## SUPPLEMENTAL TEXT

### *(I) Analytical model and parameter extraction*

In the case of conventional fluorescent reporters, we considered a two-process model for the activation of the reporter system. The first process was $Repoter_{inactive}$ in which FP was expressed but was still inactive. The second process was an activated state, $Repoter_{active}$, which corresponded to FPs after maturation and made them fluorescently competent. In this model, the [$Repoter_{inactive}$] was synthesized with production rate $k_1$, and matured with maturation rate $k_2$ into [$Repoter_{active}$] as follows:

$$DNA_{template} \xrightarrow[k_1]{} [Repoter_{inactive}] \xrightarrow[k_2]{} [Repoter_{active}] \quad \text{(Eq. S1)}$$

The evolution of concentrations of inactive and active reporters can be described based on a simple differential equation as a function of time $t$, as follows:

$$\frac{d}{dt}[Repoter_{inactive}] = k_1 DNA_{template} - k_2[Repoter_{inactive}] \quad \text{(Eq. S2)}$$

$$\frac{d}{dt}[Repoter_{active}] = k_2[Repoter_{inactive}] \quad \text{(Eq. S3)}$$

By solving these differential equations with initial conditions of $Repoter_{inactive}(t = 0) = 0$ and $Repoter_{active}(t = 0) = 0$, the concentrations of $Repoter_{inactive}(t)$ and $Repoter_{active}(t)$ can be expressed as a function of $t$ ($t \geqq 0$) as follows:

$$Repoter_{inactive}(t) = \frac{k_1}{k_2} DNA_{template}\left(1 - e^{-k_2 t}\right) \quad \text{(Eq. S4)}$$

$$Reporter_{active}(t) = k_1 \cdot DNA_{template}\left\{t + \frac{1}{k_2}\left(e^{-k_2 t} - 1\right)\right\} \quad \text{(Eq. S5)}$$

Thus, measured intensity can be described as follows:

$$Measured\ intensity\ (t) = F \cdot k_1 \cdot DNA_{template}\left\{t + \frac{1}{k_2}\left(e^{-k_2 t} - 1\right)\right\} \quad \text{(Eq. S6)}$$

, where $F$ is the fluorescent intensity or photon numbers per molecule.

In the present reporter system, we also considered a two-stage model for activation of the reporter system. First, RU was expressed with a rate constant of $k_3$. Second, expressed RU bound to PU which had already been accumulated in the reaction tube in advance, with association rate $k_4$ and dissociation rate $k_5$ to be an active reporter ($Repoter_{active}$), as follows:

$$DNA_{template} \xrightarrow[k_3]{} [RU] \quad \text{(Eq. S7)}$$

$$[RU] + [PU] \underset{k_5}{\overset{k_4}{\rightleftarrows}} [Repoter_{active}] \quad \text{(Eq. S8)}$$

Because PU is constantly expressed at the same speed as RU using the same promoter, even if most of the expressed RU associates with PU, the level of the remaining free [PU] is higher than that of $[PU]_{t=0}$. For simplicity, we postulated that the concentration of free PU was constant to get an analytical solution although we could set a model considering [PU] as a function of time *t*. The evolution of the concentration of RU and active reporter can be expressed based on an ordinary differential equation as a function of time *t*, as follows:

$$\frac{d}{dt}[RU] = k_3 \cdot DNA_{template} - k_4[RU][PU] + k_5[Repoter_{active}] \quad \text{(Eq. S9)}$$

$$\frac{d}{dt}[Repoter_{active}] = k_4[RU][PU] - k_5[Repoter_{active}] \quad \text{(Eq. S10)}$$

By solving these rate equations with initial condition of $RU(t=0) = 0$ and $Repoter_{active}(t=0) = 0$, the concentration $Repoter_{active}(t)$ can be written as a function of *t* ($t \geqq 0$) as follows:

$$Repoter_{active}(t) = \frac{k_3 k_4[PU]}{k_4[PU] + k_5} \cdot DNA_{template} \left\{ t + \frac{1}{k_4[PU] + k_5} \cdot \left( e^{-(k_4[PU] + k_5)t} - 1 \right) \right\} \quad \text{(Eq. S11)}$$

Thus, measured intensity can be described as follows:

$$Measured\ intensity\ (t) = F \cdot \frac{k_3 k_4[PU]}{k_4[PU] + k_5} \cdot DNA_{template} \left\{ t + \frac{1}{k_4[PU] + k_5} \cdot \left( e^{-(k_4[PU] + k_5)t} - 1 \right) \right\} \quad \text{(Eq. S12)}$$

, where *F* is fluorescent intensity or photon numbers per molecule.

Both the derived equations for time dependence of measured intensity for conventional and the present reporter can be expressed as a single equation by converting the constant coefficients into integrated ones, as follows:

$$Measured\ intensity\ (t) = k_{LP}\left\{ t + \frac{1}{k_M}\left( e^{-k_M t} - 1 \right) \right\} \ (t \geqq 0) \quad \text{(Eq. S13)}$$

, where $k_{LP}$ is described in the model of conventional reporter as follows:

$$k_{LP\_conventional} = F \cdot k_1 \cdot DNA_{template} \quad \text{(Eq. S14)}$$

Since $k_{LP}$ can be regarded as the light (luminescence or fluorescence) production rate (Fig. S3), and it is

expressed in the model of the present reporter as follows:

$$k_{LP\ \_\ our\ reporter} = F \cdot \frac{k_3 k_4 [PU]}{k_4 [PU] + k_5} \cdot DNA_{template} \quad \text{(Eq. S15)}$$

$k_M$ of Eq. S13 can be simply regarded as the maturation rate, because it is same as $k_2$ in the model of conventional reporter (Eq. S6). In the model of the present reporter, $k_M$ is expressed as follows:

$$k_{M\ \_\ our\ model} = k_4 [PU] + k_5 \quad \text{(Eq. S16)}$$

In summary, Eq. S13 is the final equation for analyzing the experimental results in this study. The fitting of experimental model to extract $k_{LP}$ and $k_M$ were done with a homemade software in Python.

