WHAT IS CLAIMED:

1. An immunostimulatory bacterium, comprising a plasmid that encodes a therapeutic product or products, wherein:

the bacterium comprises genome modifications, whereby the bacterium does not produce active thymidylate synthase, and requires supplementation for growth;

the genome of the immunostimulatory bacterium is modified, whereby the bacterium lacks flagella; and

the unmodified bacterium comprises flagella.

2. The immunostimulatory bacterium of claim 1, wherein:

a therapeutic product is a variant protein that is part of the cytosolic DNA/RNA sensor pathway;

the variant protein comprises amino acid modifications whereby expression of type I interferon (IFN) is constitutive when the protein is expressed in or is in a eukaryotic cell; and

amino acid modifications comprise one or more of an amino acid insertion, deletion, and replacement.

3. The immunostimulatory bacterium of claim 1, wherein:

a therapeutic product is a modified STING, IRF3, IRF5, IRF7, IRF8, MDA5, or RIG-I protein that comprises amino acid modifications, whereby expression of type I interferon (IFN) is constitutive; and

the modifications comprise one or more of an amino acid replacement, insertion, and deletion.

4. The immunostimulatory bacterium of claim 3, wherein the amino acid modifications are selected as follows:

a) in STING, with reference to SEQ ID NO: 305-309, one or more selected from among: S102P, V147L, V147M, N154S, V155M, G166E, C206Y, G207E, S102P/F279L, F279L, R281Q, R284G, R284S, R284M, R284K, R284T, R197A, D205A, R310A, R293A, T294A, E296A, R197A/D205A, S272A/Q273A, R310A/E316A, E316A, E316N, E316Q, S272A, R293A/T294A/E296A, D231A,

R232A, K236A, Q273A, S358A/E360A/S366A, D231A/R232A/K236A/R238A, S358A, E360A, S366A, R238A, R375A, N154S/R284G, and S324A/S326A;

b) in MDA5, with reference to SEQ ID NO:310, one or more of: T331I, T331R, A489T, R822Q, G821S, A946T, R337G, D393V, G495R, R720Q, R779H, R779C, L372F, and A452T;

c) in RIG-I, with reference to SEQ ID NO:311, one or both of E373A and C268F; and

d) in IRF-3, with reference to SEQ ID NO:312, S396D.

5. The immunostimulatory bacterium of any of claims 1-4, wherein the immunostimulatory bacterium comprises a plasmid that encodes an interferon, or encodes a modified STING protein that constitutively induces type I interferon, and encodes an antigen or protein from a pathogen or a tumor-associated antigen.

6. The immunostimulatory bacterium of any of claims 1-5, wherein the genome of the immunostimulatory bacterium is modified by deletion or disruption of all or of a sufficient portion of a gene or genes, whereby the bacterium produces lipopolysaccharide (LPS) with penta-acylated lipid A.

7. The immunostimulatory bacterium of any of claims 1-6, wherein the genome of the bacterium is modified so that the bacterium lacks curli fimbriae.

8. The immunostimulatory bacterium of any of claims 1-7, wherein the therapeutic product(s) is/are encoded by nucleic acid that is operatively linked to a prokaryotic promoter.

9. The immunostimulatory bacterium of claim 8, wherein the nucleic acid encoding the therapeutic product or products is transcribed in the bacterium, but cannot be translated by the bacterium.

10. The immunostimulatory bacterium of any of claims 1-7, wherein the therapeutic product(s) is/are encoded by nucleic acid that is operatively linked to a eukaryotic promoter.

11. The immunostimulatory bacterium of any claims 1-10, wherein the plasmid further comprises nucleic acid encoding an antigen from a pathogen or a tumor-associated antigen.

12. The immunostimulatory bacterium of claim 11, wherein the tumor-associated antigen is an oncofetal antigen, an oncoviral antigen, an overexpressed/accumulated antigen, a cancer-testis antigen, a linear restricted antigen, a mutated antigen, a post-translationally altered antigen, or an idiotypic antigen.

