CLAIMS

1. An engineered *Saccharomyces* strain comprising:

    a. a surface displayed binding agent, wherein the binding agent has a binding affinity to a target microorganism;

    b. any one of:

        i. an anchor moiety comprising a cell wall anchor region selected from Cwp2p, Sed1p, Ccw12p or Flo1p protein operably linked to the binding agent; or

        ii. a stem comprising a transmembrane protein operably linked to a transactivator via a conditionally cleavable peptide; wherein the surface displayed binding agent is operably linked to the stem.

2. An engineered *Saccharomyces* strain comprising a display cassette comprising:

    a. a sequence encoding a surface displayed binding agent, wherein the binding agent has a binding affinity to a target microorganism;

    b. a sequence encoding any one of:

        i. an anchor moiety comprising a cell wall anchor region selected from Cwp2p, Sed1p, Ccw12p or Flo1p protein, wherein the sequence encoding the anchor moiety and the binding agent are encoded in a single cistron; or

        ii. a stem comprising a transmembrane protein, operably linked to a transactivator via a conditionally cleavable peptide; wherein the surface displayed binding agent is operably linked to the stem.

3. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the strain is *Saccharomyces cerevisiae var. boulardii (S. boulardii).*

4. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the stem comprises a cell wall protein domain operably linked to the transmembrane protein.

5. The engineered *Saccharomyces* strain of claim 4, wherein the cell wall protein domain is Mid2 protein (Mid2p) and the transmembrane protein is Wsc1p(C8A).

6. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the target microorganism is a pathobiont, a commensal microorganism, or a pathogen.

7. The engineered *Saccharomyces* strain of claim 6, wherein the target microorganism is selected from a group consisting of *Norovirus*, *Listeria monocytogenes*, *Clostridium pathobionts, Clostridium difficile, Clostridium perfringens, Campylobacter coli,*

Staphylococcus aureus, *methicillin-resistant Staphylococcus aureus (MRSA)*, *Streptococcus pyogenes*, *Salmonella*, *P. aeruginosa*, *E. coli*.

8. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the binding agent comprises one or more of a group consisting of: peptides, proteins, antibodies, nanobodies, single chain fragments thereof, and combinations thereof.

9. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the binding agent is selected from CLM-1 (norovirus), cell binding domain of any one of ϕCP26F (PlyCP26F from *C. difficile/perfringens*), endolysin Ply500 (*Listeria monocytogenes)*, PlyP35 (*Listeria monocytogenes)*, LysGH15B (*Staphylococcus aureus)*, endolysin LysGH15 (*Staphylococcus aureus)*, LysSA97(*Staphylococcus aureus)*, ZW88 (*Staphylococcus aureus)*, phiCcolBB35 (*Campylobacter coli*), PlyC (*Streptococcus pyogenes*).

10. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the conditionally cleavable peptide is cleavable in presence of ubiquitinase.

11. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the transactivator comprises an RNA-guided DNA endonuclease enzyme Cas9, or a scRNA-binding motif and a transcription activator domain, or a RNA stem loop recognition domain recognition domain selected from MCP, PCP, or Com.

12. The engineered *Saccharomyces* strain of claim 1, wherein

    i. the transactivator is operably linked to the transmembrane protein via a first split ubiquitin fragment; and

    ii. further comprising a second split ubiquitin fragment operably linked to a transmembrane protein on a second stem;

    wherein physical interaction between the first split ubiquitin fragment and the second split ubiquitin fragment releases the transactivator.

13. The engineered *Saccharomyces* strain of claim 1 or 2, wherein the transactivator can initiate the expression of one or more of, a reporter, a biomolecule, a transcriptional activation domain or an RNA stem loop recognition domain.

14. The engineered *Saccharomyces* strain of claim 13, wherein the reporter is selected from a fluorescent protein or a luminescence protein, the biomolecule is selected from a group consisting of an antibody, a single-chain variable fragment (scFv), a nanobody, an enzyme, or an antimicrobial peptide, the transcriptional activator is VP64.

15. The engineered *Saccharomyces* strain of claim 2, wherein the display cassette further comprises a promoter and a terminator sequence.

16. The engineered yeast strain of claim 15, wherein the promoter is selected from TDH3, CCW12, WSC1, FAU1 and the terminator is selected from ADH1 or CYC1.

17. The engineered *Saccharomyces* strain of any one of claims 1-16, for use in treating gut disease.

18. The engineered *Saccharaomyces* strain of any one of claims 1-17, further comprising a second binding agent, wherein the second binding agent is capable of binding to a second target microorganism and the first binding agent is different from the second binding agent.

