

What is claimed:

1. A Pichinde virus genomic segment, wherein the genomic segment is engineered to carry a viral open reading frame (“ORF”) in a position other than the wild-type position of the ORF, wherein the Pichinde virus genomic segment is selected from the group consisting of:
 - (i) an S segment, wherein the ORF encoding the nucleoprotein (“NP”) is under control of a Pichinde virus 5’ untranslated region (“UTR”);
 - (ii) an S segment, wherein the ORF encoding the matrix protein Z (“Z protein”) is under control of a Pichinde virus 5’ UTR;
 - (iii) an S segment, wherein the ORF encoding the RNA dependent RNA polymerase L (“L protein”) is under control of a Pichinde virus 5’ UTR;
 - (iv) an S segment, wherein the ORF encoding the viral glycoprotein (“GP”) is under control of a Pichinde virus 3’ UTR;
 - (v) an S segment, wherein the ORF encoding the L protein is under control of a Pichinde virus 3’ UTR;
 - (vi) an S segment, wherein the ORF encoding the Z protein is under control of a Pichinde virus 3’ UTR;
 - (vii) an L segment, wherein the ORF encoding the GP is under control of a Pichinde virus 5’ UTR;
 - (viii) an L segment, wherein the ORF encoding the NP is under control of a Pichinde virus 5’ UTR;
 - (ix) an L segment, wherein the ORF encoding the L protein is under control of a Pichinde virus 5’ UTR;
 - (x) an L segment, wherein the ORF encoding the GP is under control of a Pichinde virus 3’ UTR;

- (xi) an L segment, wherein the ORF encoding the NP is under control of a Pichinde virus 3' UTR; and
 - (xii) an L segment, wherein the ORF encoding the Z protein is under control of a Pichinde virus 3' UTR.
- 2. The Pichinde virus genomic segment of claim 1, wherein the Pichinde virus 3' UTR is the 3' UTR of the Pichinde virus S segment or the Pichinde virus L segment, and wherein the Pichinde virus 5' UTR is the 5' UTR of the Pichinde virus S segment or the Pichinde virus L segment.
- 3. A cDNA of the Pichinde virus genomic segment of claim 1.
- 4. A DNA expression vector comprising the cDNA of claim 3.
- 5. A host cell comprising the Pichinde virus genomic segment of claim 1, the cDNA of claim 3, or the DNA expression vector of claim 4.
- 6. A Pichinde virus particle comprising the Pichinde virus genomic segment of claim 1 or 2 and a second Pichinde virus genomic segment so that the Pichinde virus particle comprises an S segment and an L segment.
- 7. The Pichinde virus particle of claim 6, wherein the Pichinde virus particle is infectious and replication competent.
- 8. The Pichinde virus particle of claim 6, wherein the Pichinde virus particle is attenuated.
- 9. The Pichinde virus particle of claim 6, wherein the Pichinde virus particle is infectious but unable to produce further infectious progeny in non-complementing cells.
- 10. The Pichinde virus particle of claim 9, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed or functionally inactivated.
- 11. The Pichinde virus particle of claim 9, wherein at least one of the four ORFs encoding GP, NP, Z protein, and L protein is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.

12. The Pichinde virus particle of claim 9, wherein only one of the four ORFs encoding GP, NP, Z protein and L protein is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.
13. The Pichinde virus particle of claim 9, wherein the ORF encoding GP is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.
14. The Pichinde virus particle of claim 9, wherein the ORF encoding NP is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.
15. The Pichinde virus particle of claim 9, wherein the ORF encoding the Z protein is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.
16. The Pichinde virus particle of claim 9, wherein the ORF encoding the L protein is removed and replaced with a heterologous ORF from an organism other than a Pichinde virus.
17. The Pichinde virus particle of anyone of claims 11 to 16, wherein the heterologous ORF encodes a reporter protein.
18. The Pichinde virus particle of anyone of claims 11 to 16, wherein the heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen.
19. The Pichinde virus particle of claim 18, wherein the heterologous ORF encoding an antigen is selected from human immunodeficiency virus antigens, papillomavirus antigens, hepatitis B virus antigens, hepatitis C virus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, tumor associated antigens, and tumor specific antigens, wherein the tumor specific antigens comprise tumor neoantigens and tumor neoepitopes.
20. The Pichinde virus particle of anyone of claims 11 to 18, wherein the growth or infectivity of the Pichinde virus particle is not affected by the heterologous ORF from an organism other than a Pichinde virus.
21. A method of producing the Pichinde virus genomic segment of claim 1, wherein said method comprises transcribing the cDNA of claim 3.