**(II) Effect of peptide-assisted complementation to YNL reporter in mammalian cells.**

To investigate the versatility of the present strategy, we measured the rise of fluorescence of the present reporters in mammalian cells, not in *E. Coli* cells. The three kinds of tested reporters described in the main text, E/SZ1, E/SZ5, and E/SZ22, were fused with the split parts of *luciferase* gene between amino acids 228 and 229 of YNL and split YNL in Cos-7 cells, which is a fibroblast-like cell line derived from African green monkey kidneys. The PU was constitutively expressed in cells and RU expression could be induced by doxycycline addition. We measured the time development of luminescence intensity after 10 ng/mL doxycycline addition. Unfortunately, the present reporters did not display a clear luminescent increase in mammalian cells (Fig. S6A). This discrepancy from *in vitro* and *E. coli* experiments could be due to the differences in intracellular environments, such as, chaperons and/or degradation mechanisms.

The split parts of luciferase between amino acids 228 and 229 self-assemble weakly to emit luminescence (Fig. S6A, *cyan*), causing an undesirable background signal. Instead, the investigated E-peptides and K-peptides [S1, S2], and the two kinds of oligomer forming systems (EF-hand motifs (EF1 domain and EF2 domain) [S3] and FKBP (FK506-binding protein)-FRB (FKBP12-rapamycin associated protein1, FRAP1 fragment) system) [S4], as a complement peptide pair work well in mammalian cells. Further, only the reporters in which PU fused to FRB and RU fused to FKBP (FRB/FKBP) or to tandem FKBP (FRB/FKBPx2) exhibited improvements in intensity and rising speed (Fig. S6B, S6C). Since the FKBP-FRB pair is a rapamycin-induced fusion system, this improvement was caused by weak self-assembly of FKBP and FRB. Hence, when adding 20 ng/mL rapamycin, the intensities and rise of luminescence in FRB/FKBP and FRB/FKBPx2 pairs improved significantly.

For the split luciferase system between amino acids 91 and 92, similar results were obtained (Fig. S7, S8B). The following were the differences from the results obtained for the 228-229 split

system: (1) The self-assembly rate of split luciferase was lower than that observed in the 228- 229 split system (Fig. S7A, *cyan*); (2) In the reporter systems where PU was fused to E-peptide and RU was fused to K-peptide (E-/K-peptides), the recovered reporting ability was the same as that in YNL (Fig. S7A, *red*); (3) All 4 reporters using the FRB-FKBP system produced complementary luminescence signals (Fig. S7B, S8B). Importantly, rapamycin induction depended on the order of FRB and FKBP and the number of FKBP (Fig. S7C), for example, the FKBP/FRB pair lost rapamycin dependency (Fig. S7BC, *red*).

By analyzing the earlier time points of these results, it is clear that unlike YNL, these two peptides pairs (FRB/FKBP and FRB/FKBPx2) were already activated (Fig. S8A). These results indicate that though the present strategy is applicable to mammalian cells, the corresponding dimerized peptides are different from *E. coli* cells. Although, the PU concentration is important to execute this model, the strength of constitutive expression promoters might not be a vital problem in cellular experiments, since at least the aforementioned two peptide pairs displayed improvement in rising speed. The reason why the screened peptide did not work in mammalian cells could be related to the differences in intracellular environment, such as chaperons and/or degradation mechanisms. More specifically, it is suggested that there are risks of undesired interactions with intracellular proteins and/or instability of reporters and labeled proteins caused by fused peptides depending on intracellular conditions of a target cell.

However, the present results do not indicate that peptide pairs that did not work in our assay cannot be functional in other models. Notably, it was recently reported that the E/K-peptide series, which showed reduced efficiency in our *in vitro* experiments, worked well as a protein labeling system in mammalian cells [S5]. The fluorescent proteins in cytosol are recruited to the protein of interest by their affinity to E-peptide/K-peptide. The fast association-dissociation cycle of the fluorescent protein and the protein of interest by the mediated peptide enables photostable labeling and spontaneous on-off switching for one of super-resolution methods, photo-activated localization microscopy (PALM). In addition, as shown in earlier literature, some E-peptide/K-peptide pairs tend to form tetramers in *in vitro* conditions [S6]. Since we used a split method strategy while they did not, not only the dimerization affinity of a peptide pair but also the appropriate directional/positional relationship of the two parts for functional complementation was required here. Even if the dimerization efficiency is high, the two parts of PU and RU are not necessarily oriented appropriately for complementation. For example, in the 91st-split YNL, the E-peptide/K-peptide pair recovered the luminescence to about the same as full-YNL (Fig. S7a, *red*), while the opposite version, the K-peptide/E-peptide pair, failed (Fig. S7a, *purple*). An additional screening step for linker lengths and/or sequences might provide a different candidate.

Thus, a best matched peptide screening system is required for different species and purposes.

In cases where other variations are needed, screening for peptides using an *in vitro* system similar to the intracellular environment of a target cell is recommended, since there is a possibility that the quaternary structure of the peptide pair might be involved.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### *Expression plasmid for in vitro cell-free experiment.*

For the construction of expression plasmids for *in vitro* cell free expression experiments, pinpoint Xa-3 vector (Promega Corporation, Madison, WI, USA) was used as a backbone. For constructing the expression plasmid of PU, unnecessary tag sequences of the vector were removed between *Pst-BglII* and replaced with an artificially synthesized sequence containing restriction enzyme sites for *HindIII*, *BamHI,* and *NheI* in order. The cDNA sequence of PU of split YNL (Venus fused with 1-91 or 1-228 a. a. of RLuc8-S257G) was amplified by PCR and cloned between *HindIII* and *BamHI* of the vector. The cDNA of dimerized peptides was artificially synthesized and fused with sequences of restriction enzyme sites at both ends, and cloned between *BamHI* and *NheI*. Thus, PU of the split YNL was fused with the dimerized peptide at its C-terminus on the vector, and was expressed under the control of *tac* promoter with *lac* operator.

For the expression plasmids of RU, YNL, Venus, and RLuc8-S257G, unnecessary tag sequences of the vector were removed between *Pst-BglII* and replaced with artificially synthesized sequences containing restriction enzyme sites for *BamHI*, *NheI*, and *HindIII* in order. The cDNA of RU of split YNL (between 92-311 or 229-311 a. a. of RLuc8-S257G), YNL, Venus, or RLuc8-S257G was amplified by PCR and cloned between *NheI* and *HindIII* of the vector. For RU, the cDNA of dimerized peptides was artificially synthesized fused to sequences of restriction enzyme sites at both ends and cloned between *BamHI* and *NheI*. Thus, RU of split YNL was fused with the dimerized peptide at N-terminus on the vector. Thus, RU, YNL, Venus, and RLuc8-S257G were under control of *tac* promoter with *lac* operator.

