MULTIPARTITE LUCIFERASE PEPTIDES AND POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims priority to U.S. Provisional Patent Application Serial No. 62/941,255 filed November 27, 2019, which is hereby incorporated by reference in its entirety.

FIELD

Provided herein are bioluminescent polypeptides and compositions and methods for the assembly of tripartite or multipartite bioluminescent complexes. In particular embodiments, a bioluminescent complex is formed upon the interaction of three or more peptide and/or polypeptide components.

BACKGROUND

Biological processes and analyte detection rely on the co-localization and interactions between molecules, macromolecules, and molecular complexes. In order to understand such processes, and to develop techniques and compounds to manipulate them for research, clinical, and other practical applications, it is necessary to have tools available to detect and monitor these co-localizations/interactions. The study of these interactions, particularly under physiological conditions (e.g., at normal expression levels for monitoring protein interactions) or in complex sample matrices (e.g. blood samples, environmental samples), requires high sensitivity.

SUMMARY

Provided herein are bioluminescent polypeptides and compositions and methods for the assembly of a tripartite or multipartite bioluminescent complex. In particular embodiments, a bioluminescent complex is formed upon the interaction of three or more peptide and/or polypeptide components.

Experiments conducted during development of embodiments herein demonstrate the assembly of a bioluminescent complex, capable of generating luminescence in the presence of an appropriate substrate (e.g., a coelenterazine or a coelenterazine analog substrate), from complementary polypeptide(s) and peptide(s) that collectively span the the length (or >75% of the length, >80% of the length, >85% of the length, >90% of the length, >95% of the

length, or more) of a luciferase base sequence (or collectively comprise at least 40% sequence identity to a luciferase base sequence (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75% >80%, >85%, >90%, >95%, or more). In some embodiments, "complementary" polyptpide(s) and peptide(s) are separate molecules that each correspond to a portion of a luciferase base sequence. Through structural complementarity, they assemble to form a bioluminescent complex.

Additional experiments were conducted during development of embodiments herein to develop monomeric bioluminescent polypeptides with enhanced characteristics (e.g., stability, luminescence, etc.).

In some embodiments, the complementary polypeptide(s) and peptide(s) are fragments of a luciferase base sequence that assmble to form a bioluminescent complex. In some embodiments, the fragments collectively comprise the full length of the luciferase base sequence. In some embodiments, the fragments collectively comprise at least 75% of the full length of the luciferase base sequence (e.g., >75% of the length, >80% of the length, >85% of the length, >90% of the length, >95% of the length, or more).

In some embodiments, the complementary polypeptide(s) and peptide(s) are variants of portions of a luciferase base sequence individually comprising at least 40% sequence identity to the corresponding portion of the luciferase base sequence (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75% >80%, >85%, >90%, >95%, or more) that assmble to form a bioluminescent complex. In some embodiments, the complementary polypeptide(s) and peptide(s) are variants of portions of a luciferase base sequence collectively comprising at least 40% sequence identity to the entire luciferase base sequence (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75% >80%, >85%, >90%, >95%, or more) that assemble to form a bioluminescent complex. In some embodiments, the fragments collectively comprise the full length of the luciferase base sequence. In some embodiments, the complementary polypeptide(s) and peptide(s) collectively comprise at least 75% of the full length of the luciferase base sequence (e.g., >75% of the length, >80% of the length, >85% of the length, >90% of the length, >95% of the length, or more).

Examples of luciferase base sequences include SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 788, and SEQ ID NO: 789. Some embodiments herein provide a polypeptide component that is a fragment of the luciferase base sequence (e.g., SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 788, and SEQ ID NO: 789) or a variant thereof (e.g., >40%, >45%,

>50%, >55%, >60%, >65%, >70%, >75% >80%, >85%, >90%, >95% sequence identity), and one or more complementary peptide(s) and/or polypeptide(s) that collectvily span the remainder of the luciferase base sequence. For example, if a luciferase base sequence is 170 amino acid residues in length, an exemplary polypeptide component may be, for example 102, 124, 133, or 148 amino acids in length, and 1, 2, 3, 4, 5, or more complementary peptides correspond to the remaining 68, 46, 37, or 22 amino acids. In some embodiments, each polypeptide component individually comprises at least 40% sequence identity (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75% >80%, >85%, >90%, >95%, or more) to the corresponding portion of the luciferase base sequence.

In some embodiments, provided herein are systems or kits comprising comprising: (a) a polypeptide component comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to a polypeptide fragment of SEQ ID NO: 788 or SEQ ID NO: 789; and (b) one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to the complementary portion of SEQ ID NO: 788 or SEQ ID NO: 789; wherein a bioluminescent signal produced by a bioluminescent complex assembled from the polypeptide component and one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when compared to a bioluminescent signal produced by the polypeptide component or one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides and the coelenterazine substrate alone. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 794. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 795. In some embodiments, the

polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 796. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 797. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 798. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 799. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 800. In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID 801. In some embodiments, the bioluminescent signal is substantially increased when the polypeptide component associates with the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides.

In some embodiments, polypeptide component and/or one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides comprise amino acid sequences that are not a naturally occurring sequences or fragments thereof. In some embodiments, polypeptide component and/or one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides comprise a non-natural amino acid, an amino acid analog, and/or peptoid amino acids. In some embodiments, the polypeptide component and/or one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides are present as fusions with one or more additional amino acid sequences. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism within a with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target. In some embodiments, provided herein are bioluminescent complexes comprising the polypeptide component and one or more complementary peptides, dipeptides, tripeptide, and/or polypeptides of the systems or kits described herein.

In some embodiments, provided herein are systems or kits comprising two or more peptide, dipeptide, tripeptide and/or polypeptide components collectively comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 788 or SEQ ID NO: 789; wherein a bioluminescent signal produced by the bioluminescent complex in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when compared to a bioluminescent

signal produced by the polypeptide or one or more complementary peptides and the coelenterazine substrate alone. In some embodiments, a system of kit comprises a polypeptide component having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, and or tripeptides collectively having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 794. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 795. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 796. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 797. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 798. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 799. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%,

80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 800. In some embodiments, the polypeptide comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 801. In some embodiments, the bioluminescent signal is substantially increased when the polypeptide associates with the one or more complementary peptides. In some embodiments, the polypeptide and/or one or more complementary peptides comprise amino acid sequences that are not a naturally occurring sequences or fragments thereof. In some embodiments, polypeptide and/or one or more complementary peptides comprise a non-natural amino acid, an amino acid analog, and/or peptoid amino acids. In some embodiments, the polypeptide and/or one or more complementary peptides are present as fusions with one or more additional amino acid sequences. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target. In some embodiments, provided herein are bioluminescent complexes comprising the two or more peptide, dipeptide, tripeptide, and/or polypeptide components of the systems or kits described herein.

Disclosed herein is a system or kit comprising two or more peptide and/or polypeptide components collectively comprising 70% or greater sequence identity to SEQ ID NO: 788 or SEQ ID NO: 789; wherein the two or more peptide and/or polypeptide componets are capable of forming a bioluminescent complex upon interacting with each other; wherein a bioluminescent signal produced by the bioluminescent complex in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when compared to a bioluminescent signal produced by the polypeptide or one or more complementary peptides and the coelenterazine substrate alone; wherein the system or kit comprises a polypeptide that corresponds structurally to 8 or fewer β strands of a Oplophorus gracilirostris luciferase.

Disclosed herein is a bioluminescent complex comprising the two or more peptide and/or polypeptide components of the system or kit as defined herein.

Disclosed herein is a method comprising contacting the bioluminescent complex as defined herein with a substrate for the bioluminescent complex.

In some embodiments, provided herein are methods comprising: (a) combining: (i)a polypeptide component comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to a polypeptide fragment of SEQ ID NO: 788 or SEQ ID NO: 789; (ii) one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to the complementary portion of SEQ ID NO: 788 or SEQ ID NO: 789; and (iii) a coelenterazine or a coelenterazine analog substrate; and (b) detecting luminescence, wherein a greater level of luminescence compared to a level of luminescence produced by the polypeptide component and a coelenterazine or a coelenterazine analog alone indicates formation of a bioluminescent complex of the polypeptide component and the one or more complementary peptides. In some embodiments, one or more of the polypeptide component and the first and second peptides are expressed in a cell, added to a cell exogenously, and/or added to a sample. In some embodiments, (i) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790 and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 794; (ii) the

polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 795; (iii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 796; (iv) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 797; (v) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 798; (vi) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 799; (vii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 800; or (viii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%,

45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID 801.

In some embodiments, provided herein are methods comprising: (a) combining: (i) two or more peptide, dipeptide, tripeptide, and/or polypeptide components collectively comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to the full length of SEQ ID NO: 788 or SEQ ID NO: 789; and (ii) a coelenterazine or a coelenterazine analog substrate; and (b) detecting luminescence, wherein a greater level of luminescence compared to a level of luminescence produced by the peptide, dipeptide, tripeptide, and/or polypeptide components and the coelenterazine or coelenterazine analog indicates formation of a bioluminescent complex of the peptide and polypeptide components. In some embodiments, one or more of the polypeptide components and the first and second peptides may be expressed in a cell, added to a cell exogenously, and/or added to a sample. In some embodiments, (i) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 794; (ii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 795; (iii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 796; (iv) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 797; (v) the polypeptide component

comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 790, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 798; (vi) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 791, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 799; (vii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 792, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 800; or (viii) the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID NO: 793, and the one or more complementary peptides, dipeptides, tripeptides, and/or polypeptides collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to SEQ ID 801.

In some embodiments, provided herein are methods of detecting an interaction between a first molecular entity and a second molecular entity, the method comprising: (a) tagging the first molecular entity with a first peptide, dipeptide, or tripeptide tag; (b) tagging the second molecular entity with a second peptide, dipeptide, or tripeptide tag; (c) combining the tagged first molecular entity and the tagged second molecular entity and/or allowing the tagged first molecular entity and the tagged second molecular entity to come into contact with one another; (d) adding one or more peptide, dipeptide, tripeptide, and/or polypeptide components, wherein the first peptide, dipeptide, or tripeptide tag, the second peptide, dipeptide, or tripeptide tag, and the peptide, dipeptide, tripeptide, and/or polypeptide components collectively comprise an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789, and capable of assembling to form a bioluminescent complex; (e) adding a coelenterazine or a coelenterazine analog substrate;

and (f) detecting a luminescent signal produced by the bioluminescent complex, wherein the magnitude of the luminescent signal correlates to the strength of the interaction between the first molecular entity and the second molecular entity. In some embodiments, the first molecular entity and/or the second molecular entity is a protein of interest or a peptide of interest and tagging comprises generating a fusion of the first molecular entity and/or the second molecular entity with the first tag and/or second tag. In some embodiments, the first molecular entity and/or the second molecular entity is a small molecule and tagging comprises directly or indirectly linking the first molecular entity and/or the second molecular entity with the first tag and/or second tag. In some embodiments, one of the first molecular entity and the second molecular entity is a drug or drug candidate and the other is a drug target or candidate drug target, and the bioluminescent signal indicates binding of the drug or drug candidate to the other is a drug target or candidate drug target. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity comprises expressing one or both within a cell and/or adding one or both to a cell.

In some embodiments, provided herein are methods of detecting an interaction between a first protein or peptide entity and a second protein or peptide entity with a cell comprising, the method comprising: (a) expressing within the cell a fusion comprising the first protein or peptide entity and a first peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a first portion of SEQ ID NO: 788 or 789; (b) expressing within the cell a fusion comprising the second protein or peptide entity and a second peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a second portion of SEQ ID NO: 788 or 789; (c) expressing within the cell one or more peptide, dipeptide, tripeptide, and/or polypeptide components comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a third portion of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789 and are configured to produce a bioluminescent complex upon interaction of the first protein or peptide entity and the second protein or peptide entity; (d) adding a

coelenterazine or a coelenterazine analog substrate to the cell; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the magnitude of the luminescent signal correlates to the strength of the interaction between the first protein or peptide entity and the second protein or peptide entity.

In some embodiments, provided herein are methods of detecting co-localization of a first molecular entity and a second molecular entity, the method comprising: (a) tagging the first molecular entity with a first peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a first portion of SEQ ID NO: 788 or 789; (b) tagging the second molecular entity with a second peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a second portion of SEQ ID NO: 788 or 789; (c) combining the tagged first molecular entity and the tagged second molecular entity in the same system; (d) adding one or more peptide, dipeptide, tripeptide, and/or polypeptide components to the system, the components having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a third portion of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789, wherein the first peptide tag, the second peptide tag, and components are configured to produce a bioluminescent complex upon co-localization of the first molecular entity and the second molecular entity; (e) adding a coelenterazine or a coelenterazine analog substrate to the system; and (f) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates co-localization of the first molecular entity and the second molecular entity within the system, and/or wherein the magnitude of the luminescent signal correlates to the amount of co-localization within the system of the first molecular entity and the second molecular entity. In some embodiments, the system comprises a cell, tissue, organ, whole organism, and/or a biochemical, non-cellular sample. In some embodiments, the first molecular entity and/or the second molecular entity is a protein of interest or a peptide of interest, and tagging comprises generating a fusion of the first molecular entity and/or the second molecular entity with the first tag and/or peptide tag. In

some embodiments, the first molecular entity and/or the second molecular entity is a small molecule and tagging comprises directly or indirectly linking the first molecular entity and/or the second molecular entity with the first tag and/or second tag. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity comprises expressing one or both within the system and/or adding one or both to the system.

In some embodiments, provided herein are methods of detecting co-localization of a first protein or peptide entity and a second protein or peptide entity with a cell comprising, the method comprising: (a) expressing within the cell a fusion comprising the first protein or peptide entity and a first peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a first portion of SEQ ID NO: 788 or 789; (b) expressing within the cell a fusion comprising the second protein or peptide entity and a second peptide, dipeptide, or tripeptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a second portion of SEQ ID NO: 788 or 789; (c) expressing with the cell one or more peptide, dipeptide, tripeptide, or polypeptide components having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a third portion of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components are configured to produce a bioluminescent complex upon co-localization of the first protein or peptide entity and the second protein or peptide entity; (d) adding a coelenterazine or a coelenterazine analog substrate to the cell; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates co-localization of the first protein or peptide entity and the second protein or peptide entity within the cell, and/or wherein the magnitude of the luminescent signal correlates to the amount of co-localization within the system of the first protein or peptide entity and the second protein or peptide entity.

In some embodiments, provided herein are methods of detecting a target molecule, wherein the target molecule displays a first antigen, epitope, or sequence and a distinct second antigen, epitope, or sequence, the method comprising: (a) contacting a sample

containing the target molecule with (i) a first primary binding moiety that recognizes the first antigen, epitope, or sequence and (ii) a second primary binding moiety that recognizes the second antigen, epitope, or sequence and allowing the first and second primary binding moieties to bind to the first and second antigens, epitopes, or sequences; (b) contacting the sample with (i) a first secondary binding moiety conjugated to a first tag and (ii) a second secondary binding moiety conjugated to second tag, wherein the first secondary binding moiety recognizes the first primary binding moiety and the second secondary binding moiety recognizes the second primary binding moiety, wherein the first or second tags comprises amino acid sequences having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with first and second portions of SEQ ID NO: 788 or 789; (c) allowing the first and second secondary binding moieties to bind to the first and second primary binding moieties; (d) contacting the sample with comprising one or more peptide, dipeptide, tripeptide, and/or polypeptide components having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a third portion of SEQ ID NO: 788 or 789; wherein the first tag, the second tag, and the components collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components are configured to produce a bioluminescent complex upon interaction; (d) contacting the sample with a coelenterazine or a coelenterazine analog substrate; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates the presence of the target molecule, and/or wherein the magnitude of the luminescent signal correlates to the amount of target molecule within the sample. In some embodiments, the binding moieties are independently selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the target molecule is a protein, nucleic acid, or small molecule. In some embodiments, the sample is *in vitro* or *in vivo*.

In some embodiments, provided herein are methods of detecting a target molecule, wherein the target molecule displays a first antigen, epitope, or sequence and a distinct second antigen, epitope, or sequence, the method comprising: (a) contacting the sample with (i) a first binding moiety conjugated to a first tag and (ii) a second binding moiety conjugated to second tag, wherein the first secondary binding moiety recognizes the first antigen, epitope, or sequence, and the second binding moiety recognizes the second antigen, epitope, or sequence, wherein the first tag comprises an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a first portion of SEQ ID NO: 788 or 789, and wherein the second tag comprises an amino acid sequence with a first portion of SEQ ID NO: 788 or 789; (b) allowing the first and second binding moieties to bind to the first and second antigens, epitope, or sequences; (c) contacting the sample with a peptide, dipeptide, tripeptide, or polypeptide component having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with a third portion of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with the entirety of SEQ ID NO: 788 or 789, wherein the first tag, the second tag, and the components are configured to produce a bioluminescent complex upon interaction; (d) contacting the sample with a coelenterazine or a coelenterazine analog substrate; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates the presence of the target molecule, and/or wherein the magnitude of the luminescent signal correlates to the amount of target molecule within the sample. In some embodiments, the binding moieties are independently selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the target molecule is a protein, nucleic acid, or small molecule. In some embodiments, the sample is *in vitro*, *in vivo,* or a biochemical sample.

In some embodiments, provided herein are peptides, dipeptides, tripeptides, and/or polypeptides listed in Table 1, Table 9, or Table 10. In some embodiments, a single peptide, dipeptide, tripeptide, or polypeptide listed in Table 1, Table 9, or Table 10 is provided (e.g., as a reagent, as a tag, etc.). In some embodiments, a pair (2) or set (e.g., 2, 3, 4, 5, or more) of peptides, dipeptides, tripeptides, and/or polypeptides listed in Table 1, Table 9, or Table 10 are provided. In particular, pairs or sets of the peptides, dipeptides, tripeptides, and/or polypeptides are provided that are complementary and are capable of forming a bioluminescent complex upon interaction (e.g., facilitated, unfacilitated) with one another.

In some embodiments, the polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to one of SEQ ID NOS: 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 117, 119, 121, 123, 125, 127, 129, 131, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829. In some embodiments, a polypeptide comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to one of SEQ ID NOS: 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 117, 119, 121, 123, 125, 127, 129, 131, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829 is provided with one or more peptides or dipeptides capable of forming a bioluminescent complex. In some embodiments, suitable fragments of SEQ ID NOS: 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 117, 119, 121, 123, 125, 127,

129, 131, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829 of polypeptides having % or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to one of SEQ ID NOS: 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 117, 119, 121, 123, 125, 127, 129, 131, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829 are provided. In some embodiments, such fragments are capable of forming bioluminescent complexes with a suitable set of peptides, dipeptides, tripeptides, polypeptides, etc. provided herein.

In some embodiments, the peptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to one of SEQ ID NOS: 900-907. In some embodiments, a polypeptide comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity to one of SEQ ID NOS: 900-907 is provided with one or more peptides, dipeptides, tripeptides, polypeptides, etc., capable of forming a bioluminescent complex.

In some embodiments, provided herein are peptides, dipeptides, tripeptides, and/or polypeptides having at least 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) seqeunce identity with one or more of the peptides, dipeptides, tripeptides, and/or polypeptides listed in Table 1, Table 9, or Table 10. In some embodiments, a single peptide, dipeptide, tripeptide, or polypeptide having at least 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) seqeunce identity with one or more of the peptides, dipeptides, tripeptides, and/or polypeptides listed in Table 1, Table 9, or Table 10 is provided (e.g., as a reagent, as a tag, etc.). In some

embodiments, a pair (2) or set (e.g., 2, 3, 4, 5, or more) of peptides, dipeptides, tripeptides, and/or polypeptides having at least 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) seqeunce identity with one or more of the peptides, dipeptides, tripeptides, and/or polypeptides listed in Table 1, Table 9, or Table 10 is provided are provided. In particular, pairs or sets of the peptides, dipeptides, tripeptides, and/or polypeptides are provided that are complementary and are capable of forming a bioluminescent complex upon interaction (e.g., facilitated, unfacilitated) with one another.

In some embodiments, provided herein are polypeptides comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with one of SEQ ID NO: 790, 791, 792, or 793. In some embodiments, the polypeptide is provided alone or as a pair/set with complementary peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, fusions of polypeptides herein with proteins of interest, intearction elements, colocalization elements, etc., are provided. In some embodiments, nucleic acids and vectors encoding the polypeptides and fusions thereof or provided.

In some embodiments, provided herein are peptides comprising SEQ ID NO: 817, 818, 819, 13, 15, 23, or 25. In some embodiments, provided herein are peptides comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with one of SEQ ID NO: 817, 818, 819, 13, 15, 23, or 25. In some embodiments, the peptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, fusions of peptides herein with proteins of interest, intearction elements, colocalization elements, etc., are provided. In some embodiments, nucleic acids and vectors encoding the peptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a peptide herein.

In some embodiments, provided herein is a β6-7-like dipeptide comprising SEQ ID NOS: 817 and 818. In some embodiments, provided herein is a β6-7-like dipeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NOS: 817 and 818. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In

some embodiments, nucleic acids and vectors encoding the dipeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a dipeptide herein.

In some embodiments, provided herein is a β7-8-like dipeptide comprising SEQ ID NOS: 818 and 819. In some embodiments, provided herein is a β7-8-like dipeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NOS: 818 and 819. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the dipeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a dipeptide herein.

In some embodiments, provided herein is a β8-9-like dipeptide comprising SEQ ID NOS: 819/23 or 819/25. In some embodiments, provided herein is a β8-9-like dipeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with NOS: 819/23 or 819/25. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the dipeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a dipeptide herein.

In some embodiments, provided herein is a β9-10-like dipeptide comprising SEQ ID NOS: 23/13, 23/15, 25/13 or 25/15. In some embodiments, provided herein is a β8-9-like dipeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with NOS: SEQ ID NOS: 23/13, 23/15, 25/13 or 25/15. In some embodiments, the dipeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the dipeptide is provided

alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the dipeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a dipeptide herein.

In some embodiments, provided herein is a β6-8-like tripeptide comprising SEQ ID NOS: 817-819. In some embodiments, provided herein is a β6-8-like tripeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with NOS: SEQ ID NOS: 817-819. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the tripeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a tripeptide herein.

In some embodiments, provided herein is a β7-9-like tripeptide comprising SEQ ID NOS: 818/819/23 or 818/819/25. In some embodiments, provided herein is a β7-9-like tripeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with NOS: SEQ ID NOS: 818/819/23 or 818/819/25. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the tripeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a tripeptide herein.

In some embodiments, provided herein is a β8-10-like tripeptide comprising SEQ ID NOS: 819/23/13, 819/23/15, 819/25/13, or 819/25/15. In some embodiments, provided herein is a β7-9-like tripeptide having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NOS: 819/23/13,

819/23/15, 819/25/13, or 819/25/15. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, the tripeptide is provided alone or as a pair/set with complementary polypeptide and/or other peptide(s), dipeptide(s), and/or tripeptide for the formation of a bioluminescent complex. In some embodiments, nucleic acids and vectors encoding the tripeptides and fusions thereof or provided. In some embodiments, molecules of interest and/or proteins of interest are tagged with a tripeptide herein.

In some embodiments, provided herein are peptides comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9, wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the peptide contacts a second peptide consisting of SEQ ID NO: 25 and a polypeptide complement consisting of SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 when compared to a bioluminescent signal produced by the peptide and the coelenterazine or coelenterazine analog substrate alone. In some embodiments, the bioluminescent signal is substantially increased when the peptide associates with the second peptide and the polypeptide complement. In some embodiments, the peptide exhibits enhancement of one or more traits compared to a peptide of SEQ ID NO: 6 and/or SEQ ID NO: 9, wherein the traits are selected from: affinity for the second peptide and the polypeptide complement or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the second peptide and the polypeptide complement. In some embodiments, the amino acid sequence is not a naturally occurring protein (e.g., not SEQ ID NO: 1), not a mutant version thereof (e.g., not SEQ ID NO: 3), not a fragment of a naturally occurring protein (e.g., not SEQ ID NOS: 5-7), and not a fragment of a mutant version thereof (e.g., not one of SEQ ID NOS: 8-10). In some embodiments, the amino acid sequence contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids. In some embodiments, a peptide is chemically conjugated to a linker, reactive moiety, detection element (e.g., fluorophore), interaction/binding element, etc.

In some embodiments, provided herein are fusion polypeptides (e.g., genetic fusions (or alternatively, chemical conjugations or synthetically produced)) comprising a peptide described in the preceding paragraph and an additional amino acid sequence or compound (e.g. small molecule drug). In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and/or a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of an antibody (e.g., polyclonal, monoclonal, and/or recombinant), antibody fragment,protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (e.g., an analyte or a protein that binds to an analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are peptides comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10, wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the peptide contacts a second peptide consisting of SEQ ID NO: 23 and a polypeptide complement consisting of SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 when compared to a bioluminescent signal produced by the peptide and the coelenterazine or coelenterazine analog substrate alone. In some embodiments, the bioluminescent signal is substantially increased when the peptide associates with the second peptide and the polypeptide complement. In some embodiments, the peptide exhibits enhancement of one or more traits compared to a peptide of SEQ ID NO: 7 and/or SEQ ID NO: 10, wherein the traits are selected from: affinity for the second peptide and the

polypeptide complement or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the second peptide and the polypeptide complement. In some embodiments, the amino acid sequence is not a naturally occurring protein (e.g., not SEQ ID NO: 1), not a mutant version thereof (e.g., not SEQ ID NO: 3), not a fragment of a naturally occurring protein (e.g., not SEQ ID NOS: 5-7), and not a fragment of a mutant version thereof (e.g., not one of SEQ ID NOS: 8-10). In some embodiments, the amino acid sequence contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids. In some embodiments, a peptide is chemically conjugated to a linker, reactive moiety, detection element (e.g., fluorophore), interaction/binding element, etc.

In some embodiments, provided herein are fusion polypeptides (e.g., genetic fusions, synthetically-produced fusions, chemical conjugates, enzymatic conjugates, etc.) comprising a peptide described in the preceding paragraph and an additional amino acid sequence. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism within a with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are compositions comprising: (a) a first peptide comprising an amino acid sequence having greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more), but less than 100% sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and

SEQ ID NO: 10; and (b) a second peptide comprising an amino acid sequence having greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more), but less than 100% sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6, SEQ ID NO: 9, and SEQ ID NO: 29; wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the first peptide contacts the second peptide and a polypeptide complement consisting of SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 when compared to a bioluminescent signal produced by the first peptide and/or the second peptide and the coelenterazine substrate alone. In some embodiments, the bioluminescent signal is substantially increased when the first peptide associates with the second peptide and the polypeptide complement. In some embodiments, the first peptide exhibits enhancement of one or more traits compared to a peptide of SEQ ID NO: 7 and/or SEQ ID NO: 10, and the second peptide exhibits enhancement of one or more traits compared to a peptide of SEQ ID NO: 6, SEQ ID NO: 9, and SEQ ID NO: 29, wherein the traits are selected from: affinity for the second peptide and the polypeptide complement or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the second peptide and the polypeptide complement. In some embodiments, the amino acid sequence of the first and/or second peptide is not a naturally occurring protein or a fragment thereof. In some embodiments, the amino acid sequence of the first and/or second peptide contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids.

In some embodiments, provided herein are compositions comprising fusion polypeptides comprising the first and second peptides of described in the preceding paragraph and an additional amino acid sequence. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction

polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism within a with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are polypeptides comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the polypeptide contacts a first peptide consisting of SEQ ID NO: 23 and a second peptide consisting of SEQ ID NO: 25 when compared to a bioluminescent signal produced by the peptide and the coelenterazine or a coelenterazine analog substrate alone. In some embodiments, the bioluminescent signal is substantially increased when the polypeptide associates with the first and second peptides. In some embodiments, the polypeptide exhibits enhancement of one or more traits compared to a polypeptide of SEQ ID NO: 5 and/or SEQ ID NO: 8, wherein the traits are selected from: affinity for the first and/or second peptides or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the first and second peptides. In some embodiments, the amino acid sequence is not a naturally occurring protein (e.g., not SEQ ID NO: 1), not a mutant version thereof (e.g., not SEQ ID NO: 3), not a fragment of a naturally occurring protein (e.g., not SEQ ID NOS: 5-7), and not a fragment of a mutant version thereof (e.g., not one of SEQ ID NOS: 8-10). In some embodiments, the amino acid sequence contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids.

In some embodiments, provided herein are fusion polypeptides (e.g., genetic fusions, synthetically-produced fusions, chemical conjugates, enzymatic conjugates, etc.) comprising a polypeptide described in the preceding paragraph and an additional amino acid sequence, nucleic acid sequence, or other fused or appended molecule. In some embodiments, the additional sequence or other molecule is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some

embodiments, the additional sequence or other molecule is a binding moiety selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional sequence or other fused or appended molecule is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional sequence or other fused or appended molecule is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism with a second co-localization polypeptide. In some embodiments, the additional sequence or other fused or appended molecule is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are polypeptides comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the polypeptide contacts a first peptide consisting of SEQ ID NO: 23 and a second peptide consisting of SEQ ID NO: 25 when compared to a bioluminescent signal produced by the peptide and the coelenterazine or coelenterazine analog substrate alone. In some embodiments, the bioluminescent signal is substantially increased when the polypeptide associates with the first and second peptides. In some embodiments, the polypeptide exhibits enhancement of one or more traits compared to a polypeptide of SEQ ID NO: 5 and/or SEQ ID NO: 8, wherein the traits are selected from: affinity for the first and/or second peptides or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the first and second peptides. In some embodiments, the amino acid sequence is not a naturally occurring protein (e.g., not SEQ ID NO: 1), not a mutant version thereof (e.g., not SEQ ID NO: 3), not a fragment of a naturally occurring protein (e.g., not SEQ ID NOS: 5-7), and not a fragment of a mutant version thereof (e.g., not one of SEQ ID NOS: 8-10). In some embodiments, the amino acid

sequence contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids.

In some embodiments, provided herein are fusion polypeptides (e.g., genetic fusions, synthetically-produced fusions, chemical conjugates, enzymatic conjugates, etc.) comprising a peptide described in the preceding paragraph and an additional amino acid sequence. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence is a binding moiety selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism with a second co-localization polypeptide. In some embodiments, the additional amino acid sequence is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are β9/β10-like dipeptides comprising an amino acid sequence having greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more), but less than 100% sequence identity with SEQ ID NO: 35 and less than 100% sequence identity with SEQ ID NO: 205 and SEQ ID NO: 206, wherein a bioluminescent signal produced in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when the peptide contacts a polypeptide complement consisting of SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 when compared to a bioluminescent signal produced by the peptide and the coelenterazine or a coelenterazine analog substrate alone. In some embodiments, a dipeptide (e.g., β_9/β_{10}-like dipeptide) associates (e.g., forms a bioluminescent complex) with a polypeptide component described herein (e.g., β_{1-8}-like polypeptide) without facilitation (e.g., from interaction

elements). In other embodiments, a dipeptide (e.g., β_9/β_{10}-like dipeptide) and polypeptide component described herein (e.g., β_{1-8}-like polypeptide) will not form a bioluminescent complex without facilitation (e.g., from interaction elements), but will associate (e.g., form a bioluminescent complex) with facilitation from appropriate interaction elements. In some embodiments, the bioluminescent signal is substantially increased when the peptide associates with the polypeptide complement. In some embodiments, the peptide exhibits enhancement of one or more traits compared to a peptide of SEQ ID NO: 205 and/or SEQ ID NO: 206, wherein the traits are selected from: affinity for the polypeptide complement or enhanced expression, solubility, stability, and/or bioluminescent activity when combined with the polypeptide complement. In some embodiments, the amino acid sequence is not a naturally occurring protein or a fragment thereof. In some embodiments, the amino acid sequence contains a non-natural amino acid, an amino acid analog, and/or peptoid amino acids.

In some embodiments, provided herein are fusion polypeptides (e.g., genetic fusions, synthetically-produced fusions, chemical conjugates, enzymatic conjugates, etc.) comprising the β9/β10-like dipeptides described herein and an additional amino acid sequence. In some embodiments, the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety. In some embodiments, the additional amino acid sequence or other fused or appended molecule is a binding moiety selected from the group consisting of antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, peptide nucleic acid, DARPin, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the additional amino acid sequence or other fused or appended molecule is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide. In some embodiments, the additional amino acid sequence or other fused or appended molecule is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism with a second co-localization

polypeptide. In some embodiments, the additional amino acid sequence or other fused or appended molecule is a protein of interest and is a candidate drug target.

In some embodiments, provided herein are nucleic acids and/or vectors coding for the peptides, polypeptides, and/or fusion polypeptides described herein. In some embodiments, provided herein are cells expressing nucleic acids and/or vectors coding for the peptides, polypeptides, and/or fusion polypeptides described herein. In some embodiments, synthetic production of the peptides, polypeptides, and/or fusion polypeptides described herein is provided. In some embodiments, the peptides, polypeptides, and/or fusion polypeptides described herein are chemically conjugated to additional moieties (e.g., interaction elements, co-localization elements, proteins of interest, molecules of interest, etc.).

In some embodiments, provided herein are bioluminescent complexes comprising: (a) a polypeptide comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8; (b) a first peptide comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; and (c) a second peptide comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10; wherein the bioluminescent complex produces substantially increased bioluminescence in the presence of a coelenterazine or a coelenterazine analog substrate when compared to a coelenterazine or a coelenterazine analog substrate in the presence of: the polypeptide alone, the first peptide alone, the second peptide alone, and any two of the polypeptide, the first peptide, and the second peptide. In some embodiments, the first peptide is a first peptide tag, wherein the second peptide is a second peptide tag, and wherein the first and second peptide tags are each linked to moieties that are independently selected from the group consisting of a molecule of interest, a peptide of interest, a protein of interest, an interaction element, a co-localization element, or a binding moiety. In some embodiments, the first peptide tag or the second peptide tag is linked to a drug or drug candidate, and the other peptide tag is linked to a drug target or candidate drug target, and wherein the intensity of the bioluminescence from the

bioluminescent complex correlates to the affinity of the drug or drug candidate for the drug target or candidate drug target. In some embodiments, the first peptide tag is linked to a first interaction element, and the second peptide tag is linked to a second interaction element, and wherein the intensity of the bioluminescence from the bioluminescent complex correlates to the affinity of the first interaction element for the second interaction element under the conditions assayed (e.g., in some embodiments, the combination of the first peptide, second peptide, polypeptide component, and substrate do not form the bioluminescent complex (and produce significant light output (e.g., above background)) in the absence of an interaction between interaction elements). In some embodiments, the first peptide tag is linked to a first co-localization element, and the second peptide tag is linked to a second co-localization element, and wherein substantially increased bioluminescence indicates co-localization, but not necessarily interaction, of the first co-localization element and the second co-localization element, under the conditions assayed.

In some embodiments, the peptides and polypeptide provided herein are not fragments of larger (e.g., pre-existing) proteins. In other embodiments, one or more peptides and/or polypeptides provided herein are fragments of larger (e.g., pre-existing) proteins.

In some embodiments, provided herein are methods comprising: (a) combining: (i) a first peptide comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9, (ii) a second peptide comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10, (iii) a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon interaction of the first molecular entity and the second molecular entity, and (iv) a coelenterazine or a coelenterazine analog substrate; and (b) detecting luminescence, wherein a greater level of luminescence compared to a level of luminescence produced by the polypeptide component and a coelenterazine or a

coelenterazine analog alone indicates formation of a bioluminescent complex of the polypeptide component and the first and second peptides. In some embodiments, one or more of the polypeptide component and the first and second peptides are expressed in a cell, added to a cell exogenously, and/or added to a sample.

In some embodiments, provided herein are methods of detecting an interaction between a first molecular entity and a second molecular entity, the method comprising: (a) tagging the first molecular entity with a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; (b) tagging the second molecular entity with a second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10; (c) combining the tagged first molecular entity and the tagged second molecular entity; (d) adding a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon interaction of the first molecular entity and the second molecular entity; (e) adding a coelenterazine or a coelenterazine analog substrate; and (f) detecting a luminescent signal produced by the bioluminescent complex, wherein the magnitude of the luminescent signal correlates with (e.g., is proportional to, is directly proportional to, etc.) the number of, strength of, favorability of, and/or stability of the interaction(s)) between the first molecular entity and the second molecular entity. In some embodiments, catalytic efficiency, substrate turnover, and/or specific activity of the resulting bioluminescent complex correlates with (e.g., is proportional to, is directly proportional to, etc.) the number of, strength of, favorability of, and/or stability of the interaction(s)) between the first molecular entity and the second molecular entity. In some embodiments, the first molecular entity and/or the second molecular entity is a protein of interest or a peptide of interest, and tagging comprises generating a fusion (or synthetic conjugation) of the first molecular entity and/or the second molecular entity with the first peptide tag and/or second

peptide tag. In some embodiments, the first molecular entity and/or the second molecular entity is a small molecule, and tagging comprises directly or indirectly linking the first molecular entity and/or the second molecular entity with the first peptide tag and/or second peptide tag. In some embodiments, one of the first molecular entity and the second molecular entity is a drug or drug candidate, and the other is a drug target or candidate drug target, and the bioluminescent signal indicates binding of the drug or drug candidate to the other is a drug target or candidate drug target. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity comprises expressing one or both within a cell and/or adding one or both to a cell. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity is performed *in vitro*, in a non-cellular sample, etc. In some embodiments, the affinity of a drug or candidate drug for a drug target or candidate drug target is determined using the systems and methods herein by titrating unlabeled drug target or candidate drug target into the system. In some embodiments, two or more of steps (a)-(f) are performed concurrently. In some embodiments, two or more of steps (a)-(f) are performed separately.

In some embodiments, provided herein are method of performing a competition assay to detect an interaction between a first molecular entity and a second molecular entity, the method comprising: (a) combining: (i) a tracer comprising the first molecular entity tagged with a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9, (ii) the second molecular entity tagged with a second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10, (iii) a coelenterazine or a coelenterazine analog substrate, (iv) a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, and (v) a sample suspected of containing untagged first molecular entity; wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex and produce a bioluminescent signal in the presence of the

coelenterazine or a coelenterazine analog substrate; (b) detecting the bioluminescent signal produced by the bioluminescent complex; and (c) comparing the bioluminescent signal produced in the presence of the sample with a control bioluminescent signal produced in the absence of the sample, wherein a decrease in the bioluminescent signal indicates the presence or amount of untagged first molecular entity in the sample. In some embodiments, the first molecular entity is a small molecule or peptide (e.g., drug or candidate drug). In some embodiments, the second molecular entity is a drug target or candidate drug target (e.g., a protein).

In some embodiments, provided herein are methods of detecting an interaction between a first protein, peptide, or molecular entity and a second protein, peptide, or molecular entity within a cell comprising, the method comprising: (a) expressing within the cell (or adding to a cell or other system (e.g., non-cellular sample)), a fusion (e.g., genetic fusion, synthetic fusion, chemical conjugation, etc.) comprising the first protein, peptide, or molecular entity and a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; (b) expressing within the cell (or adding to a cell or other system (e.g., non-cellular sample)), a fusion (e.g., genetic fusion, synthetic fusion, chemical conjugation, etc.) comprising the second protein, peptide, or molecular entity and a second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10; (c) expressing with the cell (or adding to a cell or other system (e.g., non-cellular sample)), a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon interaction of the first protein, peptide, or molecular entity and the second protein, peptide, or molecular entity; (d) adding a coelenterazine or a coelenterazine analog substrate to the cell; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the magnitude of the luminescent signal correlates to

the strength of the interaction between the first protein, peptide, or molecular entity and the second protein, peptide, or molecular entity. In some embodiments, two or more of steps (a)-(e) are performed concurrently. In some embodiments, two or more of steps (a)-(e) are performed separately.

In some embodiments, provided herein are methods of detecting co-localization of a first molecular entity and a second molecular entity, the method comprising: (a) tagging the first molecular entity with a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; (b) tagging the second molecular entity with a second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10; (c) combining the tagged first molecular entity and the tagged second molecular entity in the same system; (d) adding a polypeptide component to the system, the polypeptide components comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon co-localization of the first molecular entity and the second molecular entity; (e) adding a coelenterazine or a coelenterazine analog substrate to the system; and (f) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates co-localization of the first molecular entity and the second molecular entity within the system, and/or wherein the magnitude of the luminescent signal correlates to the amount of co-localization within the system of the first molecular entity and the second molecular entity. In some embodiments, the system comprises a cell, tissue, organ, or whole organism. In some embodiments, the first molecular entity and/or the second molecular entity is a protein of interest or a peptide of interest, and tagging comprises generating a fusion (e.g., genetic fusion, synthetic fusion, chemical conjugation, enzymatic conjugation, etc.) of the first molecular entity and/or the second molecular entity with the first peptide tag and/or second peptide tag. In some embodiments,

the first molecular entity and/or the second molecular entity is a small molecule and tagging comprises directly or indirectly linking the first molecular entity and/or the second molecular entity with the first peptide tag and/or second peptide tag. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity is performed *in vitro*, in a non-cellular sample, etc. In some embodiments, combining the tagged first molecular entity and the tagged second molecular entity comprises expressing one or both within the system and/or adding one or both to the system. In some embodiments, two or more of steps (a)-(f) are performed concurrently. In some embodiments, two or more of steps (a)-(f) are performed separately.

In some embodiments, provided herein are methods of detecting co-localization of a first protein, peptide, or molecular entity and a second protein, peptide, or molecular entity within a cell the method comprising: (a) expressing within the cell a fusion comprising the first protein or peptide entity and a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; (b) expressing within the cell a fusion comprising the second protein or peptide entity and a second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10; (c) expressing with the cell a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon co-localization of the first protein or peptide entity and the second protein or peptide entity; (d) adding a coelenterazine or a coelenterazine analog substrate to the cell; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates co-localization of the first protein or peptide entity and the second protein or peptide entity within the cell, and/or wherein the magnitude of the luminescent signal correlates to the amount of co-localization within the system of the first protein or peptide entity and the

second protein or peptide entity. In some embodiments, two or more of steps (a)-(e) are performed concurrently. In some embodiments, two or more of steps (a)-(e) are performed separately.

In some embodiments, provided herein are kits comprising: (a) a first binding moiety conjugated to a first peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9; and (b) a second binding moiety conjugated to second peptide tag comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10. In some embodiments, the first and second binding moieties are independently selected from the group consisting of an antibody (e.g., polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the first and second binding moieties are primary binding moieties configured to bind to antigens, epitopes, or sequences on the same target entity. In some embodiments, the first and second binding moieties are secondary binding moieties configured to bind to antigens, epitopes, or sequences on primary binding moieties. In some embodiments, kits further comprise a polypeptide reagent comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8. In some embodiments, kits further comprise a coelenterazine or a coelenterazine analog.

In some embodiments, provided herein are methods of detecting a target molecule, wherein the target molecule displays a first antigen, epitope, or sequence and a distinct second antigen, epitope, or sequence the method comprising: (a) contacting a sample containing the target molecule with (i) a first primary binding moiety that recognizes the first antigen, epitope, or sequence and (ii) a second primary binding moiety that recognizes the

second antigen, epitope, or sequence and allowing the first and second primary binding moieties to bind to the first and second antigens, epitopes, or sequences; (b) contacting the sample with (i) a first secondary binding moiety conjugated or fused to a first peptide tag and (ii) a second secondary binding moiety conjugated or fused to second peptide tag, wherein the first secondary binding moiety recognizes the first primary binding moiety and the second secondary binding moiety recognizes the second primary binding moiety, wherein the first or second peptide tag comprises an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 (and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9), and wherein the other of the first or second peptide tag comprises an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 (and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10) and allowing the first and second secondary binding moieties to bind to the first and second primary binding moieties; (c) contacting the sample with comprising an polypeptide component having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 (and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8), wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon interaction; (d) contacting the sample with a coelenterazine or a coelenterazine analog substrate; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates the presence of the target molecule, and/or wherein the magnitude of the luminescent signal correlates to the amount of target molecule within the sample. In some embodiments, the binding moieties are independently selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins. In some embodiments, the target

molecule is a protein, peptide, nucleic acid, chemical, or drug. In some embodiments, the sample is *in vitro* or *in vivo*.

In some embodiments, provided herein are methods of detecting a target molecule, wherein the target molecule displays a first antigen, epitope, or sequence and a distinct second antigen, epitope, or sequence, the method comprising: (a) contacting the sample with (i) a first binding moiety conjugated or fused to a first peptide tag and (ii) a second binding moiety conjugated or fused to second peptide tag, wherein the first binding moiety recognizes the first antigen, epitope, or sequence and the second binding moiety recognizes the second antigen, epitope, or sequence, wherein the first or second peptide tag comprises an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 23 (and less than 100% sequence identity with SEQ ID NO: 6 and SEQ ID NO: 9), and wherein the other of the first or second peptide tag comprises an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 25 (and less than 100% sequence identity with SEQ ID NO: 7 and SEQ ID NO: 10) and allowing the first and second binding moieties to bind to the first and second antigens, epitopes, or sequences; (c) contacting the sample with a polypeptide component having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 (and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8), wherein the first peptide tag, the second peptide tag, and the polypeptide component are configured to produce a bioluminescent complex upon interaction; (d) contacting the sample with a coelenterazine or a coelenterazine analog substrate; and (e) detecting a luminescent signal produced by the bioluminescent complex, wherein the presence of luminescent signal above background indicates the presence of the target molecule, and/or wherein the magnitude of the luminescent signal correlates to the amount of target molecule within the sample. In some embodiments, the binding moieties are independently selected from the group consisting of an antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the

analyte), and analyte binding domain(s) of proteins. In some embodiments, the target molecule is a protein, peptide, nucleic acid, chemical, or drug. In some embodiments, the sample is *in vitro* or *in vivo*.

In some embodiments, provided herein are methods comprising: (a) combining: (i) a peptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 35 and less than 100% sequence identity with SEQ ID NO: 205 and SEQ ID NO: 206, (ii) a polypeptide component comprising an amino acid sequence having 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more) sequence identity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302 and less than 100% sequence identity with SEQ ID NO: 5 and SEQ ID NO: 8, and (iii) a coelenterazine or a coelenterazine analog substrate, wherein the peptide component and the polypeptide component are configured to produce a bioluminescent complex upon interaction; and (b) detecting luminescence, wherein a greater level of luminescence compared to a level of luminescence produced by the polypeptide component and a coelenterazine or a coelenterazine analog alone indicates formation of a bioluminescent complex of the polypeptide component with the peptide. In some embodiments, the peptide is a fusion (e.g., genetic, synthetic, chemical conjugate, enzymatic conjugate, etc.) with a first interaction element, and the polypeptide component is a fusion (e.g., genetic, synthetic, chemical conjugate, enzymatic conjugate, etc.) with a second interaction element, wherein the peptide and the polypeptide component form a bioluminescent complex upon interaction of the interaction elements, but do not form a bioluminescent complex in the absence of an interaction between the interaction elements. In some embodiments, the peptide and the polypeptide component form a bioluminescent complex in the absence of facilitation (e.g., by interaction elements. In some embodiments, the peptide is a fusion or conjugate (e.g., genetic, synthetic, chemical conjugate, enzymatic conjugate, etc.) with a protein, peptide, or molecule of interest (e.g., not an interaction element) and/or the polypeptide component is a fusion or conjugate (e.g., genetic, synthetic, chemical conjugate, enzymatic conjugate, etc.) with a protein, peptide, or molecule of interest (e.g., not an interaction element). In some embodiments, the peptide component and the polypeptide component form a bioluminescent complex upon co-localization (e.g., in a sample, in a cell, in a tissue, in a subject, etc.) without facilitation by interaction elements. In some embodiments, the peptide component

and the polypeptide component form a bioluminescent complex upon facilitation by interaction elements but not without facilitation.

In some embodiments, provided herein are compositions comrpising a monomeric polypeptide comprising an amino acid sequence with greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween), but less than 100% sequence identity with SEQ ID NO: 788 or 789 and less than 100% sequence identity with SEQ ID NO: 1 or 3; wherein the polypeptide exhibits luminescence in the presence of a coelenterazine or a coelenterazine analog substrate. In some embodiments, the peptide comprising an amino acid sequence with greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequence identity with one or more of SEQ ID NOS: 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829. In some embodiments, the polypeptide further comrpises an additional amino acid sequence. In some embodiments, fusion protiens of the polypeptides described herein are provided.

In some embodiments, provided herein are nucleic acisd comprising a sequence coding for a comprising an amino acid sequence with greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween), but less than 100% sequence identity with SEQ ID NO: 788 or 789 and less than 100% sequence identity with SEQ ID NO: 1 or 3; wherein the polypeptide exhibits luminescence in the presence of a coelenterazine or a coelenterazine analog substrate. In some embodiments, a nucleic acid codes for an amino acid sequence with greater than 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequence identity with one or more of SEQ ID NOS: 780, 782, 784, 786, 802, 804, 806, 808, 813, 815, or 829. In some embodiments, nucleic acids coding for fusion protiens of the polypeptides described herein are provided.

In some embodiments, provided herein are methods of detecting bioluminescence comprising contacting a monomeric bioluminescent polypeptide described herein with a coelenterazine or a coelenterazine analog substrate and detecting luminescence.

In some embodiments, provided herein are polypeptides comrpising an amino acid sequence with greater than 40% sequence identity with SEQ ID NO: 15 linked to the N-terminal end of an amino acid sequence with greater than 40% sequence identity with one of SEQ ID NOS: 17, 21, or 302; wherein a bioluminescent signal produced in the presence of a

coelenterazine or a coelenterazine analog substrate is substantially increased when the polypeptide contacts a peptide or polytpeptide comprising an amino acid sequce consisting of SEQ ID NO: 23 when compared to a bioluminescent signal produced by the polypeptide and the coelenterazine substrate alone. In some embodiments, nucleic acids comprising a sequence coding for the polypeptides described herein are provided. In some embodiments, fusions of the polypeptides described herein and additional amino acid sequences are provided.

In some embodiments, provided herein are methods comprising (a) contacting a polypeptide comrpising an amino acid sequence with greater than 40% sequence identity with SEQ ID NO: 15 linked to the N-terminal end of an amino acid sequence with greater than 40% sequence identity with one of SEQ ID NOS: 17, 21, or 302 with a coelenterazine or a coelenterazine analog substrate and a peptide or polypeptide comprising an amino acid sequence with 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequecne identity with SEQ ID NO: 23; and (b) detecting luminescence.

In some embodiments, provided herein are systems comprising: (a) a sensor polypeptide comprising a first amino acid sequence having at least 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 11 linked to an amino acid sequence that localizes in a specific cellular location; and (b) a protein of interest linked to a peptide tag comprising an amino acid sequence having at least 40% (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequence identity to SEQ ID NO: 23; wherein a bioluminescent complex is formed between the polypeptide and the peptide tag upon colocalization within the specific cellular location. In some embodiments, the specific cellular location is selected from plasma membrane, nucleus, mitochondria, and the endoplasmic reticulum. In some embodiments, provided herein are methods comprising (a) expressing a system described herein in a cell; (b) contacting the cell with a coelenterazine or a coelenterazine analog substrate; and (c) detecting luminescnce, wherein an increase in luminescene indicates formation of the bioluminescent complex and co-localization of the polypeptide and peptide tag. In some embodiments, the method further comprises a step of inducing translocation protein of interest linked to a peptide tag to the specific cellular location.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Figure 1. Graph demonstrating that a polypeptide lacking β9 and β10 portions (LgTrip 2098; SEQ ID NO: 17) exhibits reduced background luminescence compared to LgBiT (SEQ ID NO: 11) and is activated by complementation with a peptide corresponding to β9 and β10.

Figure 2. Graph demonstrating activation of LgTrip 2098 (SEQ ID NO: 17) by separate peptide corresponding to β9 and β10, respectively.

Figure 3. Graph depicting the relative stability of exemplary LgTrip 2098 mutants.

Figure 4. Graph depicting the relative luminescent activity of amino acid site saturation at position 42 of LgTrip 2098 mutants.

Figure 5. Graph depicting the relative stability of amino acid changes at position 42 of LgTrip 2098 mutants.

Figure 6A-C. Graph depicting the relative luminescent activity of amino acid changes at (A) position 4, (B) position 30, and (C) position 106 of LgTrip 2098 mutants.

Figure 7. Graph depicting the relative luminescent activity of amino acid changes at (A) position 101, (B) position 117, (C) position 127, (D) position 120, and (E) position 126 of LgTrip 3092 mutants.

Figure 8. Graph depicting the relative stability of LgTrip 2098 (WT)(SEQ ID NO: 31), LgTrip 3092 (SEQ ID NO: 19), and LgBiT (SEQ ID NO: 11) at 37ºC.

Figure 9. Graph depicting the relative stability of LgTrip variants at (A) 42ºC and (B) 60ºC.

Figure 10. Graphs depicting (A) titration of various LgTrip variants with SmTrip9 pep286 (SEQ ID NO: 37), (B) titration of various LgTrip variants with SmTrip10 pep86 (SEQ ID NO: 25), and (C) the affinity of various LgTrip variants for SmTrip9 pep286 and SmTrip10 pep86.

Figure 11A-B. Graphs depicting the (A) stability (half-life) and (B) relative stability of various LgTrip variants at 60ºC.

Figure 12A-B. Graphs depicting the kinetic profiles of LgTrip variants in the presence of SmTrip9 pep286 (SEQ ID NO: 37) and SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) compared to NanoLuc (SEQ ID NO: 3) and LgBiT (SEQ ID NO: 11) and SmTrip10 pep86

(HiBiT; SEQ ID NO: 25) (A) assayed in TBS+0.01% BSA and (B) assayed with NanoGlo® assay buffer.

Figure 13A-B. Graphs depicting facilitated complementation of various tripartite systems via rapamycin-induced formation of a FRB/FKBP complex: (A) SmTrip10 pep86 (SEQ ID NO:25), SmTrip9 pep245 (SEQ ID NO: 23), and LgTrip 2098 (SEQ ID NO: 31); and (B) SmBiT (SEQ ID NO: 13), SmTrip9 pep245 (SEQ ID NO: 23), and LgTrip 2098 (SEQ ID NO: 31).

Figure 14A-B. Graphs comparing the stability at 37°C of LgBiT (SEQ ID NO: 11) and LgTrip 2098 (WT) (SEQ ID NO: 31 in (A) TBS+0.01% BSA and (B) Passive Lysis Buffer (PLB).

Figure 15A-B. Graphs comparing stability of LgBiT (SEQ ID NO: 11), LgTrip 3546 (SEQ ID NO: 51), and NanoLuc (SEQ ID NO: 31) at 60ºC; (A) time course and (B) half-life.

Figure 16A-B. Graphs comparing LgBiT (SEQ ID NO: 11), NanoLuc (SEQ ID NO: 3), and LgTrip 3546 (SEQ ID NO: 51), and LgTrip 2098 (WT) (SEQ ID NO: 31) (A) in the presence NaCl and (B) after 26 hour exposure to NaCl.

Figure 17A-B. Graphs comparing LgBiT (SEQ ID NO: 11), NanoLuc (SEQ ID NO: 3), and LgTrip 3546 (SEQ ID NO: 51) and LgTrip 2098 (WT) (SEQ ID NO: 31) variants (A) in the presence urea and (B) after 26 hour exposure to urea.

Figure 18A-B. Graphs comparing LgBiT (SEQ ID NO: 11), NanoLuc (SEQ ID NO: 3), and LgTrip 3546 (SEQ ID NO: 51) and LgTrip 2098 (WT) (SEQ ID NO: 31) variants (A) at varying pH and (B) after 26 hour exposure to varying pH.

Figure 19. Graph comparing the autoluminescence of LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51).

Figure 20A-B. Graph comparing the luminescence of LgBiT (SEQ ID NO: 11) + SmTrip 10 pep86 (HiBiT; SEQ ID NO: 25), LgBiT (SEQ ID NO: 11) + pep263 (SEQ ID NO: 35) (β9/β10 dipeptide), and LgTrip 3546 (SEQ ID NO: 51) + pep263 (β9/β10 dipeptide) (SEQ ID NO: 35): (A) RLU and (B) signal/background (S/B).

Figure 21A-C. Facilitated complementation of LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3546 (SEQ ID NO: 51), respectively with SmTrip10 pep86 (SEQ ID NO: 25) and SmTrip9 pep245 (SEQ ID NO: 23): (A) schematic of assay system, (B) RLU, and (C) signal/background (S/B).

Figure 22. Graph and table comparing the affinities of various SmTrip10 sequences for LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep286 (SEQ ID NO: 37).

Figure 23. Graph and table comparing the activation of LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3546 (SEQ ID NO: 51) by standard-orientation (pep263) (SEQ ID NO: 35) and inverse-orientation (pep326) (SEQ ID NO: 179) dipeptides.

Figure 24. Graph and table depicting activation of complement polypeptides by dipeptides comprising the HiBiT or SmBiT sequence. Dipeptide with HiBiT sequence pep263 (SEQ ID NO: 35) or Dipeptide with SmBiT sequence pep274 (SEQ ID NO: 147)

Figure 25A-B. (A) Graph depicting luminescence resulting from complementation of various combinations of polypeptide components (with additions or deletions relative to LgTrip 3546) with SmTrip9 pep286 (SEQ ID NO: 37) and various β10-like peptides (SmTrip10 peptides); (B) Graph depicting luminescence resulting from complementation of LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep286 (SEQ ID NO: 37) with various β10-like peptides (SmTrip10 peptides).

Figure 26A-C. (A-C) Graphs depicting luminescence produced by polypeptide/peptide combinations having overlap (relative to a base luciferase sequence) between the polypeptide component and a peptide corresponding to the β9-strand or between the β9 and β10-like peptides.

Figure 27A-B. Figures and tables depicting luminescence resulting from (A) the titration of various β9-like peptides (SmTrip9 peptides) in the present of constant LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86 (SEQ ID NO: 25) concentrations, and (B) the titration of SmTrip10 pep86 (SEQ ID NO: 25) in the presence of constant concentrations of LgTrip 3546 (SEQ ID NO: 51) and various β9-like peptides (SmTrip9 peptides).

Figure 28. Figure and table depicting luminescence resulting from the titration of various β10-like peptides (SmTrip 10 peptides) in the present of constant concentrations of LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep286 (SEQ ID NO: 37).

Figure 29A-B. Figures and tables depicting titration of β9-like peptides (A) SmTrip9 pep286 (SEQ. ID 37) and (B) SmTrip9 pep287 (SEQ ID NO: 148) in the presence of constant concentration of various β10-like peptides (SmTrip10 peptides) and LgTrip 3546 (SEQ ID NO: 51) polypeptide component.

Figure 30. Graph depicting the effect of construct orientation (β9-FKBP, FKBP-β9, β10-FKBP, FKBP-β10, β9-FRB, FRB-β9, β10-FRB, or FRB-β10) on facilitated complementation in HEK293 cells.

Figure 31. Graph depicting the effect of construct orientation (β9-FKBP, FKBP-β9, β10-FKBP, FKBP-β10, β9-FRB, FRB-β9, β10-FRB, or FRB-β10) on facilitated complementation in E. coli cells.

Figure 32. Graph depicting calculated Kd values for various β10-like peptides with LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep286 (SEQ ID NO: 37).

Figure 33. Graph and table depicting luminescence from combinations of components having varied split sites between the polypeptide component LgTrip 3546 (SEQ ID NO: 51) and the β9-like peptide.

Figure 34. Graph depicting luminescence from combinations of components with sequence gaps and/or overlaps between various LgTrip polypeptide components and SmTrip9 pep286 (SEQ ID NO: 37).

Figure 35. Graph depicting luminescence from NanoTrip component combinations with gaps and/or overlaps in sequence between the β9-like peptides (SmTrip9 peptides) and polypeptide component LgTrip 3546 (SEQ ID NO: 51) in the presence of SmTrip10 pep86 (HiBiT; SEQ ID NO: 25).

Figure 36. Table depicting a biochemical analysis of β9-like peptide (SmTrip9 peptides) length influence on β9-like peptide affinity and maximum light output with LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86.

Figure 37. Table depicting a biochemical analysis of β9-like peptide (SmTrip9 peptides) length influence on HiBiT affinity and maximum light output with LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86 (SEQ ID NO: 25).

Figure 38. Table depicting Kd and Bmax of β9-like SmTrip9 pep286 (SEQ ID NO: 37) point mutants with LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86 (SEQ ID NO: 25).

Figure 39. Table depicting the effect of various solubility tags on β9-like peptide affinity with LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep86 (SEQ ID NO: 25).

Figure 40. Table depicting the effect of various C-terminal extension sequences on β9-like or β10-like peptide affinity and maximum light output. β9-like peptide titrations (pep286 (SEQ ID NO: 37), pep292 (SEQ ID NO: 153), pep297 (SEQ ID NO: 157), pep302

(SEQ ID NO: 161)) and β10-like peptide SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) are depicted.

Figure 41. Graph depicting the effect of FRB-β10 construct linker length (15, 10, or 5 Gly/Ser residues), linker composition (with or without Ala-Ile), hexahistidine tag inclusion, and β10 composition (SmTrip10 pep86 (SEQ ID NO: 25) or SmTrip10 pep289 (SEQ ID NO: 150)) on facilitated complementation in E. coli lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 42. Graph depicting the effect of FRB-β10 construct linker length (15, 10, or 5 Gly/Ser residues), linker composition (with or without Ala-Ile), hexahistidine tag inclusion, and β10 composition SmTrip10 pep86 (SEQ ID NO: 25) or SmTrip10 pep289 (SEQ ID NO: 150) on facilitated complementation in HEK lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 43. Graph depicting the effect of β9 sequence truncations and extensions and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep86 (β10) (SEQ ID NO: 25) in E. coli lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 44. Graph depicting the effect of β9 sequence truncations and extensions and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep289 (β10) (SEQ ID NO: 150) in E. coli lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 45. Graph depicting the effect of β9 sequence truncations, extensions, and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep86 (β10) (SEQ ID NO: 25) in E. coli lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 46. Graph depicting the effect of β9 sequence truncations, extensions, and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep289 (β10) (SEQ ID NO 150) in E. coli lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 47. Graph depicting the effect of β9 sequence truncations, extensions, and construct orientation (β9-FKBP or FKBP-β9) on fold induction (facilitated complementation/spontaneous complementation) with FRB-β10 (SmTrip10 pep86 (SEQ ID NO: 25) or SmTrip10 pep289 (SEQ ID NO: 150)) in E. coli lysates (Summary of Figures 45 and 46) with LgTrip 3546 (SEQ ID NO: 51).

Figure 48. Graph depicting the effect of β9 sequence truncations and extensions and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep86 (SEQ ID NO: 25) in HEK293 lysates with LgTrip 3546 (SEQ ID NO: 51).

Figure 49. Graph depicting the effect of β9 sequence truncations and extensions and construct orientation (β9-FKBP or FKBP-β9) on facilitated complementation with FRB-SmTrip10 pep289 (SEQ ID NO: 150) in HEK293 lysates. (LgTrip 3546 (SEQ ID NO: 51).

Figure 50. Graph depicting the effect of β9 sequence truncations, extensions, and construct orientation (β9-FKBP or FKBP-β9) on fold induction (facilitated complementation/spontaneous complementation) with FRB-β10 (SmTrip10 pep86 (SEQ ID NO: 25) or SmTrip10pep289 (SEQ ID NO: 150)) in HEK293 lysates (Summary of Figures 48 and 49). (LgTrip 3546 (SEQ ID NO: 51)).

Figure 51A-D. Schematic illustrations depicting exemplary protein-protein interaction assays or analyte detection assays using binding moieties tagged with peptides.

Figure 51E-H. Schematic illustrations depicting exemplary immunoassays using components and reagents described herein: (A) direct immunoassay, (B) indirect immunoassay, (C) competition direct immunoassay, and (D) competition indirect immunoassay.

Figure 52. Schematic illustration of an exemplary multiplexed tripartite lateral flow assay. Such an assay finds use, for example, in the detection of pathogens.

Figure 53. Schematic illustration of an exemplary multiplexed tripartite lateral flow assay. Such an assay finds use, for example, in the detection of antiviral antibodies.

Figure 54. Schematic illustration of an exemplary antibody detection assay.

Figure 55. Schematic illustration of an exemplary bead-based assay.

Figure 56. Schematic illustration of an exemplary nucleic acid detection assay.

Figure 57. Graph depicting FRB-FKBP facilitated complementation in E. coli lysates with AI (Ala-Ile) dipeptide absent from linker in constructs denoted by **. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 58. Graph depicting FRB-FKBP facilitated complementation in HEK293 lysates with AI sequence dipeptide absent from linker in constructs denoted by **. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 59. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep86 and C-terminally extended FKBP-SmTrip9 peptides in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 60. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep289 and C-terminally extended FKBP-SmTrip9 peptides in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 61. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep86 and C-terminally extended FKBP-SmTrip9 peptides in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51))

Figure 62. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep86 and SmTrip9 peptide sequence trunctions and extensions in FKBP fusions in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 63. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep289 and SmTrip9 peptide sequence trunctions and extensions in FKBP fusions in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)) .

Figure 64. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep86 and SmTrip9 peptide sequence trunctions and extensions in FKBP fusions in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 65. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FRB-SmTrip10 pep86 and SmTrip9 peptide sequence trunctions and extensions in FKBP fusions in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 66. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip10 pep86 in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 67. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip10 pep289 in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 68. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip10 pep86 in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 69. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip10 pep289 in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 70. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip pep86 in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 71. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip pep289 in E. coli lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 72. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip pep86 in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 73. Graph depicting FRB-FKBP facilitation of luminescent complex formation with FKBP-SmTrip9 solubility variants and FRB-SmTrip pep289 in HEK293 lysate. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 74. Table listing affinity and Bmax of synthetic SmTrip9 solubility variants with C-terminal extensions. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 75. Table listing affinity and Bmax of synthetic SmTrip9 solubility variants with C-terminal extensions. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 76. Table listing Kd and Bmax of synthetic SmTrip9 variants with differentially blocked termini. (LgTrip 3546 (SEQ ID NO: 51)).

Figure 77. Table listing the solubility of synthetic SmTrip9 peptides.

Figure 78A-B. (A) Graph depicting the affinity of SmTrip9 pep286 (SEQ ID NO: 37) for SmTrip10 pep86 (HiBiT)/LgTrip fusions (SEQ ID NO: 210 and 212). (B) Graph depicting the affinity of SmTrip9 pep759 (SEQ ID NO: 496) for various SmTrip10 pep86 (HiBiT)/LgTrip fusions.

Figure 79. Graphs depicting bioluminescence following an 18 hour exposure to increasing detergent concentrations. NanoLuc (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), (LgTrip 3546 (SEQ ID NO: 51)).

Figure 80. Graphs depicting enzyme activity in the presence of increasing detergent concentrations. NanoLuc (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), LgTrip 3546 (SEQ ID NO: 51).

Figure 81. Graph demonstrating the reversibility of FRB-FKBP facilitated bioluminescent complex formation with LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51).

Figure 82. Table listing results of titration of various SmTrip10 peptides in the presence of constant SmTrip9 pep286 (SEQ ID NO: 37) and LgTrip 3546 (SEQ ID NO: 51) .

Figure 83. Table listing results of titration of various SmTrip10 peptides in the presence of constant SmTrip9 pep286 (SEQ ID NO: 37) and LgTrip 3546 (SEQ ID NO: 51) titration

Figure 84. Graph depicting bioluminescence from Antares-type fusions (LgTrip 3546) with SmTrip9 pep263 (SEQ ID NO: 35) and SmTrip10 pep86 (SEQ ID NO: 25) or SmTrip10 pep86 + SmTrip9 pep286 (SEQ ID NO: 37).

Figures 85A-B. Graphs depicting emission spectra from Antares-type fusions (LgTrip 3546) (SEQ ID NO: 51) with SmTrip9 pep263 (SEQ ID NO: 35) and SmTrip pep86 (HiBiT; SEQ ID NO: 25) or SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) + SmTrip9 pep286 (SEQ ID NO: 37).

Figure 86. Graphs depicting titration of LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51) with “dark” dipeptide 272 (SEQ ID NO: 146) in the presence of dipeptide pep263 (SEQ ID NO: 35).

Figure 87. Graphs comparing the inhibition of dark dipeptides 272 (SEQ ID NO: 146) and 273 (SEQ ID NO: 298) with LgTrip 3546 (SEQ ID NO: 51) and LgBiT (SEQ ID NO: 11).

Figure 88. Graph depicting inhibition of LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51) with dark BiT167 (SEQ ID NO: 300).

Figure 89. Graph depicting FRB-FKBP facilitation of luminescent complex formation in E. coli lysate with FKBP-SmTrip9 pep434 (SEQ ID NO: 230) variants’ complementation with LgTrip 3546 (SEQ ID NO: 51) and FRB-HiBiT (SEQ ID NO: 25).

