
Supplementary information

De novo design of modular and tunable protein biosensors

In the format provided by the
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Supplementary Information

Supplementary discussion:

Sensor proteins have emerged as an active area of research. Traditional ELISA methods require multiple liquid-handling steps, preventing its use at the bedside. Lateral flow immunochromatographic assays are fast and cheap, but they have limited sensitivity, reproducibility, and poor quantitative performance ¹. ELISA and lateral flow also require two binding modules for the target being sensed, one for capture and the other for readout. Sensor proteins capture analytes with excellent molecular precision and generate signals in a homogeneous way that do not require time-consuming incubation and washing steps of traditional immunoassays. A typical sensor protein is composed of two components - an analyte-binding domain for target capturing, and a reporter component to convert and amplify the actuation into a measurable signal. The main hurdle of the protein sensor construction is finding analyte binding domains that undergo sufficient conformational changes. The most commonly used binding domains (e.g., antibodies) undergo only minor structural changes of the loops upon ligand binding. Coupling an appropriate reporter with optimal geometry to amplify the conformational change is also key to a successful biosensor. Protein engineers have devoted considerable work to build biosensors for a specific application, constructing tailored protein sensors remains a major challenge ^{2,3}. Computational ⁴, synthetic ⁵, and semisynthetic ⁶ strategies have also aimed to develop generalizable platforms for arbitrary biosensors. However, computationally designing small molecule binding sites into protein interfaces and generating semisynthetic protein sensors are both quite challenging problems currently. Therefore, generalized approaches for designing biosensors with a simple and robust computational protocol that requires little empirical optimization are needed.

Herein, we select and compare several state-of-the-art sensor configurations and design strategies that seek to detect protein targets (Supplementary Fig. 1 and Table S5). These strategies generally include a protein switch at an initial “closed” state by an intramolecular β -lactamase-to-inhibitor (Supplementary Fig. 1a), Src domain-to-proline rich peptide (Supplementary Fig. 1b), or benzenesulfonamide-to-carbonic anhydrase (Supplementary Fig. 1c) interaction. Upon binding to the target, the conformational change triggers the dissociation of these interactions eventually to an “open” state, resulting in the active β -lactamase for colorimetric assay (Supplementary Fig. 1a) or a decreased BRET efficiency (Supplementary Fig. 1b,c). Both assays exhibit ideal features for POC diagnostics such as wash-free, sensitive (pM-nM), fast (min), and simple readout (camera) to detect therapeutic antibodies, protein biomarkers, and virus particles in patients’ serum or plasma ⁷⁻¹⁰. In terms of design, their target specificity can be easily switched by swapping known binding domains (epitope, affimer, or inhibitor). The length and type of semiflexible linkers play the most critical role in the success rate, which has to be re-designed or re-optimized for each binding domain swap. In some cases, the interaction partners to hold the switch at a “close” state also need to be re-evaluated ¹¹. The

abovementioned engineering work is unavoidable primarily due to the limited understanding of the dynamic nature of protein interaction, folding, and flexibility. Our generalized protein sensory system based on a *de novo* switch (Supplementary Fig. 1d) relies on the thermodynamic coupling (see Fig. 1) between a defined close state (K_{open}) and an open state (K_{LT} and K_{CK}). With our system (Table S5), the target specificity to arbitrary targets can be achieved not only by incorporating known binding domains but also *de novo* binders where we have full control over protein fold and geometry. Because there is no flexible or semi-flexible linker in our system and we are capable of designing different types of interaction to cage binding domains, the conformational change is thus decoupled from the binder-target interaction, which makes this system more structurally predictable at open state. A newly developed GraftSwitchMover in Rosetta allows sensor design in one step, bypassing the need with the other formats to empirically re-engineer sensor configuration. The intermolecular association of the LucKey with the open form of the sensor generates the luminescent signal, providing an additional tunable parameter K_{CK} that can be optimized along with K_{open} to maximize sensor dynamic range, analytical range, and sensitivity.

It is important to note that the sensors we describe here have not been extensively optimized for POC settings. Fluorescence- or colorimetric-based assays suffer from autofluorescence, light scattering, and interference in whole-blood samples, limiting these assays for immediately quantifiable POC applications¹². Background-free bioluminescence readout is ideal and ~100-fold more sensitive than fluorescence^{13,14}, but the absolute bioluminescence intensity is known to be influenced by factors in serum or whole blood not related to analyte^{15,16}. Incorporating an internal reference as we showed here or transition to a ratiometric BRET response would facilitate the adaptation of the sensors for optimal POC settings. Furthermore, the sensitivity of protein sensors described here has not been fully tuned to cover the complete linearity of the clinical diagnostic range. In addition, there are pros and cons for two-component sensors where the performance and sensitivity depend on sensor component concentrations¹⁷. This tunability can be advantageous at the developmental stage but could become problematic for accurate and reproducible quantification in clinical settings. To avoid such problems, the lucKey could be attached to lucCage through a flexible linker.