### *Expression plasmid for E. coli experiment*

For construction of expression plasmids for the *E. coli* experiment, the expression plasmids of PU for *in vitro* experiment were further modified. First, cDNA of RU (fused with dimerized peptide) was amplified by PCR from *in vitro* expression vectors and inserted between *HindIII-NcoI* sites on pPal7 vector (BioRad, CA, USA). Then, RU, including the ribosomal binding site and T7 terminator site, was amplified from pPal7 vector by PCR and inserted at *BtgI* of the PU expression plasmid by Gibson Assembly (New England Biolabs Inc., MA, USA). Next, *LacI* with *lacI* promoter was also inserted at

the *PciI* site by Gibson Assembly. Thus, the expression of PU was still under control of *tac* promoter with *lac* operator and that of RU was under the control of T7 promoter, and *LacI* expressed constitutively from *lacI* promoter. For the expression plasmid of Venus, only *LacI* with *lacI* promoter was inserted at *PciI* by Gibson Assembly.

***Peptide pair screening experiment***

For the screening of peptide pairs, we used an *in vitro* cell-free expression system of *E. coli* extract (S30 extract system for circular DNA, Promega). A total of 20 ng/μL of expression plasmids both, from a peptide library fused with PU and from a library fused with RU, were mixed with the reaction solution on ice, and then incubated at 37 °C for 1 h on a thermal cycler. Following that, the reaction tubes were kept on ice and 20 μM coelenterazine was added. The reaction mix was transferred into a 96-well plate, and luminescence was measured by a plate reader (MTP-880Lab, Corona Electric Co., Ltd, Japan). The 96-well plate contained a sample of RLuc8-S257G as a control.

***Measurement of time development of luminescence of reporters in cell-free expression system***

For the measurement of luminescence development in reporters, we used an *in vitro* cell-free expression system consisting of *E. coli* extract. The expression plasmid of PU (2, 4, 8, and 16 ng/μL for concentration dependency testing, and 4 ng/μL for others) was mixed with *E. coli* extract. Then, the reaction tubes were incubated for 1 h at 37 °C on a thermal cycler. For control samples (YNL and RLuc8-S257G), the reaction mix was incubated without any expression plasmids. After incubation, reaction tubes were transferred to ice and 4 ng/μL of the expression plasmids of RU, YNL, or RLuc8-S257G was added. The reaction solutions were dispensed and incubated at 37 °C at different time points of 0, 5, 10, 15, 20, 25, and 30 min, respectively. After incubation, the samples were kept on ice, and 20 μM coelenterazine was added to each reaction mix just before measurement, and subsequently transferred into a 96-well plate. Luminescence was measured using a plate reader (MTP-880Lab).

***E. coli growth rate evaluation in fast and slow medium.***

We used *E. coli* strain *BL21-AI* (Thermo Fisher Scientific Inc., MA, USA) which possesses T7 polymerase that can be induced by L-arabinose. The strain was transformed with an expression vector carrying the present reporter which possessed a peptide pair of E/SZ1 fused with PU and RU respectively. Cells were cultured at 37 °C in a fast medium (minimal M9 medium supplemented with 0.4% (w/v) glucose and 0.1% (w/v) casamino acids) and a slow medium (minimal M9 medium supplemented with 0.1% (w/v) succinate) with and without 0.2% (w/v) L-arabinose [S7]. A small

portion of the culture was picked up and $OD_{650nm}$ was measured per hour for 9 h to ascertain the bacterial growth kinetics.

***Measurement of the time dependent development of reporter intensity in E. coli***

For the evaluation of luminescence response of the present reporter in *E. coli* cells, we used *E. coli* strain *BL21-AI*. *BL21-AI* was transformed with the expression plasmid. Thus, it can induce expression of PU by L-arabinose and of RU by IPTG. Transformed *BL21-AI* was stored as glycerol stock at -80 $^{o}$C. Pre-incubation in fast medium was carried out for 8 h at 37 $^{o}$C. Then, cells were diluted x1000 into fresh fast medium and cultured with 0.2% (w/v) L-arabinose for 8 h. Then, the culture was spun down and diluted into a slow medium and cultured at 37 $^{o}$C. After 1 h of culturing in slow medium, 1 mM IPTG was added to the samples to induce reporter expression. For comparison, samples without IPTG were also prepared. Small portion of the culture was loaded onto a 96-well plate for measurement of luminescence or fluorescence and $OD_{650nm}$ at respective times of 1 and 0.5 h before and 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours after adding IPTG. 60 μM coelenterazine was mixed in a 96-well plate just before the respective measurements. Then, luminescence or fluorescence data and $OD_{650nm}$ were measured by a plate reader (Varioskan LUX, Thermo Fisher Scientific Inc.) at each time point. As a control, the present reporter was also expressed without L-arabinose in the fast medium but added to the slow medium at the same time with IPTG addition. Additionally, *BL21-AI* transformed by the expression plasmid of Venus was also used through the same procedure, as another control. Measured luminescence or fluorescence was normalized by dividing by $OD_{650nm}$ values of respective samples.

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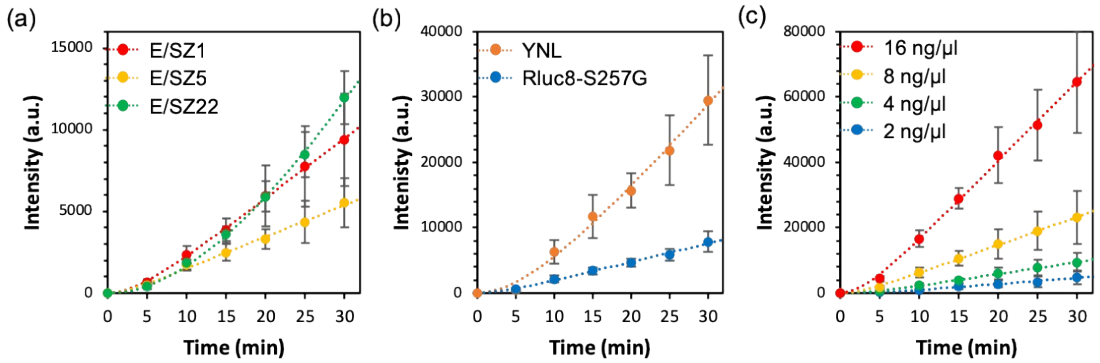


**Figure S1. Time course of luminescence production of our reporters *in vitro*.**

(a-c) Time course of luminescence increased in the present reporter system in a cell-free expression system using three peptide pairs (a), control reporters (b), and using a peptide pair of E/SZ1 with different density of template plasmid DNA of PU (c). The PU was composed of 1-228 amino acid region of RLuc8-S257G in a YNL, and the RU comprises of 229-311 amino acid region. E/SZ1, E-peptide/SYNZIP1 (a, *red*, N = 4); E/SZ5, E-peptide/SYNZIP5 (a, *yellow*, N = 4); E/SZ22, E-peptide/SYNZIP22 (a, *green*, N = 4); as formatted: "peptide fused with the PU" / "peptide fused with the RU". Dotted lines are the fitted results using a mathematical model (Eq. 1). Error bars are standard deviation.

