CLAIMS

What is claimed is

1. A method for detecting diseased epithelial tissue selected from gastrointestinal tract epithelium and bile duct epithelium, comprising:

(i) administering to the gastrointestinal tract of a subject in need thereof, a genetically engineered microorganism,

wherein the microorganism comprises an exogenous gene encoding a surface protein, wherein the surface protein specifically interacts with one or more cell membrane receptor(s),

wherein the one or more cell membrane receptor(s) are not exposed to the luminal side of epithelial cells of normal gastrointestinal tissue and/or epithelial tissue lining the bile duct, pancreatic duct, or common bile duct, *etc*.; and

wherein the one or more cell membrane receptor(s) are exposed to the luminal side of diseased epithelial cells of gastrointestinal tissue and/or epithelial tissue lining the bile duct, pancreatic duct, or common bile duct, *etc*. in the subject suffering from a disease,

wherein the surface protein promotes binding and invasion of the microorganism in the diseased epithelial cells,

wherein the microorganism comprises one or more gene(s) encoding at least one detection marker operably linked to a promoter; and

(ii) detecting the expression of the detection marker in epithelial cells to thereby detect diseased epithelial cells.

2. The method of claim 1, wherein the genetically engineered microorganism is non-pathogenic.

3. The method of claim 1 or claim 2, wherein the genetically engineered microorganism harbors at least one auxotrophic mutation, which optionally includes a deletion, inactivation, or reduced expression or activity of a gene involved in synthesis of a metabolite required for cell wall synthesis.

4. The method of any one of claims 1-3, wherein the promoter is a mammalian promoter, optionally wherein the mammalian promoter directs GI tract epithelial cell-specific expression.

5. The method of claim 4, wherein the mammalian promoter directs GI tract epithelial cell-specific expression.

6. The method of any one of claims 1-3, wherein the promoter is a microbial promoter.

7. The method of claim 6, wherein the one or more gene(s) encoding at least one detection marker further comprises an internal ribosome entry site (IRES).

8. The method of any one of claims 1-7, wherein the genetically engineered microorganism is administered via oral or rectal route.

9. The method of any one of claims 1-8, further comprising administration of a colon cleansing agent comprising a laxative, optionally wherein the colon cleansing agent is administered prior to and/or after the administration of the microorganism.

10. The method of any one of claims 1-9, wherein the at least one detection marker is selected from a fluorescent protein, a bioluminescent protein, a contrast agent for magnetic resonance imaging (MRI), a Positron Emission Tomography (PET) reporter, an enzyme reporter, a contrast agent for use in computerized tomography (CT), a Single Photon Emission Computed Tomography (SPECT) reporter, a photoacoustic reporter, an X-ray reporter, an ultrasound reporter (*e.g.* a bacterial gas vesicle), and ion channel reporters (*e.g.* cAMP activated cation channel), and a combination of any two or more thereof.

11. The method of claim 10, wherein the fluorescent protein is selected from GFP, RFP, YFP, Sirius, Sandercyanin, shBFP-N158S/L173I, Azurite, EBFP2, mKalama1, mTagBFP2, TagBFP, shBFP, ECFP, Cerulean, mCerulean3, SCFP3A, CyPet, mTurquoise, mTurquoise2, TagCFP, mTFP1, monomeric Midoriishi-Cyan, Aquamarine, TurboGFP, TagGFP2, mUKG, Superfolder GFP, Emerald, EGFP, Monomeric Azami Green, mWasabi, Clover, mNeonGreen, NowGFP, mClover3, TagYFP, EYFP, Topaz, Venus, SYFP2, Citrine, Ypet, lanRFP-ΔS83, mPapaya1, mCyRFP1, Monomeric Kusabira-Orange, mOrange, mOrange2, mKOκ, mKO2, TagRFP, TagRFP-T, RRvT, mRuby, mRuby2, mTangerine, mApple, mStrawberry, FusionRed, mCherry,

mNectarine, mRuby3, mScarlet, mScarlet-I, mKate2, HcRed-Tandem, mPlum, mRaspberry, mNeptune, NirFP, TagRFP657, TagRFP675, mCardinal, mStable, mMaroon1, mGarnet2, iFP1.4, iRFP713 (iRFP), iRFP670, iRFP682, iRFP702, iRFP720, iFP2.0, mIFP, TDsmURFP, miRFP703, miRFP709, iSplit, and miRFP670.

12. The method of claim 10, wherein the fluorescent protein is a near-infrared fluorescent protein selected from iRFP670, miRFP670, iRFP682, iRFP702, miRFP703, miRFP709, iRFP713 (iRFP), iRFP720 and iSplit.

