SUPPLEMENTARY INFORMATION

An optogenetic gene expression system with rapid activation and deactivation kinetics
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This supplementary information contains the Supplementary Figures 1-5, Supplementary Tables, legends for Supplementary Videos, Supplementary Notes, and Supplementary References.
Supplementary Figure 1. VP-EL222 is expressed in 293T cells and can function to activate luciferase expression in response to light. (a) Western blot analysis of VP-EL222 expression in cytoplasmic and nuclear fractions made from 293T cells transiently transfected with pVP-EL222. An ARNT antibody was used as a marker for nuclear localization. (b) The fold change (FC) in transcription in control versus VP-EL222 cells under dark and light conditions was
calculated by normalizing the luciferase levels (Fig. 1) to *Renilla* luciferase levels [FC_{dark or light} = (Firefly/*Renilla*)_{VP-EL222} / (Firefly/*Renilla*)_{empty or 293T}]. (n = 3 independent experiments, each performed in triplicate per condition).
Supplementary Figure 2. VP-EL222 is not activated by red light and that illumination with blue light has no negative effect on cell viability. (a) Results of cell viability assays using CellTiter-Blue reagent. 293T cells were transiently transfected with either empty vector or pVP-EL222 and pC120-Fluc and kept in the dark or illuminated with pulsing blue light (20 s on, 60 s off) for 24 hr. Subsequently, the cells were incubated with CellTiter-Blue for 2 hr and the resultant fluorescence (544 Ex/590 Em) was recorded (n = 2 independent experiments, each performed with six replicates per condition). (b, c) 293T cells were transiently transfected with either empty vector or pVP-EL222 and pC120-Fluc, at 24 hr post-transfection cells were kept in the dark or illuminated with continuous red light for 24 hr. Afterwards, luciferase levels were measured (c) and then used to calculate the fold change in gene expression (d) in cells expressing VP-EL222 versus empty vector control (n = 1 independent experiment performed with three replicates per condition). All data are represented as mean ± s.d.
Supplementary Figure 3. Light-triggered expression of luciferase protein by VP-EL222.

Western blot analysis of luciferase expression in VP-EL222 cells (+) or wild-type 293T cells (−) after illuminating with blue light or kept in the dark. Untransfected control (unt).
Supplementary Figure 4. Full Western blots and denaturing polyacrylamide gel of RT-PCR samples. (a) Blots were probed with antibodies against FLAG, VP16, and hnRNP L. EL222-VP16 was driven from an Actin promoter (Act; used in Fig. 4a) or Ubiquitin promoter (Ubi, not used in subsequent studies). Arrow indicates band of interest. (b) RT-PCR to quantify splicing of endogenous CELF2. Lane 1, wild-type JSL1 cells. Lanes 2-7, JSL1 cells containing VP16-EL222 driven from Actin promoter (Act). Lanes 4-5, wild-type JSL1 cells. Lanes 6-7, JSL1 cells containing VP16-EL222 driven from Ubiquitin promoter (Ubi). Lanes 2-5 are shown.
in Fig. 4b (lanes 2-3, right side of panel; lanes 4-5, left side of panel). Dark conditions (D); Light conditions (L); Molecular weight marker (M).
Supplementary Figure 5. VP-EL222 is only moderately toxic in zebrafish. Dose-response curve showing (a) the effect of VP-EL222 mRNA expression on zebrafish development as compared to (b) expression of a control GFP mRNA (right). Embryos were injected at the one to two-cell stage and illuminated with constant blue light until they were scored at 24 h.p.f. (at least \( n = 100 \) embryos per condition).
SUPPLEMENTARY TABLES

Supplementary Table 1. Occurrence of C120 sequence in human, mouse and zebrafish genomes. The BLASTN 2.2.28+[3] program was used to search the indicated nucleotide databases for the EL222 binding site Clone-1 20 bp (C120) sequence. For comparison, the same databases were searched for the GAL4-specific Upstream Activation Sequence (UAS). To identify the top hits in each search, we chose a cutoff Expect (E) value < 10 and required that the sequences have no gaps or mismatches to original query sequence.