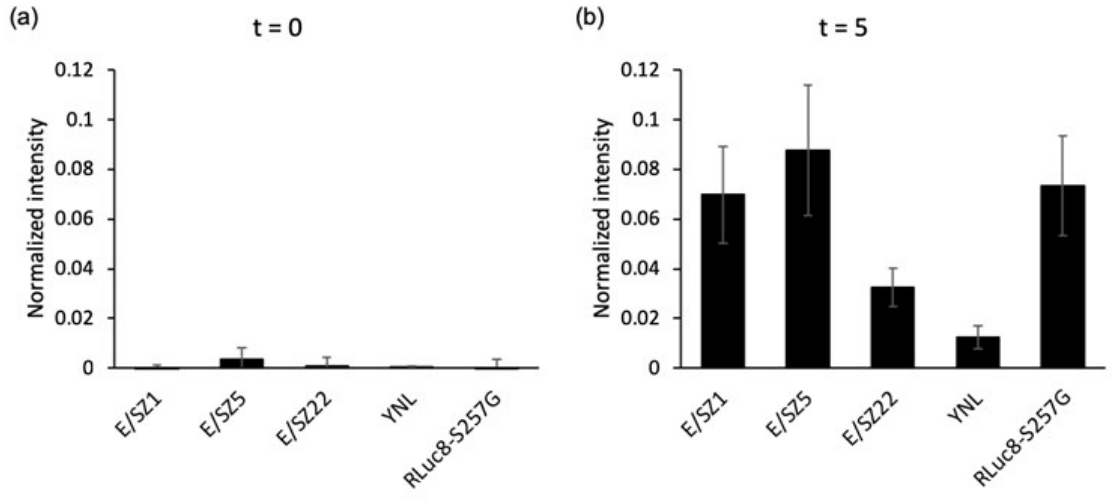


**Figure S2. Comparison between the present reporters (E/SZ1, E/SZ5, and E/SZ22) and the controls (YNL and RLuc8-S257G) at initial time points in a cell-free expression system.**

Normalized intensity of reporters in a cell-free expression system, at t = 0 (a), and t = 5 (b) in Figure S1. These intensities were normalized by their respective intensities at t = 30. The PU was composed of the region between 1-228 amino acids of Rluc8-S257G in YNL, and the RU was comprised of the region 229-311 amino acids. E/SZ1, E-peptide/SYNZIP1 (N = 4); E/SZ5, E-peptide/SYNZIP5 (N = 4); E/SZ22, E-peptide/SYNZIP22 (N = 4); as formatted: “peptide fused with the PU” / “peptide fused with the RU”.

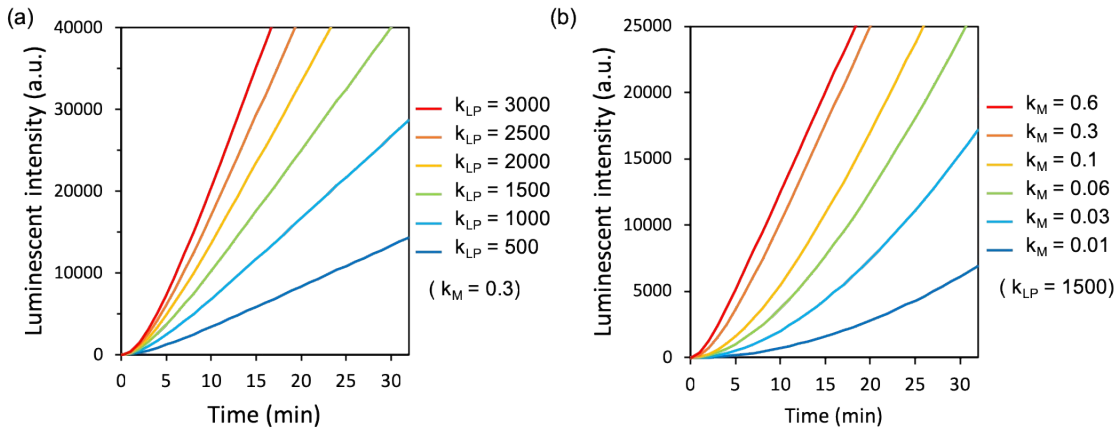


**Figure S3. Computer simulations of the effects of $k_{LP}$ and $k_M$ on luminescence intensity.**

The time development of $k_{LP}$ (a) and $k_M$ (b) were simulated based on a mathematical model (Eq. 1). $k_M$ value was fixed as 0.3 and $k_{LP}$ was varied between 6 different values in (a). $k_{LP}$ value was fixed as 1500 and $k_M$ varied with 6 different values in (b).

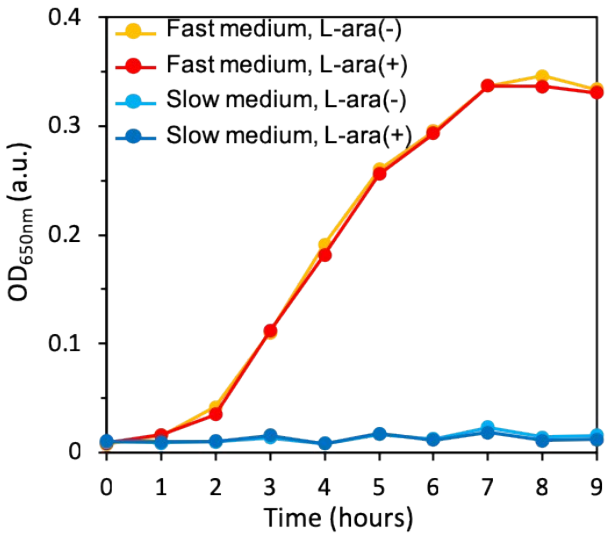


**Figure S4. Growth speeds of *BL21-AI* in a fast and slow growth medium.**

*E. coli BL21-AI* transferred by the expression plasmid of our reporter was cultured in different mediums and $OD_{650nm}$ was measured. Fast medium; minimal M9 medium supplemented with 0.4% (w/v) glucose and 0.1% (w/v) casamino acids. Slow medium; minimal M9 medium supplemented with 0.1% (w/v) succinate. L-ara(+) represents medium containing 0.2 % (w/v) L-arabinose. L-ara(-) represent medium without L-arabinose.