13. The method of claim 10, wherein the fluorescent protein is iRFP670.

14. The method of claim 10, wherein the bioluminescent protein is selected from a Ca^{+2} regulated photoprotein (*e.g.* aequorin, symplectin, *Mitrocoma* photoprotein, *Clytia* photoprotein, and *Obelia* photoprotein), North American firefly luciferase, Japanese firefly luciferase, Italian firefly luciferase, East European firefly luciferase, Pennsylvania firefly luciferase, Click beetle luciferase, railroad worm luciferase, *Renilla* luciferase, *Gaussia* luciferase, Cypridina luciferase, *Metridina* luciferase, Metrida luciferase, OLuc, and red firefly luciferase, bacterial luciferase, and active variants thereof.

15. The method of claim 14, further comprising administration of a substrate of the at least one bioluminescent protein.

16. The method of claim 10, wherein the contrast agent for use in MRI is selected from ferritin, transferrin receptor-1 (TfR1), Tyrosinase (TYR), beta-galactosidase, manganese-binding protein MntR, creatine kinase (CK), *Magnetospirillum magnetotacticum* magA, Divalent metal transporter DMT1, protamine-1 (hPRM1), urea transporter (UT-B), and ferritin receptor Timd2 (T-cell immunoglobulin and mucin domain containing protein 2), sodium iodide symporter, *E. coli* dihydrofolate reductase, norepinephrine transporter, and active variants thereof.

17. The method of claim 16, further comprising administration of a substrate of the at least one contrast agent for use in magnetic resonance imaging.

18. The method of claim 10, wherein the PET reporter is selected from thymidine kinase, deoxycytidine kinase, Dopamine 2 Receptor, estrogen receptor α surface protein binding domain,

somatostatin receptor subtype 2, carcinoembryonic antigen, a sodium iodide symporter, E. coli dihydrofolate reductase, a single-chain antibody specific to 1,4,7,10-tetraazacyclododecane-1, 4,7,10-tetraacetic acid (DOTA), or a variants thereof.

19. The method of claim 18, further comprising administration of a PET probe.

20. The method of claim 10, wherein the enzyme reporter is beta-galactosidase, chloramphenicol acetyltransferase, horseradish peroxidase, alkaline phosphatase, acetylcholinesterase, and catalase.

21. The method of claim 20, further comprising administration of a substrate of the at least one enzyme reporter.

22. The method of claim 10, wherein the Single Photon Emission Computed Tomography (SPECT) reporter is selected from sodium ion symporter, norepinephrine transporter, sodium iodide symporter, dopamine receptor, and dopamine transporter.

23. The method of claim 22, further comprising administration of one or more SPECT probe.

24. The method of any one of claims 1 to 23, wherein the one or more gene(s) encoding at least one detection marker comprises at least one intron.

25. The method of claim 24, wherein the at least one intron is a spliceosomal intron.

26. The method of any one of claims 1 to 25, wherein the detection of the abnormal cells is performed using endoscopy, colonoscopy, MRI, CT scan, PET scan, SPECT, or a combination thereof.

27. The method of any one of claims 1 to 26, wherein the surface protein comprises an invasin, intimin, or a fragment thereof.

28. The method of claim 27, wherein the invasin is selected from *Yersinia enterocolitica* invasin, *Yersinia pseudotuberculosis* invasin, *Salmonella enterica* PagN, *Candida albicans* Als3; and/or the intimin is selected from *Escherichia albertii* intimin, *Escherichia coli* intimin, and *Citrobacter rodentium* intimin.

29. The method of any one of claims 1 to 26, wherein the surface protein comprises a peptide or protein that specifically binds to the surface of cancerous and pre-cancerous cells, optionally wherein the protein is selected from a leptin, an antibody, or an antigen-binding fragment thereof.

30. The method of claim 29, wherein the surface protein is a peptide (or nanobody) that binds specifically to the surface of cancerous and pre-cancerous cells, expressed on the bacterial cell surface.

31. The method of any one of claims 1 to 30, wherein the microorganism comprises a second exogenous gene encoding a lysin that lyses the endocytotic vacuole.

32. The method of claim 31, wherein the lysin is listeriolysin O, or a derivative thereof.

33. The method of any one of claims 1 to 32, wherein the microorganism is selected from *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, and *Escherichia coli*.

34. The method of any one of claims 1 to 33, wherein the microorganism is based on *Escherichia coli* Nissle 1917 or a derivative thereof.

