGGCTGGCAAGACTCTGACCATACAAGTGAAGAGTTTGGGGATGCCGGGCAGTAC ACTTGCCATAAGGGCGGAGAAATTTCTGCCACTCACTGCTGCTGCACAAAGAAAGAGGACGGAATTTGGAGTACCGATATCCTGA AAGATCAGAAAGAGCCCAAGAAACAAACCTTCTTGCGGTGCGAAGCCAAAGAACTACTCAGGGAGATTTACTTGTGGTGGCTGACGAC GATCAGCACCGATCTGACTTCTCCGTGAATCAAGTAGGGGATCATCTGACCTCAAGGAGTCACTGTGGAGCGGCTACTCTGAGC GCTGAACGCGTAAGAGGGGCAATAAAGGAGTACGAGTATAGCGTTGAGTGCCTAGAGGATAGCGCATGCCCGCCCGCGGAAGATCAT TGCCCATTTGAAGTGGTGGATGCTGTACACAAGCTGAAGTATGAGAACTACACAAGCTCCTTCTTCACTCGGTGACATCATCAAAC AGATCCTCCTAAGAACCTCCAGCTTAAACCTCTGAAGAACTTAGACAGGTGGAATGTCTTGGAGTATCCGACACCTGGTCTACA CCACATTTCTACTTTCAGTCTCACATTTCTGCGTTCAGGTACAGGCAAGTCCAAAAGGAGAGAAAGGATCGGCTCTTTACAGATAAAA CAAGTGCCACCGTTATATGCCGGAAGAAATGCCCTCTAATTTCTGTGCGTGGCAGGACAGATACATAGCAGCTCTTGGAGTGAATGGGC CAGTGTCCCATGTTTCAGGTCATCCGGTGGTGGCGGACGCCCGGAGGGGTAGCTCCAGAAATCTCCCTGTGGCTACACCTGATCCA GGCATGTTTCCCTGTTTGCCCATAGCCAAAACCTCTGAGAGCAGTCAAGCAATGCTCCAGAAAGCTAGACAAACACTGGAATTTCT ACCCATGCACCTCCGAGGAAATAGATCAGGAGATATCACTAAGGACAAAACAAGCAGTGTGCAAGCATGCTCCCTTCCCTTGAACCTGAC AAAGAACGAGAGTTGCCCTTAATCAAGAGAAACATCTTTTCACTAGAACGGTGTGCTTGGCAAGCAGAAAAACATCTTTTATGATG GCCCTTGTCTGAGCAGTATTTATGAGGATCTCAAAATGTATCCAGGTGGATTTAAGACCATGAAATGCCAAGCTGCTGATGGACCCAA AGAGACAGATTTTCCTCGATCAGAAATATGCTGGCTGTGATGAACTGATGCGGCCCTTGAATTTTCAACAGCGAAAACCTGTTCCCA GAAAAGCAGTCTTGAAAGAACCTGACTTTTATAAAGACCAAGATCAAACTGTGTATTTCTCTCTGCAATGCCCTTTAGAAATCAGAGCAGTCACT ATAGATAGAGTGTGTCCTTACCTGAAATGCTTCCCTGATGA

Human non-optimized IL-12sc (RNA encoding CDS)	17 AUGUGUCACAGAGUUGGUAUCUCUUUGGUUUUCCUGGUUUUUUCUGGCAUCUCCCCUGUGGCCAUUUGGGAAACUGAAAGAAAGAUG UUUUAUGUCGUAGAAUUGGAUUGGUAUCCGGAUGCCCCUGGAGAAAUGGUGGUCCUACUCUGAGACACCCUCUGAAGAAUGGUAUCAC CUGGACCUUGGACAGAGCAGUGAGGUUUUAGGUCUGGCAAAACCCUGACCAUCCAAAGUCAAAAGUUUGGAGUAGUGCCUGGCCAGUAC ACCUGUCACAAAAGGAGGCGAGGUUUAAGCCAUUCGUCUCCUGUGCUUCACAAAAGGAAGUAGGAAUUUGGUCCACUGAUUUUUUAA AGGACCAAGAAACCCAAAUAUAGACUUUUAAGACUUAAGAGCGAGGCCCAAGAAUUAUUCUGGACGUUUUACCCUGUGUGGCGUGACGAC AAUCAGUACUGAUUUUGACAAUACAGUGUCAAAAGCAGAGAGGUGUUUCUGACCCCCAAAGGGGUGUACGUGCGGAGUCUAGACUCUCU CGAGAGAGUACAGAGGGGAAUCAAAGAGAUUAGUAUCUAGUGGAGUCCACGAGGACGAGUCGUCGCCCCAGCUGCUAGCAAGAGAGUC UGCCCCAUUGAGGUCAUGGUGAUGCCGUUACACAGCUCAAGCUCAAGUAUGAAAAUACACAGCAGCAGCUUCUUAUCAAGGACCAUCAAAACC UGACCCACCAAGAAACUUGCAGUCGAAAGCAUUAAGAAUUCUGGCAGGUGGAGGUCAGCUGGGAGUACCCUGACACUCUGGAGUACU CCAUUCUUAUCUCCUGACAUUCUGCGUUCAGGUCACGGCCAAAGCAAGAGAAAAGAAAGAUAGAGUCUUCACGGACCAAGAGA CCUCAGGCCAGGUAUCUGCCGCAAAAAGUCCAGCAUUAAGCGUGGGGCCAGGACCGCUUAUAUAGCUCAUUCUGGAGCGAAUUGGGC AUCUGUGCCUGCAGUGGUCUAGCGGAGGGGAGGUCUCUCUGCGGGGAUCUAGCAGAAAAACUCCGCCUGGCCACUCCAGACCCCA GGAAUGUCCCAUGCCUUCACACUCCCAAAAACUGCUGAGGGCGUCAGCAACAUGCUCAGAAAGGCCAGACAAAACUCUAGAAUUUU ACCUUUGACUUCUGAGGAAUUGAUCAUGAAGAAUAUUCACAAAAGAAUAAAAACAGCACAGGAGGCGCCUGUUUACCAUUGGAAUUAAC CAAAGUAGAGUUGCCUAAAUUCCAGAGAGACCUUCUUAUUAUAAUUAUAGGAGUUGCCUGGCCUCCAGAAAACUCUCUUUUUAUGAUG GCCUUGGCCUUUAGUAUAUUUAUGAAGACUUGAAAGUAUCCAGGUGGAGUUAUCAAAGCAUAGAAAGCUUUGGAGUGGAUCCUA AGAGGCAGAUUUUUAUGAUUCAAACAUUGCUGGCAUUAUUGAGAGCUGAGCGGCCCUUGAAUUUCAAACAGUGAGACUGUGGCCACA AAAAUCCUCCUGAAGAACCGGAUUUUUAUAAAAUAAAAUCAAAGCUCUGCAUAUCUUCUUAUGCUUUUCAGAAUUCGGGCGAGUGACU AUAUGAUGAGUAGAGCUAUCUGAAUGCUUCUGAUGA
Human non-optimized IL-12sc (RNA encoding CDS)	18 AUGUGUCACAGAGCUGGUAUCUCAUGGUUUUCCUCCUGGUAUUUCUGGCAUCUCCUUCUUGCGCAAUUCUGGGAAACUGAAAGAAAGACG UGUUAUGUCUUGAGCUGCAGCUGGUAUCCGGAUGCGCCUGGCGAGAUUGGUGUGUGACUCUGAGACACCCACAGAGGAGGAUGGGAUAC UUGGACCUUGAUCAAUCCUCCGAAGUGCUGGGUCUGGCAAGACUCUGACCAUAACAUGAAGAAAGUUUGGCGAUGCCGGGCGAGUAC ACUUGGCCAUAAAGGCGGAGAAUUCUGUCCACUCACUGCUGCUGGCACAAAGAAAGAGACGGAAUUUGGAGUAUCCGAUUAUCCUGA AAGAUCAGAAAGAGCCCAAGAACCUUCUUGCGGUGGGAAGCCAAAGAACUACUCAGGAGAAUUAUUAUUGUGUGGCGUGACGAC GAUCAGCCGUAUGACUUUCUCUGAUAUCAAAGGGAUUAUCUGACCCUCAAAGAGAUCAUUGGAGCGGCUAUCUCUGAGC GCUAGACGCUAUGAGGGGAAUAAAGGAGUACGAUAUAGCGUUGAGUCCAAAGAGUAUAGCGCAUGCCCCCGCCGCGCAAGAAUUAU UGCCCCAUUGAAGUAGGUGGAUGUGUACACAGCUGAAGUAUGAGAAUACACAAAGCUUCUUCUUAUCCUGJGACAUCAUCAAAC AGAUCCUCCUAAAGAACUCCAGCUUAAACCUUGAAGAAUCUAGACAGGUGGAAUGUGUUGGGAGUAUCCCGACACCCUGGCUAACA CCACAUCCUACUUCAGUCUCAUUCUGCGUUCAGGUACAGGCGAAAGUCCAAAAGGGAAGAAAGGAUCCGGGCUUUUACAGAUAAAA CAAUGGCCACCGUUAUUGCCGGAAGAAUGCCUUAUUUCUGUGGUGCGCAGGACAGAUUAUAUAGCAGCUUUGGAGUGAAUUGGGC CAGUGUCCCAUGUUCAGGUAUCCGGUGGUGGCGGCGAGCCUCCGGAGCGGUAUCUCCAGAAAAUCUCCUGUGGCUACACCUUGAUCCA GGCAUGUUUCCUGGUUUGCAUUAUAGCCAAAACCUCCUGAGAGCAGUCAGCAACAUGUCCAGAAAGCUAGACAAAACACUGGAAUUCU ACCCAUGCACUCCGAGGAAUUAUAGACAGGAGUAUACUAAAGGACAAAAACAAGCACUGUCGAAGCAUGCCUCCUGGGAACUGAC AAAAGACAGAGUUGCCUUAUUAUCAAAGAAAACAUUCUUAUUAUAAACGGUAUGCUUUGGCAAGCAGAAAAACUUCUUUUUAUGAUG GCCUUUGUCUGCAGUAUUUAUGAGGAUUCUCAAUUAUCCAGGUGGAGUUUAAGCAUAGAAUUGCCAAAGCUGCUGGAUGGAGCCCAA AGAGACAGAUUUUUCUGAUCAGAAUUGCUGGCGUGUAUGAUGAAUCUGAUGCAGGCCUUGAAUUUCAAACAGCGGAAACCGGUUCCCCA GAAAAGCAGUCUUGAAGAACCUGACUUUUAUUAAGCAAGAAUGAACUUGUAUUUCUUGCAUGCCUUUAAGAAUUCAGAGCAGUCAUCU AUAUGAUGAGUGAUGUCCUACCCUGAAUGCUUCUGAUGA

60

[illegible]

63

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

49	ModA murine IL-15 sushi (DNA: 5'UTR - CDS - 3'UTR)	GGCGAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAAACCCGGCCACCATGGGGCCCATGGCCCTAGAACATTTGCTCCTGCTG CTGGCCGCTGCCCTGGCCCTACACAGACAAGAGCTGGACTGGATCCACCATCGTGTCCACCTCCCGTATCTATTGAGCATGCTGACA TCCGGGTCAAGAAATTACAGTGTGAATCCAGGGAGAGGTATGTGTAACTCTGGCTTTAAGCGGAAAGCTGGAACATCCACCCCTGAT TGAGTGTGTGATCAACAAGAACACAAATGTTGCCCCACTGGACAATCCCAAGCTCCAGCTCAAGTGCATCAGAGACCCCTCCCTAGCTGGAGGG AGCGGAGGCTCTTGGCGGAGCGCGGGTCTGGAGGCTCCGGGGAAAGCGGGAAATGGATCGACGTGCGCTACGACCTGGAAGAAAGA TCGAGAGCTGTATCCAGAGCATCCACATCGACACCACTGTGTAACACAGAGACTTCCACCCAGTGCATAAAGTGAACCGCTATGAA CTGCTTCTCTGTGGAACTGCAAGTGTATCTCGACAGAGTACGAACTAGACCTTGAACGAGACAGCTGCGGAAACGCTGTGATACCTGGCC AACAGCACCTTGAGCAGCAACAAGAAAGCTGGCCGAGCGGGTCAAGAGTGCAGGAACTGGAAGAAAGACCTTACCGAGTTTC TGCAAGCTTTCATCAGGATCTGTCAGATGTTTCATCAACACCTCTTGTAGTGCAGCTCTGGTACTGCTACGACGCAATGCTAGCTG CCCTTTCTCCGTCTGGTACCCCGAGTCTCCCGAGCTCGGGTCCAGGTATGCTCCACCTCCACCTCCCTCCCTCCTCCTCCTCCTCCT GCTAGTTCAGACACCTCCCAAGCAGCAGCAATGCACTCAAAACGCTTAGCTAGCCACACCCACCGGAAACAGCAGTGTATTA CCTTTAGCAATAAAGAAAGTTTAAGCTATTAACCCAGGTTGGTCAATTTCTGTCAGCCACACCTTCGAGCTAGCAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAA
50	ModA murine IL-15 sushi (RNA)	GGCGAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAAACCCGGCCACCATGGGGCCCAUGGGCCCUAGAAACAUUGCUCCUGCUG CUGGCCGCTGCCCTGGCCCTACACAGACAAGAGCTGGAGCCUGGAUCCACCAACGUGUCCACCUCCCGUAUCUAUUGAGCAUGCUGACA UCCGGGUCAGAAUUAACAGUGAGAACUCCAGGGAGAGUAUGUCUGUAAACUCUGGCUUUAAGCGGAAAGCUGGAACAUCCACCCUGAU UGAGUGUGAUCACAAAGAACACAAUUGUUGCCACUGGACAAUCCCGAGCCUAGGUAUCAGAGACCCUCCUAGCUGGAGGG AGCGGAGGCUUGCGGGAAGCGCGGUCUGGAGGCTCCGGGGAAAGCGGGGAAUUGGAUUCGACGUGCGCUACGACCGUAGAAAGA UCGAGAGCCUGAUCAGAGCAUCCACAUCCAGACACCCUGUAACCCAGCAGCAUUCACCCAGCUGCAAAAGUGACCCGCUAUGAA CUGCUUCCUGCUGGAAACUGCAAGUGAUCUGCAACGAGUACAGCAACAUAGCCUGAAACGAGACAGUGCGGAAACGUGCUGUAACCGGCC AACAGCACCCUGAGCAGCAACAAAGACGUGGCCGAGCGGCGUACAAAGAGUGCGAGGAAACUGGAAGAAAGACCUUACCCGAGUUUC USCAGAGCUUACAGGAUCGUGCAGAUUUAUACACCUUGAUGAUCGACGUCUCCUGUAUCGCAUGCAGCAUUGCUAGCUG CCCUUCCCGUGGUAACCCGAGUUCUCCCGAGCUCGGGUCGAGUAGCCUCCAGUCCACCUCCAGCUGCCCAUCACCCACCCUCU GCUAGUUCAGACACCUCCCAAGCAGCAGCAUUGCAGCUCAAAACGCUUAGCCUAGCCACACCCACCGGAAACAGCAGUUAUA CCUUUAGCAUAAACGAAAGUUUAACUAGCUUAUAUAAACCCAGGUGGUCAUUUCUGGCCAGCCACACCCUCGAGCUAGCAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAA
51	ModB murine IL-15 sushi (amino acid)	MGAMAPRTL L L L L A A L A P T Q T R A G P G S T T C P P P V S I E H A D I R V K N Y S V N S R E R Y V C N S G F K R K A G T S T L I E C V I N K N T N V A H W T T P S L K C I R D P S L A G G S G G S G G S G G S G G N W I D V R Y D L E K I E S L I Q S I H I D T F T L Y T D S D F H P S C K V T A M N C F L L E L Q V I L H E Y S N M T L N E T V R N V L Y L A N S T L S S N K N V A E S G C K E C E E L E E K T F T E F L Q S F I R I V Q M F I N T S

70

71

[illegible]

[illegible]

74

[illegible]

[illegible]

[illegible]

[illegible]

75	ModB murine CD27L-CD40L (amino acid)	<u>MGAMAPRTLLLLAAALAPTQTRAGPGSHPEPHTAELQLNLTVPRKDPTLRWGAGPALGRSFTHGPELEEGLRIHQDGLYRLHIQVTL</u> <u>ANCSSPGSTLQHRATLAVGICSPAAHGISLLRGRFGQDCTVALQRLTYLVHGDVLCNTLTLPLPSRNADETFFGVQWICPGGGSGGGH</u> <u>PEPHTAELQLNLTVPRKDPTLRWGAGPALGRSFTHGPELEEGLRIHQDGLYRLHIQVTLANCSSPGSTLQHRATLAVGICSPAAHGISLL</u> <u>RGRFGQDCTVALQRLTYLVHGDVLCNTLTLPLPSRNADETFFGVQWICPGGGSGGGHPEPHTAELQLNLTVPRKDPTLRWGAGPALG</u> <u>RSFTHGPELEEGLRIHQDGLYRLHIQVTLANCSSPGSTLQHRATLAVGICSPAAHGISLLRGRFGQDCTVALQRLTYLVHGDVLCNTLTL</u> <u>PLPSRNADETFFGVQWICPGGGSGGGSGGGGDEDPQIAAHVSEANSNAASVLQWAKKGYTMMKSNLVMLENGKQLTVK</u> <u>REGLYVYVTQVTFCSNREPSSQRPFIVGLWLKPSSGGERILLKAANTHSSSQLCEQQSVHLGGVFELQAGASVFNVTASQVIHRVG</u> <u>FSSFGLLKLGGSGGGDEDPQIAAHVSEANSNAASVLQWAKKGYTMMKSNLVMLENGKQLTVKREGLYVYVTQVTFCSNREPS</u> <u>SQRPFIVGLWLKPSSGGERILLKAANTHSSSQLCEQQSVHLGGVFELQAGASVFNVTASQVIHRVGFSFGLLKLGGSGGGGDED</u> <u>PQIAAHVSEANSNAASVLQWAKKGYTMMKSNLVMLENGKQLTVKREGLYVYVTQVTFCSNREPSSQRPFIVGLWLKPSSGGERILL</u> <u>KAANTHSSSQLCEQQSVHLGGVFELQAGASVFNVTASQVIHRVGFSFGLLKL</u>

[illegible]

ModB murine CD27L-CD40L (RNA)	GGAAUAAACUAGUCUCAAACAACAUAUACAAAACAAAGAAUUCUACAAGCAUUCUACUUAUUGAGCAAAUUUAAAUAU UUUUUUUAAAGCAAAAGCAUUUUUUGAAAAUUUUUACCAUUUACGAAGCAUUGGCGGCCAUGGGCCCUUAGAAACAUUGCUCC UGUCUGGCGCGUGCCUUAACACAGACAAGAGCUGGACUGGAUCCCAACCGAGGCCCAACACCGGCCGAACUGCAGCUGAA CCUGACCUGGCCAGAAAAGACCCACCCUGAGAUUGGGAGUGGCCUGUCUGGGGAGAUUCUUUACACACAGGCCCCCGAGCUGGAA GAAGGCCACUGAGAAUCCACAGGACGGCCUGUAACAGACUCCACAUCCAAAGUACCCUGGCCAAACUGCAGCAGCCUGGCUCUACCC UGACGACAGAGCCACACUGGCCUGGGCAUCUGUAGCCUUGUCUCAAGAAUACAGCCUGUCUGAGAGGCAUUCUGGCCAGGACUG UACCGUGGCCCCAGCAGGCUUAUCUGGUAGUAGCCUGUCUCUACGGAUACGCCUGCUGAGAGGCAUUCUGGCCAGGACUUCUGGCCAGGACU GCCGACGAAACAUUCUUUGGAGUGCAGUGGAUUGUCUGGCGGAGGUGCCGGGGAGGACCCAGAAACCCUUAACAGCAAGAACUGC AGCUGAACCCUGACCUGGCCAGAAAAGACCCACCCUGAGAUUGGGAGUGGCCUGUCUGGGGAGAUUCUUUACACACAGGCCCCCGA GCUAGAAAGAGGCCACCCUGAGAAUCCACAGGACGGCCUGUACAGACUGCAUCCAAAGUACCCUGGCCAAACUGCAGCAGCCUGGC UCUACCCUGCAGCAGAGCCACACUGGCCUGGGCAUCUGUAGCCUUGUCUCAAGAAUACAGCCUGUCUGAGAGGCAUUCUGGCC AGGACUGUACCGUGGCCUGCAGAGGCUACUUCUGGUGCAUGGCGACUGUCUGUACCAACCTUGACACUGCCUCUGCUGCCAG CAGAAACGCCGACGAAACAUUCUUUGGAGUGCAGUGGAUUGUCUGGGGAGGCUCCGAGAGUUGGGAGCUGGCCUGUCUGGGCAGAUCCUUUACACACG GAAACUGCAGUAAACCCUGACCUGGCCAGAAAAGACCCACCCUGAGAUUGGGAGUGGCCUGUCUGGGCAGAUCCUUUACACACAG GCCCGAGCUGGAAAGAGGCCACCCUGAGAAUCCACAGGACGGCCUGUACAGACUGCAUCCAAAGUACCCUGGCCAAACUGCAGCAG CCUCUGUCUACCCUGCAGCAGAGCCACACUGGCCUGGGCAUCUGUAGCCUUGUCUCAAGAAUACAGCCUGUCUGAGAGGCAUUCUGGCC UUCGGCCAGGACUGUACCGUGGCCUGCAGAGGCUACUUCUGGUGCAUGGCGACUGUCUGUACCAACCTUGACACUGCCUCUGC UGCCACGAGAAACGCCGACGAGACCUUCUUGGCGUCCAGUGGAUUGCCCGGAGGCGGUGUAGUGGAGGUGGCGGUGCCGUGG AGGUGAAGCGCGACGAGGACCCACAGAUCCGCGCCACCGUGGUGUCUGAGGCCAACAGCAACCGCCUCUGUCUGCAGUGGGCC AAGAAAGGCUACUACCAUGAAGUCCAAACUCUGAUGUGGAAAGCGCAAGCAGCUGAACCGGAGGCGGAGGCGUGUACUUAUG UGUACACCCAAAGUACAUCUGCAGCAACCGCGAGGCCACAGCAGCCAGAGGCCUUUUUUCUGUGGGCCUGUGGCUAGAAGCCUAGCAGCGG CAGCAGAGAAUCCUGUCUGAAGGCCGCCAAACACCCACAGCAGCUCUCAGCUGUGCGGAGCAGCAGCUCUGUGCACCUUGGGAGGCGUGUUC GAGCUGCAAGCUGGGCUCUCUGUUCUGUGAAACGUGAACCGAGGCCAGCCAAAGUGAUCACAGAGUGGGCUUCAGCAGCUUUGGACUGC UCAACUGGGCGGAGGGUCCGGCGAGGCGGAGUAGAAUCCUCAGAUUGCCACAGUUGCCACCGGGUGUCUGAGGCCAACAGCAACCGCCGC CUCUGUCUGCAGUGGGCCAGAAAGGCUACUACCAUGAAGUCCAAACUCUGAUGUGGAAAGCGCAAGCAGCUGAACCGGUGAAG CGCGAGGCGCUGUAUAUGUGUACACCAAGUGACAUCUUGCAGCAACCGCGAGCCACGACAGCGCCUUAUUCUGUGGCGCUCUGU GGCUGAAGCUCUAGCAGCGGCGCAGAGAAUCCUGUCUGAAGGCCGCCAAACACCCACAGCAGCUCUCAGCUGUGCGAGCAGCAGUCUGU GCAACUGGGAGGCGUGUUCGAGCUGCAAGCUGGCGCUCUGUGUUCUGUGAACGUGACCGAGGCCAGCCAAAGUGAUCACAGAGUGGGC UUCAGCAGCUUUGGACUGUCUAAACUGGGAGGCGGCUCCGGAGCGGAGGAGUAGAAUUCUCAGAUUGCUGCCACGUGGUGUCUG AGGCCAACAGCAACGCCCCUCUGUCUGCAGUGGGCCAGAAAGGCUACUACCAUGAAGUCCAAACCTUCUGAUGCUGGAAAGCGG CAAGCAGCUGACCGUGAAGCGCGAGGGCUGUAUAUGUGUACACCAAGUGACAUCUUGCAGCAACCGCGAGCCCGCAGCAGCGAGG CCUUUUAUCUGGGCCUGUGGCUAGAGCCUAGCAGCGGCGCAGAGAGAAUCCUGUCUGAAGGCCGCCAAACACCCACAGCAGCUCUCAGC UGUGCGAGCAGCAGUCUGUGCACCCUGGGAGGCGUGUUCGAGCUGCAAGCUGGCGCUCUCUGUUCUGUGAACGUGACCGAGGCCAGGCCA AGUGAUCCACAGAGUGGGCUUCUCUCCUUCGGCCUCUCUAGAGCGUGACUCGACGUCUGGUAUCUGCAUGCACGAAUUCUAGCUGC CCUUCUCCGUCUGGUAACCCGAGUCUCCCGACCCUGGGUCCAGGUAUGUCCACCCUCCACCCUGGCCACCCACAGCAGCAGCUCUG CUAUGUUCGAGACACUCCCAAGCAGCAGCAUUGCAGCUCUAAAAACCGUAGCCUAGCCUACGCCACCCCAACCGGAAACAGCAGAGUAUAC UUUAGCAUAAACGAAAGUUUAACUAAACUUAUACUACCCCAAGGUGGUAUUUCUGGCCAGCCACCCUCCGAGCUGACGCAAAAA AAAAAAAAAAAAAAAAAAAAAGCAUUAUGACUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAA

Other sequences of the invention	
78	Poly-A AAAAAAAAAAAAAAAAAAAAAAAAAAGCAUUGACUA AAAAAAAAAAAAAAAAAAAAAA
79	Anti-PD1 Mab heavy chain EVQLLESGGV LVQPGGSLRL SCAASGTFTS NFGMTWVRQA PGKGLEWVSG ISGGGRDTYF ADSVKGRFTI SRDNSKNTLY LQMNSLKGED TAVYYCVKWG NIYFDYWQG TLVTVSAST KGPSVFPLAP CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTPVS SSLGTKTYTC VVDHKPSNTK VKRVESKYG PPCPPCPAPE FLGGPSPVFLF PPKPDKTLM I SRTPEVTCV VLVSDQEDPEV QFNWYVDGVE VHNAKTKPRE EQENSTYRVV SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLP SQEEMTKNQ SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPVLDSDGS FFYLSRLTVD KSRWQEGNVF SCVMHEALH NYHTQKSLSL SLGK
80	Anti-PD1 Mab light chain DIQMTQSPSS LSASVGDSIT ITCRASLSIN TFLNWIYQQKPK GKAPNLLIYA ASSLHGGSVPS RFSGSGSGTD FTLTIRTLPQ EDFATYYCQQ SSNTPTTFGP GTVVDPRRTV AAPSVFIFFP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKKH KYACEVTHQG LSSPVTKSFN RGE C
81	GETFSNEG
82	ISGGGRDT
83	VKWNIIYFY
84	LSINTF
85	AAS
86	QQSSNTPTT
87	Anti-PD1 Mab VH EVQLLESGGV LVQPGGSLRL SCAASGTFTS NFGMTWVRQA PGKGLEWVSG ISGGGRDTYF ADSVKGRFTI SRDNSKNTLY LQMNSLKGED TAVYYCVKWG NIYFDYWQG TLVTVS
88	Anti-PD1 Mab VL DIQMTQSPSS LSASVGDSIT ITCRASLSIN TFLNWIYQQKPK GKAPNLLIYA ASSLHGGSVPS RFSGSGSGTD FTLTIRTLPQ EDFATYYCQQ SSNTPTTFGP GTVVDPR

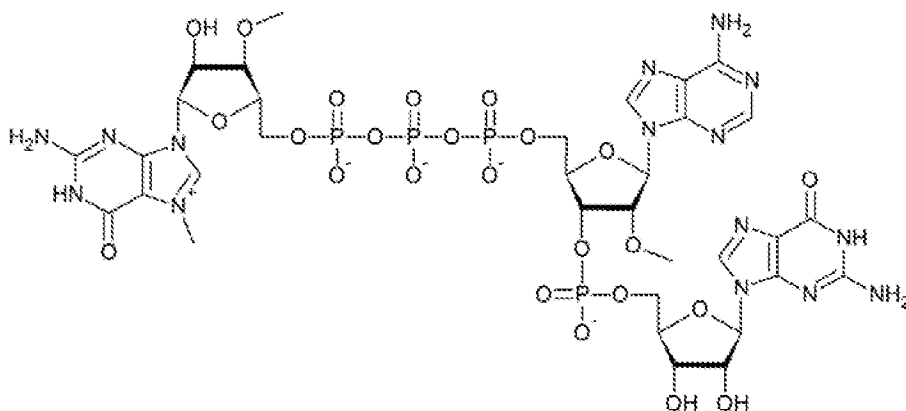
DETAILED DESCRIPTION

I. Definitions

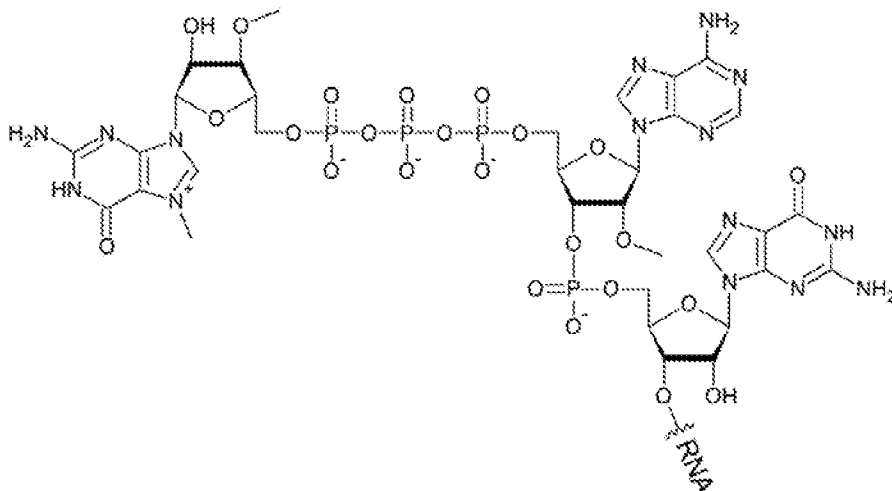
[0051] The term “ModB” describes RNA comprising a modified nucleobase in place of at least one (e.g., every) uridine and further comprising a Cap1 structure at the 5' end of the RNA. In some embodiments, the 5' UTR of a ModB RNA comprises SEQ ID NOs: 4 or 6. ModB RNA has been processed to reduce double-stranded RNA (dsRNA). The “Cap1” structure may be generated after in-vitro translation by enzymatic capping or during in-vitro translation (co-transcriptional capping).

[0052] In some embodiments, the building block cap for ModB modified RNA is as follows, which is used when co-transcriptionally capping:

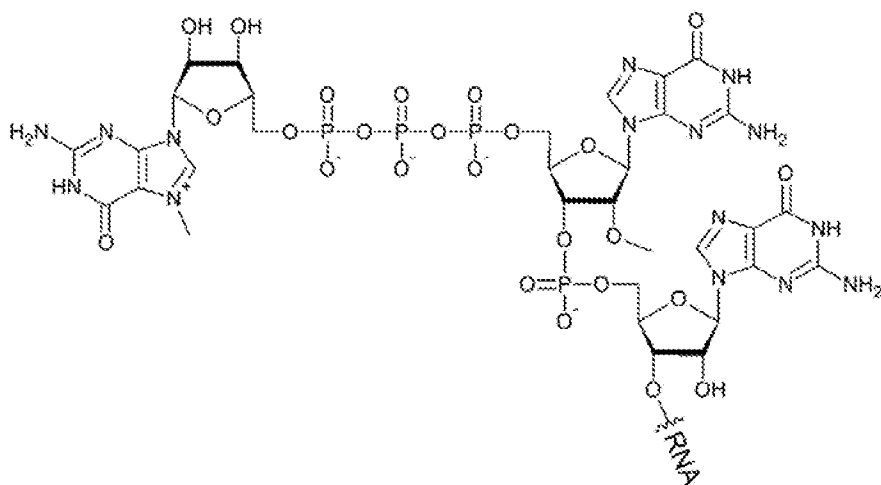
$m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ (also sometimes referred to as $m_2^{7,3'-O}G(5')ppp(5')m^{2'-O}ApG$), which has the following structure:



[0053] Below is an exemplary Cap1 RNA after co-transcriptional capping, which comprises RNA and $m_2^{7,3'-O}G(5')ppp(5')m^{2'-O}ApG$:

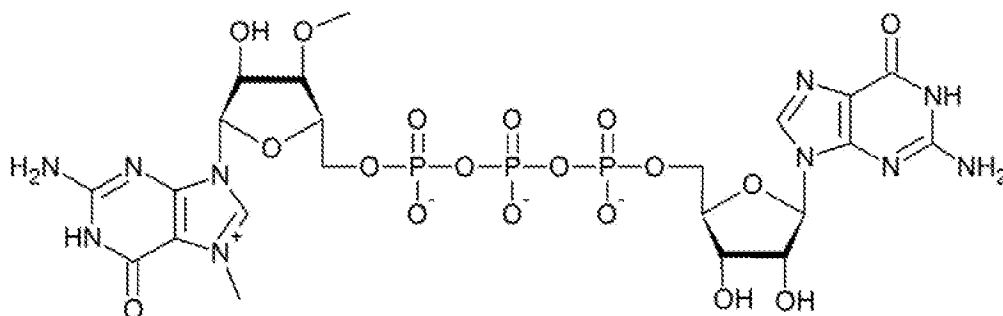


[0054] Below is another exemplary Cap1 RNA after enzymatic capping (no cap analog):

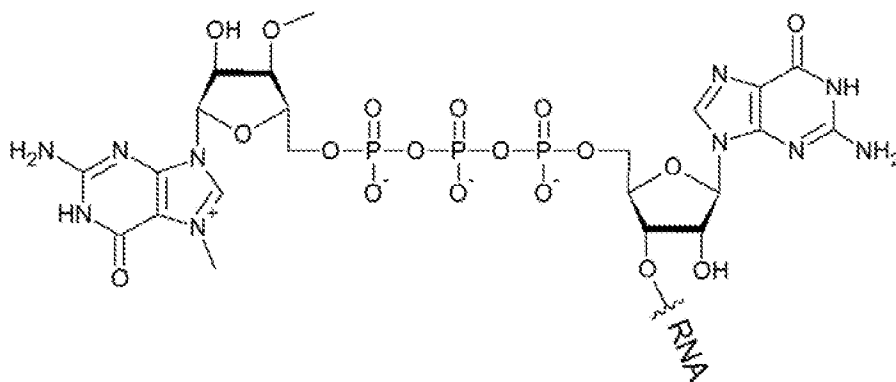


[0055] The term “ModA” describes RNA without dsRNA reduction that does not comprise a modified nucleobase in place of at least one uridine. ModA RNA comprises a Cap0 structure at the 5’ end of the RNA. The 5’ UTR of a ModA RNA may comprise SEQ ID NO: 2.

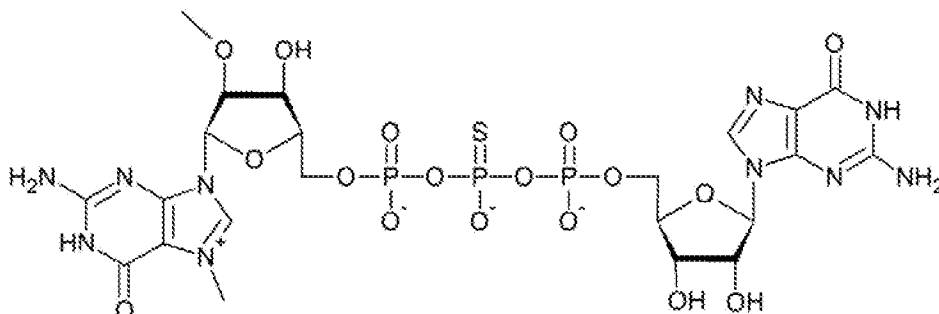
“Cap0” structures are generated during in-vitro translation (co-transcriptional capping) using, in one embodiment, the cap analog anti-reverse cap (ARCA Cap (m₂^{7,3}OG(5′)ppp(5′)G)) with the structure:



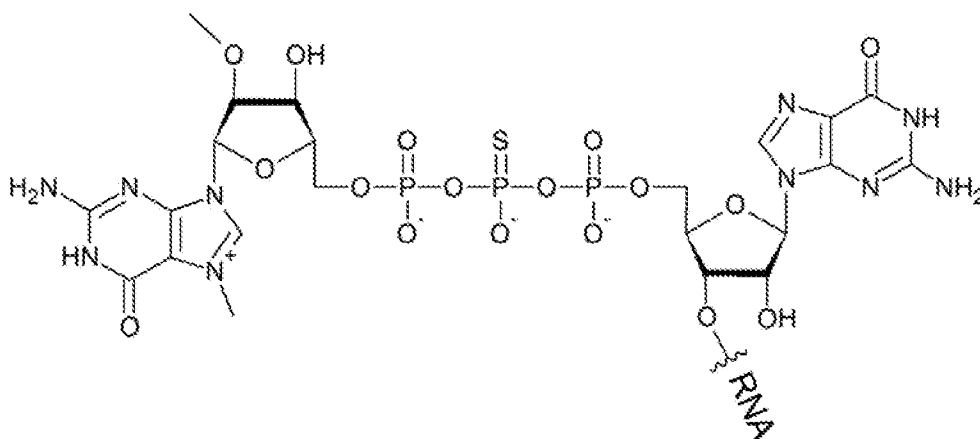
[0056] Below is an exemplary Cap0 RNA comprising RNA and $m_2^{7,3}O$ G(5')ppp(5')G:



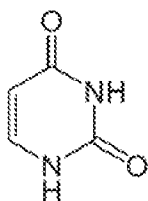
[0057] In some embodiments, the “Cap0” structures are generated during in-vitro translation (co-transcriptional capping) using the cap analog Beta-S-ARCA ($m_2^{7,2'}G(5')ppSp(5')G$) with the structure:



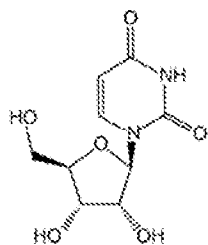
[0058] Below is an exemplary Cap0 RNA comprising Beta-S-ARCA ($m_2^{7,2'}G(5')ppSp(5')G$) and RNA.



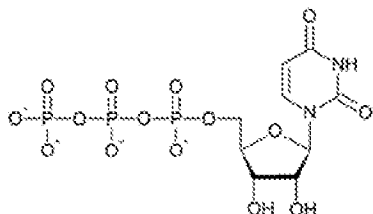
[0059] The term “uracil,” as used herein, describes one of the nucleobases that can occur in the nucleic acid of RNA. The structure of uracil is:



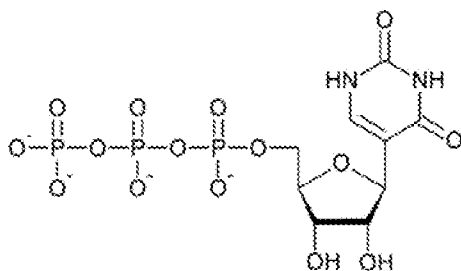
[0060] The term “uridine,” as used herein, describes one of the nucleosides that can occur in RNA. The structure of uridine is:



[0061] UTP (uridine 5'-triphosphate) has the following structure:

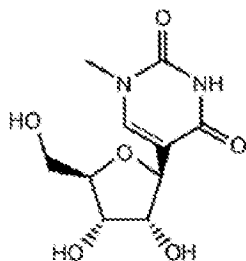


[0062] Pseudo-UTP (pseudouridine 5'-triphosphate) has the following structure:

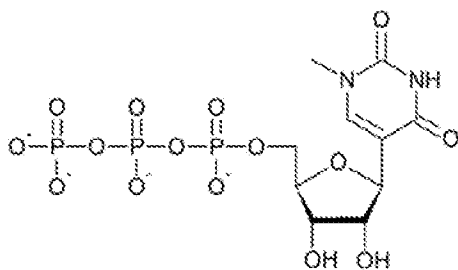


[0063] “Pseudouridine” is one example of a modified nucleoside that is an isomer of uridine, where the uracil is attached to the pentose ring via a carbon-carbon bond instead of a nitrogen-carbon glycosidic bond. Pseudouridine is described, for example, in Charette and Gray, *Life*; 49:341-351 (2000).

[0064] Another exemplary modified nucleoside is N1-methylpseudouridine (m1Ψ), which has the structure:



[0065] N1-Methylpseudo-UTP has the following structure:



[0066] As used herein, the term "poly-A tail" or "poly-A sequence" refers to an uninterrupted or interrupted sequence of adenylylate residues which is typically located at the 3' end of an RNA molecule. Poly-A tails or poly-A sequences are known to those of skill in the art, and may follow the 3' UTR in the RNAs described herein. An uninterrupted poly-A tail is characterized by consecutive adenylylate residues. In nature, an uninterrupted poly-A tail is typical. RNAs disclosed herein can have a poly-A tail attached to the free 3' end of the RNA by a template-independent RNA polymerase after transcription or a poly-A tail encoded by DNA and transcribed by a template-dependent RNA polymerase.

[0067] It has been demonstrated that a poly-A tail of about 120 A nucleotides has a beneficial influence on the levels of RNA in transfected eukaryotic cells, as well as on the levels of protein that is translated from an open reading frame that is present upstream (5') of the poly-A tail (*Holtkamp et al.*, 2006, *Blood*, vol. 108, pp. 4009-4017).

[0068] The poly-A tail may be of any length. In one embodiment, a poly-A tail comprises, essentially consists of, or consists of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 A nucleotides, and, in particular, about 120 A nucleotides. In this context "essentially consists of" means that most nucleotides in the poly-A tail, typically at least 75 %, at least 80 %, at least 85 %, at least 90 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, or at least 99 % by number of nucleotides in the poly-A tail are A nucleotides, but permits that remaining nucleotides are nucleotides other than A nucleotides, such as U nucleotides (uridylylate), G nucleotides (guanylylate), or C nucleotides (cytidylylate). In this context, "consists of" means that all nucleotides in the poly-A tail, i.e., 100 % by number of nucleotides in the poly-A tail, are A nucleotides. The term "A nucleotide" or "A" refers to adenylylate.

[0069] In some embodiments, a poly-A tail is attached during RNA transcription, e.g., during preparation of in vitro transcribed RNA, based on a DNA template comprising repeated dT nucleotides (deoxythymidylylate) in the strand complementary to the coding strand. The DNA sequence encoding a poly-A tail (coding strand) is referred to as poly(A) cassette.

[0070] In one embodiment of the present invention, the poly(A) cassette present in the coding strand of DNA essentially consists of dA nucleotides, but is interrupted by a random sequence of the four nucleotides (dA, dC, dG, and dT). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length. Such a cassette is disclosed in WO 2016/005324 A1, hereby incorporated by reference. Any poly(A) cassette disclosed in WO 2016/005324 A1 may be used in the present invention. A poly(A) cassette that essentially consists of dA nucleotides, but is interrupted by a random sequence having an equal distribution of the four nucleotides (dA, dC, dG, dT) and having a length of e.g. 5 to 50 nucleotides shows, on DNA level, constant propagation of plasmid DNA in E.coli and is still associated, on RNA level, with the beneficial properties with respect to supporting RNA stability and translational efficiency. Consequently, in one embodiment of the present invention, the poly-A tail contained in an RNA molecule described herein essentially consists of A nucleotides, but is interrupted by a random sequence of the four nucleotides (A, C, G, U). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length.

[0071] In one embodiment of the invention, no nucleotides other than A nucleotides flank a poly-A tail at its 3' end, i.e., the poly-A tail is not masked or followed at its 3' end by a nucleotide other than A.

[0072] In some embodiments, a poly-A tail comprises the sequence:

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCAUAUGACUAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAA (SEQ ID NO: 78), which is also shown in Table 2 following the 3' UTR sequence.

[0073] "RNA" and "mRNA" are used interchangeably herein.

[0074] "IFN α " is used generically herein to describe any interferon alpha Type I cytokine, including IFN α 2b and IFN α 4. In the experiments described in the Example section, human IFN α 2b and mouse IFN α 4 were utilized. Any IFN α may be incorporated into the compositions and used in the methods described herein.

[0075] The term "treatment," as used herein, covers any administration or application of a therapeutic for disease in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of the disease. For example, treatment of a solid tumor may comprise alleviating symptoms of the solid tumor, decreasing the size of the solid tumor, eliminating the solid tumor, reducing further growth of the tumor, or reducing or eliminating

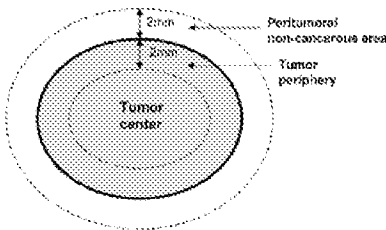
recurrence of a solid tumor after treatment. Treatment may also be measured as a change in a biomarker of effectiveness or in an imaging or radiographic measure.

[0076] The term “prevention,” as used herein, means inhibiting or arresting development of cancer, including solid tumors, in a subject deemed to be cancer free.

[0077] “Metastasis” means the process by which cancer spreads from the place at which it first arose as a primary tumor to other locations in the body.

[0078] The term “intra-tumorally,” as used herein, means into the tumor. For example, intra-tumoral injection means injecting the therapeutic at any location that touches the tumor.

[0079] The term “peri-tumorally,” or “peri-tumoral,” as used herein, is an area that is about 2-mm wide and is adjacent to the invasive front of the tumor periphery. The peri-tumoral area comprises host tissue. See, for example:



[0080] “Administering” means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

[0081] The disclosure describes nucleic acid sequences and amino acid sequences having a certain degree of identity to a given nucleic acid sequence or amino acid sequence, respectively (a reference sequence).

[0082] “Sequence identity” between two nucleic acid sequences indicates the percentage of nucleotides that are identical between the sequences. “Sequence identity” between two amino acid sequences indicates the percentage of amino acids that are identical between the sequences.

[0083] The terms “% identical”, “% identity” or similar terms are intended to refer, in particular, to the percentage of nucleotides or amino acids which are identical in an optimal alignment between the sequences to be compared. Said percentage is purely statistical, and the differences between the two sequences may be but are not necessarily randomly distributed over the entire length of the sequences to be compared. Comparisons of two sequences are usually carried out by comparing said sequences, after optimal alignment, with respect to a segment or “window of comparison”, in order to identify local regions of

corresponding sequences. The optimal alignment for a comparison may be carried out manually or with the aid of the local homology algorithm by Smith and Waterman, 1981, *Adv. App. Math.* 2, 482, with the aid of the local homology algorithm by Needleman and Wunsch, 1970, *J. Mol. Biol.* 48, 443, with the aid of the similarity search algorithm by Pearson and Lipman, 1988, *Proc. Natl Acad. Sci. USA* 88, 2444, or with the aid of computer programs using said algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.).

[0084] Percentage identity is obtained by determining the number of identical positions at which the sequences to be compared correspond, dividing this number by the number of positions compared (e.g., the number of positions in the reference sequence) and multiplying this result by 100.

[0085] In some embodiments, the degree of identity is given for a region which is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference sequence. For example, if the reference nucleic acid sequence consists of 200 nucleotides, the degree of identity is given for at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 nucleotides, in some embodiments in continuous nucleotides. In some embodiments, the degree of identity is given for the entire length of the reference sequence.

[0086] Nucleic acid sequences or amino acid sequences having a particular degree of identity to a given nucleic acid sequence or amino acid sequence, respectively, may have at least one functional property of said given sequence, e.g., and in some instances, are functionally equivalent to said given sequence. One important property includes the ability to act as a cytokine, in particular when administered to a subject. In some embodiments, a nucleic acid sequence or amino acid sequence having a particular degree of identity to a given nucleic acid sequence or amino acid sequence is functionally equivalent to said given sequence.

II. Compositions and Medical Preparations

A. Interleukin-2 (IL-2)

[0087] In some embodiments, the composition comprises a DNA sequence encoding interleukin-2 (IL-2) (SEQ ID NO: 9). In some embodiments, the DNA sequence encoding IL-2 is provided in SEQ ID NO: 10.

[0088] In some embodiments, the composition comprises a codon-optimized DNA sequence encoding IL-2. In some embodiments, the codon-optimized DNA sequence comprises or

consists of the nucleotides of SEQ ID NOs: 11. In some embodiments, the DNA sequence comprises a codon-optimized DNA sequence with 83%, 84%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

[0089] The alignment of codon optimized IL-2 to native IL-2 is shown below, where the “Q” is native IL-2 (NM_000586.3; SEQ ID NO: 10) and the “S” is codon optimized IL-2 (SEQ ID NO: 11). The percent identity is 82.79%.

```
Q:      1 ATGTACAGGATGCAACTCTGTCTTGGCATTCGACTAAGTCTTGCACTTGTGCACAAACAGT   60  
       ||||| ||||| || ||||||||| || | ||||| ||||| |||||  
S:      1 ATGTACAGAATGCAGCTGCTGTCTTGCAATTGCTCTTTCTCTGTCTGTGACAAAATTCT   60  
  
Q:     61 GCACCTACTTCAAGTCTACAAAGAAAAACACAGCTACAACGGAGCATTACTGCTGGAT   120  
       || || || || || || ||||||||| || || || || || || || ||  
S:     61 GCTCCAACATCTTCTTCAACAAAGAAAACACAGCTTCAGCTTGAACACCTTCTCTCTGAT   120  
  
Q:    121 TTACAGATGATTTTGAATGGAATTAATAATTACAGAATCCCAAACCTACCAGGATGCTC   180  
       | ||||||| ||||||| || ||||||| ||||| || || || || || || ||  
S:    121 CTTCAGATGATCTCGAATGGAATCAACAATTACAAAATCCAAAACCTGACAGAATGCTG   180  
  
Q:    181 ACATTTAAGTTTTACATGCCCAAGAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAA   240  
       ||||||| ||||||| ||||| || ||||||| ||||||| ||||||| || ||  
S:    181 ACATTTAAATTTTACATGCCAAAGAAAGCAACAGAACTGAAACACCTTCAGTGCCTTGAA   240  
  
Q:    241 GAAGAACTCAAAECTCTGGAGGAAGTGCTAAATTAGCTCAAAAGCAAAAATTTCACTTA   300  
       ||||||| ||||||| ||||||| ||| | ||||| ||||||| ||||||| |  
S:    241 GAAGAACTGAAACCTCTGGAAGAGTGCTGAATCTGCTCAGAGCAAAAATTTCACTTG   300  
  
Q:    301 AGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAAC TAAAGGGATCTGAA   360  
       ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
S:    301 AGACCAAGAGATCTGATCAGCAACATCAATGTGATTGTGCTGGAAC TGAAGGATCTGAA   360  
  
Q:    361 ACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTTGTAGAATTTCTGAACAGA   420  
       ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||  
S:    361 ACAACATTCATGTGTGAATATGCTGATGAACAGCAACAATTTGTGAATTTCTGAACAGA   420  
  
Q:    421 TGGATTACCTTTTGTCAAAGCATCATCTCAACTGACT   459  
       ||||| ||||| || ||||| ||||| ||||| ||||| ||||| |||||  
S:    421 TGGATCACATTTTGGCAGTCAATCATTTCAACACTGACA   459
```

[0090] In some embodiments, the composition comprises an RNA sequence transcribed from a DNA sequence encoding IL-2. In some embodiments, the RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NO: 10 or 11. In some embodiments, the RNA sequence comprises or consists of SEQ ID NOs: 12 or 13. In some embodiments, the RNA sequence comprises or consists of an RNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 12 or 13.

[0091] In some embodiments, one or more uridine in the IL-2 RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

[0092] In some embodiments, the IL-2 RNA comprises an altered nucleotide at the 5' end. In some embodiments, the IL-2 RNA comprises a 5' cap. Any 5' cap known in the art may be used. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage. In some

embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some embodiments, the 5' cap comprises a 2'-*O* or 3'-*O*-ribose-methylated nucleotide. In some embodiments, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some embodiments, the 5' cap comprises 7-methylguanylate. In some embodiments, the 5' cap is Cap0 or Cap1. Exemplary cap structures include m7G(5')ppp(5')G, m7,2'-*O*-mG(5')ppsp(5')G, m7G(5')ppp(5')2'-*O*-mG, and m7,3'-*O*-mG(5')ppp(5')2'-*O*-mA.

[0093] In some embodiments, the IL-2 RNA comprises a 5' untranslated region (UTR). In some embodiments, the 5' UTR is upstream of the initiation codon. In some embodiments, the 5' UTR regulates translation of the RNA. In some embodiments, the 5' UTR is a stabilizing sequence. In some embodiments, the 5' UTR increases the half-life of RNA. Any 5' UTR known in the art may be used. In some embodiments, the 5' UTR RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NOs: 1, 3, or 5. In some embodiments, the 5' UTR RNA sequence comprises or consists of SEQ ID NOs: 2, 4, or 6. In some embodiments, the 5' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 2, 4, or 6.

[0094] In some embodiments, the IL-2 RNA comprises a 3' UTR. In some embodiments, the 3' UTR follows the translation termination codon. In some embodiments, the 3' UTR regulates polyadenylation, translation efficiency, localization, or stability of the RNA. In some embodiments, the 3' UTR RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NO: 7. In some embodiments, the 3' UTR RNA sequence comprises or consists of SEQ ID NO: 8. In some embodiments, the 3' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.

[0095] In some embodiments, the IL-2 composition comprises both a 5' UTR and a 3' UTR. In some embodiments, the composition comprises only a 5' UTR. In some embodiments, the composition comprises only a 3' UTR.

[0096] In some embodiments, the IL-2 RNA comprises a poly-A tail. In some embodiments, the RNA comprises a poly-A tail of at least about 25, at least about 30, at least about 50, at least about 70, or at least about 100 nucleotides. In some embodiments, the poly-A tail comprises 200 or more nucleotides. In some embodiments, the poly-A tail comprises or consists of SEQ ID NO: 78.

[0097] In some embodiments, the RNA comprises a 5' cap, a 5' UTR, a nucleic acid encoding IL-2, a 3' UTR, and a poly-A tail, in that order.

[0098] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5.

[0099] In some embodiments, the composition comprises an RNA sequence, that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IL-2 RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00100] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00101] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. In some embodiments, one or more uridine in the IL-2 RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00102] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00103] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 10 or 11; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced.

[00104] In some embodiments, one or more uridine in the IL-2 RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00105] In some embodiments, the composition comprises an RNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 12 or 13; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 2, 4, or 6; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In some embodiments, one or more uridine in the IL-2 RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

B. Interleukin-12 single-chain (IL-12sc)

[00106] In some embodiments, the composition comprises a DNA sequence encoding interleukin-12 single-chain (IL-12sc) (e.g., SEQ ID NO: 14), which comprises IL-12 p40 (sometimes referred to as IL-12B), a linker, such as a GS linker, and IL-12 p35 (sometimes referred to as IL-12A). In some embodiments, the IL-12p40, linker, and IL-12p35 are consecutive with no intervening nucleotides. An exemplary DNA sequence encoding IL-12sc is provided in SEQ ID NO: 15.

[00107] The alignment of codon optimized IL-12 p40 to native IL-12 p40 is shown below, where the “S” is native IL-12 p40 (NM_002187.2; nucleotides 1-984 of SEQ ID NO: 15) and the “Q” is codon optimized IL-12 p40 (nucleotides 1-984 of SEQ ID NO: 16). The percent identity is 77%.

95

```

Q:      899 GCCGGAAGAATGCCCTCTATTTCTGTGCGTGCAGGACAGATACTATAGCAGCTCTTGGG 958
      ||| || ||||| ||| ||||| || ||||| || ||||| |||||
S:      899 GCCGCAAAAATGCCAGCATTAGCGTGCAGGACCAGGACCCTACTATAGCTCATCTTGGG 958

Q:      959 GTGAATGGGC--CAGTGTCCCATGTTCA 984
      ||||| || ||| ||| ||
S:      959 GCGAATGGGCATCTGTG-CCC-TG--CA 982

```

[00108] The alignment of codon optimized IL-12 p35 to native IL-12 p35 is shown below, where the “S” is native IL-12 p35 (NM_00882.3; nucleotides 1027-1623 of SEQ ID NO: 15) and the “Q” is codon optimized IL-12 p35 (nucleotides 1027-1623 of SEQ ID NO: 16). The percent identity is 80%.

```

Q:      1 AGAAATCTCCCTGTGGCTACACCTGATCCAGGCATGTTTCCCTGTTTGACCATAGCCAA 60
      ||||| ||||| ||||| || || || ||||| ||||| || || ||||| |||||
S:      1 AGAAACCTCCCGTGGCCACTCCAGACCCAGGAATGTTCCCATGCTTCACCACTCCCAA 60

Q:      61 AACCTCCTGAGAGCAGTCAGCAACATGCTCCAGAAAGCTAGACAAACACTGGAATTCTAC 120
      ||||| ||||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
S:      61 AACCTGCTGAGGGCCGTCAGCAACATGCTCCAGAAGGCCAGACAAACTCTAGAATTTTAC 120

Q:      121 CCATGCACCTCCGAGGAAATAGATCAGGAGATATCACTAAGGACAAAACAAGCACTGTC 180
      || ||||| || || || || ||||| || ||||| || || ||||| |||||
S:      121 CCTTGCACCTCTGAAGAGATTGATCATGAAGATATCACAAAAGATAAAACCAGCACAGTG 180

Q:      181 GAAGCATGCCTTCCCTTGGAACTGACAAAGAACGAGAGTTGCCCTTAATTCAAGAGAAACA 240
      || || || || || ||||| || || ||||| ||||| ||||| ||||| |||||
S:      181 GAGGCTGTATTACCATTTGAATTAACCAAGAATGAGAGTTGCCCTAAATTCCAGAGAGACC 240

Q:      241 TCTTTCATTACAAACGGTAGCTGCTTGGCAAGCAGAAAAACATCTTTTATGATGGCCCTT 300
      ||||| || || || || ||||| || ||||| || ||||| ||||| |||||
S:      241 TCTTTCATAACTAATGGGAGTTGCTTGGCTCCAGAAAGACCTCTTTTATGATGGCCCTG 300

Q:      301 TGTCTGAGCAGTATTTATGAGGATCTCAAAATGTACCAGGTGGAGTTTAAGACCATGAAT 360
      || || || ||||| || || || ||||| ||||| ||||| ||||| |||||
S:      301 TGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACCAGGTGGAGTTCAAGACCATGAAT 360

Q:      361 GCCAAGCTGCTGATGGACCCAAGAGACAGATTTTCTCGATCAGAATATGCTGGCTGTG 420
      || ||||| ||||| || ||||| ||||| || || ||||| || ||||| |||||
S:      361 GCAAAGCTTCTGATGGATCCTAAGAGGCAGATCTTCTAGATCAAAACATGCTGGCAGTT 420

Q:      421 ATTGATGAAGTATGTCAGGCTTGAATTTCAACAGCGAAACCGTTCCCAGAAAAGCAGT 480
      ||||| ||||| ||||| ||||| ||||| || || || || || || || || ||
S:      421 ATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTGAGACTGTGCCACAAAATCCTCC 480

Q:      481 CTTGAAGAACCTGACTTTTATAAGAACAGATCAAACTGTGTATTCTCTGCATGCCTTT 540
      ||||| || || ||||| || || ||||| || || || || || || || || ||
S:      481 CTTGAAGAACCGGATTTTATAAAACTAAAATCAAGCTCTGCATACTTCTTCATGCTTTC 540

Q:      541 AGAATCAGAGCAGTCACTATAGATAGAGTGATGTCTACCTGAATGCTTCC 591
      ||||| || ||||| ||||| ||||| ||||| || || ||||| |||||
S:      541 AGAATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAATGCTTCC 591

```

[00109] In some embodiments, the composition comprises a codon-optimized DNA sequence encoding IL-12sc. In some embodiments, the composition comprises a codon-

optimized DNA sequence encoding IL-12 p40. In some embodiments, the composition comprises a codon-optimized DNA sequence encoding IL-12 p35. In some embodiments, the codon-optimized DNA sequence comprises or consists of SEQ ID NO: 16. In some embodiments, the DNA sequence comprises a codon-optimized DNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16. In some embodiments, the codon-optimized DNA sequence encoding IL-12 p40 comprises the nucleotides encoding the IL-12sc-p40 (nucleotides 1-984 of SEQ ID NO: 16). In some embodiments, the codon-optimized DNA sequence encoding IL-12 p35 comprises the nucleotides encoding the IL-12sc-p35 (nucleotides 1027-1623 of SEQ ID NO: 16). In some embodiments, the codon-optimized DNA sequence encoding IL-12sc comprises the nucleotides encoding the IL-12sc-p40 (nucleotides 1-984 of SEQ ID NO: 16) and -p35 (nucleotides 1027-1623 of SEQ ID NO: 16) portions of SEQ ID NO: 16 and further comprises nucleotides between the p40 and p35 portions (e.g., nucleotides 985-1026 of SEQ ID NO: 16) encoding a linker polypeptide connecting the p40 and p35 portions. Any linker known to those of skill in the art may be used. The p40 portion may be 5' or 3' to the p35 portion.

[00110] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence encoding IL-12sc. The RNA may also be recombinantly produced. In some embodiments, the RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NOs: 15 or 16. In some embodiments, the RNA sequence comprises or consists of SEQ ID NOs: 17 or 18. In some embodiments, the RNA sequence comprises or consists of an RNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 17 or 18. In some embodiments, the RNA sequence comprises the nucleotides encoding the IL-12sc-p40 (nucleotides 1-984 of SEQ ID NOs: 17 or 18) and -p35 (nucleotides 1027-1623 of SEQ ID NOs: 17 or 18) portions of SEQ ID NOs: 17 or 18. In some embodiments, the codon-optimized RNA sequence encoding IL-12sc comprises the nucleotides encoding the IL-12sc-p40 (nucleotides 1-984 of SEQ ID NO: 18) and -p35 (nucleotides 1027-1623 of SEQ ID NO: 18) portions of SEQ ID NO: 18 and further comprises nucleotides between the p40 and p35 portions encoding a linker polypeptide connecting the p40 and p35 portions. Any linker known to those of skill in the art may be used.

[00111] In some embodiments, one or more uridine in the IL-12sc RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine

(m⁵U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine (m¹ψ).

[00112] In some embodiments, the IL-12sc RNA comprises an altered nucleotide at the 5' end. In some embodiments, the RNA comprises a 5' cap. Any 5' cap known in the art may be used. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some embodiments, the 5' cap comprises a 2'-O or 3'-O-ribose-methylated nucleotide. In some embodiments, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some embodiments, the 5' cap comprises 7-methylguanylate. In some embodiments, the 5' cap is Cap0 or Cap1. Exemplary cap structures include m7G(5')ppp(5')G, m7,2'-O-mG(5')ppsp(5')G, m7G(5')ppp(5')2'-O-mG, and m7,3'-O-mG(5')ppp(5')2'-O-mA.

[00113] In some embodiments, the IL-12sc RNA comprises a 5' untranslated region (UTR). In some embodiments, the 5' UTR is upstream of the initiation codon. In some embodiments, the 5' UTR regulates translation of the RNA. In some embodiments, the 5' UTR is a stabilizing sequence. In some embodiments, the 5' UTR increases the half-life of RNA. Any 5' UTR known in the art may be used. In some embodiments, the 5' UTR RNA sequence is transcribed from SEQ ID NOs: 1, 3, or 5. In some embodiments, the 5' UTR RNA sequence comprises or consists of SEQ ID NOs: 2, 4, or 6. In some embodiments, the 5' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 2, 4, or 6.

[00114] In some embodiments, the IL-12sc RNA comprises a 3' UTR. In some embodiments, the 3' UTR follows the translation termination codon. In some embodiments, the 3' UTR regulates polyadenylation, translation efficiency, localization, or stability of the RNA. In some embodiments, the 3' UTR RNA sequence is transcribed from SEQ ID NO: 7. In some embodiments, the 3' UTR RNA sequence comprises or consists of SEQ ID NO: 8. In some embodiments, the 3' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.

[00115] In some embodiments, the IL-12sc composition comprises both a 5' UTR and a 3' UTR. In some embodiments, the IL-12sc composition comprises only a 5' UTR. In some embodiments, the IL-12sc composition comprises only a 3' UTR.

[00116] In some embodiments, the IL-12sc RNA comprises a poly-A tail. In some embodiments, the RNA comprises a poly-A tail of at least about 25, at least about 30, at least about 50 nucleotides, at least about 70 nucleotides, or at least about 100 nucleotides. In some

embodiments, the poly-A tail comprises 200 or more nucleotides. In some embodiments, the poly-A tail comprises or consists of SEQ ID NO: 78.

[00117] In some embodiments, the RNA comprises a 5' cap, a 5' UTR, a nucleic acid encoding IL-12sc, a 3' UTR, and a poly-A tail, in that order.

[00118] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5.

[00119] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IL-12sc RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00120] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00121] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IL-12sc RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00122] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00123] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 15 or 16; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IL-12sc RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00124] In some embodiments, the composition comprises an RNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 17 or 18; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 2, 4, or 6; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In some embodiments, one or more uridine in the IL-12sc RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

C. Interferon alpha (IFN α)

[00125] In some embodiments, the composition comprises a DNA sequence encoding interferon alpha (IFN α) (e.g., SEQ ID NO: 19). An exemplary DNA sequence encoding this IFN α is provided in SEQ ID NO: 20.

[00126] The alignment of codon optimized IFN α to native IFN α is shown below, where the “S” is native IFN α (NM_000605.3; SEQ ID NO: 20) and the “Q” is codon optimized IFN α (SEQ ID NO: 21). The percent identity is 79%.

```

Q:      1 ATGGCCCTGACTTTTGCCTTCTCGTGCTTTGTTGGTGCTGAGTTGCAAAATCTTCCTGT 60
      ||||| ||||| ||||| | || ||||| | ||||| || ||||| || |||
S:      1 ATGGCCTTGACCTTTGCTTTACTGGTGCCCTCCTGGTGCTCAGCTGCAAGTCAAGCTGC 60

Q:      61 AGTGTGGGATGTGATCTGCCTCAAACCCACAGTCTGGG-ATCTAGGAGAACTGATGCT 119
      ||| || ||||| ||||| ||||| ||||| ||||| ||||| || ||||| |||
S:      61 TCTGTGGGCTGTGATCTGCCTCAAACCCACAGCCTGGGTAGC-AGGAGGACCTTGATGCT 119

Q:      120 GTTGGCACAGATGAGGAGAAT-TAGC-CTCTTTTCTGCTGAAGGATAGACATGACTTC 177
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
S:      120 CCTGGCACAGATGAGGAGAATCT--CTCTTTTCTGCTGAAGGACAGACATGACTTT 177

Q:      178 GGCTTTCCCAAGAGGAGTTTGCAATCAGTTCAGAAAGCGGAAACGATTCCCGTTCTG 237
      || ||||| ||||| ||||| ||||| ||||| ||||| || ||||| || |||||
S:      178 GGATTTCCTCAGGAGGAGTTTGCAACAGTTCAGAAAGGCTGAAACCATCCCTGTCCTC 237

Q:      238 CACGAGATGATCCAGCAGATCTCAACCTCTTTTCAAC-CAAAG-ACAGCTCAGCAGCCT 295
      || ||||| ||||| ||||| ||||| ||||| || ||||| || ||||| |||
S:      238 CATGAGATGATCCAGCAGATCTCAATCTCTT--CAGCACAAAGGACTCATCTGCTGCTT 295

Q:      296 GGGATGAGACACTGCTGGACAAATCTACACAGAACTGTATCAGCAGCTTAACGATCTGG 355
      ||||| ||||| || ||||| ||||| ||||| ||||| || ||||| || |||||
S:      296 GGGATGAGACCTCTTAGACAAATCTACACTGAACTCTACCAGCAGCTGAATGACCTGG 355

Q:      356 AGGCATGCGTGATCCAAGGGGTGGTGACTGAACTCCGCTTATGAAGGAGGACTCCA 415
      || || ||||| || ||||| ||||| ||||| || ||||| || ||||| |||||
S:      356 AAGCCTGTGTGATACAGGGGGTGGGGTGACAGAGACTCCCTGATGAAGGAGGACTCCA 415

Q:      416 TTCTGGCTGTACGGAGTACTTCCAGAGAATAACCTCTATCTGAAGGAGAGAAAGTACT 475
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||
S:      416 TTCTGGCTGTGAGGAAATACTTCCAAAGAACTACTCTATCTGAAGGAGAGAAATACA 475

Q:      476 CACCATGTGCTTGGGAAGTCGTGAGAGCCGAAATCATGAGATCCTTCAGCCTTAG-CACC 534
      || ||||| ||||| || ||||| ||||| ||||| ||||| || ||||| |||
S:      476 GCCCTGTGCTTGGGAGGTGTGAGAGCAGAAATCATGAGATC-TTTTTC-TTTGTCAAC 533

Q:      535 AATC-TCCAGGAATCTCTGAGAAGCAAGAG 564
      || || ||||| || ||||| |||||
S:      534 AAACCTGCAAGAAATTTAAGAAGTAAGGAA 564

```

[00127] In some embodiments, the composition comprises a codon-optimized DNA sequence encoding IFN α . In some embodiments, the codon-optimized DNA sequence comprises or consists of the nucleotides of SEQ ID NO: 21. In some embodiments, the DNA sequence comprises or consists of a codon-optimized DNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 21.

[00128] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence encoding IFN α . The RNA may also be recombinantly produced. In some embodiments, the RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NOs: 20 or 21. In some embodiments, the RNA

sequence comprises or consists of SEQ ID NOs: 22 or 23. In some embodiments, the RNA sequence comprises or consists of an RNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 22 or 23.

[00129] In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, each uridine in the RNA is modified. In some embodiments, each uridine in the RNA is modified with N1-methyl-pseudouridine ($m^1\psi$).

[00130] In some embodiments, the IFN α RNA comprises an altered nucleotide at the 5' end. In some embodiments, the IFN α RNA comprises a 5' cap. Any 5' cap known in the art may be used. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some embodiments, the 5' cap comprises a 2'-*O* or 3'-*O*-ribose-methylated nucleotide. In some embodiments, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some embodiments, the 5' cap comprises 7-methylguanylate. In some embodiments, the 5' cap is Cap0 or Cap1. Exemplary cap structures include $m^7G(5')ppp(5')G$, $m^7,2'O-mG(5')ppsp(5')G$, $m^7G(5')ppp(5')2'O-mG$ and $m^7,3'O-mG(5')ppp(5')2'O-mA$.

[00131] In some embodiments, the IFN α RNA comprises a 5' untranslated region (UTR). In some embodiments, the 5' UTR is upstream of the initiation codon. In some embodiments, the 5' UTR regulates translation of the RNA. In some embodiments, the 5' UTR is a stabilizing sequence. In some embodiments, the 5' UTR increases the half-life of RNA. Any 5' UTR known in the art may be used. In some embodiments, the 5' UTR RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NOs: 1, 3, or 5. In some embodiments, the 5' UTR RNA sequence comprises or consists of SEQ ID NOs: 2, 4, or 6. In some embodiments, the 5' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 2, 4, or 6.

[00132] In some embodiments, the IFN α RNA comprises a 3' UTR. In some embodiments, the 3' UTR follows the translation termination codon. In some embodiments, the 3' UTR regulates polyadenylation, translation efficiency, localization, or stability of the RNA. In some embodiments, the 3' UTR RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NO: 7. In some embodiments, the 3' UTR RNA sequence comprises or consists of SEQ ID NO: 8. In some embodiments, the 3' UTR RNA sequence is

at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.

[00133] In some embodiments, the IFN α composition comprises both a 5' UTR and a 3' UTR. In some embodiments, the composition comprises only a 5' UTR. In some embodiments, the composition comprises only a 3' UTR.

[00134] In some embodiments, the IFN α RNA comprises a poly-A tail. In some embodiments, the IFN α RNA comprises a poly-A tail of at least about 25, at least about 30, at least about 50 nucleotides, at least about 70 nucleotides, or at least about 100 nucleotides. In some embodiments, the poly-A tail comprises 200 or more nucleotides. In some embodiments, the poly-A tail comprises or consists of SEQ ID NO: 78.

[00135] In some embodiments, the RNA comprises a 5' cap, a 5' UTR, a nucleic acid encoding IFN α , a 3' UTR, and a poly-A tail, in that order.

[00136] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 20 or 21 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5.

[00137] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 20 or 21 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00138] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 20 or 21 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00139] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical

to SEQ ID NOs: 20 or 21 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00140] In some embodiments, the composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 20 or 21; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00141] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 20 or 21; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$). In some embodiments, the composition comprises an RNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 22 or 23; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 2, 4, or 6; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

D. IL-15 sushi

[00142] As used herein, the term “IL-15 sushi” describes a construct comprising the soluble interleukin 15 (IL-15) receptor alpha sushi domain and mature interleukin alpha (IL-15) as a fusion protein. In some embodiments, the composition comprises a DNA sequence encoding IL-15 sushi (SEQ ID NO: 24), which comprises the soluble IL-15 receptor alpha chain (sushi) followed by a glycine-serine (GS) linker followed by the mature sequence of IL-15. The DNA sequence encoding this IL-15 sushi is provided in SEQ ID NO: 25.

[00143] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence encoding IL-15 sushi. The RNA may also be recombinantly produced. In some embodiments, the RNA sequence is transcribed from a nucleotide sequence comprising SEQ ID NO: 25. In some embodiments, the nucleotides encoding the linker may be completely absent or replaced in part or in whole with any nucleotides encoding a suitable linker. In some embodiments, the RNA sequence comprises or consists of SEQ ID NO: 26. In some embodiments, the RNA sequence comprises an RNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 26. In some embodiments, the DNA or RNA sequence encoding IL-15 sushi comprises the nucleotides encoding the sushi domain of IL-15 receptor alpha (e.g., nucleotide 1-321 of SEQ ID NOs: 25 or 26) and mature IL-15 (e.g., nucleotide 382-729 of SEQ ID NO: 25 or 26). In some embodiments, the DNA or RNA sequence encoding IL-15 sushi comprises the nucleotides encoding the sushi domain of IL-15 receptor alpha (e.g., nucleotide 1-321 of SEQ ID NOs: 25 or 26) and mature IL-15 (e.g., nucleotide 382-729 of SEQ ID NOs: 25 or 26) and further comprises nucleotides between these portions encoding a linker polypeptide connecting the portions. In some embodiments, the linker comprises nucleotides 322-381 of SEQ ID Nos: 25 or 26. Any linker known to those of skill in the art may be used.

[00144] In some embodiments, one or more uridine in the IL-15 sushi RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00145] In some embodiments, the IL-15 sushi RNA comprises an altered nucleotide at the 5' end. In some embodiments, the IL-15 sushi RNA comprises a 5' cap. Any 5' cap known in the art may be used. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate

linkage including thiophosphate modification. In some embodiments, the 5' cap comprises a 2'-*O* or 3'-*O*-ribose-methylated nucleotide. In some embodiments, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some embodiments, the 5' cap comprises 7-methylguanylate. In some embodiments, the 5' cap is Cap0 or Cap1. Exemplary cap structures include m7G(5')ppp(5')G, m7,2' *O*-mG(5')ppsp(5')G, m7G(5')ppp(5')2' *O*-mG and m7,3' *O*-mG(5')ppp(5')2' *O*-mA.

[00146] In some embodiments, the IL-15 sushi RNA comprises a 5' untranslated region (UTR). In some embodiments, the 5' UTR is upstream of the initiation codon. In some embodiments, the 5' UTR regulates translation of the RNA. In some embodiments, the 5' UTR is a stabilizing sequence. In some embodiments, the 5' UTR increases the half-life of RNA. Any 5' UTR known in the art may be used. In some embodiments, the 5' UTR RNA sequence is transcribed from SEQ ID NOs: 1, 3, or 5. In some embodiments, the 5' UTR RNA sequence comprises or consists of SEQ ID NOs: 2, 4, or 6. In some embodiments, the 5' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 2, 4, or 6.

[00147] In some embodiments, the IL-15 sushi RNA comprises a 3' UTR. In some embodiments, the 3' UTR follows the translation termination codon. In some embodiments, the 3' UTR regulates polyadenylation, translation efficiency, localization, or stability of the RNA. In some embodiments, the 3' UTR RNA sequence is transcribed from SEQ ID NO: 7. In some embodiments, the 3' UTR RNA sequence comprises or consists of SEQ ID NO: 8. In some embodiments, the 3' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.

[00148] In some embodiments, the IL-15 sushi composition comprises both a 5' UTR and a 3' UTR. In some embodiments, the IL-15 sushi composition comprises only a 5' UTR. In some embodiments, the IL-15 sushi composition comprises only a 3' UTR.

[00149] In some embodiments, the IL-15 sushi RNA comprises a poly-A tail. In some embodiments, the RNA comprises a poly-A tail of at least about 25, at least about 30, at least about 50 nucleotides, at least about 70 nucleotides, or at least about 100 nucleotides. In some embodiments, the poly-A tail comprises 200 or more nucleotides. In some embodiments, the poly-A tail comprises or consists of SEQ ID NO: 78.

[00150] In some embodiments, the RNA comprises a 5' cap, a 5' UTR, a nucleic acid encoding IL-15 sushi, a 3' UTR, and a poly-A tail, in that order.

[00151] In some embodiments, the IL-15 sushi composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence that is at least 70%, 75%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5.

[00152] In some embodiments, the IL-15 sushi composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00153] In some embodiments, the IL-15 sushi composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00154] In some embodiments, the IL-15 sushi composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00155] In some embodiments, the IL-15 sushi composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00156] In some embodiments, the IL-15 sushi composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00157] In some embodiments, the IL-15 sushi composition comprises an RNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 2, 4, or 6; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In some embodiments, one or more uridine in the IFN α RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

E. Granulocyte-macrophage colony-stimulating factor (GM-CSF)

[00158] In some embodiments, the composition comprises a DNA sequence encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) (e.g., SEQ ID NO: 27). In some embodiments, the DNA sequence encoding GM-CSF is provided in SEQ ID NO: 28.

[00159] In some embodiments, the GM-CSF composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence encoding GM-CSF. In some embodiments, the RNA sequence is transcribed from SEQ ID NO: 28. The RNA may also be recombinantly produced. In some embodiments, the RNA sequence comprises or consists of SEQ ID NO: 29. In some embodiments, the RNA sequence comprises an RNA sequence with 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 29.

[00160] In some embodiments, one or more uridine in the GM-CSF RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine

(m⁵U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine (m¹ψ). In some embodiments, the GM-CSF RNA comprises an altered nucleotide at the 5' end. In some embodiments, the RNA comprises a 5' cap. Any 5' cap known in the art may be used. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage. In some embodiments, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some embodiments, the 5' cap comprises a 2'-O or 3'-O-ribose-methylated nucleotide. In some embodiments, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some embodiments, the 5' cap comprises 7-methylguanylate. In some embodiments, the 5' cap is Cap0 or Cap1. Exemplary cap structures include m7G(5')ppp(5')G, m7,2'-O-mG(5')ppsp(5')G, m7G(5')ppp(5')2'-O-mG and m7,3'-O-mG(5')ppp(5')2'-O-mA.

[00161] In some embodiments, the GM-CSF RNA comprises a 5' untranslated region (UTR). In some embodiments, the 5' UTR is upstream of the initiation codon. In some embodiments, the 5' UTR regulates translation of the RNA. In some embodiments, the 5' UTR is a stabilizing sequence. In some embodiments, the 5' UTR increases the half-life of RNA. Any 5' UTR known in the art may be used. In some embodiments, the 5' UTR RNA sequence is transcribed from SEQ ID NOs: 1, 3, or 5. In some embodiments, the 5' UTR RNA sequence comprises or consists of SEQ ID NOs: 2, 4, or 6. In some embodiments, the 5' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 2, 4, or 6.

[00162] In some embodiments, the GM-CSF RNA comprises a 3' UTR. In some embodiments, the 3' UTR follows the translation termination codon. In some embodiments, the 3' UTR regulates polyadenylation, translation efficiency, localization, or stability of the RNA. In some embodiments, the 3' UTR RNA sequence is transcribed from SEQ ID NO: 7. In some embodiments, the 3' UTR RNA sequence comprises or consists of SEQ ID NO: 8. In some embodiments, the 3' UTR RNA sequence is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.

[00163] In some embodiments, the GM-CSF composition comprises both a 5' UTR and a 3' UTR. In some embodiments, the composition comprises only a 5' UTR. In some embodiments, the composition comprises only a 3' UTR.

[00164] In some embodiments, the GM-CSF RNA comprises a poly-A tail. In some embodiments, the RNA comprises a poly-A tail of at least about 25, at least about 30, at least about 50 nucleotides, at least about 70 nucleotides, or at least about 100 nucleotides. In some

embodiments, the poly-A tail comprises 200 or more nucleotides. In some embodiments, the poly-A tail comprises or consists of SEQ ID NO: 78.

[00165] In some embodiments, the GM-CSF RNA comprises a 5' cap, a 5' UTR, nucleotides encoding GM-CSF, a 3' UTR, and a poly-A tail, in that order.

[00166] In some embodiments, the GM-CSF composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5.

[00167] In some embodiments, the GM-CSF composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the GM-CSF RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00168] In some embodiments, the GM-CSF composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00169] In some embodiments, the GM-CSF composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28 and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the GM-CSF RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00170] In some embodiments, the GM-CSF composition comprises a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

[00171] In some embodiments, the composition comprises an RNA sequence that is, for example, transcribed from a DNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 1, 3, or 5; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7. The RNA may also be recombinantly produced. In some embodiments, one or more uridine in the GM-CSF RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, the RNA comprises a modified nucleoside in place of each uridine. In some embodiments, the modified nucleoside is N1-methyl-pseudouridine ($m^1\psi$).

[00172] In some embodiments, the GM-CSF composition comprises an RNA sequence comprising or consisting of a nucleic acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 29; at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 2, 4, or 6; and at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In some embodiments, one or more uridine in the GM-CSF RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

F. Modifications

[00173] Each of the RNAs and compositions described herein may be modified in any way known to those of skill in the art. In some embodiments, the modifications are “ModA” or “ModB” modified as described herein.

[00174] In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside. In some embodiments, the modified nucleoside is a modified uridine.

[00175] In some embodiments, the modified uridine replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m1\psi$), or 5-methyl-uridine ($m5U$).

[00176] In some embodiments, one or more cytosine, adenine or guanine in the RNA is replaced by modified nucleobase(s). In one embodiment, the modified nucleobase replacing cytosine is 5-methylcytosine (m^5C). In another embodiment, the modified nucleobase replacing adenine is N^6 -methyladenine (m^6A). In another embodiment, any other modified nucleobase known in the art for reducing the immunogenicity of the molecule can be used.

[00177] The modified nucleoside replacing one or more uridine in the RNA may be any one or more of 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mm^5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm^5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine (τm^5s^2U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-2-thio-uridine (m^5s^2U), 1-methyl-4-thio-pseudouridine ($m^1s^4\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), 5-(isopentenylaminomethyl)uridine (inm^5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^5s^2U), α -thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^5Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^2Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^5Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^5Um), 5-carboxymethylaminomethyl-2'-O-

methyl-uridine (cmm⁵Um), 3,2'-O-dimethyl-uridine (m³Um), 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm⁵Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, 5-[3-(1-E-propenylamino)uridine, or any other modified uridine known in the art.

G. Combination Compositions

[00178] In some embodiments, the invention comprises a composition comprising more than one RNA as described herein. In some embodiments, the composition comprises two RNAs. In some embodiments, the composition comprises three RNAs. In some embodiments, the composition comprises four RNAs. In some embodiments, the composition comprises five RNAs. In some embodiments, any or all of the RNAs encoding IL-2, IL12sc, IL-15 sushi, GM-CSF, or IFN α may be replaced by IL-2, IL12sc, IL-15 sushi, GM-CSF, and/or IFN α polypeptides, e.g., in any of the compositions and formulations comprising these RNAs described herein.

[00179] In some embodiments, the modified or unmodified RNAs encoding IL-2, IL12sc, IL-15 sushi, GM-CSF, and/or IFN α may be replaced by modified or unmodified polycistronic RNAs encoding two or more polypeptides selected from IL-2, IL12sc, IL15 sushi, GM-CSF and IFN α polypeptides, e.g., in any of the compositions and formulations comprising these RNAs described herein.

[00180] Any of the combination compositions may further comprise an excipient or diluent. The excipient or diluent may be pharmaceutically acceptable for administration to a subject.

[00181] In some embodiments, a combination composition comprises RNAs with the same modifications. In some embodiments, a combination composition comprises RNAs with different modifications. In some embodiments, a combination composition comprises RNAs with ModA modification. In some embodiments, a combination composition comprises RNAs with ModB modification. In some embodiments, a combination composition comprises RNAs with ModA and ModB modifications.

[00182] In some embodiments, a composition comprising DNA or RNA encoding IL-2 and one or more of a DNA or RNA encoding IL-12sc, IFN α , IL-15 sushi, and GM-CSF is encompassed. In some embodiments, the composition comprises a DNA or RNA encoding IL-2 or codon-optimized IL-2 (SEQ ID NOs: 10-13) and one or more of a DNA or RNA encoding IL-12sc or optimized IL-12sc (SEQ ID Nos: 15-18), IFN α or optimized IFN α (SEQ ID Nos: 20-23), IL-15 sushi (SEQ ID NOs: 25-26), and GM-CSF (SEQ ID NOs: 28-29), as

described herein. In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, one or more of the RNAs in the composition further comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail as described herein in the composition section.

[00183] In some embodiments, a composition comprising DNA or RNA encoding IL-12sc and one or more of a DNA or RNA encoding IL-2, IFN α , IL-15 sushi, and GM-CSF is encompassed. In some embodiments, the composition comprises a DNA or RNA encoding IL-12sc or codon-optimized IL-12sc (SEQ ID NOs: 15-18) and one or more of a DNA or RNA encoding IL-2 or optimized IL-2 (SEQ ID NOs: 10-13), IFN α or optimized IFN α (SEQ ID NOs: 20-23), IL-15 sushi (SEQ ID NOs: 25-26), and GM-CSF (SEQ ID NOs: 28-29), as described herein. In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U).

[00184] In some embodiments, one or more of the RNAs in the composition further comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail, as described herein in the composition section.

[00185] In some embodiments, a composition comprising DNA or RNA encoding IFN α and one or more of a DNA or RNA encoding IL-2, IL-12sc, IL-15 sushi, and GM-CSF is encompassed. In some embodiments, the composition comprises a DNA or RNA encoding IFN α or codon-optimized IFN α (SEQ ID NOs: 20-23) and one or more of a DNA or RNA encoding IL-12sc or optimized IL-12sc (SEQ ID NOs: 15-18), IL-2 or optimized IL-2 (SEQ ID NOs: 10-13), IL-15 sushi (SEQ ID NOs: 25-26), and GM-CSF (SEQ ID NOs: 28-29), as described herein. In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, one or more of the RNAs in the composition further comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail as described herein in the composition section.

