CLAIMS

We claim:

1. An engineered leucine decarboxylase polypeptide comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92,%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to at least one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 38, 234, 284, 484, 594, 686, 688, and/or 766, wherein the amino acid positions of said amino acid sequence is numbered with reference to the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 38, 234, 284, 484, 594, 686, 688, and/or 766.

2. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 2.

3. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 4.

4. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 6.

5. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 8.

6. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 10.

7. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 14.

8. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said

polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 12, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 5, 14, 14/34/38/39/102/267/275/350/357, 14/39/102/127/245/267/275/349/350, 34/38/39/102/127/275/357, 34/38/39/102/275/357, 34/38/39/127/245/349/350/357, 34/38/39/127/245/350/357, 34/39/102/127/264/275/357, 34/39/102/127/275/349/357, 34/39/102/264/275/350/357, 34/39/275/349/350/357, 38/39/102/127/264/267/350/357, 38/39/102/127/267/275/349/350/357, 38/39/102/127/349/350/357, 38/39/102/127/350, 38/39/102/127/350/357, 38/39/127/245/267/357, 38/39/127/264/275, 38/39/127/264/350/357, 38/39/127/350/357, 38/39/127/357, 38/39/245/275/357, 38/39/264/267/275/350, 38/39/264/275/357, 38/39/275, 38/39/275/350, 39, 39/102/127/264/275/357, 39/102/264/275/357, 39/102/267/275/357, 39/127/245/264/267/275/350, 39/127/245/264/275/350/357, 39/127/245/357, 39/127/267/275/350/357, 39/127/267/350/357, 39/127/357, 39/245/264/267/275/357, 39/264/267/275/350, 39/275/350/357, 48, 139, 164, 196, 255, 299, 318, 324, 339, 343, 350, 353, 357, 364, 365, 379, 381, 386, 389, 391, 393, 394, 395, 397, 398, and 405, wherein the amino acid positions are numbered with reference to SEQ ID NO: 12.

9. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 38, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 48/64/164/324/343/353/357/364, 48/64/164/324/343/364, 48/64/164/353/357/364, 48/64/357/364, 64/164/324/343/353/357/364, 64/164/324/343/357/364, 64/164/353/357, 64/318/324/357/364, 64/324/353/357/364, 132/255/339/379/395, 164/196/324/357/364, 164/318/324/343/353/357, 164/318/324/357/364, 164/324/343/353/357/364, 164/324/357/364, 164/353/357/364, 164/364, 196/318/324/353/357/364, 318/343/357, 324/343/357/364, 324/353/357/364, 324/357/364, 339/379/389/394/395, 339/389/395, 339/391, 339/394/395/405, 357/364, 379/386, 379/394/395/397/404/405, 379/394/395/397/405, 389/394/395/397/405, and 394/397, wherein the amino acid positions are numbered with reference to SEQ ID NO: 38.

10. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 234, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 2, 3, 33, 48/64/255, 48/255/339,

48/255/379, 64, 64/255, 69, 161, 193, 255, 255/318/379, 259, 263, 318/339/379, 324, 324/389/394, 324/389/394/395, 324/389/394/397, 324/394, 324/394/395, 324/394/395/397, 324/395, 339, 340, 380, 382, 389, 389/394, 389/394/395, 389/394/395/397, 389/394/397, 389/395, 389/397, 390, 394, 394/395, 394/395/397, 395, 395/397, 397, 401, and 405, wherein the amino acid positions are numbered with reference to SEQ ID NO: 234.

11. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 284, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 2/64/69/324/380/382/388/389, 3/64/69/263/339/380/388, 3/64/69/389, 3/64/69/390, 3/64/379/380/390, 3/69/263/380, 3/69/324, 3/69/324/380/382/389/390, 12/135/259/263, 12/135/263/382, 12/259/263/304, 48/64/255, 64/69, 64/69/189/259/263/304, 64/69/189/259/263/304/339/340/379, 64/69/223/388, 64/69/223/388/389/390, 64/69/304/379/382, 64/69/324, 64/69/324/339/380/389/390, 64/69/339, 64/69/339/382/388/389, 64/69/339/389/390, 64/69/379/380, 64/69/380/388/390, 64/69/389, 64/69/390, 64/255/263, 64/263, 64/324/339/389/390, 69/223/263/324/382/388/390, 69/223/324/379/380/382/388/390, 69/263, 69/263/324, 69/263/339, 69/263/388, 69/263/389/390, 69/324/379/380/388, 69/324/380, 69/339/390, 69/382/390, 259/263/304, 259/263/304/339/340/379, 263/339/389/390, 263/390, and 304/340/379/380/382, wherein the amino acid positions are numbered with reference to SEQ ID NO: 284.

12. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 484, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 3/194/304, 3/259/263/304, 3/259/304, 3/259/304/324/339, 3/259/304/324/382, 3/259/304/382, 3/263/304/324, 3/263/304/324/339, 3/263/304/324/382, 3/304, 3/304/324, 16, 63, 77, 80, 87/270, 87/270/365, 87/328/365, 91, 92, 126, 140, 156, 168/270/328/338, 181, 194, 201, 256, 259, 259/263, 259/263/304, 259/263/304/324, 259/263/304/324/382, 259/263/304/379, 259/263/304/382, 259/304, 259/304/324, 259/304/324/339, 259/304/324/339/382, 259/304/382, 262, 263/304, 263/304/324, 263/304/324/339, 263/304/324/382, 263/324, 270, 270/319, 270/328/338, 270/328/338/365, 304, 304/324, 324, 328, 352, 365, 366, and 382, wherein the amino acid positions are numbered with reference to SEQ ID NO: 484.

13. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said

polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 594, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 16/63/80/126/168/366, 16/63/80/126/181/194/259/324/328/366, 16/63/126/168/270/328/366, 16/80/126/324/366, 16/80/126/366, 16/80/168, 16/80/168/270/366, 16/80/168/324, 16/80/168/366, 16/80/324, 16/91/126/168/324/366, 16/126/168/366, 16/168/259/366, 16/168/270/324/366, 16/168/324/328/366, 16/168/324/366, 16/168/366, 16/259/263/328, 16/324/328/366, 16/328/366, 80/126/168/270/366, 80/126/168/366, 80/126/181/270/324/366, 80/168/270/366, and 168/366, wherein the amino acid positions are numbered with reference to SEQ ID NO: 594.

14. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 686, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 66/76/118/141/201/300, 66/76/198/200/296/303, 66/76/198/200/300, 66/118/200/296/303/317, 66/118/296, 66/118/296/300, 66/200, 76/118/141/200/296, 76/141/198/200/201/300, 80/201/270, 80/270, 80/270/324, 89/118/200, 106/270/324/352, 118/141/200, 126, 126/201/270/324, 126/270, 141/144/198/200/300, 156/270, 156/270/324, 201/270, 201/270/352, 270, and 270/324, wherein the amino acid positions are numbered with reference to SEQ ID NO: 686.

15. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 686, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 19, 109, 123, 134, 170, 173, 187, 211, and 312, wherein the amino acid positions are numbered with reference to SEQ ID NO: 686.

16. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 688, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 19/109/123/141/170/198/200/211/270/312, 19/109/123/141/170/198/211, 19/109/123/141/170/198/211/270/312, 19/109/123/170/211/270/312, 19/109/123/198/200/211/270/312,

19/109/170/173/211/270/312, 19/109/211/270/312, 109/170/211/270/312, and 109/211/270/312, wherein the amino acid positions are numbered with reference to SEQ ID NO: 688.

17. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 766, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 5/41, 5/41/228, 33, 41, 47, 51, 55, 64, 126, 265, 267, 270, 331, 353, 357, and 384, wherein the amino acid positions are numbered with reference to SEQ ID NO: 766.

18. The engineered leucine decarboxylase polypeptide of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 766, and wherein the polypeptide sequence of said engineered leucine decarboxylase polypeptide comprises at least one substitution or substitution set at one or more amino acid positions selected from position 66, 66/118, 66/118/296, 66/118/296/300, 66/118/300, 66/296, 66/296/300, 66/300, 118, 118/296, 118/296/300, 118/300, 296, 296/300, and 300, wherein the amino acid positions are numbered with reference to SEQ ID NO: 766.

19. An engineered leucine decarboxylase polypeptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 38, 234, 284, 484, 594, 686, 688, and/or 766.

20. An engineered leucine decarboxylase polypeptide, wherein said engineered leucine decarboxylase polypeptide comprises an engineered polypeptide provided in any of Claims 1-19.

21. The engineered leucine decarboxylase polypeptide of Claim 20, wherein said engineered leucine decarboxylase polypeptide comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 38, 234, 284, 484, 594, 686, 688, and/or 766.

22. The engineered leucine decarboxylase polypeptide of any of Claims 1-18, wherein said engineered leucine decarboxylase polypeptide is a variant leucine decarboxylase polypeptide provided in any of Tables 1-2, 2-1, 3-2, 4-1, 5-1, 6-1, 7-1, 8-1, 8-2, 10-1, 11-1, and/or 11-2.

23. The engineered leucine decarboxylase polypeptide of any of Claims 1-22, wherein

said engineered leucine decarboxylase polypeptide is a Planctomycetaceae variant enzyme.

24. The engineered leucine decarboxylase polypeptide of any of Claims 1-23, wherein said engineered leucine decarboxylase polypeptide exhibits more activity on leucine than the wild-type Planctomycetaceae species leucine decarboxylase.

25. The engineered leucine decarboxylase polypeptide of any of Claims 1-24, wherein said engineered leucine decarboxylase more resistant to proteolysis than wild-type Planctomycetaceae species leucine decarboxylase.

26. The engineered leucine decarboxylase polypeptide of any of Claims 1-25, wherein said engineered leucine decarboxylase polypeptide is more thermostable than wild-type Planctomycetaceae species leucine decarboxylase.

27. The engineered leucine decarboxylase polypeptide of any of Claims 1-26, wherein said engineered leucine decarboxylase polypeptide comprises a sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to any of the even-numbered sequences of SEQ ID NOS: 16-852.