Search sequences:

- **C120 (20 bp)**: TAGTAGCCTTTAGCCATG
- **UAS (20 bp)**: GGAGGACAGTACTCCGCTC* (one extra base added to make 20 bp query)

| Organism       | Database searched     | # of sequences in database | C120 # of hits (E value <1000 / E value <10) | UAS # of hits (E value <1000 / E value <10) |
|----------------|-----------------------|----------------------------|---------------------------------------------|---------------------------------------------|
| **Homo sapiens** | Ref Seq Genomic       | 22,540                     | 200 / 14                                   | 426 / 7                                     |
| **Mus musculus** | Ref Seq Genomic       | 20,034                     | 200 / 22                                   | 486 / 0                                     |
| **Danio rerio**  | Genome (ref only)     | 4,560                      | 217 / 6                                    | 131 / 0                                     |

Top hits with E value <10, no mismatches, no gaps

| Organism       | C120                        | UAS                        |
|----------------|-----------------------------|----------------------------|
| **Homo sapiens** | 8 hits match 17 bp          | 7 hits match 17 bp         |
| **Mus musculus** | 18 hits match 16 bp         | 0 hits match 15 bp         |
| **Danio rerio**  | 6 hits match 15 bp          | 0 hits match 14 bp         |
SUPPLEMENTARY VIDEOS

Supplementary Video 1.  Z-stack of 70% epiboly embryo showing mosaic expression of mCherry after illumination with blue light. Representative zebrafish embryo after injection with both VP-EL222 mRNA and pC120-mCherry DNA and illumination with constant blue light for 5 hr beginning at 2 h.p.f. Fluorescent and brightfield images were acquired every 2.58 µm on a Digital Scanned Laser Light Sheet Microscope[1]. The two channels were merged and the z-stack converted into a video on ImageJ[2]. Mosaic expression is due to random incorporation of the pC120-mCherry DNA into cells as the embryo develops.

Supplementary Video 2. Z-stack of 70% epiboly embryo showing no expression of mCherry under dark conditions. Representative zebrafish embryo after injection with both VP-EL222 mRNA and pC120-mCherry DNA and kept in the dark for 7 hr. Fluorescent and brightfield images were acquired every 2.58 µm on a Digital Scanned Laser Light Sheet Microscope[1]. The two channels were merged and the z-stack converted into a video on ImageJ[2].

Supplementary Video 3. Localization of fluorescent mCherry in the heart of a developing zebrafish embryo at 24 h.p.f. Representative zebrafish embryo’s heart after injection of both pminiTol2-myl7-VP-EL222-C120-mCherry DNA and transposase mRNA and illumination with constant blue light for 14 hr beginning at 10 h.p.f. Fluorescent images of a single plane within the zebrafish heart were acquired by time-lapse epifluorescent microscopy (Nikon Ti-E). Frames were acquired every 144 ms for 10 s. Playback is 7 frames/s.
Supplementary Note 1. Algorithm for kinetic modeling of VP-EL222 activation
parameters. To examine the dependence of EL222-based transcriptional activation on properties of the engineered VP-EL222 protein, we developed a conceptual model for the effect of pulsed blue light activation on EL222-driven transcription. We assumed that each pulse of light triggers 3 phases of gene expression (Fig. 3a):

1). A sigmoidal buildup phase characterized by EL222 activation and dimerization, DNA binding and transcriptional activation through the point of RNA Pol II promoter clearance, described by the collective rate constant $\tau_{on}$. We checked for the need for cooperativity in this process, as implemented by Hill coefficients (h) between 1-5; while it was essential to have some degree of cooperativity (h > 1), we found minimal variation in the quality of fitting our experimental data for values between 2-5. Of these, h=4 provided the most optimal fit and was chosen for these simulations.

2). Once $t > \tau_{on}$, transcriptional activity saturates and enters a steady state phase, where transcription occurs at a maximal rate for as long as the cell is illuminated.

3). Once illumination ceases, active VP-EL222 decays as a first order exponential at a rate of $\tau_{off}$, a process we assume to be likely dominated by cleavage of the cysteine/flavin adduct within the EL222 LOV domain. Transcriptional activity falls subsequently as EL222 reverts to the monomeric dark state, free from DNA.

For the purposes of the simulation, luciferase activity was taken to reflect the sum of all transcriptional activity over time, where each EL222 binding event generates one or more
luciferase mRNAs provided that it remains on the promoter long enough for RNA polymerase commitment to generating a full-length transcript. In order for transcriptional activity to move away from initialized values and reach steady state, the model was allowed to run for 10 light-dark cycles, each 80 s long, with an illumination time between 0 and 20 s. The EL222 concentration was assumed to be at steady-state and unaffected during these ten cycles.