22. A method of generating the Pichinde virus particle of claim 6, wherein the method comprises:
- (i) transfecting into a host cell the cDNA of claim 3;
 - (ii) transfecting into the host cell a plasmid comprising the cDNA of the second Pichinde virus genomic segment;
 - (iii) maintaining the host cell under conditions suitable for virus formation; and
 - (iv) harvesting the Pichinde virus particle.
23. The method of claim 22, wherein the transcription of the L segment and the S segment is performed using a bidirectional promoter.
24. The method of claim 22, wherein the method further comprises transfecting into a host cell one or more nucleic acids encoding a Pichinde virus polymerase.
25. The method of claim 24, wherein the Pichinde virus polymerase is the L protein.
26. The method of claim 22 or 24, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP protein.
27. The method of claim 22, wherein transcription of the L segment, and the S segment are each under the control of a promoter selected from the group consisting of:
- (i) a RNA polymerase I promoter;
 - (ii) a RNA polymerase II promoter; and
 - (iii) a T7 promoter.
28. A vaccine comprising the Pichinde virus particle of claim 6 to 19 and a pharmaceutically acceptable carrier.
29. A pharmaceutical composition comprising a Pichinde virus particle of claim 6 to 19 and a pharmaceutically acceptable carrier.

30. The Pichinde virus genomic segment of claim 1 or the Pichinde virus particle of claim 6, wherein the Pichinde virus genomic segment or Pichinde virus particle is derived from the strain Munchique CoAn4763 isolate P18, or P2 strain.
31. The Pichinde virus particle of any one of claims 6 to 8, wherein the particle is tri-segmented and comprises one L segment and two S segments, wherein optionally propagation of the tri-segmented Pichinde virus particle does not result in a replication-competent bi-segmented viral particle after 70 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1) and having been infected with 10^4 PFU of the tri-segmented Pichinde virus particle.
32. The Pichinde virus particle of claim 31, wherein inter-segmental recombination of the two S segments, uniting two Pichinde virus ORFs on only one instead of two separate segments, abrogates viral promoter activity.
33. The Pichinde virus particle of any one of claims 6 to 8, wherein the particle is tri-segmented and comprises two L segments and one S segment, wherein optionally propagation of the tri-segmented Pichinde virus particle does not result in a replication-competent bi-segmented viral particle after 70 days of persistent infection in mice lacking type I interferon receptor, type II interferon receptor and recombination activating gene 1 (RAG1) and having been infected with 10^4 PFU of the tri-segmented Pichinde virus particle.
34. The Pichinde virus particle of claim 33, wherein, inter-segmental recombination of the two L segments, uniting two Pichinde virus ORFs on only one instead of two separate segments, abrogates viral promoter activity.
35. The Pichinde virus particle of claim 31, wherein one of the two S segments is selected from the group consisting of:
- (i) an S segment, wherein the ORF encoding the NP is under control of a Pichinde virus 5' UTR;
 - (ii) an S segment, wherein the ORF encoding the Z protein is under control of a Pichinde virus 5' UTR;

