CLAIMS

What is claimed is:

1. An engineered polypeptide having proline hydroxylase activity comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a reference sequence of SEQ ID NO: 4 and a residue difference at position 194 as compared to SEQ ID NO: 4, wherein said engineered polypeptide is capable of converting L-proline to trans-3-hydroxyproline.

2. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 4 and further comprising one or more residue differences as compared to SEQ ID NO: 4 at residue positions selected from: 123, 21, 28, 58/247, 65, 80, 85, 95, 98, 117, 120, 159, 185, 199, 200, 233, 237, 243, 250, 268, 281, 282, 287, 289, 307, 324, 326, 327, 330, 338, 343, 346, and 348.

3. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 4 and further comprising one or more residue differences as compared to SEQ ID NO: 4 at residue positions selected from: 21, 28, 45, 65, 95, 112, 117, 139, 177, 185, 199, 233, 243, 250, 281, 282, 287, 289, 307, 324, 326, 327, 335, 338, 343, and 346.

4. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 4 and further comprising one or more residue differences as compared to SEQ ID NO: 4 at residue positions selected from: 48/66/189, 48/66, and 66/82/85/135/189/267.

5. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 4 and further comprising one or more residue differences as compared to SEQ ID NO: 4 at residue positions selected from: 20/56/76/168/169/296, 20/56/232/294, 20/119/294/296, 56/76/119/124/147/232, 56/76/294, 76/168/232/294, 76/294/296, 76/296, 147, and 232.

6. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference

sequence SEQ ID NO: 116 and further comprising one or more residue differences as compared to SEQ ID NO: 116 at residue positions selected from: 123, 189, 195, 233, and 296.

7. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 116 and further comprising one or more residue differences as compared to SEQ ID NO: 116 at residue positions selected from: 20/21/56, 20/21/56/76/95/232/294/307/335, 20/21/56/76/147/225/232/233/281/294/296/307/335, 20/21/56/95/147/281/294/307, 20/21/56/281/307, 20/21/76/232/243, 20/21/95/232/307, 20/21/95/281/294/296, 20/21/147/189/233/243/281/307, 20/56, 20/56/76/95/281/307, 20/56/76/147/294/296/307, 20/56/95/147/294, 20/56/281, 20/76, 20/76/95/281/294/296, 20/76/95/281/296/307, 20/76/233/294/307, 20/76/243/281/294, 21/76/147/233/294/307, 21/76/147/243/296/307/335, 21/95/185/189/232/281/296, 21/95/233/243/281/296, 21/95/294/296/307/335, 21/95/307, 21/281/307, 29/76/281, 56/76/95/232/243/281, 56/76/147/281/307, 56/76/243/294, 56/76/281/294, 56/76/296, 56/76/307, 56/95/147/307/335/348, 56/95/232/233/281/294/307, 56/95/243/281, 56/147/281, 56/232/243/281, 56/232/281, 56/232/281/294/296, 56/233/281/294/296, 56/281/307, 76/95/232/243/281/307, 76/95/243/281/307/335, 76/95/294/307, 76/147, 76/147/233/243/294, 76/147/233/281/294/307, 76/147/243/294/296/307/335, 76/147/281/307, 76/189/296, 76/232/233/243/294/296/307, 76/281, 76/281/294, 76/294/296, 95/120, 95/147/335, 95/232/243/281/294/307, 95/232/281/294/296, 95/281/294/296, 95/335, 147, 147/225/232/243/281/296/307/335, 147/233/243/281/307, 147/233/281/307/335, 147/243/281, 147/307, 232/233/281/294/296/307, 232/281, 232/284/307, 233/243/281/296/307/335, 233/281/296/307, 243/281/294/296, 281, 281/294, 281/307, 307, and 335.

8. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 116 and further comprising one or more residue differences as compared to SEQ ID NO: 116 at residue positions selected from: 21/76/147/243/296/307/335, 56/76/147/281/307, and 95/147/335.

9. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 162 and further comprising one or more residue differences as compared to SEQ ID NO: 162 at residue positions selected from: 2/85/123/237, 28/115/117/120/123/268/270/343/346/348, 45/123/326, 65/117/120/123/343/346, 85/123/281/282, 114/115/117/120/123/268/271/313/326/343/346, 123/139/233/237/281/282/289/324/326, and 123/199/200/247/250/338.

10. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 322 and further comprising one or more residue differences as compared to SEQ ID NO: 322 at residue positions selected from: 26, 54, 61, 129, 132, 149, 156, 175, 189, 201, 209, 228, 236, 248, 262, 272, 277, 291, and 345.

11. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 322 and further comprising one or more residue differences as compared to SEQ ID NO: 322 at residue positions selected from: 25, 43, 54, 58, 61, 79, 129, 132, 143, 156, 163, 175, 179, 201, 209, 236, 248, 278, 291, 345, and 347.

12. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 322 and further comprising one or more residue differences as compared to SEQ ID NO: 322 at residue positions selected from: 85/117/120/135/208/270/324/343/346, 85/117/120/135/208/281/282/289, 85/117/120/270/281/289, 85/117/135/139/208, and 117/120/208/270/324/343/346.

13. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 412 and further comprising one or more residue differences as compared to SEQ ID NO: 412 at residue positions selected from: 47, 48, 56/118, 85, 95, 95/289, 113, 118, 118/247, 154, 162, 162/204, 164, 164/198/271, 168, 169, 187, 195, 243, 271, 275, 281, 314, 330, and 342.

14. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 412 and further comprising one or more residue differences as compared to SEQ ID NO: 412 at residue positions selected from: 25/129/163/236/262/345/347, 120/156/175/179/201, 129/189/236/262/277/278, 129/236/262, 156/175/179/228, and 162.

15. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 492 and further comprising one or more residue differences as compared to SEQ ID NO: 492 at residue positions selected from: 15, 17, 28, 29, 65, 135, 167, 177, 199, 208, 228, 235, 287, 294, 307, and 343.

16. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 492 and further comprising one or more residue differences as compared to SEQ ID NO: 492 at residue positions selected from: 85/187/281/347, 85/187/347, 118/120/162/175/179/330, 118/120/162/175/330, 162/175/179/330, 175/228/330, 195/347, and 278/314/347.

17. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 562 and further comprising one or more residue differences as compared to SEQ ID NO: 562 at residue positions selected from: 15, 40, 43, 44, 59, 79, 82, 149, 164, 179, 345, and 347.

18. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 562 and further comprising one or more residue differences as compared to SEQ ID NO: 562 at residue positions selected from: 29/85/177/208/228/347, 29/85/208/228/343/347, 29/177/195/228/343, 29/208/228/278/294/347, 56/195/278, 85/187/205/208/278, 113/177/187/195/208/278/294/343/347, and 177/205/208/228.

19. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 598 and further comprising one or more residue differences as compared to SEQ ID NO: 598 at residue positions selected from: 47, 162, 209, 219, 227, and 342.

20. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 598 and further comprising one or more residue differences as compared to SEQ ID NO: 598 at residue positions selected from: 17/44/179/195/250/313/345, 17/44/199/313, 43/44/195/199, 44/149/164/171/187, 44/179/195/199, 44/179/195/199/345, 79/163/164/171/187/201/286/288, 82/163/164, 82/163/164/171/187/201/203/208/286/288/320, 149/164/171/288, and 187/286.

21. The engineered polypeptide of Claim 1, comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to reference sequence SEQ ID NO: 630 and further comprising one or more residue differences as compared to SEQ ID NO: 630 at residue positions selected from: 82/164/171/203/208,

135/163/164/201/203/208, 162, 162/219/236, 162/219/313/338, 162/236/342, 162/313/342, and 164/171/201/203/282.

22. The engineered polypeptide of Claim 1, wherein said polypeptide has at least 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to at least one of the even-numbered sequences in SEQ ID NOs: 6, 8, 116-120, and 142-658.

23. The engineered polypeptide of any one of Claims 1 to 22, wherein said engineered polypeptide is capable of converting L-proline to *trans*-3-hydroxyproline with at least 1.2 fold, 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold or more the activity of the naturally occurring enzyme.

24. A polynucleotide encoding the engineered polypeptide of any of Claims 1 to 23.

25. A polynucleotide encoding the engineered polypeptide of Claim 22.

26. A polynucleotide encoding the engineered polypeptide of Claim 23.

27. The polynucleotide of any one of Claims 24 to 26, wherein said polynucleotide comprises a nucleic acid sequence optimized for expression in *E. coli*.

28. An expression vector comprising the polynucleotide of any one of Claims 24 to 27, optionally further comprising at least one control sequence.

29. The expression vector of Claim 28, wherein said vector encodes the engineered polypeptide of SEQ ID NO: 4, 116, 162, 322, 412, 492, 562, 598, and/or 630.

30. A host cell comprising the polynucleotide of any of Claims 24 to 27.

31. A host cell comprising the expression vector of Claim 28 or Claim 29.

32. The host cell of Claim 30 or Claim 31, wherein the host cell is *E. coli*.

33. A method of preparing an engineered polypeptide, comprising culturing the host cell of any one of Claims 30 to 32, under conditions suitable for expression of the polypeptide.

34. The method of Claim 33, further comprising a step of isolating the engineered polypeptide.