SUMMARY

[0006] The disclosure provides a complex comprising (a) a first monomer comprising an Fc domain fused to two FGF21 domains comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, which may be the same or different, and (b) a second monomer comprising an Fc domain fused to at least one FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, wherein at least two of the FGF21 domains of the complex comprise a free beta-klotho binding region. Optionally, (a) the first monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain. In other examples, (a) the first monomer comprises one or more FGF21 domains of the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain (optionally via a linker) followed by another FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2. In various examples, (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain. Alternatively, (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 4 fused to the C-terminus of the Fc domain. In some examples, (a) the first monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain; optionally, (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain. The disclosure also provides a complex comprising two monomers comprising the amino acid sequence of SEQ ID NO: 18, two monomers comprising the amino acid sequence of SEQ ID NO: 19, or two monomers comprising the amino acid sequence of SEQ ID NO: 20.

[0007] A complex comprising (a) a first monomer comprising an Fc domain fused at the C-terminus with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 4 and (b) a second monomer comprising an Fc domain fused at the N-terminus with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2 also is provided. In one aspect, the disclosure refers to a complex comprising (a) the peptide as described herein and (b) a second Fc domain.

[0008] The disclosure also provides a peptide comprising an Fc domain fused with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 4 at the N-terminus and an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2 at the C-terminus, as well as a peptide comprising the amino acid sequence of SEQ ID NO: 4 fused to the amino acid sequence of SEQ ID NO: 2 via a linker (e.g., a linker comprising the amino acid sequence of SEQ ID NO: 9 and/or the amino acid sequence of SEQ ID NO: 10). In one aspect, the disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the first monomer as described herein. In another aspect, the disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the second monomer as described herein. In another aspect, the disclosure refers to a system comprising an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first

monomer as described herein and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer as described herein. In another aspect, the disclosure refers to a method of producing a complex, the method comprising culturing a host cell comprising the system as described herein and harvesting the complex. In another aspect, the disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the peptide as described herein. In another aspect, the disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the first monomer as described herein or the second monomer as described herein. In another aspect, the disclosure refers to an expression vector comprising the nucleic acid as described herein. In another aspect, the disclosure refers to a host cell comprising the expression vector as described herein. In another aspect, the disclosure refers to a method of producing a complex, the method comprising culturing a host cell comprising an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer as described herein and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer as described herein, and harvesting the complex. In another aspect, the disclosure refers to a pharmaceutical composition comprising the complex or peptide as described herein. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for treating nonalcoholic steatohepatitis (NASH), treating nonalcoholic fatty liver disease (NAFLD), or reversing NASH with cirrhosis in a subject in need thereof. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for treating alcoholic steatohepatitis (ASH), alcoholic liver disease (ALD), or alcoholic fatty liver disease (AFLD) in a subject in need thereof. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for reversing liver cirrhosis or reducing fibrosis associated with NASH, NAFLD, ASH, ALD, or AFLD in a subject in need thereof. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for treating type 2 diabetes, obesity, or dyslipidemia in a subject in need thereof. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for lowering blood glucose, increasing insulin sensitivity, normalizing liver fat content, reducing uric acid, or treating craving or addiction in a subject in need thereof. In another aspect, the disclosure refers to a use of the pharmaceutical composition as described herein in the preparation of a medicament for treating metabolic dysfunction-associated steatohepatitis (MASH), treating metabolic dysfunction-associated steatotic liver disease (MASLD), treating metabolic-dysfunction associated alcoholic liver disease (MetALD), reversing MASH with cirrhosis, or reversing liver cirrhosis or reducing fibrosis associated with MASH, MAFLD, MetALD in a subject in need thereof.

[0009] The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or

1. A complex comprising (a) a first monomer comprising an Fc domain fused to two FGF21 domains comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, which may be the same or different, and (b) a second monomer comprising an Fc domain fused to at least one FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, wherein at least two of the FGF21 domains of the complex comprise a free beta-klotho binding region.

2. The complex of claim 1, wherein (a) the first monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

3. The complex of claim 2, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

4. The complex of claim 2, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain.

5. The complex of claim 2, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain.

6. The complex of claim 2, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the C-terminus of the Fc domain.

7. The complex of claim 2, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

8. The complex of claim 1, wherein (a) the first monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 4 fused to the C-terminus of the Fc domain.

9. The complex of claim 8, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 4 fused to the C-terminus of the Fc domain.

10. The complex of claim 8, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain.

11. The complex of claim 8, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

12. The complex of claim 1, wherein (a) the first monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

13. The complex of claim 12, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the N-terminus of the Fc domain and the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

14. The complex of claim 12, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 4 fused to the N-terminus of the Fc domain.

15. The complex of claim 12, wherein (b) the second monomer comprises the amino acid sequence of SEQ ID NO: 2 fused to the C-terminus of the Fc domain.

16. The complex of any one of claims 1-15, wherein one or both Fc domains comprise the amino acid sequence of SEQ ID NO: 7.