Supplementary methods

1. Thermodynamic model

The thermodynamic model shown in Fig. 1b was adapted and modified from the previously published *de novo* switch model¹⁸. The equilibrium constants were defined as K_{open} for latch opening (Equation 1), K_{CK} for the dissociation constant of the lucCage and lucKey (Equation 2 and 3), and K_{LT} for the dissociation constant of the latch and target (Equation 4 and 5). K_{R} describes the equilibrium of the reconstituted luciferase, which is determined by the reported dissociation constant of the NanoBit system (190 μM ¹⁹) and the effective local concentration (C_{eff}) of split counterparts (Equation 6 and 7). We set C_{eff} to 1 mM here as the literature suggested high micromolar to low millimolar range for intramolecular interaction partners²⁰, and our modular switch should span much shorter distance than flexible linkers. The total amount of each component is constant, so Equations 8, 9, and 10 were introduced. Given four equilibrium constants (K_{open} , K_{CK} , K_{LT} , and K_{R}) and three total concentrations ($[\text{lucCage}]_{\text{total}}$, $[\text{lucKey}]_{\text{total}}$, and $[\text{target}]_{\text{total}}$), python module sympy.solve was used to solve these ten equations numerically and find the concentration of each species at equilibrium. The total concentration of luminescent species **6** and **7** was extracted from the solution, divided by $[\text{lucCage}]_{\text{total}}$, and plotted for corresponding figures with various K_{open} for Extended Data Fig. 1a, K_{LT} for Extended Data Fig. 1b, $[\text{lucCage}]_{\text{total}}$, $[\text{lucKey}]_{\text{total}}$ for Extended Data Fig. 1c,d and K_{CK} for Extended Data Fig. 1e. The fraction of reconstituted luciferase is normalized between 0-1. R_{max} and R_{min} are the fraction of reconstituted luciferase at saturating and at low target concentration respectively. The sensor dynamic range is derived from the equation $(R_{\text{max}} - R_{\text{min}}) / R_{\text{min}} \times 100\%$. The heatmaps in Extended Data Fig. 1f and 1g were generated with a 2D array of indicated K_{open} and K_{CK} values, to plot the dynamic range and the fraction of reconstituted luciferase at saturated concentration, respectively. The code for the numerical simulations shown in this work are available at http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/model_simulation.py

Equation 1:

$$K_{\text{open}} = \frac{[2]}{[1]}$$

Equation 2:

$$K_{\text{CK}} = \frac{[2] \times [\text{lucKey}]_{\text{free}}}{[5]}$$

Equation 3:

$$K_{\text{CK}} = \frac{[3] \times [\text{lucKey}]_{\text{free}}}{[4]}$$

Equation 4:

$$K_{\text{LT}} = \frac{[6] \times [\text{target}]_{\text{free}}}{[7]}$$

Equation 5:

$$K_{\text{LT}} = \frac{[2] \times [\text{target}]_{\text{free}}}{[3]}$$

Equation 6:

$$K_{\text{R}} = \frac{190 \mu\text{M}}{C_{\text{eff}}} = \frac{[5]}{[6]}$$

Equation 7:

$$K_{\text{R}} = \frac{[4]}{[7]}$$

Equation 8:

$$[\text{lucCage}]_{\text{total}} = [1] + [2] + [3] + [4] + [5] + [6] + [7]$$

Equation 9:

$$[\text{lucKey}]_{\text{total}} = [\text{lucKey}]_{\text{free}} + [4] + [5] + [6] + [7]$$

Equation 10:

$$[\text{target}]_{\text{total}} = [\text{target}]_{\text{free}} + [3] + [4] + [7]$$

2. Computational grafting of sensing domains into lucCage

The main feature sought for when incorporating new sensing domains into lucCage is whether a target-binding interface can be blocked by the cage domain. Finding the right orientation is usually simple when grafting small peptides. In the case of globular proteins being used as sensing domains, finding the right orientation is often facilitated by finding target-binding interfaces neighboring the N-terminus of the sensing domain. In our experience, sensing domains with an α -helical segment at the N-terminus are preferable to ensure a more predictable design process, though this is not a requirement. Binding domains with other secondary structure elements have been effectively incorporated into lucCage, as shown here with the SmBiT peptide (β -strand), and the PreS1 peptide and SARS-CoV-2 epitopes (loops). These features of the lucCage platform make it compatible with diverse sensing domains, however, it can be challenging to use analyte binding domains with complex and/or distant target-binding interfaces or that are bigger in size than the lucCage itself.

The structural models of the lucCage sensors were created by grafting each sensing domain onto the latch of the lucCage scaffold (See Table S3). The design was performed using a RosettaScripts²¹ protocol, (*{name}_GraftSwitchMover_relax.xml*, See Code availability) to thread each sensing domain sequence into the model of lucCage (*lucCage.pdb*, See Code Availability). A bash script (*run_rosettascripts.sh*) was used to call RosettaScripts. This protocol uses two successive Rosetta movers: (i) GraftSwitchMover to thread the desired sensing domain sequence into a defined region of the lucCage latch (amino acids 325-359) and to select designs with the defined “important residues” that are buried in the cage/latch interface; (ii) and MultiplePoseMover to capture all models generated by GraftSwitchMover, minimize them (FastRelax²² to find the lowest energy structure given the mutations from the previous mover.) and score each output model. The resulting designs were further evaluated by eye in PyMol 2.0. This was done by selecting designs showing favorable hydrophobic packing interactions between the newly threaded sequence and the cage and discarding designs with unfavorable buried hydrophilic residues that could destabilize the closed state of the sensor (unless these residues were identified as “important residues”). At the time of publication, GraftSwitchMover is limited to inserting a sequence within the range of the input structure (i.e., the inserted sequence cannot exceed the full length of the initial lucCage model being used). In order to design sensors that would extend the latch of lucCage (e.g., insertion of cTnT), we used Rosetta Remodel²³ to model the extended C-terminus of lucCage (*{name}_Remodel_relax.xml*), which uses a blueprint file (*{name}.bp*) called by a bash script containing all necessary flags (*run_rosetta_scripts_REMODEL.sh*).