[00186] In some embodiments, a composition comprising DNA or RNA encoding IL-15 sushi and one or more of a DNA or RNA encoding IL-2, IL-12sc, IFN α , and GM-CSF is encompassed. In some embodiments, the composition comprises a DNA or RNA encoding

IL-15 sushi (SEQ ID NOs: 25-26) and one or more of a DNA or RNA encoding IL-12sc or optimized IL-12sc (SEQ ID NOs: 15-18), IFN α or optimized IFN α (SEQ ID NOs: 20-23), IL-2 or optimized IL-2 (SEQ ID NOs: 10-13), and GM-CSF (SEQ ID NOs: 28-29), as described herein. In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, one or more of the RNAs in the composition further comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail as described herein in the composition section.

[00187] In some embodiments, a composition comprising DNA or RNA encoding GM-CSF and one or more of a DNA or RNA encoding IL-2, IL-12sc, IFN α , and IL-15 sushi is encompassed. In some embodiments, the composition comprises a DNA or RNA encoding GM-CSF (SEQ ID NOs: 28-29) and one or more of a DNA or RNA encoding IL-12sc or optimized IL-12sc (SEQ ID NOs: 15-18), IFN α or optimized IFN α (SEQ ID NOs: 20-23), IL-2 or optimized IL-2 (SEQ ID NOs: 10-13), and IL-15 sushi (SEQ ID NOs: 25-26), as described herein. In some embodiments, one or more uridine in the RNA is replaced by a modified nucleoside as described herein. In some embodiments, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine ($m^1\psi$) or 5-methyl-uridine (m^5U). In some embodiments, one or more of the RNAs in the composition further comprises a 5' cap, a 5' UTR, a 3' UTR, and a poly-A tail as described herein in the composition section.

[00188] In some embodiments, the composition comprises GM-CSF, IL-2, and IL-12sc RNA. In some embodiments, the composition is modified, for example, as ModA or ModB. In some embodiments, the IL-12sc RNA is optimized as shown in SEQ ID NO: 18.

[00189] In some embodiments, the composition comprises GM-CSF, IL-15 sushi, and IL-12sc RNA. In some embodiments, the composition is modified, for example, as ModA or ModB. In some embodiments, the IL-12sc RNA is optimized as shown in SEQ ID NO: 18.

[00190] In some embodiments, the composition comprises GM-CSF, IL-2, IL-12sc, and IFN α RNA. In some embodiments, the composition is modified, for example, as ModA or ModB. In some embodiments, the IL-12sc RNA and IFN α RNA is optimized as shown in SEQ ID NOs: 18 and 23, respectively.

[00191] In some embodiments, the composition comprises GM-CSF, IL-15 sushi, IL-12sc, and IFN α RNA. In some embodiments, the composition is modified, for example, as ModA or ModB. In some embodiments, the IL-12sc RNA and IFN α RNA is optimized as shown in SEQ ID NOs: 18 and 23, respectively.

[00192] In some embodiments, the composition comprises GM-CSF, IL-15 sushi, IL-12sc, and IFN α RNA, wherein the RNAs comprise or consist of the nucleotides shown in SEQ ID Nos: 18 (IL-12sc), 23 (IFN α), 26 (IL-15 sushi), or 29 (GM-CSF). In some embodiments, the composition is modified, for example, as ModA or ModB.

[00193] In some embodiments, combinations of RNA are administered as a 1:1, 1:1:1, or 1:1:1:1 ratio based on equal RNA mass. For example, 20 μ g of IL15-sushi, 20 μ g of IL-12sc, 20 μ g of IFN α 2b and 20 μ g GM-CSF. In some embodiments, the ratio is adjusted so that different ratios by mass are administered, for example, 1:10:1:10 ratio (20 μ g, 200 μ g, 20 μ g, 200 μ g). Likewise, in some embodiments, for example, a ratio of 1:2:3:4 (20 μ g, 40 μ g, 60 μ g, 80 μ g) is used. Alternatively, rather than basing the ratio on the mass of the RNA, the ratio may be based on the molarity of the RNA.

[00194] In some embodiments, a mixture of RNAs is administered with an equal ratio of each RNA of the mixture.

[00195] In some embodiments, a mixture of RNAs is administered with an unequal ratio of each RNA of the mixture. In some embodiments, one or more RNAs are administered at a ratio that is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times greater than another RNA in the mixture. In some embodiments, one or more RNAs are administered at a ratio that is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times less than another RNA in the mixture.

[00196] In some embodiments, the compositions described herein may be a medical preparation. In some embodiments, the medical preparation comprises a kit, wherein the included RNAs may be in the same or separate vials. In some embodiments, the medical preparation further comprising instructions for use of the composition for treating or preventing a solid tumor.

[00197] In some embodiments, a kit comprising the compositions described herein is provided, wherein the included RNAs may be in the same or separate vials. In some embodiments, the kit further comprising instructions for use of the composition for treating or preventing a solid tumor.

H. Effect of IFN α addition to modified mRNA treatment

[00198] RNA can activate the immune system through stimulating various pattern recognition receptors (PRR) leading to production of Type I interferons (like IFN α). The incorporation of various modified nucleotides, or other alterations like reducing the amount of dsRNA administered, can reduce the immune stimulatory effects of RNA. Unexpectedly, as described in the Examples, inclusion of nucleotide-modified and dsRNA-reduced mRNA

encoding interferon alpha improved anti-tumor activity relative to that of mRNA that was not nucleotide modified and dsRNA-reduced. The addition of mRNA encoding interferon alpha restored a portion of the immune stimulatory effects removed by the inclusion of modified nucleotides and the dsRNA purification.

[00199] In some embodiments, RNA encoding IFN (in any form or subtype) is provided, wherein the IFN RNA is altered to have reduced immunogenicity compared to unaltered RNA. In certain embodiments, the administration of this IFN improves the anti-tumor response of non-IFN encoding RNA. In some embodiments, RNA encoding IFN α improves the anti-tumor response of other RNAs, so long as the other RNAs have been altered to reduce immunogenicity. In one embodiment, the alteration to reduce immunogenicity is a reduction in the amount of dsRNA. In some aspects, the alteration to reduce immunogenicity is the replacement of one or more uridines with a modified nucleoside. In some aspects, the alteration to reduce immunogenicity is both a reduction in the amount of dsRNA and the replacement of one or more uridines with modified nucleoside. In some embodiments, the IFN is IFN α .

[00200] In some embodiments, IFN RNA improves the anti-tumor response of modified RNAs. In some embodiments, IFN RNA improves the anti-tumor response of RNAs comprising modified nucleotides. In some embodiments, IFN RNA improves the anti-tumor response of mRNAs comprising pseudouridine. In some embodiments, IFN RNA improves the anti-tumor response of RNAs with ModB modifications.

[00201] In some embodiments, IFN RNA improves the anti-tumor response of RNAs of IL-2 (SEQ ID NO: 12 or 13), IL-12sc (SEQ ID NO: 17 or 18), IL-15 sushi (SEQ ID NO: 26) or GM-CSF (SEQ ID NO: 29). In some embodiments, the RNAs comprise ModB modifications.

[00202] In some embodiments, the IFN is IFN α .

[00203] In some embodiments, the IFN RNA construct is SEQ ID NO: 22 or 23.

III. Methods and Uses

[00204] Any of the RNAs, compositions, medical preparations and combination compositions described herein may be administered to a subject to treat cancer or a solid tumor. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is an abnormal mass of tissue that does not contain cysts or liquid areas. In some embodiments, the solid tumor may be benign or malignant. In some embodiments, the solid tumor is a pre-cancerous lesion. In some embodiments, the solid tumor occurs in lung, colon,

ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.

[00205] In some embodiments, the solid tumor is a sarcoma, carcinoma, or lymphoma. In some embodiments, the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, basal cell carcinoma, squamous cell carcinoma, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor. In some embodiments, the solid tumor is a precancerous lesion such as actinic keratosis.

[00206] In some embodiments, the RNA compositions may be delivered via injection into (e.g., intra-tumorally) or near (peri-tumorally) the tumor. In some embodiments, the RNA compositions may be delivered at or near the site of a tumor removal.

[00207] In some embodiments, the RNA compositions may be delivered via a topical solution, ointment, or cream.

[00208] In some embodiments, the RNA compositions may be delivered via a virus. In some embodiments, the RNA compositions may be delivered by infection with a virus encoding the RNA compositions, such as an oncolytic virus. In some embodiments, the RNA compositions may be delivered by an oncolytic virus.

[00209] In some embodiments, more than one administration is delivered. In some embodiments, a catheter is placed into or near the site of the tumor for multiple administrations. In some embodiments, a catheter is placed at the site of removal of a tumor for multiple administrations.

[00210] In some embodiments, the subject is human. In some embodiments, the subject is a non-human mammal such as a dog, cat, mouse, rat, rabbit, sheep, cattle, horse and pig.

[00211] In some embodiments, RNA compositions are combined with another therapy. In some embodiments, RNA compositions are combined with more than one other therapy. In some embodiments, RNA compositions are combined in a multi-modal therapy.

[00212] In some embodiments, the other therapy is surgery to excise, resect, or debulk the tumor. In some embodiments, therapeutic RNA compositions are administered during a surgery to excise, resect, or debulk the tumor.

[00213] In some embodiments, the other therapy is radiotherapy. In some embodiments, the radiotherapy is external beam radiation therapy or particle beam radiation. In some embodiments, the radiotherapy is brachytherapy involving temporary or permanent implantation of radioactive isotopes directly into the tumor via catheter or large bore needle. In some embodiments, the radioactive isotope is ¹³⁷Cesium, ¹⁹²Iridium, or radioactive iodine. In some embodiments, the radiotherapy is radioisotope preparations administered intravenously. In some embodiments, the radioisotope preparations are radioactive iodine (¹³¹I), Strontium (⁸⁹Sr), or Samarium (¹⁵³Sm).

[00214] In some embodiments, the other therapy is chemotherapy. In some embodiments, the chemotherapy is an alkylating agent, an antimetabolite, an anti-microtubule agent, a topoisomerase inhibitor, or a cytotoxic antibody.

[00215] In some embodiments, the chemotherapy comprises anti-invasion agents (e.g., metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function). In some embodiments, the chemotherapy comprises inhibitors of growth factor function (e.g., platelet derived growth factor and hepatocyte growth factor), growth factor antibodies, or growth factor receptor antibodies, (e.g., anti-erbb2 antibody trastuzumab [Herceptin™] and the anti-erbb1 antibody cetuximab™). In some embodiments, the chemotherapy is a farnesyl transferase inhibitor. In some embodiments, the chemotherapy is a tyrosine kinase inhibitor such as inhibitors of the epidermal growth factor family (e.g., EGFR family tyrosine kinase inhibitors such as gefitinib (Iressa™), erlotinib (Tarceva™), and Canertinib (CI 1033), or a serine/threonine kinase inhibitor).

[00216] In some embodiments, the chemotherapy comprises antiproliferative/antineoplastic drugs such as antimetabolites (e.g., antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, tegafur, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (e.g., anthracyclines like adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (e.g., cisplatin, carboplatin); alkylating agents (e.g., nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimetotic agents (e.g., vinca alkaloids like vincristine, vinblastine, vindesine, vinorelbine, and taxoids like taxol, taxotere); topoisomerase inhibitors (e.g., epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan, camptothecin and also irinotecan); or thymidylate synthase inhibitors (e.g., raltitrexed).

[00217] In some embodiments, the chemotherapy is an antibody-drug conjugate (ADC). In some embodiments, the ADC is an antibody linked to a cytotoxic (anticancer)

drug. In some embodiments, the ADC allows targeted delivery of cytotoxic drugs to tumor cells. In some embodiments, the ADC allows preferential deliver of cytotoxic drugs to tumor cells versus normal tissue.

[00218] In some embodiments, the chemotherapy is combination chemotherapy with a combination of different agents. In some embodiments, the combination comprises different agents that have different mechanisms of action and/or different, non-overlapping toxicities.

[00219] In some embodiments, the other therapy is an immune stimulator or immunotherapy, such as, for example, a checkpoint modulator/inhibitor. Checkpoint modulators/inhibitors are well known in the art to prevent the host immune system from attacking itself, and include, for example, CTLA-4, PD1, PDL1, GITR, OX40, LAG-3, and TIM-3. In some embodiments, the immune stimulator, immunotherapy, or checkpoint modulator/inhibitor is a monoclonal antibody. In some embodiments, the monoclonal antibody is an antibody against PD1, PDL1, CTLA-4, LAG3, OX40, CD40, CD40L, 41BB, 41BBL, GITR, CD3, CD28, CD38, or TGFbeta. In some embodiments, the monoclonal antibody is a bispecific antibody. In some embodiments, the immune stimulator is a cell-based immunotherapy. In some embodiments, the immune stimulator is a cytokine or chemokine. In some embodiments, the immune stimulator is a cancer vaccine. As a wide range of immune stimulators would be known to scientists and clinicians skilled in the art, the invention is not limited to a specific combination with a particular immune stimulator.

[00220] In some instances, any of the RNAs, RNA compositions, medical preparations, and RNA combination compositions described herein may be administered in combination with an immune stimulator, immunotherapy, or checkpoint modulator. In some instances, the RNAs, RNA compositions, and RNA combination compositions described herein are administered in combination with an antibody to a subject to treat cancer, including solid tumors. In some embodiments, the antibody is an anti-PD1 antibody, an anti-CTLA4 antibody, or a combination of an anti-PD1 antibody and anti-CTLA4 antibody. In some embodiments, the antibody is a multi-specific antibody such as, for example, a tri-specific or bi-specific antibody.

[00221] In some embodiments, the anti-PD1 antibody is a chimeric, humanized or human antibody. In some embodiments, the anti-PD-1 antibody is isolated and/or recombinant. Examples of anti-PD-1 antibodies are nivolumab, pembrolizumab, cemiplimab, MEDI0608 (formerly AMP-514; see, e.g., WO 2012/145493 and U.S. Patent No. 9,205,148), PDR001 (see, e.g., WO 2015/112900), PF-06801591 (see, e.g., WO 2016/092419), BGB-A317 (see, e.g., WO 2015/035606).

[00222] In some embodiments, the anti-PD-1 antibody is one of those disclosed in WO 2015/112800 (such as those referred to as H1M7789N, H1M7799N, H1M7800N, H2M7780N, H2M7788N, H2M7790N, H2M7791N, H2M7794N, H2M7795N, H2M7796N, H2M7798N, H4H9019P, H4xH9034P2, H4xH9035P2, H4xH9037P2, H4xH9045P2, H4xH9048P2, H4H9057P2, H4H9068P2, H4xH9119P2, H4xH9120P2, H4xH9128P2, H4xH9135P2, H4xH9145P2, H4xH8992P, H4xH8999P and H4xH9008P in Table 1 of the PCT publication, and those referred to as H4H7798N, H4H7795N2, H4H9008P and H4H9048P2 in Table 3 of the PCT publication). The disclosure of WO 2015/112800 is incorporated by reference herein in its entirety. For example, the antibodies disclosed in WO 2015/112800 and related antibodies, including antibodies and antigen-binding fragments having the CDRs, VH and VL sequences, or heavy and light chain sequences disclosed in that PCT publication, as well as antibodies and antigen-binding fragments binding to the same PD-1 epitope as the antibodies disclosed in that PCT publication, can be used in conjunction with the RNA compositions of the present invention to treat and/or prevent cancer.

[00223] In related embodiments, the anti-PD-1 antibody may comprise the heavy and light chain amino acid sequences shown below as SEQ ID NOs: 79 and 80, respectively; the VH and VL sequences in SEQ ID NOs: 87 and 88 (shown in italics), or one or more (e.g., all six) CDRs in SEQ ID NOs: 79 and 80 (shown in bold boxes). In some embodiments, an antibody comprising the following CDRs is encompassed:

HCDR1 = GFTFSNFG (SEQ ID NO: 81)
 HCDR2 = ISGGGRDT (SEQ ID NO: 82)
 HCDR3 = VKWGNIFYDY (SEQ ID NO: 83)
 LCDR1 = LSINTF (SEQ ID NO: 84)
 LCDR2 = AAS (SEQ ID NO: 85)
 LCDR3 = QQSSNTPFT (SEQ ID NO: 86).

[00224] An exemplary antibody comprising a heavy chain comprising the VH and VL sequences in SEQ ID NOs: 87 and 88 (shown in italics) is the fully human anti-PD-1 antibody known as REGN2810 (cemiplimab).

[00225] Anti-PD-1 Mab heavy chain

*EVQLLES*GGV *LVQ*PGGSLRL SCAAS**GFTFS NFG**MTWVRQA PGKGLEWVSG **ISGGGRDT**YF
ADSVKGRFTI *SRDNSKNTLY* *LQMNS*LGED TAVYYC**VKWG NIYFDY**WGQG *TLVTVSS*AST
 KGPSVFPLAP CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY
 SLSSVVTVPSSSLGTKTYTC NVDHKPSNTK VDKRVESKYG PPCPPCPAPE FLGGPSVFLF
 PPKPKDTLMI SRTPEVTCVV VDVSEDEPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV
 SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP SQEEMTKNQV

SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS FFLYSRLTVD KSRWQEGNVF
SCSVMEALH NHYTQKSLSL SLGK (SEQ ID NO: 79)

HCDR1 = GFTFSNFG (SEQ ID NO: 81)
HCDR2 = ISGGGRDT (SEQ ID NO: 82)
HCDR3 = VKWGNIFYDY (SEQ ID NO: 83)

[00226] Anti-PD-1 Mab light chain

DIQMTQSPSS LSASVGDSIT ITCRASLSIN TFLNWNYYQOKP GKAPNLLIYA AS SLHGGVPS
RFSGSGSGTD FTLTIRTLQP EDFATYYCQQ SSNTPFTFGP GTVVDERRTV AAPSVFIFPP
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK (SEQ ID NO: 80)

LCDR1 = LSINTF (SEQ ID NO: 84)
LCDR2 = AAS (SEQ ID NO: 85)
LCDR3 = QQ SSNTPFT (SEQ ID NO: 86)

[00227] In some embodiments, the RNAs, RNA compositions, and RNA combination compositions may be delivered via injection into the tumor (e.g., intratumorally), near the tumor (peri-tumorally), or near the site of a tumor removal, and the antibody may be delivered in the same manner or systemically, such as, for example, enteral or parenteral, including, via injection, infusion, and implantation. "Administered in combination" includes simultaneous or sequential administration. If sequential, administration can be in any order and at any appropriate time interval known to those of skill in the art.

[00228] In some embodiments, the other therapy is hormonal therapy. In some embodiments, the hormonal therapy is antiestrogen drugs for treatment of breast cancer or anti-androgen drugs for treating prostate cancer. Example agents include antiestrogens (e.g., tamoxifen, toremifene, raloxifene, droloxifene, idoxifene), estrogen receptor down regulators (e.g., fulvestrant), progestogens (e.g., megestrol acetate), aromatase inhibitors (e.g., anastrozole, letrozole, vorazole, exemestane), antiprogestogens, antiandrogens (e.g., flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (e.g., goserelin acetate, luprolide, buserelin), and inhibitors of 5-alpha-reductase (e.g., finasteride).

[00229] In some embodiments, the other therapy is a targeted therapy. In some embodiments, the targeted therapy is a kinase inhibitor. In some embodiments, the targeted therapy is one that inhibits activity of a gene product of a proto-oncogene. In some embodiments, the targeted therapy is an anti-angiogenic agent. In some embodiments, the targeted therapy is one directed to modulate activity of VEGF, BCR-ABL, BRAF, EGFR, c-Met, MEK, ERK, mTOR, or ALK.

- [00230] In some embodiments, the other therapy is stem cell transplantation.
- [00231] In some embodiments, therapeutic RNA compositions are delivered at the same time as another therapy.
- [00232] In some embodiments, therapeutic RNA compositions are delivered before another therapy.
- [00233] In some embodiments, therapeutic RNA compositions are delivered after another therapy.
- [00234] In some embodiments, therapeutic RNA compositions are delivered directly into the tumor, or near the tumor or the site of tumor removal together with another therapy. In some embodiments, therapeutic RNA compositions are delivered directly into the tumor, or near the tumor or site of tumor removal while another agent is delivered systemically.

IV. Pharmaceutical Formulations

- [00235] In some embodiments, any of the DNAs, RNAs, and compositions described herein are pharmaceutical formulations. In some embodiments, the pharmaceutical formulations comprise a diluent, excipient, or other pharmaceutically acceptable carrier. Thus, provided herein are pharmaceutical compositions comprising the DNA, RNA, compositions, or combinations thereof provided herein, and a pharmaceutically acceptable excipient. In certain embodiments, the pharmaceutically acceptable excipient is an aqueous solution. In certain embodiments, the aqueous solution is a saline solution. As used herein, pharmaceutically acceptable excipients are understood to be sterile. In some embodiments, a pharmaceutical composition is administered in the form of a dosage unit. For example, in certain embodiments, a dosage unit is in the form of a tablet, capsule, implantable device, or a bolus injection.
- [00236] In some embodiments, the pharmaceutical compositions provided herein may additionally contain other adjunct components conventionally found in pharmaceutical compositions. For example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents. The compositions may also contain additional, compatible, pharmaceutically-inactive materials such as excipients, diluents, and carriers.
- [00237] Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents

and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

[00238] Lipid moieties may be used to deliver the RNAs provided herein. In one method, the RNA is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In another method, RNA complexes with mono- or polycationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue.

[00239] In some embodiments, a pharmaceutical composition provided herein comprises a polyamine compound or a lipid moiety complexed with the DNA or RNA provided herein.

[00240] In some embodiments, a pharmaceutical composition provided herein comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

[00241] Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. In certain embodiments, a pharmaceutical composition provided herein comprises a RNA or combination of RNAs in a therapeutically effective amount. In certain embodiments, the therapeutically effective amount is sufficient to treat or prevent cancer in the subject being treated.

[00242] The following clauses provide numerous embodiments and are non-limiting:

Clause 1. A composition comprising RNA encoding an IL-12sc protein, RNA encoding an IL-15 sushi protein, RNA encoding an IFN α protein, and RNA encoding a GM-CSF protein.

Clause 2. The composition of clause 1, wherein the IFN α protein is an IFN α 2b protein.

Clause 3. The composition of clause 1, wherein (i) the RNA encoding an IL-12sc protein comprises the nucleotide sequence of SEQ ID NO: 17 or 18, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to

the nucleotide sequence of SEQ ID NO: 17 or 18 and/or (ii) the IL-12sc protein comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14.

Clause 4. The composition of clause 1, wherein (i) the RNA encoding an IL-15 sushi protein comprises the nucleotide sequence of SEQ ID NO: 26, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 26 and/or (ii) the IL-15 sushi protein comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 24.

Clause 5. The composition of clause 1, wherein (i) the RNA encoding an IFN α protein comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 22 or 23 and/or (ii) the IFN α protein comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 19.

Clause 6. The composition of clause 1, wherein (i) the RNA encoding a GM-CSF protein comprises the nucleotide sequence of SEQ ID NO: 29, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 29 and/or (ii) the GM-CSF protein comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

Clause 7. The composition of clause 1, wherein at least one RNA comprises a modified nucleoside in place of at least one uridine, wherein the modified nucleoside is pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ), 5-methyl-uridine (m5U), or a combination thereof.

Clause 8. The composition of clause 1, wherein each RNA comprises a modified nucleoside in place of at least one uridine, wherein the modified nucleoside is pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ), 5-methyl-uridine (m5U), or a combination thereof.

Clause 9. The composition of clause 1, wherein at least one RNA comprises the 5' cap $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$, or 3'-O-Me- $m^7G(5')ppp(5')G$, or $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$, or 3'-O-Me- $m^7G(5')ppp(5')G$.

Clause 10. The composition of clause 1, wherein at least one RNA comprises a 5' UTR comprising (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6 and/or a 3' UTR comprising (i) the nucleotide sequence of SEQ ID NO: 8, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.

Clause 11. The composition of clause 1, wherein at least one RNA comprises a poly-A tail of at least 100 nucleotides.

Clause 12. The composition of clause 11, wherein the poly-A tail comprises the poly-A tail shown in SEQ ID NO: 78.

Clause 13. The composition of clause 1, wherein one or more RNA comprises:

- a 5' cap comprising $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$;
- a 5' UTR comprising (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6;
- a 3' UTR comprising (i) the nucleotide sequence of SEQ ID NO: 8, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO:8; and
- a poly-A tail comprising at least 100 nucleotides.

Clause 14. The composition of clause 13, wherein the poly-A tail comprises SEQ ID NO: 78.

Clause 15. A pharmaceutical composition comprising the composition of clause 1 and a pharmaceutically acceptable carrier, diluent and/or excipient.

Clause 16. A method for treating or reducing the likelihood of a solid tumor comprising administering to a subject in need thereof the composition of clause 1.

Clause 17. A method for treating or reducing the likelihood of a solid tumor comprising administering to a subject in need thereof a first RNA, wherein the first RNA encodes

an IL-12sc protein, an IL-15 sushi protein, an IFN α protein, or a GM-CSF protein, and additional RNA, wherein:

- if the first RNA encodes an IL-12sc protein, then the additional RNA encodes an IL-15 sushi protein, an IFN α protein, and a GM-CSF protein;
- if the first RNA encodes an IL-15 sushi protein, then the additional RNA encodes an IL-12sc protein, an IFN α protein, and a GM-CSF protein;
- if the first RNA encodes an IFN α protein, then the additional RNA encodes an IL-15 sushi protein, an IL-12sc protein, and a GM-CSF protein; and
- if the first RNA encodes a GM-CSF protein, then the additional RNA encodes an IL-15 sushi protein, an IFN α protein, and an IL-12sc protein.

Clause 18. The method of clause 17, wherein the first RNA is administered to the subject at the same time as the additional RNA.

Clause 19. The method of clause 17, wherein the RNA is administered intra-tumorally or peri-tumorally.

Clause 20. The method of clause 17, wherein the subject is further treated with an additional therapy comprising (i) surgery to excise, resect, or debulk a tumor, (ii) immunotherapy, (iii) radiotherapy, or (iv) chemotherapy.

Clause 21. The method of clause 17, wherein the subject is further treated with a checkpoint modulator.

Clause 22. The method of clause 21, wherein the checkpoint modulator is an anti-PD1 antibody, an anti-CTLA-4 antibody, or a combination of an anti-PD1 antibody and an anti-CTLA-4 antibody.

Clause 23. The method of clause 21, wherein the RNA is administered intra-tumorally or peri-tumorally via injection, and the checkpoint modulator is administered systemically.

Clause 24. The method of clause 17, wherein the solid tumor is a sarcoma, carcinoma, or lymphoma.

Clause 25. The method of clause 17, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.

Clause 26. The method of clause 17, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma

tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.

Clause 27. The method of clause 17, wherein treating or reducing the likelihood of the solid tumor comprises reducing the size of a tumor, reducing the likelihood of a reoccurrence of cancer in remission, or reducing the likelihood of cancer metastasis in the subject.

Clause 28. A combination therapy method for treating or reducing the likelihood of a solid tumor, comprising administering to a subject in need thereof RNAs and a further therapy, wherein the RNAs encode an IL-12sc protein, an IL-15 sushi protein, an IFN α protein, and a GM-CSF protein, and the further therapy comprises immunotherapy, chemotherapy, or a checkpoint modulator.

Clause 29. The combination therapy method of clause 28, wherein the further therapy comprises an anti-PD1 antibody, an anti-CTLA-4 antibody, or a combination of an anti-PD1 antibody and an anti-CTLA-4 antibody.

Clause 30. An isolated nucleic acid comprising a sequence encoding an IL-12sc protein, wherein:

the sequence encoding the IL-12sc protein comprises a) contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 78% identity to nucleotides 1-984 of SEQ ID NO: 16 or 18, b) contiguous nucleotides having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 81% identity to nucleotides 1027-1623 of SEQ ID NO: 16 or 18, and c) nucleotides encoding a linker between the nucleotides of a) and b).

Clause 31. The nucleic acid of clause 30, which is a DNA.

Clause 32. The nucleic acid of clause 30, which is an RNA.

Clause 33. An isolated nucleic acid comprising a sequence encoding an IFN α protein, wherein:

the sequence encoding the IFN α protein has at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 21 or 23.

Clause 34. The nucleic acid of clause 33, which is a DNA.

Clause 35. The nucleic acid of clause 33, which is an RNA.

Clause 36. A method for treating or reducing the likelihood of a solid tumor comprising administering to a subject in need thereof the RNA of clause 34, wherein:

the subject is further treated with additional RNA encoding an IL-15 sushi protein, an IFN α protein, and a GM-CSF protein.

Clause 37. A method for treating or reducing the likelihood of a solid tumor comprising administering to a subject in need thereof the RNA of clause 35, wherein:
the subject is further treated with additional RNA encoding an IL-15 sushi protein, an IL-12sc protein, and a GM-CSF protein.

Clause 38. A method of producing an RNA encoding IL-12sc, comprising contacting an expression construct comprising the nucleic acid of clause 32 operably linked to a promoter with an RNA polymerase under conditions permissive for transcription.

Clause 39. A method of producing an RNA encoding IFN α , comprising contacting an expression construct comprising the nucleic acid of clause 35 operably linked to a promoter with an RNA polymerase under conditions permissive for transcription.

Clause 40. A kit comprising the composition of clause 1.

Clause 41. A kit comprising RNA encoding an IL-12sc protein, RNA encoding an IL-15 sushi protein, RNA encoding an IFN α protein, and RNA encoding a GM-CSF protein, wherein the RNAs are not in the same container.

Clause 42. The kit of clause 41, wherein each RNA is in a separate container.

Clause 43. The kit of any one of clause 40-42, further comprising instructions for use of the composition or RNAs for treating or reducing the likelihood of a solid tumor.

[00243] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about,” to the extent they are not already so modified. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00244] It is noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the,” and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term

“include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

EXAMPLES

[00245] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1 - Materials and Methods

[00246] **B16F10 Tumor Model:** Female C57BL/6J mice (Jackson Laboratory; Bar Harbor, ME), 6-8 weeks-old and weighing between 17.0 and 20.9 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA) and sterile water and housed on 12 hours light/dark cycle at 22°C ± 2°C with a relative humidity of 55% ± 15%. B16F10 cells were obtained from the American Type Culture Collection (ATCC) (Manassas, Virginia USA) (Cat No. CRL-6475) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Life technologies, Cat No. 11995) supplemented with 10% heat inactivated Fetal Bovine Serum (HI FBS) (Life technologies, Cat No. 10082-147) in 5% CO₂ at 37°C. The cells were harvested using 0.25% Trypsin-EDTA (Life technologies, Cat No. 25200-056), resuspended in Dulbecco's phosphate-buffered saline (DPBS) (Life technologies, Cat No. 14190-144), and 0.5 x 10⁶ cells/200 µl per mouse subcutaneously (SC) implanted into the right flank of female C57BL/6J mice. For dual flank tumor models on day 0, 0.5 x 10⁶ B16F10 cells/200 µl per mouse were subcutaneously (SC) implanted into the right flank and 0.25 x 10⁶ B16F10 cells/200 µl per mouse were SC implanted into the left flank. To test whether local administration of cytokine mRNA can have distant effects (see FIG 19), female C57BL/6J OlaHsd mice (Envigo, Rossdorf, Germany), 6-8 weeks of age, with a weight between 17 and 24 g, were acclimated for at least six days prior to study enrollment. Mice had free access to food (ssniff M-Z autoclavable Soest, Germany) and sterile water and housed on 12 hours light/dark cycle at 22°C ± 2°C with a relative humidity of 55% ± 10%. B16F10 cells were obtained from the American Type Culture Collection (ATCC® CRL-6475™) and cultured in DMEM, high glucose, GlutaMAX™ (Life technologies, Cat No. 31966047) supplemented with 10% Fetal Bovine Serum (FBS) (Biochrom, Cat No. S 0115) in 7.5% CO₂ at 37°C. The cells were harvested using StemPro® Accutase® Cell Dissociation Reagent (Life technologies, Cat No. A1110501), re-suspended in Dulbecco's phosphate-buffered saline (DPBS) (Life technologies, Cat No. 14190-169), and 0.3 x 10⁶

cells/100 μ l per mouse subcutaneously (SC) were implanted into the right shaven flank of female C57BL/6J mice. For seeding of distant lung tumors, B16F10_luc-gfp cells were used. These cells were derived from B16F10 by stable transfection with a plasmid coding for Luciferase and GFP. B16F10_luc-gfp cells were cultured with the same conditions as the B16F10; only 0.5 μ g/mL Puromycin was added to the culture medium. One day after SC implantation 0.3×10^6 cells/200 μ l of B16F10_luc-gfp cells were injected per mouse intravenously (IV) into the tail vein. Intratumoral mRNA injections were initiated 10-14 days after SC inoculation of the tumors. Tumor growth was assessed by caliper measurements every 2-3 days and is expressed as the product of the perpendicular diameters using the following formula: $a^2 \cdot b/2$, with $a < b$. Engraftment of luciferase-positive tumor cells in the lung was analyzed by in vivo bioluminescence imaging using the Xenogen IVIS Spectrum imaging system (Caliper Life Sciences). An aqueous solution of L-luciferin (250 μ l, 1.6 mg; BD Biosciences) was injected intraperitoneally. Emitted photons from live animals quantified 10 min later with an exposure time of 1 min. Regions of interest (ROI) were quantified as average radiance (photons $s^{-1} cm^{-2} sr^{-1}$), represented by as color-scaled images superimposed on grayscale photos of mice using the Living Image software from Caliper Life Sciences). For absolute quantification, the bioluminescence signal was blotted as total flux (photons s^{-1}). For studies performed in Figures 20C-G and 21E-I the above described model of C57BL/6J OlaHsd mice (Envigo, Rosdorf, Germany) without seeding of lung metastasis was used as well.

[00247] **CT26 tumor model:** For studies performed in Figures 2, 3, 7D-F, 8, 9, 12D and 21, female Balb/c Rj mice (Janvier, Genest-St.-Isle, France), 6-8 weeks of age, with a weight between 17 and 24 g, were acclimated for at least six days prior to study enrollment. Mice had free access to food (ssniff M-Z autoclavable Soest, Germany) and sterile water and housed on 12 hours light/dark cycle at $22^\circ C \pm 2^\circ C$ with a relative humidity of $55\% \pm 10\%$. CT26 cells were obtained from the (ATCC® CRL-2638™) and cultured in Roswell Park Memorial Institute medium (RPMI) 1640 Medium, GlutaMAX™ (Life technologies, Cat No. 61870-044) supplemented with 10% Fetal Bovine Serum (FBS) (Biochrom, Cat No. S 0115) in 5% CO₂ at $37^\circ C$. The cells were harvested using StemPro® Accutase® Cell Dissociation Reagent (Life technologies, Cat No. A1110501), resuspended in DPBS (Life technologies, Cat No. 14190-169), and 0.5×10^6 cells/100 μ l per mouse SC implanted into the right shaven flank of female Balb/c Rj mice. Intratumoral RNA injections were initiated 13-19 days after inoculation of the tumors. Tumor growth was assessed by caliper measurements

every 2-3 days and is expressed as the product of the perpendicular diameters using the following formula: $a \times b / 2$ where b is the longer of the two diameters ($a < b$).

[00248] For studies performed in Figures 6, and 7A-C female BALB/c mice (Jackson Laboratory; Bar Harbor, ME), 6-8 weeks-old and weighing between 17.0 and 20.9 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA) and sterile water and housed on 12 hours light/dark cycle at a temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$), relative humidity ($55\% \pm 15\%$). CT26 cells were obtained from the ATCC (Manassas, Virginia USA) (Cat No. CRL-2638) and cultured in RPMI-1640 (Life technologies, Cat No. 11875-093) supplemented with 10% HI FBS (Life technologies, Cat No. 10082-147) in 5% CO₂ at 37°C. The cells were harvested using 0.25% Trypsin-EDTA (Life technologies, Cat No. 25200-056), re-suspended in DPBS (Life technologies, Cat No. 14190-144), and 0.5×10^6 cells/200 μL per mouse SC implanted into the right flank of BALB/c female mice.

[00249] In CT26 tumor model in addition to tumor growth gp70-reactive CD8⁺ T-cells were measured in blood where indicated. Blood samples were taken using EDTA-coated tubes. 100 μL of blood was transferred to FACS tubes and antibody mixture was added containing T-Select H-2Ld MuLV gp70 Tetramer-SPSYVYHQF-APC (MBL (TS-M-521-2), 4 μL for 100 μL blood), anti-CD8a FITC (life technologies (MCD801), 1 μL for 100 μL blood)) and anti-CD45 V500 (BD (561487), 1 μL for 100 μL blood)). After 20 min incubation at room temperature Blood Lysis Buffer (BD (349202), 300 μL per tube) was added and incubated for further 6 min. Then samples were washed twice with PBS-EDTA buffer. FACS samples were analyzed on a FACS Canto II flow cytometer.

[00250] **MC38 tumor model:** Female C57BL/6J mice (Jackson Laboratory; Bar Harbor, ME), 6-8 weeks-old and weighing between 17.0 and 20.9 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA) and sterile water and housed on 12 hours light/dark cycle at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a relative humidity of $55\% \pm 15\%$. MC38 cells were generous gifts from Dr S. A. Rosenberg (National Institute of Health, Bethesda, MD, USA). The cell line was cultured in RPMI-1640 with L-glutamine (Gibco, Cat No. 11875) supplemented with 10% HI FBS (Gibco, Cat No. 100082) in 5% CO₂ at 37°C. The cells were harvested, re-suspended in DPBS (Gibco, Cat No. 14190), and 1×10^6 cells/200 μL per mouse SC implanted into the right flank of female C57BL/6J mice.

[00251] **A375 tumor model:** Female severe combined immune deficiency (SCID) mice (Jackson Laboratory; Bar Harbor, ME), 6-8 weeks-old and weighing between 17.0 and

20.9 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA), sterile water and housed on 12 hours light/dark cycle at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a relative humidity of $55\% \pm 15\%$. A375 cells were obtained from the ATCC (Manassas, Virginia USA) (Cat No. CRL-1619). The cell line was cultured in DMEM (Life technologies, Cat No. 11995) supplemented with 10% HI FBS (Life technologies, Cat No. 10082-147) in 5% CO_2 at 37°C . The cells were harvested using 0.25% Trypsin-EDTA (Life technologies, Cat No. 25200-056), re-suspended in DPBS (Life technologies, Cat No. 14190-144), and $3.0 \times 10^6/100 \mu\text{l}$ PBS were mixed with 100 μl BD Matrigel Matrix (BD, Cat No. 354234) and implanted SC into the right flank of female SCID mice.

[00252] KM12 (CRC) Xenograft Model: Female NOD.CB17-Prkdcscid/SCID mice (Jackson Laboratory, Bar Harbor, ME), 10-weeks-old and weighing between 17.3 g and 21.9 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA), sterile water and were housed on 12 hours light/dark cycle at $(22 \pm 2^{\circ}\text{C})$ with a relative humidity $(55 \pm 15\%)$. KM-12 cells were obtained from the American National Cancer Institute (NCI) (Cat No. 507345). The cells were grown in RPMI medium 1640 with L-glutamine (Gibco, Cat No. 11875) supplemented with 10% HI FBS (Gibco, Cat No. 10082), and incubated at 37°C with 5% CO_2 . The cells were harvested using 0.25% Trypsin- EDTA (Gibco, Cat No. 25200), re-suspended in DPBS (Gibco, Cat No. 14190), and for each mouse 5.0×10^6 cells in 200 μl DPBS with 50% matrigel (BD, Cat No. 356234) were SC implanted into the right flank of female SCID mice.

[00253] RPMI8226 (Myeloma) Xenograft Model: Female NSG mice (Jackson Laboratory, Bar Harbor, ME), 12-weeks-old and weighing between 19.8 g and 26.6 g were acclimated for at least three days prior to study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA), sterile water and were housed on 12 hours light/dark cycle at $(22 \pm 2^{\circ}\text{C})$ with a relative humidity $(55 \pm 15\%)$. RPMI8226 cells were obtained from the ATCC (Cat No. CCL-155). The cells were grown in RPMI medium 1640 with L-glutamine (Gibco, Cat No. 11875) supplemented with 10% HI FBS (Gibco, Cat No. 10082), and incubated at 37°C with 5% CO_2 . The cells were harvested using 0.25% Trypsin-EDTA (Gibco, Cat No. 25200), re-suspended in DPBS (Gibco, Cat No. 14190), and for each mouse 5.0×10^6 cells in 200 μl DPBS with 50% matrigel (BD, Cat No. 356234) were SC implanted into the right flank of female NSG mice.

[00254] NCI-N87 (Gastric) Xenograft Model: Female NOD.CB17-Prkdcscid/SCID (Jackson Laboratory, Bar Harbor, ME), 11-weeks-old and weighing between 18.3 and 22.7 g

were acclimated for at least three days before the study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA), sterile water and were housed on 12 hours light/dark cycle at ($22 \pm 2^\circ\text{C}$) with a relative humidity ($55 \pm 15\%$). NCI-N87 cells were obtained from the ATCC (Cat No. CRL-5822). The cells were grown in RPMI medium 1640 with L-glutamine (Gibco, Cat No. 11875) supplemented with 10% HI FBS (Gibco, Cat No. 10082), and incubated at 37°C with 5% CO_2 . The cells were harvested using 0.25% Trypsin-EDTA (Gibco, Cat No. 25200), re-suspended in DPBS (Gibco, Cat No. 14190), and for each mouse 3.0×10^6 cells in 200 μl DPBS with 50% matrigel (BD, Cat No. 356234) were SC implanted into the right flank of female SCID mice.

[00255] **NCI-H1975 (NSCLC) Xenograft Model:** Female NSG mice (Jackson Laboratory, Bar Harbor, ME), 10-weeks-old and weighed between 18.8 g and 26.0 g were allowed to acclimate for at least three days before study enrollment. Mice had free access to food (Harlan 2916 rodent diet, Massachusetts, USA), sterile water and were housed on 12 hours light/dark cycle at ($22 \pm 2^\circ\text{C}$) with a relative humidity ($55 \pm 15\%$). NCI-H1975 cells were obtained from the ATCC (Cat No. CRL-5908) and cultured in RPMI medium 1640 with L-glutamine (Gibco, Cat No. 11875) supplemented with 10% HI FBS (Gibco, Cat No. 10082), and incubated at 37°C with 5% CO_2 . The cells were harvested using 0.25% Trypsin-EDTA (Gibco, Cat No. 25200), re-suspended in DPBS (Gibco, Cat No. 14190), and for each mouse 5.0×10^6 cells in 200 μl DPBS with 50% matrigel (BD, Cat No. 356234) were SC implanted into the right flank of female NSG mice.

[00256] **Tumor re-challenge:** Female C57BL/6J mice were implanted with B16F10 cells as described above. Mice were treated with 4 intratumoral injections (80 μg mRNA/20 μg per target) on days 11, 13, 15, and 17 with ModB cytokine mRNA mixture (IL-15sushi, IL-12sc, GM-CSF, IFN α). After cytokine mRNA treatment 8 mice were tumor free. Four weeks after the last cytokine mRNA treatment tumor free mice were re-challenged with 0.5×10^6 B16F10 cells/200 μl per mouse by SC injection and tumor growth was monitored.

[00257] **Tumor monitoring:** Tumors were measured with a caliper twice weekly until final sacrifice. When a tumor size reached approximately 2000 mm^3 or there are animal health issues (20% area of a tumor ulcerated), animals were euthanized. Tumor regression was defined as i) tumor volume $< 20 \text{ mm}^3$ at the end of the study or ii) $T^F/T^0 < 1$, where the T^F equals the final tumor volume and T^0 equals tumor volume on the day of the first intratumoral mRNA injection.

[00258] **mRNA modification A (ModA):** Synthetic DNA fragments coding for the gene of interest were cloned into a common starting vector, comprising a 5'-UTR

(corresponding in some cases to SEQ ID NO: 1), a 3' UTR consisting of two elements called F and I (corresponding in some cases to SEQ ID NO: 7), and a poly(A)-tail of 110 nucleotides in total (A30-linker-A70 structure; corresponding in some cases to SEQ ID NO: 78). Linearization of plasmid DNA was performed downstream of the poly(dA:dT) with a class IIS restriction enzyme to generate a template with no additional nucleotide beyond the poly(dA:dT) (*see* Holtkamp et al., *Blood* 108(13):4009-172006 (2006)). Linearized plasmid DNA was subjected to in vitro transcription with T7 RNA polymerase (Thermo Fisher) as previously described (*see* Grudzien-Nogalska E et al., *Methods Mol Biol.* 969:55-72 (2013)) in the presence of 7.5 mM ATP, CTP, UTP, GTP and 6 mM D1, a beta-S-antireverse cap analogue (beta-S-ARCA, Cap0) (*see* Kuhn AN et al, *Gene Ther.* 17(8):961-71 (2010)). RNA was purified using magnetic particles (*see* Berensmeier S. *Appl Microbiol Biotechnol.* 73(3):495-504 (2006)), and RNA concentration and quality were assessed spectrophotometry and analyzed by capillary gel electrophoresis systems, respectively.

[00259] **mRNA modification B (ModB):** Synthetic DNA fragments coding for the gene of interest were cloned into a common starting vector, comprising a 5'-UTR (corresponding in some cases to the Tobacco Etch Viral leader sequences TEV, SEQ ID NO: 3), a 3' UTR consisting of two elements called F and I (corresponding in some cases to SEQ ID NO: 7), and a poly(A)-tail of 110 nucleotides in total (A30-Linker-A70 structure). Upon linearization of plasmid DNA as described above, in vitro transcription with T7 RNA polymerase (Thermo Fisher) was performed. This was carried out as described for ModA, but no cap structure was added to the reaction and UTP was substituted for N1-methyl-pseudouridinetriphosphate. RNA was then purified using magnetic particles (*Berensmeier 2006*), and subsequently Cap1 structure was enzymatically introduced using a commercially available system based on the Vaccinia virus capping enzyme (NEB) and addition of mRNA Cap 2'-O-methyltransferase (NEB). Afterwards, the RNA was subjected to a further purification procedure by Cellulose-based chromatography to remove double-stranded RNA impurities (*see* Day PR et al, *Phytopathology* 67:1393 (1977); Morris TJ et al., *Phytopathology* 69:854-858 (1979); and Castillo A et al., *Virol J.* 8:38 (2011)). RNA concentration and quality were assessed spectrophotometry and analyzed by capillary gel electrophoresis systems, respectively. Presence of dsRNA was assessed in a Northwestern dot-blot assay using dsRNA-specific J2 mAb (English & Scientific Consulting) as described in Karikó et al. *Nucleic Acids Res.* 39(21):e142 (2011).

[00260] **mRNA codon optimization:** The coding sequence of a protein may influence the efficiency as well as the accuracy of protein translation (*see* Bossi L et al., *Nature*. 286(5769):123-7 (1980) and Irwin et al., *J Biol Chem*. 270(39):22801-6 (1995)).

[00261] Therefore, different codon variants of each target were designed and tested. The design of the different codon variants for each target utilized publicly available software from Life Technologies GmbH GeneArt® (Regensburg, Germany) (*see* Raab D et al., *Syst Synth Biol*. 4(3):215-25 (2010)) and Eurofins MWG Operon (Ebersberg, Germany). In addition, codon optimization was performed manually editing each codon separately. A GC-content comparable to the wild type sequence was maintained during the optimization process.

[00262] Evaluation and selection of constructs was determined by in vitro expression performed using: (i) mRNA lipofection of HEK293T/17 cells and (ii) mRNA electroporation K562 cells.

[00263] Forty thousand (40,000) HEK293T/17 cells (ATCC® CRL-11268™) were seeded in flat bottom 96-well plates (VWR International, Cat No. 734-1794) in DMEM, high glucose, GlutaMAX™ (Life technologies, Cat No. 31966047) containing 0.5% FBS (Biochrom, Cat No. S 0115). Seeded cells in 96-well plates were incubated at 37°C, 7.5 % CO₂ for 16-18 hours. Adherent HEK293T/17 cells were transfected with RNA using Lipofectamine Messenger MAX Reagent (Invitrogen, Cat No. LMRNA) according to the manufacturer's protocol by adding 1.2 µl of the transfection reagent to 20 µl of OptiMEM (Thermo Fisher, Cat No. 31985070) in an RNase-free 1,5 ml Safe-Lock tube biopur (Eppendorf, Germany, Cat No. 0030121589); in a second tube the indicated RNA was added to 20 µl of OptiMEM. After 10 minutes of incubation the tube containing the RNA was diluted into the tube containing the Lipofectamine Messenger MAX and incubated an additional 5 minutes prior to adding 10 µl of the RNA-lipid-complex drop wise to one well of the 96-well plate containing the HEK293T/17 cell layer in 100 µl medium. The 10 µl RNA-lipid-complex contained 5 ng, 25 ng and 100 ng of target RNA respectively. The plates were placed into the incubator for 3h before an additional 140 µl of fresh medium (DMEM + 0,5% FBS) was added. The transfected cells were incubated for 15-18 hours and the supernatants were collected and analyzed for protein content by ELISA as described herein.

[00264] K562, a human cell line derived from chronic myeloid leukemia (ATCC® CCL-243™) was cultivated in RPMI 1640 Medium, GlutaMAX™ (Life technologies, Cat No. 61870-044) supplemented with 5% FBS. K562 were electroporated in a 96-well plate system as follows. Cells were washed once in X-VIVO15 medium (Lonza, Cat No. BE02-

060Q) and suspended to a final concentration of two hundred fifty thousand (250,000) cells/150 μ l in X-VIVO15. A 150 μ l of cell suspension was added per well of a 96-well plate containing 5 ng, 25 ng, or 100 ng of RNA. Cells and RNA were mixed and electroporation was performed in a 96 well Gene Pulser MX cell electroporation system from Biorad (250 V, 1 x 30 ms pulse). Immediately following electroporation, cells were transferred into a new culture plate with fresh medium without antibiotics and rested for 1 hour in the incubator at 37°C. The medium was exchanged for fresh RPMI 1640 GlutaMAX supplemented with 0.5% FCS and incubated 15-18 hours. Supernatants were harvested and analyzed for protein content by ELISA as described herein.

[00265] Protein concentrations were determined by ELISAs specific for the RNA encoded cytokine according to the manufacturer's protocol. (i) Human IL-15 sushi/IL-15 sushi R alpha Complex DuoSet ELISA (ii) Mouse IL-12sc Duo Set Development System (DY419-05) (iii) Mouse GM-CSF DuoSet ELISA Development Systems (DY415), all obtained from RnD systems, and mouse IFN α ELISA Kit (TCM) (PBL assay science, 42120-2).

[00266] For each mRNA target, the protein expression was evaluated for the wt-sequence and the different codon-optimized variants. Both data sets from lipofection of HEK293T/17 and electroporation of K562 each tested with the three different amounts of modified RNA were considered for selection of the protein coding sequence. A codon-optimized sequence was selected if an at least 1.5-fold increase of protein expression compared to WT sequence was measured. If this was not the case, the WT sequence was selected. For all constructs EarlI-restriction sequences were eliminated by mutating the DNA recognition sequence (5'-CTCTTC-3'), while preserving the WT amino acid.

[00267] **Cell lines:** HEK293 (ATCC CRL-1573) cell line and human melanoma cell lines, A101D (ATCC CRL-7798), A375 (ATCC CRL-1619), A2058 (ATCC CRL-11147), and Hs294T (ATCC HTB-140), were obtained from ATCC and cultured in DMEM (ThermoFisher Scientific, Cat 11885-084) supplemented with 10% FBS HI FBS Life Technologies, Cat. 10082) in a humidified atmosphere of 5% CO₂ at 37°C.

[00268] **mRNA transfection:** Cells were transfected using Lipofectamine MessengerMAX Reagent (Invitrogen, Cat # LMRNA001) according to the manufacturer protocol. Briefly, for each well 0.3 μ l of the transfection reagent was diluted with 5 μ l of the Opti-MEM media (Life Technologies, Cat. 31985062) and incubated for 10 min at room temperature; mRNA mixtures were diluted with Opti-MEM media (5 μ l per well) and mixed with diluted MessengerMax reagent, incubated for 5 min at room temperature and aliquoted

to the 96-well plate. Cells were diluted in complete growth media and 40,000 cells per well were added to the transfection mixtures. Cells were incubated for 24 hours at 37°C in a CO₂ incubator then media was collected and cytokine concentration was determined by Meso Scale Discovery (MSD) assay.

[00269] **Meso Scale Discovery Assay:** Cytokine concentration was determine using MSD assays: Proinflammatory Panel 1 (human) MSD kit (catalog N05049A-1) for IL12p70, Cytokine Panel 1 (human) MSD kit (catalog N05050A-1) for GM-CSF and IL-15 sushi, and Human IFN- α 2a Ultra-sensitive Kit (catalog N05050A-1) for IFN α . Data were analyzed using MSD Discovery Workbench V. 4.0.12 software and GraphPad Prism V.6.00 software.

[00270] **PBMC isolation and treatment:** Peripheral Blood Mononuclear Cells (PBMCs) were isolated by density gradient media (Ficoll-Paque) from a leukopak (Research Blood Components). 600,000 PBMCs were added per well of a 96-well plate. Cells were treated with a cytokine mixture for 24hrs and IFN γ production was measured in the cell culture media using Human IFN γ 384-Tissue Culture MSD Assay.

[00271] **Measuring CD8 response:** B16F10 tumor bearing mice received a single intratumoral injection of Immuno mRNA (IFN α , IL-15 sushi, GM-CSF, and IL-12sc, ModB) or control luciferase mRNA (Placebo). Seven days after intratumoral mRNA injection tumors were excised, processed for immunofluorescence and stained with an antibody for CD8 (gray). As shown in Figure 25, CD8 cells were present after cytokine mRNA intratumoral injection.

[00272] **Preparation of mRNA for in vivo studies:** The respective mRNA mixtures were prepared for in vivo studies by mixing equal quantities (micrograms) of mRNA in water at 2X the intended dose. The mRNA mixture was frozen at -80C until the day of intratumoral injection. On the day of injection, mRNA was thawed and mixed with an equal volume of 2X sterile Ringer's solution. The resulting 1X mRNA/Ringer solution was used for intratumoral injection.

[00273] **Gene expression analysis:** A375 tumors were homogenized in the RLT Buffer (Qiagen) using Precellys 24 homogenizer (Bertin Instruments). Total RNA was isolated with the RNeasy-96 Kit (Qiagen), following the spin protocol with the DNase treatment. RNA was eluted with nuclease-free water and quantified by ultraviolet absorbance using a NanoDrop 8000 (Thermo Scientific). cDNA synthesis was performed with the High Capacity RNA to cDNA Kit (Applied Biosystems) according to the manufacturer's recommendations. Real-time PCR were performed on a ViiATM7 (Applied Biosystems) according to standard protocol. Amplification was performed using the TaqMan Gene

Expression Master Mix (Applied Biosystems) and predesigned Taqman Assays (Applied Biosystems). Gene expression was normalized to the endogenous control GAPDH. Comparative ddCT method was used to evaluate gene expression.

Table 3

Gene	Assay ID	Lot Number	Fluorescent dye
ISG15	Hs01921425_s1	1548867	FAM
ISG54	Hs01922738_s1	1532771	FAM
Mx1	Hs00895608_m1	1539223	FAM
hGAPDH	4326317E	1311049	VIC

Example 2 - Combinations of three mRNAs reduce tumor volume in vivo

[00274] A mixture of modified mRNAs encoding GM-CSF, IL-2, and IL-12sc was injected into B16F10 tumor bearing mice and tumor growth was monitored to day 41. As shown in Figure 1, intratumoral injection of a combination of three mRNAs encoding GM-CSF, IL-2, and IL-12sc having ModA (SEQ ID NOs: 32, 38, and 56; FIG 1A), or a combination of three mRNAs encoding GM-CSF, IL-2, and IL-12sc having ModB (SEQ ID NOs: 35, 41, and 59; FIG 1B) induced regression in 6 out of the 10 mice, while mice treated with a control mRNA encoding luciferase (ModA) displayed tumor regression in 1 of 10 animals (FIG 1C). These data were confirmed in a repeat study of similar design (FIG 1D-1G). In the repeat experiment, a mixture of cytokine mRNA (GM-CSF, IL-2, IL-12sc) with ModA or ModB lead to tumor regression in 5 of 9 mice, while treatment with control mRNA in ModA and ModB displayed 2 of 9 and 0 of 9 tumor regressions, respectively.

[00275] The effects of cytokine mRNA treatment were evaluated in CT26 tumors. Mice with established CT26 tumors were injected with a cytokine mRNA mixture encoding GM-CSF, IL-2, and IL-12sc in ModA and ModB formats, respectively. Two control groups were included: i) mRNA Ringer's diluent and ii) ModA mRNA encoding firefly luciferase. A total of 6 intratumoral injections were administered on days 19, 21, 24, 26, 28 and 31. As shown in Figure 2, both GM-CSF, IL-2, and IL-12sc mRNA ModA (SEQ ID NOs: 56, 32, and 38; FIG2A) and ModB (SEQ ID NOs: 59, 35, and 41; FIG 2B) resulted in tumor regression in 5 and 6 out of 8 mice, respectively, while no tumors treated with control mRNA in ModA (FIG 2C) or Ringer's solution (FIG 2D) displayed tumor regression.

[00276] The cytokine mRNA mixture encoding IL-15 sushi, GM-CSF and IL-12sc (ModB; SEQ ID NOs: 53, 59, and 41) and IL-2, GM-CSF and IL-12sc (ModB; SEQ ID NOs:

35, 59, and 41) were evaluated for anti-tumor activity in the CT26 tumor model. Tumors received intratumoral mRNA injections on days 13, 15, 18, 20 and 22 after tumor inoculation. As shown in Figure 3, intratumoral injection of either the IL-2 mixture (FIG 3A) or IL-15 sushi mixture (FIG 3B) resulted in tumor regression in 5 out of 10 or 11 tumor-bearing animals, respectively (FIG 3A and B), whereas in the control group injected with luciferase mRNA (ModB) no tumor regression was observed (FIG 3C).

[00277] Anti-tumor activity of the mRNA mixture of IL-2, IL-12sc, and GM-CSF (FIG 4A-ModA [SEQ ID NOs: 32, 38, and 56], 4B-ModB [SEQ ID NOs: 35, 41, and 59]), or IL-15 sushi, IL-12sc, and GM-CSF or (FIG 4C-ModA [SEQ ID NOs: 50, 38, and 56], 4D-ModB [SEQ ID NOs: 53, 41, and 59]) was further evaluated in the B16F10 tumor model. Mice with B16F10 tumors were injected intratumorally with mRNA on days 11, 13, 15, and 17. Treatment of B16F10 tumors with the cytokine mRNA mixture that encoded IL-15 sushi, IL-12sc, GM-CSF resulted in tumor regression in 3 and 4 of 8 mice in ModA and ModB, respectively. Tumors treated with an mRNA mixture of IL-2, IL-12sc, and GM-CSF (ModA or ModB) had tumor regression in 4 out of 8 mice, while no tumor regression was noted for mice treated with ModA or ModB mRNA encoding luciferase (FIG 4E-ModA, FIG 4F-ModB).

Example 3 – Combinations of four mRNAs reduce tumor volume in vivo

[00278] We next tested the effect of adding a fourth mRNA to the cytokine mRNA mixture. B16F10 tumor bearing mice received four intratumoral injections of ModB cytokine mRNA mixture encoding: i) GM-CSF, IL-2, IL-12sc (SEQ ID NOs: 59, 35, and 41; FIG 5A), ii) GM-CSF, IL-15 sushi, IL-12sc (SEQ ID NOs: 59, 53, and 41; FIG 5B) and iii) GM-CSF, IL-15 sushi, IL-12sc, IFN α (SEQ ID NOs: 59, 53, 41, and 47; FIG 5C), and tumor growth was monitored to day 45. Each of the cytokine mRNA mixtures had an anti-tumor effect with 4 out of 8 tumors regressing following intratumoral injection of cytokine mRNA mixtures of GM-CSF, IL-2, and IL-12sc or GM-CSF, IL-15 sushi, and IL-12sc, and 7 out of 8 tumors regressed upon treatment with GM-CSF, IL-15 sushi, IL-12sc, and IFN α . Mice treated with control mRNA (ModB) exhibited no tumor regression (FIG 5D).

[00279] The anti-tumor activity of GM-CSF, IL-2, IL-12sc, and IFN α was examined in three different murine in vivo tumor models, CT26, B16F10 and MC38. Tumor bearing mice received 4-6 intratumoral injections of ModB cytokine mRNA encoding IL-2, IL-12sc, GM-CSF and IFN α (SEQ ID NOs: 35, 41, 59, and 47) or a control ModB mRNA encoding firefly luciferase. Anti-tumor activity was assessed in each tumor model. Mice treated with this combination of cytokine mRNA had 4/8, 7/8 and 5/5 regressing tumors in the CT26 (FIG

6A), B16F10 (FIG 6C) and MC38 (FIG 6E) models, respectively. No tumor regression was observed in the comparator tumor bearing mice treated with luciferase mRNA (FIGs 6B [CT26], 6D [B16F10], and 6F [MC38]).

[00280] The anti-tumor activity of a four-component cytokine mRNA mixture encoding each of IL-2, IL-12sc, GM-CSF and IFN α (ModB, SEQ ID NOs: 35, 41, 59, and 47) or IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB, SEQ ID NOs: 53, 41, 59, and 47) was evaluated in the CT26 tumor model. Tumor bearing mice were treated with 6 intratumoral injections and tumor growth was monitored. Treatment with both IL-2, IL-12sc, GM-CSF and IFN α (FIG 7A) and IL-15 sushi, IL-12sc, GM-CSF and IFN α (FIG 7B) effectively induced tumor regression in 4/8 and 8/8 mice, respectively, while no tumor regressions were observed for mice treated with the control mRNA (FIG 7C). These data were confirmed in a repeat study of similar design (FIG 7D-F).

[00281] A study in CT26 tumor model was conducted in which individual components were systematically removed from the mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB, SEQ ID NOs: 53, 41, 59, and 47). CT26 tumors were injected with cytokine mRNA on days 12, 15, 19 and 22 after inoculation. Tumors treated with four injections of mRNA encoding IL-15 sushi, IL-12sc, GM-CSF and IFN α induced regression in all 10 treated tumors (FIG 8A). Tumors treated with the ModB mRNA mixtures of i) IL-15 sushi, IL-12sc and IFN α , ii) IL-15 sushi, GM-CSF and IFN α , iii) IL-12sc, GM-CSF and IFN α , iv) IL-15 sushi, IL-12sc, and GM-CSF resulted in regression of 8, 6, 8, 7 out of 10 tumors, respectively (FIG 8B-E). No tumors treated with control mRNA (ModB) displayed tumor regression (FIG 8F). To analyze tumor growth kinetics, mean tumor volumes were calculated for each treatment group up to day 33 (FIG 8G). The smallest mean tumor volume was observed for mice treated with the mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α , while the largest mean tumor volume was observed in the luciferase treated animals. Tumor growth repression T/C (Tumor/Control based on mean tumor volume) was plotted to day 19 (FIG 8H) for each of the treatment groups. The four-component mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α exhibited the largest T/C.

[00282] Analogous to the study shown in Figure 8, a study was conducted in which individual components were systematically removed from the mRNA mixture of IL-2, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 35, 41, 59, and 47). CT26 tumors received 6 intratumoral mRNA injections on days 19, 21, 23, 26, 28 and 30. Treatment with the cytokine mRNA mixture of IL-2, IL-12sc, GM-CSF and IFN α resulted in tumor regression in 4 of 10 animals led to complete tumor regression in 4 animals and stable disease beyond day 40 in

two individuals (FIG 9A). Tumors treated with the ModB 3-component mRNA mixtures of i) IL-2, IL-12sc, and IFN α , ii) IL-2, GM-CSF and IFN α , iii) IL-12sc, GM-CSF and IFN α , and iv) IL-12sc, GM-CSF and IL-2 resulted in regression of 2, 3, 3 and 4 tumors, respectively (FIG 9B-E) and CT26 tumors treated with a control luciferase mRNA displayed no tumor regression (FIG 9F).

[00283] To analyze tumor growth kinetics, mean tumor volumes were calculated for each treatment group up to day 36. The smallest mean tumor volume was observed for mice treated with the mixture of IL-2, IL-12sc, GM-CSF and IFN α , while the largest mean tumor volume was observed in the luciferase treated animals (FIG 10 A). Tumor growth repression T/C (Tumor/Control based on mean tumor volume) was plotted to day 30 (FIG 10B) for each of the treatment groups. The four-component mixture of IL-2, IL-12sc, GM-CSF and IFN α exhibited the largest T/C.

[00284] The anti-tumor response from the different cytokine mRNA mixtures in Figure 9 were further analyzed as follows: The tumor volume change (ΔV) as defined by the difference in tumor volume between the measurement at the end of experiment or termination (V_t) and the measurement at the beginning of the experiment (V_b) ($\Delta V = V_t - V_b$), was calculated for all mice in each of the six treatment groups, including mice treated with 4 component cytokine mRNA mixture (IL-2, IL-12sc, GM-CSF and IFN α), mice treated with 3 component mixture missing one of the four cytokines and mice treated with control luciferase mRNA. Mean (μ) and standard deviation (σ) of a trimmed data (removing top and bottom 10% of the original data) on tumor volume change was calculated for control luciferase group and the Shapiro-Wilk normality test of this trimmed data showed it approximately followed a normal distribution. A Z score ($Z = (\Delta V - \mu) / \sigma$) was then calculated for tumor volume change data in each individual mouse from all treatment groups. A ratio (R) between each tumor volume change value and the trimmed mean of control group was calculated ($R = \Delta V / \mu$). Given the definitions, we consider the Z score as “significance of tumor volume reduction” and R as “extent of tumor volume reduction”. To identify a mouse that shows a significantly smaller tumor volume increase than that in control luciferase group (in other words “significant tumor reduction” as compared with control group), a cutoff of $Z \leq -1.645$ ($p(Z \leq -1.645) = 0.05$) and $R \leq 0.15$ was applied. The results showed that the number of mice in each treatment group that has significantly smaller tumor volume increase than control group as follows: i) 6 out of 8 mice for the cytokine mRNA mixture of IL-2, IL-12sc, GM-CSF and IFN α (ModB). ii) 2 of 8 mice for IL-2, GM-CSF and IFN α (ModB), iii)

3 of 8 for IL-12sc, GM-CSF and IFN α and IL-2, IL-12sc, and IFN α and iv) 4 of 8 mice treated with the cytokine mRNA mixture of IL-2, IL-12sc, and GM-CSF (ModB) (FIG 11).

[00285] Female C57BL/6J mice were implanted with B16F10 cells as described above. Mice were treated with 4 intratumoral injection (8 μ g mRNA/2 μ g per target) on days 11, 15, 19, and 23 with ModB cytokine mRNA mixture (IL-15 sushi, IL-12sc, GM-CSF, IFN α) or control luciferase mRNA. Treatment with the 4-component mixture of cytokine mRNA resulted in tumor rejection in 6/10 treated mice. See, FIG 24B. In comparison, no tumor free mice were observed in any of the groups treated with a single mRNA (FIGS 24C-F). The combination of IL-15 sushi, IL-12sc, GM-CSF, IFN α led to increased overall survival with 60% of the mice tumor free at Day 70, while all tumors of mice treated with a single cytokine mRNA progressing to the stage where the animals needed to be euthanized (FIG 24G).

Luciferase control is shown in FIG 24A.

Example 4 – Cytokine mRNA Protects Against Tumor Re-Challenge

[00286] To evaluate the effect of the cytokine mRNA mixture on the development of immunological memory, re-challenge experiments were performed. Briefly, B16F10 tumor bearing mice were treated with a cytokine mRNA mixture of IL-15sushi, IL-12sc, GM-CSF, and IFN α (Mod B; SEQ ID NOs: 53, 41, 59, and 47). A portion of the cytokine mRNA treatment B16F10 tumors completely regressed leading to tumor free animals. These tumor free animals were then re-challenged with B16F10 cells as a way to assess adaptive immune memory and 9 naïve mice were implanted with B16F10 tumor cells as a positive control for tumor engraftment (FIG 12A). All 9 naïve mice engrafted with B16F10 cells developed tumors, whereas all eight tumor-free mice rejected the B16F10 cells and did not exhibit growth of B16F10 tumors (FIG 12B). A portion of mice previously treated with cytokine mRNA develop localized vitiligo at the original site of the tumor (FIG. 12C). This experiment was essentially repeated and results are shown in FIG 33.

[00287] To evaluate the effect of the cytokine mRNA mixture on the development of immunological memory, a re-challenge experiment was performed using the CT26 tumor model. Therefore, CT26 tumor bearing mice were treated with a cytokine mRNA mixture of IL-15sushi, IL-12sc, GM-CSF, and IFN α (Mod B; SEQ ID NOs: 53, 41, 59, and 47). A portion of the cytokine mRNA treatment CT26 tumors completely regressed leading to tumor free animals. Three tumor free animals were then re-challenged with CT26 tumor cells and three tumor free animals were then re-challenged with CT26 tumor cells, in which the gp70 epitope (CT26- Δ gp70) was knocked out. 9 and 10, respectively, naïve mice were implanted

with CT26 tumor cells and CT26- Δ gp70 as a positive control for tumor engraftment. On day 21 after tumor inoculation 8 out of 9 naïve mice had engrafted with CT26 tumor cells developed tumors and all 10 naïve mice engrafted with CT26- Δ gp70 tumor cells developed tumors whereas all three tumor-free mice in each group rejected the CT26 and CT26- Δ gp70 cells and did not exhibit growth of CT26 tumors and CT26- Δ gp70, respectively (FIG 12D). This experiment shows that immunological memory upon cytokine mRNA injection in the CT26 tumor model is not restricted to T-cells specific for the immunodominant epitope gp70.

Example 5 – Systemic Anti-tumor Activity of Cytokine mRNA

[00288] To evaluate the ability of local intratumoral cytokine mRNA to exert a systemic anti-tumor response, mice were engrafted with B16F10 tumor cells on both the left and right flanks (FIG 13A). Mice bearing bilateral B16F10 tumors received four intratumoral injections with control mRNA encoding luciferase or a cytokine mRNA mixture encoding IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47). The right tumor was injected with mRNA at three different dose levels (80 μ g, 8 μ g, and 0.8 μ g mRNA corresponding to 20 μ g, 2 μ g and 0.2 μ g mRNA/target), while tumors on the left flank were untreated. Dose dependent anti-tumor activity was observed in both the injected (FIG 13B) and uninjected (FIG 13C) tumors with tumor growth inhibition ranging from 88% in the uninjected tumor to 96% in the injected tumor. Groups treated with cytokine mRNA treatment had increased median survival compared to groups treated with the Luciferase control mRNA (FIG 13D).

[00289] To further evaluate the ability of local intratumoral cytokine mRNA to exert a systemic anti-tumor response, mice were engrafted with B16F10 tumor cells on the right flank and received an IV injection of Luciferase-expressing B16F10 cells for induction of tumors in the lung (FIG 19A). On day 11, 14 and 18 after SC tumor implantation mice bearing B16F10 tumors received in total three intratumoral injections with cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47) into the flank tumor only, while tumors in the lung were untreated. The control group received intratumoral injection of equal amounts of control mRNA without any coding sequence. Mice were sacrificed for endpoint analysis on day 20, at which time lungs were taken out and weighed. Figure 19B shows exemplarily bioluminescence measurements of four animals and pictures of the according lungs taken out in order to visualize the dark tumor nodes. Tumor growth of SC tumors was strongly suppressed by injection of cytokine mRNA mixture, whereas tumors injected with control mRNA grew progressively as depicted in

Figure 19C showing mean tumor volume of 15 mice in each group. Lung tumor growth was suppressed in animals which received intratumoral cytokine mRNA injection in SC tumors when compared to animals treated with control mRNA; Figure 19D shows total flux analysis of bioluminescence measurements of all 15 animals per group on day 20, which is a correlate for tumor burden due to Luciferase-expressing tumor cells; line indicates median and asterisk indicates $p < 0.05$ analyzed by T-test. Additionally, lungs of animals treated with cytokine mRNA had significantly less weight (FIG 19E, line indicates median). Higher weight of lungs of animals treated with control RNA resulted from higher tumor burden.

Example 6 – Human Cytokine mRNA

[00290] To evaluate in vitro expression of the human cytokine mRNA, an mRNA mixture encoding the human cytokines IL-15 sushi, IL-12sc, GM-CSF, and IFN α 2b (SEQ ID Nos: 26, 18, 29, and 23) (ModB) were transfected into the HEK293 cell line along with four melanoma tumor cell lines (A375, A101D, A2058 and Hs294T) (FIG 14A). The cytokine mRNA mixture exhibited dose dependent expression and secretion across a panel of five human cell lines (FIG 14B-F).

[00291] The pharmacodynamic effects of the human mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b were evaluated in vitro with human peripheral blood mononuclear cells (PBMC). In short, human cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b (ModB) or the individual cytokine mRNAs encoding IL-12sc, IFN α 2b, IL-15 sushi or GM-CSF (ModB) were transfected in HEK293 cells and the conditioned media was collected at 24 hrs, diluted and added to human PBMC (FIG 15A). The median IFN γ levels from 6 donors treated with the cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b was 5623 pg/mL, while treatment with the individual cytokine mRNA for IL-12sc, IFN α 2b, IL-15 sushi or GM-CSF induced median IFN γ levels of 534, 67, 17, and 4 pg/mL, respectively (FIG 15B).

[00292] *In vivo* expression of the human cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b (ModB) and IL-2, IL-12sc, GM-CSF and IFN α 2b (ModB) was monitored in the A375 human melanoma xenograft. Tumor bearing mice received a single injection of cytokine mRNA and tumor samples were collected at 2 hrs, 4 hrs, 8 hrs, 24 hrs, 48 hrs and 72 hrs after mRNA injection. Tumor lysates were prepared and expression of the individual human cytokines IFN α 2b (FIG 16A), IL-2 (FIG 16B), IL-12sc (FIG 16C), IL-15 sushi (FIG 16D), GM-CSF (FIG 16E) were evaluated.

[00293] Time dependent expression was observed for each of the individual cytokines with the maximal concentration (Cmax) occurring between 2-8 hrs for the mixtures of IL-15

sushi, IL-12sc, GM-CSF and IFN α 2b (Table 4) and IL-2, IL-12sc, GM-CSF and IFN α 2b (Table 5).

Table 4: Pharmacokinetic results for the IL-15 sushi mixture

Tumor	GM-CSF	IFN α 2b	IL-12sc	IL-15sushi
$t_{1/2}$ (hrs)	12.4	9.59	15.2	10.2
Tmax (hrs)	4	4	8	4
Cmax (pg/mg)	1591	4862	3767	2639

Table 5: Pharmacokinetic results for the IL-2 mixture

Tumor	GM-CSF	IFN α 2b	IL-12sc	IL-2
$t_{1/2}$ (hrs)	14.8	10.2	15.5	12.8
Tmax (hrs)	2	2	8	2
Cmax (pg/mg)	2487	10685	4961	4274

[00294] Induction of the human interferon alpha genes ISG15, ISG54 and MX1 were monitored in the A375 tumors as a pharmacodynamics marker at 2h, 4h, 8h, 24h, 48h and 72h following mRNA injection of the cytokine mRNA mixtures of IL-15 sushi, IL-12sc, GM-CSF and IFN α 2b (ModB) and IL-2, IL-12sc, GM-CSF and IFN α 2b (ModB). Compared to control treated tumors, A375 tumors treated with cytokine mRNA displayed greater than 100-fold induction of ISG15 (FIG 17A), ISG54 (FIG 17B) and MX1 (FIG 17C) with peak induction occurring by 8 hrs after intratumoral mRNA injection.

Example 7 – Interferon effect

[00295] B16F10-tumor-bearing mice received intratumoral injections of ModA (“standard”) cytokine mRNA encoding IL-2, Flt3 ligand (FLT3L), 41BBL (also known as CD137L or tumor necrosis factor superfamily member 9), and CD27L-CD40L (this comprises a fusion protein of the soluble domain of CD27L also known as CD70, and CD40L; both the CD27L and the CD40L is comprised of three soluble domains of either CD27L or CD40L, all separated by GS-Linker sequences (FIG 18A, SEQ ID NOs: 32, 62, 68, and 74) or ModB (“modified”) cytokine mRNA encoding IL-2, FLT3L, 41BBL, and CD27L-CD40L (FIG 18B, SEQ ID NOs: 35, 65, 71, and 77). In addition, either ModA mRNA encoding IFN α (SEQ ID NO: 44) or ModB mRNA encoding IFN α (SEQ ID No: 47) was added to the ModA or ModB mRNA mixes, respectively (FIGS 18D and 18E). Anti-tumor activity was assessed.

[00296] Mice treated with this combination of ModA mRNA had 4/9 mice tumor-free without IFN α (FIG 18B) and 3/9 mice tumor-free with IFN α (FIG 18D). Therefore, treatment with IFN α mRNA did not appear to increase the response to the cytokines when mRNA was dosed in the ModA form.

[00297] In contrast, mice treated with the combination of ModB mRNA had 1/9 mice tumor-free without IFN α (FIG 18C) and 7/9 mice tumor-free with IFN α (FIG 18E). Thus, treatment with IFN α mRNA increased the response to the mixture of cytokines when mRNA was dosed in the ModB form.

Example 8 – Cytokine mRNA in Combination with Antibodies

[00298] To evaluate the effect of intratumoral injection of cytokine mRNA in combination with systemic administration of antibodies, mice were engrafted with B16F10 or MC38 tumor cells on both the left and right flanks. Mice received four intratumoral injections with cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47) into only one of the flank tumors on Days 11, 15, 19, 23, while the other flank tumor was left untreated. Mice also received intraperitoneal injection anti-PD1 antibody (Sanofi murinized version of rat IgG2a anti-mouse PD-1 clone RMP1-14 at 5mg/kg) on Days 10, 13, 16, 19, 22, 25. Groups were as follows: 1) control mRNA (80 μ g total mRNA; 50 μ L intratumoral injection at 1.6mg/mL plus control isotype antibody (clone MOPC-21 (BioLegend); 5mg/kg); 2) control mRNA plus anti-PD1 antibody; 3) cytokine mRNA plus control isotype antibody; and 4) cytokine mRNA plus anti-PD1. Overall survival was monitored in both the B16F10 (FIG 20A) and MC38 (FIG 20B) tumor models. In both tumor models the highest overall survival was observed with the combination of cytokine mRNA and anti-PD-1 treatment with 60% of mice bearing B16F10 and 80% of MC38 bearing mice tumor free at the end of the study. In the B16F10 tumor model 10% of mice treated with anti-PD-1 or cytokine mRNA alone were tumor free, while in the MC38 model 40% of mice treated with anti-PD-1 and 30% of mice treated with cytokine alone were tumor free. The results indicate strong antitumor activity associated with cytokine mRNA and PD-1 combination.

[00299] To further evaluate the ability of local intratumoral cytokine mRNA in combination with the PD-1 antibody to exert a systemic anti-tumor response, mice were engrafted with B16F10 tumor cells on the right flank and received one day later an IV injection of Luciferase-expressing B16F10 cells for induction of lung metastasis. On day 11, 14 and 17 after IV tumor implantation mice bearing B16F10 tumors received in total three

intratumoral injections with cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47) into the flank tumor only, while tumors in the lung were untreated. On the same day mice also received intraperitoneal (IP) injections of PD-1 antibody (Sanofi murinized version of rat IgG2a anti-mouse PD-1 clone RMP1-14 at 10 mg/kg). Groups were as follows: 1) control mRNA (40 μ g total mRNA; 50 μ L intratumoral injection of control isotype antibody (clone MOPC-21 (BioLegend); 10 mg/kg) (FIG 20C); 2) control mRNA plus anti-PD1 antibody (FIG 20D); 3) cytokine mRNA plus control isotype antibody (FIG 20E); and 4) cytokine mRNA plus anti-PD1 (FIG 20F). Tumor growth of SC tumors was monitored (FIGS 20C-F) as well as survival (FIG 20G). Overall survival in this model was determined by tumor burden due to SC tumors as well as lung pseudometastasis tumor (not shown in this Figure); in some mice the SC tumor was rejected, while lung metastasis grew progressively. The highest overall survival was observed with the combination of cytokine mRNA and anti-PD-1 treatment. 6-7 % of mice treated with cytokine mRNA alone were tumor free, while mice that had received anti-PD-1 alone or control mRNA + isotype antibody were all sacrificed at day 22 due to high tumor burden. The results indicate strong antitumor activity associated with cytokine mRNA and PD-1 combination in this B16F10 tumor model with lung pseudo-metastasis, while anti-PD-1 antibody alone did not show any anti-tumor activity.

[00300] To further evaluate the effect of intratumoral injection of cytokine mRNA in combination with systemic administration of antibodies, mice were engrafted with CT26 tumor cells on right flanks. Mice received four intratumoral injections with cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47) on day 11, 14, 18 and 21 after tumor inoculation. On the same day mice also received intraperitoneal (IP) injections of an anti-CTLA-4 antibody (100 μ g/200 μ L per mouse; clone 9H10 from InVivoMAb) or the isotype control antibody (100 μ g/200 μ L per mouse; Armenian hamster IgG from BioXCell). Groups were as follows: 1) cytokine mRNA plus anti-CTLA-4 antibody (FIG 21A); 2) cytokine mRNA plus isotype control antibody (FIG 21B); 3) control mRNA plus anti-CTLA-4 antibody (FIG 21C) and 4) control mRNA plus isotype control antibody (FIG 21D). Combination therapy of intratumoral cytokine mRNA and IP-injected anti-CTLA-4 resulted in strongest anti-tumoral activity with 12 tumor-free mice out of 16 mice on day 55 after tumor inoculation (FIG 21A). Treatment with either cytokine mRNA plus isotype control antibody (FIG 21B) or control mRNA plus anti-CTLA-4 antibody (FIG 21C) induced less anti-tumoral activity with 5 and 7 tumor-free mice at the end of the study, respectively. In comparison, in the group that received control mRNA plus

isotype control antibody (FIG 21D), only one tumor-free mouse remained at the conclusion of the study.

[00301] To further evaluate the effect of intratumoral injection of cytokine mRNA in combination with an anti-CTLA-4 antibody, mice were engrafted with B16F10 tumor cells on right flanks. Mice received three intratumoral injections with cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 53, 41, 59, and 47) on day 13, 17 and 20 after tumor inoculation. On day 13, 17, 20 and 24 after tumor inoculation mice also received intraperitoneal (IP) injections of an anti-CTLA-4 antibody (100 μ g/200 μ L per mouse; clone 9H10 from InVivoMAb) or the isotype control antibody (100 μ g/200 μ L per mouse; Armenian hamster IgG from BioXCell). Tumor growth of SC tumors as well as survival was monitored. Groups were as follows: 1) cytokine mRNA plus anti-CTLA-4 antibody (FIG 21 E); 2) cytokine mRNA plus isotype control antibody (FIG 21F); 3) control mRNA plus anti-CTLA-4 antibody (FIG 21G) and 4) control mRNA plus isotype control antibody (FIG 21H). Combination therapy of intratumoral cytokine mRNA and IP-injected anti-CTLA-4 resulted in strongest anti-tumoral activity with 6 tumor-free mice out of 9 mice on day 60 after tumor inoculation (FIG 21E). Treatment with cytokine mRNA plus isotype control antibody (FIG 21F) induced less anti-tumoral activity with 2 tumor-free mice out of 9 mice. In comparison, in the two groups that either received control mRNA plus anti-CTLA-4 antibody (FIG 21G) or control mRNA plus isotype control antibody (FIG 21H), no tumor-free mouse remained at the conclusion of the study. Percent survival is depicted in FIG 21I, showing the highest overall survival in the combination of cytokine mRNA and anti-CTLA-4 treatment with 67% of mice bearing tumors at the end of the study (day 70), while 33% of mice treated with cytokine mRNA alone were tumor free. The results indicate strong antitumor activity associated with cytokine mRNA alone and cytokine mRNA and anti-CTLA-4 antibody in this B16F10 tumor model, in which anti-CTLA-4 antibody alone did not show any anti-tumor activity.

Example 9 – mRNA cytokine injection in multiple cancer types

[00302] To evaluate the effect of intratumoral injection of cytokine mRNA in various types of cancer, five xenograft mouse models – KM12 (CRC), RPMI8226 (Myeloma), NCI-N87 (Gastric), A375 (Melanoma), and NCI-H1975 (NSCLC) – were tested as described in Example 1. Mice bearing KM12 (CRC), RPMI8226 (Myeloma), NCI-N87 (Gastric), A375 (Melanoma), and NCI-H1975 (NSCLC) tumors received a single intratumoral injection with 8 μ g (2 μ g/target) human cytokine mRNA mixture of IL-15 sushi, IL-12sc, GM-CSF and

IFN α (ModB; SEQ ID NOs: 26, 18, 29, and 23) and the encoded cytokines were assessed in the tumor at 24 hours. Expression of each of the 4 cytokines of IL-15 sushi (FIG 22D), IL-12sc (FIG 22A), GM-CSF (FIG 22C) and IFN α (FIG 22B) was detected in all five of the xenograft models with the highest cytokines levels observed in NCI-H1975, followed by A375, NCI-N87, RPMI8226, and KM12.

Example 10 – Dose dependent serum expression of cytokines after intratumoral mRNA cytokine injection

[00303] The effect of different intratumoral mRNA doses on the expression of the encoded cytokines was examined in the serum of mice engrafted with a single A375 tumor on the right flank. Mice received a single intratumoral injection of a cytokine mRNA mixture of human IL-15 sushi, IL-12sc, GM-CSF and IFN α (ModB; SEQ ID NOs: 26, 18, 29, and 23). At 6 hours after intratumoral mRNA injection, serum was collected and cytokine expression was analyzed by Meso Scale Discovery assay. Dose dependent expression of each of the mRNA encoded cytokines was observed in the serum from the highest dose of 80 μ g (20 μ g) to the lowest dose of 0.0256 μ g (0.0064 μ g). Results are shown in Figures 23A-D.

Example 11 – Cytokine mRNA leads to expansion of gp70+ CD8 T cells

[00304] Mice bearing a single CT26 tumor on one flank received a four intratumoral injections of a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (ModB; SEQ ID NOs: 53, 41, 59, and 47). Blood was collected 13 days after first intratumoral mRNA administration and T cells specific for the gp70 tumor antigen were quantified by flow cytometry. Frequency of T cells specific for the gp70 tumor antigen in blood were strongly increased in mice upon intratumoral injection of mRNA cytokines compared to mice that had received control RNA.

Example 12 – Cytokine mRNA induces multiple pro-inflammatory pathways and increases immune infiltrate in both treated and untreated tumors.

[00305] Mice bearing B16F10 tumors on the left and right flank received a single intratumoral injection of 80 μ g of mRNA (20 μ g/target) into right tumor which was initiated with 0.5×10^6 cells (treated), while the tumor initiated with 0.25×10^6 cells remained untreated. At seven days after intratumoral injection of mRNA, both tumors were collected, and RNA was isolated and subjected to RNA sequencing analysis. As shown in FIGS. 27A-C, treatment with a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID NOs: 59, 53, 41, and 47) upregulated multiple proinflammatory pathways including a range of IFN γ response genes. The upregulation of proinflammatory/IFN γ

related pathways occurred in both the treated and untreated tumors, supporting the notion that local intratumoral treatment has systemic immune modulatory effects.