Predicted luciferase activities for a given $\tau_{on}, \tau_{off}$ pair were normalized and compared to experimental values. For illumination times of 0, 2, 5, 10 and 20 s, the difference between the predicted and actual value for luciferase activity was computed and squared (Fig. 3b). The sum of these five errors was assumed to reflect the overall quality of the prediction. To determine $\tau_{on}, \tau_{off}$ pairs that gave the least squared error, a simple grid search was implemented in MATLAB version R2012a (code provided as Supplementary Note 2), where the error function was evaluated at all combinations of $\tau_{on}$ and $\tau_{off}$ for values of $\tau_{on} = 1$-$100$ s and $\tau_{off} = 1$-$100$ s (Fig. 3e shows expansion of values between $\tau_{on} = 1$-$10$ s and $\tau_{off} = 1$-$100$ s).

Supplementary Note 2. MATLAB code for kinetic model (provided as a separate file).
Supplementary Note 3. DNA and plasmid sequence information.

AN45[4]   GGCCCCGAGGTCCAGCAACGCCATCCCTTTGTCGCAAGCGAC
C120[5]   TAGGTCATTTTAGTCCATG

1. p(AN45)-Fluc construct
This construct was used for transient transfections. Three copies of the 45 bp version of the AN45 DNA sequence were cloned into the pGL4.23 vector (Promega) using XhoI (5’end) and HindIII (3’end).

Bold= restriction sites
Underlined= AN45 repeats
Yellow= minimal TATA-box promoter
Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTAAGTCCGGTACCTGACGTAGCTAGCCCCGAGGTCGACGCGCAGCAGACGGGGTACGCCGACGGCCGGAGGCTGCAGCCACCGGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGCCGAC
C120[5]   TAGGTCATTTTAGTCCATG

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Bold= restriction sites
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Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTAAGTCCGGTACCTGACGTAGCTAGCCCCGAGGTCGACGCGCAGCAGACGGGGTACGCCGACGGCCGGAGGCTGCAGCCACCGGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGCCGAC
C120[5]   TAGGTCATTTTAGTCCATG

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Bold= restriction sites
Underlined= AN45 repeats
Yellow= minimal TATA-box promoter
Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTAAGTCCGGTACCTGACGTAGCTAGCCCCGAGGTCGACGCGCAGCAGACGGGGTACGCCGACGGCCGGAGGCTGCAGCCACCGGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGCCGAC
C120[5]   TAGGTCATTTTAGTCCATG

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Vector sequence:

GGCCTAAGTCCGGTACCTGACGTAGCTAGCCCCGAGGTCGACGCGCAGCAGACGGGGTACGCCGACGGCCGGAGGCTGCAGCCACCGGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGCCGAC
C120[5]   TAGGTCATTTTAGTCCATG

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Bold= restriction sites
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Vector sequence:

GGCCTAAGTCCGGTACCTGACGTAGCTAGCCCCGAGGTCGACGCGCAGCAGACGGGGTACGCCGACGGCCGGAGGCTGCAGCCACCGGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGCCGAC
C120[5]   TAGGTCATTTTAGTCCATG

1. p(AN45)-Fluc construct
This construct was used for transient transfections. Three copies of the 45 bp version of the AN45 DNA sequence were cloned into the pGL4.23 vector (Promega) using XhoI (5’end) and HindIII (3’end).

Bold= restriction sites
Underlined= AN45 repeats
Yellow= minimal TATA-box promoter
Green= Firefly Luciferase open reading frame

Vector sequence:
2. pcDNA-C120-empty construct

Five copies of the 20 bp version of the Clone-1 DNA sequence were cloned into was cloned into the pcDNA3.1(+) vector (Invitrogen) using BlgII (5’end) and BamHI (3’end).

