

CLAIMS

1. A genetically modified ascomycetous filamentous fungus for producing an exogenous protein of interest, the genetically modified filamentous fungus comprises at least one cell having reduced expression and/or protease activity of KEX2 and/or ALP7, the at least one cell comprising an exogenous polynucleotide encoding the protein of interest.
2. The genetically modified ascomycetous filamentous fungus of claim 1, having reduced expression and/or activity of KEX2.
3. The genetically modified ascomycetous filamentous fungus of claim 1, having reduced expression and/or activity of ALP7.
4. The genetically modified ascomycetous filamentous fungus of claim 1, having reduced expression and/or activity of KEX2 and ALP7.
5. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 4, wherein KEX2 comprises an amino acid sequence having at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99%, or 100% identity to the amino acid sequence of *Thermothelomyces heterothallica* KEX2.
6. The genetically modified ascomycetous filamentous fungus of claim 5, wherein the *Thermothelomyces heterothallica* KEX2 comprises the amino acids of SEQ ID NO: 14.
7. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 6, wherein ALP7 comprises an amino acid sequence having at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99%, or 100% identity to the amino acid sequence of *Thermothelomyces heterothallica* ALP7.
8. The genetically modified ascomycetous filamentous fungus of claim 7, wherein the *Thermothelomyces heterothallica* ALP7 comprises the amino acids of SEQ ID NO: 13.
9. The genetically modified ascomycetous filamentous fungus of any one of claims

- 1 to 8, having reduced expression and/or activity of at least one additional protease.
10. The genetically modified ascomycetous filamentous fungus of claim 9, wherein the additional protease is selected from the group consisting of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, and ALP4.
11. The genetically modified ascomycetous filamentous fungus of claim 10, said fungus having reduced expression and/or activity of at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 proteases selected from the group consisting of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, and ALP4.
12. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 11, having reduced expression and/or activity of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, ALP4 and KEX2.
13. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 11, having reduced expression and/or activity of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, ALP4 and ALP7.
14. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 13, having reduced expression and/or activity of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, ALP4, ALP7 and KEX2.
15. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 14, having reduced expression and/or activity of at least one additional protease selected from the group consisting of ALP5, ALP6, SRP3, SRP5, and SRP8.
16. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 15, wherein said genetically modified ascomycetous filamentous fungus produces the protein of interest in an increased amount compared to the amount produced by its non-genetically modified parent ascomycetous filamentous fungus strain cultured under similar conditions.
17. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 16, wherein the protein produced by said genetically modified ascomycetous filamentous fungus has increased stability compared to said protein produced by

the non-genetically modified parent ascomycetous filamentous fungus strain cultured under similar conditions.

18. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 17, wherein the ascomycetous filamentous fungus is of a genus within *Pezizomycotina*.
19. The genetically modified ascomycetous filamentous fungus of claim 18 said ascomycetous filamentous fungus is of a genus selected from the group consisting of *Thermothelomyces*, *Myceliophthora*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Rasamsonia*, *Chrysosporium*, *Corynascus*, *Fusarium*, *Neurospora*, and *Talaromyces*.
20. The genetically modified ascomycetous filamentous fungus of claim 19, said ascomycetous filamentous fungus is of a species selected from the group consisting of *Thermothelomyces heterothallica*, *Myceliophthora lutea*, *Aspergillus nidulans*, *Aspergillus funiculosus*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Trichoderma viride*, *Rasamsonia emersonii*, *Penicillium chrysogenum*, *Penicillium verrucosum*, *Sporotrichum thermophile*, *Corynascus fumimontanus*, *Corynascus thermophilus*, *Chrysosporium lucknowense*, *Fusarium graminearum*, *Fusarium venenatum*, *Neurospora crassa*, and *Talaromyces piniphilus*.
21. The genetically modified ascomycetous filamentous fungus of claim 20, said ascomycetous filamentous fungus is a *Thermothelomyces heterothallica* strain comprising rDNA sequence having at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% or 100% identity to the nucleic acid sequence set forth in SEQ ID NO: 20.
22. The genetically modified ascomycetous filamentous fungus of claim 21, wherein the ascomycetous filamentous fungus is *Thermothelomyces heterothallica* C1.
23. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 22, wherein the at least one exogenous polynucleotide is a DNA construct or an expression vector further comprising at least one regulatory element operable in said ascomycetous filamentous fungus.