19. A method of constructing an engineered *Saccharomyces* cell, the method comprising:

    i. providing a *Saccharomyces* cell;

    ii. providing a display cassette of any one of claims 2-18; and

    integrating the display cassette into an intergenic site of the *Saccharomyces* cell genome.

20. The method of constructing an engineered *Saccharomyces* cell of claim 19, wherein the strain is *S. boulardii* and the intergenic site is selected from Chr VII, XII, XV, XVI, or combinations thereof.

21. Use of the engineered *Saccharomyces* strain of any one of claims 1-18 in the manufacture of a medicament for treating a norovirus infection.

22. The engineered *Saccharomyces* strain of any one of claims 1-18, for use in the treatment of a norovirus infection.

23. Use of the engineered *Saccharomyces* strain of any one of claims 1-16 in the manufacture of a medicament for treating gut disease.

CLAIMS

What is claimed is:

1. An engineered microorganism comprising:

at least one binding agent displayed on a surface of the engineered microorganism, wherein the binding agent has a binding affinity to a target microorganism.

2. The engineered microorganism of claim 1, wherein the binding agent is operably linked to the engineered microorganism via an anchor protein.

3. The engineered microorganism of claim 2, wherein the anchor protein is a cell wall protein anchor region.

4. The engineered microorganism of claim 3, wherein the cell wall protein anchor region is a Flo1p anchor region.

5. The engineered microorganism of claim 1, wherein the binding agent is operably linked to the engineered microorganism via a stem comprising a transmembrane protein.

6. The engineered microorganism of claim 5, wherein the transmembrane protein is selected from Wsc1p (C8A).

7. The engineered microorganism of claim 5, wherein the stem comprises a cell wall protein domain operably linked to the transmembrane protein, wherein the cell wall protein domain is capable of extending the stem into extracellular space.

8. The engineered microorganism of claim 7, wherein the cell wall protein domain is Mid2 protein (Mid2p).

9. The engineered microorganism of claim 1, comprising a first binding agent and a second binding agent, wherein the first binding agent is capable of binding to a first target microorganism and the second binding agent is capable of binding to a second target microorganism and the first binding agent is different from the second binding agent.

10. The engineered microorganism of claim 1, wherein the engineered microorganism is Saccharomyces yeast.

11. The engineered microorganism of claim 10, wherein the engineered microorganism is selected from *Saccharomyces cerevisiae* var. *boulardii* (*S. boulardii*).

12. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is a pathobiont, a commensal microorganism, or a pathogen.

13. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is a bacteria, a fungi, a virus, or a eukaryote.

14. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is selected from the group consisting of Norovirus, *Listeria monocytogenes, Clostridium* pathobionts*, Clostridium difficile, Clostridium perfringens, Campylobacter coli, Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA)*, Streptococcus pyogenes, Salmonella, P. aeruginosa, E. coli*, and any other biological entity having a binding region that can be targeted by a binding agent, such as scFvs, receptors, or binding domains.

15. The engineered microorganism of any one of claims 1 to 10, wherein the binding agent specifically binds to the target microorganism.

16. The engineered microorganism of any one of claims 1 to 10, wherein the binding agent comprises one or more of the group consisting of: peptides, proteins, antibodies, nanobodies, single chain fragments thereof, and combinations thereof.

17. The engineered microorganism of any one of claims 1 to 10, wherein the binding agent comprises one or more of the group consisting of: a cell wall binding domain of a target microorganism and a cell wall binding domain of an endolysin.

18. The engineered microorganism of claim 2, wherein the anchor protein comprises a cell wall protein anchor region in a single cistron bracketed by a promoter and a terminator.

19. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is norovirus and the binding agent is CLM-1.

20. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is *Clostridium* pathobionts (*C. difficile/perfringens*) and the binding agent is cell binding domain of ΦCP26F (PlyCP26F).

21. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is *Listeria monocytogenes* and the binding agent is cell binding domain 500 of endolysin Ply500 or cell wall binding domain of PlyP35.

22. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is *Streptococcus pyogenes* and the binding agent is PlyC binding domain of endolysin PlyC.

23. The engineered microorganism of any one of claims 1 to 10, wherein the target microorganism is methicillin-resistant *Staphylococcus aureus* (MRSA) or *Staphylococcus aureus* and the binding agent is LysGH15B, a cell wall binding domain of endolysin LysGH15, LysSA97, or ZW88.

24. The engineered microorganism of any one of claims 1 to 10, the target microorganism is *Campylobacter coli* and the binding agent is a cell wall binding domain of phiCcolBB35.