13. The immunostimulatory bacterium of any of claims 1-12 that comprises genome modifications whereby the bacterium lacks flagella, is *msbB*$^{-}$/*pagP*$^{-}$, and lacks curli fimbriae, wherein genome modifications include deletions, insertions, replacements, and transpositions.

14. The immunostimulatory bacterium of claim 13 that comprises modifications that render the bacterium *asd*$^{-}$, whereby the bacterium does not replicate *in vivo*, and comprises nucleic acid encoding aspartate dehydrogenase (*asd*) on the plasmid under control of a prokaryotic promoter for growth of the bacteria *in vitro*.

15. An immunostimulatory bacterium, comprising a plasmid encoding a therapeutic product or products, wherein:

nucleic acid encoding the therapeutic product(s) is operatively linked to a prokaryotic promoter; and

the therapeutic product(s) encoded by the plasmid is/are selected from:

a tumor-associated antigen; or

a tumor-associated antigen and a modified STING protein that constitutively induces type I interferon; or

a tumor-associated antigen and interferon alpha, or a tumor-associated antigen and interferon beta.

16. The immunostimulatory bacterium of claim 15, wherein the tumor-associated antigen is an oncofetal antigen, an oncoviral antigen, an overexpressed/accumulated antigen, a cancer-Testis antigen, a linear restricted

antigen, a mutated antigen, a post-translationally altered antigen, or an idiotypic antigen.

17. The immunostimulatory bacterium of claim 16, wherein the tumor-associated antigen (TAA) is selected from among the following antigens:

a) an oncofetal antigen selected from among carcinoembryonic antigen (CEA), immature laminin receptor, and tumor-associated glycoprotein 72 (TAG-72);

b) an oncoviral TAA selected from HPV E6 and HPV E7;

c) an overexpressed/accumulated TAA selected from among epidermal Growth Factor Receptor (EGFR), Wilms' tumor protein, calcium-activated chloride channel 2, cyclin-B1, 9D7, epidermal cell adhesion molecule (EpCAM), Ephrin type-A receptor 3 (EphA3), human epidermal growth factor receptor 2 (HER2/Neu), telomerase, mesothelin, stomach-cancer–associated protein tyrosine phosphatase 1 (SAP-1), and survivin;

d) a cancer testis TAA selected from among BAGE (B melanoma antigen) family, CAGE family, GAGE family, MAGE (melanoma antigen) family, SAGE (sarcoma antigen) family, PAGE family, XAGE family, CT9, CT10, New York esophageal squamous cell carcinoma 1 (NY-ESO-1), LAGE-1, preferentially expressed antigen in melanoma (PRAME), and synovial sarcoma/X breakpoint 2 (SSX-2);

e) a linear-restricted TAA selected from melanoma antigen recognized by T-cells 1 (Melan-A/MART-1), gp100/Pmel17, tyrosinase, tyrosine-related protein (TRP)-1, TRP-2, polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen (PSA);

f) a mutated TAA selected from among β-catenin, BRCA1, BRCA2, cyclin dependent kinase 4 (CDK4), chronic myelogenous leukemia tumor antigen 66 (CML66), fibronectin, melanoma antigen recognized by T-cells 2 (MART-2), p53, Ras, and TGF-β receptor type II (TGF-βRII);

g) a post-translationally altered TAA that is mucin 1, cell surface associated (MUC1); and

h) an idiotypic TAA that is immunoglobulin Ig or T-cell Receptor (TCR).

18. The immunostimulatory bacterium of any of claims 1-17 that comprises genome modifications whereby the bacterium is *pagP*$^{-}$/*msbB*$^{-}$ and lacks flagella, wherein the wild-type bacterium has flagella.