35. The method of claim 34, wherein the *Escherichia coli* Nissle 1917 or the derivative thereof harbors a plasmid pMUT1 and/or a plasmid pMUT2, and/or derivatives thereof.

36. The method of claim 34, wherein the *Escherichia coli* Nissle 1917 or the derivative thereof is cured of the plasmid pMUT1 and/or the plasmid pMUT2.

37. The method of any one of claims 1 to 36, wherein the gene encoding the surface protein is integrated at a genomic site.

38. The method of any one of claims 1 to 37, wherein the second gene encoding the lysin is integrated at a genomic site.

39. The method of claim 37 or claim 38, wherein the gene encoding the surface protein, and the second gene encoding the lysin are integrated at a single genomic site, optionally the single genomic site is an integration site of a bacteriophage, and/or a naturally occurring plasmid.

40. The method of any one of claims 1 to 39, wherein the one or more gene(s) encoding at least one detection marker is integrated at a genomic site.

41. The method of claim 40, the gene encoding the surface protein, the second gene encoding the lysin and the gene(s) encoding at least one detection marker are integrated at a single genomic site genomic site, optionally the single genomic site is an integration site of a bacteriophage, and/or a naturally occurring plasmid.

42. The method of any one of claims 1 to 36, wherein the gene encoding the surface protein, and /or the second gene encoding the lysin are inserted on a plasmid, which is optionally selected from the plasmid pMUT1, the plasmid pMUT2, and/or a derivative thereof.

43. The method of any one of claims 1 to 36 or 42, wherein the one or more gene(s) encoding at least one detection marker is inserted on the plasmid.

44. The method of any one of claims 1 to 36 or 42, wherein the one or more gene(s) encoding at least one detection marker is inserted on a second plasmid, which is optionally selected from the plasmid pMUT1, the plasmid pMUT2, and/or a derivative thereof.

45. The method of claim 44, wherein the microorganism is *Escherichia coli* Nissle 1917 or a derivative thereof; and/or the plasmid or the second.

46. The method of claim 44 or claim 45 wherein the plasmid and/ or the second plasmid comprises a selection mechanism.

47. The method of claim 46, wherein the selection mechanism is selected from an antibiotic resistance marker, a toxin-antitoxin system, a marker causing complementation of a mutation in an essential gene, a cis acting genetic element and a combination of any two or more thereof.

48. The method of claim 46, wherein the antibiotic resistance marker is selected from kanamycin resistance gene and tetracycline resistance gene.

49. The method of claim 46, wherein the toxin-antitoxin system is selected from a *hok/sok* system of plasmid R1, *parDE* system of plasmid RK2, *ccdAB* of F plasmid, *flmAB* of F plasmid, *kis/kid* system of plasmid R1, *XCV2162- ptaRNA1* of *Xanthomonas* campestris, *ataT-ataR* of

enterohemorragic *E. coli* or *Klebsiella*, *toxIN* system of *Erwinia carotovora*, *parE-parD* system of *Caulobacter crescentus*, *fst-RNAII* from *Enterococcus faecalis* plasmid AD1, ε-ζ system of *Bacillus subtilis* plasmid pSM19035 and a combination of any two or more thereof.

50. The method of claim 46, wherein the essential gene encodes

an enzyme involved in biosynthesis of an essential nutrient or a cell wall component; and/or

a house-keeping function.

51. The method of claim 50, wherein the essential gene is selected from *dapA*, *dapD*, *murA*, *alr*, *dadX*, *murI*, *dapE*, *thyA* and a combination of any two or more thereof, optionally wherein the essential genes are a combination of *alr* and *dadX*, and optionally wherein the plasmid and/ or the second plasmid is selected by complementation of the *alr* and *dadX* mutations by a functional *alr* gene present on the plasmid and/ or the second plasmid.

52. The method of claim 50, wherein the house-keeping function is selected from *infA*, a gene encoding a subunit of an RNA polymerase, a DNA polymerase, an rRNA, a tRNA, a cell division protein, a chaperon protein, and a combination of any two or more thereof.

53. The method of claim 47, wherein the cis acting genetic element is ColE1 *cer* locus or *par* locus from pSC101.

54. The method of any one of claims 1-53, wherein the microorganism harbors at least one nutritional auxotrophic mutation selected from *dapA*, *dapD*, *dapE*, *murA*, *alr*, *dadX*, *murI*, *thyA*, *aroC*, *ompC*, and *ompF*, optionally wherein the strain harbors a combination of *dapA*, *alr* and *dadX* auxotrophic mutations.