For grafting mini-protein binders with a pre-defined tertiary structure (i.e., Bot.671.2, SpaC, the Her2 affibody, and LCB1) we first identified the primary interaction surface of the binding protein to its target and identified the main secondary structure elements involved in it. We identified the main amino acid sequence of the N-terminal secondary structure element and

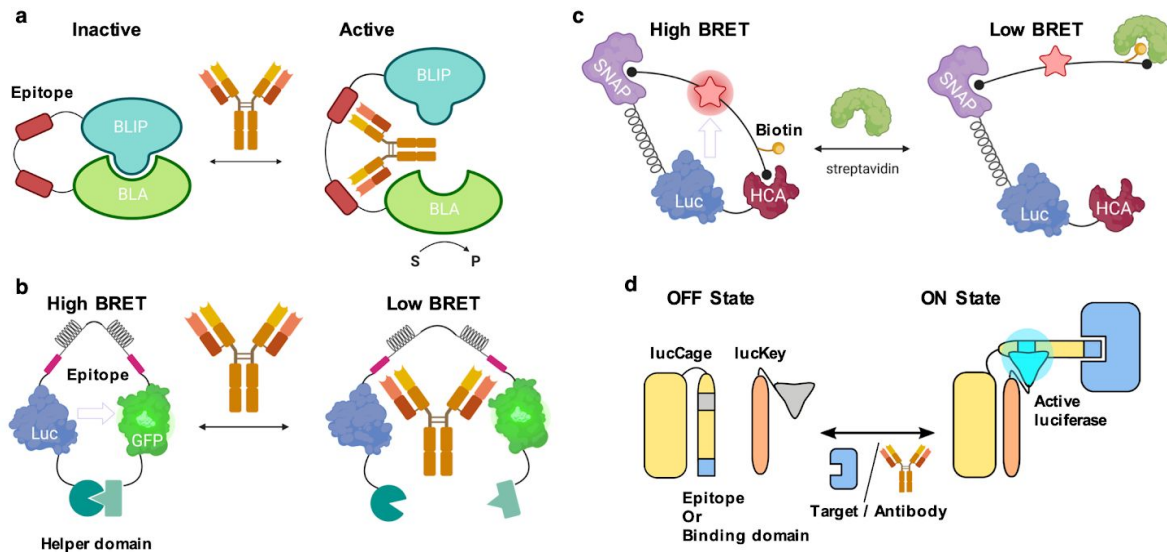
threaded it into lucCage ({name}_GraftSwitchMover_relax.xml), to obtain output protein models including the partial sequence of a sensing domain onto the latch. Then, we used an additional script ({name}_MergePDB_relax.xml) to align the resulting lucCage designs with the model (or crystal structure) of the full-length sensing domain, create a complete model including the full sensing domain in the context of the switch, and energy-minimize the designs. The input sensing domains used are named as: Her2_3mzw.pdb, Bot_5vid.pdb, ProA_4WWI.pdb, LCB1_7jzu.pdb, and delta4LCB1_7jzu.pdb. Finally, the best designs were selected by eye using PyMol 2.0. The design models and RosettaScripts code used in this work have been deposited to http://files.ipd.uw.edu/pub/de_novo_design_of_tunable_biosensors_2021/designcode_and_models.zip.

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Supplementary Fig. 1. Working mechanisms of recent biosensor platforms to detect protein targets. **a**, An antibody-sensing enzyme-inhibitor switch. The initially inactive TEM1- β -lactamase (BLA) is tethered to its inhibitor protein (BLIP) via a semi-flexible linker. Antibody binding to two epitopes in the semi-flexible linker triggers the dissociation of BLA-BLIP, leading to active BLA as the reporter²⁴. **b**, LUMinescent AntiBody Sensor platform, LUMABS. High BRET efficiency is achieved when the luciferase (Luc) donor and the GFP acceptor are in close proximity mediated by an interaction between a Src Homology 3 domain and a proline-rich peptide (helper domain). Binding of an antibody to epitope sequences in the linker disrupts the interaction between these helper domains, resulting in loss of BRET efficiency⁷. **c**, Chemical ligand-associated steric hindrance platform, CLASH. The intramolecular tether contains one benzenesulfonamide ligand for human carbonic anhydrase (HCA) binding and one benzylguanine moiety for SNAP-tag. One additional synthetic biotin on the tether can bind to streptavidin as a specific effector protein, which increases the overall steric hindrance and prevents the interaction of benzenesulfonamide to the HCA domain, leading to a low BRET efficiency open state²⁵. **d**, Modular biosensor platform based on a *de novo* switch. A structurally preorganized lucCage containing a caged sensing domain (or epitope) stabilizes the complex in a closed state. The sensing domain binding to the target of interest (or the epitope binding to the antibody of interest) facilitates the association of lucCage and lucKey into an open state, which is reported by bioluminescence emission after split-luciferase reconstitution.

Table S1. X-ray data collection and structure refinement statistics.