Figure 90. Graph depicting FRB-FKBP facilitation of luminescent complex formation in E. coli lysate with SmTrip9 pep434 (SEQ ID NO: 230) variants’ complementation with LgTrip 3546 (SEQ ID NO: 51) and FRB-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO: 150).

Figure 91. (A) Graph depicting FRB-FKBP facilitation of luminescent complex. formation in HEK293 lysate with SmTrip9 pep435 (SEQ ID NO: 231) and SmTrip9 pep434 (SEQ ID NO: 230) variants’ complementation with LgTrip 3546 (SEQ ID NO: 51) and FRB-

SmTrip10 pep86 (HiBiT; SEQ ID NO: 25). (B) Graph depicting FRB-FKBP facilitation of luminescent complex formation in HEK293 lysate with SmTrip9 pep435 (SEQ ID NO: 231) and SmTrip9 pep434 (SEQ ID NO: 230) variants' complementation with LgTrip 3546 (SEQ ID NO: 51) and FRB-SmTrip10 pep289 (SEQ ID NO: 150).

Figure 92. Table depicting the results of a FRB-FKBP assay screen with SmTrip9s 823 and 840.

Figure 93. Table listing Kd and Bmax of synthetic SmTrip9 pep435 (SEQ ID NO: 231) and SmTrip9 pep434 (SEQ ID NO: 230) variants with LgTrip 3546 (SEQ ID NO: 51).

Figure 94. Graph demonstration of wt LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3546 (SEQ ID NO: 51) with pep263 (SEQ ID NO: 35) or pep331 (SEQ ID NO: 301) as bioluminescence reagents for detecting endogenously tagged (e.g., by CRISPR/Cas9) GAPDH.

Figure 95. Exemplary SmTrip10 chemical conjugates. (A) Example of SmTrip10 with N-terminal cysteine modification for disulfide bond formation on solvent exposed or protected cysteine targets on proteins/peptides/DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules that have been prepared with maleimide for reaction with a thiol such as cysteine or N-hydroxysuccinimide esters (NHS-ester) for reaction with an amine such as lysine. (B) Examplary SmTrip10 with N-terminal azido-lysine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with unstrained or strained alkyne targets separately installed on proteins/peptides/ DNA and RNA oligonucleotides/small molecules. (C) Examplary SmTrip10 with N-terminal N-hydroxylsuccinimide ester (NHS-ester) for general conjugation to nucleophiles (e.g., lysines, other primary amines) on proteins/peptides/ DNA and RNA oligonucleotides/small molecules. Nucleophiles can be present on unmodified proteins/oligos/small molecules or may be chemically added for the purposes of this conjugation. (D) Examplary SmTrip10 with an N-terminal propargyl glycine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with azide, diazo, or tetrazine targets separately introduced chemically or biologically on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (E) Examplary SmTrip10 with a N-terminal propargyl glycine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") and a C-terminal fluorophore (e.g., BODIPY dye).

Figure 96. Exemplary SmTrip9 pep286 chemical conjugates. (A) Example of SmTrip9-286 with C-terminal azido-lysine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with unstrained or strained alkyne targets separately introduced chemically or biologically on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (B) Example of SmTrip9 pep286 with C-terminal propargyl glycine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with azide, diazo, tetrazine targets separately introduced chemically or biologically on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (C) Example of SmTrip9 pep286 with C-terminal cysteine modification for disulfide bond formation on solvent exposed or protected cysteine targets on proteins/peptides/DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules that have been prepared with maleimide handles or N-hydroxysuccinimide esters. (D) Example of SmTrip9 pep286 with C-terminal cysteine modification and a N-terminal BODIPY dye. The dye is not limited to BODIPY and could be any fluorophore, BRET partner, or FRET dye/quencher partner. Dyes can be incorporated with any other combination of conjugation handles prepared on the C-terminus. (E) Example of SmTrip9 pep286 with C-terminal N-hydroxysuccinimide esters (NHS-ester) for general conjugation to nucleophilic targets (e.g., lysines) on proteins/peptides/DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules. (F) Example of SmTrip9 pep286 with C-terminal N-hydroxysuccinimide ester (NHS-ester) for general conjugation to nucleophilic targets (i.e. lysines) on proteins/peptides/DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules.

Figure 97. Exemplary SmTrip9 pep521 chemical conjugates. (A) Example of SmTrip9 pep521 with C-terminal azido-lysine modification and a N-terminal BODIPY dye. The dye is not limited to BODIPY and could be any fluorophore, BRET partner, or FRET dye/quencher partner. Dyes can be incorporated with any other combination of conjugation handles prepared on the C-terminus. (B) Example of SmTrip9 pep521with C-terminal azido-lysine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with unstrained or strained alkyne targets separately introducedchemically or biologically on proteins/peptides/ DNA and RNA oligonucleotides/small molecules. (C) Example of SmTrip9 pep521 with C-terminal propargyl glycine modification for copper

catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with azide, diazo, tetrazine targets separately introduced chemically or biologicallyon proteins/peptides/ DNA and RNA oligonucleotides/small molecules. (D) Example of SmTrip9 pep521 with C-terminal cysteine modification for disulfide bond formation on solvent exposed or protected cysteine targets on proteins/peptides/DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules that have been prepared with maleimide handles or an NHS-ester. (E) Example of SmTrip9 pep521 with C-terminal N-hydroxysuccinimide ester (NHS-ester) for general conjugation to nucleophilic targets (i.e. lysines) on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (F) Example of SmTrip9 pep521 with C-terminal N-hydroxysuccinimide ester (NHS-ester) for general conjugation to nucleophilic targets (i.e. lysines) on proteins/peptides/ DNA and RNA oligonucleotides/small molecules.

Figure 98. Exemplary SmTrip9 pep524 chemical conjugates. (A) Example of SmTrip9 pep524 with C-terminal azido-lysine modification and a N-terminal BODIPY dye. The dye is not limited to BODIPY and could be any fluorophore, BRET partner, or FRET dye/quencher partner. Dyes can be incorporated with any other combination of conjugation handles on the C-terminus. (B) Example of SmTrip9 pep524 with C-terminal azido-lysine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with unstrained or strained alkyne targets separately introduced chemically or biologically on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (C) Example of SmTrip9 pep524 with C-terminal propargyl glycine modification for copper catalyzed or copper free 1,3-dipolar cycloaddition reactions ("Click") with azide, diazo, tetrazine targets separately introduced chemically or biologically on proteins/peptides/DNA and RNA oligonucleotides/small molecules. (D) Example of SmTrip9 pep524 with C-terminal cysteine modification for disulfide bond formation on solvent exposed or protected cysteine targets on proteins/peptides/ DNA and RNA oligonucleotides/small molecules or proteins/peptides/DNA and RNA oligonucleotides/small molecules that have been prepared with maleimide handles or an NHS-ester. (E) Example of SmTrip9 pep524 with C-terminal N-hydroxysuccinimide ester (NHS-ester) for general conjugation to nucleophilic targets (i.e. lysines) on proteins/peptides/ DNA and RNA oligonucleotides/small molecules. (F) Example of SmTrip9 pep524 with C-terminal N-hydroxysuccinimide ester (NHS-ester) for general

conjugation to nucleophilic targets (i.e. lysines) on proteins/peptides/DNA and RNA oligonucleotides/small molecules.

Figure 99A-B. Exemplary peptide-oligomer probes. Peptides displaying reactive azido groups are conjugated to oligonucleotides displaying reactive alkyne groups to form exemplary peptide-oligomer probes.(A) Peptide oligomer conjugate of SmTrip9 pep286 (w/azido group) conjugated to a DNA oligomer containing 5'-terminal alkyne functionality via a copper "click" 1,3-cycloaddition. (B) Peptide oligomer conjugate of SmTrip10 pep86 (HiBiT) (w/azido group) conjugated to a DNA oligomer containing 3'-terminal alkyne functionality via a copper "click" 1,3-cycloaddition.

Figure 100. Graph depicting a screen of SmTrip9 G147 site-saturation variants.

Figure 101. Graph depicting a screen of SmTrip9 K148 site-saturation variants.

Figure 102. Graph depicting a screen of SmTrip9 M149 site-saturation variants.

Figure 103. Graph depicting a screen of SmTrip9 L150 site-saturation variants.

Figure 104. Graph depicting a screen of SmTrip9 F151 site-saturation variants.

Figure 105. Graph depicting a screen of SmTrip9 R152 site-saturation variants.

Figure 106. Graph depicting a screen of SmTrip9 V153 site-saturation variants.

Figure 107. Graph depicting a screen of SmTrip9 T154 site-saturation variants.

Figure 108. Graph depicting a screen of SmTrip9 I155 site-saturation variants.

Figure 109. Graph depicting a screen of SmTrip9 N156 site-saturation variants.

Figure 110. Graph depicting a screen of SmTrip9 S157 site-saturation variants.

Figure 111. Graph depicting a screen of SmTrip9 W158 site-saturation variants.

Figure 112. Graph depicting a screen of SmTrip9 K149 site-saturation variants.

Figure 113. Table of the results of FRB-FKBP facilitated complementation in E. coli lysates with SmTrip9 pep435/434.

Figure 114. Table of the results of FRB-FKBP facilitated complementation in E. coli lysates with SmTrip9 pep435/434.

Figure 115. Table of the results of FRB-FKBP facilitated complementation in E. coli lysates with SmTrip9 pep435/434.

Figure 116. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 117. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 118. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 119. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 120. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 121. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 122. Table of the results FRB-FKBP facilitated complementation assay screen with combinational SmTrip9 variants.

Figure 123. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 124. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 125. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 126. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 127. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 128. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 129. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 130. Table of Kd and Bmax of SmTrip9 synthetic peptides.

Figure 131. Table of Solubility of synthetic SmTrip9 peptides.

Figure 132. Graph of biochemical co-titration of SmTrip9 synthetic peptides and pep289.

Figure 133. Garph of biochemical co-titration of SmTrip9 synthetic peptides and pep289.

Figure 134. Graph of biochemical co-titration of SmTrip9 and SmTrip 10 synthetic peptides.

Figure 135. Graph of biochemical co-titration of pep521 and alternative SmTrip 10 synthetic peptides.

Figure 136. SDS PAGE gel of strand removal (purification) from LgTrip 3546 template.

Figure 137. Graphs of strand removal proteins with various combinations of peptides.

Figure 138. graphs of strands 6, 7, 8, 9, or 10 removal (purification) from LgTrip 3546 template.

Figure 139. Graphs of Kd and Bmax values of the dipeptide titrations.

Figure 140. Schematic depicting the approach taken to develop a solution-based homogeneous, quantitative assay for anti-TNFa biologic agents Remicade, Humira, and Enbrel using tripartite protein G and TNFa fusion proteins.

Figure 141. Graphs depicting quantitative analysis of TNFa inhibitor dose responses via facilitated complementation with SmTrip9 pep521-protein G (SEQ ID NO: 268) and TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins with purified LgTrip 3546 (SEQ ID NO: 51) in a solution-based homogeneous assay.

Figure 142. Graphs depicting quantitative analysis of 10nM infliximab via facilitated complementation with SmTrip9 pep521-protein G (SEQ ID NO: 268) and TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins with purified LgTrip 3546 (SEQ ID NO: 51) in the presence of complex sample matrices including human serum and urine using a solution-based homogenous assay.

Figure 143. Graph depicting the binding kinetics of signal generation measuring 100pM of infliximab via facilitated complementation with SmTrip9 pep521-protein G (SEQ ID NO: 268) and TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins with purified LgTrip 3546 (SEQ ID NO: 51) in a solution-based homogenous assay.

Figure 144. Graph depicting signal generation measuring 10nM of infliximab via facilitated complementation of different SmTrip9 pep(X)-protein G variants and TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins with purified LgTrip 3546 (SEQ ID NO: 51) in a solution-based homogenous assay.

Figure 145. Schematic depicting the approach taken to develop a homogenous cell-based, quantitative assay for anti-EGFR biologic agents panitumumab and cetuximab using SmTrip9-protein G fusion proteins and HEK293 cells expressing SmTrip10 pep289-EGFR (SEQ ID NO:150).

Figure 146. Graph depicting quantitation of Panitumumab via facilitated complementation with SmTrip9 pep521-protein G (SEQ ID NO: 268) fusion protein and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells with purified LgTrip 3546 (SEQ ID NO: 51) in a cell-based homogeneous assay.

Figure 147. Graph depicting the real time binding kinetics of signal generation measuring Cetuximab via facilitated complementation with SmTrip9 pep521-protein G (SEQ

ID NO: 268) fusion protein and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells with purified LgTrip 3546 (SEQ ID NO: 51) in a cell-based homogeneous assay.

Figure 148. Graph depicting signal generation measuring 1nM of panitumumab via facilitated complementation of different SmTrip9 pep(X)-protein G variants and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells paired with purified LgTrip 3546 (SEQ ID NO: 51) in a cell-based homogenous assay.

Figure 149. Graph depicting quantitation of panitumumab dose response via facilitated complementation of different SmTrip9 pep(X)-protein G variants and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells paired with purified LgTrip 3546 (SEQ ID NO: 51) in a cell-based homogenous assay.

Figure 150. Graphs depicting quantitation of human IL-1beta using Halotag-SmTrip9 pep521 (SEQ ID NO: 268) and HaloTag-SmTrip10 pep289 (SEQ ID NO: 150) chemically-labeled paired antibodies in a solution-based homogeneous assay. Real-time binding kinetics for human Troponin using NanoTrip chemically labeled paired antibodies.

Figure 151. Graphs depicting real time binding kinetics for quantitation of human Troponin using Halotag-SmTrip9 pep521 (SEQ ID NO: 268) and HaloTag-SmTrip10 pep289 (SEQ ID NO: 150) chemically labeled paired antibodies in a solution-based homogeneous assay.

Figure 152. Specialized peptides responsible to direct proteins to specific cellular compartments were fused to LgBiT-HaloTag. (A) LgBiT-membrane sensor: LgBiT is in green and nucleus is in blue. (B) LgBiT-nuclear sensor: LgBiT is in green and nucleus is in blue. (C) LgBiT-mitochondria sensor: LgBiT is in green, MitoTracker is in red, and nucleus is in blue. (D) LgBiT-ER sensor: LgBiT is in green, ER marker is in red, and nucleus is in blue.

Figure 153. Translocation assay. POI is endogenously tagged with HiBiT. Upon stimulation, the POI translocates to a different cellular compartment, for example the nucleus. A LgBiT-nuclear sensor could be used to detect this translocation event as the HiBiT meets LgBiT resulting in luminescence signal.

Figure 154. Membrane translocation assay with wild-type LgBiT sensor. PKCα-HiBiT cell line was transfected with wild-type LgBiT-membrane sensor. Due to the strong

interaction between LgBiT and HiBiT, the spontaneous complementation occurs, leading to no response to PMA stimuli.

Figure 155. Membrane translocation assay with LgBiT* sensor (SEQ ID NO: 979). PKCα-HiBiT cell line was transfected with different amount of DNA encoding LgBiT*-membrane sensor. Upon PMA treatment, PKCα-HiBiT migrates to the plasma membrane, where the LgBiT*-membrane sensor anchors. The assembly between HiBiT and LgBiT* produces luminescence signal, and the signal is proportional to the amount of PKCα-HiBiT on the membrane. The assay is sensitive and robust. Titration of PMA yielded similar EC_{50} (EC_{50} = 2.0 nM) regardless of the amount of sensor input. Fold response is varied between 12- to 19-fold depending on the amount of sensor input.

Figure 156. Nuclear translocation assay with LgBiT* sensor. p65-HiBiT cell line was transfected with DNA encoding LgBiT*-nuclear sensor. Addition of TNFα recruits p65 to the nucleus where LgBiT*-nuclear sensor localizes. Complementation occurs between HiBiT and LgBiT* to produce light. The signal intensity reflects the concentration of p65 in the nucleus. Titration of TNFα yielded EC_{50} of 0.7 ng/mL with fold-response of 4. Real-time measurement showed that it requires 30 min to reach the maximum accumulation of p65 in the nucleus upon stimulation.

Figure 157A-B. (A) Graph and (B) table depicting affinity and Bmax of LgBiT mutants with HiBiT.

Figure 158. Graph depicting affinity of LgBiT mutant lysates for HiBiT.

Figure 159. Graph and table depicting the relative bioluminescence, produced in the presence of substrate, of various combinations of truncated luciferase polypeptide and peptide components.

Figure 160. LgTrip was diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol and pep289 was added to 25 uM. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT.

Figure 161. LgTrip was diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol and pep289 was added to 25 uM. This solution was used as the diluent for 5-fold serial dilution

series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT.

Figure 162. Dipeptides were diluted to 5 uM and diluted serially 5-fold using TBS + 0.01% BSA + 0.01% Tergitol with 0.2 nM of LgTrip as the diluent. Samples were incubated 10 minutes at room temperature and added to assay plates in triplicate. One-to-one vol:vol of TBS + 0.01% BSA + 0.01% Tergitol with 20x diluted live cell substrate was added to samples, and plates were read on a GloMax® luminometer after 10 minutes.

Figure 163. LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 4 hours at 37°C with shaking. Ten microliters of each induction was diluted into 250 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5). Eighty microliters of lysates were diluted further in 2 ml lysis buffer. A 10-fold dilution series of pep788 (SEQ ID 414) was performed stating at 10 μM peptide using Nano-Glo with 50 uM furimazine as the diluent. Peptide dilutions and lysates were mixed 1:1 vol:vol, incubated 10 min at room temperature, and luminescence was read.

Figure 164. LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 4 hours at 37°C with shaking. Ten microliters of each induction was diluted into 250 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5). Eighty microliters of lysates were diluted further in 2 ml lysis buffer. A 5-fold dilution series of pep759 (SEQ ID 496) was performed stating at 50 μM peptide using Nano-Glo with 50 uM furimazine and 50 μM pep289 (SEQ ID 826) as the diluent. Peptide dilutions and lysates were mixed 1:1 vol:vol, incubated 10 min at room temperature, and luminescence was read.

Figure 165. LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at 80°C

for 1.5 hrs in a thermocycler. Controls were incubated on ice. Samples were equilibrated to room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID 414) in TBS + 0.01% BSA + 20x diluted live cell substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read.

Figure 166. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT.

Figure 167. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT.

Figure 168. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (12.5 uM) and titration of VS-HiBiT.

Figure 169. MagneHis purified LgTrip variants were diluted to 20 nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in thermal cycler. Samples were removed at various

time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 µl in 495 µl) in TBS+0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of TBS + 0.01% BSA +20x diluted live cell substrate furimazine + 400 nM pep788 (SEQ ID NO: 414). Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated by non-linear regression.

Figure 170. Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variants, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes.

Figure 171. Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes.

Figure 172. LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at 70°C for 1.5 hrs in a thermocycler. Controls were incubated on ice. Samples were equilibrated to

room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID 414) in TBS + 0.01% BSA + 20x diluted live cell furimazine substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read.

Figure 173. LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at two temperature gradients, either 75-100°C for 10 min or 50-75°C for 1.5 hr, in a Veritas thermocycler. Controls were incubated on ice. Samples were equilibrated to room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID 414) in TBS + 0.01% BSA + 20x diluted live cell furimazine substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read.

Figure 174. MagneHis purified LgTrip variants were diluted to 20 nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in a thermal cycler. Samples were removed at various time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 in 495µl) in TBS+0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of TBS + 0.01% BSA + 20x diluted live cell substrate furimazine + 400 nM pep788 (SEQ ID NO: 414). Samples were incubated for 10 minutes and then read on GMM+.

Figure 175. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (12.5 uM) and titration of VS-HiBiT.

Figure 176. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (12.5 uM) and titration of VS-HiBiT.

Fiogure 177. LgTrip variants were purified using the Promega MagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT.

Figure 178. LgTrip variants were purified according to using the Promega MagnaHisMagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT.

Figure 179. LgTrip variantss were purified according to using the Promega MagnaHisMagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-

HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT.

Figure 180. LgTrip variants were purified according to using the Promega MagnaHisMagneHis™ Protein Purification system according to the manufacturer's protocoland diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT.

Figure 181. LgTrip variant cultures were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin and induced ~20 hr at 25°C with shaking. Cells were diluted 1000x in PLB assay reagent (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 20 min. Five-fold serial dilutions of SmTrip9 pep840 were performed in Nano-Glo + 50 uM furimazine + 25 uM pep289 and mixed 1:1 vol:vol with LgTrip lysates. Samples were incubated 10 min at room temperature and read on GloMax® luminometer.

Figure 182. LgTrip variants were purified according to using the Promega MagnaHisMagneHis™ Protein Purification system according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT.

Figure 183. MagneHis purified LgTrip variants were diluted to 20 nM in TBS + 0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in a thermal cycler. Samples were removed at various time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 in 495µl) in TBS + 0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of

TBS + 0.01% BSA + 20x diluted live cell substrate furimazine + 200 nM pep900 (SEQ ID NO: 907). Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated by non-linear regression.

Figure 184. Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax after 5 minutes.

Figure 185. LgTrip variant cultures were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin and induced ~20 hr at 25°C with shaking. Cells were diluted 5000x in 0.3x PLB assay reagent and lysed for 20 min. Five-fold serial dilutions of dipeptides pep788, pep900, or SmTrip9 pep840 with saturating pep289 were performed in Nano-Glo + 50 uM furimazine and mixed 1:1 vol:vol with LgTrip variant lysates. Samples were incubated 10 min at room temperature and read on GloMax® luminometer. Bmax was calculated by non-linear regression.

Figure 186. LgTrip variants were diluted to 20 nM in 2 ml TBS+0.01% BSA, and 100ul of each sample aliquoted into duplicate rows of 96-well PCR plates (two plates). Plates were incubated for 3 hrs at high (75-100°C) or low (50-75°C) temperature gradient in a Veritas thermocycler. Samples were placed at 70°C and then aliquots moved to RT at various timepoints. Samples were equilibrated to RT. Samples at each timepoint were mixed with pipette and then diluted 1:100 into TBS + 0.01% BSA(5ul into 495ul). 25ul of each sample was aliquoted into a white assay plate, and 25ul of 200nM pep788 or pep900 in TBS + 0.01% BSA + 20x dil LCS added. The plate was incubated for 10 minutes and then luminescence read on GMM+.

Figure 187. Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C

with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, and +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes.

Figure 188. LgTrip 3546 was diluted to 1nM in OptiMEM+10% FBS. 12μM solutions of strand 9 peptides 521 and 693 were prepared in OptiMem+10% FBS. Each strand 9 dilution was used to prepare 3-fold dilution series of each strand 10 peptide starting at 20μM. (pep86=HiBiT, pep289=VS HiBiT, pep691=HiBiT RR, pep692=VSHiBiT RR). 90μl of each dilution series was transferred to a white assay plate and then 10μl of the 1μM stock of LgTrip 3546 added. The plate was placed on an orbital shaker set to 600RPM for 30 minutes. Detection reagent of OptiMEM + 10% FBS consisting of 10mM DTT and 50uM Furimazine was prepared, and 11μl added to the samples. The plate was placed on orbital shaker and mixed for 5 minutes at room temperature. The plate was read on a GloMax® Multi+ luminometer. Kd and Bmax were calculated using GraphPad Prism one site specific binding.

Figure 189. Purification of Proteins. Cultures for each sample were started from an isolated colony in LB + 30ug/ml KAN and grown for 20 hours at 37°C. Cultures were diluted 1:100 (500ul in 50ml) of LB supplemented with 30ug/ml KAN, 0.1% Rhamnose, and 0.15% glucose. Cultures were grown for 20 hours at 25°C. Cells were spun and resuspended in 9ml 100mM HEPES pH 7.5 + 1ml FastBreak Lysis Buffer + 200ul RQ DNAse I and placed on orbital mixer for 30 minutes at 4°C (An aliquot of the total lysate was saved). Each sample was spun (7000RPM 15 minutes) to clear lysate, and MagneHis™ purification system (Promega V8550) used to purify proteins. 1ml of MagneHis™ magnetic resin was added to each cleared lysate and placed on orbital mixer for 10 minutes. Each sample was washed three times with wash buffer and then 500ul of elution buffer was used 2 times to recover samples. The samples were dialyzed into 1X TBS using 10,000 MWCO dialysis units (Thermo 88404) for 2 hours.

Figure 190. Luminescnece comparison of NanoLuc (ATG-462) with monomeric LgBiT-SmBiT proteins. Purified protein was diluted to 0.2nM in TBS + 0.01% BSA. 50ul of

each sample was combined with 50ul of 50uM Fz in Nano-Glo® buffer (N113, N112), and luminescence measured on GMM+ luminometer three minutes after substrate addition. NanoLuc was ~2X brighter compared to monomeric constructs

Figure 191. Km determination. Purified protein was diluted to 0.2nM in TBS + 0.01% BSA. A 2-fold dilution series of Fz in Nano-Glo® buffer starting at 50uM (40ul in 2ml) was prepared, then 1ml to 1ml dilutions. 50ul of each sample in duplicate was combined with 50ul of the titration series, incubated for three minutes at RT, and then luminescence read on GMM+

Figure 192. Stability of monomeric LgBiT-SmBiT protein compared to NanoLuc (ATG-462). Each sample was diluted to 2nM in TBS + 0.01% BSA, aliquoted into multiple wells of a 96-well PCR plate, and placed in a thermocycler set for 30 minutes with temperature gradient. After a 30-minute incubation, 5ul of each sample was combined with 45ul of TBS + 0.01% BSA, 50ul of Fz diluted into Nano-Glo® buffer added, incubated for 3 minutes, and luminescence measured on a GMM+ luminometer . Temperature gradient A: 54°, 57°, 60°, 63°, 66°, and 70°C. Temperature gradient B: 55°, 60°, 65°, 70°, 75°, and 80°C. Temperature gradient C: 65°, 70°, 75°, 80°, 85°, and 90°C.

Figure 193. Thermostability comparison. Each sample was diluted to 2nM in TBS + 0.01% BSA, aliquoted into multiple wells of two-96-well PCR plateand placed in thermocyclers set for 30 minutes with temperature gradient. (75°, 80°, 85°, 90°, 95°, and 100°C). After a 30-minute incubation, 5ul of each sample was combined with 45ul of TBS + 0.01% BSA, 50ul of Fz diluted into Nano-Glo® buffer added, incubated for 3 minutes, and luminescence read a GMM+ luminomether. Position 159G provides enhanced stability compared to 159S.

Figure 194. Stbility comparison of LgBiT-SmBiT variants with NanoLuc. Samples were diluted to 2nM, aliquoted into wells of a 96-well PCR plate, and placed in a thermocycler set to 60°C. At various timepoints, an aliquot was removed and placed on ice. After all samples were incubated, samples were allowed to equilibrate to RT, and 5ul of each sample combined with 45ul of TBS + 0.01% BSA. 50ul of Nano-Glo® buffer + 50uM Fz was added, the plated incubated 3 minutes, and then luminescence measure on GMM+ luminometer. The 60°C stability curve is consistent with temperature gradients curves with LgBiT-SmBiT slightly more stable compared to NanoLuc, and LgBiT-HiBiT significantly more stable compared to NanoLuc.

Figure 195. Stability of monomeric LgBiT-SmBiT at elevated temperature. Samples were diluted to 200nM in TBS + 0.01% BSA and then further diluted to 0.2nM (4ul in 4ml). 50ul of ATG-462 or ATG-3564 was combined with 50ul of Nano-Glo® buffer + 50uM Fz or 50ul of TBS + 0.01%BSA + 20uM Fz in wells of a thin wall PCR tray, and the tray placed in a Veritas thermocycler set with gradient 55°, 60°, 65°, 70°, 75°, and 80°C. At various timepoints, samples were removed, and luminescence measure on GMM+ luminometer.

Figure 196. Stability of monomeric LgBiT-SmBiT at elevated temperature for 30 minutes. Samples were diluted to 200nM in TBS + 0.01% BSA and then further diluted to 0.2nM (4ul in 4ml). 50ul of ATG-462 or ATG-3564 was combined with 50ul of Nano-Glo® buffer + 50uM Fz or 50ul of TBS + 0.01%BSA + 20uM Fz into wells of a thin wall PCR tray, and the tray placed in a Veritas thermocycler set with gradient 55°, 60°, 65°, 70°, 75°, and 80°C. Luminescence was measure at 30 minutes.

Figure 197. Measurement of Km and Vmax for purified NanoLuc® mutants. Purified protein was diluted to 0.2nM in TBS+0.01% BSA. A 2-fold dilution series of Fz in Nano-Glo® buffer starting at 50uM (40ul in 2ml), then 1ml to 1ml dilutions. 50ul of each sample in duplicate was combined with 50ul of the titration series, incubated for three minutes at RT, and luminescence read on GMM+ luminometer.

Figure 198. Thermal challenge.

Figure 199. Thermal challenge with stable variants. Each sample was diluted to 2nM in TBS + 0.01% BSA, aliquoted into multiple wells of two-96-well PCR plates, and placed in a thermocyclers set for 30 minutes with temperature gradient. (70°, 75°, 80°, 85°, 90°, and 95°C). After 30-minute incubation, 5ul of each sample was combined with 45ul of TBS + 0.01% BSA, 50ul of Fz diluted into Nano-Glo® buffer added, incubated for 3 minutes, and luminescence measured on a GMM+ luminometer.

Figure 200. Comparison of pep521 and pep 40 with detector proteins. Each protein was diluted to 20nM in TBS + 0.01% BSA. A 3X dilution series of pep521 and pep840 was prepared in TBS + 0.01% BSA starting at 20uM. 50ul of each enzyme dilution in duplicate was combined with 50ul of each peptide titration and incubated for 10 minutes on shaker for pre-equilibration. An assay buffer was prepared by diluting Nano-Glo Live Cell Substrate (LCS; Promega N205) 30-fold into TBS + 0.01% BSA, 100ul added to each well, incubated 5 minutes, and luminescece measured on GMM+ luminometer. The background reading was obtained from samples that contained no peptide.

Figure 201. Comparison of strand 9 detector proteins with pep840. Each protein was diluted to 200nM in TBS + 0.01% BSA. A 3X dilution series of pep840 was prepared in TBS + 0.01% BSA starting at 0.5uM. 50ul of each enzyme dilution in quadruplicate was combined with 50ul of each peptide titration and incubated for 10 minutes on shaker for pre-equilibration. An assay buffer was prepared by diluting Nano-Glo LCS (Promega N205) 30-fold into TBS + 0.01% BSA, 100ul added to each well, incubated 5 minutes, and luminescence measured on GMM+ luminometer. The background reading was obtained from samples that contained no peptide.

Figure 202. Test linker series. Overnight cultures of each sample were prepared in LB + 100ug/ml amp. The following day, cultures were diluted 1:20 (150ul to 3ml) in LB + 0.1% Rhamnose + 100ug/ml amp, grown for 4 hours at 37°C, and then lysed with PLB lysis buffer (0.3X Passive Lysis Buffere (Promega) + 25mM HEPES pH 7.5) (500ul of lysate to 4.5ml of PLB lysis buffer). To assay, lysates were diluted 1:100 into TBS + 0.01% BSA. A 3X dilution series of strand 9 (pep840) starting at 10nM was prepared,50ul combined with 50ul of the diluted lysate for each sample, and incubated for 20 minutes at RT on orbital shaker set to 600rpm. 100ul of NanoGlo® buffer +50uM Furimazine (N113) was added, and luminescence measured on GMM+ luminometer. Each clone, with the exception of ATG-5485, 5AA linker,produced similar RLU values to ATG-4992.

Figure 203. Linker comparison: 8GS (ATG-4992) vs. 11GS (ATG-5490). ATG-4992 and ATG-5490 were purified using the MagneHis™ purification system and diluted to 100nM in CO_2 independent media + 10% FBS. A 3-fold serial dilution of pept840 was prepared starting at 10nM in Nano-Glo® buffer + 50uM furimazine. 50ul of each enzyme dilution was combined in quadruplicate with 50ul the peptide titration. Luminescence was measured over time in a GMM+ luminometer. The data plotted is from the 50-minute kinetic read. ATG-5490 had a ~2-fold higher signal compared to ATG-4992

Figure 204. Kd of mutants of ATG-4992 and ATG-5490. Overnight cultures of each sample in LB+100ug/ml amp were prepared. The following day, cultures were diluted 1:20 (150ul to 3ml) in LB + 0.1% Rhamnose + 100ug/ml amp, grown for 4 hours at 37°C, and lysed with PLB lysis buffer (500ul of lysate to 4.5ml of PLB lysis buffer). To assay, lysates were diluted 1:100 into TBS + 0.01% BSA. A 3X dilution series of strand 9 (pep840) was prepared starting at 40uM, 50ul combined with 50ul of the diluted lysate for each sample, and

incubated for 20 minutes at RT on orbital shaker set to 600rpm. 100ul of Nano-Glo® buffer + 50uM Furimazine (N113), and luminescence measured on GMM+ luminometer.

Figure 205. Comparison of mutants of ATG-4992 and ATG-5490. Proteins were purified (MagneHis™ Purification System, Promega) and diluted to 100nM in CO_2 independent media + 10% FBS. A 3-fold dilution series was prepared with pep840 starting at 2nM in Nano-Glo® buffer + 50uM furimazine. 50ul of each enzyme dilution was combined in triplicate with 50ul of each peptide titration in the Furimazine/NanoGlo solution. Luminescence was measured every 5 minutes. Data is plotted from the 60-minute timepoint. Clones ATG-5515 and ATG-5517 had lower calculated Kd values and showed higher RLU values when paired with pep840 and slightly higher signal to background

Figure 206. Biochemical analysis. Samples were purified using MagneHis™ Protein Purification System (Promega V8500) and diluted to 0.2nM in TBS + 0.01% BSA. A 3X dilution series of VS-HiBiT peptide (pep289) was prepared in TBS + 0.01% Tergitol. One series was prepared starting at 400nM and the other at 20uM. 50ul of each diluted enzyme (0.2nM) was combined with 50ul of each peptide titration and incubated on shaker for 10 minutes. 100ul of Fz, diluted 250- fold in TBS + 0.01% BSA was added, the plate placed on shaker for 5 minutes, and luminescence measured on a GMM+ luminometer. Kd and Bmax was calculated using GraphPad prism one site specific binding regression.

Figure 207. Activity at various pH. Each sample of purified enzyme was diluted to 0.2nM in TBS + 0.01% BSA, 20uM VS HiBiT (pep289) was added to each solution, and incubated at RT for 20 minutes. 990ul of each pH buffer was aliquoted into wells of a deep well plate, 10ul of furimazine (Promega, N113) added, and 50ul of each enzyme/peptide dilution with 50ul of the pH buffer/furimazine solution added. Samples were incubated for 12 minutes at RT, and luminescence measured on GMM+ luminometer. Data was normalized to the pH 8.49 sample.

Figure 208. Biochemical comparison of ATG-5823, ATG-5824, ATG-5825, and ATG-5146 (pep 263). Proteins (ATG-5823, ATG-5824, and ATG-5825) were purified using MageHis™ purification system (Promega). ATG-5146 was purified using an AKTA with Nickel Sepharose column. Purified proteins were diluted first to 200nM in TBS + 0.01% BSA and then further diluted to 0.2nM in TBS + 0.01% BSA. A three-fold dilution series of pep263 was prepared starting at 100nM in TBS + 0.01% BSA + 0.02% Tergitol. 50ul of each enzyme was combined with 50ul of the peptide dilution series. Samples were incubated

on orbital shaker (600RPM) for 10 minutes. After incubation, 100ul of LCS (N205 Promega) was diluted 1:30 into TBS + 0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence was measured luminescence was measured on a GloMaxMulti+.

Figure 209. Biochemical comparison of ATG-5826 and ATG-5827 with pep86. Proteins were purified using MagneHis™ purification system (Promega V8500). Purified proteins were diluted first to 200nM in TBS + 0.01% BSA and then further diluted to 0.2nM in TBS + 0.01% BSA. Two, 2-fold titration series of pep86 were prepared starting at 1uM and 100nM in TBS + 0.01% BSA + 0.02% Tergitol. 50ul of ATG-5826 and ATG-5827 were combined with 50ul of the peptide dilution series that started at 1uM. 50ul of LgBiT protein (Promega N401C) was combined with the peptide titration series that started at 100nM. Samples were incubated on orbital shaker (600RPM) for 10 minutes. After incubation, 100ul of LCS (N205 Promega) was diluted 1:30 into TBS + 0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence was measured luminescence was measured on a GloMaxMulti+.

Figure 210. Biochemical comparison of ATG-5826 and ATG-5827 with pep114. Proteins were purified using MagneHis™ purification system (Promega V8500). Purified proteins were diluted first to 200nM in TBS + 0.01% BSA and then further diluted to 0.2nM in TBS + 0.01% BSA. Two, 2-fold titration series of pep114 was prepared starting at 1mM in TBS + 0.01% BSA + 0.02% Tergitol. 50ul of ATG-5826, ATG-5827, and LgBiT protein (Promega N401C) were combined with the peptide titration series. Samples were incubated on orbital shaker (600RPM) for 30 minutes. After incubation, 100ul of LCS (N205 Promega) was diluted 1:30 into TBS + 0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence was measured luminescence was measured on a GloMaxMulti+.

Figure 211. SDS PAGE analysis. Each protein was diluted to 0.1ug/ml in TBS+1X SDS loading dye. Samples were heated to 70°C for 5 minutes and then 3ul was loaded to an SDS PAGE gel.

Figure 212. Exemplary assay formats for the detection of SARS-COV-2.

Figure 213. Exemplary reagents used to screen reactive peptides. SulfoSE reacts with lysines on target proteins, PEG6 linker and pyridinium sulfonic acid (PSA) provide solubility, and PSA has UV signature that can be used to measure labeling density.

Figure 214. Spike-in antibody titration of alternative labeling strategies for detection of SARS-COV-2.

Figure 215. Purified SARS-CoV-2 Nucleocapsid antigen detection.

Figure 216. Samples tested with the lyophilized antigen assay in handheld assay. Patient nasal swab was placed into reagent tube breaking the foil seal at the bottom of the tube. Buffer containing the reagent cap was placed onto the reagent tube containing sample, which locks. The buffer capsule was cracked to release and then shook. The reagent tube was inserted into a handheld luminometer, and the sample analyzed.

Figure 217. Monomeric NanoBiT with Fluoro-Fz. Each enzyme to be tested was diluted into TBS + 0.01% BSA. A titration series with either FZ (N205) or JRW-1677 was prepared in either TBS (starting at 20uM) or NanoGlo (starting at 25uM). Each substrate was serially diluted with either TBS + 0.01% BSA or Nano-Glo® buffer (Promega N112). 50ul of each enzyme dilution was combined with 50ul of each substrate titration, incubated for 3 minutes, and then measured luminescence on GMM+ luminometer. Kinetic parameters for Fz and JRW-1667 were calculated. NanoLuc (ATG-462) produced higher RLU values with both Fz and JRW-1667 (~10-20 fold) compared to the Monomeric NanoBiT constructs when TBS was used to dilute substrates. NanoLuc and the monomeric constructs showed similar RLU values for Fz/NanoGlo buffer, but only NanoLuc showed improved luminescence with JRW-1667. Although the monomeric NanoBiT constructs showed lower RLU values with both Fz and JRW-1667, RLU values were similar for the two buffers and two substrates. Km values are lower in TBS + 0.01% BSA compared to NanoGlo, but generally the Km values were similar for each condition except ATG-3562 (Monomeric LgBiT-SmBiT), which showed a lower calculated Km for all conditions tested.

Figure 218. HaloTag-LgBiT* - mammalian cell expression and fluorescence imaging. Immunofluoresence images of HaloTag-LgBiT* in HeLa cells showed its ubiquitous distribution in the cells. Left: red channel only, Middle: blue channel only; Right: overlay.

Figure 219. Nuclear sensor - mammalian cell expression and fluorescence imaging. Immunofluoresence images of nuclear sensor in HeLa cells showed its nuclear localization. Left: red channel only, Middle: blue channel only; Right: overlay.

Figure 220. Mitochondrial sensor - mammalian cell expression and fluorescence imaging. Immunofluoresence images of mitochondria sensor in HeLa cells showed its

localization in mitochondria matrix. DNA stain in blue. Left: red channel only, Middle: green channel only; Right: overlay.

Figure 221. ER sensor - mammalian cell expression and fluorescence imaging. Immunofluorescence images of endoplasmic reticulumn sensor (ER) in Hela cells showed its ER localization. DNA stain in blue. Left: green channdel only; Middle: red channel only; Right: overlay.

Figure 222. Golgi sensor - mammalian cell expression and fluorescence imaging. Immunofluorescence images of Golgi sensor in Hela cells showed its Golgi localization. Golgi-GFP marker only stained for cis-Golgi. A portion of our Golgi sensor did not co-localize with Golgi-GFP marker, suggesting our Golgi sensor might reside in both cis- and trans-Golgi. DNA stain in blue. Left: red channdel only; Middle: green channel only; Right: overlay.

Figure 223. Lysosome sensor - mammalian cell expression and fluorescence imaging. Immunofluoresence images of lysosome sensor in HeLa cells showed its localization to the lysosome. DNA stain in blue. Left: red channel only, Middle: green channel only; Right: overlay.

Figure 224. Membrane sensor - mammalian cell expression and fluorescence imaging. Immunofluoresence images of membrane sensor in HeLa cells showed its localization to the plasma membrane. DNA stain in blue. Left: red channel only, Middle: green channel only; Right: overlay.

Figure 225. Membrane sensor - mammalian cell expression and luinescence assays. Translocation of PKCα under PMA stimulation. PKCα-HiBiT clone (HeLa) was transiently transfected with membrane sensors. Transfected cells were treated with phorbol-12-myristate-13 acetate (PMA) and measured kinetically. All 3 membrane sensors resulted in similar EC_{50}, suggesting the affinity of LgBiT variants to HiBiT do not effect translocation events of PKCα.

Figure 226. Membrane/ HaloTag-LgBiT sensor - mammalian cell expression and luinescence assays. Reversibility of HiBiT-LgBiT* complex. Increased PMA concentration recruits more PKCα to the plasma membrane, resulting in higher luminescence signal, as more PKCα-HiBiT complementing with LgBiT* membrane sensor. Conversely, less PKCα accumulates in the cytosol; thus, decreasing luminescence signal as pairing with HaloTag-LgBiT (cytosolic sensor).

Figure 227. Translocation event of PKCα. Kinetic measurement of PKCα translocation event. Accumulation of PKCα to the plasma membrane can be measured kinetically, when paired with membrane sensor. Under PMA stimulation, PKCα reaches its maximum accumulation to the plasma membrane at t =16 min.

DEFINITIONS

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments described herein, some preferred methods, compositions, devices, and materials are described herein. However, before the present materials and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols herein described, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the embodiments described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. However, in case of conflict, the present specification, including definitions, will control. Accordingly, in the context of the embodiments described herein, the following definitions apply.

As used herein and in the appended claims, the singular forms “a”, “an” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a peptide” is a reference to one or more peptides and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term “and/or” includes any and all combinations of listed items, including any of the listed items individually. For example, “A, B, and/or C” encompasses A, B, C, AB, AC, BC, and ABC, each of which is to be considered separately described by the statement “A, B, and/or C.”

As used herein, the term “comprise” and linguistic variations thereof denote the presence of recited feature(s), element(s), method step(s), etc. without the exclusion of the presence of additional feature(s), element(s), method step(s), etc. Conversely, the term “consisting of” and linguistic variations thereof, denotes the presence of recited feature(s), element(s), method step(s), etc. and excludes any unrecited feature(s), element(s), method

step(s), etc., except for ordinarily-associated impurities. The phrase "consisting essentially of" denotes the recited feature(s), element(s), method step(s), etc. and any additional feature(s), element(s), method step(s), etc. that do not materially affect the basic nature of the composition, system, or method. Many embodiments herein are described using open "comprising" language. Such embodiments encompass multiple closed "consisting of" and/or "consisting essentially of" embodiments, which may alternatively be claimed or described using such language.

As used herein, the term "substantially" means that the recited characteristic, parameter, and/or value need not be achieved exactly, but that deviations or variations, including for example, tolerances, measurement error, measurement accuracy limitations and other factors known to skill in the art, may occur in amounts that do not preclude the effect the characteristic was intended to provide. A characteristic or feature that is substantially absent (e.g., substantially non-luminescent) may be one that is within the noise, beneath background, below the detection capabilities of the assay being used, or a small fraction (e.g., <1%, <0.1%, <0.01%, <0.001%, <0.00001%, <0.000001%, <0.0000001%) of the significant characteristic (e.g., luminescent intensity of a bioluminescent protein or bioluminescent complex).

As used herein, the term "bioluminescence" refers to production and emission of light by a chemical reaction catalyzed by, or enabled by, an enzyme, protein, protein complex, or other biomolecule (e.g., bioluminescent complex). In typical embodiments, a substrate for a bioluminescent entity (e.g., bioluminescent protein or bioluminescent complex) is converted into an unstable form by the bioluminescent entity; the substrate subsequently emits light.

As used herein the term "complementary" refers to the characteristic of two or more structural elements (e.g., peptide, polypeptide, nucleic acid, small molecule, etc.) of being able to hybridize, dimerize, or otherwise form a complex with each other. For example, a "complementary peptide and polypeptide" are capable of coming together to form a complex. Complementary elements may require assistance (facilitation) to form a complex (e.g., from interaction elements), for example, to place the elements in the proper conformation for complementarity, to place the elements in the proper proximity for complementarity, to co-localize complementary elements, to lower interaction energy for complementary, to overcome insufficient affinity for one another, etc.

As used herein, the term "complex" refers to an assemblage or aggregate of molecules (e.g., peptides, polypeptides, etc.) in direct and/or indirect contact with one another. In one aspect, "contact," or more particularly, "direct contact" means two or more molecules are close enough so that attractive noncovalent interactions, such as Van der Waal forces, hydrogen bonding, ionic and hydrophobic interactions, and the like, dominate the interaction of the molecules. In such an aspect, a complex of molecules (e.g., peptides and polypeptide) is formed under assay conditions such that the complex is thermodynamically favored (e.g., compared to a non-aggregated, or non-complexed, state of its component molecules). As used herein the term "complex," unless described as otherwise, refers to the assemblage of two or more molecules (e.g., peptides, polypeptides or a combination thereof).

As used herein, the term "non-luminescent" refers to an entity (e.g., peptide, polypeptide, complex, protein, etc.) that exhibits the characteristic of not emitting a detectable amount of light in the visible spectrum (e.g., in the presence of a substrate). For example, an entity may be referred to as non-luminescent if it does not exhibit detectable luminescence in a given assay. As used herein, the term "non-luminescent" is synonymous with the term "substantially non-luminescent. In some embodiments, an entity is considered "non-luminescent" if any light emission is sufficiently minimal so as not to create interfering background for a particular assay.

As used herein, the terms "non-luminescent peptide" and "non-luminescent polypeptide" refer to peptides and polypeptides that exhibit substantially no luminescence (e.g., in the presence of a substrate), or an amount that is beneath the noise (e.g., 100-fold, 200-fold, 500-fold, $1x10^3$-fold, $1x10^4$-fold, $1x10^5$-fold, $1x10^6$-fold, $1x10^7$-fold, etc.) when compared to a significant signal (e.g., a bioluminescent complex) under standard conditions (e.g., physiological conditions, assay conditions, etc.) and with typical instrumentation (e.g., luminometer, etc.). In some embodiments, such non-luminescent peptides and polypeptides assemble, according to the criteria described herein, to form a bioluminescent complex.

As used herein, the term "interaction element" refers to a moiety that assists or facilitates the bringing together of non-luminescent elements to form a bioluminescent complex. In some embodiments, a pair of interaction elements (a.k.a. "interaction pair") is attached to a pair of non-luminescent elements (e.g., non-luminescent peptides), and the attractive interaction between the two interaction elements facilitates formation of the bioluminescent complex; although the present invention is not limited to such a mechanism,

and an understanding of the mechanism is not required to practice the invention. Interaction elements may facilitate formation of the bioluminescent complex by any suitable mechanism (e.g., bringing non-luminescent elements into close proximity, placing a non-luminescent element in proper conformation for stable interaction, reducing activation energy for complex formation, combinations thereof, etc.). An interaction element may be a protein, polypeptide, peptide, small molecule, cofactor, nucleic acid, lipid, carbohydrate, antibody, etc. An interaction pair may be made of two of the same interaction elements (i.e., homopair) or two different interaction elements (i.e., heteropair). In the case of a heteropair, the interaction elements may be the same type of moiety (e.g., polypeptides) or may be two different types of moieties (e.g., polypeptide and small molecule). In some embodiments, in which complex formation by the interaction pair is studied, an interaction pair may be referred to as a "target pair" or a "pair of interest," and the individual interaction elements are referred to as "target elements" (e.g., "target peptide," "target polypeptide," etc.) or "elements of interest" (e.g., "peptide of interest," "polypeptide or interest," etc.).

As used herein, the term "low affinity" describes an intermolecular interaction between two or more (e.g., three) entities that is too weak to result in significant complex formation between the entities, except at concentrations substantially higher (e.g., 2-fold, 5-fold, 10-fold, 100-fold, 1000-fold, or more) than physiologic or assay conditions, or with facilitation from the formation of a second complex of attached elements (e.g., interaction elements).

As used herein, the term "high affinity" describes an intermolecular interaction between two or more (e.g., three) entities that is of sufficient strength to produce detectable complex formation under physiologic or assay conditions, without facilitation from the formation of a second complex of attached elements (e.g., interaction elements).

As used herein, the term "co-localization element" refers to a moiety that facilitates co-localization of non-luminescent elements. In some embodiments, a set of non-luminescent elements has sufficient affinity to form a complex when the non-luminescent elements are co-localized at sufficient concentration. In such embodiments, a set (e.g., pair) of co-localization elements (a.k.a. "co-localization pair") is attached to a pair of non-luminescent elements (e.g., non-luminescent peptides), and the co-localization (e.g., within a cellular compartment, within a tissue, within a solution, on a solid matrix support, etc.) of the two co-localization elements facilitates co-localization of the non-luminescent elements,

thereby facilitating formation of the bioluminescent complex; although the present invention is not limited to such a mechanism, and an understanding of the mechanism is not required to practice the invention. In some embodiments, due to the capacity of the non-luminescent elements to self-assemble into a luminescent complex, the co-localization elements need not directly interact to facilitate complex formation. A co-localization element may be a protein, polypeptide, peptide, small molecule, cofactor, nucleic acid, lipid, carbohydrate, antibody, etc. A co-localization pair may be made of two of the same co-localization elements (i.e., homopair) or two different co-localization elements (i.e., heteropair). In the case of a heteropair, the co-localization elements may be the same type of moiety (e.g., polypeptides) or may be two different types of moieties (e.g., polypeptide and small molecule). In some embodiments, in which the localization of the co-localization pair is studied, a co-localization pair may be referred to as a "target pair" or a "pair of interest," and the individual co-localization elements are referred to as "target elements" (e.g., "target peptide," "target polypeptide," etc.) or "elements of interest" (e.g., "peptide of interest," "polypeptide or interest," etc.).

As used herein, the term "coelenterazine" refers to naturally-occurring ("native") coelenterazine. As used herein, the term "coelenterazine analog" or "coelenterazine derivative" refers to synthetic (e.g., derivative or variant) and natural analogs thereof, including furimazine, coelenterazine-n, coelenterazine-f, coelenterazine-h, coelenterazine-hcp, coelenterazine-cp, coelenterazine-c, coelenterazine-e, coelenterazine-fcp, bis-deoxycoelenterazine ("coelenterazine-hh"), coelenterazine-i, coelenterazine-icp, coelenterazine-v, and 2-methyl coelenterazine, in addition to those disclosed in WO 2003/040100; U.S. Application Ser. No. 12/056,073 (paragraph [0086]); U.S. Pat. No. 8,669,103; WO 2012/061529, U.S. Pat. Pub. 2017/0233789 and U.S. Pat. Pub. 2018/0030059; the disclosures of which are incorporated by reference herein in their entireties. In some embodiments, coelenterazine analogs include pro-substrates such as, for example, those described in U.S. Application Ser. No. 12/056,073; U.S. Pub. No. 2012/0707849; U.S. Pub. No. 2014/0099654; herein incorporated by reference in their entireties.

As used herein, the term "preexisting protein" refers to an amino acid sequence that was in physical existence prior to a certain event or date. A "peptide that is not a fragment of a preexisting protein" is a short amino acid chain that is not a fragment or sub-sequence of a

protein (e.g., synthetic or naturally-occurring) that was in physical existence prior to the design and/or synthesis of the peptide.

As used herein, the term "fragment" refers to a peptide or polypeptide that results from dissection or "fragmentation" of a larger whole entity (e.g., protein, polypeptide, enzyme, etc.), or a peptide or polypeptide prepared to have the same sequence as such. Therefore, a fragment is a subsequence of the whole entity (e.g., protein, polypeptide, enzyme, etc.) from which it is made and/or designed. A peptide or polypeptide that is not a subsequence of a preexisting whole protein is not a fragment (e.g., not a fragment of a preexisting protein). A peptide or polypeptide that is "not a fragment of a preexisting bioluminescent protein" is an amino acid chain that is not a subsequence of a protein (e.g., natural or synthetic) that: (1) was in physical existence prior to design and/or synthesis of the peptide or polypeptide, and (2) exhibits substantial bioluminescent activity.

As used herein, the term "subsequence" refers to peptide or polypeptide that has 100% sequence identify with a portion of another, larger peptide or polypeptide. The subsequence is a perfect sequence match for a portion of the larger amino acid chain.

The term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomers, unless otherwise indicated, if their structures allow such stereoisomeric forms.

Natural amino acids include alanine (Ala or A), arginine (Arg or R), asparagine (Asn or N), aspartic acid (Asp or D), cysteine (Cys or C), glutamine (Gln or Q), glutamic acid (Glu or E), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), leucine (Leu or L), Lysine (Lys or K), methionine (Met or M), phenylalanine (Phe or F), proline (Pro or P), serine (Ser or S), threonine (Thr or T), tryptophan (Trp or W), tyrosine (Tyr or Y) and valine (Val or V).

Unnatural amino acids include, but are not limited to, pentafluorophenylalanine ("Z"), azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, naphthylalanine ("naph"), aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine ("tBuG"), 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline ("hPro" or "homoP"), hydroxylysine, allo-hydroxylysine, 3-hydroxyproline ("3Hyp"), 4-hydroxyproline ("4Hyp"), isodesmosine, allo-isoleucine, N-methylalanine ("MeAla" or "Nime"), N-alkylglycine ("NAG") including N-methylglycine, N-

methylisoleucine, N-alkylpentylglycine (“NAPG”) including N-methylpentylglycine. N-methylvaline, naphthylalanine, norvaline (“Norval”), norleucine (“Norleu”), octylglycine (“OctG”), ornithine (“Orn”), pentylglycine (“pG” or “PGly”), pipecolic acid, thioproline (“ThioP” or “tPro”), homoLysine (“hLys”), and homoArginine (“hArg”). Unnatural reactive amino acids are described in, for example, Boutureira, O. and G. J. Bernardes (2015) "Advances in chemical protein modification." Chem Rev 115(5): 2174-2195; herein incorporated by reference in its entirety.

The term “amino acid analog” refers to a natural or unnatural amino acid where one or more of the C-terminal carboxy group, the N-terminal amino group and side-chain bioactive group has been chemically blocked, reversibly or irreversibly, or otherwise modified to another bioactive group. For example, aspartic acid-(beta-methyl ester) is an amino acid analog of aspartic acid; N-ethylglycine is an amino acid analog of glycine; or alanine carboxamide is an amino acid analog of alanine. Other amino acid analogs include methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone. Amino acid analogs may comprise amino acids with various protecting groups (Isidro-Llobet, A., et al. (2009). "Amino Acid-Protecting Groups." Chemical Reviews 109(6): 2455-2504; herein incorporated by reference in its entirety).

As used herein, unless otherwise specified, the terms “peptide” and “polypeptide” refer to polymer compounds of two or more amino acids joined through the main chain by peptide amide bonds (--C(O)NH--). The term “peptide” typically refers to short amino acid polymers (e.g., chains having fewer than 30 amino acids), whereas the term “polypeptide” typically refers to longer amino acid polymers (e.g., chains having more than 30 amino acids).

As used herein, unless otherwise specified, the term “dipeptide” refers to a peptide or small polypeptide (e.g., <70 amino acids, <60 amino acids, <50 amino acids, etc.) comprising two peptide segments (e.g., corresponding to two beta strands of a luciferase (e.g., a “β9/β10 dipeptide,” corresponding to the β9 and β10 strands of an OgLuc luciferase polypeptide), fused/attached diretly or indirectly (e.g., via a linker (e.g., peptide linker (e.g., 1-10 amino acids (e.g., a single glycine)))).

As used herein, unless otherwise specified, the term “tripeptide” refers to a peptide or small polypeptide (e.g., <100 amino acids, <90 amino acids, <80 amino acids, etc.)

comprising three peptide segments (e.g., corresponding to three beta strands of a luciferase (e.g., a "β8-10 tripeptide," corresponding to the β8-10 strands of an OgLuc luciferase polypeptide), fused/attached diretly or indirectly (e.g., via a linker (e.g., peptide linker (e.g., 1-10 amino acids (e.g., a single glycine)))).

As used herein, terms "peptidomimetic" and "peptide mimetic" refer to peptide-like or polypeptide-like molecules that emulate a sequence derived from a protein or peptide. A peptidomimetic may contain amino acids analogs, peptoid amino acids, and/or non-amino acid components either exclusively or in combination with amino acids (e.g., natural or non-natural amino acids). Examples of peptidomimitecs include chemically modified peptides/polypeptides, peptoids (side chains are appended to the nitrogen atom of the peptide backbone rather than to the α-carbons), β-peptides (amino group bonded to the β carbon rather than the α carbon), etc.

As used herein, the term "peptoid" refers to a class of peptidomimetics where the side chains are functionalized on the nitrogen atom of the peptide backbone rather than to the α-carbon.

As used herein, the term "artificial" refers to compositions and systems that are designed or prepared by man and are not naturally occurring. For example, an artificial peptide, peptoid, or nucleic acid is one comprising a non-natural sequence (e.g., a peptide without 100% identity with a naturally-occurring protein or a fragment thereof).

As used herein, a "conservative" amino acid substitution refers to the substitution of an amino acid in a peptide or polypeptide with another amino acid having similar chemical properties such as size or charge. For purposes of the present disclosure, each of the following eight groups contains amino acids that are conservative substitutions for one another:

1) Alanine (A) and Glycine (G);
2) Aspartic acid (D) and Glutamic acid (E);
3) Asparagine (N) and Glutamine (Q);
4) Arginine (R) and Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), and Valine (V);
6) Phenylalanine (F), Tyrosine (Y), and Tryptophan (W);
7) Serine (S) and Threonine (T); and
8) Cysteine (C) and Methionine (M).

Naturally occurring residues may be divided into classes based on common side chain properties, for example: polar positive (or basic) (histidine (H), lysine (K), and arginine (R)); polar negative (or acidic) (aspartic acid (D), glutamic acid (E)); polar neutral (serine (S), threonine (T), asparagine (N), glutamine (Q)); non-polar aliphatic (alanine (A), valine (V), leucine (L), isoleucine (I), methionine (M)); non-polar aromatic (phenylalanine (F), tyrosine (Y), tryptophan (W)); proline and glycine; and cysteine. As used herein, a "semi-conservative" amino acid substitution refers to the substitution of an amino acid in a peptide or polypeptide with another amino acid within the same class.

In some embodiments, unless otherwise specified, a conservative or semi-conservative amino acid substitution may also encompass non-naturally occurring amino acid residues that have similar chemical properties to the natural residue. These non-natural residues are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include, but are not limited to, peptidomimetics and other reversed or inverted forms of amino acid moieties. Embodiments herein may, in some embodiments, be limited to natural amino acids, non-natural amino acids, and/or amino acid analogs.

Non-conservative substitutions may involve the exchange of a member of one class for a member from another class.

As used herein, the term "sequence identity" refers to the degree two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have the same sequential composition of monomer subunits. The term "sequence similarity" refers to the degree with which two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have similar polymer sequences. For example, similar amino acids are those that share the same biophysical characteristics and can be grouped into the families, e.g., acidic (e.g., aspartate, glutamate), basic (e.g., lysine, arginine, histidine), non-polar (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan) and uncharged polar (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). The "percent sequence identity" (or "percent sequence similarity") is calculated by: (1) comparing two optimally aligned sequences over a window of comparison (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), (2) determining the number of positions containing identical (or similar) monomers (e.g., same amino acids occurs in both sequences, similar amino acid occurs in both sequences) to yield the number of matched positions, (3) dividing the number of matched positions by the total number of positions in the comparison

window (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), and (4) multiplying the result by 100 to yield the percent sequence identity or percent sequence similarity. For example, if peptides A and B are both 20 amino acids in length and have identical amino acids at all but 1 position, then peptide A and peptide B have 95% sequence identity. If the amino acids at the non-identical position shared the same biophysical characteristics (e.g., both were acidic), then peptide A and peptide B would have 100% sequence similarity. As another example, if peptide C is 20 amino acids in length and peptide D is 15 amino acids in length, and 14 out of 15 amino acids in peptide D are identical to those of a portion of peptide C, then peptides C and D have 70% sequence identity, but peptide D has 93.3% sequence identity to an optimal comparison window of peptide C. For the purpose of calculating "percent sequence identity" (or "percent sequence similarity") herein, any gaps in aligned sequences are treated as mismatches at that position.

Any peptide/polypeptides described herein as having a particular percent sequence identity or similarity (e.g., at least 70%) with a reference sequence ID number, may also be expressed as having a maximum number of substitutions (or terminal deletions) with respect to that reference sequence. For example, a sequence having at least Y% sequence identity (e.g., 90%) with SEQ ID NO:Z (e.g., 100 amino acids) may have up to X substitutions (e.g., 10) relative to SEQ ID NO:Z, and may therefore also be expressed as "having X (e.g., 10) or fewer substitutions relative to SEQ ID NO:Z."

As used herein, the term "wild-type," refers to a gene or gene product (e.g., protein, polypeptide, peptide, etc.) that has the characteristics (e.g., sequence) of that gene or gene product isolated from a naturally occurring source, and is most frequently observed in a population. In contrast, the term "mutant" or "variant" refers to a gene or gene product that displays modifications in sequence when compared to the wild-type gene or gene product. It is noted that "naturally-occurring variants" are genes or gene products that occur in nature, but have altered sequences when compared to the wild-type gene or gene product; they are not the most commonly occurring sequence. "Artificial variants" are genes or gene products that have altered sequences when compared to the wild-type gene or gene product and do not occur in nature. Variant genes or gene products may be naturally occurring sequences that are present in nature, but not the most common variant of the gene or gene product, or "synthetic," produced by human or experimental intervention.

As used herein, the term “physiological conditions” encompasses any conditions compatible with living cells, e.g., predominantly aqueous conditions of a temperature, pH, salinity, chemical makeup, etc. that are compatible with living cells.

As used herein, the term “sample” is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum, and the like. Sample may also refer to cell lysates or purified forms of the enzymes, peptides, and/or polypeptides described herein. Cell lysates may include cells that have been lysed with a lysing agent or lysates such as rabbit reticulocyte or wheat germ lysates. Sample may also include cell-free expression systems. Environmental samples include environmental material such as surface matter, soil, water, crystals, and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

As used herein, the terms “fusion,” “fusion polypeptide,” and “fusion protein” refer to a chimeric protein containing a first protein or polypeptide of interest (e.g., substantially non-luminescent peptide) joined to a second different peptide, polypeptide, or protein (e.g., interaction element).

As used herein, the terms “conjugated” and “conjugation” refer to the covalent attachment of two molecular entities (e.g., post-synthesis and/or during synthetic production). The attachment of a peptide or small molecule tag to a protein or small molecule, chemically (e.g., “chemically” conjugated) or enzymatically, is an example of conjugation.

The term "binding moiety" refers to a domain that specifically binds an antigen or epitope independently of a different epitope or antigen binding domain. A binding moiety may be an antibody, antibody fragment, a receptor domain that binds a target ligand, proteins that bind to immunoglobulins (e.g., protein A, protein G, protein A/G, protein L, protein M), a binding domain of a proteins that bind to immunoglobulins (e.g., protein A, protein G, protein A/G, protein L, protein M), oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins etc. Table A provides a lists of exemplary binding moieties that could be used singly or in various combinations in methods, systems, and assays (e.g., immunoassays) herein.

Table A. Exemplary binding moieties

Binding Moiety A	Binding Moiety B
Protein A	Protein A
Ig Binding domain of protein A	Ig binding domain of protein A
Protein G	Protein G
Ig Binding domain of protein G	Ig binding domain of protein G
Protein L	Protein L
Ig Binding domain of protein L	Ig binding domain of protein L
Protein M	Protein M
Ig Binding domain of protein M	Ig binding domain of protein M
polyclonal antibody against analyte X	polyclonal antibody: same antibody or second polyclonal antibody recognizing same target analyte X
monoclonal antibody	monoclonal antibody recognizing different epitope on same target analyte X
recombinant antibody	recombinant antibody recognizing different epitope on same target analyte X
scFv	scFv recognizing different epitope on same target analyte X
variable light chain (V_L) of antibody (monoclonal, recombinant, polyclonal) recognizing target analyte X	variable heavy chain (V_H) of same antibody (monoclonal, recombinant, polyclonal) recognizing target analyte X
protein (e.g. receptor) binding domain 1 that binds to analyte X	protein (e.g. receptor) binding domain 2 that binds to analyte X
(Fab) fragment	(Fab) fragment from different antibody recognizing different epitope to same target analyte X
Fab' fragment	Fab' from different antibody recognizing different epitope to same target analyte X
Fv fragment	Fv from different antibody recognizing different epitope to same target analyte X
F(ab')2 fragment	F(ab')2 from different antibody recognizing different epitope to same target analyte X
oligonucleotide probe	oligonucleotide probe to same DNA or RNA target but recognizing non-overlapping sequence
DARPin	DARPin recognizing non-overlapping domain of same target
peptide nucleic acid	peptide nucleic acid recognizing same DNA or RNA target but non-overlapping sequence
aptamer	aptamer binding to same target analyte X but recognizing non-overlapping sequence or epitope
affimer	aptamer binding to same target analyte X but recognizing different epitope

As used herein, the term “antibody” refers to a whole antibody molecule or a fragment thereof (e.g., fragments such as Fab, Fab', and $F(ab')_2$, variable light chain, variable heavy chain, Fv, it may be a polyclonal or monoclonal or recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, etc. As used herein, when an antibody or

other entity "specifically recognizes" or "specifically binds" an antigen or epitope, it preferentially recognizes the antigen in a complex mixture of proteins and/or macromolecules, and binds the antigen or epitope with affinity which is substantially higher than to other entities not displaying the antigen or epitope. In this regard, "affinity which is substantially higher" means affinity that is high enough to enable detection of an antigen or epitope which is distinguished from entities using a desired assay or measurement apparatus. Typically, it means binding affinity having a binding constant (K_a) of at least 10^7 M^{-1} (e.g., $>10^7$ M^{-1}, $>10^8$ M^{-1}, $>10^9$ M^{-1}, $>10^{10}$ M^{-1}, $>10^{11}$ M^{-1}, $>10^{12}$ M^{-1}, $>10^{13}$ M^{-1}, etc.). In certain such embodiments, an antibody is capable of binding different antigens so long as the different antigens comprise that particular epitope. In certain instances, for example, homologous proteins from different species may comprise the same epitope.

As used herein, the term "antibody fragment" refers to a portion of a full-length antibody, including at least a portion of the antigen binding region or a variable region. Antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, variable light chain, variable heavy chain, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. See, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; herein incorporated by reference in its entirety. In certain embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies (e.g., papain digestion and pepsin digestion of antibody) produced by recombinant DNA techniques, or chemical polypeptide synthesis. For example, a "Fab" fragment comprises one light chain and the C_{H1} and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab'" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the C_{H1} and C_{H2} domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "$F(ab')_2$" molecule. An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203; herein incorporated by reference in their entireties. In certain instances, a single variable region (e.g., a heavy chain variable region or

a light chain variable region) may have the ability to recognize and bind antigen. Other antibody fragments will be understood by skilled artisans.

As used herein, the term "peptide tag" refers to a peptide that may be attached (e.g., post-synthesis or during synthetic production) or fused to another entity (e.g., protein of interest, molecule of interest, interaction element, co-localization element, etc.). The peptide tag may or may not be attached to another entity. Typically, as used herein, a peptide tag is capable of forming a bioluminescent complex with another peptide tag and a polypeptide under appropriate conditions. In embodiments in which a peptide tag is attached to another entity, a peptide tag is chemically conjugated to another molecule (e.g., peptide, polypeptide, nucleic acid, other small molecules or macromolecules), chemically synthesized to be a part of another molecule, or genetically fused to another peptide or polypeptide molecule, etc.