55. The method of any one of claims 44-54, wherein the plasmid or the second plasmid comprises at least one binding site for a DNA binding protein (*e.g.* SV40 enhancer, transcription factor binding sites, defined bacterial operators).

56. The method of claim 55, wherein the DNA binding protein comprises one or more nuclear localization signal(s) (NLS).

57. The method of claim 55 or claim 56, wherein the NLS is SV40 T antigen NLS sequence (KKKRKV).

58. The method of any one of claims 55-57, wherein the DNA binding protein is NFκB.

59. The method of any one of claims 55-58, wherein the microorganism comprises a gene encoding the DNA binding protein comprises one or more nuclear localization signal(s) (NLS).

60. The method of any one of claims 1 to 59, wherein the disease is selected from a precancerous lesion, cancer, ulcerative colitis, Crohn's disease, Barrett's esophagus, irritable bowel syndrome and irritable bowel disease.

61. The method of claim 60, wherein the precancerous lesion comprises a polyp selected from sessile polyp, serrated polyp (*e.g.* hyperplastic polyps, sessile serrated adenomas/polyps, and traditional serrated adenoma), sessile serrated polyp, flat polyp, sub-pedunculated polyp , pedunculated polyp, and a combination thereof.

62. The method of claim 60 or claim 61, wherein the polyp is a diminutive polyp.

63. The method of claim 60, wherein the precancerous lesion comprises a biliary intraepithelial neoplasm (BilIN) selected from BilIN-1, BilIN-2, BilIN-3, and cholangiocarcinoma.

64. The method of claim 60, wherein the precancerous lesion comprises a pancreatic intraepithelial neoplasm (PanIN) selected from PanIN -1, PanIN -2, PanIN -3 and pancreatic ductal adenocarcinoma (PDAC).

65. The method of any one of claims 60-61 or 63-64, wherein the precancerous lesion has a size of from about 0.05 mm to about 30 mm.

66. The method of claim 63, wherein the precancerous lesion has a size of less than about 0.1 mm, less than about 0.25 mm, less than about 0.5 mm, less than about 1 mm, less than about 2 mm, less than about 5 mm, less than about 8 mm, less than about 10 mm, less than about 15 mm, less than about 20 mm, less than about 25 mm, or less than about 30 mm.

~~67. The method of claim 60, wherein the cancer comprises a polyp, an adenoma, or a frank cancer.~~

~~68. The method of claim 60, wherein the cancer comprises Lynch syndrome, familial adenomatous polyposis, hereditary non-polyposis colon cancer (HNPCC), or a sporadic cancer.~~

~~69. The method of any one of claims 1 to 68, wherein the method is used for the diagnosis, prognosis, or evaluation of a disease or disorder in a subject.~~

~~70~~1. A genetically engineered microorganism comprising a gene encoding a surface protein, wherein the surface protein specifically interacts with one or more cell membrane(s) receptor,

wherein the one or more cell membrane receptor(s) are not exposed to the luminal side of epithelial cells of normal gastrointestinal tissue and/or epithelial tissue lining the bile duct, pancreatic duct, or common bile duct, *etc*.; and

wherein the one or more cell membrane receptor(s) are exposed to the luminal side of epithelial cells of diseased gastrointestinal tissue and/or epithelial tissue lining the bile duct, pancreatic duct, or common bile duct, *etc*.,

wherein the surface protein promotes binding and invasion of epithelial cells of diseased gastrointestinal tissue and/or epithelial tissue lining the bile duct, pancreatic duct, or common bile duct, *etc*.,

wherein the microorganism comprises one or more gene(s) encoding at least one detection marker operably linked to a promoter.

~~71. The genetically engineered microorganism of claim 70, wherein the genetically engineered microorganism is non-pathogenic.~~

~~72. The genetically engineered microorganism of claim 70 or claim 71, wherein genetically engineered microorganism harbors at least one auxotrophic mutation, which optionally includes a deletion, inactivation, or reduced expression or activity of a gene involved in synthesis of a metabolite required for cell wall synthesis.~~

~~73~~2. The genetically engineered microorganism of ~~any one of~~ claim~~s 70-72~~1, wherein the promoter is a mammalian promoter~~.~~

~~74. The genetically engineered microorganism of claim 73, wherein the mammalian promoter directs GI tract epithelial cell-specific expression.~~

~~75. The genetically engineered microorganism of any one of claims 70-72, wherein the promoter is~~ or a microbial promoter.