[00306] Causal network analysis (part of Ingenuity pathway analysis tool) was performed on 3298 genes that were differentially expressed (1699 up-regulated and 1599 down-regulated) between cytokine mRNA treatment and control in injected flank and 4973 genes (2546 up-regulated and 2427 down-regulated) in un-injected flank to identify changes in signaling pathways that could explain the observed changes in gene expression. Z scores were calculated to indicate the changes in pathways, with signs of score indicating the direction of change (positive sign suggests the activation whereas negative sign suggests inhibition).

[00307] Hierarchical clustering on expression of 328 genes regulated by IFNG (295 up-regulation and 33 down-regulation) was performed in both control and cytokine mRNA treated samples in both injected and un-injected tumors. Expression of each gene across samples were z-score normalized. The similarity metric is based on Pearson's correlation coefficient and complete-linkage is used to generate dendrogram. See, **Table 6**.

[00308] Relative abundance of infiltrated immune cells is determined by calculating the average expression of immune cell –type specific gene signatures.

[00309] Table 6

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
ABI1	Activate	1.6776	0.0073	0.0995	1.5218	0.0354	0.1551
ACOD1	Activate	31.6293	3.9652E-06	0.0024	6.1017	0.0096	0.0911
ADGRG2	Activate	1.8174	0.0248	0.2076	1.3902	0.2293	0.4662
ADORA2B	Activate	2.9657	0.0044	0.0752	1.4261	0.352	0.6138
AIF1	Activate	6.0406	0.0005	0.0201	4.7598	0.0032	0.057
AIM2	Activate	9.9707	0.000085324	0.0086	3.4835	0.0276	0.1361
ANGPTL4	Activate	2.0707	0.0293	0.2265	2.0904	0.0368	0.1586
APOL6	Activate	10.3994	0.0017	0.0418	10.1341	0.0031	0.0564
APP	Activate	2.0312	0.0006	0.0238	2.2382	0.0003	0.0276
ARFGAP3	Activate	1.6535	0.0146	0.1517	1.0201	0.9237	1
ASS1	Activate	3.5414	0.0084	0.1093	-1.0202	0.9668	1
ATF3	Activate	5.5284	0.0006	0.0231	-1.1007	0.8409	1
B2M	Activate	4.7005	0.000092097	0.0089	2.7946	0.0085	0.0859

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
BACH1	Activate	2.2944	0.0098	0.1179	1.3281	0.3821	0.6482
BATF2	Activate	4.0479	0.0211	0.1885	1.6468	0.4201	0.6889
BCL2L11	Activate	4.2973	0.0057	0.0868	1.1465	0.7946	1
BCL3	Activate	4.4411	0.0167	0.1639	2.5676	0.1413	0.3455
BLNK	Activate	4.2753	0.0003	0.0158	3.2008	0.0044	0.0643
BST1	Activate	16.9081	1.3371E-06	0.0016	6.6164	0.0007	0.0334
BTG1	Activate	3.5307	0.0001	0.0102	-1.1775	0.5968	0.8607
C10A	Activate	5.3999	0.0029	0.0588	1.3506	0.5911	0.8558
C10B	Activate	5.955	0.0104	0.1219	3.531	0.0786	0.2408
C10C	Activate	6.2282	0.0009	0.0307	2.7013	0.0707	0.2284
C2	Activate	2.2866	0.0367	0.2598	2.5908	0.0242	0.1281
C3	Activate	10.2057	0.0043	0.0734	11.6428	0.0044	0.0648
C4B	Activate	3.3143	0.0154	0.1562	1.3973	0.5045	0.7768
C5AR1	Activate	6.4343	0.000061281	0.0074	1.8122	0.1729	0.3925

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
CAMK4	Activate	2.5261	0.0011	0.0335	2.0726	0.012	0.1009
CASP1	Activate	8.2891	0.0079	0.1049	6.7759	0.0214	0.1263
CASP4	Activate	3.9281	0.0015	0.0389	1.2492	0.5969	0.8608
CCL11	Activate	6.225	0.0028	0.0579	9.5207	0.0007	0.0334
CCL17	Activate	3.9738	0.0268	0.2146	3.0432	0.0868	0.2549
CCL2	Activate	5.0444	0.0331	0.2446	1.9159	0.4045	0.6716
CCL22	Activate	5.4223	0.000050981	0.0069	3.6151	0.002	0.0467
CCL3	Activate	8.2215	0.0007	0.0259	4.7966	0.0129	0.1045
CCL4	Activate	8.7234	0.0008	0.0287	2.8285	0.1042	0.2846
CCL5	Activate	19.1375	0.000004935	0.0027	9.4224	0.0004	0.0299
CCL7	Activate	4.7053	0.0068	0.0952	-1.2497	0.6964	0.9457
CCL8	Activate	9.3589	0.0113	0.1283	3.9524	0.1286	0.3243
CCR1	Activate	5.333	0.0012	0.0349	1.7221	0.2823	0.5325
CCR2	Activate	5.8007	0.0025	0.0546	2.1931	0.1766	0.3975

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
CCR5	Activate	10.8409	0.000010504	0.004	4.8697	0.0025	0.0512
CCR2	Activate	3.9822	0.002	0.0465	3.6428	0.0056	0.071
CD14	Activate	5.6413	0.0025	0.0546	1.413	0.5406	0.8104
CD2	Activate	13.539	0.0001	0.0095	5.0981	0.0138	0.1086
CD274	Activate	11.0684	0.000096803	0.0091	3.4411	0.038	0.1616
CD38	Activate	5.5697	0.0005	0.0212	1.4645	0.4221	0.6908
CD40	Activate	11.0285	0.0003	0.0158	1.2537	0.7201	0.9637
CD40LG	Activate	3.1168	0.0068	0.0952	1.1976	0.6673	0.9224
CD68	Activate	6.1325	0.000040468	0.0063	3.6801	0.0029	0.0544
CD74	Activate	30.4664	0.000057089	0.0072	5.9291	0.0291	0.1392
CD80	Activate	4.1003	0.0005	0.0217	3.5579	0.0026	0.0517
CD86	Activate	6.3927	0.0008	0.027	8.2257	0.0004	0.0289
CDKN2A	Activate	1.9017	0.0117	0.1312	-1.2679	0.3579	0.6202
CEBPB	Activate	4.4389	0.0007	0.0253	2.1429	0.0784	0.2404

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
CELSR1	Activate	1.6953	0.0001	0.0107	1.1455	0.3001	0.5534
CERS6	Activate	2.2538	0.0064	0.0927	1.717	0.0763	0.2363
CFB	Activate	9.003	0.0001	0.0109	6.2886	0.0018	0.0444
CH25H	Activate	4.3757	0.007	0.097	1.9042	0.2435	0.4858
CHRNE	Activate	1.6587	0.0293	0.2263	1.0536	0.8254	1
CIITA	Activate	6.2751	0.0008	0.0287	2.736	0.0651	0.2172
CLEC2D	Activate	4.9639	0.0002	0.0135	2.6567	0.0223	0.1263
CLEC4E	Activate	25.396	2.9823E-07	0.001	7.0201	0.0009	0.0355
CLIC4	Activate	2.2909	0.000025847	0.0053	1.4654	0.0399	0.1658
CMPPK2	Activate	2.6988	0.0048	0.0785	1.9213	0.0687	0.2249
CORO1A	Activate	5.8653	0.0076	0.1019	2.1612	0.2494	0.4929
CP	Activate	2.7012	0.0222	0.1945	1.2703	0.5885	0.8544
CSF1	Activate	5.3648	0.0071	0.0977	2.9314	0.0916	0.2624
CSF2	Activate	2.4249	0.0053	0.0828	1.8261	0.0635	0.2145

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
CSF2RB	Activate	7.8798	0.000014063	0.0041	3.3417	0.008	0.0833
CTSB	Activate	2.9396	0.0192	0.1786	-1.5854	0.3271	0.5867
CTSD	Activate	1.7662	0.0058	0.0873	1.5654	0.0351	0.1546
CTSH	Activate	6.6723	0.0004	0.0189	3.0998	0.0341	0.1523
CTSS	Activate	14.2913	0.000052305	0.0069	2.0554	0.2402	0.4813
CXCL1	Activate	2.4631	0.0443	0.2909	2.3701	0.0679	0.223
CXCL10	Activate	4.0615	0.0183	0.174	3.84	0.0314	0.1453
CXCL11	Activate	6.3825	0.0032	0.0623	2.1745	0.2167	0.4494
CXCL12	Activate	4.1992	0.0099	0.1191	3.9497	0.0188	0.1263
CXCL16	Activate	11.7476	0.000012898	0.0041	2.7724	0.052	0.1919
CXCL2	Activate	17.8737	0.000065478	0.0076	2.0435	0.2897	0.5414
CXCL3	Activate	2.5623	0.0157	0.1584	-1.1159	0.7808	1
CXCL9	Activate	10.5652	0.002	0.0475	6.7433	0.0152	0.1139
CYBB	Activate	10.2429	0.0001	0.0102	2.4115	0.1288	0.3246

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
CYLD	Activate	2.1952	0.0005	0.0217	1.0366	0.8685	1
CYSLTR2	Activate	4.6876	0.0015	0.0389	-1.3886	0.4896	0.7624
DAPK1	Activate	2.9566	0.0202	0.1837	2.3734	0.0752	0.2341
DAXX	Activate	2.5923	0.0255	0.2101	2.7152	0.0271	0.1349
DDIT3	Activate	2.2027	0.0286	0.2233	2.4229	0.0212	0.1263
DDX58	Activate	2.2084	0.0054	0.084	1.2566	0.422	0.6908
DPP4	Activate	4.4101	0.047	0.3002	2.2805	0.2869	0.5383
DTX3L	Activate	3.4716	0.000027857	0.0053	1.6387	0.0759	0.2356
EBI3	Activate	5.2118	0.0003	0.0153	2.8451	0.0203	0.1263
ECE1	Activate	3.9089	0.0033	0.0624	1.8673	0.1782	0.3996
EGLN3	Activate	2.9572	0.0465	0.2989	1.0391	0.9453	1
EGR2	Activate	2.4479	0.0155	0.157	2.5764	0.0158	0.1159
ERAP1	Activate	2.5669	0.0033	0.0634	2.2811	0.0134	0.1068
FAM26F	Activate	14.7052	0.0008	0.0279	26.6887	0.0002	0.0227

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
FAS	Activate	7.0896	0.0003	0.0169	4.7216	0.0052	0.0695
FCER1G	Activate	6.7045	0.0007	0.0254	4.8631	0.0059	0.0728
FGF2	Activate	2.9272	0.0257	0.2106	1.8257	0.2249	0.4606
FGL2	Activate	12.456	9.3027E-06	0.0038	4.9116	0.0036	0.0599
FPR2	Activate	26.1833	0.000096227	0.0091	4.1758	0.0744	0.2328
FTH1	Activate	1.6855	0.0413	0.2791	2.2166	0.0045	0.0657
GBP2	Activate	7.2783	0.0007	0.0248	6.7229	0.0017	0.0437
GBP3	Activate	6.2795	0.0006	0.0222	3.0356	0.0358	0.156
GBP4	Activate	18.7632	0.0002	0.0139	6.7981	0.0154	0.1146
GBP5	Activate	18.254	0.0003	0.0157	7.5534	0.0118	0.1
GBP6	Activate	6.4537	0.0004	0.0187	8.0969	0.0002	0.0266
GBP7	Activate	4.3285	0.0017	0.0427	2.3295	0.0713	0.2296
GCH1	Activate	3.9933	0.0057	0.0867	-2.2318	0.1144	0.3009
GNA13	Activate	1.843	0.0129	0.1403	1.2404	0.3874	0.6539

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
GSDMD	Activate	5.5069	0.000067203	0.0076	4.0075	0.0013	0.0403
GZMB	Activate	10.9342	0.001	0.0323	11.0118	0.0018	0.0442
H2-M3	Activate	6.3613	0.0003	0.0169	-1.2831	0.6129	0.8754
H2-Q7	Activate	13.035	0.00007123	0.0078	13.2107	0.0001	0.0209
H2-T23	Activate	9.9835	0.0035	0.0651	1.093	0.9091	1
HCAR2	Activate	5.1405	0.0007	0.0254	3.0731	0.0206	0.1263
HCK	Activate	7.0952	0.000025322	0.0053	4.1767	0.0019	0.0455
HIF1A	Activate	1.661	0.0261	0.2122	1.117	0.6345	0.8942
HMOX1	Activate	2.9191	0.0049	0.0797	2.2892	0.0345	0.1532
ICAM1	Activate	5.8185	0.0005	0.0217	4.9939	0.0023	0.0487
ICOSL	Activate	1.9545	0.0086	0.1107	1.8592	0.0203	0.1263
IDO1	Activate	2.1676	0.007	0.097	1.5151	0.1535	0.3644
IFI27	Activate	2.1198	0.0058	0.088	1.1685	0.5669	0.8345
IFI44	Activate	10.2739	0.0001	0.0093	5.8893	0.0032	0.057

Gene	IFNG_regulation	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
IFIH1	Activate	3.3922	0.0001	0.0093	1.8886	0.0369	0.1588
IFIT1	Activate	4.7651	0.000038724	0.0062	2.3488	0.0191	0.1263
IFIT2	Activate	2.6547	0.0381	0.266	1.6098	0.3269	0.5865
IFIT3	Activate	6.6433	0.0003	0.0153	2.3076	0.0981	0.2746
IFITM1	Activate	13.8818	0.0017	0.0427	4.0409	0.096	0.2703
IFITM3	Activate	5.3416	0.000049885	0.0069	4.6318	0.0003	0.0278
IFNG	Activate	15.8295	0.000030559	0.0055	5.3984	0.0086	0.0862
IFNGR1	Activate	4.6529	0.0003	0.0169	1.1624	0.713	0.9579
IKBKE	Activate	3.6191	0.000070796	0.0078	2.1509	0.0151	0.1135
IL10RA	Activate	6.6362	0.0006	0.0225	2.2744	0.1259	0.3198
IL12B	Activate	1.5869	0.0237	0.2019	1.3356	0.1698	0.3882
IL12RB1	Activate	3.6563	0.0032	0.0623	2.7374	0.0261	0.1323
IL12RB2	Activate	10.8737	0.0001	0.0105	2.7693	0.0898	0.2596
IL15RA	Activate	5.6222	0.0002	0.0124	3.263	0.01	0.0929

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
IL18BP	Activate	13.3799	0.000016505	0.0045	2.6518	0.0808	0.2445
IL18R1	Activate	10.5794	0.0004	0.0172	4.1773	0.0288	0.1387
IL18RAP	Activate	12.8979	1.6368E-06	0.0016	3.9032	0.0058	0.0721
IL1A	Activate	5.0611	0.0002	0.014	2.016	0.1006	0.2792
IL1B	Activate	7.1785	0.0017	0.0427	2.9628	0.0846	0.2505
IL1RL1	Activate	3.2501	0.0421	0.2825	2.7191	0.0999	0.2778
IL1RN	Activate	11.4696	0.000016637	0.0045	3.5493	0.0183	0.1256
IL27	Activate	5.0377	0.0003	0.0145	2.9655	0.0136	0.1077
IL2RA	Activate	12.629	0.000036735	0.0059	9.0232	0.0004	0.03
IL3RA	Activate	2.0071	0.0463	0.2977	-2.3554	0.0222	0.1263
INHBA	Activate	7.0079	0.0006	0.0238	8.1176	0.0005	0.0316
IRF1	Activate	4.8896	0.0002	0.0136	4.2429	0.0011	0.0383
IRF2	Activate	3.0856	0.0001	0.0102	2.3475	0.0038	0.0609
IRF4	Activate	3.6001	0.0009	0.0303	2.7396	0.0107	0.0955

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
IRF5	Activate	6.3959	0.0039	0.0697	6.2724	0.0066	0.0767
IRF7	Activate	4.6951	0.0016	0.0408	2.6708	0.0468	0.1806
IRGM1	Activate	3.5604	0.0205	0.1855	3.8887	0.0196	0.1263
ISG15	Activate	6.1635	0.0003	0.0158	2.64	0.0488	0.1845
ISG20	Activate	3.3567	0.0012	0.0349	3.9719	0.0006	0.0322
ITGA4	Activate	2.0304	0.0397	0.272	2.0083	0.055	0.1984
ITGAL	Activate	16.3545	0.000032377	0.0056	6.1759	0.0055	0.0707
ITGAM	Activate	8.5704	0.0093	0.1151	3.5033	0.1371	0.3385
ITGAX	Activate	3.9189	0.0101	0.1202	2.0482	0.185	0.4084
ITGB2	Activate	7.7479	0.0002	0.0124	6.9547	0.0006	0.0332
JAK2	Activate	1.6647	0.0461	0.2976	1.3678	0.2378	0.4779
JUN	Activate	1.7589	0.0035	0.0649	-1.1739	0.4037	0.6708
KLF4	Activate	1.907	0.0409	0.2778	1.156	0.6546	0.9116
KLF6	Activate	2.4625	0.0037	0.0672	2.1351	0.0182	0.125

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
KYNU	Activate	3.8871	0.008	0.1057	4.8859	0.0039	0.061
LAG3	Activate	7.4221	0.0002	0.0114	2.156	0.1298	0.3262
LAT2	Activate	2.6441	0.028	0.2208	1.5322	0.3475	0.6094
LCN2	Activate	4.3524	0.0296	0.2275	16.3586	0.0003	0.0276
LCP2	Activate	8.4235	0.0004	0.0193	4.07	0.0205	0.1263
LGALS9	Activate	1.6825	0.0051	0.0817	1.6728	0.0085	0.086
LST1	Activate	7.6841	0.02	0.1832	1.6707	0.5648	0.8328
LTA	Activate	2.7386	0.0055	0.0851	2.4968	0.0157	0.1157
LTB	Activate	8.1723	0.0014	0.0379	2.3655	0.1846	0.4079
LY6E	Activate	5.1119	0.0028	0.0572	2.8148	0.061	0.2104
LY96	Activate	2.4218	0.0302	0.2305	1.5311	0.3103	0.5661
LYN	Activate	5.1219	0.001	0.0325	1.6841	0.2851	0.536
MAP3K8	Activate	7.131	0.0009	0.0302	1.0205	0.9717	1
MEFV	Activate	10.8378	0.000019758	0.0047	2.3756	0.0951	0.2688

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
MMP12	Activate	6.4662	0.0035	0.0647	5.0068	0.015	0.1131
MX1	Activate	6.1489	0.0128	0.1395	2.6469	0.1916	0.4171
MX2	Activate	3.9804	0.0005	0.0205	1.8666	0.1068	0.2889
NAMPT	Activate	2.341	0.0066	0.0943	-1.4194	0.2673	0.514
NAPSA	Activate	4.3452	0.0003	0.0149	4.7434	0.0003	0.0272
NEURL3	Activate	5.943	0.0009	0.0291	1.9354	0.2066	0.4363
NFKB1	Activate	1.8089	0.0051	0.0815	1.0738	0.7351	0.9751
NFKBIA	Activate	4.9409	0.000013016	0.0041	2.4668	0.0097	0.0912
NFKBIZ	Activate	2.328	0.0073	0.0995	2.0257	0.0308	0.1436
NGF	Activate	3.1687	0.0065	0.0933	3.4968	0.0055	0.0706
NLR5	Activate	3.4173	0.0109	0.1261	2.2706	0.0985	0.2752
NLRP3	Activate	5.11	0.0007	0.0254	3.4525	0.0111	0.0975
NMI	Activate	2.0435	0.0228	0.1979	1.6117	0.1416	0.3459
NOD2	Activate	3.5157	0.000018135	0.0045	2.5449	0.0013	0.0396

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
NOS2	Activate	26.5216	2.2445E-06	0.0017	24.8128	7.9556E-06	0.0117
NOTCH1	Activate	2.2952	0.0301	0.2303	1.4949	0.3078	0.5625
NR1D1	Activate	1.7995	0.0049	0.0799	1.8784	0.0045	0.065
NUPR1	Activate	2.8421	0.001	0.0318	1.7353	0.0807	0.2444
OAS2	Activate	3.8997	0.0003	0.0154	2.3682	0.0204	0.1263
OAS3	Activate	14.5317	7.7153E-06	0.0034	4.0924	0.0123	0.1019
P2RY14	Activate	16.6314	0.0005	0.0204	4.8513	0.0471	0.1812
P2RY6	Activate	5.352	0.0001	0.0103	2.2094	0.0606	0.2093
PARP9	Activate	3.1267	0.0179	0.1716	-1.1573	0.7644	0.9977
PARVG	Activate	6.3047	0.0025	0.0539	1.2154	0.7444	0.9822
PDCD1L G2	Activate	11.6201	2.1928E-06	0.0017	3.9612	0.0045	0.065
PF4	Activate	6.7372	0.0284	0.2226	-2.2437	0.3655	0.6285
PIM1	Activate	4.0708	0.0003	0.0169	2.0732	0.0575	0.2034
PLA2G1 6	Activate	4.8619	0.001	0.0309	1.3802	0.4886	0.7613

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
PLAU	Activate	3.8214	0.00003401	0.0058	2.1662	0.0131	0.1056
PLEK	Activate	5.9274	0.0049	0.0805	2.9561	0.0921	0.2632
PMAIP1	Activate	3.4224	0.0002	0.0134	2.6199	0.0041	0.0627
PRDM1	Activate	3.5702	0.0297	0.2283	4.0335	0.0251	0.13
PRKCD	Activate	4.5574	2.2524E-07	0.001	2.0325	0.0066	0.0768
PRKCQ	Activate	2.1379	0.0065	0.0932	2.1615	0.0089	0.087
PSMB10	Activate	3.6197	0.005	0.0807	1.4724	0.3976	0.6643
PSMB8	Activate	3.9093	0.0032	0.0615	2.652	0.0388	0.1637
PSMB9	Activate	7.4132	0.0005	0.0197	4.5308	0.009	0.0877
PSME1	Activate	6.1609	0.0001	0.0096	3.9584	0.0037	0.06
PTAFR	Activate	8.5218	0.000052564	0.0069	2.2614	0.1024	0.2821
PTGES	Activate	3.3225	0.0241	0.2039	1.61	0.3819	0.6479
PTGS2	Activate	10.9036	0.0075	0.1017	3.9538	0.1309	0.3282
PTX3	Activate	5.271	0.0253	0.2091	1.8833	0.4052	0.672

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
RAB20	Activate	2.29	0.0257	0.2106	1.1261	0.7537	0.9893
RAC2	Activate	7.9519	0.000098427	0.0091	3.7008	0.0123	0.1021
RIPK1	Activate	1.6822	0.0456	0.2959	-1.1344	0.6373	0.8964
RSAD2	Activate	3.916	0.0003	0.0152	1.6141	0.184	0.407
RTP4	Activate	4.8033	0.0025	0.0537	2.5839	0.0696	0.2269
RUNX2	Activate	5.3191	0.0001	0.0095	2.2697	0.0491	0.185
RUNX3	Activate	5.5652	0.0012	0.0354	5.5875	0.002	0.0467
S100A10	Activate	2.1419	0.001	0.0318	-1.0647	0.7799	1
S100A8	Activate	10.9785	0.0065	0.0931	11.8118	0.0079	0.0832
S100A9	Activate	5.1245	0.0058	0.0879	4.0622	0.0228	0.1263
S1PR3	Activate	2.8084	0.0426	0.2844	4.3054	0.0083	0.0851
SAMHD1	Activate	6.8165	0.000028539	0.0053	5.2676	0.0004	0.0289
SELL	Activate	9.0266	0.000036704	0.0059	2.6848	0.0503	0.1879
SEMA4A	Activate	4.5461	0.0002	0.0134	7.1376	0.000014188	0.0118

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
SEPT3	Activate	1.9813	0.0021	0.0484	1.2827	0.2581	0.5036
SERPINB9	Activate	3.6968	0.0068	0.0955	1.3544	0.5306	0.8014
SERPINE1	Activate	2.7348	0.0131	0.142	1.0382	0.9268	1
SERPING1	Activate	8.5956	0.0003	0.0154	5.6206	0.0042	0.063
SHARPIN	Activate	1.8222	0.0003	0.0169	-1.1856	0.2897	0.5414
SLAMF1	Activate	4.2282	0.000066073	0.0076	1.8422	0.0753	0.2344
SLC11A1	Activate	8.1631	0.0002	0.0114	6.6594	0.0009	0.0364
SLC15A3	Activate	13.0122	0.0001	0.0093	4.2688	0.0238	0.1278
SLC16A9	Activate	2.2466	0.0273	0.2172	1.6072	0.21	0.4406
SLC28A2	Activate	6.903	0.0001	0.0096	1.4304	0.4466	0.7176
SLFN5	Activate	3.7058	0.0022	0.0493	2.1361	0.0772	0.2383
SMAD7	Activate	3.1247	0.0209	0.187	-1.1526	0.7767	1
SOCS1	Activate	3.9437	0.011	0.1267	3.3365	0.0325	0.1481
SOD2	Activate	2.6759	0.0047	0.0774	3.1489	0.0021	0.0475

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
SOD3	Activate	2.1257	0.0158	0.1591	1.4255	0.2663	0.5128
SP100	Activate	4.8282	7.0055E-06	0.0032	2.8699	0.0019	0.0452
SP110	Activate	3.7976	0.0057	0.0867	1.1957	0.71	0.9556
SP1	Activate	10.5793	0.000055966	0.0071	6.5856	0.0014	0.0409
SPP1	Activate	2.5268	0.0212	0.1893	2.4021	0.0384	0.1624
STAT1	Activate	4.2736	0.0089	0.1122	3.9844	0.0176	0.1229
STAT2	Activate	5.4847	0.000013182	0.0041	3.019	0.0035	0.0589
STAT4	Activate	6.3819	0.0038	0.069	1.1576	0.8175	1
STAT6	Activate	1.7399	0.0097	0.1171	1.7712	0.0116	0.0993
STX11	Activate	3.6303	0.0058	0.088	1.7593	0.2306	0.4679
TAP1	Activate	4.588	0.001	0.0323	2.7092	0.0329	0.1493
TAP2	Activate	2.8268	0.0148	0.1526	1.5315	0.3257	0.585
TAPBP	Activate	2.5416	0.0274	0.2176	1.7971	0.1801	0.4019
TAPBPL	Activate	3.0796	0.0031	0.0609	2.4014	0.0251	0.13

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
TBX21	Activate	3.8788	0.0008	0.0273	4.1566	0.0008	0.0354
TCIRG1	Activate	2.7899	0.0005	0.0217	1.2508	0.4321	0.7008
TGFBR2	Activate	1.9044	0.031	0.2346	1.2596	0.451	0.7222
THBS1	Activate	2.8135	0.005	0.0807	2.9522	0.0055	0.0707
THEMIS2	Activate	9.3368	0.0011	0.0339	6.5099	0.0079	0.0832
THY1	Activate	5.5745	0.0036	0.0666	3.0999	0.0597	0.2073
TLR1	Activate	3.9534	0.0044	0.0751	4.9709	0.002	0.0463
TLR2	Activate	4.2854	0.0004	0.0173	1.0613	0.8785	1
TLR7	Activate	3.6076	0.0317	0.2384	1.1789	0.7874	1
TLR8	Activate	7.9426	0.0012	0.0349	1.5453	0.4846	0.7574
TLR9	Activate	7.0125	0.0004	0.0182	5.5003	0.0026	0.052
TMEM50B	Activate	2.2646	0.0029	0.0584	1.6005	0.089	0.2584
TNF	Activate	15.5761	0.000001549	0.0016	3.6025	0.014	0.1094
TNFAIP2	Activate	4.2629	0.0043	0.0737	1.3184	0.5837	0.8504

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
TNFRSF1A	Activate	3.7342	0.0089	0.1124	3.2927	0.0236	0.1273
TNFRSF14	Activate	3.3507	0.0151	0.1547	2.0443	0.1615	0.3761
TNFRSF1B	Activate	6.9767	0.0004	0.0187	3.0014	0.0433	0.1723
TNFSF10	Activate	5.9466	0.0008	0.0286	2.8254	0.0505	0.1883
TNFSF12	Activate	4.0396	0.0003	0.0145	3.0636	0.0039	0.061
TNFSF13	Activate	3.7405	0.0229	0.1979	-1.7547	0.3434	0.6048
TNFSF13B	Activate	4.2939	0.00009167	0.0089	1.0175	0.96	1
TRAFD1	Activate	7.5123	0.000066493	0.0076	1.4364	0.4415	0.7113
TRIM21	Activate	3.1067	0.0069	0.096	-1.1129	0.7984	1
TXK	Activate	3.1599	0.0017	0.0418	1.8261	0.0992	0.2766
UBD	Activate	39.4489	3.1101E-06	0.0021	20.5238	0.0001	0.0203
UBE2L6	Activate	3.418	0.002	0.0464	4.4962	0.0005	0.031
USP18	Activate	5.4886	0.0004	0.0192	1.697	0.258	0.5036
VCAM1	Activate	7.3103	0.0003	0.0163	1.2409	0.6812	0.9337

Gene	IFNG_ regulation	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.F oldChange	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPVal ue	Treated_ cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
WARS	Activate	1.5033	0.0098	0.1179	1.8802	0.0003	0.0284
ZFP36	Activate	3.0067	0.0051	0.0815	-1.1887	0.6585	0.9153
AHCY	Inhibit	-2.0639	0.0374	0.2628	-1.8643	0.0878	0.2566
AQP1	Inhibit	-2.6893	0.0012	0.0354	-2.9843	0.0008	0.0346
AZGP1	Inhibit	-3.6306	0.0039	0.0701	-3.72	0.0054	0.0705
CSE1L	Inhibit	-1.9528	0.0448	0.2931	-4.9001	0.000053442	0.0177
DHX9	Inhibit	-1.9049	0.0192	0.1787	-1.3891	0.2439	0.4863
EMID1	Inhibit	-2.5566	0.047	0.3003	-1.9661	0.1701	0.3887
FKBP6	Inhibit	-4.1867	0.0043	0.0733	-2.2618	0.1075	0.2898
FLT4	Inhibit	-1.7478	0.0248	0.2076	-1.6367	0.0588	0.2058
GMPR	Inhibit	-2.9853	0.027	0.2154	-5.5877	0.0017	0.0432
GNAO1	Inhibit	-2.2497	0.0179	0.1721	-1.0647	0.8565	1
HSP90A	Inhibit	-1.9951	0.007	0.0974	-1.1404	0.6083	0.8715
ID1	Inhibit	-2.9218	0.004	0.0713	-2.402	0.023	0.1263
IGFBP4	Inhibit	-3.3872	0.0211	0.1885	-3.0044	0.0473	0.1815
MIF	Inhibit	-3.5707	0.0052	0.0828	-1.3189	0.5425	0.8124
MSH2	Inhibit	-1.909	0.0328	0.2435	-1.7932	0.0664	0.2198
MYC	Inhibit	-1.8764	0.0225	0.1962	-1.5059	0.1507	0.36
PBK	Inhibit	-4.8198	0.0008	0.0287	-10.4275	0.000012076	0.0117
PCDH17	Inhibit	-2.2868	0.0029	0.0588	-1.1447	0.6223	0.8835
PLCG1	Inhibit	-1.6389	0.0439	0.2895	1.263	0.3564	0.6189
POLR2F	Inhibit	-1.7137	0.0139	0.1473	-2.1058	0.0019	0.0456
POMP	Inhibit	-1.5182	0.047	0.3002	-1.3884	0.1354	0.336
PRPF8	Inhibit	-2.0788	0.0159	0.1593	-2.1397	0.0179	0.1239
PTGES2	Inhibit	-1.9137	0.0278	0.2201	-1.0746	0.811	1

Gene	IFN γ regulation	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Treated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FoldChange	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.RawPValue	Untreated_cytokine mRNA mix/IgG1 vs Luc mRNA/IgG1.FDR_BH
RAD18	Inhibit	-2.1129	0.0032	0.0615	-1.7251	0.0354	0.1551
SF3A1	Inhibit	-1.598	0.0042	0.0729	-1.2281	0.2111	0.4418
SLC12A2	Inhibit	-2.8366	0.0039	0.0697	-3.5987	0.0011	0.0373
SMTN	Inhibit	-1.8432	0.0398	0.2725	-1.2994	0.3927	0.6593
SQLE	Inhibit	-4.0941	0.0396	0.2717	-4.8892	0.0296	0.1404
SREBF2	Inhibit	-2.3929	0.0032	0.0615	-3.5164	0.0001	0.0209
SSBP1	Inhibit	-1.5752	0.0094	0.1156	1.0074	0.9665	1
TMEM158	Inhibit	-1.6002	0.044	0.2897	1.4394	0.1352	0.3357
TYMP	Inhibit	-2.6432	0.0448	0.2928	1.469	0.4412	0.7111
UNC5B	Inhibit	-2.3093	0.0267	0.2143	-1.5396	0.2666	0.5132

Example 13 – Cytokine mRNA Increases CD4+ and CD8+ T Cells in Both Treated and Untreated Tumors

[00310] Mice bearing B16F10 tumors on the left and right flank received a single intratumoral injection of 80 µg of mRNA (20 µg/target) into right tumor which was initiated with 0.5×10^6 cells only one of the tumors (treated), while the other tumor initiated with 0.25×10^6 cells remained untreated. At seven days post intratumoral mRNA injection, both the tumors were collected and processed for IHC (Immunofluorescence microscopy) staining with antibodies for CD4+, CD8+, and FoxP3+ cells. Mice from tumors in Figures 28A and 28B were treated with cytokine mRNA, while mice from tumors in Figures 28C and D were treated with control mRNA. Panels A and C are from the tumors injected with mRNA, while panels B and D are from the corresponding contralateral tumor not injected. (FIGS 28A-D). For both the cytokine mRNA treated and control mRNA treated groups 5 tumors injected with RNA and the corresponding 5 contralateral tumors uninjected were subjected to immunofluorescent staining for CD4+, CD8+, FOXP3+ cells. The relative frequency and ratio of cells are plotted in Figures 28E, F, G. The results indicate that a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID NOs: 59, 53, 41, and 47) increases CD8+ and CD4+ T cells infiltration leading to altering the CD8+/Treg ratio. An increase in immune infiltration occurred in both the treated and untreated tumors, supporting the notion that local intratumoral treatment has systemic immune modulatory effects.

Example 14 – mRNA is expressed in both tumor and infiltrating immune cells

[00311] Mice bearing a single B16F10 tumor received a single intratumoral injection with mRNA encoding the Thy1.1 protein (FIG. 29A –G). Approximately 18hrs after intratumoral injection the tumor was dissociated, stained with a panel of antibodies and flow cytometry was performed to define cells expressing Thy1.1 protein. The results indicate that both tumor and immune cells take up and express the mRNA.

Example 15 – Dose dependent tumor expression and PD response following intratumoral cytokine mRNA injection

[00312] Mice bearing B16F10 tumors received a single mRNA injection with 80, 8 or 0.8 µg of a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID Nos: 59, 53, 41, and 47). Approximately 6 hrs after the intratumoral injection, the tumor was removed and lysed, and levels of IL-15 sushi, GM-CSF, IFN α , and IL-12sc, IFN γ and IP-10 were quantified in the tumor lysate. Figures 30A-F show that the cytokine mRNA was expressed intratumorally in a dose-dependent manner.

[00313] In a separate experiment, mice bearing B16F10 tumors received a single mRNA injection with 80, 8 or 0.8ug of a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID Nos: 59, 53, 41, and 47). At seven days following intratumoral cytokine mRNA injection, the tumors were dissociated, stained with a panel of antibodies, and analyzed by flow cytometry. The antibodies used were against murine: CD45, CD4, CD3, CD8, CD279, IFN γ , TNF α , FOXP3, Granzyme B). The results indicate that treatment with the cytokine mRNA mixture altered the CD8 $^{+}$ /Treg ratio (FIG 31A-B), led to increased frequency of polyfunctional CD8 $^{+}$ T cells in the tumor microenvironment (FIG 31C-D), increased PD-L1 on infiltrating myeloid cells (FIG 31E), and increased levels of PD-1 on infiltrating CD8 $^{+}$ T cells (FIG 31F).

[00314] In a further experiment, mice bearing B16F10 tumors on the left and right flank received a single intratumoral injection of a cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID Nos: SEQ ID NOs: 59, 53, 41, and 47) or control mRNA into only one of the tumors (treated), while the other tumor remained untreated. At seven days post intratumoral mRNA injection, the injected tumor was collected and processed for flow cytometry staining with antibodies for CD45 $^{+}$, CD8 $^{+}$, CD3 $^{+}$, and Granzyme B. The results indicate that the cytokine mRNA mixture increased the frequency of intratumoral Granzyme B CD8 $^{+}$ T cells in the tumor (FIG 31G-H).

Example 16 – Intratumorally injected mRNA is primarily expressed in the tumor

[00315] Mice bearing B16F10 tumors received a single intratumoral injection of 50 μ g mRNA encoding firefly luciferase. At 6 and 24-hour time points, 3 mice were sacrificed and tumor, liver, spleen, tumor draining lymph node (TDLN) and non-tumor draining lymph node (NDLN) were analyzed ex vivo for luciferase expression. Figures 32A-B show that luciferase expression was highest in the tumor, in which expression was greater than 100-fold above any other tissue.

Example 17 – CD4 $^{+}$, CD8 $^{+}$, and NK cells contribute to the anti-tumor activity of cytokine mRNA in B16F10 model

[00316] Groups of mice bearing B16F10 tumors were treated with 100 μ g of depleting antibodies (anti-CD4, anti-CD8, anti-NK1.1) by intraperitoneal injection once a week for 4 weeks total. Antibody mediated cellular depletion was initiated one day prior to treatment with an 80 μ g cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID Nos: 59, 53, 41, and 47). The effect of antibody depletion on overall survival was monitored. The results, shown in Figure 34, indicate that individual depletion CD8 $^{+}$, CD4 $^{+}$, or NK cells

reduced, to varying degrees, the anti-tumor activity and overall survival of the cytokine mRNA.

Example 18 – Antitumor activity of cytokine mRNAs is not observed in IFN-gamma-deficient mice

[00317] WT C57BL6J mice and C57BL6J mice deficient for the murine IFN γ (IFN γ KO) were implanted with B16F10 tumor cells as described in Example 1. Mice were treated by intratumoral injection with 80 μ g (20 μ g/target) cytokine mRNA mixture of IL-15 sushi, GM-CSF, IFN α , and IL-12sc (SEQ ID Nos: 59, 53, 41, and 47) or 80 μ g control mRNA, and overall survival was monitored. The results, depicted in Figure 35, indicate that mice lacking IFN γ did not exhibit a detectable antitumor response when treated with the cytokine mRNA.

We claim:

1. A composition or medical preparation comprising RNA encoding an IL-12sc protein, RNA encoding an IL-15 sushi protein, RNA encoding an IFN α protein, and RNA encoding a GM-CSF protein.
2. The composition according to claim 1.
3. The medical preparation or composition of claim 1 or 2, wherein the IFN α protein is an IFN α 2b protein.
4. The medical preparation or composition of any one of claims 1- 3, wherein (i) the RNA encoding an IL-12sc protein comprises the nucleotide sequence of SEQ ID NO: 17 or 18, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17 or 18 and/or (ii) the IL-12sc protein comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14.
5. The medical preparation or composition of any one of claims 1-4, wherein (i) the RNA encoding an IL-15 sushi protein comprises the nucleotide sequence of SEQ ID NO: 26, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 26 and/or (ii) the IL-15 sushi protein comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 24.
6. The medical preparation or composition of any one of claims 1-5, wherein (i) the RNA encoding an IFN α protein comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 22 or 23 and/or (ii) the IFN α protein comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 19.
7. The medical preparation or composition of any one of claims 1-6, wherein (i) the RNA encoding a GM-CSF protein comprises the nucleotide sequence of SEQ ID NO: 29, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 29 and/or (ii) the GM-CSF protein comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid

- sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.
8. The medical preparation or composition of any one of claims 1-7, wherein at least one RNA comprises a modified nucleoside in place of at least one uridine.
 9. The medical preparation or composition of any one of claims 1-7, wherein at least one RNA comprises a modified nucleoside in place of each uridine.
 10. The medical preparation or composition of any one of claims 1-7, wherein each RNA comprises a modified nucleoside in place of at least one uridine.
 11. The medical preparation or composition of any one of claims 1-7, wherein each RNA comprises a modified nucleoside in place of each uridine.
 12. The medical preparation or composition of any one of claims 8-11, wherein the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine ($m1\psi$), and 5-methyl-uridine ($m5U$).
 13. The medical preparation or composition of any one of claims 7-11, wherein at least one RNA comprises more than one type of modified nucleoside, wherein the modified nucleosides are independently selected from pseudouridine (ψ), N1-methyl-pseudouridine ($m1\psi$), and 5-methyl-uridine ($m5U$).
 14. The medical preparation or composition of claim 12, wherein the modified nucleoside is N1-methyl-pseudouridine ($m1\psi$).
 15. The medical preparation or composition of any one of claims 1-14, wherein at least one RNA comprises the 5' cap $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$.
 16. The medical preparation or composition of any one of claims 1-15, wherein each RNA comprises the 5' cap $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$.
 17. The medical preparation or composition of any one of claims 1-16, wherein at least one RNA comprises a 5' UTR comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.
 18. The medical preparation or composition of any one of claims 1-17, wherein each RNA comprises a 5' UTR comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.

19. The medical preparation or composition of any one of claims 1-18, wherein at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
20. The medical preparation or composition of any one of claims 1-19, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
21. The medical preparation or composition of any one of claims 1-20, wherein at least one RNA comprises a poly-A tail.
22. The medical preparation or composition of any one of claims 1-21, wherein each RNA comprises a poly-A tail.
23. The medical preparation or composition of claim 21 or 22, wherein the poly-A tail comprises at least 100 nucleotides.
24. The medical preparation or composition of claim 21 or 22, wherein the poly-A tail comprises the poly-A tail shown in SEQ ID NO: 78.
25. The medical preparation or composition of any one of claims 1-24, wherein one or more RNA comprises:
 - a. a 5' cap comprising $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$;
 - b. a 5' UTR comprising (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6;
 - c. a 3' UTR comprising (i) the nucleotide sequence of SEQ ID NO: 8, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8; and
 - d. a poly-A tail comprising at least 100 nucleotides.
26. The medical preparation or composition of claim 25, wherein the poly-A tail comprises SEQ ID NO: 78.
27. The medical preparation or composition of any one of claims 1 to 26, which is a pharmaceutical composition comprising the RNAs.
28. The medical preparation or composition of claim 27, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

29. The medical preparation or composition of any one of claims 1 to 28, wherein the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.
30. The medical preparation or composition of any one of claims 1 to 29 for pharmaceutical use.
31. The medical preparation or composition of claim 30, wherein the pharmaceutical use comprises a therapeutic or prophylactic treatment of a disease or disorder.
32. The medical preparation or composition of any one of claims 1 to 31 for use in a method for treating or preventing a solid tumor.
33. The medical preparation or composition of claim 32, wherein the solid tumor is a sarcoma, carcinoma, or lymphoma.
34. The medical preparation or composition of any one of claims 32 or 33, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.
35. The medical preparation or composition of any one of claims 32-34, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.
36. The medical preparation or composition of any one of claims 1-35, wherein the RNA is for intra-tumoral or peri-tumoral administration.
37. The medical preparation or composition of any one of claims 1-36, wherein the RNA is formulated for injection.
38. The medical preparation or composition of any one of claims 1-36, wherein the RNA is for use by injection.
39. The medical preparation or composition of any one of claims 1-38, which is for administration to a human.
40. The medical preparation or composition of any one of claims 32-39, wherein treating or preventing the solid tumor comprises reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject.
41. A kit comprising the composition of any one of claims 1-29.

42. The medical preparation of any one of claims 1 or 3-29, wherein the medical preparation is a kit.
43. The medical preparation of claim 42, wherein the RNAs are in separate vials.
44. The kit of any one of claims 40-43, further comprising instructions for use of the composition for treating or preventing a solid tumor.
45. RNA for use in a method for treating or preventing a solid tumor in a subject, wherein the method comprises administering RNA encoding an IL-12sc protein, RNA encoding an IL-15 sushi protein, RNA encoding an IFN α protein, and RNA encoding a GM-CSF protein.
46. Use of RNA encoding an IL-12sc protein, an IL-15 sushi protein, an IFN α protein, and a GM-CSF protein for the treatment of solid tumor.
47. The RNA or use of any one of claims 45-46, wherein the IFN α protein is an IFN α 2b protein.
48. The RNA or use of any one of claims 45-47, wherein (i) the RNA encoding an IL-12sc protein comprises the nucleotide sequence of SEQ ID NO: 17 or 18, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17 or 18 and/or (ii) the IL-12sc protein comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14.
49. The RNA or use of any one of claims 45-48, wherein (i) the RNA encoding an IL-15 sushi protein comprises the nucleotide sequence of SEQ ID NO: 26, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 26 and/or (ii) the IL-15 sushi protein comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 24.
50. The RNA or use of any one of claims 45-49, wherein (i) the RNA encoding an IFN α protein comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 22 or 23 and/or (ii) the IFN α protein comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 19.

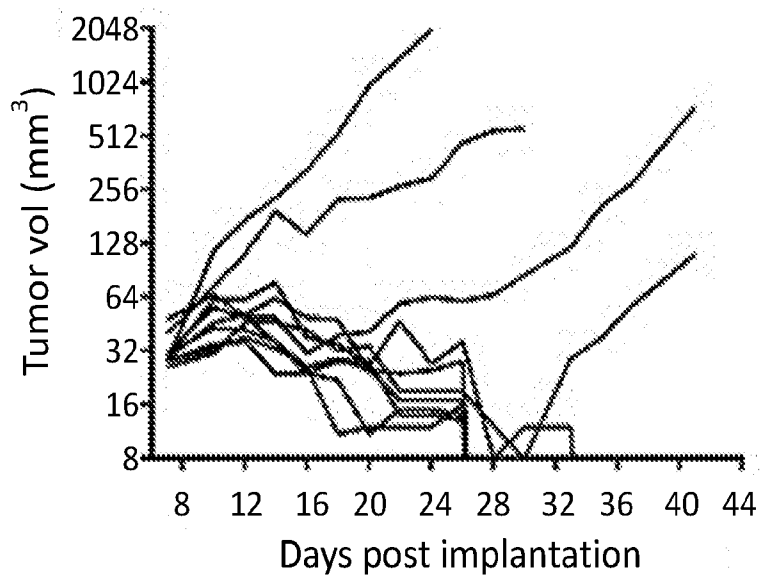
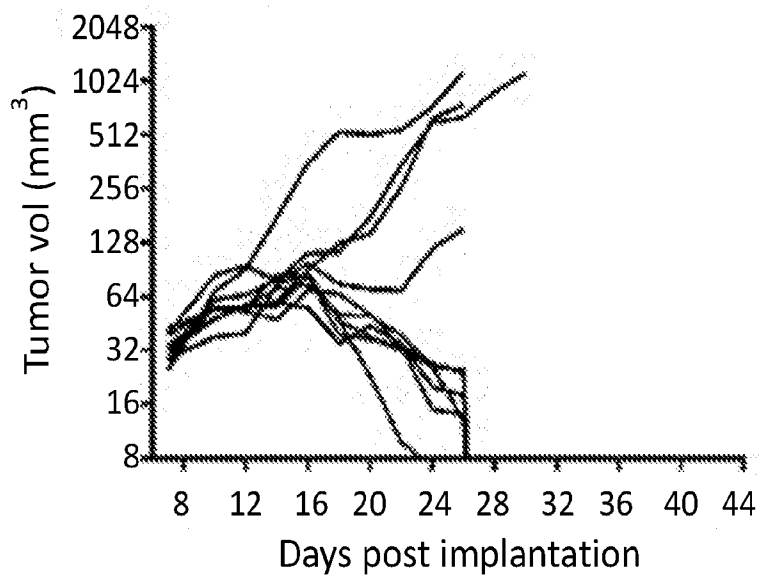
51. The RNA or use of any one of claims 45-50, wherein (i) the RNA encoding a GM-CSF protein comprises the nucleotide sequence of SEQ ID NO: 29, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 29 and/or (ii) the GM-CSF protein comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.
52. The RNA or use of any one of claims 45-51, wherein at least one RNA comprises a modified nucleoside in place of at least one uridine.
53. The RNA or use of any one of claims 45-52, wherein at least one RNA comprises a modified nucleoside in place of each uridine.
54. The RNA or use of any one of claims 45-53, wherein each RNA comprises a modified nucleoside in place of at least one uridine.
55. The RNA or use of any one of claims 45-54, wherein each RNA comprises a modified nucleoside in place of each uridine.
56. The RNA or use of any one of claims 52-55, wherein the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine ($m1\psi$) and 5-methyl-uridine ($m5U$).
57. The RNA of any one of claims 52-55, wherein at least one RNA comprises more than one type of modified nucleoside, wherein the modified nucleosides are independently selected from pseudouridine (ψ), N1-methyl-pseudouridine ($m1\psi$), and 5-methyl-uridine ($m5U$).
58. The RNA or use of claim 56, wherein the modified nucleoside is N1-methyl-pseudouridine ($m1\psi$).
59. The RNA or use of any one of claims 45-58, wherein at least one RNA comprises the 5' cap $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$.
60. The RNA or use of any one of claims 45-59, wherein at each RNA comprises the 5' cap $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or 3'-O-Me- $m^7G(5')ppp(5')G$.
61. The RNA or use of any one of claims 45-60, wherein at least one RNA comprises a 5' UTR comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.

62. The RNA or use of any one of claims 45-61, wherein each RNA comprises a 5' UTR comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6.
63. The RNA or use of any one of claims 45-62, wherein at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
64. The RNA or use of any one of claims 45-63, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 8, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8.
65. The RNA or use of any one of claims 45-64, wherein at least one RNA comprises a poly-A tail.
66. The RNA or use of any one of claims 43-65, wherein each RNA comprises a poly-A tail.
67. The RNA or use of claim 65 or 66, wherein the poly-A tail comprises at least 100 nucleotides.
68. The RNA or use of claim 65 or 66, wherein the poly-A tail comprises the poly-A tail shown in SEQ ID NO: 78.
69. The RNA or use of any one of claims 45-68, wherein one or more RNA comprises:
 - a. a 5' cap comprising $m_2^{7,3'}\text{-O}Gppp(m_1^{2'}\text{-O})ApG$ or $3'\text{-O-Me-}m^7G(5')ppp(5')G$;
 - b. a 5' UTR comprising (i) a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 4, and 6;
 - c. a 3' UTR comprising (i) the nucleotide sequence of SEQ ID NO: 8, or (ii) a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 8; and
 - d. a poly-A tail comprising at least 100 nucleotides.
70. The RNA or use of claim 69, wherein the poly-A tail comprises SEQ ID NO: 78.
71. The RNA or use of any one of claims 45 to 70, wherein the method further comprises administering a further therapy.

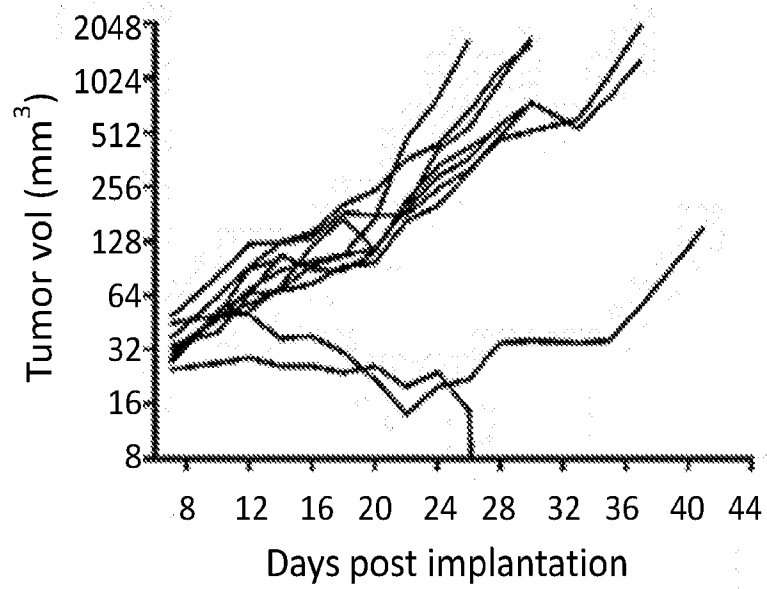
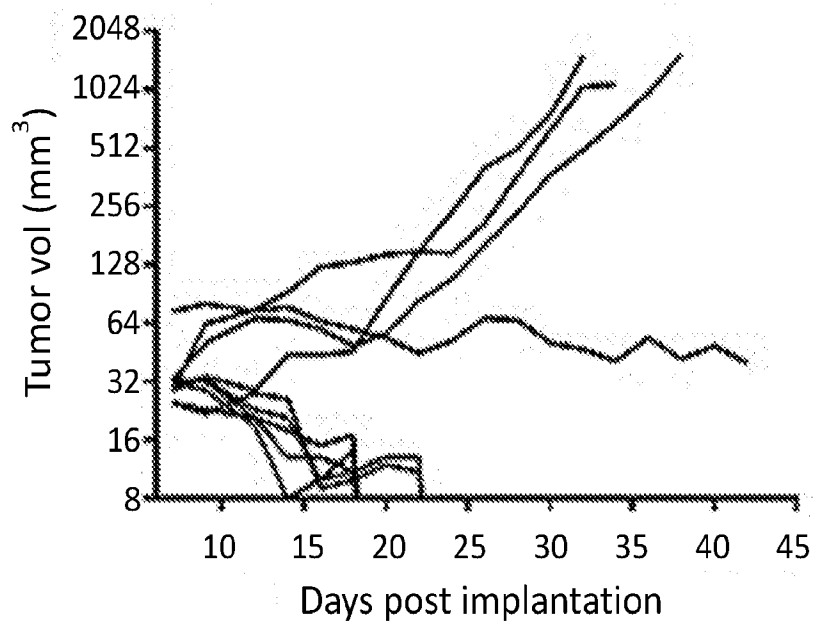
72. The RNA or use of claim 71, wherein the further therapy comprises one or more selected from the group consisting of: (i) surgery to excise, resect, or debulk a tumor, (ii) immunotherapy, (iii) radiotherapy, and (iv) chemotherapy.
73. The RNA or use of claim 71 or 72, wherein the further therapy comprises administering a further therapeutic agent.
74. The RNA or use of claim 73, wherein the further therapeutic agent is an anti-cancer therapeutic agent.
75. The RNA or use of claim 73 or 74, wherein the further therapeutic agent is a checkpoint modulator.
76. The RNA or use of claim 75, wherein the checkpoint modulator is an anti-PD1 antibody, an anti-CTLA-4 antibody, or a combination of an anti-PD1 antibody and an anti-CTLA-4 antibody.
77. The RNA or use of any one of claims 45-76, wherein the solid tumor is a sarcoma, carcinoma, or lymphoma.
78. The RNA or use of any one of claims 45-77, wherein the solid tumor is in the lung, colon, ovary, cervix, uterus, peritoneum, testicles, penis, tongue, lymph node, pancreas, bone, breast, prostate, soft tissue, connective tissue, kidney, liver, brain, thyroid, or skin.
79. The RNA or use of any one of claims 45-78, wherein the solid tumor is an epithelial tumor, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, prostate tumor, ovarian tumor, renal cell tumor, gastrointestinal tract tumor, hepatic tumor, colorectal tumor, tumor with vasculature, mesothelioma tumor, pancreatic tumor, breast tumor, sarcoma tumor, lung tumor, colon tumor, brain tumor, melanoma tumor, small cell lung tumor, neuroblastoma tumor, testicular tumor, carcinoma tumor, adenocarcinoma tumor, glioma tumor, seminoma tumor, retinoblastoma, or osteosarcoma tumor.
80. The RNA or use of any one of claims 45-79, wherein the RNA is administered intra-tumorally or peri-tumorally.
81. The RNA or use of any one of claims 45-80, wherein the RNA is formulated for injection.
82. The RNA or use of any one of claims 73-81, wherein the further therapeutic agent is administered systemically.
83. The RNA or use of any one of claims 45-82, wherein the subject is a human.
84. The RNA or use of any one of claims 45-83, wherein the RNAs are administered at the same time.

85. The RNA or use of any one of claims 45-84, wherein the RNAs are administered via injection, wherein the RNAs are mixed together in liquid solution prior to injection.
86. The RNA or use of any one of claims 45-85, wherein the RNAs are administered by administering a composition comprising a combination of the RNAs.
87. The RNA or use of any one of claims 45 to 86, wherein treating or preventing a solid tumor comprises reducing the size of a tumor, preventing the reoccurrence of cancer in remission, or preventing cancer metastasis in a subject.
88. A method for treating or reducing the likelihood of a solid tumor comprising administering to a subject in need thereof a first RNA, wherein the first RNA encodes an IL-12sc protein, an IL-15 sushi protein, an IFN α protein, or a GM-CSF protein and the subject is further treated with additional RNA, wherein:
 - a. if the first RNA encodes an IL-12sc protein, then the additional RNA encodes an IL-15 sushi protein, an IFN α protein, and a GM-CSF protein;
 - b. if the first RNA encodes an IL-15 sushi protein, then the additional RNA encodes an IL-12sc protein, an IFN α protein, and a GM-CSF protein;
 - c. if the first RNA encodes an IFN α protein, then the additional RNA encodes an IL-15 sushi protein, an IL-12sc protein, and a GM-CSF protein; and
 - d. if the first RNA encodes a GM-CSF protein, then the additional RNA encodes an IL-15 sushi protein, an IFN α protein, and an IL-12sc protein.

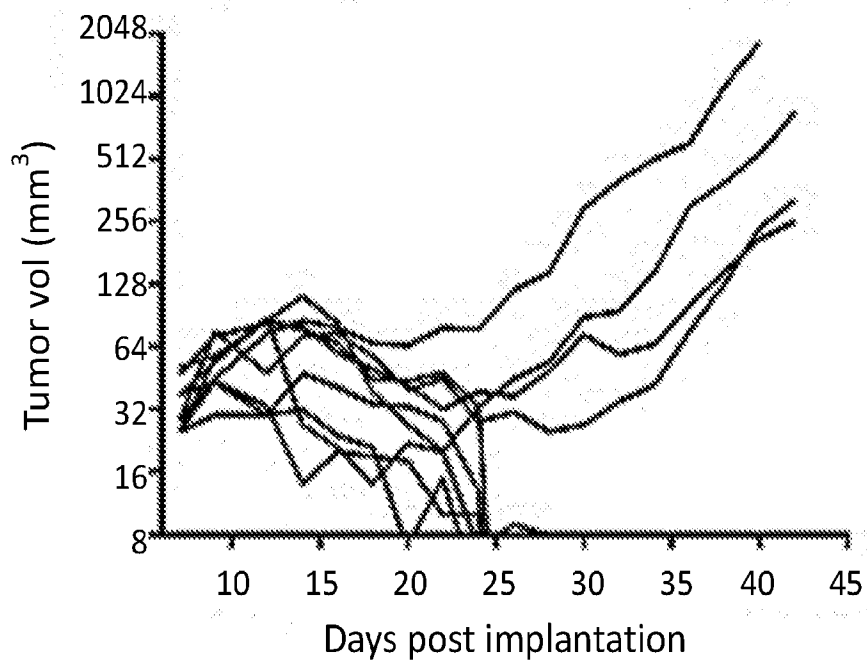
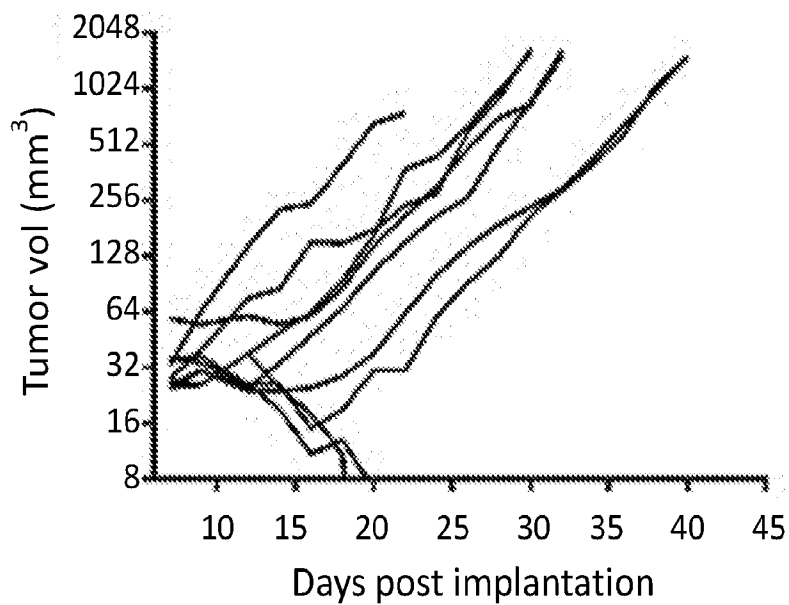
1/86

*Fig. 1A**Fig. 1B*

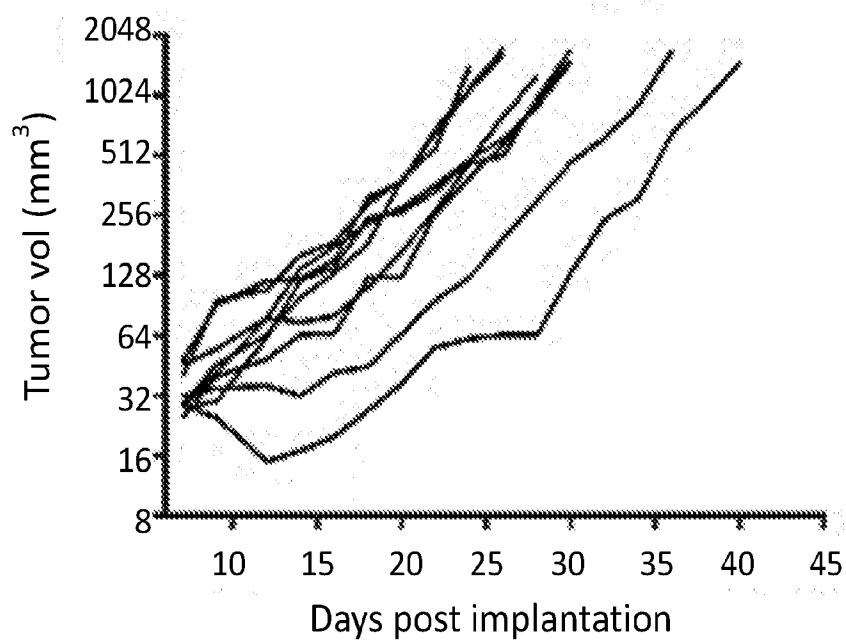
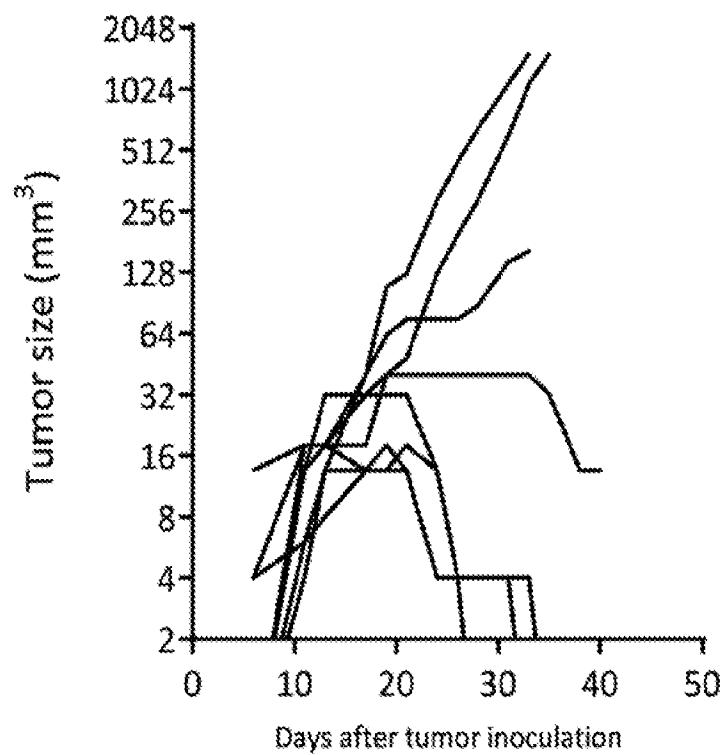
2/86

*Fig. 1C**Fig. 1D*

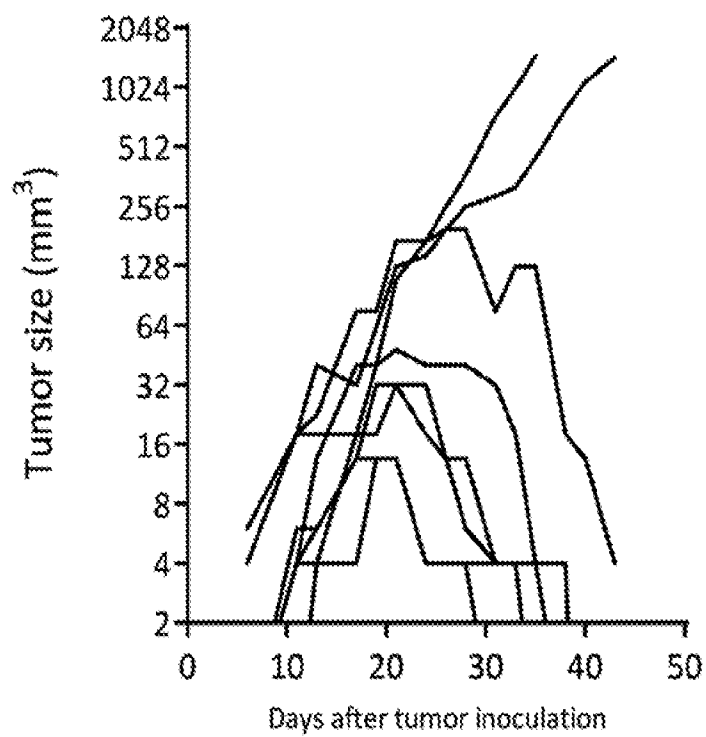
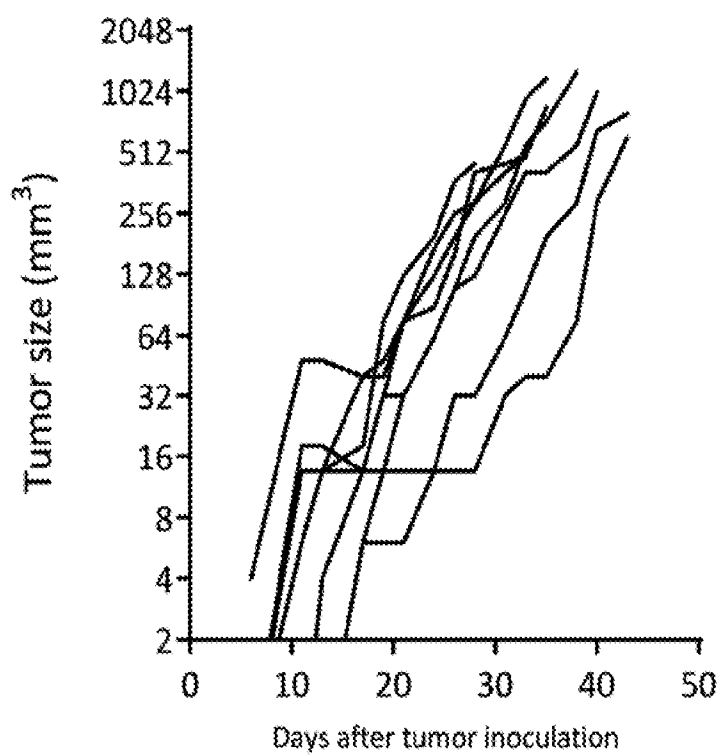
3/86

*Fig. 1E**Fig. 1F*

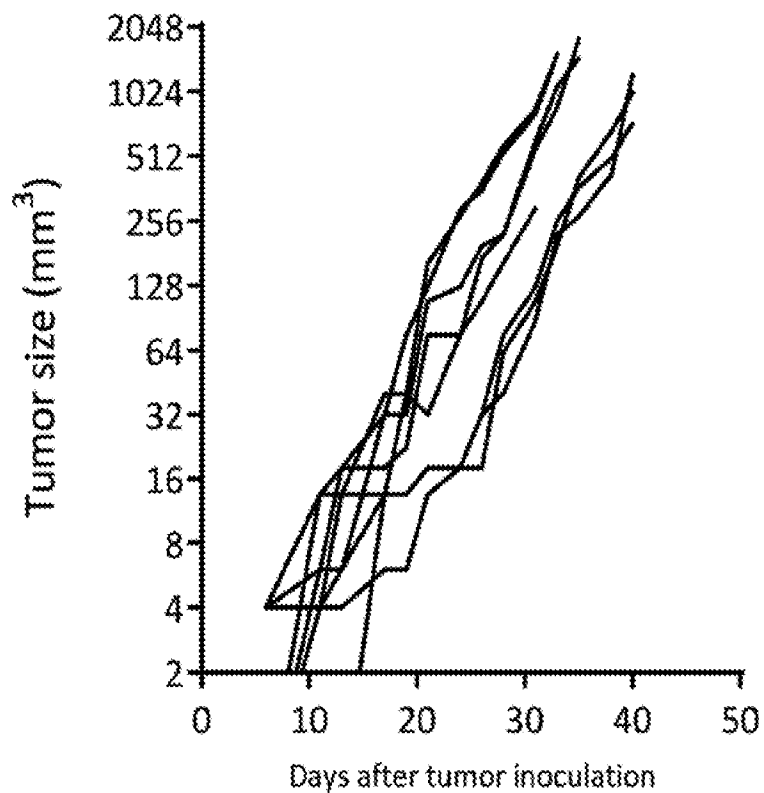
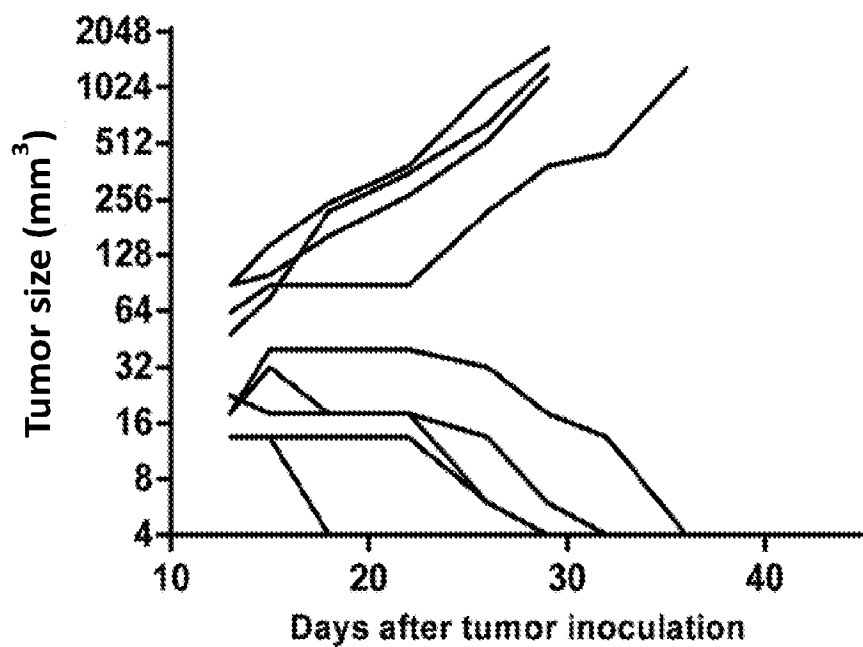
4/86

*Fig. 1G**Fig. 2A*

5/86

*Fig. 2B**Fig. 2C*

6/86

*Fig. 2D**Fig. 3A*

7/86

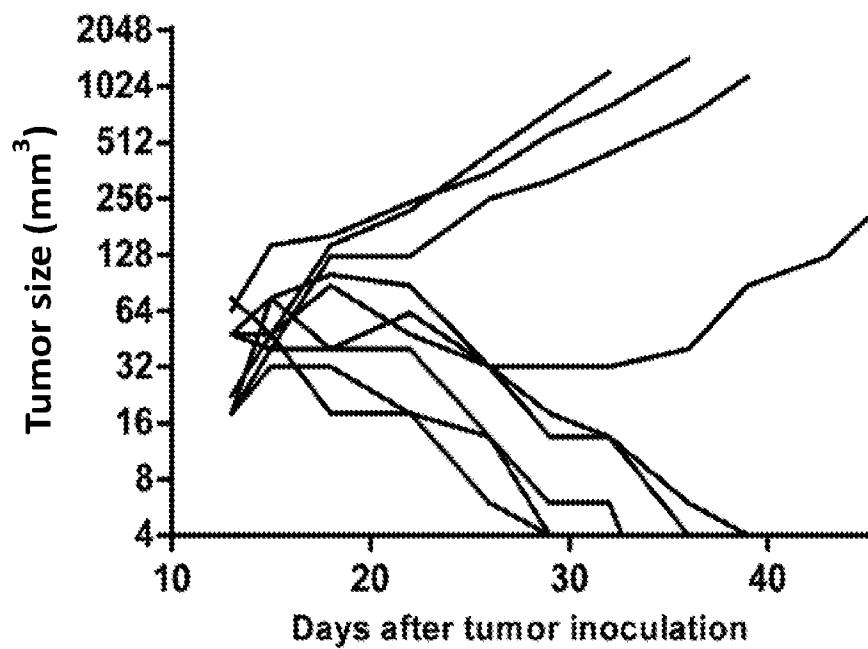


Fig. 3B

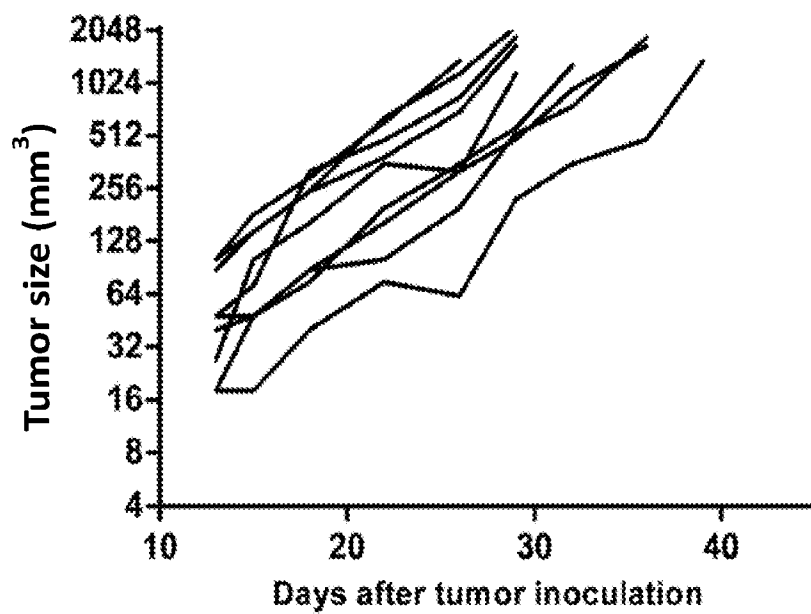
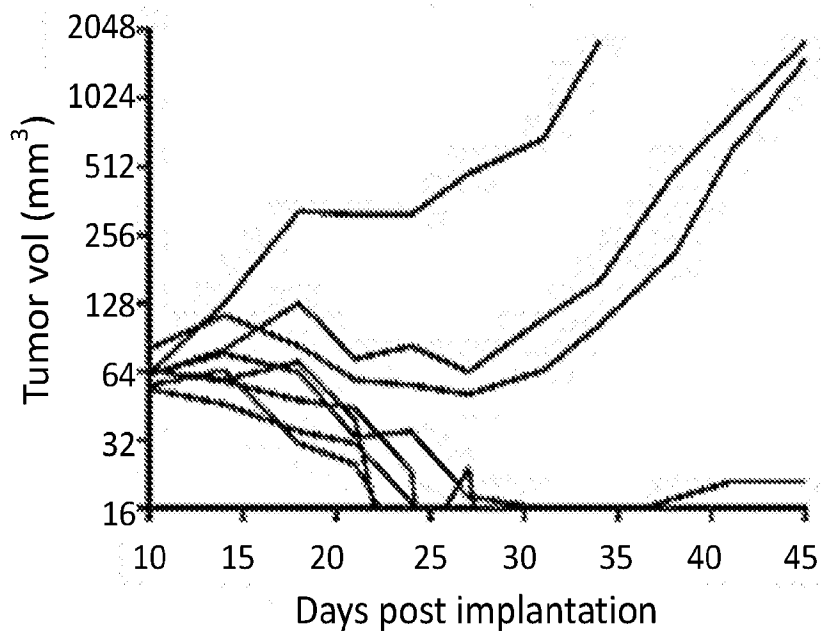
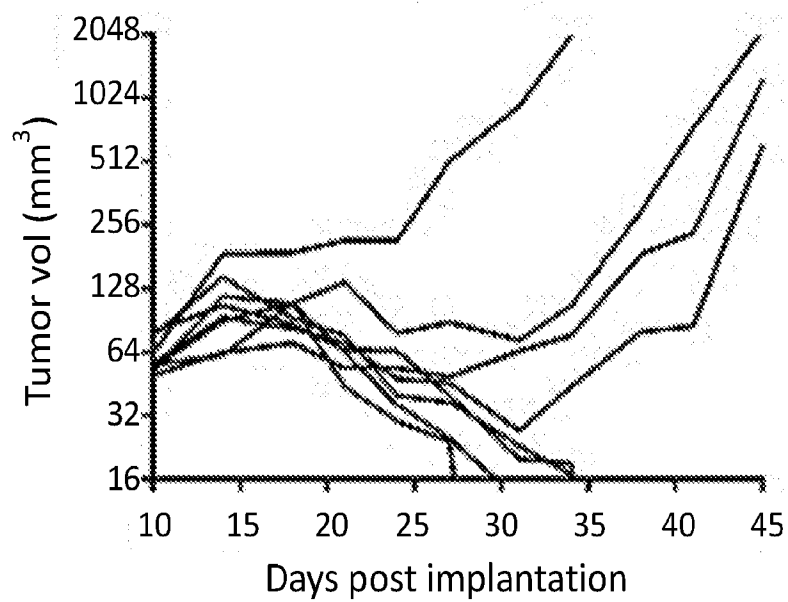
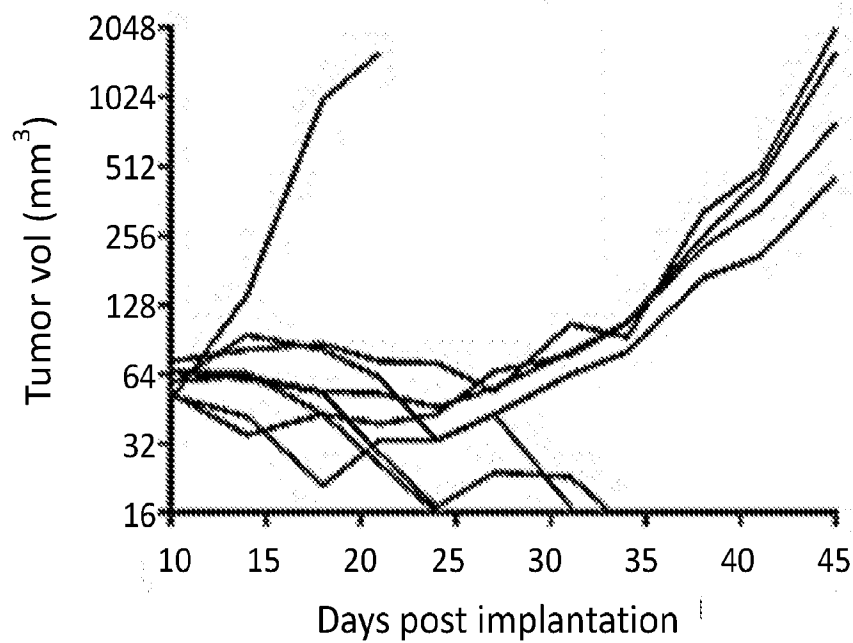
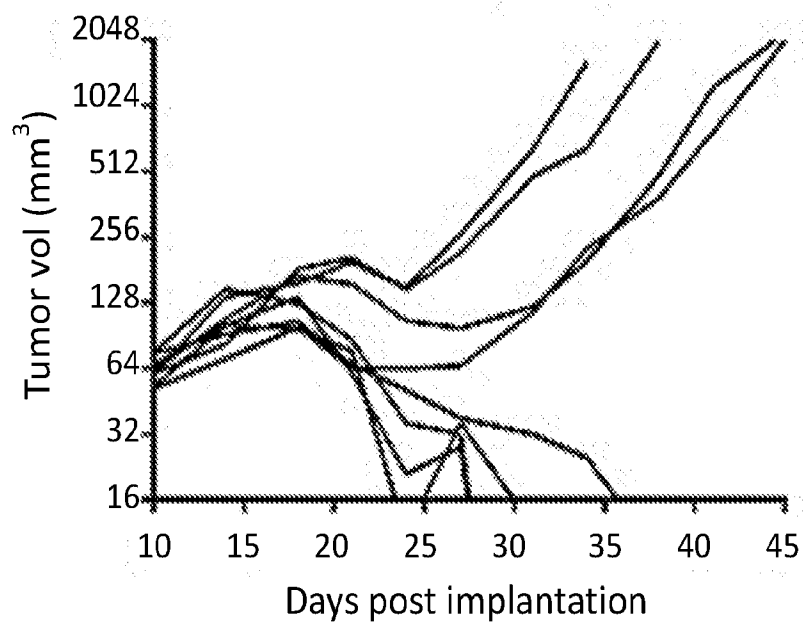


Fig. 3C

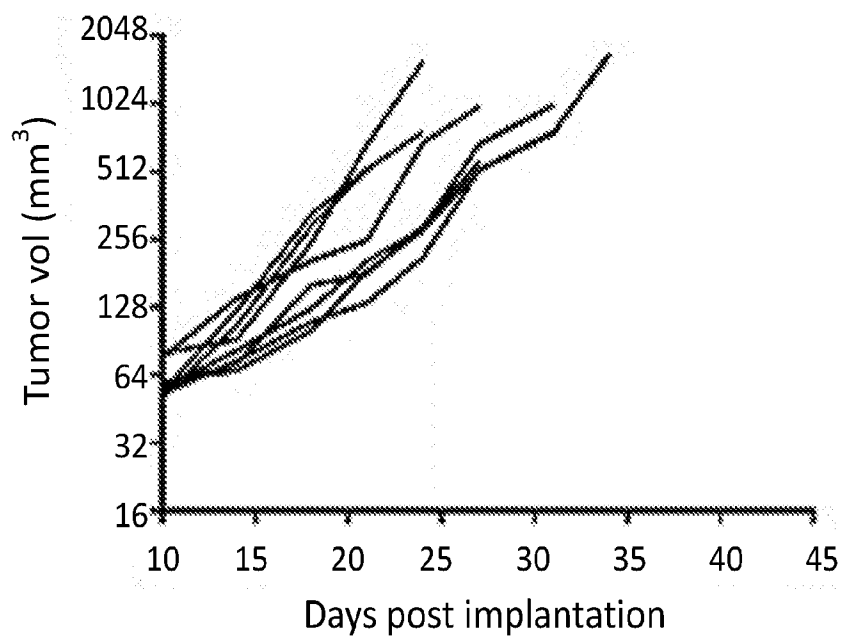
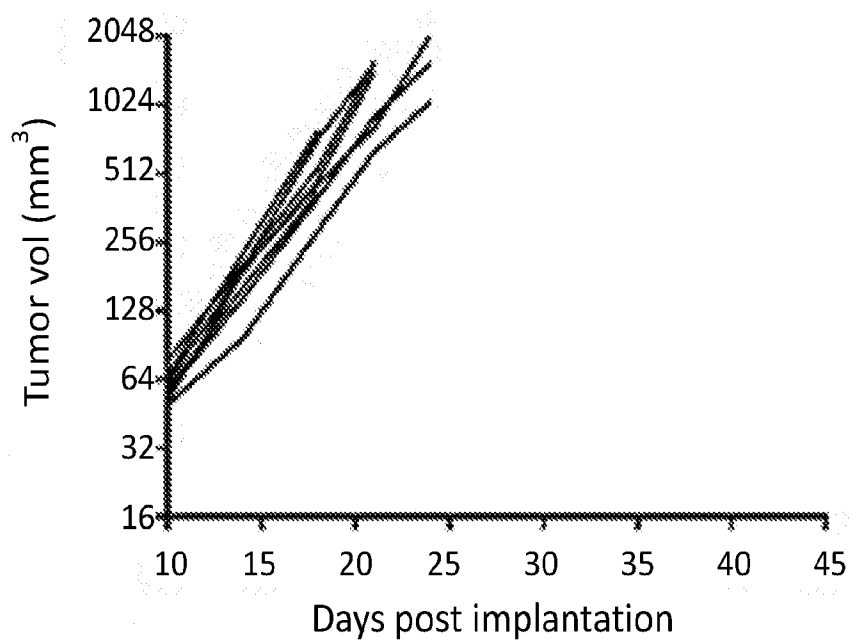
8/86