- (iii) an S segment, wherein the ORF encoding the L protein is under control of a Pichinde virus 5' UTR;
 - (iv) an S segment, wherein the ORF encoding the GP is under control of a Pichinde virus 3' UTR;
 - (v) an S segment, wherein the ORF encoding the L is under control of a Pichinde virus 3' UTR; and
 - (vi) an S segment, wherein the ORF encoding the Z protein is under control of a Pichinde virus 3' UTR.
36. The Pichinde virus particle of claim 33, wherein one of the two L segments is selected from the group consisting of:
- (i) an L segment, wherein the ORF encoding the GP is under control of a Pichinde virus 5' UTR;
 - (ii) an L segment, wherein the ORF encoding the NP is under control of a Pichinde virus 5' UTR;
 - (iii) an L segment, wherein the ORF encoding the L protein is under control of a Pichinde virus 5' UTR;
 - (iv) an L segment, wherein the ORF encoding the GP is under control of a Pichinde virus 3' UTR;
 - (v) an L segment, wherein the ORF encoding the NP is under control of a Pichinde virus 3' UTR; and
 - (vi) an L segment, wherein the ORF encoding the Z protein is under control of a Pichinde virus 3' UTR.
37. The Pichinde virus particle of claim 35 or 36, wherein the Pichinde virus 3' UTR is the 3' UTR of the Pichinde virus S segment or the Pichinde virus L segment, and wherein the Pichinde virus 5' UTR is the 5' UTR of the Pichinde virus S segment or the Pichinde virus L segment.

38. The Pichinde virus particle of claim 31, wherein the two S segments comprise (i) one or two heterologous ORFs from an organism other than a Pichinde virus; or (ii) one or two duplicated Pichinde virus ORFs; or (iii) one heterologous ORF from an organism other than a Pichinde virus and one duplicated Pichinde virus ORF.
39. The Pichinde virus particle of claim 33, wherein the two L segments comprise (i) one or two heterologous ORFs from an organism other than a Pichinde virus; or (ii) two duplicated Pichinde virus ORFs; or (iii) one heterologous ORF from an organism other than a Pichinde virus and one duplicated Pichinde virus ORF.
40. The Pichinde virus particle of claim 38 or 39, wherein the heterologous ORF encodes an antigen derived from an infectious organism, tumor, or allergen.
41. The Pichinde virus particle of claim 40, wherein the heterologous ORF encoding an antigen is selected from human immunodeficiency virus antigens, papillomavirus antigens, hepatitis B virus antigens, hepatitis C virus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, tumor associated antigens, and tumor specific antigens, wherein the tumor specific antigens comprise tumor neoantigens and tumor neoepitopes.
42. The Pichinde virus particle of claim 38 or 39, wherein at least one heterologous ORF encodes a fluorescent protein.
43. The Pichinde virus particle of claim 42, wherein the fluorescent protein is green fluorescent protein or red fluorescent protein.
44. The Pichinde virus particle of any one of claims 31 to 41, wherein the Pichinde virus particle comprises all four Pichinde virus ORFs, and wherein the Pichinde virus particle is infectious and replication competent.
45. The Pichinde virus particle of any one of claims 31 to 43, wherein the Pichinde virus particle lacks one or more of the four Pichinde virus ORFs, wherein the Pichinde virus particle is infectious but unable to produce further infectious progeny in non-complementing cells.
46. The Pichinde virus particle of any one of claims 31 to 43, wherein the Pichinde virus particle lacks one of the four Pichinde virus ORFs, wherein the Pichinde virus particle

is infectious but unable to produce further infectious progeny in non-complementing cells.