25. The engineered microorganism of any one of claims 1 to 10, further comprising a transactivator operably linked to a transmembrane protein via a conditionally cleavable peptide.

26. The engineered microorganism of claim 25, wherein the conditionally cleavable peptide is cleavable when in the presence of ubiquitinase.

27. The engineered microorganism of claim 25, wherein the transactivator comprises an RNA-guided DNA endonuclease enzyme.

28. The engineered microorganism of claim 27, wherein the RNA-guided DNA endonuclease enzyme is nuclease-null Cas9 (dCas9).

29. The engineered microorganism of claim 27, wherein the RNA-guided DNA endonuclease enzyme corresponds to a display unit integrated into an intergenic site.

30. The engineered microorganism of claim 29, wherein the engineered microorganism is *S. boulardii* and the intergenic site is selected from Chr VII, XII, XV, XVI, or combinations thereof.

31. The engineered microorganism of claim 25, wherein

a first split ubiquitin fragment is operably linked to transmembrane protein;

a transactivator is operably to the transmembrane protein via a second split ubiquitin fragment; and

physical interaction between the first split ubiquitin fragment and the second split ubiquitin fragment releases the transactivator.

32. The engineered microorganism of claim 31, wherein

the first split ubiquitin fragment comprises a N-terminal 37 amino acid residues of ubiquitin is operably linked to transmembrane protein; and

the second split ubiquitin fragment comprises a C-terminal 42 amino acid residues of ubiquitin.

33. The engineered microorganism of claim 25, wherein the transactivator comprises a scRNA-binding motif and a transcription activator capable of initiating expression of a reporter protein.

34. The engineered microorganism of claim 33, wherein the reporter protein is selected from a fluorescent protein, such as GFP, RFP, BFP, or a luminescence protein, such as luciferase.

35. The engineered microorganism of claim 25, wherein the transactivator comprises a transcriptional activation domain.

36. The engineered microorganism of claim 35, wherein the transcriptional activation domain comprises VP64.

37. The engineered microorganism of claim 25, wherein the transactivator comprises an RNA stem loop recognition domain and the RNA stem loop recognition domain is fused to an intracellular enzyme-cleavable-binding fragment.

38. The engineered microorganism of claim 37, wherein the RNA stem loop recognition domain is selected from MCP, PCP, or Com.

39. The engineered microorganism of claim 37, wherein the intracellular enzyme-cleavable-binding fragment is a ubiquitin fragment.

40. The engineered microorganism of any one of claims 1 to 10, wherein the engineered microorganism comprises a reporter gene.

41. The engineered microorganism of claim 40, wherein the reporter gene is selected from GFP, RFP, BFP, luminescence proteins, or combinations thereof.

42. The engineered microorganism of any one of claims 1 to 10, wherein the engineered microorganism is selected from a microorganism that does not colonize in a mammalian gut.

43. Use of the engineered microorganism of any one of claims 1 to 42 in the manufacture of a medicament for targeted sensing, detecting, or killing of commensal or pathogenic gut microbes.

44. Use of the engineered microorganism of any one of claims 1 to 42 in the manufacture of a medicament for treating a gut disease.

45. Use of the engineered microorganism of any one of claims 1 to 42 in the manufacture of a medicament for regulating commensal gut microbiota.

46. A method of constructing an engineered microorganism comprising:

providing a microorganism;

providing a display cassette; and

integrating the display cassette into an intergenic site of the microorganism genome;

wherein,

the display cassette comprises a sequence for expression of a binding agent; a promoter capable of expressing the binding agent; a terminator; an anchor; and a secretion signal peptide.

47. The method of claim 46, wherein the anchor comprises a cell wall protein anchor region in a single cistron bracketed by a constitutive promoter and a terminator.

48. The method of claim 46, wherein the anchor is selected from Cwp2p, Sed1p, Ccw12p, or Flo1p from *Saccharomyces* yeasts.

49. The method of claim 46, wherein the anchor is selected from Flo1p.

50. A method of constructing an engineered microorganism comprising:
providing a microorganism;
providing a display cassette; and
integrating the display cassette into an intergenic site of the microorganism genome;
wherein,
the display cassette comprises a sequence for expression of a binding agent; a constitutive promoter; a terminator; a stem comprising a transmembrane domain of a microorganism cell wall protein; a secretion signal peptide; and an intracellular enzyme-cleavable-binding fragment operably linked to a transactivator.

51. The method of claim 50, wherein the transmembrane domain of a microorganism cell wall protein is selected from Wsc1p (C8A).

52. The method of claim 50, wherein the display cassette further comprises a cell wall protein domain operably linked to the stem, wherein the cell wall protein domain is capable of extending the stem into extracellular space.