*Fig. 4A**Fig. 4B*

9/86

*Fig. 4C**Fig. 4D*

10/86

*Fig. 4E**Fig. 4F*

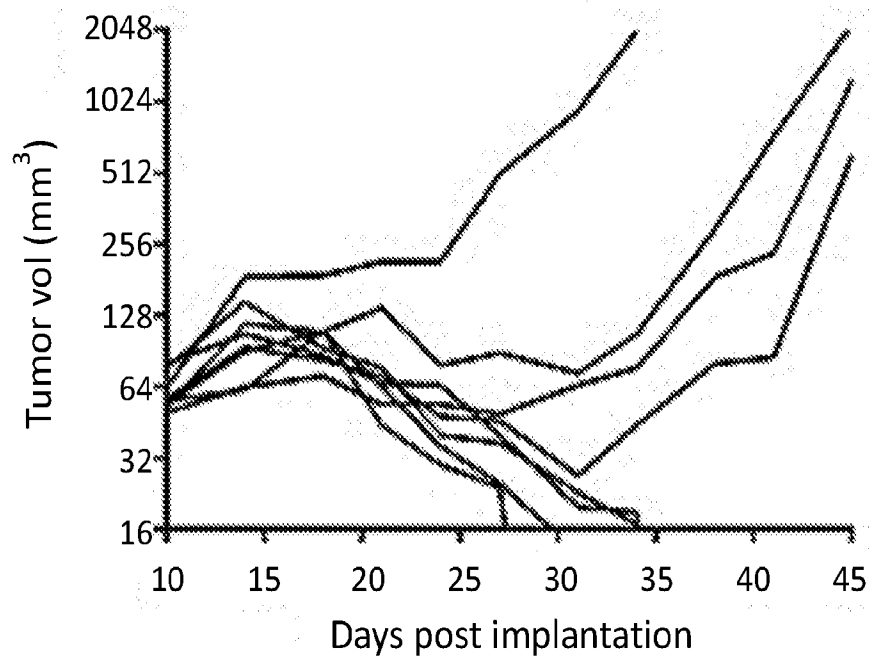


Fig. 5A

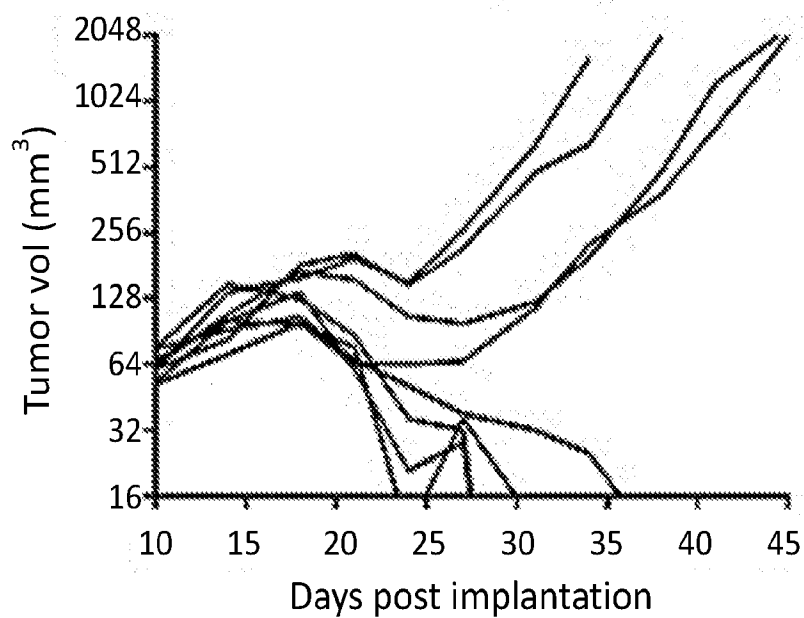
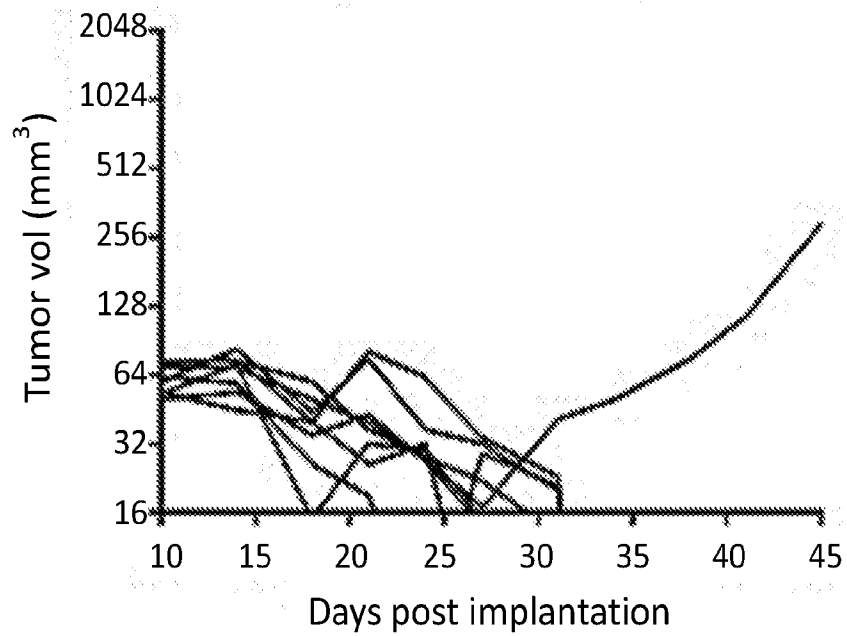
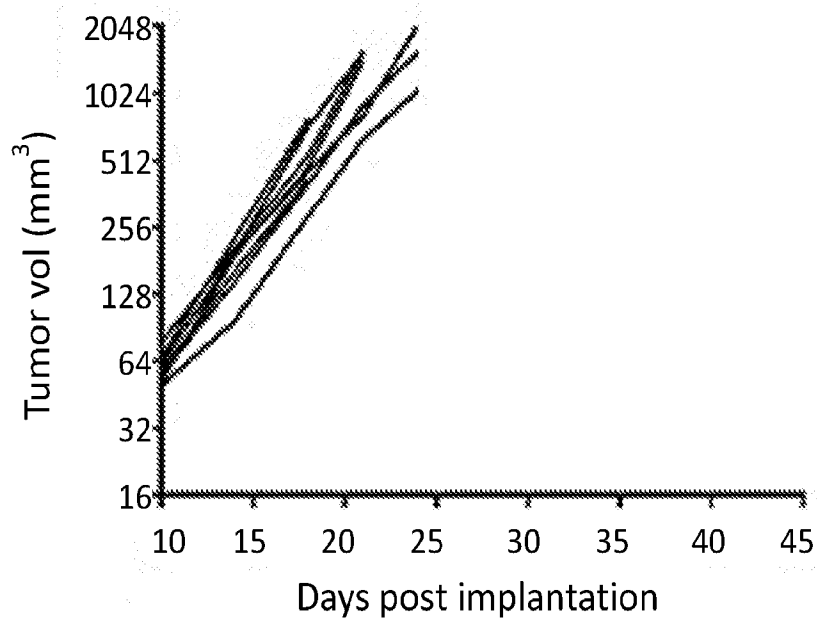
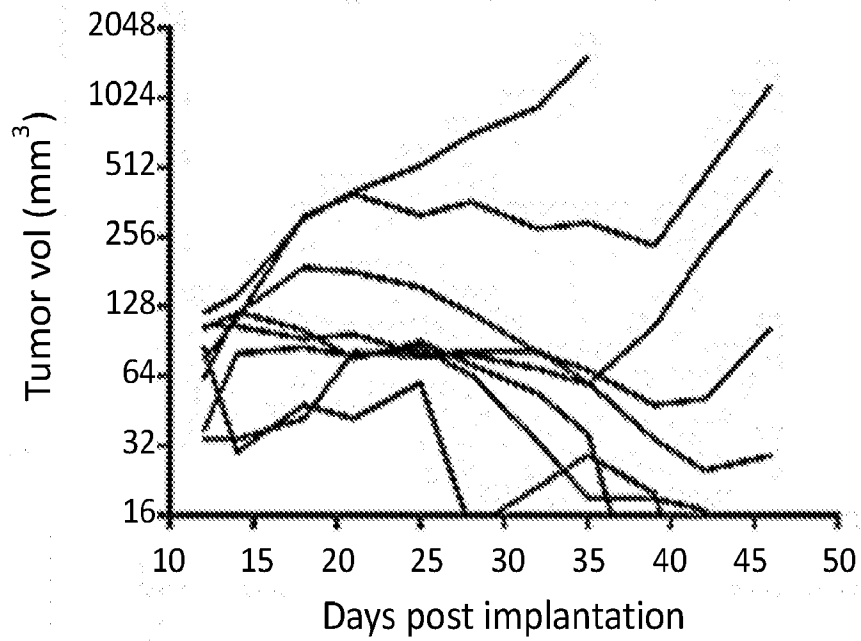
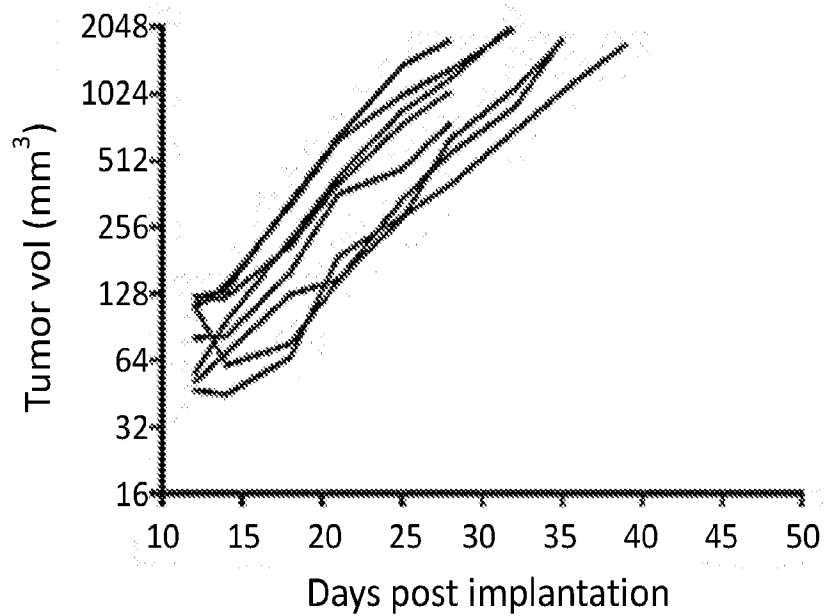


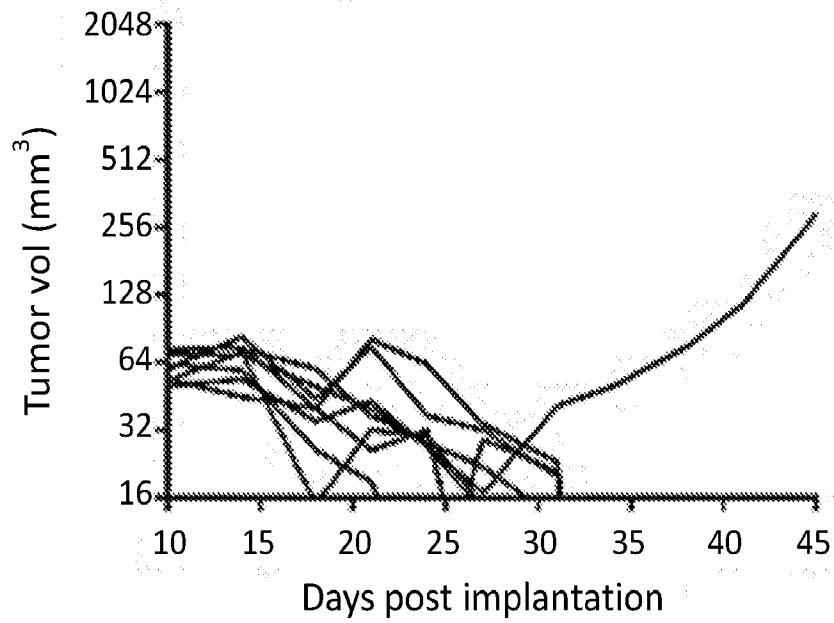
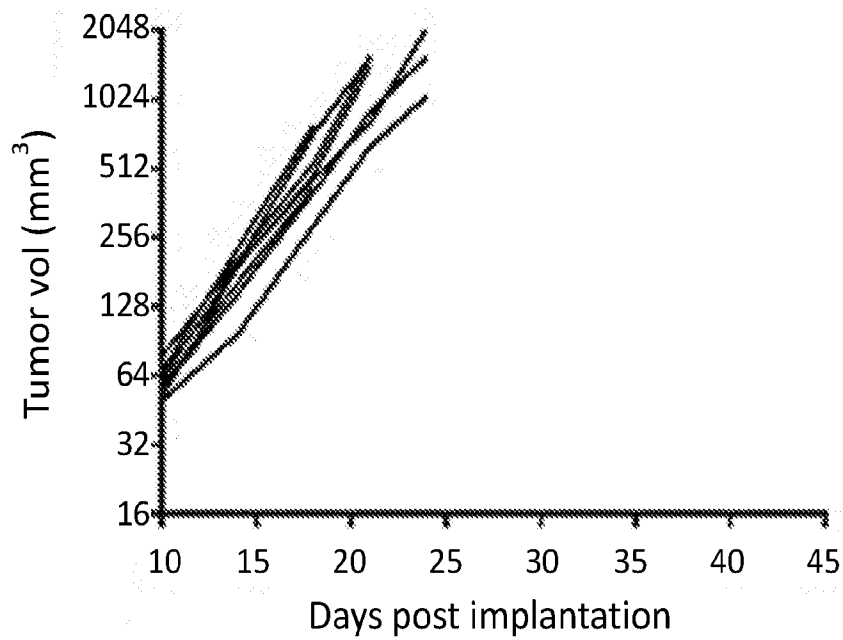
Fig. 5B

12/86

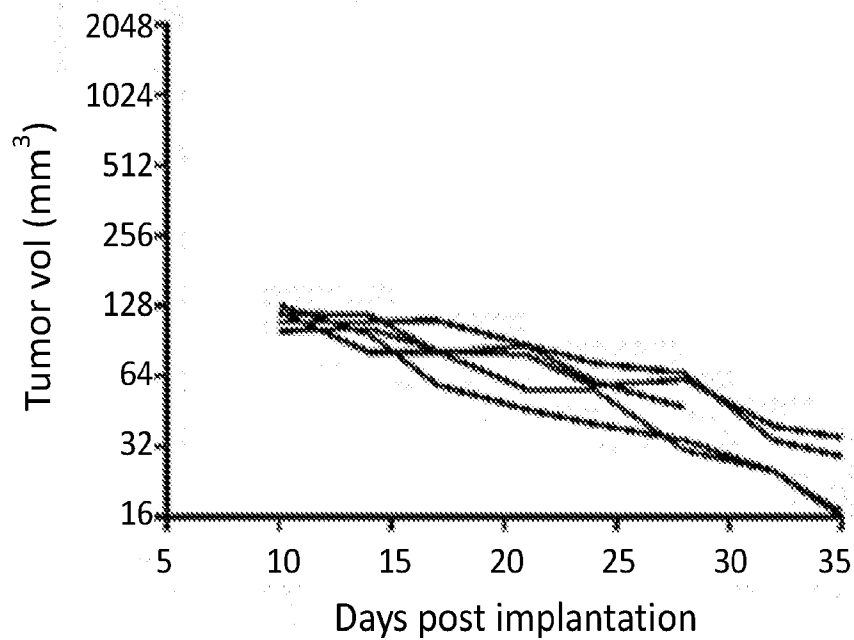
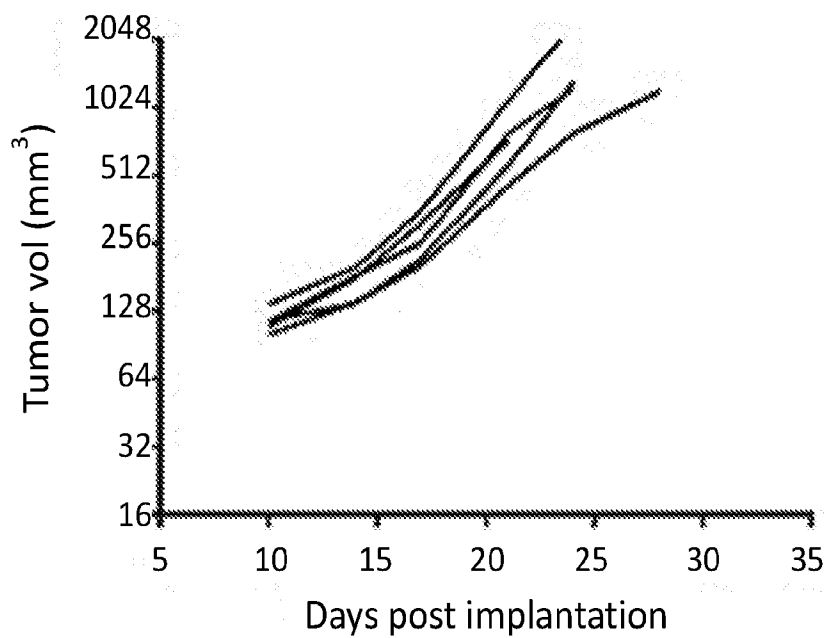
*Fig. 5C**Fig. 5D*

*Fig. 6A**Fig. 6B*

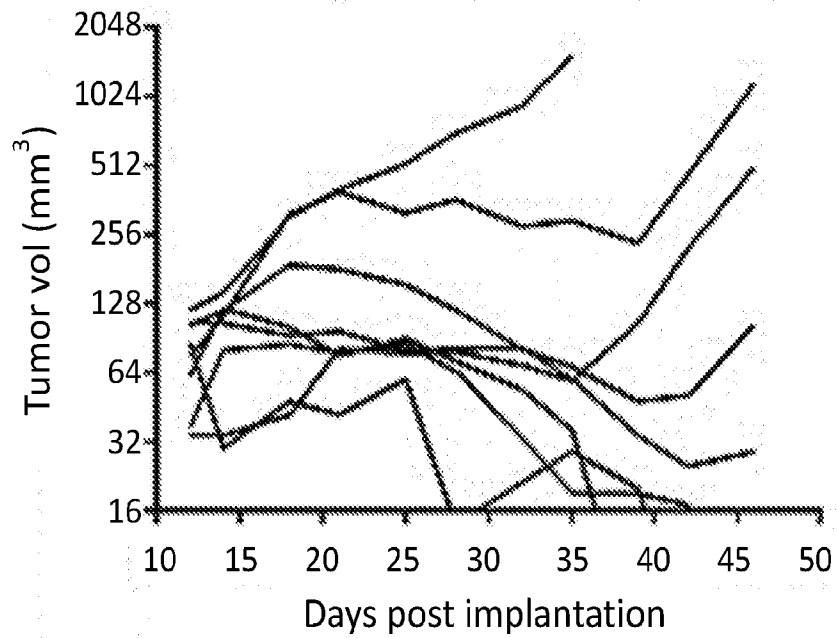
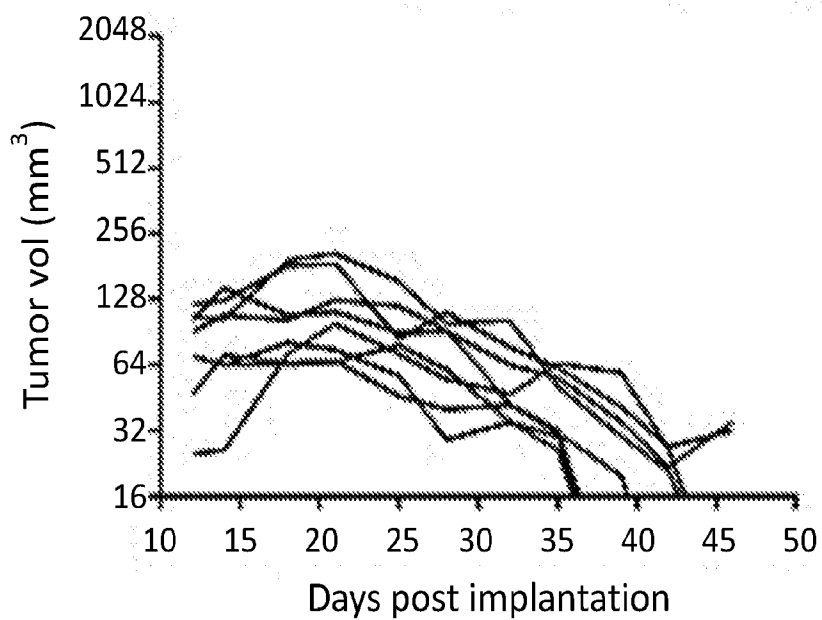
14/86

*Fig. 6C**Fig. 6D*

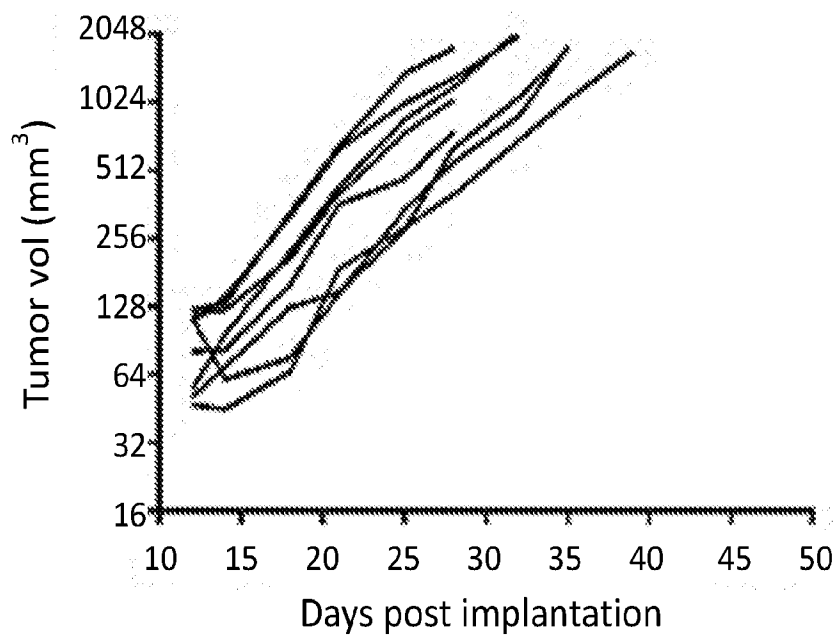
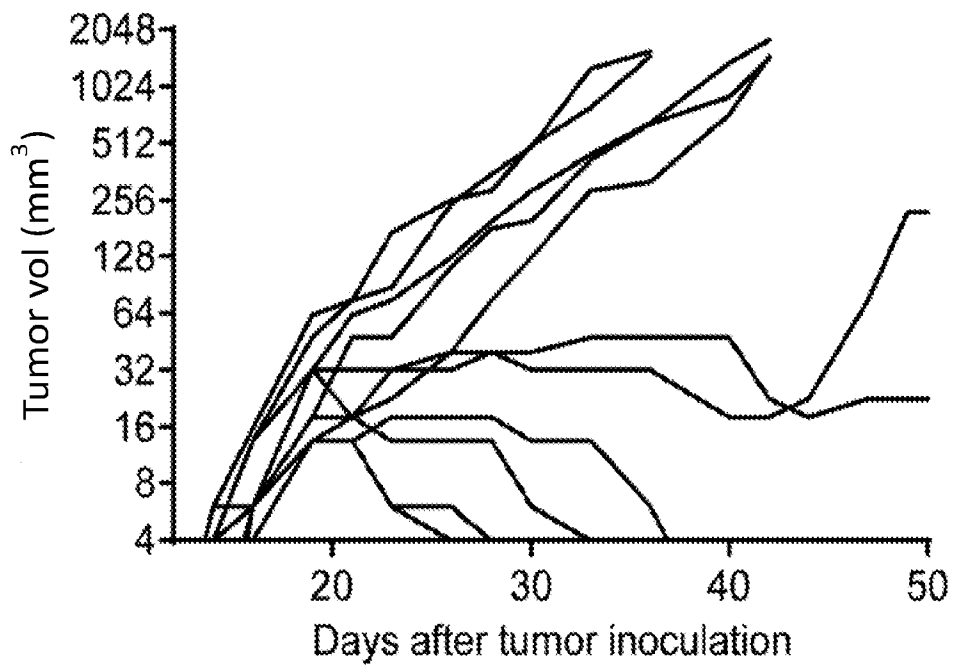
15/86

*Fig. 6E**Fig. 6F*

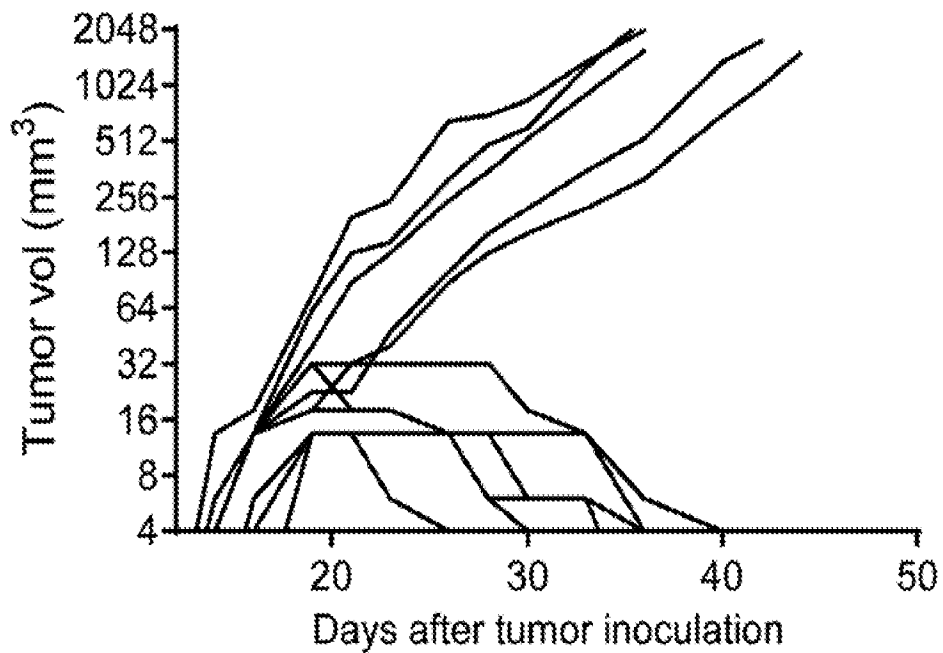
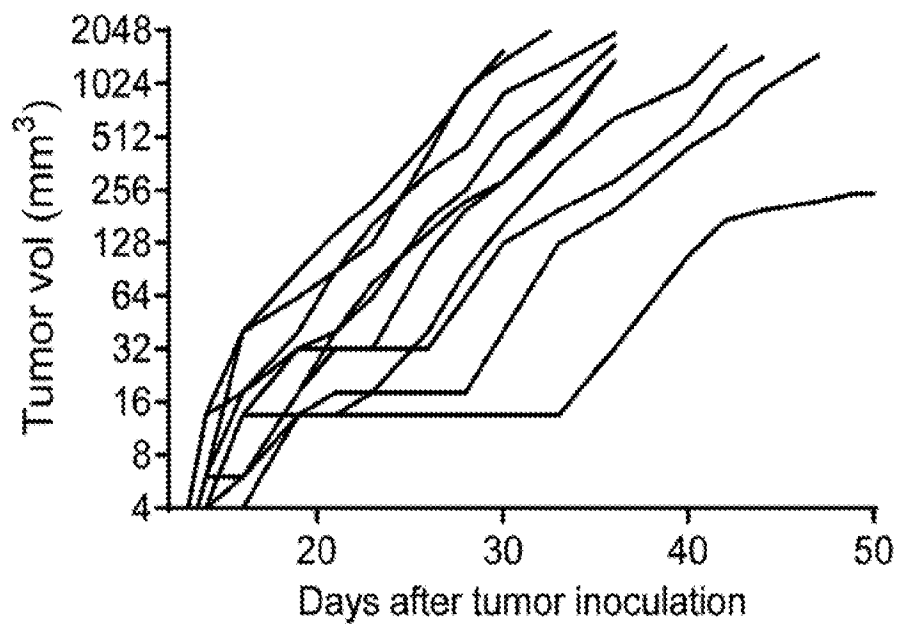
16/86

*Fig. 7A**Fig. 7B*

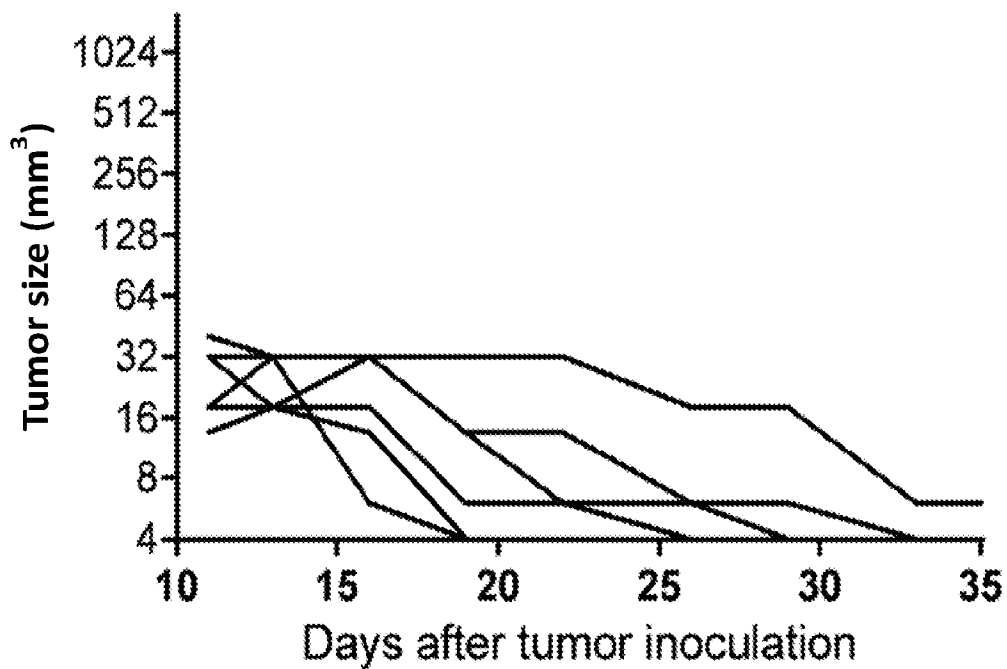
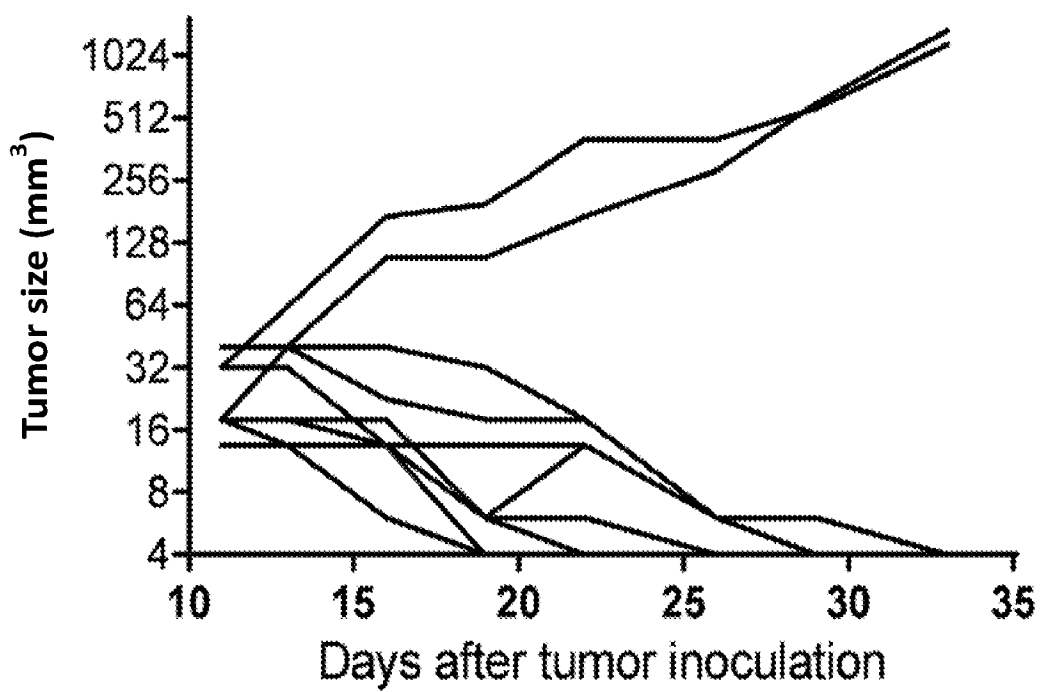
17/86

*Fig. 7C**Fig. 7D*

18/86

*Fig. 7E**Fig. 7F*

19/86

*Fig. 8A**Fig. 8B*

20/86

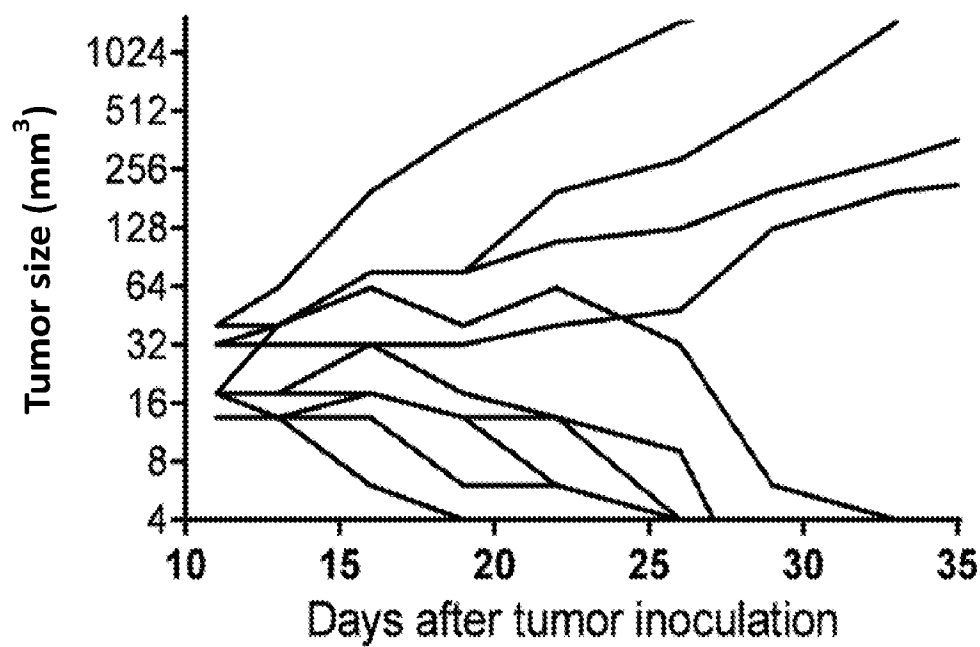


Fig. 8C

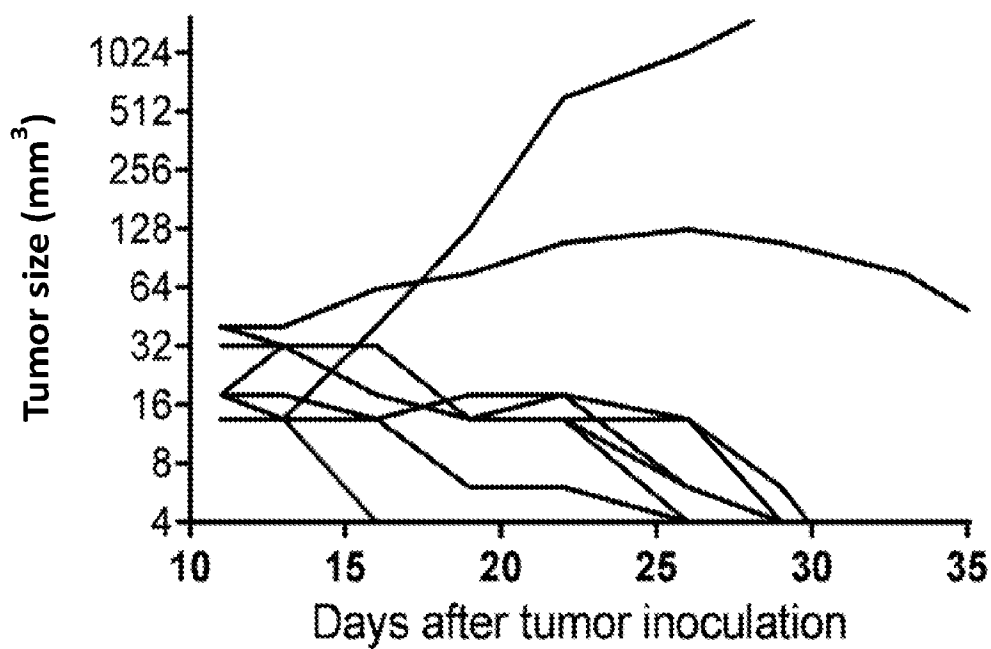


Fig. 8D

21/86

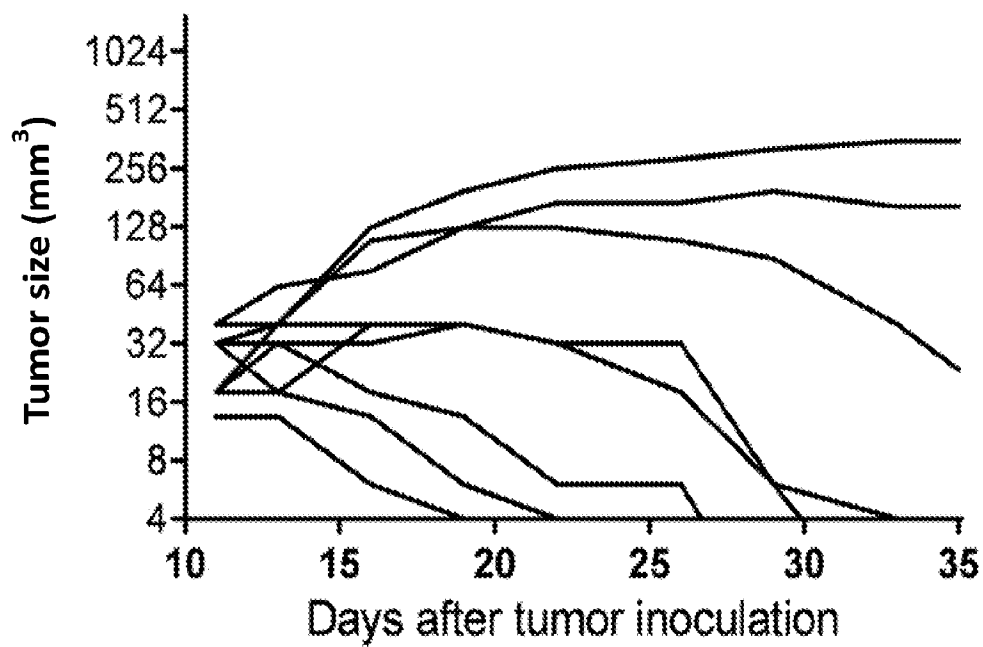


Fig. 8E

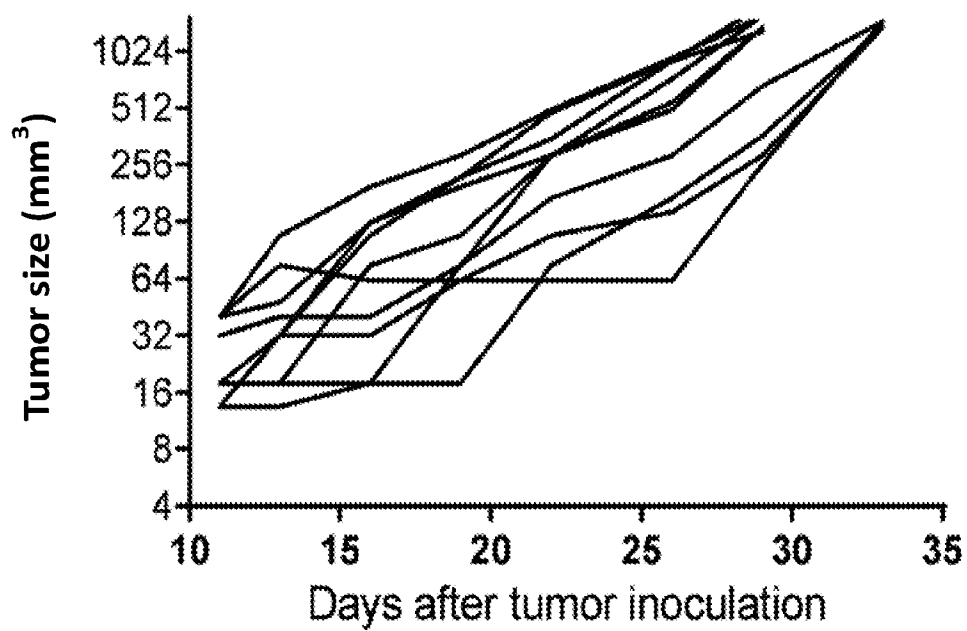


Fig. 8F

22/86

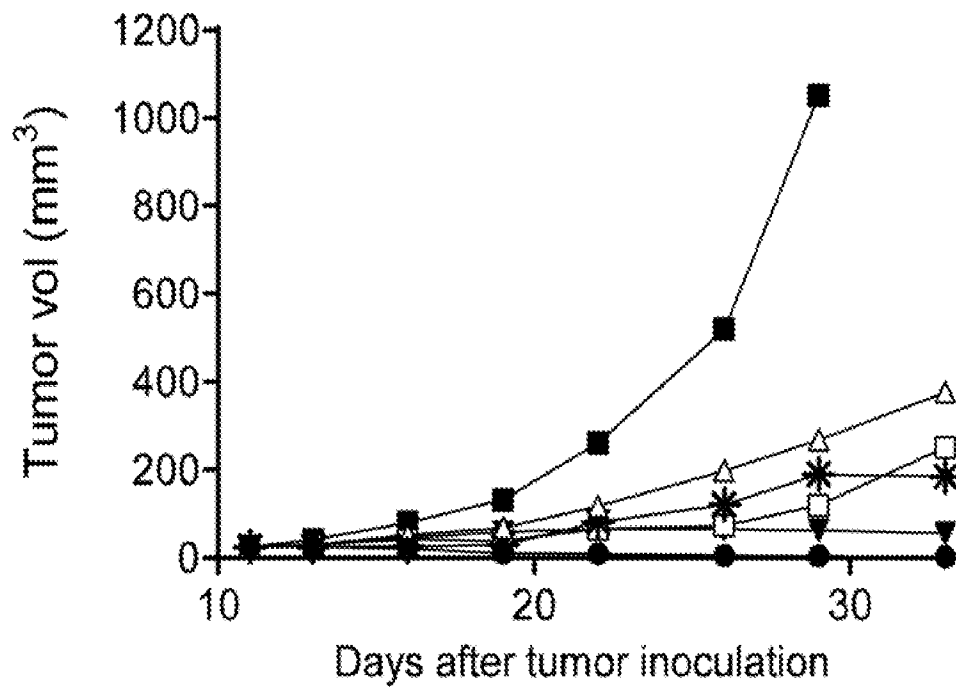


Fig. 8G

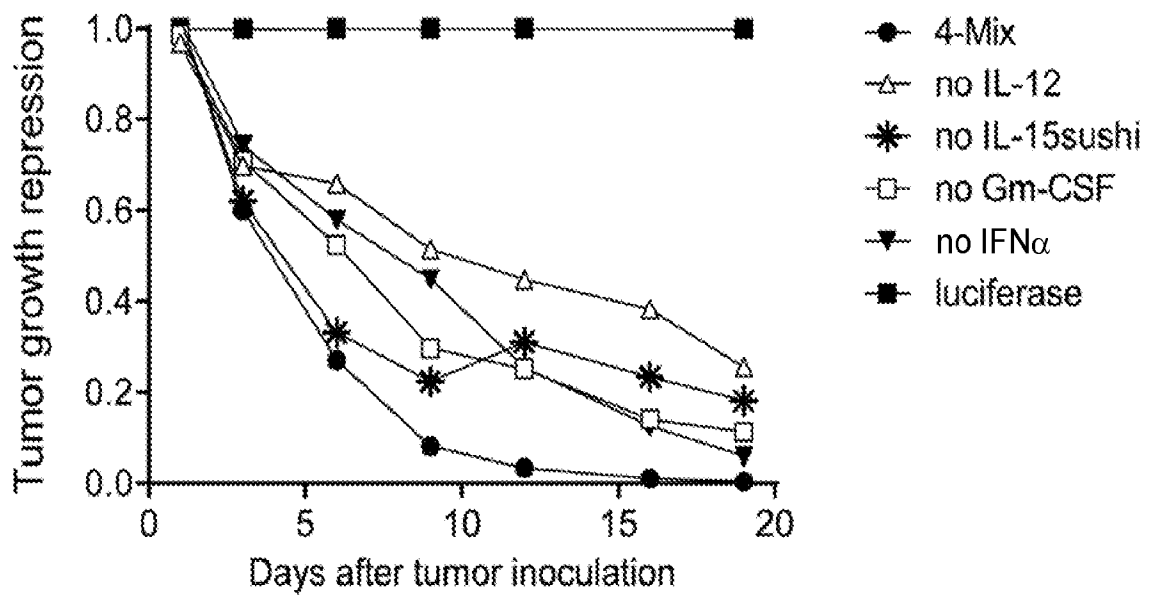
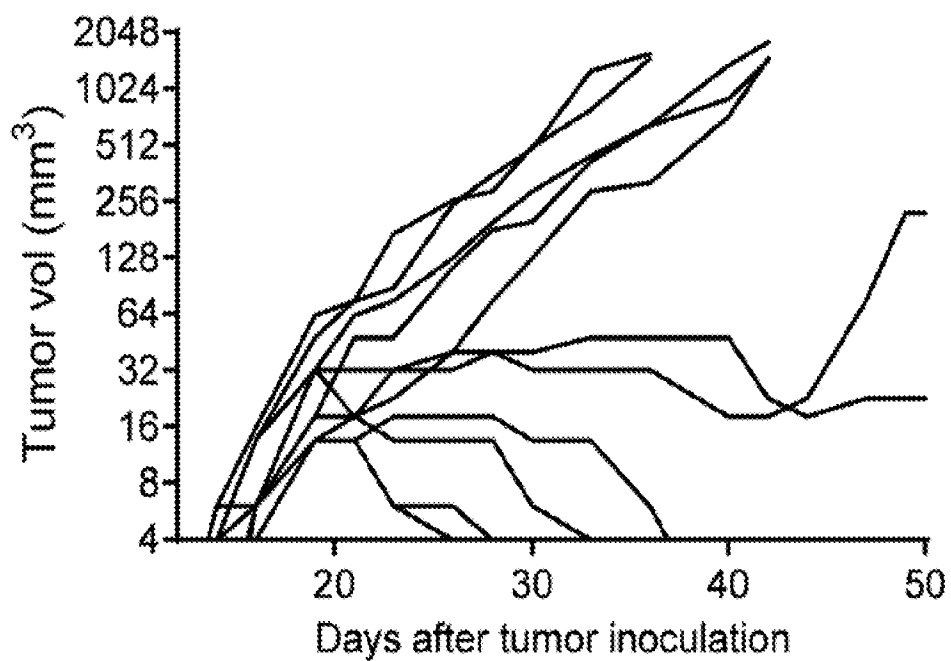
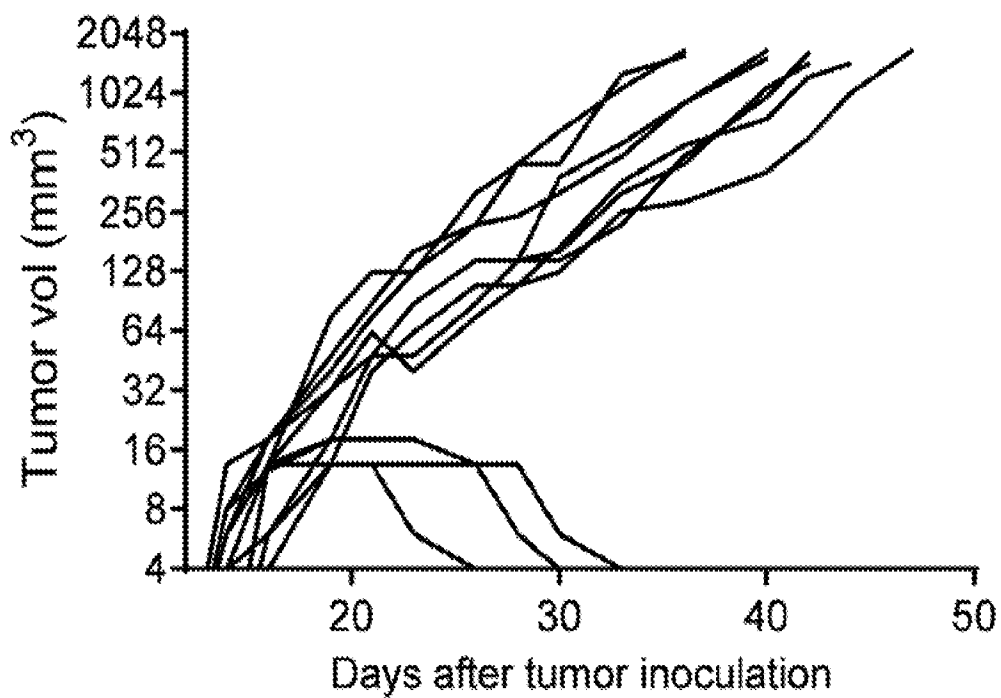
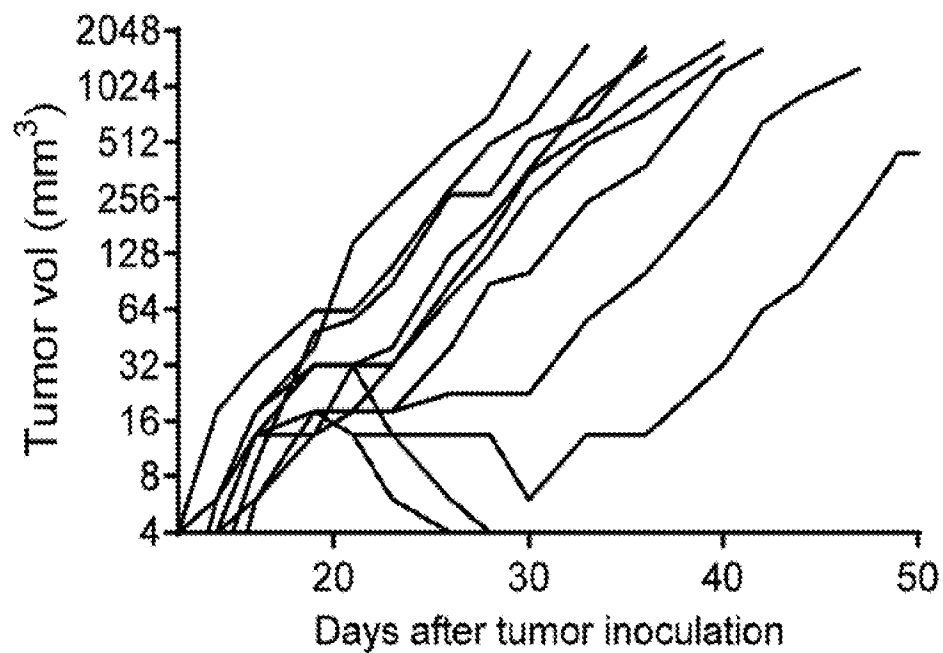
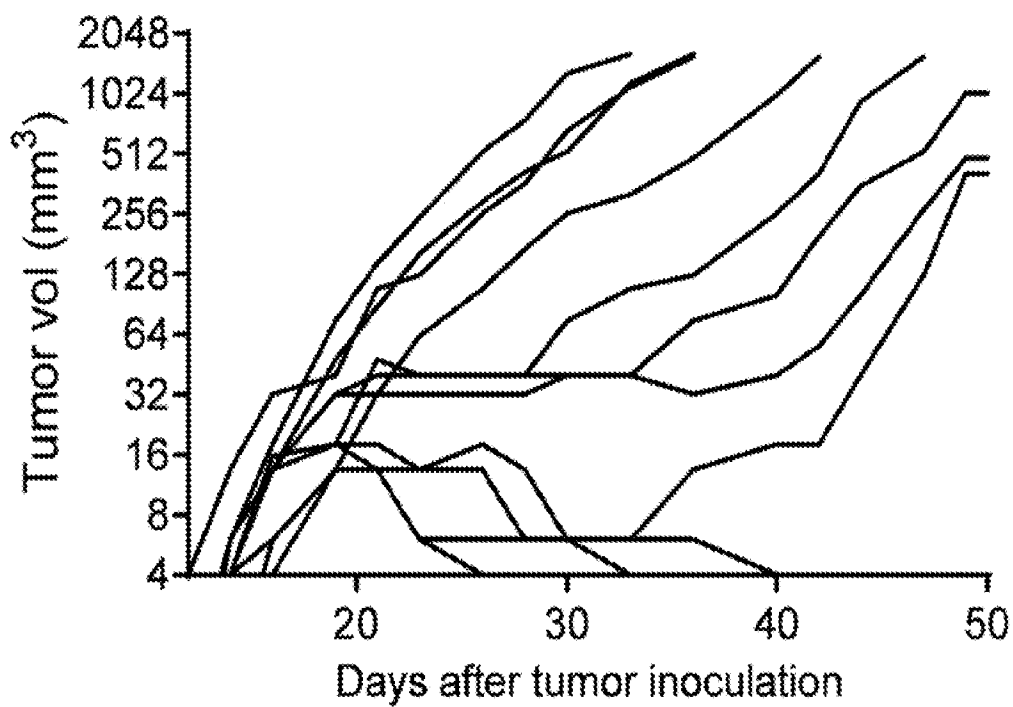


Fig. 8H

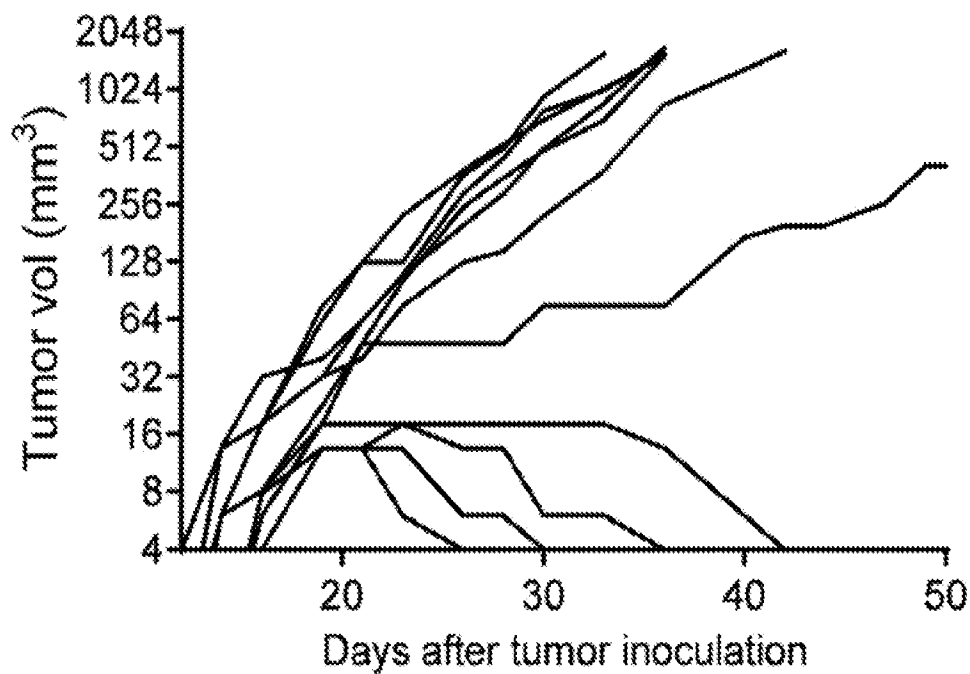
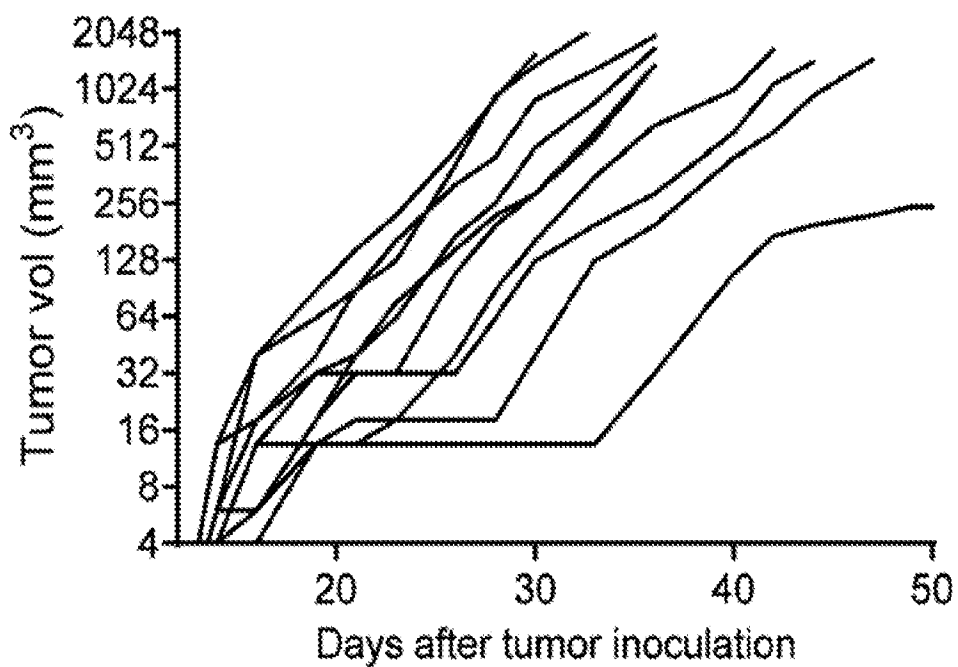
23/86

*Fig. 9A**Fig. 9B*

24/86

*Fig. 9C**Fig. 9D*

25/86

*Fig. 9E**Fig. 9F*

26/86

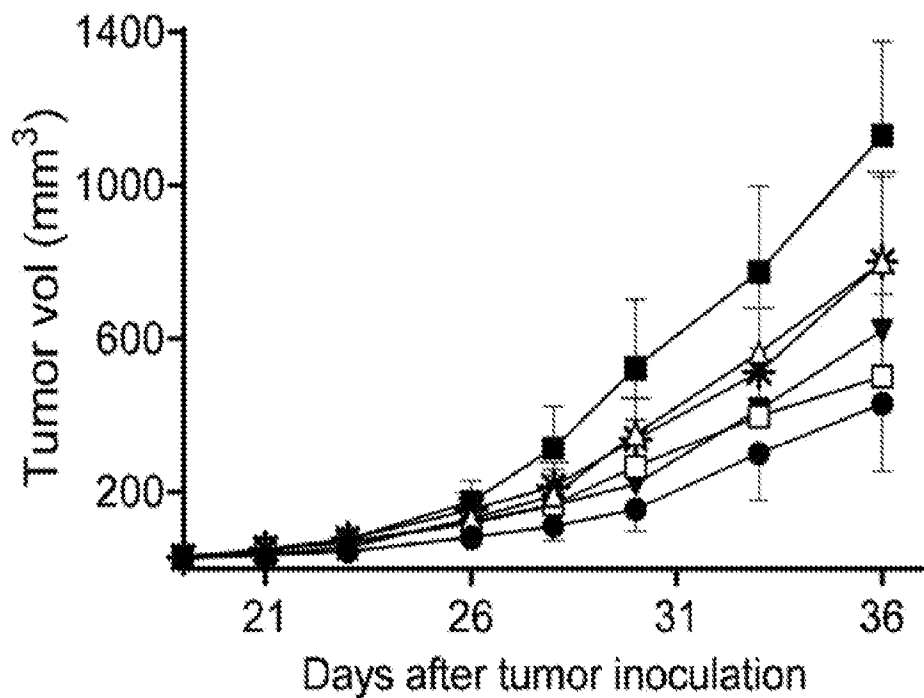


Fig. 10A

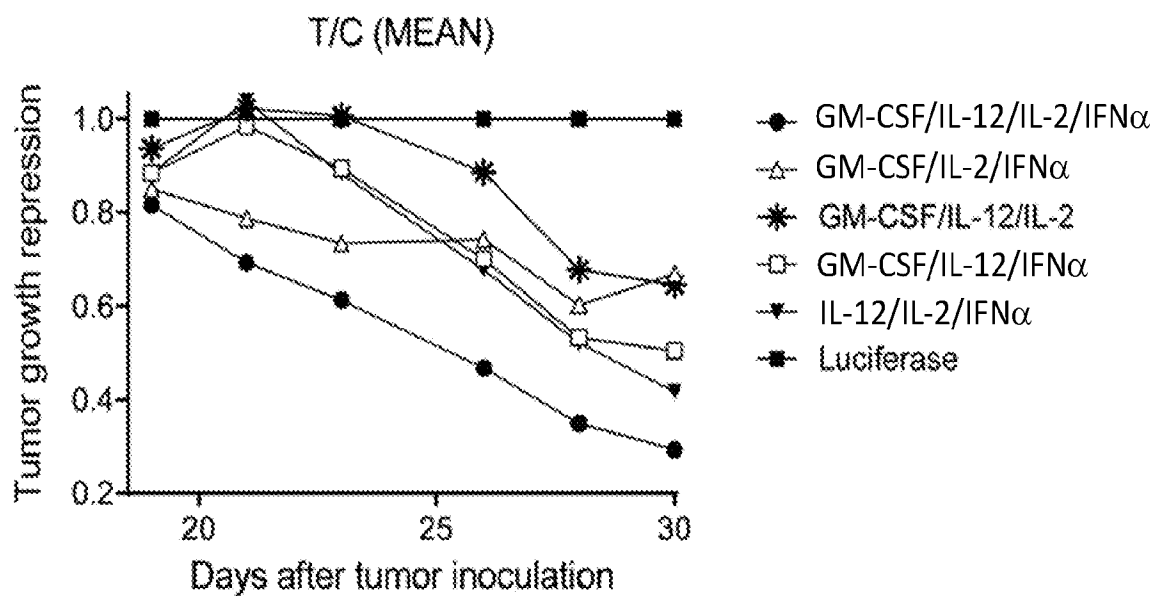
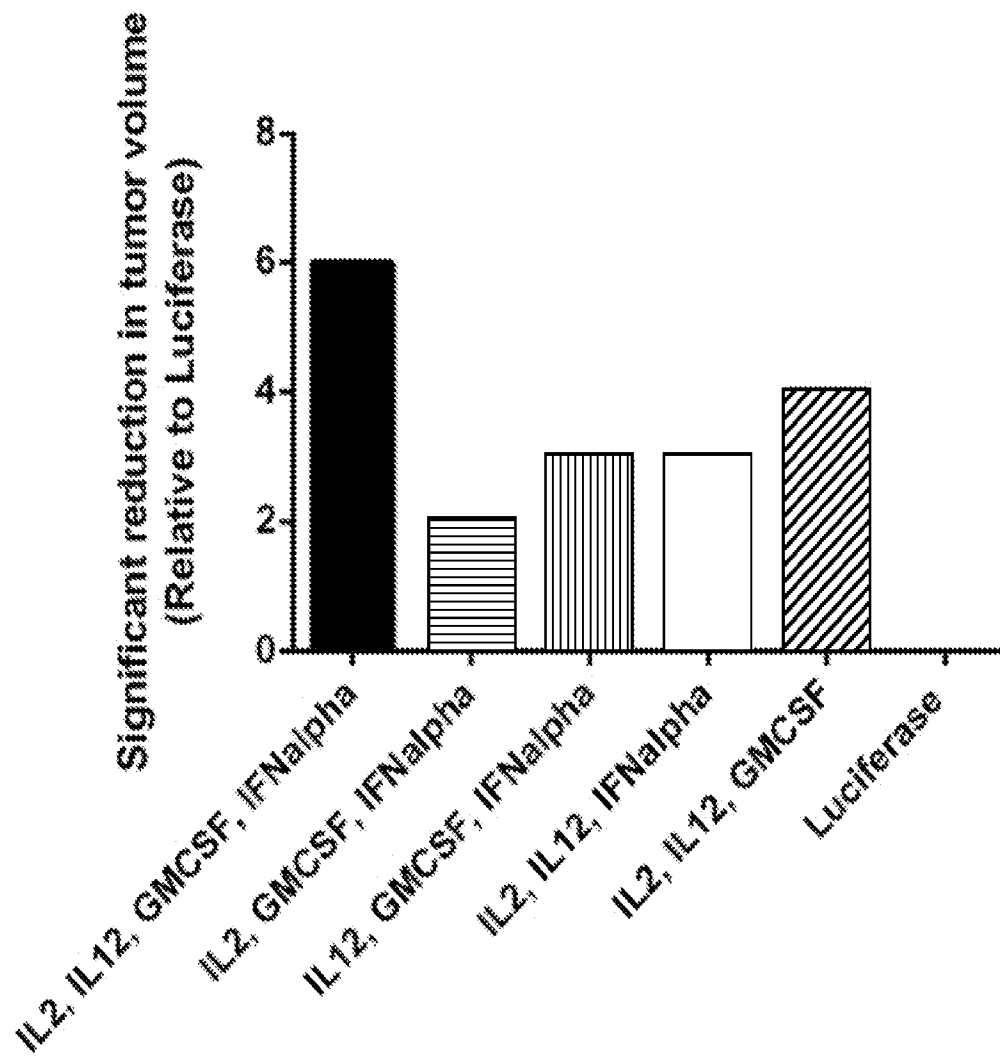
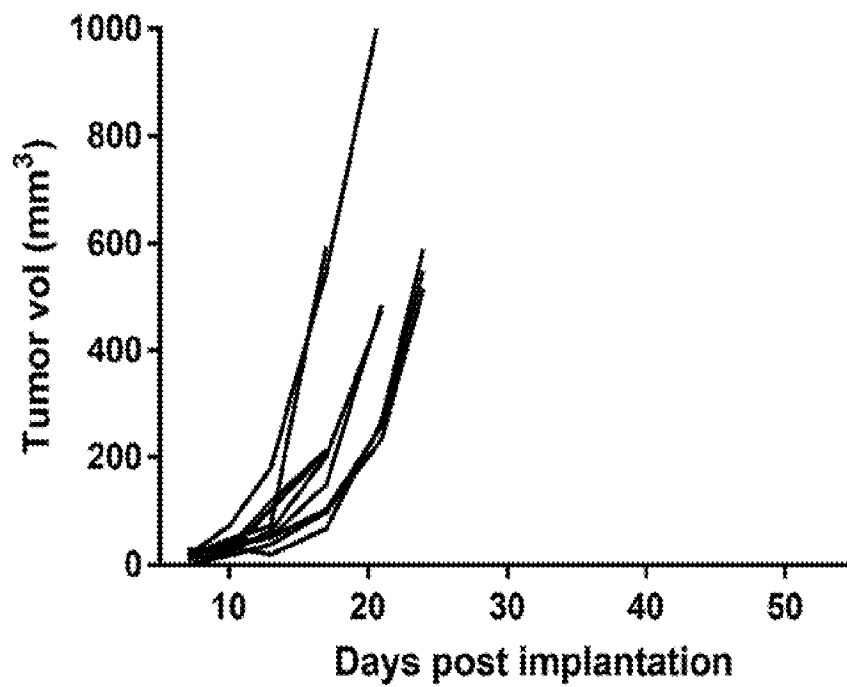
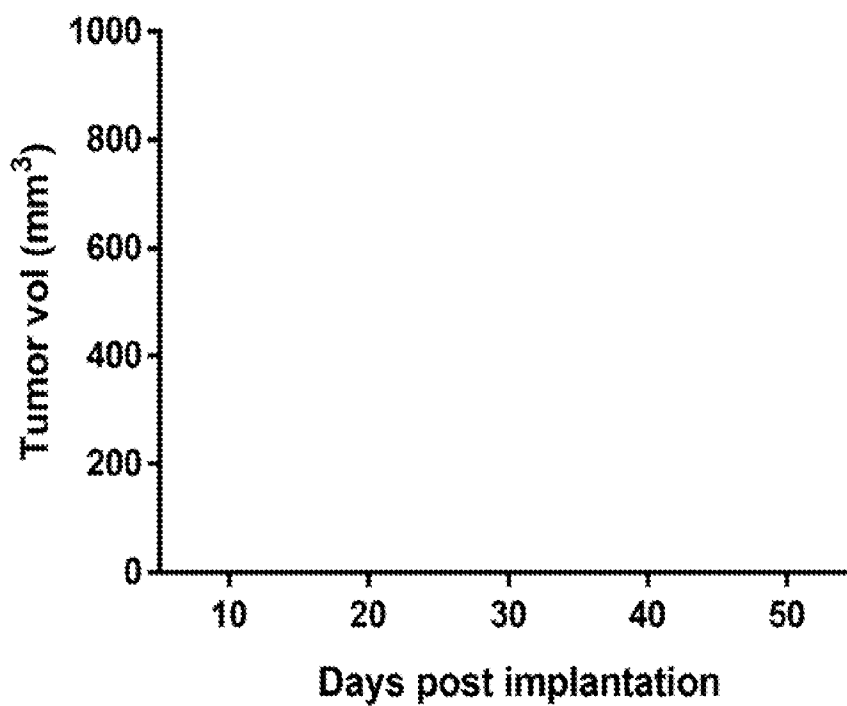


Fig. 10B

*Fig. 11*

28/86

*Fig. 12A**Fig. 12B*

Localized vitiligo at tumor site

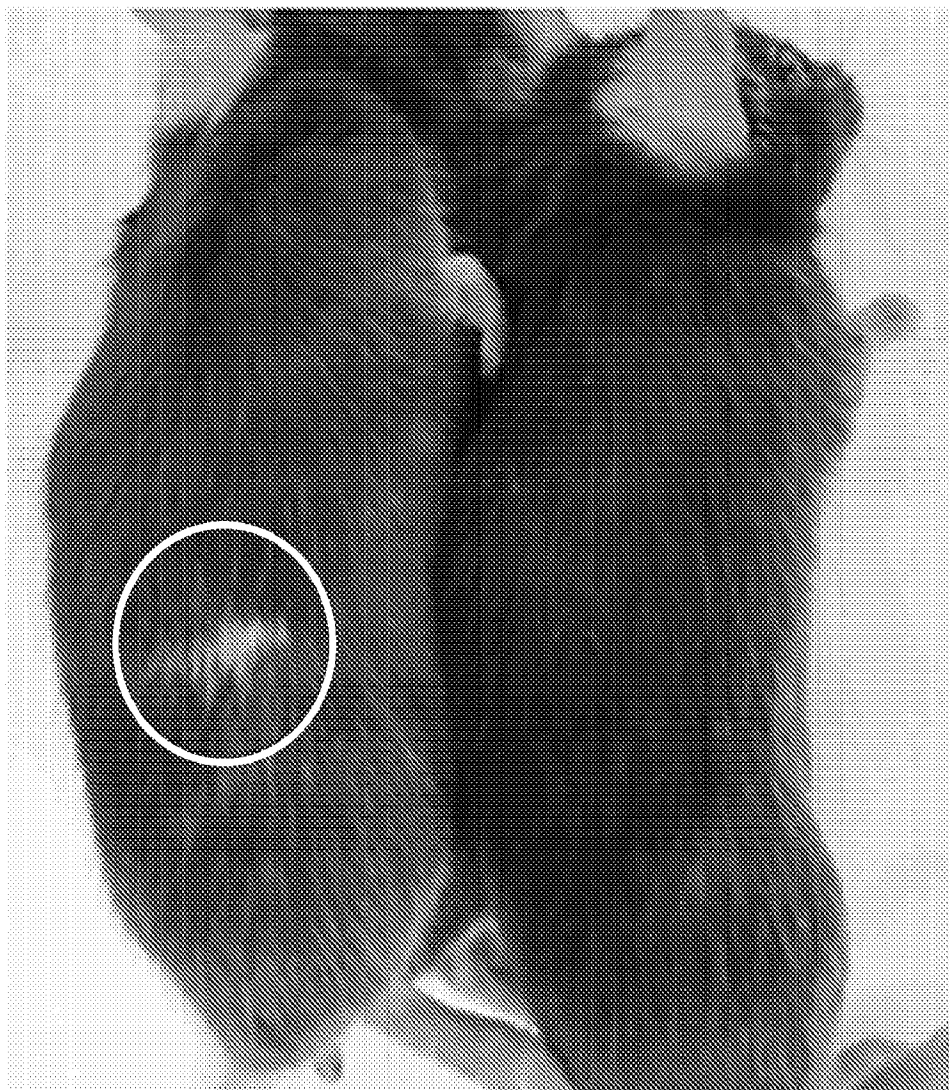


Fig. 12C

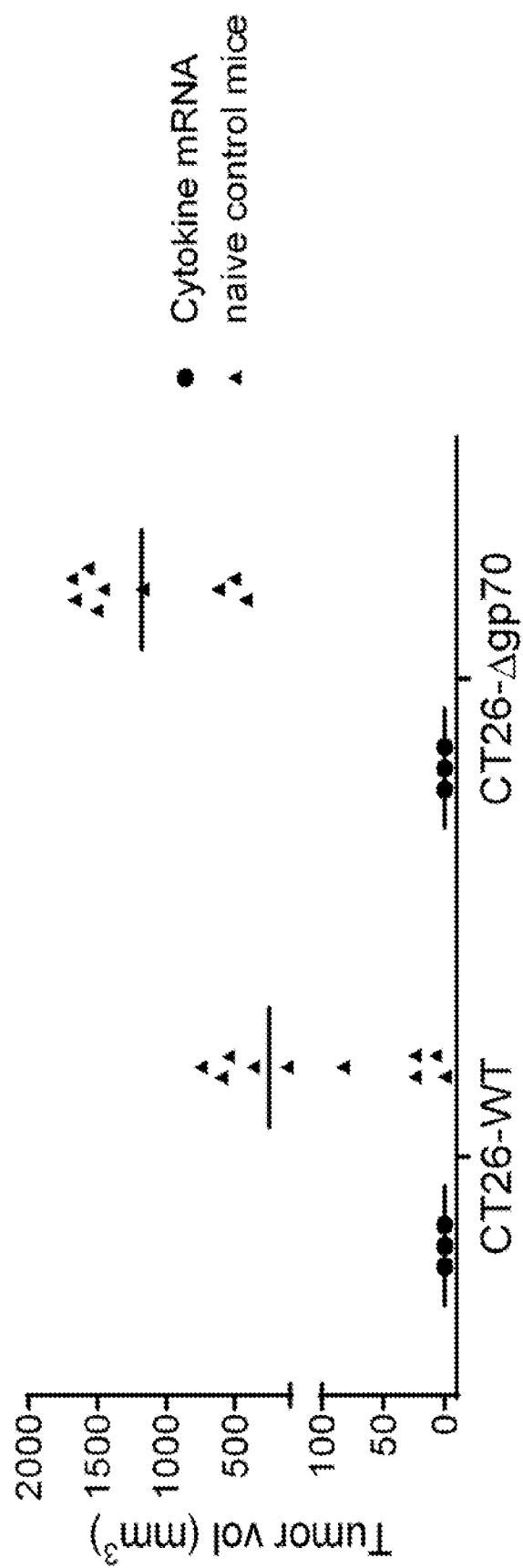
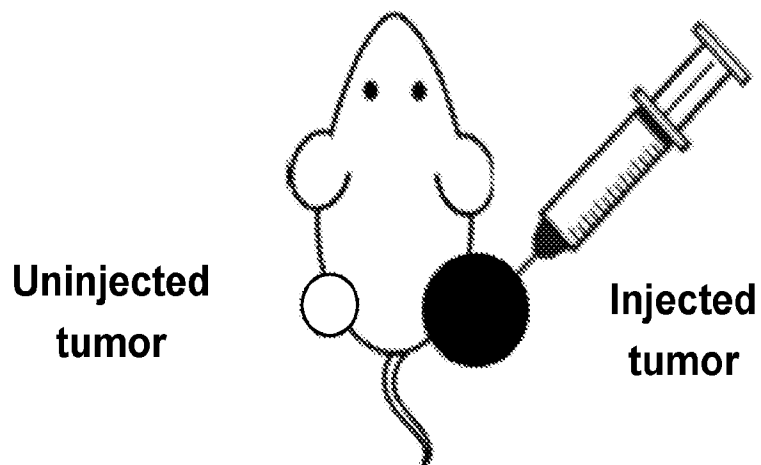
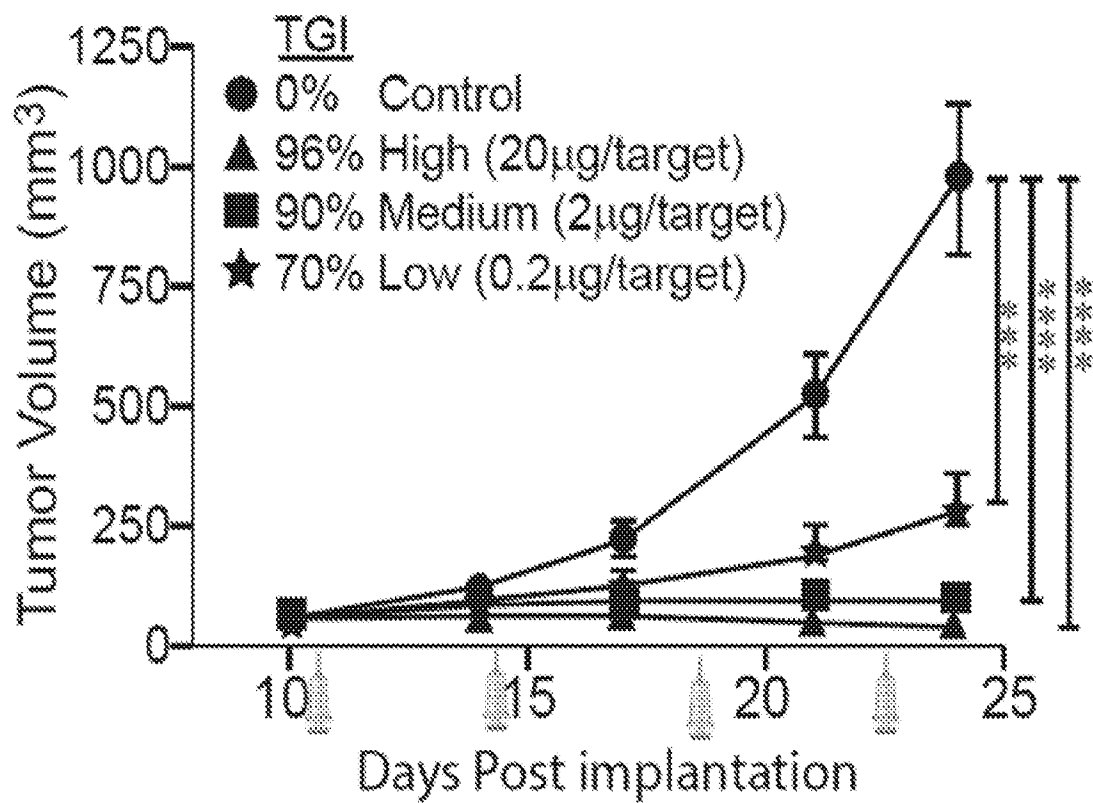
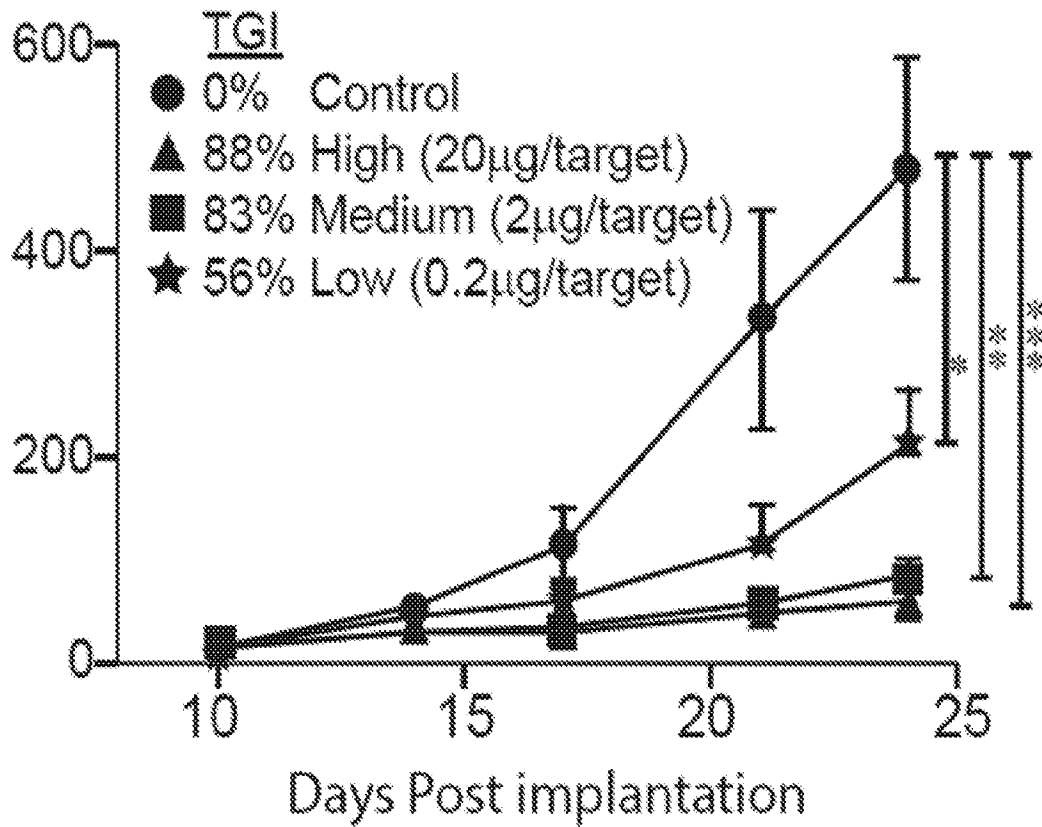
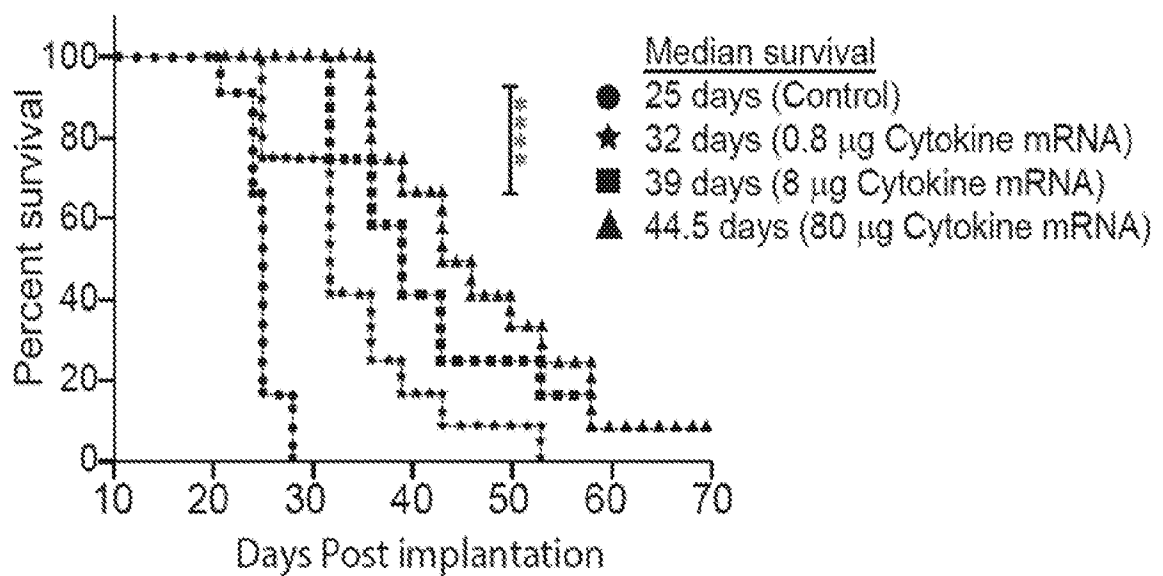


Fig. 12D

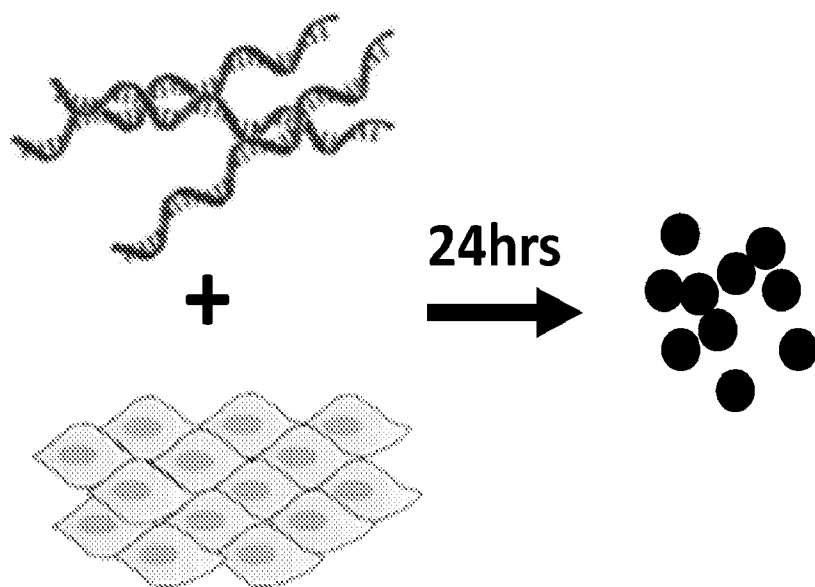
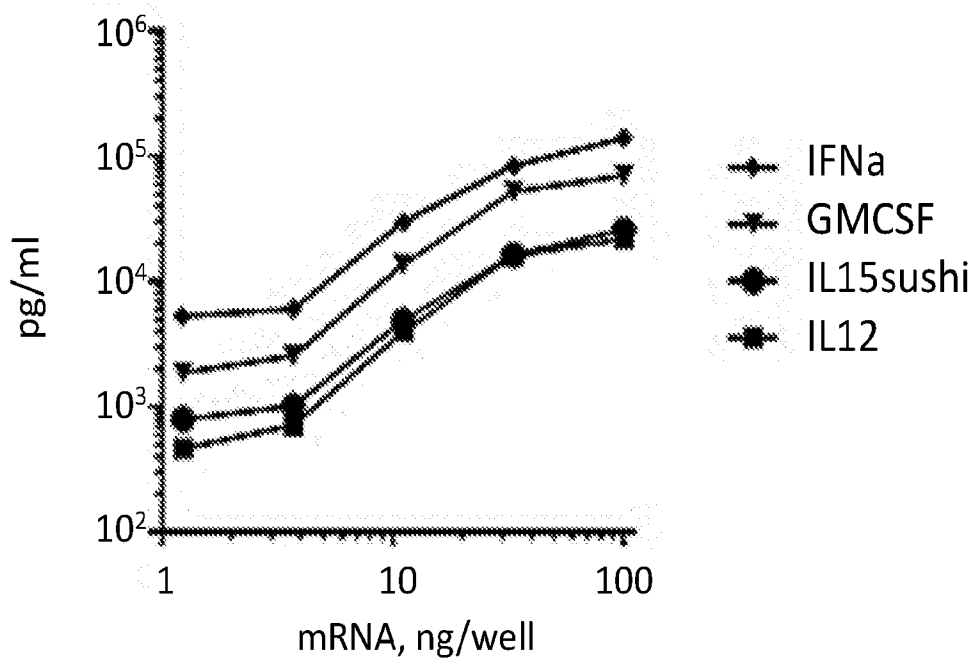
31/86