47. The Pichinde virus particle of claim 45 or 46, wherein the Pichinde virus lacks the GP ORF.
48. The Pichinde virus particle of claim 6, wherein the particle comprises one L segment and two S segments, wherein a first S segment is engineered to carry an ORF encoding GP in a position under control of a Pichinde virus 3' UTR and an ORF encoding a first gene of interest in a position under control of a Pichinde virus 5' UTR and a second S segment is engineered to carry an ORF encoding NP in a position under control of a Pichinde virus 3' UTR and an ORF encoding a second gene of interest in a position under control of a Pichinde virus 5' UTR.
49. The Pichinde virus particle of claim 6, wherein the particle comprises one L segment and two S segments, wherein a first S segment is engineered to carry an ORF encoding GP in a position under control of a Pichinde virus 5' UTR and an ORF encoding a first gene of interest in a position under control of a Pichinde virus 3' UTR and a second S segment is engineered to carry an ORF encoding NP in a position under control of a Pichinde virus 5' UTR and an ORF encoding a second gene of interest in a position under control of a Pichinde virus 3' UTR.
50. The Pichinde virus particle of claim 48 or 49, wherein the gene of interest encodes an antigen derived from an infectious organism, tumor, or allergen.
51. The Pichinde virus particle of claim 50, wherein the gene of interest encodes an antigen selected from human immunodeficiency virus antigens, papillomavirus antigens, hepatitis B virus antigens, hepatitis C virus antigens, varizella zoster virus antigens, cytomegalovirus antigens, mycobacterium tuberculosis antigens, tumor associated antigens, and tumor specific antigens, wherein the tumor specific antigens comprise tumor neoantigens and tumor neoepitopes.
52. The Pichinde virus particle of claim 48 or 49, wherein at least one gene of interest encodes a fluorescent protein.

53. The Pichinde virus particle of claim 52, wherein the fluorescent protein is green fluorescent protein or red fluorescent protein.
54. A cDNA of the Pichinde virus particle genome of any one of claims 31, 33, 35, 36, 48 or 49.
55. A DNA expression vector comprising the cDNA of claim 54.
56. A host cell comprising the Pichinde virus particle of claim 31 or 33, the cDNA of claim 54, or the vector of claim 55.
57. The Pichinde virus particle of any one of claims 31 to 44, 48, or 49, wherein the Pichinde virus particle is attenuated.
58. A method of generating the Pichinde virus particle of claim 31, wherein the method comprises:
- (i) transfecting into a host cell one or more cDNAs of the L segment and two S segments;
 - (ii) maintaining the host cell under conditions suitable for virus formation; and
 - (iii) harvesting the Pichinde virus particle.
59. A method of generating the Pichinde virus particle of claim 33, wherein the method comprises:
- (i) transfecting into a host cell one or more cDNAs of two L segments and one S segment;
 - (ii) maintaining the host cell under conditions suitable for virus formation; and
 - (iii) harvesting the Pichinde virus particle.
60. The method of claim 58, wherein the transcription of one L segment and two S segments is performed using a bidirectional promoter.

61. The method of claim 59, wherein the transcription of two L segments and one S segment is performed using a bidirectional promoter.
62. The method of claim 58 or 59, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding a Pichinde virus polymerase.
63. The method of claim 62, wherein the Pichinde virus polymerase is the L protein.
64. The method of claim 58, 59, 60 or 61, wherein the method further comprises transfecting into the host cell one or more nucleic acids encoding the NP protein.
65. The method of claim 58, wherein transcription of the L segment, and the two S segments are each under the control of a promoter selected from the group consisting of:
- (i) a RNA polymerase I promoter;
 - (ii) a RNA polymerase II promoter; and
 - (iii) a T7 promoter.
66. The method of claim 59, wherein transcription of two L segments, and the S segment are each under the control of a promoter selected from the group consisting of:
- (i) a RNA polymerase I promoter;
 - (ii) a RNA polymerase II promoter; and
 - (iii) a T7 promoter.
67. The Pichinde virus particle of any one of claims 31 to 49, wherein the tri-segmented Pichinde virus particle has the same tropism as the bi-segmented Pichinde virus particle.
68. The Pichinde virus particle of any one of claims 31 to 43 or 45 to 49, wherein the Pichinde virus particle is replication deficient.
69. A vaccine comprising the Pichinde virus particle of any one of claims 31 to 51, 67 or 68 and a pharmaceutically acceptable carrier.

70. A pharmaceutical composition comprising the Pichinde virus particle of any one of the claims 31 to 51, 67 or 68 and a pharmaceutically acceptable carrier.
71. The Pichinde virus particle of any one of claims 31 to 51, 67 or 68, wherein the Pichinde virus is strain Munchique CoAn4763 isolate P18, or P2 strain.