CLAIMS

WHAT IS CLAIMED IS:

1. An engineered RNA ligase, or a functional fragment thereof, comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference sequence corresponding to residues 12 to 346 of an even-numbered SEQ ID NO. of SEQ ID NOs: 14 and 24-582, or to a reference sequence corresponding to an even-numbered SEQ ID NO. of SEQ ID NOs: 14 and 24-582, wherein the amino acid sequence comprises one or more substitutions relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 14, 42, or 204, or relative to the reference sequence corresponding to SEQ ID NO: 14, 42, or 204.

2. The engineered RNA ligase of claim 1, wherein the amino acid sequence of the engineered RNA ligase comprises at least a substitution at amino acid position 2, 14, 15, 18, 33, 39, 63, 69, 81, 83, 85, 86, 89, 95, 116, 117, 119, 138, 142, 144, 149, 154, 156, 159, 171, 175, 177, 181, 185, 186, 195, 202, 212, 214, 224, 226, 230, 247, 256, 257, 260, 266, 275, 280, 283, 285, 288, 291, 296, 303, 306, 307, 310, 314, 315, 316, 317, 326, 330, 331, 333, 334, 335, 337, 339, 342, or 345, or combinations thereof, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 14, or relative to the reference sequence corresponding to SEQ ID NO: 14.

3. The engineered RNA ligase of claim 1 or claim 2, wherein the amino acid sequence of the engineered RNA ligase comprises at least a substitution or substitution set at amino acid position(s) 177/260/307/345, 171/175/183/280/283/303, 95/159/177/260/288, 175/183/247/280/283/296, 177/307, 81/171/183/335, 175/183, 81/175/183/280/296/335/339, 81/183/247/266, 95/177, 171/280/283/296/303, 85/177/260/288/333/345, 177/291/307/333, 81/171/175/183/316/337, 81/171/266/280/283/335/339, 177, 95/159/177/260/280/288/306/345, 171/175/335, 177/260/30/333, 14/95/177/288/307, 95/177/345, 95/159/177/260/285/288, 266/296, 81/283/335/337, 95/159/177/260/342/345, 63/171/175/183/266/280/296/316, 280/335/337, 81/171/280, 81/171/175/296/316, 171/175/316/335, 171/280/283/296/316, 171/283/335, 63/171/175/183/266/283/303/316, 171/247/266/283/296/303/316/335, 63/81/171/175/183/266/280/283/337, 81/171/303/316/335, 288/307, 14/95/159/177/345, 81/171/175/183/247/266/280/283, 81/247/266, 280, 85/177/260, 63/183, 175/183/247/280/283/316, 291, 247, 171/280/283, 81/171/175/280/316, 333, 171/296/316, 81/171, or 280/316/335, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 14, or relative to the reference sequence corresponding to SEQ ID NO: 14.

4. The engineered RNA ligase of claim 1, wherein the amino acid sequence of the engineered RNA ligase comprises at least a substitution at amino acid position 2, 14, 15, 18, 33, 39, 63, 69, 81, 83, 85, 86, 89, 95, 116, 117, 119, 138, 142, 144, 149, 154, 156, 159, 171, 175, 177, 181, 185, 186, 195, 202, 212, 214, 224, 226, 230, 247, 256, 257, 260, 266, 275, 280, 283, 285, 288, 291, 296, 303, 306, 307, 310, 314, 315, 316, 317, 326, 330, 331, 333, 334, 335, 337, 339, 342, or 345, or combinations thereof, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 42 or 204, or relative to the reference sequence corresponding to SEQ ID NO: 42 or 204.

