Claims

1. A recombinant Gram-negative bacterial strain which comprises
i) a first polynucleotide molecule comprising a nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of a nucleotide sequence encoding a delivery signal from a bacterial effector protein, wherein the nucleotide sequence encoding the delivery signal from a bacterial effector protein is operably linked to a promoter;
ii) a second polynucleotide molecule comprising a nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of a nucleotide sequence encoding a delivery signal from a bacterial effector protein, wherein the nucleotide sequence encoding the delivery signal from a bacterial effector protein is operably linked to a promoter;
iii) a third polynucleotide molecule comprising a nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of a nucleotide sequence encoding a delivery signal from a bacterial effector protein, wherein the nucleotide sequence encoding the delivery signal from a bacterial effector protein is operably linked to a promoter; and
iv) a fourth polynucleotide molecule comprising a nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of a nucleotide sequence encoding a delivery signal from a bacterial effector protein, wherein the nucleotide sequence encoding the delivery signal from a bacterial effector protein is operably linked to a promoter,
wherein said first and said second polynucleotide molecule are located on a vector comprised by said Gram-negative bacterial strain and said third and said fourth polynucleotide molecule are located on a chromosome of said Gram-negative bacterial strain or on an extra-chromosomal genetic element comprised by said Gram-negative bacterial strain, with the proviso that the extra-chromosomal genetic element is not the vector on which the said first and said second polynucleotide molecule are located.

2. The recombinant Gram-negative bacterial strain of claim 1, wherein the nucleotide sequence encoding a heterologous protein or a fragment thereof of

the first polynucleotide molecule and the nucleotide sequence encoding a heterologous protein or a fragment thereof of the third polynucleotide molecule encode the same heterologous protein or a fragment thereof.

3. The recombinant virulence attenuated Gram-negative bacterial strain of claim 1 or 2, wherein the nucleotide sequence encoding a heterologous protein or a fragment thereof of the second polynucleotide molecule and the nucleotide sequence encoding a heterologous protein or a fragment thereof of the fourth polynucleotide molecule encode the same heterologous protein or a fragment thereof.

4. The recombinant Gram-negative bacterial strain of any one of claims 1-3, wherein the nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of the nucleotide sequence encoding a delivery signal from a bacterial effector protein of the first polynucleotide molecule and the nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of the nucleotide sequence encoding a delivery signal from a bacterial effector protein of the second polynucleotide molecule are operably linked to the same promoter.

5. The recombinant Gram-negative bacterial strain of any one of claims 1-4, wherein the nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of the nucleotide sequence encoding a delivery signal from a bacterial effector protein of the third polynucleotide molecule and the nucleotide sequence encoding a heterologous protein or a fragment thereof fused in frame to the 3'end of the nucleotide sequence encoding a delivery signal from a bacterial effector protein of the fourth polynucleotide molecule are operably linked to two different promoters.

6. The recombinant Gram-negative bacterial strain of any one of claims 1-5, wherein a fragment of a heterologous protein contains between 100 and 800 amino acids and does have the same functional properties as the heterologous protein from which it is derived.

7. The recombinant Gram-negative bacterial strain of any one of claims 1-6, wherein the delivery signal from a bacterial effector protein is a polypeptide sequence which can be recognized by the secretion and translocation system of the recombinant Gram-negative bacterial strain and directs the delivery of a protein from the recombinant Gram-negative bacterial strain to eukaryotic cells.

8. The recombinant Gram-negative bacterial strain of any one of claims 1-7, wherein the delivery signal from a bacterial effector protein is selected from the group consisting of a bacterial T3SS effector protein or a N-terminal fragment thereof, a bacterial T4SS effector protein or a N-terminal fragment thereof and a bacterial T6SS effector protein or a N-terminal fragment thereof.

9. The recombinant Gram-negative bacterial strain of any one of claims 1-7, wherein the delivery signal from a bacterial effector protein is a bacterial T3SS effector protein comprising a bacterial T3SS effector protein or a N-terminal fragment thereof.

10. The recombinant Gram-negative bacterial strain of claim 9, wherein the N-terminal fragment of a bacterial T3SS effector protein includes at least the first 10 amino acids of the bacterial T3SS effector protein.

11. The recombinant Gram-negative bacterial strain of any one of claims 1-10, wherein the vector is a medium copy number plasmid.

12. The recombinant Gram-negative bacterial strain of any one of claims 1-11, wherein the extra-chromosomal genetic element is an endogenous virulence plasmid.

13. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first, second, third and fourth polynucleotide molecule is, independently of each other, selected from the group consisting of proteins involved in induction or regulation of an interferon (IFN) response, proteins involved in apoptosis or apoptosis regulation, cell cycle regulators, ankyrin repeat proteins, cell signalling proteins, reporter proteins, transcription

factors, proteases, small GTPases, GPCR related proteins, nanobody fusion constructs and nanobodies, bacterial T3SS effectors, bacterial T4SS effectors and viral proteins, or a fragment thereof.

14. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first, second, third and fourth polynucleotide molecule is, independently of each other, selected from the group consisting of proteins involved in induction or regulation of a type I IFN response selected from the group consisting of cGAS, STING, TRIF, TBK1, IKKepsilon, IRF3, TREX1, VPS34, ATG9a, DDX3, LC3, DDX41, IFI16, MRE11, DNA-PK, RIG1, MDA5, LGP2, IPS-1/MAVS/Cardif/VISA, Trim25, Trim32, Trim56, Riplet, TRAF2, TRAF3, TRAF5, TANK, IRF3, IRF7, IRF9, STAT1, STAT2, PKR, TLR3, TLR7, TLR9, DAI, IFI16, IFIX, MRE11, DDX41, LSm14A, LRRFIP1, DHX9, DHX36, DHX29, DHX15, Ku70, cyclic dinucleotide generating enzymes (cyclic-di-AMP, cyclic-di-GMP and cyclic-di-GAMP cyclases) as WspR, DncV, DisA and DisA-like, CdaA, CdaS and cGAS, or a fragment thereof; proteins involved in apoptosis or apoptosis regulation selected from the group consisting of pro-apoptotic proteins, anti-apoptotic proteins, inhibitors of apoptosis-prevention pathways and inhibitors of pro-survival signalling or pathways; cell cycle regulators selected from the group consisting of cyclins, cyclin dependent kinases (CDKs), CDK activating kinases, Cdk inhibitors, CDK substrates, anaphase promoting complex/cyclosome and cell-cycle-checkpoint proteins; ankyrin repeat proteins; cell signalling proteins selected from the group consisting of cytokine signalling proteins, survival factor signaling proteins, death signalling proteins, growth factor signalling proteins, hormone signalling proteins, chemokine signalling proteins, and extracellular matrix/Wnt/Hedgehog signalling proteins; reporter proteins selected from the group consisting of fluorescent proteins, luciferases and enzymatic reporter proteins; transcription factors; proteases; small GTPases; GPCR related proteins selected from the group consisting of G-protein coupled receptors, G protein complexes, kinases, adaptor proteins, signal transducers/regulators and transcription factors; nanobody fusion constructs selected from the group consisting of nanobodies fused to protein degrading

domains, nanobodies fused to cell signalling proteins or parts thereof, nanobodies fused to identical or other nanobodies, nanbodies fused to reporter proteins and nanobodies fused to subcellular localization signals; nanobodies; bacterial T3SS effectors; bacterial T4SS effectors and viral proteins; or a fragment thereof.

15. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first, second, third and fourth polynucleotide molecule is, independently of each other, a protein involved in induction or regulation of an interferon (IFN) response, or a fragment thereof.

16. The recombinant Gram-negative bacterial strain of any one of claims 1-12 and 15, wherein a protein involved in induction or regulation of an interferon (IFN) response, or a fragment theeof, is a protein involved in induction or regulation of a type I IFN response selected from the group consisting of cGAS, STING, TRIF, TBK1, IKKepsilon, IRF3, TREX1, VPS34, ATG9a, DDX3, LC3, DDX41, IFI16, MRE11, DNA-PK, RIG1, MDA5, LGP2, IPS-1/MAVS/Cardif/VISA, Trim25, Trim32, Trim56, Riplet, TRAF2, TRAF3, TRAF5, TANK, IRF3, IRF7, IRF9, STAT1, STAT2, PKR, TLR3, TLR7, TLR9, DAI, IFI16, IFIX, MRE11, DDX41, LSm14A, LRRFIP1, DHX9, DHX36, DHX29, DHX15, Ku70, or a fragment thereof, cyclic dinucleotide generating enzymes (cyclic-di-AMP, cyclic-di-GMP and cyclic-di-GAMP cyclases) as WspR, DncV, DisA and DisA-like, CdaA, CdaS and cGAS, or a fragment thereof.

17. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first, second, third and fourth polynucleotide molecule is, independently of each other, selected from the group consisting of the RIG-I-like receptor (RLR) family, other CARD domain containing proteins involved in antiviral signaling and type I IFN induction, and cyclic dinucleotide generating enzymes such as cyclic-di-AMP, cyclic-di-GMP and cyclic-di-GAMP cyclases selected from the group consisting of WspR, DncV, DisA and

DisA-like, CdaA, CdaS and cGAS, leading to stimulation of STING, or a fragment thereof.

18. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first, second, third and fourth polynucleotide molecule is, independently of each other, selected from the group consisting of the RIG-I-like receptor (RLR) family, other CARD domain containing proteins selected from the group consisting of MAVS, CRADD/RAIDD, RIPK2/RIP2, CARD6, NOD1 and NOD2, or a fragment therof, and cyclic dinucleotide generating enzymes such as cyclic-di-AMP, cyclic-di-GMP and cyclic-di-GAMP cyclases selected from the group consisting of WspR, DncV, DisA and DisA-like, CdaA, CdaS and cGAS, leading to stimulation of STING, or a fragment thereof.

19. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first and the third polynucleotide molecule is cGAS or a fragment thereof, preferably a fragment of cGAS.

20. The recombinant Gram-negative bacterial strain of any one of claims 1-12, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the first and the third polynucleotide molecule is a fragment of cGAS as shown in SEQ ID NO:10.

21. The recombinant Gram-negative bacterial strain of any one of claims 1-12 or 18, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the second and the fourth polynucleotide molecule is RIG1 or a fragment thereof, preferably a fragment of RIG1 comprising a CARD domain.

22. The recombinant Gram-negative bacterial strain of any one of claims 1-12 or 19-20, wherein the heterologous protein or a fragment thereof encoded by the nucleotide sequence of the second and the fourth polynucleotide molecule is a

fragment of RIG1 comprising a CARD domain as shown in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3.

23. The recombinant Gram-negative bacterial strain of any one of claims 1-22, wherein the recombinant Gram-negative bacterial strain is a *Yersinia* strain.

24. The recombinant Gram-negative bacterial strain of any one of claims 1-23, for use as a medicament.

25. ~~The~~ Use of the recombinant Gram-negative bacterial strain of any one of claims 1-23, in the manufacture of a medicament for ~~use in a method of treating~~the treatment of cancer in a subject, ~~the method comprising administering~~wherein an effective amount of the medicament is to be administered to the subject ~~said recombinant Gram-negative bacterial strain, wherein the recombinant Gram-negative bacterial strain is administered in an amount that is sufficient to treat the subject~~.