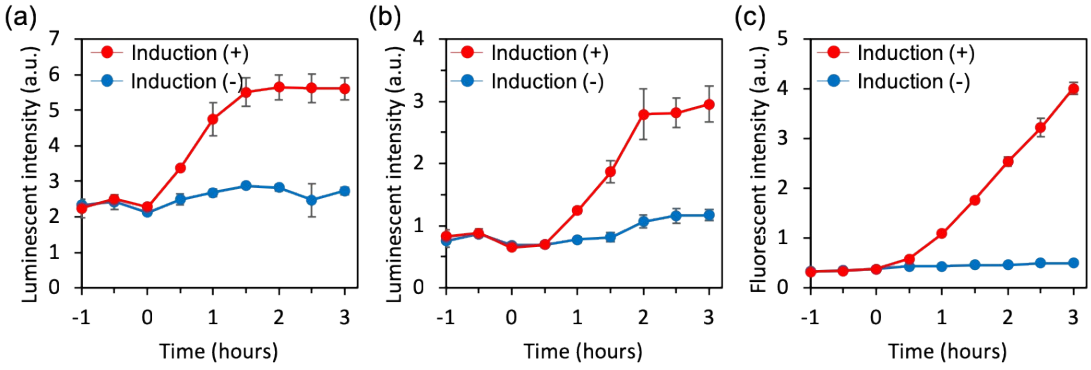


**Figure S5. Comparison of induction response in *E. coli* among the present reporter and Venus.**
(a-c) Time course of luminescence intensity of our reporter with pre-induction of PU for 8 h (a), our reporter without pre-induction of PU but induced at t = 0 (b), and of Venus as a conventional fluorescence reporter (c). Induction of RU or Venus was started at t = 0. Luminescence or fluorescence intensity was normalized by $OD_{650nm}$. Error bars are standard deviation (N = 3).

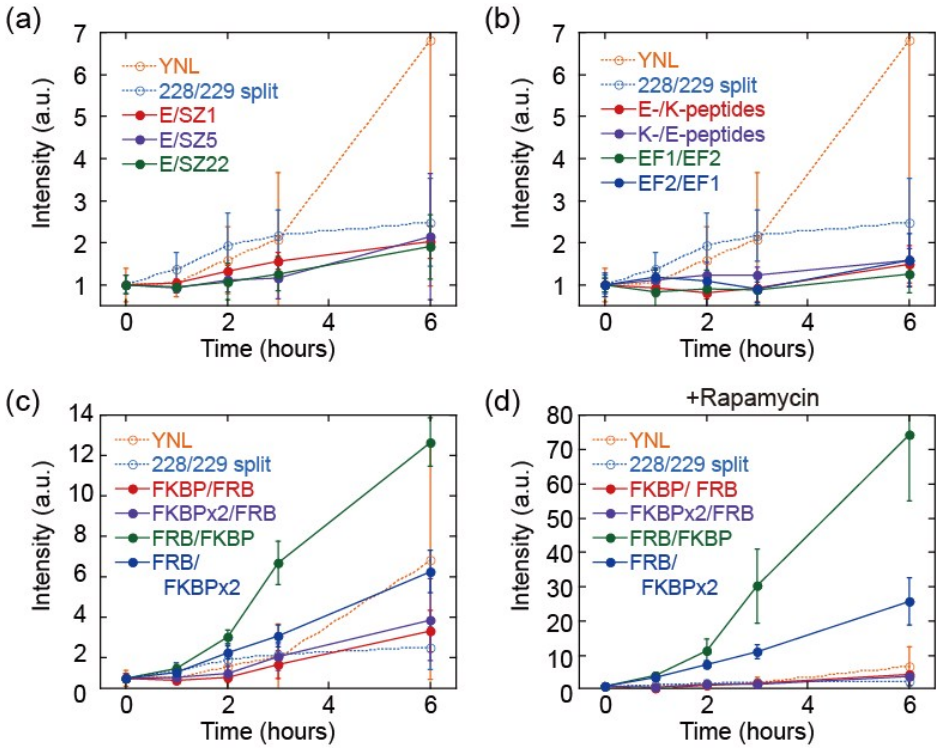


**Figure S6. Reporter sensitivity of split YNL at 228th residue with protein fragment-assisted complementation.**

(a-c) Time course of luminescence intensity of the present reporters in Cos-7 cells after doxycycline addition. YNL, yellow nano-lantern (*orange*, N = 42); 228/229 split, the reporter in which PU is comprised of the region between 1-228 amino acids of RLuc8-S257G in split YNL, and RU is comprised of the region between 229-311 amino acids (*cyan*, N = 9); E/SZ1, E-peptide/SYNZIP1 (a, *red*, N = 6); E/SZ5, E-peptide/SYNZIP5 (a, *purple*, N = 6); E/SZ22, E-peptide/SYNZIP22 (a, *green*, N = 6); E-/K-peptides, E-peptide/K-peptides (b, *red,* N = 6); K-/E-peptides, K-peptide/E-peptides (b, *purple*, N = 6); EF1/EF2, EF1 domain/EF2 domain (b, *green*, N = 6) ; EF2/EF1, EF2 domain/EF1 domain (b, *blue*, N = 6); FKBP/FRB, FKBP/FRB (c, *red,* N = 10); FKBPx2/FRB, tandem FKBP/FRB (c, *purple*, N = 10); FRB/FKBP, FRB/FKBP (c, *green*, N = 10) ; FRB/FKBPx2, FRB/ tandem FKBP (c, *blue*, N = 10); as formatted: "peptide fused with the PU" / "peptide fused with the RU"). (d) Time course of luminescence intensity of the present reporters in Cos-7 cells after doxycycline addition with rapamycin induction (each N = 4). Simplified notations are the same as (c). Error bars are standard deviation.