5. The engineered RNA ligase of claim 1, wherein the amino acid sequence of the engineered RNA ligase comprises a substitution or substitution set at amino acid positions(s) 149/156/195, 15/149/186/224/317, 15/156/195/224/226/317/331, 15/195/317/331, 149/224/331, 15/195/224/226/317/331, 15/33/86/149/154/195/317, 15/86/154/156/186/195/224/226/331, 15/149/224/317/331, 15/154/156/186/317/331, 15/33/149/224/226/331, 15/186/224/226/317/331, 15/33/149/186/224/331, 195/224/331, 15/86/156/186/195/226/317/331, 15/195/22/331, 156/186/224/226/331, 15/149/154/156/224/226, 33/156/186/195/331, 15/86/149/154/156/317/331, 15/186/317/331, 15/86/149/154/195/331, 15/33/149/154/156/226, 15/149/154/186/317/331, 195/331, 15/86/156/186/195/331, 149/156/224/226/331, 15/149/154/156/186/317/331, 15/154/186/195, 15/86/195/224/226, 230, 15/33/156/331, 15/33/156/195/224/226, 33/149/156/317, 15/33/186/195, 334, 15/154/156/224/317, 15/149/186/331, 15/86/149/186/195/317, 15/33/154/195, 15/156/186/331, 15/149/186/317, 15/33/156/186/214/224/331, 15/86/186/224/226/317, 15/154/331, 15/149/154/226, 15/33/195/226, 15/224/317/331, 15/86/186/195/317, 226/317/331, 330, 89, 15/149/186, 156/195/331, 156, 15/86/156/186/331, 275, 15/33/186/224, 15/186/317, 15/33/186/226/317, 15/149/154/156/331, 15/154/226/317, 15/86/156/186/195/317, 257, 15/154/156/186, 33/186/224, 335, 15/86/156/186/195/224/331, 154/156/317, 39, 2, 15/224/226/331, 15/33/149/224, 89/307, 15/154/156/317, 15/186/331, 15/33/154/317, 15/33/195/224/317, 15/156/224/226, 154/186/331, 185, 15/18/149/186/195, 86/149/154/156, 15/33/149/331, 326, 202, 119, 212, 15/33/156/317, 280, 142, 15/149, 15/33/86/156/195, 315, 256, 15/33/156/186, 138, 117, 116, 316, 69, 310, 317, 15/195/331, 15/33/331, 314, or 144, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 42, or relative to the reference sequence corresponding to SEQ ID NO: 42; or

wherein the amino acid sequence of the engineered RNA ligase comprises at least a substitution set at amino acid positions(s) 15/149/154/156/186/195/331, 149/154/186/195/224/317/331, 15/33/149/154/186/195, 149/156/186/195/224/226/317, 15/149/156/195/224/226, 15/33/86/149/154/156/186/195/226/331, 15/149/156/186/195/224/317/331, 15/33/149/154/195/317, 15/149/154/195/224/317, 15/149/156/186/224/226/317/331, 15/33/149/154/156/186/195/224/226/317/331, 149/195/224, 86/149/154/156/186/195/331, 15/33/149/156/195/224/317/331, 15/149/154/156/186/195/317/331, 15/33/149/154/156/186/224/331, 15/149/154/156/195/224, 15/149/154/156/195/224/226, 15/149/154/156/186/195/224/317, 33/149/156/186/224/331, 15/33/86/149/195/224/331, 15/86/149/154/156/186/195/331, 149/186/195/331, 15/154/156/186/226/331, 15/149/154/186/195/224/226, 15/149/154/156/195/224/331, 15/33/149/154/156/195/317/331, 15/33/154/156/186/195/224/317/331, 15/149/186/195/224/331, 15/33/149/154/156/195/224/331, 15/149/154/156/224/226/317/331, 15/33/149/156/195/331, 15/149/154/156/186/224/226/331, 154/156/195/224/226, 15/33/86/149/154/156/186/195/224/317/331, 15/149/154/156/186/195/224/226, 15/33/149/195/317/331, 15/149/154/224/331, 149/154/156/195/224/226/331, 15/149/154/195/331, 15/149/154/156/186/195, 15/33/149/154/156/186/195, 15/33/86/149/154/195/224/317/331, or 149/186/195/224/226/331, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 42, or relative to the reference sequence corresponding to SEQ ID NO: 42.