~~763~~. The genetically engineered microorganism of claim ~~752~~, wherein the promoter is a microbial promoter and the gene encoding the detection marker further encodes an internal ribosome entry site (IRES).

~~77. The genetically engineered microorganism of any one of claims 70-76, wherein the microbial promoter is inducible and/or repressible.~~

~~784~~. The genetically engineered microorganism of any one of claims ~~701~~-~~773~~, wherein the at least one detection marker is selected from a fluorescent protein, a bioluminescent protein, a contrast agent for magnetic resonance imaging (MRI), a Positron Emission Tomography (PET) reporter, an enzyme reporter, a contrast agent for use in computerized tomography (CT), a Single Photon Emission Computed Tomography (SPECT) reporter, a photoacoustic reporter, an X-ray reporter, an ultrasound reporter, and ion channel reporters (*e.g.* cAMP activated cation channel).

~~79. The genetically engineered microorganism of claim 78, wherein the fluorescent protein is selected from GFP, RFP, YFP, Sirius, Sandercyanin, shBFP-N158S/L173I, Azurite, EBFP2, mKalama1, mTagBFP2, TagBFP, shBFP, ECFP, Cerulean, mCerulean3, SCFP3A, CyPet, mTurquoise, mTurquoise2, TagCFP, mTFP1, monomeric Midoriishi-Cyan, Aquamarine, TurboGFP, TagGFP2, mUKG, Superfolder GFP, Emerald, EGFP, Monomeric Azami Green, mWasabi, Clover, mNeonGreen, NowGFP, mClover3, TagYFP, EYFP, Topaz, Venus, SYFP2, Citrine, Ypet, lanRFP-ΔS83, mPapaya1, mCyRFP1, Monomeric Kusabira-Orange, mOrange, mOrange2, mKOκ, mKO2, TagRFP, TagRFP-T, RRvT, mRuby, mRuby2, mTangerine, mApple, mStrawberry, FusionRed, mCherry, mNectarine, mRuby3, mScarlet, mScarlet-I, mKate2, HcRed-Tandem, mPlum, mRaspberry, mNeptune, NirFP, TagRFP657, TagRFP675, mCardinal, mStable, mMaroon1, mGarnet2, iFP1.4, iRFP713 (iRFP), iRFP670, iRFP682, iRFP702, iRFP720, iFP2.0, mIFP, TDsmURFP, miRFP703, miRFP709 and miRFP670.~~

805. The genetically engineered microorganism of claim 784, wherein the fluorescent protein is a near-infrared fluorescent protein selected from iRFP670, miRFP670, iRFP682, iRFP702, miRFP703, miRFP709, iRFP713 (iRFP), iRFP720 and iSplit.

81. The genetically engineered microorganism of claim 80, wherein the fluorescent protein is iRFP670.

82. The genetically engineered microorganism of claim 78, wherein the bioluminescent protein is selected from a Ca^{+2} regulated photoprotein (*e.g.* aequorin, symplectin, *Mitrocoma* photoprotein, *Clytia* photoprotein, and *Obelia* photoprotein), North American firefly luciferase, Japanese firefly luciferase, Italian firefly luciferase, East European firefly luciferase, Pennsylvania firefly luciferase, Click beetle luciferase, railroad worm luciferase, *Renilla* luciferase, *Gaussia* luciferase, Cypridina luciferase, *Metridina* luciferase, Metrida luciferase, OLuc, and red firefly luciferase, bacterial luciferase, and active variants thereof.

83. The genetically engineered microorganism of claim 78, wherein the contrast agent for use in MRI is selected from ferritin, transferrin receptor-1 (TfR1), Tyrosinase (TYR), beta-galactosidase, manganese-binding protein MntR, creatine kinase (CK), *Magnetospirillum magnetotacticum* magA, Divalent metal transporter DMT1, protamine-1 (hPRM1), urea transporter (UT-B), and ferritin receptor Timd2 (T-cell immunoglobulin and mucin domain containing protein 2), sodium iodide symporter, *E. coli* dihydrofolate reductase, norepinephrine transporter, and active variants thereof.

84. The genetically engineered microorganism of claim 78, wherein the PET reporter is selected from thymidine kinase, deoxycytidine kinase, Dopamine 2 Receptor, estrogen receptor α surface protein binding domain, somatostatin receptor subtype 2, carcinoembryonic antigen, a sodium iodide symporter, *E. coli* dihydrofolate reductase, a single-chain antibody specific to 1,4,7,10-tetraazacyclododecane-1, 4,7,10-tetraacetic acid (DOTA), or a variants thereof.