Data Collection	SelMET-sCageHA_267-1S(E99Y/T144Y/I30M)
Space group	C2
Unit cell dimensions	
a, b, c (Å)	178.993 60.127 71.799
a, β , γ (°)	90, 112.463, 90
Wavelength (Å)	0.9794
Resolution (Å)	50-2.03 (2.03-2.00) ^a
R_{sym}	6.6 (16.5) ^a
$I/\sigma(I)$	24.0 (3.5) ^a
Completeness (%), $>1\sigma$	70.6 (33.8) ^a
Redundancy	2.5 (1.6) ^a
Refinement	
Resolution (Å)	46.09-1.99 (2.06-1.99) ^a
No. of reflections	37603
$R_{\text{work}} / R_{\text{free}}$	0.2078/0.2515
R.m.s deviations	
bond (Å) / angle (°)	0.007/0.910
Average B-values (Å ²)	38.19
Ramachandran plot (%)	
Favored / Additional allowed	97.7/2.3
Generously allowed	0.0

^aThe numbers in parentheses are the statistics from the highest resolution shell.

Table S2. Summary of biosensors in this work

Biosensor	Analytical target	Dynamic range^a	LOD (nM)	Detection range (nM)
lucCageBim	Bcl-2	360%	0.2	0.2-12.5
lucCageBoT	Botulinum neurotoxin B	130%	0.4	0.4-50
lucCageProA	Fc domain	350%	0.39	0.39-12.5
lucCagHer2	Her2 receptor	380%	0.23	0.23-25
lucCageTrop	Troponin I	250%	0.009	0.009-0.3
lucCageHBV	Anti-HBV antibody (HzKR127-3.2)	98%	2	2-100
lucCageHBV α	Anti-HBV antibody (HzKR127-3.2)	225%	0.26	0.26-100
lucCageHBV+ HzKR127-3.2	PreS1	80%	0.6	0.6-100
lucCageSARS2-M	anti-SARS-Cov-M	50%	2.9	2.9-250
lucCageSARS2-N	anti-SARS-Cov-NP	70%	3.0	3.0-100
lucCageRBD	SARS-CoV-2 RBD	1700%	0.015	0.015-6

^aDefined as intensimetric change ($\Delta E/E_{\min}$) of total bioluminescence intensity. $\Delta E = E_{\max} - E_{\min}$ where $E_{\max}$ is the maximal bioluminescence intensity at saturating target concentration and $E_{\min}$ is the emission in the absence of the analytical target.

Table S3. List of sensing domains used in this work

Biosensor	Sensing domain	Sensing domain sequence
lucCageBim	Bim	EIWIAQELRRIGDEFNAYYA
lucCageBoT	Bot.0671.2	MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAERI IRKYELE
lucCageProA	Protein A domain C (SpaC)	EQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKE LLAEAKKLNDQAQPK
lucCagHer2	Her2 affibody	EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSAN LLAEAKKLNDQAQPK
lucCageTrop	cTnI + cTnC	EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYEI NVLNRINDNQKVSCTKDDSKGKSEEELSDLFRMFD KNADGYIDLEELKIMLQATGETITEDDIEELMKDGD KNNDGRIDYDEFLEFMKGVE
lucCageHBV	preS1 (a.a. 35–46)	GANSNNPDWDFN
lucCageHBV α	preS1 (a.a. 35–46) 2x	GANSNNPDWDFNGSGGGSSGFGANSNNPDWDFNPN
lucCageSARS2-M	SARS-CoV-2 nucleocapsid protein (a.a. 369-382) 2x	MADSNGTITVEELKKLLEGGSGGMADSNGTITVEEL KKLLE
lucCageSARS2-N	SARS-CoV-2 membrane protein (a.a. 1-17) 2x	KKDKKKKADETQALGGSGGKKDKKKKADETQAL
lucCageRBD	LCB1	ILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGD ERLLEEAEERLLEEVER

Table S4. Comparison of background intensity and dynamic range between the lucCage sensors under the same experimental conditions

Biosensor	No target (% $\pm$ SD)	Saturating target (% $\pm$ SD)	Fold Activation
lucCageBim	6.685 $\pm$ 0.056	21.689 $\pm$ 0.223	3.24
lucCageBoT	1.261 $\pm$ 0.030	2.419 $\pm$ 0.022	1.92
lucCageProA	11.403 $\pm$ 0.030	42.503 $\pm$ 0.786	3.73
lucCagHer2	2.989 $\pm$ 0.010	9.562 $\pm$ 0.300	3.20
lucCageTrop	8.773 $\pm$ 0.057	30.717 $\pm$ 0.420	3.50
lucCageHBV α	62.945 $\pm$ 0.896	87.414 $\pm$ 0.385	1.39
lucCageSARS2-M	22.286 $\pm$ 0.432	28.357 $\pm$ 0.836	1.27
lucCageRBD	7.340 $\pm$ 0.126	100 $\pm$ 1.731	13.62

Percentages calculated assuming activation of lucCageRBD at saturating target concentration as 100% luciferase intensity.

Experimental conditions: (1nM lucCage + 1nM lucKey in 1:1 of buffer 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20 Nano-Glo assay buffer). These conditions may differ from optimal experimental conditions for some lucCage sensors, hence, the reduced dynamic range for some of them was observed.