*Fig. 13A**Fig. 13B*

32/86

**Fig. 13C****Fig. 13D**

33/86

*Fig. 14A**Fig. 14B*

34/86

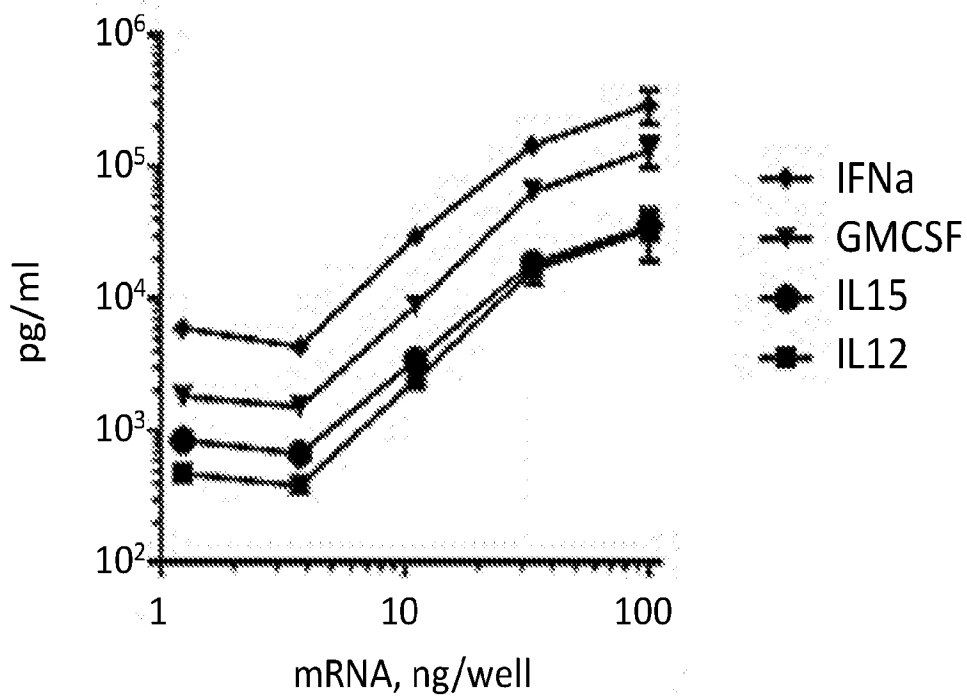


Fig. 14C

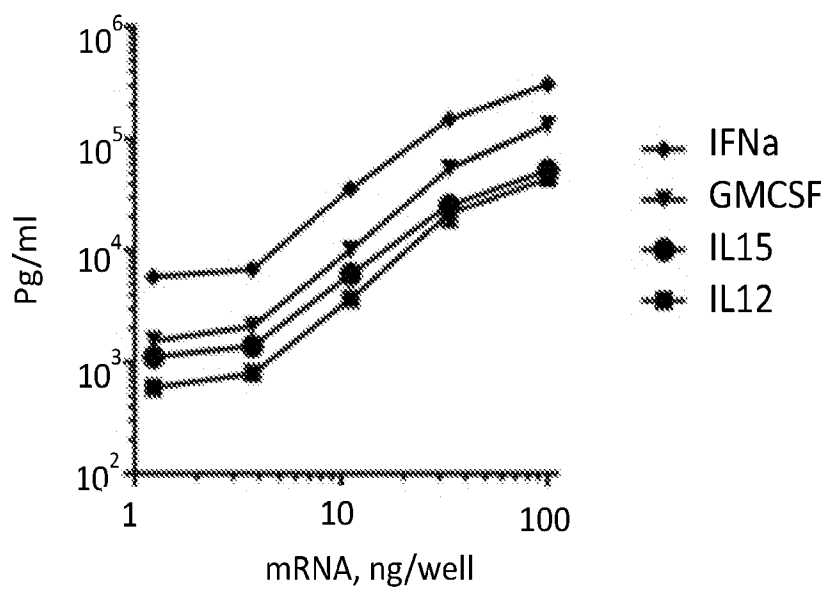


Fig. 14D

35/86

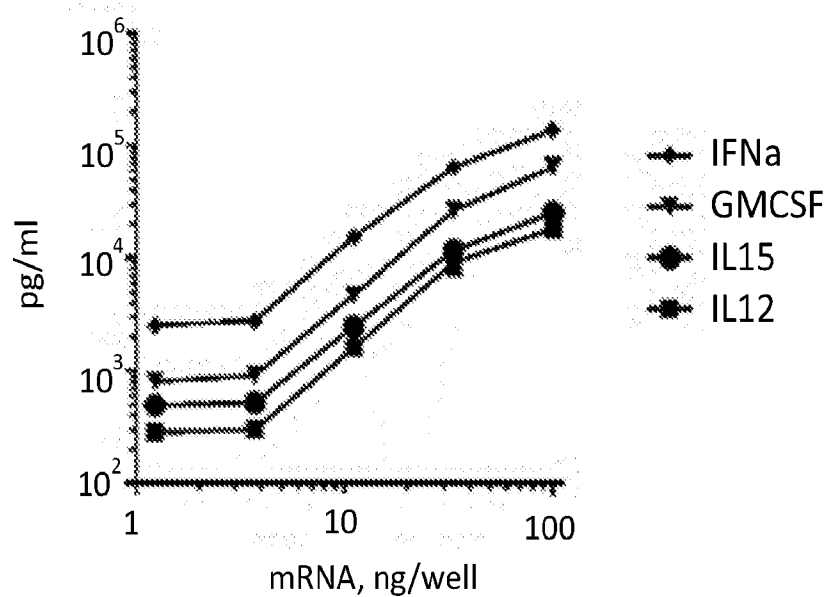


Fig. 14E

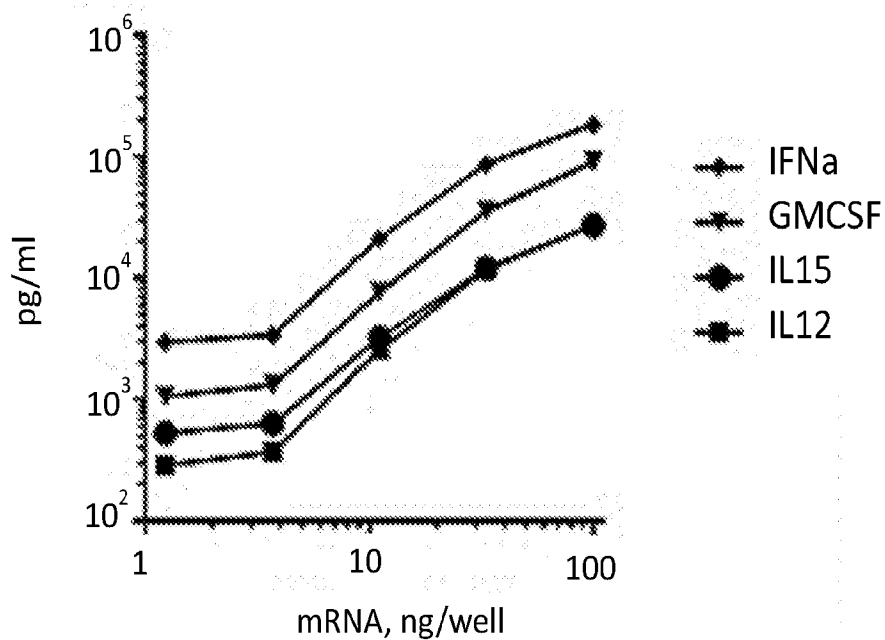
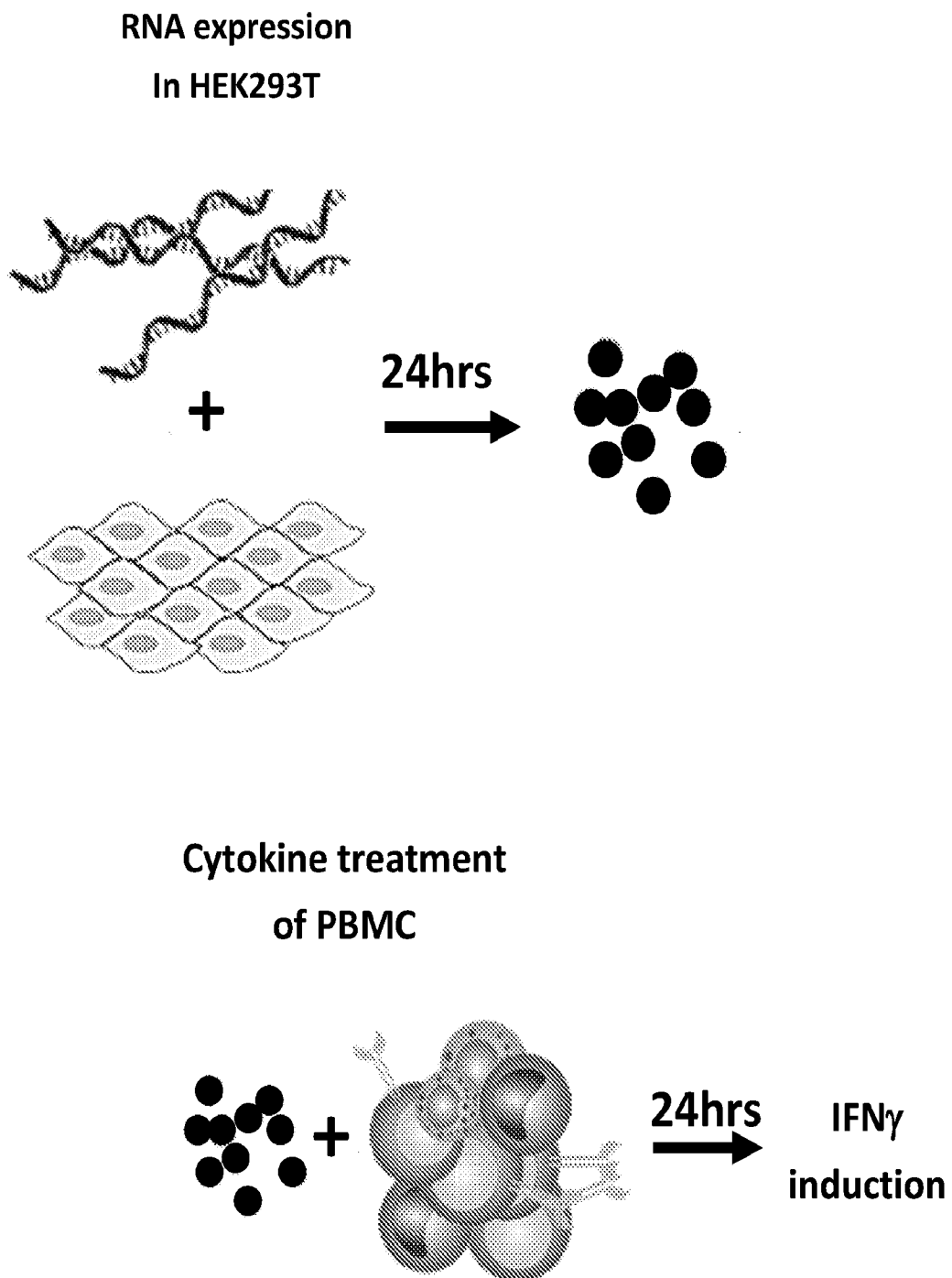
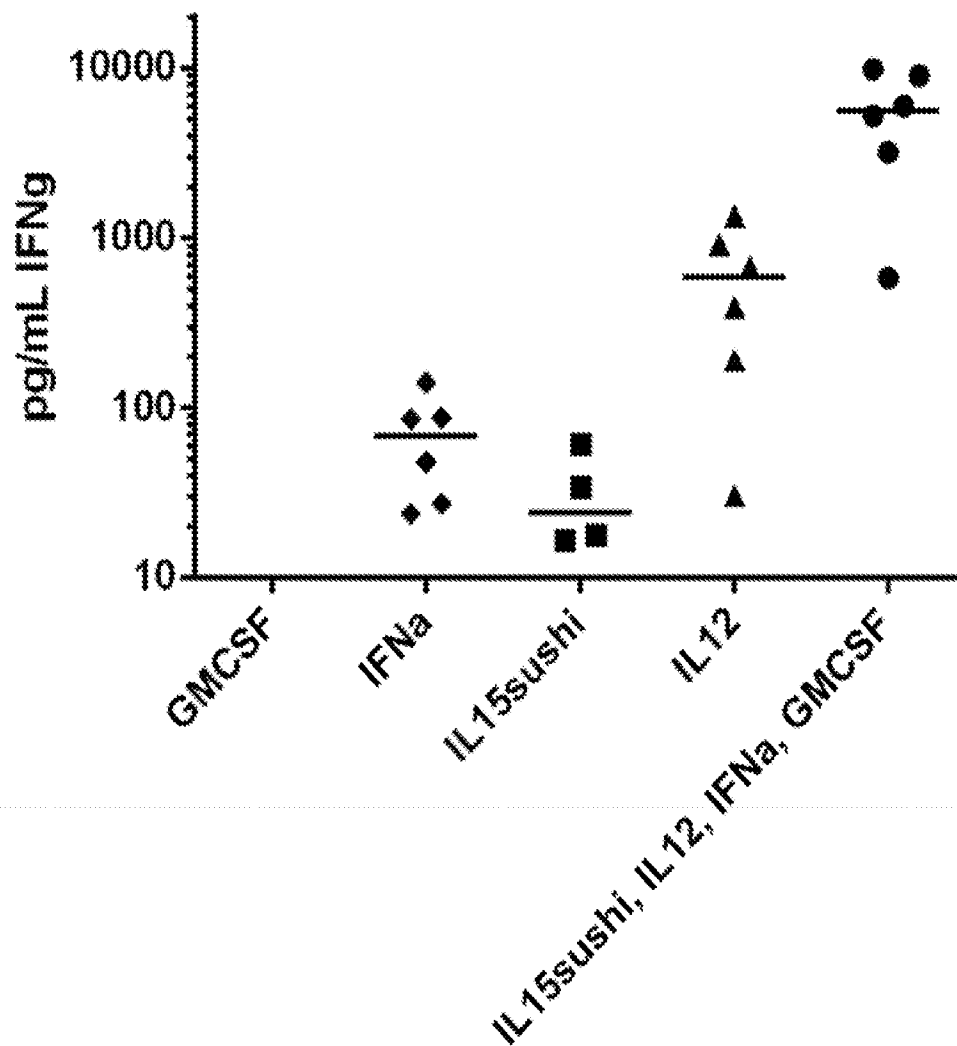


Fig. 14F

*Fig. 15A*

37/86

*Fig. 15B*

38/86

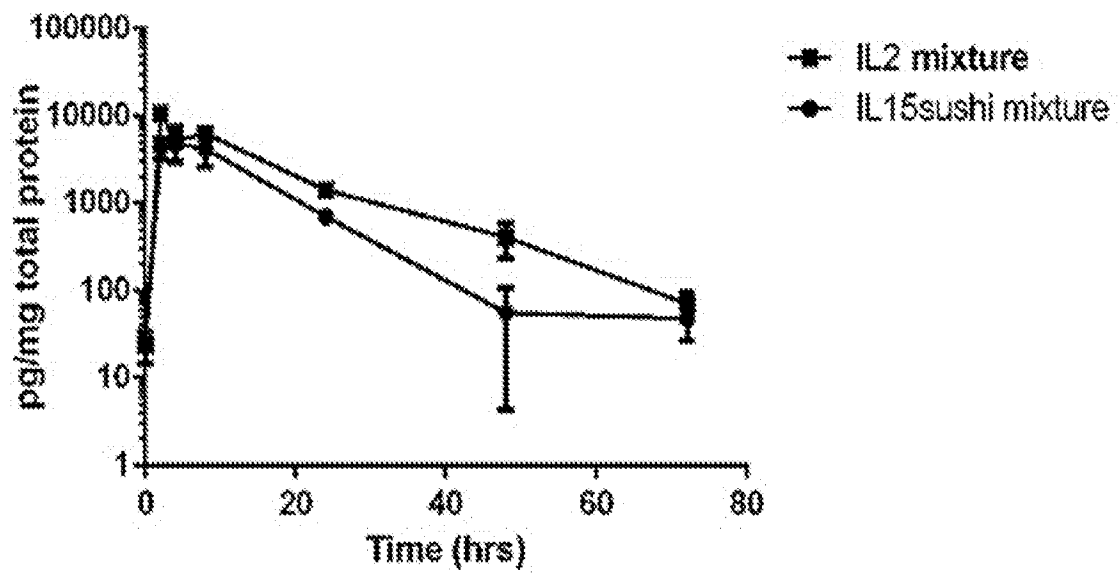


Fig. 16A

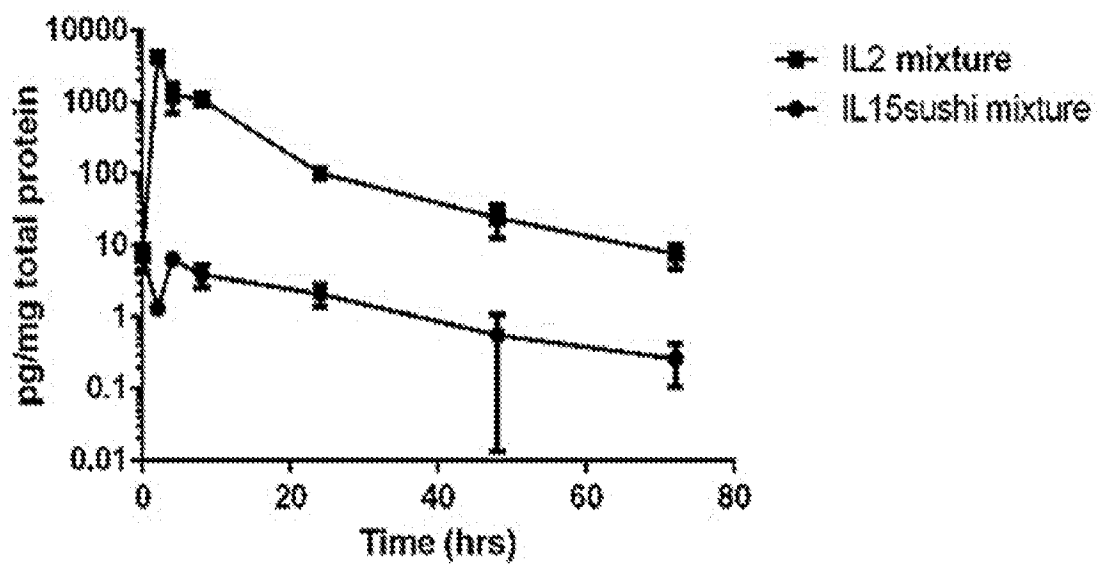


Fig. 16B

39/86

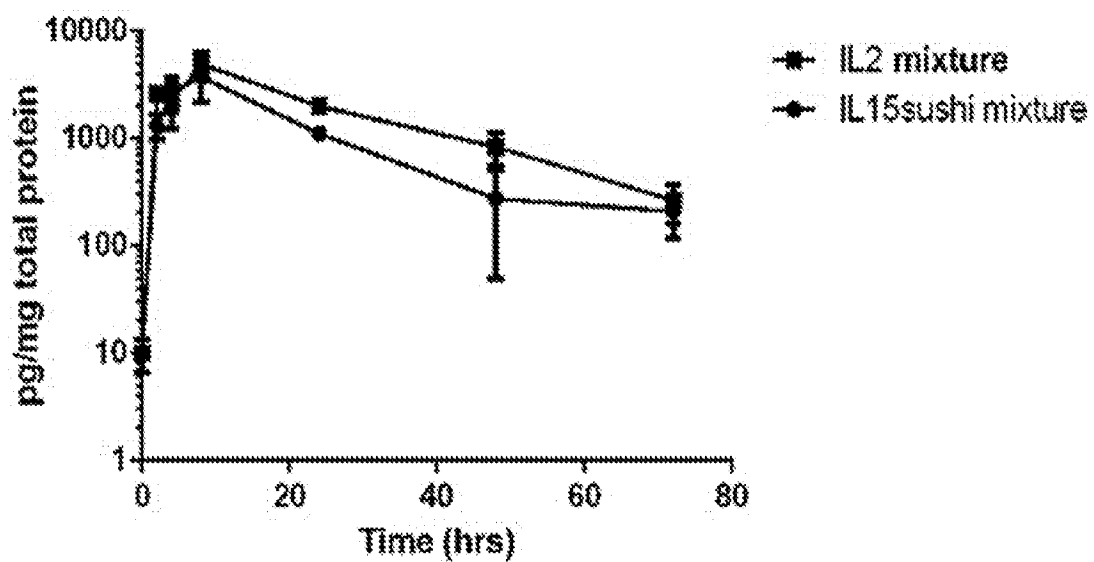


Fig. 16C

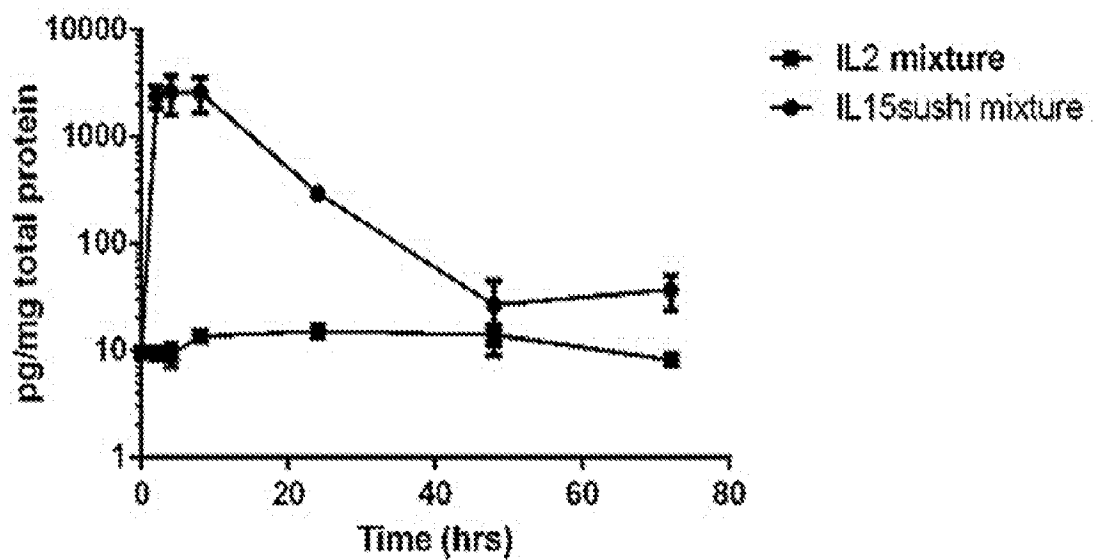


Fig. 16D

40/86

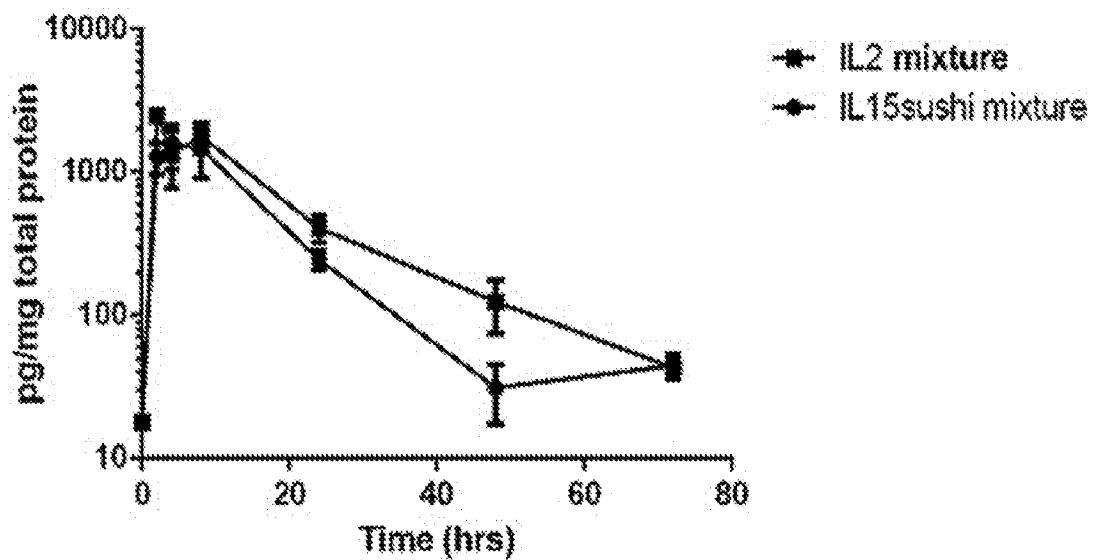


Fig. 16E

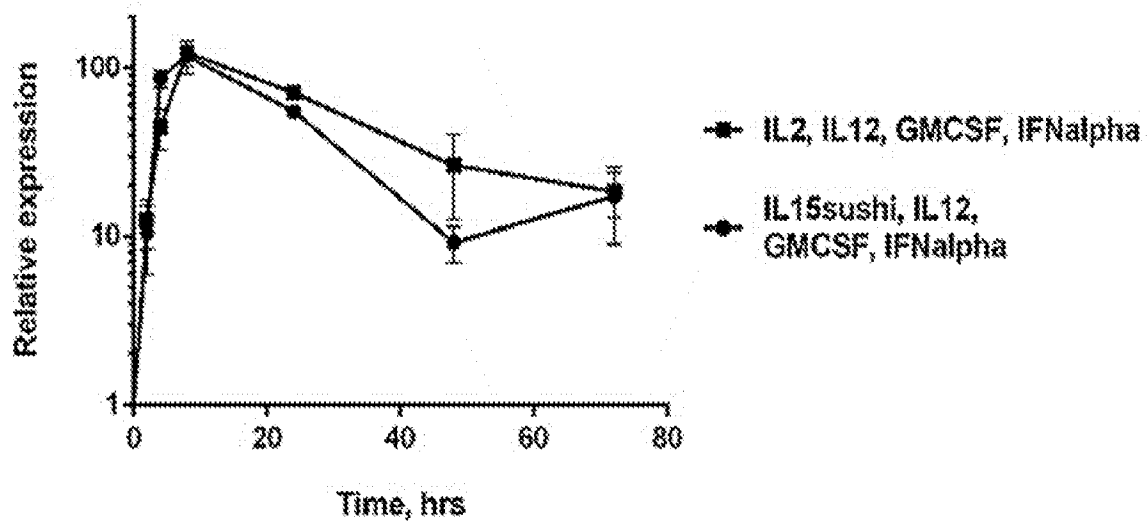


Fig. 17A

41/86

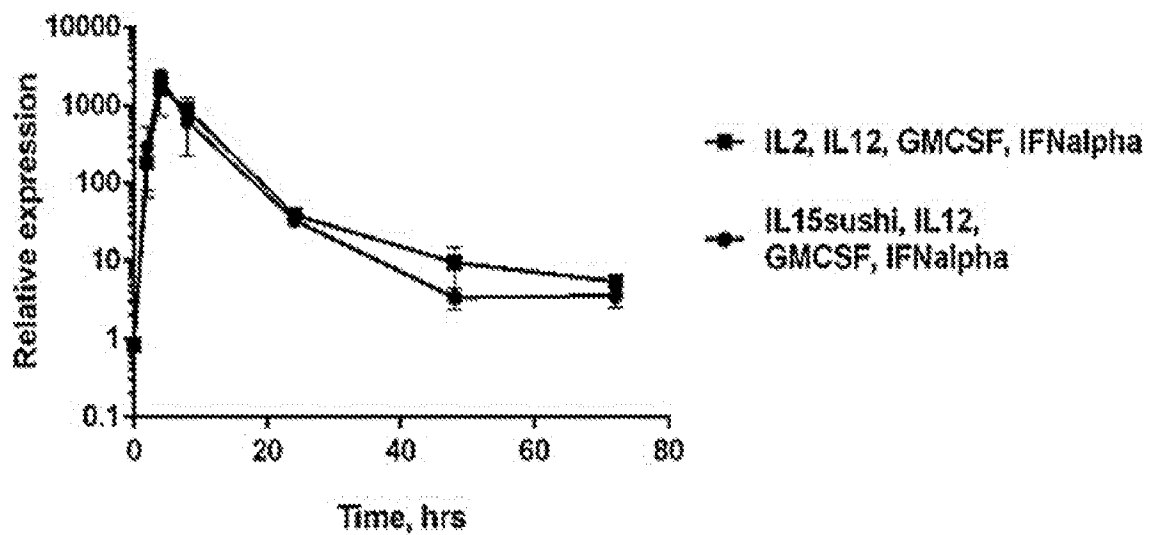


Fig. 17B

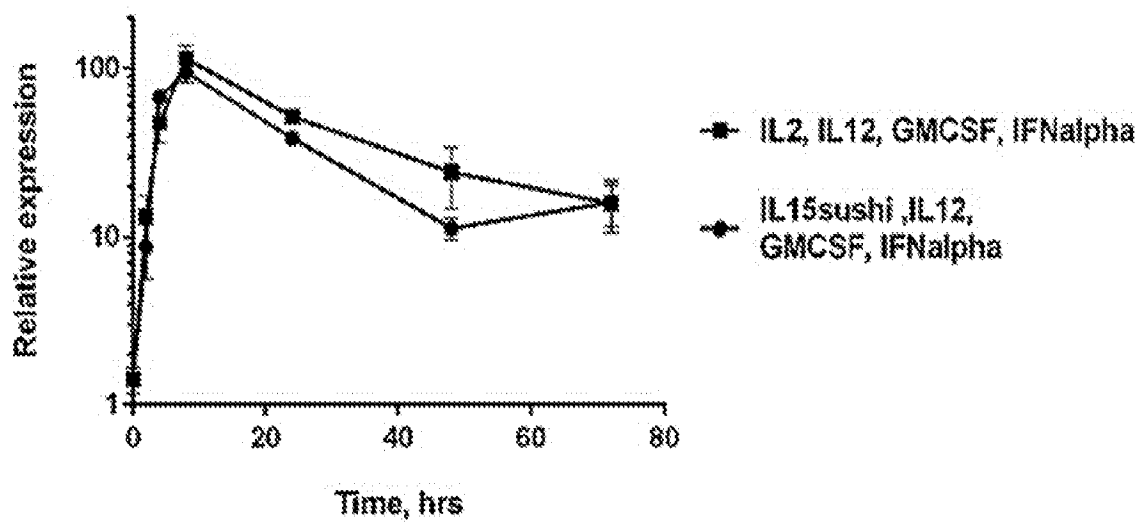
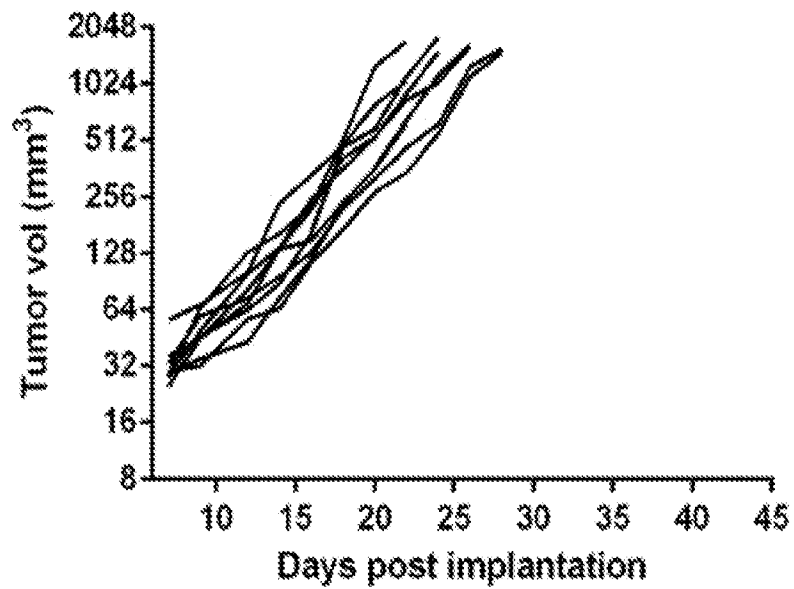
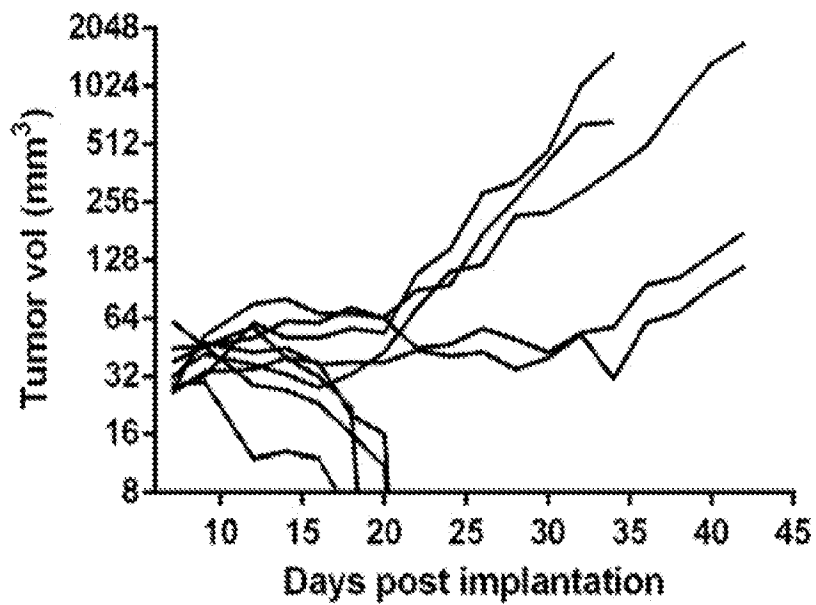
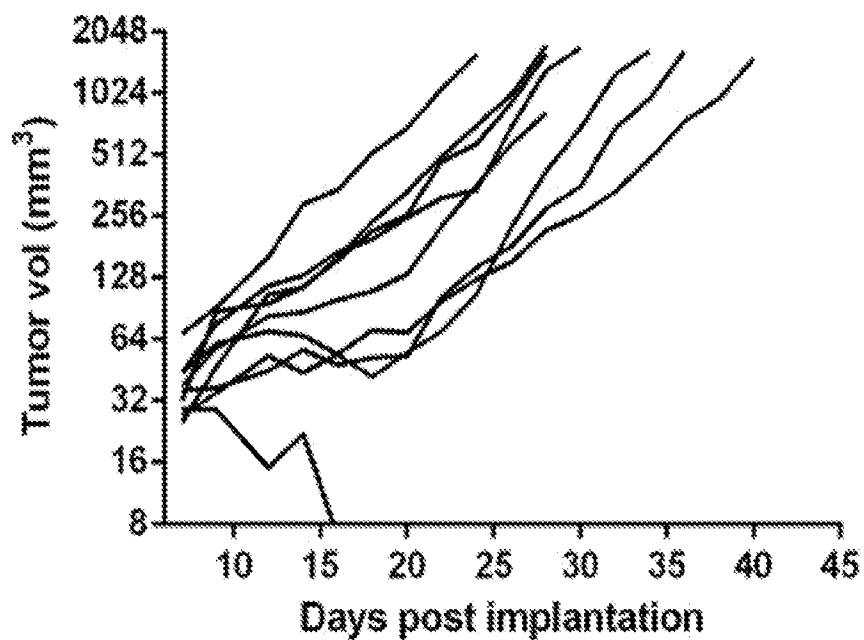
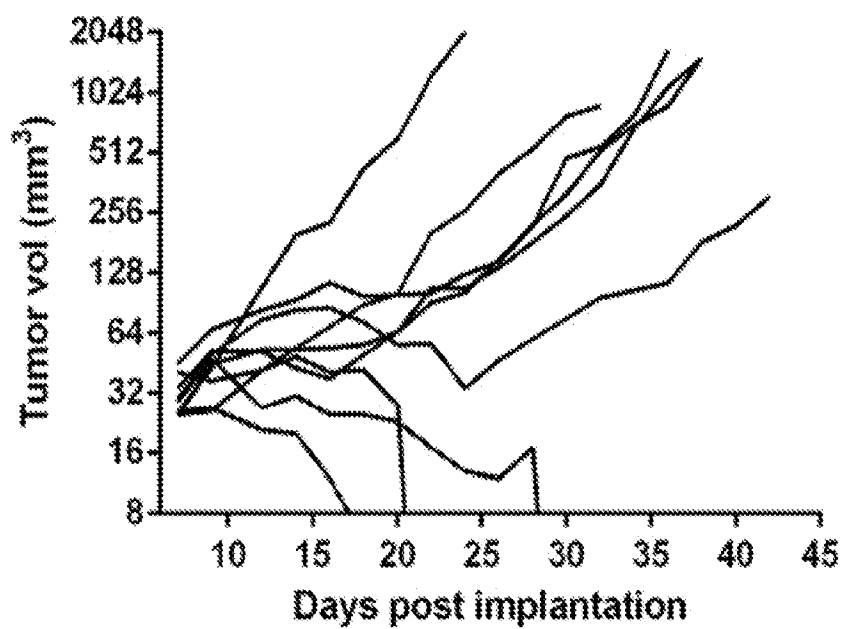


Fig. 17C

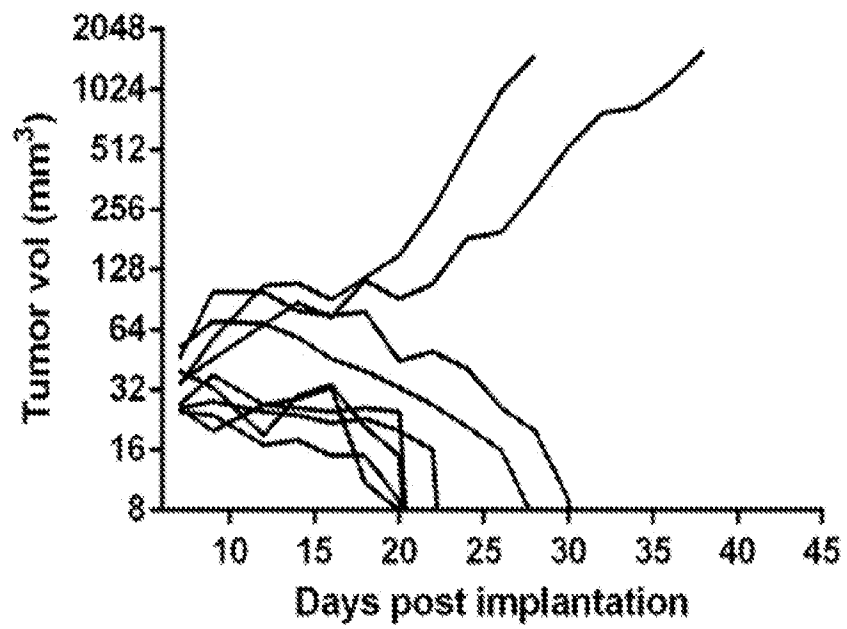
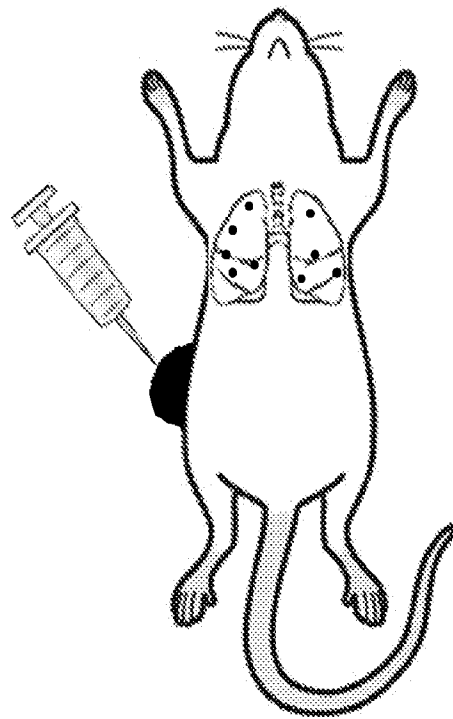
42/86

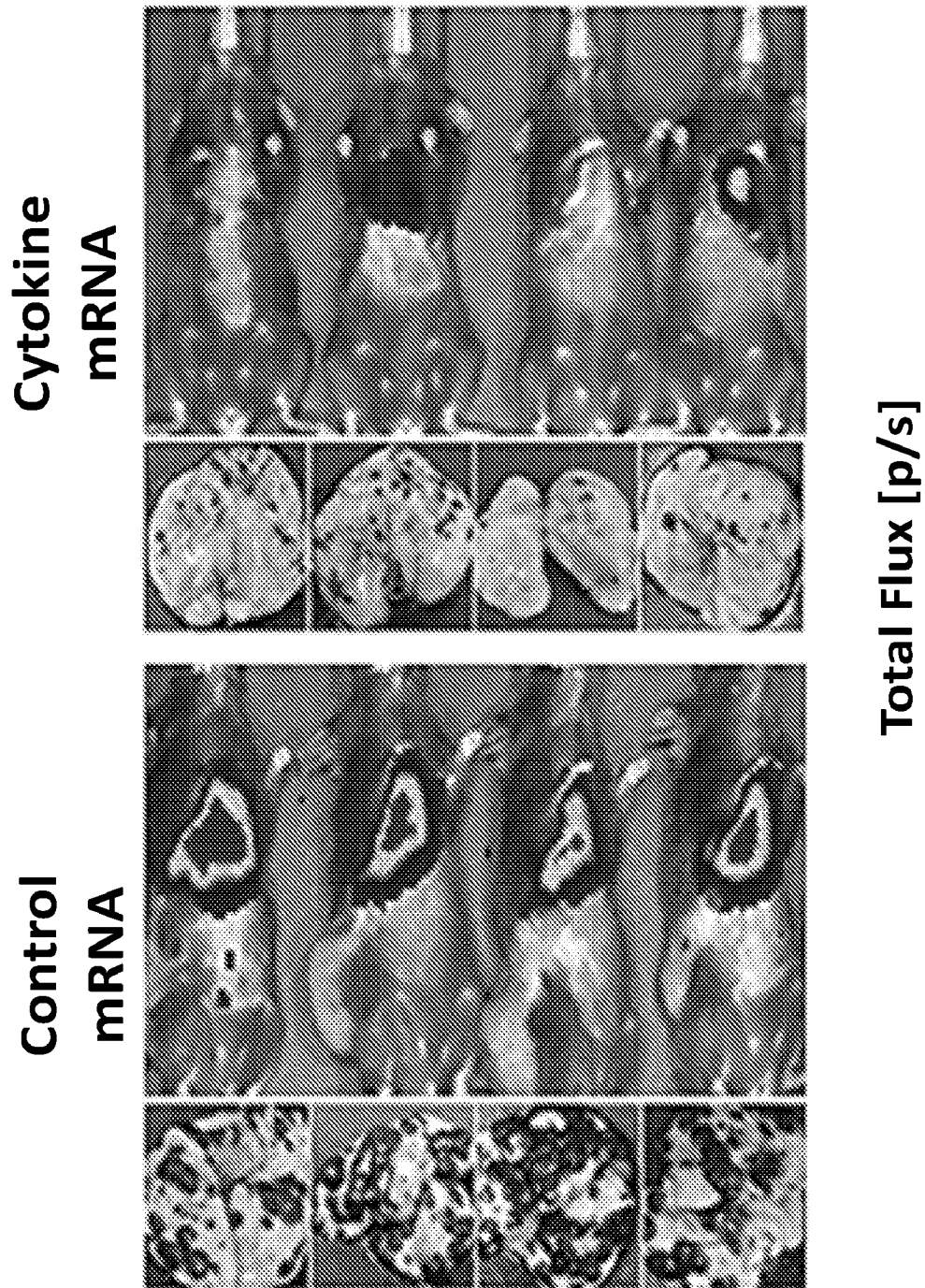
*Fig. 18A**Fig. 18B*

43/86

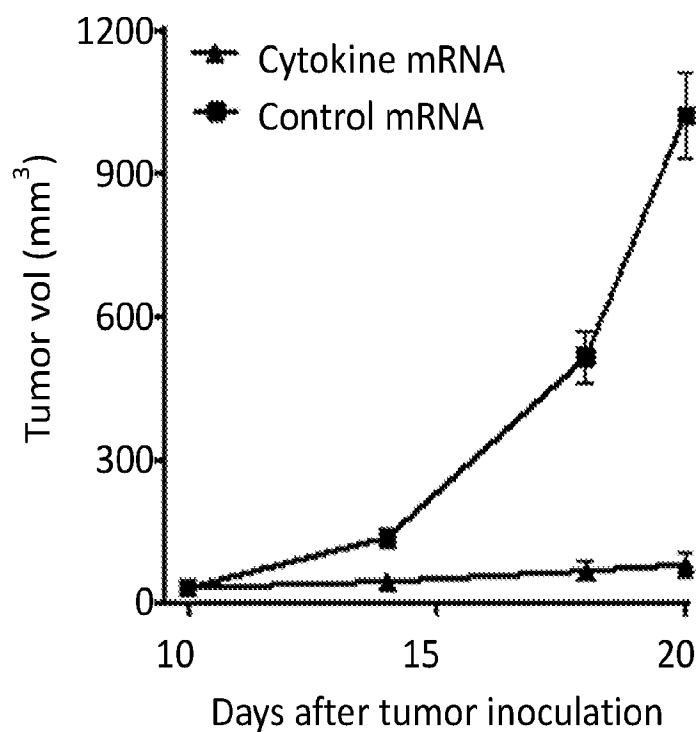
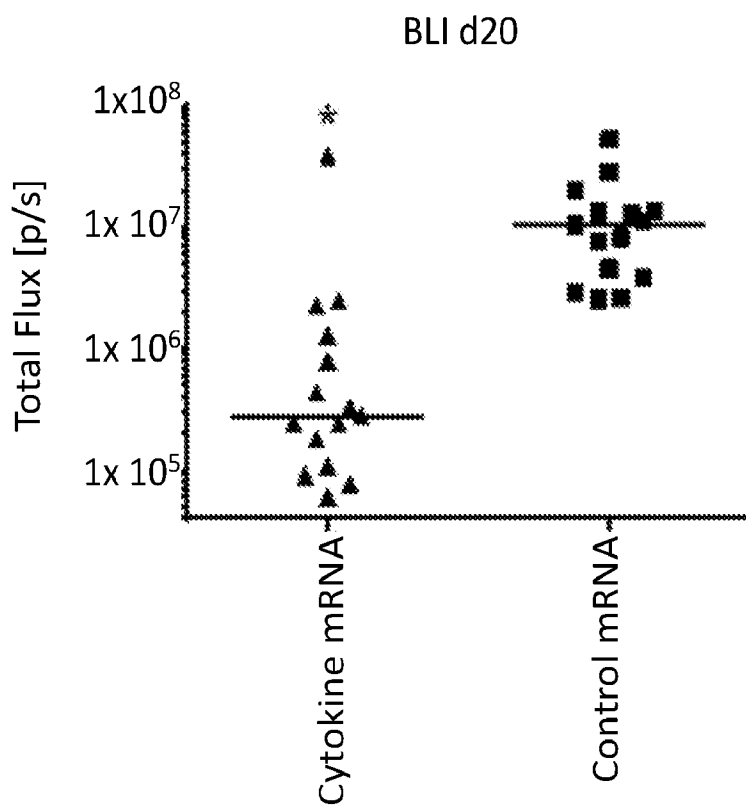
*Fig. 18C**Fig. 18D*

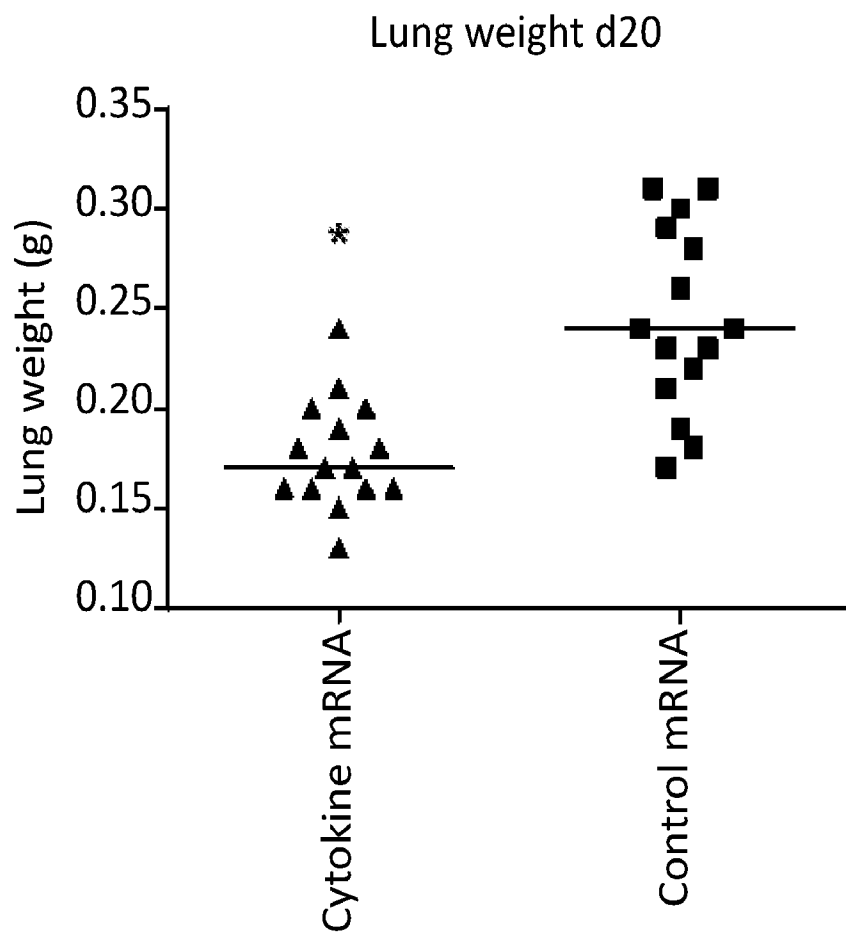
44/86

*Fig. 18E**Fig. 19A*

*Fig. 19B*

46/86

*Fig. 19C**Fig. 19D*

*Fig. 19E*

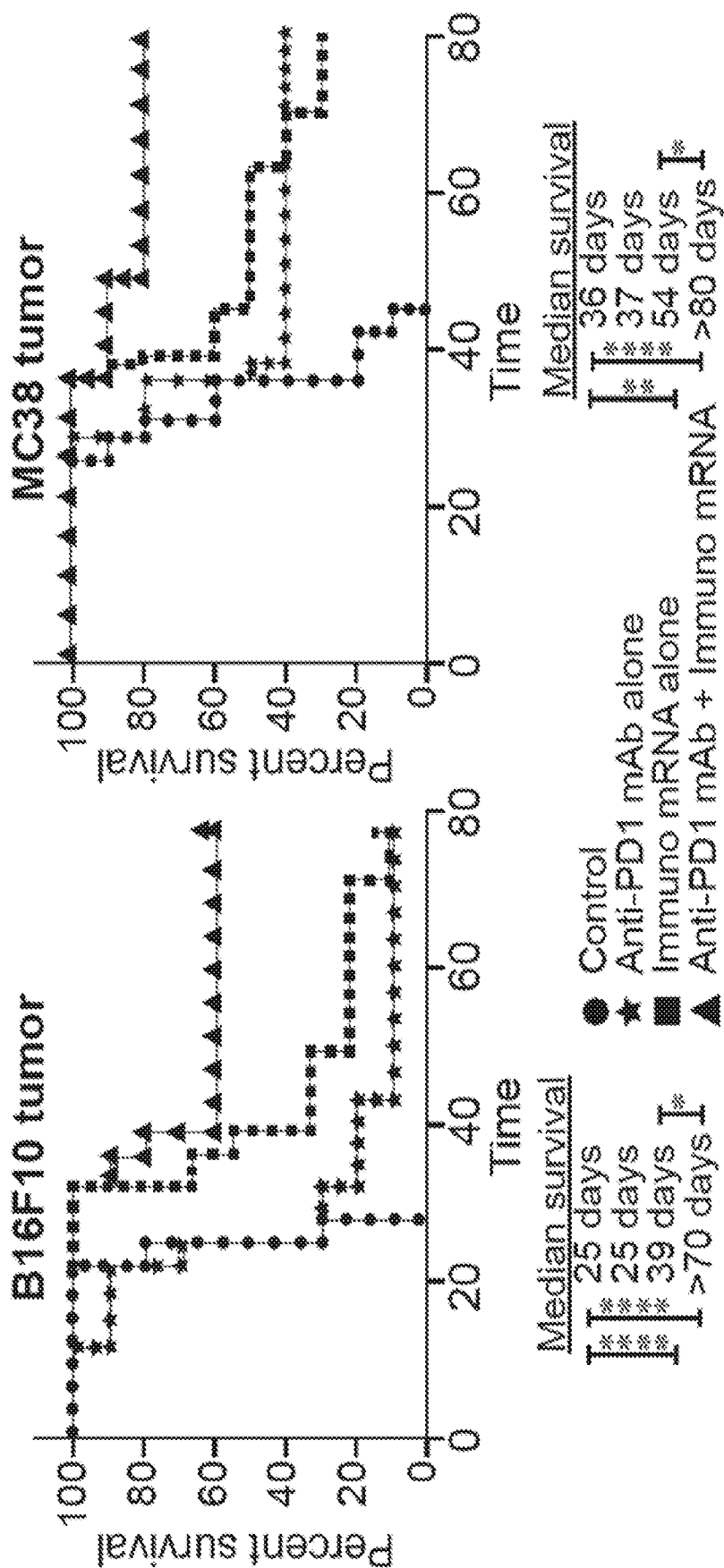
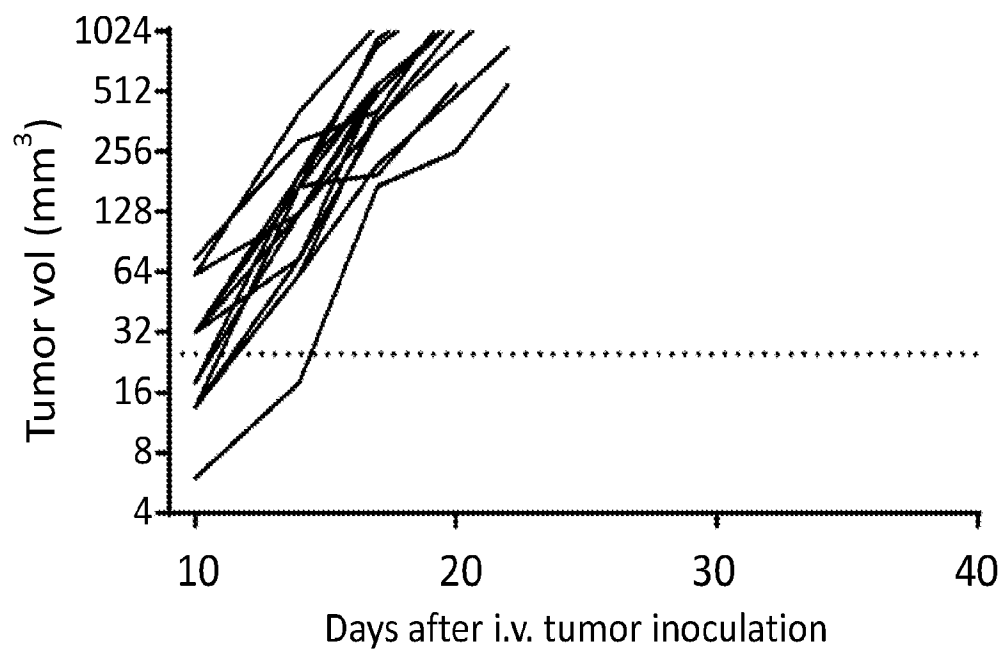
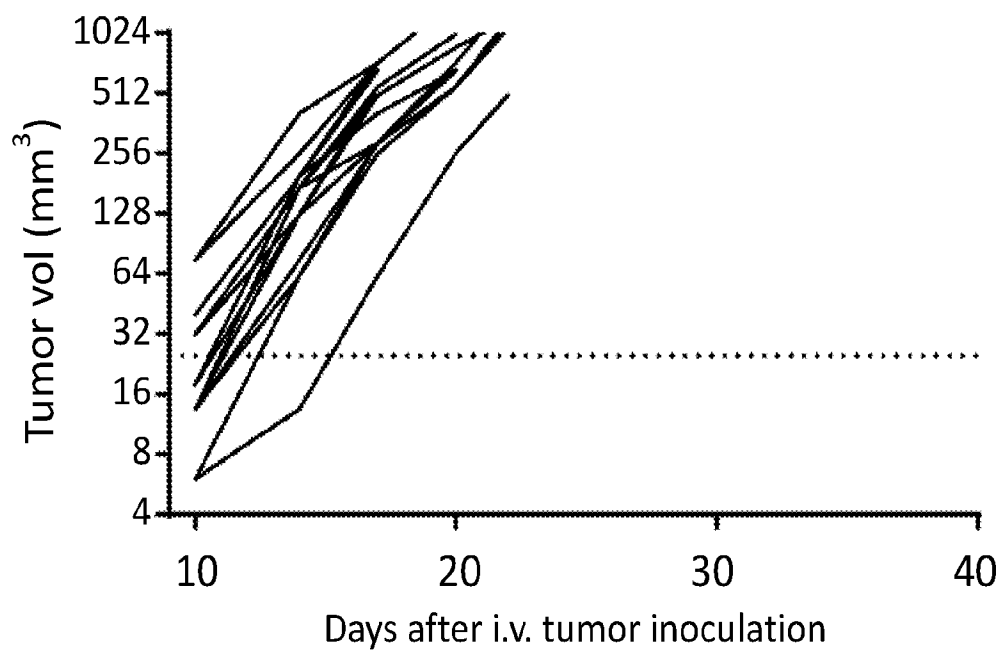


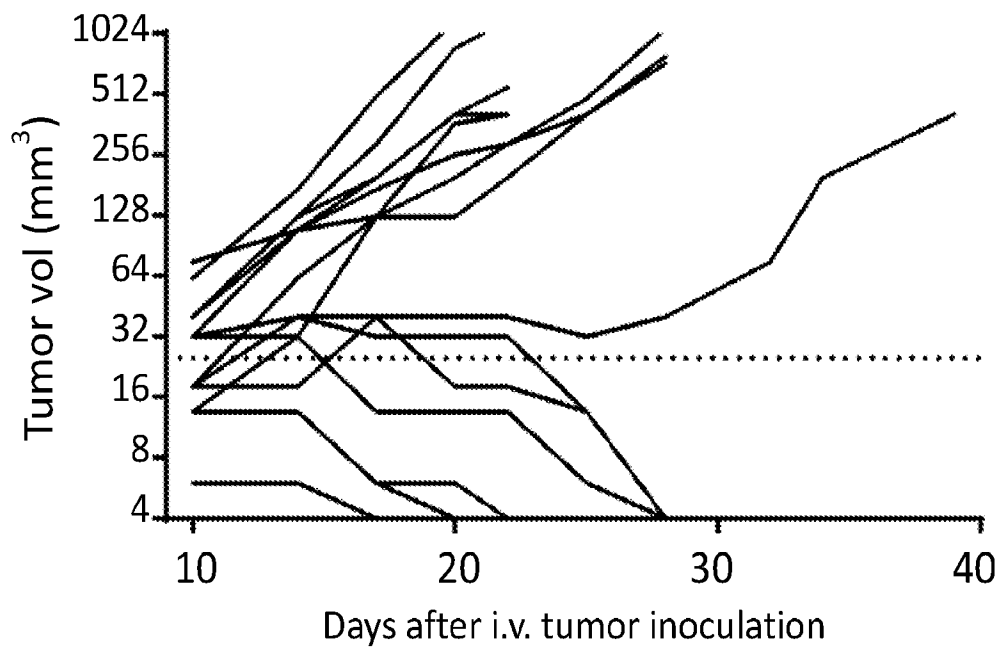
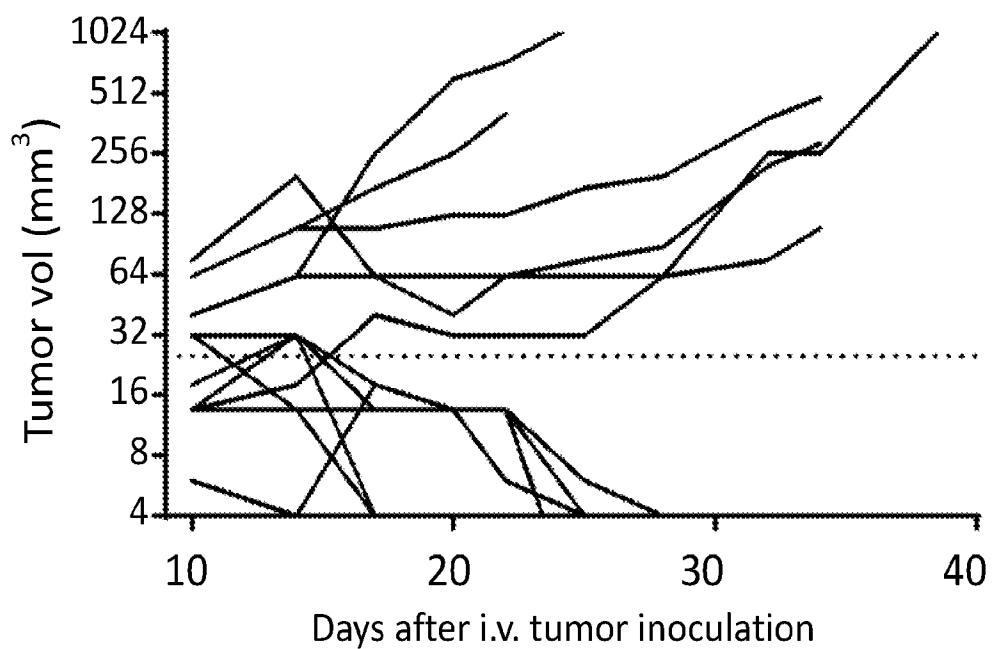
Fig. 20A

Fig. 20B

49/86

*Fig. 20C**Fig. 20D*

50/86

*Fig. 20E**Fig. 20F*

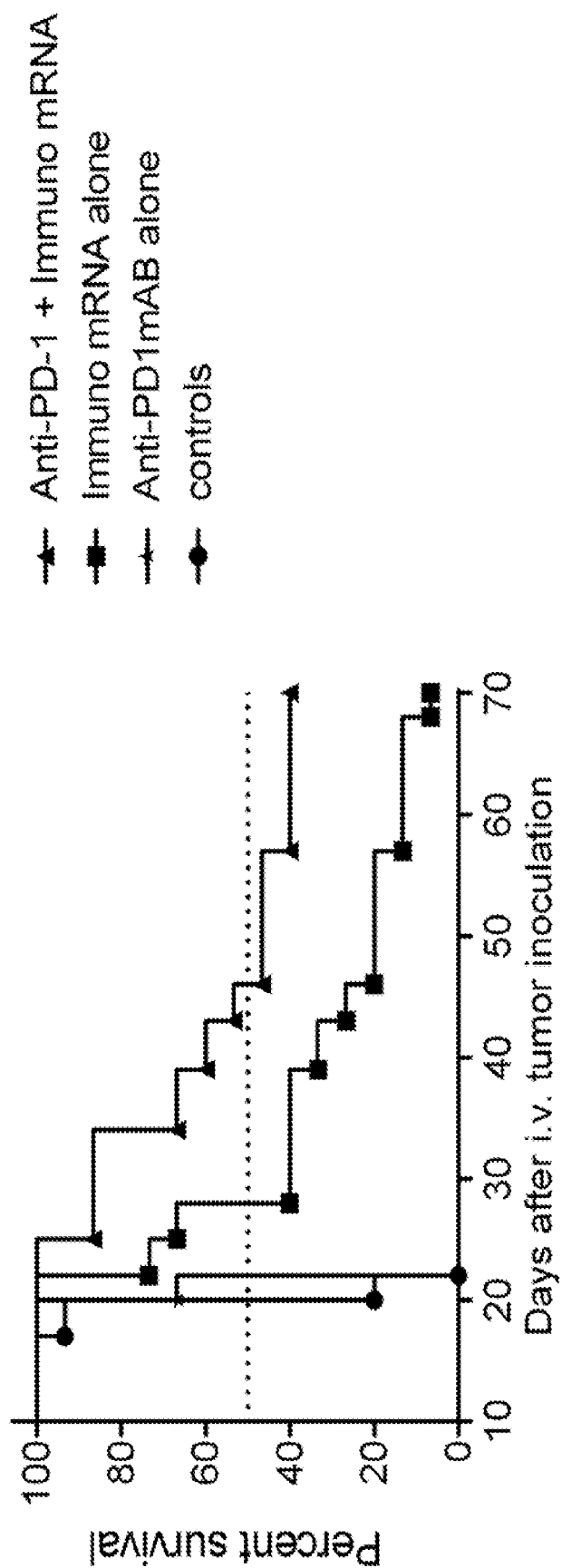
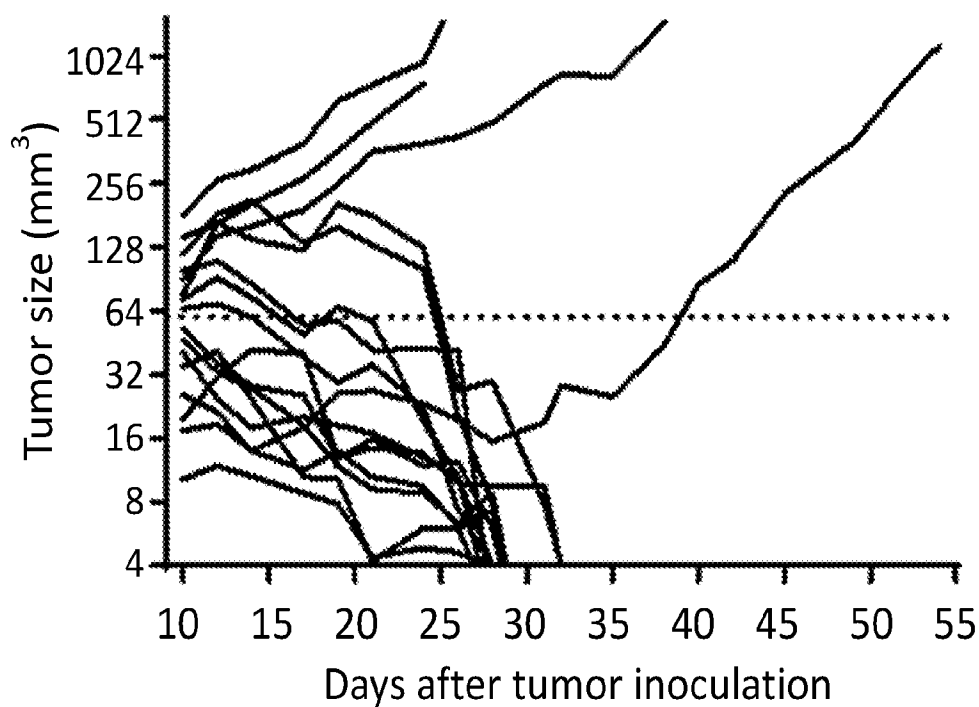
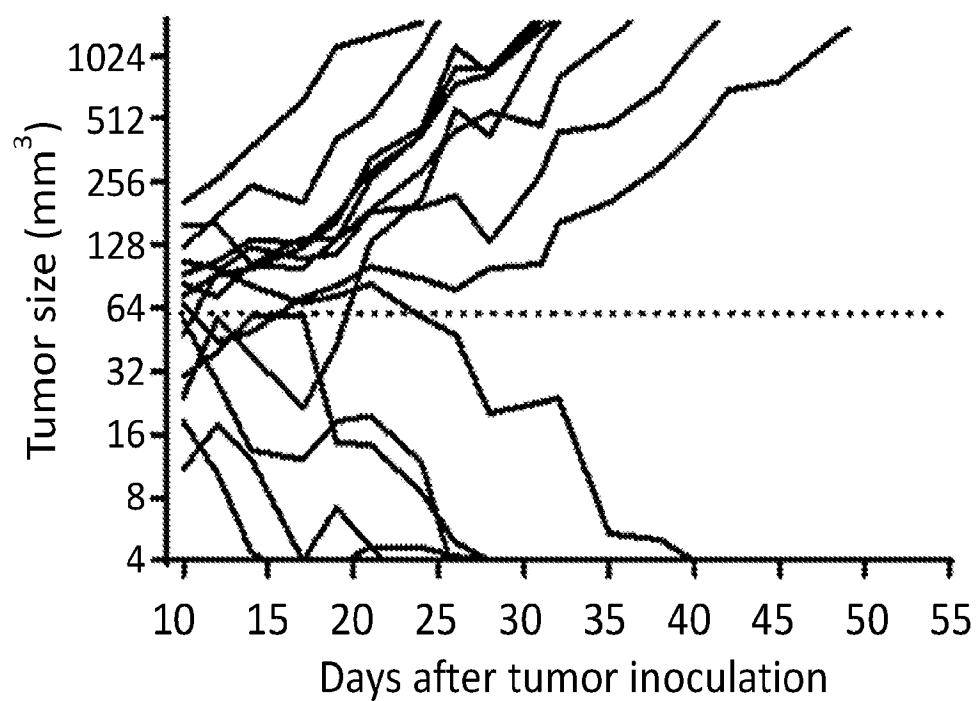
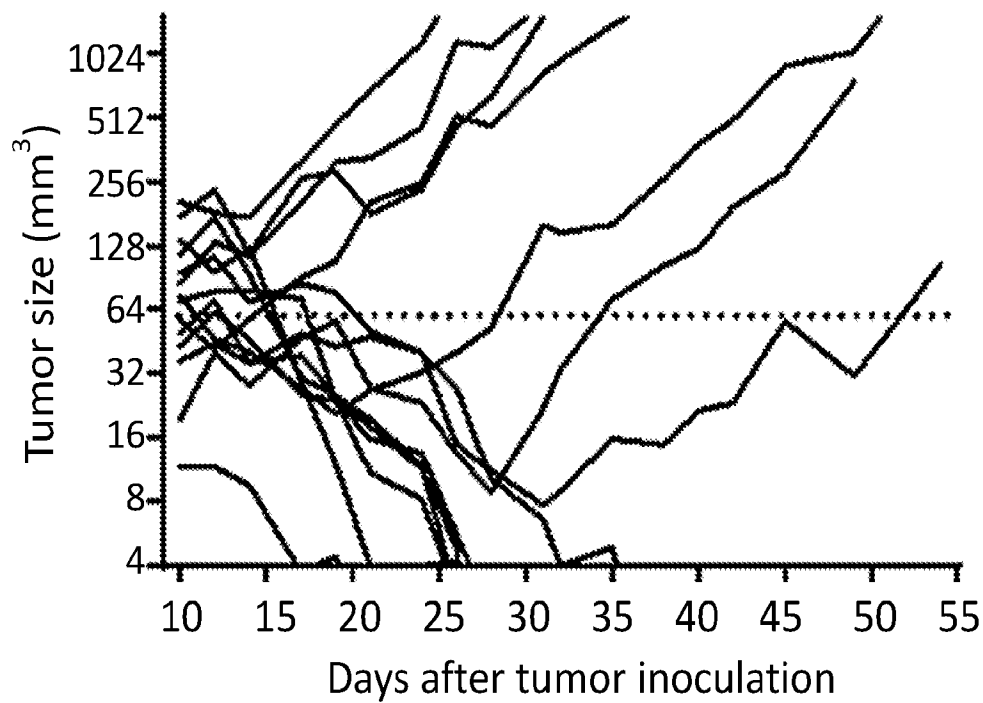
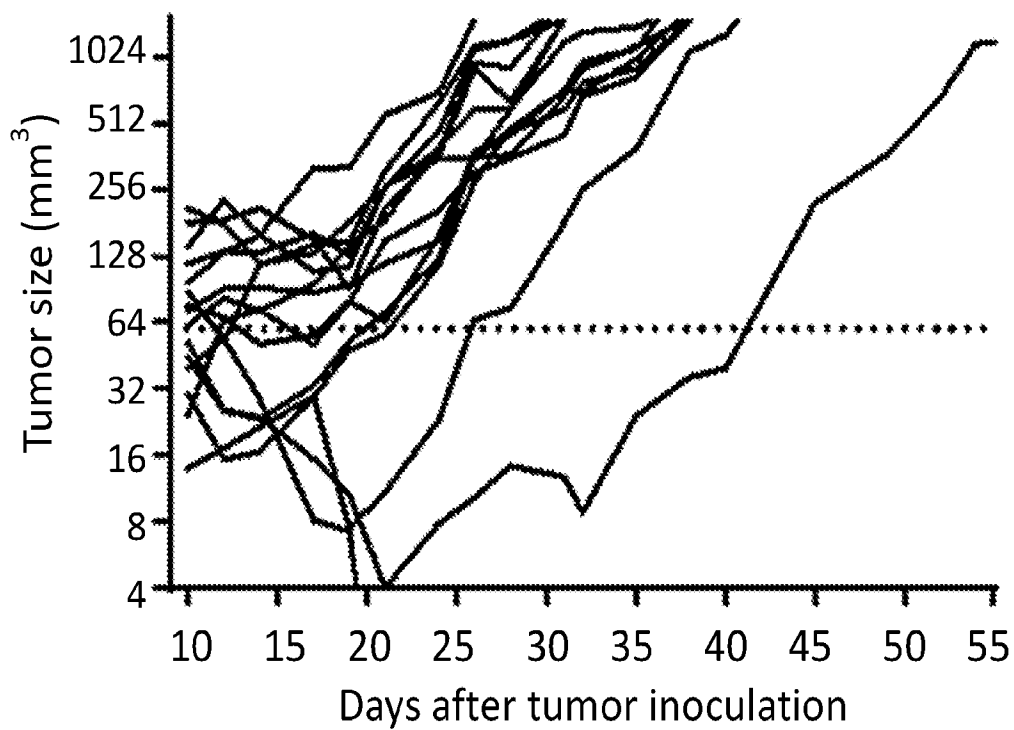


Fig. 20G

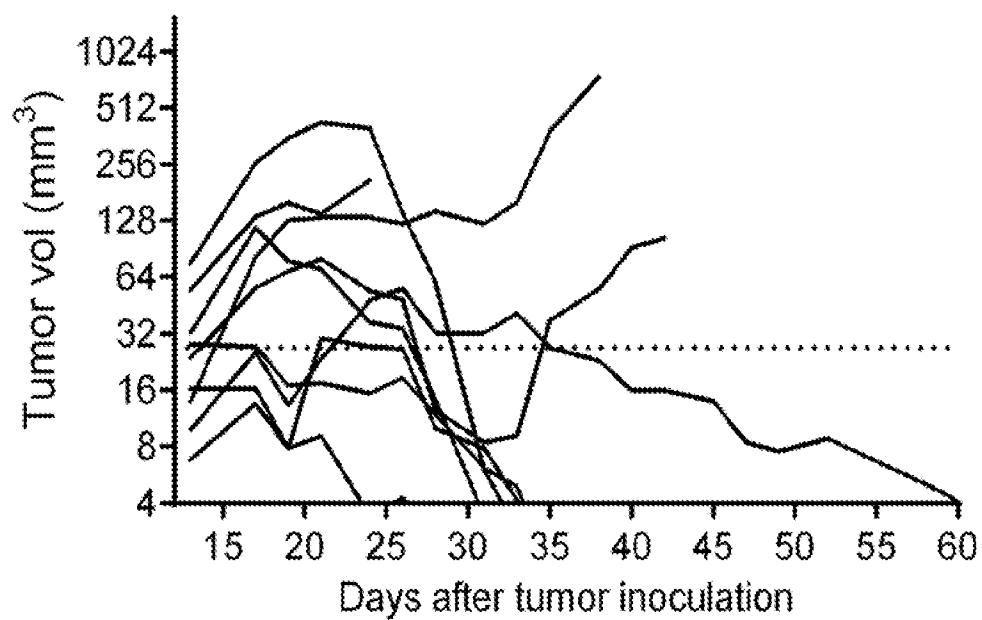
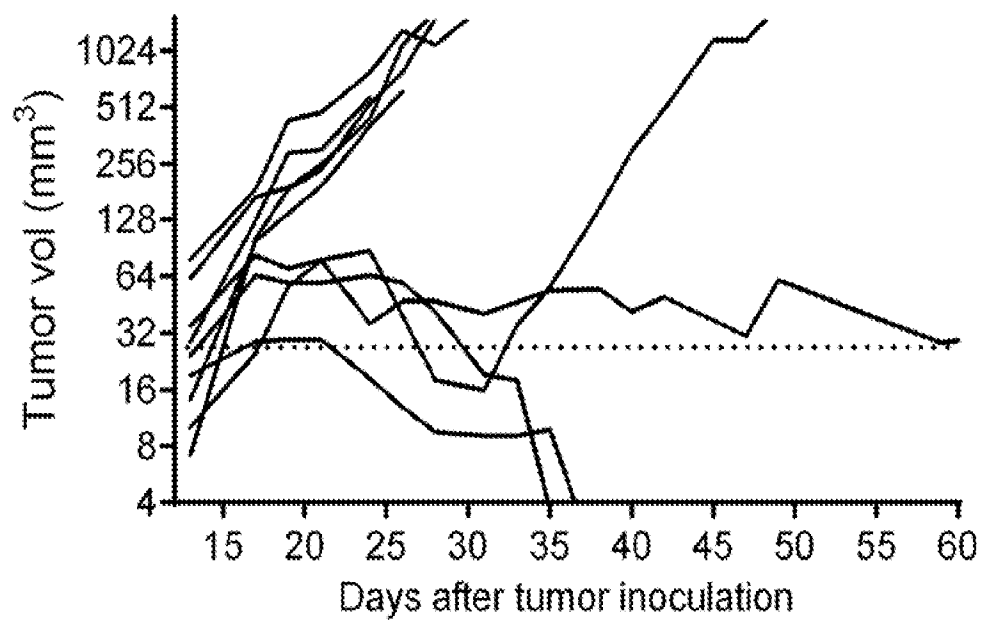
52/86

*Fig. 21A**Fig. 21B*

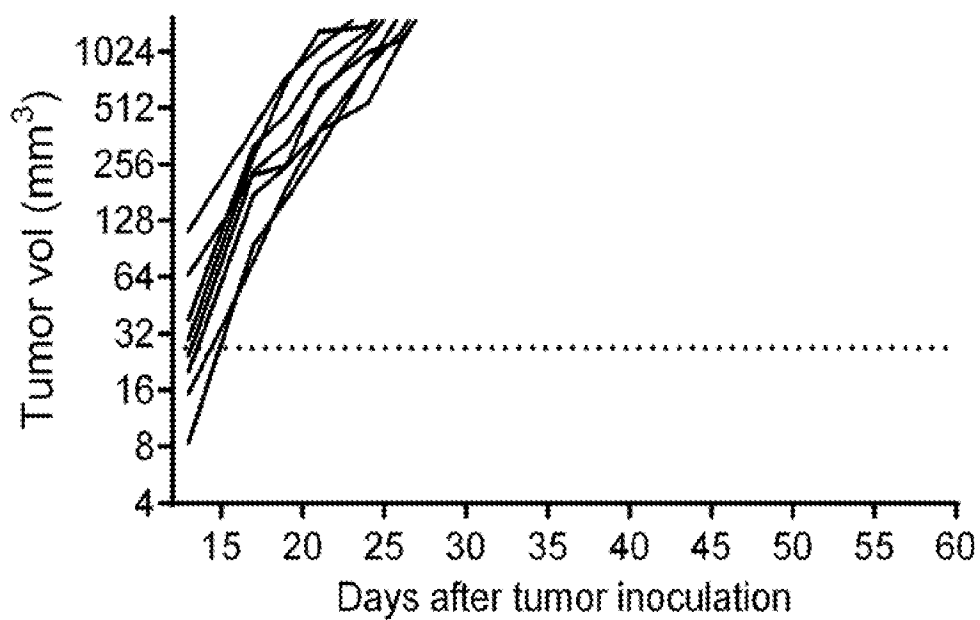
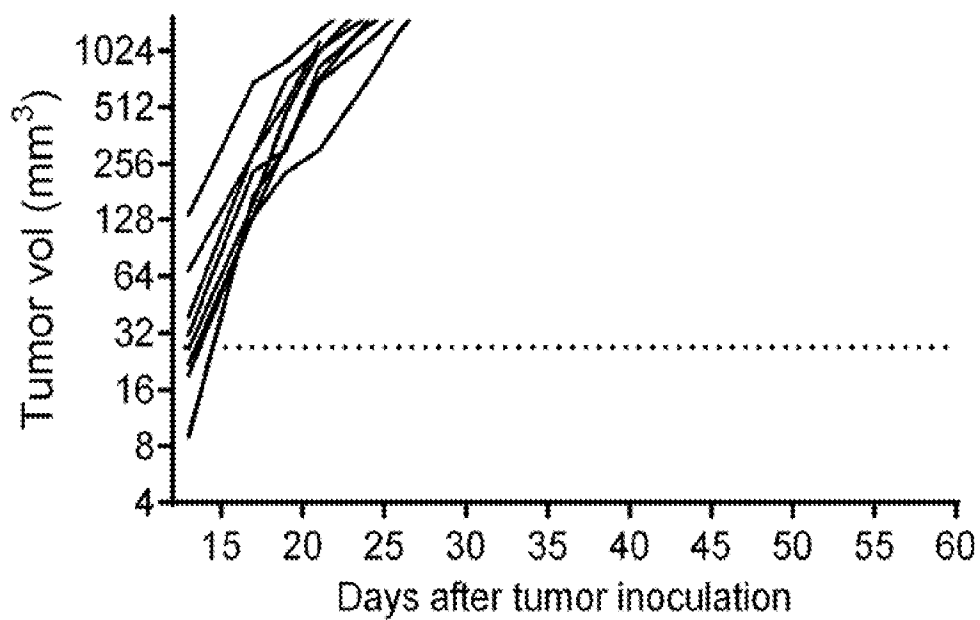
53/86