6. The engineered RNA ligase of claim 1, wherein the amino acid sequence of the engineered RNA ligase comprises at least a substitution or substitution set at amino acid position(s)

2/39/69/144/156/316/330, 2/39/69/119/185/316, 69/212/256/330, 39/119/256/316/326/330, 69/116/119/185/330, 2/39/119/144/156/159/202/256/316/326/330, 81/183/185/230, 2/39/69/116/144/156/185, 2/14/39/69/116/119/159/185/256/326, 2/116/119/316, 116/119/144/185/314/316, 39/69/116/144/181/185/256/330, 2/39/69/119/144/156/185/314, 14/39/69/144/156/212/256/314/316/326/330, 39/69/144/156/185/316/330, 156/185/334/335, 39/63/156/230/257/275/330/335, 156/266/316/330/334/335, 39/63/156/183/185/230/330/334, 69/144/316, 116/119/156/202/212/326/330, 39/69/119/138/181/185/316/326, 39/81/156/230, 2/14/69/116/119/144/159/326/330, 39/156/334/345, 230/266/334, 2/116/144/156/159, 156/183/185/230, 2/14/116/316/326/330, 39/63/156/230/316/345, 69/138/144/159/185/202/316, 39/156/257/266/316/334, 156/257/275/334, 183, 89/156/185/230/316/345, 2/14/119, 144/156/316/330, 39/81/185/316/334, 89/230/266/345, 39/156/230/275/316, 39/156/230/266/334/335, 156/185/275/316/330, 119/156/202, 230/257/275/316/330/345, 230, 81/156/330/335/345, 156/183/230/266, 39/185, 39/81/183/275/316/334/345, 156/230/257/316, 39/116/156, or 183/185/257/275/316/330/334, wherein the amino acid positions are relative to the reference sequence corresponding to residues 12 to 346 of SEQ ID NO: 204, or relative to the reference sequence corresponding to SEQ ID NO: 204.

7. The engineered RNA ligase of any one of claims 1-6, wherein the engineered RNA ligase has RNA ligase 2 activity.

8. The engineered RNA ligase of any one of claims 1-7, having at least one improved property as compared to a reference RNA ligase, optionally wherein the improved property is selected from i) increased activity, ii) increased stability, iii) increased thermostability, iv) increased product yield, v) increased activity on polynucleotides ligase substrates with phosphorothioate internucleotide linkages, vi) increased activity on oligonucleotides with 2'-modifications, vii) increased substrate tolerance, or any combination of i), ii), iii), iv), v), vi), and vii).

9. A recombinant polynucleotide comprising a polynucleotide sequence encoding an engineered RNA ligase of any one of claims 1-8.

10. The recombinant polynucleotide of claim 9, wherein the polynucleotide sequence has at least 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a reference polynucleotide sequence corresponding to nucleotide residues 34 to 1038 of an odd numbered SEQ ID NO. of SEQ ID NOs: 23-581, or to a reference polynucleotide sequence corresponding an odd numbered SEQ ID NO. of SEQ ID NOs: 23-581, wherein the recombinant polynucleotide encodes an RNA ligase.

11. The recombinant polynucleotide of claim 9, comprising

(a) a polynucleotide sequence comprising nucleotide residues 34 to 1038 of SEQ ID NO. 13, 41, or 203, or a polynucleotide sequence comprising SEQ ID NOs: 13, 41, or 203, or

(b) a polynucleotide sequence comprising nucleotide residues 34 to 1038 of an odd numbered SEQ ID NO. of SEQ ID NOs: 23-581, or a polynucleotide sequence comprising an odd numbered SEQ ID NO. of SEQ ID NOs: 23-581.