As used herein, the term "polypeptide component" is used synonymously with the term "polypeptide component of a bioluminescent complex." Typically, as used herein, a polypeptide component is capable of forming a bioluminescent complex with a pair of peptide tags, under appropriate conditions.

As used herein, the term "an Oplophorus luciferase" ("an OgLuc") refers to a luminescent polypeptide having significant sequence identity, structural conservation, and/or the functional activity of the luciferase produce by and derived from the deep-sea shrimp *Oplophorus gracilirostris*. In particular, an OgLuc polypeptide refers to a luminescent polypeptide having significant sequence identity, structural conservation, and/or the functional activity of the mature 19 kDa subunit of the Oplophorus luciferase protein complex (e.g., without a signal sequence) such as SEQ ID NOs: 1 (WT OgLuc) and 3 (NanoLuc), which comprises 10 β strands (β1, β2, β3, β4, β5, β6, β7, β8, β9, β10) and utilize substrates such as coelenterazine or coelenterazine derivatives to produce luminescence.

As used herein, the term "β9-like peptide" refers to a peptide (or peptide tag) comprising significant sequence identity, structural conservation, and/or the functional activity of the β (beta) 9 strand of an OgLuc polypeptide. In particular, a β9-like peptide is a peptide capable of structurally complementing an OgLuc polypeptide lacking a β9 strand resulting in enhanced luminescence of the complex compared to the OgLuc polypeptide in the absence of the β9-like peptide. Other "βX-like peptides" may be similarly named (e.g., β1-like, β2-like, β3-like, β4-like, β5-like, β6-like, β7-like, β8-like, β9-like).

As used herein, the term "β10-like peptide" refers to a peptide (or peptide tag) comprising significant sequence identity, structural conservation, and/or the functional activity of the β (beta) 10 strand of an OgLuc polypeptide. In particular, a β10-like peptide is a peptide capable of structurally complementing an OgLuc polypeptide lacking a β10 strand resulting in enhanced luminescence of the complex compared to the OgLuc polypeptide in the absence of the β10-like peptide. Other "βX-like peptides" may be similarly named (e.g., β1-like, β2-like, β3-like, β4-like, β5-like, β6-like, β7-like, β8-like, β9-like).

As used herein, the term "β_{1-8}-like polypeptide" refers to a polypeptide bearing sequence and structural similarity to β (beta) strands 1-8 of an OgLuc polypeptide, but lacking β (beta) strands 9 and 10. Other "β_{Y-Z}-like polypeptides" may be similarly named (e.g., β_{1-4}-like polypeptide, β_{2-8}-like polypeptide, β_{5-10}-like polypeptide, etc.).

As used herein, the term "NANOLUC" refers to an artificial luciferase or bioluminescent polypeptide produced commercially by the Promega Corporation and corresponding to SEQ ID NO: 3.

As used herein, the term "LgBiT" refers to a polypeptide corresponding to β_{1-9}-like polypeptide that finds use in, for example, binary complementation to form a bioluminescent complex and corresponds to SEQ ID NO: 11.

As used herein, the term "SmBiT" refers to a peptide corresponding to β_{10}-like peptide that finds use in, for example, binary complementation to form a bioluminescent complex, but has low affinity for LgBiT (e.g., requires facilitation for complex formation) and corresponds to SEQ ID NO: 13.

As used herein, the term "HiBiT" refers to a peptide corresponding to β_{10}-like peptide that finds use in, for example, binary complementation to form a bioluminescent complex, but has low affinity for LgBiT (e.g., requires facilitation for complex formation) and corresponds to SEQ ID NO: 15. HiBiT is has the same sequence as "SmHiTrip10" (SEQ ID NO: 25) and "pep86," terms which may be used interchangeably (also SmTrip10 pep86, etc.).

As used herein, the term "LgTrip" refers to a polypeptide corresponding to β_{1-8}-like polypeptide that corresponds to SEQ ID NO: 17 and finds use in, for example, tripartite complementation with β_9-like and β_{10}-like peptides to form a bioluminescent complex, or bianry complementation, with a β_{9-10}-like dipeptide to form a bioluminescent complex. LgTrip variants include: LgTrip 2098 (w/ His tag: SEQ ID NO: 31; w/o His tag: SEQ ID NO: 304) and LgTrip 3546 (w/ His tag: SEQ ID NO: 51; w/o His tag: SEQ ID NO: 302).

As used herein, the term "SmTrip10" refers to a peptide corresponding to β_{10}-like peptide that finds use in, for example, tripartite complementation to form a bioluminescent complex.

As used herein, the term "SmTrip9" refers to a peptide corresponding to β_{9}-like peptide that finds use in, for example, tripartite complementation to form a bioluminescent complex.

DETAILED DESCRIPTION

Provided herein are bioluminescent polypeptides and compositions and methods for the assembly of a tripartite or multipartite bioluminescent complex. In particular embodiments, a bioluminescent complex is formed upon the interaction of three or more peptide and/or polypeptide components.

Experiments conducted during development of embodiments herein demonstrate that a tripartite luciferase comprising two small peptide elements (e.g., a β10-like peptide and β9-like peptide) and one polypeptide element (e.g., β_{1-8}-like polypeptide) assemble to form a luminescent complex. Experiments conducted during development of embodiments herein further demonstrate the formation of a bioluminescent complex from up to five fragments of a luciferase (or variants of such fragments), such as a polypeptide fragment (or variants thereof) and one or more peptide, dipeptide, or tripeptide fragments (or variants of such fragments).

The commercially-available NANOLUC luciferase (Promega Corporation) comprises 10 β (beta) strands (β1, β2, β3, β4, β5, β6, β7, β8, β9, β10). U.S. Pat. No. 9,797,889 (herein incorporated by reference in its entirety) describes development and use of a complementation system comprising a β_{1-9}-like polypeptide and a β_{10}-like peptide (the actual polypeptide and peptide sequences in U.S. Pat. No. 9,797,889 differ from the corresponding sequences in NANOLUC and wild-type native OgLuc).

In experiments conducted during development of embodiments herein, a β_{1-9}-like polypeptide was further split by removal of the β9 strand. The remaining portion (a β_{1-8}-like polypeptide) is referred to herein as LgTrip 2098 (SEQ ID NO: 17; or SEQ ID NO: 31 (with His tag)). Experiments attempted to reconstitute a luminescent complex from LgTrip and two peptides corresponding to the β9 (SmTrip9 pep245; SEQ ID NO: 23) and β10 (SmTrip10 pep86; HiBit, a β10 sequence optimized for use in a high affinity bipartite system; SEQ ID NO: 15) strands. Experiments demonstrated that LgTrip 2098 (SEQ ID NO: 17; or SEQ ID

NO: 31 (with His tag)) expressed poorly in *E. coli*, was unstable, and was susceptible to surface inactivation. Experiments were conducted during development of embodiments herein to develop artificial variants that exhibit one or more (e.g., all) of enhanced stability, enhanced expression, enhanced activity, enhanced molecular interactions, etc., and is capable of being used in a system to reconstitute a bioluminescent complex with peptides corresponding to the β9 (e.g., β9-like peptides (e.g., SmTrip9 pep245; SEQ ID NO: 23)) and β10 (e.g., β10-like peptides (e.g., SmTrip10 pep86; HiBiT; SEQ ID NO: 25)) strands. Experiments conducted during development of embodiments herein demonstrate, for example, that LgTrip 3092 (SEQ ID NO: 19) or LgTrip 3546 (SEQ ID NO: 51) are capable of forming a luminescent complex with suitable β9-like (e.g., SmTrip9 pep245; SEQ ID NO: 23) and β10-like (e.g., SmTrip10 pep86; HiBiT; SEQ ID NO: 25) peptides. Experiments were conducted during development of embodiments herein to develop artificial polypeptide components (e.g., SEQ ID NOs: 19, 21, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, and additional variants thereof) and peptide tags (e.g., the peptides listed in Table 1 and additional variants thereof) with enhanced characteristics for luminescent complex reconstitution.

Further experiments conducted during development of embodiments herein demonstrate that NANOLUC-based bioluminescent complexes can be formed using constructs comprising other polypeptide components (e.g., β_{1-5}-like, β_{1-6}-like, β_{1-7}-like, etc.) and corresponding combinations of complimentary peptides (e.g., β_{6}-like, β_{7}-like, β_{8}-like, β_{9}-like, β_{10}-like), dipeptides (e.g., β_{6-7}-like, β_{7-8}-like, β_{8-9}-like, β_{9-10}-like), tripeptides (e.g., β_{6-8}-like, β_{7-9}-like, β_{8-10}-like), polypeptides (e.g., β_{6-10}-like, β_{6-9}-like, β_{7-10}-like, etc.) derived from the NANOLUC-based, NanoBiT-based, and NanoTrip-based systems, polypeptides, and peptide described herein. The experiments conducted during development of embodiments herein demonstrate the formation of a bioluminescent complex from two or more (e.g., 2, 3, 4, 5, etc.) peptide and polypeptide components that collectively comprise the full lenth of a luciferase contruct (e.g., a full lngth luciferase polypeptide comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 788 or 789).

In some embodiments, provided herein are compositions and methods for the assembly of a bioluminescent complex from two peptide tags (e.g., β9-like (e.g., SmTrip9)

and β10-like (e.g., SmTrip10) peptides) and a polypeptide component (e.g., β_{1-8}-like (e.g., LgTrip) polypeptide).

In some embodiments, provided herein are compositions and methods for the assembly of a bioluminescent complex from a polypeptide component (e.g., a β_{1-5}-like, β_{1-6}-like , β_{1-7}-like , or β_{1-8}-like polypeptide), and complementary peptide(s) (e.g., β_6-like, β_7-like, β_8-like, β_9-like, β_{10}-like), dipeptide(s) (e.g., β_{6-7}-like, β_{7-8}-like, β_{8-9}-like, β_{9-10}-like), tripeptide (e.g., β_{6-8}-like, β_{7-9}-like, β_{8-10}-like), and/or polypeptides (e.g., β_{6-10}-like, β_{6-9}-like, β_{7-10}-like, etc.).

In some embodiments, one or more (e.g., two, three, four, five, etc.) of the peptide tags and the polypeptide component are not fragments of a preexisting protein (e.g., are not structurally-complementary subsequences of a known polypeptide sequence). However, in other embodiments, one or more of the peptide tags and the polypeptide component may be fragments of a known or existing protein, polypeptide, or peptide. In certain embodiments, the bioluminescent activity of the polypeptide component (of the bioluminescent complex) is enhanced (e.g., 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 10^2-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold, or more) via structural complementation with the two peptide tags. In some embodiments, provided herein are peptide (peptide tags)/polypeptide elements that are capable of assembling into a bioluminescent complex for the purpose of, for example, detecting and monitoring molecular interactions (e.g., protein-protein, protein-DNA, protein-RNA interactions, RNA-DNA, protein-small molecule, RNA-small-molecule, DNA-DNA, RNA-RNA, PNA-DNA, PNA-RNA, etc.). In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptide thereof) are fused, or otherwise linked to interaction elements. In particular embodiments, the peptide/dipeptide/tripeptide tags and polypeptide components, when for the purpose of detecting/monitoring molecular interactions, do not form a complete bioluminescent complex without facilitation by the interaction between interaction elements. However, upon interaction (e.g., binding) of the interaction elements to each other (or to a target molecule or complex), formation of the bioluminescent complex is facilitated. In some embodiments, the bioluminescent signal from the bioluminescent complex (or the capacity to produce such a signal in the presence of substrate) serves as a reporter for the formation of a complex by the interaction elements. If an interaction complex is formed, then a bioluminescent complex is

formed, and a bioluminescent signal is detected/measured/monitored (e.g., in the presence of substrate). If an interaction complex fails to form (e.g., due to unfavorable conditions, due to unstable interaction between the interaction elements, due to incompatible interaction elements), then a bioluminescent complex does not form, and a bioluminescent signal is not produced (e.g., in the presence of substrate). In some embodiments, the bioluminescent signal from the bioluminescent complex (or the capacity to produce such a signal in the presence of substrate) serves as a reporter for the binding of the interaction elements to a target. If target-binding occurs, then a bioluminescent complex is formed and a bioluminescent signal is detected/measured/monitored (e.g., in the presence of substrate). If target-binding fails to occur (e.g., due to unfavorable conditions, due to unstable interaction between an interaction element and target, due to the absence of target, etc.), then a bioluminescent complex does not form and a bioluminescent signal is not produced.

In certain embodiments, interaction elements are two molecules of interest (e.g., protein(s) of interest, small molecule(s) of interest, etc.). For example, assays can be performed to detect the interaction of two molecules of interest by tethering each one to separate peptide/dipeptide/tripeptide tag (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof). If the molecules of interest interact (e.g., transiently interact, stably interact, etc.), the peptide/dipeptide/tripeptide tags are brought into close proximity in a suitable conformation, and a bioluminescent complex is formed between the peptide/dipeptide/tripeptide tags and the polypeptide component of the bioluminescent complex (and bioluminescent signal is produced/detected (e.g., in the presence of substrate)). In the absence of an interaction between the molecules of interest, the peptide/dipeptide/tripeptide tags are not brought into close proximity and/or arranged in an orientation to facilitate complex formation with the polypeptide component of the bioluminescent complex, the bioluminescent complex is not formed, and a bioluminescent signal is not produced (in the presence of substrate). Such embodiments can be used to study the effect of inhibitors on complex formation, the effect of mutations on complex formation, the effect of conditions (e.g., temperature, pH, etc.) on complex formation, the interaction of a small molecule (e.g., potential therapeutic) with a target molecule, etc.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and

tripeptide thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are provided that are capable of assembling into a bioluminescent complex without facilitation by interaction elements. In such embodiments, a bioluminescent complex will form when the peptide/dipeptide/tripeptide tags and polypeptide component are together within the same sample, subcellular compartment, cell, tissue, etc. (e.g., co-localized). In some embodiments, provided herein peptide/dipeptide/tripeptide (tags)/polypeptide elements that are capable of assembling into a bioluminescent complex for use in detecting and monitoring co-localization (e.g., without molecular interaction) of molecular elements (e.g., protein(s), nucleic acid(s), small molecule(s), lipid, carbohydrate, cellular structure, etc.). In some embodiments, a bioluminescent complex is formed from peptide/dipeptide/tripeptide tags and a polypeptide component that collectively span a full β1-like to β10-like sequence. In some embodiments, the peptide/dipeptide/tripeptide tags are fused or otherwise linked to co-localization elements. In particular embodiments, particularly for the purpose of detecting/monitoring co-localization (e.g., without molecular interaction), the peptide/dipeptide/tripeptide tags and polypeptide components are capable of forming a bioluminescent complex without facilitation (e.g., without interaction elements). Upon co-localization (e.g., within the same cell, on the same surface, with the same cellular compartment, within the same tissue, etc.) of the co-localization elements (e.g., fused to the peptide/dipeptide/tripeptide tags), formation of the bioluminescent complex (from the peptide/dipeptide/tripeptide tags and the polypeptide component), with or without interaction of the co-localization elements, is facilitated. In some embodiments, the bioluminescent signal from the bioluminescent complex (or the capacity to produce such a signal in the presence of substrate) serves as a reporter for co-localization of the co-localization elements. If the co-localization elements co-localize, then a bioluminescent complex of the polypeptide component and the peptide/dipeptide/tripeptide tags fused to the co-localization elements is formed, and a bioluminescent signal is detected/measured/monitored (e.g., in the presence of substrate). If the co-localization elements do not co-localize, then a bioluminescent complex does not form, and a bioluminescent signal is not produced (e.g., in the presence of substrate).

In certain embodiments, the co-localization pair comprises two molecules of interest (e.g., protein(s) of interest, small molecule(s) of interest, etc.). For example, assays can be performed to detect the co-localization (e.g., within a cell, within a cellular compartment, within a tissue, etc.) of two molecules of interest by tethering each one to a separate

dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof). If the molecules of interest co-localize, the peptide tags are brought into close proximity in a suitable conformation, and a bioluminescent complex is formed with the polypeptide component polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide), and bioluminescent signal is produced/detected (e.g., in the presence of substrate). In the absence of co-localization of the molecules of interest, the polypeptide components and peptide/dipeptide/tripeptide tags tags do not interact to form a complex, and a bioluminescent signal is not produced (e.g., in the presence of substrate). Such embodiments can be used to study co-localization of molecules of interest under various conditions.

In some embodiments, systems, assays, and devices comprising dipeptide/tripeptide tags tags e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are provided for the detection of an analyte (e.g., small molecule, peptide, protein, antibody, nucleic acid, etc.) in a sample. In some embodiments, peptide/dipeptide/tripeptide tags are tethered or fused with detection/binding agents (e.g., binding moiety, binding sequence, etc.) that recognize the target analyte, target analytes, secondary analytes that are bound by the target analyte, secondary binding agents that bind to primary binding agents, etc. In some embodiments, various combinations of peptide/dipeptide/tripeptide tags tethered/fused to the aforementioned detection/binding agents are used in assays and devices for the detection/quantification/identification of analytes in a sample. Exemplary systems that find use in assays and devices are depicted in, for example, Figures 51-56 and described herein.

In some embodiments, provided herein are compositions and methods for the assembly of a bioluminescent complex from a dipeptide (e.g., a β9/β10-like dipeptide) and a polypeptide component (e.g., $\beta_{1\text{-}8}$-like (e.g., LgTrip) polypeptide). In some embodiments, the dipeptide and the polypeptide component are not fragments of a preexisting protein (e.g., are not structurally-complementary subsequences of a known polypeptide sequence). However, in other embodiments, the dipeptide and/or the polypeptide component may be fragments of a known or existing protein, polypeptide, or peptide. In certain embodiments, the bioluminescent activity of the polypeptide component (of the bioluminescent complex) is

enhanced (e.g., 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 10^2-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold, or more) via structural complementation with the dipeptide. In some embodiments, a β_{1-8}-like polypeptide exhibits lower background luminescence than a β_{1-9}-like polypeptide. In some embodiments, a β_{1-8}-like polypeptide exhibits increased thermal and chemical stability compared to a β_{1-9}-like polypeptide.

In some embodiments, provided herein are bioluminescent complexes, including but not limited to those comprising any of the following combinations of peptide, dipeptide, tripeptide, and polypeptide components:

- β1-5-like polypeptide + β6-like peptide + β7-like peptide + β8-like peptide + β9-like peptide + β10-like peptide;
- β1-5-like polypeptide + β6-like peptide + β7-like peptide + β8-like peptide + β9/10-like dipeptide;
- β1-5-like polypeptide + β6-like peptide + β7/8-like dipeptide + β9/10-like dipeptide;
- β1-5-like polypeptide + β6/7/8-like tripeptide + β9/10-like dipeptide;
- β1-5-like polypeptide + β6-like peptide + β7/8/9-like tripeptide + β10-like peptide;
- β1-6-like polypeptide + β7-like peptide + β8-like peptide + β9-like peptide + β10-like peptide;
- β1-6-like polypeptide + β7-like peptide + β8-like peptide + β9/10-like dipeptide;
- β1-6-like polypeptide + β7/8-like dipeptide + β9/10-like dipeptide;
- β1-6-like polypeptide + β6/7/8-like dipeptide + β9-like peptide + β10-like peptide;
- β1-6-like polypeptide + β7/8/9-like tripeptide + β10-like peptide;
- β1-7-like polypeptide + β8-like peptide + β9-like peptide + β10-like peptide;
- β1-7-like polypeptide + β8-like peptide + β9/10-like dipeptide;
- β1-7-like polypeptide + 8/9-like dipeptide + β10-like peptide;
- β1-7-like polypeptide + β8/9/10-like tripeptide;
- β1-8-like polypeptide + β9-like peptide + β10-like peptide;
- β1-8-like polypeptide + β9/10-like dipeptide;
- β1-5-like polypeptide + β6-10-like polypeptide;
- β1-5-like polypeptide + β6-9-like polypeptide + β10-like peptide; and
- β1-5-like polypeptide + β7-10-like polypeptide + β6-like peptide.

The above combinations are not limiting and other combinations of peptide, dipeptide, tripeptide, and polypeptide components are within the scope herein.

In some embodiments, a β1-5-like polypeptide comprises positions 1-102 of SEQ ID NO: 788. In some embodiments, a β1-6-like polypeptide comprises positions 1-124 of SEQ ID NO: 788. In some embodiments, a β1-7-like polypeptide comprises positions 1-133 of SEQ ID NO: 788. In some embodiments, a β1-8-like polypeptide comprises positions 1-148 of SEQ ID NO: 788.

In some embodiments, a set of β5-10-like peptides/dipeptide/tripeptides/polypeptide collectively comprise positions 103-170 of SEQ ID NO: 788 or 789. In some embodiments, a set of β6-10-like peptides/dipeptide/tripeptides/polypeptide collectively comprise positions 125-170 of SEQ ID NO: 788 or 789. In some embodiments, a set of β7-10-like peptides/dipeptide/tripeptides/polypeptide collectively comprise positions 134-170 of SEQ ID NO: 788 or 789. In some embodiments, a set of β8-10-like peptides/dipeptide/tripeptides/polypeptide collectively comprise positions 149-170 of SEQ ID NO: 788 or 789.

In some embodiments, one or more components of a bioluminescent complex span partial beta strands of the base luciferases (e.g., OgLuc, NANOLUC, SEQ ID NO: 788, SEQ ID NO: 789, etc.) described herein. The separations between peptide, dipeptide, tripeptide, and polypeptide components may reside at the split points between the beta strands or may appear at a position -1, -2, -3, -4, -5, +1, +2, +3, +4, +5, or more from the split points identified by the sequences herein. In some embodiments, peptide, dipeptide, tripeptide, and polypeptide components that span the full sequence of a base luciferases (e.g., OgLuc, NANOLUC, SEQ ID NO: 788, SEQ ID NO: 789, etc.) described herein are capable of forming a bioluminescent complex, even if the split points for the components are not between the beta strands.

For example, a split site between β5 and β6 may occur between positions 102 and 103 of SEQ ID NO: 788, or in some embodiments such a split site may occur at a position up to 5 residues before or after that position (e.g., after position 96, 97, 98, 99, 100, 101, 103, 104, 105, 106, 107). In some embodiments, a split site between β6 and β7 may occur between positions 124 and 125 of SEQ ID NO: 788, or in some embodiments such a split site may occur at a position up to 5 residues before or after that position (e.g., after position 118, 119, 120, 121, 122, 123, 125, 126, 127, 128, 129). In some embodiments, a split site between β7 and β8 may occurr between positions 133 and 134 of SEQ ID NO: 788, or in some embodiments such a split site may occur at a position up to 5 residues before or after that

position (e.g., after position 127, 128, 129, 130, 131, 132, 134, 135, 136, 137, 138). In some embodiments, a split site between β8 and β9 may occur between positions 148 and 149 of SEQ ID NO: 788, or in some embodiments such a split site may occur at a position up to 5 residues before or after that position (e.g., after position 142, 143, 144, 145, 146, 147, 149, 150, 151, 152, 153).

In some embodiments, two peptide, dipeptide, tripeptide, and polypeptide components that are sequentially adjacent within the base luciferase (e.g., OgLuc, NANOLUC, SEQ ID NO: 788, SEQ ID NO: 789, etc.) sequence comprise all of the amino acids of that corresponding portion of the base sequence. In some embodiments, one or more (e.g., 1, 2, 3, 4, 5, or more) amino acids adjaecnt to the split point in the base sequence are absent from the corresponding peptide, dipeptide, tripeptide, and/or polypeptide components.

In some embodiments, provided herein are peptides comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of the following:

- β6-like – GVTPNKLNYFGRPYEGIAVFDG (SEQ ID NO: 802);
- β7-like – KKITTTGTL (SEQ ID NO: 803
- β8-like – WNGNKIIDERLITPD (SEQ ID NO: 804
- β9-like – GSMLFRVTINS (SEQ ID NO: 805
- β10-like (Hi affinity) – VSGWRLFKKIS (SEQ ID NO: 806 and
- β10-like (Lo affinity) – VTGYRLFEEIL (SEQ ID NO: 807

In some embodiments, provided herein are dipeptides comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of the following:

- β6/7-like – GVTPNKLNYFGRPYEGIAVFDGKKITTTGTL (SEQ ID NO: 808
- β7/8-like – KKITTTGTLWNGNKIIDERLITPD (SEQ ID NO: 809);
- β8/9-like – WNGNKIIDERLITPDGSMLFRVTINS (SEQ ID NO: 810);
- β9/10-like (Hi affinity) – GSMLFRVTINSVSGWRLFKKIS (SEQ ID NO: 811); and
- β9/10-like (Lo affinity) – GSMLFRVTINSVTGYRLFEEIL (SEQ ID NO: 812).

In some embodiments, provided herein are tridipeptides comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of the following:

- β6/7/8-like – GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD (SEQ ID NO: 813);
- β7/8/9-like – KKITTTGTLWNGNKIIDERLITPDGSMLFRVTINS (SEQ ID NO: 814);
- B8/9/10-like (Hi affinity) – WNGNKIIDERLITPDGSMLFRVTINSVSGWRLFKKIS (SEQ ID NO: 815); and
- B8/9/10-like (Lo affinity) – WNGNKIIDERLITPDGSMLFRVTINSVTGYRLFEEIL (SEQ ID NO: 816).

In some embodiments, provided herein are polypeptide comprising 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of the following:

- β1-5-like – MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID (SEQ ID NO: 790);
- β6-10-like (Hi affinity) – GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDGSMLFRVTINSVSGWRLFKKIS (SEQ ID NO: 794);
- β6-10-like (Lo affinity) – GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDGSMLFRVTINSVTGYRLFEEIL (SEQ ID NO: 798);
- β6-9-like – GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDGSMLFRVTINS (SEQ ID NO: 829);
- β7-10-like (Hi affinity) – KKITTTGTLWNGNKIIDERLITPDGSMLFRVTINSVSGWRLFKKIS (SEQ ID NO: 795); and

- β7-10-like (Lo affinity) –

KKITTTTGTLWNGNKIIDERLITPDGSMLFRVTINSVTGYRLFEEIL (SEQ ID NO: 799).

In some embodiments, a polypeptide component (e.g., of a set of peptides/polypeptides, or a bioluminescent complex, etc.) comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of SEQ ID NOS: 788, 789, 790, 791, 792, and 793.

In some embodiments, peptide/dipeptide/tripeptide components (e.g., tags) (e.g., of a set of peptides/polypeptides, or a bioluminescent complex, etc.) collectively comprise 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) sequence identity with one of SEQ ID NOS: 794, 795, 796, 797, 798, 799, 800, and 801.

In some embodiments, provided herein are sets of components and complexes of the peptides, dipeptides, tripeptides, and polypeptides listed above. In particular embodiments, sets of components are selected that span all ten of the beta strands of a base luciferase sequence.

In some embodiments, the interaction, co-localization, detection, and other methods, assays, and technologies described for use with the two-peptide tag systems herein (e.g., β9-like (e.g., SmTrip9) peptide, β10-like (e.g., SmTrip10) peptides and polypeptide component ((e.g., β1-8-like (e.g., LgTrip) polypeptide)), also find use with the dipeptide systems described herein (e.g., β9/10-like dipeptide and polypeptide component). In some embodiments, a dipeptide has high affinity for a polypeptide component; in such embodiments, a bioluminescent complex forms when the dipeptide and polypeptide component are brought into contact (e.g., co-localize, are added to the sample sample, etc.) without facilitation. In some embodiments, a dipeptide has low affinity for a polypeptide component; in such embodiments, a bioluminescent complex will not form when the dipeptide and polypeptide component are brought into contact (e.g., co-localize, are added to the sample sample, etc.) without facilitation. Like the two-peptide tag systems herein (e.g., β9-like (e.g., SmTrip9) peptide, β10-like (e.g., SmTrip10) peptides and polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide)), dipeptide/polypeptide pairs of varying affinities may be selected for different applications. In some embodiments, systems, methods, and assays for two-component complementation systems are described in U.S. Pat. No. 9,797,890 (herein incorporated by reference in its entirety), and all such systems, methods, and assays find use with the dipeptide/polypeptide systems described herein.

In some embodiments, the interaction, co-localization, detection, and other methods, assays, and technologies described for use with the two-peptide tag systems herein (e.g., β9-like (e.g., SmTrip9) peptide, β10-like (e.g., SmTrip10) peptides and polypeptide component ((e.g., β1-8-like (e.g., LgTrip) polypeptide)), also find use with systems comprising any suitbale combination fo peptides, dipeptides, trippeptides, and polypeptides, as described herein. In some embodiments, the components have high affinity for one another; in such embodiments, a bioluminescent complex forms when the components are brought into contact (e.g., co-localize, are added to the sample sample, etc.) without facilitation. In some embodiments, one or more of the components have low affinity for one or more of the other components; in such embododiments, a bioluminescent complex will not form when the components are brought into contact (e.g., co-localize, are added to the sample sample, etc.) without facilitation. Like the two-peptide tag systems herein (e.g., β9-like (e.g., SmTrip9) peptide, β10-like (e.g., SmTrip10) peptides and polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide)), the other systems described herein may be provided with varying affinities for different applications. In some embodiments, systems, methods, and assays for two-component complementation systems are described in U.S. Pat. No. 9,797,890 (herein incorporated by reference in its entirety), and all such systems, methods, and assays find use with the various peptide, dipeptide, tripeptide, and polypeptide systems described herein.

In some embodiments, provided herein are complementary panels of interchangeable peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) that have variable affinities and luminescence upon formation of bioluminescent complexes therefrom (e.g., a high-affinity/high-luminescence, a moderate-affinity/high-luminescence, a low-affinity/moderate-luminescence, etc.). Utilizing different combinations of peptide/dipeptide/tripeptide tags and polypeptide components provides an adaptable system comprising various sets ranging from lower to higher affinities, luminescence, expression level, stability, solubility, and other variable characteristics. This adaptability allows the detection/monitoring/identification/quantification of analytes, molecular interactions, co-localization, and/or other characteristics to be fine-tuned to the specific molecule(s) of interest and/or conditions to be studied and expands the range of molecular interactions and/or co-localizations that can be detected/monitored/identified/quantified to

include interactions with very high or low affinities. Further provided herein are methods by which non-luminescent elements and panels of non-luminescent elements are developed and tested.

In some embodiments, due to the small size of the tags (e.g., peptide tags) herein (e.g., compared to larger polypeptides and proteins), they are resistant to denaturation (they have no tertiary structure required for function).

In some embodiments, peptide/dipeptide/tripeptide tags and a polypeptide components may be selected based on the molecules or proteins of interest to be studied. In some embodiments, different peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) may require different strength, duration, and/or stability of an interaction complex (e.g., complex of interaction elements) to result in bioluminescent complex formation. In some embodiments, a highly stable interaction complex is required to produce a detectable bioluminescent signal (e.g., in the presence of a substrate). In other embodiments, even a weak or transient interaction complex results in bioluminescent complex formation. In still other embodiments, a bioluminescent complex will form in the absence of an interaction complex as long as the peptide/dipeptide/tripeptide tags and polypeptide component are co-localized. In some embodiments, the strength or extent of an interaction complex is directly proportional to the strength of the resulting bioluminescent signal. Some peptide/dipeptide/tripeptide tags/polypeptide component sets produce a detectable signal when combined with an interaction complex with a high millimolar dissociation constant (e.g., K_d>100 mM). Other peptide/dipeptide/tripeptide tags/polypeptide component sets require an interaction pair with a low millimolar (e.g., K_d<100 mM), micromolar (e.g., K_d<1 mM), nanomolar (e.g., K_d<1 μM), or even picomolar (e.g., K_d<1 nM) dissociation constant in order to produce a bioluminescent complex with a detectable signal.

In some embodiments, the peptide/dipeptide/tripeptide tags and/or polypeptide components herein are not fragments of a pre-existing protein (e.g., a pre-existing bioluminescent protein). In some embodiments, none of the peptide/dipeptide/tripeptide tags and polypeptide component used to form a complex are fragments of a pre-existing protein (e.g., the same pre-existing protein, a pre-existing bioluminescent protein, etc.). In some embodiments, neither the peptide tags (e.g., β9-like (e.g., SmTrip9) and β10-like (e.g.,

SmTrip10) peptides; β9/β10-like dipeptides; etc.) nor polypeptide component (e.g., β_{1-8}-like (e.g., LgTrip) polypeptide)) that assemble together to form a bioluminescent complex are fragments of a pre-existing protein (e.g., the same pre-existing protein, a pre-existing bioluminescent protein, etc.). In some embodiments, the peptide/dipeptide/tripeptide tags or polypeptide component of a bioluminescent complex for use in embodiments of the present invention is not a subsequence of a preexisting protein. In some embodiments, non-luminescent elements for use in embodiments described herein do not comprise structurally-complementary subsequences of a preexisting protein.

In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) herein are non-luminescent or substantially non-luminescent in isolation (e.g., in the presence or absence of substrate). In some embodiments, the peptide/dipeptide/tripeptide tags herein are non-luminescent or substantially non-luminescent when associated together, in the absence of the polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) (e.g., in the presence or absence of substrate). In some embodiments, a polypeptide component is non-luminescent or substantially non-luminescent in isolation (e.g., in the presence or absence of substrate). In some embodiments, a single peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and the polypeptide component are non-luminescent or substantially non-luminescent in the absence of the second or third or fourth peptide/dipeptide/tripeptide tag (e.g., in the presence or absence of substrate). In certain embodiments, when placed in suitable conditions (e.g., physiological conditions), multiple peptide/dipeptide/tripeptide tags and a polypeptide component interact to form a bioluminescent complex and produce a bioluminescent signal in the presence of substrate.

In certain embodiments, an interaction element and/or co-localization element and a peptide/dipeptide/tripeptide tag are attached, fused, linked, connected, etc. In typical embodiments, a first peptide/dipeptide/tripeptide tag and a first interaction element (or first co-localization element) are attached to each other, and a second peptide/dipeptide/tripeptide tag and a second interaction element (or second co-localization element) are attached to each other. Attachment of peptide/dipeptide/tripeptide tags to interaction elements (or co-localization elements) may be achieved by any suitable mechanism, chemistry, linker, etc.

The interaction elements (or co-localization elements) and peptide/dipeptide/tripeptide tags are typically attached through covalent connection, but non-covalent linking of the two elements is also provided. In some embodiments, the peptide/dipeptide/tripeptide tags and interaction elements (or co-localization elements) are directly connected and, in other embodiments, they are connected by a linker. In some embodiments, the peptide/dipeptide/tripeptide tags and interaction elements (or co-localization elements) are provided as genetic/recombinant fusions. In some embodiments, endogenous tagging with the peptide/dipeptide/tripeptide tags herein (e.g., under endogenous regulatory control), allows for monitoring of normal cellular functions with the tools described herein. For example, a protien of interest may be endogenously tagged (e.g., using CRISPR/Cas9) with a high affinity β9/β10-like dipeptide, and then spontaneous complementation with LgTrip (or a variant thereof) is monitored in a cell, animal, lysate, etc. In other embodiments, the peptide tags and interaction elements (or co-localization elements) are connected by chemical modification/conjugation, such as by Native chemical ligation, Staudinger ligation, "traceless" Staudinger ligation, amide coupling, methods that employ activated esters, methods to target lysine, tyrosine and cysteine residues, imine bond formation (with and without *ortho*-boronic acid), boronic acid/diol interactions, disulfide bond formation, copper/copper free azide, diazo, and tetrazine "click" chemistry, UV promoted thiolene conjugation, diazirine photolabeling, Diels-Alder cycloaddition, metathesis reaction, Suzuki cross-coupling, thiazolidine (Step-4) coupling, streptavidin/biotin complementation, HaloTag/chloroalkane substrate complementation, etc. In some embodiments, peptide/dipeptide/tripeptide tags and interaction elements (or co-localization elements) are produced synthetically (e.g., solid-state synthesis, solution-phase synthesis, etc.). In some embodiments, interaction elements (or co-localization elements) are produced (e.g., synthetically or recombinantly) or obtained (e.g., from crude lysate, extracted proteins, purified proteins, etc.) by any suitable means.

In some embodiments, in which the interaction element (or co-localization element) is a peptide or polypeptide, a peptide/dipeptide/tripeptide tag (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and an interaction element (or co-localization element) are contained within a single amino acid chain. In some embodiments, a single amino acid chain comprises, consists of, or consists essentially of a peptide/dipeptide/tripeptide tag and an

interaction element (or co-localization element). In some embodiments, a single amino acid chain comprises, consists of, or consists essentially of a peptide/dipeptide/tripeptide tag, an interaction element (or co-localization element), optionally one or more an N-terminal sequence, a C-terminal sequence, regulatory elements (e.g., promoter, translational start site, etc.), and a linker sequence. In some embodiments, the peptide/dipeptide/tripeptide tag and interaction element (or co-localization element) are contained within a fusion polypeptide. In some embodiments, the first fusion of peptide/dipeptide/tripeptide tag and interaction element (or co-localization element) and the second fusion of peptide/dipeptide/tripeptide tag and interaction element (or co-localization element) are expressed separately; however, in other embodiments, a fusion protein is expressed that comprises or consist of both of the interaction (or co-localization) and peptide/dipeptide/tripeptide tags.

In some embodiments, a first fusion protein comprising a first peptide/dipeptide/tripeptide tag (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and first interaction element as well as a second fusion protein comprising a second peptide/dipeptide/tripeptide tag (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and second interaction element are expressed within the same cells. In some embodiments, a first fusion protein comprising a first peptide/dipeptide/tripeptide tag and first co-localization element as well as a second fusion protein comprising a second peptide/dipeptide/tripeptide tag and second co-localization element are expressed within the same cells. In some embodiments, the first and second fusion proteins are purified and/or isolated from the cells. In some embodiments, the interaction and/or co-localization of the fusion proteins is assayed within the cells. In some embodiments, the interaction and/or co-localization of the fusion proteins is assayed within a lysate of the cells. In other embodiments, first and second fusion proteins are expressed in separate cells and combined (e.g., following purification and/or isolation, following fusion of the cells or portions of the cells, by transfer of a fusion protein from one cell to another, or by secretion of one or more fusion proteins into the extracellular medium) for signal detection. In some embodiments, one or more fusion proteins are expressed in cell lysate (e.g., rabbit reticulocyte lysate) or in a cell-free system. In some embodiments, one or more fusion proteins are expressed from the genome of a virus or other cellular pathogen. In some embodiments, the polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-

like (e.g., LgTrip) polypeptide) and any other peptide/dipeptide/tripeptide components (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) for complex formation (with the first and second fusion proteins) is expressed in the same cell or cell lysate as one or both of the tag-containing fusion proteins. In some embodiments, the peptide/dipeptide/tripeptide/polypeptide components for complex formation with the peptide/dipeptide/tripeptide tags (within the first and second fusion proteins) are expressed in a different cell or cell lysate as one or both of the peptide-tag-containing fusion proteins. In some embodiments, the peptide/dipeptide/tripeptide/polypeptide components for complex formation with the peptide/dipeptide/tripeptide tags (within the first and second fusion proteins) is added to a cell, cell lysate, or other sample comprising the peptide-tag-containing fusion proteins.

In some embodiments, the systems (e.g., peptide/dipeptide/tripeptide tags, peptide/dipeptide/tripeptide/polypeptide components, substrates, vectors, etc.) and methods herein find use in the analysis of a sample (e.g., detection/quantification/identification/monitoring of co-localization, a molecular interaction, a target, etc.). In some embodiments, one or more of the components of a system herein are added to and/or provided or expressed within a sample. Suitable samples that may find use in embodiments herein include, but are not limited to: blood, plasma, sera, urine, saliva, cells, cell lysates, tissues, tissue homogenates, purified nucleic acids, stool, vaginal secretions, cerebrospinal fluid, allantoic fluid, water, biofilm, soil, dust, food, beverage, agriculture products, plants, etc.

In certain embodiments, nucleic acids, DNA, RNA, vectors, etc. are provided that encode the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide), fusion polypeptides, fusion proteins, etc. described herein. Such nucleic acids and vectors may be used for expression, transformation, transfection, injection, etc.

In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and interaction, co-localization element, or binding agent are connected

by a linker. In some embodiments, a linker connects the signal and interaction or co-localization elements while providing a desired amount of space/distance between the elements. In some embodiments, a linker allows both the signal and interaction elements to form their respective complexes (e.g., luminescent complex and interaction complex) simultaneously. In some embodiments, a linker assists the interaction element in facilitating the formation of a luminescent complex. In some embodiments, when an interaction complex is formed, the linkers that connect each peptide/dipeptide/tripeptide tag to their respective interaction elements position the peptide tags at the proper distance and conformation to form a bioluminescent complex. In some embodiments, an interaction or co-localization element and peptide/dipeptide/tripeptide tag are held in close proximity (e.g., <4 monomer units) by a linker. In some embodiments, a linker provides a desired amount of distance (e.g., 1, 2, 3, 4, 5, 6…10… 20, or more monomer units) between peptide tags and interaction elements (e.g., to prevent undesirable interactions between peptide/dipeptide/tripeptide tags and interaction or co-localization elements, for steric considerations, to allow proper orientation of non-luminescent element upon formation of interaction complex, to allow propagation of a complex-formation from interaction complex to luminescent complex, etc.). In certain embodiments, a linker provides appropriate attachment chemistry between the peptide/dipeptide/tripeptide tags and interaction elements. A linker may also improve the synthetic process of making the peptide/dipeptide/tripeptide tag and interaction or co-localization element (e.g., allowing them to be synthesized as a single unit, allowing post synthesis connection of the two elements, etc.).

In some embodiments, a linker is any suitable chemical moiety capable of linking, connecting, or tethering a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) or polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) to an interaction element or co-localization element. In some embodiments, a linker is a polymer of one or more repeating or non-repeating monomer units (e.g., nucleic acid, amino acid, carbon-containing polymer, carbon chain, etc.). When a peptide/dipeptide/tripeptide tag and an interaction, co-localization element, or binding agent are part of a fusion protein, a linker (when present) is typically an amino acid chain. When a peptide/dipeptide/tripeptide tag and interaction element, co-localization element, or binding agent are tethered together after the expression of the individual elements, a linker may

comprise any chemical moiety with functional (or reactive) groups at either end that are reactive with functional groups on the peptide tag and interaction or co-localization elements, respectively. Any suitable moiety capable of tethering the signal and interaction elements, co-localization element, and/or binding agent may find use as a linker.

A wide variety of linkers may be used. In some embodiments, the linker is a single covalent bond. In some embodiments, the linker comprises a linear or branched, cyclic or heterocyclic, saturated or unsaturated, structure having 1-20 nonhydrogen atoms (e.g., C, N, P, O and S) and is composed of any combination of alkyl, ether, thioether, imine, carboxylic, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds. In some embodiments, linkers are longer than 20 non-hydrogen atoms (e.g. 21 non-hydrogen atoms, 25 non-hydrogen atoms, 30 non-hydrogen atoms, 40 non-hydrogen atoms, 50 non-hydrogen atoms, 100 non-hydrogen atoms, etc.) In some embodiments, the linker comprises 1-50 non-hydrogen atoms (in addition to hydrogen atoms) selected from the group of C, N, P, O, and S (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40,41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 non-hydrogen atoms).

The scope of embodiments herein is not limited by the types of linkers available. The peptide/dipeptide/tripeptide tags, polypeptide components, and interaction elements, co-localization elements, or binding agents are linked either directly (e.g. linker consists of a single covalent bond) or linked via a suitable linker. Embodiments are not limited to any particular linker group. A variety of linker groups are contemplated, and suitable linkers could comprise, but are not limited to, alkyl groups, methylene carbon chains, ether, polyether, alkyl amide linker, a peptide linker, a modified peptide linker, a Poly(ethylene glycol) (PEG) linker, a streptavidin-biotin or avidin-biotin linker, polyaminoacids (e.g. polylysine), functionalized PEG, polysaccharides, glycosaminoglycans, dendritic polymers (WO93/06868 and by Tomalia et al. in Angew. Chem. Int. Ed. Engl. 29:138-175 (1990), herein incorporated by reference in their entireties), PEG-chelant polymers (W94/08629, WO94/09056 and WO96/26754, herein incorporated by reference in their entireties), oligonucleotide linker, phospholipid derivatives, alkenyl chains, alkynyl chains, disulfide, or a combination thereof. In some embodiments, the linker is cleavable (e.g., enzymatically (e.g., TEV protease site), chemically, photoinduced, etc.

In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide), recognition element, interaction element, co-localization element, binding agent, analyte, substrate, etc. is attached (e.g., via any suitable chemistry) to, or contained within, a solid surface or matrix. In some embodiments, one or more system components are attached (e.g., via any suitable chemistry) to, or contained within, a solid surface or matrix and other components are added (e.g., in solution (e.g., in a sample)) to the solid surface or matrix. Suitable solid surfaces include, but are not limited to: beads (e.g., magnetic beads), chips, tubes, plates, particles, membranes, paper, etc. In some embodiments, solid surfaces/matrix is made of any suitable materials, such as: Ahlstrom CytoSep, Cellulose nitrate, Cellulose acetate, Cellulose (e.g., Whatman FTA-DMPK-A, B, and C cards; Whatman ET 3/Chr; Whatman protein saver 903 cards; Whatman Grade 1 filter paper; Whatman FTA Elute; Ahlstrom 226 specimen collection paper; etc.), Noviplex Plasma Prep Cards, Polypropylene membrane, PVDF, Nitrocellulose membrane (Millipore Nitrocellular Hi Flow Plus) Polytetrafluoroethylene film, Mixed cellulose esters, Glass fiber media (e.g., Whatman uniflter plates glass fiber filter membrane, Agilent dried matrix spotting cards, Ahlstrom grade 8950, etc.), Plastic (e.g., Polyester, Polypropylene, Polythersulfene, poly (methacrylate), Acrylic polymers, polytetrafluoreten, etc.), natural and synthetic polymers (e.g., mixture of polymers, co-block polymers, etc.), sugars (e.g., pullulan, trehalose, maltose, sucrose, cellulose, etc.), polyamides (e.g., natural (e.g., wool, silk, etc.), synthetic (e.g., aramids, nylon, etc.), etc.), metals (e.g., aluminum, cadmium, chromium, cobalt, copper, iron, manganese, nickel, platinum, palladium, rhodium, silver, gold, tin, titanium, tungsten, vanadium, zinc, etc.), alloys (e.g., alloys of aluminium (e.g., Al-Li, alumel, duralumin, magnox, zamak, etc.), alloys of iron (e.g., steel, stainless steel, surgical stainless steel, silicon steel, tool steel, cast iron, Spiegeleisen, etc.), alloys of cobalt (e.g., stellite, talonite, etc.), alloys of nickel (e.g., German silver, chromel, mu-metal, monel metal, nichrome, nicrosil, nisil, nitinol, etc.), alloys of copper (e.g., beryllium copper, billon, brass, bronze, phosphor bronze, constantan, cupronickel, bell metal, Devarda's alloy, gilding metal, nickel silver, nordic gold, prince's metal, tumbaga, etc.), alloys of silver (e.g., sterling silver, etc.), alloys of tin (e.g., Britannium, pewter, solder, etc.), alloys of gold (electrum, white gold, etc.), amalgam, etc.), ELISPot plates, Immunoassay plates, Tissue culture plates, etc.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) of a luminescent complex are provided with less than 100% sequence identity and/or similarity to any portion of an existing luciferase (e.g., a firefly luciferase, a Renilla luciferase, an Oplophorus luciferase, enhanced Oplophorus luciferases as described in U.S. Pat. App. 2010/0281552 and U.S. Pat. App. 2012/0174242, herein incorporated by reference in their entireties). Certain embodiments involve the formation of bioluminescent complexes of peptide/dipeptide/tripeptide tags and a polypeptide component with less than 100% sequence identity with all or a portion (e.g., 8 or more amino acids, less than about 25 amino acids for peptides) of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). Certain embodiments involve the formation of bioluminescent complexes from peptide/dipeptide/tripeptide tags and a polypeptide component with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity with all or a portion (e.g., 8 or more amino acids, less than about 25 amino acids for peptides) of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide/dipeptide/tripeptide tags and a polypeptide component are provided with less than 100% sequence similarity with a portion (e.g., 8 or more amino acids, less than about 25 amino acids for peptides) of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide/dipeptide/tripeptide tags and a polypeptide component are provided with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence similarity with a portion (e.g., 8 or more amino acids, less than about 25 amino acids for peptides) of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide/dipeptide/tripeptide tags are provided that have less than 100% sequence identity and/or similarity with about a 25 amino acid or less portion of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence), wherein two of such peptides form a bioluminescent

complex when combined under appropriate conditions (e.g., stabilized by an interaction pair, brought into proximity by co-localization elements, etc.) with a polypeptide component having less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity and/or similarity with another portion SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide/dipeptide/tripeptide tags are provided that have less than 100% sequence identity and/or similarity with about a 25 amino acid or less portion of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence), wherein a pair of such peptide tags form a bioluminescent complex when combined under appropriate conditions (e.g., stabilized by an interaction pair, brought into proximity by co-localization elements, etc.) with a polypeptide component having less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity and/or similarity with another portion SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide/dipeptide/tripeptide tags are provided that have less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity and/or similarity with about a 25 amino acid or less portion of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence), wherein a pair of such peptides form a bioluminescent complex when combined under appropriate conditions (e.g., stabilized by an interaction pair, brought into proximity by co-localization elements, etc.) with a polypeptide having less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity and/or similarity with another portion of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). Similarly, polypeptide components are provided that have less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with a portion of SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence), wherein such polypeptide components form a bioluminescent complex when

combined under appropriate conditions with a pair of peptide tags having less than 100%, but optionally more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity and/or similarity with another portion SEQ ID NO: 1 (e.g., complete wild type Oplophorus luciferase sequence) and/or SEQ ID NO: 3 (e.g., complete NANOLUC sequence). In some embodiments, peptide tags with less than 100% sequence identity or similarity with SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, and/or SEQ ID NO: 10 are provided. In some embodiments, peptide tags with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO:9, and/or SEQ ID NO: 10 are provided. In some embodiments, peptide tags with less than 100% sequence identity or similarity with SEQ ID NO: 23, SEQ ID NO: 25, and/or SEQ ID NO: 29 are provided. In some embodiments, peptide tags with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 23, SEQ ID NO: 25, and/or SEQ ID NO: 29 are provided. In some embodiments, polypeptide components with less than 100% sequence identity or similarity with SEQ ID NO: 5 and/or SEQ ID NO: 8 are provided. In some embodiments, polypeptide components with less than 100% sequence identity or similarity with SEQ ID NO: 17 and/or SEQ ID NO: 27 are provided. In some embodiments, polypeptide components with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 5, SEQ ID NO: 8, and/or SEQ ID NO: 27 are provided. In some embodiments, polypeptide components with less than 100%, but more than 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 17 are provided.

In some embodiments, one or more (e.g., all) peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) in a set, kit, or system herein comprise 100% sequence identity with a portion of a luciferase (e.g., SEQ ID NO: 1, SEQ ID NO: 3, etc.).

In some embodiments, peptide tags (e.g., β9-like (e.g., SmTrip9) and β10-like (e.g., SmTrip10) peptides; β9/β10-like dipeptides; etc.) that find use in embodiments of the present invention include peptides with one or more amino acid substitutions, deletions, or additions from SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 29. In some embodiments, a peptide tag comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 13. In some embodiments, a peptide tag comprises 6 or fewer (e.g., 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 13. In some embodiments, a peptide tag comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 25. In some embodiments, a peptide tag comprises 6 or fewer (e.g., 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 25. In some embodiments, a peptide tag comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 23. In some embodiments, a peptide tag comprises 6 or fewer (e.g., 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 23. In some embodiments, a peptide tag comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 25. In some embodiments, a peptide tag comprises 6 or fewer (e.g., 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 25.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) that find use in embodiments of the present invention include the peptides, dipeptides, tripeptides, and polypeptides disclosed herein and in the tables provided herein. In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or

dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) that find use in embodiments of the present invention comprise one or more amino acid substitutions, deletions, or additions relative to the peptides, dipeptides, tripeptides, and polypeptides disclosed herein and in the tables provided herein. In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) that find use in embodiments of the present invention comprise at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with the peptides, dipeptides, tripeptides, and polypeptides disclosed herein and in the tables provided herein.

In some embodiments, dipeptides and tripeptides that find use in embodiments herein comprise any suitable combinations of the peptides described herein and/or listed in the tables herein.

In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) or a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) is linked (e.g., chemically) or fused to one or more additional elements (e.g., recognition element, interaction element, co-localization element, detectable element (e.g., a fluorophore (e.g., to facilitate BRET)), protein of interest, HALOTAG, etc.). In some embodiments, a peptide/dipeptide/tripeptide tag or polypeptide component is linked or fused to a cyOFP (e.g., in an Antares construct such as those described in U.S. Pat. No. 9,908,918; herein incorporated by reference in its entirety) or other fluorescent protein (e.g., to facilitate BRET). In some embodiments, a peptide/dipeptide/tripeptide tag or polypeptide component comprises one or more chemical modifications and/or unnatural amino acids or amino acid analogs to facilitate chemical conjugation of the polypeptide component with additional elements. In some embodiments, provided herein is a single peptide/dipeptide/tripeptide tag or polypeptide component fused to an acceptor fluorescent protein. In some embodiments, two or more peptide/dipeptide/tripeptide and/or polypeptide components are fused to an acceptor fluorescent protein (e.g., sandwhich fusion). In some embodiments, a peptide/dipeptide/tripeptide tag or polypeptide component is fused to two or

more acceptor fluorescent protein (e.g., sandwhich fusion). In some embodiments, a LgTrip polypeptide (e.g., a $\beta_{1\text{-}8}$-like polypeptide desccribed herein) is fused to a single fluorescent protein (e.g., cyOFP) or placed between two fluorescent proteins (e.g., two copies of a cyOFP) in a sandwhcih fusion.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) that find use in the present invention incorporate reactive groups suitable for chemical conjugation to an additional element (e.g., recognition element, interaction element, etc.). These reactive groups may be present on the N-terminus, C-terminus, or within the sequence. These reactive groups may optionally be attached to the peptide with a linker. In some cases, these peptide/dipeptide/tripeptide bearing reactive groups may be synthesized using standard synthesis and incorporated on an unnatural amino acid bearing the desired group. In some cases, the reactive group may be present on a natural amino acid (e.g. the sulfhydryl of cysteine). The additional element intended to react with a peptide tag bearing a reactive group may be a protein, an antibody, a nucleic acid, a small molecule such as a drug or a fluorophore or a surface. The peptide/dipeptide/tripeptide tag may incorporate a reactive group that is designed to react specifically with a reactive partner that has been chemically or biologically introduced on the additional element using bioorthogonal, or click, chemistry. An exemplary click reaction is copper catalyzed click where the peptide tag bears an alkyne or an azide, and the additional element bears the complementary group. Mixing these two species together in the presence of an appropriate copper catalyst causes the peptide to be covalently conjugated to the additional element through a triazole. Many other bioorthogonal reactions have been reported (for example Patterson, D. M., et al. (2014). "Finding the Right (Bioorthogonal) Chemistry." ACS Chemical Biology 9(3): 592-605.), and peptide tags and additional elements incorporating complementary reactive species are embodiments of the present invention.

Another embodiment of the present invention are peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and/or a polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) bearing reactive groups that react with naturally occurring amino acids. Exemplary reactive groups include maleimides for reaction with cysteine and succinimidyl esters for reaction with lysine. A

more comprehensive list of reactive groups can be found in Koniev, O. and A. Wagner (2015). "Developments and recent advancements in the field of endogenous amino acid selective bond forming reactions for bioconjugation." Chem Soc Rev 44: 5495-5551. These reactive groups may be chemically or biologically introduced on a peptide/dipeptide/tripeptide/polypeptide through peptide synthesis or through other chemical modification of a peptide tag. In some embodiments, the peptide tag exists in a protected form (Isidro-Llobet, A., et al. (2009). "Amino Acid-Protecting Groups." Chemical Reviews 109(6): 2455-2504; herein incorporated by reference in its entirety), preventing the peptide/dipeptide/tripeptide/polypeptide itself from reacting with the reactive group. These reactive groups may react with a protein in a selective fashion or in a random fashion, yielding either one conjugate or a mixture of conjugates. In some embodiments, either a defined single conjugate or a mixture can be used successfully in this invention.

Examples of peptides (e.g., β9-like (e.g., SmTrip9) and β10-like (e.g., SmTrip10) peptides; β9/β10-like dipeptides; etc.) described herein bearing reactive groups suitable for chemical conjugation to an additional element (e.g., recognition element, interaction element, etc.) are displayed in Figures 95-98. Other combinations of reactive groups and peptides/dipeptides/tripeptides/polypeptides are within the scope herein.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) described herein is fused or conjugated to a detectable element such as a fluorophore or fluorescent protein. In such embodiments, complementation to form the bioluminescent complex, and the resultant bioluminescence, results in BRET and excitement of/emission from the attached detectable element (e.g., fluorophore or fluorescent protein). In such embodiments, the bioluminescent complex is a BRET energy donor, and the detectable element (e.g., fluorophore or fluorescent protein) attached to a component of the complex (e.g., peptide tag or polypeptide component) is the BRET energy acceptor.

Suitable fluorophores for use in a BRET system with the tripartite/multipartite complementation systems described herein include, but are not limited to: xanthene derivatives (e.g., fluorescein, rhodamine, Oregon green, eosin, Texas red, etc.), cyanine derivatives (e.g., cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, merocyanine, etc.), naphthalene derivatives (e.g., dansyl and prodan derivatives), oxadiazole

derivatives (e.g., pyridyloxazole, nitrobenzoxadiazole, benzoxadiazole, etc.), pyrene derivatives (e.g., cascade blue), oxazine derivatives (e.g., Nile red, Nile blue, cresyl violet, oxazine 170, etc.), acridine derivatives (e.g., proflavin, acridine orange, acridine yellow, etc.), arylmethine derivatives (e.g., auramine, crystal violet, malachite green, etc.), tetrapyrrole derivatives (e.g., porphin, phtalocyanine, bilirubin, etc.), CF dye (Biotium), BODIPY (Invitrogen), ALEXA FLOUR (Invitrogen), DYLIGHT FLUOR (Thermo Scientific, Pierce), ATTO and TRACY (Sigma Aldrich), FluoProbes (Interchim), DY and MEGASTOKES (Dyomics), SULFO CY dyes (CYANDYE, LLC), SETAU AND SQUARE DYES (SETA BioMedicals), QUASAR and CAL FLUOR dyes (Biosearch Technologies), SURELIGHT DYES (APC, RPE, PerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phyco-Biotech), autofluorescent proteins (e.g., YFP, RFP, mCherry, mKate), quantum dot nanocrystals, etc. In some embodiments, a fluorophore is a rhodamine analog (e.g., carboxy rhodamine analog), such as those described in U.S. Pat. App. Ser. No. 13/682,589, herein incorporated by reference in its entirety.

In other embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) described herein is fused or conjugated to a detectable element such as a fluorophore or fluorescent protein (e.g., green fluorescent protein (GFP), enhanced GFP (EGFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and variants thereof. In other embodiments, a peptide tag or polypeptide component described herein is fused or conjugated to a cyan-excitable orange-red fluorescent protein (CyOFP), such as those described in U.S. Pat. No. 9,908,918; herein incorporated by reference in its entirety. In some embodiments, the CyOFP and BRET systems described in U.S. Pat. No. 9,908,918 find use with the peptide tags and/or polypeptide components described herein (e.g., CyOFP-(β_9-like peptide), CyOFP-(β_{10}-like peptide), CyOFP-(β_{1-8}-like polypeptide), CyOFP-(β_{9-10}-like peptide), CyOFP-(β_9-like peptide)-CyOFP, CyOFP-(β_{10}-like peptide)-CyOFP, CyOFP-(β_{1-8}-like polypeptide)-CyOFP, CyOFP-(β_{9-10}-like peptide) - CyOFP, etc.). In some embodiments, such systems comprising CyOFP linked to peptide/dipeptide/tripeptide tags and/or polypeptide components herein may be referred to herein as “Antares constructs” or “Antares systems.” Such BRET systems are particularly useful in certain imaging applications (Schaub, F. X., et al. (2015) "Fluorophore-NanoLuc

BRET Reporters Enable Sensitive In Vivo Optical Imaging and Flow Cytometry for Monitoring Tumorigenesis." Cancer Research 75(23): 5023-5033; herein incorporated by reference in its entirety).

In other embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) described herein are linked to fluorophores (e.g., directly or via a linker) for use in a constitutive BRET system (e.g., an Antares-like system). In constitutive BRET systems, the emission spectrum is shifted from the bioluminescence spectrum toward that of the fluorophore (e.g., for better sensitivity, lower scattering, desired emission wavelength, etc.). In other embodiments, peptide/dipeptide/tripeptide tags and/or polypeptide components described herein find use as functional sensors (e.g., for monitoring cellular/intracellular/intercellular processes (e.g., for detecting calcium flux or voltage (Suzuki, K., et al. (2016). "Five colour variants of bright luminescent protein for real-time multicolour bioimaging." Nature Communications 7: 13718.; Inagaki, S., et al. (2017). "Genetically encoded bioluminescent voltage indicator for multi-purpose use in wide range of bioimaging." Sci Rep 7: 42398; herein incorporated by reference in their entireties)), for imaging, for optogenetics, etc.).

In some embodiments, two or more of the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are attached to a single interaction element that can access multiple conformations. In one conformation, the peptide/dipeptide/tripeptide tags and the polypeptide are unable to form a luminescent complex. Upon changing conformation, i.e., in response to a stimulus, the peptide/dipeptide/tripeptide tags are brought into a conformation where they can form a bioluminescent complex. As an example, a SmTrip9 peptide and a SmTrip10 peptide can be conjugated to calmodulin such that they do not form a luminescent complex even in the presence of LgTrip and furimazine. Upon exposure to calcium, the conformational change of calmodulin bring the SmTrip9 peptide and SmTrip10 peptide into a position whereupon addition of LgTrip makes a complex that is bioluminescent in the presence of

furimazine. Many other biosensors for calcium and other stimuli (pH, voltage, etc.) are known in the literature.

In some embodiments, systems herein find use in multiplexable analyte detection. In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) that find use in the present invention are conjugated to both an interaction element and a reporter element. In some embodiments, the interaction element is an antibody or the like, and the reporter element is a small molecule fluorophore. In some embodiments, antibodies to different pathogens (e.g. Zika virus, Dengue virus, etc.) are conjugated to a peptide/dipeptide/tripeptide tag (e.g., a SmTrip9 peptide) and a fluorophore with a different and distinguishable wavelength. In this embodiment, the luminescent complex that is formed upon the antibody binding to its antigen emits light at the emission wavelength of the bound fluorophore due to bioluminescence resonance energy transfer. This allows the antibodies to all be present in the same well, device, etc., and the identity of the antigen detected to be determined by the color of the light emitted by the luminescent complex formed.

In some embodiments, polypeptide components (e.g., β1-8-like (e.g., LgTrip) polypeptide) that find use in embodiments of the present invention include polypeptides with one or more amino acid substitutions, deletions, or additions from SEQ ID NO: 5 and/or SEQ ID NO: 8. In some embodiments, a polypeptide component comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 11. In some embodiments, polypeptide component comprises 100 or fewer (e.g., 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 11. In some embodiments, a polypeptide component comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 17. In some embodiments, a polypeptide component comprises 100 or fewer (e.g., 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 17. In some embodiments, a polypeptide component comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%,

>98%, >99%) sequence identity or similarity with SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302. In some embodiments, a polypeptide component comprises 100 or fewer (e.g., 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 17, SEQ ID NO: 21, and/or SEQ ID NO: 302. In some embodiments, a polypeptide component comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 788. In some embodiments, polypeptide component comprises 100 or fewer (e.g., 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 788. In some embodiments, a polypeptide component comprises at least 40% (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >98%, >99%) sequence identity or similarity with SEQ ID NO: 789. In some embodiments, polypeptide component comprises 100 or fewer (e.g., 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or ranges there between) substitutions (e.g., conservative substitutions, semi-conservative substitutions, non-conservative substations, etc.) relative to SEQ ID NO: 789.

In some embodiments, a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) is linked (e.g., chemically) or fused to one or more additional elements (e.g., recognition element, interaction element, co-localization element, detectable element (e.g., a fluorophore (e.g., to facilitate BRET)), protein of interest, HALOTAG, etc.). In some embodiments, a polypeptide component is linked or fused to a cyOFP (e.g., in an Antares construct such as those described in U.S. Pat. No. 9,908,918; herein incorporated by reference in its entirety) or other fluorescent protein (e.g., to facilitate BRET). In some embodiments, a polypeptide component comprises one or more chemical modifications and/or unnatural amino acids or amino acid analogs to facilitate chemical conjugation of the polypeptide component with additional elements.

In some embodiments, a peptide tag (e.g., β9-like (e.g., SmTrip9) and β10-like (e.g., SmTrip10) peptides; β9/β10-like dipeptides; etc.) and/or peptide component is not identical to and/or is not exact subsequences of one or more (e.g., all) of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID

NO: 17, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 51, SEQ ID NO: 302 (or any combinations thereof). In other embodiments, a peptide tag and/or peptide component is identical to and/or is an exact subsequences one or more (e.g., all) of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and/or SEQ ID NO: 29, SEQ ID NO: 51, SEQ ID NO: 302 (or any combinations thereof).

In some embodiments, a polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide) corresponds to and comprises substantial sequence identity (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >99%, 100%) with a portion of SEQ ID NO: 3. For example, in some embodiments, a polypeptide component corresponds to, and comprises substantial sequence identity (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >99%, 100%) with positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 through 142, 143, 144, 145, 146, 147, 148, 149, 150, 152, 153 of SEQ ID NO: 3 (e.g., positions 1-148).

In some embodiments, a peptide tag (β10-like (e.g, SmTrip10) peptide) corresponds to and comprises substantial sequence identity (e.g., >40%, >50%, >60%, >70%, >80%, >90%, 100%) with a portion of SEQ ID NO: 3. For example, in some embodiments, a peptide tag corresponds to, and comprises substantial sequence identity (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >99%, 100%) with positions 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 163, or 164 through 166, 167, 168, 169, 170, or 171 of SEQ ID NO: 3 (e.g., positions 160-171).

In some embodiments, a peptide tag (β9-like (e.g., SmTrip9) peptide) corresponds to and comprises substantial sequence identity (e.g., >40%, >50%, >60%, >70%, >80%, >90%, 100%) with a portion of SEQ ID NO: 3. For example, in some embodiments, a peptide tag corresponds to, and comprises substantial sequence identity (e.g., >40%, >45%, >50%, >55%, >60%, >65%, >70%, >75%, >80%, >85%, >90%, >95%, >99%, 100%) with positions 142, 143, 144, 145, 146, 147, 148, 149, 150, 152, 153 through 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 163, or 164 of SEQ ID NO: 3.

In some embodiments, a polypeptide component e.g., β1-8-like (e.g., LgTrip) polypeptide), a first peptide tag (β9-like (e.g., SmTrip9) peptide) and a second peptide tag (β10-like (e.g., SmTrip10) peptide) together correspond to and comprise substantial sequence

identity (e.g., >40%, >50%, >60%, >70%, >80%, >90%, 100%) with at least 90% of the length of SEQ ID NO: 3.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) comprise one or more substitutions relative to SEQ ID NO: 1 and/or SEQ ID NO: 3. For example, in some embodiments, a polypeptide component comprises 40% or greater (e.g., 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%) sequence identity with SEQ ID NO: 3 or a portion thereof (e.g., SEQ ID NO: 11, SEQ ID NO: 17, etc.), but comprise a substitution at one or more of positions 4, 30, 42, and/or 106 relative to SEQ ID NO: 17. In some embodiments, a polypeptide component comprises an E4D substitution relative to SEQ ID NO: 17. In some embodiments, a polypeptide component comprises an A, D, E, G, K, L, M, N, Q, S, T, V, or Y at position 30 relative to SEQ ID NO: 17. In some embodiments, a polypeptide component comprises an A, C, F, G, I, L, M, S, T, or V at position 42 relative to SEQ ID NO: 17. In some embodiments, a polypeptide component comprises a D, K, or Q at position 106 relative to SEQ ID NO: 17.