19. The immunostimulatory bacterium of any of claims 1-18 that is a *Salmonella* strain.

20. An immunostimulatory bacterium, comprising a plasmid containing nucleic acid encoding a therapeutic product, wherein:

the nucleic acid encoding the therapeutic product is operatively linked to a prokaryotic promoter; and

the bacterium comprises genome modifications that reduce or eliminate recognition of the bacterium by or activation of TLR2, and optionally TLR4 and/or TLR5, whereby induction of type I IFN is not inhibited compared to the bacterium without the genome modifications.

21. The immunostimulatory bacterium of claim 20, wherein the nucleic acid encodes RNA that cannot be translated by prokaryote ribosomes, but can be translated by eukaryotic ribosomes, whereby the bacterium, when cultured *in vitro,* comprises RNA transcribed from the nucleic acid encoding the therapeutic product.

22. The immunostimulatory bacterium of any of claims 8, 15, 20 and 21, wherein the nucleic acid encodes RNA that lacks a Shine-Dalgarno sequence and/or includes an Internal Ribosome Entry Site (IRES), and/or a translational read through 2A peptide.

23. The immunostimulatory bacterium of any of claims 20-22, wherein the nucleic acid encodes a modified STING protein that constitutively induces type I interferon, and encodes a tumor-associated antigen and/or an antigen from a pathogen.

24. An immunostimulatory bacterium, comprising a plasmid encoding a combination of heterologous products, wherein:

the genome of the immunostimulatory bacterium is modified by deletion or disruption of all or of a sufficient portion of a gene or genes, whereby the bacterium has attenuated recognition by TLR2, and optionally attenuated recognition of one or both of TLR4 and TLR5;

one plasmid encoded product is a protein that is part of a cytosolic DNA/RNA sensor pathway that leads to expression of type I interferon (IFN) and the product is modified whereby expression of type I interferon is constitutive; and

a second encoded product is an antigen, an epitope or epitopes from an antigen, a protein for immunization against a pathogen or tumor, an anti-viral therapeutic, or an anti-pathogenic bacteria therapeutic.

25. The immunostimulatory bacterium of claim 24, wherein the genome of the bacterium is modified whereby the bacterium does not have curli fimbriae, if the wild-type bacterium contains flagella, the bacterium lacks flagella, and the bacterium has penta-acylated lipid A.

26. The immunostimulatory bacterium of any of claims 1-25, wherein:

the bacterium comprises genome modifications, whereby the phenotype of the resulting bacterium is selected from among modifications comprising:

a) *asd*$^-$, *csgD*$^-$, *purI*$^-$, *msbB*$^-$, flagellin$^-$, and *pagP*$^-$;

b) *thyA*$^-$, *csgD*$^-$, *purI*$^-$, *msbB*$^-$, flagellin$^-$, and *pagP*;

c) *ansB*$^-$, *asd*$^-$, *csgD*$^-$, *purI*$^-$, *msbB*$^-$, flagellin$^-$, and *pagP*;

d) *ansB*$^-$, *thyA*$^-$, *csgD*$^-$, *purI*$^-$, *msbB*$^-$, flagellin$^-$, and *pagP*$^-$; and

e) *asd*$^-$, *ansB*$^-$, *thyA*$^-$, *csgD*$^-$, *purI*$^-$, *msbB*$^-$, flagellin$^-$, and *pagP*$^-$,

wherein, if the bacterium is *asd*$^-$, it optionally encodes aspartate dehydrogenase (*asd*) on the plasmid under control of a prokaryotic promoter for expression of *asd in vitro*.

27. The immunostimulatory bacterium of any of claims 24-26, wherein a therapeutic product encoded on the plasmid is an anti-viral therapeutic or an anti-pathogenic bacteria therapeutic.

28. The immunostimulatory bacterium of any of claims 24-27, wherein a therapeutic product encoded on the plasmid is an anti-viral therapeutic that is a viral

antigen whose expression results in an immune-protective response against a virus, or is an antibody that binds to or interacts with a viral antigen, whereby a virus is inhibited or blocked, or anti-viral immunity results.