85. The genetically engineered microorganism of claim 78, wherein the enzyme reporter is beta-galactosidase, chloramphenicol acetyltransferase, horseradish peroxidase, alkaline phosphatase, acetylcholinesterase, and catalase.

~~86. The genetically engineered microorganism of claim 78, wherein the Single Photon Emission Computed Tomography (SPECT) reporter is selected from sodium ion symporter, norepinephrine transporter, sodium iodide symporter, dopamine receptor, and dopamine transporter.~~

~~876~~. The genetically engineered microorganism of any one of claims ~~701~~ to ~~865~~, wherein the one or more gene(s) encoding at least one detection marker comprises at least one intron.

~~887~~. The genetically engineered microorganism of any one of claims ~~701~~ to ~~876~~, wherein the surface protein is an invasin, or a fragment thereof.

~~89. The genetically engineered microorganism of claim 88, wherein the invasin is selected from *Yersinia enterocolitica* invasin, *Yersinia pseudotuberculosis* invasin, *Salmonella enterica* PagN, *Candida albicans* Als3; and/or the intimin is selected from *Escherichia albertii* intimin, *Escherichia coli* intimin, and *Citrobacter rodentium* intimin.~~

~~90. The genetically engineered microorganism of any one of claims 70 to 87, wherein the surface protein comprises a peptide or protein that specifically binds to the surface of cancerous and pre-cancerous cells, optionally wherein the protein is selected from a leptin, an antibody, or an antigen-binding fragment thereof.~~

~~918~~. The genetically engineered microorganism of any one of claims ~~701~~ to ~~907~~, wherein the microorganism comprises a second exogenous gene encoding a lysin that lyses the endocytotic vacuole~~.~~

~~92. The genetically engineered microorganism of claim 91~~, optionally wherein the lysin is listeriolysin O, or a derivative thereof.

~~93. The genetically engineered microorganism of any one of claims 70 to 92, wherein the microorganism is selected from *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, and *Escherichia coli*.~~

~~94. The genetically engineered microorganism of any one of claims 70 to 93, wherein the microorganism is a probiotic *Escherichia coli* strain or a derivative thereof.~~

~~95~~9. The genetically engineered microorganism of any one of claims ~~7~~01 to ~~9~~48, wherein the microorganism is *Escherichia coli* Nissle 1917 or a derivative thereof.

~~96. The genetically engineered microorganism of claim 95, wherein the *Escherichia coli* Nissle 1917 or the derivative thereof harbors a plasmid pMUT1 and/or a plasmid pMUT2, and/or derivatives thereof.~~

~~97. The genetically engineered microorganism of claim 95, wherein the *Escherichia coli* Nissle 1917 or the derivative thereof is cured of the plasmid pMUT1 and/or the plasmid pMUT2.~~

~~98~~10. The genetically engineered microorganism of any one of claims ~~7~~01 to 9~~7~~, wherein the gene encoding the surface protein is integrated at a genomic site.

~~99~~11. The genetically engineered microorganism of any one of claims ~~7~~08-~~98~~10, wherein the second gene encoding the lysin is integrated at a genomic site.

~~100. The genetically engineered microorganism of claim 99, wherein the gene encoding the surface protein, and the second gene encoding the lysin are integrated at a single genomic site, optionally the single genomic site is an integration site of a bacteriophage, and/or a naturally occurring plasmid.~~

~~101. The genetically engineered microorganism of any one of claims 70-100, wherein the one or more gene(s) encoding at least one detection marker is integrated at a third genomic site.~~

1~~0~~2. The genetically engineered microorganism of claim 1~~0~~1, wherein the gene encoding the surface protein, the second gene encoding the lysin and the gene(s) encoding at least one detection marker are integrated at a single genomic site genomic site.

1~~0~~3. The genetically engineered microorganism of any one of claims ~~70-97~~8 or 9, wherein the gene encoding the surface protein, and /or the second gene encoding the lysin are inserted on a plasmid.

~~104. The genetically engineered microorganism of any one of claims 70-97 or 103, wherein the one or more gene(s) encoding at least one detection marker is inserted on the plasmid, wherein the plasmid is optionally a single copy plasmid, or a multi-copy plasmid.~~

1054. The genetically engineered microorganism of claim 10413, wherein the one or more gene(s) encoding at least one detection marker is inserted on a plasmid different from a plasmid that harbors the gene encoding the surface protein and/or the gene encoding the lysin.