In some embodiments, a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) is an artificial sequence that comprises 70% or greater (e.g., 75%, 80%, 85%, 90%, 95%, 100%, or ranges there between) sequence identity and/or sequence similarity with one or more of SEQ ID NOS: 19, 21, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, and 131 (or the r β1-5-like, β1-6-like, or β1-7-like portion thereof). In some embodiments, a polypeptide component is an artificial sequence that comprises all or a portion (e.g., 50 amino acids, 60 amino acids, 70 amino acids, 80 amino acids, 90 amino acids, 100 amino acids, 110 amino acids, 120 amino acids, 130 amino acids, 140, or more, or ranges there between) of one of SEQ ID NOs: 19, 21, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, and 131 (or the r β1-5-like, β1-6-like, or β1-7-like portion thereof). In some embodiments, a polypeptide component is a sequence consisting of one of SEQ ID NOs: 19, 21, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117,

119, 121, 123, 125, 127, 129, and 131 (or the r β1-5-like, β1-6-like, or β1-7-like portion thereof).

In some embodiments, a peptide/dipeptide/tripeptide tag ((e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) is an artificial sequence that comprises 70% or greater (e.g., 75%, 80%, 85%, 90%, 95%, 100%, or ranges there between) sequence identity and/or sequence similarity with one or more of the peptide sequences listed in Table 1, Table 9, Table 10, or dipeptide/tripeptide combinations thereof. In some embodiments, a peptide/dipeptide/tripeptide tag component is an artificial sequence that comprises all or a portion (e.g., 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14, or more, or ranges there between) of one of the peptide sequences listed in Table 1, Table 9, Table 10, or dipeptide/tripeptide combinations thereof. In some embodiments, a peptide/dipeptide/tripeptide tag component is a sequence consisting of one of the peptide sequences listed in Table 1, Table 9, Table 10, or dipeptide/tripeptide combinations thereof.

Although referred to herein as peptide/dipeptide/tripeptide e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof), in some embodiments, one or more of the peptide/dipeptide/tripeptide components of a bioluminescent complex within the scope herein are not attached to an interaction element, co-localization element, binding agent, protein of interest, molecule of interest, or any other moiety. In some embodiments, one or both of the peptide/dipeptide/tripeptide components interact with the polypeptide and other peptide/dipeptide/tripeptide components to form a luminescent complex without being fused or otherwise tethered to another element.

In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) of a luminescent complex, a co-localization element, and/or an interaction element comprises a synthetic peptide/polypeptide, a peptide/polypeptide containing one or more non-natural amino acids, a peptide/polypeptide containing one or more amino acid analogs, a peptide/polypeptide mimetic, a conjugated synthetic peptide (e.g., conjugated to a functional group (e.g., fluorophore, luminescent substrate, etc.)), etc.

Provided herein are compositions and methods that are useful in a variety of fields including basic research, medical research, molecular diagnostics, etc. Although the reagents and assays described herein are not limited to any particular applications, and any useful application should be viewed as being within the scope of the present invention, the following are exemplary assays, kits, fields, experimental set-ups, etc. that make use of the presently claimed invention.

Typical applications that make use of embodiments herein involve the monitoring/detection of protein dimerization (e.g., heterodimers, homodimers), protein-protein interactions, protein-RNA interactions, protein-DNA interactions, antibody (or other recognition element) binding to a target, nucleic acid hybridization, protein-small molecule interactions, analyte quantitation or detection, or any other combinations of molecular entities. In an exemplary embodiment, a first entity of interest is attached to a first peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a second entity of interest is attached to the second peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof). If a detectable signal is produced under the particular assay conditions (e.g., in the presence of a polypeptide component of the luminescent complex (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) and a coelenterazine or a coelenterazine analog substrate), then interaction and/or co-localization of the first and second entities is inferred. Such assays are useful for monitoring molecular interactions and/or localization under any suitable conditions (e.g., in vitro, in vivo, in situ, whole animal, etc.), and find use in, for example, drug discovery, elucidating molecular pathways, studying equilibrium or kinetic aspects of complex assembly, high throughput screening, proximity sensor, etc.

In some embodiments, the systems and methods provided herein are useful for the detection, quantification, analysis, characterization, etc. of: an analyte, analytes, co-localization of analytes, and/or molecular interaction of analytes. In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) is tethered/fused to an analyte. In some embodiments, a peptide/dipeptide/tripeptide tag is tethered/fused to a recognition element agent that binds to a target analyte.

Suitable analytes that find use (e.g., are analyzed) in embodiments herein include, but are not limited to: nucleic acids (e.g., DNA, RNA, miRNA, etc.), proteins (ex: bacterial antigens, viral antigens, biomarkers, antibodies, etc.), small molecules, toxins, biomarkers, environmental or food contaminants, surfactants, pathogens (e.g., viral antigens and proteins, bacterial antigens and proteins, etc.), drugs (e.g., therapeutic drugs, drugs of abuse, etc.), vitamins, cytokines, antibodies (e.g., autoantibodies, infectious disease exposure, therapeutic drug monitoring, anti-HLA transplantation rejection, etc.), cells, cell receptor proteins, biomarker based diagnostics, cell free nucleic acids and non-cell free nucleic acids (e.g., DNA, RNA, mRNA, miRNA, etc.), nucleic acid SNPs, extracted nucleic acids, non-amplified nucleic acid samples, genomic DNA, ssDNA, bacterial resistance genes, immunocomplexes (e.g., antigen:antibody complex; antigen:complement complex, etc.), blood sugars, hormones, metabolites, microbes, parasites, enzymes, transcription factors, metal ions/heavy metals, etc.

Suitable recognition elements or binding moieties that find use (e.g., fused/tethered to a peptide tag, binding to an analyte, etc.) in embodiments herein, include, but are not limited to: antibodies (e.g., monoclonal, polyclonal, recombinant, animal derived, autoantibody, biotherapeutic, etc.), antibody variable heavy chain, antibody variable light chain, antibody binding fragment (Fab) [F(ab)'2], camelid, single chain variable fragment (scFv), monomeric proteins, receptor domains, affibodies, monobodies, natural and derivatized nucleic acid aptamers (e.g., RNA aptamer, DNA aptamer, chemical modified aptamer, etc.), peptide nucleic acids (PNA), locked nucleic acids (LNA), hexitol nucleic acids (HNA), protein A, G, L, M and/or domains thereof, sequence specific oligonucleotide probes (e.g., DNA probe, RNA probe, etc.), small molecule drug, antibody-oligonucleotide conjugates, darpins, nanobodies, affimers, adhirons, anticalins, phage, magnetic particles (e.g., labeled directly or labeled with a tagged recognition element), nanoparticles (e.g., polystyrene nanospheres, etc.) labeled directed or labeled with a tagged recognition element, streptavidin, antigens, etc. In some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) is linked to an oligonucleotide recognition element or binding moiety. Such constructs may find use in nucleic acid (e.g., DNA, RNA, etc.) complementation and/or detection. Exemplary peptide/oligomer probes are depicted in

Figure 99. In such exempalry constructs, a peptide/dipeptide/tripeptide comprising a reactive group (e.g., azido group (e.g., N-terminal, C-terminal, internal, etc.) or other reactive group herein) is conjugated to an oligonucleotide comprising a complementary reactive group (e.g., alkyne group (e.g., 5'-terminal, 3'-terminal, interanl, etc.) or other reactive group herein). In an exemplary embodiment, peptide oligonuceleotide probes are prepard by combing components and reagents (e.g., oligonucleotide (1 mg,161 nmol, in water); triethylammonium acetate buffer (40 uL, 1M in water); aminoguanidine hydrochloride (8 uL, 50 mM in water); peptide (2.8 mg, 1.93 umol, in DMSO); copper(II) TBTA solution (10 mM in 1:1 water/DMSO); ascorbic acid solution (50 mM in water); final volume is 300 uL, 1:1 Water:DMSO); vortexing and heat for 30 min at 60ºC; filtering using Illustra NAP-5 column; exchanging buffer into TE buffer that is RNase and DNase free; and storing at -20ºC. In embodiments, in which a molecular interaction is being monitored/detected, peptide/dipeptide/tripeptide tags and a polypeptide component are selected that have affinities for each other such that a significant increase in signal is detectable/measurable upon interaction (e.g., binding) of the associated first and second entities. In some embodiments, one or both (or more) peptide/dipeptide/tripeptide tags have sufficiently low affinity for the other peptide tag and/or the polypeptide component that only background luminescence is detected in the absence of the interaction (e.g., binding) between the associated first and second entities. In other embodiments, the peptide/dipeptide/tripeptide tags and polypeptide component will form a complex and produce a signal in the absence of interaction between the associated first and second entities, but the signal is increased (e.g., 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 10^2-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold, or more or ranges there between) upon interaction (e.g., binding) of the associated first and second entities.

In embodiments in which a co-localization is being monitored/detected, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are selected that have affinities for each other, such that a signal from the luminescent complex is detectable/measurable even in the absence of an interaction (e.g., binding) of the associated first and second entities. In such embodiments, if the associated first and second entities co-localize (e.g., in the same tissue, in the same cell, in the same

subcellular compartment, etc.), the peptide/dipeptide/tripeptide tags and polypeptide component will form a complex and emit a signal (in the presence of coelenterazine or a coelenterazine analog), whether or not the first and second entities interact with each other. In some embodiments, two or more (e.g., both, all) of the peptide/dipeptide/tripeptide tags have sufficiently high affinity for the other components that luminescence is detected in the absence of the interaction (e.g., binding) between the associated first and second entities. In some embodiments, no significant increase in signal is detected upon interaction of the first and second entities. In other embodiments, the peptide/dipeptide/tripeptide tags and polypeptide component will form a complex and produce a signal in the absence of interaction between the associated first and second entities, but the signal is increased (e.g., 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 10^2-fold, 10^3-fold, 10^4-fold, 10^5-fold, 10^6-fold, or more or ranges there between) upon interaction (e.g., binding) of the associated first and second entities.

In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) having known characteristics (e.g., spectral characteristics, mutual affinity, etc.) are used to elucidate the affinity of, or understand the interaction of, an interaction pair of interest. In other embodiments, a well-characterized interaction pair is used to determine the characteristics (e.g., spectral characteristics, mutual affinity, etc.) of one or more elements of a set of peptide/dipeptide/tripeptide tags and a polypeptide component. In some embodiments, peptide/dipeptide/tripeptide tags and a polypeptide component having known characteristics (e.g., spectral characteristics, mutual affinity, etc.) are used to characterize/monitor the co-localization of a co-localization par of interest (e.g., under desired conditions).

Embodiments described herein may find use in drug screening and/or drug development. For example, the interaction of a small molecule drug or an entire library of small molecules with a target protein of interest (e.g., therapeutic target) is monitored under one or more relevant conditions (e.g., physiological conditions, disease conditions, etc.). Such an assay may comprise a first peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) attached to a drug candidate (or a library of candidates) and a second

peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) attached to a therapeutic target; luminescence in the present of the polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) and substrate indicates interaction and/or co-localization of the candidate and target.

Some embodiments herein find use in the diagnostic or criminal setting for monitoring for drugs (e.g., drugs of abuse in human) as well as for therapeutic drug monitoring of patients in biological samples. For example, two peptide/dipeptide/tripeptide tagged binding moieties (e.g., binding moieties separately tagged with peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) that recognize a drug analyte facilitate such embodiments. In some embodiments, a competitive dispalcement assay utilizing a peptide/dipeptide/tripeptide-tagged target in a system described herein to identify untagged target in a sample finds use in embodiments herein. Some embodiments find use in detecting environmental contamination, for example, soil samples, water supply, etc. being contaminated by a specific drug or other specific contaminant (e.g., small molecule contaminant).

In other embodiments, the ability of a drug (e.g., small molecule drug) or an entire library of drugs (e.g., small molecules) to enhance or inhibit the interactions between two entities (e.g., receptor and ligand, protein-protein, etc.) is assayed (e.g., by gain or loss of the bioluminescent signal). In some embodiments, drug screening applications are carried out in a high through-put format to allow for the detection of the binding of thousands, or tens of thousands, of different molecules to a target, or to test the effect of those molecules on the binding of other entities.

In some embodiments, provided herein is the detection of molecular interactions in living organisms (e.g., bacteria, yeast, eukaryotes, mammals, primates, human, etc.) and/or cells. In some embodiments, pep peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) fused to interaction (target) polypeptides are co-expressed in a cell or whole organism, and a signal is detected in the presence of a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) and substrate (e.g., coelenterazine or coelenterazine analog), wherein the signal is correlated to the formation of

the interaction complex. In some embodiments, cells are transiently and/or stably transformed or transfected with vector(s) coding for fusions comprising peptide tags and interaction elements. In some embodiments, CRISPR is utilized to generate cells that express fusions comprising peptide/dipeptide/tripeptide tags and interaction elements. In some embodiments, fusions (e.g., of a cellular target and a peptide/dipeptide/tripeptide or polypeptide described herein) generated by CRISPR replace endogenous protein (e.g., non-fused cellular target) and are regulated in a similar manner to endogenous protein. In some embodiments, such endogenous taging is used to monitor the level of the endogenously tagged protein, especially in complex systems such as live cells, whole organisms, etc. In some embodiments, transgenic organisms are generated that code for the necessary fusions (e.g., fusions comprising peptide tags and interaction elements) for carrying out the assays described herein. In other embodiments, vectors are injected into whole organisms.

In some embodiments, provided herein is the detection of molecular co-localization in living organisms (e.g., bacteria, yeast, eukaryotes, mammals, primates, human, etc.) and/or cells. In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) fused to co-localization (target) polypeptides are co-expressed in a cell or whole organism, and a signal is detected in the presence of a polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) and substrate (e.g., coelenterazine or coelenterazine analog), wherein the signal is correlated to the co-localization of the co-localization elements. In some embodiments, cells are transiently and/or stably transformed or transfected with vector(s) coding for fusions comprising peptide tags and co-localization elements. In some embodiments, CRISPR is utilized to generate cells that express fusions comprising peptide tags and co-localization elements. In some embodiments, transgenic organisms are generated that code for the necessary fusions (e.g., fusions comprising peptide tags and co-localization elements) for carrying out the assays described herein. In other embodiments, vectors are injected into whole organisms.

In certain embodiments, cells are engineered to express one or more peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof), polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide), or fusions thereof (e.g., with cellular targets) using gene transfer technology or

other engineering techniques. For example, the cells may be genetically engineered to express one or more peptide/dipeptide/tripeptide tags, polypeptide components, or fusions thereof (e.g., with cellular targets) using gene editing methodologies such as CRISPR (clustered regularly interspaced short palindromic repeat). The terms "CRISPR" or "CRISPR-Cas9," as used herein, refer to the various CRISPR-Cas9 and -CPF1 (and other) systems that can be programmed to target specific stretches of a genome and to edit DNA at precise locations. CRISPR-Cas9 gene editing systems are based on the RNA-guided Cas9 nuclease from the type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system (see, e.g., Jinek et al., Science, 337: 816 (2012); Gasiunas et al, Proc. Natl. Acad. Set U.S.A., 109, E2579 (2012); Garneau et al., Nature, 468: 67 (2010); Deveau et al., Annu. Rev. Microbiol, 64: 475 (2010); Horvath and Barrangou, Science, 327: 167 (2010); Makarova et al., Nat. Rev. Microbiol., 9, 467 (2011); Bhaya et al., Annu. Rev. Genet., 45, 273 (201 1); and Cong et al., Science, 339: 819-823 (2013); herein incorporated by reference in their entireties). CRISPR gene editing systems have been developed to enable targeted modifications to a specific gene of interest in eukaryotic cells (see, e.g., Cong et al., supra; Xiao-Jie et al., J. Med. Genet., 52(5): 289-96 (2015); U.S. Patent 8,697,359; Xie et al., Genome Res., 24(9): 1526-1533 (2014); Huang et al., Stem Cells, 33(5): 1470-1479 (2015); Smith et al., Molecular Therapy, 23(3): 570- 577 (2015); and U.S. Patent Application Publication 2014/0068797; herein incorporated by reference in their entireties). Methods for utilizing CRISPR technology for gene editing are described in, for example, Barrangou et al., Science 315, 1709-1712 (2007); Bolotin et al., Microbiology, 151, 2551-2561 (2005); Brouns et al., Science 321, 960-964 (2008); Cong et al., supra; Deltcheva et al., Nature 471, 602-607 (2011); Gasiunas et al., supra; Hale et al., Cell 139, 945-956 (2009); Jinek et al., Science 337, 816-821 (2012); Makarova et al., Biology Direct 2006, 1:7 (2006); Mali et al., Science 339, 823-826 (2013); Marraffini et al., Science 322, 1843-1845 (2008); Mojica et al., J Mol Evol 60, 174-182 (2005); Pourcel et al., Microbiology 151, 653-663 (2005); and Sapranauskas et al., Nucl. Acids Res. 39, gkr606–gkr9282 (2011); herein incorporated by reference in their entireties.

In some embodiments, one or more peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) are employed as a protein tag (e.g., within cells, within a whole animal). In such embodiments, the complement components to the

peptide/dipeptide/tripeptide tag(s) (e.g., polypeptide components, the other peptide/dipeptide/tripeptide tag, substrate) are applied to the system (e.g., cells, animal, etc.) (e.g., as part of a reagent) to detect/quantify the presence of tagged proteins.

In some embodiments, the small size of the peptide tags herein (e.g., β9-like (e.g., SmTrip9) and β10-like (e.g., SmTrip10) peptides) is useful for protein tagging.

In some embodiments, the components of the bioluminescent complexes herein (e.g., peptide/dipeptide/tripeptide tags herein (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof), polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are stable enough to exist in a suitable buffer for extended periods of time (e.g., in the presence of coelenterazine or a coelenterazine analog (e.g., furimazine) substrate). In certain embodiments, components of the bioluminescent complexes herein (e.g., peptide/dipeptide/tripeptide tags, polypeptide components, etc.) exhibit minimal detectable luminescence in the absence of the complementing components (e.g., even in the presence of coelenterazine or coelenterazine analog (e.g., furimazine) substrate). In some embodiments, optimized buffer conditions are utilized to meet criteria necessary for protein tagging.

The compositions and methods provided herein, as well as any techniques or technologies based thereon find use in a variety of applications and fields.

Provided herein are methods for the design and/or optimization of peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide), and the bioluminescent complexes that form therefrom. Any suitable method for the design of non-luminescent pairs/groups that are consistent with embodiments described herein, and/or panels thereof, is within the scope herein. In some embodiments, characteristics of peptide/dipeptide/tripeptide tags and polypeptide components, and combinations thereof are optimized by substitutions (e.g., substitution of natural amino acids, non-natural amino acids, amino acid analogs, etc.); such characteristics include, but are not limited to structural stability (e.g., of the peptide/dipeptide/tripeptide tag or polypeptide component, of a complex, etc.), expression, stickiness (e.g., to tubes, wells, etc.), brightness

(or complexes formed therefrom), affinity for other components of the bioluminescent complex, solubility, thermal and chemical stability, low autoluminescence, etc.

In certain embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and a polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are designed de novo to lack luminescence individually and exhibit luminescence upon association. In such embodiments, the strength of the interaction between the non-luminescent elements is insufficient to produce a bioluminescent signal in the absence of interaction elements to facilitate formation of the bioluminescent complex. In other embodiments, peptide/dipeptide/tripeptide tags and polypeptide components and/or bioluminescent complexes thereof are rationally designed, for example, using a bioluminescent protein as a starting point. For example, such methods may comprise: (a) aligning the sequences of three or more related proteins; (b) determining a consensus sequence for the related proteins; (c) providing fragments (e.g., one or more peptides/dipeptides/tripeptides and a polypeptide) of a bioluminescent protein that is related to the ones from which the consensus sequence was determined, wherein the fragments are individually substantially non-luminescent but exhibit luminescence upon interaction of the fragments; and (d) testing the fragments for the absence of luminescence when unassociated and luminescence upon association of the non-luminescent pair. In some embodiments, the fragments are mutated at one or more positions (e.g., in vitro, in silico, etc.), wherein said mutations alter the sequences of the fragments and result in optimization of characteristics.

In some embodiments, a peptide/dipeptide/tripeptide tag is a 'dark peptide,' or one that forms a complex with the other peptide tag and polypeptide components (e.g., with low or high affinity), but produces minimal or no luminescence. In some embodiments, a high affinity dark peptide/dipeptide/tripeptide finds use in inverse complementation or gain of signal assays for biosensors or for measuring inhibitors. In some embodiments, a low affinity dark peptide/dipeptide/tripeptide is used to bring down background luminescence of a complex for the detection of binding of a high affinity bright peptide/dipeptide/tripeptide tag to the complex.

In some embodiments, a peptide/dipeptide/tripeptide tag is a 'quencher peptide,' or one that contains a quencher moiety (e.g., DAB), and the quencher absorbs the light/energy

produced by either or both of a polypeptide component (e.g., the signal produced independent of a complementing peptide/dipeptide/tripeptide tags) and/or bioluminescent complex.

In some embodiments, the luminescent complexes herein find use in systems, methods, assays, devices, etc. that utilize BRET between the complex and a fluorophore (e.g., small molecule fluorophore, fluorescent protein (e.g., cyOFP)). In some embodiments, a fluorophore (e.g., small molecule fluorophore, fluorescent protein (e.g., cyOFP)) is linked or fused to an analyte, cellular target, etc. In some embodiments, a fluorophore (e.g., small molecule fluorophore, fluorescent protein (e.g., cyOFP)) is linked or fused to a peptide/dipeptide/tripeptide tag and/or polypeptide component. In some embodiments, energy is transferred from a bioluminescent complex to an energy acceptor. In certain embodiments, an energy acceptor is a fluorophore or other detectable chromophore. Suitable fluorophores include, but are not limited to: xanthene derivatives (e.g., fluorescein, rhodamine, Oregon green, eosin, Texas red, etc.), cyanine derivatives (e.g., cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, merocyanine, etc.), naphthalene derivatives (e.g., dansyl and prodan derivatives), oxadiazole derivatives (e.g., pyridyloxazole, nitrobenzoxadiazole, benzoxadiazole, etc.), pyrene derivatives (e.g., cascade blue), oxazine derivatives (e.g., Nile red, Nile blue, cresyl violet, oxazine 170, etc.), acridine derivatives (e.g., proflavin, acridine orange, acridine yellow, etc.), arylmethine derivatives (e.g., auramine, crystal violet, malachite green, etc.), tetrapyrrole derivatives (e.g., porphin, phtalocyanine, bilirubin, etc.), CF dye (Biotium), BODIPY (Invitrogen), ALEXA FLOUR (Invitrogen), DYLIGHT FLUOR (Thermo Scientific, Pierce), ATTO and TRACY (Sigma Aldrich), FluoProbes (Interchim), DY and MEGASTOKES (Dyomics), SULFO CY dyes (CYANDYE, LLC), SETAU AND SQUARE DYES (SETA BioMedicals), QUASAR and CAL FLUOR dyes (Biosearch Technologies), SURELIGHT DYES (APC, RPE, PerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phyco-Biotech), autofluorescent proteins (e.g., YFP, RFP, mCherry, mKate), quantum dot nanocrystals, etc. In some embodiments, a fluorophore is a rhodamine analog (e.g., carboxy rhodamine analog), such as those described in U.S. patent application Ser. No. 13/682,589, herein incorporated by reference in its entirety. In some embodiments, a fluorophore is a small molecule fluorophore; embodiments herein reciting a fluorophore may be read as or limited to a small molecule fluorophore. In some embodiments, a fluorophore is a fluorescent protein (e.g., cyOFP, GFP, CFP, etc.;

embodiments herein reciting a fluorophore may be read as or limited to a fluorescent protein (e.g., cyOFP, GFP, CFP, etc.).

In various embodiments, the bioluminescent complexes described herein, and components thereof, find use in a variety of different immunoassay concepts. For example, in some embodiments, a peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) is tethered/fused to a primary or secondary antibody to provide a method of detection for a particular analyte. As another example, a peptide tag is tethered/fused to an antibody-binding protein (e.g., protein A or protein G) and used to detect a specific antibody bound to a particular analyte (e.g., wherein the analyte is bound to the complementary peptide tag). As another example, a peptide/dipeptide/tripeptide tag is tethered/fused to streptavidin and used to detect a specific biotinylated antibody bound to a particular analyte (e.g., wherein the analyte is bound to the complementary peptide tag). As yet another example, peptide/dipeptide/tripeptide tags are tethered/fused to primary and secondary antibodies, where the primary antibody recognizes a particular analyte, and the secondary antibody recognizes the primary antibody. As still another example, a peptide/dipeptide/tripeptide tag is tethered/fused to an analyte and used in a competitive sandwich ELISA format. A peptide/dipeptide/tripeptide tag is tethered/fused conjugated to an analyte may also be used to detect antibodies capable of binding the analyte.

Various embodiments herein find use in small molecule detection via immunoassay. Exemplary embodiments comprise the use of a small molecule directly (e.g., identical or similar to the target small molecule) labeled with a first peptide/dipeptide/tripeptide described herein and a binding moiety for the target small molecule fused or linked to a peptide/dipeptide/tripeptide described herein. In the presence of polypeptide component and substrate (e.g., coelenterazine or coelenterazine analog), a bioluminescent signal is produced by the system. When the system is exposed to a sample (e.g., biological sample, environmental sample, etc.), the bioluminescent signal will be reduced if the small molecule target is present in the sample (the labeled small molecule will be competed out of the complex allowing, in some cases, quantitation of the small molecule target). Alternative configurations for such assays are also within the scope herein. In some embodiments, the target small molecule is a toxin (e.g., mycotoxin, etc.), metabolite (e.g., amino acid, glucose molecule, fatty acid, nucleotide, cholesterol, steroid, etc.), vitamin (e.g., vitamin A, vitamin

B1, vitamin B2, Vitamin B3, vitamin B5, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin H or vitamin K, etc.), coenzyme or cofactor (e.g., coenzyme A, coenzyme B, coenzyme M, coenzyme Q, cytidine triphosphate, acetyl coenzyme A, reduced nicotinamide adenine dinucleodtide (NADH), nicotinamide adenine (NAD+), nucleotide adenosine monophosphoate, nucleotide adenosine triphosphate, glutathione, heme, lipoamide, molybdopterin, 3'-phosphoadenosine-5'-phsphosulfate, pyrroloquinoline quinone, tetrahydrobiopterin, etc.), biomarker or antigen (e.g., erythropoietin (EPO), ferritin, folic acid, hemoglobin, alkaline phosphatase, transferrin, apolipoprotein E, CK, CKMB, parathyroid hormone, insulin, cholesteryl ester transfer protein (CETP), cytokines, cytochrome c, apolipoprotein AI, apolipoprotein AII, apolipoprotein BI, apolipoprotein B-100, apolipoprotein B48, apolipoprotein CII, apolipoprotein CIII, apolipoprotein E, triglycerides, HD cholesterol, LDL cholesterol, lecithin cholesterol acyltransferase, paraxonase, alanine aminotransferase (ALT), asparate transferase (AST), CEA, HER-2, bladder tumor antigen, thyroglobulin, alpha-fetoprotein, PSA, CA 125, CA 19.9, CA 15.3, leptin, prolactin, osteoponitin, CD 98, fascin, troponin I, CD20, HER2, CD33, EGFR, VEGFA, etc.), drug (cannabinoid (e.g., tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), etc.), opioid (e.g., heroin, opium, fentanyl, etc.), stimulant (e.g., cocaine, amphetamine, methamphetamine, etc.), club drug (e.g., MDMA, flunitrazepam, gama-hydroxybutyrate, etc.), dissociative drug (e.g., ketamine, phencyclidine, salvia, dextromethorphan, etc.), hallucinogens (e.g., LSD, mescaline, psilocybin, etc.), etc.), explosive (e.g., 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), pentaerythritol tetranitrate (PETN), etc.), toxic chemical (e.g., tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), 2-(dimethylamino)ethyl N, N-dimethylphosphoramidofluroidate (GV), VE, VG, VM, VP, VR, VS, or VX nerve agent), etc.

The systems and methods described herein find use in a wide variety of applications and formats. The following are non-exhaustive exemplary examples of methods and formats utilizing the systems described herein.

- In some embodiments, provided herein are intracellular two protein systems for dynamic protein-protein interaction analysis with SmTrip peptide-labeled proteins expressed as fusions via traditional transfection or endogenously tagged proteins via CRISPR; LgTrip can be used as a detection reagent either by co-transfection, of LgTrip, providing a stable cell line expressing LgTrip, or providing LgTrip in the

detection reagent and adding it to lysed cells expressing SmTrip-labeled proteins.(Figure 51A).

- In some embodiments, provided herein are intracellular three protein systems for dynamic protein-protein interaction analysis with SmTrip- and LgTrip-labeled proteins expressed as fusions via traditional transfection or as endogenously-tagged proteins generated via CRISPR (Figure 51B).
- In some embodiments, provided herein are target specific assays to measure analyte X with binding moiety A and binding moiety B (See Table A; purified genetic fusions or chemically conjugated SmTrip9 or SmTrip10 peptide) for a gain of signal assay (e.g. diagnostic test, non-cellular, etc.) (Figure 51C).
- In some embodiments, provided herein are target specific competition assays for analyte measurement through loss of signal (e.g. diagnostic test, noncellular, etc.) (Figure 51D). Such a system use a purified binding moiety A (e.g., purified genetic fusion or chemically conjugated comprising synthetic SmTrip9 or SmTrip10 peptide) that binds the tagged target analyte to generate light in the presence of LgTrip and a coelenterazine substrate or coelenterazine analog, which may be provided as part of a detection reagent. In the presence of sample analyte X, SmTrip9 or SmTrip10 peptide will compete with the sample analyte X to cause a loss of signal specific to the presence of the sample analyte in the sample and proportional to the concentration of the analyte.
- In some embodiments, two or three of the peptide tags peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) are linked (or fused) to recognition elements for proximal, but non-overlapping (mutually exclusive or distinct), epitopes on the same target analyte. A signal generated from the luminescent complex (e.g., in the presence of a substrate) indicates the presence of the target analyte.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in immunoassays

or various formats. Immunoassays employing the peptide/dipeptide/tripeptide tags and polypeptide components herein are not limited to full length antibodies and may also employ antibody fragments or non-antibody binding moieties (e.g., DARPins, aptamers, affimers, etc.). In an exemplary direct immunoassay (See, e.g., Figure 51E), two monoclonal or recombinant antibodies (mAbs or rAbs) against an analyte are labeled with β9-like (e.g., SmTrip9) and β10-like (e.g., SmTrip10) peptide tags; a polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide) of the luminescent complex is included as part of detection reagent (e.g., with substrate). For an exemplary indirect immunoassay (See, e.g., Figure 51F), generic reagents labeled with β9-like (e.g., SmTrip9) and β10-like (SmTrip10) peptide tags are used in combination with any paired antibody system specific to an analyte (e.g., mAb or rAb +Biotin-pAb, Biotin-mAb, or Biotin-rAb etc.); a polypeptide component of the luminescent complex is included as part of detection reagent (e.g., with substrate). An exemplary competition direct immunoassay (See, e.g., Figure 51G) is provided by labeling one antibody with a first peptide tag (β9- (e.g, SmTrip9) or β10-like (e.g., SmTrip10) peptide) and labeling a analyte with a second peptide tag (β10- (e.g., SmTrip10) or β9-like (e.g., SmTrip9) peptide); a polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide) of the luminescent complex is included as part of detection reagent (e.g., with substrate); loss of signal indicates the presence of unlabeled target analyte. To provide a competition indirect immunoassay (See, e.g., Figure 51H), one antibody is labeled with a first peptide tag (β9-(e.g., SmTrip9) or β10-like (e.g., SmTrip10) peptide), a generic binding reagent (e.g., streptavidin) is labeled with a second peptide tag (β10-(e.g., SmTrip10) or β9-like (e.g., SmTrip9) peptide), and analyte is labeled with a binding moiety for the generic binding reagent (e.g., biotin); a polypeptide component of the luminescent complex is included as part of detection reagent (e.g., with substrate); loss of signal indicates the presence of unlabeled test analyte. Alternative immunoassays utlizing other peptide/dipeptide/tripeptide/polypeptide combinations described herein are within the scpe of the present invention.

- In some embodiments, provided herein are homogeneous assays using peptide/dipeptide/tripeptide tag-labelled (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and

tripeptides thereof) recognition elements with the polypeptide component (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) as component of a detection reagent (e.g., along with a substrate for the luminescent complex).

- In some embodiments, provided herein are homogeneous assays utilizing peptide/dipeptide/tripeptide-tag-labelled (e.g., SmTrip9, SmTrip10, etc.) and/or polypeptide-component-labelled (e.g., LgTrip variants) recognition elements. In some embodiments, homogeneous assays are provided for detection/quantification of a single analyte or multiple analytes.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in sandwich hybridization assays (e.g. non-target amplified, amplified, etc.).
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in the detection of analyte(s) in liquid/solution phase or solid phase.
- In some embodiments, the pepeptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in surface-based assays (e.g., plate-based (e.g., microtiter plate), paper-based (e.g., Whatman protein saver 903 cards), plastic-based, swab-based, cuvette-based, membrane-based (e.g., PVDF, nitrocellulose, etc.), etc.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in lateral flow and other capillary driven based methods. In some embodiments, such lateral flow assays allow multiplexed detection/identification/characterization of analytes (e.g.,

pathogens). In some embodiments, lateral flow assays find use in performing immunoassays described herein.

- An exemplary multiplexed tripartite lateral flow assay for the detection and identification of pathogens using tripartite antibody fusions in a direct immunoassay is depicted in Figure 52. In this example, a set of monoclonal or recombinant antibodies (mAbs or rAbs), each fused to a peptide tag (e.g., β10-like (e.g., SmTrip10) peptides) are added to a liquid sample, the sample is passed over a detection window comprising a second set of mAbs or rAbs, each fused to a peptide tag (e.g., β9-like (e.g., SmTrip9) peptides), immobilized in lanes within the detection window, and each recognizing a distinct epitope on the same target as one of the mAbs or rAbs in the liquid sample. When the liquid sample is passed through the detection window in the presence of a polypeptide component and substrate (e.g., preloaded in the detection window, added with the sample, added separately to the device, etc.), luminescence in a particular lane indicates the binding of mAbs or rAbs to separate epitopes on a target, and thereby provide for detection and identification of the target. The above described assay, and alternatives thereof utilizing the systems and methods herein, may find use in providing various detection panels (e.g., Respiratory Panel: Streptococcus, Pseudomonas, Mycobacterium, Staphylococcus; Urinary Tract Panel: E. Coli, Klebsiella, Enterobacter, Streptococcus; Food Borne Panel: Shigella, Campylobacter, Salmonella, E. Coli, Listeria; Waste Water Management: Coliform panel; Panel for strain identification within one type of bacteria; etc.), as well as for other applications (e.g., toxin detection).
- An exemplary multiplexed tripartite lateral flow assay for the detection and identification of anti-viral antibodies, e.g., for disease diagnosis, using tripartite antibody fusions in a direct immunoassay is depicted in Figure 53. In this example, a sample is added to the lateral flow device and allowed to flow into a conjugation zone (e.g., pad). The conjugation zone comprises a generic antibody-binding agent (e.g., Protein L), tethered or fused to a first peptide tag (e.g., β10-like (e.g., SmTrip10) peptide). If antibodies are present in the sample, they will be bound by the labeled antibody-binding agent. A detection

window of the device comprises separate lanes, each comprising distinct immobilized viral antigens tethered or fused to a second peptide tag (e.g., β9-like (e.g., SmTrip9) peptide). As the labeled antibody flows from the conjugation zone into the detection window, the antibodies will bind to appropriate antigens, binging the peptide tags into proximity and producing a luminescent signal in the presence of the polypeptide component and substrate (e.g., preloaded in the detection window, added with the sample, added separately to the device, etc.). Such a device and assay would allow detection and discrimination of multiple viruses and viral infections using a single device/assay. For example, Zika, Dengue, and Chicungkunga could all be independently detected using a single test.

- In some embodiments, the details of the above lateral flow assays are carried out in a plate-based format for solution phase assay (e.g., with the binding moiety combinations in wells provided with a map). In some embodiments, such an assay is performed in a multiplexed dot blot/spot array assay format. In some embodiments, any multiplexed assays described herein in a particular format (e.g., lateral flow) may also be performed in alterantive formats described herein or understood in the field (e.g., dot blot, spot array, etc.).
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in aerosol-based detection (e.g., (1) protease to lyse cells, (2) spray detection reagents, (3) visualize to detect/quantify).
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use with isothermal amplification of nucleic acids.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-

like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use with rapid cycling PCR detection of nucleic acids.

- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in the detection of protein-protein interaction (e.g., between 2 proteins, between 3 proteins, etc.).
- In some embodiments, analysis of the assays and methods described herein is performed using stationary or portable devices and readers, a luminometer plate reader or handheld reader, smart phone camera or CCD camera, etc.
- In some embodiments, analyte is detected/quantified via gain of signal through recognition elements via luminometer or imaging based techniques.
- In some embodiments, analyte is detected/quantified measured via loss of signal through competitive displacement via luminometer or imaging based techniques.
- In some embodiments, systems and methods herein allow for multiple tags on each recognition element, either genetically or through chemical conjugation, thereby providing signal amplification by adding increasing stoichiometry of peptide tags per recognition element.
- In some embodiments, systems and methods herein find use in detection of native proteins in heterogeneous solutions.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in nucleic acid detection, for example, peptide-tagged complimentary recognition elements hybridize to a nucleic acid target sequence in tandem.
- In some embodiments, assays are provided herein for the detection of an antibody (e.g., antibody as analyte). One such assay is depicted in Figure 54. A first peptide tag (e.g., β9-like (e.g., SmTrip9) peptide) is fused or tethered to an antibody-binding protein (e.g., Protein L) and a second peptide tag (e.g., β10-like (e.g., SmTrip10) peptide) is fused or tethered to the analyte that the antibody is specific to; a polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide) of the luminescent

complex is included as part of detection reagent (e.g., with substrate); presence of the analyte specific antibody in a sample results in complex formation and luminescence.

- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in FISH-like applications utilizing bioluminescence or BRET for detection/quantification.
- In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in detection of nucleic acids (e.g., single stranded and/or double stranded DNA and/or RNA) via, for example amplification-free detection of nucleic acids. For example, as depicted in Figure 56, a pair of peptide tag-labelled nucleic acid probes, when hybridized to nearby locations on a nucleic acid target, will allow formation of a luminescent complex, facilitated by complementation with the nucleic acid target. Such and assay could be performed on a solid surface, in solution, for a single nucleic acid target, or multiplexed (e.g., using an array).
- In some embodiments, peptide/dipeptide/tripeptide tags (e.g., β6-like, β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use in lab-on-chip and/or microfluidics applications.
- In some embodiments, systems and methods herein find use in heterogeneous assays, such as, immunoassays (e.g., PCR amplification combined with homogeneous immunoassay analysis).

In some embodiments, a peptide/dipeptide/tripeptide-based sensor is provided is which a chemical (e.g., removal of a blocking moiety) or enzymatic (e.g., proteolytic cleavage) event is required to render a peptide tag capable of bioluminescent complex formation. For example, a protease is required to cleave a blocked dipeptide (e.g., incapable of bioluminescent complex formation) into two non-blocked peptides capable of complementation. In some embodiments, the peptide/dipeptide/tripeptide tags (e.g., β6-like,

β7-like, β8-like, β9-like (e.g., SmTrip9), and/or β10-like (e.g., SmTrip10) peptides, and/or dipeptides and tripeptides thereof) and polypeptide components (e.g., β1-5-like, β1-6-like, β1-7-like, β1-8-like (e.g., LgTrip) polypeptide) herein find use with bead-based assays, utilizing magnetic enrichment for increased assay sensitivity. One such assay is depicted in Figure 55. In such an assay, a magnetic particle is conjugated to a first peptide tag (e.g., β9-like (e.g., SmTrip9) peptide) and to a first binding agent directed to a first epitope on an analyte; a non-magnetic particle (e.g., polystyrene particle) is conjugated to a second peptide tag (e.g., β10-like (e.g., SmTrip10) peptide) and to a second binding agent directed to the first epitope or a second epitope on an analyte. The beads are combined with a sample, along with a polypeptide component (e.g., β1-8-like (e.g., LgTrip) polypeptide) of the luminescent complex. Magnetic separation is used to capture the magnetic beads and any components of the sample or other reagents bound thereto. Luminescence of the magnetically-captured elements is then detected in the presence of substrate for the luminescent complex. If the analyte is present in the sample, both the magnetic and non-magnetic beads will be captured, resulting in the capture of the luminescent complex. In the absence of analyte, the non-magnetic beads will not be captured, and the luminescent complex will not be formed. The above applications and formats are exemplary and non-limiting. Other embodiments consistent with the description herein are within the scope of the present invention. Systems comprising and method utilizing peptides, dipeptides, and polypeptides bearing structural (although not necessarily sequence identity) and functional correlation to portions of NanoLuc® commercial luciferase and/or natural luciferase from *Oplophorus gracilirostris*, and bioluminescent complexes formed by complementation thereof, are described herein. In particualr, detailed description is provided of complementaion between β_{1-8}-like (e.g., LgTrip) polypeptides and either β_{9}-like (e.g., SmTrip9) and β_{10}-like (e.g., SmTrip10) peptides or $\beta_{9/10}$-like dipeptides. However, embodiments herein are not limited to complementaion between β_{1-8}-like polypeptides (e.g., LgTrip) and β_{9}-like (e.g., SmTrip9) and β_{10}-like (e.g., SmTrip10) peptides or $\beta_{9/10}$-like dipeptides. In some embodiments, eptides, dipeptides, and polypeptides bearing structural (although not necessarily sequence identity) and functional correlation to portions of NanoLuc® commercial luciferase and/or natural luciferase from *Oplophorus gracilirostris* are provided. For example, also provided herein are systems and methods for complementation between a β_{1-5}-like polypeptide and β_{6-10}-like polypeptide; between a β_{1-2}-like dipeptide and β_{3-10}-like polypeptide; between a β_{1}-like

peptide, β_2-like peptide and β_{3-10}-like polypeptide; between a β_{7-8}-like dipeptide and $\beta_{9-10/1-6}$-like polypeptide fusion; between a β_{1-7}-like polypeptide and β_8-like, β_9-like, and β_{10}-like peptides; and/or between a β_{1-6}-like polypeptide and β_7-like, β_8-like, β_9-like, and β_{10}-like peptides.

In some embodiments, the peptides, dipeptides, triprptides, and/or polypeptides herein find use in translocation assays. In some embodiments, a translocation assay is composed of two components: a complementary polypeptide sensor (e.g., LgBiT-based, LgTrip-based, etc.) and a peptide/dipeptide/tripeptide-tagged protein of interest (POI). A variety of LgBiT sensors were genrated that localize at specific cellular compartments such as plasma membrane, nucleus, mitochondria and endoplasmic reticulum (ER) (Figure 152). These LgBiT sensors can be introduced to cells via transfection or establishment of stable cell lines. The POI is endogenously tagged with peptide/dipeptide/tripeptide complementary to the polypeptide (e.g., LgBiT) . Under stimuli, the POI translocates to a different cellular compartment where the polypeptide (e.g., LgBiT) sensor resides, complementation occurs leading to the assembly of pepdtide/polypeptide compl;ex (e.g., HiBiT•LgBiT) to yield luminescence signal (Figure 153). Thus, the translocation activity of POI is quantitatively measured via luminescence output. Experiments conducted during development of embodiments of the translocation assay are described in Example 89.

As described further herein, translocation assays can be designed as part of a modular system that includes, for example, the following components: 1) Cell lines expressing HiBiT-tagged proteins from endogenous loci; and 2) a LgBiT localization sensor. This principal design allows for the correlation of a luminescent signal with the presence of the target protein at a specific cellular localization. The sensors can include localization-specific sequences fused to a LgBiT affinity variant, as well as to HaloTag for validation and imaging of the target compartment. Localization-specific sequences can be short peptides that are derived from known protein cellular markers. In some embodiments, when short peptides are not specific enough to exclude the LgBiT sensor to the cellular compartment of choice, full length protein marker can be used to fuse to the sensor. DNA sequences encoding LgBiT localization sensors can be cloned into a circular double-stranded DNA plasmid, which can be delivered into HiBiT cell line. In some embodiments, the delivery method can be via lipid based transfection using Fugene HD transfection reagent, or via viral transduction using Lenti viral particles or BacMam. In some embodiments, when transfected/transduced, cells are

treated by an agonist, and HiBiT-tagged protein translocates to the cellular compartment where LgBiT sensor resides; complementation occurs and produces luminescence. In some embodiment, the agonists are small molecules that stimulate translocation of the target protein.

EXPERIMENTAL

Example 1

Further truncated version of (LgBiT) is activated by peptide

The luminescence of LgBiT (background and in the presence of complementary SmTrip10 pep86)(SEQ ID NO: 15, 25) was compared with a further truncated polypeptide (LgTrip 2098, SEQ ID NO: 17) lacking both the β10 and β9 strands of the full-length luciferase (background and in the presence of complementary pep263 (SEQ. ID 35)) (Figure 1).

E. coli KRX harboring LgBiT (SEQ ID NO: 11) or LgTrip 2098 (SEQ ID NO: 17) were grown for 20 h from a single colony in LB+amp (50 ug/mL) at 30ºC (275 rpm) in a volume of 50 mL. From these cultures, 100x dilutions were made into the same media and the cultures grown at 37C (275 rpm) for 3 h and then cooled to 25ºC before adding rhamnose (inducing agent for protein overexpression) to a final concentration of 0.2%. Cultures were then grown (induced) for 22 h at 25ºC (275 rpm) at which time cultures were harvested, and the resulting pellets stored at -20ºC until processing. To lyse cells, pellets were removed from -20ºC, resuspended in 50 mL of PBS pH 7.2, and taken through 3 sequential freeze thaw cycles (-70°C to 22°C), centrifuged to produce soluble fractions, and then kept cold (on ice) until assaying. Lysates and peptide(s) (25 nm final concentration) were incubated together for 10 minutes at 25ºC prior to the addition of NanoGlo® reagent . After addition of reagent, plates were incubated for another 5 min at 35ºC and read over time to measure luminescence (RLU) using a Tecan Infinite F500 plate reader.

Experiments conducted during development of embodiments herein demonstrate that both LgBiT (SEQ ID NO: 11) and LgTrip 2098 (SEQ ID NO: 17) produce some background luminescence, but the level is much higher for LgBiT. Data shows that both LgBiT and LgTrip 2098 produce more luminescence in the presence of their respective complementary peptide. The magnitude of the gain in signal in the presence of peptide is greater for LgTrip 2098. These data demonstrate that the further truncated LgBiT (and with the A51G

substitution) is activated by a single complementary peptide corresponding to the β10 and β9 beta strands that are absent from LgTrip 2098.

Example 2

LgTrip 2098 is activated by pair of separate β9 and β10-like peptides

The luminescence of LgTrip 2098 (SEQ ID NO: 31) was monitored over time in the presence of separate peptides corresponding to the β10 and β9 portions of the full-length luciferase (SmTrip10 pep86 (SEQ ID NO: 25) and SmTrip9 pep245) (SEQ ID NO: 23) (Figure 2). Similar experimental protocols were used as in Example 1; however, a 10x concentrated lysate was used, Peptides SmTrip10 pep86 and SmTrip9 pep245 were used at 500 nM, and 0.001% Prionex added to reactions. Experiments conducted during development of embodiments herein demonstrate that LgTrip 2098 (SEQ ID NO: 31) is activated by the addition of SmTrip10 pep86 and SmTrip9 pep245. Controls with no peptides added or only one of the peptides added produced near the background of the plate reader.

Example 3

LgTrip mutagenesis – round 1 (luminescence)

Overnight cultures used for sequencing were used to inoculate cultures (30ul of cells in 3ml of media+0.1% Rhamnose+0.15% glucose). Cells were grown overnight at 25ºC for 20 hours. Cells were diluted 10ul into 250ul of Passive Lysis Buffer (PLB) and allowed to lyse for 5 minutes. The lysate was mixed and then diluted 1:100 into PLB lysis buffer (0.3X PLB, 25mM HEPES pH 7.5, 0.001U/ml RQ DNase 1 (10ul in 990ul). 50ul of the diluted lysate was combined with 50ul of NanoGlo® buffer + 2uM pep263 (SmTrip9-10 dipeptide) (SEQ ID NO: 35) at a 1uM final concentration (saturating dipeptide concentration). Samples were incubated for 5 minutes, read on GloMax® Multi+ (GMM+) luminometer, and normalized to LgTrip 2098 (SEQ ID NO: 31) (Table 2).

Table 2. Relative luminescence of LgTrip variants compared to LgTrip 2098.

Clone #	Cell plate	Sequence	Secondary screen(normalize to 2098)
#7		F1L	1.1
#10		Q42L	1.8
#14		I44V, E63D, L142Q	2.5
#16		L30S	8.3
#19		N17D	3.0
#22		Y16C, I56T	2.6
#35		L142Q	1.8
#38		T2S, M106K	1.8
#39		E4D, V27A	3.7
#42		E4D	2.5

Example 4

LgTrip mutagenesis – round 1 (stability)

Experiments were conducted during development of embodiments herein to determine the stability of HisLink purified LgTrip mutants. LgTrip 2098 (SEQ ID NO: 31) , LgTrip 2098 (RH) (SEQ ID NO: 31) (column purified LgTrip 2098), #10, #14, #16, #19, #22, #35, #38, #39, and #42 polypeptides (Table 3) were diluted 1:1000 into PLB lysis buffer (2ul into 2ml). 100ul of each sample was transferred into one column of wells in a 96-well PCR tray. Samples were incubated at 37ºC, and aliquots were remove at various time-points. Samples were placed on ice when thermal treatment was complete. When all samples were processed, the PCR tray was equilibrated to room temperature. Samples were mixed and then diluted 1:100 in PLB lysis buffer (5ul into 495ul buffer). 50ul of each sample was combined with 50ul of NanoGlo® buffer reagent + 2uM pep263. The plate was incubated for 5 minutes and then read on GMM+. Results are depicted in Figure 3. Stability studies identified position 42 of LgTrip 2098 (SEQ ID NO: 31) as a position of interest for further analysis.

Table 3. Experimental nomenclature for LgTrip mutants (mutations relative to LgTrip 2098).

Clone #	Sequence
#10	Q42L
#14	I44V, E63D, L142Q
#16	L30S
#19	N17D
#22	Y16C, I56T
#35	L142Q
#38	T2S, M106K
#39	E4D, V27A
#42	E4D

Example 5

Position 42 site saturation (luminescence)

Experiments were conducted during development of embodiments herein to optimize the identity of the amino acid at position 42 of LgTrip 2098 (SEQ ID NO: 31) (Figure 4). *E. Coli* cultures (3ml) were prepared for each sample and grown overnight at 37°C in LB media+100ug/ml ampicillin. Cultures were then diluted in quadruplicate at a 20X concentration (10µl in 200µl) into induction media (LB+ampicllin+0.1% Rhamnose). Samples were grown at 37°C for 6 hours. Samples were then lysed with 0.3X PLB, 25mM HEPES pH 7.5, and 0.001U/ml RQ1 DNase (10µl of cells to 250µl of Lysis buffer). 50µl of the lysate was then combined with 50µl of NanoGlo® buffer+50µM furimazine+20nM of dipeptide 263 (SEQ ID NO: 35). Samples were measured on a BMG Clariostar luminometer. RLU values were normalized to LgTrip 2098 (SEQ ID NO: 31).) (Figure 4)

Example 6

37°C stability of purified LgTrip position 42 mutants

Experiments were conducted during development of embodiments herein to determine the stability of position 42 in LgTrip 2098 mutants (Figure 5). Polypeptides were diluted to 20nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 37°C in thermal cycler. Samples were removed at various time-points, placed on ice, and then allowed to equilibrate to room temperature. Samples were diluted to 0.2nM (5 in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each diluted sample was combined with 50µl of 50µM Furimazine+6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated

for 10 minutes and then read on GMM+. Half-life was calculated by non-linear regression (Figure 5).

Example 7

Site saturation of LgTrip

Experiments were conducted during development of embodiments herein to optimize the identity of the amino acid at various positions of LgTrip 2098 (SEQ ID NO: 31) (Figure 6). *E. coli* cultures (3ml) were prepared for each sample and grown overnight at 37°C in LB media+100µg/ml ampicillin. Cultures were then diluted in quadruplicate to a 20X concentration (10µl in 200µl) into induction media (LB+ampicllin+0.1% Rhamnose). Samples were grown at 37°C for 6 hours. Samples were then lysed with 0.3X PLB+25mM HEPES pH 7.5+0.001U/ml DNase (10µl of cells to 250µl of Lysis buffer). 50µl of the lysate was then combined with 50µl of NanoGlo® buffer+50µM furimazine+20nM of dipeptide 263 (SEQ ID NO: 35). Samples were measured on a BMG Clariostar luminometer. RLU values were normalized to LgTrip 2098 (SEQ ID NO: 31)(Figure 6).

Example 8

Mutations on LgTrip 3092 template

Experiments were conducted during development of embodiments herein to determine the effect of various amino acid substitutions relative to the LgTrip 3092 (SEQ ID NO: 19) variant (Table 4). *E. coli* cultures (3ml) were prepared for each sample and grown overnight at 37°C in LB media+100ug/ml ampicillin. Cultures were then diluted in quadruplicate to a 20X concentration (10µl in 200µl) into induction media (LB+ampicllin+0.1% Rhamnose). Samples were grown at 37°C for 6 hours. Samples were then lysed with 0.3X PLB+25mM HEPES pH 7.5+0.001U/ml DNase. (10µl of cells to 250µl of Lysis buffer). 50µl of the lysate was then combined with 50µl of NanoGlo® buffer+50µM furimazine+2nM of dipeptide 263 (SEQ ID NO: 35). The mutant lysates were further diluted 1:100 in PLB (5µl in 495µl). 50µl of the diluted lysate was added to 50µl of NanoGlo® buffer+50µM furimazine+6µM pep263 or 50µl of TBS+20µM LCS (furimazine)+6µM pep263 (SEQ ID NO: 35). Samples were measured on a GMM+ after a 10 minute incubation. RLU values were normalized to LgTrip 3092 (SEQ ID NO: 19)

Table 4. Relative luminescence of LgTrip variants compared to LgTrip 3092.

Sample	LCS 6uM 263	Nglo 6uM 263	Nglo 2nM 263
ATG 3092	1.0	1.0	1.0
V127A	4.2	3.5	3.7
Y16S	1.3	1.3	3.1
V119A	1.4	1.2	2.0
V127A+T128A	4.9	3.7	3.7
I117N	2.9	2.1	2.5
F120S	2.0	1.6	2.1
G122D	1.2	1.3	2.3
N105S	1.2	1.4	1.6
T126S	2.3	1.4	2.1
G101E	2.3	1.4	3.9
V36E+V102D+E115D	2.4	1.4	1.7

Example 9

Site saturation of LgTrip 3092 template

Experiments were conducted during development of embodiments herein to optimize the identity of the amino acid at various positions of LgTrip 3092 (SEQ ID NO: 19). *E. coli* cultures (3ml) were prepared for each sample and grown overnight at 37°C in LB media+100ug/ml ampicillin. Cultures were then diluted in quadruplicate to a 20X concentration (10µl in 200µl) into induction media (LB+ampicllin+0.1% Rhamnose). Samples were grown at 37°C for 6 hours. Samples were then lysed with 0.3X PLB+25mM HEPES pH 7.5+0.001U/ml DNase (10µl of cells to 250µl of Lysis buffer). 50µl of the lysate was then combined with 50µl of NanoGlo® buffer+50µM furimazine+2nM of dipeptide 263 (1nM final). Samples were measured on a BMG Clariostar luminometer. RLU values were normalized to LgTrip 3092 (Figure 7).

Example 10

Stability of LgTrip 2098 and LgTrip 3092 compared to LgBiT

Experiments were conducted during development of embodiments herein to compare the stability of positions in LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3092 (SEQ ID NO: 33) to LgBiT (SEQ ID NO: 11) (Figure 8). Purified LgTrip 2098, LgTrip 3092, and LgBiT samples were diluted to 20nM in TBS+0.01% BSA. 100µl of each sample was aliquoted into 200µl thin walled PCR tubes. Samples were incubated at 37°C in thermal cycler. Samples

were removed at various time-points and placed on ice. Samples were equilibrated to RT and then diluted to 0.2nM (5µl in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each sample was diluted with 50µl of 50µM Furimazine +6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated for 10 minutes and then read on GMM+ (Figure 8).

Example 11

Stability of LgTrip variants at 42°C and 60°C

Experiments were conducted during development of embodiments herein to compare the stability of LgTrip variants at 42°C and 60°C (Figure 9). The LgTrip variant samples were diluted to 20nM in TBS+0.01% BSA. 100µl aliquots were added into 200µl thin walled PCR tubes. Samples were incubated at 42°C or 60°C in thermal cycler. Samples were removed at various time-points and placed on ice. Samples were equilibrated to RT and then each diluted to 0.2nM (5µl in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each diluted sample was combined with 50µl of 50uM Furimazine+6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated for 10 minutes and then read on GMM+ (Figure 9).

Example 12

Affinity of LgTrip variants with SmTrip9 and SmTrip10

Experiments were conducted during development of embodiments herein to determine the affinity of various LgTrip variants for the SmTrip9- and SmTrip10-like peptides (Figure 10).

For the SmTrip9 pep286 (SEQ ID NO: 37) titration, purified LgTrip samples were diluted to 2nM in TBS+0.01% BSA+0.005% IGEPAL. Assay reagent containing TBS+0.01% BSA+0.005% IGEPAL+20µM furimazine+200µM SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) was prepared. 4uM of SmTrip9 pep286 (SEQ ID NO: 37) was added to the assay reagent and then serially diluted 500µl to 500µl in assay reagent containing Furimazine+200µM SmTrip10 pep86 (HiBiT; SEQ ID NO: 15). 25ul of each peptide titration was added to 25ul of diluted LgTrip solution. Luminescence was read on a plate reader at 10 and 15 minutes.

For the SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) titration, purified LgTrip samples were diluted to 2nM in TBS+0.01% BSA+0.005% IGEPAL. Assay reagent containing TBS+0.01% BSA+0.005% IGEPAL+20µM furimazine+4µM SmTrip9 pep286

(SEQ ID NO: 37) was prepared. 200uM of SmTrip10 pep86 (SmHiTrip; SEQ ID NO:25) was added to then assay reagent and then serially diluted 500µl to 500µl in assay reagent containing Furimazine+4µM SmTrip9 pep286 (SEQ ID NO: 37). 25ul of each peptide titration was added to 25ul of diluted LgTrip solutions. Luminescence was read on plate reader at 10 and 15 minutes.

Example 13

Stability of LgTrip variants (60°C)

Experiments were conducted during development of embodiments herein to compare the stability of LgTrip variants at 60°C (Figure 11). Purified LgTrip mutants were diluted to 20nM in TBS+0.01% BSA. 100µl of each sample was aliquoted into 200µl thin walled PCR tubes. Samples were incubated at 60°C in thermal cycler and then were removed at various time-points, placed on ice, equilibrated to room temperature, and then diluted to 0.2nM (5µl in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each diluted sample was combined with 50µl of 50µM Furimazine+6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated using GraphPad Prism non-linear regression (One-Phase Decay plateau set to zero).

Example 14

Comparison of kinetic profiles of LgBiT and LgTrip variants

Experiments were conducted during development of embodiments herein to compare the kinetic profiles of various LgTrip variants with NanoLuc® luciferase (SEQ ID NO: 3) and a LgBiT (SEQ ID NO: 11)/HiBiT (SmTrip10 pep86) (SEQ ID NO: 25) two component system (Figure 12). NanoLuc, LgBiT, LgTrip 2098 (SEQ ID NO: 31), LgTrip 3092 (SEQ ID NO: 33), and LgTrip 3546 (SEQ ID NO: 51) were diluted to 20µM in TBS+0.01% BSA+0.005% IGEPAL. Samples were diluted 1:100 (2µl in 198µl) and then 1:1000 (10µl in 10ml) or $10E^{-5}$ dilution total to a 0.2nM final concentration. To the LgBiT polypeptide, 200nM of SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) was added. To the LgTrip variants, 200µM SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) and 20µM SmTrip9 pep286 (SEQ ID NO: 37) were added. Samples were incubated for 15 minutes, 50ul of each enzyme/peptide dilution combined with either TBS+0.01% BSA+20µM Live Cell Substrate (LCS; Promega Cat. No. N205) or NanoGlo® buffer+50µM Furimazine, and immediately read on a GMM+ luminometer.

Example 15

Detecting protein-protein interactions with a tripartite system

Experiments were conducted during development of embodiments herein to demonstrate the use of a tripartite complementation system in detecting protein-protein interactions (Figure 13). Lysates containing FRB and FKBP fused to one each of SmTrip9 pep245 and SmTrip10 pep86 (Figure 13A) or SmTrip9 pep245 and SmBiT (Figure 13B) were added to purified LgTrip 2098 (SEQ ID NO: 31). Formation of the FRB/FKBP complex was induced with rapamycin and facilitated complementation of the tripartite system was monitored by luminescence.

Cell and lysate preparation. Cultures of each FRB-FKBP construct were grown overnight in LB+100ug/ml ampicillin. Cultures were induced (30µl in 3ml of culture) in LB+0.1% rhamnose+0.15% glucose+100ug/ml ampicillin and grow for 24 hours at 25ºC. 200µl of 10X Fastbreak Cell Lysis Reagent (Promega) was added to 2ml of culture 0.001U/ml RQ1 DNase. Cultures were incubated for 30 min at 4°C on a rotating mixer and then spun at 3500 rpm for 30 min. at 4°C. Cleared lysate was removed and placed into new tubes, frozen, and stored at -70°C.

Assay. Lysates were thawed, diluted 1:10 into TBS+0.1% BSA, and appropriate lysates combined. The lysates were divided, and 30nM rapamycin was added to one of the aliquots. 25µl of each lysate was combined with 25µl of LgTrip 2098 (SEQ ID NO: 31), diluted to 200nM in TBS+0.01% BSA, and incubated for one hour. 50µl of NanoGlo® Buffer+50µM furimazine was added, and luminescence was read on GMM+.

Example 16

Random library preparation and screening

A random library of variant LgTrip polypeptides (using template LgTrip 2098) (SEQ ID NO: 31) was generated and screened for complementation with the β9/β10 dipeptide (SEQ ID NO: 35)(pep263).

Template DNA from LgTrip 2098 (SEQ ID NO: 32) was diluted to 10ug/ml in water. Diversify™ PCR Random Mutagenesis Kit (63070-ClonTech) was used to prepare a random library of mutants. Library amplification products were band isolated and purified using WIZARD SV Gel and PCR Clean-Up System (A9281; Promega), cloned into pF4Ag, transformed into KRX competent cells (Promega), and plated onto LB agarose plates. Colonies were picked and place into wells of a 96-well plates with LB+ampicillin, and

samples were grown overnight at 37ºC with shaking. Overnight cultures were diluted 1:20 into induction media (LB+0.1% Rhamnose+0.15% glucose+100ug/ml ampicillin), and cultures were grown for 2-6 hours at 37ºC. 10ul of cells were added to 250ul of PLB lysis buffer (0.3X PLB, 25mM HEPES pH 7.0, 0.001U/ml DNase). 50ul of cell lysate was combined with 50ul of assay buffer (NanoGlo® buffer+50uM Furimazine+0.2nM of pep263). Plates were incubated for 5 minutes after reagent addition and then samples were read on ClarioStar luminometer. Clones that had improved luminescence compared to the template clone were selected for additional screening.

Approximately 6,000 LgTrip 2098-based variant clones were further screened, and favorable mutation sites were evaluated with site saturation mutagenesis. Favorable mutations following saturation mutagenesis were combined to derive the LgTrip clone LgTrip 3092 (SEQ ID NO: 19). Screening was repeated using LgTrip 3092 (SEQ ID NO: 19) as a template, and the resulting clone was LgTrip 3546 (SEQ ID NO: 51).

Example 17

Purification of LgTrip clones

50ml cultures of LgTrip mutants were induced in LB+0.1% Rhamnose+0.15% Glucose+amp. Cultures were spun and re-suspended in 4.5ml of Hepes pH 7.5+0.001U/ml DNase. 500ul of FastBreak™ Cell Lysis Reagent (Promega; V8571) was added, and samples were incubated on a rotating mixer for 1 hour at 4ºC. Samples were spun to clear lysate, and supernatant was transferred to a new tube. Using the HisLink™ Spin Protein Purification System, 500ul of HisLink™ Protein Purification Resin (Promega; V8821) was added to the samples, incubated for 2 hours at 4ºC on a rotating mixer, washed with HisLink wash/binding buffer, and eluted with elution buffer. Slide-A-Lyzer dialysis columns were used to exchange buffer to TBS.

Example 18

Stability comparison

Experiments were conducted during development of embodiments herein to compare the stability of activity of LgBiT (SEQ ID NO: 11) and LgTrip 2098 (SEQ ID NO: 31) over time (Figure 14). Diluted purified LgTrip 2098 and LgBiT to 20nM in TBS+0.01% BSA or in 0.3X PLB+25mM HEPES pH 7.5. Aliquoted 100µl of each sample into 200µl thin walled PCR tubes. Incubated samples at 37°C in thermal cycler, removed at various time-points, and placed on ice. Samples were equilibrated to room temperature and then diluted each

sample to 0.2nM (5µl in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each diluted sample was combined with 50µl of 50µM Furimazine+6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated for 10 minutes and then read on GMM+. Calculated half-life using GraphPad Prism non-linear regression (One-Phase Decay plateau set to zero).

Example 19

Stability of LgTrip in TBS+minimal BSA carrier

Experiments were conducted during development of embodiments herein to determine the stability of the activity of NanoLuc® (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), and LgTrip 3546 (SEQ ID NO: 51) in TBS and a minimal BSA carrier over time (Figure 15). NanoLuc, LgBiT, and LgTrip 3546 were diluted to 20nM in TBS+0.01% BSA. 100µl of each were aliquoted into 200µl thin walled PCR tubes. Samples were incubated at 60°C in thermal cycler, removed at various time-points, and placed on ice. Samples were equilibrated to room temperature and diluted to 0.2nM (5µl in 495µl) in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5). 50µl of each diluted sample was combined with 50µl of 50µM Furimazine+6µM pep263 (SEQ ID NO: 35) in NanoGlo® buffer. Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated using GraphPad Prism non-linear regression (One-Phase Decay plateau set to zero).

Example 20

Effect of salt on activity

Experiments were conducted during development of embodiments herein to determine the effect of salt concentration on the activity of NanoLuc® (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11) LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3546 (SEQ ID NO: 51) (Figure 16).

To test activity in the presence of salt, each enzyme was diluted to 1uM in TBS+0.01% BSA+0.01% Tergitol, and further diluted to 2nM in TBS+0.01% BSA+0.01% Tergitol . 4uM of pep 263 (SEQ ID NO: 35) was added to LgBiT, LgTrip 3546 (SEQ ID NO: 51), and LgTrip 2098 (SEQ ID NO: 31) and incubated for 30 minutes. A two-fold titration series was prepared for each, starting with 5M NaCl in 25mM Tris pH 7.5+0.01% Tergitol. 10uM furimazine was added to each sample of the titration series, and 5ul of each enzyme or enzyme+pep263 (SEQ ID NO: 35) were combined with 45ul of each substrate additive mixture. Plates were incubated for 3 minutes and then read on GMM+.

To test the effect of prolonged exposure to salt, each enzyme was diluted to 1uM, and a two-fold titration series was prepared starting with 5M NaCl in 25mM Tris pH 7.5+0.01% Tergitol. 2ul of each enzyme was added to 198ul of the NaCl titration (10nM final of each enzyme). The "no" additive control was TBS+0.01% BSA+0.01% Tergitol. Samples were incubated for 26 hours. After incubation, samples were diluted 1:10,000 into TBS+0.01% BSA+0.01% Tergitol (10ul in 990 two times). 4uM pep263 (SEQ ID NO: 35) was added to LgTrip 2098 (SEQ ID NO: 31), LgTrip 3546 (SEQ ID NO: 51), and LgBiT (SEQ ID NO: 11) in the second dilution. 50ul of each sample was combined with 50ul of NanoGlo® buffer+50uM Furimazine. Plates were incubated for 3 minutes and then read on GMM+.

Example 21

Effect of urea on activity

Experiments were conducted during development of embodiments herein to determine the effect of urea concentration on the activity of NanoLuc (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), LgTrip 2098 (SEQ ID NO: 31), and LgTrip 3546 (SEQ ID NO: 51) (Figure 17).

To test activity in the presence of urea, each enzyme was diluted to 1uM in TBS+0.01% BSA+0.01% Tergitol and further diluted to 2nM in TBS+0.01% BSA+0.01% Tergitol. 4uM of pep263 (SEQ ID NO: 35) was added to LgBiT (SEQ ID NO: 11), LgTrip 3546 (SEQ ID NO: 51), and LgTrip 2098 (SEQ ID NO: 31) and incubated for 30 minutes. A two-fold titration series was prepared for each, starting with 10M urea in 25mM Tris pH 7.5+0.01% Tergitol. 10uM furimazine was added to each sample of the titration series, and 5ul of each enzyme or enzyme+pep263 (SEQ ID NO: 35) were combined with 45ul of each substrate additive mixture. Plates were incubated for 3 minutes and then read on GMM+.