12. An expression vector comprising a recombinant polynucleotide of any one of claims 9-11.

13. A host cell comprising an expression vector of claim 12, wherein the host cell is selected from the group comprising a prokaryotic cell, a eukaryotic cell, a bacterial cell, a fungal cell, an insect cell or a mammalian cell.

14. A method of producing an engineered RNA ligase polypeptide in a host cell comprising culturing a host cell of claim 13, under suitable culture conditions such that the engineered RNA ligase is produced.

15. A composition comprising an RNA ligase of any one of claims 1-8.

16. A method of ligating at least a first polynucleotide strand and a second polynucleotide strand, comprising contacting a first polynucleotide strand and a second polynucleotide strand with an engineered RNA ligase of any one of claims 1-8 in presence of a nucleotide substrate under conditions suitable for ligation of the first polynucleotide strand to the second polynucleotide strand, wherein the first polynucleotide strand comprises a ligatable 5'-end and the second polynucleotide strand comprises a 3'-end ligatable to the 5'-end of the first polynucleotide.

17. The method of claim 16, further comprising a third polynucleotide strand, wherein the first polynucleotide strand and second polynucleotide strand hybridize adjacent to one another on the third polynucleotide strand to position the 5'-end of the first polynucleotide strand adjacent to the 3'-end of the second polynucleotide strand; optionally

wherein the third polynucleotide strand is continuous with the first polynucleotide strand or second polynucleotide strand or

wherein the third polynucleotide strand is continuous with the first polynucleotide strand and second polynucleotide strand to form a single continuous polynucleotide substrate.

18. The method of claim 17, wherein the third polynucleotide strand comprises a splint or bridging polynucleotide, wherein the 5'-terminal sequence of the first polynucleotide strand and the 3'-terminal sequence of the second polynucleotide strand hybridize adjacent to one another on the splint or bridging polynucleotide to position the 5'-end of the first polynucleotide strand adjacent to the 3'-end of the second polynucleotide strand; or

wherein the first and third polynucleotide strands hybridize to each other to form a first double stranded polynucleotide fragment, and the second polynucleotide strand hybridizes to a fourth polynucleotide strand to form a second double stranded fragment, wherein the first and second double stranded fragments have complementary ends that can base pair to form substrates for the engineered RNA ligase.

19. The method of any one of claims 16-18, wherein the first polynucleotide strand and/or second polynucleotide strand comprises RNA or a mixture of RNA and DNA, the 3'-end of the second polynucleotide strand is a 3'-OH, the 5'-end of the first polynucleotide strand is a 5'-phosphate, and/or the internucleotide linkage comprises a phosphate linkage.

20. The method of any one of claims 16-19, wherein the first polynucleotide strand and/or second polynucleotide strand comprise one or more nucleotide analogs, wherein the nucleotide analog comprises a modified nucleobase, modified nucleoside sugar residue, modified nucleobase or nucleobase analog, modified internucleotide linkage, and/or modified 5'-end phosphate group, optionally wherein the modified 5' end phosphate group is a phosphate analog, the modified nucleoside sugar residue is a 2'-O-alkyl, a 2'-fluoro, a b-D-ribo LNA, or a a-L-ribo-LNA (locked nucleic acids), the modified nucleobase or nucleobase analog is xanthine, hypoxanthine, inosine, 7-methylguanine, 2,6-diaminopurine, 5-methylcytosine, 5-hydroxycytosine, 5-bromocytosine, 5-iodocytosine, 2-thiothymine, 5-fluorouracil, 5-bromouracil, 8-bromoguanine, 8-aminoguanine, or 8-aza-7-deazaguanine, the internucleotide linkage is a phosphorothioate, phosphoacetate, phosphoramidate, methylphosphonate, or phosphonocarboxylate, and/or the nucleotide substrate is ATP or dATP.

21. The method of claim 20, wherein the modified 5'-end phosphate group is a phosphate analog, optionally wherein the phosphate analog is a phosphorothioate or monomethylphosphate.