29. The immunostimulatory bacterium of any of claims 24-28, wherein:

the encoded protein that is part of a cytosolic DNA/RNA sensor pathway is a modified STING protein that constitutively induces type I interferon; and

another encoded product or products is/are a tumor-associated antigen and/or an antigen from a pathogen.

30. The immunostimulatory bacterium of claim 29, wherein the encoded products are encoded as a polycistronic message under control of a single prokaryotic promoter for expression in the bacterium.

31. The immunostimulatory bacterium of any of claims 1-30 that encodes a therapeutic, wherein the therapeutic is an anti-bacterial therapeutic that is a bacterial antigen whose expression results in an immune-protective response against the bacterial pathogen, or the anti-bacterial therapeutic is an antibody that binds to or interacts with a bacterial antigen, whereby the pathogenic bacterium is inhibited or blocked, or anti-pathogenic bacterium immunity results.

32. The immunostimulatory bacterium of any of claims 1-30, wherein the immunostimulatory bacterium encodes a therapeutic for treating an infectious agent that causes persistent infection, or for treating an infectious agent that causes chronic infection, or for treating an infectious agent that causes latent infection, or for treating an infectious agent that causes slow infection.

33. The immunostimulatory bacterium of claim 32, wherein the infectious agent is a virus selected from among T-Cell leukemia viruses, Epstein-Barr virus, cytomegalovirus, herpesviruses, varicella zoster virus, measles virus, papovaviruses, hepatitis virus type A, B, C, D and E, adenoviruses, parvoviruses, human immunodeficiency virus (HIV), coronaviruses, smallpox virus, poliovirus, influenza

virus, rotavirus, yellow fever virus, mumps virus, rubella virus, and papillomaviruses; or is a bacterium; or is a prion; or is a protozoan.

34. The immunostimulatory bacterium of any of claims 1-33 that is a strain designated as YS1646Δ*asd*/ΔFLG/Δ*pagP*/Δ*ansB*/Δ*csgD*/F-Δ*purI*, or YS1646Δ*asd*/ΔFLG/Δ*pagP*/Δ*ansB*/Δ*csgD*/F-Δ*purI*/Δ*thyA*, wherein F-Δ*purI* refers to a deletion of the full-length *purI* gene.

35. An immunostimulatory bacterium, comprising a plasmid containing nucleic acid operatively linked to a prokaryotic promoter, wherein:

the nucleic acid comprises DNA that lacks sequences necessary for translation by a prokaryote or comprises sequences that prevent translation by a prokaryote, whereby the RNA encoded by the DNA is produced in the bacterium, but cannot be translated by the bacterium;

the RNA encodes a therapeutic product or is a therapeutic product;

the bacterium has genomic modifications whereby infection is substantially or is restricted to phagocytic cells; and

the genome of the immunostimulatory bacterium is modified by deletion or disruption of all or of a sufficient portion of the gene *ansB*, encoding L-asparaginase II, whereby the bacterium is $ansB^-$ and does not express active L-asparaginase II.

36. The immunostimulatory bacterium of claim 35, wherein the genome of the bacterium is modified by deletion or disruption of all or of a sufficient portion of the gene *csgD*, whereby the bacterium is $csgD^-$ and does not activate the synthesis of curli fimbriae.

37. The immunostimulatory bacterium of claim 35 or 36, wherein the genome of the bacterium is further modified by deletion or disruption of all or of a sufficient portion of genes, whereby the bacterium is $ansB^-/csgD^-/msbB^-/pagP^-$.

38. An immunostimulatory bacterium, comprising a plasmid encoding a therapeutic product, wherein:

the product is a bi-specific T-cell engager antibody that binds DLL3 and CD3;

the immunostimulatory bacterium lacks flagella and comprises LPS that is penta-acylated; and

the wild-type bacterium comprises flagella.

39. The immunostimulatory bacterium of claim 38, wherein the bi-specific T-cell engager antibody comprises a heavy chain and light chain of an anti-DLL3 antibody and of an anti-CD3 antibody.