To test the effect of prolonged exposure to urea, each enzyme was diluted to 1uM, and a two-fold titration series was prepared starting with 10M urea in 25mM Tris pH 7.5+0.01% Tergitol. 2ul of each enzyme was added to 198ul of the urea titration (10nM final of each enzyme). The "no" additive control was TBS+0.01% BSA+0.01% Tergitol. Samples were incubated for 26 hours. After incubation, samples were diluted 1:10,000 into TBS+0.01% BSA+0.01% Tergitol (10ul in 990 ul two times). 4uM pep263 (SEQ ID NO: 35) was added to LgTrip 2098 (SEQ ID NO: 31), LgTrip 3546 (SEQ ID NO: 51), and LgBiT (SEQ ID NO: 11) in the second dilution. 50ul of each sample was combined with 50ul of

NanoGlo® buffer+50uM Furimazine. Plates were incubated for 3 minutes and then read on GMM+.

The results demonstrate that NanoLuc and NanoBiT are more susceptible to inactivation by urea compared to LgTrip 3546, while LgTrip 2098 is the least effected by urea. The exposure results demonstrate that LgTrip 3546, LgTrip 2098, and LgBiT regain activity upon prolonged treatment with urea indicating that activity of these polypeptide may be negatively affected by contaminating proteins, and that denaturation of these contaminants enhances activity.

Example 22

Effect of pH on activity

Experiments were conducted during development of embodiments herein to determine the effect of pH on the activity of NanoLuc® (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), LgTrip 2098 (WT) (SEQ ID NO: 31), and LgTrip 3546 (SEQ ID NO: 51) (Figure 18).

A universal buffer was prepared containing 25mM of each: NaCitrate, MES, PIPES, HEPES, TAPS, and Thiourea in 0.5% Tergitol. The buffer was divided into 8 aliquots of 20 ml, and NaOH or HCl was added to create a pH titration series.

To test effect of pH on activity, each enzyme was diluted to 1uM and then diluted to 0.4nM in 3ml of TBS+0.01% BSA+0.01% Tergitol. 4uM pep263 (SEQ ID NO: 35) was added to LgBiT, LgTrip 2098, and LgTrip 3546. Assay reagent for each pH buffer tested (Table 5) (20ul of furimazine in 980 of buffer). 10ul of each enzyme/peptide dilution was combined with 50ul of assay reagent. Plates were incubated for 3 minutes and read on GMM+.

Table 5. Buffers.

Component	MW(g/mol)	g
Na Citrate	294.1	1.47
MES	195.24	0.98
PIPES	302.37	1.51
Hepes	238.3	1.19
TAPS	243.3	1.22
Thiourea	76.12	0.53

To test the effect of prolonged exposure varying pH, each enzyme was diluted to 1uM in TBS+0.01% BSA+0.01% Tergitol, which was then diluted to 20 nM in each buffer. T=0 samples were mixed and then diluted 1:10 into 200mM HEPES pH 7.5+0.01% BSA+0.01% Tergitol and stored at 4ºC. T=8 samples were mixed and then diluted 1:10 into 200mM HEPES pH 7.5+0.01% BSA+0.01% Tergitol store at 4ºC. T=24 samples were mixed and then diluted 1:10 into 200mM HEPES pH 7.5+0.01% BSA+0.01% Tergitol store at 4ºC. To perform the assay, LgTrip and LgBiT were diluted 1:10 in TBS+0.01% BSA+0.01% Tergitol+4uM SmTrip10 pep286 (SEQ ID NO: 35), and NanoLuc was diluted into TBS+0.01% BSA+0.01% Tergitol. 40ul of each sample was diluted with 40ul of NanoGlo® buffer+40uM furimazine, incubated for 3 minutes, and then read on GMM+.

The results indicate that LgTrip is resistant to a wide pH range.

Example 23

Autoluminescence

Experiments were conducted during development of embodiments herein to compare the autoluminescence of LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51). Each was diluted to 3uM in DPBS+0.01% BSA. Three-fold serial dilutions were prepared of each in DPBS+0.01% BSA (300 ul to 700ul) and placed in wells of a 96-well plate. The last row of the plate contained the furimazine controls (n=12). 50ul of each titration (or controls) were combined with 50ul of NanoGlo® buffer+50uM furimazine, incubated for 3 minutes, and then read on GMM+. LgTrip (i.e., LgTrip 3546) exhibited significantly reduced autoluminescence compared to LgBiT (Figure 19).

Example 24

Complementation of LgTrip with β9/β10 dipeptide

Experiments were conducted during development of embodiments herein to determine the capacity of a β9/β10 dipeptide (e.g., pep263) (SEQ ID NO: 35) to form a bioluminescent complex with either LgTrip 3546 (SEQ ID NO: 51) or LgBiT (SEQ ID NO: 11). The luminescence of such a complex was compared with the luminescence of complexes of LgTrip 3546 with β9/β10 dipeptide and LgBiT with either SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) or the dipeptide, pep263 (SEQ ID NO: 35) (Figure 20). SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) and pep263 (SEQ ID NO: 35) were diluted to 2nM in H_2O. A 3X dilution series was prepared of each peptide in TBS+0.01% BSA starting at 300nM. Solutions of 200nM LgTrip 3546 and LgBiT were prepared in NanoGlo®

buffer+50uM furimazine (NanoGlo® reagent). 50ul of each titration were combined with 50ul of each NanoGlo® reagent, and luminescence was read after a five minute incubation. Signal/background was calculated by dividing the amount of peptide dependent RLU by the background reading. Results demonstrate that the dipeptide has a K_d ~2-3X higher than HiBiT which accounts for lower RLU at low peptide concentration. The background of LgBiT decreases signal to background. RLU values for LgBiT/dipeptide and LgTrip/dipeptide were equal.

Example 25

Comparison of LgTrip 2098 & LgTrip 3546 complementation with SmTrip10 and SmTrip9

Experiments were conducted during development of embodiments herein to demonstrate complementation of LgTrip 2098 (SEQ ID NO: 31) & LgTrip 3546 (SEQ ID NO: 51), respectively, with SmTrip10 and SmTrip9 peptides, facilitated by the rapamycin-induced binding of SmTrip9 pep245-bound FKBP to SmTrip10 pep86-bound Frb (Figure 21). FKBP-SmTrip9 pep245, SmTrip10 pep86-Frb, and LgTrip 3546 or LgTrip 2098 were transiently transfected into HEK293 cells (20,000 cells per well/96-well plate). Samples were exposed to serial dilutions of rapamycin (to induce FKBP/Frb complex formation) and 10μM furimazine, and luminescence was measured. Results demonstrate that the affinity of SmTrip10 pep86 is ~10X lower for LgTrip 3546 compared to LgTrip 2098.

Example 26

Affinity of various SmTrip10 sequences

Experiments were conducted during development of embodiments herein forming luminescent complexes between various SmTrip10 pep286 (HiBiT; SEQ ID NO: 25) sequences and LgTrip 3546 (SEQ ID NO: 51)/SmTrip9 pep286 (SEQ ID NO: 37) (Figure 22). Enzymes were diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, serial dilutions (100ul into 900ul) were prepared in TBS+0.01% BSA+0.01% Tergitol (2X to make 2nM), and 2nM sample was diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500ul into 4.5ml). A 2X dilution series was prepared of each SmTrip10-like peptide in TBS+0.01% BSA+0.01% Tergitol+20uM of SmTrip9 pep286 (SEQ ID NO: 37) starting at 100uM. 50ul of diluted LgTrip 3546 (SEQ ID NO: 51) was combined with 50ul of the peptide titration and incubated for 10 minutes at room temperature. 100ul of TBS+0.01% BSA+0.01%

Tergitol+20uM Furimazine was added to each sample, samples incubated for 10 minutes, and then read on GMM+.

Example 27

Inverse dipeptide

Experiments were conducted during development of embodiments herein to compare the capacity of dipeptides having opposite beta strand order (e.g., β9-β10 vs. β10-β9) to activate complement polypeptides (Figure 23). LgTrip 3546 (SEQ ID NO: 51) and LgTrip 2098 (SEQ ID NO: 31) were diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, and serial dilutions of each were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol. The 2nM sample was diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500ul into 4.5ml). 20uM stocks of each dipeptide (pep326 (SEQ ID NO: 179) and pep263 (SEQ ID NO: 35)) were prepared in TBS+0.01% BSA+0.01% Tergitol. 2X serial dilutions of each peptide were prepared TBS+0.01% BSA+0.01% Tergitol (250ul in 250ul). 50ul diluted LgTrip 2098 and LgTrip 3546 was combined with 50ul of the each peptide series and incubated at room temperature for 20 minutes. 100ul of LCS (Live cell substrate; Promega Catalog No. N205) in TBS (20uM) was added, and samples were incubated for 3 minutes and then read on GMM+.

Example 28

Comparison of dipeptides comprised of SmTrip9 (SEQ ID NO: 23) and either SmHiTrip (SEQ ID NO: 25) or SmBiT (SEQ ID NO: 13) for the SmTrip10 component

Experiments were conducted during development of embodiments herein to compare the capacity of dipeptides comprising the SmHiTrip (SEQ ID NO:25) or SmBiT (SEQ ID NO:13) sequence to activate complement polypeptides (Figure 24). LgBiT, LgTrip 2098, and LgTrip 2899 (SEQ ID NO: 364) were diluted to 200nM into TBS+0.01% BSA. Polypeptides were further diluted 1:100 into NanoGlo® buffer+50uM Furimazine (30ul in 3 ml). Pep263 (SEQ ID NO: 35) and pep274 (SEQ ID NO: 147) were diluted into TBS+0.01% BSA to 5uM. 50ul of each LgBiT/LgTrip dilution were combined with 50ul of peptide dilution, incubated 5 minutes, and then read on GMM+.

Example 29

Additions/deletions of C-terminus of LGTrip 3546

Experiments were conducted during development of embodiments herein to determine the effect of C-terminal additions/deletions and/or corresponding

additions/deletions to peptide tags on complementation and luminescence (Figure 25). Exemplary tested peptides and polypeptides are listed in Table 6.

Table 6. Peptide tags and polypeptide components comprising additions/deletions

SmTrip9 SmTrip10
LITPDGSMLFRVTINSVSGWRLFKKIS Note: SmTrip9 peptides contain additional SSWKR sequence at their N-termini.

LgTrip	C-term	SmTrip9	ID	SmTrip10	ID
ATG-3575 (aka LgTrip+GS)	--LITPDGS	MLFRVTINS	292	VSGWRLFKKIS	86
ATG-3572 (aka LgTrip+G)	--LITPDG	SMLFRVTINS	291	VSGWRLFKKIS	86
ATG-3573 (aka LgTrip-D)	--LITP	DGSMLFRVTINS	293	VSGWRLFKKIS	86
ATG-3574 (aka LgTrip-PD	--LIT	PDGSMLFRVTINS	294	VSGWRLFKKIS	86
ATG-3575 (aka LgTrip+GS)	--LITPDGS	MLFRVTINSV	297	SGWRLFKKIS	219
ATG-3572 (aka LgTrip+G)	--LITPDG	SMLFRVTINSV	296	SGWRLFKKIS	219
ATG-3573 (aka LgTrip-D)	--LITP	DGSMLFRVTINSV	298	SGWRLFKKIS	219
ATG-3574 (aka LgTrip-PD	--LIT	PDGSMLFRVTINSV	299	SGWRLFKKIS	219
ATG-3575 (aka LgTrip+GS)	--LITPDGS	MLFRVTINSVS	302	GWRLFKKIS	206
ATG-3572 (aka LgTrip+G)	--LITPDG	SMLFRVTINSVS	301	GWRLFKKIS	206
ATG-3573 (aka LgTrip-D)	--LITP	DGSMLFRVTINSVS	303	GWRLFKKIS	206
ATG-3574 (aka LgTrip-PD	--LIT	PDGSMLFRVTINSVS	304	GWRLFKKIS	206
ATG-3575 (aka LgTrip+GS)	--LITPDGS	MLFRVTIN	308	SVSGWRLFKKIS	157
ATG-3572 (aka LgTrip+G)	--LITPDG	SMLFRVTIN	307	SVSGWRLFKKIS	157
ATG-3573 (aka LgTrip-D)	--LITP	DGSMLFRVTIN	309	SVSGWRLFKKIS	157
ATG-3574 (aka LgTrip-PD)	--LIT	PDGSMLFRVTIN	310	SVSGWRLFKKIS	157
ATG-3575 (aka LgTrip+GS)	--LITPDGS	MLFRVTI	312	NSVSGWRLFKKIS	158
ATG-3572 (aka LgTrip+G)	--LITPDG	SMLFRVTI	311	NSVSGWRLFKKIS	158
ATG-3573 (aka LgTrip-D)	--LITP	DGSMLFRVTI	313	NSVSGWRLFKKIS	158
ATG-3574 (aka LgTrip-PD)	--LIT	PDGSMLFRVTI	314	NSVSGWRLFKKIS	158
ATG-3546 (aka LgTrip)	--LITPD	GSMLFRVTINSV	295	SGWRLFKKIS	219
ATG-3546 (aka LgTrip)	--LITPD	GSMLFRVTINSVS	300	GWRLFKKIS	206
ATG-3546 (aka LgTrip)	--LITPD	GSMLFRVTIN	305	SVSGWRLFKKIS	157
ATG-3546 (aka LgTrip)	--LITPD	GSMLFRVTI	306	NSVSGWRLFKKIS	158

Addition/deletion polypeptides were grown in 50ml cultures, pelleted, and resuspended in 10ml of 100mm HEPES pH 7.5+0.001U/ml DNase. 1ml of Fastbreak Cell Lysis Reagent (Promega Corporation) and 1ml of HisLink Resin (Promega Corporation) were added and incubated on a rotating shaker for 3 hours at 4ºC. Resin was allowed to settle, and samples were washed 4X with 100mM HEPES pH 7.5+10mM Imidazole. Polypeptides were eluted twice into 500ul HisLink Elution buffer. Thermo dialysis tubes were used to equilibrate to 1X TBS.

Enzymes were diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, and serial dilutions were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol. 2nM samples were diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500ul into 4.5ml). SmTrip9- and SmTrip10-like peptides were combined with a polypeptide complement according to Table 7 and incubated for 10 minutes at room temperature. 100ul of

TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, incubated for10 minutes, and then read on GMM+.

Table 7. Polypeptide/peptide combinations tested.

Group 1	SmTrip 9	SmTrip10
3546	286	86
3575	292	86
3572	291	86
3573	293	86
3574	294	86

Group 2	SmTrip 9	SmTrip10
3546	286	86
3575	297	219
3572	296	219
3573	298	219
3574	299	219

Group 3	SmTrip 9	SmTrip10
3546	286	86
3575	302	206
3572	302	206
3573	303	206
3574	304	206

Group 4	SmTrip 9	SmTrip10
3546	286	86
3575	312	158
3572	312	158
3573	312	158
3574	312	158

Group 5	SmTrip 9	SmTrip10
3546	286	86
3546	295	219
3546	300	206
3546	305	157
3546	306	158

Example 30

Polypeptide/peptide and/or peptide/peptide overlap

Experiments were conducted during development of embodiments herein to determine the sequence overlap between the polypeptide component and a peptide corresponding to the β-strand or between the two peptides (Figure 26). In such experiments, a polypeptide component and peptide, or the two peptides, each comprise amino acids corresponding to the same amino acids in a base luciferase.

Polypeptides were diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol. Serial dilutions (100ul into 900ul) were prepared in TBS+0.01% BSA+0.01% Tergitol. 2nM of each polypeptide sample were diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500 into 4.5ml). Polypeptides and peptides were combined, i.e., 50ul of each LgTrip mutant with 50ul of the peptide, according to Table 8. Reactions were incubated for 10 minutes at room

temperature. Next, 100ul of TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, and reactions were incubated for 10 more minutes prior to reading on a GMM+ luminometer.

Table 8. Polypeptide/peptide combinations tested.

Group1	SmTrip9	SmTrip10
3546	286	86
3546	286	219
3546	286	206
3546	286	157
3546	286	158

Group2	SmTrip9	SmTrip10
3575	286	86
3575	286	219
3575	286	206
3575	286	157
3575	286	158

Group3	SmTrip9	SmTrip10
3546	293	86
3546	293	219
3546	293	206
3546	293	157
3546	293	158

Group4	SmTrip9	SmTrip10
3575	293	86
3575	293	86
3575	293	86
3575	293	86
3575	293	86

Group5	SmTrip9	SmTrip10
3546	294	86
3546	294	219
3546	294	206
3546	294	157
3546	294	158

Group6	SmTrip9	SmTrip10
3575	294	86
3575	294	219
3575	294	206
3575	294	157
3575	294	158

Example 31

The identity of the β9-peptide alters the affinity of the β10-peptide

Experiments were conducted during development of embodiments herein to determine the sequence overlap between the polypeptide component and a peptide corresponding to the β-strand or between the two peptides (Figure 27). These results show that the sequence of the β9 strand peptide (SmTrip9) can impact the affinity of the β10 strand peptide (SmTrip10).

SmTrip9 titration

LgTrip 3546 (SEQ ID NO: 51) was diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, and serial dilutions were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol. 2nM polypeptide samples were diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500into 4.5ml).

A 2X dilution series was prepared of each SmTrip9 peptide in TBS+0.01% BSA+0.01% Tergitol+100uM of SmTrip10 pep86 (SEQ ID NO: 25) starting at 20uM. 50ul of diluted LgTrip 3546 (SEQ ID NO: 51) was combined with 50ul of each peptide titration. Reactions were incubated for 10 minutes at room temperature. 100ul of TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, the reaction was incubated for 10 more minutes, and read on a GMM+.

SmTrip10 titration

LgTrip 3546 (SEQ ID NO: 51) was diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, and serial dilutions were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol. 2nM polypeptide samples were diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500into 4.5ml).

A 2X dilution series was prepared of SmTrip10 pep86 (SEQ ID NO: 25) in TBS+0.01% BSA+0.01% Tergitol+20uM of SmTrip9-like peptides starting at 100uM. 50ul of diluted LgTrip 3546 was combined with 50ul of each peptide titration. Reactions were incubated for 10 minutes at room temperature. 100ul of TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, reaction was incubated for 10 more minutes, and then read on GMM+.

Results (Figure 27)

SmTrip 9 peptide variants were titrated in the presence of constant SmTrip10 ep86 (SEQ ID 15) (Figure 27a), and then SmTrip10 pep86 was titrated in the presence of saturating amounts of each SmTrip9 variant peptide. (Figure 27b) This shows that the affinity of the SmTrip10 sequence can be altered depending on the SmTrip9 sequence. The experiments demonstrate that identity of the β9-like peptide (e.g., SmTrip9) can influence the affinity of the β10-like peptide (e.g., SmTrip10) for the polypeptide component. SmTrip9 pep293 (SEQ ID NO: 154) and SmTrip9 pep294 (SEQ ID NO: 155) sequences have overlap with the C-terminus of LgTrip 3546 (SEQ ID NO: 51) and show a decrease in affinity compared to SmTrip9 pep286 (SEQ ID NO: 37) (no overlap), but also decrease affinity of SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) for LgTrip 3546. SmTrip9 pep298 (SEQ ID NO: 158) and SmTrip9 pep299 (SEQ ID NO: 159) sequences overlap with the C-terminus of LgTrip 3546 and the N-terminus of SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) and decrease the affinity of SmTrip10 pep86 (HiBiT) for or LgTrip 3546.

Example 32

Effect of β10-peptide identity on the affinity of the β10 peptide component to the polypeptide and β9-peptide.

Experiments were conducted during development of embodiments herein to determine the how sequence overlaps or sequence gaps between the polypeptide component and a peptide corresponding to the β-strands or between the two peptides influence the affinity of the β10-like (e.g., SmTrip10) peptides (Figure 28). LgTrip 3546 (SEQ ID NO: 51) was diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, and serial dilutions were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol. 2nM polypeptide samples were diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500 into 4.5ml). A 2X dilution series was prepared of each SmTrip10-like peptide in TBS+0.01% BSA+0.01% Tergitol+20uM of SmTrip9 pep286 (SEQ ID NO: 37) starting at 100uM. 50ul of diluted LgTrip 3546 was combined with 50ul of each peptide titration. Reactions were incubated for 10 minutes at room temperature. 100ul of TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, reaction was incubated for 10 more minutes, and then read on GMM+.

Example 33

Measure of affinity of β9-like peptides in the presence of various saturating β10-like peptides

Experiments were conducted during development of embodiments herein to determine the how the affinity of β9-like (e.g., SmTrip9) peptides are impacted in the presence of constant concentrations of various β10-like (e.g., SmTrip10) peptides with LgTrip 3546 (SEQ ID NO: 51) (Figure 29). Polypeptide component LgTrip 3546 was diluted to 200nM in TBS+0.01% BSA+0.01% Tergitol, serial dilutions were prepared (100ul into 900ul) in TBS+0.01% BSA+0.01% Tergitol (2X to make 2nM), and 2nM sample was diluted 1:10 into TBS+0.01% BSA+0.01% Tergitol (500µl into 4.5ml). A 2X dilution series was prepared of each β9-like peptide (SmTrip9 pep286 (SEQ ID NO: 37) and SmTrip9 pep287 (SEQ ID NO: 148)) in TBS+0.01% BSA+0.01% Tergitol+100uM of each SmTrip10-like peptide starting at 20uM. 50ul of diluted LgTrip 3546 (SEQ ID NO: 51) was combined with 50ul of each peptide titration. The reactions were incubated for 10 minutes at room temperature, 100ul of TBS+0.01% BSA+0.01% Tergitol+20uM furimazine was added, and the reactions were incubated for another 10 minutes and then read on GMM+.

Example 34

Effect of construct orientation on facilitated complementation in HEK293 cells

Experiments were conducted during development of embodiments herein to determine the effect the orientation of interaction elements (FRB and FKBP) relative to the peptide tags on complementation with LgTrip 3546 (SEQ ID NO: 51) (Figure 30). Un-induced signal greater than 100,000 RLU is indicative of background contamination, which decreases the apparent fold-response.

HEK293 cells were grown overnight at 37ºC with 5% CO_2. Cells were transfected with 3 ug DNA (SmTrip9 pep245-FKBP, FKBP-SmTrip9 pep245, SmTrip10 pep86-FKBP, FKBP-SmTrip10 pep86, SmTrip9 pep245-FRB, FRB-SmTrip9 pep245, SmTrip10 pep86-FRB, or FRB-SmTrip10 pep86 construct) per well using FuGENE protocol. Cells were washed in DPBS. 1 ml DPBS was added, cells were frozen at -80C for ~10 min, and thawed at room temperature. Lysates were cleared by centrifugation for 10 minutes, diluted 1:10 in TBS+200nM LgTrip (+/- 30nM RAP), and incubated for 30 min at room temperature. 50ul of each sample was combined with 50ul of TBS + 20uM Furimazine, and luminescence was read at 5 minutes.

Example 35

Effect of construct orientation on facilitated complementation in E. coli

Experiments were conducted during development of embodiments herein to determine the effect the orientation of interaction elements (FRB and FKBP) relative to the peptide tags on complementation with LgTrip 3546 (SEQ ID NO: 51) (Figure 31).

Overnight cultures of each construct were prepared in LB+100ug/ml ampicillin. Cultures were diluted 1:100 into induction media (LB+amp+0.1% rhamnose+0.15% glucose, cells were grown for 20 hours at 25ºC, and lysed with PLB lysis buffer (0.3X PLB, 25mM Hepes pH 7.5, 0.001U/ul Rq1 DNase; 250ul of cells, 750ul PLB) for 15 minutes. Cells were diluted 5X into CO_2 independent media+10% FBS that contains 200nM LgTrip 3546 and +/- 30nM RAP. Reactions were incubated for 30 minutes at room temperature, combined with equal volumes of NanoGlo+50uM furimazine (50ul to 50ul), incubated for 5 minutes, and then read on GMM+

Example 36

Kd measurement for various β10-like peptides

Experiments were conducted during development of embodiments herein to measure Kd values for various β10-like peptides with LgTrip 3546 (SEQ ID NO: 51) and SmTrip9

pep286 (SEQ ID NO: 37) (Figure 32). A solution was prepared of 20uM SmTrip9 pep286 (SEQ ID NO: 37) in TBS+0.005%+0.01% BSA. 3X serial dilutions were prepared of SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25), SmTrip10 pep288 (SEQ ID NO: 149), SmTrip10 pep289 (SEQ ID NO: 150), and SmTrip10 pep290 (SEQ ID NO: 151) (150ul in 350ul TBS+0.01% BSA+286 solution starting at 100uM). 20nM LgTrip 3546 solutions were prepared in TBS+0.01% BSA, and then diluted 1:10 in TBS+0.01% BSA. 25ul of each peptide solution was combined with 2.5ul of the LgTrip 3546 solutions. Reactions were incubated for 10 minutes, 28ul of TBS+20uM LCS (Promega Catalog No. N205) was added, incubated for 10 minutes, and then read on GMM+. This experiment shows that the addition of either "V" or "VS" to the N-terminus of SEQ ID NO: 25 increases the affinity of the SmTrip10-like peptide compared to SmTrip10 pep86 (HiBiT).

Example 37

Effect of Polypeptide/β9 split site on luciferase light output

Experiments were conducted during development of embodiments herein to analyze the effect of moving the split site between the polypeptide component and the SmTrip9-like peptide (Figure 33). Polypeptide components with varied C-terminal extensions or deletions were diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol, and 50 uM SmTrip10 pep86 (SEQ ID NO: 25) was added to each. SmTrip9 pep286 (SEQ ID NO: 37) was added to 10 uM in the SmTrip10 pep86+LgTrip solutions, and samples were incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added at 1:1, and luminescence was read. All synthetic SmTrip9 peptides contained the N-terminal solubility tag SSWKR.

Example 38

Effect of sequence gaps and overlaps between LgTrip C-terminus and SmTrip9 pep286 on luciferase light output

Experiments were conducted during development of embodiments herein to analyze the effect of gaps and/or overlaps between the polypeptide component and the SmTrip9-like peptide (Figure 34). Polypeptide components with varied C-terminal extensions or deletions were diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol, and 50 uM SmTrip10 pep86 (SEQ ID NO: 25) was added to each. 10 uM of a SmTrip9 pep286 was added to SmTrip10 pep86+LgTrip solutions, and the reactions were incubated for 10 minutes. TBS+0.01%

BSA+0.01% Tergitol+20uM furimazine detection reagent was added at 1:1, and luminescence was read.

Example 39

Effect of SmTrip9 sequence gaps and overlaps with LgTrip 3546 and SmTrip10 pep 86 (HiBiT) on luciferase light output

Experiments were conducted during development of embodiments herein to analyze the effect of gaps and/or overlaps between the SmTrip9-like peptide and the polypeptide component (e.g., LgTrip) and/or SmTrip10-like peptide (Figure 35). LgTrip 3546 (SEQ ID NO: 51) was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol, and 50 uM SmTrip10 pep86 (SEQ ID NO: 25) was added to each. SmTrip9 pep286 (SEQ ID NO: 37) was added to 10 uM in SmTrip10 pep86+LgTrip solutions, and samples were incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added at 1:1, and luminescence was read. All synthetic SmTrip9 peptides contained the N-terminal solubility tag, SSWKR.

Example 40

Biochemical analysis (Kd and Bmax) of SmTrip9 peptide length variants

Experiments were conducted during development of embodiments herein to analyze complementation of SmTrip9 peptides of different lengths with LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 15) (Figures 36-37).

SmTrip9 titration (Figure 36)

LgTrip 3546 (SEQ ID NO: 51) polypeptide was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 100 uM SmTrip10 pep86 was prepared in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions of each SmTrip9-like peptide were added to the SmTrip10 pep86 solution. 2X serial dilutions were prepared of each SmTrip9 peptide solution using the SmTrip10 pep86 solution as a diluent. Peptide dilutions and LgTrip 3546 solution were combined 1:1 and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added (1:1), and luminescence was detected.

HiBiT (SmTrip10) titration (Figure 37)

LgTrip 3546 (SEQ ID NO: 51) polypeptide was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 20 uM SmTrip9-like peptide solutions were prepared in TBS+0.01% BSA+0.01% Tergitol for each SmTrip9-like peptide to be tested. 100 uM solutions of SmTrip10 pep86 was added to each SmTrip9-like peptide solution. 2X serial dilutions were

prepared of SmTrip10 pep86 using each SmTrip9-like peptide solution as a diluent. Peptide dilutions and LgTrip 3546 solution were combined, 1:1, and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added (1:1), and luminescence was detected.

Example 41

Biochemical affinity and Bmax of SmTrip9 pep286 point mutants

Experiments were conducted during development of embodiments herein to analyze the affinity of SmTrip9 pep286 (SEQ ID NO: 37) point mutants for LgTrip 3546 (SEQ ID NO: 51) and SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25). LgTrip 3546 polypeptide was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 100 uM SmTrip10 pep86 was prepared in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions of each SmTrip9-like peptide were added to the SmTrip10 pep86 solution. 2X serial dilutions were prepared of each SmTrip9-like peptide solution using the SmTrip10 pep86 solution as a diluent. Equal volumes of peptide dilutions and LgTrip solution were combined and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added (1:1, vol:vol), and luminescence was detected (Figure 38) to determine Kd and Bmax of each SmTrip9-like peptide.

Example 42

Effect of SmTrip9 solubility tags on biochemical affinity and Bmax

Experiments were conducted during development of embodiments herein to analyze the affinity of SmTrip9-like peptides with alternative solubility tags (Figure 39). LgTrip 3546 (SEQ ID NO: 51) was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 100 uM SmTrip10/pep86 solution was prepared in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions were prepared of each SmTrip9-like peptide in the SmTrip10 pep86 solution. 2X serial dilutions were prepared of each SmTrip9-like peptide using the SmTrip10 pep86 solution as a diluent. Equal volumes of peptide dilutions were combined with the LgTrip 3546 solution, and reactions were incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added 1:1 vol:vol to the reactions, and luminescence was read after 10 minutes of incubation.

Example 43

C-terminal extension sequences

Experiments were conducted during development of embodiments herein to analyze the affinity of SmTrip9-like peptides with C-terminal sequence extensions (Figure 40).

SmTrip9 peptide titration

LgTrip 3546 (SEQ ID NO: 51) polypeptide was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 100 uM SmTrip10 pep86 was prepared in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions of each SmTrip9-like peptide were added to the SmTrip10 pep86 solution. 2X serial dilutions were prepared of each SmTrip9-like peptide solution using the SmTrip10 pep86 solution as a diluent. Peptide dilutions and LgTrip 3546 solution were combined 1:1 and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added (1:1), and luminescence was detected.

SmTrip10 pep 86 (HiBiT) titration

LgTrip 3546 (SEQ ID NO: 51) polypeptide was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 20 uM SmTrip9-like peptide solutions were prepared in TBS+0.01% BSA+0.01% Tergitol for SmTrip9-like peptide to be tested. 100 uM solutions of SmTrip10 pep86 (SEQ ID NO: 25) was added each SmTrip9-like solution. 2X serial dilutions were prepared of SmTrip10 pep86 using each SmTrip9-like peptide solution as a diluent. Peptide dilutions and LgTrip 3546 solution were combined 1:1 and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM furimazine detection reagent was added (1:1), and luminescence was detected.

Example 44

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 variants and FKBP fused SmTrip9 pep245 in KRX E. coli lysates

Overnight cultures of FRB-SmTrip10 variants, FKBP-SmTrip9 pep245, and SmTrip9 pep245-FKBP were grown in LB+100ug/ml ampicillin from glycerol stocks. Cells were diluted 1:100 in LB + 0.15% glucose + 0.1% rhamnose + Amp, and shook for 20 hours at 25ºC. Cultures were diluted 1:4 in PLB and incubated 15 min at room temperature to lyse cells. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo® buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results for fold induction (+rap signal/-rap signal) are depicted in Figure 41. FRB-SmTrip10 variant peptide constructs possessed varied linker lengths, linker

content (with or without alanine-isoleucine), and either contained or lacked a hexahistidine tag.

Example 45

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 variants and FKBP fused SmTrip9 pep245 in HEK lysates

Overnight cultures of FRB-SmTrip10 variants, FKBP-SmTrip9 pep245, and SmTrip9 pep245-FKBP were grown at 37ºC with 5% CO_2. Cells were transfected with 1 ug DNA (FKBP or FRB construct) per well using FuGENE protocol. Cells were washed in 1 ml DPBS. 1 ml DPBS was added and cultures were frozen at -80ºC for 10 min. Cultures were thawed at room temperature to lyse cells. Lysates were cleared by centrifugation for 10 min and diluted 2-fold in PLB. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo®buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results for fold induction (+rap signal/-rap signal) are depicted in Figure 42. FRB-SmTrip10 variant peptide constructs possessed varied linker lengths, linker content (with or without alanine-isoleucine), and either contained or lacked a hexahistidine tag.

Example 46

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 pep86 (HiBiT)/SmTrip10 pep289 (VS-HiBiT) and SmTrip9 sequences fused to FKBP in both orientations in KRX E. coli lysates

Overnight cultures for FRB-SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) or FRB-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO: 150) and SmTrip9-like peptide sequences fused to FKBP were grown in LB+100ug/ml ampicillin from glycerol stocks. Cells were diluted 1:100 in LB+0.15% glucose+0.1% rhamnose+Amp and shaken for 20 hours at 25ºC. Cultures were diluted 1:4 in PLB and incubated 15 min at room temperature to lyse. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo® buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results are depicted in Figures 43-47.

Example 47

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 pep86 (HiBiT)/SmTrip10 pep289 (VS-HiBiT) and SmTrip9 sequences fused to FKBP in both orientations in HEK lysates

Overnight cultures for FRB-SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) or FRB-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO: 150) and SmTrip9-like peptide sequences fused to FKBP were grown at 37ºC with 5% CO_2. Cells were transfected with 1 ug DNA (FKBP or FRB construct) per well using FuGENE protocol. Cells were washed in 1 ml DPBS. 1 ml DPBS was added and cultures were frozen at -80ºC for 10 min. Cultures were thawed at room temperature to lyse cells. Lysates were cleared by centrifugation for 10 min, and diluted 2-fold in PLB. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo® buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results are depicted in Figures 48-50.

Example 48

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 pep86 (HiBiT)/SmTrip10 pep289 (VS-HiBiT) and SmTrip9 sequences fused to FKBP in E. coli lysates

Overnight cultures for FRB-SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) or FRB-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO: 150) and SmTrip9-like peptide sequences fused to FKBP were grown in LB+100ug/ml ampicillin from glycerol stocks. Cells were diluted 1:100 in LB + 0.15% glucose + 0.1% rhamnose + Amp and shook for 20 hours at 25ºC. Cultures were diluted 1:4 in PLB and incubated 15 min at room temperature to lyse. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo® buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results are depicted in Figures 57, 59, 60, 62-63, 66-67, and 70-71. In Figure 57, ** indicates that alanine-isoluecine (AI) in the linker directly upstream of SmTrip9 peptides or SmTrip10 peptides has been removed. Alanine-isoluecine is absent from

C-terminal FKBP or FRB fusions with SmTrip9 peptide or SmTrip10 peptides, respectively, in all subsequent figures.

Example 49

Measurement of FRB-FKBP facilitated complementation using FRB-SmTrip10 pep86 (HiBiT)/SmTrip10 pep289 (VS-HiBiT) and SmTrip9 sequences fused to FKBP in HEK293 lysates

Overnight cultures for FRB-SmTrip10 pep86 (HiBiT; SEQ ID NO: 25) or FRB SmTrip10 pep289(VS-HiBiT; SEQ ID NO: 150) and SmTrip9-like peptide sequences fused to FKBP were grown at 37ºC with 5% CO_2. Cells were transfected with 3 ug DNA (FKBP or FRB construct) per well using FuGENE protocol. Cells were washed in 1 ml DPBS. 1 ml DPBS was added and cultures were frozen at -80ºC for 10 min. Cultures were thawed at room temperature to lyse cells. Lysates were cleared by centrifugation for 10 min, and diluted 2-fold in PLB. SmTrip9/SmTrip10 peptide combinations were mixed 1:1 (vol:vol). Mixtures were diluted 1:5 into PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin, and reactions were incubated for 30 minutes at room temperature. Each reaction was combined with 50ul of NanoGlo® buffer+50uM Furimazine, and luminescence was measured at 5 minutes. Results are depicted in Figures 58, 61, 64-65, 68-69, and 72-73. In Figure 58, ** indicates that alanine-isoluecine (AI) in the linker directly upstream of SmTrip9 peptides or SmTrip10 peptides has been removed. Alanine-isoluecine is absent from C-terminal FKBP or FRB fusions with SmTrip9 peptides or SmTrip10 peptides, respectively, in all subsequent figures.

Example 50

Biochemical analysis (Kd and Bmax) of varied SmTrip9 sequences

Results are depicted in Figures 74-76.

SmTrip9-like Peptide Titrations

LgTrip 3546 (SEQ ID NO: 51) was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 100 uM SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) was prepared in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions were prepared of each SmTrip9-like peptide in the SmTrip10 pep86 solution. 2X serial dilutions of each SmTrip9-like peptide were prepared using the SmTrip10 pep86 solution as a diluent. Peptide dilutions were combined with LgTrip 3546 (SEQ ID NO: 51) solution, 1:1, and incubated for 10 minutes.

TBS+0.01% BSA+0.01% Tergitol+20uM Furimazine (Fz) detection reagent was added, 1:1. Luminescence was read at 10 min.

SmTrip10 pep86 (HiBiT) Titrations

LgTrip 3546 (SEQ ID NO: 51) was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 20 uM SmTrip9-like peptide solutions were prepared in TBS+0.01% BSA+0.01% Tergitol. SmTrip10 pep86 was added to 100 uM in each SmTrip9-like peptide solution. 2X serial dilutions of each SmTrip10 pep86 (SEQ ID NO: 25) were prepared using the SmTrip9-like peptide solutions as a diluent. Peptide dilutions were combined with LgTrip 3546 (SEQ ID NO: 51) solution 1:1 and incubated for 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM Furimazine (Fz) detection reagent was added, 1:1. Luminescence was read at 10 min.

Example 51

Solubility of Synthetic SmTrip9 peptides

Synthetic peptides were synthesized by Peptide2.0 with termini blocked (N-terminal acetylation and C-terminal amidation) unless otherwise noted. Peptides were dissolved in nuclease-free water to ~1 mM and mixed on rotater at 4ºC for 30 min. Following centrifugation for 10 min at top speed, peptides were diluted 1:50 in water and quantified on NanoDrop. Peptides were stored at -20°C until use. Peptides were deemed soluble if they remained in solution after 3 freeze/thaw cycles in which peptides were thawed in a 22ºC water bath, kept at 4ºC, and frozen at -20°C. Solubility of synthetic peptides is depicted in Figure 77.

Example 52

Circulalry permuted LgBiT

SmTrip9/pep286 affinity and Bmax for SmTrip10 pep 86 (HiBiT)-LgTrip 3546 fusions

A fusion polypeptide comprising a SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) sequence fused to the front of LgTrip 3546 (SEQ ID NO: 51) was generated and experiments were conducted to monitor complex formation and luminescence of the SmTrip10 pep86 HiBiT-LgTrip fusions with SmTrip9 pep286 (SEQ ID NO: 37) (Figure 78).

Overnight cultures were grown in LB+100ug/ml ampicillin from glycerol stocks. Cells were diluted 1:100 in LB+0.15% glucose+0.1% rhamnose+Amp and shaken for 20 hr at 25ºC. 800 µl culture was lysed in FastBreak and each SmTrip10 pep86-LgTrip fusion was purified using the HisLink protocol. 2-fold SmTrip9 pep286 (SEQ ID NO: 37) serial dilutions

starting at 10 uM were made in TBS + 0.01% Tergitol + 0.01% BSA containing 0.2 nM SmTrip10 pep86-LgTrip fusion (ATG 3745 (SEQ ID NO: 211) or ATG 3746 (SEQ ID NO: 213)). Reactions were pre-incubated for 10 minutes at room temperature. TBS+0.01% Tergitol+0.01% BSA with 20 uM Furimazine (Fz) was added to samples 1:1 (vol:vol). Luminescence was read on GloMax® luminometer at 10 min.

SmTrip9/pep759 affinity for vaious SmTrip10 pep 86 (HiBiT)-LgTrip 3546 fusions

From pellets of induced cell culture, pellets were resuspended in 1/10 of the original culture volume (e.g. a 50 mL culture would be resuspended in 5 mL) using 1X TBS + 0.01% BSA. A lysis buffer was prepared using 100 parts Fast Break® Buffer, 10 Parts RQ1 RNAse free DNAse, and 1 part 1 M DTT (e.g. 650 µL Fast Break® Buffer + 65 µL RQ1 RNAse free DNAse, and 6.5 µL 1 M DTT or equivalent scaling). 1 part Lysis buffer was added to 9 parts cell suspension (e.g. 33.3 µL Lysis buffer + 300 uL suspension) in a 15 mL tube. Incubated at 4ºC for 30 minutes while mixing (using a rotary shaker). A 4 µM solution of pep 759 was prepared in 1X TBS + 0.01 % BSA. 50 uL of 4 µM pep759 was added to 50 µL of each lysate in a 96 well plate in triplicate. 50 µL of each lysate was separately mixed with 50 µL of 1X TBS + 0.01 % BSA buffer in triplicate. NanoGlo® Reagent aws prepared by mixing 100 parts NanoGlo® Buffer with 1 part Furimazine (e.g. 10 mL buffer + 100 uL furimazine). 100 uL of NanoGlo® reagent was added to each well. Luminescence was measured using Glomax® Multi instrument kinetic cycles. Luminescence measurements were compared after about 29 minutes. Luminescence readings for samples with pep759 were divided by the corresponding measurement of the same lysate without pep759. Results are depicted in figure 78B. Two batches of cultures were used to generate data: one was from inductions of 50 mL cultures (the right side, ATG-4808 through and including ATG-4632) and the other was from inductions of 3 mL cultures (left side, starting with ATG-4815 through and including ATG-3746). Some constructs were present in both tests (ATG-2623, ATG-3745, ATG-3746, ATG-4632).

Example 53

Detergent titration

Experiments were conducted during development of embodiments herein to determine the impact of various detergents on NanoLuc (SEQ ID NO: 3), LgBiT (SEQ ID NO: 11), and LgTrip 3546 (SEQ ID NO: 51) complexes with the dipeptide, pep263 (SEQ ID NO: 35).

Exposure experiments

500ul of 20mM SDS or 2mM CDTA or 5% Tergitol was added to a deep well plate. 3X serial dilutions were prepared of each detergent in TBS+0.01% BSA (150ul in 350ul). 100ul of each dilution was combined with 100ul of either 2nM NanoLuc, LgBiT, or LgTrip, and samples were incubated for 18 hours. Samples were diluted 1:100 in TBS+0.01% BSA (5ul in 495 ul). 50ul of each sample was combined in triplicate with 50ul of TBS+0.01% BSA+20uM Furimazine (Fz) for NanoLuc or TBS+0.01% BSA+20uM Furimazine (Fz)+2uM pep263 for LgBiT and LgTrip. Luminescence of samples was read on GMM+ 3 minutes after reagent addition. Results of prolonged exposure to detergent on LgBiT, LgTrip 3546, and NanoLuc are depicted in Figure 79.

Activity experiments

20ml of 20uM Fz was prepared in TBS+0.01% BSA. 2ml of 20mM SDS and 2mM of CDTA and 5% Tergitol were added to a deep well plate. 20uM Fz was added to each sample (8ul). 2X serial dilutions were prepared of each detergent in 20uM Fz solution (1ml to 1ml). A solution of 400pM NanoLuc in TBS+0.01% BSA was prepared. A solution of 400pM LgBiT+1uM pep263 (SEQ ID NO: 35) in TBS+0.01% BSA was prepared. A solution of 400pM LgTrip 3546 (SEQ ID NO: 51)+1uM pep263 (SEQ ID NO: 35) in TBS+0.01% BSA was prepared. 50ul of each enzyme solution was combined with 50ul of the detergent titrations, placed in luminometer, and read after a 3 minute incubation at RT. Results of LgBiT, LgTrip and NanoLuc activity in the presence of detergent are depicted in Figure 80.

Example 54

Reversibility of FRB-FKBP facilitated complex formation

Experiments were conducted during development of embodiments herein to demonstrate the reversibility of bioluminescent complex formation. Media was aspirated from a T75 growth flask of HEK293 cells. Cells were washed with 10ml of DPBS and trypsinized by adding 3ml of Tryple Express Trypsin. After a 3 minute incubation at 37ºC,

10ml of growth media (DMDM+10% FBS) was added to the flask, mixing cells with pipette. Cells were pelleted at 200 rpm for 5 minutes. Media was aspirated and replaced by fresh media. Cells were counted on a T20 cell counter and diluted to 200,000 cells/ml. 3ml of the cell suspension was added to each well of a six well plate. Cells were grown overnight at 37ºC with 5% CO_2. To transfect the cells, DNA was diluted for each construct to a concentration of 100ng/ul and 3.3ug of DNA was added in a final volume of 155ul of OptiMEM for each construct (FKBP-SmBiT, FRB-LgBiT, FRB-SmTrip10 pep86 (HiBiT), FKBP-SmTrip9 pep245). 9.9ul of FugeneHd was added to the diluted DNA and incubated for 15 minutes. 150ul of each DNA complex was then added to cells plated in a 6 well plate. Cells were grown overnight at 37ºC with 5% CO_2. After aspirating media, cells were washed once with DPBS (Life Technologies Cat. No. 14190) and then frozen in a fresh 1ml of DPBS at -80ºC. The samples were then thawed to lyse cells. FRB and FKBP constructs for NanoBiT (FKBP-SmBiT+FRB-LgBiT) and NanoTrip (FRB-SmTrip10 pep86 (HiBiT)+FKBP-SmTrip9 pep245+200nM purified LgTrip 3546 (SEQ ID NO: 51)) were combined and incubated with 30nM Rapamyacin for 30 minutes. A dilution series of FK506 was prepared in DMSO starting at 10mM. 3-fold serial dilutions were performed in DMSO (30ul into 70ul). 200ul of each FRB-FKBP combination was aliquoted into 8wells of a 96 well PCR tray. Upon addition of 2ul of the FK506 dilution series, each sample was incubated at 37ºC for 6 hours. 50ul of each sample was combined with 50ul of TBS+0.01% BSA+20uM Furimazine (Fz), incubated for 3 minutes, and read on GMM+. Results are depicted in Figure 81.

Example 55

LgTrip/SmTrip9 titration with SmTrip10 peptides

Experiments were conducted during development of embodiments herein to analyze titrations of LgTrip 3546 (SEQ ID NO: 51) and SmTrip9 pep286 (SEQ ID NO: 37) with various SmTrip10 peptides. Data was normalized to SmTrip10 pep86 (HiBiT) values. SmTrip10 pep86 (HiBiT) is SmHiTrip10 (SEQ ID NO: 25).

Peptide stocks were diluted to 250uM in water. A SmTrip9 pep286 (SEQ ID NO: 37) solution (10uM in final reaction) was prepared in OptiMEM+10% FBS. A 2-fold serial dilution of each SmTrip10 peptide was performed in the OptiMEM solution containing SmTrip9 pep286. The highest concentration of the SmTrip10 peptide was 15uM (final in reaction). A 10X stock (1nM) of LgTrip 3546 (SEQ ID NO: 51) was prepared in

OptiMEM+10% FBS, and 10ul was added to 90ul of each of the SmTrip10 peptide titrations. Samples were incubated for 30 minutes on an orbital shaker set to 500rpm. 2ml of detection reagent (OptiMEM+10% FBS+20ul of 1M DTT+80ul of 5mM Furimazine) was prepared. 10ul of detection reagent was added to each LgTrip 3546:peptide solution, and plates were placed on an orbital shaker. Plates were read at 5 minutes and 10 minutes. SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) and SmTrip10 pep289 (SEQ ID NO: 150) were used as controls on each of the 4 plates. Results are depicted in the tables of Figures 82-83.

Example 56

Antares constructs

Experiments were conducted during development of embodiments herein to demonstrate the complementation systems described herein in the context of the Antares BRET system comprising one or more CyOFP fluorescent proteins linked to a component of the systems described herein.

Samples were purified using HisLink Resin: 10ml of 100mM HEPES pH 7.5, 1ml of FastBreak Cell Lysis Reagent and 50u of DNase were added, and samples were placed on rotating mixer for 45 minutes and then spun at 7,000rpm for 20 minutes. Next, 1ml of HisLink resin was added to each sample, and samples were washed 3X with 5ml of binding wash buffer, eluted with 300ul of elution buffer, and dialyzed against TBS (2 hours, TBS replace, 2 more hours). Samples were diluted to 100nM in TBS+0.01% BSA and then further diluted to 0.4nM by adding 4ul to 996ul of TBS+0.01% BSA. 3X serial dilutions were prepared by transferring 300ul to 700ul . 10ml of 2uM dipeptide pep263 (SEQ ID NO: 35) was prepared in TBS+0.01% BSA. 10ml of 400pM SmTrip10 pep86 (SEQ ID NO: 25) was prepared in TBS+0.01% BSA. 10ml of 1uM SmTrip9 pep286 (SEQ ID NO: 37) and 10uM SmTrip10 pep86 were prepared. 50ul of each enzyme was combined with either TBS or dipeptide solution (all samples in triplicate on two plates). Antares fusions with LgBiT and LgTrip 3546 samples were combined with SmTrip9 pep286+SmTrip10 pep86. Samples were incubated for 1 hour at RT. 100ul of 20um furimazine was added in TBS+0.01% BSA+2mM ATT. Plates were incubated for 3 minutes and then read on GMM+. Results are depicted in graphs of Figures 84-85.

Example 57

"Dark" dipeptide 272

Experiments were conducted during development of embodiments herein to compare titration series with "Dark" dipeptide 272 (SEQ ID NO: 146) with LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51) in the presence of 0.1nM pep 263. LgBiT and LgTrip 3456 were diluted to 200nM in TBS+0.01% BSA and +/- 0.4nM of dipeptide pep263 (SEQ ID NO: 35) and incubated for 10 minutes. A 3X dilution series of dipeptide pep272 was prepared starting at 40nM (at this concentration, LgBiT showed inhibition at high concentrations, so Kd value could not be calculated; a new titration series was created starting at 4nM pep272 for LgBiT to obtain a Kd value). 50ul of the peptide dilution series was added to an assay plate followed by addition of 50ul of the LgBiT and LgTrip 3546 dilutions. Samples were incubated for 1 hour at room temperature. After addition of 100ul of NanoGlo+50uM Furimazine (Fz), plates were incubated for 5 minutes and luminescence was read on GMM+. Results are depicted in Figure 86.

Example 58

Comparison of dark dipeptides pep272 and pep273

LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51) were diluted to 200nM in TBS + 0.01% BSA with +/- 0.4nM of dipeptide pep263 (SEQ ID NO: 35) or +/- 0.4nM didpeptide pep264 (SEQ ID NO: 299) and incubated for 10 minutes. 3X dilution series of didpeptide pep272 (SEQ ID NO: 146) and dipeptide pep273 (SEQ ID NO: 298) were prepared starting at 40nM using the dipeptide pep263 dilution as a diluent for pep272 and the dipeptide pep264 dilution as a diluent for pep273. 50ul of the LgBiT and LgTrip 3546 dilutions was combined with 50ul of the pep272/273 titration series and incubated at room temperature for 2 hours. After addition of 100ul of NanoGlo® buffer+50uM Fz, plates were incubated at room temperature for 5 minutes, and luminescence was read on GMM+. Results are depicted in Figure 87.

Example 59

DarkBiT pep167

Solutions with 200nM LgBiT (SEQ ID NO: 11) and LgTrip 3546 (SEQ ID NO: 51) were prepared. 0.2nM SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25) was added to LgBiT solutions, and 1uM of SmTrip9 pep286 (SEQ ID NO: 37) with 200nM of SmTrip10 pep86 was added to LgTrip 3546 solutions. A dark bit (pep167) (SEQ ID NO: 300) titration was prepared starting at 12uM in TBS+0.01% BSA. 50ul of the dark bit titration was combined

with 50ul of the LgBiT or LgTrip 3546/pep167 dilutions and incubated for 1 hour. After addition of 100ul of NanoGlo® buffer+50uM Furimazine (Fz), plates were incubated 10 minutes, and luminescence was read on GMM+. Results are depicted in Figure 88.

Example 60

FRB-FKBP facilitated complementation in E. coli lysates with SmTrip9 pep435/434 variants

Cultures were grown overnight in LB+100ug/ml ampicillin from glycerol stocks, and cells were diluted 1:100 in LB + 0.15% glucose + 0.1% rhamnose + Amp. After 20 hr shaking at 25ºC, cells were diluted 1:4 in PLB and incubated 15 min at room temperature to lyse. Lysates of SmTrip9/SmTrip10 peptide combinations of interest were mixed 1:1 vol:vol and diluted 1:5 in PLB+ 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin. Samples were incubated for 30 minutes at room temperature and combined 1:1 (vol:vol) with NanoGlo® buffer containing 50uM Furimazine. Luminescence was read at 5 minutes. Results are depicted in Figures 89-90.

Example 61

FRB-FKBP facilitated complementation

FRB-FKBP facilitated complementation in HEK lysates with SmTrip9 pep435 and pep434 variants

600,000 cells were added to each well of 6-well plates in DMEM+1% FBS. Cells were grown overnight at 37ºC with 5% CO_2 and transfected with 3 µg DNA (FKBP or FRB construct) per well using FuGENE protocol. Following overnight incubation at 37ºC with 5% CO_2, cells were washed with DPBS. After aspiration, 1 ml of fresh DPBS was added to each well and plates were frozen at -80ºC for ~10 min. Plates were thawed at room temperature to lyse cells and lysates were cleared by centrifuging 10 min and removing supernatant. Lysates were diluted 2-fold in PLB and SmTrip9/SmTrip10 peptide combinations of interest were mixed 1:1 (vol:vol). Mixtures were then diluted 1:5 in PLB + 200 nM LgTrip 3546 (SEQ ID NO: 51) with or without 30 nM rapamycin. Samples were incubated for 30 minutes at room temperature and combined 1:1 vol:vol with NanoGlo® buffer containing 50uM Furimazine. Luminescence was read at 5 minutes. Results are depicted in Figure 91.

FRB-FKBP assay screen with SmTrip9s 823 and 840

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1%

rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM LgTrip 3546, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of NanoGlo containing 50 uM furimazine was added to assay plates wells and luminescence was read on GloMax after 5 minutes. Results are depicted in Figure 92.

Example 62

Determination of Kd of pep434 and pep435 variants

LgTrip 3546 (SEQ ID NO: 51) was diluted to 0.2 nM in TBS+0.01% BSA+0.01% Tergitol. 20 uM solutions of each SmTrip9-like peptides were prepared with 100 uM SmTrip10 pep86 (SEQ ID NO: 25) in TBS+0.01% BSA+0.01% Tergitol. 2-fold serial dilutions of each SmTrip9-like peptide were performed using the 100 uM SmTrip10 pep86 solution as a diluent. Peptide dilutions were combined with LgTrip 3546 solution 1:1 (vol:vol) and incubated 10 minutes. TBS+0.01% BSA+0.01% Tergitol+20uM Furimazine (Fz) detection reagent was added to LgTrip/peptide solutions 1:1 vol:vol and luminescence was read at 10 min. Results are depicted in Figure 93.

Example 63

Detection of CRISPR-tagged dipeptide-GAPDH using LgTrip 3546

Experiments were conducted during development of embodiments herein to demonstrate that both LgTrip 2098 (SEQ ID NO: 31) and LgTrip 3546 (SEQ ID NO: 51) find use as bioluminescence reagents for detecting endogenously tagged GAPDH (Tagged with SmTrip10 pep86 (SmHiTrip; SEQ ID NO: 25).

HeLa cells were edited using CRISPR/Cas9 to express endogenous GAPDH C-terminal fusions to the indicated peptide. The edited HeLa cells were plated at a density of approximately 20,000 cells per well of a solid white assay plate in 100 µl of DMEM/10% FBS. Cells were then incubated in the presence of 100 µl of NanoGlo® HiBiT Lytic Buffer (Promega) containing NanoGlo® HiBiT Lytic Buffer and 200 nM of LgTrip for 10 min. Luminescence was recorded using a GloMax® Discover with 0.5s integration time. Relative cell numbers were determined using the CellTiter® Glo Luminescent Cell Viability Assay (Promega) according to manufacturer's protocol. Data are represented as average relative light units normalized to cell number, with variability expressed as standard deviation**.**

Results are depicted in Figure 94.

Example 64

Site-Saturation Screen of SmTrip9

Experiments were conducted during development of embodiments herein to identify beneficial amino acid substitutions in SmTrip9.

Genetic site-saturation libraries were generated using primers with randomized codons at the indicated positions in SmTrip9. KRX *E. coli* was transformed with pooled genetic variants, plated onto LB + ampicillin agar, and grown overnight at 37°C. Individual colonies were picked and placed into 96-well culture plates containing LB + 100 ug/ml ampicillin. Cultures were grown overnight at 37°C with shaking. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. Assay reagent was prepared by adding 444 nM LgTrip (SEQ ID NO: 51), 90x diluted FRB-VS-HiBiT culture, and +/- 35 nM rapamycin to 25 mM HEPES with 0.3x Passive Lysis Buffer (PLB) and DNase. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of NanoGlo® buffer containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes.

Results are depicted in Figures 100-112.

Example 65

FRB-FKBP facilitated complementation in E. coli lysates with SmTrip9 pep435/434 variants

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. Cultures were diluted 1:4 in PLB and incubated 15 min at room temperature to lyse cells. SmTrip9 and SmTrip10 dilutions were mixed 1:1 (vol:vol) for combinations of interest. Mixtures were diluted 1:5 into PLB + 200 nM LgTrip , with or without 30 nM rapamycin. Samples were incubated 30 min at room temperature. Fifty microliters of NanoGlo® buffer containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes. Results are depicted in Figures 113-115.

Example 66

FRB-FKBP Facilitated Complementation Assay Screen with Combinational SmTrip9 variants

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10s were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM LgTrip, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of NanoGlo® buffer containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes. Results are depicted in Figures 116-122.

Example 67

Determination of Kd and Bmax of SmTrip9 synthetic peptides

LgTrip was diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol and pep289 was added to 25 uM. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT. Results are depicted in Figures 123-130.

Example 68

Determination of Solubility of Synthetic SmTrip9 peptides

Synthetic peptides were ordered from Peptide2.0 with termini blocked (N-terminal acetylation and C-terminal amidation) unless otherwise noted. Peptides were dissolved in nuclease-free water and stored at -20°C. Stocks were thawed in 22°C water bath, centrifuged, and kept at 4°C until use. Results are depicted in Figures 131.

Example 69

Biochemical co-titration of SmTrip9 synthetic peptides and pep289

LgTrip was diluted to 200 nM in 25 mM HEPES with 0.3x Passive Lysis Buffer (PLB) and DNase. SmTrip9 peptides and pep289 were diluted to 100 uM and co-titrated serially 6-fold in PLB. Samples were incubated 10 minutes at room temperature. Most concentrated samples were diluted 50-100-fold in PLB. Samples were aliquoted in triplicate into assay plates and mixed 1:1 vol:vol with NanoGlo® buffer + 50uM furimazine. Luminescence was read after 10 minutes on ClarioStar or GloMax® instruments. Results are depicted in Figures 132-133.

Example 70

Biochemical co-titration of SmTrip9 and SmTrip 10 synthetic peptides

LgTrip was diluted to 200 nM in 25 mM HEPES with 0.3x Passive Lysis Buffer (PLB) and DNase. SmTrip9 and SmTrip10 peptides were diluted to 100 uM and co-titrated serially 6-fold in PLB. Samples were incubated 10 minutes at room temperature. Most concentrated samples were diluted 50-100-fold in PLB. Samples were aliquoted in triplicate into assay plates and mixed 1:1 vol:vol with NanoGlo® buffer + 50uM furimazine. Luminescence was read after 10 minutes on ClarioStar or GloMax® instruments. Results are depicted in Figure 134.

Example 71

Biochemical co-titration of pep521 and alternative SmTrip 10 synthetic peptides

LgTrip was diluted to 200 nM in 25 mM HEPES with 0.3x Passive Lysis Buffer (PLB) and DNase. SmTrip10 peptides and pep521 were diluted to 100 uM and co-titrated serially 6-fold in PLB. Samples were incubated 10 minutes at room temperature. Most concentrated samples were diluted 50-100-fold in PLB. Samples were aliquoted in triplicate into assay plates and mixed 1:1 vol:vol with NanoGlo® buffer + 50uM furimazine. Luminescence was read after 10 minutes on ClarioStar or GloMax® instruments. Results are depicted in Figure 135.

Example 72

Strand removal (purification) from LgTrip 3546 template

A single colony from each clone was grown for 18 hours at 37°C in LB+100ug/ml ampicillin. The overnight culture was diluted 1:100 into 50ml of Terrific Broth+0.1% Rhamnose+100ug/ml ampicillin. After 48 hours of growth at 15°C, cells were pelleted and resuspended in 10ml of 100mM HEPES pH 7.5+0.001U/ml DNase. 1ml of FastBreak® Lysis Buffer was added to each sample, and then samples incubated on a rotating mixer at 4°C for 1 hour. A cleared lysate was prepared by centrifugation of 7,000 RPM for 10 minutes.

Purification of the strands using the MagneHis purification system: 300ul µl of MagneHis resin (Promega) was added to each sample, and then samples mixed 20 times and placed on a magnetic stand. The supernatant was removed, and the resin was washed two times with column wash buffer. Samples were eluted in 600ul of elution buffer. Samples were then placed in a dialysis apparatus to exchange with TBS. Identification of the strand removal proteins was observed via SDS PAGE as depicted in Figure 136.

Example 73

Strand Removal Proteins with Various Combinations of Peptides

200µl of OptiMEM+10% FBS was added to multiple wells of a multi-well plate. Peptide combinations were added to a final concentration of 10µM with each to be assayed separately with each strand removal protein. Each strand removal protein was diluted to 20nM (2nM for LgTrip 3546) in OptiMEM+10% FBS. 20µl of each strand removal peptide was added to the designated peptide combination, samples e mixed, and 45µl aliquoted in triplicate into wells of a white assay plate (Costar 3600). After 15-minute incubation at RT, 5µl of detection reagent (100uM Fz (Promega LCS N205)) was added to each sample. Samples were placed on an orbital shaker for 30 seconds, and then luminescence was measured every 2 minutes for 1 hour. Luminescence is reported as peak height of the kinetic read. Background is OptiMEM+10% FBS+detection reagent.

As demonstrated in Figure 137, there was no signal over background for strand removal proteins 7, 8, 9, 10 when added as separate peptides. Two of the three peptide combinations gave ~2X signal over background ((8+9) dipeptide+7+10) or ((7+8)dipeptide+9+10). One of the 3 peptide combinations gave ~10X signal over background (((9+10) dipeptide+7+8) The two dipeptide combination of (10+9)+(7+8) gave signal of ~4.5 logs over background. It is likely that the peptide combinations that gave the

greatest signal have the highest affinity. Lower affinity combinations could produce light in a facilitated complementation assay. Figure 137D demonstrates that peptides with alternative split sites (e.g., mid beta strand) are capable of forming a biolumiunescent complex.

Example 74

Strands 6, 7, 8, 9, or 10 Removal (purification) from LgTrip 3546 template

400µl of OptiMEM+10% FBS was added to multiple wells of a deep well 96-well plate. Peptide combinations were added to a final concentration of 10µM each peptide to be assayed separately with either ATG-3929 or LgTrip. The peptide solutions were then divided. To one of the peptide aliquots, 20ul of either 20nM ATG-3929 or 2nM LgTrip was added to the designated peptide combination, samples mixed, and 45µl of the +/- peptide samples aliquoted in triplicate into wells of a white assay plate (Costar 3600). After a 15-minute incubation at RT, 5µl of detection reagent (100uM Fz in OptiMEM+10% FBS (Promega LCS N205)) was added to each sample. Samples were placed on an orbital shaker for 5 minutes. Background for each sample is OptiMEM+10% FBS+peptide dilutions+detection reagent.

As demonstrated in Figure 138, sample ATG-3929 with strands (9+10)+(7+8)+6 shows ~2X signal over background. On the other hand, the sample with two peptides (6+7+8)+(9+10) showed ~300X over background.

Note that spontaneous complementation is not visible for samples with more than 3 peptides. It is possible that the affinity is not high enough affinity of the peptides is not high enough to produce light. It is possible that if the peptides are brought together through facilitated complementation with a fusion partner that it would be possible to obtain signal.

Example 75

Dipeptide Titrations

Dipeptides were diluted to 5 uM and diluted serially 5-fold using TBS + 0.01% BSA + 0.01% Tergitol with 0.2 nM of LgTrip as the diluent. Samples were incubated 10 minutes at room temperature and added to wells of assay plates in triplicate. One-to-one vol:vol of TBS + 0.01% BSA + 0.01% Tergitol with 20x diluted live cell substrate was added to samples and plates were read on a GloMax luminometer after 10 minutes. Figure 139A-B demonstate the Kd and Bmax values from the dipeptide titrations.

Fold response of Binary NanoTrip in mammalian cells

Growth media was removed from confluent flasks of cells. (HEK293 and Hela). Cells were washed with 10ml of DPBS and then 3ml of TrypLE Express trypsin was added to cells. Cells were incubated for 3 minutes at 37°C. 10ml of growth media was added and then cells were spun at 200RCF for 5 minutes. Media was replaced and cells were resuspend in 10ml of growth media. Cells were counted and diluted to 200,000/ml. 100ul of cells were plated into each well of a white assay plates and grow overnight at 37°C with CO_2. The next day 100ng/ul DNA from FRB and FKBP fusions of LgTrip (3546) and various dipeptide in each orientation were combined. 263 samples started at 1:10 dilution in carrier DNA or 10ng/ul. DNA samples were then diluted serially into carrier DNA (10ul to 90µl in 100ng/ul carrier DNA) Next 20ul of each DNA dilution was added to 83ul of OptiMEM. Samples were mixed and then 6.6 ul of Viafect transfection reagent was added to each sample. Samples were incubated for 20 minutes at RT and then 5ul of transfection complex was added to 6 wells of cells for each FRB-FKBP orientation. Plates were then grown overnight at at 37°C with CO_2. The next day Rapamycin (RAP) was added to 3 of the wells for each sample to a final concentration of 100nM. Samples were placed on orbital shaker for 1 minute and then Incubated at $_{37C}$ for 30 minutes. After incubation, 100ul of NanoGlo+50uM Fz was added to each sample (+RAP and –RAP) and then samples were placed on orbital shaker for 5 minutes. Luminescent measurements were acquired using a Glomax Discover luminometer. Fold response was calculated by dividing RLU values from the +RAP sample by the RLU values from the –RAP samples. Results are depicted I Figure 139C-E. Dipeptide fusions that have lower affinity to LgTrip produce a greater fold response compared to samples with higher affinity.

Example 76

Development of a Tripartite Quantitative Assay for anti-TNFa Biologic Agents using Tripartite Fusion Proteins

Infliximab (Remicade), Adalimumab (Humira), and Etanercept (Enbrel) are TNFa inhibitors that all bind human TNFa and also all contain a human IgG1 Fc. A quantitative assay was deveeloped for all 3 TNFa inhibitors by expressing and purifying SmTrip9- or SmTrip10-protein G and TNFa fusion proteins which serve as the binding components to the TNFa inhibitor (Figure 140). The Protein G fusion protein will bind to the conserved IgG1 Fc region of the TNFa inhibitor. The Inhibitor will bind to the TNFa fusion protein bringing the SmTrip9 and SmTrip10 into close proximity. In the presence of LgTrip, the

bioluminescent complex will form creating the signal that is proportional to the amount of TNFa inhibitor present. All reporter tag configurations were tested with SmTrip9 or SmTrip10 expressed on the N- or C-terminal of Protein G and TNFa with either a 4gly-ser or 15gly-ser linker. The optimal pairing resulting from screening all orientations was SmTrip9-15gly/ser-protein G with TNFa-15gly/ser-SmTrip10.

