CLAIMS

What is claimed is:

1. A polypeptide comprising an amino acid sequence with at least 70% sequence identity with SEQ ID NO:1 and comprising at least one amino acid substitution relative to SEQ ID NO: 1 selected from T2S, A4E/S/R/G/D/T/L, Q11R/V/I/L/K/T, Q20R, E23V, S28P, A33K, L34M, V44I/L, V45E, G51V, A54F/T/V/G/S/W/L/I**,** F68S/Y/V, L72Q, M75R/K/Q/G/T/A, I76V, F77W, V79I, K89E, I90T/V, L92S/A/G/Q, I99V, P104L, P115E/I/Q/L/V/G/H/R/S/C/A/T, Q124K, N135K, Y138V/I/N/T/L/C/R/M/K, D139E, I143L, N144K, C164S, N166R/K/A/L/P/Q/S, I167V, or 169L ~~at a position corresponding to position 2, 4, 11, 20, 23, 28, 33, 34, 44, 45, 51, 54, 68, 72, 75, 76, 77, 89, 90, 92, 99, 104, 115, 124, 135, 138, 139, 143, 144,166, 167, or 169~~ of SEQ ID NO:1, wherein the polypeptide is a luciferase that is capable of utilizing coelenterazine as a substrate to generate luminescence, and wherein the polypeptide has at least one of enhanced luminescence, enhanced signal stability, and enhanced protein stability relative to a luciferase comprising the amino acid sequence of SEQ ID NO: 1, wherein the modified luciferase polypeptide has luciferase activity.

2. The polypeptide of claim 1, wherein the at least one amino acid substitution relative to SEQ ID NO: 1 is selected from A4E/S/R/G/D/T/L, Q11R/V/I/L/K/T, Q20R, S28P, A33K, A54F/T/V/G/S/W/L/I**,** F68S/Y/V, M75R/K/Q/G/T/A, P115E/I/Q/L/V/G/H/R/S/C/A/T, Q124K, Y138V/I/N/T/L/C/R/M/K, I143L, or N166R/K/A/L/P/Q/S ~~at a position corresponding to at least one of positions 4, 11, 20, 28, 33, 54, 68, 75, 115, 124, 138, 143, or 166~~ of SEQ ID NO:1.

3. The polypeptide of claim 1, comprising Q11R/V/I/L/K/T, A33K, V44I/L, A54F/T/V/G/S/W/L/I**,** P115E/I/Q/L/V/G/H/R/S/C/A/T, Q124K, Y138V/I/N/T/L/C/R/M/K, I143L, and N166R/K/A/L/P/Q/S amino acid substitutions relative to SEQ ID NO: 1 ~~at positions corresponding to positions 11, 33, 44, 54, 115, 124, 138, and 166 of SEQ ID NO:1~~.

4. The polypeptide of claim 3, wherein the amino acid at position 11 is arginine, the amino acid at position 33 is lysine, the amino acid at position 44 is isoleucine, the amino acid at position 54 is phenylalanine, the amino acid at position 115 is glutamic acid, the amino acid at position 124 is lysine, the amino acid at position 138 is isoleucine, and the amino acid at position 166 is arginine.

5. The polypeptide of claim 3, further comprising V45E, P104L, N135K, D139E, and I167V amino acid substitutions relative to SEQ ID NO: 1 ~~at positions corresponding to positions 45, 104, 135, 139, and 167 of SEQ ID NO:1~~.

6. The polypeptide of claim 5, wherein the amino acid at position 45 is glutamic acid, the amino acid at position 104 is leucine, the amino acid at position 135 is lysine, the amino acid at position 139 is glutamic acid, and the amino acid at position 167 is valine.

7. The polypeptide of claim 3, further comprising S28P, L34M, G51V, I99V, and I143L amino acid substitutions relative to SEQ ID NO: 1 ~~at positions corresponding to positions 28, 34, 51, 99 and 143 of SEQ ID NO:1~~.

8. The polypeptide of claim 7, wherein the amino acid at position 28 is proline, the amino acid substitution made at position 34 is methionine, the amino acid substitution made at position 51 is valine, the amino acid substitution made at position 99 is valine, and the amino acid substitution made at position 143 is leucine.

9. The polypeptide of claim 3, further comprising an amino acid substitution relative to SEQ ID NO: 1 selected from Q20R, A54F/T/V/G/S/W/L/I**,** F68S/Y/V, L72Q, M75R/K/Q/G/T/A, I76V, F77W, V79I, K89E, I90T/V, L92S/A/G/Q, and C164S ~~at one or more positions corresponding to positions 20, 54, 68, 72, 75, 77, 79, 89, 90, 92, and 164 of SEQ ID NO:1~~.

10. The polypeptide of claim 9, further comprising one or more of an arginine at position 20, an isoleucine at position 54, a serine at position 68, a glutamine at position 72, a lysine at

position 75, a tryptophan at position 77, an isoleucine at position 79, a glutamic acid at position 89, either a threonine or valine at position 90, an amino acid selected from the group consisting of glycine, glutamine, serine and alanine at position 92, and a serine at position 164.

11. The polypeptide of claim 3, further comprising E23V, S28P, I143L, and N166R/K/A/L/P/Q/S amino acid substitutions relative to SEQ ID NO: 1 ~~at positions corresponding to positions 23, 28, 143, and 166 of SEQ ID NO:1~~.

12. The polypeptide of claim 11, wherein the amino acid at position 23 is valine, the amino acid at position 28 is proline, the amino acid at position 143 is valine, and the amino acid at position 166 is arginine.

13. The polypeptide of claim 1, wherein the amino acid substitutions relative to SEQ ID NO: 1 are A4E/S/R/G/D/T/L, L34M, I76V, and N166R/K/A/L/P/Q/S ~~at positions corresponding to positions 4, 34, 76, and 166 of SEQ ID NO:1~~.

14. The polypeptide of claim 13, wherein the amino acid at position 4 is serine, the amino acid at position ~~35~~ 34 is methionine, the amino acid at position 76 is valine, and the amino acid at position 166 is arginine.

15. The polypeptide of claim 1, wherein the amino acid substitutions relative to SEQ ID NO: 1 are G51V, I99V, and N166R/K/A/L/P/Q/S ~~at positions corresponding to positions 51, 99, and 166 of SEQ ID NO:1~~.

16. The polypeptide of claim 15, wherein the amino acid at position 51 is valine, the amino acid at position 99 is valine, and the amino acid at position ~~177~~ 166 is arginine.

17. A fusion protein comprising the polypeptide of claim 1 linked to a polypeptide of interest.