24. The genetically modified ascomycetous filamentous fungus of any one of claims 1 to 23, wherein the protein of interest is selected from the group consisting of an antigen, an antibody, an enzyme, a vaccine, and structural protein.
- 5 25. The genetically modified ascomycetous filamentous fungus of claim 24, wherein the protein of interest is an antibody.
26. The genetically modified ascomycetous filamentous fungus of any one of claims 1-25, wherein the protein of interest is fused to a tag.
- 10 27. The genetically modified ascomycetous filamentous fungus of claim 26, wherein the tag is selected from the group consisting of Spytag, HA-tag, chitin binding protein (CBP), maltose binding protein (MBP), Strep-tag, glutathione-S-transferase (GST), FLAG-tag, C-tag, ALFA-tag, V5-tag, Myc-tag, Spot-tag, T7-tag, NE-tag, and poly(His) tag.
28. The genetically modified ascomycetous filamentous fungus of claim 24, wherein the protein of interest is a viral component.
- 15 29. The genetically modified ascomycetous filamentous fungus of claim 28, wherein the viral component is the receptor binding domain (RBD) of SARS-CoV2 spike domain or a fragment thereof.
- 20 30. The genetically modified ascomycetous filamentous fungus of any one of claims 26-29, wherein the protein of interest comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, and SEQ ID NO: 57.
31. The genetically modified ascomycetous filamentous fungus of claim 28, wherein the viral component is an antigen protein from rift valley fever virus (RVFV).
- 25 32. The genetically modified ascomycetous filamentous fungus of claim 28, wherein the viral component is of an influenza virus protein.
33. The genetically modified ascomycetous filamentous fungus of claim 24, wherein the protein of interest is fibrinogen.
34. A method for producing a fungus capable of producing a protein of interest, the method comprising transforming at least one cell of the fungus with at least one

exogenous polynucleotide encoding the protein of interest; said at least one cell of the fungus having reduced expression and/or protease activity of KEX2 and/or ALP7.

35. The method of claim 34, said method further comprises engineering the fungus to have inhibited expression and/or protease activity of KEX2 or ALP7.
36. The method of claim 35, said method further comprises engineering the fungus to have inhibited expression and/or activity of at least one additional protease selected from the group consisting of ALP1, PEP4, ALP2, PRT1, SRP1, ALP3, PEP1, MTP2, PEP5, MTP4, PEP6, and ALP4 in the at least one cell.
37. The method of any one of claims 34 to 36, wherein the genetically modified fungus produces the protein of interest in an elevated amount compared to the amount produced by a corresponding parent untransformed fungus strain cultured under similar conditions.
38. The method of any one of claims 34 to 37, wherein the ascomycetous filamentous fungus is of a genus within *Pezizomycotina*.
39. The method of claim 38, wherein the ascomycetous filamentous fungus is of a genus selected from the group consisting of *Thermothelomyces*, *Myceliophthora*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Rasamsonia*, *Chrysosporium*, *Corynascus*, *Fusarium*, *Neurospora*, and *Talaromyces*.
40. The method of claim 39, wherein the ascomycetous filamentous fungus is of a species selected from the group consisting of *Thermothelomyces heterothallica*, *Myceliophthora lutea*, *Aspergillus nidulans*, *Aspergillus funiculosus*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Trichoderma viride*, *Rasamsonia emersonii*, *Penicillium chrysogenum*, *Penicillium verrucosum*, *Sporotrichum thermophile*, *Corynascus fumimontanus*, *Corynascus thermophilus*, *Chrysosporium lucknowense*, *Fusarium graminearum*, *Fusarium venenatum*, *Neurospora crassa* and *Talaromyces piniphilus*.
41. The method of claim 40, wherein the ascomycetous filamentous fungus is a *Thermothelomyces heterothallica* strain comprising rDNA sequence having at

least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% or 100% identity to the nucleic acid sequence set forth in SEQ ID NO: 20.

42. The method of claim 41, wherein the ascomycetous filamentous fungus is *Thermothelomyces heterothallica* C1.
- 5 43. A method of producing at least one protein of interest, the method comprising culturing the genetically modified fungus of any one of claims 1 to 33 in a suitable medium; and recovering the produced protein of interest.
- 10 44. The method of claim 43, wherein the medium comprises a carbon source selected from the group consisting of glucose, sucrose, xylose, arabinose, galactose, fructose, lactose, cellobiose, glycerol and any combination thereof.
45. The method of any one of claims 34-44, wherein the at least one protein of interest is a viral component.
46. The method of claim 45, wherein the viral component is of coronavirus.
47. The method of claim 46, wherein the coronavirus is SARS-CoV-2.
- 15 48. A protein produced by a method according to any one of claims 34 to 47.
49. A combination of at least two proteins of claim 48.
50. The combination of claim 49, wherein each of the at least two proteins is a viral component of a coronavirus and wherein each viral component is of different coronavirus variant.