Methods for making the fusion proteins

A fusion protein comprising of SmTrip9 pep521 (SEQ ID NO: 268) sequence followed by a linker of 15 glycine-serine repeat was fused to the N-terminus of Protein G was expressed and purified. A second fusion protein comprising of SmTrip10 pep289 (SEQ ID NO: 150) sequence was fused to the C terminus of human TNFa separated by a linker of 15 glycine-serine repeat was also expressed and purified. Streak plates from glycerol stocks of KRX transformed E. coli cells were created on LB plates with Ampicillin (100ug/ml) and allowed to incubate overnight at 37°C. A single colony was inoculated into 3mls of SOC media + AMP and incubated shaking (275 rpm) overnight at 37°C. The cells were lysed and the plasmid DNA was collected. Shuffle competent E. coli cells were transformed with 100ng of plasmid DNA, spread onto pre-warmed selection plates, and allowed to incubate overnight at 30°C. A colony was selected and inoculated into a 50ml volume of LB containing ampicillin. The cultures were incubated overnight at 37°C shaking before being diluted 1:100 into 500mL of LB medium containing ampicillin. These flasks were allowed to incubate at 37°C while shaking until the OD600 reached 0.6-0.8. Cells were induced by addition of IPTG at a final concentration of 1mM and allowed to incubate overnight at 25°C while shaking. Cells were harvested, centrifuged, and resuspended in 50mL extraction and lysis buffer at 4°C with mixing. Three cycles of freeze/thaw were performed followed by addition of RQI DNase. The total lysate was transferred to a think 50mL centrifuge tube and spun at 10,000xg for 30 minutes at 4°C. 20mM Imidazole/350mM NaCl was added prior to loading onto a nickel column. Fusion proteins were washed and eluted off the columns in a 5 step elution process with increasing imidazole. Samples were dialyzed against TBS and final stock proteins were stored in 50% glycerol in TBS at -20°C.

Example 77

Homogeneous Quantitative Analysis of TNFa Inhibitors Infliximab, Adalimumab, and Etanercept using SmTrip9 pep521-protein G and TNFa-SmTrip10 pep289 Fusion Proteins

Experiments were conducted during development of embodiments herein to determine the ability of NanoTrip fusion proteins to quantitate TNFa inhibitors in a homogeneous assay. The results show that protein G and TNFa NanoTrip fusion proteins together with LgTrip display great sensitivity and range for quantitating infliximab, adalimumab, and etanercept.

A 2X stock of the TNFa inhibitors was generated in assay buffer, serially diluted 1:2 to create a dose response, and 50ul/well was added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521-protein G (SEQ ID NO: 268) (final 10nM) + TNFa-SmTrip10 pep289 (SEQ ID NO: 150) (final 10nM) was created in assay buffer, and 50ul/well added. Plates were allowed to incubate at room temperature for 90 minutes. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, allowed to incubate for ~ 5 minutes, and luminescence measured using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate/plate, and n=3 independent experiments run. Data as demonstrated in Figure 141 was analyzed for limit of detection (LOD), limit of quantitation (LOQ), and upper limit of quantitation (ULOQ).

Example 78

Homogenous Quantitative Analysis of Infliximab in Complex Sample Matrices such as Human Serum and Urine

Experiments were conducted during development of embodiments herein to determine the ability of NanoTrip fusion proteins to quantitate infliximab in the presence of the complex sample matrices of normal human IgG depleted serum, normal pooled human AB serum, and pooled normal human urine in a homogenous assay. Results indicate that the NanoTrip system was largely unaffected by the presence of urine nor the presence of serum proteins with the exception of endogenous IgG as expected.

A 2X stock containing 20nM Infliximab in presence of the human sample matrix to be tested was created by diluting with assay buffer, and 50ul/well added to a non-binding

surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521-protein G (SEQ ID NO: 268) (final 10nM) + TNFa-SmTrip10 pep289 (SEQ ID NO: 150) (final 10nM) was created in assay buffer, and 50ul/well added. Plates were allowed to incubate at room temperature for 90 minutes. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, allowed to incubate for ~ 5 minutes, and luminescence measured using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate. Data as demonstrated in Figure 142 is displayed as signal/background.

Example 79

Kinetic Analysis of Signal Generation via Facilitated Complementation of SmTrip9 pep521-protein G (SEQ ID NO: 268) and TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) Fusion Proteins with Purified LgTrip 3546 (SEQ ID NO: 51) in the Presence of 100pM of Infliximab in a Solution Phase, Homogenous Assay.

Experiments were conducted during development of embodiments herein to determine the binding kinetics of the Protein G/TNFa NanoTrip system to quantitate 100pM of Infliximab in a solution phase, homogenous assay. Results show that signal generation is immediate and sustained indicating rapid binding kinetics of the fusion proteins to infliximab as well as LgTrip to the SmTrip9 and SmTrip10 fusion proteins.

A 2X stock of Infliximab (100pM final) was generated in assay buffer, and 50ul/well added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521-protein G (SEQ ID NO: 268) (final 10nM) + TNFa-SmTrip10 pep289 (SEQ ID NO: 150) (final 10nM) was created in assay buffer, and 50ul/well added. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, and 25ul/well added to the plate for a final concentration of 10uM. All reagents were added, and the plate immediately placed on a GloMax® Discover to read luminescence over time. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate.

Results are depicted in Figure 143.

Example 80

Testing SmTrip9-Protein G Variants for Their Ability to Measure Infliximab via Facilitated Complementation with TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) Fusion Proteins Purified LgTrip 3546 (SEQ ID NO: 51) in a Solution Phase, Homogenous Assay

Experiments were conducted during development of embodiments herein to determine the ability of other SmTrip9 variants expressed as a fusion proteins to protein G to measure Infliximab via facilitated complementation with TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins purified LgTrip 3546 (SEQ ID NO: 51) in a solution phase, homogenous assay. Results show that all of the SmTrip9 pep(x)-Protein G variants tested were able to generate signal.

A 2X stock of Infliximab (10nM final) was generated in assay buffer, and 50ul/well added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep(x)-Protein G (final 10nM) + TNFa-SmTrip10 pep289 (SEQ ID NO: 150) (final 10nM) was created in assay buffer, and 50ul/well added. Plates were allowed to incubate at room temperature for 90 minutes. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, allowed to incubate for ~ 5 minutes, and luminescence was measured using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate. Results are depicted in Figure 144.

Example 81

Homogeneous Quantitative Infliximab Testing SmTrip9 pep(X)-Protein G Variants and TNFa-SmTrip10 pep289 Fusion Proteins

Experiments were conducted during development of embodiments herein to demonstrate the ability of different SmTrip9 pep(X)-Protein G variants to quantitate Infliximab via facilitated complementation with TNFa-SmTrip10 pep289 (VS-HiBiT; SEQ ID NO:150) fusion proteins with purified LgTrip 3546 (SEQ ID NO: 51) in a solution phase, homogeneous assay. Results show that all SmTrip9 variants were able to quantitate infliximab.

A 2X stock of Infliximab (10nM final) was generated in assay buffer, and 50ul/well added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9

pep(x)-protein G (final 10nM) + TNFa-SmTrip10 pep289 (SEQ ID NO: 150) (final 10nM) was created in assay buffer, and 50ul/well added. Plates were allowed to incubate at room temperature for 90 minutes. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, allowed to incubate for ~ 5 minutes, and luminescence was measured using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate.

Example 82

Development of a Tripartite Quantitative Assay for anti-EGFR Biologic Agents Using Tripartite Fusion Proteins in a Cell-Based Assay.

We developed a quantitative, cell-based assay for panitumumab and cetuximab representing a phase separation or surface chemistry like assay. Using purified SmTrip9-Protein G fusion proteins that will bind to the conserved human IgG Fc region of the EGFR inhibitor, the Inhibitor will bind to the SmTrip10-EGFR fusion protein that is expressed on the cell surface bringing the SmTrip9 and SmTrip10 into close proximity. In the presence of LgTrip, the bioluminescent complex will form creating the signal that is proportional to the amount of EGFR inhibitor present. All reporter tag configurations were tested with SmTrip9 or SmTrip10 expressed on the N- or C-terminal of protein G or on the N terminal of EGFR with either a 4gly-ser or 15gly-ser linker. The optimal pairing resulting from screening all orientations was SmTrip9-4gly/ser-protein G with EGFR-15gly/ser-SmTrip10.

Results are depicted in Figure 145.

Example 83

Quantitation of Panitumumab via Facilitated Complementation with SmTrip9 pep521-protein G (SEQ ID NO: 268) Fusion Protein and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) Expressing Cells with Purified LgTrip 3546 (SEQ ID NO: 51) in a Cell-Based Homogeneous Assay.

Experiments were conducted during development of embodiments herein to determine the ability of NanoTrip fusion proteins to quantitate the EGFR inhibitor panitumumab in a cell-based homogeneous assay. The results show that SmTrip9 pep521-protein G (SEQ ID NO: 268) purified protein, SmTrip10 pep289-EGFR (SEQ ID NO:150) expressing cells, and LgTrip 3546 (SEQ ID NO: 51) display great sensitivity and range for quantitating panitumumab.

HEK293 cells were maintained in growth medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C/5% CO_2 in a humidified tissue culture incubator. Transient reverse transfection were performed by first diluting the expression construct for the SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) into Opti-MEM containing carrier DNA (PGEM-3ZF(-)) at a mass ratio of 1:10. The transfection reagent:DNA complex was prepared by adding FuGENE HD transfection reagent at a ratio of 1:3 (mg DNA per mL FuGENE HD) followed by 15 minutes incubation at room temperature. The resulting transfection:DNA complex was then mixed with a HEK293 cell suspension (2 x 10^5 cells/ml) in growth medium at a ratio of 1:20 (vol/vol), followed by incubation for 18-20 hours at 37°C/5% CO_2 in humidified tissue culture incubator.

HEK293 cells expressing the SmTrip10 pep289-EGFR (SEQ ID NO: 150) fusion protein were harvested using Trypsin-EDTA, washed in growth medium, and resuspended in Opti-MEM at a concentration of 4.5 x 10^5 cells/ml. 50ul of cells/well (20,000 cells/well) are added to a non-binding surface, solid white 96 well plate (Costar 3600). A 4X stock of Panitumumab was generated in Opti-MEM, serially diluted in Opti-MEM to create dose response, and 25ul/well added. A 4X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521-protein G (SEQ ID NO: 268) (final 5nM) was created in Opti-MEM, and 25ul/well added. Plates were allowed to incubate for 1 hour at 37°C. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, and luminescence was measured on a GloMax® Discover. Samples were tested in triplicate. N=3 independent experiments.

Results are depicted in figure 146.

Example 84

Real-Time Binding Kinetic Analysis of Signal Generation via Facilitated Complementation of SmTrip9 pep521-Protein G (SEQ ID NO: 268) Purified Fusion Protein and SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) Expressing HEK293 Cells Paired with Purified LgTrip 3546 (SEQ ID NO: 51) in the Presence of Increasing Doses of Cetuximab in a Cell-Based Homogeneous Assay.

Experiments were conducted during development of embodiments herein to determine the binding kinetics of the Protein G/EGFR NanoTrip system to quantitate Cetuximab in a cell-based homogenous assay. Results show that the luminescent signal

increases with time in accordance with the formation of the luciferase complex. Signal generation is also dose dependent.

HEK293 cells were maintained in growth medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C/5% CO_2 in a humidified tissue culture incubator. Transient reverse transfection were performed by first diluting the expression construct for the SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) into Opti-MEM containing carrier DNA (PGEM-3ZF(-)) at a mass ratio of 1:10. The transfection reagent:DNA complex was prepared by adding FuGENE HD transfection reagent at a ratio of 1:3 (mg DNA per mL FuGENE HD) followed by 15 minutes incubation at room temperature. The resulting transfection:DNA complex was then mixed with a HEK293 cell suspension (2 x 10^5 cells/ml) in growth medium at a ratio of 1:20 (vol/vol), followed by incubation for 18-20 hours at 37°C/5% CO_2 in humidified tissue culture incubator.

HEK293 cells expressing the SmTrip10 pep289-EGFR fusion protein were harvested using Trypsin-EDTA, washed in growth medium, and resuspended in Opti-MEM at a concentration of 4.5 x 10^5 cells/ml. 50ul of cells/well (20,000 cells/well) were added to a non-binding surface, solid white 96 well plate (Costar 3600). A 4X stock of cetuximab was generated in Opti-MEM, and 25ul/well added. A 4X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 10uM) + SmTrip9 pep521-protein G (SEQ ID NO: 268) (final 780pM) was created in Opti-MEM, and 25ul/well added. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, and 25ul/well added to the plate for a final concentration of 10uM. All reagents were added, and the plate was immediately placed on a GloMax® Discover to read luminescence over time. Samples were tested in triplicate.

Results are depicted in Figure 147.

Example 85

Testing SmTrip9-Protein G Variants for Their Ability to Measure Panitumumab via Facilitated Complementation with SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) Expressing Cell Paired with Purified LgTrip 3546 (SEQ ID NO: 51) in a Cell-Based Homogenous Assay.

Experiments were conducted during development of embodiments herein to determine the ability of other SmTrip9 variants expressed as a fusion proteins to protein G to measure Panitumumab via facilitated complementation with SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells paired with purified LgTrip 3546 (SEQ ID NO: 51)

in a cell-based homogenous assay. Results show that all of the SmTrip9 pep(x)-protein G variants tested were able to generate signal.

HEK293 cells were maintained in growth medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C/5% CO_2 in a humidified tissue culture incubator. Transient reverse transfection were performed by first diluting the expression construct for the SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) into Opti-MEM containing carrier DNA (PGEM-3ZF(-)) at a mass ratio of 1:10. The transfection reagent:DNA complex was prepared by adding FuGENE HD transfection reagent at a ratio of 1:3 (mg DNA per mL FuGENE HD) followed by 15 minutes incubation at room temperature. The resulting transfection:DNA complex was then mixed with a HEK293 cell suspension (2 x 10^5 cells/ml) in growth medium at a ratio of 1:20 (vol/vol), followed by incubation for 18-20 hours at 37°C/5% CO_2 in humidified tissue culture incubator.

HEK293 cells expressing the SmTrip10 pep289-EGFR (SEQ ID NO: 150) fusion protein were harvested using Trypsin-EDTA, washed in growth medium, and resuspended in Opti-MEM at a concentration of 4.5 x 10^5 cells/ml. 50ul of cells/well (20,000 cells/well) were added to a non-binding surface, solid white 96 well plate (Costar 3600). A 4X stock of Panitumumab (final 1nM) was generated in Opti-MEM, and 25ul/well added. A 4X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep(X)-protein G (final 10nM) was created in Opti-MEM, and 25ul/well added. Plates were allowed to incubate for 1 hour at 37C. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, and luminescence was measured on a GloMax® Discover. Samples were tested in triplicate. N=3 independent experiments. Results are depicted in Figure 148.

Example 86

Testing SmTrip9-Protein G Variants for Their Ability to Measure Panitumumab via Facilitated Complementation with SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) Expressing Cell Paired with Purified LgTrip 3546 (SEQ ID NO: 51) in a cell-Based Homogenous Assay.

Experiments were conducted during development of embodiments herein to determine the ability of other SmTrip9 variants expressed as a fusion proteins to protein G to measure panitumumab via facilitated complementation with SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) expressing cells paired with purified LgTrip 3546 (SEQ ID NO: 51)

in a cell-based homogenous assay. Results show that all of the SmTrip9 pep(x)-protein G variants tested were able to quantitate panitumumab in a dose response analysis.

HEK293 cells were maintained in growth medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C/5% CO_2 in a humidified tissue culture incubator. Transient reverse transfection were performed by first diluting the expression construct for the SmTrip10 pep289-EGFR (VS-HiBiT; SEQ ID NO:150) into Opti-MEM containing carrier DNA (PGEM-3ZF(-)) at a mass ratio of 1:10. The transfection reagent:DNA complex was prepared by adding FuGENE HD transfection reagent at a ratio of 1:3 (mg DNA per mL FuGENE HD) followed by 15 minutes incubation at room temperature. The resulting transfection:DNA complex was then mixed with a HEK293 cell suspension (2 x 10^5 cells/ml) in growth medium at a ratio of 1:20 (vol/vol), followed by incubation for 18-20 hours at 37°C/5% CO_2 in humidified tissue culture incubator.

HEK293 cells expressing the SmTrip10 pep289-EGFR (SEQ ID NO: 150) fusion protein were harvested using Trypsin-EDTA, washed in growth medium, and resuspended in Opti-MEM at a concentration of 4.5 x 10^5 cells/ml. 50ul of cells/well (20,000 cells/well) were added to a non-binding surface, solid white 96 well plate (Costar 3600). A 4X stock of Panitumumab (final 1nM) was generated in Opti-MEM, and 25ul/well added. A 4X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep(X)-protein G (final 10nM) was created in Opti-MEM, and 25ul/well added. Plates were allowed to incubate for 1 hour at 37°C. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, and luminescence was measured on a GloMax® Discover. Samples were tested in triplicate. Results are depicted in Figure 149.

Example 87

Quantitation of Human IL-1beta using NanoTrip Chemically Labeled-Paired Antibodies

Experiments were conducted during development of embodiments herein to demonstrate the use of paired monocloncal antibodies that have been chemically conjugated with NanoTrip peptides to quantitation human IL-1beta. This model system consists of two monoclonal mouse antibodies that recognize IL-1beta at different epitopes. HaloTag-SmTrip9 pep521 (SEQ ID NO: 268) was chemically conjugated to one of the antibodies, and HaloTag-SmTrip10 pep289 (SEQ ID NO: 150) was chemically conjugated to the other

antibody. In the presence of IL-1beta, the two antibodies bind to the IL-1beta thus bringing the two tags in close proximity. Addition of LgTrip 3546 (SEQ ID NO: 51) completes the complementation, and a luminescent signal is generated.

HaloTag-SmTrip9 and HaloTag-SmTrip10 fusion proteins are expressed and purified. Anti-IL-1beta mouse monoclonal antibody clone 508A 4A2 (Thermo) is labeled with the HaloTag-SmTrip9 pep521 (SEQ ID NO: 268) and anti-IL-1beta mouse monoclonal antibody clone 508A 7G8 (Thermo) is labeled with the HaloTag-SmTrip10 pep289 (SEQ ID NO: 150). The unlabeled antibodies are prepped by first doing a buffer exchange into 10mM $NaHCO_3$ (pH 8.5) using a Zeba column. Antibodies are then primed with a 20-fold excess of HaloTag® Succinimidyl Ester (04) Ligand (Promega) and allowed to incubate at room temperature for 2 hours. A buffer exchange is done 2x using Zeba columns to remove free linker. The primed antibodies are incubated with a 4-fold excess of HaloTag-SmTrip9 or HaloTag-SmTrip10 overnight at 4C while mixing. HaloLink® Resin is used to remove any free HaloTag® fusion proteins.

A 2X stock of recombinant human IL-1beta was generated in assay buffer, serially diluted 1:2 to create a dose response, and 50ul/well added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521 labeled 4A2 clone (SEQ ID NO: 268) (final 100ng/ml) + SmTrip10 pep289 labeled 7G8 clone (SEQ ID NO: 150) (final 100 ng/ml) was created in assay buffer, and 50ul/well added. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well added to the plate for a final concentration of 10uM, and luminescence measured in real-time using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate. Data displayed is the signal that was read at the 20 minute time point.

Results are depicted in Figure 150.

Example 88

Real-time Binding Kinetics for Human Troponin using NanoTrip Chemically-labeled Paired Antibodies

Experiments were conducted during development of embodiments herein to demonstrate the use of paired monocloncal antibodies that have been chemically conjugated with NanoTrip peptides to quantitation human Troponin. This model system consists of two

monoclonal mouse antibodies that recognize Troponin at different epitopes. HaloTag-SmTrip9 pep521 (SEQ ID NO: 268) was chemically conjugated to one of the antibodies, and HaloTag-SmTrip10 pep289 (SEQ ID NO: 150) was chemically conjugated to the other antibody. In the presence of Troponin, the two antibodies bind to the Troponin thus bringing the two tags in close proximity. Addition of LgTrip 3546 (SEQ ID NO: 51) completes the complementation and a luminescent signal is generated.

HaloTag-SmTrip9 and HaloTag-SmTrip10 fusion proteins are expressed and purified. Anti-troponin mouse monoclonal antibody 10-T79C (Fitzgerald) is labeled with the HaloTag-SmTrip10 pep289 (SEQ ID NO: 150), and anti-troponin mouse monoclonal antibody 10-T79F (Fitzgerald) is labeled with the HaloTag-SmTrip9 pep521 (SEQ ID NO: 268). The unlabeled antibodies are prepped by first doing a buffer exchange into 10mM NaHCO3 (pH 8.5) using a Zeba column. Antibodies are then primed with a 20-fold excess of HaloTag® Succinimidyl Ester (04) Ligand (Promega) and allowed to incubate at room temperature for 2 hours. A buffer exchange is done 2x using Zeba columns to remove free linker. The primed antibodies are incubated with a 4-fold excess of HaloTag-SmTrip9 or HaloTag-SmTrip10 overnight at 4°C while mixing. HaloLink® Resin is used to remove any free HaloTag® fusion proteins.

A 2X stock of recombinant human Troponin (final 1ug/ml) was generated in assay buffer, and 50ul/well added to a non-binding surface treated, 96 well solid-white plate (Costar 3600). A 2X master mix of the purified LgTrip 3546 (SEQ ID NO: 51) (final concentration 1uM) + SmTrip9 pep521 labeled 10-T79F clone (SEQ ID NO: 268) (final 1ug/ml) + SmTrip10 pep289 labeled 10-T79C clone (SEQ ID NO: 150) (final 1ug/ml) was created in assay buffer, and 50ul/well added. A 5X stock of Nano-Glo® Live Cell Substrate in assay buffer, 25ul/well was added to the plate for a final concentration of 10uM, and luminescence was measured in real-time using a GloMax® Discover. Assay buffer consisted of Blocker BSA (10%) (Thermo) diluted in PBS (pH 7.0) to a final of 0.01% BSA in PBS. Samples were tested in triplicate.

Results are depicted in Figure 151.

Example 89

Translocation assay

HiBiT exhibits a very high affinity for the LgBiT polypeptide (K_D = 1 nM) and other similar complementary polypeptides. The strong interaction between the two fragments

would drive complementation without any stimuli (Figure 154), which would be unsuitable for a translocation assay. A study was conducted to determine the optimal affinity between two components (e.g., peptide and polypeptide) of a translocation assays. The optimal affinity was found to be in the range of 280 nM to 1300 nM. A quadruple mutations in LgBiT (E11K/I44M/N135V/L150S), referred to as LgBiT*, reduces its interaction with HiBiT by ~1000 fold (K_D = 1296 nM), renderring the HiBiT/LgBiT* pair well-suited for a translocation assay. Two different translocation assays were designed and tested.

A membrane translocation assay was developed to measure PKCα translocation from cytosol to the plasma membrane under PMA stimulus. PKCα was endogenously tagged with HiBiT at the C-terminus in HeLa cells. The clones of edited cells were isolated, and the best clone with the highest luminescence signal was chosen to perform the assay. LgBiT*-membrane sensor was introduced to the clone using transfection method. Addition of PMA recruits PKCα-HiBiT to the plasma membrane, where HiBiT meets LgBiT* to produce light. Titration of PMA yielded 12- to 19-fold increase in response depending on the amount of LgBiT* transfected (Figure 155).

A nuclear translocation assay was developed using measuring p65 movement from cytosol to the nucleus under TNFα stimulus. The nuclear translocation assay was set up similar to the membrane translocation assay. Specifically, p65 was endogenously tagged at the C-terminus in HeLa cells, and LgBiT*-nuclear sensor was introduced to p65-HiBiT cell line via transfection method. Treatment of TNFα promotes translocation of p65-HiBiT to the nucleus, where complementation occurs between HiBiT and LgBiT* to yield luminescence signal. Titration of TNFα resulted in 4-fold increase in response (Figure 156A). The assay allows measurement of protein translocation in real time. As shown in Figure 156B, it takes approximately 30 minutes for p65 to migrate to the nucleus upon stimulation of TNFα, which is consistent with findings in the literature.

Example 90

Comparison Kd and Bmax values of LgBiT mutants with HiBiT

A solution of HiBiT peptide was prepared starting at 1.22uM in OptiMEM+10% FBS. Serially diluted the peptide dilution 3-fold into OptiMEM+10% FBS. (300ul in 700ul). Diluted purified LgBiT or LgBiT mutant into OptiMEM+10% FBS to a concentration of 2nM. 90ul of the peptide solution was combined with 10ul of the LgBiT dilution (0.2nM LgBiT final). Samples were incubated on an orbital shaker for 30 minutes, and then 11ul of

100uM furimazine in OptiMEM+10% FBS added. Samples were placed on an orbital shaker for 5 minutes, and then luminescence read using a GloMax Multi+ luminometer. Bmax and Kd was calculated with GraphPad Prism using one site specific binding non-linear regression (Figure 157A-B).

Example 91

Affinity of LgBiT mutant lysates for HiBiT

Grew 37°C overnight cultures of LgBiT and each LgBiT mutant. Diluted each culture 1:100 into LB+0.1% Rhamnose and 0.15% glucose. Grew for 20 hours at 25°C. Lysates of each culture were prepared by diluting equal volumes of induced cultures with PLB lysis buffer. (PLB lysis buffer is 0.3X PLB+25mM HEPES pH 7.5). Each lysate was then diluted10,000X into PLB lysis buffer. A dilution series of synthetic HiBiT peptide starting at 300nM was prepared into NanoGlo® Assay buffer+50uM furimazine. 50ul of each diluted lysate was combined with 50ul of the peptide/NanoGlo® titration. Samples were incubated for 3 minutes, and then luminescence read samples on a GloMax® multi+ luminometer (Figure 158).

Example 92

Bioluminescence from complexes of truncated LgTrip3546 with complementary polypeptide

495ul of OptiMEM+10% FBS was aliquoted into deep well plate. Peptides 846 and 847 were diluted to 20uM in 500ul of OptiMEM+10% FBS. Two-fold serial dilutions were prepared for each peptide. Then 200ul of each dilution series was transferred to a new row and then ATG-3929 was added (2nM final) to the 846 titration series and ATG-4794 was added to the 847 titration. As a control 250nM pep 263 was added to 0.2nM of LgTrip 3546. 50ul of each sample was aliquoted in triplicate into a white assay plate and then plate was incubated with shaking for 10 minutes. After incubation 6ul of 10X FZ LCS (167ul of LCS in 833ul of OptiMEM+10% FBS. Samples were mixed for 30 sec on orbital shaker then read immediately, and several times after to get peak luminescence for each condition. The mixtures of 846+ATG-3929 and 847+ATG-4794 peaked at a concentration of 1.25uM peptide. Results are depicted in Figure 159.

Example 93

B9 Titration

LgTrip was diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol, and pep289 was added to 25 uM. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT. Results are depicted in Figure 160.

Example 94

Pep289 Titration

LgTrip was diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol, and pep289 was added to 25 uM. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (20x Kd) and titration of VS-HiBiT. Results are depicted in Figure 161.

Example 95

Dipeptide Affinity Determination

Dipeptides pep263, pep788, and pep900 were diluted to 5 uM and diluted serially 5-fold using TBS + 0.01% BSA + 0.01% Tergitol with 0.2 nM of LgTrip as the diluent. Samples were incubated 10 minutes at room temperature and added to assay plates in triplicate. One-to-one vol:vol of TBS + 0.01% BSA + 0.01% Tergitol with 20x diluted live cell furimazine substrate was added to samples, and plates were read on a GloMax® luminometer after 10 minutes. Results are depicted in Figure 162.

Example 96

Bmax Determination with LgTrip Variants and Pep788

LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 4 hours at 37°C with shaking. Ten microliters of each induction sample was diluted into 250 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5). Eighty microliters of lysates were diluted further in 2 ml lysis buffer. A 10-fold dilution series of pep788 (SEQ ID 414) was performed stating at 10 μM peptide using Nano-Glo with 50 uM furimazine as the

diluent. Peptide dilutions and lysates were mixed 1:1 vol:vol, incubated 10 min at room temperature, and luminescence was read. Results are depicted in Figure 163.

Example 97

Bmax Determination with LgTrip Variants and Pep759

LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 4 hours at 37°C with shaking. Ten microliters of each induction sample was diluted into 250 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5). Eighty microliters of lysates were diluted further in 2 ml lysis buffer. A 5-fold dilution series of pep759 (SEQ ID 496) was performed stating at 50 μM peptide using Nano-Glo with 50 uM furimazine and 50 μM pep289 (SEQ ID 826) as the diluent. Peptide dilutions and lysates were mixed 1:1 vol:vol, incubated 10 min at room temperature, and luminescence was read. Results are depicted in Figure 164.

Example 98

Thermal Stability of LgTrip Variants

LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at 80°C for 1.5 hrs in a thermocycler. Controls were incubated on ice. Samples were equilibrated to room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID 414) in TBS + 0.01% BSA + 20x diluted live cell furimazine substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read. Results are depicted in Figure 165.

Example 99

Kd and Bmax Determinations of LgTrip Variants with Pep788

LgTrip variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep788. This solution was used as the diluent for 5-fold serial

dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT. Results are depicted in Figure 166.

Example 100

Kd and Bmax Determinations of LgTrip Variants with Pep840

LgTrips variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep840. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT. Results are depicted in Figure 167.

Example 101

Kd and Bmax Determinations of LgTrip Variants with Pep289 and Saturating Pep840

LgTrip variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289 + 12.5 uM Pep840. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT. Results are depicted in Figure 168.

Example 102

Half-Life Determination of LgTrip Variants

MagneHis purified LgTrip variants were diluted to 20 nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in thermal cycler. Samples were removed at various time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 in 495µl) in TBS+0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of TBS + 0.01% BSA +20x diluted live cell substrate furimazine + 400 nM pep788 (SEQ ID NO: 414). Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated by non-linear regression. Results are depicted in Figure 169.

Example 103

Rapamycin Assay with LgTrip Variants

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10 were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes. Results are depicted in Figures 170-171.

Example 104

Thermal Stability of the Lysates of LgTrip Variants

LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at 70°C for 1.5 hrs in a thermocycler. Controls were incubated on ice. Samples were equilibrated to room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID

414) in TBS + 0.01% BSA + 20x diluted live cell substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read. Results are depicted in Figure 172.

Example 105

Thermal Stability of the Lysates of LgTrip Variants at Different Temperature Gradients

LgTrip variants were grown overnight at 37°C in LB with 100 ug/ml ampicillin. Cells were diluted 20-fold into induction media (LB with 100 ug/ml ampicillin and 0.1% rhamnose w/v) and induced 20 hours at 25°C with shaking. Twenty microliters of each induction was diluted into 40 ul lysis buffer (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 15 min at room temperature. Lysates were diluted 1,000-fold into 1X TBS + 0.01% BSA. Fifty microliters of each sample was transferred into a PCR plate and incubated at two temperature gradients, either 75-100°C for 10 min or 50-75°C for 1.5 hr, in a Veritas thermocycler. Controls were incubated on ice. Samples were equilibrated to room temperature and diluted 1:100 into 1X TBS + 0.01% BSA. Twenty-five microliters of each sample were transferred into assay plates and mixed with 25 ul of 400nM pep788 (SEQ ID 414) in TBS + 0.01% BSA + 20x diluted live cell furimazine substrate. Samples were incubated 10 minutes at room temperature, and luminescence was read. Results are depicted in Figure 173.

Example 106

Thermal Stability of Purified LgTrip Variants

MagneHis purified LgTrip variants were diluted to 20 nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in thermal cycler. Samples were removed at various time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 in 495µl) in TBS+0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of TBS + 0.01% BSA +20x diluted live cell substrate furimazine + 400 nM pep788 (SEQ ID NO: 414). Samples were incubated for 10 minutes and then read on GMM+. Results are depicted in Figure 174.

Example 107

Kd and Bmax Determinations of LgTrip Variants with Pep521 and Saturating VS-HiBiT

LgTrip variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA

+ 0.01% Tergitol + 25 uM pep521. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. Results are depicted in Figure 175.

Example 108

Kd and Bmax Determinations of LgTrip Variants with Pep840 and Saturating VS-HiBiT

LgTrip variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep840. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. Results are depicted in Figure 176.

Example 109

Kd and Bmax Determinations of SmTrip9 Variants with LgTrip Variant ATG-3546 or ATG-5146 and Saturating Pep289

LgTrip ATG-3546 and ATG-5146 were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min and luminescence was read. Results are depicted in Figures 177 and 178.

Example 109

Kd and Bmax Determinations of Pep289 with LgTrip Variant ATG-3546 or ATG-5146 and Saturating SmTrip9

LgTrip ATG-3546 and ATG-5146 were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at

room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min and luminescence was read. To determine VS-HiBiT Kd, the same protocol was followed, but with saturating SmTrip9 (25 uM) and titration of VS-HiBiT. Results are depicted in Figures 179 and 180.

Example 110

Kd and Bmax Determinations of Lysates of LgTrip Variants with Pep840 Titration

LgTrip cultures were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin and induced ~20 hr at 25°C with shaking. Cells were diluted 1000x in PLB assay reagent (0.3X PLB + 25mM HEPES pH 7.5) and lysed for 20 min. Five-fold serial dilutions of SmTrip9 pep840 were performed in NanoGlo + 50 uM furimazine + 25 uM pep289 and mixed 1:1 vol:vol with LgTrip lysates. Samples were incubated 10 min at room temperature and read on GloMax® luminometer. Results are depicted in Figures 181.

Example 111

Kd and Bmax Determinations of Purified LgTrip Variants with Pep840 Titration

LgTrip variants were purified using the Promega MagneHis™ Protein Purification System according to the manufacturer's protocol and diluted to 0.2 nM in TBS + 0.01% BSA + 0.01% Tergitol + 25 uM pep289. This solution was used as the diluent for 5-fold serial dilution series of SmTrip9 peptides. Samples were equilibrated 10 min at room temperature and aliquoted into assay plates in triplicate. TBS + 0.01% BSA + 0.01% Tergitol containing 20 uM furimazine was added to samples in 1:1 vol:vol ratio. Plates were incubated 10 min, and luminescence was read. Results are depicted in Figures 182.

Example 112

Half-Life Determination of Purified LgTrip Variants

MagneHis purified LgTrip variants were diluted to 20 nM in TBS+0.01% BSA. In triplicate, 100µl aliquots of each sample were loaded into 200µl thin walled PCR tubes. Samples were incubated at 70°C in thermal cycler. Samples were removed at various time-points and equilibrated to room temperature. Samples were diluted to 0.2 nM (5 in 495µl) in TBS+0.01% BSA. 25 µl of each diluted sample was combined with 25 µl of TBS + 0.01%

BSA +20x diluted live cell substrate furimazine + 200 nM pep900 (SEQ ID NO: 907). Samples were incubated for 10 minutes and then read on GMM+. Half-life was calculated by non-linear regression. Results are depicted in Figures 183.

Example 113

Rapamycin Assay with LgTrip Variants ATG-3546 and ATG-5146

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10 were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes. Results are depicted in Figures 184.

Example 114

Bmax Determinations of Lystates of LegTrip Variants with Pep788, Pep900, or Pep840

LgTrip variant cultures were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin and induced ~20 hr at 25°C with shaking. Cells were diluted 5000x in 0.3x PLB assay reagent and lysed for 20 min. Five-fold serial dilutions of dipeptides pep788, pep900, or SmTrip9 pep840 with saturating pep289 were performed in Nano-Glo + 50 uM furimazine and mixed 1:1 vol:vol with LgTrip lysates. Samples were incubated 10 min at room temperature and read on GloMax® luminometer. Bmax was calculated by non-linear regression. Results are depicted in Figures 185.

Example 115

Thermal Stability of LgTrip Variants at Different Temperature Gradients

LgTrip variants were diluted to 20 nM in 2 ml TBS+0.01% BSA. 100ul of each sample was aliquoted into duplicate rows of 96-well PCR plates (make two plates). Plates were incubated for 3 hrs at high (75-100°C) or low (50-75°C) temperature gradients in a Veritas thermocycler. Samples were placed at 70°C and then aliquots moved to rt at various

timepoints. Samples were mixed at each timepoint with a pipette and then diluted 1:100 into TBS + 0.01% BSA. (5ul into 495ul). 25ul of each sample was then aliquoted into a white assay plate. 25ul of 200nM pep788 or pep900 in TBS + 0.01% BSA + 20x diluted live cell furimaizine substrate was added. The plate was incubated for 10 minutes and then read on GMM+. Results are depicted in Figures 186.

Example 116

Rapamycin Assay with LgTrip Variants

Cultures of FKBP_SmTrip9 variants and FRB-SmTrip10 were grown overnight in LB + 100 ug/ml ampicillin at 37°C. Cells were diluted 1:20 in LB with 0.15% glucose, 0.1% rhamnose, and 100ug/ml ampicillin. Cultures were induced ~20 hr at 25°C with shaking. PLB assay reagent was prepared with 444 nM of a MagneHis purified LgTrip variant, 90x diluted FRB-SmTrip10 culture, +/- 35 nM Rapamycin. Ninety microliters of assay reagent was added to each well of 96-well assay plates. FKBP_SmTrip9 cultures were diluted 1:10 in PLB, and 10 ul was added to assay plates. Samples were incubated 30 min at room temperature. One hundred microliters of Nano-Glo containing 50 uM furimazine was added to assay plates wells, and luminescence was read on GloMax® luminometer after 5 minutes. Results are depicted in Figures 187.

Example 117

Kd and Bmax Determinations of Pep691 and Pep692

LgTrip 3546 was diluted to 1nM in OptiMEM+10% FBS. 12µM solutions of strand 9 peptides 521 and 693 were prepared in OptiMem+10% FBS. Each strand 9 dilution was used to prepare 3-fold dilution series of each strand 10 peptide starting at 20µM. (pep86=HiBiT, pep289=VS HiBiT, pep691=HiBiT RR, pep692=VSHiBiT RR). 90µl of each dilution series was transferred to a white assay plate and then 10µl of the 1µM stock of LgTrip 3546 added. The plate was placed on an orbital shaker set to 600RPM for 30 minutes. Detection reagent of OptiMEM+10% FBS consisting of 10mM DTT and 50uM Furimazine was prepared, and 11µl added to the samples. The plate was placed on orbital shaker and mixed for 5 minutes at room temperature. The plate was read on a GloMax® Multi+ luminometer. Kd and Bmax were calculated using GraphPad Prism one site specific binding. Results are depicted in Figures 188.

Example 118

Purification of Monomeric LgBiT-SmBiT Clones

50ml cultures of an isolated colony of each monomeric LgBiT-SmBiT clone were grown in LB+30ug/ml Kanamycin for 20 hours at 37°. Cultures were then diluted 1:100 (500ul in 50ml) in LB supplmented with 30ug/ml Kanamycin, 0.1% Rhamnose, and 0.15% Glucose and grown at 25°C for 20 hours. Cultures were spun and re-suspended in 9ml of 100mMHepes pH 7.5+1ml FastBreak™ Cell Lysis Reagent (Promega; V8571)+200ul RQ DNase 1 (Promega). Samples were incubated on a orbital mixer for 30 min. at 4ºC. A aliquot was saved for "Total Lysate" sample. Samples were spun to clear lysate (7000RPM for 15 minutes), and supernatant was transferred to a new tube. Using the HisLink™ Spin Protein Purification System (Promega; V8550), 1ml of HisLink™ Protein Purification Resin was added to each cleared lysated, incubated for 10 minutes at 4ºC on an orbital mixer, washed 3x with HisLink wash/binding buffer, and eluted with 500ul elution buffer two times to recover samples.

Figure 189 demonstrates the amount of soluble and purified protein from each monomeric LgBiT-SmBiT clone, and Table 12 lists the constructs used.

Table 12. Constructs

ATG-462	NanoLuc	G
ATG-3561	Monomeric LgBiT-SmBiT	S
ATG-3562	Monomeric LgBiT-SmBiT	G
ATG-3563	Monomeric LgBiT-HiBiT	S
ATG-3564	Monomeric LgBiT-HiBiT	G

Example 119

Luminescence Determination of Monomeric LgBiT-SmBiT Clones

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 0.2nM in TBS+0.01% BSA. Diluted protein was then combined with 50ul of Furimazine (Promega; N113) in NanoGlo® buffer (Promega; N112). Luminescence was read on a GMM+ 3 minutes after substrate addition.

Figure 190 demonstrates that NanoLuc (ATG-462) is 2x brighter than the monomeric LgBiT-SmBiT proteins.

Example 120

Substrate Utilization by Monomeric LgBiT-SmBiT Clones

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 0.2nM in TBS+0.01% BSA. A 2-fold dilution series, starting at 50uM (40ul in 2ml) then 1ml to 1ml, of Furimazine (Promega; N113) in NanoGlo® buffer (Promega; N112) was prepared. 50ul of each purified monomeric LgBiT-SmBiT was combined, in duplicate, with 50ul of the tirated substrate series. Samples were incubated for 3 minutes at RT, and luminescence read on a GMM.

Figure 191 demonstrates that each monomeric LgBiT-SmBiT protein utilizes Furimazine similarly.

Example 121

Temperature Gradient

Experiments were conducted during development of embodiments herein to determine the impact of temperature on monomeric LgBiT-SmBiT variants.

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 2nM in TBS+0.01% BSA. Each diluted sample was then aliquoted into multiple wells of a 96-well PCR plate. The plate was placed in a thermocycler set for 30 minutes with a temperature gradient:

Temperature gradient A: 54, 57, 60, 63, 66, 70°C,

Temperature gradient B: 55, 60, 65, 70, 75, 80°C, or

Temperature gradient C: 65, 70, 75, 80, 85, 90°C.

After the 30 minute incubation, 5ul of each sample was combined wth 45ul of TBS+0.01% BSA, 50ul of Furimazine in NanoGlo® buffer added, incubated for 3 minutes at RT, and luminescence detected on a GMM+.

Figure 192 demonstrates that the monomeric LgBiT-SmBiT protein are significantly more stable compared to NanoLuc (ATG-462).

Example 122

Temperature Challenge

Experiments were conducted during development of embodiments herein to determine the impact of high temperature on monomeric LgBiT-SmBiT variants.

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 2nM in TBS+0.01% BSA. Each diluted sample was then aliquoted into multiple wells of a 96-well PCR plate. The plate was placed in a thermocycler set for 30 minutes with a temperature gradient of 75, 80, 85, 90, 95, 100°C.

After the 30 minute incubation, 5ul of each sample was combined wth 45ul of TBS+0.01% BSA, 50ul of Furimazine in NanoGlo® buffer added, incubated for 3 minutes at RT, and luminescence detected on a GMM+.

Figure 193 demonstrates that the 159G amino acid change (ATG-3563) provides enhanced thermostability over the 159S amino acid change (ATG-3564) in the monomeric LgBiT-SmBiT protein are significantly more stable compared to NanoLuc (ATG-462).

Example 123

Stability of Monomeric LgBiT-SmBiT at 60°C

Experiments were conducted during development of embodiments herein to determine the stability of monomeric LgBiT-SmBiT variants at 60°C.

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 2nM in TBS+0.01% BSA. Each diluted sample was then aliquoted into multiple wells of a 96-well PCR plate, and the placed placed in a thermocycler set at 60°C. At various timepoints, aliquots were removed and kept on ice. After all sample timepoints had been collected, samples were equilibrated to RT.

Once equilibrated, 5ul of each sample was combined wth 45ul of TBS+0.01% BSA, 50ul of Furimazine in NanoGlo® buffer added, incubated for 3 minutes at RT, and luminescence detected on a GMM+.

Figure 194 demonstrates consistent results with the temperature gradient assay. The monomeric LgBiT-SmBiT variants are more stable than NanoLuc.

Example 124

Stability of Monomeric LgBiT-SmBiT with Reagent at Elevated Temperature

Experiments were conducted during development of embodiments herein to determine the stability of monomeric LgBiT-SmBiT variants with a reagent at elevated temperature.

Purified monomeric LgBiT-SmBiT proteins from Example 118 were diluted to 200nM in TBS+0.01% BSA and then further diluted to 0.2nM (4ul in 4ml). 50ul of ATG-462 or ATG-3564 was mixed with either 50ul of 50uM Furimazine in NanoGlo™ buffer or 50ul of 20uM Furimazine in TBS+0.01% BSA and placed into wells of a thin-walled 96-well PCR plate. The tray was placed in a Veritas thermocycler set at a temperature gradient of 55, 60, 65, 70, 75, 80°C. At various timepoints (FIG. 195) or after 30 (FIG. 196) minutes, aliquots were removed, and luminescence detected on a GMM+.

Example 125

Kd and Vmax Determinations of NanoLuc Variants

Purified NanoLuc variants were diluted to 0.2nM in TBS+0.01% BSA. A 2-fold dilution series of Furimazine in NanoLuc® buffer starting at 50uM (40ul in 2ml), and then 1ml to 1ml dilutions. 50ul of sample, in duplicate, was mixed with the 50ul of the titration series. Samples were inclubated for 3 minutes at RT, and luminescence detected on a GMM+ (FIG. 197).

Example 126

Temperature Challenge of NanoLuc Variants

Experiments were conducted during development of embodiments herein to determine the impact of high temperature on NanoLuc variants.

Purified NanoLuc variant proteins were diluted to 2nM in TBS+0.01% BSA. Each diluted sample was then aliquoted into multiple wells of two 96-well PCR plates. The plates were placed in a thermocycler set for 30 minutes with a temperature gradient of 60, 65, 70, 75, 80, 85°C.

After the 30 minute incubation, 5ul of each sample was combined wth 45ul of TBS+0.01% BSA, 50ul of Furimazine in NanoGlo® buffer added, incubated for 3 minutes at RT, and luminescence detected on a GMM+ (FIG. 198).

The temperature challenge was repeated at a higher temperature gradient 70, 75, 80, 85, 90, 95°C with the most stable clones identified in the above temperature challenges (FIG. 199).

Example 127

Variants on ATG-5333 and ATG-5344 Screen in the Context of a Fusion for Cell-Based Translocation Assay

a) Luminescence Detection

Cultures of each variant were started from a single colony picked into 200ul LB+ampicillin media into wells of 96 well plates and grown at 37°C for 20 hours with shaking. The following day induction cultures were prepared by diluting 10ul of culture into 200ul of LB+100ug/ml ampicillin+0.1% Rhamnose and grown for 3 hours at 37°C. A lysate was prepared from the induced cultures as follows: 10ul of cells was transferred to 190ul of Passive Lysis Buffer (PLB)(0.3X PLB+25mM HEPES pH 7.5) and incubated for 5 minutes. 50ul of lysate was transferred into two assay plates and then 50ul of PLB assay buffer+20uM Furimazine with either 6uM or 0.2uM (3uM or 0.1uM final) of Pep289 was added. Assay plates were incubated for 5 minutes, and then luminescence was measured. A ratio was calculated by dividing RLU from the 3uM samples by the RLU values of the 0.1uM samples.

Table 13. Variants on ATG-5333

ATG#	3uM	0.1uM	ratio
5338	1.1	0.30	3.7
5340	1.3	0.50	2.6
5407	1.9	2.70	0.7
5408	0.3	0.06	5.0
5411	1.4	1.40	1.0
5413	3.6	2.98	1.2
5414	1.09	0.54	2.0
5416	1.34	0.28	4.8
5417	1.62	0.80	2.0
5418	1.61	0.77	2.1
5419	1.47	0.36	4.1
5420	0.63	0.10	6.3
5421	1.97	1.40	1.4

Table 14. Variants on ATG-5344

ATG#	3uM	0.1uM	ratio
5338	1.1	0.30	3.7
5340	1.3	0.50	2.6
5407	1.9	2.70	0.7
5408	0.3	0.06	5.0
5411	1.4	1.40	1.0
5413	3.6	2.98	1.2
5414	1.09	0.54	2.0
5416	1.34	0.28	4.8
5417	1.62	0.80	2.0
5418	1.61	0.77	2.1
5419	1.47	0.36	4.1
5420	0.63	0.10	6.3
5421	1.97	1.40	1.4

b) Kd and Bmax Calculation with Pep289

2ml of induction media (LB+100ug/ml AMP+0.1% Rhamnose) was innoculated with 100ul of an overnight culture of each variant. Cells were grown for 3 hours at 37°C. 250ul of the cells were diluted in 5ml of PLB and incubated for ~10 minutes at RT. 20ml of 20uM Fz in PLB (80ul) was prepared, and three 3X titration series of Pep289 prepared in the Fz reagent (50uM in 2ml) (10ul of 5mM sample in 990ul, then 300ul in 700ul, and then the 3-dilution series combined. 50ul of cell lysate was combined with 50ul of Pep289 titration, incubated for 5 minutes, and then luminescence read on GMM+.

Table 15. Calculated Bmax and Kd for LgBiT mutants

ATG#	Sequence	Bmax	Kd
5333	LgBiT'	1	1
5339	K11N	0.58	13.0
5340	R152Q	0.9	2.3
5341	V135A	0.3	2.3
5408	N156D	0.4	20.2
5409	H57Q	0.88	2.2
5432	L3H	0.2	44.0
5433	T13S	0.42	3.8
5434	P93H	0.75	1.0
5435	F120L	0.30	2.5
5437	S157R	1.0	0.6
5438	H86L	0.82	1.1
5439	M149V	0.07	51.3
5456	K11L	0.3	0.6
5457	K11R	0.67	1.0
5458	K11Y	0.4	1.2
5459	K11N+R152Q	0.4	16.9
5460	K11N+N156D	0.3	25.9
5491	K11Q	0.9	0.6
5492	K11M	0.3	0.6
5493	K11H	0.7	2.8
5494	K11F	0.3	2.2
5495	K11W	0.2	2.7
5505	V135A+R152Q	2.0	1.2
5506	V135A+R152Q+N156D	1.5	4.1
5507	P93H+V135A	1.9	0.5
5508	P93H+R152Q	0.8	3.0
5509	P93H+R152Q+N156D	0.7	6.8
5510	P93H+N135A+R152Q	1.7	1.5
5511	P93H+N135G+R152Q	0.9	3.9

Example 128

Site saturation of ATG-5534 template

Experiments were conducted during development of embodiments herein to optimize the identity of the amino acid at various positions of ATG-5534 (SEQ ID NO: 978).

a) E. coli cultures were started from a single colony picked into 200ul LB+ampicillin media into wells of 96-well plates and grown at 37°C for 20 hours with shaking. The following day, induction cultures were prepared by diluting 10ul of culture into 200ul of LB+100ug/ml ampicillin+0.1% Rhamnose and grown for 3 hours at 37°C. A lysate was prepared from the induced cultures as follows: 10ul of cells was transferred to 190ul of PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5) and incubated for 5 minutes. 50ul of lysate was transferred into two assay plates and then 50ul of PLB assay buffer+20uM Furimazine with either 6uM or 0.2uM (3uM or 0.1uM final) of pep289 was added. Assay plates were incubated for 5 minutes, and then luminescence was measured. Bmax and Kd values were determined for each mutant.

Table 16. Calculated Bmax and Kd for site saturation at position 44 of LgBiT mutant ATG-5810

ATG#	Mutant	Bmax	Kd
5654	M44V	**1.8**	**0.4**
5655	M44I	**4.3**	**0.13**
5676	M44K	0.03	19.4
5677	M44E	0.02	15.7
5678	M44A	0.4	2.1
5679	M44C	0.4	1.1
5680	M44W	0.4	0.1
5681	M44G	0.1	11.9
5682	M44H	0.4	2.5
5683	M44S	0.2	2.9
5684	M44Q	0.4	0.8
5685	M44R	0.001	0.0
5686	M44T	0.4	1.3
5687	M44Y	0.2	1.1
5688	M44L	0.9	0.8
5689	M44P	0.0	3.4
5690	M44F	0.8	1.0

b) Comparison in E.coli and mammalian cells

i) E. Coli (Kd and Bmax)

2ml of induction media (LB+100ug/ml AMP+0.1% Rhamnose) was inoculated with 100ul of an overnight culture for each mutant. Cells were grown for 3 hours at 37°C in a tube. 250ul of cells were diluted in 5ml of PLB lysis buffer and incubated for ~10 minutes at RT. 20ml of 20uM Furimazine in PLB lysis buffer (80ul) was prepared, and three 3X titration series of pep289 in the Furimazine reagent (50uM in 2ml) (10ul of 5mM sample in 990ul, then 300ul in 700ul with the 3-dilution series combined). 50ul of cell lysate was mixed with 50ul of pep289 titration, incubated for 5 minutes at RT, and then luminescence read on GMM+.

ii) Mammalian Cell Expression

a) Transfection protocol: Media from HeLa cells (PKCα-HiBiT clone) that were grown to confluency in a T-150 flask was aspriated, and cells washed with 10ml DPBS. (Life Technologies 14190). The DPBS was aspirated, and 4ml of TryPLE Express Trypsin (Life Technologies 12604) added. Cells were incubated for 2-3 minutes at 37°C, then resuspend in 16ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194).

Cells were spun at 200RPM for 5 minutes, supernatant aspirated, and 20ml of DMEM+10% FBS added. Cells were counted and then diluted to 1,000,000 cells per ml. 1mL of cells with 3mL of DMEM+10%FBS were plated into 6 cm dishes and incubated for 24h.

b) Transfection complex: For each DNA sample to be tested, 10ug of DNA (1ug of DNA encoding membrane sensor, and 9 ug of carrier DNA) (Promega E4882) combined and mixed with 400ul of OptiMem (Life Technologies 11058), 30µl of FugeneHD transfection reagent (Promega E2311) added, and incubated for 10 min at ambient temperature. Transfection complex was added to the plated cells and incubated for 24 h.

c) Cell-based luminescence assay: Media was aspirated from the transfected cells HeLa cells, and then the cells were washed with 5ml DPBS. (Life Technologies 14190). The DPBS was aspirated, and 0.75ml of TryPLE Express Trypsin (Life Technologies 12604) added. Cells were incubated for 2-3 minutes at 37°C, and then resuspended in 4ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194). 100µl of each sample was added to each well of a white 96 well assay plate (Corning 3917). For each sample, 30 wells in total were used, 10 wells per row with 3 rows. Cells were incubated for another 16-24h.

The following morning, growth media was aspirated and replaced with 90µl of CO_2 independent media (Life Technologies 18045)+10% FBS containing 1.1x NanoGlo® Live Cell Substrate (Promega N2012), incubated for 10 min, and 10µl of titrated PMA compound added to each well. (See, e.g., FIGS. 225-227). Luminescence was detected on a GlomaxMulti+ luminometer set to 37°C with a kinetic run over the course of 1 hour. Unless otherwise stated, the reported luminescence is the peak height of the kinetic run.

Table 17. RLU, S/B, Bmax, and Kd Values Normalized to ATG-5534

		Membrane sensor		*E. Coli*	
Sample	**Mutation**	**RLU**	**S/B**	**Bmax**	**Kd**
ATG-5534	WT	1	4.5	1	1
ATG-5652	Y16F+Q20P+Q152H	0.3	5.2	1.7	2.3
ATG-5653	M106R+Y114F	0.6	3.8	2	1
ATG-5654	M44V	4.4	2.4	1.8	0.4
ATG-5655	M44I	5.2	2.1	4.3	0.13
ATG-5656	M106R	1	3.2	3.5	0.76
ATG-5657	M106T	0.7	3.6	2.2	0.77
ATG-5658	M106R	0.8	4.1	3.2	0.61
ATG-5659	M106K+K136E	0.7	4.4	3.1	1.58
ATG-5660	V36G	0.8	3.3	3.7	1.1
ATG-5661	M106L+I138K	0.6	4.2	3.7	0.68
ATG-5688	M44L	1.72	3.1	0.89	0.76
ATG-5690	M44F	0.24	3.5	0.79	0.98

Example 129

Strand 9 detector polypeptides

A polypetide construct was prepared by placing a strand 10 sequence at the N-terminus of a LgTrip polypeptide. This configuration produces increased luminescence in the presence of strand 9 sequences. Experiments were conducted during development of embodiments herein to determine the limits of detection for the strand 9 peptides being tested (pep521 and pep840).

a) Strand 9 Detector Proteins with pep521 and pep840

Each strand 9 detector protein was diluted to 20nM in TBS+0.01% BSA. A 3X dilution series of pep521 and pep840 was diluted in TBS+0.01% BSA starting at 20uM. 50ul of each enzyme dilution in duplicate was combined with 50ul of each peptide titration and incubated for 10 minutes on a shaker for pre-equilibration. An assay buffer was prepared by diluting Nano-Glo Live Cell Substrate (Furimazine; Promega; N205) 30-fold into TBS+0.01% BSA, 100ul added to each well, incubated for 5 minutes at RT, and luminescence then read on GMM+. The background reading was obtained from samples that contained no peptide.

FIG. 200 shows that all three strand 9 detector proteins bound more tightly to pep840 than to pep521.

b) Strand 9 Detector Proteins with pep840

Each strand 9 detector protein was diluted to 200nM in TBS+0.01% BSA. A 3X dilution series of pep840 was diluted in TBS+0.01% BSA starting at 0.5uM. 50ul of each enzyme dilution in quadruplicate was combined with 50ul of each peptide titration and incubated for 10 minutes on a shaker for pre-equilibration. An assay buffer was prepared by diluting Nano-Glo Live Cell Substrate (Furimazine; Promega; N205) 30-fold into TBS+0.01% BSA, 100ul added to each well, incubated for 5 minutes at RT, and luminescence then read on GMM+. The background reading was obtained from samples that contained no peptide.

Regarding FIG. 20, the panel on the left shows a titration of Strand 9 peptide 840 in the presence of 50nM of three strand 9 detector constructs. The panel on the right shows the background (no peptide 840) for each strand 9 detector and the signal to background ratio at 0.1nM peptide 840 for each strand 9 detector.

Example 130

Linker Test Series

a) Overnight cultures of circularly-permuted, strand 9 detector variants were diluted to 1:20 (150ul to 3ml) in LB+0.1% rhamnose+100ug/ml ampicilin, grown for 4 hours at 37°C, and then lysed in PLB lysis buffer (0.3X PLB+25mM HEPES pH 7.5) (500ul of lysate to 4.5ml of PLB lysis buffer). To assay, lysates were diluted 1:100 into TBS+0.01% BSA. A 3x dilution series of pep840 (strand 9) was prepared starting at 10nM. 50ul of each dilute lysate was combined with 50ul of peptide titration and incubated for 20 minutes at RT on a shaker set at 600rpm. 100ul Nano-Glo® buffer + 50uM Furimazine was added to each well, and luminescence then read on GMM+. The linkers used for each construct tested were: ATG-4992, 8GS; ATG-5485, 5GS; ATG-5486, 6GS; ATG-5487, 7GS; ATG-5488, 9GS; ATG-5489, 10GS; and ATG-5490, 11GS.

FIG. 202 shows that each clone, other than ATG-5485, which has a 5AA linker, produced similar luminescence to ATG-4992. The experiments demonstrate that linker length between strand 10, and LgTrip did not play a significant role in the detection of strand 9 sequences.

b) 8GS Linker (ATG-4992) vs. 11GS (ATG-5490) Linker

ATG-4992 and ATG-5490 proteins were purified using the MagneHis purification system. (Promega). Purified protein for 4992 and 5490 were diluted to 100nM in CO_2 independent media+10% FBS. A 3-fold serial dilution of pep840 was prepared starting at 10nM in Nano-Glo® buffer + 50uM furimazine(Promega N113). 50ul of each enzyme dilution was combined in quadruplicate with 50ul the peptide titration. Luminescence was measured over time on a GMM+ luminometer.

FIG. 203 show the data plotted is from the 50-minute kinetic read and that 5490, which has the longer linker, provided 2-fold more luminescence over 4992.

Example 131

Determination of Kd of ATG-4992 and ATG-5490 variants

Overnight cultures of each variant were prepared in LB+100ug/ml ampicillin. The following day, cultures were diluted 1:20 (150ul to 3ml) in LB+0.1% Rhamnose+100ug/ml ampicillin. Cultures were grown for 4 hours at 37°C and then lysed with PLB lysis buffer (0.3X PLB (Promega)+25mM HEPES pH 7.5)(500ul of lysate to 4.5ml of PLB lysis buffer). To assay, lysates were diluted 1:100 into TBS+0.01% BSA. A 3X dilution series of strand 9 (pep840) was prepared starting at 40uM. 50ul of the titration series was combined with 50ul

of the each diluted lysate, incubated for 20 minutes at RT on orbital shaker set to 600rpm, 100ul of Nano-Glo® buffer + 50uM Furimazine (Promega; N113) added, and luminescence read on GMM+. Results are depicted in FIG. 204.

Example 132

Comparison of ATG-4992 and ATG-5490 variants

ATG-4992 and ATG-5490 variant proteins were purified using the MagneHis purification system. (Promega). Purified proteins were then diluted to 100nM in CO_2 independent media+10% FBS. A 3-fold serial dilution of pep840 was prepared starting at 2nM in Nano-Glo® buffer + 50uM furimazine(Promega N113). 50ul of each enzyme dilution was combined in triplicate with 50ul the peptide titration. Luminescence was measured every 5 minutes on a GMM+ luminometer. Results are depicted in FIG. 205 as data plotted from the 60-minute timepoint and show that variants 5515 and 5517 have lower calculated Kd values (Example 131) and show higher RLU signal to background values when paired with pep840.

Example 133

Screen of ATG-4166 Variants

Table 18. LgBiT sequences in different backbones. (C-terminal His Tag for protein purification or sensor fusion (pH domain-GSSG-HaloTag-GSSG-LgBiT mutant).

pF1A (His tag for purification)	Equivalent sequence (sensor backbone)	Sequence compared to LgBiT
ATG-2623	LgBiT	LgBiT
ATG-4166	ATG-5333	LgBiT+E11K+I44M+N135V+L150S
ATG-5810	ATG-5534	LgBiT+E11K+I44M+L150S+P93H+V135A+R152Q
ATG-5819	ATG-5655	LgBiT+E11K+L150S+P93H+N135A+R152Q
ATG-5820	ATG-5654	LgBiT+E11K+I44V+L150S+P93H+N135A+R152Q
ATG-5821	ATG-5690	LgBiT+E11K+I44F+L150S+P93H+N135A+R152Q
ATG-5822	ATG-5688	LgBiT+E11K+I44L+L150S+P93H+N135A+R152Q

i) Bmax and Kd Determination

ATG-4166 variant proteins were purified using the MagneHis purification system. (Promega). Purified proteins were then diluted to 0.2nM in TBS+0.01% BSA. A 3-fold serial dilution of pep289 (VS-HiBiT) was prepared in TBS+0.01% Tergitol with one series starting at 400nM and the other at 20uM. 50ul of each enzyme dilution (0.2nM) was combined with 50ul the peptide titration and incubated on a shaker for 10 minutes. 100ul of Furimazine (N113) diluted 250-fold in TBS+0.0.1% Tergitol was added, and samples again placed on a shaker for 5 miuntes. Luminescence was measured on a GMM+ luminometer. Bmax and Kd were calculated, and results depicted in FIG. 206.

ii) Activity at Various pH

ATG-4166 variant proteins were diluted to 0.2nM in TBS+0.01% BSA. 20uM pep289 (VS-HiBiT) to each variant protein sample and incubate at RT for 20 minutes. 990ul of each pH buffer solution was added to a well a deep well plate. 10ul of Furimazine (N113) was added to each well, and 50ul of each variant protein/peptide sample added to each well. The plate was incubated at RT for 12 minutes, and luminescence was measured on a GMM+ luminometer. Activity was calculated, and results depicted in FIG. 207.

pH Buffer Series Preparation: Components listed in the table below were mixed in 400ml of water. 30ml of the buffer was added to twelve 50ml tubes, and either NaOH or HCl used to create the necessary pH.

Table 19. Formulation for universal pH buffer

Component	Stock	Amount	Unit	Concentration (400ml)
Na Citrate	Powder	5.88	g	50mM
MES Hydrate	Powder	3.9	g	50mM
PIPES	Powder	6.05	g	50mM
HEPES	1M	20	ml	50mM
TAPS	Powder	4.87	g	50mM
Tergitol	100%	1	ml	0.25
Mazu	100%	0.1	ml	0.025
ATT	powder	57	mg	1mM

Example 134

Screen of ATG-5823, ATG-5824, and ATG-5825 Variants

Variant proteins (ATG-5823,ATG-5824, and ATG-5825) were purified using Magne His purification system (Promega). ATG-5146 was purified using an AKTA with Nickel Sepharose column. Purified proteins were diluted first to 200nM in TBS+0.01% BSA and

then further diluted to 0.2nM in TBS+0.01% BSA. A three-fold dilution series of pep263 was prepared starting at 100nM in TBS+0.01% BSA+0.02% tergitol. 50ul of each enzyme was combined with 50ul of the peptide dilution series. Samples were incubated on orbital shaker (600RPM) for 10 minutes. After incubation, 100ul of LCS (N205; Promega) was diluted 1:30 into TBS+0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence measured on a GloMaxMulti+. Bmax and Kd were calculated, and results depicted in FIG. 208 show similar Bmax and Kd for these variants.

Example 135

Screen of ATG-5826 and ATG-5827 Variants

i. pep86

Variant proteins (ATG-5826 and ATG-5827) were purified using MagneHis purification system (Promega V8500). Purified proteins were diluted first to 200nM in TBS+0.01% BSA and then further diluted to 0.2nM in TBS+0.01% BSA. Two 2-fold titration series of pep86 were prepared starting at 1uM and 100nM in TBS+0.01% BSA+0.02% Tergitol. 50ul of ATG-5826 and ATG-5827 were combined with 50ul of the peptide dilution series that started at 1uM. 50ul of LgBiT protein (Promega; N401C) was combined with the peptide titration series that started at 100nM. Samples were incubated on orbital shaker (600RPM) for 10 minutes. After incubation 100ul of LCS (N205; Promega) was diluted 1:30 into TBS+0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence measured on a GloMaxMulti+. Bmax and Kd were calculated, and results depicted in FIG. 209 show similar Bmax and Kd for these variants. Clones with 760 sequence for strand 9 showed a significantly higher Kd. This shows that variants such as these two containing the 760 sequence have higher Kd valued.

ii. pep114

Variant proteins (ATG-5826 and ATG-5827) were purified using MageHis purification system (Promega V8500). Purified proteins were diluted first to 200nM in TBS+0.01% BSA and then further diluted to 0.2nM in TBS+0.01% BSA. Two 2-fold titration series of pep114 was prepared starting at 1mM in TBS+0.01% BSA+0.02% tergitol. 50ul of ATG-5826, ATG-5827, and LgBiT protein (Promega N401C) were combined with the peptide titration series. Samples were incubated on orbital shaker (600RPM) for 30 minutes. After incubation, 100ul of LCS (N205 Promega) was diluted 1:30 into TBS+0.01% BSA and added to each sample. Samples were incubated for 3 minutes at RT, and then luminescence measured on a

GloMaxMulti+. Bmax and Kd were calculated, and results depicted in FIG. 210 show similar trend for Bmax values compared to pep86 (ATG-5826>LgBiT>ATG-5827). Both ATG-5826 and ATG-5827 have slightly lower calculated Kd values compared to LgBiT/pep114, but significantly higher Kd values compared to LgBiT/HiBiT(pep86).

Example 136

SDS-PAGE of ATG-5823, ATG-5824, ATG-5825, ATG-5826, and ATG-5827 Variants

Each variant protein was diluted to 0.1ug/ml in TBS+1X SDS loading dye. Samples were heated to 70°C for 5 minutes and then 3ul (0.3ug) was loaded to an SDS PAGE gel (BioRad Criterion). Results depicted in FIG. 211.

Example 137

Spike-in Antibody Titration Using 3 Labeling Methods:

NanoTrip, Sulfhydryl-Trip Labeling, and NHS-CA-HaloTag-Trip labeling

Figure 212 provides a demonstration of each labeling method.

i) NanoTrip-Genetic fusion

A two-fold antibody titration starting at 4ug/mL was prepared in PBS+0.01% BSA or PBS+20% human serum leaving the 24th well as the "no antibody" control. (Pool equal amounts of Sino antibodies D1-D5). 50ul of each antibody titration was added to wells of a white assay plate in triplicate. A master mix for each combination with each strand 9 and 10 protein and 2μM of LgTrip was prepared. 125ng/ml was used for ATG-5547 and ATG-5546 and 500ng/ml for ATG-5541. 50ul of the master mix for each combination (ATG-5546+ATG-5541 or ATG5547+ATG-5541) was added to the antibody titrations and incubated for 60 minutes at RT. A detection reagent was made by diluting NanoGlo® Live Cell Substate (N205) 1:30 in PBS+0.01% BSA, and 100ul added to each sample. Plates were incubated for three minutes, and then luminescence measured on a GloMax Discover. RLU readings were divided by the "no antibody" control to obtain Signal/Background readings.

ii) Sulfhydryl-Trip and NHS-CA-HaloTag-Trip labeling

FIG. 214 demonstrates that three different NanoTrip detection methods are capable of detecting SARS-COV/COV2 antibody.