*Fig. 21C**Fig. 21D*

54/86

*Fig. 21E**Fig. 21F*

55/86

*Fig. 21G**Fig. 21H*

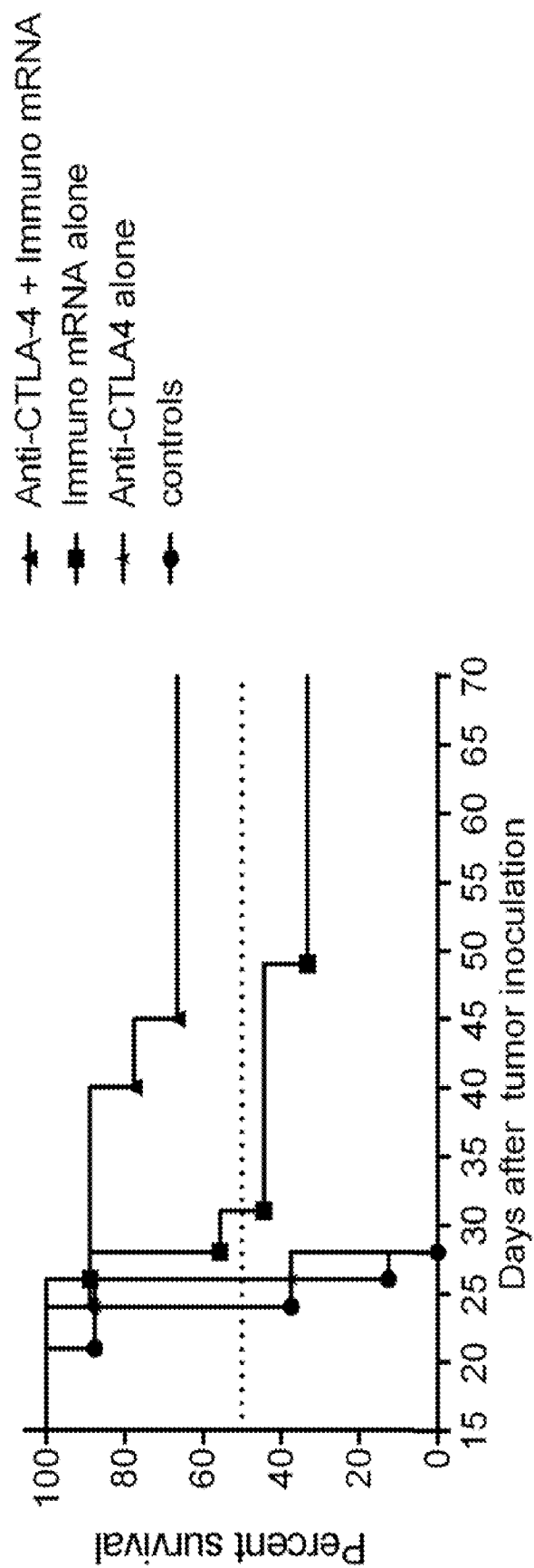


Fig. 21I

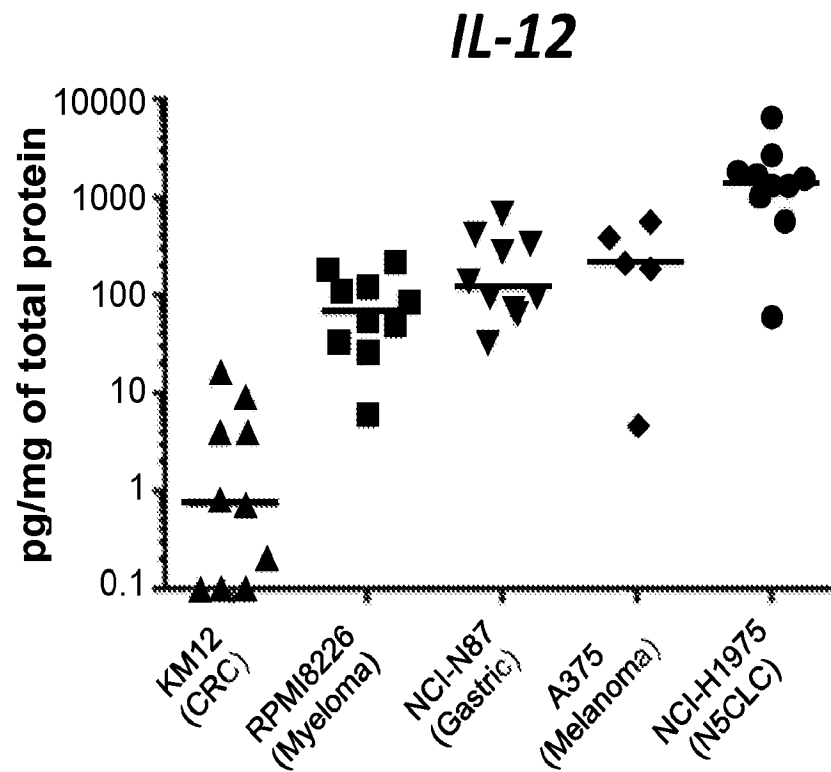


Fig. 22A

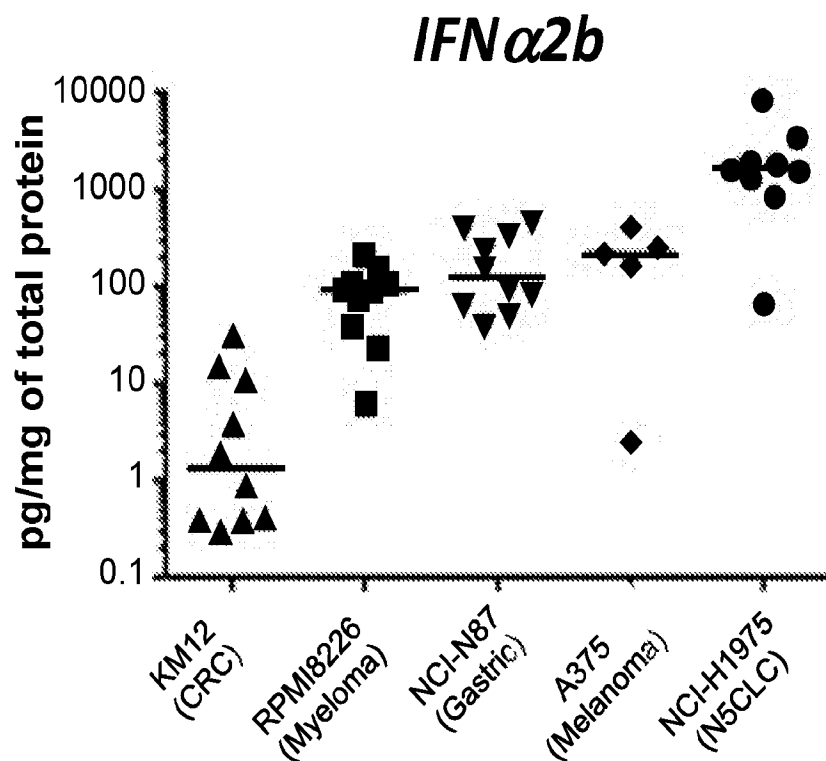


Fig. 22B

58/86

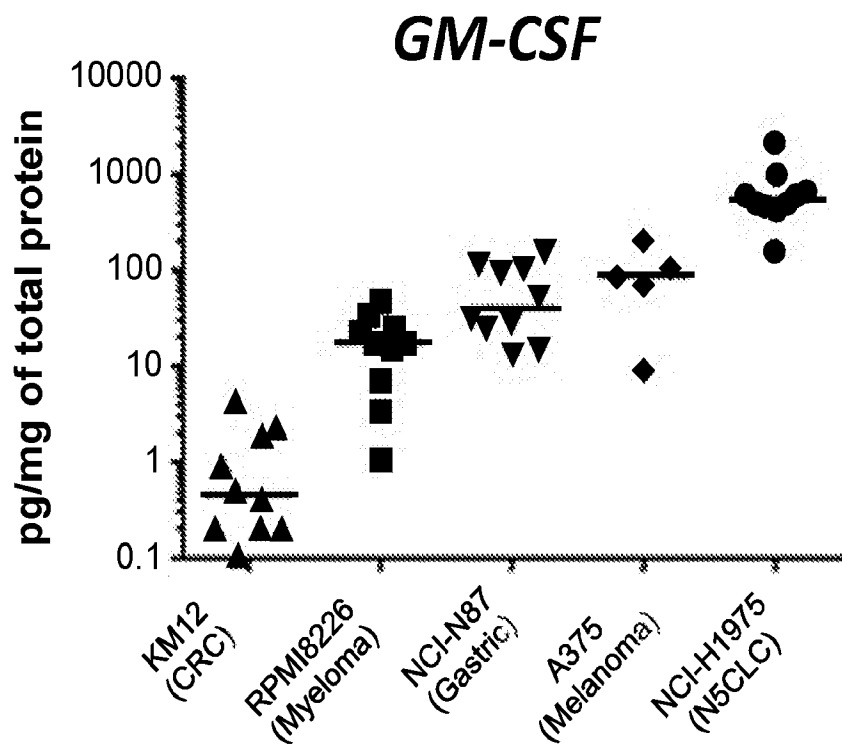


Fig. 22C

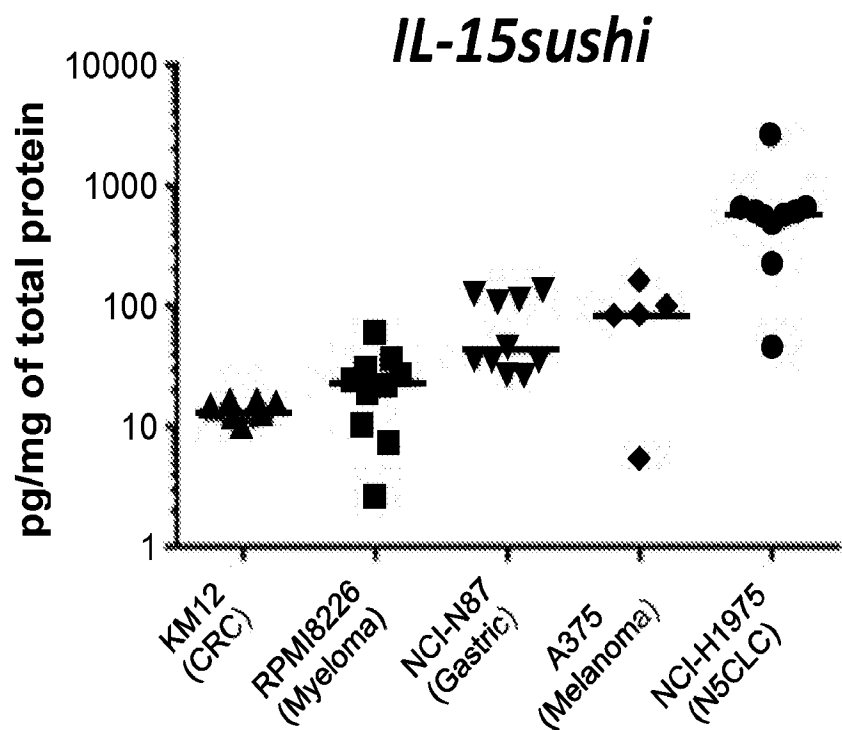
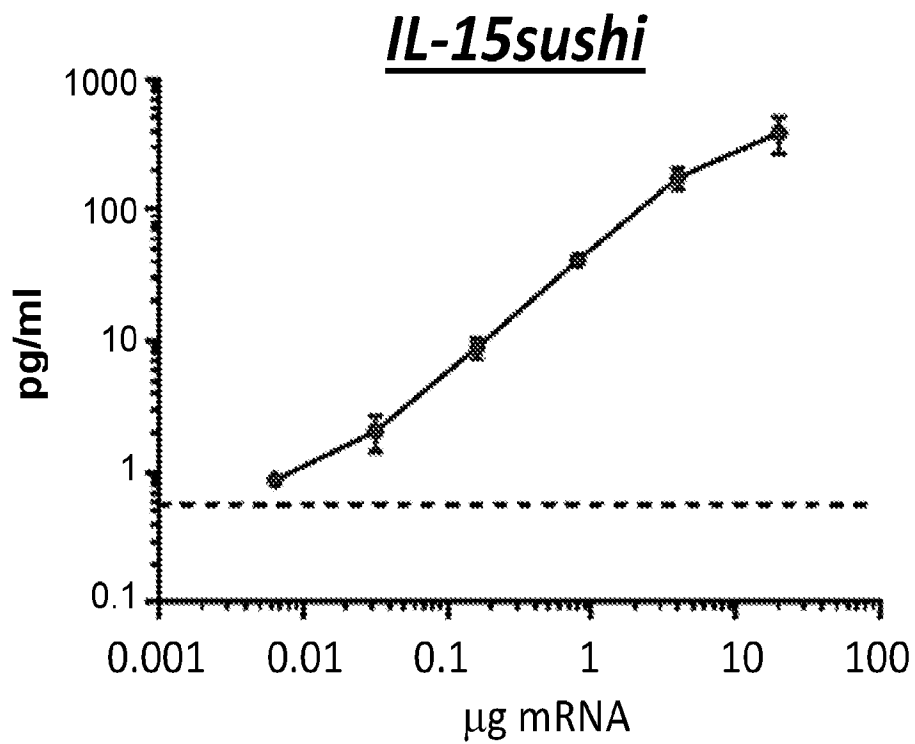
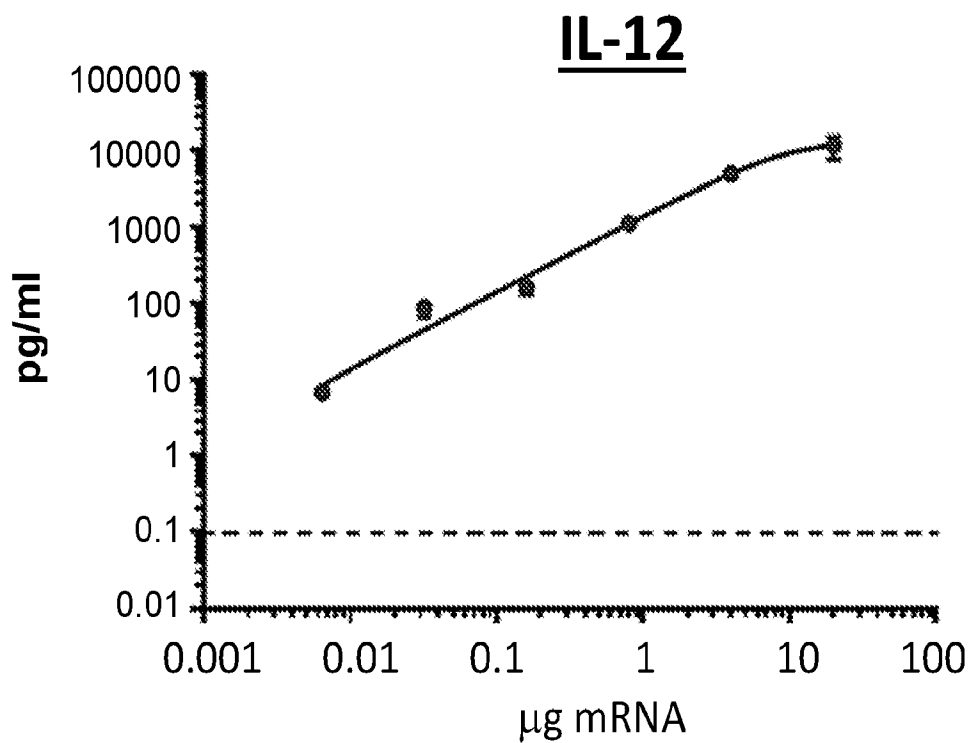
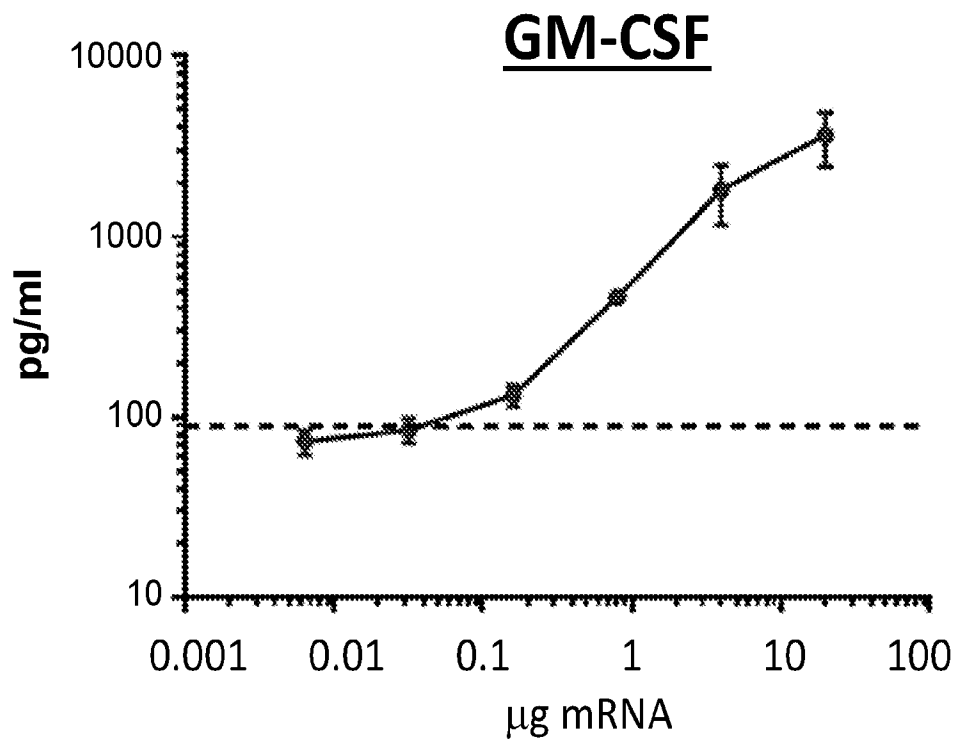
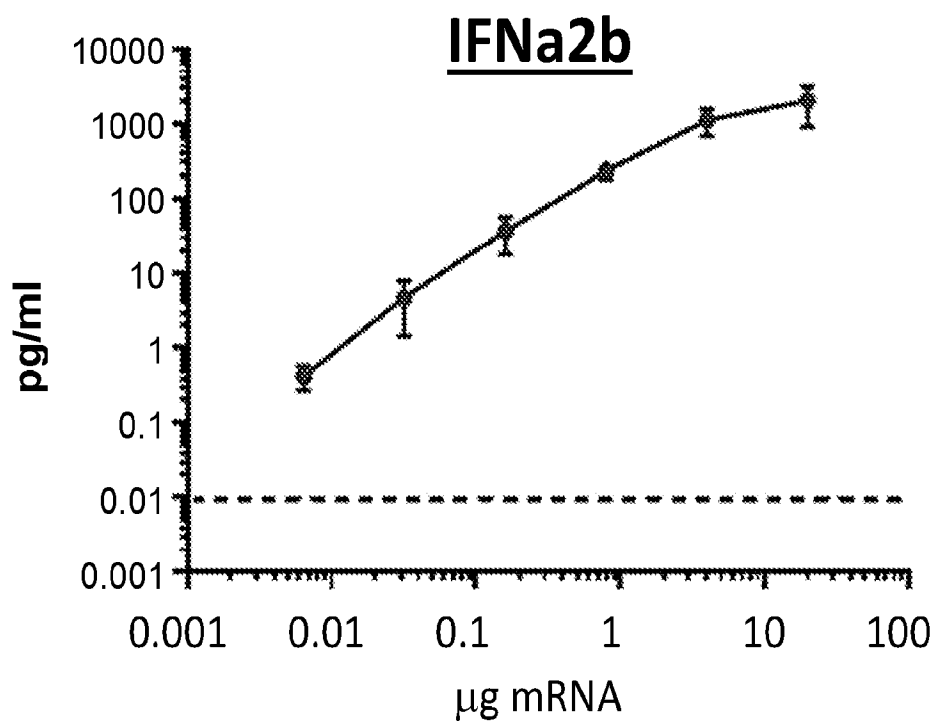


Fig. 22D

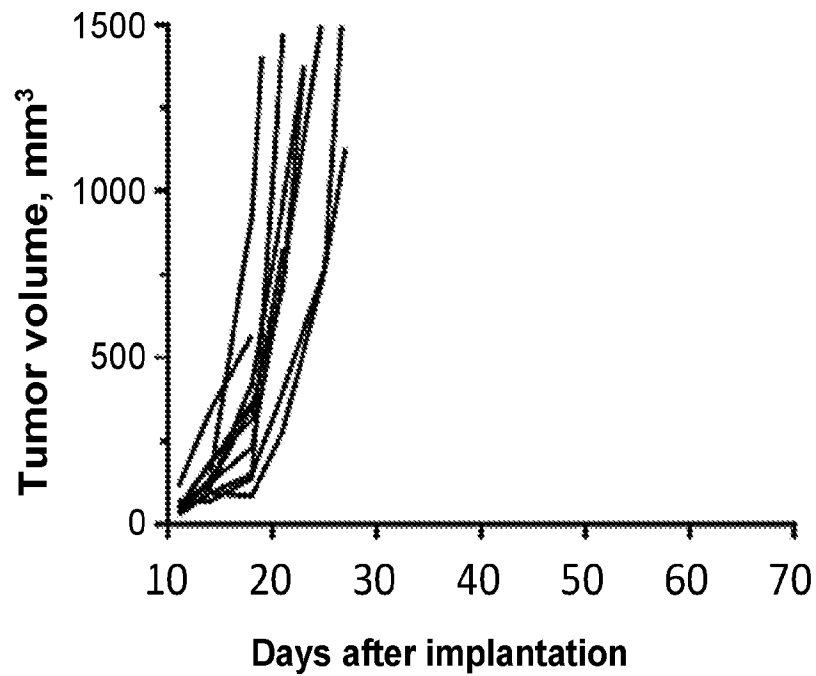
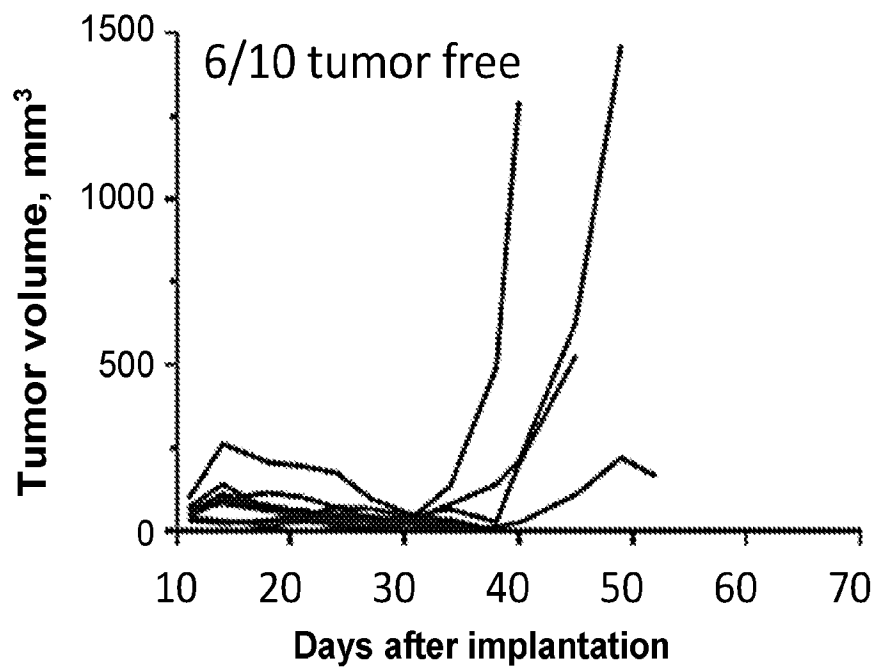
59/86

*Fig. 23A**Fig. 23B*

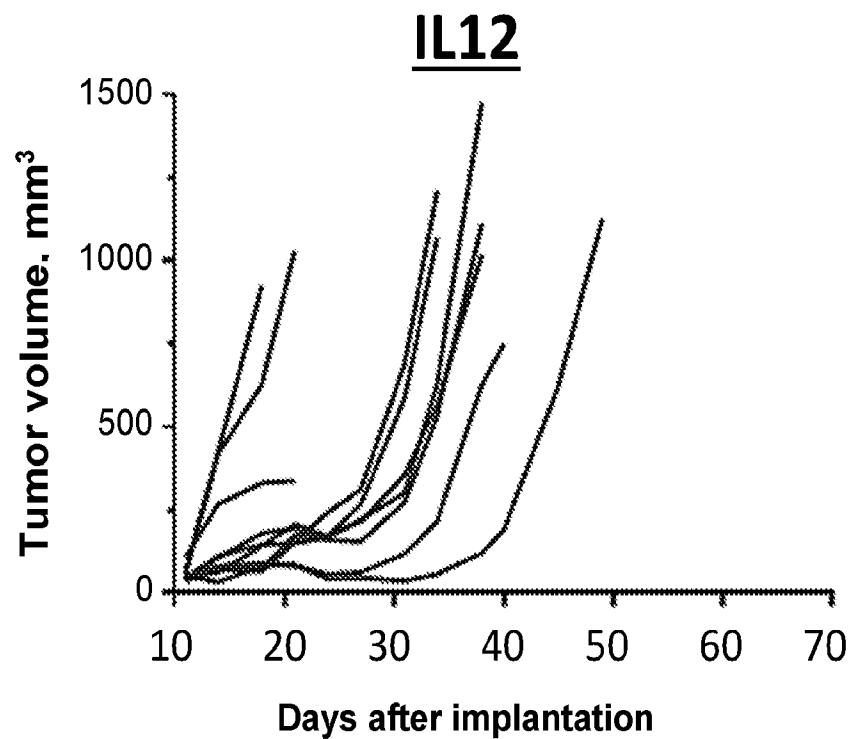
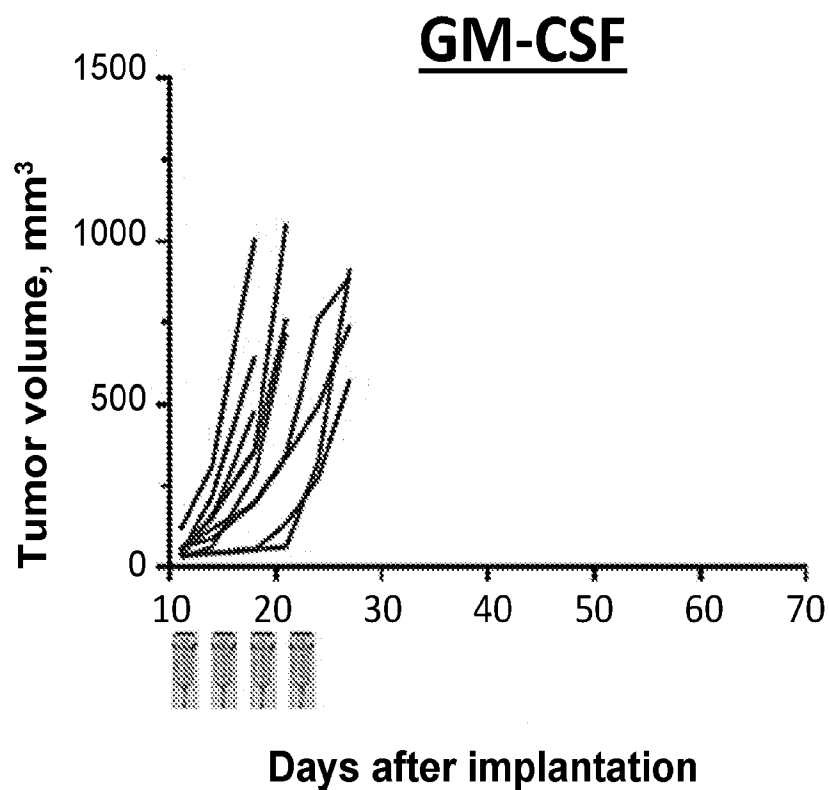
60/86

*Fig. 23C**Fig. 23D*

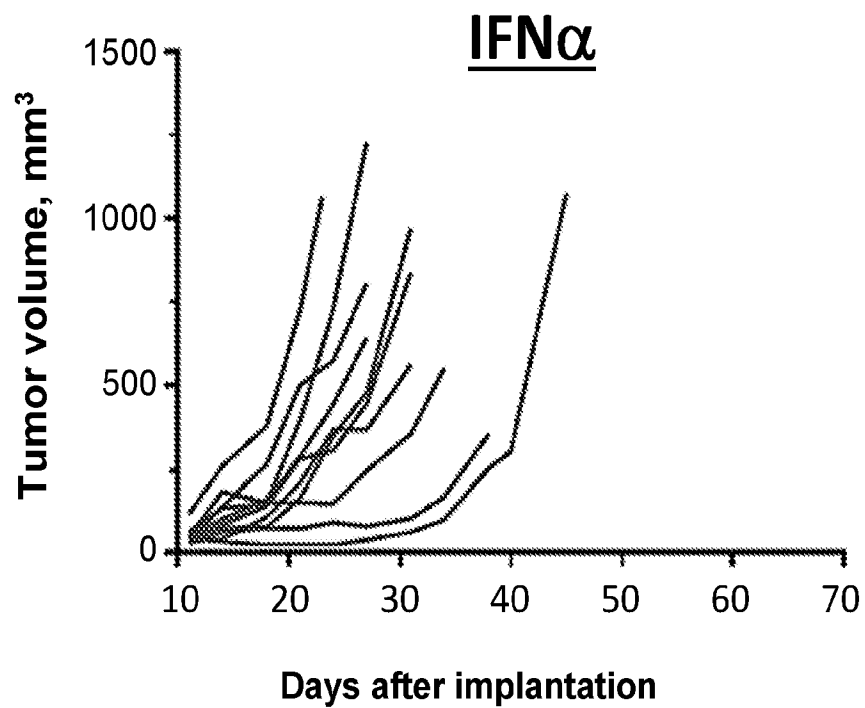
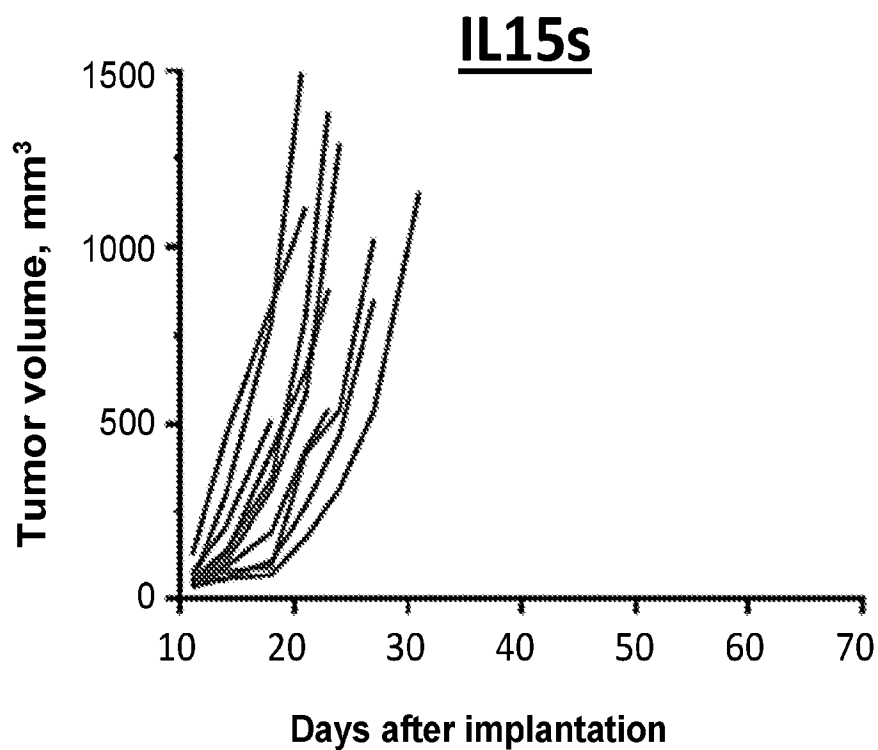
61/86

Luc*Fig. 24A*Cytokine mixture*Fig. 24B*

62/86

*Fig. 24C**Fig. 24D*

63/86

*Fig. 24E**Fig. 24F*

64/86

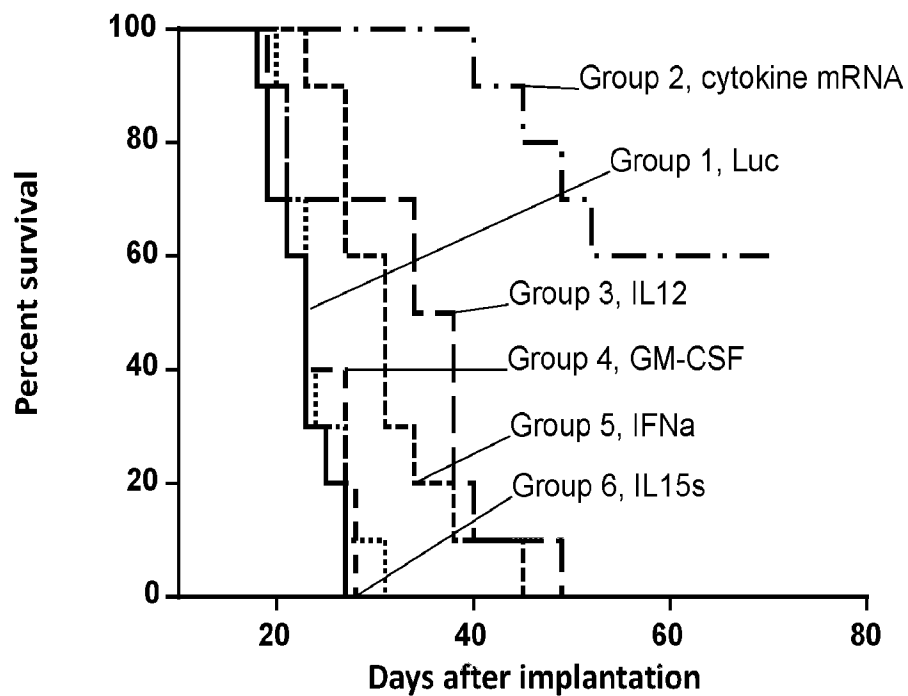


Fig. 24G

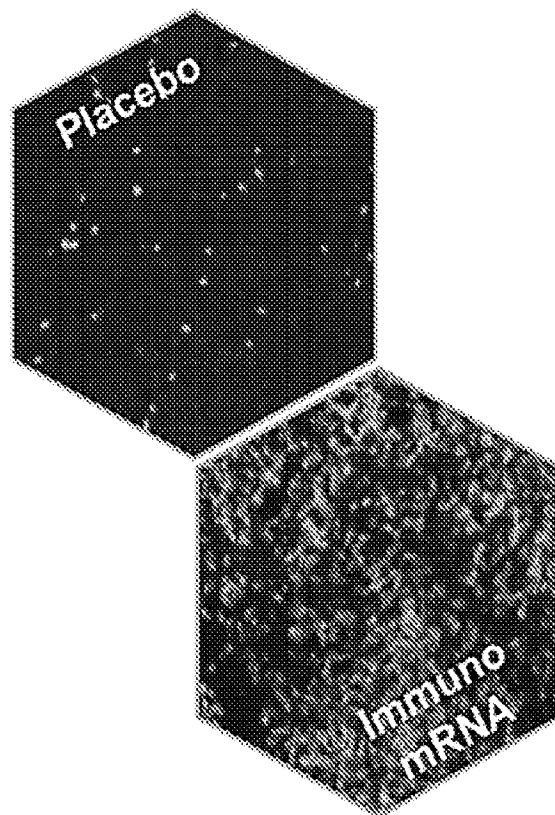
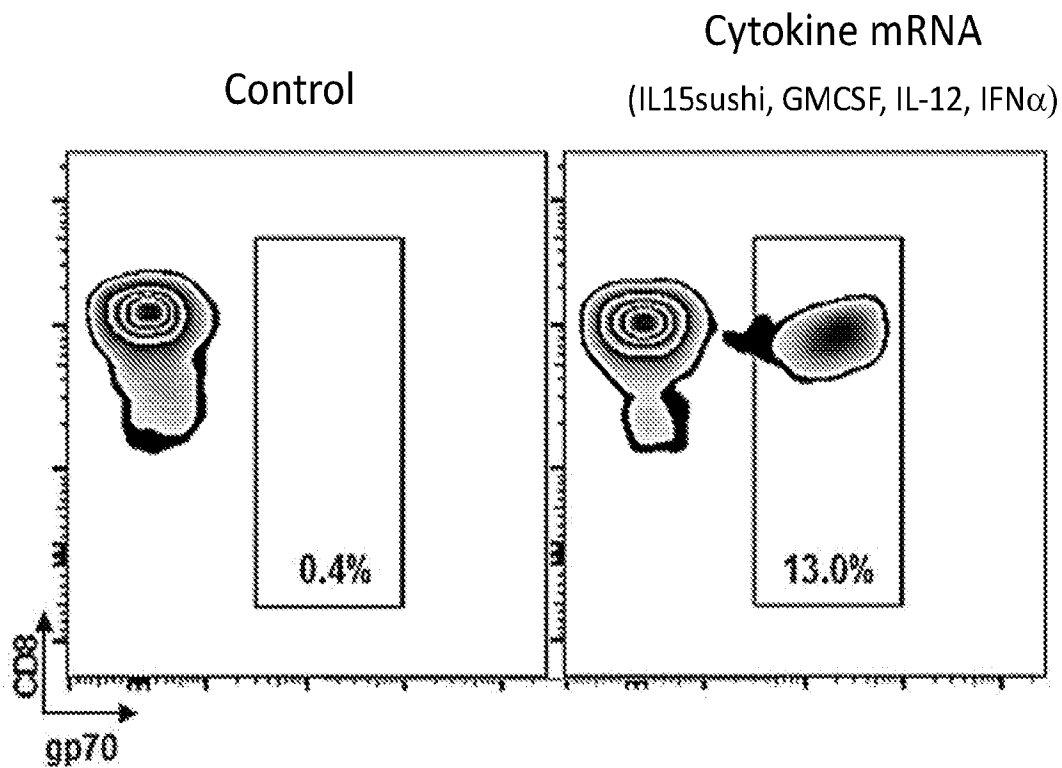
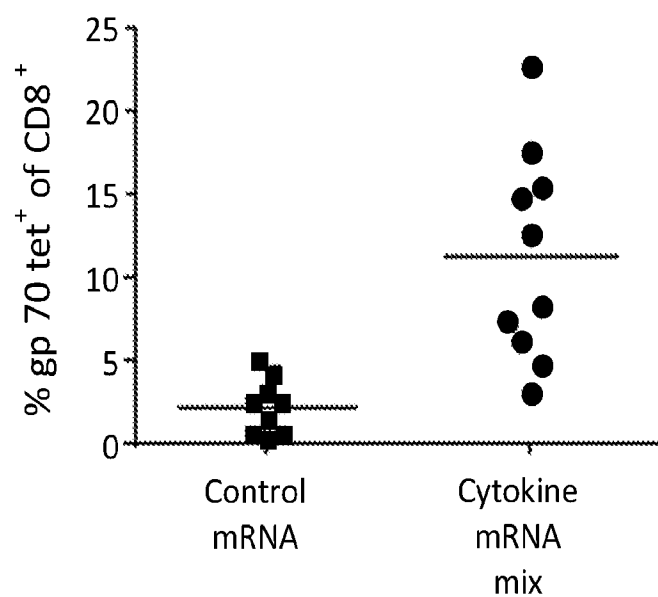


Fig. 25

65/86

*Fig. 26A**Fig. 26B*

Increased antigen-specific
T cells in blood

*Fig. 26C*

66/86

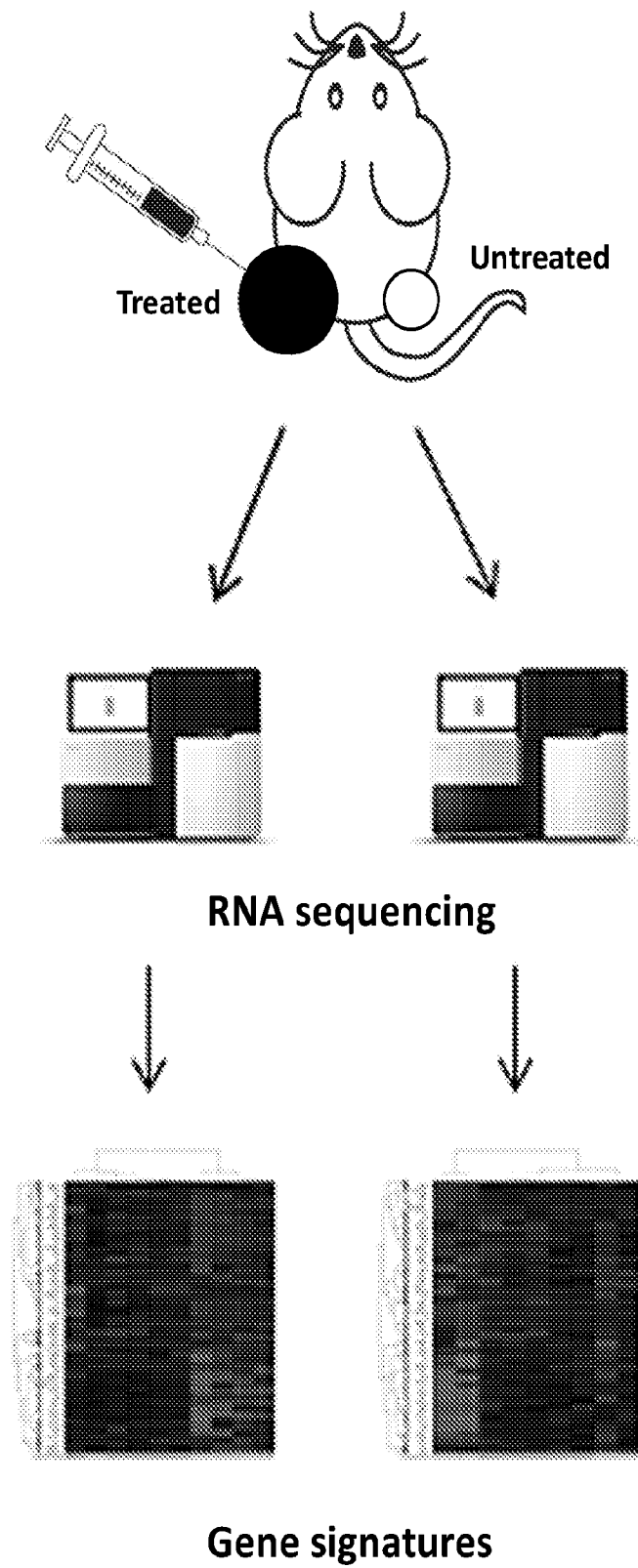


Fig. 27A

67/86

lipopolysaccharide	
IFNG	
TNF	
poly rI:rC-RNA	
IL1B	
NFkB (complex)	
Interferon alpha	
tretinoin	
phorbol myristate acetate	
E. coli B5 lipopolysaccharide	
IRF7	
STAT1	
IFNA2	
TLR3	
CD40LG	
TLR9	
IL2	
MYD88	
E. coli B4 lipopolysaccharide	
TLR4	
RELA	
IKBKB	
IL1	
NUPR1	
salmonella Minnesota R595 lipopolysaccharide	
TLR7	
TICAM1	
IL1A	
IL18	
IRF3	
RARA	
MYCN	
FOXM1	
VCAN	
L-dopa	
Bay 11-7082	
SOCS1	
GFI1	
SCAP	
N-acetyl-L-cysteine	
alefacept	
U0126	
pyrrolidine dithiocarbamate	
TBX2	
NKX2-3	
Alpha catenin	
RABL6	
MYC	
IL1RN	
TRIM24	
SB203580	
IL10RA	

Fig. 27B

68/86

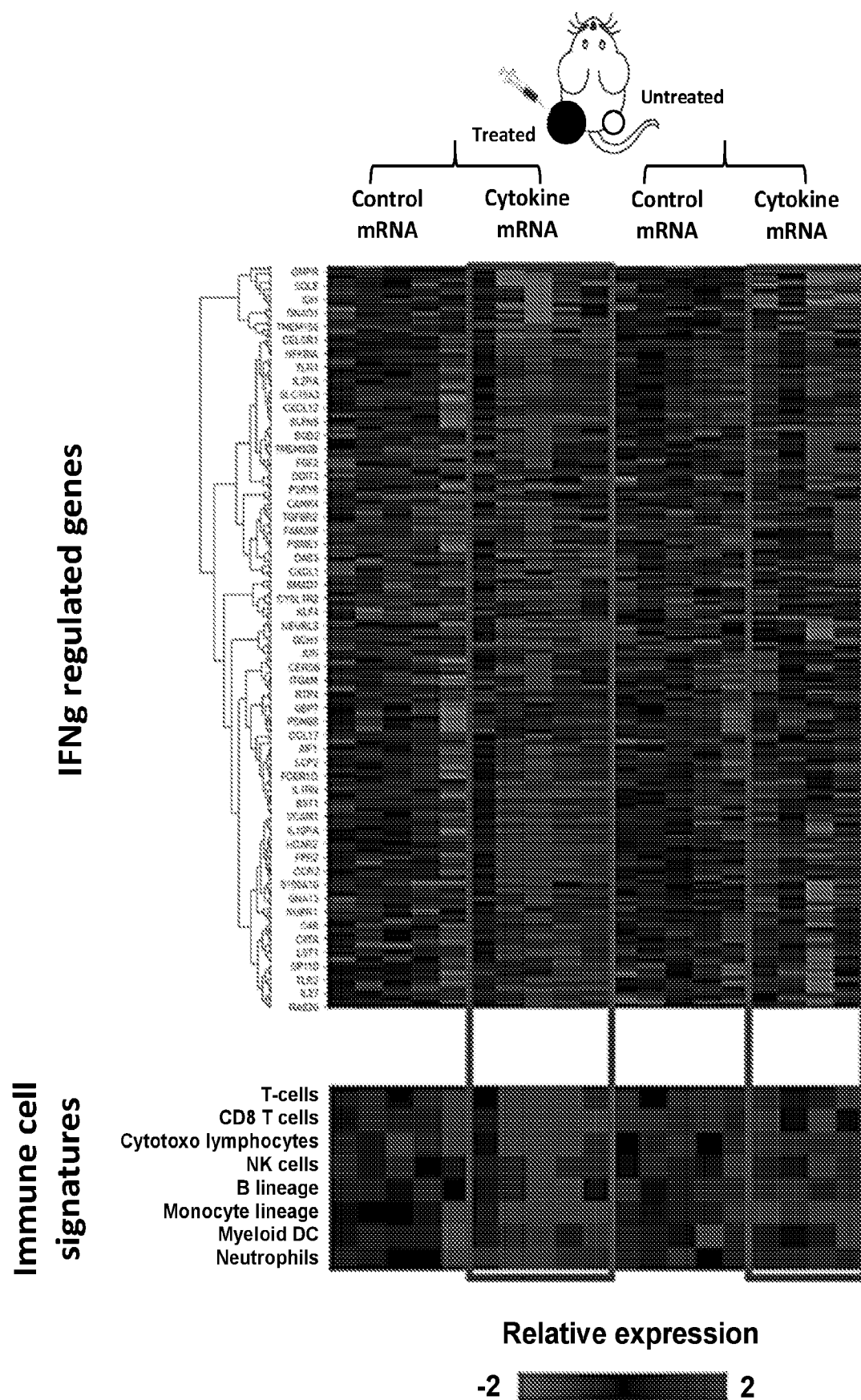


Fig. 27C

Fig. 28B

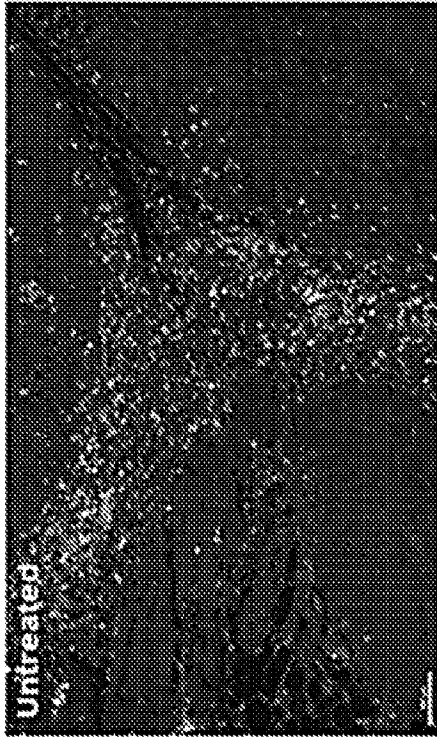


Fig. 28A

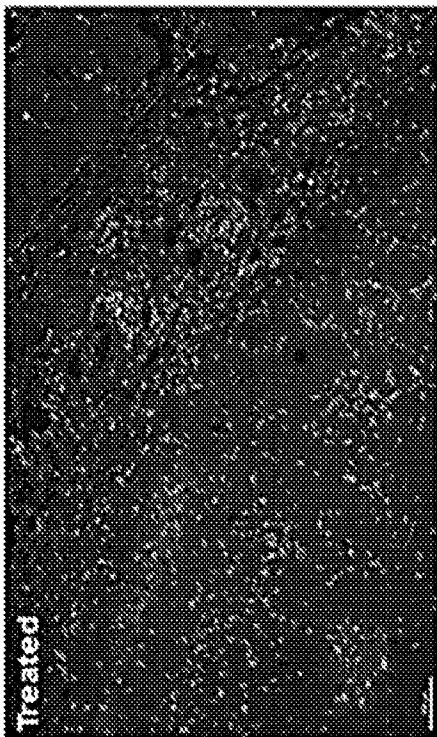


Fig. 28D

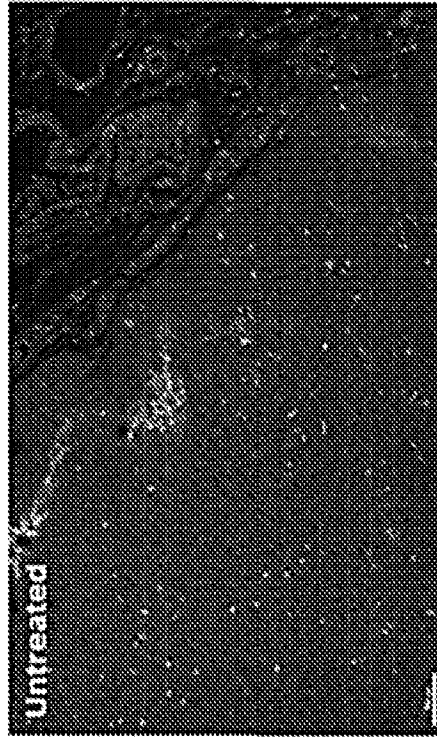
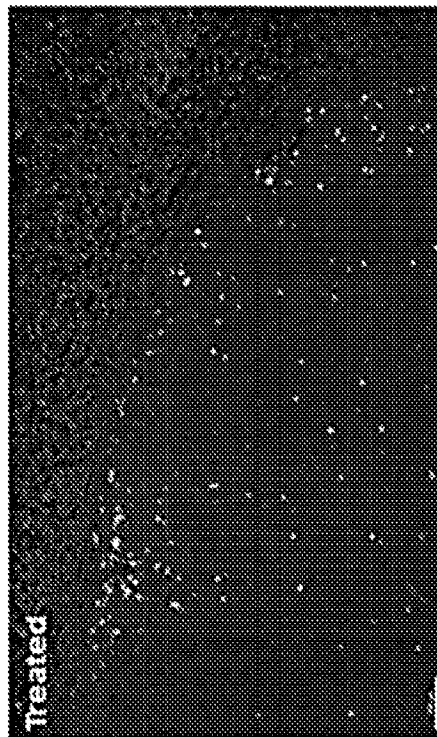


Fig. 28C



CD4+ CD8+ FOXP3 Nuclei

70/86

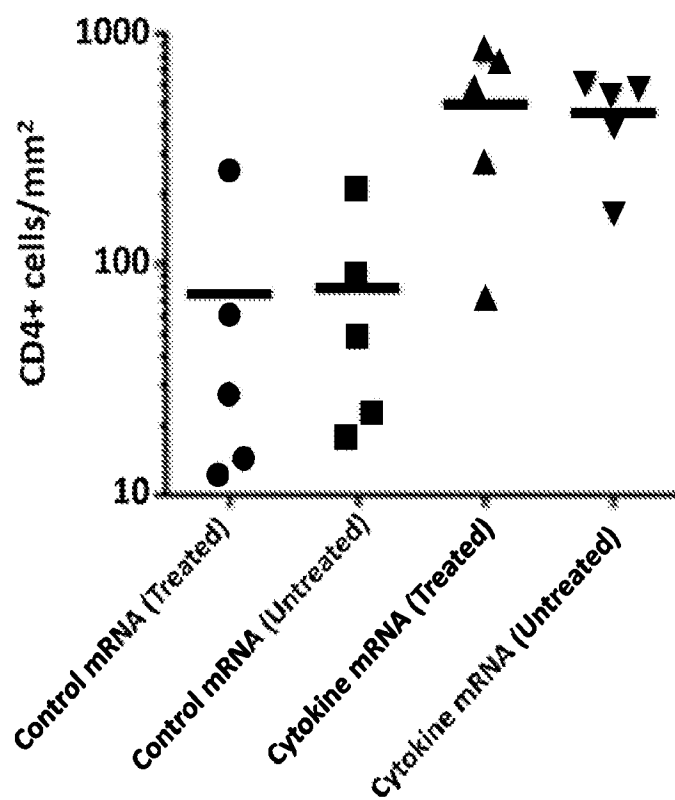


Fig. 28E

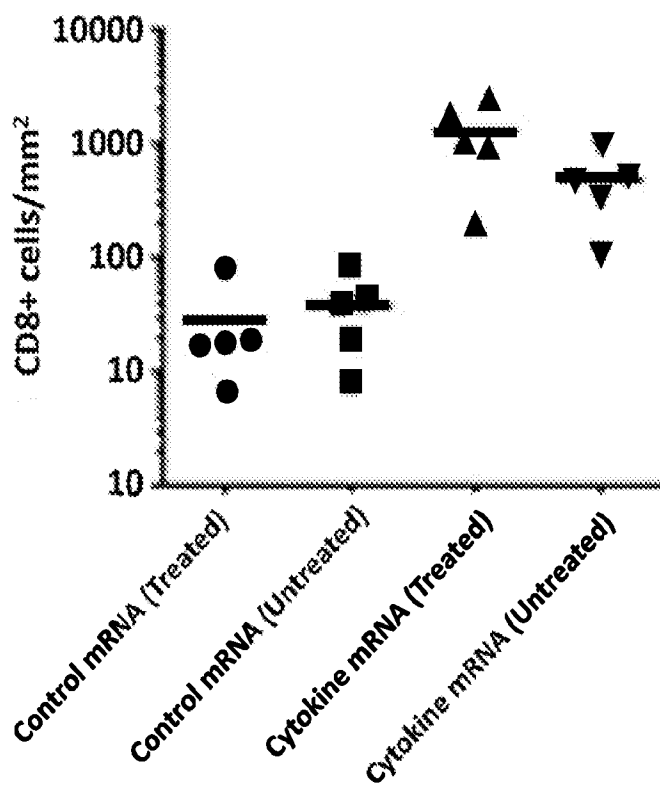
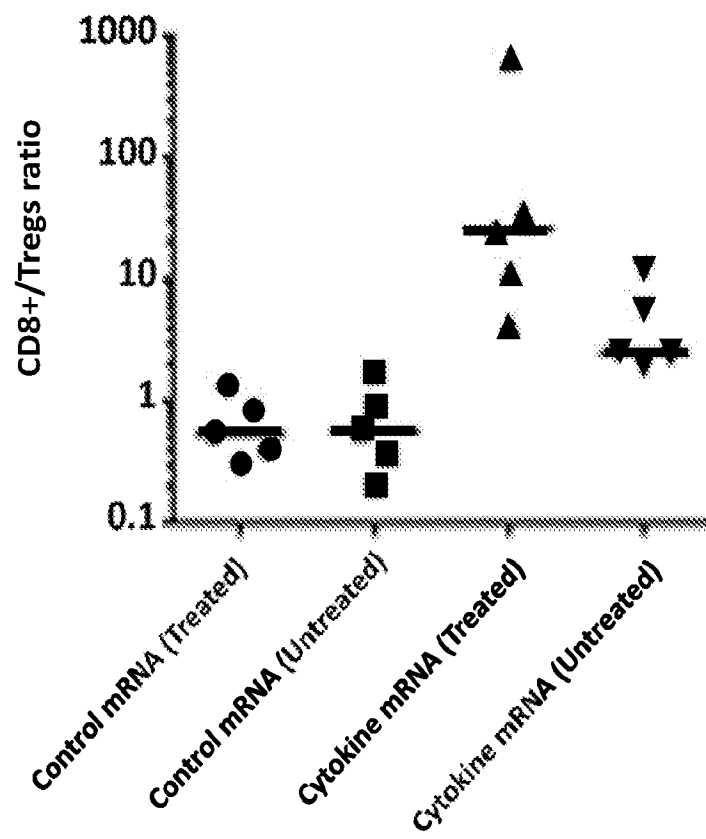


Fig. 28F

*Fig. 28G*

72/86

Study design
(B16F10 tumor)

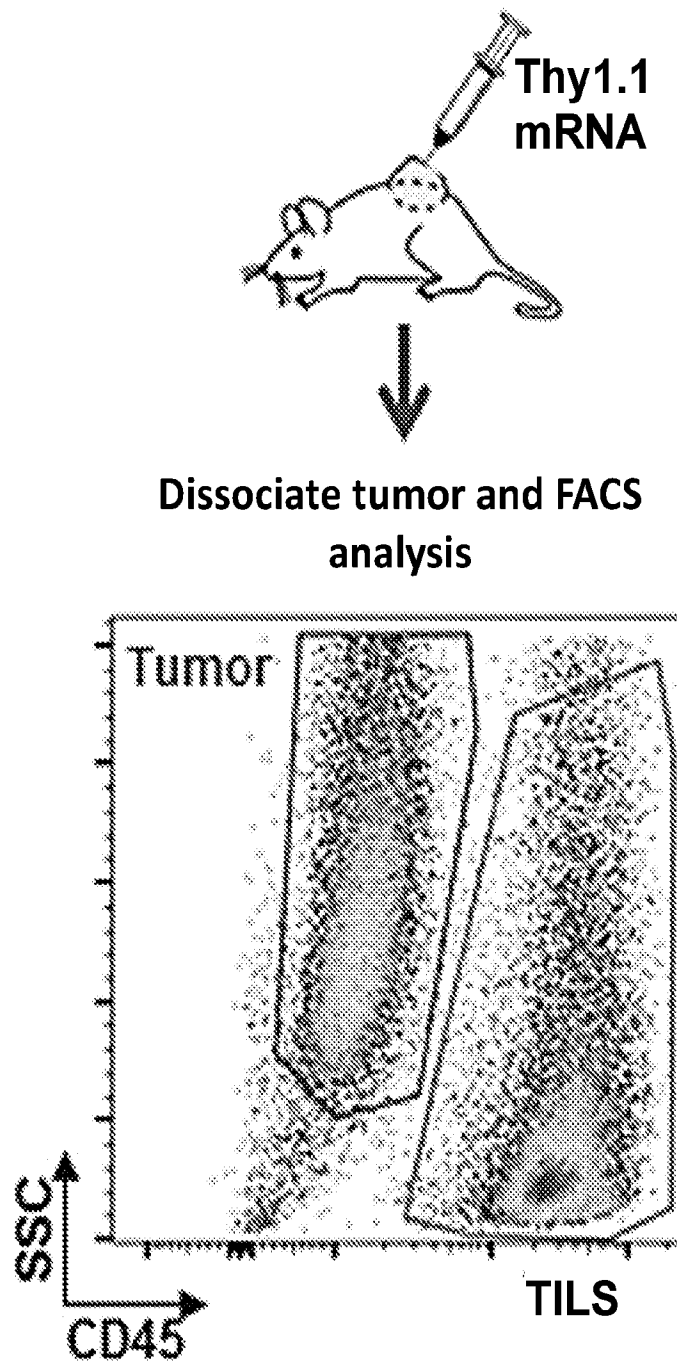
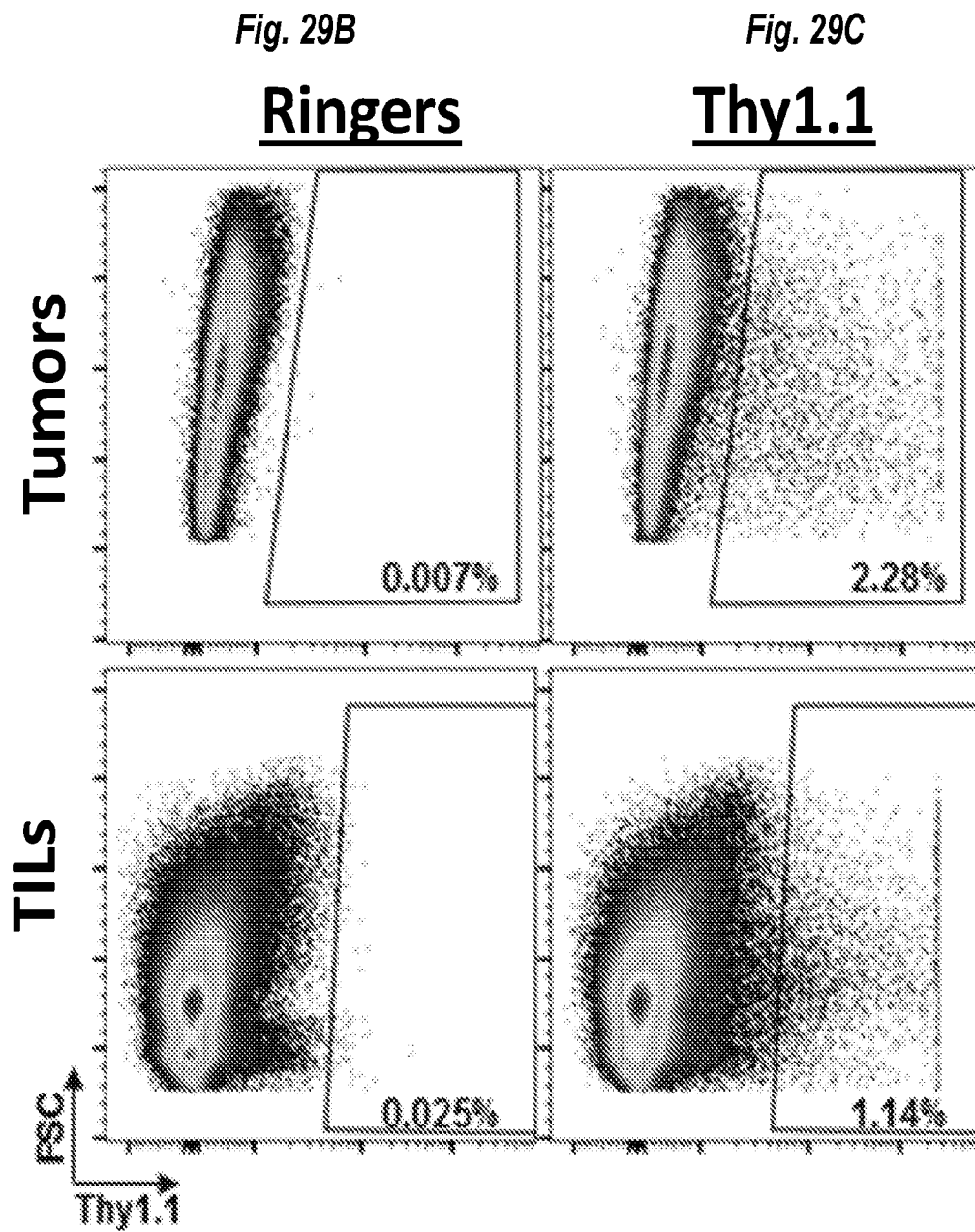


Fig. 29A

**Fig. 29D****Fig. 29E**

74/86

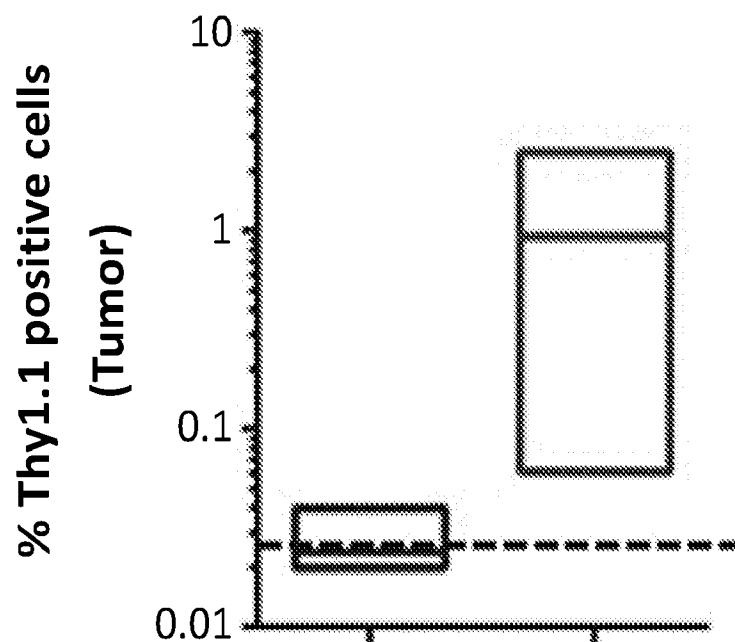


Fig. 29F

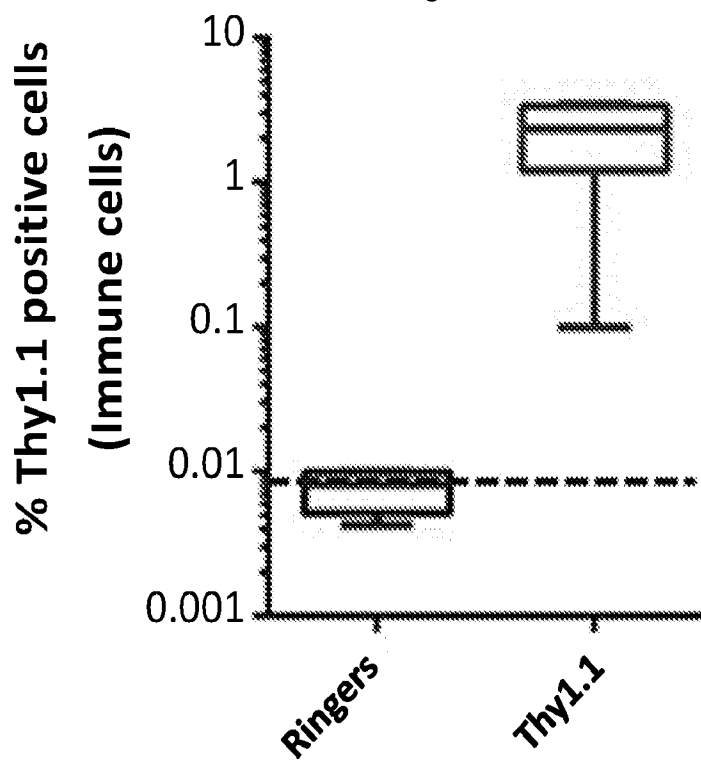
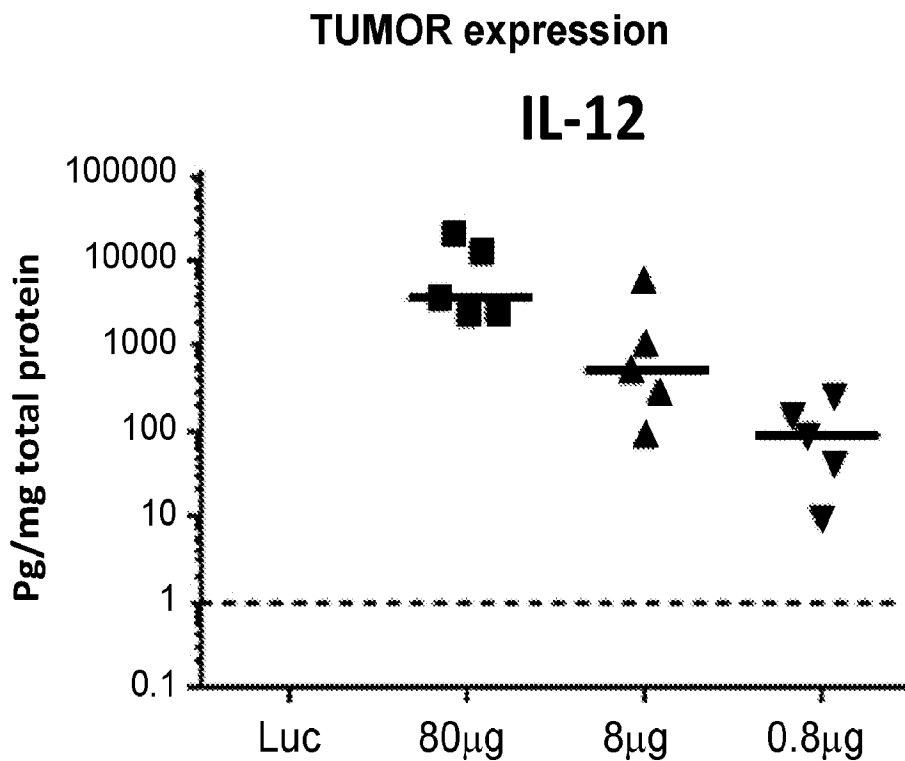
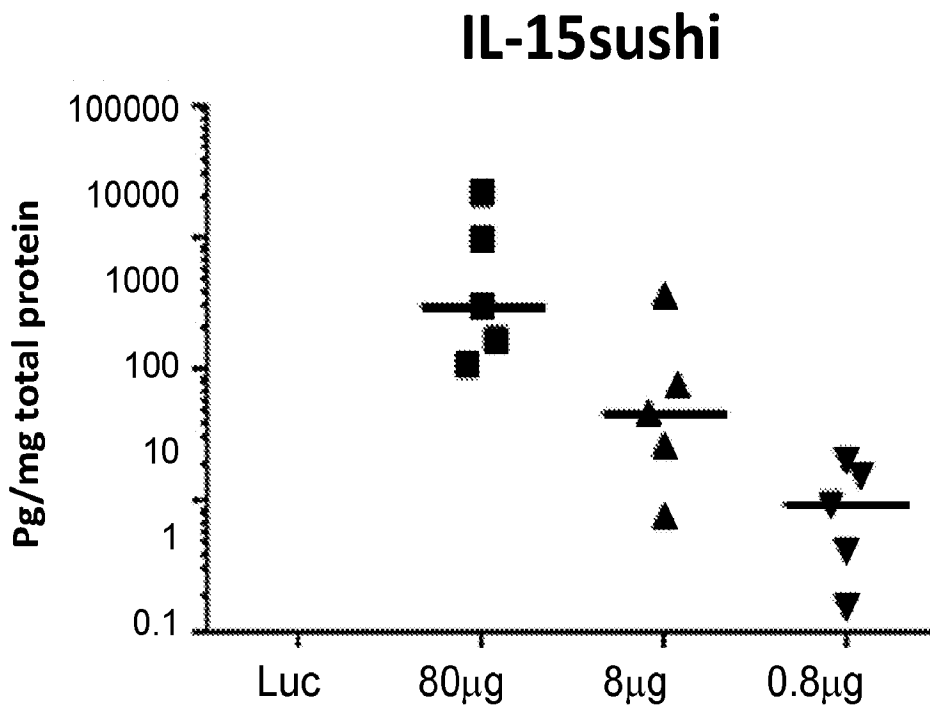
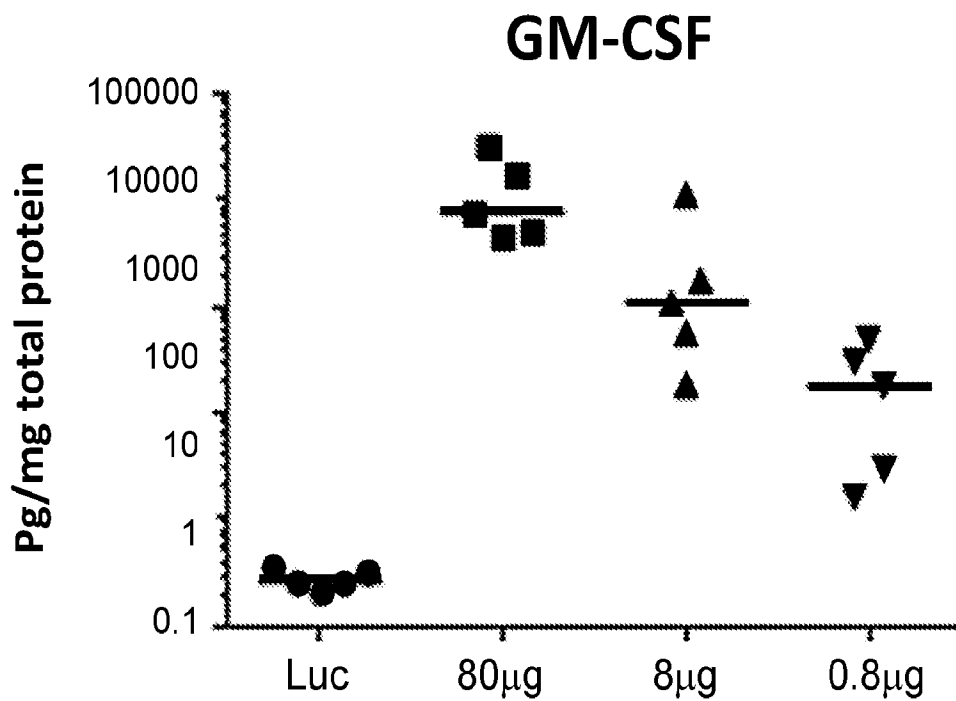
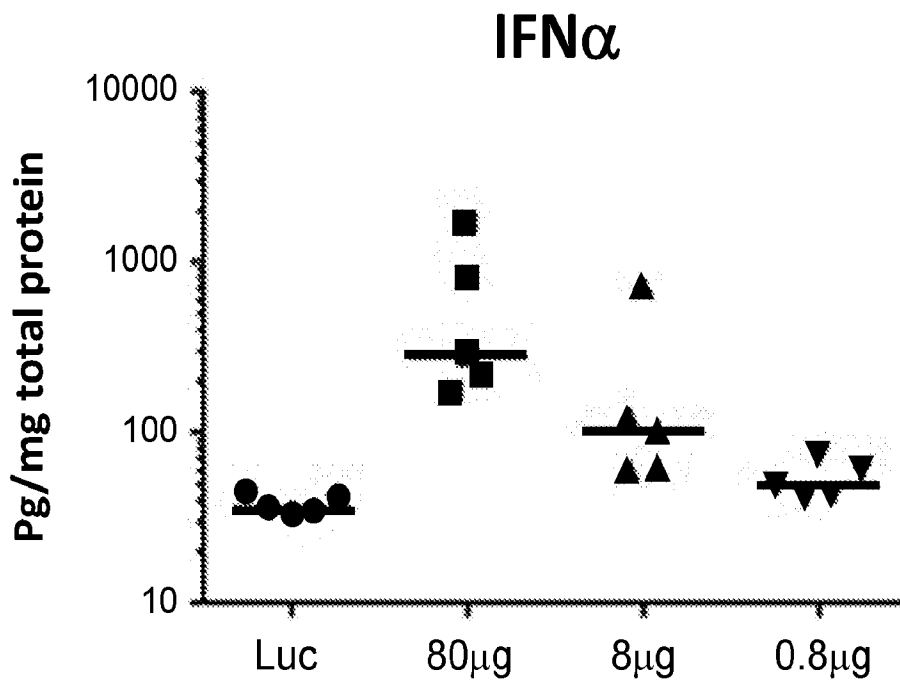


Fig. 29G

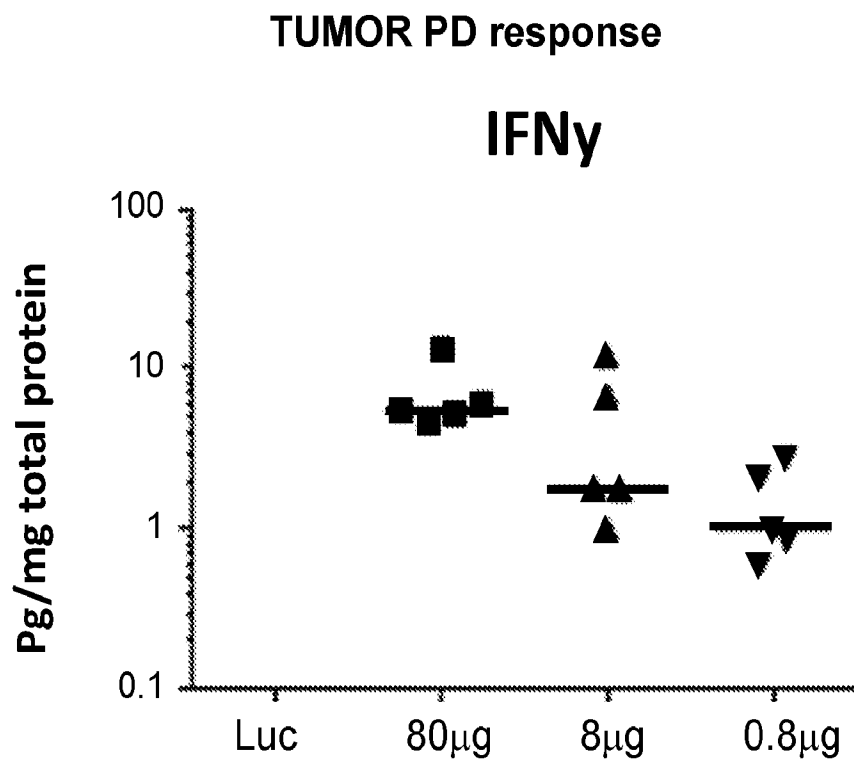
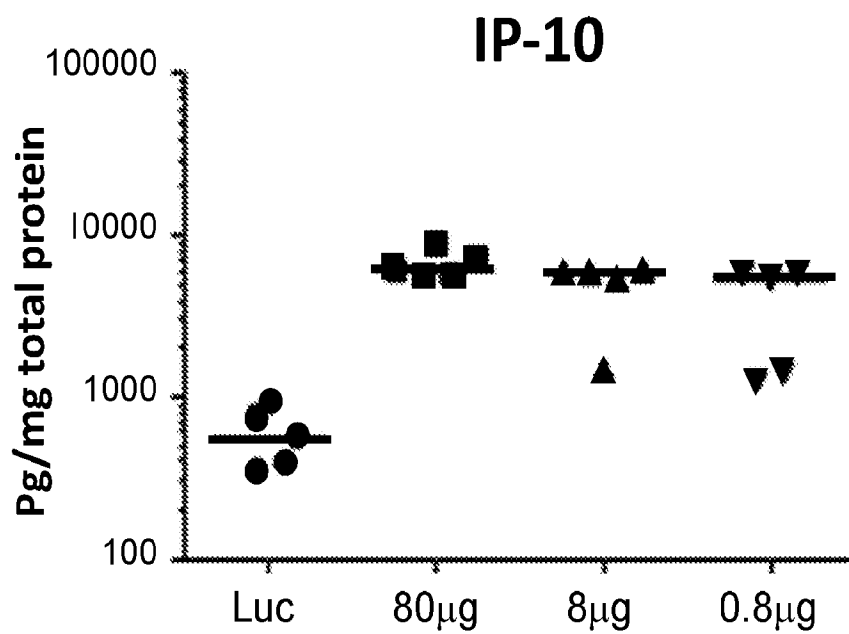
75/86

*Fig. 30A**Fig. 30B*

76/86

*Fig. 30C**Fig. 30D*

77/86

*Fig. 30E**Fig. 30F*

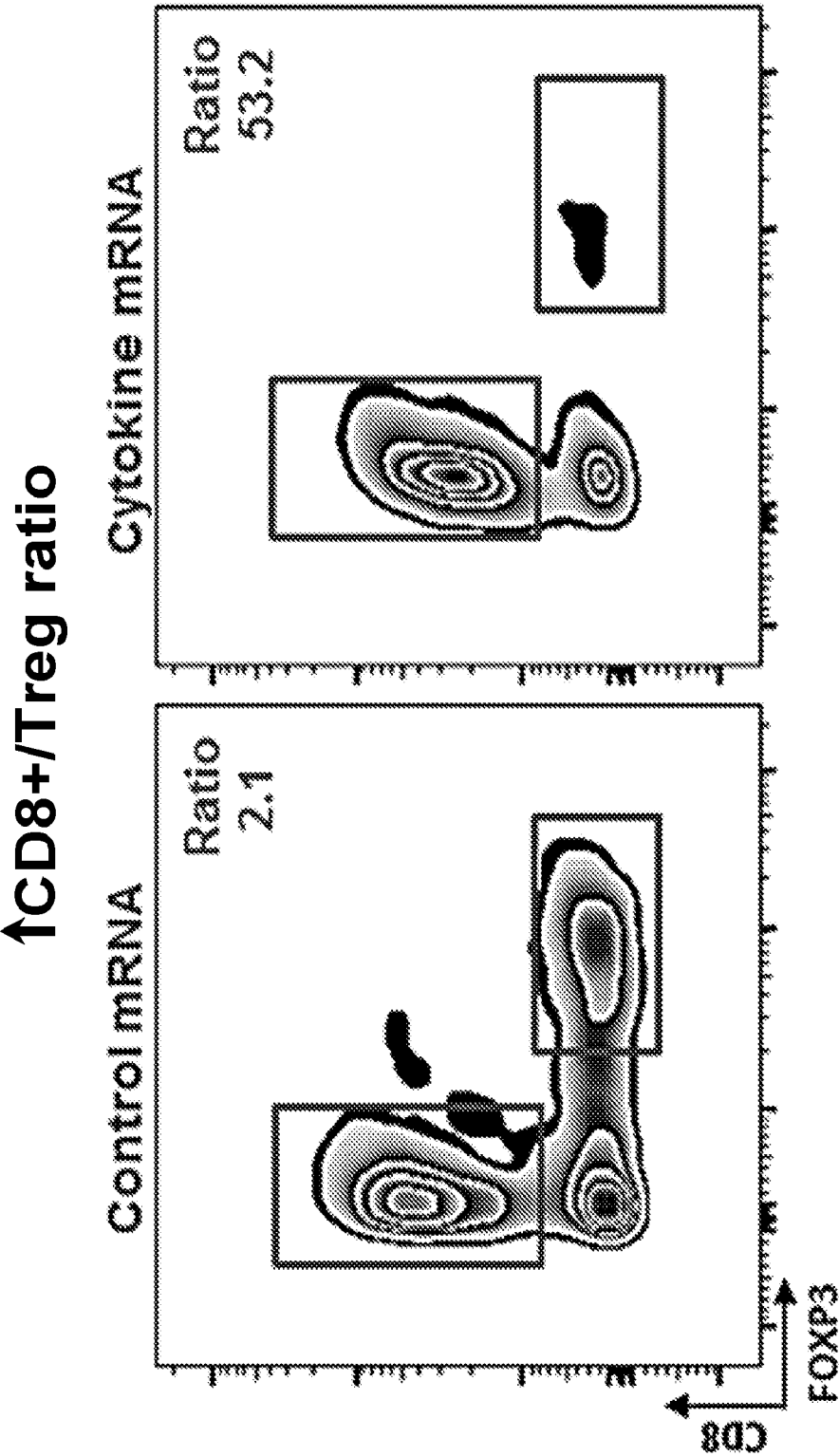


Fig. 31A

Fig. 31B

↑Polyfunctional CD8+ T cells

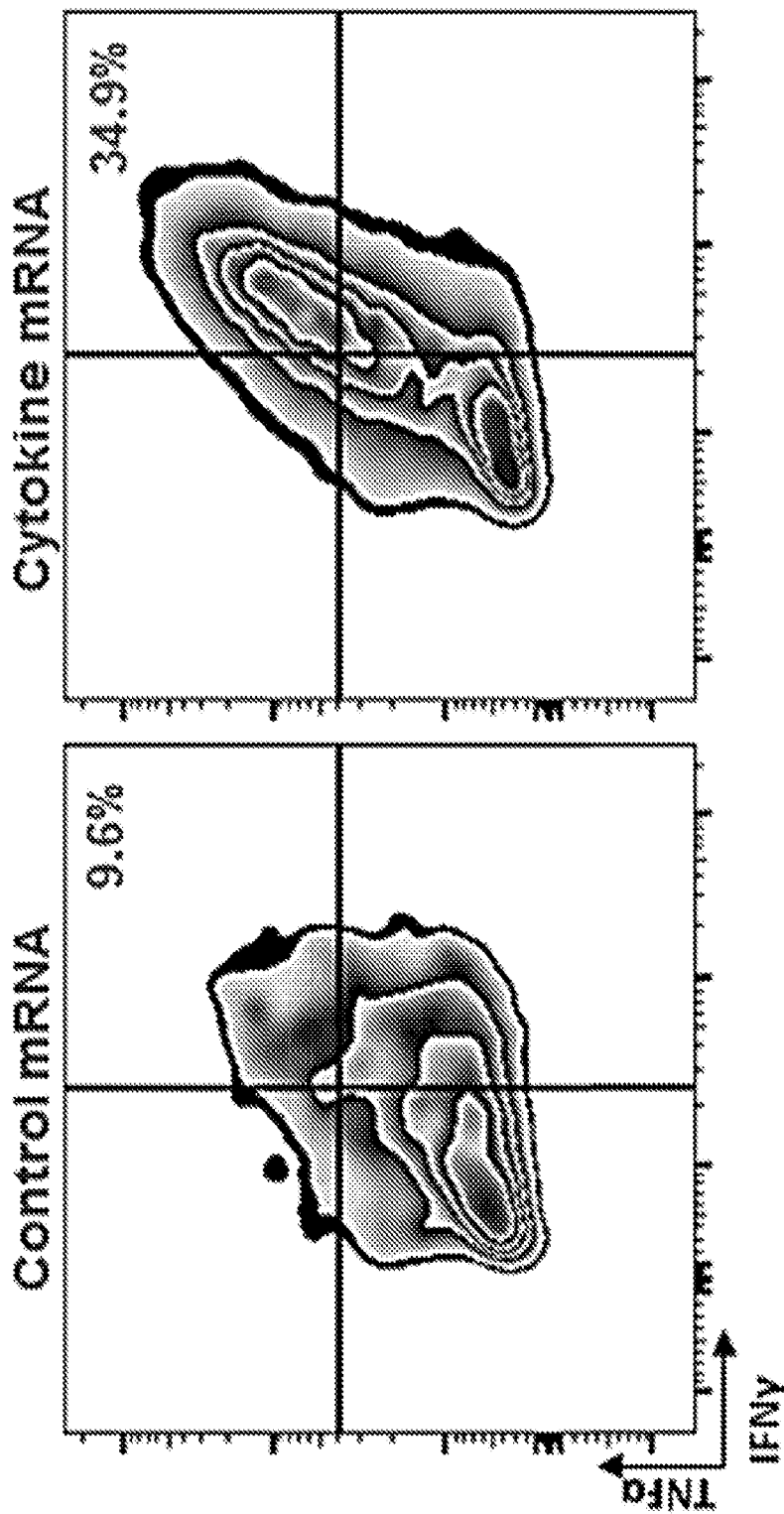


Fig. 31C

Fig. 31D

↑PD-L1 on tumor infiltrating cells (CD11B+)

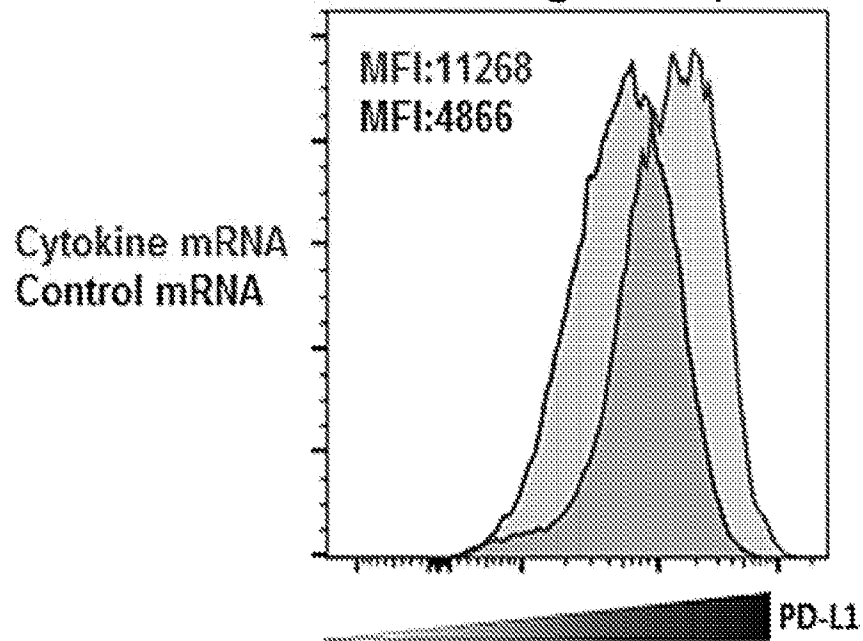


Fig. 31E

↑PD-1 on tumor infiltrating CD8+

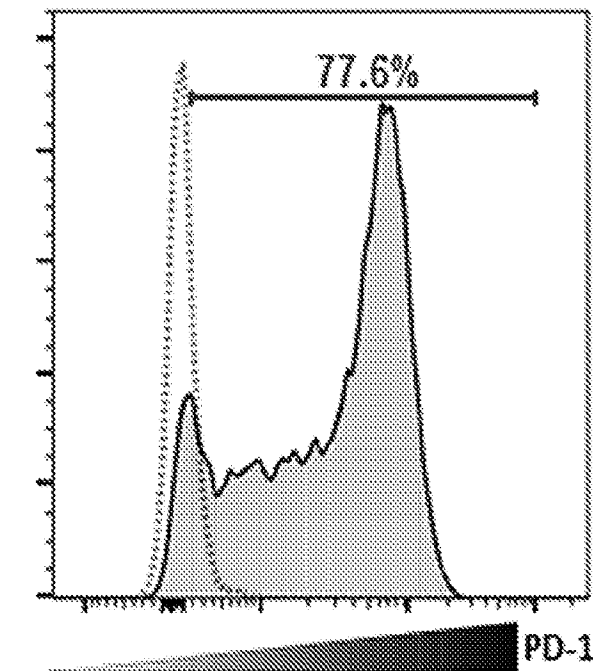
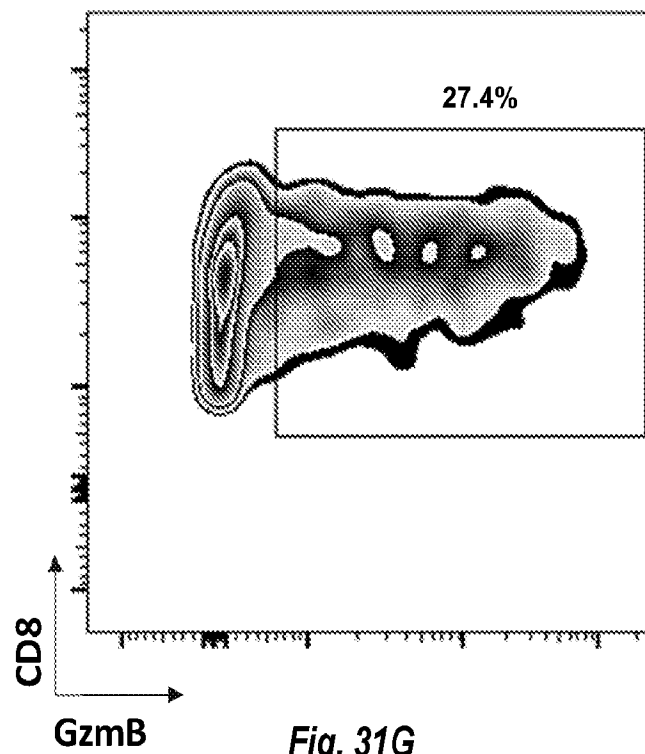


