CLAIMS

We claim:

1. An engineered guanylate kinase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, or a functional fragment thereof, wherein the polypeptide sequence of said engineered guanylate kinase comprises a substitution at position 144 and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

2. The engineered guanylate kinase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, and wherein the polypeptide sequence of said engineered guanylate kinase further comprises at least one substitution or substitution set at one or more positions in said polypeptide sequence selected from the group consisting of 82/115/193/200, 42, 41, 60, 77, 83, 115, 116, 142, 150, 153, 157, and 176, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

3. The engineered guanylate kinase of Claim 1, wherein said polypeptide sequence of said engineered guanylate kinase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, and wherein said polypeptide sequence of said engineered guanylate kinase further comprises at least one substitution or substitution set at one or more positions selected from the group consisting of: 82/115/193/200, 82, 82/100, 82/100/115/116, 82/100/115/116/160/193, 82/100/116/160, 82/100/116/200, 82/100/160/200, 82/100/193, 82/100/200/203, 82/115/116, 82/115/116/193/200, 82/115/116/172, 82/115/160, 82/115/193, 82/116, 82/116/160, 82/116/193, 82/116/193, 82, 82/193, 82/160/172, 82/160/179, 82/160/193/200, 82/172, 82/193, 82/193/203, 82/200, 91, 100/172, 100/193, 115, 115/160, 115/193, 115/193, 115/200, 193, 193/200, and 200, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 4.

4. The engineered guanylate kinase of Claim 1, wherein said polypeptide sequence of said engineered guanylate kinase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 46, and wherein said polypeptide sequence of said engineered guanylate kinase further comprises at least one substitution or substitution set at one or more positions selected from the group consisting of: 41, 41/42, 41/42/91, 41/42/91/134, 41/42/91/134/160, 41/42/91/160, 41/42/91/172, 41/42/134, 41/42/134/144, 41/42/134/144/160, 41/42/134/150, 41/42/134/150/160, 41/42/134/160, 41/42/134/160/172, 41/42/144, 41/42/160, 41/42/160/172, 41/42/172, 42, 42/91, 42/91/134, 42/91/134/172, 42/91/172, 42/134, 42/134/172, 42/144, and 42/160, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 46.

5. The engineered guanylate kinase of Claim 1, wherein said polypeptide sequence of said engineered guanylate kinase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 46, and wherein said polypeptide sequence of said engineered guanylate kinase further comprises at least one substitution or substitution set at one or more positions selected from the group consisting of 9/41/42, 9/41/42/60/115, 9/41/42/115/150/153, 9/41/42/115/153, 9/41/42/115/153/157, 9/41/42/115/157, 9/42/115/150/157/176, 9/60/150/153, 41/42, 41/42/60, 41/42/60/77/153/157, 41/42/60/115, 41/42/60/115/144, 41/42/60/115/150, 41/42/60/115/150/153, 41/42/60/115/150/153/157, 41/42/60/115/150/153/176, 41/42/60/115/150/157, 41/42/60/115/153/176, 41/42/60/115/176, 41/42/60/150/153, 41/42/60/153, 41/42/60/153/157, 41/42/60/157, 41/42/77, 41/42/77/115, 41/42/77/115/153, 41/42/77/115/153/157, 41/42/77/115/157, 41/42/77/150, 41/42/77/153, 41/42/79/115/150/157, 41/42/83/150/153, 41/42/115, 41/42/115/150, 41/42/115/150/153, 41/42/115/150/153/157, 41/42/115/150/157/176, 41/42/115/153, 41/42/115/153/157, 41/42/115/157, 41/42/115/176, 41/42/150/153, 41/42/153, 41/42/153/157, 41/42/153/157/176, 41/42/157, 41/42/176, 41/60/77/115/150/157, 41/150, 41/150/157, 42, 42/60, 42/60/77/150/153, 42/60/115/150/153, 42/60/115/153, 42/60/150/153/157, 42/60/157, 42/60/176, 42/77/115/150/157, 42/115, 42/115/150/153, 42/150, 42/150/153, 42/150/153/157, 42/153, 42/153/157, 42/157, 60/150/153, 150/153, 150/153/176, 150/157, and 153/157, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 46.

6. The engineered guanylate kinase of Claim 1, wherein said engineered guanylate kinase comprises a variant engineered guanylate kinase set forth in SEQ ID NO: 4 and/or SEQ ID NO: 46.

7. The engineered guanylate kinase of Claim 1, wherein said engineered guanylate kinase comprises a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of at least one engineered guanylate kinase variant set forth in the even numbered sequences of SEQ ID NOs: 6-552.

8. The engineered guanylate kinase of Claim 1, wherein said engineered guanylate kinase comprises a polypeptide sequence set forth in one of the even numbered sequences of SEQ ID NOs: 6-552.

9. The engineered guanylate kinase of any one of Claims 1-8, wherein said engineered guanylate kinase exhibits increased activity on a substrate selected from the group consisting of 3'F-3'-deoxyguanosine-5'-(1-thio)-monophosphate (F-thioGMP **(6)**) and/or Sp-2'F-ara-adenosine-5'-(1-thio)-triphosphate (F-thioATP **(3)**) as compared to SEQ ID NO:4.

10. The engineered guanylate kinase of any one of Claims 1-8, wherein said engineered guanylate kinase exhibits increased production of Sp-3'F-3'-deoxyguanosine-5'-(1-thio)-diphosphate (F-thioGDP **(7)**) as compared to SEQ ID NO:4.

11. The engineered guanylate kinase of any one of Claims 1-10, wherein said engineered guanylate kinase is purified.

12. A composition comprising at least one engineered guanylate kinase of any one of Claims 1-11.

13. A polynucleotide sequence encoding at least one engineered guanylate kinase of any one of Claims 1-11.

14. The polynucleotide sequence of Claim 13, wherein said polynucleotide sequence is operably linked to a control sequence.

15. The polynucleotide sequence of Claim 13 or Claim 14, wherein said polynucleotide sequence is codon optimized.

16. The polynucleotide sequence of any one of Claims 13-15, wherein said polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOs: 5-551.

17. An expression vector comprising at least one polynucleotide sequence of any one of Claims 13-16.

18. A host cell comprising at least one expression vector of Claim 17.

19. A host cell comprising at least one polynucleotide sequence of any one of Claims 13-16.

20. A method of producing an engineered guanylate kinase in a host cell, comprising culturing the host cell of Claim 18 or Claim 19, under suitable conditions, such that at least one engineered guanylate kinase is produced.