Sino Biologicals (SARS-CoV/SARS-CoV2 spike)Antibodies:

D1	40150-D001	HA14MA0604
D2	40150-D002	HA14FE2802
D3	40150-D003	HA14FE2803
D4	40150-D004	MA14AP0203
D5	40150-D005	HA14FE2502

About 50ul 2X RBD reagents + 2X LgTrip prepared in 0.01% PBSA were added to wells of a 96-well assay plated. For the NHS-CA-HaloTag-Trip labeling, RBD reagent was prepared as a final concentration/well of 8ng/ml RBD-HaloTag-HiBiT + 16 ng/ml RBD-HaloTag-SmTrip9 (pep840) + 1uM LgTrip ATG-5146. For Suldhydryl-Trip labeling, RBD reagent was prepared as a final concentration/well of 15ng/ml RBD-HiBiT + 15 ng/ml SulfoSE-PEG6-RBD-SmTrip9 (pep840)-PSA (FIG. 213) + 1uM LgTrip ATG-5146. 50ul 2X pooled antibodies prepared in either 0.01% PBSA or 20% serum diluted in 0.01% PBSA was added to each sample and incubated for 45 minutes. 20uM NanoGlo® Live Cell Substrate was prepared in 0.01% PBSA, 100ul added to each well, and total luminescence read on a luminometer.

Example 138

SARS-CoV-2 Nucleocapsid Titration

Anti-nucleocapsid Ab clone 9547 (Meridian Biosciences) and anti-nucleocapsid Ab clone 9548 (Meridian Biosciences) were labeled with HaloTag-SmTrip9(pep840) and HaloTag-VSHiBiT, respectfully. 25ul/well of a 4x cocktail of Abs + LgTrip ATG-5146 was added to wells of a non-binding surface, solid white 96 well microtiter plate (Costar 3600) for a final concentration/well of 30ng/ml Ab-SmTrip9 + 60 ng/ml Ab-HiBiT + 1uM LgTrip ATG-5146. 25ul/well of a 4x solution of recombinant nucleocapsid protein (Meridian Biosciences Cat # 9560) was added to each well followed by the addition of 50ul/well of a 2x solution of NanoGlo® Live Cell Substrate for final concentration/well of 10uM substrate. Plates were incubate for 15 minutes, and total luminescence measured on GlowMax luminometer.

FIG. 215 demonstrates ternary NanoLuc-labeled antibodies detect SARS-CoV-2 Nucleocapsid protein.

Example 139

Point of Care Swabs for SARS-CoV-2 Nucleocapsid Protein in Nasopharyngeal Swab Samples

A stock solution containing 120 ng/ml Anti-nucleocapsid antibody HaloTag-SmTrip9, 240 ng/ml Anti-nucleocapsid antibody HaloTag-HiBiT, 4uM LgTrip ATG-5146, 40uM

Furimazine in ethanol, 1.2mM azo-thiothymine (ATT), 1.2 mM ascorbic acid, 0.6% pullulan w/v, 4.8 mM HEPES buffer pH 8.0, 21.6 mM Glycine, 4.8mM histidine, 6 mg/ml sucrose, and 0.0024% Polysorbate 80 was created. 100ul of the stock solution was dispensed into plastic swab jackets and loaded onto the lyophilizer (Virtis Genesis 12EL) with shelves pre-set to 4.0°C. Upon evacuation of the system, the lyophilization process was performed between shelf temperatures of -25°C and +25°C. The ice sublimation phase lasted 8hr, and the bound water desorption phase lasted 16hr. At the end of the lyophilization process, the swabs were back filled with N° and sealed by inserting a second plastic swab jacket manually.

Swabs containing the lyophilized assay reagents were then rehydrated with 100ul of nasopharyngeal swab samples + 300ul PBS containing 0.01% BSA. Total luminescence was measured on a handheld luminometer (Prom4ega) at times 15, 30, 45, and 60 minutes and plotted.

FIG. 216 show the results from 3 PCR confirmed negative (NS46, NS47, and NS52) and 3 PCR confirmed positive samples (PS46, PS49, and PS56).

Example 140

Monomeric NanoBiT with Fluoro-Fz

Each enzyme to be tested was diluted into TBS+0.01% BSA. A titration series with either Furimazine (N205) or JRW-1677 was made either TBS (start at 20uM) or NanoGlo® buffer (start at 25uM; Promega N112). Each substrate was serially diluted with either TBS+0.01% BSA or NanoGlo® buffer. 50ul of each enzyme dilution as combined with 50ul of each substrate titration. Plates were incubated for 3 minutes, and then luminescence measured on GMM+ luminometer. GraphPad Prism was used to generate a non-linear regression using Michaelis-Menten Least squares fit.

FIG. 217 provide the kinetic parameters (Vmax and Km) for Furimazine and JRW-1667. NanoLuc (ATG-462) produced higher RLU values with both Furimazine and JRW-1667 (~10-20 fold) compared to the Monomeric NanoBiT constructs when TBS is used to dilute substrates. NanoLuc and the monomeric constructs showed similar RLU values in Furimazin/NanoGlo® buffer, but only NanoLuc showed improved luminescence with JRW-1667. Although the monomeric NanoBiT constructs showed lower RLU values with both Furimazine and JRW-1667, RLU values were similar for the two buffers and two substrates.

Km values were lower in TBS+0.01% BSA compared to NanoGlo, but generally the Km values were similar for each condition except ATG-3562 (Monomeric LgBiT-SmBiT), which shows a lower calculated Km for all conditions tested.

Example 141

Mammalian cell expression and fluorescence imaging

Transfection protocol: Preparing cells: Aspirated media from HeLa cells that were grown to confluency in a T-75 flask and washed cells with 10ml DPBS. (Life Technologies 14190). Aspirated DPBS and added 2ml of TryPLE Express Trypsin (Life Technologies 12604). Incubated cells for 2-3 minutes at 37°C then resuspended cells in 8ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194). Spun cells at 200RPM for 5 minutes. Aspirated supernatant and added 10ml of DMEM+10% FBS. Counted cells and then diluted to 100,000 cells per ml. Plated 3 mL of cells to each well of 6 well plate. Incubated cells for 24 h.

Transfection complex: For each DNA sample to be tested, combined and mixed 2.5µg of DNA (0.25µg of DNA encoding sensor, and 2.25 µg of carrier DNA) (Promega E4882) with 100ul of OptiMem (Life Technologies 11058). Next added 7.5µl of Fugene HD transfection reagent (Promega E2311) and incubated transfection complex for 10 min at ambient temperature. Added transfection complex to each well containing plated cells. Incubated cells for 24 h.

Fluorescence Imaging: Replated transfected cells to 8-well chamber slides (MatTek glass bottom). Aspirated media from transfected cells HeLa cells and washed cells with 3ml DPBS. (Life Technologies 14190). Aspirated DPBS and add 0.5ml of TryPLE Express Trypsin (Life Technologies 12604). Incubatde cells for 2-3 minutes at 37°C then resuspended cells in 3ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194). Counted cells and then diluted to 50,000 cells per mL. Plated 500µl of each well. For cellular markers that are delivered by BacMam transduction, after 3h of plating, add 10% (v/v) of CellLight Plasma Membrane-GFP (Thermo Fisher C10607) or CellLight Golgi-GFP (Thermo Fisher C10592) or CellLight Lysosome-GFP (Thermo Fisher C10507). Incubated cells for another 16-24h.

The following morning, aspirated growth media and replaced with 400µl of FluoroBrite DMEM+10%FBS (Life Technologies A1896701)+10% FBS. Prepared 5X dilution of Janelia Fluor 549 HaloTag (Promega GA1110) in FluoroBrite DMEM+10%FBS,

and add 100 µl to each well. Incubated for 30 min. Washed cells with 500µl FluoroBrite DMEM+10%FBS for 30 min. Cells were stained with appropriate cellular markers. Staining procedure was followed by manufacture recommendation. Stained mitochondria with MitoTracker Green FM (Thermo Fisher M7514). Stain endoplasmic reticulum with ER-Tracker Red (Thermo Fisher E34250). Counterstain cells with the nuclear probe NucBlue Live ReadyProbes reagent (Thermo Fisher R37605). Image cells with C2 laser scanning confocal microscope (Nikon).

Results are depicted in images of Figures 218-224.

Example 142

Mammalian cell expression and luminescence assays

Transfection protocol: Preparing cells: Aspirated media from HeLa cells (PKCα-HiBiT clone) that were grown to confluency in a T-150 flask and wash cells with 10ml DPBS. (Life Technologies 14190). Aspirated DPBS and added 4ml of TryPLE Express Trypsin (Life Technologies 12604). Incubatde cells for 2-3 minutes at 37°C then resuspended cells in 16ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194). Spun cells at 200RPM for 5 minutes. Aspirated supernatant and added 20ml of DMEM+10% FBS. Counted cells and then diluted to 1,000,000 cells per ml. Plated 1 mL of cells and 3 mL of DMEM+10%FBS in 6 cm dish. Incubated cells for 24 h.

Transfection complex: For each DNA sample to be tested, combined and mixed 10ug of DNA (1ug of DNA encoding membrane sensor, and 9 ug of carrier DNA) (Promega E4882) with 400ul of OptiMem (Life Technologies 11058). Next, added 30µl of FugeneHD transfection reagent (Promega E2311) and incubated transfection complex for 10 min at ambient temperature. Added transfection complex to 6 cm dish containing plated cells. Incubated cells for 24 h.

Cell-based luminescence assay: Replated transfected cells to white 96 well assay plate. Aspirated media from transfected cells HeLa cells in 6 cm dish and washed cells with 5ml DPBS. (Life Technologies 14190). Aspirated DPBS and add 0.75ml of TryPLE Express Trypsin (Life Technologies 12604). Incubated cells for 2-3 minutes at 37°C then resuspended cells in 4ml of growth media (DMEM Life Technologies 11995)+10% FBS (VWR 89510-194). Plated 100µl of each sample in 30 wells of a white 96 well assay plate. Incubated cells for another 16-24h.

The following morning, aspirated growth media and replaced with 90µl of CO2 independent media (Life Technologies 18045)+10% FBS containing 1.1X NanoGlo Live Cell Substrate (Promega N2012). Incubated for 10 min. Added 10µl of titrated PMA compound to each well. (Final PMA concentration is presented in the graph). Placed plate in a GlomaxMulti+ luminometer set to 37°C and ran a kinetic over the course of 1 hours. Unless otherwise stated, the reported luminescence is the peak height of the kinetic run.

Results depicted in Figures 225-227.

Example 143

LgTrip 3546 Optimization

Experiments were conducted during development of embodiments herein to optimize the identity of the amino acid at various positions of LgTrip 3546 (SEQ ID NO: 51), as shown below in Table 20. *E. coli* cultures (200µl) were prepared for each sample and grown overnight at 37°C in LB media+100µg/ml ampicillin. Cultures were then diluted in quadruplicate to a 20X concentration (10µl in 200µl) into induction media (LB+ampicllin+0.1% Rhamnose). Samples were grown at 37°C for 3 hours. Samples were then lysed with 0.3X PLB+25mM HEPES pH 7.5+0.001U/ml DNase (10µl of cells to 250µl of Lysis buffer). 50µl of the lysate was then combined with 50µl of NanoGlo® buffer+50µM furimazine+50nM of dipeptide 788 (SEQ ID NO: 51). Samples were measured on a BMG Clariostar luminometer. RLU values were normalized to LgTrip 3546 (SEQ ID NO: 51).

Table 20. Amino Acid Substitutions for LgTrip 3546 Optimization

```
01       10         20         30         40         50         60         70
||        |          |          |          |          |          |          |
VFTLDDFVGDW EQTAAYNLDQ VLEQGGVSSL LQNLAVSVTP IMRIVRSGEN ALKIDIHVII PYEGLSADQM

71      80         90        100        110        120        130        140
|        |          |          |          |          |          |          |
AQIEEVFKVV YPVDDHHFKV ILPYGTLVID GVTPNKLNYF GRPYEGIAVF DGKKITTTGT LWNGNKIIDE

141  146
|    |
RLITPD
```

Amino acid substitution	Fold-improvement over 3546
V0D	1.90
T13E	2.62
S28P	2.12
S29G	1.83
S29P	2.02
L30F	1.66

L30T	1.65
L30Y	1.95
T39K	1.81
K53S	1.58
Y62N	1.46
G64E	1.88
Y81E	1.97
H86D	2.52
H87N	1.83
K89E	1.89
Y94I	2.18
L97V	1.85
I99A	1.60
I99L	1.49
I99M	1.57
V102E	1.85
R112H	2.37
E115D	2.38
I117Q	2.06
I117T	1.76
V119A	1.66
V119D	1.72
V119E	1.62
V119G	1.81
V119N	1.89
K123E	1.95
K123G	1.57
W132P	1.96
L142C	1.93
T144D	1.81
P145D	1.48

SEQUENCES

The following polypeptide sequences each comprise an N-terminal methionine residue or corresponding ATG codon; polypeptide sequences lacking the N-terminal methionine residue or corresponding ATG codon are also within the scope herein and are incorporated herein by reference.

The following peptide sequences (and the peptide sequences of Table 1) each lack an N-terminal methionine residue; peptide sequences comprising an N-terminal methionine residue are also within the scope herein and are incorporated herein by reference.

Some embodiments described herein make reference to a His-tagged (or non-His-tagged) sequence within Table 1; alterantive embodiments utilizing a non-His-tagged (or His-

tagged) version of the sequence, either appearing in Table 1 or not listed, are within the scope herein.

Table 1. Exemplary peptide, dipeptide, and polypeptide sequences.

SEQ ID NO	Name	Sequence
1	WT OgLuc	MFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGV SVTPIQKVVLSGENGLKADIHVIIPYEGLSGFQMGLIEMIF KVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRPYPGI AVFDGKQITVTGTLWNGNKIYDERLINPDGSLLFRVTIN GVTGWRLCENILA
2	WT OgLuc	atggtgtttaccttggcagatttcgttggagactggcaacagacagctggatacaaccaag atcaagtgttagaacaaggaggattgtctagtctgttccaagccctgggagtgtcagtcac cccaatccagaaagttgtgctgtctggggagaatgggttaaaagctgatattcatgtcatca tcccttacgagggactcagtggttttcaaatgggtctgattgaaatgatcttcaaagttgttta cccagtggatgatcatcatttcaagattattctccattatggtacactcgttattgacggtgtg acaccaaacatgattgactactttggacgcccttaccctggaattgctgtgtttgacggcaa gcagatcacagttactggaactctgtggaacggcaacaagatctatgatgagcgcctgat caacccagatggttcactcctcttccgcgttactatcaatggagtcaccggatggcgccttt gcgagaacattcttgcc
3	NanoLuc	MKHHHHHHAIAMVFTLEDFVGDWRQTAGYNLDQVLEQ GGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLS GDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPN MIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINP DGSLLFRVTINGVTGWRLCERILAV
4	NanoLuc	atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttgg ggactggcgacagacagccggctacaacctggaccaagtccttgaacagggaggtgtg tccagtttgtttcagaatctcggggtgtccgtaactccgatccaaaggattgtcctgagcgg tgaaaatgggctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcggcga ccaaatgggccagatcgaaaaaatttttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgcactatggcacactggtaatcgacggggttacgccgaacatgatcgact atttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacag gaccctgtggaacggcaacaaaattatcgacgagcgcctgatcaaccccgacggctcc ctgctgttccgagtaaccatcaacggagtgaccggctggcggctgtgcgaacgcattctg gcggtt
5	WT OgLuc Lg	MFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGV SVTPIQKVVLSGENGLKADIHVIIPYEGLSGFQMGLIEMIF KVVYPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRPYPGI AVFDGKQITVTGTLWNGNKIYDERLINPD
6	WT OgLuc β9	GSLLFRVTIN
7	WT OgLuc β10	GVTGWRLCENILA

8	WT NanoLuc Lg	MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLG VSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIF KVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEG IAVFDGKKITVTGTLWNGNKIIDERLINPD
9	WT NanoLuc β9	GSLLFRVTINV
10	WT NanoLuc β10	GVTGWRLCERILA
11	LgBit	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTI NSHHHHHH
12	LgBit	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccac
13	SmBit	VTGYRLFEEIL
14	SmBit	gtgaccggctaccggctgttcgaggagattctg
15	HiBit	VSGWRLFKKIS
16	HiBit	gtgagcggctggcggctgttcaagaagattagc
17	LgTrip 2098	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPD
18	LgTrip 2098	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgCcctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgac
19	LgTrip 3092 His	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPD

20	LgTrip 3092 His	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
21	LgTrip 3092	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPD
22	LgTrip 3092	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgac
23	SmTrip9	GSMLFRVTINS
24	SmTrip9	ggctccatgctgttccgagtaaccatcaacagc
25	SmHiTrip10	VSGWRLFKKIS
26	SmHiTrip10	gtgagcggctggcggctgttcaagaagattagc
27	5P-B9	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLFQNLA VSVTPIQRIVLSGENALKIDIHVIIPYEGLSADQMAQIEKIF KVVYPVDDHHFKVILHYGTLVIDGVTPNMINYFGRPYEG IAVFDGKKITVTGTLWNGNKIIDERLITPD
28	5P-B9	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgtttcagaatctcgccgtgtccgtaac tccgatccaaaggattgtcctgagcggtgaaaatgccctgaagatcgacatccatgtcatc atcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaaaaaatttttaaggtg gtgtaccctgtggatgatcatcactttaaggtgatcctgcactatggcacactggtaatcga cggggttacgccgaacatgatcaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacg agcgcctgatcacccccgac
29	5P(147-157)	GSMLFRVTINV
30	5P(147-157)	ggctccatgctgttccgagtaaccatcaac
31	LgTrip 2098 His	MKHHHHHHVFTLEDFVGDWEQTAAYNLDQVLEQGGVS SLLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQ MAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLN YFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPD

32	LgTrip 2098 His	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
35	SmTrip9/10 Dipeptide (pep263)	GSMLFRVTINSVSGWRLFKKIS
36	SmTrip9/10 Dipeptide (pep263)	ggctccatgctgttccgagtaaccatcaacagcgtgagcggctggcggctgttcaagaag attagc
37	SmTrip9+ (pep286)	SSWKRGSMLFRVTINS
38	SmTrip9+ (pep286)	Agcagctggaagcgcggctccatgctgttccgagtaaccatcaacagc
39	LgTrip 3440	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGDTPNKL NYFGRPYDGIAVFDGKKITVTGTLWNGNKIIDERLITPD
40	LgTrip 3440	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggatacgccgaacaagctgaactatttcggacgg ccgtatgatggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
41	LgTrip 3121	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPSKL NYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPD
42	LgTrip 3121	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgagcaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
43	LgTrip 3482	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGFAVFDGKKITVTGTLWNGNKIIDERLITPD

44	LgTrip 3482	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcttcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgga acggcaacaaaattatcgacgagcgcctgatcacccccgac
45	LgTrip 3497	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVCDGKKITVTGTLWNGNKIIDERLITPD
46	LgTrip 3497	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgtgcgacggcaaaaagatcactgtaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
47	LgTrip 3125	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKISVTGTLWNGNKIIDERLITPD
48	LgTrip 3125	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcgggcggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatctctgtaacagggaccctgtgga acggcaacaaaattatcgacgagcgcctgatcacccccgac
49	LgTrip 3118	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITATGTLWNGNKIIDERLITPD
50	LgTrip 3118	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgcaacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
51	LgTrip 3546	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD

52	LgTrip 3546	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgac
53	LgTrip 3546+G (ATG 3572)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDG
54	LgTrip 3546+G (ATG 3572)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggc
55	LgTrip 3546-D (ATG 3573)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITP
56	LgTrip 3546-D (ATG 3573)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcaccccc
57	LgTrip 3546-PD (ATG 3574)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLIT
58	LgTrip 3546-PD (ATG 3574)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacc
59	LgTrip 3546+GS (ATG 3575)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDGS

60	LgTrip 3546+GS (ATG 3575)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggcagc
61	-V_LgBiT (ATG3618)	MFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV SVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVF KVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTI NSHHHHHH
62	-V_LgBiT (ATG3618)	atgttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggac caagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactc cgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatca tcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtg gtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcga cggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacg agcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatc accatcaccactaa
63	-VF_LgBiT (ATG3619)	MTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFK VVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGI AVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINS HHHHHH
64	-VF_LgBiT (ATG3619)	atgacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggacca agtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccg atccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcc cgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgt accctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacgg ggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcga cggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagc gcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatcacc atcaccactaa
65	-VFT_LgBiT (ATG3620)	MLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSV TPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIA VFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINSH HHHHH
66	-VFT_LgBiT (ATG3620)	atgctcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagt ccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatc caaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccg tatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtac cctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggt tacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacgg caaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcc

		tgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatca ccactaa
67	-VFTL_LgBiT (ATG3621)	MEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVT PIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVV YPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAV FDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINSHH HHHH
68	-VFTL_LgBiT (ATG3621)	atggaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaa ggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatga aggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgt ggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacg ccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaa aagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcacca ctaa
69	(M)FKKIS-GSSG-LgBiT (ATG3632)	MFKKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNML NYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDG SMLFRVTINSHHHHHH
70	(M)FKKIS-GSSG-LgBiT (ATG3632)	atgttcaagaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggact gggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagt ttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaa atgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaat ggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtg atcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcg gacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggacc ctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgct gttccgagtaaccatcaacagccatcatcaccatcaccactaa
71	(M)KKIS-GSSG-LgBiT (ATG3633)	MKKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVS SLLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQ MAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLN YFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGS MLFRVTINSHHHHHH
72	(M)KKIS-GSSG-LgBiT (ATG3633)	atgaagaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgg gaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagttt gctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaa tgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatg gcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgat cctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcgga cggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccct

		gtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgtt ccgagtaaccatcaacagccatcatcaccatcaccactaa
73	(M)KIS-GSSG-LgBiT (ATG3634)	MKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSS LLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQM AQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNY FGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSM LFRVTINSHHHHHH
74	(M)KIS-GSSG-LgBiT (ATG3634)	atgaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaac agacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgc agaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccc tgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccc agatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctg ccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacgg ccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtg gaacggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttcc gagtaaccatcaacagccatcatcaccatcaccactaa
75	(M)IS-GSSG-LgBiT (ATG3635)	MISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSL LQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMA QIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYF GRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSML FRVTINSHHHHHH
76	(M)IS-GSSG-LgBiT (ATG3635)	atgattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacaga cagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcaga atctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccctga agatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccaga tcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccct atggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaac ggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagta accatcaacagccatcatcaccatcaccactaa
77	(M)S-GSSG-LgBiT (ATG3636)	MSGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLL QNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMA QIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYF GRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSML FRVTINSHHHHHH
78	(M)S-GSSG-LgBiT (ATG3636)	atgagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagaca gccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaat ctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccctgaag atcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatc gaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctat ggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtat gaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacg

		gcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaa ccatcaacagccatcatcaccatcaccactaa
79	LgTrip + GSM (ATG3722)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SM
80	LgTrip + GSM (ATG3722)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggcagcatgtaa
81	LgTrip + GSML (ATG3723)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SML
82	LgTrip + GSML (ATG3723)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggcagcatgctgtaa
83	LgTrip + GSMLF (ATG3724)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SMLF
84	LgTrip + GSMLF (ATG3724)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggcagcatgctgttctaa

85	LgTrip – TPD (ATG3725)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLI
86	LgTrip – TPD (ATG3725)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatctaa
87	LgTrip – ITPD (ATG3726)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERL
88	LgTrip – ITPD (ATG3726)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgtaa
89	LgTrip – LITPD (ATG3727)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDER
90	LgTrip – LITPD (ATG3727)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgctaa
91	FRB-15GS-AI-86 (ATG1634)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGA IVSGWRLFKKIS
92	FRB-15GS-AI-86 (ATG1634)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgt actttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgat ggaacggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagcgatcgtgagcggctggcggctgttca agaagattagctaa

93	FRB-15GS-AI-289 (ATG3586)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGA IVSVSGWRLFKKIS
94	FRB-15GS-AI-289 (ATG3586)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgt actttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgat ggaacggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagcgatcgttagcgttagcggctggcgcct gttcaagaagatcagctaa
95	FRB-15GS-AI-86-His6 (ATG3743)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGA IVSGWRLFKKISHHHHHH
96	FRB-15GS-AI-86-His6 (ATG3743)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgt actttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgat ggaacggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagcgatcgtgagcggctggcggctgttca agaagattagccatcatcaccatcaccactaa
97	FRB-15GS-AI-289-His6 (ATG3744)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGA IVSVSGWRLFKKISHHHHHH
98	FRB-15GS-AI-289-His6 (ATG3744)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgt actttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgat ggaacggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagcgatcgttagcgtgagcggctggcggc tgttcaagaagattagccatcatcaccatcaccactaa
99	His6-FRB-5GS-86 (ATG3760)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGVSG WRLFKKIS
100	His6-FRB-5GS-86 (ATG3760)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtgtgagcggctggcggctgttcaagaagattagctaa
101	His6-FRB-10GS-86 (ATG3761)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGGGS GGVSGWRLFKKIS

102	His6-FRB-10GS-86 (ATG3761)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtggcgggagcggtggcgtgagcggctggcggctgttcaaga agattagctaa
103	His6-FRB-15GS-86 (ATG3762)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGGGS GGSSSGGVSGWRLFKKIS
104	His6-FRB-15GS-86 (ATG3762)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtggcgggagcggtggctcgagcagcggtggagtgagcggct ggcggctgttcaagaagattagctaa
105	His6-FRB-5GS-289 (ATG3763)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGVSV SGWRLFKKIS
106	His6-FRB-5GS-289 (ATG3763)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtgttagcgttagcggctggcgcctgttcaagaagatcagctaa
107	His6-FRB-10GS-289 (ATG3764)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGGGS GGVSVSGWRLFKKIS
108	His6-FRB-10GS-289 (ATG3764)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtggcgggagcggtggcgttagcgttagcggctggcgcctgtt caagaagatcagctaa
109	His6-FRB-15GS-289 (ATG3765)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKG MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE WCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGGGS GGSSSGGVSVSGWRLFKKIS

110	His6-FRB-15GS-289 (ATG3765)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcc tggaagaggcatctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgct ggagcccttgcatgctatgatggaacggggcccccagactctgaaggaaacatcctttaa tcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatgaaatc agggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatc agtggtggttcaggtggtggcgggagcggtggctcgagcagcggtggagttagcgttag cggctggcgcctgttcaagaagatcagctaa
111	SmTrip9-FKBP fusion template (ATG780)	M— GSMLFRVTINS — SSSGGGGSGGGSSGGGVQVETISPGDGRTFPKRGQTCVV HYTG MLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQ MSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELL KLE
112	SmTrip9-FKBP fusion template (ATG780)	atgggctccatgctgttccgagtaaccatcaacagctcgagttcaggtggtggcgggagc ggtggagggagcagcggtggaggagtgcaggtggaaaccatctccccaggagacgg gcgcaccttccccaagcgcggccagacctgcgtggtgcactacaccgggatgcttgaag atggaaagaaatttgattcctcccgggacagaaacaagccctttaagtttatgctaggcaa gcaggaggtgatccgaggctgggaagaaggggttgcccagatgagtgtgggtcagag agccaaactgactatatctccagattatgcctatggtgccactgggcacccaggcatcatc ccaccacatgccactctcgtcttcgatgtggagcttctaaaactggaataa
113	FKBP-SmTrip9 fusion template (ATG777)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFD SSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLT ISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGSGGGGS GGSSSGGAI— GSMLFRVTINS
114	FKBP-SmTrip9 fusion template (ATG777)	Atgggagtgcaggtggaaaccatctccccaggagacgggcgcaccttccccaagcgc ggccagacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaatttgattcc tcccgggacagaaacaagcccttaagtttatgctaggcaagcaggaggtgatccgagg ctgggaagaaggggttgcccagatgagtgtgggtcagagagccaaactgactatatctc cagattatgcctatggtgccactgggcacccaggcatcatcccaccacatgccactctcgt cttcgatgtggagcttctaaaactggaaggtggttcaggtggtggcgggagcggtggctc gagcagcggtggagcgatcggctccatgctgttccgagtaaccatcaacagc
115	LgBiT (ATG2623)	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTI NSHHHHHH
116	LgBiT (ATG2623)	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
133	pep78	NVSGWRLFKKISN
134	pep79	NVTGYRLFKKISN

135	pep80	VSGWRLFKKISN
136	pep81	SGWRLFKKISN
137	pep82	GWRLFKKISN
138	pep99	VTGYRLFEKIS
139	pep219	SGWRLFKKIS
140	pep225	VSGWRL
141	pep226	VSGWRLF
142	pep227	VSGWRLFK
143	pep228	VSGWRLFKK
144	pep229	VSGWRLFKKI
145	pep243	VSGWRLYKKIS
146	pep272	GSMLFRVTINSVSGWALFKKIS
147	pep274	GSMLFRVTINSVTGYRLFEEIL
148	pep287 (WT SmTrip9)+Cterm solubility tag	GSMLFRVTINSSSWKR
149	pep288	VSGVSGWRLFKKIS
150	pep289	VSVSGWRLFKKIS
151	pep290	VVSGWRLFKKIS
152	pep291	SSWKRSMLFRVTINS
153	pep292	SSWKRMLFRVTINS
154	pep293	SSWKRDGSMLFRVTINS
155	pep294	SSWKRPDGSMLFRVTINS
156	pep296	SSWKRSMLFRVTINSV
157	pep297	SSWKRMLFRVTINSV
158	pep298	SSWKRDGSMLFRVTINSV
159	pep299	SSWKRPDGSMLFRVTINSV
160	pep301	SSWKRSMLFRVTINSVS
161	pep302	SSWKRMLFRVTINSVS
162	pep303	SSWKRDGSMLFRVTINSVS
163	pep304	SSWKRPDGSMLFRVTINSVS
164	pep305	SSWKRGSMLFRVTIN
165	pep306	SSWKRGSMLFRVTI
166	pep307	SSWKRSMLFRVTIN
167	pep308	SSWKRMLFRVTIN
168	pep309	SSWKRDGSMLFRVTIN
169	pep310	SSWKRPDGSMLFRVTIN
170	pep311	SSWKRSMLFRVTI
171	pep312	SSWKRMLFRVTI
172	pep313	SSWKRDGSMLFRVTI

173	pep314	SSWKRPDGSMLFRVTI
174	pep316	VSGWRLFKKISVFTL
175	pep317	VSGWRLFKKISVFT
176	pep318	VSGWRLFKKISVF
177	pep319	VSGWRLFKKISV
178	pep320	VSGWRLCKKIS
179	pep326	VSGWRLFKKISGSMLFRVTINS
180	pep380	SSWKRLFRVTINS
181	pep383	SSWKRFRVTINS
182	pep386	SSWKRRVTINS
183	pep389	SSWKRTPDGSMLFRVTINS
184	pep392	SSWKRITPDGSMLFRVTINS
185	pep395	SSWKRLITPDGSMLFRVTINS
186	pep396	SSRGSMLFRVTINSWK
187	pep397	SKRGSMLFRVTINSWS
188	pep398	SWRGSMLFRVTINS
189	pep400	SSRGSMLFRVTIWK
190	pep401	SSWKRGSMLYRVTINS
191	pep402	SSWKRGSMLWRVTINS
192	pep403	SSWKRGSMLHRVTINS
193	pep404	SSWKRGSLLFRVTINS
194	pep405	SSWKRGSKLFRVTINS
195	pep406	SSWKRGSRLFRVTINS
196	pep407	SSWKRGSFLFRVTINS
197	pep408	SSWKRGSWLFRVTINS
198	pep409	SSWKRGSMLFRVSINS
199	pep410	SSWKRGSMLFRVQINS
200	pep411	SSWKRGSMLFRVNINS
201	SmTrip9-286 with cysteine	SSWKRGSMLFRVTINSC
202	HiBit with cysteine	CVSGWRLFKKIS
203	SmTrip9-286 with azide	SSWKRGSMLFRVTINSK(Aza)
204	HiBit with azide	(aza)KVSGWRLFKKIS
205	WT OgLuc dipeptide	GSLLFRVTINGVTGWRLCENILA
206	WT NanoLuc dipeptide	GSLLFRVTINVGVTGWRLCERILA
207	pep157	SVSGWRLFKKIS
208	pep158	NSVSGWRLFKKIS

209	pep206	GWRLFKKIS
210	HiBiT-His-LgTrip3546 (ATG 3745)	Atggtgagcggctggcggctgttcaagaagattagccaccatcaccatcaccatcatcac ttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaa gtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgat catgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatccc gtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgta ccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggg gttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgac ggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagc gcctgatcacccccgactaa
211	HiBiT-His-LgTrip3546 (ATG 3745)	MVSGWRLFKKISHHHHHHHHFTLDDFVGDWEQTAAYN LDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIH VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTL VIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNK IIDERLITPD
212	His-HiBiT-GSSG-LgTrip3546 (ATG 3746)	Atgaaacatcaccatcaccatcatgtgagcggctggcggctgttcaagaagattagcgg cagctccggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaa cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtcc gtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccat gtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgttt aaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggt aatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcg ccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaatt atcgacgagcgcctgatcacccccgactaa
213	His-HiBiT-GSSG-LgTrip3546 (ATG 3746)	MKHHHHHHVSGWRLFKKISGSSGFTLDDFVGDWEQTA AYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALK IDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLW NGNKIIDERLITPD
214	FRB-15GS-86, no AI in linker (ATG3768)	Atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttg tactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatga tggaacggggccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagtgagcggctggcggctgttcaagaaga ttagctaa
215	FRB-15GS-86, no AI in linker (ATG3768)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGV SGWRLFKKIS
216	FRB-15GS-289 (ATG3769)	Atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttg tactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatga tggaacggggccccagactctgaaggaaacatcctttaatcaggcctatggtcgagattt aatggaggcccaagagtggtgcaggaagtacatgaaatcagggaatgtcaaggacctc acccaagcctgggacctctattatcatgtgttccgacgaatcagtggtggttcaggtggtg gcgggagcggtggctcgagcagcggtggagttagcgttagcggctggcgcctgttcaa gaagatcagctaa

217	FRB-15GS-289 (ATG3769)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK SGNVKDLTQAWDLYYHVFRRISGGSGGGGSGGSSSGGV SVSGWRLFKKIS
218	FKBP-SmTrip9 fusion template, no AI in linker (ATG3770)	atgggagtgcaggtggaaaccatctccccaggagacgggcgcaccttccccaagcgcg gccagacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaatttgattcctc ccgggacagaaacaagccctttaagtttatgctaggcaagcaggaggtgatccgaggct gggaagaaggggttgcccagatgagtgtgggtcagagagccaaactgactatatctcca gattatgcctatggtgccactgggcacccaggcatcatcccaccacatgccactctcgtctt cgatgtggagcttctaaaactggaaggtggttcaggtggtggcgggagcggtggctcga gcagcggtgga
219	FKBP-SmTrip9 fusion template, no AI in linker (ATG3770)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFD SSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLT ISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGSGGGGS GGSSSGG
220	295	GSMLFRVTINSV
221	300	GSMLFRVTINSVS
222	412	MLFRVTINSVSG
223	413	MLFRVTINSVSGW
224	415	MLFRVTINSVSGWK
225	416	MLFRVTINSVSGWR
226	418	GSMLFRVTINSVSG
227	419	GSMLFRVTINSVSGW
228	422	GSMLFRVTINSVSGWR
229	423	GSMLFRVTINSVSGWK
230	434	GSMLFRVTIWK
231	435	GSMLFRVTINSWK
232	477	MLFRVTINSWK
233	478	MLFRVTINSWS
234	479	MLFRVTIWS
235	480	MLFRVTIWK
236	481	MLFRVKINS
237	482	GSMLFRVTINSWS
238	483	GSMLFRVKINS
239	484	GSMLFRVTIWS
240	485	MLFRVNINS
241	486	MLFRVWINS
242	487	LLFRVKINS
243	488	FLFRVTINS
244	295	SSWKRGSMLFRVTINSV
245	300	SSWKRGSMLFRVTINSVS

246	412	SSWKRMLFRVTINSVSG
247	413	SSWKRMLFRVTINSVSGW
248	414	SSWKRMLFRVTINSVSGWR
249	415	SSWKRMLFRVTINSVSGWK
250	417	MLFRVTINSVSGWK
251	418	SSWKRGSMLFRVTINSVSG
252	419	SSWKRGSMLFRVTINSVSGW
253	420	SSWKRGSMLFRVTINSVSGWR
254	421	SSWKRGSMLFRVTINSVSGWK
255	424	SSWKRGSYLFRVTINS
256	425	SSWKRGSMLFRVKINS
257	426	SSWKRGSMLFRVRINS
258	427	SSWKRGSMLFRVWINS
259	428	SSKRGSMLFRVTIWSV
260	429	SSKRGSMLFRVTIWSVS
261	430	SSWRGSMLFRVTIKS
262	431	KRSSGSMLFRVTIWS
263	432	SSKRMLFRVTIWS
264	433	KRSSMLFRVTIWS
265	445	GSMKFRVTINSWK
266	450	GSMLFRKTINSWK
267	455	GSMLFRVTKNSWK
268	521	GKMLFRVTINSWK
269	522	GKMLFRVTIWK
270	523	GSMKFRVTINSWK
271	524	GSMKFRVTIWK
272	525	GRMLFRVTINSWK
273	526	GRMLFRVTIWK
274	527	GSMRFRVTINSWK
275	528	GSMRFRVTIWK
276	529	GDMLFRVTINSWK
277	530	GDMLFRVTIWK
278	531	GSMDFRVTINSWK
279	532	GSMDFRVTIWK
280	533	GEMLFRVTINSWK
281	535	GSMEFRVTINSWK
282	536	GSMEFRVTIWK
283	538	GSMLFRVTIWKVK
284	539	GSMLFRVTIWSVK
285	540	GSMLFRVTIWSK
286	541	GSMLFRVTIWKWK

287	542	GSMLFRVTIWKK
288	245	GSMLFRVTINS
289	292.x	MLFRVTINS
290	297.x	MLFVTINSV
291	302.x	MLFRVTINSVS
292	305.x	GSMLFRVTIN
293	306.x	GSMLFRVTI
294	307.x	SMLFRVTIN
295	308.x	MLFRVTIN
296	312.x	MLFRVTI
297	399	SSKRGSMLFRVTIWS
298	273	GSMLFRVTINSGVSGWALFKKIS
299	264	GSMLFRVTINSGVSGWRLFKKIS
300	167	VSGWALFKKIS
301	331	GSMLFRVTINSVSGVSGWRLFKKIS
302	LgTrip 3546 (no His6)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPD
303	LgTrip 3546 (no His6)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgac
304	LgTrip 2098 (no His6)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPD
305	LgTrip 2098 (no His6)	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgac
306	157	SVSGWRLFKKIS
307	158	NSVSGWRLFKKIS
308	206	GWRLFKKIS
309	264	GSMLFRVTINSGVSGWRLFKKIS
310	489	GSMLFRVTINSWK (N-term unblocked)

311	490	GSMLFRVTINSWK (C-term unblocked)
312	491	GSMLFRVTINSWK (Both unblocked)
313	492	GSMLFRVTINKWK
314	493	GSMLFRVTIKSWK
315	494	GSMLFRVTINRWK
316	495	GSMLFRVTIRSWK
317	496	GSMLFRVTINDWK
318	497	GSMLFRVTIDSWK
319	498	GSMLFRVTINEWK
320	499	GSMLFRVTIESWK
321	465	GSMRFRVTINSWK (Both termini unblocked)
322	466	GSMDFRVTINSWK (Both termini unblocked)
323	467	GSMEFRVTINSWK (Both termini unblocked)
324	468	GSMLFRRTINSWK (Both termini unblocked)
325	469	GSMLFRDTINSWK (Both termini unblocked)
326	470	GSMLFRETINSWK (Both termini unblocked)
327	472	GSMLFRVTDNSWK (Both termini unblocked)
328	473	GSMLFRVTENSWK (Both termini unblocked)
329	474	GSMKFRVTINSWK (Both termini unblocked)
330	475	GSMLFRKTINSWK (Both termini unblocked)
331	476	GSMLFRVTKNSWK (Both termini unblocked)
332	436	GSMLFRVTINS (N-term unblocked)
333	437	GSMLFRVSINS (N-term unblocked)
334	438	GSMLFRVNINS (N-term unblocked)
335	439	GSKLFRVTINS (N-term unblocked)
336	440	GSRLFRVTINS (N-term unblocked)
337	441	GSMWFRVTINS (N-term unblocked)
338	442	GSMSFRVTINS (N-term unblocked)
339	443	GSMNFRVTINS (N-term unblocked)
340	444	GSMKFRVTINS (N-term unblocked)
341	446	GSMLFRWTINS (N-term unblocked)
342	447	GSMLFRSTINS (N-term unblocked)
343	448	GSMLFRNTINS (N-term unblocked)
344	449	GSMLFRKTINS (N-term unblocked)
345	451	GSMLFRVTWNS (N-term unblocked)
346	452	GSMLFRVTSNS (N-term unblocked)
347	453	GSMLFRVTNNS (N-term unblocked)
348	454	GSMLFRVTKNS (N-term unblocked)
349	456	GSMLFRVTIKS (N-term unblocked)

350	Antares ATG 3802	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCT HEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGS KVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTAT QDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPS TETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKS KKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVAR YSNLGGGFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLF QNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMG QIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFG RPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLF RVTINGVTGWRLCERILARHELIKENMRSKLYLEGSVNG HQFKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILAT HFMYGSKVFIKYPADLPDYFKQSFPEGFTWERVMVFED GGVLTATQDTSLQDGELIYNVKVRGVNFPANGPVMQKK TLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLHVN FKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQY EHAVARYSNLGGGMDELYK
351	Antares ATG 3802	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatg cggtctaaactgtacctcgagggctccgtcaatgggcaccagtttaagtgtacccacgag ggtgagggaaagccctatgaggggaagcagacaaaccgcatcaaggtcgtcgaaggg ggacccctcccgtttgcctttgatatcttggctactcactttatgtacggaagcaaagttttcat aaagtatcctgccgaccttcctgattattttaaacagtcatttcccgagggtttcacatggga aagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggc ctgtaatgcagaagaagaccctgggatgggagccgtccaccgaaaccatgtaccctgct gatggtgggctggagggccgatgtgacaaggctctgaagctcgttggaggtggtcatttg cacgtaaatttcaagacaacttacaagagcaaaaaacccgtaaaaatgcccggggttcatt acgttgacagaaggcttgaacgcataaaggaagctgataacgagacatacgtggagcag tacgagcacgccgttgcccggtactcaaacctgggggtggctttacactggaggattttg tgggagattggagacagacagccggctacaatctggatcaggtgctggaacaaggagg agtgtcttctctgtttcagaatctgggagtgagcgtgacacctatccagaggatcgtgctgt ctggcgagaatggactgaagatcgatattcacgtgatcatcccctacgaaggcctgtctgg agaccagatgggccagattgagaagatcttcaaagtggtgtatcctgtggacgatcacca cttcaaggtgatcctgcactacggcaccctggtgattgatggagtgacacctaacatgatc gactacttcggaagaccttacgagggaatcgccgtgttcgacggaaagaagatcaccgt gacaggaacactgtggaatggaaacaagatcatcgacgagcggctgatcaaccctgatg gatctctgctgttcagagtgaccatcaacggagtgacaggatggagactgtgcgagaga attctggctagacatgagctaatcaaggaaaatatgagaagtaagctatacttagaggggt ccgtcaacggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaaggta agcagactaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgatattctg gccactcactttatgtatgggtctaaggtctttattaaataccccgctgatttgccagactactt taaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggagg cgttcttactgcaactcaggatacttccttgcaagacggggaactgatctacaacgttaagg tccgcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggggtggg agccctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgtgataag gcattgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatataaaagta aaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaa

		gaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaact tgggcggaggaatggatgaactgtacaag
352	Antares (LgBiT) ATG 3803	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCT HEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGS KVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTAT QDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPS TETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKS KKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVAR YSNLGGGFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLL QNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMA QIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYF GRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSML FRVTINSRHELIKENMRSKLYLEGSVNGHQFKCTHEGEG KPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIK YPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTS LQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTETM YPADGGLEGRCDKALKLVGGGHLHVNFKTTYKSKKPV KMPGVHYVDRRLERIKEADNETYVEQYEHAVARYSNL GGGMDELYK

353	Antares (LgBiT) ATG 3803	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatg cggtctaaactgtacctcgagggctccgtcaatgggcaccagtttaagtgtacccacgag ggtgagggaaagccctatgaggggaagcagacaaaccgcatcaaggtcgtcgaaggg ggacccctcccgtttgcctttgatatcttggctactcactttatgtacggaagcaaagttttcat aaagtatcctgccgaccttcctgattattttaaacagtcatttcccgagggtttcacatggga aagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggc ctgtaatgcagaagaagaccctgggatgggagccgtccaccgaaaccatgtaccctgct gatggtgggctggagggccgatgtgacaaggctctgaagctcgttggaggtggtcatttg cacgtaaatttcaagacaacttacaagagcaaaaaacccgtaaaaatgcccggggttcatt acgttgacagaaggcttgaacgcataaaggaagctgataacgagacatacgtggagcag tacgagcacgccgttgcccggtactcaaacctgggggtggcttcacactcgaagatttc gttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggag gtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagc gccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcat cactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgc tgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgt aacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgac ggctccatgctgttccgagtaaccatcaacagcagacatgagctaatcaaggaaaatatg agaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatgaag gtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggt cctctgccattcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaa taccccgctgatttgccagactactttaaacagtccttccctgaaggattcacatgggagcg ggtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttccttgcaagac ggggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatggtccagtg atgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcggacg gcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcat gtgaatttcaagactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacg tggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatg agcacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
354	Antares (LgTrip 3546) ATG 3804	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCT HEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGS KVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTAT QDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPS TETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKS KKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVAR YSNLGGGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLL QNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMA QIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYF GRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDRHELI KENMRSKLYLEGSVNGHQFKCTHEGEGKPYEGKQTNRI KVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFKQ SFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVK VRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGR CDKALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDR RLERIKEADNETYVEQYEHAVARYSNLGGGMDELYK

355	Antares (LgTrip 3546) ATG 3804	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatg cggtctaaactgtacctcgagggctccgtcaatgggcaccagtttaagtgtacccacgag ggtgagggaaagccctatgaggggaagcagacaaaccgcatcaaggtcgtcgaaggg ggacccctcccgtttgcctttgatatcttggctactcactttatgtacggaagcaaagttttcat aaagtatcctgccgaccttcctgattattttaaacagtcatttcccgagggtttcacatggga aagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggc ctgtaatgcagaagaagaccctgggatgggagccgtccaccgaaaccatgtaccctgct gatggtgggctggagggccgatgtgacaaggctctgaagctcgttggaggtggtcatttg cacgtaaatttcaagacaacttacaagagcaaaaaacccgtaaaaatgcccggggttcatt acgttgacagaaggcttgaacgcataaaggaagctgataacgagacatacgtggagcag tacgagcacgccgttgcccggtactcaaacctgggggggtggcttcacactcgacgatttc gttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggag gtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagc gccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcat cactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaag ctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcact accacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccg acagacatgagctaatcaaggaaaatatgagaagtaagctatacttagaggggtccgtca acggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaaggtaagcaga ctaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgatattctggccact cactttatgtatgggtctaaggtctttattaaatacccgctgatttgccagactactttaaaca gtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttctt actgcaactcaggatacttccttgcaagacggggaactgatctacaacgttaaggtccgc ggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggggtgggagcc ctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgtgataaggcat tgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatataaaagtaaaaa accagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaagaag ccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttggg cggaggaatggatgaactgtacaag
356	ATG 3815	MKHHHHHHFTLEDFVGDWEQTAAYNLDQVLEQGGVSS LLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQM AQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNY FGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSM LFRVTINSGGSGGSSGELIKENMRSKLYLEGSVNGHQFK CTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMY GSKVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLT ATQDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGW EPSTETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTY KSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAV ARYSNLGGGMDELYK

357	ATG 3815	atgaaacatcaccatcaccatcatttcacactcgaagatttcgttggggactgggaacaga cagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcaga atctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccctga agatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccaga tcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccct atggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaac ggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagta accatcaacagcggaggctcaggtggatcctcaggtgagctaatcaaggaaaatatgag aagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatgaaggt gaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcc tctgccattcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaata ccccgctgatttgccagactactttaaacagtccttccctgaaggattcacatgggagcgg gtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttccttgcaagacg gggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatggtccagtgat gcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcggacggc gggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtg aatttcaagactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtgg atcgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatgag cacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
358	ATG 3816	MKHHHHHHFTLEDFVGDWEQTAAYNLDQVLEQGGVSS LLQNLAVSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQM AQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNY FGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSM LFRVTINSRHELIKENMRSKLYLEGSVNGHQFKCTHEGE GKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFI KYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDT SLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTET MYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKSKKP VKMPGVHYVDRRLERIKEADNETYVEQYEHAVARYSN LGGGMDELYK
359	ATG 3816	Atgaaacatcaccatcaccatcatttcacactcgaagatttcgttggggactgggaacaga cagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcaga atctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccctga agatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccaga tcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccct atggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaac ggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagta accatcaacagcagacatgagctaatcaaggaaaatatgagaagtaagctatacttagag gggtccgtcaacggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaa ggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgata ttctggccactcactttatgtatgggtctaaggtctttattaaataccccgctgatttgccagac tactttaaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatg gaggcgttcttactgcaactcaggatacttccttgcaagacggggaactgatctacaacgtt aaggtccgcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggg gtgggagccctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgt gataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatata

		aaagtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
360	ATG 3817	MKHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDGGSGGSSGELIKENMRSKLYLEGSVNGHQFKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVARYSNLGGGMDELYK
361	ATG 3817	Atgaaacatcaccatcaccatcatttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacggaggctcaggtggatcctcaggtgagctaatcaaggaaaatatgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaataccccgctgatttgccagactactttaaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttccttgcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
362	ATG 3818	MKHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDRHELIKENMRSKLYLEGSVNGHQFKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVARYSNLGGGMDELYK

363	ATG 3818	Atgaaacatcaccatcaccatcatttcacactcgacgatttcgttggggactgggaacaga cagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcaga atctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaa gatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagat cgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccct atggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaac ggcaacaaaattatcgacgagcgcctgatcacccccgacagacatgagctaatcaagga aaatatgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcact catgaaggtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcga gggcggtcctctgccattcgctttcgatattctggccactcactttatgtatgggtctaaggtc tttattaaataccccgctgatttgccagactactttaaacagtccttccctgaaggattcacat gggagcgggtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttcctt gcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatg gtccagtgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccct gcggacggcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggcc accttcatgtgaatttcaagactacatataaaagtaaaaaaccagtcaagatgcctggagtg cactacgtggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtaga gcaatatgagcacgccgtggctcgttattccaacttgggcggaggaatggatgaactgta caag
364	LgTrip 2899 (LgTrip 2098+Q42L)	MKHHHHHHVFTLEDFVGDWEQTAAYNLDQVLEQGGVS SLLQNLAVSVTPILRIVRSGENALKIDIHVIIPYEGLSADQ MAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLN YFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPD
365	LgTrip 2899 (LgTrip 2098+Q42L)	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaaca gaccgccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcctaaggattgtccggagcggtgaaaatgccctg aagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccag atcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcc ctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggcc gtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtgga acggcaacaaaattatcgacgagcgcctgatcacccccgac
820	ATG-3930	atgAAACATCACCATCACCATCATgtcTTCACACTCGACG ATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAA CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCAT GAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATC GACATCCATGTCATCATCCCGTATGAAGGTCTGAGCG CCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGT GGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCC TGCCCTATGGCACACTGGTAATCGACGGGGTTACGCC GAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGC ATCGCCGTGTTCGACGGCTAA

821	ATG-3930	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDG
822	SmTrip9- 15GS-ProteinG (ATG 4002)	gggagctccGGTGGTGGCGGGAGCGGAGGTGGAGGctcgAG CGGTATGACGTATAAGTTAATCCTTAATGGTAAAACA TTGAAAGGCGAGACAACTACTGAAGCTGTTGATGCTG CTACTGCAGAAAAAGTCTTCAAACAATACGCTAACGA CAACGGTGTTGACGGTGAATGGACTTACGACGATGCG ACGAAAACCTTTACGGTCACCGAAAAACCAGAAGTGA TCGATGCGTCTGAATTAACACCAGCCGTGACAACTTA CAAACTTGTTATTAATGGTAAAACATTGAAAGGCGAA ACAACTACTGAGGCTGTTGATGCTGCTACTGCAGAGA AGGTGTTCAAACAATATGCGAATGACAACGGTGTTGA CGGTGAGTGGACTTACGACGATGCGACTAAGACCTTT ACAGTTACTGAAAAACCAGAAGTGATCGATGCGTCTG AGTTAACACCAGCCGTGACAACTTACAAACTTGTTATT AATGGTAAAACATTGAAAGGCGAAACAACTACTAAA GCAGTAGACGCAGAAACTGCGGAGAAGGCCTTCAAA CAATACGCTAACGACAACGGTGTTGATGGTGTTTGGA CTTATGATGATGCCACAAAAACCTTTACGGTAACTGA GCATCATCACCATCACCACTAA
823	SmTrip9- 15GS-ProteinG (ATG 4002)	GSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDA ATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVI DASELTPAVTTYKLVINGKTLKGETTTEAVDAATAEKVF KQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTP AVTTYKLVINGKTLKGETTTKAVDAETAEKAFKQYAND NGVDGVWTYDDATKTFTVTEHHHHHH
830	ATG-3929	atgAAACATCACCATCACCATCATgtcTTCACACTCGACG ATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAA CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCAT GAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATC GACATCCATGTCATCATCCCGTATGAAGGTCTGAGCG CCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGT GGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCC TGCCCTATGGCACACTGGTAATCGACGGGGTTACGCC GAACAAGCTGAACTATTTCGGATAA

831	ATG-3929	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsg enalkidihviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnk lnyfg
832	ATG-3930	atgAAACATCACCATCACCATCATgtcTTCACACTCGACG ATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAA CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCAT GAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATC GACATCCATGTCATCATCCCGTATGAAGGTCTGAGCG CCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGT GGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCC TGCCCTATGGCACACTGGTAATCGACGGGGTTACGCC GAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGC ATCGCCGTGTTCGACGGCTAA
833	ATG-3930	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsg enalkidihviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnk lnyfgrpyegiavfdg
834	ATG-3931	atgAAACATCACCATCACCATCATgtcTTCACACTCGACG ATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAA CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCAT GAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATC GACATCCATGTCATCATCCCGTATGAAGGTCTGAGCG CCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGT GGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCC TGCCCTATGGCACACTGGTAATCGACGGGGTTACGCC GAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGC ATCGCCGTGTTCGACGGCAAAAAGATCACTACCACAG GGACCCTGTAA
835	ATG-3931	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsg enalkidihviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnk lnyfgrpyegiavfdgkkitttgtl

836	ATG-3932	atgAAACATCACCATCACCATCATgtcTTCACACTCGACG ATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAA CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGT TTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCAT GAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATC GACATCCATGTCATCATCCCGTATGAAGGTCTGAGCG CCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGT GGTGTACCCTGTGGATGATCATCACTTTAAGGTGATCC TGCCCTATGGCACACTGGTAATCGACGGGGTTACGCC GAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGC ATCGCCGTGTTCGACGGCAAAAAGATCACTACCACAG GGACCCTGTGGAACGGCTAA
837	ATG-3932	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsg enalkidihviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnk lnyfgrpyegiavfdgkkitttgtlwng
838	ATG-4808	Atggtttccgtgagcggctggcggctgttcaagaagattagcttcacactcgacgatttcg ttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggagg tgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcg ccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatc actttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagct gaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactac cacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgact aa
839	ATG-4808	Mvsvsgwrlfkkisftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimri vrsgenalkidihviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgv tpnklnyfgrpyegiavfdgkkitttgtlwngnkiiderlitpd
840	ATG-4809	Atggtttccgtgagcggctggcggctgttcaagaagattagcggcagctccggtttcaca ctcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatga ggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatga aggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgt ggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacg ccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaa aaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctga tcacccccgactaa

841	ATG-4809	MVSVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQ VLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIP YEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIID ERLITPD
842	ATG-4810	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacct ggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgta actccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtc atcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaag gtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatc gacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgt gttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcg acgagcgcctgatcacccccgactaa
843	ATG-4810	MVSVSGWRLFKKISGSSGGSSGFTLDDFVGDWEQTAAY NLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDI HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG TLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNG NKIIDERLITPD
844	ATG-4811	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacag ccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatct cgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagat cgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcga agaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatgg cacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatga aggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggc aacaaaattatcgacgagcgcctgatcacccccgactaa
845	ATG-4811	MVSVSGWRLFKKISGSSGGSSGGSSGFTLDDFVGDWEQ TAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVI LPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPD
846	ATG-4812	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactg ggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtt tgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaa atgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaat ggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtg atcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcg

		gacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggac cctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa
847	ATG-4812	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGFTLDDFVGD WEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRS GENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDH HFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKIT TTGTLWNGNKIIDERLITPD
848	ATG-4813	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtggctcgagcggtttcacactcgacgatt tcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacaggga ggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccg gagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgag cgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatca tcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaag ctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcact accacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccg actaa
849	ATG-4813	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDD FVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMR IVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDG KKITTTGTLWNGNKIIDERLITPD
850	ATG-4814	Atggtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcg gtggctcgagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttg gggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgt gtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagc ggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgcc gaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcact ttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctga actatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactacca cagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa

851	ATG-4814	MVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFV GDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIV RSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKI TTTGTLWNGNKIIDERLITPD
852	ATG-4815	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacgtttccgtgagcggctggcggctgttcaagaagattagc taa
853	ATG-4815	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDVSVSGWRLF KKIS
854	ATG-4816	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctcgagcggtgtttccgtgagcggctggcggctgtt caagaagattagctaa
855	ATG-4816	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDGSSGVSVSG WRLFKKIS
856	ATG-4817	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac

		gagcgcctgatcacccccgacggctcgagcggtggctcgagcggtgtttccgtgagcg gctggcggctgttcaagaagattagctaa
857	ATG-4817	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDGSSGGSSGV SVSGWRLFKKIS
858	ATG-4818	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggt ggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctcgagcggtggctcgagcggtgtgagcggctgg cggctgttcaagaagattagctaa
859	ATG-4818	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYE GIAVFDGKKITTTGTLWNGNKIIDERLITPDGSSGGSSGV SGWRLFKKIS
860	ATG-4819	Atggtttccgtgagcggctggcggctgttcaagaagattagcttcacactcgacgatttcg ttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggagg tgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcg ccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatc actttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagct gaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactac cacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgac catcaccatcaccatcattaa
861	ATG-4819	MVSVSGWRLFKKISFTLDDFVGDWEQTAAYNLDQVLEQ GGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGL SADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTP NKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLIT PDHHHHHH

862	ATG-4820	Atggtttccgtgagcggctggcggctgttcaagaagattagcggcagctccggtttcaca ctcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatga ggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatga aggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgt ggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacg ccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaa aaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctga tcacccccgaccatcaccatcaccatcattaa
863	ATG-4820	MVSVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQ VLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIP YEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID GVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIID ERLITPDHHHHHH
864	ATG-4821	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacct ggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgta actccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtc atcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaag gtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatc gacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgt gttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcg acgagcgcctgatcacccccgaccatcaccatcaccatcattaa
865	ATG-4821	MVSVSGWRLFKKISGSSGGSSGFTLDDFVGDWEQTAAY NLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDI HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG TLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNG NKIIDERLITPDHHHHHH
866	ATG-4822	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacag ccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatct cgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagat cgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcga agaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatgg cacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatga

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867	ATG-4822	MVSVSGWRLFKKISGSSGGSSGGSSGFTLDDFVGDWEQ TAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVI LPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDHHHHHH
868	ATG-4823	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactg ggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtt tgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaa atgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaat ggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtg atcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcg gacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggac cctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgaccatcaccatc accatcattaa
869	ATG-4823	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGFTLDDFVGD WEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRS GENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDH HFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKIT TTGTLWNGNKIIDERLITPDHHHHHH
870	ATG-4824	Atggtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcg gtggctcgagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttg gggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgt gtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagc ggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgcc gaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcact ttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctga actatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactacca cagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgaccat caccatcaccatcattaa

871	ATG-4824	MVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFV GDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIV RSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKI TTTGTLWNGNKIIDERLITPDHHHHHH
872	ATG-4825	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtggctcgagcggtttcacactcgacgatt tcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacaggga ggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccg gagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgag cgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatca tcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaag ctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcact accacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccg accatcaccatcaccatcattaa
873	ATG-4825	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDD FVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMR IVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDG KKITTTGTLWNGNKIIDERLITPDHHHHHH
874	ATG-4826	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaac agacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgc agaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacgtttccgtgagcggctg gcggctgttcaagaagattagctaa
875	ATG-4826	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDV SVSGWRLFKKIS

876	ATG-4827	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaac agacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgc agaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggctcgagcggtgtttc cgtgagcggctggcggctgttcaagaagattagctaa
877	ATG-4827	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SSGVSVSGWRLFKKIS
878	ATG-4828	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaac agacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgc agaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggctcgagcggtggct cgagcggtgtgagcggctggcggctgttcaagaagattagctaa
879	ATG-4828	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SSGGSSGVSGWRLFKKIS
880	ATG-4829	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaac agacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgc agaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtgg aacggcaacaaaattatcgacgagcgcctgatcacccccgacggctcgagcggtggct cgagcggtgtttccgtgagcggctggcggctgttcaagaagattagctaa

881	ATG-4829	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDG SSGGSSGVSVSGWRLFKKIS
882	ATG-2623	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
883	ATG-2623	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTI NSHHHHHH
884	ATG-3745	atggtgagcggctggcggctgttcaagaagattagccaccatcaccatcaccatcatcact tcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaa gtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgat catgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatccc gtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgta ccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggg gttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgac ggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagc gcctgatcacccccgactaa
885	ATG-3745	MVSGWRLFKKISHHHHHHHHHFTLDDFVGDWEQTAAYN LDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIH VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTL VIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNK IIDERLITPD
886	ATG-3746	atgaaacatcaccatcaccatcatgtgagcggctggcggctgttcaagaagattagcggc agctccggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaac ctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccg taactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgt catcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaa ggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaa tcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgcc

		gtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattat cgacgagcgcctgatcacccccgactaa
887	ATG-3746	MKHHHHHHVSGWRLFKKISGSSGFTLDDFVGDWEQTA AYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALK IDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLW NGNKIIDERLITPD
888	ATG-4632	atggtgagcggctggcggctgttcaagaagattagcggcagctccggtttcacactcgac gatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaaca gggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggatt gtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggat gatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccga acaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaag atcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcac ccccgaccatcaccatcaccatcattaa
889	ATG-4632	MVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVL EQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYE GLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGV TPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERL ITPDHHHHHH
890	ATG-2757	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacgagaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
891	ATG-2757	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNENKIIDERLITPDGSMLFRVTI NSHHHHHH

892	ATG-2760	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcgttaaaattatcgacg agcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatc accatcaccactaa
893	ATG-2760	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYE GIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMLFRVTI NSHHHHHH
894	ATG-3882	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
895	ATG-3882	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRV TINSHHHHHH
896	ATG-3901	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa

897	ATG-3901	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRV TINSHHHHHH
898	ATG-3945	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacgacgtcaaaattatcgac gagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
899	ATG-3945	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNDVKIIDERLITPDGSMLFRV TINSHHHHHH
890	ATG-3984	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacgacgtcaaaattatcgac gagcgcctgatcacccccgacggctccatgtccttccgagtaaccatcaacagccatcat caccatcaccactaa
891	ATG-3984	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNDVKIIDERLITPDGSMSFRV TINSHHHHHH
892	ATG-4147	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgac

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893	ATG-4147	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMSFRV TINSHHHHHH
894	ATG-4166	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctg gaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggatggtccggagcggtgaaaatgccctgaagatcgacatccatgtca tcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaagg tggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcgtcaaaattatcgac gagcgcctgatcacccccgacggctccatgtccttccgagtaaccatcaacagccatcat caccatcaccactaa
895	ATG-4166	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEE VFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRV TINSHHHHHH
908	ATG-5037	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGTGTTCGACGGCAAAAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCACCCCCGACTAA

909	ATG-5037	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGHPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD
910	ATG-5038	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGG CATCGCCGTGTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCACCCCCGACTAA
911	ATG-5038	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGEKITTTGTLWNGNKIIDERLITPD
912	ATG-5039	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGG CATCGCCGTGTTCGACGGCAAAAAGATCACTACCACA GGGACCCTGCCTAACGGCAACAAAATTATCGACGAGC GCCTGATCACCCCCGACTAA

913	ATG-5039	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLPNGNKIIDERLITPD
914	ATG-5040	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGG CATCGCCGTGTTCGACGGCAAAAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATCCCGACTAA
915	ATG-5040	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLIDPD
916	ATG-5041	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGG CATCGCCGTGTTCGACGGCAAAAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCACCGATGACTAA

917	ATG-5041	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITDD
918	ATG-5135	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGTGTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCACCCCCGACTAA
919	ATG-5135	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLITPD
920	ATG-5146	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGTGTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATCCCGACTAA

921	ATG-5146	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPD
922	ATG-5158	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGTGTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATGATGACTAA
923	ATG-5158	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDDD
924	ATG-5260	ATGAAACATCACCATCACCATCATGATTTCACACTCG ACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTA CAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCC AGTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGAT CATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAG ATCGACATCCATGTCATCATCCCGTATGAAGGTCTGA GCGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAA GGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG ATCCTGCCCATCGGCACACTGGTAATCGACGGGGTTA CGCCGAACAAGCTGAACTATTTCGGACACCCGTATGA AGGCATCGCCGTGTTCGACGGCGAGAAGATCACTACC ACAGGGACCCTGTGGAACGGCAACAAAATTATCGACG AGCGCCTGATCGATCCCGACTAA

925	ATG-5260	MKHHHHHHDFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDGVTPNKL NYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPD
926	ATG-5266	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCATCGGCACACTGGTAATCGACGGGGAGACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGTGTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATCCCGACTAA
927	ATG-5266	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDGETPNKL NYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPD
928	ATG-5267	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCATCGGCACACTGGTAATCGACGGGGTTACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGATTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATCCCGACTAA

929	ATG-5267	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDGVTPNKL NYFGHPYEGIADFDGEKITTTGTLWNGNKIIDERLIDPD
930	ATG-5278	ATGAAACATCACCATCACCATCATGTCTTCACACTCGA CGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCA GTTTGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATC ATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGG TGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC CTGCCCATCGGCACACTGGTAATCGACGGGGAGACGC CGAACAAGCTGAACTATTTCGGACACCCGTATGAAGG CATCGCCGATTTCGACGGCGAGAAGATCACTACCACA GGGACCCTGTGGAACGGCAACAAAATTATCGACGAGC GCCTGATCGATCCCGACTAA
931	ATG-5278	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDGETPNKL NYFGHPYEGIADFDGEKITTTGTLWNGNKIIDERLIDPD
932	ATG-4794	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaaca gacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccct gaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggccca gatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgc cctatggcacactggtaatcgac
933	ATG-4794	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSAD QMAQIEEVFKVVYPVDDHHFKVILPYGTLVID

Table 9. Exemplary peptide sequences.