Table S5. Summary of recent biosensor platforms to detect protein targets

Platform	Enzyme–inhibitor switch	LUMABS	CLASH	lucCage-lucKey (this work)
Switch module	BLA-BLIP	SH3-PRP	HCA-Nluc-p30-SNAP	Two components (lucCage and lucKey)
Switch tunability	Introducing point mutations	Replacing helper domain	Changing the affinity of tethered ligands	Computational interface design
Binder geometry	Small linear epitope or affimer	Small linear epitope or mimotope	Small molecule-target interaction	<i>De novo</i> binders, affibody, linear epitope, natural binders
No. of Binding sites required	2	2	1	1
Linker module	Semi-flexible or flexible linker	Semi-flexible linker	30-proline linker	No linker
Readout	Colorimetric by nitrocefin	BRET (450/517nm)	BRET (450/570nm)	Intensiometric in luminescence
Target tunability	Swapping binding motif	Swapping binding motif	Changing ligand	Swapping binding motif
Sensitivity	pM	pM	nM	pM
Sensitivity tunability	Monovalent affinity & avidity	Monovalent affinity & avidity	Mutations in the receptor protein	Monovalent affinity & avidity (K_{LT})
Dynamic range tunability	Linker optimization	Replacing helper domain	Geometry optimization	Cage-latch & Cage-key interface design (K_{open} & K_{CK}). Key length.
Number of sensors built	3: Herceptin, hCRP, plant virus	7: therapeutic antibodies	2: streptavidin, DHFR	10: Bcl-2, BoNT/B, Fc, Her2, cTnI, anti-HBV, HBV PreS1, SARS-CoV-2 RBD, anti-SARS-CoV-N, anti-SARS-CoV-M

Table S6. Amino acid sequences

(The sequences below contain a 6His-TEV tag for protein purification purposes (MGSHHHHHHGSGSENLYFQG)

(The Cage is in parenthesis) The region after the parenthesis constitutes the latch.

The SmBit sequence (VTGYRLFEEIL) is underlined.

The sensing domains are in **bold**

>lucCage: The SmBiT sequence (VTGYRLFEEIL) is underlined.

MGSHHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAAERLIREAAAASEKISRE

> lucKey: The LgBit sequence is underlined

MGSHHHHHHGSGSENLYFQSGMVF~~TLED~~FDVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKID
IHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTNMLNYFGRPYEGIAVFDGKKITVTGTLWNGN
KIIDERLITPDGSMFLFRVTINSGGSGGGGGSGGGSGGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAAERL
IREAAAASEKISRE

> truncated lucKey: MGS-(His)6-TEV site-linker-LgBit-linker-latch sequence

MGSHHHHHHGSGSENLYFQSGMVF~~TLED~~FDVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKID
IHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTNMLNYFGRPYEGIAVFDGKKITVTGTLWNGN
KIIDERLITPDGSMFLFRVTINSGGSGGGGGSGGGSGGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAAERL

lucCageBim variants (Bcl2 sensors)

- SmBit sequence: VTGYRLFEEIL
- BIM sequence: **EIWIAQELRRIGDEFNAYYA**

>nluc301_bim331

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRSKEIIDEAERAIRAAKRE
SERIIIEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLSRE**EIWIAQELRRIGDEFNAYYA**AASEKISRE

>nluc308_bim331

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRSKEIIDEAERAIRAAKRE
SERIIIEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARVTGYRLFEEILRIVEDAERLSRE**EIWIAQELRRIGDEFNAYYA**AASEKISRE

>nluc312_bim331

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRSKEIIDEAERAIRAAKRE
SERIIIEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSRE**EIWIAQELRRIGDEFNAYYA**AASEKISRE

>nluc315_bim331

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRSKEIIDEAERAIRAAKRE
SERIIIEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAIARVKVTGYRLFEEILRLSRE**EIWIAQELRRIGDEFNAYYA**AASEKISRE

>nLuc301_bim339

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLsREAAAASEKIE**IWIAQELRRIGDEFNAYYAE**

>nLuc308_bim339

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARVVTGYRLFEEILRIVEDAERLsREAAAASEKIE**IWIAQELRRIGDEFNAYYAE**

>nLuc312_bim339

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAASEKIE**IWIAQELRRIGDEFNAYYAE**

>nLuc315_bim339

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAIARVKVTGYRLFEEILRLsREAAAASEKIE**IWIAQELRRIGDEFNAYYAE**

>nLuc301_bim343

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLsREAAAASEKISREAE**IWIAQELRRIGDEFNAYYA**

>nLuc308_bim343

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARVVTGYRLFEEILRIVEDAERLsREAAAASEKISREAE**IWIAQELRRIGDEFNAYYA**

>nLuc312_bim343

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAASEKISREAE**IWIAQELRRIGDEFNAYYA**

>nLuc315_bim343

MGSHHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRE
SERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAIARVKVTGYRLFEEILRLsREAAAASEKISREAE**IWIAQELRRIGDEFNAYYA**

lucCageTrop variants (cardiac Troponin I sensors)

- SmBiT sequence: VTGYRLFEEIL
- Variants of cardiac troponin T (cTnT) used sequences:
 - cTnTf1:226-**EDQLREKAKELWQTI**-240
 - cTnTf2:226-**EDQLREKAKELWQTIYN**-242
 - cTnTf3:226-**EDQLREKAKELWQTIYNLEAE**-246
 - cTnTf4:226-**EDQLREKAKELWQTIYNLEAEKFD**-249
 - cTnTf5:226-**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYEINVLNRINDNQ**-252
 - cTnTf6:226-**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYEINVLNRINDNQKFKQQKYEINVLNRINDNQ**-272

>336-cTnTf4-K342A (jp625_1fix_nluc312_cTnT336_K342A_359end)
MGSHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAAS**EDQLREaAKELWQTIYNLEAEKFD**

>336-cTnTf6-K342A (jp626_1fix-nluc312_cTnT336_K342A_362end)
MGSHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAAS**EDQLREaAKELWQTIYNLEAEKFDLQE**