17. The complex of any one of claims 1-16, wherein one or more FGF21 domains are fused to an Fc domain via a linker comprising the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

18. The complex of claim 1 comprising two monomers comprising the amino acid sequence of SEQ ID NO: 18.

19. The complex of claim 1 comprising two monomers comprising the amino acid sequence of SEQ ID NO: 19.

20. The complex of claim 1 comprising two monomers comprising the amino acid sequence of SEQ ID NO: 20.

21. A peptide comprising an Fc domain fused with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 4 at the N-terminus and an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2 at the C-terminus.

22. The peptide of claim 21, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO: 7.

23. The peptide of claim 21 or claim 22, wherein the FGF21 domain comprising SEQ ID NO: 4 is fused to the Fc domain via a linker comprising the amino acid sequence of SEQ ID NO: 10 and the FGF21 domain comprising SEQ ID NO: 2 is fused to the Fc domain via a linker comprising the amino acid sequence of SEQ ID NO: 9.

24. The peptide of claim 21, comprising the amino acid sequence of SEQ ID NO: 21.

25. A complex comprising (a) the peptide of any one of claims 21-24 and (b) a second Fc domain.

26. A complex comprising (a) a first monomer comprising an Fc domain fused at the C-terminus with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 4 and (b) a second monomer comprising an Fc domain fused at the N-terminus with an FGF21 domain comprising the amino acid sequence of SEQ ID NO: 2.

27. The complex of claim 26, wherein at least one Fc domain comprises the amino acid sequence of SEQ ID NO: 7.

28. The complex of claim 26 or claim 27, wherein the FGF21 domain comprising SEQ ID NO: 4 is fused to the Fc domain of the first monomer via a linker comprising the amino acid sequence of SEQ ID NO: 10 and the FGF21 domain comprising SEQ ID NO: 2 is fused to the Fc domain of the second monomer via a linker comprising the amino acid sequence of SEQ ID NO: 9.

29. The complex of claim 26, wherein the first monomer comprises the amino acid sequence of SEQ ID NO: 27 and the second monomer comprises the amino acid sequence of SEQ ID NO: 28.

30. A peptide comprising the amino acid sequence of SEQ ID NO: 4 fused to the amino acid sequence of SEQ ID NO: 2 via a linker comprising the amino acid sequence of SEQ ID NO: 9 and/or the amino acid sequence of SEQ ID NO: 10.

31. The complex or peptide of any one of claims 1-30, wherein the Fc domain is an IgG1 Fc domain.

32. A nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 1-20.

33. A nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 1-20.

34. A system comprising an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 1-20 and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 1-20.

35. The system of claim 34, comprising

(a) an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 1-7 and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 1-7;

(b) an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 8-11 and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 8-11; or

(c) an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 12-15 and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 12-15.

36. A method of producing a complex, the method comprising culturing a host cell comprising the system of claim 35 and harvesting the complex.

37. A nucleic acid comprising a nucleotide sequence encoding the peptide of any one of claims 21-24 and 30.

38. A nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 26-29 or the second monomer of any one of claims 26-29.

39. An expression vector comprising the nucleic acid of claim 32, 33, 37, or claim 38.

40. A host cell comprising the expression vector of claim 39.

41. A method of producing a complex, the method comprising culturing a host cell comprising an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the first monomer of any one of claims 26-29 and an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding the second monomer of any one of claims 26-29, and harvesting the complex.

42. A pharmaceutical composition comprising the complex or peptide of any one of claims 1-30.

43. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for treating nonalcoholic steatohepatitis (NASH), treating nonalcoholic fatty liver disease (NAFLD), or reversing NASH with cirrhosis in a subject in need thereof.

44. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for treating alcoholic steatohepatitis (ASH), alcoholic liver disease (ALD), or alcoholic fatty liver disease (AFLD) in a subject in need thereof.

45. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for reversing liver cirrhosis or reducing fibrosis associated with NASH, NAFLD, ASH, ALD, or AFLD in a subject in need thereof.

46. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for treating type 2 diabetes, obesity, or dyslipidemia in a subject in need thereof.

47. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for lowering blood glucose, increasing insulin sensitivity, normalizing liver fat content, reducing uric acid, or treating craving or addiction in a subject in need thereof.

48. The use of any one of claims 43-48, wherein pharmaceutical composition comprises the complex of any one of claims 18-20.

49. Use of the pharmaceutical composition of claim 42 in the preparation of a medicament for treating metabolic dysfunction-associated steatohepatitis (MASH), treating metabolic dysfunction-associated steatotic liver disease (MASLD), treating metabolic-dysfunction associated alcoholic liver disease (MetALD), reversing MASH with cirrhosis, or reversing liver cirrhosis or reducing fibrosis associated with MASH, MAFLD, MetALD in a subject in need thereof.

50. The complex of any of claims 1-17, wherein the first monomer and/or the second monomer comprises two or more tandem FGF21 domains comprising SEQ ID NO: 2 and/or SEQ ID NO: 4 fused to the N-terminus of the Fc domain.

51. The complex or peptide of any one of claims 1-15, 17-21, 23-26, and 28-30, wherein the Fc domain comprises the amino acid sequence of any one of SEQ ID NO: 76-83.