1065. The genetically engineered microorganism of any one of claims 708-1054, wherein the microorganism is *Escherichia coli* Nissle 1917 or a derivative thereof and one or more of the genes encoding the surface protein, the lysin, and the detection marker are carried on plasmid pMUT1 or the plasmid pMUT2, and/or a derivatives thereof.

1076. The genetically engineered microorganism of any one of claims 7013 to 1065 wherein the plasmid(s) carrying one or more of the gene encoding the surface protein, the lysin, and the detection marker further comprises a selection mechanism.

1087. The genetically engineered microorganism of claim 1076, wherein the selection mechanism is selected from an antibiotic resistance marker, a toxin-antitoxin system, a marker causing complementation of a mutation in an essential gene, a cis acting genetic element and a combination of any two or more thereof.

~~109. The genetically engineered microorganism of claim 107 or claim 108, wherein the antibiotic resistance marker is selected from kanamycin resistance gene and tetracycline resistance gene.~~

~~110. The genetically engineered microorganism of any one of claims 70-109, wherein the toxin-antitoxin system is selected from a *hok/sok* system of plasmid R1, *parDE* system of plasmid RK2, *ccdAB* of F plasmid, *flmAB* of F plasmid, *kis/kid* system of plasmid R1, *XCV2162- ptaRNA1* of *Xanthomonas* campestris, *ataT-ataR* of enterohemorragic *E. coli* or *Klebsiella*, *toxIN* system of *Erwinia carotovora*, *parE-parD* system of *Caulobacter crescentus*, *fst-RNAII* from *Enterococcus faecalis* plasmid AD1, ε-ζ system of *Bacillus subtilis* plasmid pSM19035 and a combination of any two or more thereof.~~

~~111. The genetically engineered microorganism of any one of claims 70-110, wherein the essential gene encodes~~

~~an enzyme involved in biosynthesis of an essential nutrient or a cell wall component; and/or~~

~~a house-keeping function.~~

~~112~~18. The genetically engineered microorganism of claim 1~~11~~7, wherein the essential gene is selected from *dapA*, *dapD*, *murA*, *alr*, *dadX*, *murI*, *dapE*, *thyA* and a combination of any two or more thereof, optionally wherein the essential genes are a combination of *alr* and *dadX*, and optionally wherein the plasmid and/ or the second plasmid is selected by complementation of the *alr* and *dadX* mutations by a functional *alr* gene present on the plasmid and/ or the second plasmid.

~~113. The genetically engineered microorganism of claim 111, wherein the house-keeping function is selected from *infA*, a gene encoding a subunit of an RNA polymerase, a DNA polymerase, an rRNA, a tRNA, a cell division protein, a chaperon protein, and a combination of any two or more thereof.~~

~~114. The genetically engineered microorganism of any one of claims 70-113, wherein the cis acting genetic element is ColE1 *cer* locus or *par* locus from pSC101.~~

1~~15~~9. The genetically engineered microorganism of any one of claims ~~70~~1-1~~14~~8, wherein the microorganism harbors at least one nutritional auxotrophic mutation selected from *dapA*, *dapD*, *dapE*, *murA*, *alr*, *dadX*, *murI*, *thyA*, *aroC*, *ompC*, and *ompF*, optionally wherein the strain harbors a combination of *dapA alr* and *dadX* auxotrophic mutations.

~~116. The genetically engineered microorganism of any one of claims 100-115, wherein the plasmid or the second plasmid comprises at least one binding site for a DNA binding protein.~~

~~117. The genetically engineered microorganism of claim 116, wherein the DNA binding protein comprises one or more nuclear localization signal(s) (NLS).~~

~~118. The genetically engineered microorganism of claim 116 or claim 117, wherein the NLS is SV40 T antigen NLS sequence (KKKRKV).~~

~~119. The genetically engineered microorganism of any one of claims 116 to 118, wherein the DNA binding protein is NFκB.~~

~~120. The genetically engineered microorganism of any one of claims 116 to 119, wherein the microorganism comprises a gene encoding the DNA binding protein comprises one or more nuclear localization signal(s) (NLS).~~

~~121~~10. ~~A method of diagnosis and/or treatment of a disease in a subject, the method comprising:~~

~~(i) administering to the gastrointestinal tract of the subject~~ Use of the genetically engineered microorganism of any one of claims ~~70~~1 to 1~~20~~9 in the preparation of a composition for detecting diseased epithelial cells, wherein the composition comprising the genetically engineered microorganism is to be administered to the subject; wherein ~~and~~

~~(ii) detecting the~~ expression of the detection marker is to be detected for ~~to~~ thereby detecting diseased epithelial cells to diagnose a disease in the subject,

optionally wherein a treatment is to be administered to ~~the method further comprises administering a treatment to~~ the subject.