>336-cTnTf6-K342A (jp627_1fix-nluc312_cTnT336_K342A_0001_382end)
MGSHHHHHGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAAS**EDQLREaAKELWQTIYNLEAEKFDLQEKFKQQKYEINVLNRINDNQ**

>339-cTnTf3 (jp628_1fix-nluc312_cTnT339_359end)
MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAASEKI**EDQLREKAKELWQTIYNLEAE**

>339-cTnTf5 (jp629_1fix-nluc312_cTnT339_0001_365end)
MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAASEKI**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYEINVLNRINDNQ**

>339-cTnTf6 (jp630_1fix-nluc312_cTnT339_0001_385end)
MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
ELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAAKRE
SERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLD
LLRLASEL) TDPDEARKAI AVTGYRLFEEILDAERLsREAAAASEKI**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYEINVLNRINDNQKFKQQKYEINVLNRINDNQ**

>343-cTnTf2 (jp631_1fix-nluc312_cTnT343_359end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREA**EDQLREKAKELWQTIYN**

>343-cTnTf5 (jp632_1fix-nluc312_cTnT343_0001_369end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREA**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQK
YEINVLNRNRINDNQ**

>343-cTnTf6 (jp633_1fix-nluc312_cTnT343_0001_389end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREA
EDQLREKAKELWQTIYNLEAEKFDLQEKFKQKQKYEINVLNRNRINDNQKFKQKQKYEINVLNRNRINDNQ

>345-cTnTf1 (jp634_1fix-nluc312_cTnT345_359end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAER**EDQLREKAKELWQTI**

>345-cTnTf5 (jp635_1fix-nluc312_cTnT345_0001_371end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAER**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQ
QKYEINVLNRNRINDNQ**

>345-cTnTf6 (jp636_1fix-nluc312_cTnT345_0001_391end)
MGSHHHHHGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERII
EEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
SEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAER**EDQLREKAKELWQTIYNLEAEKFDLQEKFKQ
QKYEINVLNRNRINDNQKFKQKQKYEINVLNRNRINDNQ**

lucCageBot variants (Botulinum neurotoxin B sensors)

- Bot.0671.2 sequence: **MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE**

>BoNTB_338_1S
GSHHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAI AETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEIL**DAERLSREAAAASEKMFAE**

LKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*

> BoNTB_341_1S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRM
FAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*

>BoNTB_342_1S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*

>BoNTB_345_1S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AER**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE***

>BoNTB_348_2S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIR**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE***

>BoNTB_349_2S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIRE**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE***

>BoNTB_352_2S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIREAA**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE***

>BoNTB_355_2S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKISRE
AERSIREAAAASE**MF~~AE~~LKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE***

>BoNTB_GGG_2S

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKISRE
AERSIREAAAASEKISREGGG**MF~~AE~~LKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRK
YELE***

>BoNTB_GGG_2S_fullBotBinder

GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKISRE
AERSIREAAAASEKISREGGGSHMQ**MF~~AE~~LKAKFFLEIGDRDAARNALRKAGYSDEEA
RIIRKYELE***

lucCageProA variants (Fc domain biosensors)

- Staphylococcus aureus Protein A domain C (SpaC) sequence:
EQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK

>SpaC_360GGG

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKIS
REAERSIREAAAASEKISREGGGFN**KEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***

>SpaC_354-2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKIS
REAERSIREAAAASE**EQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***

>SpaC_351_2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD~~DAERLS~~SREAAAASEKIS

REAERSIREAA**EQQNAFYEILHLPNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPK***

>SpaC_350_2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
REAERSIREAA**EQQNAFYEILHLPNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPK***

>SpaC_347_2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
REAERSI**EQQNAFYEILHLPNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPK***

>SpaC_347_1S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
REAERLI**EQQNAFYEILHLPNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPK***

lucCageHer2 variants (Fc domain biosensors)

- Her2 affibody sequence:

EMRNAYWEIALLPNLNNQOKRAFIRSLYDDPSQSANLLAEAKKLNDAQAPK

>AffiHer2_347_1S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
REAERLI**EMRNAYWEIALLPNLNNQOKRAFIRSLYDDPSQSANLLAEAKKLNDAQAPK***

>AffiHer2_347_2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
REAERSI**EMRNAYWEIALLPNLNNQOKRAFIRSLYDDPSQSANLLAEAKKLNDAQAPK***
E

>AffiHer2_350_2S

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAIATQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE

EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS
REAERSIREAA**EMRNAYWEIALLPNLNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

>AffiHer2_351_2S

MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAI AETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS
REAERSIREAA**EMRNAYWEIALLPNLNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

>AffiHer2_354-2S

MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAI AETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS
REAERSIREAAA**SEEMRNAYWEIALLPNLNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

>AffiHer2_360GGG

MGSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAI AETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRLAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS
REAERSIREAAAASEKISREGGVDNKFNK**EMRNAYWEIALLPNLNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

lucCageHBVvariants (antiHBV antibody and PreS1 sensors)

- anti-HBV epitope sequence 1X: **GANSNNPDWDFN**
- anti-HBV epitope sequence 2X: **GANSNNPDWDFNGGSGGGSSGFGANSNNPDWDFNPN**

>lucCageHBV336

GSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
KRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLL
LKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
EL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASE**GANSNNPDWDFN**

>lucCageHBV338

GSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
KRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLL
LKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
EL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKI**GANSNNPDWDFN**

>lucCageHBV340

GSHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
KRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLL
LKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
EL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISR**GANSNNPDWDFN**