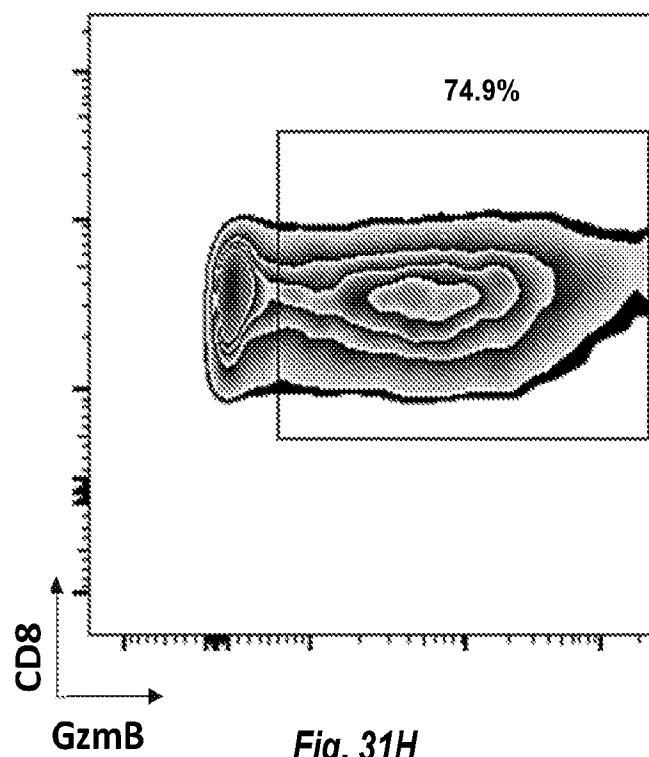
Fig. 31F

81/86

Control mRNA

*Fig. 31G*

Cytokine mRNA

*Fig. 31H*

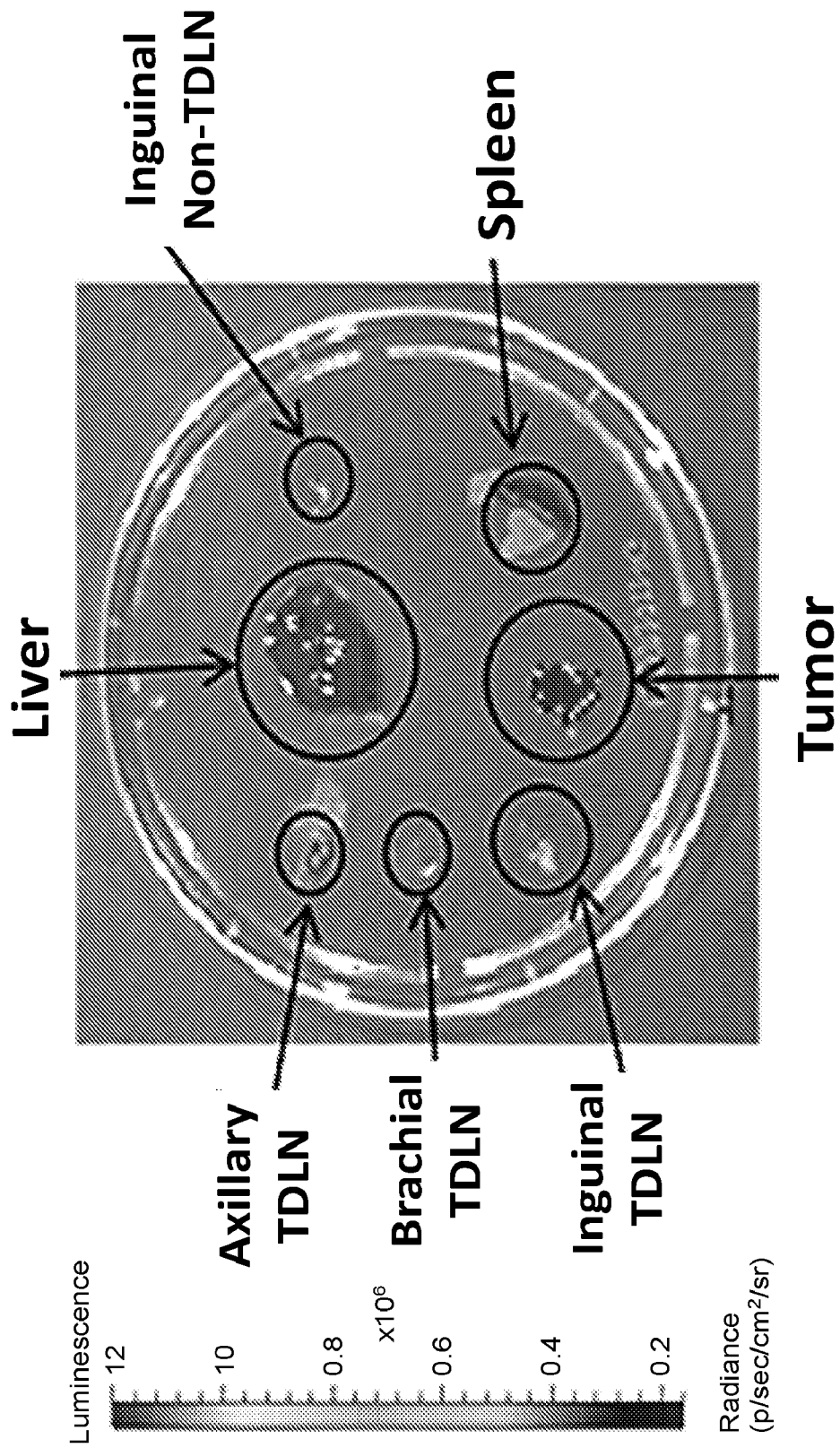


Fig. 32A

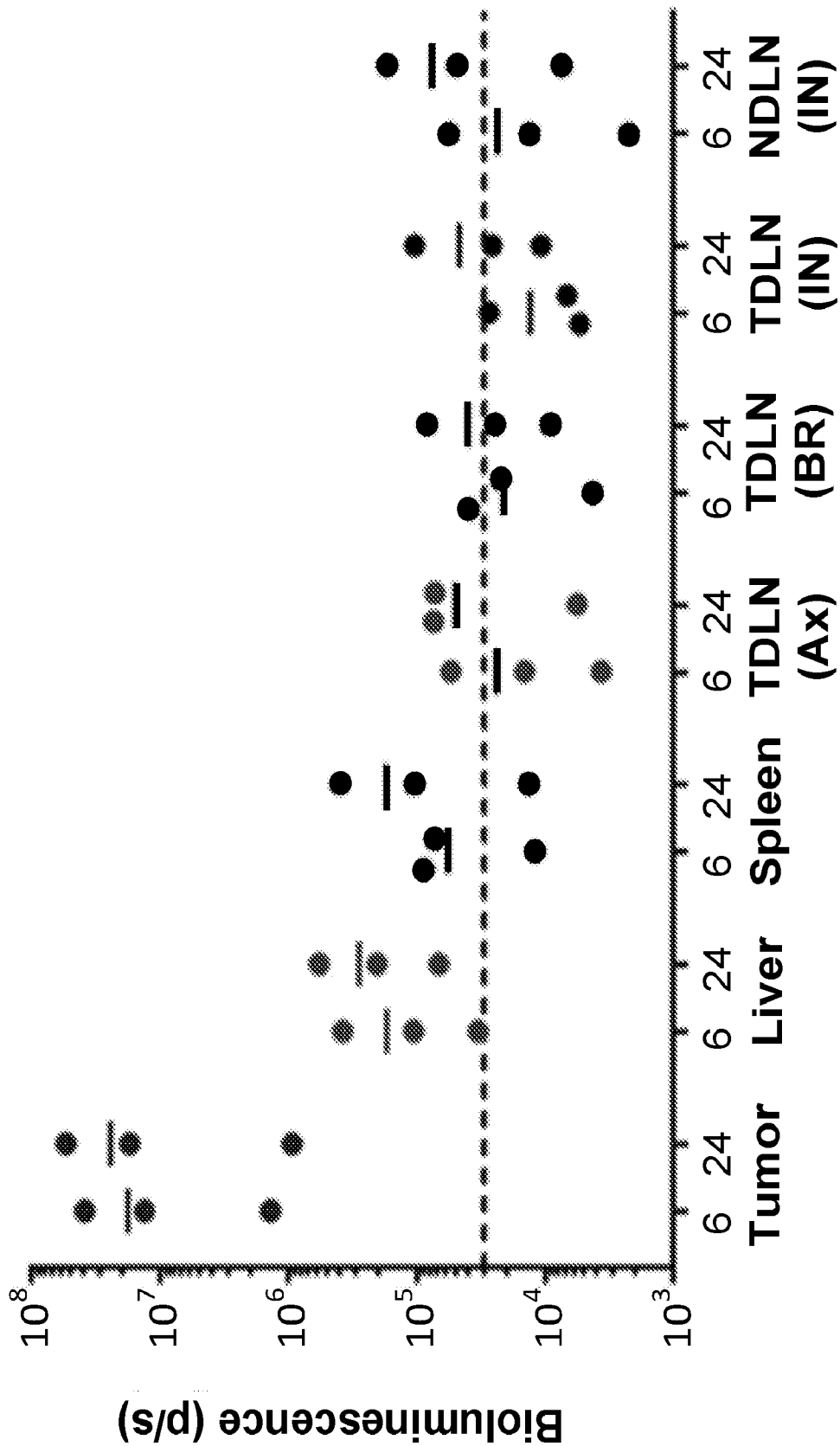


Fig. 32B

Resistance to tumor rechallenge

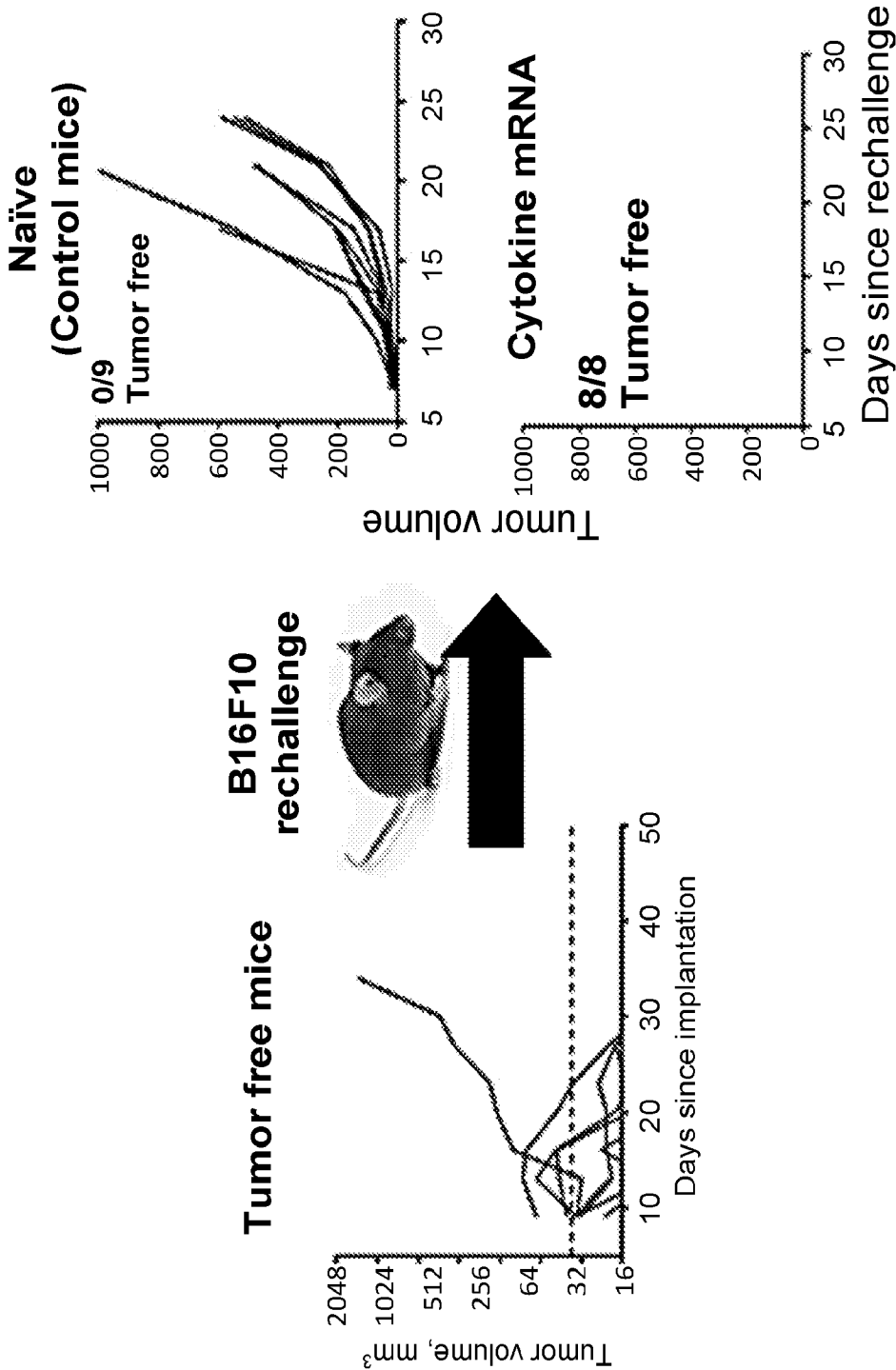
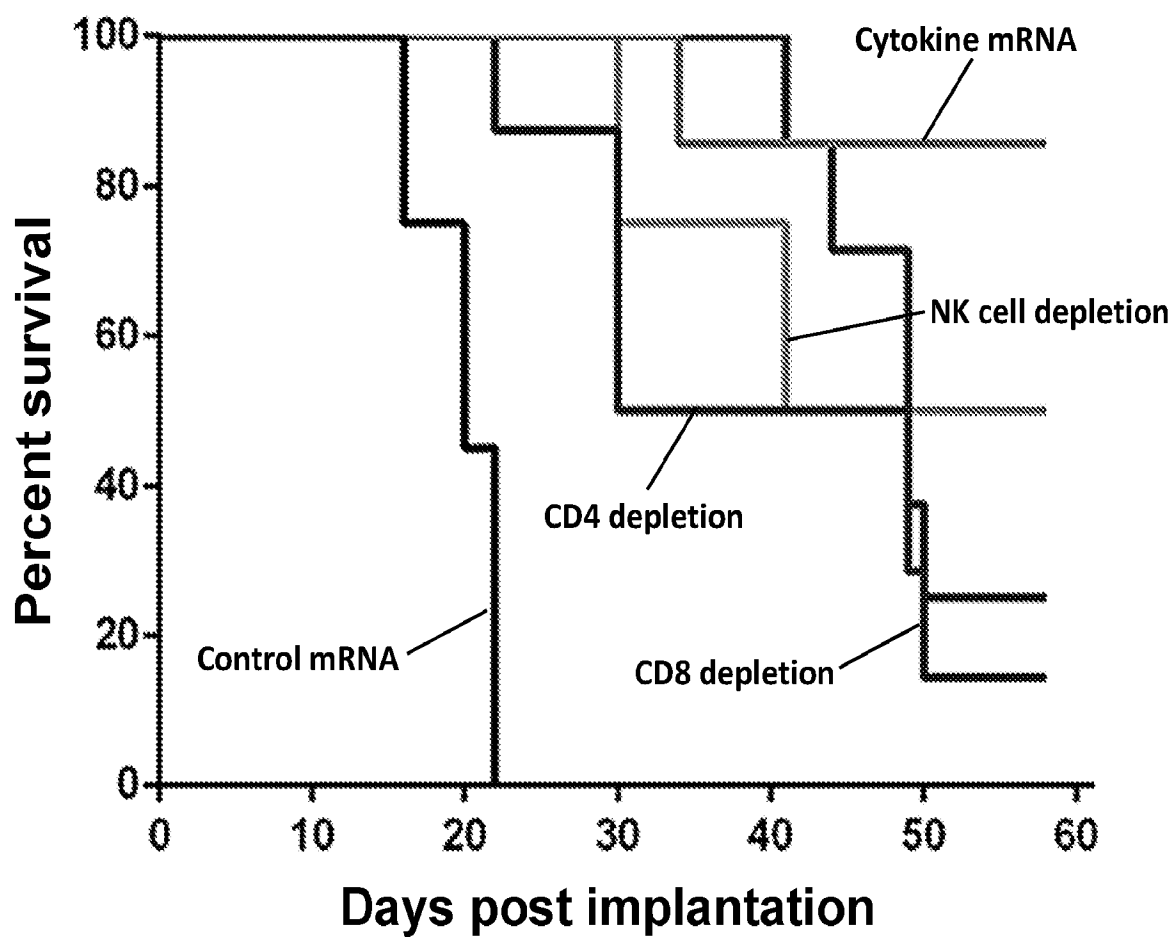


Fig. 33

*Fig. 34*

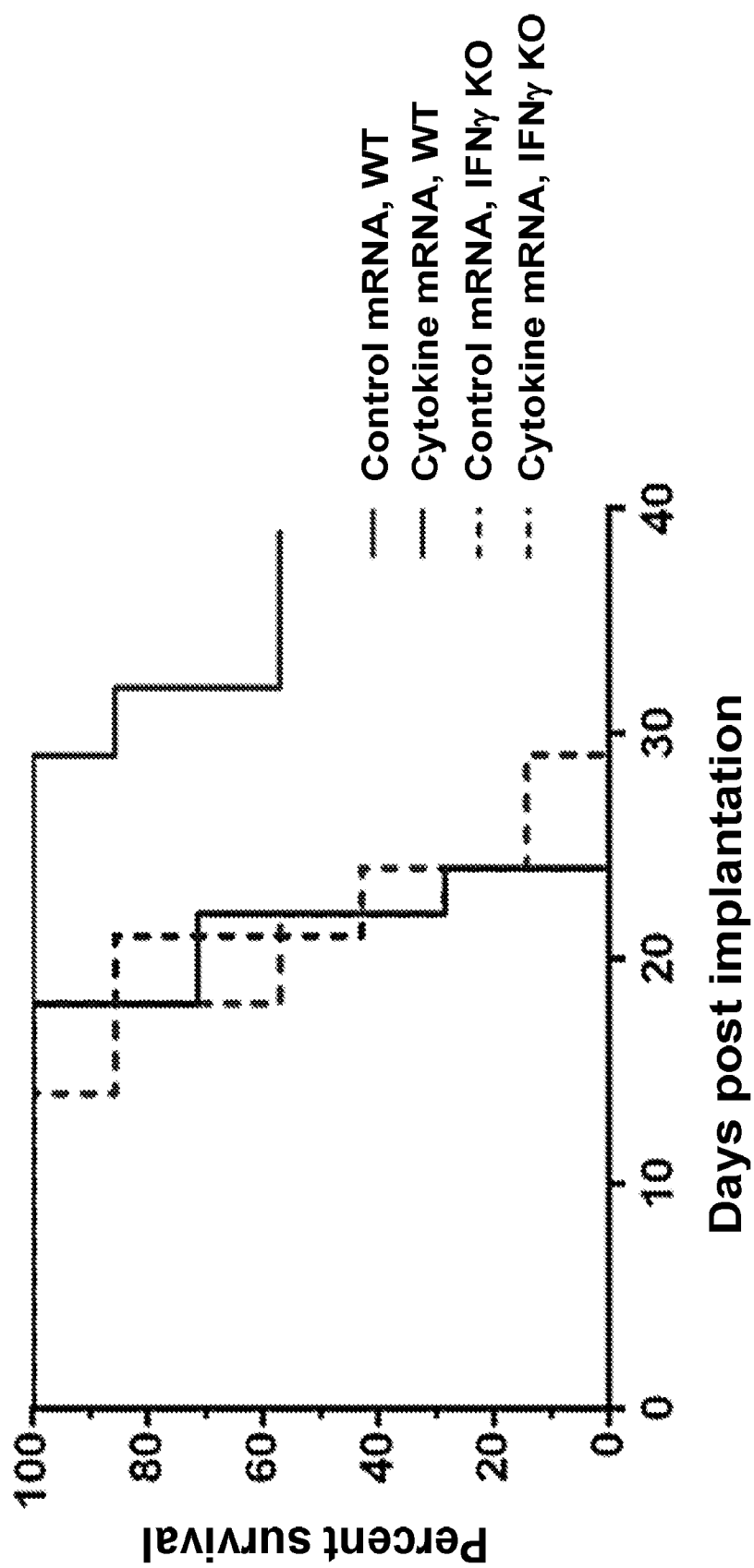


Fig. 35

2018-02-20_01183-0001-00PCT_Seq_List_ST25_FINAL_ST25.txt
SEQUENCE LISTING

<110> Sanofi
BioNTech RNA Pharmaceuticals GMBH

<120> Therapeutic RNA

<130> 01183-0001-00PCT

<150> US 62/464,981

<151> 2017-02-28

<150> US 62/597,527

<151> 2017-12-12

<150> EP 17306089.8

<151> 2017-08-23

<160> 88

<170> PatentIn version 3.5

<210> 1

<211> 52

<212> DNA

<213> Artificial sequence

<220>

<223> ModA 5' UTR

<400> 1

gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca cc

52

<210> 2

<211> 52

<212> RNA

<213> Artificial sequence

<220>

<223> ModA 5' UTR

<400> 2

gggcgaacua guauucuucu gguccccaca gacucagaga gaacccgcca cc

52

<210> 3

<211> 145

<212> DNA

<213> Artificial sequence

<220>

<223> ModB 5' UTR

<400> 3

ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60

tctacttcta ttgcagcaat ttaaattcatt tcttttaaag caaaagcaat tttctgaaaa 120

ttttcaccat ttacgaacga tagcc 145

<210> 4

<211> 145

<212> RNA

<213> Artificial sequence

<220>

<223> ModB 5' UTR

<400> 4

ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaUCAAGCAU 60

UCUACUUCUA uugcagcaau uuaaaUCAUU ucuuuuaaag caaaagcaau uuucugaaaa 120

uuuucaccau uuacgaacga uagcc 145

<210> 5

<211> 52

<212> DNA

<213> Artificial sequence

<220>

<223> Alternative Mod 5' UTR

<400> 5

agacgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca cc 52

<210> 6

<211> 52

<212> RNA

<213> Artificial sequence

<220>

<223> Alternative Mod 5' UTR

<400> 6

agacgaacua guauucuucu ggucgccaca gacucagaga gaacccgcca cc 52

<210> 7

<211> 427

<212> DNA

<213> Artificial sequence

<220>

<223> ModA/B 3' UTR

<400> 7

```

ctcgagctgg tactgcatgc acgcaatgct agctgcccct ttcccgtcct gggtagccccg      60
agtctcccc gacctcgggt cccaggtatg ctccacctc cacctgcccc actcaccacc      120
tctgctagtt ccagacacct cccaagcacg cagcaatgca gctcaaaacg cttagcctag      180
ccacaccccc acgggaaaca gcagtgatta accttttagca ataaacgaaa gttaactaa      240
gctatactaa cccaggggtt ggtcaatttc gtgccagcca caccgagacc tgggtccagag      300
tcgctagccg cgtcgctaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagca tatgactaaa      360
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      420
aaaaaaaaa                                     427

```

<210> 8

<211> 427

<212> RNA

<213> Artificial sequence

<220>

<223> ModA/B 3' UTR

<400> 8

```

cucgagcugg uacugcaugc acgcaaugcu agcugccccc uucccguccu gggtagccccg      60
agucuccccc gaccucgggu cccagguaug cuccaccuc caccugcccc acucaccacc      120
ucugcuaguu ccagacaccu cccaagcacg cagcaaugca gcucaaaacg cuuagccuag      180
ccacaccccc acgggaaaca gcagugauua accuuuagca auaaacgaaa guuuuacuaa      240
gcuauacuaa cccagggguu ggucauuuuc gugccagcca caccgagacc ugguccagag      300
ucgcuagccg cgucgcuaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagca uaugacuaaa      360
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      420
aaaaaaaaa                                     427

```

<210> 9

<211> 153

<212> PRT

<213> Homo sapiens

<400> 9

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
 1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
 20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
 35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
 50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
 65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
 85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
 100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
 115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
 130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
 145 150

<210> 10

<211> 465

<212> DNA

<213> Homo sapiens

<400> 10

atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaaacagt 60

```

gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat      120
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc      180
acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa      240
gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta      300
agaccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa      360
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga      420
tggattacct tttgtcaaag catcatctca aactgactt gatga                        465

```

```

<210> 11
<211> 465
<212> DNA
<213> Artificial sequence

```

```

<220>
<223> Human optimized IL-2

```

```

<400> 11
atgtacagaa tgcagctgct gtcttgcatt gctctttctc ttgtcttgt gacaaattct      60
gctccaacat cttcttcaac aaagaaaaca cagcttcagc ttgaacacct tcttcttgat      120
cttcagatga ttctgaatgg aatcaacaat tacaaaaatc caaaactgac aagaatgctg      180
acatttaaatt tttacatgcc aaagaaagca acagaactga aacaccttca gtgccttgaa      240
gaagaactga aacctctgga agaagtgtg aatctggctc agagcaaaaa ttttcacctg      300
agaccaagag atctgatcag caacatcaat gtgattgtgc tggaactgaa aggatctgaa      360
acaacattca tgtgtgaata tgctgatgaa acagcaacaa ttgtggaatt tctgaacaga      420
tggatcacat tttgccagtc aatcatttca aactgacat gatga                        465

```

```

<210> 12
<211> 465
<212> RNA
<213> Homo sapiens

```

```

<400> 12
auguacagga ugcaacuccu gucuugcauu gcacuaaguc uugcacuugu cacaaacagu      60
gcaccuacuu caaguucuac aaagaaaaca cagcuacaac uggagcauuu acugcuggau      120

```

```

uuacagauga uuuugaauagg aaauuaauaau uacaagaauuc ccaaacucac caggaugcuc      180
acauuuuagu uuuacaugcc caagaaggcc acagaacuga aacaucuua gugucuagaa      240
gaagaacuca aaccucugga ggaagugcua aaauuagcuc aaagcaaaaa cuuucacuua      300
agacccaggg acuuauucag caauaucaac guaauaguuc uggaacuaaa gggaucugaa      360
acaacauuca ugugugaaua ugcugaugag acagcaacca uuguagaauu ucugaacaga      420
uggauuaccu uuugucaaag caucaucuca acacugacuu gauga                        465

```

```

<210> 13
<211> 465
<212> RNA
<213> Artificial sequence

```

```

<220>
<223> Human optimized IL-2

```

```

<400> 13
auguacagaa ugcagcugcu gucuugcauu gcucuuucuc uugcucuugu gacaaaauuc      60
gcuccaacau cuucuuaac aaagaaaaca cagcuucagc uugaacaccu ucuucuugau      120
cuucagauga uucugaauagg aaucaacaau uacaaaaauc caaacugac aagaugcug      180
acauuuuuuu uuuacaugcc aaagaaagca acagaacuga aacaccuua gugccuugaa      240
gaagaacuga aaccucugga agaagugcug aaucuggcuc agagcaaaaa uuuucaccug      300
agaccaagag aucugaucag caacaucaau gugauugugc uggaacugaa aggaucugaa      360
acaacauuca ugugugaaua ugcugaugaa acagcaacaa uuguggaauu ucugaacaga      420
uggaucacau uuugccaguc aaucuuuua acacugacau gauga                        465

```

```

<210> 14
<211> 539
<212> PRT
<213> Artificial sequence

```

```

<220>
<223> Human IL-12sc

```

```

<400> 14

```

```

Met Cys His Gln Gln Leu Val Ile Ser Trp Phe Ser Leu Val Phe Leu
1          5          10         15

```

Ala Ser Pro Leu Val Ala Ile Trp Glu Leu Lys Lys Asp Val Tyr Val
20 25 30

Val Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu
35 40 45

Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln
50 55 60

Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
65 70 75 80

Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
85 90 95

Leu Ser His Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp
100 105 110

Ser Thr Asp Ile Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe
115 120 125

Leu Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp
130 135 140

Leu Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg
145 150 155 160

Gly Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser
165 170 175

Ala Glu Arg Val Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu
180 185 190

Cys Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile
195 200 205

Glu Val Met Val Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr
210 215 220

Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn
225 230 235 240

Leu Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp
245 250 255

Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr
260 265 270

Phe Cys Val Gln Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg
275 280 285

Val Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala
290 295 300

Ser Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser
305 310 315 320

Glu Trp Ala Ser Val Pro Cys Ser Gly Ser Ser Gly Gly Gly Gly Ser
325 330 335

Pro Gly Gly Gly Ser Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro
340 345 350

Gly Met Phe Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val
355 360 365

Ser Asn Met Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys
370 375 380

Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser
385 390 395 400

Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys
405 410 415

Leu Asn Ser Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala
420 425 430

Ser Arg Lys Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr
435 440 445

Glu Asp Leu Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys
450 455 460

Leu Leu Met Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu
465 470 475 480

Ala Val Ile Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr
485 490 495

Val Pro Gln Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys
500 505 510

Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr
515 520 525

Ile Asp Arg Val Met Ser Tyr Leu Asn Ala Ser
530 535

<210> 15

<211> 1623

<212> DNA

<213> Artificial sequence

<220>

<223> Human non-optimized IL-12sc

<400> 15

atgtgtcacc agcagttggt catctcttgg ttttccctgg tttttctggc atctcccctc	60
gtggccatat gggaactgaa gaaagatggt tatgtcgtag aattggattg gtatccggat	120
gcccctggag aaatggtggt cctcacctgt gacaccctg aagaagatgg tatcacctgg	180
accttggacc agagcagtga ggtcttaggc tctggcaaaa ccctgaccat ccaagtcaaa	240
gagtttggag atgctggcca gtacacctgt cacaaggag gcgagggttct aagccattcg	300
ctcctgctgc ttcacaaaaa ggaagatgga atttggtcca ctgatatttt aaaggaccag	360
aaagaacca aaaataagac ctttctaaga tgcgaggcca agaattattc tggacgtttc	420

```

acctgctggt ggctgacgac aatcagtact gatttgacat tcagtgtcaa aagcagcaga      480
gggtcttctg accccaagg ggtgacgtgc ggagctgcta cactctctgc agagagagtc      540
agaggggaca acaaggagta tgagtactca gtggagtgcc aggaggacag tgcctgcca      600
gctgctgagg agagtctgcc cattgaggtc atggtggatg ccgttcacaa gctcaagtat      660
gaaaactaca ccagcagctt cttcatcagg gacatcatca aacctgaccc acccaagaac      720
ttgcagctga agccattaaa gaattctcgg caggtggagg tcagctggga gtaccctgac      780
acctggagta ctccacattc ctacttctcc ctgacattct gcgttcaggt ccagggaag      840
agcaagagag aaaagaaaga tagagtcttc acggacaaga cctcagccac ggtcatctgc      900
cgcaaaaatg ccagcattag cgtgcgggcc caggaccgct actatagctc atcttgagc      960
gaatgggcat ctgtgccctg cagtggctct agcggagggg gaggctctcc tggcggggga     1020
tctagcagaa acctccccgt ggccactcca gaccaggaa tgttcccatg ccttcaccac     1080
tcccaaaacc tgctgagggc cgtcagcaac atgctccaga aggccagaca aactctagaa     1140
ttttaccctt gcacttctga ggaaattgat catgaagata tcacaaaaga taaaaccagc     1200
acagtggagg cctgtttacc attggaatta accaagaatg agagttgcct aaattccaga     1260
gagacctctt tcataactaa tgggagttgc ctggcctcca gaaagacctc ttttatgatg     1320
gccctgtgcc ttagtagtat ttatgaagac ttgaagatgt accaggtgga gttcaagacc     1380
atgaatgcaa agcttctgat ggatcctaag aggagatct ttctagatca aaacatgctg     1440
gcagttattg atgagctgat gcaggccctg aatttcaaca gtgagactgt gccacaaaaa     1500
tcctcccttg aagaaccgga tttttataaa actaaaatca agctctgcat acttcttcat     1560
gctttcagaa ttcgggcagt gactattgat agagtgatga gctatctgaa tgcttcctga     1620
tga                                                                           1623

```

<210> 16

<211> 1623

<212> DNA

<213> Artificial sequence

<220>

<223> Human optimized IL-12sc

<400> 16

atgtgtcacc agcagctggt gatctcatgg ttctccctgg tatttctggc atctcctctt	60
gtcgcaatct gggaactgaa gaaagacgtg tatgtcgttg agctcgactg gtatccggat	120
gcgcttggcg agatggtggt gctgacctgt gacaccccag aggaggatgg gatcacttgg	180
acccttgatc aatcctccga agtgctcggg tctggcaaga ctctgaccat acaagtgaaa	240
gagtttggcg atgccgggca gtacacttgc cataagggcg gagaagttct gtcccactca	300
ctgctgctgc tgcacaagaa agaggacgga atttggagta ccgatatcct gaaagatcag	360
aaagagccca agaacaaaac cttcttgctg tgcgaagcca agaactactc aggagattt	420
acttgttggt ggctgacgac gatcagcacc gatctgactt tctccgtgaa atcaagtagg	480
ggatcatctg accctcaagg agtcacatgt ggagcggcta ctctgagcgc tgaacgcgta	540
agaggggaca ataaggagta cgagtatagc gttgagtgcc aagaggatag cgcattgcccc	600
gccgccgaag aatcattgcc cattgaagtg atggtggatg ctgtacacaa gctgaagtat	660
gagaactaca caagctcctt cttcatccgt gacatcatca aaccagatcc tcctaagaac	720
ctccagctta aacctctgaa gaactctaga caggtggaag tgtcttggga gtatcccgac	780
acctggtcta caccacattc ctacttcagt ctcacattct gcgttcaggt acagggcaag	840
tcmetaagg agaagaagga tcgggtcttt acagataaaa caagtgccac cgttatatgc	900
cggagaatg cctctatttc tgtgcgtgcg caggacagat actatagcag ctcttggagt	960
gaatgggcca gtgtcccatg ttcagggtca tccggtggtg gcggcagccc cggaggcgggt	1020
agctccagaa atctccctgt ggctacacct gatccaggca tgtttccctg tttgcacat	1080
agccaaaacc tcctgagagc agtcagcaac atgctccaga aagctagaca aacactggaa	1140
ttctacccat gcacctccga ggaaatagat cacgaggata tcactaagga caaaacaagc	1200
actgtcgaag catgccttcc cttggaactg acaaagaacg agagttgcct taattcaaga	1260
gaaacatctt tcattacaaa cggtagctgc ttggcaagca gaaaaacatc ttttatgatg	1320
gccctttgtc tgagcagtat ttatgaggat ctcaaaatgt accaggtgga gttaagacc	1380
atgaatgcca agctgctgat ggacccaaag agacagattt tcctcgatca gaatatgctg	1440
gctgtgattg atgaactgat gcaggccttg aatttcaaca gcgaaaccgt tccccagaaa	1500
agcagtcttg aagaacctga cttttataag accaagatca aactgtgtat tctcctgcat	1560

gccttttagaa tcagagcagt cactatagat agagtgatgt cctacctgaa tgcttcctga 1620
tga 1623

<210> 17
<211> 1623
<212> RNA
<213> Artificial sequence

<220>
<223> Human non-optimized IL-12sc

<400> 17
augugucacc agcaguuggu caucucuugg uuuucccugg uuuuucuggc aucuccccuc 60
guggccauau gggaacugaa gaaagauguu uaugucguag aaugggauug guauccggau 120
gccccuggag aaugguggu ccucaccugu gacaccccug aagaagaugg uauccaccug 180
accuuggacc agagcaguga ggucuaggc ucuggcaaaa ccugaccuau ccaagucaaa 240
gaguuggag augcuggcca guacaccugu caciaaggag gcgagguucu aagccauucg 300
cuccugcugc uucacaaaaa ggaagaugga auuuggucca cugauuuuuu aaaggaccag 360
aaagaacca aaaauaagac cuuucuaaga ugcgaggcca agaauuauuc uggacguuuc 420
accugcuggu ggcugacgac aaucaguacu gauuugacau ucagugucua aagcagcaga 480
gggucuucug accccaagg ggugacgugc ggagcugcua cacucucugc agagagaguc 540
agaggggaca acaaggagua ugaguacua guggagugcc aggaggacag ugccugccca 600
gcugcugagg agagucugcc cauugagguc augguggaug ccguucacaa gcucaaguau 660
gaaaacuaca ccagcagcuu cuucaucagg gacaucaua aaccugaccc acccaagaac 720
uugcagcuga agccauuaaa gaauucucgg cagguggagg ucagcuggga guaccugac 780
accuggagua cuccacauuc cuacuuccc cugacauucu gcguucaggu ccagggcaag 840
agcaagagag aaaagaaaga uagagucuuc acggacaaga ccucagccac ggucaucugc 900
cgcaaaaug ccagcauuag cgugcgggcc caggaccgcu acuaauagcuc aucuuggagc 960
gaaugggcau cugugcccug caguggcucu agcggagggg gaggcucucc uggcggggga 1020
ucuagcagaa accuccccgu ggccacucca gaccaggaa uguucccaug ccuucaccac 1080
ucccaaaacc ugcugagggc cgucagcaac augcuccaga aggccagaca aacucuagaa 1140

```

uuuuacccuu gcacuucuga ggaaauugau caugaagaua ucacaaaaga uaaaaccagc      1200
acaguggagg ccuguuuacc auuggaauua accaagaauug agaguugccu aaauuccaga      1260
gagaccucuu ucauaacuaa ugaggaguugc cuggccucca gaaagaccuc uuuuauaug      1320
gcccuugucc uuaguaguau uuaugaagac uugaagaugu accaggugga guucaagacc      1380
augaauugcaa agcuucugau ggauccuaag aggcagaucu uucuagauga aaacaugcug      1440
gcaguuauug augagcugau gcaggcccug aauuucaaca gugagacugu gccacaaaaa      1500
uccucccuug aagaaccgga uuuuuauaaa acuaaaaauca agcucugcau acuucuucan      1560
gcuuucagaa uucgggcagu gacuauugau agagugauga gcuauugaa ugcuccuga      1620
uga                                                                           1623

```

```

<210> 18
<211> 1623
<212> RNA
<213> Artificial sequence

```

```

<220>
<223> Human optimized IL-12sc

```

```

<400> 18
augugcacc agcagcuggu gaucucaugg uucucccugg uauuucuggc aucuccucuu      60
gucgcaaucu gggaacugaa gaaagacgug uaugucguug agcucgacug guauccggau      120
gcgccuggcg agaugguggu gcugaccugu gacaccccag aggaggauug gaucacuugg      180
acccuugauc aauccuccga agugcucggg ucuggcaaga cucugaccan acaagugaaa      240
gaguuuaggc augccgggca guacacuugc cauaagggcg gagaaguucu gucccacuca      300
cugcugcugc ugcacaagaa agaggacgga auuuggagua ccgauauccu gaaagaucag      360
aaagagccca agaacaaaac cuucuugcgg ugcgaagcca agaacuacuc agggagauuu      420
acuuguuggu ggcugacgac gaucagcacc gaucugacuu ucuccgugaa aucaaguagg      480
ggaucaucug acccucaagg agucacaugu ggagcggcua cucugagcgc ugaacgcgua      540
agaggggaca auaaggagua cgaguauagc guugagugcc aagaggauag cgcaugcccc      600
gccgccgaag aaucanugcc cauugaagug augguggaug cuguacacaa gcugaaguau      660
gagaacuaca caagcuccuu cuucauccgu gacaucan aaccagauc uccuaagaac      720

```

cuccagcuua aaccucugaa gaacucuaga cagguggaag ugucuuggga guaucccgac	780
accuggucua caccacauuc cuacuucagu cucacauucu gcguucaggu acagggcaag	840
uccaaaaggg agaagaagga ucgggucuuu acagauaaaa caagugccac cguuauaugc	900
cggaagaaug ccucuauuuc ugugcgugcg caggacagau acuauagcag cucuuggagu	960
gaaugggcca guguccaug uucaggguca uccgguggug gcggcagccc cggaggcggu	1020
agcuccagaa aucucccugu ggcuacaccu gauccaggca uguuucccug uuugcaccu	1080
agccaaaacc uccugagagc agucagcaac augcuccaga aagcuagaca aacacuggaa	1140
uucuacccau gcaccuccga ggaaauagau cacgaggaua ucacuaagga caaaacaagc	1200
acugucgaag caugccuucc cuuggaacug acaaagaacg agaguugccu uaauucaaga	1260
gaaacauuu ucauuacaaa cgguagcugc uuggcaagca gaaaaacauc uuuuauaug	1320
gcccuuuguc ugagcaguau uuaugaggau cucaaaaugu accaggugga guuuagacc	1380
augaaucca agcugcugau ggacccaaag agacagauuu uccucgauca gaauaugcug	1440
gcugugauug augaacugau gcaggccuug aauuucaaca gcgaaaccgu uccccagaaa	1500
agcagucuug aagaaccuga cuuuuauaag accaagauca aacuguguau ucuccugcau	1560
gccuuuagaa ucagagcagu cacuauagau agagugaugu ccuaccugaa ugcuuuccga	1620
uga	1623

<210> 19
 <211> 188
 <212> PRT
 <213> Homo sapiens

<400> 19

Met	Ala	Leu	Thr	Phe	Ala	Leu	Leu	Val	Ala	Leu	Leu	Val	Leu	Ser	Cys
1				5					10					15	

Lys	Ser	Ser	Cys	Ser	Val	Gly	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu
			20					25					30		

Gly	Ser	Arg	Arg	Thr	Leu	Met	Leu	Leu	Ala	Gln	Met	Arg	Arg	Ile	Ser
		35					40					45			

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
 50 55 60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
 65 70 75 80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
 85 90 95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
 100 105 110

Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
 115 120 125

Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
 130 135 140

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
 145 150 155 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
 165 170 175

Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
 180 185

<210> 20
 <211> 570
 <212> DNA
 <213> Homo sapiens

<400> 20
 atggccttga cctttgcttt actggtggcc ctcttggtgc tcagctgcaa gtcaagctgc 60
 tctgtgggct gtgatctgcc tcaaaccac agcctgggta gcaggaggac cttgatgctc 120
 ctggcacaga tgaggagaat ctctcttttc tcctgcttga aggacagaca tgactttgga 180
 tttccccagg aggagtttgg caaccagttc caaaaggctg aaaccatccc tgtcctccat 240
 gagatgatcc agcagatctt caaccttttc agcacaaagg actcatctgc tgcttgggat 300

gagaccctcc tagacaaatt ctacactgaa ctctaccagc agctgaatga cctggaagcc 360
 tgtgtgatac aggggggtggg ggtgacagag actcccctga tgaaggagga ctccattctg 420
 gctgtgagga aatacttcca aagaatcact ctctatctga aagagaagaa atacagccct 480
 tgtgcctggg aggttgtcag agcagaaatc atgagatcct tttctttgtc aacaaacttg 540
 caagaaagtt taagaagtaa ggaatgatga 570

<210> 21
 <211> 570
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Human optimized IFN-alpha-2b

<400> 21
 atggccctga cttttgccct tctcgtggct ttgttggtgc tgagttgcaa atcttcctgt 60
 agtgtcggat gtgatctgcc tcaaaccac agtctgggat ctaggagaac actgatgctg 120
 ttggcacaga tgaggagaat tagcctcttt tcctgcctga aggatagaca tgacttcggc 180
 tttccccaag aggagtttgg caatcagttc cagaaagcgg aaacgattcc cgttctgcac 240
 gagatgatcc agcagatcct caacctcttt tcaaccaaag acagctcagc agcctgggat 300
 gagacactgc tggacaaatt ctacacagaa ctgtatcagc agcttaacga tctggaggca 360
 tgcgtgatcc aaggggttgg tgtgactgaa actccgctta tgaaggagga ctccattctg 420
 gctgtacgga agtacttcca gagaataacc ctctatctga aggagaagaa gtactcacca 480
 tgtgcttggg aagtcgtgag agccgaaatc atgagatcct tcagccttag caccaatctc 540
 caggaatctc tgagaagcaa agagtgatga 570

<210> 22
 <211> 570
 <212> RNA
 <213> Homo sapiens

<400> 22
 auggccuuga ccuuugcuuu acugguggcc cuccuggugc ucagcugcaa gucaagcugc 60
 ucugugggcu gugaucugcc ucaaaccac agccugggua gcaggaggac cuugaugcuc 120
 cuggcacaga ugaggagaau cucucuuuuc uccugcuuga aggacagaca ugacuuugga 180

uuuucccagg aggaguuugg caaccaguuc caaaaggcug aaaccauucc uguccuccau	240
gagaugaucc agcagaucuu caaccuuuuc agcacaaagg acucaucugc ugcuuuggau	300
gagaccucc uagacaaau cuacacugaa cucuaccagc agcugaauga ccuggaagcc	360
ugugugauac aggggguggg ggugacagag acuccccuga ugaaggagga cuccauucug	420
gcugugagga aaacuucca aagaucacu cucuauauga aagagaagaa auacagccu	480
ugugccuggg agguugucag agcagaauc augagaucuu uuucuuuguc aacaaacuug	540
caagaaaguu uaagaagua ggaugauga	570

<210> 23
 <211> 570
 <212> RNA
 <213> Artificial sequence

<220>
 <223> Human optimized IFN-alpha-2b

<400> 23 auggcccuga cuuuugccu ucucguggcu uuguuggugc ugaguugcaa aucuuccugu	60
agugucggau gugaucugcc ucaaaccac agucugggau cuaggagaac acugaugcug	120
uuggcacaga ugaggagaau uagccucuuu uccugccuga aggauagaca ugacuucggc	180
uuuccccaag aggaguuugg caaucaguuc cagaaagcgg aaacgauucc cguucugcac	240
gagaugaucc agcagaucuu caaccucuuu ucaaccaaag acagcucagc agccugggau	300
gagacacugc uggacaaau cuacacagaa cuguauagc agcuuaacga ucuggaggca	360
ugcgugauc aagggguugg ugugacugaa acuccguua ugaaggagga cuccauucug	420
gcuguacgga aguacuucca gagaauaacc cucuauauga aggagaagaa guacucacca	480
ugugcuuggg aagucgugag agccgaauc augagaucuu ucagccuag caccaaucuc	540
caggaaucuc ugagaagcaa agagugauga	570

<210> 24
 <211> 241
 <212> PRT
 <213> Artificial sequence

<220>

<223> Human IL-15 sushi

<400> 24

Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala
 1 5 10 15

Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly Ile Thr
 20 25 30

Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
 35 40 45

Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
 50 55 60

Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala
 65 70 75 80

Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp
 85 90 95

Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Gly Gly Gly Ser Gly
 100 105 110

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Gln Asn
 115 120 125

Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln
 130 135 140

Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro
 145 150 155 160

Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val
 165 170 175

Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn
 180 185 190

Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr
 195 200 205

Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys
 210 215 220

Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr
 225 230 235 240

Ser

<210> 25
 <211> 729
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Human IL-15 sushi

<400> 25
 atggcccgcg ggcgggcgcg cggctgccgg accctcggtc tcccggcgct gctactgctg 60
 ctgctgctcc ggccgccggc gacgcggggc atcacgtgcc ctccccccat gtccgtggaa 120
 cacgcagaca tctgggtcaa gagctacagc ttgtactcca gggagcggta catttgtaac 180
 tctggtttca agcgtaaagc cggcacgtcc agcctgacgg agtgcgtgtt gaacaaggcc 240
 acgaatgtcg cccactggac aacccccagt ctcaaagca ttagagaccc tgccctggtt 300
 caccaaaggc cagcgccacc cgggggagga tctggcggcg gtgggtctgg cgggggatct 360
 ggcggaggag gaagcttaca gaactgggtg aatgtaataa gtgatttgaa aaaaattgaa 420
 gatcttattc aatctatgca tattgatgct actttatata cggaagtga tgttcacccc 480
 agttgcaaag taacagcaat gaagtgcttt ctcttgagat tacaagttat ttcacttgag 540
 tccggagatg caagtattca tgatacagta gaaaatctga tcaccttagc aaacaacagt 600
 ttgtcttcta atgggaatgt aacagaatct ggatgcaaag aatgtgagga actggaggaa 660
 aaaaatatta aagaatTTTT gcagagTTTT gtacatatgt tccaaatgtt catcaacact 720
 tcttgatga 729

<210> 26
 <211> 729
 <212> RNA
 <213> Artificial sequence

<220>
 <223> Human IL-15 sushi

<400> 26
 auggccccgc ggcgggcgcg cggcugccgg acccucgguc ucccggcgcu gcuacugcug 60
 cugcugcuucc ggccgccggc gacgcggggc aucacugucc cuccccccau guccguggaa 120
 cacgcagaca ucugggucaa gagcuacagc uuguacucca gggagcggua cauuuguaac 180
 ucugguuuca agcguaaagc cggcacgucc agccugacgg agugcguguu gaacaaggcc 240
 acgaaugucg cccacuggac aacccccagu cucaaauca uuagagaccc ugcccugguu 300
 caccaaaggc cagcgccacc cgggggagga ucuggcggcg gugggucugg cgggggaucu 360
 ggcggaggag gaagcuuaca gaacugggug aauguaauaa gugauuugaa aaaaauugaa 420
 gaucuuauuc aaucuaugca uauugaugcu acuuuauaua cggaaguga uguucacccc 480
 aguugcaaag uaacagcaau gaagugcuuu cucuuggagu uacaaguauu uucacuugag 540
 uccggagaug caaguauuca ugauacagua gaaaauauga ucauccuagc aaacaacagu 600
 uugucuucua augggaugu aacagaauca ggaugcaaag aaugugagga acuggaggaa 660
 aaaaauauua aagaauuuuu gcagaguuuu guacauauug uccaaauguu caucaacacu 720
 ucuugauga 729

<210> 27
 <211> 144
 <212> PRT
 <213> Homo sapiens

<400> 27

Met Trp Leu Gln Ser Leu Leu Leu Leu Gly Thr Val Ala Cys Ser Ile
 1 5 10 15

Ser Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His
 20 25 30

Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp

35

40

45

Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe
 50 55 60

Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys
 65 70 75 80

Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met
 85 90 95

Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser
 100 105 110

Cys Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys
 115 120 125

Asp Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu
 130 135 140

<210> 28

<211> 438

<212> DNA

<213> Homo sapiens

<400> 28

atgtggctcc agagcctgct gctcttgggc actgtggcct gctccatctc tgcacccgcc 60

cgctcgccca gccccagcac gcagccctgg gagcatgtga atgccatcca ggaggcccgg 120

cgtctgctga acctgagtag agacactgct gctgagatga atgaaacagt agaagtcatc 180

tcagaaatgt ttgacctcca ggagccgacc tgcctacaga cccgcctgga gctgtacaag 240

cagggcctgc ggggcagcct caccaagctc aagggcccct tgaccatgat ggccagccac 300

tacaagcagc actgccctcc aaccccggaa acttcctgtg caaccagat tatcaccttt 360

gaaagtttca aagagaacct gaaggacttt ctgcttgtca tcccctttga ctgctgggag 420

ccagtccagg agtgatga 438

<210> 29

<211> 438

<212> RNA

<213> Homo sapiens

<400> 29

```

auguggcucc agagccugcu gcucuugggc acuguggccu gcuccaucuc ugcacccgcc      60
cgucgcccc a gcccagcac gcagcccugg gagcauguga augccaacca ggaggcccgg      120
cgucugcuga accugaguag agacacugcu gcugagauga augaaacagu agaagucauc      180
ucagaaaugu uugaccucca ggagccgacc ugccuacaga cccgccugga gcuguacaag      240
cagggccugc ggggcagccu caccaagcuc aagggccccc ugaccaugau ggccagccac      300
uacaagcagc acugcccucc aaccccggaa acuuccugug caaccagau uaucacuuu      360
gaaaguuuca aagagaaccu gaaggacuuu cugcuuguca ucccccuuuga cugcugggag      420
ccaguccagg agugauga                                         438
    
```

<210> 30

<211> 161

<212> PRT

<213> Artificial sequence

<220>

<223> ModA IL-2 (human IL-2 in combination with a mouse optimized secretion sequence)

<400> 30

```

Met Arg Val Thr Ala Pro Arg Thr Leu Ile Leu Leu Leu Ser Gly Ala
1           5           10           15
    
```

```

Leu Ala Leu Thr Glu Thr Trp Ala Gly Ser Gly Ser Ala Pro Thr Ser
          20           25           30
    
```

```

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
          35           40           45
    
```

```

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
          50           55           60
    
```

```

Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu
65           70           75           80
    
```

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
85 90 95

Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
100 105 110

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
115 120 125

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
130 135 140

Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu
145 150 155 160

Thr

<210> 31
<211> 959
<212> DNA
<213> Artificial sequence

<220>
<223> ModA IL-2 (5'UTR-CDS-3'UTR)

<400> 31
gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatgagagt 60
gaccgcccc agaaccctga tcctgctgct gtctggcgcc ctggccctga cagagacatg 120
ggccggaagc ggatccgcac ctacttcaag ttctacaaag aaaacacagc tacaactgga 180
gcatttactt ctggatttac agatgatttt gaatggaatt aataattaca agaatcccaa 240
actcaccagg atgctcatat ttaagtttta catgcccag aaggccacag aactgaaaca 300
tcttcagtgt ctagaagaag aactcaaacc tctggaggaa gtgctaaatt tagctcaaag 360
caaaaacttt cacttaagac ccagggactt aatcagcaat atcaacgtaa tagttctgga 420
actaaaggga tctgaaacaa cattcatgtg tgaatatgct gatgagacag caaccattgt 480
agaatttctg aacagatgga ttaccttttg tcaaagcatc atctcaacac tgacttgact 540
cgagagctcg ctttcttgct gtccaatttc tattaaggt tcctttgttc cctaagtcca 600

actactaaac tgggggatat tatgaagggc cttgagcatc tggattctgc ctaataaaaa	660
acatttattt tcattgctgc gtcgagagct cgctttcttg ctgtccaatt tctattaaag	720
gttcctttgt tccctaagtc caactactaa actgggggat attatgaagg gccttgagca	780
tctggattct gcctaataaa aaacatttat tttcattgct gcgtcgagac ctggtccaga	840
gtcgctagca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa catatgacta aaaaaaaaaa	900
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	959

<210> 32
 <211> 959
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModA IL-2

<400> 32 gggcgaacua guauucuucu ggucgccaca gacucagaga gaacccgcca ccaugagagu	60
gaccgcccc agaaccuga uccugcugcu gucuggcgcc cuggcccuga cagagacaug	120
ggccggaagc ggauccgcac cuacucaag uucuacaaag aaaacacagc uacaacugga	180
gcauuuacuu cuggauuuac agaugauuuu gaauggaauu aaauuuuaca agaaucccaa	240
acucaccagg augcucacau uuaaguuuuu caugcccaag aaggccacag aacugaaaca	300
ucuucagugu cuagaagaag aacucaaacc ucuggaggaa gugcuaaaau uagcucaaag	360
caaaaacuuu cacuuagac ccagggacuu aaucagcaau aucaacguua uaguucugga	420
acuaaaggga ucugaaacaa cauucaugug ugaauaugcu gaugagacag caaccauugu	480
agaauuucug aacagaugga uuaccuuuug ucaaagcauc aucucaacac ugacuugacu	540
cgagagcucg cuuucuugcu guccaauuuc uauuaaaggu uccuuuguuc ccuaagucca	600
acuacuaaac ugggggauau uaugaagggc cuugagcauc uggauucugc cuaauaaaaa	660
acauuuuuuu ucauugcugc gucgagagcu cgcuuucuug cuguccaaau ucuauuaaag	720
guuccuuugu ucccuaaguc caacuacuaa acugggggau auuaugaagg gccuugagca	780
ucuggauucu gccuaauaaa aaacauuuau uuucauugcu gcgucgagac cugguccaga	840
gucgcuagca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa cauaugacua aaaaaaaaaa	900

aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 959

<210> 33
 <211> 161
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModB IL-2

<400> 33

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala
 1 5 10 15

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Ala Pro Thr Ser
 20 25 30

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
 35 40 45

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
 50 55 60

Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu
 65 70 75 80

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
 85 90 95

Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
 100 105 110

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
 115 120 125

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
 130 135 140

Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu
 145 150 155 160

Thr

<210> 34
 <211> 1039
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModB IL-2 (5'UTR-CDS-3'UTR)

<400> 34
 ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60
 tctacttcta ttgcagcaat ttaaatacatt tcttttaaaag caaaagcaat tttctgaaaa 120
 ttttcaccat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct 180
 gctggccgct gccctggccc ctacacagac aagagctgga cctggatccg cacctacttc 240
 aagttctaca aagaaaacac agctacaact ggagcattta cttctggatt tacagatgat 300
 tttgaatgga attaataatt acaagaatcc caaactcacc aggatgctca catttaagtt 360
 ttacatgccc aagaaggcca cagaactgaa acatcttcag tgtctagaag aagaactcaa 420
 acctctggag gaagtgctaa atttagctca aagcaaaaac tttcacttaa gaccagggga 480
 cttaatcagc aatatcaacg taatagttct ggaactaaag ggatctgaaa caacattcat 540
 gtgtgaatat gctgatgaga cagcaacat tgtagaattt ctgaacagat ggattacctt 600
 ttgtcaaagc atcatctcaa cactgacttg actcgacgtc ctggtactgc atgcacgcaa 660
 tgctagctgc ccctttcccg tcctgggtac ccgaggtctc ccccgacctc ggggtcccagg 720
 tatgctccca cctccacctg cccactcac cacctctgct agttccagac acctcccaag 780
 cacgcagcaa tgcagctcaa aacgcttagc ctagccacac cccacggga aacagcagtg 840
 attaaccttt agcaataaac gaaagtttaa ctaagctata ctaaccccag ggttggtcaa 900
 tttcgtgcca gccacacct cgagctagca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 960
 catatgacta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
 aaaaaaaaaa aaaaaaaaaa 1039

<210> 35
 <211> 1039
 <212> RNA
 <213> Artificial sequence

 <220>
 <223> ModB IL-2

 <400> 35
 ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaucaagcau 60
 ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa 120
 uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu 180
 gcuggccgcu gcccgggccc cuacacagac aagagcugga ccuggaucg caccuacuuc 240
 aaguucuaca aagaaaacac agcuacaacu ggagcauuua cuucuggauu uacagaugau 300
 uuugaaugga auuaauauu acaagaaucc caaacucacc aggaugcuca cauuuaaguu 360
 uuacaugccc aagaaggcca cagaacugaa acaucuucag ugucuagaag aagaacucua 420
 accucuggag gaagugcuua auuuagcuca aagcaaaaac uuucacuuua gaccagggga 480
 cuuaaucagc aaaucaacg uaauaguucu ggaacuaaag ggaucugaaa caacauucau 540
 gugugaauau gcugaugaga cagcaaccau uguagaauuu cugaacagau ggauuaccuu 600
 uuugcaaagc aucaucuaa cacugacuug acucgacguc cugguacugc augcacgcaa 660
 ugcuagcugc cccuuucccg uccuggguac cccgagucuc ccccgaccuc gggucccagg 720
 uaugcuccca ccuccaccug ccccacucac caccucugcu aguuccagac accucccaag 780
 cacgcagcaa ugcagcucaa aacgcuuagc cuagccacac cccacggga aacagcagug 840
 auuaaccuuu agcaauaaac gaaaguuuaa cuaagcuaua cuaaccccag gguuggucaa 900
 uuucgugcca gccacacccu cgagcuagca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 960
 cauugacua aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
 aaaaaaaaaa aaaaaaaaaa 1039

<210> 36
 <211> 544
 <212> PRT
 <213> Artificial sequence

<220>

<223> ModA murine IL-12

<400> 36

Met Arg Val Thr Ala Pro Arg Thr Leu Ile Leu Leu Leu Ser Gly Ala
 1 5 10 15

Leu Ala Leu Thr Glu Thr Trp Ala Gly Ser Gly Ser Met Trp Glu Leu
 20 25 30

Glu Lys Asp Val Tyr Val Val Glu Val Asp Trp Thr Pro Asp Ala Pro
 35 40 45

Gly Glu Thr Val Asn Leu Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile
 50 55 60

Thr Trp Thr Ser Asp Gln Arg His Gly Val Ile Gly Ser Gly Lys Thr
 65 70 75 80

Leu Thr Ile Thr Val Lys Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys
 85 90 95

His Lys Gly Gly Glu Thr Leu Ser His Ser His Leu Leu Leu His Lys
 100 105 110

Lys Glu Asn Gly Ile Trp Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn
 115 120 125

Lys Thr Phe Leu Lys Cys Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr
 130 135 140

Cys Ser Trp Leu Val Gln Arg Asn Met Asp Leu Lys Phe Asn Ile Lys
 145 150 155 160

Ser Ser Ser Ser Ser Pro Asp Ser Arg Ala Val Thr Cys Gly Met Ala
 165 170 175

Ser Leu Ser Ala Glu Lys Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys
 180 185 190

Tyr Ser Val Ser Cys Gln Glu Asp Val Thr Cys Pro Thr Ala Glu Glu
 195 200 205

Thr Leu Pro Ile Glu Leu Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr
 210 215 220

Glu Asn Tyr Ser Thr Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp
 225 230 235 240

Pro Pro Lys Asn Leu Gln Met Lys Pro Leu Lys Asn Ser Gln Val Glu
 245 250 255

Val Ser Trp Glu Tyr Pro Asp Ser Trp Ser Thr Pro His Ser Tyr Phe
 260 265 270

Ser Leu Lys Phe Phe Val Arg Ile Gln Arg Lys Lys Glu Lys Met Lys
 275 280 285

Glu Thr Glu Glu Gly Cys Asn Gln Lys Gly Ala Phe Leu Val Glu Lys
 290 295 300

Thr Ser Thr Glu Val Gln Cys Lys Gly Gly Asn Val Cys Val Gln Ala
 305 310 315 320

Gln Asp Arg Tyr Tyr Asn Ser Ser Cys Ser Lys Trp Ala Cys Val Pro
 325 330 335

Cys Arg Val Arg Ser Val Pro Gly Val Gly Val Pro Gly Val Gly Arg
 340 345 350

Val Ile Pro Val Ser Gly Pro Ala Arg Cys Leu Ser Gln Ser Arg Asn
 355 360 365

Leu Leu Lys Thr Thr Asp Asp Met Val Lys Thr Ala Arg Glu Lys Leu
 370 375 380

Lys His Tyr Ser Cys Thr Ala Glu Asp Ile Asp His Glu Asp Ile Thr
 385 390 395 400

Arg Asp Gln Thr Ser Thr Leu Lys Thr Cys Leu Pro Leu Glu Leu His
 405 410 415

Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu Thr Ser Ser Thr Thr Arg
 420 425 430

Gly Ser Cys Leu Pro Pro Gln Lys Thr Ser Leu Met Met Thr Leu Cys
 435 440 445

Leu Gly Ser Ile Tyr Glu Asp Leu Lys Met Tyr Gln Thr Glu Phe Gln
 450 455 460

Ala Ile Asn Ala Ala Leu Gln Asn His Asn His Gln Gln Ile Ile Leu
 465 470 475 480

Asp Lys Gly Met Leu Val Ala Ile Asp Glu Leu Met Gln Ser Leu Asn
 485 490 495

His Asn Gly Glu Thr Leu Arg Gln Lys Pro Pro Val Gly Glu Ala Asp
 500 505 510

Pro Tyr Arg Val Lys Met Lys Leu Cys Ile Leu Leu His Ala Phe Ser
 515 520 525

Thr Arg Val Val Thr Ile Asn Arg Val Met Gly Tyr Leu Ser Ser Ala
 530 535 540

<210> 37
 <211> 2111
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModA murine IL-12 (5'UTR-CDS-3'UTR)

<400> 37
 gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatgagagt 60
 gaccgcccc agaaccctga tcctgctgct gtctggcgcc ctggccctga cagagacatg 120
 ggccggaagc ggatccatgt gggagctgga gaaagacgtt tatgtttag aggtggactg 180
 gactcccgat gccctggag aaacagtga cctcacctgt gacacgcctg aagaagatga 240

catcacctgg acctcagacc agagacatgg agtcataggc tctggaaaaga ccctgaccat	300
cactgtcaaa gagtttctag atgctggcca gtacacctgc cacaaaggag gcgagactct	360
gagccactca catctgctgc tccacaagaa ggaaaatgga atttggtcca ctgaaatttt	420
aaaaaatctt aaaaacaaga ctttcttgaa gtgtgaagca ccaaattact ccggacgggt	480
cacgtgctca tggctgggtgc aaagaaacat ggacttgaag ttcaacatca agagcagtag	540
cagttcccct gactctcggg cagtgcacatg tggaatggcg tctctgtctg cagagaaggt	600
cacactggac caaagggact atgagaagta ttcagtgtcc tgccaggagg atgtcacctg	660
cccaactgcc gaggagaccc tgcccattga actggcggtg gaagcacggc agcagaataa	720
atatgagaac tacagcacca gcttcttcat caggacatc atcaaaccag acccgcccaa	780
gaacttgacg atgaagcctt tgaagaactc acaggtggag gtcagctggg agtaccctga	840
ctcctggagc actccccatt cctacttctc cctcaagttc tttgttcgaa tccagcgcaa	900
gaaagaaaag atgaaggaga cagaggaggg gtgtaaccag aaagggtcgt tcctcgtaga	960
gaagacatct accgaagtcc aatgcaaagg cggaatgtc tgcgtgcaag ctcaggatcg	1020
ctattacaat tcctcatgca gcaagtgggc atgtgttccc tgcagagtcc gatcggttcc	1080
tggagtaggg gtacctggag tgggcagggt cataccggtc tctggacctg ccagggtgtct	1140
tagccagtcc cgaaacctgc tgaagaccac agatgacatg gtgaagacgg ccagagaaaa	1200
gctgaaacat tattcctgca ctgctgaaga catcgatcat gaagacatca cacgggacca	1260
aaccagcaca ttgaagacct gtttaccact ggaactacac aagaacgaga gttgcctggc	1320
tactagagag acttcttcca caacaagagg gagctgcctg ccccccacaga agacgtcttt	1380
gatgatgacc ctgtgccttg gtagcatcta tgaggacttg aagatgtacc agacagagtt	1440
ccaggccatc aacgcagcac ttcagaatca caaccatcag cagatcattc tagacaaggg	1500
catgctgggt gccatcgatg agctgatgca gtctctgaat cataatggcg agactctgcg	1560
ccagaaacct cctgtgggag aagcagaccc ttacagagtg aaaatgaagc tctgcatcct	1620
gcttcacgcc ttcagcaccg gcgtcgtgac catcaacagg gtgatgggct atctgtccag	1680
cgcctaatag ctcgagagct cgctttcttg ctgtccaatt tctattaaag gttcctttgt	1740
tccctaagtc caactactaa actgggggat attatgaagg gccttgagca tctggattct	1800

gcctaataaa aaacatttat tttcattgct gcgtcgagag ctcgctttct tgctgtccaa	1860
tttctattaa aggttccttt gttccctaag tccaactact aaactggggg atattatgaa	1920
gggccttgag catctggatt ctgcctaata aaaaacattt attttcattg ctgcgtcgag	1980
acctggtcca gagtcgctag caaaaaaaaaa aaaaaaaaaa aaaaaaaaaa agcatatgac	2040
taaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	2100
aaaaaaaaaa a	2111

<210> 38

<211> 2111

<212> RNA

<213> Artificial sequence

<220>

<223> ModA murine IL-12

<400> 38

gggcgaacua guauucuucu ggucgccaca gacucagaga gaacccgcca ccaugagagu	60
gaccgcccc agaaccuga uccugcugcu gucuggcgcc cuggcccuga cagagacaug	120
ggccggaagc ggauccaugu gggagcugga gaaagacguu uauguuguag agguggacug	180
gacucccgau gccccuggag aaacagugaa ccucaccugu gacacgccug aagaagauga	240
caucaccugg accucagacc agagacaugg agucauaggc ucuggaaaaga cccugaccu	300
cacugucaaa gaguuucuag augcuggcca guacaccugc cacaaggag gcgagacucu	360
gagccacuca caucugcugc uccacaagaa ggaaaugga auuuggucca cugaaauuuu	420
aaaaauuuc aaaaacaaga cuuuccugaa gugugaagca ccaauuacu ccggacgguu	480
cacgugcuca uggcuggugc aaagaaacau ggacuugaag uucaacauca agagcaguag	540
caguucccu gacucucggg cagugacaug uggaauggcg ucucugucug cagagaaggu	600
cacacuggac caaagggacu augagaagua uucagugucc ugccaggagg augucaccug	660
cccaacugcc gaggagaccc ugcccuauga acuggcguug gaagcacggc agcagaauaa	720
auaugagaac uacagcacca gcuucuucac cagggacauc aucaaaccag acccgcccaa	780
gaacuugcag augaagccuu ugaagaacuc acagguggag gucagcuggg aguaccuga	840
cuccuggagc acucccauu ccuacuucuc ccucaaguuc uuuguucgaa uccagcgcaa	900

gaaagaaaag augaaggaga cagaggaggg guguaaccag aaaggugcgu uccucguaga	960
gaagacau cu accgaagucc aaugcaaagg cgggaauguc ugcgugcaag cucaggau cu	1020
cuauuaca au uccucaugca gcaagugggc auguguuccc ugcagagucc gaucggau cu	1080
uggaguaggg guaccuggag ugggcagggg cauaccgguc ucuggaccug ccaggugucu	1140
uagccagucc cgaaaccugc ugaagaccac agaugacaug gugaagacgg ccagagaaaa	1200
gcugaaac au uauuccugca cugcugaaga caucgauc au gaagacau ca cacgggacca	1260
aaccagcaca uugaagaccu guuuaccacu ggaacuacac aagaacgaga guugccuggc	1320
uacuagagag acuucuucca caacaagagg gagcugccug ccccccacaga agacgucuuu	1380
gaugaugacc cugugccuug guagcaucua ugaggacuug aagauguacc agacagagu u	1440
ccaggccauc aacgcagcac uucagaauc aaccaucag cagauc au uagacaaggg	1500
caugcuggug gccaucgaug agcugaugca gucucuga au cauaauggcg agacucugcg	1560
ccagaaaccu ccugugggag aagcagaccc uuacagagug aaaaugaagc ucugcauccu	1620
gcuucacgcc uucagcaccg gcgucgugac caucaacagg gugaugggcu aucuguccag	1680
cgccuaau ag cucgagagcu cgcuuu cu cugucca au ucuauuaaag guuccuuugu	1740
ucccuaguc caacuacua acugggggau auuaugaagg gccuugagca ucuggau cu	1800
gccuaauaaa aaacauuu au uuuc au gcgucgagag cucgcuu cu ugcuguccaa	1860
uuuc au uaa agguuccu guucccuag uccaacuac aaacuggggg auuuuagaa	1920
gggccuugag caucuggau cu gccuaau aaaaaac au auuuu au cu gcgucgag	1980
accuggucca gagucgcuag caaaaaaaaa aaaaaaaaaa aaaaaaaaaa agcauagac	2040
uaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	2100
aaaaaaaaa a	2111

<210> 39
 <211> 544
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModB murine IL-12

<400> 39

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala
 1 5 10 15

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Met Trp Glu Leu
 20 25 30

Glu Lys Asp Val Tyr Val Val Glu Val Asp Trp Thr Pro Asp Ala Pro
 35 40 45

Gly Glu Thr Val Asn Leu Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile
 50 55 60

Thr Trp Thr Ser Asp Gln Arg His Gly Val Ile Gly Ser Gly Lys Thr
 65 70 75 80

Leu Thr Ile Thr Val Lys Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys
 85 90 95

His Lys Gly Gly Glu Thr Leu Ser His Ser His Leu Leu Leu His Lys
 100 105 110

Lys Glu Asn Gly Ile Trp Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn
 115 120 125

Lys Thr Phe Leu Lys Cys Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr
 130 135 140

Cys Ser Trp Leu Val Gln Arg Asn Met Asp Leu Lys Phe Asn Ile Lys
 145 150 155 160

Ser Ser Ser Ser Ser Pro Asp Ser Arg Ala Val Thr Cys Gly Met Ala
 165 170 175

Ser Leu Ser Ala Glu Lys Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys
 180 185 190

Tyr Ser Val Ser Cys Gln Glu Asp Val Thr Cys Pro Thr Ala Glu Glu
 195 200 205

Thr Leu Pro Ile Glu Leu Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr
 210 215 220

Glu Asn Tyr Ser Thr Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp
 225 230 235 240

Pro Pro Lys Asn Leu Gln Met Lys Pro Leu Lys Asn Ser Gln Val Glu
 245 250 255

Val Ser Trp Glu Tyr Pro Asp Ser Trp Ser Thr Pro His Ser Tyr Phe
 260 265 270

Ser Leu Lys Phe Phe Val Arg Ile Gln Arg Lys Lys Glu Lys Met Lys
 275 280 285

Glu Thr Glu Glu Gly Cys Asn Gln Lys Gly Ala Phe Leu Val Glu Lys
 290 295 300

Thr Ser Thr Glu Val Gln Cys Lys Gly Gly Asn Val Cys Val Gln Ala
 305 310 315 320

Gln Asp Arg Tyr Tyr Asn Ser Ser Cys Ser Lys Trp Ala Cys Val Pro
 325 330 335

Cys Arg Val Arg Ser Val Pro Gly Val Gly Val Pro Gly Val Gly Arg
 340 345 350

Val Ile Pro Val Ser Gly Pro Ala Arg Cys Leu Ser Gln Ser Arg Asn
 355 360 365

Leu Leu Lys Thr Thr Asp Asp Met Val Lys Thr Ala Arg Glu Lys Leu
 370 375 380

Lys His Tyr Ser Cys Thr Ala Glu Asp Ile Asp His Glu Asp Ile Thr
 385 390 395 400

Arg Asp Gln Thr Ser Thr Leu Lys Thr Cys Leu Pro Leu Glu Leu His
 405 410 415

Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu Thr Ser Ser Thr Thr Arg
420 425 430

Gly Ser Cys Leu Pro Pro Gln Lys Thr Ser Leu Met Met Thr Leu Cys
435 440 445

Leu Gly Ser Ile Tyr Glu Asp Leu Lys Met Tyr Gln Thr Glu Phe Gln
450 455 460

Ala Ile Asn Ala Ala Leu Gln Asn His Asn His Gln Gln Ile Ile Leu
465 470 475 480

Asp Lys Gly Met Leu Val Ala Ile Asp Glu Leu Met Gln Ser Leu Asn
485 490 495

His Asn Gly Glu Thr Leu Arg Gln Lys Pro Pro Val Gly Glu Ala Asp
500 505 510

Pro Tyr Arg Val Lys Met Lys Leu Cys Ile Leu Leu His Ala Phe Ser
515 520 525

Thr Arg Val Val Thr Ile Asn Arg Val Met Gly Tyr Leu Ser Ser Ala
530 535 540

<210> 40
<211> 2191
<212> DNA
<213> Artificial sequence

<220>
<223> ModB murine IL-12 (5'UTR-CDS-3'UTR)

<400> 40
ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60
tctacttcta ttgcagcaat ttaaatacatt tcttttaaag caaaagcaat tttctgaaaa 120
ttttcaccat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct 180
gctggccgct gccctggccc ctacacagac aagagctgga cctggatcca tgtgggagct 240
ggagaaagac gtttatgttg tagaggtgga ctggactccc gatgccctg gagaaacagt 300

gaacctcacc tgtgacacgc ctgaagaaga tgacatcacc tggacctcag accagagaca	360
tggagtcata ggctctggaa agaccctgac catcactgtc aaagagtttc tagatgctgg	420
ccagtacacc tgccacaaag gaggcgagac tctgagccac tcacatctgc tgctccacaa	480
gaaggaaaat ggaatttggt ccactgaaat tttaaaaaat ttcaaaaaca agactttcct	540
gaagtgtgaa gcaccaaatt actccggacg gttcacgtgc tcatggctgg tgcaaagaaa	600
catggacttg aagttcaaca tcaagagcag tagcagttcc cctgactctc gggcagtgac	660
atgtggaatg gcgtctctgt ctgcagagaa ggtcacactg gaccaaaggg actatgagaa	720
gtattcagtg tcctgccagg aggatgtcac ctgcccact gccgaggaga ccctgccccat	780
tgaactggcg ttggaagcac ggcagcagaa taaatatgag aactacagca ccagcttctt	840
catcagggac atcatcaaac cagacccgcc caagaacttg cagatgaagc ctttgaagaa	900
ctcacaggtg gaggtcagct gggagtaccc tgactcctgg agcactcccc attcctactt	960
ctccctcaag ttctttgttc gaatccagcg caagaaagaa aagatgaagg agacagagga	1020
ggggtgtaac cagaaaggtg cgttcctcgt agagaagaca tctaccgaag tccaatgcaa	1080
aggcgggaaat gtctgcgtgc aagctcagga tcgctattac aattcctcat gcagcaagtg	1140
ggcatgtgtt ccctgcagag tccgatcggt tcctggagta ggggtacctg gagtgggcag	1200
ggtcataccg gtctctggac ctgccaggtg tcttagccag tcccgaacc tgctgaagac	1260
cacagatgac atggtgaaga cggccagaga aaagctgaaa cattattcct gcactgctga	1320
agacatcgat catgaagaca tcacacggga ccaaaccagc acattgaaga cctgtttacc	1380
actggaacta cacaagaacg agagttgcct ggctactaga gagacttctt ccacaacaag	1440
agggagctgc ctgccccac agaagacgtc tttgatgatg accctgtgcc ttggtagcat	1500
ctatgaggac ttgaagatgt accagacaga gttccaggcc atcaacgcag cacttcagaa	1560
tcacaacat cagcagatca ttctagacaa gggcatgctg gtggccatcg atgagctgat	1620
gcagtctctg aatcataatg gcgagactct gcgccagaaa cctcctgtgg gagaagcaga	1680
cccttacaga gtgaaaatga agctctgcat cctgcttcac gccttcagca cccgcgtcgt	1740
gaccatcaac aggggtgatgg gctatctgtc cagcgcctaa tagctcgacg tcctggtact	1800
gcatgcacgc aatgctagct gcccttttcc cgtcctgggt accccgagtc tccccgacc	1860

tcgggtccca ggtatgctcc cacctccacc tgccccactc accacctctg ctagttccag	1920
acacctccca agcacgcagc aatgcagctc aaaacgctta gcctagccac acccccacgg	1980
gaaacagcag tgattaacct ttagcaataa acgaaagttt aactaagcta tactaacccc	2040
agggttggtc aatttcgtgc cagccacacc ctcgagctag caaaaaaaaa aaaaaaaaaa	2100
aaaaaaaaaa agcatatgac taaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	2160
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a	2191

<210> 41

<211> 2191

<212> RNA

<213> Artificial sequence

<220>

<223> ModB murine IL-12

<400> 41

ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaucaagcau	60
ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa	120
uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu	180
gcuggccgcu gcccuggccc cuacacagac aagagcugga ccuggaucca ugugggagcu	240
ggagaaagac guuuanguug uagaggugga cuggacuccc gaugccccug gagaaacagu	300
gaaccucacc ugugacacgc cugaagaaga ugacaucacc uggaccucag accagagaca	360
uggagucuaa ggcucuggaa agaccucgac caucacuguc aaagaguuuu uagaugcugg	420
ccaguacacc ugccacaaag gaggcgagac ucugagccac ucacaucugc ugcuccacaa	480
gaaggaaaau ggaauuuggu ccacugaaaau uuuaaaaaa uucaaaaaa agacuuuccu	540
gaagugugaa gcaccaaauu acuccggacg guucacgugc ucauggcugg ugcaaagaaa	600
cauggacuug aaguucaaca ucaagagcag uagcaguucc ccugacucuc gggcagugac	660
auguggaaug gcgucucugu cugcagagaa ggucacacug gaccaaaggg acuaugagaa	720
guauucagug uccugccagg aggaugucac cugcccaacu gccgaggaga ccugcccau	780
ugaacuggcg uuggaagcac ggcagcagaa uaaauaugag aacuacagca ccagcuucuu	840
caucagggac aucaucaaac cagacccgcc caagaacuug cagaugaagc cuuugaagaa	900

cucacaggug gaggucagcu gggaguaccc ugacuccugg agcacucccc auuccuacuu	960
cucccucaag uucuuuguuc gaauccagcg caagaaagaa aagaugaagg agacagagga	1020
gggguguaac cagaaaggug cguuccucgu agagaagaca ucuaccgaag uccaauugcaa	1080
aggcgggaau gucugcgugc aagcucagga ucgcuaauuac aaauccucau gcagcaagug	1140
ggcauguguu ccugcagag uccgaucggu uccuggagua gggguaccug gagugggcag	1200
ggucauaccg gucucuggac cugccaggug ucuuagccag ucccgaacc ugcugaagac	1260
cacagaugac auggugaaga cggccagaga aaagcugaaa cauuauuccu gcacugcuga	1320
agacaucgau caugaagaca ucacacggga ccaaaccagc acauugaaga ccuguuuacc	1380
acuggaacua cacaagaacg agaguugccu ggcuacuaga gagacuucuu ccacaacaag	1440
agggagcugc cugccccac agaagacguc uuugaugaug acccugugcc uugguagcau	1500
cuaugaggac uugaaugu accagacaga guuccaggcc aucaacgcag cacuucagaa	1560
ucacaaccau cagcagauca uucuagacaa gggcaugcug guggccaucg augagcugau	1620
gcagucucug aaucuaaau gcgagacucu gcgccagaaa ccuccugugg gagaagcaga	1680
cccuacaga gugaaaauga agcucugcau ccugcuucac gccuucagca cccgcgucgu	1740
gaccaucaac agggugaugg gcuaucuguc cagcgccuaa uagcucgacg uccugguacu	1800
gcaugcacgc aaugcuagcu gccccuuucc cguccugggu accccgaguc uccccgacc	1860
ucggguccca gguaugcucc caccuccacc ugccccacuc accaccucug cuaguuccag	1920
acaccuccca agcacgcagc aaugcagcuc aaaacgcuua gccuagccac acccccacgg	1980
gaaacagcag ugauuaaccu uuagcaauaa acgaaaguuu aacuaagcua uacuaacccc	2040
aggguugguc aaauucgugc cagccacacc cucgagcuag caaaaaaaaaa aaaaaaaaaa	2100
aaaaaaaaaa agcauugac uaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	2160
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a	2191

<210> 42

<211> 190

<212> PRT

<213> Artificial sequence

<220>

<223> ModA murine IFN-alpha-4

<400> 42

Met Arg Val Thr Ala Pro Arg Thr Leu Ile Leu Leu Leu Ser Gly Ala
 1 5 10 15

Leu Ala Leu Thr Glu Thr Trp Ala Gly Ser Gly Ser Cys Asp Leu Pro
 20 25 30

His Thr Tyr Asn Leu Gly Asn Lys Arg Ala Leu Thr Val Leu Glu Glu
 35 40 45

Met Arg Arg Leu Pro Pro Leu Ser Cys Leu Lys Asp Arg Lys Asp Phe
 50 55 60

Gly Phe Pro Leu Glu Lys Val Asp Asn Gln Gln Ile Gln Lys Ala Gln
 65 70 75 80

Ala Ile Leu Val Leu Arg Asp Leu Thr Gln Gln Ile Leu Asn Leu Phe
 85 90 95

Thr Ser Lys Asp Leu Ser Ala Thr Trp Asn Ala Thr Leu Leu Asp Ser
 100 105 110

Phe Cys Asn Asp Leu His Gln Gln Leu Asn Asp Leu Lys Ala Cys Val
 115 120 125

Met Gln Glu Pro Pro Leu Thr Gln Glu Asp Ser Leu Leu Ala Val Arg
 130 135 140

Thr Tyr Phe His Arg Ile Thr Val Tyr Leu Arg Lys Lys Lys His Ser
 145 150 155 160

Leu Cys Ala Trp Glu Val Ile Arg Ala Glu Val Trp Arg Ala Leu Ser
 165 170 175

Ser Ser Thr Asn Leu Leu Ala Arg Leu Ser Glu Glu Lys Glu
 180 185 190

<210> 43
 <211> 1049
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> ModA murine IFN-alpha-4 (5'UTR-CDS-3'UTR)

 <400> 43
 gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgccca ccatgagagt 60
 gaccgcccc agaaccctga tcctgctgct gtctggcgcc ctggccctga cagagacatg 120
 ggccggaagc ggatcctgtg acctgcctca cacttataac ctcgggaaca agagggcctt 180
 gacagtcctg gaagaaatga gaagactccc ccctctttcc tgcctgaagg acaggaagga 240
 ttttggattc cccttgagga aggtggataa ccaacagatc cagaaggctc aagccatcct 300
 tgtgctaaga gatcttacct agcagatctt gaacctcttc acatcaaaag acttgtctgc 360
 tacttggaat gcaactctcc tagactcatt ctgcaatgac ctccatcagc agtcaatga 420
 tctcaaagcc tgtgtgatgc aggaacctcc tctgaccag gaagactccc tgctggctgt 480
 gaggacatac ttccacagga tcaactgtgta cctgagaaag aagaaacaca gcctctgtgc 540
 ctgggaggtg atcagagcag aagtctggag agccctctct tcctcaacca acttgctggc 600
 aagactgagt gaggagaagg agtgataact cgagagctcg ctttcttgct gtccaatttc 660
 tattaaaggt tcctttgttc cctaagtcca actactaaac tgggggatat tatgaagggc 720
 cttgagcatc tggattctgc ctaataaaaa acatttattt tcattgctgc gtcgagagct 780
 cgctttcttg ctgtccaatt tctattaaag gttcctttgt tccctaagtc caactactaa 840
 actgggggat attatgaagg gccttgagca tctggattct gcctaataaa aaacatttat 900
 tttcattgct gcgtcgagac ctggtccaga gtcgctagca aaaaaaaaaa aaaaaaaaaa 960
 aaaaaaaaaa catatgacta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1049

<210> 44
 <211> 1049
 <212> RNA
 <213> Artificial sequence

<220>

<223> ModA murine IFN-alpha-4

<400> 44

```

gggcgaacua guauucuucu ggucgccaca gacucagaga gaacccgccca ccaugagagu      60
gaccgcccccc agaaccuga uccugcugcu gucuggcgcc cuggcccuga cagagacaug      120
ggccggaagc ggauccugug accugccuca cacuuauaac cucgggaaca agagggccuu      180
gacaguccug gaagaaauga gaagacuccc cccucuuucc ugccugaagg acaggaagga      240
uuuuggauuc cccuuggaga agguggauaa ccaacagauc cagaaggcuc aagccauccu      300
ugugcuaaga gaucuuaccc agcagauuuu gaaccucuuc acaucaaag acuuugucugc      360
uacuuggaau gcaacucucc uagacucauu cugcaaugac cuccaucagc agcucaauga      420
ucucaaagcc ugugugaugc aggaaccucc ucugaccag gaagacuccc ugcuggcugu      480
gaggacauac uuccacagga ucacugugua ccugagaaag aagaaacaca gccucugugc      540
cugggaggug aucagagcag aagucuggag agcccucucu uccucaacca acuuugcuggc      600
aagacugagu gaggagaagg agugauaacu cgagagcucg cuuucuugcu guccaauuuc      660
uauuaaaggu uccuuuguuc ccuaagucca acuacuaaac ugggggauau uaugaagggc      720
cuugagcauc uggauucugc cuaauaaaaa acauuuuuuu ucauugcugc gucgagagcu      780
cgcuuucuug cuguccaauu ucuauuaaag guuccuuugu ucccuaaguc caacuacuaa      840
acugggggau auuaugaagg gccuugagca ucuggauucu gccuaauaaa aaacauuuau      900
uuucauugcu gcgucgagac cugguccaga gucgcuagca aaaaaaaaaa aaaaaaaaaa      960
aaaaaaaaag cauugacua aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1020
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1049

```

<210> 45

<211> 190

<212> PRT

<213> Artificial sequence

<220>

<223> ModB murine IFN-alpha-4

<400> 45

```

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Ala Ala Ala
1           5           10          15

```

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Cys Asp Leu Pro
20 25 30

His Thr Tyr Asn Leu Gly Asn Lys Arg Ala Leu Thr Val Leu Glu Glu
35 40 45

Met Arg Arg Leu Pro Pro Leu Ser Cys Leu Lys Asp Arg Lys Asp Phe
50 55 60

Gly Phe Pro Leu Glu Lys Val Asp Asn Gln Gln Ile Gln Lys Ala Gln
65 70 75 80

Ala Ile Leu Val Leu Arg Asp Leu Thr Gln Gln Ile Leu Asn Leu Phe
85 90 95

Thr Ser Lys Asp Leu Ser Ala Thr Trp Asn Ala Thr Leu Leu Asp Ser
100 105 110

Phe Cys Asn Asp Leu His Gln Gln Leu Asn Asp Leu Lys Ala Cys Val
115 120 125

Met Gln Glu Pro Pro Leu Thr Gln Glu Asp Ser Leu Leu Ala Val Arg
130 135 140

Thr Tyr Phe His Arg Ile Thr Val Tyr Leu Arg Lys Lys Lys His Ser
145 150 155 160

Leu Cys Ala Trp Glu Val Ile Arg Ala Glu Val Trp Arg Ala Leu Ser
165 170 175

Ser Ser Thr Asn Leu Leu Ala Arg Leu Ser Glu Glu Lys Glu
180 185 190

<210> 46

<211> 1129

<212> DNA

<213> Artificial sequence

<220>

<223> ModB murine IFN-alpha-4 (5'UTR-CDS-3'UTR)