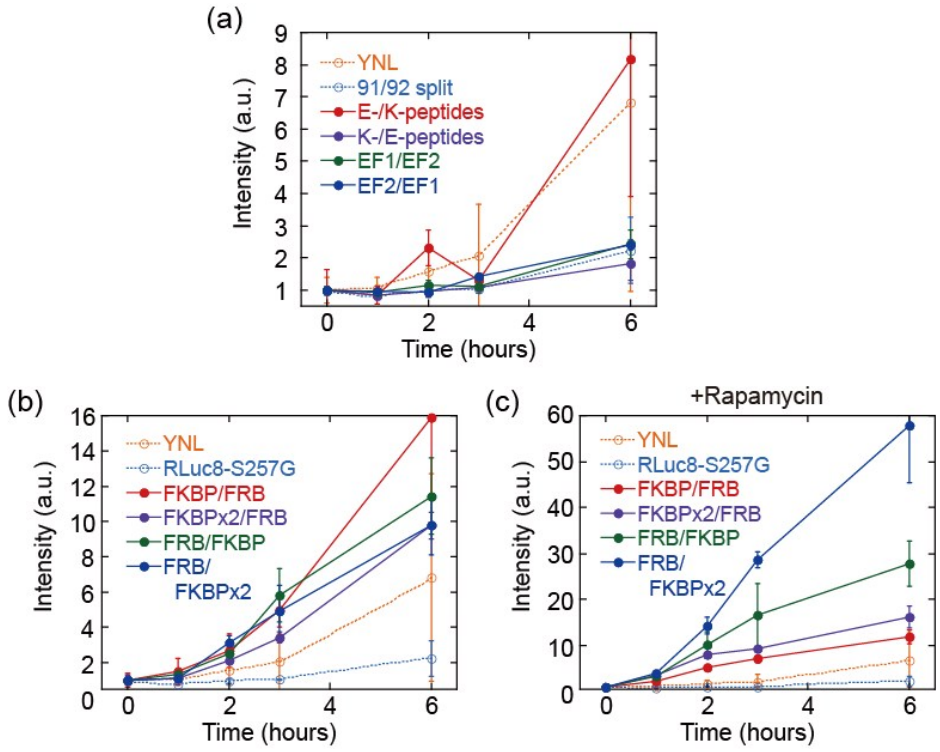


**Figure S7. Reporter sensitivity of split YNL at 91st residue with protein fragment-assisted complementation.**

(a, b) Time course of luminescence intensity of the present reporters in Cos-7 cells after doxycycline addition. YNL, yellow nano-lantern (*orange*, N = 42); 91/92 split, the reporter in which PU is comprised of the region between amino acids 1-91 of RLuc8-S257G in YNL, and RU is comprised of the region between amino acids 92-311 (*cyan*, N = 4); K-/E-peptides, K-peptide/E-peptides (a, *purple*, N = 3); EF1/EF2, EF1 domain/EF2 domain (a, *green*, N = 3) ; EF2/EF1, EF2 domain/EF1 domain (a, *blue*, N = 3); FKBP/FRB, FKBP/FRB (b, *red,* N = 7); FKBPx2/FRB, tandem FKBP/FRB (b, *purple*, N = 7); FRB/FKBP, FRB/FKBP (b, *green*, N = 7) ; FRB/FKBPx2, FRB/ tandem FKBP (b, *blue*, N = 7); as formatted: “peptide fused with the PU” / “peptide fused with the RU”. (c) Time course of luminescence intensity of the present reporters in Cos-7 cells after doxycycline addition with rapamycin induction (each N = 4). Simplified notations are the same as (b). Error bars indicate standard deviation.

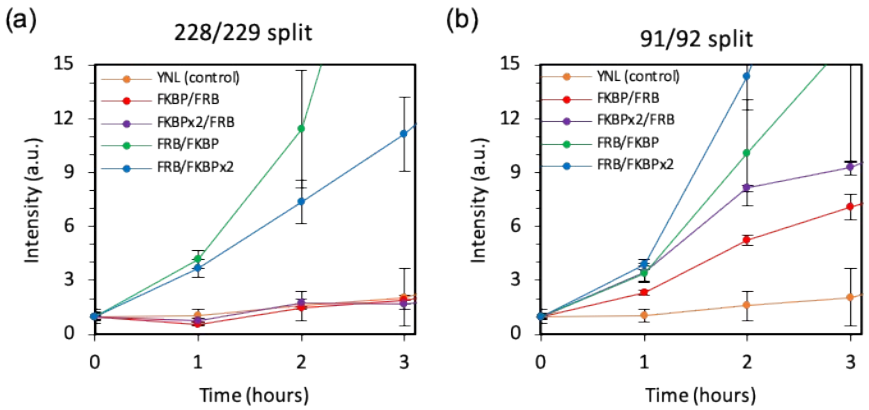


**Figure S8. Closing up the initial response of our reporter with known well-working peptides in mammalian cells.**

(a, b) Extraction of earlier time points of Fig. S6d (a), and Fig. S7c (b).