Pep ID	SEQ ID NO.	Sequence
86	824	VSGWRLFKKIS
229	825	VSGWRLFKKI
289	826	VSVSGWRLFKKIS
521	827	GKMLFRVTINSWK
543	366	WNGNKIIDERLITPD
544	367	KKITTTGTLWNGR
545	368	RPYEGIAVFDGK
591	369	GKMLFRVTIWKVSVSGWRLFKKIS
592	370	GKMLFRVTIWKVSGWRLFKKIS
593	371	GSMKFRVTINSWKVSVSGWRLFKKIS
594	372	GSMKFRVTINSWKVSGWRLFKKIS
595	373	GSMKFRVTINSWKNVTGYRLFKKISN
596	374	GSMKFRVTINSWKVTGYRLFEKIS
597	375	GSMKFRVTIWKVSVSGWRLFKKIS
598	376	GSMKFRVTIWKVSGWRLFKKIS
599	377	GRMLFRVTINSWKVSVSGWRLFKKIS
600	378	GRMLFRVTINSWKVSGWRLFKKIS
601	379	GRMLFRVTIWKVSVSGWRLFKKIS
602	380	GRMLFRVTIWKVSGWRLFKKIS
603	381	GSMLFRVTINSVSVSGWRLFKKIS
604	382	GSMLFKVTINSVSGWRLFKKIS
605	383	GSMLFQVTINSVSGWRLFKKIS

606	384	GSMLFEVTINSVSGWRLFKKIS
607	385	GSMLFNVTINSVSGWRLFKKIS
608	386	GRPYEGIAVFDGKKITTTGTL
609	387	GSMKFRVTINSWKVTGYRLFEKES
610	388	GSMKFRVTINSWKVEGYRLFEKIS
611	389	KKITTTGTLWNGNKIIDERLITPD
612	390	WNGNKIIDERLITPDGSMLFRVTINS
671	391	GKMLFRVTIQKWK
668	392	GKMLFRVTIGKWK
727	393	GKMLFRVTIGRWK
669	394	GKMLFRVTIGNWK
674	395	GKMLFRVTIQNWK
702	396	GKMLFRVTIDKWK
703	397	GKMLFRVTIEKWK
705	810	EKMLFRVTIESWK
724	811	EKLLFRVTIESWK
725	812	EKLLFRVTIESYK
730	398	GKMLFRVTIERWK
731	399	GKMLFRVTIDRWK
738	400	DKMLFRVTIQKWK
739	401	DKMLFRVTIGKWK
848	402	DKMLFRVTIGRWK
740	403	DKMLFRVTIGNWK
741	404	DKMLFRVTIQNWK

732	405	DKMLFRVTIDKWK
742	406	DKMLFRVTIEKWK
735	407	DKMLFRVTIERWK
733	408	DKMLFRVTIDRWK
759	816	DKLLFTVTIEKYK
798	409	RPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPD
849	410	EKMLFRVTIQKWK
708	411	EKMLFRVTIGKWK
709	412	EKMLFRVTIGRWK
775	413	DKMLFTVTIQKVSGWRLFKKIS
788	414	DKLLFTVTIEKVSGWRLFKKIS
789	415	DKLLFTVTIEKWKVSGWRLFKKIS
790	416	DKLLFTVTIEKYKVSGWRLFKKIS
792	417	DKLLFTVTIEKYKVSVSGWRLFKKIS
795	418	KKMLFRVTIQKVSGWRLFKKIS
797	419	KKMLFRVTIQKWKVSVSGWRLFKKIS
796	420	KKMLFRVTIQKWKVSGWRLFKKIS
804	421	DKLLFTVTIGKVSGWRLFKKIS
805	422	DKLLFTVTIGKYKVSGWRLFKKIS
806	423	DKLLFTVTIGKYKVSVSGWRLFKKIS
807	424	DKLLFTVTIGKWKVSVSGWRLFKKIS
808	425	DKLLFTVTIQKVSGWRLFKKIS
813	426	KKMLFTVTIQKVSGWRLFKKIS
816	427	KKLLFRVTIQKVSGWRLFKKIS

825	428	DKLLFTVTIEKVSGWRLFKKI
826	429	DKLLFTVTIEKYKVSVSGWRLFKKI
827	430	DRLLFTVTIERVSGWRLFKKIS
831	431	EKLLFTVTIEKVSGWRLFKKIS
832	432	KKLLFTVTIGKVSGWRLFKKIS
833	433	GSMRFRVTINSWRVTGYRLFERES
834	434	GSMKFRVTINSVTGYRLFEKES
844	435	KKITTTGTLWNGNKIID
845	436	ERLITPDGSMLFRVTINSVSGWRLFKKIS
846	437	GRPYEGIAVDFGKKITTTGTLWNGNKIIDERLITPDGSML FRVTINSVSGWRLFKKIS
847	438	GVTPNKLNYFGRPYEGIAVDFGKKITTTGTLWNGNKIID ERLITPDGSMLFRVTINSVSGWRLFKKIS
850	439	EKMLFRVTIGNWK
851	440	EKMLFRVTIQNWK
706	441	EKMLFRVTIDKWK
707	442	EKMLFRVTIEKWK
737	443	EKMLFRVTIERWK
736	444	EKMLFRVTIDRWK
760	445	KKMLFRVTIQKWK
852	446	KKMLFRVTIGKWK
853	447	KKMLFRVTIGRWK
854	448	KKMLFRVTIGNWK
855	449	KKMLFRVTIQNWK
856	450	KKMLFRVTIDKWK

857	451	KKMLFRVTIEKWK
858	452	KKMLFRVTIERWK
859	453	KKMLFRVTIDRWK
860	454	RKMLFRVTIQKWK
861	455	RKMLFRVTIGKWK
862	456	RKMLFRVTIGRWK
863	457	RKMLFRVTIGNWK
864	458	RKMLFRVTIQNWK
865	459	RKMLFRVTIDKWK
866	460	RKMLFRVTIEKWK
867	461	RKMLFRVTIERWK
868	462	RKMLFRVTIDRWK
656	463	EQMLFRVTINSWK
869	464	SRMLFRVTINSWK
533	465	GEMLFRVTINSWK
690	466	GKMKFRVTINSWK
678	467	GKMLFRVKINSWK
679	468	GKMLFRVRINSWK
681	469	GKMLFRVDINSWK
663	470	GKMLFRVTIDSWK
743	471	GKMLFRVTINKWK
714	472	EKMLFKVTIQKWK
870	473	EKMLFTVTIQKWK
871	474	EKMLFKVTIDKWK

872	475	EKMLFTVTIDKWK
873	476	EKMLFKVTIGRWK
744	477	DKMLFKVTIQKWK
745	478	DKMLFTVTIQKWK
874	479	DKMLFKVTIDKWK
875	480	DKMLFTVTIDKWK
876	481	GKMLFKVTIEKWK
877	482	GKMLFTVTIEKWK
748	483	DKMLFKVTIGKWK
749	484	DKMLFTVTIGKWK
878	485	DKMLFKVTIGNWK
879	486	DKMLFKVTIQNWK
781	487	GKMLFKVTINKWK
782	488	GKMLFTVTINKWK
752	489	DKMLFKVTIEKWK
753	490	DKMLFTVTIEKWK
750	491	DKLLFKVTIGKWK
786	492	DKMLFTVTINKWK
756	493	DKLLFTVTIQKWK
757	494	DKLLFTVTIQKYK
758	495	DKLLFTVTIEKWK
759	496	DKLLFTVTIEKYK
793	497	DKLLFTVTIGKWK
794	498	DKLLFTVTIGKYK

799	499	DKLLFTVTINKWK
800	500	DKLLFTVTINKYK
780	501	GKMLFRVTINS
765	502	DKMLFTVTIQK
779	503	DKMLFKVTIQK
820	504	DKLLFTVTIGK
819	505	DKMLFTVTIGK
822	506	DKMLFTVTIEK
821	507	DKLLFTVTIEK
627	508	*DKMLFRVTINSWK
628	509	*EKMLFRVTINSWK
629	510	*RKMLFRVTINSWK
630	511	*KKMLFRVTINSWK
631	512	*HKMLFRVTINSWK
632	513	*GLMLFRVTINSWK
633	514	*GQMLFRVTINSWK
634	515	*GTMLFRVTINSWK
635	516	*GKLLFRVTINSWK
636	517	*GKMLFKVTINSWK
637	518	*GKMLFRVTIQSWK
638	519	*GKMLFRVTIDSWK
639	520	*GKMLFRVTIGSWK
640	521	*GKMLFRVTINTWK
641	522	*GKMLFRVTINNWK

642	523	*GKMLFRVTINQWK
643	524	*GKMLFRVTINPWK
644	525	*GKMLFRVTINKWK
645	526	*GKMLFRVTINSWQ
646	527	*GKMLFRVTINSWN
647	528	*GKMLFRVTINSWT
648	529	*GKMLFRVTINSWH
649	530	*GKMLFRVTINSWP
650	531	*GKMLFRVTINSWR
677	532	GKMKFRVTIDSWK
680	533	GKMLFRVEINSWK
682	534	GKMLFRVQINSWK
683	535	GKMKFRVKINSWK
684	536	GKMKFRVRINSWK
685	537	GKMKFRVEINSWK
686	538	GKMKFRVDINSWK
687	539	GKMKFRVQINSWK
688	540	GKMKFRVNINSWK
689	541	GKMKFRVSINSWK
613	542	GKMLFRVNINSWK
614	543	GKMLFRVSINSWK
615	544	GKMLFRVWINSWK
616	545	GKMSFRVTINSWK
617	546	GKMWFRVTINSWK

618	547	GKMNFRVTINSWK
619	548	GSMLFRVTINSYK
620	549	GKMLFRVTINSYK
621	550	GKMLFRVTIKSWK
622	551	GKMLFRVTIESWK
716	552	GKMKFRVTIQSWK
717	553	GKMKFRVTIESWK
718	554	GKMKFRVTIKSWK
719	555	GKMKFRVTIRSWK
651	556	RLMLFRVTINSWK
652	557	RQMLFRVTINSWK
653	558	KLMLFRVTINSWK
654	559	KQMLFRVTINSWK
655	560	ELMLFRVTINSWK
657	561	DLMLFRVTINSWK
658	562	DQMLFRVTINSWK
659	563	DKMLFRVTINSWK
660	564	EKMLFRVTINSWK
661	565	RKMLFRVTINSWK
662	566	KKMLFRVTINSWK
665	567	GKMLFRVTIGSWK
667	568	GKMLFRVTINKWK
670	569	GKMLFRVTISKWK
671	570	GKMLFRVTIQKWK

672	571	GKMLFRVTITKWK
673	572	GKMLFRVTIKKWK
675	573	GKMLFKVTINSWK
676	574	RLMLFRVTIGKWK
701	575	GKMLFRVTINRWK
710	576	EKMLFTVTIGKWK
711	577	EKLLFTVTIGKWK
712	578	EKMLFTVTIGRWK
720	579	EKMLFTVTIEKWK
722	580	DKMLFRVTIESWK
726	581	EKLLFRVTIGKYK
746	582	DKLLFKVTIQKWK
747	583	DKLLFKVTIQKYK
751	584	DKLLFKVTIGKYK
754	585	DKLLFKVTIEKWK
755	586	DKLLFKVTIEKYK
761	587	KKLLFRVTIQKWK
762	588	DRMLFRVTIQRWR
766	589	ERMLFRVTIGRWR
768	590	GRMLFRVTINRWR
770	591	DRMLFRVTIERWR
783	592	DKMLFKVTIQKYK
784	593	DKMLFRVTINKWK
785	594	DKMLFKVTIEKYK

787	595	DKMLFKVTINKWK
693	900	GRMLFRVTINSWR
691	901	VSGWRLFRRIS
895	902	GRLLFVVVIERYR
937	903	VSGWRLFRRISC
938	904	GRMLFRVTINSWRC
939	905	GRLLFTVTIERYRC
840	906	GKLLFVVVIEKYK
900	907	GKLLFVTIEKVSGWRLFKKIS
*Terminus unblocked		

Table 10. Exemplary luciferase base sequences

Pep ID	**SEQ ID NO.**	**Sequence**
LgTrip 3546 – WT strand 9 – HiBiT	788	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID**GVTPNKLNYFGRPYEGIAV FDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VSG WRLFKKIS**
LgTrip 3546 – WT strand 9 – SmBiT	789	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID**GVTPNKLNYFGRPYEGIAV FDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VTG YRLFEEIL**
LgTrip 3546 (1-5)	790	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID

LgTrip 3546 (1-6)	791	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID**GVTPNKLNYFGRPYEGIAV FDG**
LgTrip 3546 (1-7)	792	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID**GVTPNKLNYFGRPYEGIAV FDG**KKITTTGTL
LgTrip 3546 (1-8)	793	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVID**GVTPNKLNYFGRPYEGIAV FDG**KKITTTGTL**WNGNKIIDERLITPD**
LgTrip 3546 (strands 6-8) – WT strand 9 – HiBiT	794	**GVTPNKLNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIID ERLITPD**GSMLFRVTINS**VSGWRLFKKIS**
LgTrip 3546 (strands 7-8) – WT strand 9 – HiBiT	795	KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VSGWR LFKKIS**
LgTrip 3546 (strand 8) – WT strand 9 – HiBiT	796	**WNGNKIIDERLITPD**GSMLFRVTINS**VSGWRLFKKIS**
WT strand 9 – HiBiT	797	GSMLFRVTINS**VSGWRLFKKIS**

LgTrip 3546 (strands 6-8) – WT strand 9 – SmBiT	798	**GVTPNKLNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIID ERLITPD**GSMLFRVTINS**VTGYRLFEEIL**
LgTrip 3546 (strands 7-8) – WT strand 9 – SmBiT	799	KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VTGYRL FEEIL**
LgTrip 3546 (strand 8) – WT strand 9 – SmBiT	800	**WNGNKIIDERLITPD**GSMLFRVTINS**VTGYRLFEEIL**
WT strand 9 – SmBiT	801	GSMLFRVTINS**VTGYRLFEEIL**
β6-like	817	**GVTPNKLNYFGRPYEGIAVFDG**
β7-like	818	KKITTTGTL
β8-like	819	**WNGNKIIDERLITPD**

Table 11. Exemplary polypeptides

Name	**Polypeptide construct description**
ATG-2623	LgBiT-6His
ATG-3745	HiBiT-8His-LgTrip
ATG-3746	6His-HiBiT-4GS-LgTrip
ATG-4632	HiBiT-4GS-LgTrip-6His
ATG-4808	VS-HiBiT-0GS-LgTrip

ATG-4809 VS-HiBiT-4GS-LgTrip

ATG-4810 VS-HiBiT-8GS-LgTrip

ATG-4811 VS-HiBiT-12GS-LgTrip

ATG-4812 VS-HiBiT-16GS-LgTrip

ATG-4813 VS-HiBiT-20GS-LgTrip

ATG-4814 HiBiT-20GS-LgTrip

ATG-4815 LgTrip-0GS-VS-HiBiT

ATG-4816 LgTrip-4GS-VS-HiBiT

ATG-4817 LgTrip-8GS-VS-HiBiT

ATG-4818 LgTrip-8GS-HiBiT

ATG-4819 VS-HiBIT-0GS-LgTrip-6His

ATG-4820 VS-HiBiT-4GS-LgTrip-6His

ATG-4821 VS-HiBiT-8GS-LgTrip-6His

ATG-4822 VS-HiBiT-12GS-LgTrip-6His

ATG-4823 VS-HiBiT-16GS-LgTrip-6His

ATG-4824 HiBiT-20GS-LgTrip-6His

ATG-4825 VS-HiBiT-20GS-LgTrip-6His

ATG-4826 6His-LgTrip-0GS-VS-HiBiT

ATG-4827 6His-LgTrip-4GS-VS-HiBiT

ATG-4828 6His-LgTrip-8GS-HiBiT

ATG-4829 6His-LgTrip-8GS-VS-HiBiT

Mutations on 5333

ATG-5538 (I41T) Nucleotide (SEQ ID NO: 934)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGACCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA

TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5538 (I41T) Amino Acid (SEQ ID NO: 935)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPTQRMVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGR
PYEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5339 (K11N) Nucleotide (SEQ ID NO: 936)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5339 (K11N) Amino Acid (SEQ ID NO: 937)

VFTLEDFVGDWNQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5340 (R152Q) Nucleotide (SEQ ID NO: 938)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAAGTAACCATCAACAGC

ATG-5340 Amino Acid (SEQ ID NO: 939)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFQVTINS

ATG-5407 V135A Nucleotide (SEQ ID NO: 940)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5407 V135A Amino Acid (SEQ ID NO: 941)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFRVTINS

ATG-5408 D156N Nucleotide (SEQ ID NO: 942)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCGACAGC

ATG-5408 D156N Amino Acid (SEQ ID NO: 943)
VFTLEDFVGDWNQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTIDS

ATG-5409 H57Q Nucleotide (SEQ ID NO: 944)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG

TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCAAGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCC
AGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGT
GATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAA
CTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCAC
TGTAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCAC
CCCCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5409 H57Q Amino Acid (SEQ ID NO: 945)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IQVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5411 N33K+I155V Nucleotide (SEQ ID NO: 946)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAAACTCGCC
GTGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAG
ATCGACATCCACGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCC
AGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGT
GATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAA
CTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCAC
TGTAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCAC
CCCCGACGGCTCCATGTCCTTCCGAGTAACCGTCAACAGC

ATG-5411 N33K+I155V Amino Acid (SEQ ID NO: 947)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQKLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTVNS

ATG-5412 I54V+L127A Nucleotide (SEQ ID NO: 948)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGG
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
CAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5412 I54V+L127A Amino Acid (SEQ ID NO: 949)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKV
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGR
PYEGIAVFDGKKITATGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5413 M44L+V135A Nucleotide (SEQ ID NO: 950)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGTTGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5413 M44L+V135A Amino Acid (SEQ ID NO: 951)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRLVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFRVTINS

ATG-5414 V119A Nucleotide (SEQ ID NO: 952)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGCGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5414 V119A (Amino Acid) (SEQ ID NO: 953)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAAFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5416 D9A+H57Q Nucleotide (SEQ ID NO: 954)

GTCTTCACACTCGAAGATTTCGTTGGGGCCTGGAAACAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT

CGACATCCAAGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5416 D9A+H57Q (Amino Acid) (SEQ ID NO: 955)

VFTLEDFVGAWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IQVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5417 N33D+I41T Nucleotide (SEQ ID NO: 956)

GTCTTCACACTCGAAGATTTCGTAGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGGATCTCGCCG
TGTCCGTAACTCCGACCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5417 N33D+I41T Amino Acid (SEQ ID NO: 957)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQDLAVSVTPTQRMVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGR
PYEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5418 Q32R+I155T Nucleotide (SEQ ID NO: 958)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAGACAGACTGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCGGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCACCAACAGC

ATG-5418 Q32R+I155T mino Acid (SEQ ID NO: 959)

VFTLEDFVGDWRQTAAYNLDQVLEQGGVSSLLRNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTTNS

ATG-5419 D19V+M106T+V120L Nucleotide (SEQ ID NO: 960)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGTCCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACACGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGCTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5419 D19V+M106T+V120L Amino Acid (SEQ ID NO: 961)

VFTLEDFVGDWKQTAAYNLVQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNTLNYFGRP
YEGIAVLDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5420 I41N+E63G Nucleotide (SEQ ID NO: 962)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGAACCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGGAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TGACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5420 I41N+E63G Amino Acid (SEQ ID NO: 963)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPNQRMVRSGENALKI
DIHVIIPYGGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5421 N50S Nucleotide (SEQ ID NO: 964)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAACCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAGTGCCCTGAAGA

TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5421 N50S Amino Acid (SEQ ID NO: 965)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGESALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5432 L3H Nucleotide (SEQ ID NO: 966)

GTCTTCACACACGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5432 Amino Acid (SEQ ID NO: 967)

VFTHEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5433 T13S Nucleotide (SEQ ID NO: 968)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGTCAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5433 T13S Amino Acid (SEQ ID NO: 969)

VFTLEDFVGDWKQSAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5434 P93H Nucleotide (SEQ ID NO: 970)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5434 P93H Amino Acid (SEQ ID NO: 117)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5435 F120L Nucleotide (SEQ ID NO: 118)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGCTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATAGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5435 F120L Amino Acid (SEQ ID NO: 119)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVLDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5437 S157R Nucleotide (SEQ ID NO: 120)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA

TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACCGC

ATG-5437 S157R Amino Acid (SEQ ID NO: 121)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINR

ATG-5438 H86L Nucleotide (SEQ ID NO: 122)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCTTCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5438 H86L Amino Acid (SEQ ID NO: 123)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDLHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5439 M149V Nucleotide (SEQ ID NO: 124)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCGTGTCCTTCCGAGTAACCATCAACAGC

ATG-5439 M149V Amino Acid (SEQ ID NO: 125)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSVSFRVTINS

ATG-5440 I59V Nucleotide (SEQ ID NO: 126)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTTGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCGTCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5440 I59V Amino Acid (SEQ ID NO: 127)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVVIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGR
PYEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5441 D19A Nucleotide (SEQ ID NO: 128)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGCCCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5441 D19A Amino Acid (SEQ ID NO: 129)

VFTLEDFVGDWKQTAAYNLAQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5442 Q69L+T144S Nucleotide (SEQ ID NO: 130)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA

TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCTAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTATTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCTCCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5442 Q69L+T144S Amino Acid (SEQ ID NO: 131)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADLMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLISPDGSMSFRVTINS

ATG-5456 K11Y Nucleotide (SEQ ID NO: 132)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGTACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5456 K11Y Nucleotide Amino Acid (SEQ ID NO: 596)

VFTLEDFVGDWYQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5457 K11R Nucleotide (SEQ ID NO: 597)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGGCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5457 K11R Amino Acid (SEQ ID NO: 598)

VFTLEDFVGDWRQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5458 K11L Nucleotide (SEQ ID NO: 599)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGCTGCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5458 K11L Amino Acid (SEQ ID NO: 600)

VFTLEDFVGDWLQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5459 R152Q Nucleotide (SEQ ID NO: 601)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5459 R152Q Amino Acid (SEQ ID NO: 602)

VFTLEDFVGDWNQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFQVTINS

ATG-5460 N156D Nucleotide (SEQ ID NO: 603)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT

CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCGACAGC

ATG-5460 N156D Amino Acid (SEQ ID NO: 604)

VFTLEDFVGDWNQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTIDS

ATG-5491 K11Q Nucleotide (SEQ ID NO: 605)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGCAGCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5491 K11Q Amino Acid (SEQ ID NO: 606)

VFTLEDFVGDWQQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5492 K11M Nucleotide (SEQ ID NO: 607)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGATGCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5492 K11M Amino Acid (SEQ ID NO: 608)

VFTLEDFVGDWMQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5493 K11H Nucleotide (SEQ ID NO: 609)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGCACCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5493 K11H Amino Acid (SEQ ID NO: 610)

VFTLEDFVGDWHQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5494 K11F Nucleotide (SEQ ID NO: 611)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGTTCCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5494 K11F Amino Acid (SEQ ID NO: 612)

VFTLEDFVGDWFQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5495 K11W Nucleotide (SEQ ID NO: 613)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGTGGCAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGAT

CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5495 K11W Amino Acid (SEQ ID NO: 614)

VFTLEDFVGDWWQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFRVTINS

ATG-5505 V135A+R152Q Nucleotide (SEQ ID NO: 615)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATAGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTACGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5505 V135A+R152Q Amino Acid (SEQ ID NO: 616)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5506 V135A+R152Q+N156D Nucleotide (SEQ ID NO: 617)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATAGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTACGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCGACAGC

ATG-5506 V135A+R152Q+N156D Amino Acid (SEQ ID NO: 618)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTIDS

ATG-5507 P93H+V135A Nucleotide (SEQ ID NO: 619)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATAGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTACGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCGAGTAACCATCAACAGC

ATG-5507 P93H+V135A Amino Acid (SEQ ID NO: 620)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFRVTINS

ATG-5508 P93H+R152Q Nucleotide (SEQ ID NO: 621)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5508 P93H+R152Q Amino Acid (SEQ ID NO: 622)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFQVTINS

ATG-5509 P93H+R152Q+N156D Nucleotide (SEQ ID NO: 623)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA

TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGTCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCGACAGC

ATG-5509 P93H+R152Q+N156D Amino Acid (SEQ ID NO: 624)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGVKIIDERLITPDGSMSFQVTIDS

ATG-5510 P93H+V135A+R152Q Nucleotide (SEQ ID NO: 625)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATAGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTACGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5510 P93H+V135A+R152Q Amino Acid (SEQ ID NO: 626)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5511 P93H+V135G+R152Q Nucleotide (SEQ ID NO: 627)

GTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATAGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGAACT
ATTTCGGACGGCCGTACGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
TAACAGGGACCCTGTGGAACGGCGGCAAAATTATCGACGAGCGCCTGATCACCC
CCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5511 P93H+V135G+R152Q Amino Acid (SEQ ID NO: 628)

VFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKID
IHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGGKIIDERLITPDGSMSFQVTINS

Mutations on 5344

ATG-5534 P93H+V135A+R152Q (codon optimized 5510) Nucleotide (SEQ ID NO: 629)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGAtaaactcg

ATG-5534 P93H+V135A+R152Q (codon optimized 5510) Amino Acid (SEQ ID NO: 630)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5535 P93H+V135A+R152Q+N156D Nucleotide (SEQ ID NO: 631)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAGACTCG

ATG-5535 P93H+V135A+R152Q+N156D Amino Acid (SEQ ID NO: 632)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTID

ATG-5536 E4D+V135A+R152Q Nucleotide (SEQ ID NO: 633)

TTCACTTTGGACGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACCATATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5536 E4D+V135A+R152Q Amino Acid (SEQ ID NO: 634)

FTLDDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5537 E4D+Q42M+P93H+V135A+R152Q Nucleotide (SEQ ID NO: 635)

TTCACTTTGGACGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATAATGCGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCGT

ATG-5537 E4D+Q42M+P93H+V135A+R152Q Amino Acid (SEQ ID NO: 636)

FTLDDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

<u>Mutations on 5534</u>

ATG-5652 5534+Y16F Q20P Q152H Nucleotide (SEQ ID NO: 637)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATTCAACTTAG
ACCCAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT

AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGCTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCACGTTACGATAAACTCG

ATG-5652 5534+Y16F Q20P Q152H Amino Acid (SEQ ID NO: 638)

FTLEDFVGDWKQTAAFNLDPVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFHVTINS

ATG-5653 5534+M106R,Y114F,E4E Nucleotide (SEQ ID NO: 639)

TTCACTTTGGAGGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACAGGTTAAACTATT
TCGGTAGACCTTTTGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTA
CTGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAG
ACGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5653 5534+M106R,Y114F,E4E Amino Acid (SEQ ID NO: 640)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNRLNYFGRPF
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5654 5534+M44V Nucleotide (SEQ ID NO: 641)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTGTGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
AGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5654 5534+M44V (amino acid) (SEQ ID NO: 642)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRVVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5655 5534+M44I Nucleotide (SEQ ID NO: 643)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATAGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5655 5534+M44I Amino Acid (SEQ ID NO: 644)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5656 5534+A35A,P40P,M106R Nucleotide (SEQ ID NO: 645)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCTGTTAG
TGTTACGCCAATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACAGGTTAAACTATT
TCGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTA
CTGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAG
ACGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5656 5534+A35A,P40P,M106R amino acid (SEQ ID NO: 646)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNRLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5657 5534+M106T Nucleotide (SEQ ID NO: 647)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACACGTTAAACTATT
TCGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTA
CTGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAG
ACGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5657 5534+M106T amino acid (SEQ ID NO: 648)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNTLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5659 5534+M106K+L30L+K136E Nucleotide (SEQ ID NO: 649)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTGTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACAAGTTAAACTATT
TCGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTA
CTGGTACGTTATGGAACGGAGCCGAGATAATAGACGAGAGATTAATAACGCCAG
ACGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5659 5534+M106K+L30L+K136E amino acid (SEQ ID NO: 650)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNKLNYFGRP
YEGIAVFDGKKITVTGTLWNGAEIIDERLITPDGSMSFQVTINS

ATG-5660 5534+V36G,N50N,A14A Nucleotide (SEQ ID NO: 651)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCGGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGGTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAACGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5660 5534+V36G,N50N,A14A amino acid (SEQ ID NO: 652)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAGSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5661 5534+A15A,A67A,M106L,L107L,I138K Nucleotide (SEQ ID NO: 653)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCGTACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCTGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACTTGTTGAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAAAAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5661 5534+A15A,A67A,M106L,L107L,I138K amino acid (SEQ ID NO: 654)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNLLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIKDERLITPDGSMSFQVTINS

ATG-5662 5534+D5G, A35T, L92F, D100V, R141G, L1142S Nucleotide (SEQ ID NO: 655)

TTCACTTTGGAAGGTTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGACAGTTAG
TGTTACGCCTATACAACGTATGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTTCACTATGGAACTTTAGTTATAGTCGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGGGATCAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5662 5534+D5G, A35T, L92F, D100V, R141G, L1142S amino acid (SEQ ID NO: 656)

FTLEGFVGDWKQTAAYNLDQVLEQGGVSSLLQNLTVSVTPIQRMVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIFHYGTLVIVGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDEGSITPDGSMSFQVTINS

ATG-5676 5534+M44K Nucleotide (SEQ ID NO: 657)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTAAGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGAtaaactcg

ATG-5676 5534+M44K amino acid (SEQ ID NO: 658)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRKVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5677 5534+M44E Nucleotide (SEQ ID NO: 659)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTGAGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5677 5534+M44E amino acid (SEQ ID NO: 660)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQREVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5678 5534+M44A Nucleotide (SEQ ID NO: 661)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTGCGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5678 5534+M44A amino acid (SEQ ID NO: 662)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRAVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5679 M44C Nucleotide (SEQ ID NO: 663)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTTGTGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5679 M44C amino acid (SEQ ID NO: 664)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRCVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5680 M44W Nucleotide (SEQ ID NO: 665)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTTGGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCGT

ATG-5680 M44W amino acid (SEQ ID NO: 666)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRWVRSGENALKIDI
HVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRP
YEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5681 M44G Nucleotide (SEQ ID NO: 667)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG

TGTTACGCCTATACAACGTGGGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5681 M44G amino acid (SEQ ID NO: 668)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRGVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5682 M44H Nucleotide (SEQ ID NO: 669)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTCATGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5682 M44H amino acid (SEQ ID NO: 670)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRHVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5683 M44S Nucleotide (SEQ ID NO: 671)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTAGTGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5683 M44S amino acid (SEQ ID NO: 672)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRSVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5684 M44Q Nucleotide (SEQ ID NO: 673)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTCAGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5684 M44Q Amino acid (SEQ ID NO: 674)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRQVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5685 5534+M44R Nucleotide (SEQ ID NO: 675)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTCGGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5685 5534+M44R amino acid (SEQ ID NO: 676)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRRVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5686 5534+M44T Nucleotide (SEQ ID NO: 677)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTACGGTGAGATCGGGAGAAAATGCATTAAAAATAGA

CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5686 5534+M44T amino acid (SEQ ID NO: 678)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRTVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5687 5534+M44Y Nucleotide (SEQ ID NO: 679)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTTATGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5687 5534+M44Y Amino acid (SEQ ID NO: 680)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRYVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5688 5534+M44L Nucleotide (SEQ ID NO: 681)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTCTGGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5688 5534+M44L amino acid (SEQ ID NO: 682)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRLVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5689 5534+M44P Nucleotide (SEQ ID NO: 683)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTCCTGTGAGATCGGGAGAAAATGCATTAAAAATAGA
CATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGAT
AGAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATA
TTACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTT
CGGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTAC
TGGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGA
CGGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5689 5534+M44P amino acid (SEQ ID NO: 684)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRPVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5590 5534+M44F Nucleotide (SEQ ID NO: 685)

TTCACTTTGGAAGATTTCGTGGGTGACTGGAAACAGACTGCAGCATACAACTTAG
ACCAAGTGTTGGAACAGGGTGGAGTTAGTTCGCTTTTACAGAATTTGGCAGTTAG
TGTTACGCCTATACAACGTTTTGTGAGATCGGGAGAAAATGCATTAAAAATAGAC
ATACATGTGATAATACCTTATGAAGGATTATCGGCAGACCAAATGGCACAGATA
GAAGAGGTTTTTAAAGTTGTTTACCCAGTTGATGATCATCACTTTAAAGTTATATT
ACACTATGGAACTTTAGTTATAGACGGTGTGACTCCTAACATGTTAAACTATTTC
GGTAGACCTTATGAAGGAATAGCAGTTTTCGACGGAAAGAAAATAACGGTTACT
GGTACGTTATGGAACGGAGCCAAGATAATAGACGAGAGATTAATAACGCCAGAC
GGAAGTATGAGTTTCCAGGTTACGATAAACTCG

ATG-5590 5534+M44F Amino acid (SEQ ID NO: 686)

FTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRFVRSGENALKIDIH
VIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPY
EGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5788 Nucleotide (SEQ ID NO: 687)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag

gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5788 Amino Acid (SEQ ID NO: 688)

MKHHHHHHHAIAMVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5789 Nucleotide (SEQ ID NO: 689)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacaagtccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5789 Amino Acid (SEQ ID NO: 690)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDKSMLFRVTINGVS
GWRLFKKIS

ATG-5790 Nucleotide (SEQ ID NO: 691)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacaaggttagcggctggcgcctgt
tcaagaagatcagc

ATG-5790 Amino Acid (SEQ ID NO: 692)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINKVS
GWRLFKKIS

ATG-5791 Nucleotide (SEQ ID NO: 693)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5791 Amino Acid (SEQ ID NO: 694)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5792 Nucleotide (SEQ ID NO: 695)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
aagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5792 Amino Acid (SEQ ID NO: 696)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNKLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5793 Nucleotide (SEQ ID NO: 697)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc

aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatccagggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5793 Amino Acid (SEQ ID NO: 698)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTIQGVS
GWRLFKKIS

ATG-5794 Nucleotide (SEQ ID NO: 699)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacaagaagatgctgttccgagtaaccatccagaaggttagcggctggcgcctg
ttcaagaagatcagc

ATG-5794 Amino Acid (SEQ ID NO: 700)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDKKMLFRVTIQKVS
GWRLFKKIS

ATG-5795 Nucleotide (SEQ ID NO: 701)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5795 Amino Acid (SEQ ID NO: 702)

MKHHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIM
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5796 Nucleotide (SEQ ID NO: 703)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggca
acaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgtt
caagaagatcagc

ATG-5796 Amino Acid (SEQ ID NO: 704)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGHPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5797 Nucleotide (SEQ ID NO: 705)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggcaagatgctgttccgagtaaccatcaacggcgttagcggctggcgcctg
ttcaagaagatcagc

ATG-5797 Amino Acid (SEQ ID NO: 706)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGKMLFRVTINGVS
GWRLFKKIS

ATG-5798 Nucleotide (SEQ ID NO: 707)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcgatcccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5798 Amino Acid (SEQ ID NO: 708)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLIDPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5799 Nucleotide (SEQ ID NO: 709)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5799 Amino Acid (SEQ ID NO: 710)

MKHHHHHHAIAMDFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5800 Nucleotide (SEQ ID NO: 711)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5800 Amino Acid (SEQ ID NO: 712)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5801 Nucleotide (SEQ ID NO: 713)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag

gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcccatcggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5801 Amino Acid (SEQ ID NO: 714)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5802 Nucleotide (SEQ ID NO: 715)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgtgaccggctggcggctg
tgcgaacgcattctg

ATG-5802 Amino Acid (SEQ ID NO: 716)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVT
GWRLCERIL

ATG-5803 Nucleotide (SEQ ID NO: 717)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcccatcggcacactggtaatcgacggggttacgccgaac
aagctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactaccacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcgatcccgacaagaagatgctgttccgagtaaccatccagaaggttagcggctggcgcctgt
tcaagaagatcagc

ATG-5803 Amino Acid (SEQ ID NO: 718)

MKHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIM
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDG

VTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPDKKMLFRVTIQKVS
GWRLFKKIS

ATG-5804 Nucleotide (SEQ ID NO: 719)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcccatcggcacactggtaatcgacggggttacgccgaac
aagctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactaccacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcgatcccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5804 Amino Acid (SEQ ID NO: 720)

MKHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIM
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPIGTLVIDG
VTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPDKKMLFRVTIQKVS
GWRLFKKIS

Example 52-Circularly permuted LgBiT

ATG-4992 PEP78-8GS-LGTRIP3546-6HIS Nucleotide (SEQ ID NO: 721)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAA
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCACCCCCGACCATCACCATCACCATCAT

ATG-4992 PEP78-8GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 722)

MNVSGWRLFKKISNGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHH
H

ATG-4993 PEP79-8GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 723)

ATGAACGTGACCGGCTACCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAA
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCACCCCCGACCATCACCATCACCATCAT

ATG-4993 PEP79-8GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 724)

MNVTGYRLFKKISNGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHH
H

ATG-4994 PEP99-8GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 725)

ATGGTGACCGGCTACCGGCTGTTCGAGAAGATTAGCGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-4994 PEP99-8GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 726)

MVTGYRLFEKISGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV
SVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG
TLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-4995 PEP128-8GS-LGTRIP3546-6HIS Nucleotide (SEQ ID NO: 727)

ATGGTGACCGGCTACCGGCTGTTCGAGAAGATTCTGGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA

AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-4995 PEP128-8GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 728)

MVTGYRLFEKILGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV
SVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG
TLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-4996 HIBIT-8GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 729)

ATGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-4996 HIBIT-8GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 730)

MVSGWRLFKKISGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV
SVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG
TLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-4997 HIBIT-STOG-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 731)

ATGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTGGCGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-4997 HIBIT-STOG-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 732)

MVSGWRLFKKIGGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA
VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY
GTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-4998 HIBIT-ISTOSG-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 733)

ATGGTGAGCGGCTGGCGGCTGTTCAAGAAGTCGGGCGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-4998 HIBIT-ISTOSG-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 734)

MVSGWRLFKKSGGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA
VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY
GTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-4999 HIBIT-DELETE IS-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 735)

ATGGTGAGCGGCTGGCGGCTGTTCAAGAAGGGCTCGAGCGGTGGCTCGAGCGGT
TTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAACCTG
GACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGT
CCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGATCG
ACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCAGAT
CGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTGATC
CTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATT
TCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTACCA
CAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCACCCCCG
ACCATCACCATCACCATCAT

ATG-4999 HIBIT-DELETE IS-8GS-LGTRIP-3546-6HIS nucleotide amino acid (SEQ ID NO: 736)

MVSGWRLFKKGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS
VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGT
LVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5000 HIBIT-DELETE S-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 737)

ATGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTGGCTCGAGCGGTGGCTCGAGC
GGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAAC
CTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCG
TGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGA
TCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAAC
TATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACT
ACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCACC
CCCGACCATCACCATCACCATCAT

ATG-5000 HIBIT-DELETE S-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 738)

MVSGWRLFKKIGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS
VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGT
LVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5001 VSHIBIT-ISTOSG-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 739)

ATGGTTTCCGTGAGCGGCTGGCGGCTGTTCAAGAAGTCGGGCGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAA
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5001 VSHIBIT-ISTOSG-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 740)

MVSVSGWRLFKKSGGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQN
LAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL
PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHH
HH

ATG-5002 VSHIBIT-STOG-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 741)

ATGGTTTCCGTGAGCGGCTGGCGGCTGTTCAAGAAGATTGGCGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAA
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5002 VSHIBIT-STOG-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 742)

MVSVSGWRLFKKIGGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHH
H

ATG-5003 VSHIBIT-DELETE IS-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 743)

ATGGTTTCCGTGAGCGGCTGGCGGCTGTTCAAGAAGGGCTCGAGCGGTGGCTCG
AGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-5003 VSHIBIT-DELETE IS-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 744)

MVSVSGWRLFKKGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA
VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY
GTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5004 VSHIBIT-DELETE S-8GS-LGTRIP-3546-6HIS nucleotide (SEQ ID NO: 745)

ATGGTTTCCGTGAGCGGCTGGCGGCTGTTCAAGAAGATTGGCTCGAGCGGTGGCT
CGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCT
ACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCT
CGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTG
AAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATG
GCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTA

AGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGC
TGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGA
TCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGA
TCACCCCCGACCATCACCATCACCATCAT

ATG-5004 VSHIBIT-DELETE S-8GS-LGTRIP-3546-6HIS amino acid (SEQ ID NO: 746)

MVSVSGWRLFKKIGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA
VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY
GTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5485 PEP78-5GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 747)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
TCGTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTACAACC
TGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT
GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGAAGAT
CGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCCCA
GATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGGTG
ATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAAC
TATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACT
ACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCACC
CCCGACCATCACCATCACCATCAT

ATG-5485 PEP78-5GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 748)

MNVSGWRLFKKISNGSSGSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS
VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGT
LVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5486 PEP78-6GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 749)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGAT
CACCCCCGACCATCACCATCACCATCAT

ATG-5486 PEP78-6GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 750)

MNVSGWRLFKKISNGSSGGSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA
VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY
GTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH

ATG-5487 PEP78-7GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 751)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCCGCCT
ACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCT
CGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCTG
AAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATG
GCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTA
AGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAACAAGC
TGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGA
TCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGA
TCACCCCCGACCATCACCATCACCATCAT

ATG-5487 PEP78-7GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 752)

MNVSGWRLFKKISNGSSGGSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHH
H

ATG-5488 PEP78-9GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 753)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACA
GCCGCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGC
AGAATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAA
ATGCCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGA
CCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCA
TCACTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCG
AACAAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGC
AAAAAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAG
CGCCTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5488 PEP78-9GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 754)

MNVSGWRLFKKISNGSSGGSSGGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQ
NLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVI
LPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHH
HHH

ATG-5489 PEP78-10GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 755)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTGGTAGCTTCACACTCGACGATTTCGTTGGGGACTGGGAACAG
ACAGCCGCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTG
CTGCAGAATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTG
AAAATGCCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC
CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGA
TCATCACTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACG
CCGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGAC
GGCAAAAAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGAC
GAGCGCCTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5489 PEP78-10GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 756)

MNVSGWRLFKKISNGSSGGSSGGSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQ
NLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVI
LPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHH
HHH

ATG-5490 PEP78-11GS-LGTRIP3546-6HIS nucleotide (SEQ ID NO: 757)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTGGTAGCTCGTTCACACTCGACGATTTCGTTGGGGACTGGGAAC
AGACAGCCGCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTT
TGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGG
TGAAAATGCCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC
GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGAT
GATCATCACTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTA
CGCCGAACAAGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCG
ACGGCAAAAAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCG
ACGAGCGCCTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5490 PEP78-11GS-LGTRIP3546-6HIS amino acid (SEQ ID NO: 758)

MNVSGWRLFKKISNGSSGGSSGGSSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLL
QNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFK
VILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHH
HHHH

ATG-5513 4992+R112H+K123E nucleotide (SEQ ID NO: 759)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA

AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAG
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5513 4992+R112H+K123E amino acid (SEQ ID NO: 760)

MNVSGWRLFKKISNGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLITPDHHHHH
H

ATG-5514 4992+R112H+T144D nucleotide (SEQ ID NO: 761)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACACCCGTATGAAGGCATCGCCGTGTTCGACGGCAAA
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCGATCCCGACCATCACCATCACCATCAT

ATG-5514 4992+R112H+T144D amino acid (SEQ ID NO: 762)

MNVSGWRLFKKISNGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGHPYEGIAVFDGKKITTTGTLWNGNKIIDERLIDPDHHHHH
H

ATG-5515 4992+R112H+K123E+T144D nucleotide (SEQ ID NO: 763)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTTTCACACTCGACGATTTCGTTGGGGACTGGGAACAGACAGCC
GCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAG
AATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATG
CCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCA
AATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCA
CTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTACGCCGAAC
AAGCTGAACTATTTCGGACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAG
AAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGC
CTGATCGATCCCGACCATCACCATCACCATCAT

ATG-5515 4992+R112H+K123E+T144D amino acid (SEQ ID NO: 764)

MNVSGWRLFKKISNGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP
YGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPDHHHHH
H

ATG-5516 5490+R112H+K123E nucleotide (SEQ ID NO: 765)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTGGTAGCTCGTTCACACTCGACGATTTCGTTGGGGACTGGGAAC
AGACAGCCGCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTT
TGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGG
TGAAAATGCCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC
GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGAT
GATCATCACTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTA
CGCCGAACAAGCTGAACTATTTCGGACACCCGTATGAAGGCATCGCCGTGTTCG
ACGGCGAGAAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCG
ACGAGCGCCTGATCACCCCCGACCATCACCATCACCATCAT

ATG-5516 5490+R112H+K123E amino acid (SEQ ID NO: 766)

MNVSGWRLFKKISNGSSGGSSGGSSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLL
QNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFK
VILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLITPDHH
HHHH

ATG-5517 5490+R112H+K123E+T144D nucleotide (SEQ ID NO: 767)

ATGAACGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCAACGGCTCGAGCGGT
GGCTCGAGCGGTGGTAGCTCGTTCACACTCGACGATTTCGTTGGGGACTGGGAAC
AGACAGCCGCCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTT
TGCTGCAGAATCTCGCCGTGTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGG
TGAAAATGCCCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC
GCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGAT
GATCATCACTTTAAGGTGATCCTGCCCTATGGCACACTGGTAATCGACGGGGTTA
CGCCGAACAAGCTGAACTATTTCGGACACCCGTATGAAGGCATCGCCGTGTTCG
ACGGCGAGAAGATCACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCG
ACGAGCGCCTGATCGATCCCGACCATCACCATCACCATCAT

ATG-5517 5490+R112H+K123E+T144D amino acid (SEQ ID NO: 768)
MNVSGWRLFKKISNGSSGGSSGGSSFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLL
QNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFK
VILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTLWNGNKIIDERLIDPDHH
HHHH

ATG-5810 Nucleotide (SEQ ID NO: 769)

ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAGCAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCCAAAGGATGGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTGTAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGAT
CACCCCCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5810 Amino Acid (SEQ ID NO: 770)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5819 Nucleotide (SEQ ID NO: 771)

ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAGCAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCCAAAGGATAGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTGTAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGAT
CACCCCCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5819 Amino Acid (SEQ ID NO: 772)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5820 Nucleotide (SEQ ID NO: 773)

ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAGCAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCCAAAGGGTGGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA

GGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTGTAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGAT
CACCCCCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5820 Amino Acid (SEQ ID NO: 774)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRVVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5821 Nucleotide (SEQ ID NO: 775)

ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAGCAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCCAAAGGTTTGTCCGGAGCGGTGAAAATGCCCTGAA
GATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGGCC
CAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAAGG
TGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCTGA
ACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCA
CTGTAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGATCA
CCCCCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5821 Amino Acid (SEQ ID NO: 776)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRFVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

ATG-5822 Nucleotide (SEQ ID NO: 777)

ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGAAGCAGACAGCCGCCTAC
AACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCG
CCGTGTCCGTAACTCCGATCCAAAGGCTGGTCCGGAGCGGTGAAAATGCCCTGA
AGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGCCGACCAAATGG
CCCAGATCGAAGAGGTGTTTAAGGTGGTGTACCCTGTGGATGATCATCACTTTAA
GGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGCT
GAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGAT
CACTGTAACAGGGACCCTGTGGAACGGCGCCAAAATTATCGACGAGCGCCTGAT
CACCCCCGACGGCTCCATGTCCTTCCAGGTAACCATCAACAGC

ATG-5822 Amino Acid (SEQ ID NO: 778)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRLVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

Monomeric LgBiT sequences:

ATG-5872 Nucleotide (SEQ ID NO: 779)

Atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
aagctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5872 Amino Acid (SEQ ID NO: 780)

MKHHHHHHHAIAMVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNKLNYFGHPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5873 Nucleotide (SEQ ID NO: 781)

atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga
gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
tgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaaca
agctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggca
acaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgtt
caagaagatcagc

ATG-5873 Amino Acid (SEQ ID NO: 782)

MKHHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNKLNYFGRPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5874 Nucleotide (SEQ ID NO: 783)

atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga

gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
tgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaaca
agctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggca
acaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgtt
caagaagatcagc

ATG-5874 Amino Acid (SEQ ID NO: 784)

MKHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNKLNYFGHPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5875 Nucleotide (SEQ ID NO: 785)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
atgctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5875 Amino Acid (SEQ ID NO: 786)

MKHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGHPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5876 Nucleotide (SEQ ID NO: 787)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgaagatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
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aagctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5876 Amino Acid (SEQ ID NO: 802)

MKHHHHHHAIAMDFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG

VTPNKLNYFGHPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5877 Nucleotide (SEQ ID NO: 803)

Atgaaacatcaccatcaccatcatgcgatcgccatggatttcacactcgacgatttcgttggggactgggaacagacagccgcctaca
acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgg
agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagag
gtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaac
aagctgaactatttcggacacccgtatgaaggcatcgccgtgttcgacggcgagaagatcactgtaacagggaccctgtggaacggc
aacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgt
tcaagaagatcagc

ATG-5877 Amino Acid (SEQ ID NO: 804)

MKHHHHHHAIAMDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNKLNYFGHPYEGIAVFDGEKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5878 Nucleotide (SEQ ID NO: 805)

Atgaaacatcatcaccatcaccacgcagaaatcggtactggctttccattcgacccccattatgtggaagtcctgggcgagcgcatgc
actacgtcgatgttggtccgcgcgatggcacccctgtgctgttcctgcacggtaacccgacctcctcctacgtgtggcgcaacatcatc
ccgcatgttgcaccgacccatcgctgcattgctccagacctgatcggtatgggcaaatccgacaaaccagacctgggttatttcttcgac
gaccacgtccgcttcatggatgccttcatcgaagccctgggtctggaagaggtcgtcctggtcattcacgactggggctccgctctggg
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cagaatttgcccgcgagaccttccaggccttccgcaccaccgacgtcggccgcaagctgatcatcgatcagaacgtttttatcgagggt
acgctgccgatgggtgtcgtccgcccgctgactgaagtcgagatggaccattaccgcgagccgttcctgaatcctgttgaccgcgagc
cactgtggcgcttcccaaacgagctgccaatcgccggtgagccagcgaacatcgtcgcgctggtcgaagaatacatggactggctgc
accagtcccctgtcccgaagctgctgttctggggcaccccaggcgttctgatcccaccggccgaagccgctcgcctggccaaaagcc
tgcctaactgcaaggctgtggacatcggcccgggtctgaatctgctgcaagaagacaacccggacctgatcggcagcgagatcgcg
cgctggctgtcgacgctcgagatttccggcgagccaaccactagcgataacggctccatgctgttccgagtaaccatcaacagcgtta
gcggctggcgcctgttcaagaagatcagc

ATG-5878 Amino Acid (SEQ ID NO: 806)

MKHHHHHHAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVW
RNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHD
WGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLII
DQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVAL
VEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQED
NPDLIGSEIARWLSTLEISGEPTTSDNGSMLFRVTINSVSGWRLFKKIS

ATG-5879 Nucleotide (SEQ ID NO: 807)

atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga
gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
tgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacat
gctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaa
caaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgttc
aagaagatcagc

ATG-5879 Amino Acid (SEQ ID NO: 808)

MKHHHHHHHAIAMVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5880 Nucleotide (SEQ ID NO: 809)

atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactggcgccagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga
gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
tgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacat
gctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaa
caaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgttc
aagaagatcagc

ATG-5880 Amino Acid (SEQ ID NO: 813)

MKHHHHHHHAIAMVFTLEDFVGDWRQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5881 Nucleotide (SEQ ID NO: 814)

atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga
gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
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caaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgttc
aagaagatcagc

ATG-5881 Amino Acid (SEQ ID NO: 815)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPFEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5882 Nucleotide (SEQ ID NO: 828)

atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaa
cctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccgga
gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagagg
tgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacat
gctgaactatttcggacggccgtgggaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggca
acaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacggcgttagcggctggcgcctgtt
caagaagatcagc

ATG-5882 Amino Acid (SEQ ID NO: 829)

MKHHHHHHAIAMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQ
RIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDG
VTPNMLNYFGRPWEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTINGVS
GWRLFKKIS

ATG-5546 IL6-3xFlag-optRBD-15GS-SmTrip9 (840) Nucleotide (SEQ ID NO: 971)

atgaactccttctccacaagcgccttcggtccagttgccttctccctgggcctgctcctggtgttgcctgctgccttccctgccccagacta
caaagaccatgacggtgattataaagatcatgacatcgattacaaggatgacgatgacaagagagtccaacctactgaatctattgttag
atttcctaatattactaatctttgtccttttggcgaggtgttcaatgccaccagattcgcctctgtgtacgcctggaaccggaagcggatca
gcaattgcgtggccgactactccgtgctgtacaactccgccagcttcagcaccttcaagtgctacggcgtgtcccctaccaagctgaac
gacctgtgcttcacaaacgtgtacgccgacagcttcgtgatccggggagatgaagtgcggcagattgcccctggacagacaggcaa
gatcgccgactacaactacaagctgcccgacgacttcaccggctgtgtgattgcctggaacagcaacaacctggactccaaagtcgg
cggcaactacaattacctgtaccggctgttccggaagtccaatctgaagcccttcgagcgggacatctccaccgagatctatcaggcc
ggcagcacccctgtaacggcgtggaaggcttcaactgctacttcccactgcagtcctacggctttcagcccacaaatggcgtgggct
atcagccctacagagtggtggtgctgagcttcgaactgctgcatgcccctgccacagtgtgcggccctaagaaaagcaccaatctcgt
gaagaacaaatgcgtgaacttcgggagctccggtggtggcgggagcggaggtggaggctcgagcggtggcaagctcctgttcacg
gtaaccatcgagaagtataag

ATG-5546 Amino Acid (SEQ ID NO: 972)

MNSFSTSAFGPVAFSLGLLLVLPAAFPAPDYKDHDGDYKDHDIDYKDDDDKRVQPT
ESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYG
VSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNS
NNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSY
GFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFGSSGGGGSGG
GGSSGGKLLFTVTIEKYK

ATG-5541 nucleotide IL6-VSHiBiT-optRBD-3xFlag (SEQ ID NO: 973)

atgaactccttctccacaagcgccttcggtccagttgccttctccctgggcctgctcctggtgttgcctgctgccttccctgccccagtctc
cgtgagcggctggcggctgttcaagaagattagcgggagctccggtggtggcgggagcggaggtggaggctcgagcggtagagt
ccaacctactgaatctattgttagatttcctaatattactaatctttgtccttttggcgaggtgttcaatgccaccagattcgcctctgtgtacg
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cgtgtcccctaccaagctgaacgacctgtgcttcacaaacgtgtacgccgacagcttcgtgatccggggagatgaagtgcggcagatt
gcccctggacagacaggcaagatcgccgactacaactacaagctgcccgacgacttcaccggctgtgtgattgcctggaacagcaa
caacctggactccaaagtcggcggcaactacaattacctgtaccggctgttccggaagtccaatctgaagcccttcgagcgggacatc
tccaccgagatctatcaggccggcagcacccttgtaacggcgtggaaggcttcaactgctacttcccactgcagtcctacggctttca
gcccacaaatggcgtgggctatcagccctacagagtggtggtgctgagcttcgaactgctgcatgcccctgccacagtgtgcggccct
aagaaaagcaccaatctcgtgaagaacaaatgcgtgaacttcgactacaaagaccatgacggtgattataaagatcatgacatcgatta
caaggatgacgatgacaag

ATG-5541 Amino Acid (SEQ ID NO: 974)

MNSFSTSAFGPVAFSLGLLLVLPAAFPAPVSVSGWRLFKKISGSSGGGGSGGGGSSGR
VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTF
KCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVI
AWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFP
LQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFDYKDH
DGDYKDHDIDYKDDDDK

ATG-5547 Nucleotide IL6-SmTrip9 (840)-optRBD-3xFlag (SEQ ID NO: 975)

atgaactccttctccacaagcgccttcggtccagttgccttctccctgggcctgctcctggtgttgcctgctgccttccctgccccaggca
agctcctgttcacggtaaccatcgagaagtataaggggagctccggtggtggcgggagcggaggtggaggctcgagcggtagagt
ccaacctactgaatctattgttagatttcctaatattactaatctttgtccttttggcgaggtgttcaatgccaccagattcgcctctgtgtacg
cctggaaccggaagcggatcagcaattgcgtggccgactactccgtgctgtacaactccgccagcttcagcaccttcaagtgctacgg
cgtgtcccctaccaagctgaacgacctgtgcttcacaaacgtgtacgccgacagcttcgtgatccggggagatgaagtgcggcagatt
gcccctggacagacaggcaagatcgccgactacaactacaagctgcccgacgacttcaccggctgtgtgattgcctggaacagcaa
caacctggactccaaagtcggcggcaactacaattacctgtaccggctgttccggaagtccaatctgaagcccttcgagcgggacatc
tccaccgagatctatcaggccggcagcacccttgtaacggcgtggaaggcttcaactgctacttcccactgcagtcctacggctttca
gcccacaaatggcgtgggctatcagccctacagagtggtggtgctgagcttcgaactgctgcatgcccctgccacagtgtgcggccct
aagaaaagcaccaatctcgtgaagaacaaatgcgtgaacttcgactacaaagaccatgacggtgattataaagatcatgacatcgatta
caaggatgacgatgacaag

ATG-5547 Amino Acid (SEQ ID NO: 976)

MNSFSTSAFGPVAFSLGLLLVLPAAFPAPGKLLFTVTIEKYKGSSGGGGSGGGGSSGR
VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTF
KCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVI
AWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFP
LQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFDYKDH
DGDYKDHDIDYKDDD

ATG-5534 Nucleotide (SEQ ID NO: 977)

atggactcgggccgggacttcctgaccctgcacggcctacaggatgatgaggatctacaggcgctgctgaagggcagccagctcct
gaaggtgaagtccagctcatggaggagagagcggttctacaagttgcaggaggactgcaagaccatctggcaggagtcccgcaag
gtcatgcggaccccggagtcccagctgttctccatcgaggacattcaggaggtgcgaatggggcaccgcacggagggtctggagaa
gttcgcccgtgatgtgcccgaggaccgctgcttctccattgtcttcaaggaccagcgcaatacactagacctcatcgccccatcgccag
ctgatgcccagcactgggtgctggggctgcacaagatcatccaccactcaggctccatggaccagcgtcagaagctacagcactgg
attcactcctgcttgcgaaaagctgacaaaaacaaggacaacaagatgagcttcaaggagctgcagaacttcctgaaggagctcaac
atccagctcggctcttcaggcgcagaaatcggtactggctttccattcgacccccattatgtggaagtcctgggcgagcgcatgcacta
cgtcgatgttggtccgcgcgatggcacccctgtgctgttcctgcacggtaacccgacctcctcctacgtgtggcgcaacatcatcccgc
atgttgcaccgacccatcgctgcattgctccagacctgatcggtatgggcaaatccgacaaaccagacctgggttatttcttcgacgacc
acgtccgcttcatggatgccttcatcgaagccctgggtctggaagaggtcgtcctggtcattcacgactggggctccgctctgggtttcc
actgggccaagcgcaatccagagcgcgtcaaaggtattgcatttatggagttcatccgccctatcccgacctgggacgaatggccaga
atttgcccgcgagaccttccaggccttccgcaccaccgacgtcggccgcaagctgatcatcgatcagaacgtttttatcgagggtacgc
tgccgatgggtgtcgtccgcccgctgactgaagtcgagatggaccattaccgcgagccgttcctgaatcctgttgaccgcgagccact
gtggcgcttcccaaacgagctgccaatcgccggtgagccagcgaacatcgtcgcgctggtcgaagaatacatggactggctgcacc
agtcccctgtcccgaagctgctgttctggggcaccccaggcgttctgatcccaccggccgaagccgctcgcctggccaaaagcctgc
ctaactgcaaggctgtggacatcggcccgggtctgaatctgctgcaagaagacaacccggacctgatcggcagcgagatcgcgcgc
tggctgtcgacgctggagatttccggcggctcgagcggtgtcTTCACTTTGGAAGATTTCGTGGGTGACTG
GAAACAGACTGCAGCATACAACTTAGACCAAGTGTTGGAACAGGGTGGAGTTAG
TTCGCTTTTACAGAATTTGGCAGTTAGTGTTACGCCTATACAACGTATGGTGAGA
TCGGGAGAAAATGCATTAAAAATAGACATACATGTGATAATACCTTATGAAGGA
TTATCGGCAGACCAAATGGCACAGATAGAAGAGGTTTTTAAAGTTGTTTACCCAG
TTGATGATCATCACTTTAAAGTTATATTACACTATGGAACTTTAGTTATAGACGGT
GTGACTCCTAACATGTTAAACTATTTCGGTAGACCTTATGAAGGAATAGCAGTTT
TCGACGGAAAGAAAATAACGGTTACTGGTACGTTATGGAACGGAGCCAAGATAA
TAGACGAGAGATTAATAACGCCAGACGGAAGTATGAGTTTCCAGGTTACGAtaaact
cg

ATG-5534 Amino Acid (SEQ ID NO: 978)

MDSGRDFLTLHGLQDDEDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESR
KVMRTPESQLFSIEDIQEVRMGHRTEGLEKFARDVPEDRCFSIVFKDQRNTLDLIAPSP
ADAQHWVLGLHKIIHHSGSMDQRQKLQHWIHSCLRKADKNKDNKMSFKELQNFLK
ELNIQLGSSGAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVW
RNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHD
WGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLII
DQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVAL
VEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQED
NPDLIGSEIARWLSTLEISGGSSGVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQ
NLAVSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV
ILHYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGS
MSFQVTINS

LgBiT* Amino Acid (SEQ ID NO: 979)

MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKI
DIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFG
RPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

HaloTag-LgBiT* Amino Acid (SEQ ID NO: 980)

MAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAP
THRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFH
WAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGT
LPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDW
LHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEI
ARWLSTLEISGGSSGVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPI
QRMVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVI
DGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS

LgBiT*-Membrane Sensor Amino Acid (SEQ ID NO: 981)

MDSGRDFLTLHGLQDDEDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIWQESR
KVMRTPESQLFSIEDIQEVRMGHRTEGLEKFARDVPEDRCFSIVFKDQRNTLDLIAPSP
ADAQHWVLGLHKIIHHSGSMDQRQKLQHWIHSCLRKADKNKDNKMSFKELQNFLK
ELNIQLGSSGAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVW
RNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHD
WGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLII
DQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVAL
VEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQED
NPDLIGSEIARWLSTLEISGGSSGVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQ
NLAVSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV
ILHYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGS
MSFQVTINS

LgBiT*-Nuclear Sensor Amino Acid (SEQ ID NO: 982)

MPKKKRKVAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWR
NIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDW
GSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQ
NVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVE
EYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNP
DLIGSEIARWLSTLEISGGSSGVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNL
AVSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL
HYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMS
FQVTINSPKKKRKVPKKKRKVPPPVSKRESKSRSRSKSPPKSPEEEGAVSS

LgBiT*-Mitochondria Sensor Amino Acid (SEQ ID NO: 983)

MSVLTPLLLRGLTGSARRLPVPRAKIHSLGDPMSVLTPLLLRGLTGSARRLPVPRAKI
HSLGKLMSVLTPLLLRGLTGSARRLPVPRAKIHSLGDPMSVLTPLLLRGLTGSARRLP
VPRAKIHSLGKLRILQSTVPRARDPPVATMAEIGTGFPFDPHYVEVLGERMHYVDVG
PRDGTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVR
FMDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPE
FARETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREP
LWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKS
LPNCKAVDIGPGLNLLQEDNPDLIGSEIARWLSTLEISGGSSGVFTLEDFVGDWKQTA

AYNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMA
QIEEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVT
GTLWNGAKIIDERLITPDGSMSFQVTINS

LgBiT*-Endoplasmic Reticulum Sensor Amino Acid (SEQ ID NO: 984)

MLLSVPLLLGLLGLAVAVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSV
TPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTL
VIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSMLFRVTI
NSGSSGMAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRN
IIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWG
SALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQN
VFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEE
YMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPD
LIGSEIARWLSTLEISGKDEL

LgBiT*-Golgi Sensor Amino Acid (SEQ ID NO: 985)

MAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAP
THRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFH
WAKRNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGT
LPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDW
LHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEI
ARWLSTLEISGGSSGVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPI
QRMVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILHYGTLVI
DGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGAKIIDERLITPDGSMSFQVTINS
SGLRSRGEPQQSFSEAQQQLCNTRQEVNELRKLLEEERDQRVAAENALSVAEEQIRR
LEHSEWDSSRTPIIGSCGTQEQALLIDLTSNSCRRTRSGVGWKRVLRSLCHSRTRVPL
LAAIYFLMIHVLLILCFTGHL

LgBiT*-Lysosome Sensor Amino Acid (SEQ ID NO: 986)

MAAPGSARRPLLLLLLLLLLGLMHCASAAMFMVKNGNGTACIMANFSAAFSVNYD
TKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTLNFTRNATRYS
VQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQVHMNNVTVT
LHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPSVDKYNVSGTN
GTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTLELHSEGTTV
LLFQFGMNASSSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGNSYKCNAEE
HVRVTKAFSVNIFKVWVQAFKVEGGQFGSVEECLLDENSMLIPIAVGGALAGLVLIV
LIAYLVGRKRSHAGYQTIKDPPVATMAEIGTGFPFDPHYVEVLGERMHYVDVGPRD
GTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFM
DAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDEWPEFA
RETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPL
WRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSL
PNCKAVDIGPGLNLLQEDNPDLIGSEIARWLSTLEISGGSSGVFTLEDFVGDWKQTAA
YNLDQVLEQGGVSSLLQNLAVSVTPIQRMVRSGENALKIDIHVIIPYEGLSADQMAQI
EEVFKVVYPVDDHHFKVILHYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGT
LWNGAKIIDERLITPDGSMSFQVTINS

CLAIMS

1. A system or kit comprising two or more peptide and/or polypeptide components collectively comprising 70% or greater sequence identity to SEQ ID NO: 788 or SEQ ID NO: 789; wherein the two or more peptide and/or polypeptide componets are capable of forming a bioluminescent complex upon interacting with each other; wherein a bioluminescent signal produced by the bioluminescent complex in the presence of a coelenterazine or a coelenterazine analog substrate is substantially increased when compared to a bioluminescent signal produced by the polypeptide or one or more complementary peptides and the coelenterazine substrate alone; wherein the system or kit comprises a polypeptide that corresponds structurally to 8 or fewer β strands of a *Oplophorus gracilirostris* luciferase.

2. The system or kit of claim 1, comprising a polypeptide component having 70% or greater sequence identity to SEQ ID NO: 790 and one or more complementary peptides, dipeptides, and or tripeptides collectively having 70% or greater sequence identity to SEQ ID NO: 794.

3. The system or kit of claim 1, wherein the polypeptide comprises 70% or greater sequence identity to SEQ ID NO: 791 and the one or more complementary peptides collectively comprise 70% or greater sequence identity to SEQ ID NO: 795.

4. The system or kit of claim 1, wherein the polypeptide comprises 70% or greater sequence identity to SEQ ID NO: 792 and the one or more complementary peptides collectively comprise 70% or greater sequence identity to SEQ ID NO: 796.

5. The system or kit of claim 1, wherein the polypeptide comprises 40% or greater sequence identity to SEQ ID NO: 793 and the one or more complementary peptides collectively comprise 40% or greater sequence identity to SEQ ID NO: 797.

6. The system or kit of claim 1, wherein the polypeptide comprises 70% or greater sequence identity to SEQ ID NO: 790 and the one or more complementary peptides collectively comprise 70% or greater sequence identity to SEQ ID NO: 798.

7. The system or kit of claim 1, wherein the polypeptide comprises 70% or greater sequence identity to SEQ ID NO: 791 and the one or more complementary peptides collectively comprise 70% or greater sequence identity to SEQ ID NO: 799.

8. The system or kit of claim 1, wherein the polypeptide comprises 70% or greater sequence identity to SEQ ID NO: 792 and the one or more complementary peptides collectively comprise 70% or greater sequence identity to SEQ ID NO: 800.

9. The system or kit of claim 1, wherein the polypeptide comprises 40% or greater sequence identity to SEQ ID NO: 793 and the one or more complementary peptides collectively comprise 40% or greater sequence identity to SEQ ID NO: 801.

10. The system or kit of claim 1, wherein one or more of the polypeptide and/or peptide components comprise a non-natural amino acid, an amino acid analog, and/or peptoid amino acids.

11. The system or kit of claim 1, wherein one or more of the polypeptide and/or peptide components are present as fusions with one or more additional amino acid sequences.

12. The system or kit of claim 11, wherein the additional amino acid sequence is selected from the group consisting of a protein of interest, an interaction element, a co-localization element, and a binding moiety.

13. The system or kit of claim 11, wherein the additional amino acid sequence is a binding moiety selected from the group consisting of antibody (polyclonal, monoclonal, and/or recombinant), antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, an Ig binding domain of protein L, protein M, an Ig binding domain of protein M, oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins.

14. The system or kit of claim 11, wherein the additional amino acid sequence is a first interaction polypeptide that is configured to form a complex with a second interaction polypeptide upon contact of the first interaction polypeptide and the second interaction polypeptide.

15. The system or kit of claim 11, wherein the additional amino acid sequence is a first co-localization polypeptide that is configured to co-localize within a cellular compartment, a cell, a tissue, or an organism within a with a second co-localization polypeptide.

16. The system or kit of claim 11, wherein the additional amino acid sequence is a protein of interest and is a candidate drug target.

17. A bioluminescent complex comprising the two or more peptide and/or polypeptide components of the system or kit of claim 1.

18. A method comprising contacting the bioluminescent complex of claim 17 with a substrate for the bioluminescent complex.

19. The method of claim 18 wherein the substrate is coelenterazine or furimazine.