<400> 46

```

ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat      60
tctacttcta ttgcagcaat ttaaatacatt tcttttaaag caaaagcaat tttctgaaaa    120
ttttcaccat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct     180
gctggccgct gccctggccc ctacacagac aagagctgga cctggatcct gtgacctgcc     240
tcacacttat aacctcgga acaagagggc cttgacagtc ctggaagaaa tgagaagact      300
ccccctctt tcctgcctga aggacaggaa ggattttgga ttccccttgg agaaggtgga     360
taaccaacag atccagaagg ctcaagccat ctttgtgcta agagatctta cccagcagat     420
tttgaacctc ttcacatcaa aagacttgtc tgctacttgg aatgcaactc tcctagactc     480
attctgcaat gacctccatc agcagctcaa tgatctcaaa gcctgtgtga tgcaggaacc     540
tcctctgacc caggaagact ccctgctggc tgtgaggaca tacttccaca ggatcactgt     600
gtacctgaga aagaagaaac acagcctctg tgcctgggag gtgatcagag cagaagtctg     660
gagagccctc tcttcctcaa ccaacttgct ggcaagactg agtgaggaga aggagtgata     720
actcgacgtc ctgggtactgc atgcacgcaa tgctagctgc ccctttcccg tcctgggtac     780
cccgagtctc ccccgacctc gggtcccagg tatgctccca cctccacctg cccactcac      840
cacctctgct agttccagac acctcccaag cacgcagcaa tgcagctcaa aacgcttagc     900
ctagccacac cccacggga aacagcagtg attaaccttt agcaataaac gaaagttaa     960
ctaagctata ctaaccccag ggttggtcaa tttcgtgcca gccacaccct cgagctagca    1020
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa catatgacta aaaaaaaaaa aaaaaaaaaa    1080
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    1129

```

<210> 47

<211> 1129

<212> RNA

<213> Artificial sequence

<220>

<223> ModB murine IFN-alpha-4

<400> 47

```

ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaUCAAGCAU      60

```

ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa	120
uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu	180
gcuggccgcu gcccuiggccc cuacacagac aagagcugga ccuggauccu gugaccugcc	240
ucacacuuau aaccucggga acaagagggc cuugacaguc cuggaagaaa ugagaagacu	300
ccccccucuu uccugccuga aggacaggaa ggauuuugga uuccccuugg agaaggugga	360
uaaccaacag auccagaagg cucaagccau ccuugugcua agagaucuaa cccagcagau	420
uuugaaccuc uucacaucaa aagacuuguc ugcuaucugg aaugcaacuc uccuagacuc	480
auucugcaau gaccuccauc agcagcucaa ugaucuaaaa gccuguguga ugcaggaacc	540
uccucugacc caggaagacu cccugcuggc ugugaggaca uacuuccaca ggaucacugu	600
guaccugaga aagaagaaac acagccucug ugccuggggag gugaucagag cagaagucug	660
gagagcccuc ucuuccucaa ccaacuugcu ggcaagacug agugaggaga aggagugaua	720
acucgacguc cugguacugc augcacgcaa ugcuaugcugc cccuuucccg uccuggguac	780
cccgagucuc ccccgaccuc gggucccagg uaugcuccca ccuccaccug ccccacucac	840
caccucugcu aguuccagac accucccaag cacgcagcaa ugcagcucaa aacgcuuagc	900
cuagccacac cccacggga aacagcagug auuaaccuuu agcaauaaac gaaaguuuaa	960
cuaagcuaua cuaaccccag gguuggucaa uuucgugcca gccacacccu cgagcuagca	1020
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa cauugacua aaaaaaaaaa aaaaaaaaaa	1080
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	1129

<210> 48

<211> 232

<212> PRT

<213> Artificial sequence

<220>

<223> ModA murine IL-15 sushi

<400> 48

Met	Gly	Ala	Met	Ala	Pro	Arg	Thr	Leu	Leu	Leu	Leu	Ala	Ala	Ala
1				5				10				15		

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Thr Thr Cys Pro
 20 25 30

Pro Pro Val Ser Ile Glu His Ala Asp Ile Arg Val Lys Asn Tyr Ser
 35 40 45

Val Asn Ser Arg Glu Arg Tyr Val Cys Asn Ser Gly Phe Lys Arg Lys
 50 55 60

Ala Gly Thr Ser Thr Leu Ile Glu Cys Val Ile Asn Lys Asn Thr Asn
 65 70 75 80

Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp Pro Ser
 85 90 95

Leu Ala Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly
 100 105 110

Ser Gly Gly Ser Gly Gly Asn Trp Ile Asp Val Arg Tyr Asp Leu Glu
 115 120 125

Lys Ile Glu Ser Leu Ile Gln Ser Ile His Ile Asp Thr Thr Leu Tyr
 130 135 140

Thr Asp Ser Asp Phe His Pro Ser Cys Lys Val Thr Ala Met Asn Cys
 145 150 155 160

Phe Leu Leu Glu Leu Gln Val Ile Leu His Glu Tyr Ser Asn Met Thr
 165 170 175

Leu Asn Glu Thr Val Arg Asn Val Leu Tyr Leu Ala Asn Ser Thr Leu
 180 185 190

Ser Ser Asn Lys Asn Val Ala Glu Ser Gly Cys Lys Glu Cys Glu Glu
 195 200 205

Leu Glu Glu Lys Thr Phe Thr Glu Phe Leu Gln Ser Phe Ile Arg Ile
 210 215 220

Val Gln Met Phe Ile Asn Thr Ser

225

230

<210> 49

<211> 1162

<212> DNA

<213> Artificial sequence

<220>

<223> ModA murine IL-15 sushi (5'UTR-CDS-3'UTR)

<400> 49

```

gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgccca ccatgggcgcg      60
catggcccct agaacattgc tcctgctgct ggccgctgcc ctggccccta cacagacaag      120
agctggacct ggatccacca cgtgtccacc tcccgtatct attgagcatg ctgacatccg      180
ggtcaagaat tacagtgtga actccaggga gaggtatgtc tgtaactctg gctttaagcg      240
gaaagctgga acatccaccc tgattgagtg tgtgatcaac aagaacacaa atgttgccca      300
ctggacaact cccagcctca agtgcacag agacccctcc ctagctggag ggagcggagg      360
ctctggcgga agcggcggggt ctggaggctc cgggggaagc ggcggaatt ggatcgacgt      420
gcgctacgac ctggaaaaga tcgagagcct gatccagagc atccacatcg acaccaccct      480
gtacaccgac agcgacttcc accccagctg caaagtgacc gctatgaact gcttcctgct      540
ggaactgcaa gtgacacctg acgagtacag caacatgacc ctgaacgaga cagtgcggaa      600
cgtgctgtac ctggccaaca gcaccctgag cagcaacaag aacgtggccg agagcggctg      660
caaagagtgc gaggaactgg aagaaaagac cttcaccgag tttctgcaga gcttcatcag      720
gatcgtgcag atgttcatca acacctcttg atgagtcgac gtcctggtac tgcatgcacg      780
caatgctagc tgcccctttc ccgtcctggg taccctgagt ccccccgac ctcgggtccc      840
aggtatgctc ccacctccac ctgccccact caccacctct gctagttcca gacacctccc      900
aagcacgcag caatgcagct caaacgctt agcctagcca ccccccacg ggaaacagca      960
gtgattaacc tttagcaata aacgaaagtt taactaagct atactaacc cagggttggt     1020
caatttcgtg ccagccacac cctcgagcta gcaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1080
aagcatatga ctaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1140
aaaaaaaaaa aaaaaaaaaa aa                                             1162

```

<210> 50
 <211> 1162
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModA murine IL-15 sushi

<400> 50
 gggcgaacua guauucuucu ggucccccaca gacucagaga gaacccgccca ccauggggcgc 60
 cauggcccccua agaacaauugc uccugcugcu ggccgcugcc cuggcccccua cacagacaag 120
 agcugggaccu ggauccacca cguguccacc ucccguauucu auugagcaug cugacaucgg 180
 ggucaagaau uacaguguga acuccaggga gagguauguc uguaacucug gcuuuuagcg 240
 gaaagcugga acauccaccc ugauugagug ugugaucaac aagaacacaa auguugccca 300
 cuggacaacu cccagccuca agugcaucag agacccuccu cuagcuggag ggagcggagg 360
 cucuggcgga agcggcgggg cuggaggcuc cgggggaagc ggcggaauu ggaucgacgu 420
 gcgcuacgac cuggaaaaga ucgagagccu gauccagagc auccacaucg acaccacccu 480
 guacaccgac agcgacuucc accccagcug caaagugacc gcuaugaacu gcuuccugcu 540
 ggaacugcaa gugaucugc acgaguacag caacaugacc cugaacgaga cagugcggaa 600
 cgugcuguac cuggccaaca gcaccucgag cagcaacaag aacguggccg agagcggcug 660
 caaagagugc gaggaacugg aagaaaagac cuucaccgag uuucugcaga gcuucaucag 720
 gaucgugcag auguucacua acaccucuug augagucgac guccugguac ugcaugcacg 780
 caaugcuagc ugccccuuuc ccguccuggg uaccccgagu cuccccgac cucggguccc 840
 agguaugcuc ccaccuccac cugccccacu caccaccucu gcuaguucca gacaccuccc 900
 aagcacgcag caaugcagcu caaaacgcuu agccuagcca ccccccacg ggaaacagca 960
 gugauuaacc uuuagcaaua aacgaaaguu uaacuaagcu auacuaaccc cagggguuggu 1020
 caauuucgug ccagccacac ccucgagcua gcaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1080
 aagcauuga cuaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1140
 aaaaaaaaaa aaaaaaaaaa aa 1162

<210> 51

<211> 232

<212> PRT

<213> Artificial sequence

<220>

<223> ModB murine IL-15 sushi

<400> 51

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Ala Ala Ala
1 5 10 15

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Thr Thr Cys Pro
20 25 30

Pro Pro Val Ser Ile Glu His Ala Asp Ile Arg Val Lys Asn Tyr Ser
35 40 45

Val Asn Ser Arg Glu Arg Tyr Val Cys Asn Ser Gly Phe Lys Arg Lys
50 55 60

Ala Gly Thr Ser Thr Leu Ile Glu Cys Val Ile Asn Lys Asn Thr Asn
65 70 75 80

Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp Pro Ser
85 90 95

Leu Ala Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly
100 105 110

Ser Gly Gly Ser Gly Gly Asn Trp Ile Asp Val Arg Tyr Asp Leu Glu
115 120 125

Lys Ile Glu Ser Leu Ile Gln Ser Ile His Ile Asp Thr Thr Leu Tyr
130 135 140

Thr Asp Ser Asp Phe His Pro Ser Cys Lys Val Thr Ala Met Asn Cys
145 150 155 160

Phe Leu Leu Glu Leu Gln Val Ile Leu His Glu Tyr Ser Asn Met Thr
165 170 175

Leu Asn Glu Thr Val Arg Asn Val Leu Tyr Leu Ala Asn Ser Thr Leu
180 185 190

Ser Ser Asn Lys Asn Val Ala Glu Ser Gly Cys Lys Glu Cys Glu Glu
195 200 205

Leu Glu Glu Lys Thr Phe Thr Glu Phe Leu Gln Ser Phe Ile Arg Ile
210 215 220

Val Gln Met Phe Ile Asn Thr Ser
225 230

<210> 52
<211> 1255
<212> DNA
<213> Artificial sequence

<220>
<223> ModB murine IL-15 sushi (5'UTR-CDS-3'UTR)

<400> 52
ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60
tctacttcta ttgcagcaat ttaaattcatt tcttttaaag caaaagcaat tttctgaaaa 120
ttttcaccat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct 180
gctggccgct gccctggccc ctacacagac aagagctgga cctggatcca ccacgtgtcc 240
acctcccgta tctattgagc atgctgacat ccgggtcaag aattacagtg tgaactccag 300
ggagaggtat gtctgtaact ctggctttta gcggaaagct ggaacatcca ccctgattga 360
gtgtgtgatc aacaagaaca caaatgttgc ccactggaca actcccagcc tcaagtgcac 420
cagagacccc tccctagctg gagggagcgg aggctctggc ggaagcggcg ggtctggagg 480
ctccggggga agcggcgga attggatcga cgtgcgctac gacctggaaa agatcgagag 540
cctgatccag agcatccaca tcgacaccac cctgtacacc gacagcgact tccaccccag 600
ctgcaaagtg accgctatga actgcttcct gctggaactg caagtgatcc tgcacgagta 660
cagcaacatg accctgaacg agacagtgcg gaacgtgctg tacctggcca acagcaccct 720
gagcagcaac aagaacgtgg ccgagagcgg ctgcaaagag tgcgaggaac tggaagaaaa 780

gaccttcacc gagtttctgc agagcttcat caggatcgtg cagatgttca tcaacacctc	840
ttgatgagtc gacgtcctgg tactgcatgc acgcaatgct agctgcccct ttcccgtcct	900
gggtaccccc agtctccccc gacctcgggt cccaggtatg ctcccacctc cacctgcccc	960
actcaccacc tctgctagtt ccagacacct cccaagcacg cagcaatgca gctcaaaacg	1020
cttagcctag ccacaccccc acgggaaaca gcagtgatta accttttagca ataaacgaaa	1080
gtttaactaa gctatactaa ccccaggggt ggtcaatttc gtgccagcca caccctcgag	1140
ctagcaaaaa aaaaaaaaaa aaaaaaaaaa aaaaagcata tgactaaaaa aaaaaaaaaa	1200
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa	1255

<210> 53

<211> 1255

<212> RNA

<213> Artificial sequence

<220>

<223> ModB murine IL-15 sushi

<400> 53

ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaucaagcau	60
ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa	120
uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu	180
gcuggccgcu gcccuggccc cuacacagac aagagcugga ccuggaucca ccacgugucc	240
accucccgua ucuauugagc augcugacau ccgggucaag aaauacagug ugaacuccag	300
ggagagguau gucuguaacu cuggcuuuua gcggaaagcu ggaacaacca ccugauuga	360
gugugugauc aacaagaaca caaanguugc ccacuggaca acucccagcc ucaagugcau	420
cagagacccc ucccuagcug gagggagcgg aggcucuggc ggaagcggcg ggucuggagg	480
cuccggggga agcggcgga auuggaucga cgugcgcuac gaccuggaaa agaucgagag	540
ccugauccag agcauccaca ucgacaccac ccuguacacc gacagcgacu uccaccccag	600
cugcaaagug accgcuauga acugcuuccu gcuggaacug caagugauc ugcacgagua	660
cagcaacaug acccugaacg agacagugcg gaacgugcug uaccuggcca acagcacccu	720
gagcagcaac aagaacgugg ccgagagcgg cugcaaagag ugcgaggaac uggaagaaaa	780

gaccuucacc gaguuucugc agagcuucau caggauugug cagauguuca ucaacaccuc 840
uugaugaguc gacguccugg uacugcaugc acgcaaugcu agcugccccc uucccguccu 900
ggguaccccc agucuccccc gaccucgggu cccagguaug cucccaccuc caccugcccc 960
acucaccacc ucugcuaguu ccagacaccu cccaagcacg cagcaaugca gcucaaaacg 1020
cuuagccuag ccacaccccc acgggaaaca gcagugauua accuuuagca auaaacgaaa 1080
guuuuacuaa gcuaauacuaa ccccaggggu ggucaauuuc gugccagcca caccucgag 1140
cuagcaaaaa aaaaaaaaaa aaaaaaaaaa aaaaagcaua ugacuaaaaa aaaaaaaaaa 1200
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 1255

<210> 54
<211> 141
<212> PRT
<213> Artificial sequence

<220>
<223> ModA murine GM-CSF

<400> 54

Met Trp Leu Gln Asn Leu Leu Phe Leu Gly Ile Val Val Tyr Ser Leu
1 5 10 15

Ser Ala Pro Thr Arg Ser Pro Ile Thr Val Thr Arg Pro Trp Lys His
20 25 30

Val Glu Ala Ile Lys Glu Ala Leu Asn Leu Leu Asp Asp Met Pro Val
35 40 45

Thr Leu Asn Glu Glu Val Glu Val Val Ser Asn Glu Phe Ser Phe Lys
50 55 60

Lys Leu Thr Cys Val Gln Thr Arg Leu Lys Ile Phe Glu Gln Gly Leu
65 70 75 80

Arg Gly Asn Phe Thr Lys Leu Lys Gly Ala Leu Asn Met Thr Ala Ser
85 90 95

Tyr Tyr Gln Thr Tyr Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu Thr
 100 105 110

Gln Val Thr Thr Tyr Ala Asp Phe Ile Asp Ser Leu Lys Thr Phe Leu
 115 120 125

Thr Asp Ile Pro Phe Glu Cys Lys Lys Pro Gly Gln Lys
 130 135 140

<210> 55
 <211> 908
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModA murine GM-CSF (5'UTR-CDS-3'UTR)

<400> 55
 gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatgtggct 60
 gcagaacctg ctgttcctgg gcatcgtggt gtacagcctg agcgccccca ccaggagccc 120
 catcaccgtg accaggccct ggaagcacgt ggaggccatc aaggaggccc tgaacctgct 180
 ggacgacatg cccgtgaccc tgaacgagga ggtggaggtg gtgagcaacg agttcagctt 240
 caagaagctg acctgcgtgc agaccaggct gaagatcttc gagcagggcc tgaggggcaa 300
 cttaccaag ctgaagggcg ccctgaacat gaccgccagc tactaccaga cctactgccc 360
 cccaccccc gagaccgact gcgagacca ggtgaccacc tacgccgact tcatcgacag 420
 cctgaagacc ttctgaccg acatccccct cgagtgaag aagcccggcc agaagtgatg 480
 actcgagctg gtactgcatg cacgcaatgc tagctgcccc tttcccgtcc tgggtacccc 540
 gagtctcccc cgacctcggg tcccaggtat gctccacct ccacctgccc cactcaccac 600
 ctctgctagt tccagacacc tccaagcac gcagcaatgc agctcaaaac gcttagccta 660
 gccacacccc cacgggaaac agcagtgatt aacctttagc aataaacgaa agtttaacta 720
 agctatacta accccagggg tgggtcaattt cgtgccagcc acaccgagac ctgggtccaga 780
 gtcgctagcc gcgtcgctaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa atatgactaa 840
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 900
 aaaaaaaaaa 908

<210> 56
 <211> 908
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModA murine GM-CSF

<400> 56
 gggcgaacua guauucuucu ggucccccaca gacucagaga gaacccgccca ccauguggcu 60
 gcagaaccug cuguuccugg gcaucguggu guacagccug agcgccccca ccaggagccc 120
 caucaccgug accaggcccu ggaagcacgu ggaggccauc aaggaggccc ugaaccugcu 180
 ggacgacaug cccgugaccc ugaacgagga gguggaggug gugagcaacg aguucagcuu 240
 caagaagcug accugcgugc agaccaggcu gaagauucuuc gagcagggcc ugaggggcaa 300
 cuucaccaag cugaagggcg cccugaacau gaccgccagc uacuaccaga ccuacugccc 360
 cccaccccc gagaccgacu gcgagaccca ggugaccacc uacgccgacu ucaucgacag 420
 ccugaagacc uuccugaccg acauccccuu cgagugcaag aagcccggcc agaagugaug 480
 acucgagcug guacugcaug cacgcaaugc uagcugcccc uuucccgucc uggguacccc 540
 gagucucucc cgaccucggg ucccagguau gcucccaccu ccaccugccc cacucaccac 600
 cucugcuagu uccagacacc ucccagcac gcagcaaugc agcucaaaac gcuuagccua 660
 gccacacccc cacgggaaac agcagugauu aaccuuuagc aauaaacgaa aguuuaacua 720
 agcuauacua accccagggg uggucaauuu cgugccagcc acaccgagac cugguccaga 780
 gucgcuagcc gcgucguua aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa auaugacuaa 840
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 900
 aaaaaaaaaa 908

<210> 57
 <211> 141
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModB murine GM-CSF

<400> 57

Met Trp Leu Gln Asn Leu Leu Phe Leu Gly Ile Val Val Tyr Ser Leu
1 5 10 15

Ser Ala Pro Thr Arg Ser Pro Ile Thr Val Thr Arg Pro Trp Lys His
20 25 30

Val Glu Ala Ile Lys Glu Ala Leu Asn Leu Leu Asp Asp Met Pro Val
35 40 45

Thr Leu Asn Glu Glu Val Glu Val Val Ser Asn Glu Phe Ser Phe Lys
50 55 60

Lys Leu Thr Cys Val Gln Thr Arg Leu Lys Ile Phe Glu Gln Gly Leu
65 70 75 80

Arg Gly Asn Phe Thr Lys Leu Lys Gly Ala Leu Asn Met Thr Ala Ser
85 90 95

Tyr Tyr Gln Thr Tyr Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu Thr
100 105 110

Gln Val Thr Thr Tyr Ala Asp Phe Ile Asp Ser Leu Lys Thr Phe Leu
115 120 125

Thr Asp Ile Pro Phe Glu Cys Lys Lys Pro Gly Gln Lys
130 135 140

<210> 58

<211> 1001

<212> DNA

<213> Artificial sequence

<220>

<223> ModB murine GM-CSF (5'UTR-CDS-3'UTR)

<400> 58

ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60

tctacttcta ttgcagcaat ttaaattcatt tcttttaaag caaaagcaat tttctgaaaa 120

ttttcaccat ttacgaacga tagccatgtg gctgcagaac ctgctgttcc tgggcatcgt 180

```

ggtgtacagc ctgagcgccc ccaccaggag ccccatcacc gtgaccaggc cctggaagca      240
cgtggaggcc atcaaggagg ccctgaacct gctggacgac atgcccgtga ccctgaacga      300
ggaggtggag gtggtgagca acgagttcag cttcaagaag ctgacctgcg tgcagaccag      360
gctgaagatc ttcgagcagg gcctgagggg caacttcacc aagctgaagg gcgccctgaa      420
catgaccgcc agctactacc agacctactg cccccccacc cccgagaccg actgcgagac      480
ccaggtgacc acctacgccg acttcacga cagcctgaag accttcctga ccgacatccc      540
cttcgagtgc aagaagcccc gccagaagtg atgactcgag ctggtactgc atgcacgcaa      600
tgctagctgc ccctttcccg tcctgggtac cccgagtctc ccccgacctc ggggtcccagg      660
tatgctccca cctccacctg cccactcac cacctctgct agttccagac acctcccaag      720
cacgcagcaa tgcagctcaa aacgcttagc ctagccacac ccccacggga aacagcagtg      780
attaaccttt agcaataaac gaaagtttaa ctaagctata ctaaccccag ggttggtcaa      840
tttcgtgcca gccacaccga gacctggtcc agagtcgcta gccgcgtcgc taaaaaaaaa      900
aaaaaaaaaa aaaaaaaaaa agcatatgac taaaaaaaaa aaaaaaaaaa aaaaaaaaaa      960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a                                1001

```

```

<210> 59
<211> 1001
<212> RNA
<213> Artificial sequence

```

```

<220>
<223> ModB murine GM-CSF

```

```

<400> 59
ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaucaagcau      60
ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa      120
uuuucaccau uuacgaacga uagccaugug gcugcagaac cugcuguucc ugggcaucgu      180
gguguacagc cugagcgccc ccaccaggag ccccaucacc gugaccaggc ccuggaagca      240
cguggaggcc aucaaggagg ccugaaccu gcuggacgac augcccguga ccugaacga      300
ggagguggag guggugagca acgaguucag cuucaagaag cugaccugcg ugagaccag      360
gcugaagauc uucgagcagg gccugagggg caacuucacc aagcugaagg gcgccucgaa      420

```

```

caugaccgcc agcuacuacc agaccuacug cccccccacc cccgagaccg acugcgagac      480
ccaggugacc accuacgccg acuucaucga cagccugaag accuuccuga ccgacauccc      540
cuucgagugc aagaagcccg gccagaagug augacucgag cugguacugc augcacgcaa      600
ugcuagcugc cccuuucccg uccuggguac cccgagucuc ccccgaccuc gggucccagg      660
uauvcuccca ccuccaccug ccccacucac caccucugcu aguuccagac accucccaag      720
cacgcagcaa ugcagcucaa aacgcuuagc cuagccacac ccccacggga aacagcagug      780
auuaaccuuu agcaauaaac gaaaguuuua cuaagcuaua cuaaccccag gguuggucaa      840
uuucgugcca gccacaccga gaccuggucc agagucgcu gccgcgucgc uaaaaaaaaa      900
aaaaaaaaaa aaaaaaaaaa agcauauagc uaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a                                1001

```

<210> 60
 <211> 187
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModA FLT3L (human FLT3L in combination with a mouse optimized
 secretion sequence)

<400> 60

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala
 1 5 10 15

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Thr Gln Asp Cys
 20 25 30

Ser Phe Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg
 35 40 45

Glu Leu Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser
 50 55 60

Asn Leu Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu
 65 70 75 80

Ala Gln Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met
85 90 95

Gln Gly Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys
100 105 110

Cys Ala Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn
115 120 125

Ile Ser Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys
130 135 140

Pro Trp Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys
145 150 155 160

Gln Pro Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu
165 170 175

Glu Ala Thr Ala Pro Thr Ala Pro Gln Pro Pro
180 185

<210> 61
<211> 1027
<212> DNA
<213> Artificial sequence

<220>
<223> ModA FLT3L (5'UTR-CDS-3'UTR)

<400> 61
gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatgggctgc 60
catggcccct agaacattgc tcctgtgtgt ggccgctgcc ctggccccta cacagacaag 120
agctggacct ggatccaccc aggactgcag cttccagcac tcccctatct cctccgactt 180
cgccgtgaag atccgggagc tgtccgatta cctgtgtgcag gactaccctg tgaccgtggc 240
cagcaacctg caggacgaag aactgtgtgg cggcctgtgg cggctgggtgc tggcccagcg 300
gtggatggaa cggctgaaaa ccgtggccgg ctccaagatg cagggcctgc tcgagcgggt 360
gaacaccgag atccacttcg tgaccaagtg cgccttccag cctcctcctt cctgcctgcg 420

```

gttcgtgcag accaacaatct cccggctgct gcaggaaacc tccgagcagc tggtcgccct 480
gaagccttgg atcaccgggc agaacttctc ccggtgtctg gaactccagt gtcagcccga 540
ctcctccacc ctgcctcctc cctgggtccc caggcctctg gaagccaccg cccctaccgc 600
cccacagcct ccttgatagg tcgacgtcct ggtactgcat gcacgcaatg ctagctgccc 660
ctttcccgctc ctgggtaccc cgagtctccc ccgacctcgg gtcccaggta tgctcccacc 720
tccacctgcc ccactcacca cctctgctag ttccagacac ctcccaagca cgcagcaatg 780
cagctcaaaa cgcttagcct agccacaccc ccacgggaaa cagcagtgat taaccttag 840
caataaacga aagtttaact aagctatact aaccccaggg ttggtcaatt tcgtgccagc 900
cacaccctcg agctagcaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagca tatgactaaa 960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
aaaaaaaaaa 1027

```

```

<210> 62
<211> 1027
<212> RNA
<213> Artificial sequence

```

```

<220>
<223> ModA FLT3L

```

```

<400> 62
gggcgaacua guauucuucu ggucccccaca gacucagaga gaacccgcca ccaugggcgc 60
cauggcccccua agaacauugc uccugcugcu ggccgcugcc cuggccccua cacagacaag 120
agcuggaccu ggauccaccc aggacugcag cuuccagcac uccccuauuc ccuccgacuu 180
cgccgugaag auccgggagc uguccgauua ccugcugcag gacuacccug ugaccguggc 240
cagcaaccug caggacgaag aacugugugg cggccugugg cggcuggugc uggcccagcg 300
guggauggaa cggcugaaaa ccguggccgg cuccaagaug cagggccugc ucgagcgggu 360
gaacaccgag auccacuucg ugaccaagug cgccuuccag ccuccuccuu ccugccugcg 420
guucgugcag accaacaucu cccggcugcu gcaggaaacc uccgagcagc uggucgccccu 480
gaagccuugg aucaccgggc agaacuucuc ccggugucug gaacuccagu gucagcccga 540
cuccuccacc cugccuccuc ccuggucccc caggccucug gaagccaccg ccccuaccgc 600

```



```

ccccacagccu ccuugauagg ugcacguccu gguacugcau gcacgcaaug cuagcugccc      660
cuuuucccguc cuggguaccc cgagucuccc ccgaccucgg gucccaggua ugcucccacc      720
uccaccugcc ccacucacca ccucugcuag uuccagacac cucccaagca cgcagcaaug      780
cagcucaaaa cgcuuagccu agccacaccc ccacgggaaa cagcagugau uaaccuuuag      840
caauaaacga aaguuaacu aagcuauacu aaccccaggg uuggucauuu ucgugccagc      900
cacaccucg agcuagcaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaagca uaugacuaaa      960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     1020
aaaaaaa                                           1027

```

<210> 63
 <211> 187
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModB FLT3L (human FLT3L in combination with a mouse optimized
 secretion sequence)

<400> 63

```

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala
1           5           10           15

```

```

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser Thr Gln Asp Cys
          20           25           30

```

```

Ser Phe Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg
          35           40           45

```

```

Glu Leu Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser
50           55           60

```

```

Asn Leu Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu
65           70           75           80

```

```

Ala Gln Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met
          85           90           95

```

Gln Gly Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys
 100 105 110

Cys Ala Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn
 115 120 125

Ile Ser Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys
 130 135 140

Pro Trp Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys
 145 150 155 160

Gln Pro Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu
 165 170 175

Glu Ala Thr Ala Pro Thr Ala Pro Gln Pro Pro
 180 185

<210> 64
 <211> 1120
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModB FLT3L (5'UTR-CDS-3'UTR)

<400> 64
 ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60
 tctacttcta ttgcagcaat ttaaattcatt tcttttaaag caaaagcaat tttctgaaaa 120
 ttttcacat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct 180
 gctggccgct gccctggccc ctacacagac aagagctgga cctggatcca ccaggactg 240
 cagcttccag cactccccta tctcctccga cttcgccgtg aagatccggg agctgtccga 300
 ttacctgctg caggactacc ctgtgaccgt ggccagcaac ctgcaggacg aagaactgtg 360
 tggcggcctg tggcggctgg tgctggccca gcggtgatg gaacggctga aaaccgtggc 420
 cggctccaag atgcagggcc tgctcgagcg ggtgaacacc gagatccact tcgtgaccaa 480
 gtgcgcttc cagcctcctc cttcctgcct gcggttcgtg cagaccaaca tctcccggtc 540
 gctgcaggaa acctccgagc agctggtcgc cctgaagcct tggatcacc ggagaactt 600

ctccccggtgt ctggaactcc agtgtcagcc cgactcctcc accctgcctc ctccctggtc	660
ccccaggcct ctggaagcca ccgcccctac cgccccacag cctccttgat aggtcgacgt	720
cctggtactg catgcacgca atgctagctg cccctttccc gtcctgggta ccccgagtct	780
cccccgacct cgggtcccag gtatgctccc acctccacct gccccactca ccacctctgc	840
tagttccaga cacctcccaa gcacgcagca atgcagctca aaacgcttag cctagccaca	900
cccccacggg aaacagcagt gattaacctt tagcaataaa cgaaagtta actaagctat	960
actaacccca gggttggtca atttcgtgcc agccacaccc tcgagctagc aaaaaaaaaa	1020
aaaaaaaaaa aaaaaaaaaa gcatatgact aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	1080
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	1120

<210> 65
 <211> 1120
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModB murine FLT3L

<400> 65	
ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaUCAAGCAU	60
UCUACUUCUA uugcagcaau uuaaaUCAUU uuuuuuaaag caaaagcaau uuucugaaaa	120
uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu	180
gcuggccgcU gcccuggccc cuacacagac aagagcugga ccuggaucca cccaggacug	240
cagcuuccag cacuccccua ucuccuccga cuucgccgug aagauccggg agcuguccga	300
uuaccugcug caggacuacc cugugaccgu ggccagcaac cugcaggacg aagaacugug	360
uggcggccug uggcggcugg ugcuggccca gcgguggaug gaacggcuga aaaccguggc	420
cggcuccaag augcagggcc ugcucgagcg ggugaacacc gagauccacu ucgugaccaa	480
gugcgccuuc cagccuccuc cuuccugccu gcgguucgug cagaccaaca ucucccggu	540
gcugcaggaa accuccgagc agcuggucgc ccugaagccu uggaucaccc ggcagaacuu	600
cucccgguu cuggaacucc agugucagcc cgacuccucc acccugccuc cucccggu	660
ccccaggccu cuggaagcca ccgccccuac cgccccacag ccuccuugau aggucgacgu	720

```

ccugguacug caugcacgca augcuagcug ccccuuuccc guccugggua ccccgagucu      780
cccccgaccu cggguccag guaugcucc accuccaccu gccccacuca ccaccucugc      840
uaguuccaga caccuccaa gcacgcagca augcagcuca aaacgcuuag ccuagccaca      900
cccccacggg aaacagcagu gauuaaccuu uagcaauaaa cgaaaguuua acuaagcuau      960
acuaacccca gggguugguca auuucgugcc agccacaccc ucgagcuagc aaaaaaaaaa    1020
aaaaaaaaaa aaaaaaaaaa gcauugacu aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa    1080
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                        1120

```

<210> 66
 <211> 309
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModA murine 41BBL

<400> 66

Met Asp Gln His Thr Leu Asp Val Glu Asp Thr Ala Asp Ala Arg His
 1 5 10 15

Pro Ala Gly Thr Ser Cys Pro Ser Asp Ala Ala Leu Leu Arg Asp Thr
 20 25 30

Gly Leu Leu Ala Asp Ala Ala Leu Leu Ser Asp Thr Val Arg Pro Thr
 35 40 45

Asn Ala Ala Leu Pro Thr Asp Ala Ala Tyr Pro Ala Val Asn Val Arg
 50 55 60

Asp Arg Glu Ala Ala Trp Pro Pro Ala Leu Asn Phe Cys Ser Arg His
 65 70 75 80

Pro Lys Leu Tyr Gly Leu Val Ala Leu Val Leu Leu Leu Leu Ile Ala
 85 90 95

Ala Cys Val Pro Ile Phe Thr Arg Thr Glu Pro Arg Pro Ala Leu Thr
 100 105 110

Ile Thr Thr Ser Pro Asn Leu Gly Thr Arg Glu Asn Asn Ala Asp Gln
115 120 125

Val Thr Pro Val Ser His Ile Gly Cys Pro Asn Thr Thr Gln Gln Gly
130 135 140

Ser Pro Val Phe Ala Lys Leu Leu Ala Lys Asn Gln Ala Ser Leu Cys
145 150 155 160

Asn Thr Thr Leu Asn Trp His Ser Gln Asp Gly Ala Gly Ser Ser Tyr
165 170 175

Leu Ser Gln Gly Leu Arg Tyr Glu Glu Asp Lys Lys Glu Leu Val Val
180 185 190

Asp Ser Pro Gly Leu Tyr Tyr Val Phe Leu Glu Leu Lys Leu Ser Pro
195 200 205

Thr Phe Thr Asn Thr Gly His Lys Val Gln Gly Trp Val Ser Leu Val
210 215 220

Leu Gln Ala Lys Pro Gln Val Asp Asp Phe Asp Asn Leu Ala Leu Thr
225 230 235 240

Val Glu Leu Phe Pro Cys Ser Met Glu Asn Lys Leu Val Asp Arg Ser
245 250 255

Trp Ser Gln Leu Leu Leu Leu Lys Ala Gly His Arg Leu Ser Val Gly
260 265 270

Leu Arg Ala Tyr Leu His Gly Ala Gln Asp Ala Tyr Arg Asp Trp Glu
275 280 285

Leu Ser Tyr Pro Asn Thr Thr Ser Phe Gly Leu Phe Leu Val Lys Pro
290 295 300

Asp Asn Pro Trp Glu
305

<210> 67
 <211> 1393
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModA murine 41BBL (5'UTR-CDS-3'UTR)

<400> 67
 gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatggacca 60
 gcacacactt gatgtggagg ataccgcgga tgccagacat ccagcaggta cttcgtgccc 120
 ctcggatgcg gcgctcctca gagataccgg gctcctcgcg gacgctgcgc tcctctcaga 180
 tactgtgcgc cccacaaatg ccgcgctccc cacggatgct gcctaccctg cggttaatgt 240
 tcgggatcgc gaggccgcgt ggccgcctgc actgaacttc tgttcccgcc acccaaagct 300
 ctatggccta gtcgctttgg ttttgctgct tctgatcgcc gcctgtgttc ctatcttcac 360
 ccgcaccgag cctcggccag cgctcacaat caccacctcg cccaacctgg gtacccgaga 420
 gaataatgca gaccaggtca cccctgtttc ccacattggc tgccccaaca ctacacaaca 480
 gggctctcct gtgttcgcca agctactggc taaaaaccaa gcatcgttgt gcaatacaac 540
 tctgaactgg cacagccaag atggagctgg gagctcatac ctatctcaag gtctgaggta 600
 cgaagaagac aaaaaggagt tgggtggtaga cagtcccggg ctctactacg tatttttggga 660
 actgaagctc agtccaacat tcacaaacac aggccacaag gtgcagggct gggctctctct 720
 tgttttgcaa gcaaagcctc aggtagatga ctttgacaac ttggccctga cagtggaact 780
 gttcccttgc tccatggaga acaagttagt ggaccgttcc tggagtcaac tgttgctcct 840
 gaaggctggc caccgcctca gtgtgggtct gagggcttat ctgcatggag cccaggatgc 900
 atacagagac tgggagctgt cttatcccaa caccaccagc tttggactct ttcttgtgaa 960
 acccgacaac ccatgggaat gatagggatc cgatctggta ctgcatgcac gcaatgctag 1020
 ctgccccttt cccgtcctgg gtaccccgag tctccccga cctcgggtcc caggatatgct 1080
 cccacctcca cctgccccac tcaccacctc tgctagttcc agacacctcc caagcacgca 1140
 gcaatgcagc tcaaaacgct tagcctagcc acacccccac gggaaacagc agtgattaac 1200
 cttagcaat aaacgaaagt ttaactaagc tatactaacc ccagggttgg tcaatttcgt 1260

gccagccaca ccctcgagct agcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaagcatatg	1320
actaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	1380
aaaaaaaaaa aaa	1393

<210> 68
 <211> 1393
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModA murine 41BBL

<400> 68	
gggcgaacua guauucuucu ggucccccaca gacucagaga gaacccgccca ccauggacca	60
gcacacacuu gauguggagg auaccgcgga ugccagacau ccagcaggua cuucgugccc	120
cucggaugcg gcgcuccuca gagauaccgg gcuccucgcg gacgcugcgc uccucucaga	180
uacugugcgc cccacaaaug ccgcgcuccc cacggauugu gccuaccug cgguuaaugu	240
ucgggaucgc gaggccgcgu ggccgccugc acugaacuuc uguuuccgcc acccaaagcu	300
cuauggccua gucgcuuugg uuuugcugcu ucugaucgcc gccuguguuc cuaucuucac	360
ccgcaccgag ccucggccag cgcucacaau caccaccucg cccaaccugg guacccgaga	420
gaauaaugca gaccaggua cccuguuuc ccacauuggc ugccccaca cuacacaaca	480
gggcucuccu guguucgcca agcuacuggc uaaaaaccaa gcaucguugu gcaauacaac	540
ucugaacugg cacagccaag auggagcugg gagcucauac cuaucucaag gucugaggua	600
cgaagaagac aaaaaggagu uggugguaga cagucccggg cucuacuacg uauuuuugga	660
acugaagcuc aguccaaca ucacaaacac aggccacaag gugcaggguu gggucucucu	720
uguuuugcaa gcaaagccuc agguagauga cuuugacaac uggcccuga caguggaacu	780
guucccuugc uccauggaga acaaguugu ggaccguucc uggagucaac uguugcuccu	840
gaaggcuggc caccgccuca guguggguu gagggcuuau cugcauggag cccaggaugc	900
auacagagac ugggagcugu cuuaucccaa caccaccagc uuuggacucu uucuugugaa	960
acccgacaac ccaugggaau gauagggauc cgaucuggua cugcaugcac gcaaugcuag	1020
cugccccuuu cccguccugg guaccccag ucucuccga ccucgggucc cagguaugcu	1080

```

cccaccucca ccugccccac ucaccaccuc ugcuguuucc agacaccucc caagcacgca      1140
gcaaugcagc ucaaaacgcu uagccuagcc acacccccac gggaaacagc agugauuaac      1200
cuuuagcaau aaacgaaagu uuaacuaagc uauacuaacc ccaggguugg ucaauuucgu      1260
gccagccaca cccucgagcu agcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaagcauag      1320
acuaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      1380
aaaaaaaaaa aaa                                                         1393

```

<210> 69
 <211> 309
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModB murine 41BBL

<400> 69

Met Asp Gln His Thr Leu Asp Val Glu Asp Thr Ala Asp Ala Arg His
 1 5 10 15

Pro Ala Gly Thr Ser Cys Pro Ser Asp Ala Ala Leu Leu Arg Asp Thr
 20 25 30

Gly Leu Leu Ala Asp Ala Ala Leu Leu Ser Asp Thr Val Arg Pro Thr
 35 40 45

Asn Ala Ala Leu Pro Thr Asp Ala Ala Tyr Pro Ala Val Asn Val Arg
 50 55 60

Asp Arg Glu Ala Ala Trp Pro Pro Ala Leu Asn Phe Cys Ser Arg His
 65 70 75 80

Pro Lys Leu Tyr Gly Leu Val Ala Leu Val Leu Leu Leu Leu Ile Ala
 85 90 95

Ala Cys Val Pro Ile Phe Thr Arg Thr Glu Pro Arg Pro Ala Leu Thr
 100 105 110

Ile Thr Thr Ser Pro Asn Leu Gly Thr Arg Glu Asn Asn Ala Asp Gln
 115 120 125

Val Thr Pro Val Ser His Ile Gly Cys Pro Asn Thr Thr Gln Gln Gly
 130 135 140

Ser Pro Val Phe Ala Lys Leu Leu Ala Lys Asn Gln Ala Ser Leu Cys
 145 150 155 160

Asn Thr Thr Leu Asn Trp His Ser Gln Asp Gly Ala Gly Ser Ser Tyr
 165 170 175

Leu Ser Gln Gly Leu Arg Tyr Glu Glu Asp Lys Lys Glu Leu Val Val
 180 185 190

Asp Ser Pro Gly Leu Tyr Tyr Val Phe Leu Glu Leu Lys Leu Ser Pro
 195 200 205

Thr Phe Thr Asn Thr Gly His Lys Val Gln Gly Trp Val Ser Leu Val
 210 215 220

Leu Gln Ala Lys Pro Gln Val Asp Asp Phe Asp Asn Leu Ala Leu Thr
 225 230 235 240

Val Glu Leu Phe Pro Cys Ser Met Glu Asn Lys Leu Val Asp Arg Ser
 245 250 255

Trp Ser Gln Leu Leu Leu Leu Lys Ala Gly His Arg Leu Ser Val Gly
 260 265 270

Leu Arg Ala Tyr Leu His Gly Ala Gln Asp Ala Tyr Arg Asp Trp Glu
 275 280 285

Leu Ser Tyr Pro Asn Thr Thr Ser Phe Gly Leu Phe Leu Val Lys Pro
 290 295 300

Asp Asn Pro Trp Glu
 305

<210> 70

<211> 1486

<212> DNA

<213> Artificial sequence

<220>

<223> ModB murine 41BBL (5'UTR-CDS-3'UTR)

<400> 70

```

ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat      60
tctacttcta ttgcagcaat ttaaattcatt tcttttaaag caaaagcaat tttctgaaaa      120
ttttcaccat ttacgaacga tagccatgga ccagcacaca cttgatgtgg aggataccgc      180
ggatgccaga catccagcag gtacttcgtg cccctcggat gcggcgctcc tcagagatac      240
cgggctcctc gcggacgtg cgctcctctc agatactgtg cgccccacaa atgccgcgct      300
ccccacggat gctgcctacc ctgcggttaa tgttcgggat cgcgaggccg cgtggccgcc      360
tgactgaac ttctgttccc gccacccaaa gctctatggc ctagtcgctt tggttttgct      420
gcttctgata gccgcctgtg ttcctatctt caccgcacc gagcctcggc cagcgctcac      480
aatcaccacc tcgccaacc tgggtacccg agagaataat gcagaccagg tcacccctgt      540
ttccacatt ggctgcccc aactacaca acagggtctt cctgtgttcg ccaagctact      600
ggctaaaaac caagcatcgt tgtgcaatac aactctgaac tggcacagcc aagatggagc      660
tgggagctca tacctatctc aaggtctgag gtacgaagaa gacaaaaagg agttggtggt      720
agacagtccc gggctctact acgtattttt ggaactgaag ctcatgcaa cattcacaaa      780
cacaggccac aaggtgcagg gctgggtctc tcttgttttg caagcaaagc ctgaggtaga      840
tgactttgac aacttggtccc tgacagtgga actgttcctt tgctccatgg agaacaagtt      900
agtggaccgt tcctggagtc aactgttgct cctgaaggct ggccaccgcc tcagtgtggg      960
tctgagggct tatctgcatg gagcccagga tgcatacaga gactgggagc tgtcttatcc     1020
caacaccacc agctttggac tctttcttgt gaaacccgac aacccatggg aatgataggg     1080
atccgatctg gtactgcatg cacgcaatgc tagctgcccc tttcccgtcc tgggtacccc     1140
gagtctcccc cgacctcggg tcccaggtat gtccccacct ccacctgccc cactcaccac     1200
ctctgctagt tccagacacc tccaagcac gcagcaatgc agctcaaaac gcttagccta     1260
gccacacccc cacgggaaac agcagtgatt aacctttagc aataaacgaa agtttaacta     1320

```

agctatacta accccagggt tgggtcaattt cgtgccagcc acaccctcga gctagcaaaa	1380
aaaaaaaaa aaaaaaaaaa aaaaaagcat atgactaaaa aaaaaaaaaa aaaaaaaaaa	1440
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa	1486

<210> 71
 <211> 1486
 <212> RNA
 <213> Artificial sequence

<220>
 <223> ModB murine 41BBL

<400> 71	
ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaUCAAGCAU	60
UCUACUUCUA UUGCAGCAAU UUAUAUCAUU UCUUUUAAAG CAAAAGCAAU UUUCUGAAAA	120
UUUUCACCAU UUACGAACGA UAGCCAUGGA CCAGCACACA CUUGAUGUGG AGGAUACCGC	180
GGaugccaga cauccagcag guacuucgug cccucggau gcggcgcuCC UCAGAGAUAC	240
CGGGCUCCUC gcggacgcug cgcuccucuc agauacugug cgccccacaa augccgcgcu	300
CCCCACGGAU gcugccuacc cugcgguuua uguucgggau cgcgaggccg cguggccgcc	360
UGCACUGAAC uucuguuCCC GCCACCCAAA gcucuauggc cuagucgcuu ugguuuugcu	420
gcuucugauc gccgccugug uuccuauuu caccgcacc gagccucggc cagcgcucac	480
aaucaccacc ucgccaacc ugguuacccg agagaauaau gcagaccagg ucacccugU	540
UUCCACAUU ggcugcccca acacuacaca acagggcucu ccuguguucg ccaagcuacu	600
ggcuaaaaac caagcaucgu ugugcaauac aacucugaac ugGCACAGCC aagauggagc	660
ugggagcuca uaccuauCUC aaggucugag guacgaagaa gacaaaaagg aguugguggu	720
agacaguCCC gggcucuacu acguauuuuu ggaacugaag cucaguccaa cauucacaaa	780
cacaggccac aaggugcagg gcugggucuc ucuuguuuug caagcaaagc cucagguaga	840
ugacuuugac aaCuUGGCC ugacagugga acuguuCCCU ugcuccaugg agaacaaguu	900
aguggaccgu uccuggaguc aacuguugcu ccugaaggcu ggccaccgcc ucaguguggg	960
ucugagggcu uaucugcaug gagcccagga ugcauacaga gacugggagc ugucuuaucc	1020
caacaccacc agcuuuggac ucuuucuugu gaaacccgac aacccauggg aaugauaggg	1080

```

auccgaucug guacugcaug cacgcaaugc uagcugcccc uuucccgucc ugggguacccc      1140
gagucucuccc cgaccucggg ucccagguau gcucccaccu ccaccugccc cacucaccac      1200
cucugcuagu uccagacacc ucccaagcac gcagcaaugc agcucaaaac gcuuagccua      1260
gccacacccc cacgggaaac agcagugauu aaccuuuagc aauaaaacgaa aguuuaacua      1320
agcuauacua accccagggg uggucaauuu cgugccagcc acaccucga gcuagcaaaa      1380
aaaaaaaaa aaaaaaaaaa aaaaaagcau augacuaaaa aaaaaaaaaa aaaaaaaaaa      1440
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa      1486

```

<210> 72
 <211> 935
 <212> PRT
 <213> Artificial sequence

<220>
 <223> ModA murine CD27L-CD40L

<400> 72

Met Arg Val Thr Ala Pro Arg Thr Leu Ile Leu Leu Leu Ser Gly Ala
 1 5 10 15

Leu Ala Leu Thr Glu Thr Trp Ala Gly Ser Gly Ser His Pro Glu Pro
 20 25 30

His Thr Ala Glu Leu Gln Leu Asn Leu Thr Val Pro Arg Lys Asp Pro
 35 40 45

Thr Leu Arg Trp Gly Ala Gly Pro Ala Leu Gly Arg Ser Phe Thr His
 50 55 60

Gly Pro Glu Leu Glu Glu Gly His Leu Arg Ile His Gln Asp Gly Leu
 65 70 75 80

Tyr Arg Leu His Ile Gln Val Thr Leu Ala Asn Cys Ser Ser Pro Gly
 85 90 95

Ser Thr Leu Gln His Arg Ala Thr Leu Ala Val Gly Ile Cys Ser Pro
 100 105 110

Ala Ala His Gly Ile Ser Leu Leu Arg Gly Arg Phe Gly Gln Asp Cys
115 120 125

Thr Val Ala Leu Gln Arg Leu Thr Tyr Leu Val His Gly Asp Val Leu
130 135 140

Cys Thr Asn Leu Thr Leu Pro Leu Leu Pro Ser Arg Asn Ala Asp Glu
145 150 155 160

Thr Phe Phe Gly Val Gln Trp Ile Cys Pro Gly Gly Gly Ser Gly Gly
165 170 175

Gly His Pro Glu Pro His Thr Ala Glu Leu Gln Leu Asn Leu Thr Val
180 185 190

Pro Arg Lys Asp Pro Thr Leu Arg Trp Gly Ala Gly Pro Ala Leu Gly
195 200 205

Arg Ser Phe Thr His Gly Pro Glu Leu Glu Glu Gly His Leu Arg Ile
210 215 220

His Gln Asp Gly Leu Tyr Arg Leu His Ile Gln Val Thr Leu Ala Asn
225 230 235 240

Cys Ser Ser Pro Gly Ser Thr Leu Gln His Arg Ala Thr Leu Ala Val
245 250 255

Gly Ile Cys Ser Pro Ala Ala His Gly Ile Ser Leu Leu Arg Gly Arg
260 265 270

Phe Gly Gln Asp Cys Thr Val Ala Leu Gln Arg Leu Thr Tyr Leu Val
275 280 285

His Gly Asp Val Leu Cys Thr Asn Leu Thr Leu Pro Leu Leu Pro Ser
290 295 300

Arg Asn Ala Asp Glu Thr Phe Phe Gly Val Gln Trp Ile Cys Pro Gly
305 310 315 320

Gly Gly Ser Gly Gly Gly His Pro Glu Pro His Thr Ala Glu Leu Gln
325 330 335

Leu Asn Leu Thr Val Pro Arg Lys Asp Pro Thr Leu Arg Trp Gly Ala
340 345 350

Gly Pro Ala Leu Gly Arg Ser Phe Thr His Gly Pro Glu Leu Glu Glu
355 360 365

Gly His Leu Arg Ile His Gln Asp Gly Leu Tyr Arg Leu His Ile Gln
370 375 380

Val Thr Leu Ala Asn Cys Ser Ser Pro Gly Ser Thr Leu Gln His Arg
385 390 395 400

Ala Thr Leu Ala Val Gly Ile Cys Ser Pro Ala Ala His Gly Ile Ser
405 410 415

Leu Leu Arg Gly Arg Phe Gly Gln Asp Cys Thr Val Ala Leu Gln Arg
420 425 430

Leu Thr Tyr Leu Val His Gly Asp Val Leu Cys Thr Asn Leu Thr Leu
435 440 445

Pro Leu Leu Pro Ser Arg Asn Ala Asp Glu Thr Phe Phe Gly Val Gln
450 455 460

Trp Ile Cys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
465 470 475 480

Gly Gly Ser Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val Val Ser
485 490 495

Glu Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys Gly
500 505 510

Tyr Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys Gln
515 520 525

Leu Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val Thr
530 535 540

Phe Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val Gly
545 550 555 560

Leu Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu Lys Ala
565 570 575

Ala Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val His
580 585 590

Leu Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val Asn
595 600 605

Val Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser Phe
610 615 620

Gly Leu Leu Lys Leu Gly Gly Gly Ser Gly Gly Gly Gly Asp Glu Asp
625 630 635 640

Pro Gln Ile Ala Ala His Val Val Ser Glu Ala Asn Ser Asn Ala Ala
645 650 655

Ser Val Leu Gln Trp Ala Lys Lys Gly Tyr Tyr Thr Met Lys Ser Asn
660 665 670

Leu Val Met Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Glu Gly
675 680 685

Leu Tyr Tyr Val Tyr Thr Gln Val Thr Phe Cys Ser Asn Arg Glu Pro
690 695 700

Ser Ser Gln Arg Pro Phe Ile Val Gly Leu Trp Leu Lys Pro Ser Ser
705 710 715 720

Gly Ser Glu Arg Ile Leu Leu Lys Ala Ala Asn Thr His Ser Ser Ser
725 730 735

Gln Leu Cys Glu Gln Gln Ser Val His Leu Gly Gly Val Phe Glu Leu
740 745 750

Gln Ala Gly Ala Ser Val Phe Val Asn Val Thr Glu Ala Ser Gln Val
755 760 765

Ile His Arg Val Gly Phe Ser Ser Phe Gly Leu Leu Lys Leu Gly Gly
770 775 780

Gly Ser Gly Gly Gly Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val
785 790 795 800

Val Ser Glu Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys
805 810 815

Lys Gly Tyr Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly
820 825 830

Lys Gln Leu Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln
835 840 845

Val Thr Phe Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile
850 855 860

Val Gly Leu Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu
865 870 875 880

Lys Ala Ala Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser
885 890 895

Val His Leu Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe
900 905 910

Val Asn Val Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser
915 920 925

Ser Phe Gly Leu Leu Lys Leu
930 935

<210> 73
 <211> 3281
 <212> DNA
 <213> Artificial sequence

<220>
 <223> ModA murine CD27L-CD40L (5'UTR-CDS-3'UTR)

<400> 73
 gggcgaacta gtattcttct ggtccccaca gactcagaga gaacccgcca ccatgagagt 60
 gaccgcccc agaaccctga tcctgctgct gtctggcgcc ctggccctga cagagacatg 120
 ggccggaagc ggatcccacc ccgagcccca caccgccgaa ctgcagctga acctgaccgt 180
 gccagaaaag gacccacccc tgagatgggg agctggccct gctctgggca gacaccttac 240
 acacggcccc gagctggaag aaggccacct gagaatccac caggacggcc tgtacagact 300
 gcacatccaa gtgaccctgg ccaactgcag cagccctggc tctaccctgc agcacagagc 360
 cacactggcc gtgggcatct gtagccctgc tgctcacgga atcagcctgc tgagaggcag 420
 attcggccag gactgtaccg tggccctgca gaggtgacc tatctggtgc atggcgacgt 480
 gctgtgcacc aacctgacac tgcctctgct gcccagcaga aacgccgacg aaacattctt 540
 tggagtgcag tggatttgtc ctggcggagg gtccggggga ggacaccag aacctcatac 600
 agctgaactg cagctgaacc tgaccgtgcc cagaaaggac cccaccctga gatggggagc 660
 tggccctgct ctgggcagat cctttacaca cggccccgag ctggaagaag gccacctgag 720
 aatccaccag gacggcctgt acagactgca catccaagt accctggcca actgcagcag 780
 ccctggctct accctgcagc acagagccac actggccgtg ggcatctgta gccctgctgc 840
 tcacggaatc agcctgctga gaggcagatt cggccaggac tgtaccgtgg ccctgcagag 900
 gctgacctat ctggtgcatg gcgacgtgct gtgcaccaac ctgacactgc ctctgctgcc 960
 cagcagaaac gccgacgaaa cattcttttg agtgcagtgg atttgtcctg ggggaggctc 1020
 cggaggcgga caccctgaac ctcatcacgc tgaactgcag ctgaacctga ccgtgcccag 1080
 aaaggacccc accctgagat ggggagctgg ccctgctctg ggcatatcct ttacacacgg 1140
 ccccgagctg gaagaaggcc acctgagaat ccaccaggac ggctgtaca gactgcacat 1200
 ccaagtgacc ctggccaact gcagcagccc tggctctacc ctgcagcaca gagccacact 1260

ggccgtgggc atctgtagcc ctgctgctca cggaatcagc ctgctgagag gcagattcgg	1320
ccaggactgt accgtggccc tgcagaggct gacctatctg gtgcatggcg acgtgctgtg	1380
caccaacctg aactgcctc tgctgcccag cagaaacgcc gacgagacct tcttcggcgt	1440
ccagtggatc tgccccggag gcggtggtag tggagggtggc ggggccgggtg gaggtggaag	1500
cggcgacgag gacccccaga tcgccgcccga cgtgggtgtct gaggccaaca gcaacgccgc	1560
ctctgtgctg cagtgggcca agaaaggcta ctacaccatg aagtccaacc tcgtgatgct	1620
ggaaaacggc aagcagctga ccgtgaagcg cgagggcctg tactatgtgt acaccaagt	1680
gacattctgc agcaaccgcg agcccagcag ccagaggcct tttatcgtgg gcctgtggct	1740
gaagcctagc agcggcagcg agagaatcct gctgaaggcc gccaacaccc acagcagctc	1800
tcagctgtgc gagcagcagt ctgtgcacct gggaggcgtg ttcgagctgc aagctggcgc	1860
ttccgtgttc gtgaacgtga ccgaggccag ccaagtgatc cacagagtgg gcttcagcag	1920
ctttggactg ctcaaactgg gcggagggtc cggcggaggc ggagatgaag atcctcagat	1980
tgctgcccac gtggtgtctg aggccaacag caacgccgcc tctgtgctgc agtgggcca	2040
gaaaggctac tacaccatga agtccaacct cgtgatgctg gaaaacggca agcagctgac	2100
cgtgaagcgc gagggcctgt actatgtgta cacccaagtg acattctgca gcaaccgcga	2160
gcccagcagc cagaggcctt ttatcgtggg cctgtggctg aagcctagca gcggcagcga	2220
gagaatcctg ctgaaggccg ccaacacca cagcagctct cagctgtgcg agcagcagtc	2280
tgtgcacctg ggaggcgtgt tcgagctgca agctggcgct tccgtgttcg tgaacgtgac	2340
cgaggccagc caagtgatcc acagagtggg cttcagcagc tttggactgc tcaaactggg	2400
aggcggctcc ggaggcggag gagatgaaga tcctcagatt gctgcccacg tgggtgtctga	2460
ggccaacagc aacgccgcct ctgtgctgca gtgggccaag aaaggctact acaccatgaa	2520
gtccaacctc gtgatgctgg aaaacggcaa gcagctgacc gtgaagcgcg agggcctgta	2580
ctatgtgtac acccaagtga cattctgcag caaccgcgag cccagcagcc agaggccttt	2640
tatcgtgggc ctgtggctga agcctagcag cggcagcgag agaatcctgc tgaaggccgc	2700
caacaccac agcagctctc agctgtgcga gcagcagtct gtgcacctgg gaggcgtgtt	2760
cgagctgcaa gctggcgctt ccgtgttcgt gaacgtgacc gaggccagcc aagtgatcca	2820

cagagtgggc ttctcctcct tcggcctcct gaagctgtga ctcgagagct cgctttcttg	2880
ctgtccaatt tctattaaag gttcctttgt tccctaagtc caactactaa actgggggat	2940
attatgaagg gccttgagca tctggattct gcctaataaa aaacatttat tttcattgct	3000
gcgtcgagag ctcgctttct tgctgtccaa tttctattaa aggttccttt gttccctaag	3060
tccaactact aaactggggg atattatgaa gggccttgag catctggatt ctgcctaata	3120
aaaaacattt attttcattg ctgcgtcgag acctgggtcca gagtcgctag caaaaaaaaa	3180
aaaaaaaaaa aaaaaaaaaa agcatatgac taaaaaaaaa aaaaaaaaaa aaaaaaaaaa	3240
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a	3281

<210> 74

<211> 3281

<212> RNA

<213> Artificial sequence

<220>

<223> ModA murine CD27L-CD40L

<400> 74

gggcgaacua guauucuucu ggucccccaca gacucagaga gaacccgccca ccaugagagu	60
gaccgcccc agaaccuga uccugcugcu gucuggcgcc cuggcccuga cagagacaug	120
ggccggaagc ggaucccacc ccgagcccca caccgccgaa cugcagcuga accugaccgu	180
gcccagaaag gacccaccc ugagaugggg agcuggcccu gcucugggca gauccuuuac	240
acacggcccc gagcuggaag aaggccaccu gagaauccac caggacggcc uguacagacu	300
gcacauccaa gugaccugc ccaacugcag cagcccuggc ucuaccugc agcacagagc	360
cacacuggcc gugggcaucu guagcccugc ugcucacgga aucagccugc ugagaggcag	420
auucggccag gacuguaccg uggcccugca gaggcugacc uaucuggugc auggcgacgu	480
gcugugcacc aaccugacac ugccucugcu gcccagcaga aacgccgacg aaacauucuu	540
uggagugcag uggauuuguc cuggcggagg guccggggga ggacaccag aaccucauac	600
agcugaacug cagcugaacc ugaccugcc cagaaaggac cccaccuga gauggggagc	660
uggcccugcu cugggcagau ccuuuacaca cggccccgag cuggaagaag gccaccugag	720
aauccaccag gacggccugu acagacugca cauccaagug acccuggcca acugcagcag	780

cccuggcucu acccugcagc acagagccac acuggccgug ggcaucugua gcccugcugc	840
ucacggaauc agccugcuga gaggcagauu cggccaggac uguaccgugg cccugcagag	900
gcugaccuau cuggugcaug gcgacgugcu gugcaccaac cugacacugc cucugcugcc	960
cagcagaaac gccgacgaaa cauucuuugg agugcagugg auuuguccug ggggaggcuc	1020
cggaggcgga caccugaac cucauacagc ugaacugcag cugaaccuga ccgugcccag	1080
aaaggacccc acccugagau ggggagcugg cccugcucug ggcagauccu uuacacacgg	1140
ccccgagcug gaagaaggcc accugagaau ccaccaggac ggccuguaca gacugcacau	1200
ccaagugacc cuggccaacu gcagcagccc uggcucuacc cugcagcaca gagccacacu	1260
ggccgugggc aucuguagcc cugcugcuca cggaaucagc cugcugagag gcagauucgg	1320
ccaggacugu accguggccc ugcagaggcu gaccuauucug gugcauggcg acgugcugug	1380
caccaaccug acacugccuc ugcugcccag cagaaacgcc gacgagaccu ucuucggcgu	1440
ccaguggauc ugccccggag gcgguuguag uggagguggc ggguccggug gagguggaag	1500
cggcgacgag gacccccaga ucgccgcca cguggugucu gaggccaaca gcaacgccgc	1560
cucugugcug cagugggcca agaaaggcua cuacaccaug aaguccaacc ucgugaugcu	1620
ggaaaacggc aagcagcuga ccgugaagcg cgagggccug uacuaugugu acacccaagu	1680
gacauucugc agcaaccgcg agcccagcag ccagaggccu uuuaucgugg gccuguggcu	1740
gaagccuagc agcggcagcg agagaauccu gcugaaggcc gccaacaccc acagcagcuc	1800
ucagcugugc gagcagcagu cugugcaccu gggaggcgug uucgagcugc aagcuggcg	1860
uuccguguuc gugaacguga ccgaggccag ccaagugauc cacagagugg gcuucagcag	1920
cuuuggacug cucaaacugg gcgagggguc cggcggaggc ggagaugaag auccucagau	1980
ugcugcccac guggugucug aggccaacag caacgccgcc ucugugcugc agugggcca	2040
gaaaggcuac uacaccauga aguccaaccu cgugaugcug gaaaacggca agcagcugac	2100
cgugaagcgc gagggccugu acuaugugua caccacagug acauucugca gcaaccgcga	2160
gcccagcagc cagaggccuu uuauucgugg ccuguggcug aagccuagca gcggcagcga	2220
gagaauccug cugaaggccg ccaacaccca cagcagcucu cagcugugcg agcagcaguc	2280
ugugcaccug ggaggcgugu ucgagcugca agcuggcgcu uccguguucg ugaacgugac	2340

```

cgaggccagc caagugaucc acagaguggg cuucagcagc uuuggacugc ucaaacuggg      2400
aggcggcucc ggaggcggag gagaugaaga uccucagauu gcugcccacg uggugucuga      2460
ggccaacagc aacgccgccu cugugcugca gugggccaag aaaggcuacu acaccaugaa      2520
guccaaccuc gugaugcugg aaaacggcaa gcagcugacc gugaagcgcg agggccugua      2580
cuauguguac acccaaguga cauucugcag caaccgcgag cccagcagcc agaggccuuu      2640
uaucgugggc cuguggcuga agccuagcag cggcagcgag agaauccugc ugaaggccgc      2700
caacacccac agcagcucuc agcugugcga gcagcagucu gugcaccugg gaggcguguu      2760
cgagcugcaa gcuggcgcuu ccguguucgu gaacgugacc gaggccagcc aagugaucca      2820
cagagugggc uucuccuccu ucggccuccu gaagcuguga cucgagagcu cgcuuucuug      2880
cuguccaauu ucuauuaaag guuccuuugu ucccuaaguc caacuacuaa acugggggau      2940
auuaugaagg gccuugagca ucuggauucu gccuaauaaa aaacauuuau uuucauugcu      3000
gcgucgagag cucgcuuucu ugcuguccaa uuucuauuaa agguuccuuu guucccuaag      3060
uccaacuacu aaacuggggg auauuaugaa gggccuugag caucuggauu cugccuaaua      3120
aaaaacauuu auuuucauug cugcgucgag accuggucca gagucgcuag caaaaaaaaaa      3180
aaaaaaaaaa aaaaaaaaaa agcauugac uaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      3240
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a                        3281

```

<210> 75

<211> 935

<212> PRT

<213> Artificial sequence

<220>

<223> ModB murine CD27L-CD40L

<400> 75

```

Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala
1           5           10          15

```

```

Leu Ala Pro Thr Gln Thr Arg Ala Gly Pro Gly Ser His Pro Glu Pro
          20          25          30

```

His Thr Ala Glu Leu Gln Leu Asn Leu Thr Val Pro Arg Lys Asp Pro
 35 40 45

Thr Leu Arg Trp Gly Ala Gly Pro Ala Leu Gly Arg Ser Phe Thr His
 50 55 60

Gly Pro Glu Leu Glu Glu Gly His Leu Arg Ile His Gln Asp Gly Leu
 65 70 75 80

Tyr Arg Leu His Ile Gln Val Thr Leu Ala Asn Cys Ser Ser Pro Gly
 85 90 95

Ser Thr Leu Gln His Arg Ala Thr Leu Ala Val Gly Ile Cys Ser Pro
 100 105 110

Ala Ala His Gly Ile Ser Leu Leu Arg Gly Arg Phe Gly Gln Asp Cys
 115 120 125

Thr Val Ala Leu Gln Arg Leu Thr Tyr Leu Val His Gly Asp Val Leu
 130 135 140

Cys Thr Asn Leu Thr Leu Pro Leu Leu Pro Ser Arg Asn Ala Asp Glu
 145 150 155 160

Thr Phe Phe Gly Val Gln Trp Ile Cys Pro Gly Gly Gly Ser Gly Gly
 165 170 175

Gly His Pro Glu Pro His Thr Ala Glu Leu Gln Leu Asn Leu Thr Val
 180 185 190

Pro Arg Lys Asp Pro Thr Leu Arg Trp Gly Ala Gly Pro Ala Leu Gly
 195 200 205

Arg Ser Phe Thr His Gly Pro Glu Leu Glu Glu Gly His Leu Arg Ile
 210 215 220

His Gln Asp Gly Leu Tyr Arg Leu His Ile Gln Val Thr Leu Ala Asn
 225 230 235 240

Cys Ser Ser Pro Gly Ser Thr Leu Gln His Arg Ala Thr Leu Ala Val
 245 250 255

Gly Ile Cys Ser Pro Ala Ala His Gly Ile Ser Leu Leu Arg Gly Arg
 260 265 270

Phe Gly Gln Asp Cys Thr Val Ala Leu Gln Arg Leu Thr Tyr Leu Val
 275 280 285

His Gly Asp Val Leu Cys Thr Asn Leu Thr Leu Pro Leu Leu Pro Ser
 290 295 300

Arg Asn Ala Asp Glu Thr Phe Phe Gly Val Gln Trp Ile Cys Pro Gly
 305 310 315 320

Gly Gly Ser Gly Gly Gly His Pro Glu Pro His Thr Ala Glu Leu Gln
 325 330 335

Leu Asn Leu Thr Val Pro Arg Lys Asp Pro Thr Leu Arg Trp Gly Ala
 340 345 350

Gly Pro Ala Leu Gly Arg Ser Phe Thr His Gly Pro Glu Leu Glu Glu
 355 360 365

Gly His Leu Arg Ile His Gln Asp Gly Leu Tyr Arg Leu His Ile Gln
 370 375 380

Val Thr Leu Ala Asn Cys Ser Ser Pro Gly Ser Thr Leu Gln His Arg
 385 390 395 400

Ala Thr Leu Ala Val Gly Ile Cys Ser Pro Ala Ala His Gly Ile Ser
 405 410 415

Leu Leu Arg Gly Arg Phe Gly Gln Asp Cys Thr Val Ala Leu Gln Arg
 420 425 430

Leu Thr Tyr Leu Val His Gly Asp Val Leu Cys Thr Asn Leu Thr Leu
 435 440 445

Pro Leu Leu Pro Ser Arg Asn Ala Asp Glu Thr Phe Phe Gly Val Gln
 450 455 460

Trp Ile Cys Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 465 470 475 480

Gly Gly Ser Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val Val Ser
 485 490 495

Glu Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys Gly
 500 505 510

Tyr Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys Gln
 515 520 525

Leu Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val Thr
 530 535 540

Phe Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val Gly
 545 550 555 560

Leu Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu Lys Ala
 565 570 575

Ala Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val His
 580 585 590

Leu Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val Asn
 595 600 605

Val Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser Phe
 610 615 620

Gly Leu Leu Lys Leu Gly Gly Gly Ser Gly Gly Gly Gly Asp Glu Asp
 625 630 635 640

Pro Gln Ile Ala Ala His Val Val Ser Glu Ala Asn Ser Asn Ala Ala
 645 650 655

Ser Val Leu Gln Trp Ala Lys Lys Gly Tyr Tyr Thr Met Lys Ser Asn
660 665 670

Leu Val Met Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Glu Gly
675 680 685

Leu Tyr Tyr Val Tyr Thr Gln Val Thr Phe Cys Ser Asn Arg Glu Pro
690 695 700

Ser Ser Gln Arg Pro Phe Ile Val Gly Leu Trp Leu Lys Pro Ser Ser
705 710 715 720

Gly Ser Glu Arg Ile Leu Leu Lys Ala Ala Asn Thr His Ser Ser Ser
725 730 735

Gln Leu Cys Glu Gln Gln Ser Val His Leu Gly Gly Val Phe Glu Leu
740 745 750

Gln Ala Gly Ala Ser Val Phe Val Asn Val Thr Glu Ala Ser Gln Val
755 760 765

Ile His Arg Val Gly Phe Ser Ser Phe Gly Leu Leu Lys Leu Gly Gly
770 775 780

Gly Ser Gly Gly Gly Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val
785 790 795 800

Val Ser Glu Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys
805 810 815

Lys Gly Tyr Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly
820 825 830

Lys Gln Leu Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln
835 840 845

Val Thr Phe Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile
850 855 860

Val Gly Leu Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu
865 870 875 880

Lys Ala Ala Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser
885 890 895

Val His Leu Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe
900 905 910

Val Asn Val Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser
915 920 925

Ser Phe Gly Leu Leu Lys Leu
930 935

<210> 76
<211> 3361
<212> DNA
<213> Artificial sequence

<220>
<223> ModB murine CD27L-CD40L (5'UTR-CDS-3'UTR)

<400> 76
ggaataaact agtctcaaca caacatatac aaaacaaacg aatctcaagc aatcaagcat 60
tctacttcta ttgcagcaat ttaaattcatt tctttttaag caaaagcaat tttctgaaaa 120
ttttcaccat ttacgaacga tagccatggg cgccatggcc cctagaacat tgctcctgct 180
gctggccgct gccctggccc ctacacagac aagagctgga cctggatccc accccgagcc 240
ccacaccgcc gaactgcagc tgaacctgac cgtgcccaga aaggaccca ccctgagatg 300
gggagctggc cctgctctgg gcagatcctt tacacacggc cccgagctgg aagaaggcca 360
cctgagaatc caccaggacg gcctgtacag actgcacatc caagtgaccc tggccaactg 420
cagcagccct ggctctaccc tgcagcacag agccacactg gccgtgggca tctgtagccc 480
tgctgctcac ggaatcagcc tgctgagagg cagattcggc caggactgta ccgtggccct 540
gcagaggctg acctatctgg tgcattggcg cgtgctgtgc accaacctga cactgcctct 600
gctgcccagc agaaacgccg acgaaacatt ctttggagtg cagtggattt gtcctggcgg 660
agggtccggg ggaggacacc cagaacctca tacagctgaa ctgcagctga acctgaccgt 720

gcccagaaag gacccacccc tgagatgggg agctggccct gctctgggca gatcctttac	780
acacggcccc gagctggaag aaggccacct gagaatccac caggacggcc tgtacagact	840
gcacatccaa gtgaccctgg ccaactgcag cagccctggc tctaccctgc agcacagagc	900
cacactggcc gtgggcatct gtagccctgc tgctcacgga atcagcctgc tgagaggcag	960
attcggccag gactgtaccg tggccctgca gaggctgacc tatctggtgc atggcgacgt	1020
gctgtgcacc aacctgacac tgcctctgct gcccagcaga aacgccgacg aaacattctt	1080
tggagtgcag tggatttgtc ctgggggagg ctccggaggc ggacaccctg aacctcatac	1140
agctgaactg cagctgaacc tgaccgtgcc cagaaaggac cccaccctga gatgggggagc	1200
tggccctgct ctgggcagat cctttacaca cggccccgag ctggaagaag gccacctgag	1260
aatccaccag gacggcctgt acagactgca catccaagt accctggcca actgcagcag	1320
ccctggctct accctgcagc acagagccac actggccgtg ggcatctgta gccctgctgc	1380
tcacggaatc agcctgctga gaggcagatt cggccaggac tgtaccgtgg ccctgcagag	1440
gctgacctat ctggtgcatg gcgacgtgct gtgcaccaac ctgacactgc ctctgctgcc	1500
cagcagaaac gccgacgaga ccttcttcgg cgtccagtgg atctgccccg gaggcggtgg	1560
tagtggaggt ggcgggtccg gtggaggtgg aagcggcgac gaggaccccc agatcgccgc	1620
ccacgtggtg tctgaggcca acagcaacgc cgcctctgtg ctgcagtggg ccaagaaagg	1680
ctactacacc atgaagtcca acctcgtgat gctggaaaac ggcaagcagc tgaccgtgaa	1740
gcgcgagggc ctgtactatg tgtacacca agtgacattc tgcagcaacc gcgagcccag	1800
cagccagagg ctttttatcg tgggcctgtg gctgaagcct agcagcggca gcgagagaat	1860
cctgctgaag gccgccaaca cccacagcag ctctcagctg tgcgagcagc agtctgtgca	1920
cctgggaggc gtgttcgagc tgcaagctgg cgcttccgtg ttcgtgaacg tgaccgaggc	1980
cagccaagtg atccacagag tgggcttcag cagctttgga ctgctcaaac tgggcggagg	2040
gtccggcgga ggcggagatg aagatcctca gattgctgcc cacgtggtgt ctgaggccaa	2100
cagcaacgcc gcctctgtgc tgcagtgggc caagaaaggc tactacacca tgaagtccaa	2160
cctcgtgatg ctggaaaacg gcaagcagct gaccgtgaag cgcgagggcc tgtactatgt	2220
gtacacccaa gtgacattct gcagcaaccg cgagcccagc agccagaggc cttttatcgt	2280

```

gggcctgtgg ctgaagccta gcagcggcag cgagagaatc ctgctgaagg ccgccaacac      2340
ccacagcagc tctcagctgt gcgagcagca gtctgtgcac ctgggaggcg tgttcgagct      2400
gcaagctggc gcttccgtgt tcgtgaacgt gaccgaggcc agccaagtga tccacagagt      2460
gggcttcagc agctttggac tgctcaaact gggaggcggc tccggaggcg gaggagatga      2520
agatcctcag attgctgccc acgtggtgtc tgaggccaac agcaacgccg cctctgtgct      2580
gcagtgggcc aagaaaggct actacaccat gaagtccaac ctcgtgatgc tggaaaacgg      2640
caagcagctg accgtgaagc gcgagggcct gtactatgtg tacaccaag tgacattctg      2700
cagcaaccgc gagcccagca gccagaggcc ttttatcgtg ggctgtggc tgaagcctag      2760
cagcggcagc gagagaatcc tgctgaaggc cgccaacacc cacagcagct ctcagctgtg      2820
cgagcagcag tctgtgcacc tgggaggcgt gttcgagctg caagctggcg cttccgtgtt      2880
cgtgaacgtg accgaggcca gccaagtgat ccacagagtg ggcttctcct ctttcggcct      2940
cctgaagctg tgactcgacg tcctggtact gcatgcacgc aatgctagct gcccctttcc      3000
cgtcctgggt accccgagtc tccccgacc tcgggtccca ggtatgctcc cacctccacc      3060
tgccccactc accacctctg ctagttccag acacctccca agcacgcagc aatgcagctc      3120
aaaacgctta gcctagccac acccccacgg gaaacagcag tgattaacct ttagcaataa      3180
acgaaagttt aactaagcta tactaacccc agggttggtc aatttcgtgc cagccacacc      3240
ctcgagctag caaaaaaaaaa aaaaaaaaaa aaaaaaaaaa agcatatgac taaaaaaaaa      3300
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa      3360
a                                                                 3361

```

<210> 77

<211> 3361

<212> RNA

<213> Artificial sequence

<220>

<223> ModB murine CD27L-CD40L

<400> 77

```

ggaauaaacu agucucaaca caacauauac aaaacaaacg aaucucaagc aaucaagcau      60

```

```

ucuacuucua uugcagcaau uuaaaucuu uuuuuuaag caaaagcaau uuucugaaaa      120

```

uuuucaccau uuacgaacga uagccauggg cgccauggcc ccuagaacau ugcuccugcu	180
gcuggccgcu gcccuiggccc cuacacagac aagagcugga ccuggauccc accccgagcc	240
ccacaccgcc gaacugcagc ugaaccugac cgugcccaga aaggacccca cccugagaug	300
gggagcuggc ccugcucugg gcagauccuu uacacacggc cccgagcugg aagaaggcca	360
ccugagaau caccaggacg gccuguacag acugcacau caagugaccc uggccaacug	420
cagcagcccu ggcucuaccc ugcagcacag agccacacug gccgugggca ucuguagccc	480
ugcugcucac ggaucagcc ugcugagagg cagauucggc caggacugua ccguggcccu	540
gcagaggcug accuauucgg ugcuauggcg cgugcugug accaaccuga cacugccucu	600
gcugcccagc agaaacgcc acgaaacau cuuuggagug caguggauuu guccuggcgg	660
aggguccggg ggaggacacc cagaaccuca uacagcugaa cugcagcuga accugaccgu	720
gcccagaaa gacccccacc ugagaugggg agcuggcccu gcucugggca gauccuuuac	780
acacggcccc gagcuggaag aaggccaccu gagaauccac caggacggcc uguacagacu	840
gcacauccaa gugaccucgg ccaacugcag cagcccuggc ucuaccucug agcacagagc	900
cacacuggcc gugggcaucu guagcccugc ugcucacgga aucagccugc ugagaggcag	960
auucggccag gacuguaccg uggcccugca gaggcugacc uaucuggugc auggcgacgu	1020
gcugugcacc aaccugacac ugccucugcu gccagcaga aacgccgacg aaacauucuu	1080
uggagugcag uggauuuguc cugggggagg cuccggaggc ggacaccucg aaccucauac	1140
agcugaacug cagcugaacc ugaccgugcc cagaaaggac cccaccucga gauggggagc	1200
uggcccugcu cugggcagau ccuuuacaca cggccccgag cuggaagaag gccaccugag	1260
aauccaccag gacggccugu acagacugca cauccaagug acccuggcca acugcagcag	1320
cccuggcucu acccugcagc acagagccac acuggccgug ggcaucugua gccucugc	1380
ucacggaau agccugcuga gaggcagauu cggccaggac uguaccgugg cccugcagag	1440
gcugaccuau cuggugcaug gcgacugcu gugcaccaac cugacacugc cucugcugcc	1500
cagcagaaac gccgacgaga ccuucuucgg cguccagugg aucugccccg gaggcggugg	1560
uaguggaggu ggcggguccg guggaggugg aagcggcgac gaggaacccc agaucgccgc	1620
ccacguggug ucugaggcca acagcaacgc cgccucugug cugcaguggg ccaagaaagg	1680

cuacuacacc	augaagucca	accucgugau	gcuggaaaac	ggcaagcagc	ugaccgugaa	1740
gcgcgagggc	cuguacuaug	uguacaccca	agugacauuc	ugcagcaacc	gcgagcccag	1800
cagccagagg	ccuuuuaucg	ugggccugug	gcugaagccu	agcagcggca	gcgagagaau	1860
ccugcugaag	gccgccaaca	cccacagcag	cucucagcug	ugcagcagc	agucugugca	1920
ccugggagggc	guguucgagc	ugcaagcugg	cgcuuccgug	uucgugaacg	ugaccgagggc	1980
cagccaagug	auccacagag	ugggcuucag	cagcuuugga	cugcucaaac	ugggcggagg	2040
guccggcgga	ggcggagaug	aagauccuca	gauugcugcc	cacguggugu	cugaggccaa	2100
cagcaacgcc	gccucugugc	ugcagugggc	caagaaaggc	uacuacacca	ugaaguccaa	2160
ccucgugaug	cuggaaaacg	gcaagcagcu	gaccgugaag	cgcgagggcc	uguacuauugu	2220
guacacccaa	gugacauucu	gcagcaaccg	cgagcccagc	agccagaggc	cuuuuaucgu	2280
gggcccugugg	cugaagccua	gcagcggcag	cgagagaau	cugcugaagg	ccgccaacac	2340
ccacagcagc	ucucagcugu	gcgagcagca	gucugugcac	cugggaggcg	uguucgagcu	2400
gcaagcuggc	gcuuccgugu	ucgugaacgu	gaccgaggcc	agccaaguga	uccacagagu	2460
gggcuucagc	agcuuuggac	ugcucaaacu	gggaggcggc	uccggaggcg	gaggagauga	2520
agauccucag	auugcugccc	acgugguguc	ugaggccaac	agcaacgccg	ccucugugcu	2580
gcagugggcc	aagaaaggcu	acuacaccu	gaaguccaac	cucgugaugc	uggaaaacgg	2640
caagcagcug	accgugaagc	gcgagggccu	guacuauug	uacacccaag	ugacauucug	2700
cagcaaccgc	gagcccagca	gccagaggcc	uuuuaucgug	ggccuguggc	ugaagccuag	2760
cagcggcagc	gagagaaucc	ugcugaaggc	cgccaacacc	cacagcagcu	cucagcugug	2820
cgagcagcag	ucugugcacc	ugggaggcgu	guucgagcug	caagcuggcg	cuuccguguu	2880
cgugaacgug	accgaggcca	gccaagugau	ccacagagug	ggcuucuccu	ccuucggccu	2940
ccugaagcug	ugacucgacg	uccugguacu	gcaugcacgc	aaugcuagcu	gccccuuucc	3000
cguccugggu	accccaguc	ucccccgacc	ucggguccca	gguaugcucc	caccuccacc	3060
ugccccacuc	accaccucug	cuaguuccag	acaccuccca	agcacgcagc	aaugcagcuc	3120
aaaacgcuaa	gccuagccac	acccccacgg	gaaacagcag	ugauuaaccu	uuagcaauaa	3180
acgaaaguuu	aacuaagcua	uacuaacccc	aggguugguc	aauuucgugc	cagccacacc	3240

cucgagcuag caaaaaaaaa aaaaaaaaaa aaaaaaaaaa agcauauagac uaaaaaaaaa 3300
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 3360
 a 3361

<210> 78
 <211> 110
 <212> RNA
 <213> Artificial sequence

<220>
 <223> Poly-A

<400> 78
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa gcauauagacu aaaaaaaaaa aaaaaaaaaa 60
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 110

<210> 79
 <211> 444
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Anti-PD1 Mab heavy chain

<400> 79

Glu Val Gln Leu Leu Glu Ser Gly Gly Val Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
 20 25 30

Gly Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Gly Gly Gly Arg Asp Thr Tyr Phe Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Lys Gly Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190

Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn
 195 200 205

Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
 210 215 220

Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe
 225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 245 250 255

Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
 260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 290 295 300

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 305 310 315 320

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
 325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln
 340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 385 390 395 400

Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu
 405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
 435 440

<210> 80

<211> 214

<212> PRT

<213> Artificial sequence

<220>

<223> Anti-PD1 Mab light chain

<400> 80

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Ser Ile Thr Ile Thr Cys Arg Ala Ser Leu Ser Ile Asn Thr Phe
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu His Gly Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg Thr Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Ser Asn Thr Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Val Val Asp Phe Arg Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 81
 <211> 8
 <212> PRT
 <213> Artificial sequence

<220>
 <223> HCDR1

<400> 81

Gly Phe Thr Phe Ser Asn Phe Gly
 1 5

<210> 82
 <211> 8
 <212> PRT
 <213> Artificial sequence

<220>
 <223> HCDR2

<400> 82

Ile Ser Gly Gly Gly Arg Asp Thr
 1 5

<210> 83
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> HCDR3

<400> 83

Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr
 1 5 10

<210> 84
 <211> 6
 <212> PRT
 <213> Artificial sequence

<220>
 <223> LCDR1

<400> 84

Leu Ser Ile Asn Thr Phe
1 5

<210> 85

<211> 3

<212> PRT

<213> Artificial sequence

<220>

<223> LCDR2

<400> 85

Ala Ala Ser
1

<210> 86

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> LCDR3

<400> 86

Gln Gln Ser Ser Asn Thr Pro Phe Thr
1 5

<210> 87

<211> 117

<212> PRT

<213> Artificial sequence

<220>

<223> Anti-PD1 Mab VH

<400> 87

Glu Val Gln Leu Leu Glu Ser Gly Gly Val Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
20 25 30

Gly Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Gly Gly Gly Arg Asp Thr Tyr Phe Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Gly Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 88
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Anti-PD1 Mab VL

<400> 88

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Ile Thr Ile Thr Cys Arg Ala Ser Leu Ser Ile Asn Thr Phe
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu His Gly Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg Thr Leu Gln Pro
65 70 75 80

2018-02-20_01183-0001-00PCT_Seq_List_ST25_FINAL_ST25.txt

Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Ser	Asn	Thr	Pro	Phe
				85					90					95	

Thr	Phe	Gly	Pro	Gly	Thr	Val	Val	Asp	Phe	Arg
			100					105		