~~122. A method of selecting a subject suffering from or suspected to be suffering from a disease for a treatment, the method comprising:~~

~~(i) administering to the gastrointestinal tract of the subject the genetically engineered microorganism of any one of claims 70 to 120;~~

~~(ii) detecting elevated expression of the detection marker compared to surrounding normal epithelial cells; and~~

~~(iii) selecting the subject for treatment if expression of the detection marker is observed compared to surrounding normal epithelial cells.~~

2~~123~~. The ~~method~~use of claim ~~12~~10 ~~or claim 122~~, wherein the disease is selected from a precancerous lesion, cancer, ulcerative colitis, Crohn's disease, Barrett's esophagus, irritable bowel syndrome and irritable bowel disease.

~~124~~2. The ~~method~~use of claim ~~123~~1, wherein the precancerous lesion comprises:

a polyp selected from sessile polyp, serrated polyp (*e.g.* hyperplastic polyps, sessile serrated adenomas/polyps, and traditional serrated adenoma), sessile serrated polyp, flat polyp, sub-pedunculated polyp , pedunculated polyp, and a combination thereof;

a biliary intraepithelial neoplasm (BilIN) selected from BilIN-1, BilIN-2, BilIN-3, and cholangiocarcinoma; and/or

a pancreatic intraepithelial neoplasm (PanIN) selected from PanIN -1, PanIN -2, PanIN -3 and pancreatic ductal adenocarcinoma (PDAC).

~~1~~253. The ~~method~~use of claim ~~1~~242, wherein the polyp is a diminutive polyp.

~~126. The method of claim 123 or 124, wherein the precancerous lesion has a size of from about 0.05 mm to about 30 mm.~~

~~1~~274. The ~~method~~use of claim 2126 ~~or 22~~, wherein the precancerous lesion has a size of less than about 0.1 mm, less than about 0.25 mm, less than about 0.5 mm, less than about 1 mm, less than about 2 mm, less than about 5 mm, less than about 8 mm, less than about 10 mm, less than about 15 mm, less than about 20 mm, less than about 25 mm, or less than about 30 mm.

~~128. The method of claim 123, wherein the cancer comprises a polyp, an adenoma, or a frank cancer.~~

~~1~~295. The ~~method~~use of claim 2123, wherein the cancer comprises Lynch syndrome, familial adenomatous polyposis, hereditary non-polyposis colon cancer (HNPCC), or a sporadic cancer.

~~130~~26. The ~~method~~use of claim 2123, wherein the cancer is selected from squamous cell carcinoma of anus, low-grade squamous intraepithelial lesions (LSIL) of anus, high-grade squamous intraepithelial lesions (HSIL) of anus, colorectal cancer, colorectal adenocarcinoma, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, colorectal polyposis (*e.g.* Peutz–Jeghers syndrome, juvenile polyposis syndrome, MUTYH-associated polyposis, familial adenomatous polyposis/Gardner's syndrome, and Cronkhite-Canada syndrome), carcinoid, pseudomyxoma peritonei, duodenal adenocarcinoma, premalignant adenoma of small bowel, distal bile duct carcinomas, biliary intraepithelial neoplasm (BilIN), BilIN-1, BilIN-2, BilIN-3 or cholangiocarcinoma, pancreatic ductal adenocarcinoma (PDAC), pancreatic intraepithelial neoplasm (PanIN), PanIN -1, PanIN -2, PanIN -3, gastric carcinoma, signet ring cell carcinoma (SRCC), gastric lymphoma (MALT lymphoma), linitis plastic (Brinton's disease), and squamous cell carcinoma of esophagus and adenocarcinoma.

~~131~~27. ~~A method for treating a cancer in a patient, comprising:~~

~~(i) administering to the gastrointestinal tract of the subject the genetically engineered microorganism of any one of claims 70 to 100;~~

~~(ii) detecting the expression of the detection marker to thereby detecting diseased epithelial cells; and~~Use of claim 20, wherein the disease is cancer and a treatment is to be administered to a subject ~~(iii) administering a treatment~~ if the expression of the detection marker is observed.

~~132. The method of any one of claims 121 to 131, wherein the treatment is surgery or administration of a therapeutic agent selected from the group consisting of a chemotherapeutic agent, a cytotoxic agent, an immune checkpoint inhibitor, an immunosuppressive agent, a sulfa drug, a corticosteroid, an antibiotic and a combination of any two or more thereof.~~