40. The immunostimulatory bacterium of claim 38 or 39, wherein the bi-specific T-cell engager antibody comprises combinations of a) - f), whereby the resulting construct can bind to each of DLL3 and CD3:

a) a light chain that comprises amino acid residues 154-260 of SEQ ID NO: 487, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

b) a heavy chain that comprises the sequence of amino acid residues set forth as amino acid residues 22-138 of SEQ ID NO: 487, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

c) a light chain that comprises a sequence of amino acid residues set forth as amino acid residues 155-261 of SEQ ID NO: 489, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

d) a heavy chain that comprises a sequence of amino acid residues set forth as amino acid residues 22-139 of SEQ ID NO: 489, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

e) a heavy and light chain, wherein:

the light chain comprises a sequence of amino acid residues set forth as amino acid residues 155-261 of SEQ ID NO: 485, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

the heavy chain comprises a sequence of amino acid residues set forth as amino acid residues 22-139 of SEQ ID NO: 485, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

f) a heavy and light chain of an anti-CD3 antibody, wherein:

the light chain of the anti-CD3 antibody comprises a sequence of amino acid residues set forth as amino acid residues 398-504 of SEQ ID NO: 485, or

a humanized variant thereof, or a variant having at least 95 % sequence identity thereto; and

the heavy chain of the anti-CD3 antibody comprises a sequence of amino acid residues set forth as amino acid residues 267-382 of SEQ ID NO: 485, or a humanized variant thereof, or a variant having at least 95 % sequence identity thereto.

41. An immunostimulatory bacterium, comprising a plasmid that encodes two or more therapeutic products under control of a single promoter, wherein:

the therapeutic products are selected from among:

a) an immunostimulatory protein that confers or contributes to an anti-tumor immune response in the tumor microenvironment;

b) one or more of a protein that is part of a cytosolic DNA/RNA sensor pathway, wherein the protein is a variant that results in constitutive expression of a type I IFN; and

c) an anti-cancer antibody or antigen-binding portion thereof; and

the encoding nucleic acids are separated by an IRES sequence or 2A peptides, and each nucleic acid encoding each product is optionally operatively linked to nucleic acid encoding a signal sequence, whereby, upon translation of the encoded mRNA, each product is separately expressed and secreted from a cell comprising the bacterium and/or plasmid.

42. The immunostimulatory bacterium of claim 41, wherein the immunostimulatory protein is a co-stimulatory molecule that lacks a cytoplasmic domain or a sufficient portion thereof, for expression on an antigen-presenting cell (APC), whereby the truncated co-stimulatory molecule is capable of constitutive immunostimulatory signaling to a T-cell through co-stimulatory receptor engagement and is unable to counter-regulatory signal to the antigen presenting cell (APC).

43. The immunostimulatory bacterium of claim 41 or claim 42, wherein the immunostimulatory bacterium comprises a plasmid that encodes the at least two therapeutic products, wherein the products are selected from among a cytokine, a protein that constitutively induces a type I IFN, a co-stimulatory molecule, and an

anti-cancer antibody or antigen-binding portion thereof, and also encodes an antigen or an antigenic protein.

44. An immunostimulatory bacterium that is a strain designated as YS1646Δ*asd*/ΔFLG/Δ*pagP*/Δ*ansB*/Δ*csgD*/F-Δ*purI*, or YS1646Δ*asd*/ΔFLG/Δ*pagP*/Δ*ansB*/Δ*csgD*/F-Δ*purI*/Δ*thyA*, wherein F-Δ*purI* refers to deletion of the full-length of the *purI* gene.