28. The engineered leucine decarboxylase polypeptide of Claim 27, wherein said leucine decarboxylase polypeptide comprises a sequence at least 90%, identical to any of the even-numbered sequences of SEQ ID NOS: 16-852.

29. The engineered leucine decarboxylase polypeptide of Claim 28, wherein said leucine decarboxylase polypeptide comprises any of the even-numbered sequences of SEQ ID NOS: 16-852.

30. The engineered leucine decarboxylase polypeptide of any of Claims 1-29, wherein said engineered leucine decarboxylase polypeptide is purified.

31. A composition comprising at least one engineered leucine decarboxylase polypeptide provided in any of Claims 1-30.

32. An engineered polynucleotide sequence encoding at least one engineered leucine decarboxylase polypeptide of any of Claims 1-31.

33. The engineered polynucleotide sequence of Claim 32, wherein said polynucleotide

sequence comprises a sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to any of the odd-numbered sequences of SEQ ID NOS: 15-851.

34. The engineered polynucleotide sequence of Claim 32, wherein said polynucleotide sequence comprises a sequence at least 90% or more identical to any of the odd-numbered sequences of SEQ ID NOS: 15-851.

35. The engineered polynucleotide sequence of Claim 32, wherein said polynucleotide sequence comprises any of the odd-numbered sequences of SEQ ID NOS: 15-851.

36. The engineered polynucleotide sequence of any of Claims 32-35, wherein said sequence is operably linked to a control sequence.

37. The engineered polynucleotide sequence of any of Claims 32-36, wherein said polynucleotide is codon-optimized.

38. An expression vector comprising at least one engineered polynucleotide sequence of any of Claims 32-37, and at least one control sequence.

39. The expression vector of Claim 38, wherein said control sequence comprises a promoter.

40. The expression vector of Claim 39, wherein said promoter is a heterologous promoter.

41. A host cell transformed with at least one polynucleotide sequence of any of Claims 32-37, and/or comprising the expression vector of any of Claims 38-40.

42. The host cell of Claim 41, wherein said host cell is *E. coli*.

43. A method of producing an engineered leucine decarboxylase polypeptide in a host cell comprising culturing a host cell comprising at least one polynucleotide encoding at least one engineered leucine decarboxylase polypeptide of any of Claims 1-30, and/ or at least one polynucleotide sequence of any of Claims 32-37, and/or at least one expression vector of any of any of Claims 38-40, under suitable culture conditions, such that at least one engineered leucine decarboxylase polypeptide is produced.

44. The method of Claim 43, comprising culturing a host cell comprising at least one polynucleotide encoding at least one engineered leucine decarboxylase polypeptide of any of Claims 1 to 30, under suitable culture conditions, such that at least one engineered leucine decarboxylase polypeptide is produced.

45. The method of Claim 43 and/or 44, further comprising recovering at least one engineered leucine decarboxylase polypeptide from the culture and/or host cells.

46. The method of any of Claims 43-45, further comprising the step of purifying said at least one engineered leucine decarboxylase polypeptide.

47. A composition comprising at least one engineered polynucleotide of any of Claims 32-37.

48. The composition of Claim 47, wherein said composition is a pharmaceutical composition.

49. The composition of Claim 48, further comprising at least one pharmaceutically acceptable excipient and/or carrier.

50. The composition of any of Claims 31, 48, and 49, wherein said composition is suitable for the treatment of maple syrup urine disease.

51. The composition of any of Claims 31, and 47-50, wherein said composition is suitable for oral administration to a human.

52. The composition of any of Claims 31, and 47-51, wherein said composition is in the form of a pill, tablet, capsule, gelcap, liquid, or emulsion.

53. The composition of Claim 52, wherein said pill, tablet, capsule, or gelcap further comprises an enteric coating.

54. The composition of any of Claims 31, and 47-52, wherein said composition is suitable for parenteral injection into a human.

55. The composition of any of Claims 31, and 47-54, wherein said composition is

coadministered with at least one additional therapeutically effective compound.

56. The composition of Claim 55, wherein said composition comprises at least one additional therapeutically effective compound.

57. A method for treating and/or preventing the symptoms of maple syrup urine disease in a subject, comprising providing a subject having maple syrup urine disease, and providing the composition of any of Claims 31, and 47-56, to said subject.

58. The method of Claim 57, wherein said symptoms of maple syrup urine disease are ameliorated.

59. The method of Claims 57 and/or 58, wherein said subject is able to eat a diet that is less restricted in its in isoleucine, leucine, and/or valine content than diets required by subjects who have not been provided at least one composition comprising at least one engineered leucine decarboxylase polypeptide set forth in Claims 1-30.

60. The method of any of Claims 57-59, wherein said subject is able to eat a diet that is less restricted in its in isoleucine, leucine, and/or valine content than diets required by subjects who have not been provided at least one composition comprising at least one engineered leucine decarboxylase polypeptide produced using the methods set forth in Claims 43-46.

61. The method of any of Claims 57-60, wherein said subject is an infant, child, young adult, or adult.

62. Use of the compositions provided in any of Claims 31, and/or 47-56.