| Peptide name | Peptide sequence |
|---|---|
| WinZip-A1 | VAQLEEKVKTLRAQNYELKSRVQRLREQVAQL |
| WinZip-B1 | VDELQAEVDQLQDENYALKTKVAQLRKKVEKL |
| K-peptide | KVSALKEKVSALKEKVSALKEKVSALKEKVSALKE |
| E-peptide | EVSALEKEVSALEKEVSALEKEVSALEKEVSALEK |
| K3-peptide | KIAALKEKIAALKEKIAALKE |
| K4-peptide | KIAALKEKIAALKEKIAALKEKIAALKE |
| K5-peptide | KIAALKEKIAALKEKIAALKEKIAALKEKIAALKE |
| E3-peptide | EIAALEKEIAALEKEIAALEK |
| E4-peptide | EIAALEKEIAALEKEIAALEKEIAALEK |
| E5-peptide | EIAALEKEIAALEKEIAALEKEIAALEKEIAALEK |
| SYNZIP1 | NLVAQLENEVASLENENETLKKKNLHKKDLIAYLEKEIANLRKKIEE |
| SYNZIP2 | ARNAYLRKKIARLKKDNLQLERDEQNLEKIIANLRDEIARLENEVASHEQ |
| SYNZIP3 | NEVTTLENDAAFIENENAYLEKEIARLRKEKAALRNRLAHKK |
| SYNZIP4 | QKVAELKNRVAVKLNRNEQLKNKVEELKNRNAYLKNELATLENEVARLENDVAE |
| SYNZIP5 | NTVKELKNYIQELEERNAELKNLKEHLKFAKAELEFELAAHKFE |
| SYNZIP6 | QKVAQLKNRVAYKLKENAKLENIVARLENDNANLEKDIANLEKDIANLERDVAR |
| SYNZIP10 | NLLATLRSTAAVLENENHVLEKEKEKLRKEKEQLLNKLEAYK |
| SYNZIP13 | QKVEELKNKIAELENRNAVKKNRVAHLKQEIAYLKDELAAHEFE |
| SYNZIP15 | FENVTHEFILATLENENAKLRRLEAKLERELARLRNEVAWL |
| SYNZIP17 | NEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYK |
| SYNZIP18 | SIAATLENDLARLENENARLEKDIANLERDLAKLEREEAYF |
| SYNZIP19 | NELESLENKKEELKNRNEELKQKREQLKQKLAALRNKLDAYKNRL |
| SYNZIP22 | KRIAYLRKKIAALKKDNANLEKDIANLENEIERLIKEIKTLENEVASHEQ |
| SYNZIP23 | ALRAELKAKIALLRADNWALKRKAKDLRRLLRRLRNKAEELK |
| WinZip-A1 rv | LQAVQERLRQVRSKLEYNQARLTKVKEELQAV |
| WinZip-B1 rv | LKEVKKRLQAVKTKLAYNEDQLQDVEAQLEDV |
| K-peptide rv | EKLASVKEKLASVKEKLASVKEKLASVKEKLASVK |
| E-peptide rv | KELASVEKELASVEKELASVEKELASVEKELASVE |
| K3-peptide rv | EKLAAIKEKLAAIKEKLAAIK |
| K4-peptide rv | EKLAAIKEKLAAIKEKLAAIKEKLAAIK |
| K5-peptide rv | EKLAAIKEKLAAIKEKLAAIKEKLAAIKEKLAAIK |
| E3-peptide rv | KELAAIEKELAAIEKELAAIE |
| E4-peptide rv | KELAAIEKELAAIEKELAAIEKELAAIE |
| E5-peptide rv | KELAAIEKELAAIEKELAAIEKELAAIEKELAAIE |
| SYNZIP1 rv | EEIKKRLNAIEKELYAILDKKHLNKKKLTENENELSAVENELQAVLN |
| SYNZIP2 rv | QEHSAVENELRAIEDRLNAIIKELNQEDRELQLNDKKLRAIKKRLYANRA |
| SYNZIP3 rv | KKHALRNRLAAKEKRLRAIEKELYANENEIFAADNELTTVEN |
| SYNZIP4 rv | EAVDNELRAVENELTALENKLYANRNKLEEVKNKLQENRNLKVAVRNKLEAVKQ |
| SYNZIP5 rv | EFKHAALEFELEAKAFKLHEKLNKLEANREELEQIYNKLEKVTN |
| SYNZIP6 rv | RAVDRELNAIDKELNAIDKELNANDNELRAVINELKANEKLKYAVRNKLQAVKQ |
| SYNZIP10 rv | KYAELKNLLQEKEKRLKEKEKELVHNENELVAATSRLTALLN |
| SYNZIP13 rv | EFEHAALEDKLYAIEQKLHAVRNKKVANRNELEAIKNKLEEVKQ |
| SYNZIP15 rv | LWAVENRLRALERELKAELRRLKANENELTALIFEHTVNEF |
| SYNZIP17 rv | KYAEIEKRLNAIKQKLQERKQKLQEIRNRLEAKKSKLEEKEN |
| SYNZIP18 rv | FYAEERELKALDRELNAIDKELRANENELRALDNELTAAIS |
| SYNZIP19 rv | LRNKYADLKNRLAALKQKLQERKQKLEENRNKLEEKKNELSELEN |
| SYNZIP22 rv | QEHSAVENELTKIEKILREIENELNAIDKELNANDKKLAAIKKRLYAIRK |
| SYNZIP23 rv | KLEEAKNRLRRLLRRLDKAKRKLAWNDARLLAIKAKLEARL |

*rv represents reversed sequence.