53. The method of claim 50, comprising:
a first pair of binding agents and a second pair of binding agents; and
a first transactivator and a second transactivator; wherein

the first pair of binding agents are capable of binding to a first target microorganism and the second pair of binding agents are capable of binding to a second target microorganism and the first pair of binding agents are different from the second pair of binding agents;

the first pair of binding agents are operably linked to a first pair of intracellular enzyme-cleavable-binding fragment via a first transmembrane protein, wherein one of the first pair of the first intracellular enzyme-cleavable-binding fragment is operably linked to the first transactivator;

the second binding agent is operably linked to a second pair of intracellular enzyme-cleavable-binding fragment via a second transmembrane protein, wherein one of the second pair of the second intracellular enzyme-cleavable-binding fragment is operably linked to a second transactivator; and

one of the first pair of intracellular enzyme-cleavable-binding fragments and one of the second pair of intracellular enzyme-cleavable-binding fragments have the same binding protein in the N-terminal region, but have different transactivators in the C-terminal region, which are capable of physically interacting with target microorganisms, and releasing the first or the second transactivators.

54. The method of any one of claims 46 or 50, wherein the binding agent comprises one or more of the group consisting of: peptides, proteins, antibodies, nanobodies, single chain fragments thereof, and combinations thereof.

55. The method any one of claims 46 or 50, wherein the binding agent comprises one or more of the group consisting of: a cell wall binding domain of a target microorganism and a cell wall binding domain of an endolysin.

56. The method any one of claims 46, 50, or 53 wherein the binding agent is selected from CLM-1; ZW88; cell binding domain of ΦCP26F (PlyCP26F); cell binding domain 500 of endolysin Ply500; PlyC binding domain of endolysin PlyC; LysGH15B, a

cell wall binding domain of endolysin LysGH15 or LysSA97; or cell wall binding domain of phiCcoIBB35.

57. The method of any one of claims 50 to 56, wherein the transactivator is operably linked to a transmembrane protein via a conditionally cleavable peptide.

58. The method of claim 50, wherein the transactivator comprises an RNA-guided DNA endonuclease enzyme.

59. The method of claim 58, wherein the RNA-guided DNA endonuclease enzyme corresponds to a display unit integrated into an intergenic site.

60. The method of any one of claims 50 to 59, wherein a nuclease-null Cas9 (dCas9) and a scaffold RNA recruit the transactivator to corresponding synthetic promoters.

61. The method of claim 50, wherein the transactivator comprises a scRNA-binding motif and a transactivator capable of initiating the expression of a reporter or biomolecule.

62. The method of any one of claims 50 to 60, wherein the transactivator comprises a transcriptional activation domain.

63. The method of claim 50, wherein the transactivator comprises an RNA stem loop recognition domain fused to an intracellular enzyme-cleavable-binding fragment.

64. The method of claim 50, comprising initiating transcription of target reporter proteins.

65. The method of claim 64, wherein the target reporter proteins are selected from a fluorescent protein and a luminescence protein.

66. The method of claim 64, wherein the target reporter proteins are selected from the group consisting of: GFP, RFP, BFP, luciferase, or combinations thereof.

67. The method of any one of claims 46 or 50, wherein the microorganism is selected from *S. boulardii* and the intergenic site is selected from Chr VII, XII, XV, or XVI, or a combination thereof; and corresponding guide RNAs for Cas9 yield stable expression of target proteins without interference in native transcriptions of the microorganism.

68. The method of any one of claims 46 or 50, wherein frameworks for stable expression of target proteins do not require an inducer.

69. The method of any one of claims 46 or 50, comprising a strong constitutive promoter or weak promoter of the engineered microorganism.

70. The method of claim 69, wherein the strong constitutive promoter is selected from *TDH3* or *CCW12* promoters of *S. boulardii*.

71. The method of claim 69, wherein the weak promoter is selected from *WSC1* or *FAU1* promoters of *S. boulardii.*

72. The method of claim 69, further comprising a terminator.

73. The method of claim 72, wherein the terminator is selected from *ADH1* or *CYC1* terminators of *S. boulardii*.

74. The engineered microorganism of any one of claims 1 to 42 for use as a medicament.

~~75. The engineered microorganism of any one of claims 1 to 42 for use in targeted sensing, detecting, or killing of commensal or pathogenic gut microbes.~~

~~76. The engineered microorganism of any one of claims 1 to 42 for use in treating a gut disease.~~

~~77. The engineered microorganism of any one of claims 1 to 42 for use in regulating commensal gut microbiota.~~