>lucCageHBV342
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
 KRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLL
 LKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
 EL) TDPDEARKAI AVTGYRLFEEILDAERLSREAAAASEKISREAG**GANSNNPDWDFN**

>lucCageHBV344
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
 KRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLL
 LKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
 EL) TDPDEARKAI AVTGYRLFEEILDAERLiREAAAASEKISREAER**GANSNNPDWDFN**

>lucCageHBV346
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
 KRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLL
 LKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
 EL) TDPDEARKAI AVTGYRLFEEILDAERLiREAAAASEKISREAERLi**GANSNNPDWDFN**

>lucCageHBV348
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
 KRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLL
 LKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
 EL) TDPDEARKAI AVTGYRLFEEILDAERLiREAAAASEKISREAERLiRE**GANSNNPDWDFN**

>lucCageHBV350
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDP
 KRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLL
 LKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLAS
 EL) TDPDEARKAI AVTGYRLFEEILDAERLiREAAAASEKISREAERLiREAA**GANSNNPDWDFN**

>lucCageHBVa (2xepitope)
 MGSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
 PKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKL
 LLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIE
 EEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLA
 SEL) TDPDEARKAI AVTGYRLFEEILDAERLiREAAAASEKISREAER**GANSNNPDWDFN**GGSGGGSGFG**GANSNNPDW
 DFNPN**

lucCageSARS2N variants (anti-SARS-CoV-2 Nucleocapsid protein antibodies sensors)

- SARS-Cov-2 Nucleocapsid protein epitope peptides used:
 - N6: **PKDKKKKKADETQALPQRQKKGSGGPKDKKKKKADETQALPQRQK**
 - N62: **KKDKKKKKADETQALGGSGGKKDKKKKKADETQAL**

>lucCageSARS2-N6_368-388_339
 GSHHHHHHGSSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKAI AETQDLNLRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAEIRA AKRESERIEEA

RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEK**PKKDKKKKADETQALPQRQKKG**
GSGGPKKDKKKKADETQALPQRQKK*

>lucCageSARS2-N6_368-388_346
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEKISRE
AERS**PKKDKKKKADETQALPQRQKKGSGGPKKDKKKKADETQALPQRQKK***

>lucCageSARS2-N6_368-388_353
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEKISRE
AERSIREAAAA**PKKDKKK**
KADETQALPQRQKKGDRADLRKTKRRKPTKPKHCRNVKKS

>lucCageSARS2-N62_369-382_336
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAA**SKKDKKKKADETQALGGSGGKKDKK**
KKKADETQAL*

>lucCageSARS2-N62_369-382_340
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEKI**KKDKKKKADETQALGGSGGKK**
DKKKKADETQAL*

>lucCageSARS2-N62_369-382_343
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEKISRE**AKKDKKKKADETQALGGS**
GGKKDKKKKADETQAL*

>lucCageSARS2-N62_369-382_347
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEILD~~DAERLSREAAAASEKISRE
AERSI**KKDKKKKADETQA**
LGSGGGKKDKKKKADETQAL*

>lucCageSARS2-N62_369-382_350
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI AETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAAASEKISREAERSIREA**KKDKKKKADE**
TQALGSGSGGKKDKKKKADETQAL*

>lucCageSARS2-N62_369-382_354
GSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI AETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAAS**KKDKKK**
KADETQALGSGSGGKKDKKKKADETQAL*

lucCageSARS2M variants (anti-SARS-Cov-2 Membrane protein antibodies sensors)

- SARS-Cov-2 Membrane protein epitope peptides used:
 - M1_1-31:**MADSNGTITVEELKKLLEQWNLVIGFLFTWIGSGGMADSNGTITVEELKKLLEQWNLVIGFLFTWI**
 - M3_1-17:**MADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE**
 - M4_8-24:**ITVEELKKLLEQWNLVIGSGGITVEELKKLLEQWNLVI**

>lucCageSARS2-M1_1-31_334
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAA**MADSNGTITVEELKKLLEQWNLVIGFLFTWIGSGGMA**
DSNGTITVEELKKLLEQWNLVIGFLFTWI*

>lucCageSARS2-M1_1-31_337
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAAASE**MADSNGTITVEELKKLLEQWNLVIGFLFTWIGSGG**
MADSNGTITVEELKKLLEQWNLVIGFLFTWI*

>lucCageSARS2-M1_1-31_341
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAA**ASEKISRMADSNGTITVEELKKLLEQWNLVIGFLFTWI**
GGSGGMADSNGTITVEELKKLLEQWNLVIGFLFTWI*

>lucCageSARS2-M1_1-31_343
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVVTGYRLFEEILDAERLSREAAAASEKISRE**MADSNGTITVEELKKLLEQWNLVIGFLFTWI**
IGSGGMADSNGTITVEELKKLLEQWNLVIGFLFTWI*

>lucCageSARS2-M3_1-17_341
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAAASEKISR**MADSNGTITVEELKKLLEG**
GSGGMADSNGTITVEELKKLLE*

>lucCageSARS2-M3_1-17_343
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAAASEKISRE**MADSNGTITVEELKKLL**
EGSGGMADSNGTITVEELKKLLE*

>lucCageSARS2-M3_1-17_348
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAAASEKISRE**MADSNGTITVEE**
LKKLLEGGSGGMADSNGTITVEELKKLLE*

>lucCageSARS2-M3_1-17_350
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAAASEKISRE**MADSNGTITV**
EELKKLLEGGSGGMADSNGTITVEELKKLLE*