**Table S1. Sequences of dimerized peptides used in this study.**

| Rank | Peptide fused with PU | Peptide fused with RU | Split region | Intensity | □ | Rank | Peptide fused with PU | Peptide fused with RU | Split region | Intensity |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | E-peptide rv | SYNZIP5 | 228/229 | 1.96 | □ | 47 | K-peptide | SYNZIP5 | 228/229 | 0.18 |
| 2 | E-peptide | SYNZIP1 | 228/229 | 1.04 | □ | 48 | K5-peptide | WinZip-B1 rv | 228/229 | 0.18 |
| 3 | E3-peptide rv | SYNZIP5 | 228/229 | 1.01 | □ | 49 | E4-peptide rv | SYNZIP1 | 228/229 | 0.17 |
| 4 | E-peptide | SYNZIP5 | 228/229 | 0.73 | □ | 50 | E4-peptide | WinZip-B1 | 228/229 | 0.17 |
| 5 | E4-peptide | WinZip-B1 | 91/92 | 0.71 | □ | 51 | E-peptide | SYNZIP15 | 228/229 | 0.17 |
| 6 | E-peptide | SYNZIP22 | 228/229 | 0.62 | □ | 52 | WinZip-B1 rv | K3-peptide | 228/229 | 0.16 |
| 7 | E-peptide rv | SYNZIP4 | 228/229 | 0.59 | □ | 53 | SYNZIP22 | SYNZIP2 | 228/229 | 0.15 |
| 8 | E3-peptide rv | SYNZIP1 | 228/229 | 0.52 | □ | 54 | E3-peptide rv | SYNZIP13 | 228/229 | 0.15 |
| 9 | E4-peptide rv | SYNZIP5 | 228/229 | 0.43 | □ | 55 | SYNZIP22 | SYNZIP22 | 228/229 | 0.15 |
| 10 | E3-peptide rv | SYNZIP4 | 228/229 | 0.42 | □ | 56 | E3-peptide rv | SYNZIP2 | 228/229 | 0.15 |
| 11 | E4-peptide rv | SYNZIP3 | 228/229 | 0.37 | □ | 57 | E-peptide | SYNZIP1 rv | 228/229 | 0.15 |
| 12 | WinZip-B1 rv | SYNZIP4 | 228/229 | 0.37 | □ | 58 | WinZip-A1 | E5-peptide | 228/229 | 0.14 |
| 13 | E4-peptide rv | SYNZIP4 | 228/229 | 0.36 | □ | 59 | SYNZIP19 | SYNZIP22 | 228/229 | 0.14 |
| 14 | E4-peptide | SYNZIP1 | 228/229 | 0.36 | □ | 60 | E-peptide rv | SYNZIP1 rv | 228/229 | 0.14 |
| 15 | WinZip-B1 rv | E3-peptide | 228/229 | 0.35 | □ | 61 | E4-peptide | SYNZIP17 rv | 228/229 | 0.14 |
| 16 | E-peptide | WinZip-B1 | 228/229 | 0.34 | □ | 62 | K3-peptide | SYNZIP2 | 228/229 | 0.14 |
| 17 | WinZip-A1 rv | SYNZIP22 | 228/229 | 0.34 | □ | 63 | WinZip-B1 rv | SYNZIP2 | 228/229 | 0.14 |
| 18 | E-peptide | K3-peptide | 228/229 | 0.34 | □ | 64 | E4-peptide rv | SYNZIP22 | 228/229 | 0.14 |
| 19 | WinZip-B1 rv | SYNZIP22 | 228/229 | 0.33 | □ | 65 | E-peptide | E3-peptide | 228/229 | 0.14 |
| 20 | E-peptide | SYNZIP2 | 228/229 | 0.32 | □ | 66 | E3-peptide | SYNZIP5 | 228/229 | 0.14 |
| 21 | E3-peptide | SYNZIP1 | 228/229 | 0.32 | □ | 67 | E-peptide | WinZip-A1 rv | 228/229 | 0.13 |
| 22 | E-peptide | SYNZIP4 | 228/229 | 0.32 | □ | 68 | E4-peptide | SYNZIP22 | 228/229 | 0.13 |
| 23 | E-peptide rv | SYNZIP1 | 228/229 | 0.31 | □ | 69 | E-peptide rv | WinZip-A1 rv | 228/229 | 0.13 |
| 24 | E-peptide | WinZip-B1 rv | 228/229 | 0.31 | □ | 70 | E3-peptide rv | WinZip-B1 rv | 228/229 | 0.13 |
| 25 | E-peptide rv | SYNZIP22 | 228/229 | 0.30 | □ | 71 | E-peptide rv | SYNZIP2 | 228/229 | 0.12 |
| 26 | E4-peptide | SYNZIP4 | 228/229 | 0.30 | □ | 72 | E3-peptide | WinZip-B1 | 228/229 | 0.12 |
| 27 | E4-peptide | SYNZIP5 | 228/229 | 0.29 | □ | 73 | SYNZIP4 | SYNZIP22 | 228/229 | 0.12 |
| 28 | K3-peptide | E3-peptide | 228/229 | 0.29 | □ | 74 | SYNZIP10 rv | SYNZIP22 | 228/229 | 0.12 |
| 29 | SYNZIP22 | SYNZIP4 | 228/229 | 0.28 | □ | 75 | E-peptide | SYNZIP18 | 228/229 | 0.12 |
| 30 | E3-peptide rv | WinZip-B1 | 228/229 | 0.28 | □ | 76 | WinZip-A1 | WinZip-B1 rv | 228/229 | 0.12 |
| 31 | E3-peptide rv | SYNZIP22 | 228/229 | 0.27 | □ | 77 | WinZip-A1 | SYNZIP5 | 228/229 | 0.12 |
| 32 | WinZip-A1 | SYNZIP22 | 228/229 | 0.27 | □ | 78 | E-peptide rv | E-peptide | 228/229 | 0.11 |
| 33 | WinZip-A1 rv | E3-peptide | 228/229 | 0.26 | □ | 79 | E3-peptide rv | SYNZIP6 | 228/229 | 0.11 |
| 34 | E-peptide rv | WinZip-B1 | 228/229 | 0.26 | □ | 80 | SYNZIP3 | SYNZIP22 rv | 228/229 | 0.11 |
| 35 | WinZip-A1 | SYNZIP1 | 228/229 | 0.25 | □ | 81 | K3-peptide | SYNZIP1 | 228/229 | 0.11 |
| 36 | E3-peptide rv | SYNZIP3 | 228/229 | 0.24 | □ | 82 | K4-peptide | SYNZIP4 | 228/229 | 0.11 |
| 37 | E4-peptide rv | SYNZIP13 | 228/229 | 0.22 | □ | 83 | WinZip-A1 rv | SYNZIP18 | 228/229 | 0.11 |
| 38 | WinZip-B1 rv | SYNZIP1 | 228/229 | 0.22 | □ | 84 | K4-peptide | SYNZIP22 | 228/229 | 0.11 |
| 39 | K3-peptide | SYNZIP22 | 228/229 | 0.21 | □ | 85 | E-peptide rv | SYNZIP6 | 228/229 | 0.11 |
| 40 | WinZip-A1 | E3-peptide | 228/229 | 0.21 | □ | 86 | E4-peptide rv | SYNZIP22 rv | 228/229 | 0.11 |
| 41 | K-peptide | SYNZIP22 | 228/229 | 0.21 | □ | 87 | SYNZIP17 rv | SYNZIP22 | 228/229 | 0.10 |
| 42 | WinZip-A1 rv | SYNZIP4 | 228/229 | 0.21 | □ | 88 | WinZip-A1 | SYNZIP18 | 228/229 | 0.10 |
| 43 | WinZip-A1 rv | SYNZIP2 | 228/229 | 0.21 | □ | 89 | E3-peptide rv | SYNZIP1 rv | 228/229 | 0.10 |
| 44 | E4-peptide | SYNZIP2 | 228/229 | 0.20 | □ | 90 | E3-peptide | SYNZIP2 | 228/229 | 0.10 |
| 45 | E-peptide rv | WinZip-B1 rv | 228/229 | 0.19 | □ | 91 | E4-peptide | K5-peptide | 91/92 | 0.10 |
| 46 | WinZip-A1 rv | SYNZIP1 | 228/229 | 0.18 | □ | 92 | K5-peptide | K4-peptide | 228/229 | 0.10 |

**Table S2. List of efficient reconstruction peptide pairs.**