45. A genome modified bacterium, comprising genome modifications, whereby the response by toll-like receptors (TLRs) 2, 4, and 5 is reduced compared to the bacterium without the genome modifications, wherein:

the bacterium comprises further genomic modifications whereby it is auxotrophic for a required nutrient or factor so that it is unable to replicate in a eukaryotic host, but can replicate *in vitro* when supplied with the nutrient or factor;

the bacterium comprises a plasmid containing nucleic acid encoding a product, or the bacterium comprises RNA encoding the product;

the product encoded by the nucleic acid or RNA is an antigenic sequence or sequence from a pathogen that is a pathogenic virus, bacterium, prion, or parasite, or is a tumor-associated antigen, whereby, upon expression of the encoded antigenic sequence in the host, the host develops an immune-protective response or immunizing response against the pathogenic virus, bacterium, prion, parasite, or tumor-associated antigen, or the product is a therapeutic product against a pathogen or tumor;

expression of the antigenic sequence(s) is/are under control of a prokaryotic promoter so that RNA encoding the product is produced in the bacterium;

nucleic acid encoding the antigen comprises regulatory sequences that inhibit or prevent translation of the encoded RNA by bacterial ribosomes, but that do not inhibit or prevent translation of the encoded RNA by eukaryotic host ribosomes, whereby translation is de-coupled from transcription in the bacterium; and

the resulting bacterium is selective for infecting phagocytic cells when administered to a eukaryotic subject, and delivers the nucleic acid into the phagocytic cells, wherein the RNA is translated into the product.

46. The bacterium of claim 45 that comprises nucleic acid encoding a TLR8 agonist.

47. The bacterium of claim 45 or 46 that is *msbB*$^{-}$/*pagP*$^{-}$, lacks flagella, and is *asd*$^{-}$ or *thyA*$^{-}$ or both *asd*$^{-}$ and *thyA*$^{-}$.

48. The bacterium of any of claims 1-18 and 20-47 that is a species or strain of *Escherichia*, *Listeria*, *Mycobacteria,* or *Salmonella.*

49. A vaccine, comprising nucleic acid that encodes an antigen or protein or epitope from a pathogen or tumor, wherein:

the vaccine elicits an immune response against the pathogen or tumor;

the pathogen is a respiratory pathogen that infects the respiratory system including the lungs and/or naso-pharynx;

the tumor is a lung tumor;

the vaccine is formulated for inhalation through the nose or lungs;

the vaccine delivers the nucleic acid to phagocytic macrophages to convert the immunosuppressive phagocytic macrophages to immunostimulatory, phagocytic macrophages that are capable of in situ antigen cross-presentation to CD8+ T-cells, and of migration to lymph nodes to prime CD4+ and CD8+ T-cells; and

the vaccine does not activate a TLR2, TLR4 and/or TRL5 response sufficient to decease or inhibit type I IFN.

50. The vaccine of claim 49, wherein the pathogen is a virus.

51. The vaccine of claim 49 or 50, wherein the virus is a corona virus or influenza virus.

52. A pharmaceutical composition, comprising the bacterium of any of claims 1-48 in a pharmaceutically acceptable vehicle.

53. The bacterium of any of claims 1-48 or pharmaceutical composition of claim 52, wherein the bacterium is a *Salmonella typhimurium*.

54. The bacterium of any of claims 1-48 and 53 or vaccine of any of claims 49-51 for use for treatment of or immunization against infection with a pathogen or against a tumor, wherein the bacterium comprises or encodes an antigen, protein, or epitope thereof from the pathogen or tumor.

55. The bacterium or vaccine of claim 54, wherein the bacterium encodes the antigen, protein, or epitope thereof under control of a promoter recognized by the bacterium; and the bacterium comprises a genome modification(s), whereby it does not replicate *in vivo*.

56. Use of the bacterium of any of claims 1-48 and 53 in the manufacture of a medicament for the treatment of cancer or for immunization against or for treatment of a pathogen.

57. The bacterium of any of claims 1-48 and 53-56, or the vaccine of any of claims 49-51, wherein an encoded therapeutic product is a cytokine.

58. The bacterium or vaccine of claim 57, wherein the cytokine is IL-15/IL-15R alpha chain complex.

59. The bacterium of any of claims 1-48 and 53 for use in therapy.