>lucCageSARS2-M4_8-24_334
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAA**ITVEELKKLLEQWNLVIGSGGITVE**
ELKKLLEQWNLVI*

>lucCageSARS2-M4_8-24_340
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLKKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLSREAAAASEKIS**ITVEELKKLLEQWNLVIGGS**
GGITVEELKKLLEQWNLVI*

>lucCageSARS2-M4_8-24_341
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR

KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI AETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI A V T G Y R L F E E I L D A E R L S R E A A A A S E K I S R I T V E E L K K L L E Q W N L V I G G
S G G I T V E E L K K L L E Q W N L V I *

>lucCageSARS2-M4_8-24_348
MGSHHHHHHGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI AETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAI A V T G Y R L F E E I L D A E R L S R E A A A A S E K I S R E A E R S I R I T V E E L K K L L E Q
W N L V I G G S G G I T V E E L K K L L E Q W N L V I *

sCage-HA variants

>HB1.9549.2

TFACRIAAKIAAEFGYSEEQIKELLKNAGCSEDEARDAVEYLR

>sCage
MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR
AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSG (SELARELLRAHAQLQRL
NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIR

>sCageHA_267-1S:

MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR
AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSG (SELARELLRAHAQLQRL
NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVKRESNRIVEDAERLGGG**TFACRIAAKIAAEFGYSEE
QIKELLKNAGCSEDEARDAVEYLR**

>SCageHA_256

MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR
AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSG (SELARELLRAHAQLQRL
NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVKGGG**TFACRIAAKIAAEFGYSEEQIKELLKNAGCSE
DEARDAVEYLR**

>SCageHA_260_1S

MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR
AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSG (SELARELLRAHAQLQRL
NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARSKRESKRGGG**TFACRIAAKIAAEFGYSEEQIKELLKN
AGCSEDEARDAVEYLR**

>SCageHA_263_1S

MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR
AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSG (SELARELLRAHAQLQRL
NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARSKRESKRIVEGGG**TFACRIAAKIAAEFGYSEEQIKEL
LKNAGCSEDEARDAVEYLR**

>SCageHA_267_1S

MGSHHHHHHGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDE
IKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIR

AVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKGGSGG (SELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARSKRESKRIVEDAERGGG**TFACRIA****AKIAAEFGYSEEQIKELLKNAGCSEDEARDAVEYLR**

>SCageHA_270_2S

MGSHHHHHHGGSGSENLYFQGGG (SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKGGSGG (SELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARSKRESKRIVEDAERLSRGGG**TFACRIA****AKIAAEFGYS****EEQIKELLKNAGCSEDEARDAVEYLR**

lucCageRBD variants (SARS-CoV2 Spike Protein Receptor binding domain (RBD) biosensors)

- LCB1: **DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R
- LCB1_delta4: **ILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R

>lucCageRBD_336

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEIL~~DAERLSREAAAAS**DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R*

>lucCageRBD_340

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEIL~~DAERLSREAAAASEKIS**DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R*

>lucCageRBD_344

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEIL~~DAERLSREAAAASEKIS**REAEDKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R*

>lucCageRBD_347

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEIL~~DAERLSREAAAASEKIS**REAERSIDKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R*

>lucCageRBD_351

MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIATQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAARKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAI~~AVTGYRLFEEIL~~DAERLSREAAAASEKIS**REAERSIREAADKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE**R*

>lucCageRBD_354
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISREAERSIREAAAAS**DKEWILQKIYEIMRLLDELG
HAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEER***

>lucCageRBD_GGG_360
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISREAERSIREAAAASEKISREGGG**DKEWILQKIYE
IMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEER***

>lucCageRBDdelta4_336
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAAS**ILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDER
LLEEAERLLEEVEER***

>lucCageRBDdelta4_340
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS**ILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKG
GDERLLEEAERLLEEVEER***

>lucCageRBDdelta4_344
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISREA**ILQKIYEIMRLLDELGHAEASMRVSDLIYE
FMKGDERLLEEAERLLEEVEER***

>lucCageRBDdelta4_347
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISREAERSI**ILQKIYEIMRLLDELGHAEASMRVSDI
IYEFMKGDERLLEEAERLLEEVEER***

>lucCageRBDdelta4_348
MGSHHHHHHGGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISREAERSIR**ILQKIYEIMRLLDELGHAEASMRVSD
LIYEFMKGDERLLEEAERLLEEVEER***

>lucCageRBDdelta4_351

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAA**ILQKIYEIMRLDELGHAEASMR**
VSDLIYEFMKGDERLLEEAERLLEEVEVER*

>lucCageRBDdelta4_354
MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAAS**ILQKIYEIMRLDELGHAEASMR**
VSDLIYEFMKGDERLLEEAERLLEEVEVER*

>lucCageRBDdelta4_357
MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAASEKI**ILQKIYEIMRLDELGH**
AEASMRVSDLIYEFMKGDERLLEEAERLLEEVEVER*

>lucCageRBDdelta4_GGG_360
MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVEL
TDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELA
KLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESER
IIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLR
LASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAASEKISREGGG**ILQKIYEIMRL**
LDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEVER*

TARGET SEQUENCES:

> GST-PreS1 domain:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMATIRYIADKH
NMLGGCPKERAIEISMLEGAVLDIRYGVSRAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDA
LDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSMGGWSSKPQQG
MGTNLSVPNPLGFFPDHQLDPAFGANANNPDWDFNPNKDHWPDGN