**Bold**= restriction sites
**Underlined**= C120 repeats
Yellow = minimal TATA-box promoter

Vector sequence:

GACCGGATCCGGGAGATCTTCGCTAGCCTCGAGTATTCTAATGCATCCTCGGTTATATGGTAGGCTGCTGCATAGCTGCCCTGCATCTTCTGGGTTACGCGCTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTCTGCAGCGCCCGCGCTCCCGGCTACTGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACAC...
For the purposes of clarity, the two reporter gene constructs (pGL4.23-C120-Fluc and pcDNA-C120-Fluc) used in this study are both referred to as pC120-Fluc in the text. Nevertheless, the specific experiments in which each construct was used are clearly listed in the Methods section. In general, pGL4.23-C120-Fluc was used for transient transfections, and pcDNA-C120-Fluc was used for transfection of VP-EL222 stable cell line. The sequences for both constructs are listed below.

a) pGL4.23-C120-Fluc

This construct was used for transient transfections. Five copies of the 20 bp version of the Clone-
1 DNA sequence were cloned into the pGL4.23 vector (Promega) using XhoI (5’end) and HindIII (3’end).

**Bold= restriction sites**

**Underlined= C120 repeats**

**Yellow= minimal TATA-box promoter**

**Green= Firefly Luciferase open reading frame**

**Vector sequence:**

```
GGCTAACTGGCCGCTACCTGAGCTGCAGTTAGCTAGGTCAGTGGCCTAGGTTTAGCCATAGCTGT
AGGTAGCTTTAGTCCATGTCGACAGATCTACCTAGGCTGAAAGCCACCAGGAAAGATGCCAAACTTA
AGAAGGCCCGCGCCCATCTACCCACCCGCGGAGCCAGCGCGGACACGCAGTCAGCTACCAAGCCAC
CATGACGCTACCGCCTTCGTTCCCGGACACCCGCGGCCGCGGCAGCGGCGACGAGCCAGCCTGG
TGGAGGAGCCCGCCGAGGCTTGAGGCTGAGGCTGAGGCTGAGGCTGAGGCTGAGGCTGAGGCTG
CTTTAGTCCATGTCGACAGATCTACCTAGGCTGAAAGCCACCAGGAAAGATGCCAAACTTA
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b) pcDNA-C120-Fluc

This construct was used for transfection of VP-El222 stable cell line. The Firefly luciferase ORF (\textit{luc2} from Promega) was cloned into the pcDNA-C120-empty vector using EcoRI (5’end) and XbaI (3’end).

\textbf{Bold}= restriction sites
\textbf{Underlined}= C120 repeats
\textbf{Yellow}= minimal TATA-box promoter
\textbf{Green}= Firefly Luciferase open reading frame

\textbf{Vector sequence:}

\begin{verbatim}
GACGGGATCCGGGAGATCTTCGCTAGCCTCGAG
\end{verbatim}
4. pcDNA-C120-mCherry construct

The mCherry ORF was cloned into the pcDNA-C120-empty vector using EcoRI (5’end) and XbaI (3’end).

**Bold= restriction sites**

**Underlined= C120 repeats**

**Yellow= minimal TATA promoter**

**Blue= mCherry ORF**

**Vector sequence:**

```
GACGGATCGGGAGATCTTCGCTAGCCTTGAGGTAGGTTAGCTTTATAGCCTACTGCACTTCTAGTGAGTACCT
CTTGAGTGACTACATCGCTGATGAGCTTGCTTCTCCTATCATGAGGTAGATTTTGGGAGGGAATACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGACATGGAGGGCTCCGTGAACGGCCACGAGTTTCATCTACAAGGTGACCTGCGCGGCACCAACTTCCCCTCCGACGGCGAGGGCCGCCCTGACCAAGGACTACAGAGGCTGAA
GCTGACATGGGCGCTGGGAGGCGATGTAACGTGCTGCCAGGCGCAAGCTGACAGGCGATCTACATGAGCCTTCCTCAAGGTATACCCCTACAGGAGAGGACCTTCCCCAGGATTTAGTTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTT
TCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACCTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC
ATCTCAATTAGT
CAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGT
CAGCAACCAG
```
GCTCCCGGAGCTTTGATATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCAGCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGGACGAGGCAAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAATCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCATTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGA
GCTTGGCGGCGAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATAGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACCTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTG

AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTTCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTAGCAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATAGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACCTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTG
TTGAGATCCAGTTGATGAACCCACTCGTGCAACCCAACTGATCTTCAGCATCTTTTACTTTCA
CCAGCGTTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCCGC
AGCCGGAAATGGTGAATCTACATCTCTCTTCCTTTTTCAATATATTATGGAAGCATTITATCAGGGTTAT
TGCTTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACATAGGGTTCGCCGCAGCA
CATTTCCCCGAAAAATGCCCACCTGACGTC

5. p(UAS)$_5$-Fluc construct

This construct was used for transient transfections. Five consensus GAL4 binding sites (amplified from pG5-SEAP vector (Clontech)) were cloned into the pGL4.23 vector (Promega) using KpnI (5’end) and HindIII (3’end).

Bold= restriction sites
Underlined= UAS repeats
Yellow= minimal TATA-box promoter
Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTAAACTGGCGGGTACCATGACCATGATTACGCGCCAAAGCTAATTTCCGGATCCGACCTCGG
AGGACAGTACTCCGCTCGAGGACAGTACTCCGCTCGAGGACAGTACTCCGCTCGAGGACAGTACTCCGCTCGG
AAGCTT
AGACACTAGAGGGTATA
TAATGGAAGCTCGACTTCCAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCATAAGAAGGGCC
CAGCGCCATTCTACCCACTCGAAGACGGGACCGCCGGCGAGCAG
CTGCACAAAGCATGAAGCGCTACGCCCTGGTGCCCGGCACCATCGCCTTTACCGACGCACATA
TCGAGGTTGAGCATATTACCTACCGCAGTACTCTCGAGATGAGCGTTCGGCTGGCAGAAGCTATGAA
GCGCTATGGGCTGAATACAAACCATCGGATCGTGGTGTGCAGCGAGAATAGCTTGCAGTTCTTC
ATGCCCGTGTGTGGTTCGCTTCATCGTGTTGGTGTGCAGGACACCTCAACAGCAGTGCAGGAAAAGGG
GGCTGCAAAAGCATGACTCCGGATTCTACGCGGACTTCTGCGTATGCAGGGCCCAACCGTCTGCAG
GGTTCAGTGTGCAGGCTACGTTAACAACCCCGAGGCTACAAAGCTCTCATCGACAGGACGGCTGGCTGC
ACAGCGGCGACATCGCCTACTGGGACGAGGACGAGC
ACTTCTTCGACGCTCTGCTGCAGGTCGAGGACTTCTGCGTATGGATGGTTACACCTACGCGGACTTCTGCGT
GGCCTAGGAGTGGGACTGGTGGGACTTGGACACCGGTAAGACACTGGGTGTGAACCAGCGCGG
CGAGCTGTGCGTCGAGTCTACGCGGACTTCTGCGTATGGATGGTTACACCTACGCGGACTTCTGCGT
GGCCTAGGAGTGGGACTGGTGGGACTTGGACACCGGTAAGACACTGGGTGTGAACCAGCGCGG
CGAGCTGTGCGTCGAGTCTACGCGGACTTCTGCGTATGGATGGTTACACCTACGCGGACTTCTGCGT

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6. pVP-empty construct

Purchased from Clontech

Yellow= nuclear localization sequence
Green = VP16 activation domain

Vector sequence:

```
TATGTATCATACACATACGATTTAGGTGACACTATAGAACTCGACTGTGGAATGTGTGTCAGTT
AGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCAGTGGTGAAGATCCTCCAGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCAT
CTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATG
CAAAGCATGCATCATTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGC
AGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCC
AGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAG
```
7. pVP-EL222 construct

The EL222 ORF (amino acids 14-222) was cloned into the pVP16 vector (Clontech) using EcoRI (5’end) and SpeI/XbaI (3’end).

Bold= restriction sites
Yellow= nuclear localization sequence
Green= VP16 activation domain
Blue= EL222 sequence (aa 14-222)

Vector sequence:
ACTGACTCGCTGGCCTGGCTGTCTGCTGGCAGGCAGGATCTCAGTCACACTCAAGGGCGGCTAA
TACGGTTATATCCACAGAAATCCAGGCTAGATACAGGATCAGAAGAACTGAGCGACAAAGCAGA
GGCCAGGAAACCCTAAAAGGGCCTGGTCTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
ACTAGCTTACCAAGTCAAGGCAGAGGATCTCAGTCACACTCAAGGGCGGCTAA

The VP-EL222 ORF from the pVP-EL222 vector was cloned into the pIRES-puro vector (Clontech) using EcoRV (5’ end) and BamHI (3’ end).

Bold = restriction sites
Yellow = nuclear localization sequence
Green = VP16 activation domain
Blue = EL222 sequence (aa 14-222)

Vector sequence:
SUPPLEMENTARY REFERENCES

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